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ABSTRACT BOOK

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PLENARY INVITED LECTURES

Plenary Lecture
PL01
Robust high resolution MRI in anatomical and diffusion investigations by spatiotemporal encoding
Lucio Frydman[*1]
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Abstract: Over the last decade we and others have introduced and perfected so-called spatiotemporal encoding (SPEN) methodologies to collect multidimensional NMR spectra and images in a single scan. This talk will focus on the potential of this technique to deliver superior imaging information, particularly in comparison with established methods such as spin-echo EPI in the realm of diffusion MRI, and with fast-spin-echo/RARE in multi-shot anatomical MRI. Achievements that will be described include the acquisition of diffusion in vivo images at <100µm in-plane resolutions in challenging preclinical areas, hitherto unavailable characterizations of cancerous tissues by DWI and DTI in preclinical models and in patients, and sub-mm anatomical measurements in humans with unprecedented acceleration factors.

Plenary Lecture

PL02

Heavy mice and light things: using solid-state NMR spectroscopy to understand biological tissues in health and disease

Melinda Duer[*1]

[1]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

Abstract: The extracellular matrix (ECM) forms the bulk of our structural tissues and provides them with their particular mechanical properties. At the microscopic level, it provides the scaffold which supports cells but more intriguingly, at the molecular level, it provides the communication system between the cells in the tissue and the signals that drives the individual behaviour of cells. Ultimately, if we can understand how the extracellular matrix structure dictates the behaviour of cells, then we can develop ways to treat diseases such as cancer, by changing the extracellular matrix to drive the necessary change in cell behaviour. However, understanding the molecular level properties of the extracellular matrix has been hampered by the lack of methods to study tissues at the atomic scale. In this talk, I will describe the various solid-state NMR spectroscopy approaches my group has taken over the last decade to tackle these complex questions.

The first requirement is native-like tissues in which we can control isotope labelling patterns so that we can record assignable multidimensional NMR spectra. Using multidimensional solid-state correlation NMR spectra (^{13}C-^{13}C, ^{13}C-^{15}N) as fingerprints of the underlying molecular structures in isotope-labelled native tissues has allowed us to develop laboratory-grown tissues that have very similar molecular structures to native tissues, and thus represent demonstrably good models of native tissue. The refined laboratory-grown tissues can then be manipulated by growing them with isotope labels in specific components to allow detailed study of structure and function of the various extracellular matrix components. For instance, ^{13}C, ^{15}N labelling Gly and Pro in the collagen component has led to the unexpected conclusion that Gly-Pro-Hyp triplets in collagen act like springs allowing the collagen helix to flex, rather than these triplets being rigid structures as they have long been assumed to be.

We have now coupled this approach with NMR methods to examine calcified tissues in health, such as bone, and in diseases such as vascular calcification (hardening of the arteries). In calcified tissues, the extracellular matrix incorporates stacks of ordered nanocrystals of a complex calcium phosphate phase. Using an NMR crystallography approach, we have put forward a new model for the structure of bone mineral, and now have an understanding of what chemical species control bone mineral strength. In combination with the methods described above, we now also have an intriguing insight into what initiates hardening of the arteries, which leads to a potential route to prevent this condition, the major cause of cardiovascular disease worldwide.

Plenary Lecture

PL03

Isotope labeling in higher eukaryotes makes more proteins amenable to NMR: applications to signal transmission in GPCRs

Stephan Grzesiek[*1], Anne Grahl[1], Christian Opitz[1], Shin Isogai[1], Bastian Franke[1], Rajesh Sonti[1], Laraya Abiko[1]
[1]Biozentrum, University of Basel, Basel, Switzerland

Abstract: We extensively use isotope labeling in insect cells for NMR studies of proteins, which cannot be expressed in functional form in *E. coli*. Selective labeling is achieved easily by supplementing drop-out media with specifically ^{15}N- and ^{13}C-labeled amino acids [1,2]. Recently, also uniform labeling by ^{15}N, ^{13}C, and ^{2}H has become economically feasible by providing amino acids in form of isotope-labeled yeast or algal extracts [3-7]. Expression yields can be as high as ~80 mg/L even for 'difficult' soluble proteins such as kinases and ~2 mg/L of G protein coupled receptors (GPCRs). We will discuss practical aspects, limitations and NMR applications to detect allosteric signaling in Abelson kinase [1,2,8,9] and GPCRs.
In particular, we have recently shown that GPCR motions can be followed from ^{1}H-^{15}N resonances at virtually any backbone site in a thermostabilized mutant of the turkey β_1-adrenergic receptor [10]. We will describe current progress in the functional understanding of GPCR signal transmission based on relaxation data.
We will also discuss general insights on the effects of deuterium on life derived from large-scale proteomics data on the adaption of *E. coli* to a perdeuterated medium.

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Plenary Lecture

PL04

Time-Resolved NMR to characterize biomolecular refolding

Harald Schwalbe[*1]

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Abstract: Time-Resolved NMR to characterize biomolecular refolding Harald SchwalbeInstitute for Organic Chemistry and Chemical Biology, Center for Biomolecular Resonance, J.W. Goethe University of Frankfurt Max-von-Laue-Str.7, D-60438 Frankfurt am Main, schwalbe@nmr.uni-frankfurt.de ABSTRACT. In my presentation, the development and application of NMR methods to follow the kinetics of biomolecular folding in real-time will be presented. Different technologies for initiation of refolding reactions including mixing, light application and temperature jump will be discussed. Key applications to understand soluble and membrane protein folding, RNA and DNA refolding will be discussed to show the information obtainable by time-resolved NMR.

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Plenary Lecture

PL05

Probing actinide–ligand interactions by EPR spectroscopy

Eric McInnes[*1]

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Abstract: Our understanding of the bonding regimes in actinide (5f) materials lags behind that of the rest of the Periodic Table. However, this has become an increasingly important topic as early actinide (particularly uranium) chemistry has developed towards (for example) small molecule activation and materials synthesis, in addition to its obvious technological importance. As an example, some key chemical differences between actinides and lanthanides, and between different actinides, are thought to be due to minor differences in covalency in the metal-ligand bonding, yet covalency in the actinides is still a topic of much debate.

EPR spectroscopy, being specifically sensitive to the environment of unpaired electrons, should be a powerful tool to probe actinide-ligand interactions but (with some notable exceptions) have rarely been used beyond classic studies of actinide-doped mineral lattices in the 1970s. Even more surprisingly there is an almost complete lack of pulsed EPR studies of actinides in the literature which, given the broad linewidths in continuous wave (c.w.) EPR of such species, means that there are very few studies of ligand nuclear hyperfine interactions.

The fantastic developments in non-aqueous actinide chemistry, providing many new materials for study, makes this an opportune time to revisit this part of the Periodic Table with modern EPR methods. In this talk I will summarise our recent work on molecular actinide species including Th(III), U(III) and U(V) coordination and organometallic complexes, including comparison with lanthanide (4f) analogues where possible. These include examples where subtle differences in crystal field lead to gross changes in electronic ground state detectable by c.w. EPR, and investigation of ligand hyperfines by pulsed EPR methods including ESEEM (electron spin echo envelope modulation) and HYSCORE (hyperfine sublevel correlation; a 2D ESEEM technique).

Plenary Lecture

PL06

Order, disorder, and interactions in conjugated polymers and blends from solid-state NMR

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Abstract: The properties of conjugated polymer materials depend on complex compositional, architectural, and processing factors that influence the structures and interactions of their backbones and sidechains. These are further complicated by interactions with intercalated species that are introduced to impart charge transport properties, which are required for photovoltaic or transistor applications. In particular, the presence, causes, and consequences of local order and disorder in such systems are often subtle and have been challenging to establish. Nevertheless, advances in syntheses and molecular characterization of conjugated polymers increasingly enable molecular-level features to be measured and correlated with their macroscopic physicochemical properties. Solid-state NMR, especially two-dimensional techniques, provide improved resolution that yields detailed information on the local environments, interactions, and distributions of backbone, sidechain, and intercalated moieties in conjugated polymer materials. NMR analyses, together with X-ray scattering, molecular modelling, and macroscopic property measurements, yield insights on pi-pi interactions, backbone conformations, sidechain interdigitation, and structure-function relationships associated with bulk heterojunction materials. Recent results will be presented for conjugated polymers and conjugated polymer-fullerene blends, with emphases on the molecular interactions and distributions of compositional or structural order and disorder that influence the properties and performances of devices based on these materials

Plenary Lecture

PL07

Hyperpolarized Xenon-129 MRI Contrast Agents for Molecular Imaging

Ivan J. Dmochowski[*1], Serge Zemerov[1], Benjamin Roose[1]

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Abstract: For the past decade, our laboratory has explored the exciting potential of ^{129}Xe, which is spin-1/2, and can be hyperpolarized (HP) to give a 10^4-10^5 signal enhancement over the room-temperature Boltzmann population of nuclear spins. HP ^{129}Xe can be delivered to living organisms via inhalation or Xe-solution injection, and has been employed for imaging the lungs and brain of living mammals, including human. Moreover, the significant polarizability of xenon generates significant affinity for hydrophobic void spaces, which has been exploited for characterization of the cavities within inorganic zeolites as well as small-molecule organic hosts. Our laboratory has developed water-soluble cryptophane molecules with the highest-measured xenon affinity, and characterized both the thermodynamics and kinetics of these interactions. Using a NMR technique known as HP ^{129}Xe chemical exchange saturation transfer (Hyper-CEST), cryptophanes and other Xe host molecules can be detected at 1 picomolar concentration. Because Hyper-CEST is such a remarkably sensitive technique, structures with low-affinity Xe binding sites can be identified that are otherwise invisible by direct detection of HP ^{129}Xe NMR peaks. We have used this technique to study Xe diffusion within biomaterials. And, this opens the possibility of developing genetically-encoded protein reporters to visualize specific molecular processes by ^{129}Xe NMR/MRI. Most notably, the genetically encoded green fluorescent protein (GFP) reporter has enabled biomolecular imaging via fluorescence microscopy, but such reporters have been conspicuously lacking for *in vivo* magnetic resonance imaging (MRI). In 2016, we identified TEM-1 β-lactamase (bla) as a single-protein reporter for Hyper-CEST, with significant saturation contrast at 0.1 μM. Xenon exchange interactions with an allosteric site in bla gives rise to a unique saturation peak at 255 ppm, well removed (~60 ppm downfield) from the ^{129}Xe-H_2O peak. Useful saturation contrast is observed for bla expressed in bacterial cells and mammalian cells. More recently, we have identified maltose binding protein (MBP) and other periplasmic binding proteins as genetically encoded reporters for quantifying small molecule analytes in solution. Importantly, the Hyper-CEST effect is only observed in the MBP "closed" conformation, upon maltose binding. Opportunities for *in vivo* molecular imaging will be discussed.

Plenary Lecture

PL08

How Quantitative NMR Enables New Metabolomics Methods

Daniel Raftery[*1,2]

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Abstract: Highly complex biological samples present challenging analysis problems for the field of metabolomics. Ideally, platforms that provide broad metabolome coverage allow the opportunity for deep insights into biological problems, while excellent quantitation ensures good data quality and allows an ability to compare across studies. However, these goals can be difficult to achieve on a routine basis because the highly complex sample matrix often precludes reliable measurements of many metabolites and complicates quantitation efforts. Due to its exquisite quantitative capabilities and reproducibility, NMR spectroscopy has much to offer the field of metabolomics, especially in the area of new methods development.

We can exploit NMR's quantitative abilities using a simple protein precipitation procedure that allows the absolute quantitation of over 70 metabolites using a single standard compound. These metabolites, including some at even sub-micromolar concentrations, span a broad range of classes and pathways, including organic and amino acids, as well as energy metabolites and co-enzymes (NAD+, NADH, NADP+, NADPH), which we show can be measured simultaneously in a variety of tissue extracts and even whole human blood. This quantitative NMR approach is also useful for calibrating quantitative mass spectrometry measurements, *without the use of internal or external metabolite standards* and has led to some unusual findings about the stability of well-known metabolites such as glutamine and others.

Combining NMR's quantitative capabilities with statistical methods has led to an improved method to aid identification of metabolites in complex samples. In a new approach we call "eRANSY", a new solvent extraction protocol and Ratio Analysis is used to simplify the NMR spectra dramatically to allow for improved unknown identification. Finally, we are using an old econometrics approach called seemingly unrelated regression (SUR) that allows us to model the effects of so-called confounding factors such as age, BMI, gender, and etc., which have a significant effect on the measured metabolite levels. We show that SUR can unravel these effects well, and by building models using groups of biologically related metabolites we can achieve improved differentiation of patients in the context of colon cancer and polyp detection.

Plenary Lecture

PL09

Hyperfine spectroscopy and distance measurements to study ATP induced conformational changes

Daniella Goldfarb[*1]

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Abstract: ATP dependent conformational changes represent a central motif in the catalytic cycle of many classes of important proteins with very different functions. In such systems full understanding of the protein mechanism requires detailed information about local structural rearrangements accruing at the ATPase site, the large scale conformational changes and their coupling. We are exploring this by combining EPR based hyperfine spectroscopy with pulse dipolar spectroscopy at high field (W-band, 95 GHz, ~3.5 T). The ATPase site, where Mg^{2+}-ATP binds and is hydrolyzes, is studied by substituting the essential Mg^{2+} co-factor with paramagnetic Mn^{2+} and exploring its coordination shell with the hyperfine spectroscopic techniques ENDOR (electron-electron double resonance) and ELDOR (electron-electron double resonance) –detected NMR. By targeting the ^{31}P nuclear couplings of the nucleotide phosphates we track the hydrolysis process, while the interaction with the protein is probed via ^{14}N coupling with protein residues. The conformational change in the different states of hydrolysis is followed by double electron-electron resonance (DEER) distance measurements between two spin labels situated at strategic location of the proteins, which are sensitive to the conformational changes induced by the binding and hydrolysis of ATP. The spin labels we employ are nitroxide or Gd(II) based and occasionally also the substituted Mn^{2+} itself can be used. The possibility of using triple labeling for extracting three distances is a new possibility to be explored. The application of this approach will be demonstrated on a membrane transporter, an RNA helicase and a folding chaperone.

Plenary Lecture

PL10

Non-covalent and covalent bonding from a multinuclear solid-state magnetic resonance perspective: structure, symmetry, and dynamic processes

David Bryce[*1]

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Abstract:

We report on recent advances from our laboratory in the framework of the following two broad research themes: (i) non-covalent electrophilic interactions studied via solid-state NMR spectroscopy and (ii) dynamics and equivalence from *J* splittings associated with pairs of quadrupolar nuclei in solids.

The hydrogen bond is widely recognized as a ubiquitous non-covalent interaction. More recently, a number of related interactions, named after the electrophilic site, have taken on increased prominence in chemistry, biochemistry, materials science, and crystal engineering. For example, the halogen bond results from the donation of electrons from a Lewis base (Y) towards the electron-deficient σ-hole found on the halogen atom opposite a covalent bond, i.e., R-X···Y. We report on multinuclear powder and single-crystal solid-state magnetic resonance studies of a range of cocrystals and materials featuring halogen bonds, chalcogen bonds, and tetrel bonds [1]. In addition to establishing relationships between the various NMR parameters and the nature of these electrophilic interactions, we also describe a case study focussing on real-time in-situ kinetic monitoring via ^{31}P CP/MAS NMR of mechanochemical halogen bond formation in the NMR rotor [2]. The catalysis of dynamical processes via halogen bonding, as studied by deuterium NMR, will also be discussed.

In the second part of the talk, we describe two-dimensional double-quantum filtered *J*-resolved solid-state NMR experiments as applied to homonuclear pairs of quadrupolar nuclei. Such experiments provide valuable information on crystallographic symmetry, bond order, electronic structure, and molecular dynamics. Results for ^{11}B-^{11}B and ^{71}Ga-^{71}Ga spin pairs in singly, doubly, and triply-bonded systems will be presented [3,4]. The crucial role of dynamics in the interpretation of the experimental data will be highlighted for a series of synthetically important electron-precise dianionic diboranes featuring two-centre two-electron bonds [5].

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Plenary Lecture

PL11

Relaxing hardware constraints: shifting the burden to software to make faster, portable and motion tolerant images

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Abstract: The textbook formulation of MRI is typically framed with uniform fields, well-controlled linear gradients and stationary objects. If violated, corrections are imposed to nudge the image back into place. If instead we replace image reconstruction with a more general optimization-based strategy, the increased computational burden can buy us important benefits by relaxing the hardware requirements. We show 3 recent projects which utilize this approach. The image reconstruction is formed as a general optimization problem with a forward model that relates the image pixels (typically formatted as one long vector) to the acquired kspace data (also one long vector, with the different RF Rx coil's data concatenated). For standard Fourier imaging the model can be inverted with the inverse DFT. The strategy for the more general case poses a data-consistency optimization problem asking "what is the data vector optimally consistent (in the least squares sense) to the acquired data and the forward model (encoding matrix)?" which is iteratively solved and possibly subject to some prior knowledge constraints (such as image sparsity in some domain).

In two of our applications, we expand this formalism to solve a joint optimization problem. Namely we solve for both the image pixels (typically about 10^6 - 10^7 unknowns) and some "nuisance parameters" which are also unknown. These nuisance parameters are not of direct interest, but if not accounted for in the forward model lead to artifacts. In our first example, these parameters represent the unknown miscalibration of the gradient trajectory. The traditional approach would either calibrate the scanner so these error parameters were zero or measure them and include them in the forward model so only the image unknowns need to be estimated. By jointly solving for both the image and these nuisance variables, the problem can be eliminated without adding a complex service calibration or measurement system (such as field probes or motion trackers.)

The first experiment applies this to an acquisition with a tight cork-screw trajectory.(1) For highly under-sampled acquisitions this provides nearly incoherent aliasing (including in the readout direction) which is highly advantageous for parallel imaging and/or compressive sensing reconstruction. However, small errors in the trajectory lead to image artifacts that varied significantly from scanner to scanner. A service calibration could eliminate the problem, but was not easily generalized to all image orientations. Similarly field probes could measure these errors, but add costly hardware. Instead, the errors are parameterized by a small number of unknowns and jointly estimated along with the object using the data-consistency framework.(1)

In the second experiment, we set our sites on the "nuisance variable" of head motion during a clinical Turbo Spin Echo (TSE, aka FSE) acquisition.(2) In the TAMER method, we form the forward model to include rotation and translation matrices which describe the rigid body position of the head at the time of every shot (TSE echo train). Without trackers or navigators, we must treat these as unknowns. Fortunately modern array coils encode the motion of the head through changes of the signal at the individual detectors. Thus a joint estimation of both image pixels and motion parameters is possible. The joint optimization is computationally burdensome, so a reduced pixel set must be found to speed up the iterations. Validation for the method in a rotating pineapple is shown in Fig. 2.

Using a general forward-model inverse approach, we demonstrate a 140 kg permanent magnet (B_0 = 0.1T) with a highly inhomogeneous field (~0.5%) as a potential portable brain imager. We eliminate switching gradients and instead rotate the inhomogeneous field pattern taking spin-echoes at each rotation angle. We reconstruct the image using the generalized method described above.

Finally, Machine Learning approaches appear poised to either completely take over the model-based reconstruction, offering a very general form of model, or perhaps less scary, do some of the more difficult and computation-time consuming steps.

References and Acknowledgements: The work was done with the authors referenced below.
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Image:

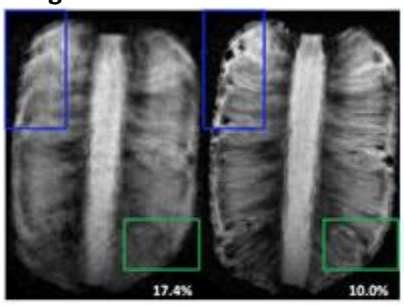

Fig. 2 The TAMER method applied to a rotating pineapple.

Fig. 3 Rotating Halbach magnet. The field inhomogenieties serve as image encoding fields.

Plenary Lecture
PL12
The Quantum Science of Atoms on Surfaces –
Single spin Electron Spin Resonance

Andreas Heinrich[*1]
[1]Center for Quantum Nanoscience, Institute for Basic Science, Seoul, Korea, Republic Of

Abstract: The scanning tunneling microscope is an amazing tool because of its atomic-scale spatial resolution. This can be combined with the use of low temperatures, culminating in precise atom manipulation and spectroscopy with microvolt energy resolution. In this talk we will apply these techniques to the investigation of the quantum spin properties of magnetic atoms sitting on thin insulating films.
We will start our exploration with the understanding of the magnetic states of transition metal atoms measured by Spin Excitation Spectroscopy (*Science* 2004) and including magnetic anisotropy (*Science 2006*).
Then we will explore the superposition of quantum states which is inherent to spin resonance techniques. We recently demonstrated the use of electron spin resonance on single Fe atoms on MgO (*Science* 2015). This technique combines the power of STM of atomic-scale spectroscopy with the unprecedented energy resolution of spin resonance techniques, which is about 10,000 times better than normal tunneling spectroscopy. ESR-STM can be used to build engineered dimers with dipolar and exchange interactions (*PRL* 2017). Finally, we will discuss the role of nuclear spins in ESR-STM (submitted).

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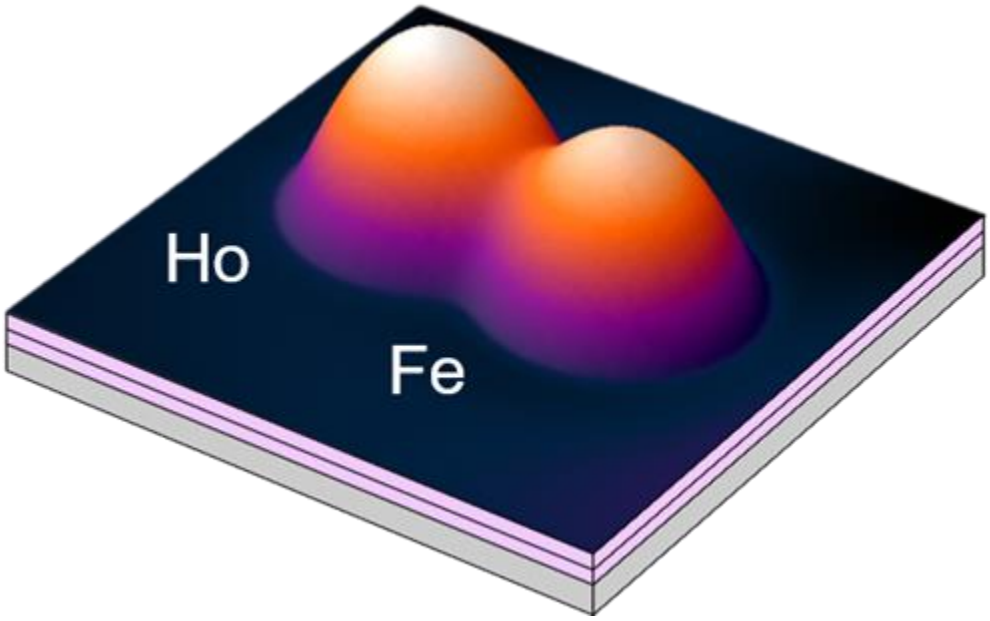
Image:



Plenary Lecture
PL13
Nuclear Spins Far From Equilibrium
Malcolm H. Levitt[*] [1]
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Abstract: Most of the nuclear magnetic resonance (NMR) methods applied today employ nuclear spin systems which remain very close to a state of thermal equilibrium with respect to the molecular environment. It is now possible to prepare substances in which the nuclear spin systems are very far from thermal equilibrium. Such systems may, in some cases, give rise to NMR signals which are highly enhanced with respect to signals obtained under routine conditions. There are also modes of non-equilibrium nuclear spin order which are non-magnetic and do not give rise to NMR signals, but which are relatively long-lived, allowing the non-equilibrium state to be maintained for a relatively long time. Methods exist for extracting hyperpolarized spin order from such non-magnetic non-equilibrium long-lived states, on demand. In some cases, highly non-equilibrium nuclear spin order even gives rise to non-magnetic effects, such as a change in the dielectric constant of the material.

I will review the types of non-equilibrium spin order that exist, describe how they are prepared, how they may be interconverted, and how they may be applied. I will present some of the following:

- Ortho-para conversion of fullerene-encapsulated water at room temperature
- Quantum-rotor-polarization effects in ^{17}O-labelled fullerene-encapsulated water
- Dissolution DNP with rapid transfer of the polarized solid to a second magnet ("bullet-DNP")
- Dynamic nuclear polarization of long-lived states in methyl groups
- Parahydrogen-induced polarization of ^{13}C nuclei using ultralow field cycling
- Storage of nuclear spin order by precipitation of a solid from solution
- A master equation for nuclear spin dynamics, outside the weak-order approximation

TUTORIALS

Tutorial
TU1
Pure shift NMR
Gareth Morris[*1]
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Abstract: NMR is unique among spectroscopies in the freedom it allows us to manipulate the form and information content of spectra, switching off and on different classes of spin interaction, including the chemical shift and spin-spin coupling. Since the early days of NMR we have taken for granted the ability to use a spin echo to refocus the effects of the chemical shift, to use broadband heteronuclear decoupling to suppress the effects of heteronuclear couplings, or to suppress homonuclear dipolar spin-spin couplings in the solid state with windowed pulse sequences such as WAHUHA. Until relatively recently, however, one such class of experiments proved elusive, that of broadband decoupling of scalar homonuclear interactions. The goal of producing proton spectra containing only chemical shift information, with a single peak for each chemically distinct site, was identified by Primas and Ernst back in the 1960s, but only in the last decade has it proved possible to obtain such "pure shift" spectra with high enough quality to be of real use [1-4].
This tutorial will examine the difficulties encountered in designing such experiments and the different approaches that have been used to overcome them, show how pure shift methods have developed to become routine tools, illustrate the current state of the art in pure shift NMR techniques, and discuss how to circumvent some of the limitations of current methods.

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Normal (left) and pure shift (right) TOCSY spectra of estradiol

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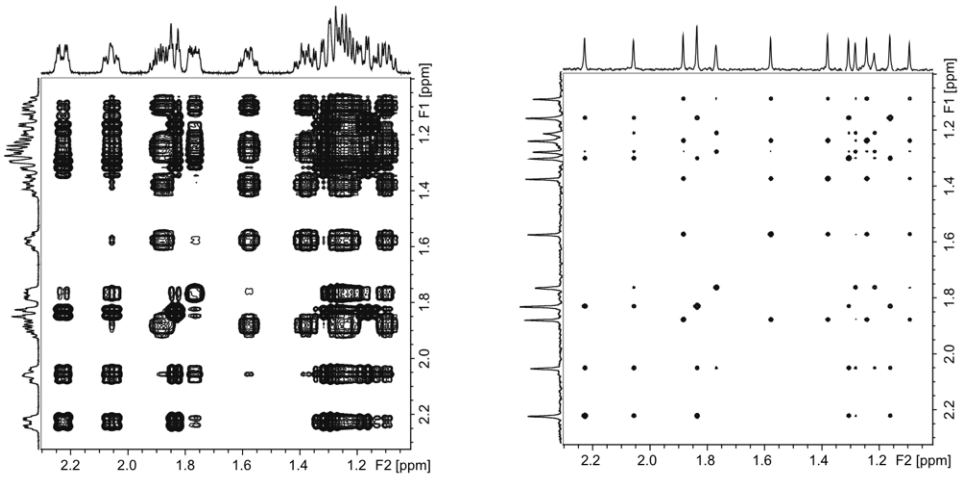

Tutorial
TU2
Deconstructing DNP
Anne Lesage[*1], Sami Jannin[1]
[1]High-field NMR Center, Institute of Analytical Sciences, Villeurbanne, France

Abstract: In the form of a scientific battle, *dramatically* opposing dissolution- and MAS-DNP, we will give DNP a *super* hard time. Throughout the presentation, we will *increasingly* deconstruct DNP, down to its very roots… questioning its existence, emphasizing its limitations, disclosing the unsaid from under the carpet with a *10'000-fold enhanced* bad faith. Only after that, we will eventually try to look on the bright side of DNP and gently reconstruct it.

PARALLEL INVITED LECTURES

Parallel Lecture
IL01
Covalent epitope masking in allergenic proteins
Martin Tollinger[*1]
[1]University of Innsbruck, Innsbruck, Austria

Abstract: In the temperate climate zone of the northern hemisphere, an estimated 100 million people are allergic to birch pollens, and more than 80% of these individuals subsequently develop cross-allergies to certain kinds of fruits and nuts, in particular apples and hazelnuts, in their lifetimes. This allergic cross-reactivity is caused by antibody (IgE) binding to homologous proteins in birch pollens, fruits and nuts. We show that in their natural environment, the causating allergenic proteins can be covalently modified at specific sites, which leads to (partial) obstruction and masking of the antibody binding epitopes on the protein surface. Using a combination of different isotope labeling approaches, solution NMR structure determination and ESI mass spectrometry, the chemical nature of these covalent modifications is elucidated and the modification site(s) are identified. Extraction of the protein allergens from natural sources is employed to verify the in vitro results. To assess the protein surface area that is covered by these chemical modifications and to study overlap with antibody binding epitopes in detail, paramagnetic relaxation and NOE measurements are used. In addition, the effects of these covalent modifications on antibody (IgE) binding affinities are probed by multiplex allergen testing of birch pollen and food allergic patients in a clinical study, revealing a clear correlation between reduced IgE binding affinities and the NMR spectroscopic and mass spectrometric data.

Parallel Lecture

IL02

Controlled explosions for studying protein folding by real time NMR

Cyril Charlier[1], Joseph Courtney[1], Reid Alderson[1], Philip Anfinrud[1], Ad Bax[* 1]

[1]Laboratory of Chemical Physics, National Institutes of Health, Bethesda, United States

Abstract: The equilibrium between a protein's folded and unfolded state is strongly impacted by hydrostatic pressure, to an extent determined by the difference in volume of the unfolded and folded states. Many proteins, in particular larger proteins, can be unfolded by applying a modest amount (≤3 kbar) of hydrostatic pressure. Smaller proteins often can be mutated to generate small internal cavities, such that they also have a large volume difference between the folded and denatured states, and can be unfolded at pressures that are within reach inside an NMR spectrometer. Rapidly decompression inside the NMR magnet, associated with significant volume expansion of the solvent, and hydraulic fluid traveling at the speed of sound, enables a range of different experiments to monitor the actual folding process under native conditions by two- and three-dimensional NMR. Measurements on ubiquitin show a strong temperature dependence of the folding rate constant, but a much weaker temperature dependence of the unfolding rates at high pressure, responsible for the shift to the unfolded states at lower temperatures. Remarkably, after a jump from high to atmospheric pressure, the spectrum of the unfolded state disappears at a rate that can be up to an order of magnitude faster than the appearance of the folded spectrum, providing evidence of molten-globule meta-stable intermediate states invisible to NMR. However, novel NMR experiments permit probing of the structural properties of these intermediates and show evidence for a state with non-native contacts on the pathway towards the folded state. This observation goes against the widely held concept that native contacts define the path towards the folded structure, a hypothesis that formed the basis for many hundreds of protein folding studies.

Parallel Lecture

IL03

NMR Crystallography Driven by Machine Learned Chemical Shifts.

Lyndon Emsley[*1]

[1]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: We will discuss new approaches to determining structures in materials that have so far been difficult to address using high-resolution multi-dimensional NMR methods.

Specifically, we will address the challenge of how to achieve high throughput calculation of chemical shifts in molecular solids, enabling rapid DNP enhanced NMR crystallography.

Machine learning methods have recently emerged as a way to overcome the need for time consuming first-principles calculations. However, the large chemical and combinatorial space spanned by molecular solids, together with the strong dependency of chemical shifts of atoms on their environment, poses a challenge for machine learning methods. Here we propose a machine learning method based on local environments to predict chemical shifts in molecular solids within DFT accuracy (RMSE of 0.49 ppm (^{1}H), 4.3ppm (^{13}C), 13.3 ppm (^{15}N), and 17.7 ppm (^{17}O) with R^2 of 0.97 for ^{1}H, 0.99 for ^{13}C, 0.99 for ^{15}N, and 0.99 for ^{17}O). We also show that the trained model is able to correctly determine, based on the match between experimentally-measured and ML-predicted shifts, structures of cocaine and the drug 4-[4-(2-adamantylcarbamoyl)-5-tert-butylpyrazol-1-yl]benzoic acid in a chemical shift based NMR crystallography approach.

Parallel Lecture

IL04

Para-Hydrogen based Hyperpolarization for quantitative NMR analysis at nanomolar concentrations

Marco Tessari[*1], Lisanne Sellies[1], Ruud Aspers[1], Martin Feiters[2], Floris Rutjes[2]
[1]Solid State NMR, [2]Synthetic Organic Chemistry, Radboud University, Nijmegen, Netherlands

Abstract: Nuclear spin hyperpolarization (e.g., Dynamic Nuclear Polarization (DNP), Para-Hydrogen Induced Polarization (PHIP), etc.) has gained a widespread interest over the last years as a tool to enhance NMR sensitivity. Particularly, SABRE[1] is a hyperpolarization technique based on the reversible association of substrate molecules and parahydrogen (p-H_2) to an iridium complex in solution. A transient scalar coupling network within this complex allows the transfer of the spin-order from p-H_2 to the nuclear spin of the substrate molecules, resulting in NMR signals enhanced up to three orders of magnitude. SABRE has so far been reported for different classes of substrates, such as nitrogenous heteroaromatics, sulphur heteroaromatic compounds, nitriles, Schiff bases and diazirines. Several such SABRE-active moieties appear in the structures of drugs, odorants and metabolites, giving rise to an interest in devising analytical approaches for their detection.

We have developed a PHIP approach based on the reversible association to iridium catalysts that allows the detection of SABRE substrates at nanomolar concentration. Importantly, the iridium catalyst can act as an NMR chemosensor, selectively increasing the NMR signals of the target compounds while removing the large background originating from the complex matrix.[2, 3] This technique has been successfully applied to complex mixtures, such as body fluid extracts, in which hundreds SABRE substrates at low- or sub-micromolar concentrations could be simultaneously detected. Importantly, as previously demonstrated, a quantitative determination of dilute components in hyperpolarized PHIP spectra is possible under suitable experimental conditions. The latest applications of this PHIP-based NMR chemosensor to chemical analysis of complex mixtures will be presented, and different quantification methods of hyperpolarized spectra will be discussed.

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Parallel Lecture

IL05

Processing of high-resolution 2D spectra. Towards a fully automated extractions of NMR parameters

Damien Jeannerat* [1]

[1]Department of organic chemistry, University of Geneva, Geneve, Switzerland

Abstract: The fully automated analysis of NMR spectra is an important but still ambitious objective. In principle, computer programs should be able to extract signals from 1D spectra, analyze their structures and provide list of peaks, integrals, coupling constants. They should then be able to combine this information in a consistent manner with the list of peaks extracted from 2D spectra such as COSY, HSQC and HMBC. Ideally, the outcome of this treatment should be *the* chemical structure of the compound under scrutiny.

But in reality, the analysis of 1D ^{1}H, 1D ^{13}C and 2D spectra, face specific difficulties making it more effective for software to assist the users in the exploitation NMR spectra than attempt (and risk to fail) to do it fully automatically. We will not discuss all these difficulties and but present two processing tools that should contribute to approaching the objective.

One very general problem with one and two-dimensional spectra is to distinguish signals, artifacts and noise. We will present a simple visualization tool facilitating their discrimination and provide an improved criterion for setting the threshold for peak picking and automatic choice of the level of the contours used to plot 2D spectra.

We will also discuss a problem which is specific to very high resolution 2D spectra. In these spectra the B_0 field inhomogeneity causes the elongation of signals parallel to the spectral diagonal making it difficult to extract coupling constants from top-resolution DQF-COSY or DIAG spectra (see Figure 1). We will present how 2D line shape deconvolution techniques can be finely tuned to make them more robust and effective. It will produce spectra where the effect of the B_0 field is eliminated and, when favorable, the resolution is further improved while keeping deconvolution artifacts under control.

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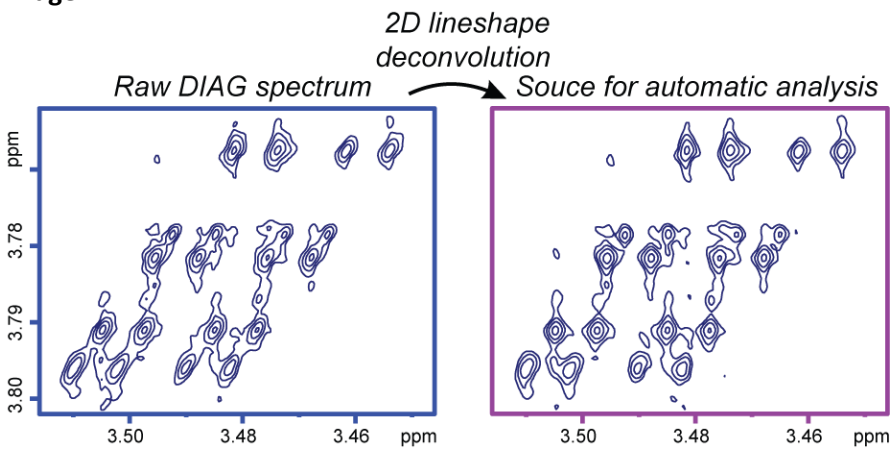

Parallel Lecture

IL06

Spin dynamics under MAS-DNP: theory and experiments

Frederic Mentink-Vigier[*1], Shimon Vega[2], Gaël De Paëpe[3]

[1]CIMAR, National High Magnetic Field Laboratory, Tallahassee, United States, [2]Chemical Physics, Weizmann Institute of Science, Rehovot, Israel, [3]INAC, CEA, Grenoble, France

Abstract: The advent of high field Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP) is opening prospects for solid-state NMR. From the early developments and after the introduction of commercial systems[1], the sensitivity gain obtained has kept increasing. The introduction of nitroxide biradicals as efficient polarizing agents starting with TOTAPOL[2] has allowed obtaining very large polarization gains with faster polarization times without theoretical understanding. Indeed before 2012, the Cross-Effect mechanism (CE) driving the DNP process under MAS was only analyzed in terms of static Hamiltonians and could not explain such results. The first simulations accounting for the time dependence[3],[4] revealed the complexity of the CE. It turned out to be based on fast energy level anticrossings induced by the MAS modulation.

The simulations of small spin systems (three-five spins) is particularly challenging from both a numerical and analysis standpoint. The ongoing analysis is unveiling the interplay between the biradical's properties (interaction strengths, relaxation times, orientations) and the experimental conditions (MAS frequency, magnetic field, microwave power). This ongoing work gives insights on the observed MAS/field dependence[5] and reveals an unexpected effect: the nuclear depolarization.[6],[7]

Here we will present the main conclusions obtained from the small spin system simulations highlighting the relevant experimental parameters. As an illustration of the simulations, we'll show how they explain the spin rate dependence and how the nuclear depolarization relates to the CE mechanism. We then present the extension of this model to a many spin system,[8] stressing the link between the simulations' outcome and the experimental observations. Finally we will show how these models compare with existing biradicals AMUPol/TEKPol and how we used them to design new nitroxide biradicals: AsymPol.

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Parallel Lecture
IL07
To assign or not to assign: the ABC transporter BmrA

Anja Böckmann[*1], Denis Lacabanne[2,3], Cedric Orelle[1], Thomas Wiegand[3], Lauriane Lecoq[1], Britta Kunert[1], Jean-Michel Jault[1], Beat H Meier[3]
[1]MMSB, CNRS/Université de Lyon 1, [2]MMSB, CNRS, Lyon, France, [3]Physical Chemistry, ETH, Zurich, Switzerland

Abstract: Large and complex biomolecules, and their assemblies, push the current limits of biomolecular NMR. While spectral fingerprints can often be established, sequential assignments cannot be achieved anymore for some of them, due to low protein molar amounts in the rotor, and, in some cases, also poor transfer efficiencies, e.g. limited by dynamics, compromising signal-to-noise in higher-dimensional spectra.
We will show the potential (and limitations) of workarounds, including selective labelling and paramagnetic NMR [1-5], allowing to obtain information on the different states the transporter accesses along its functional export cycle.

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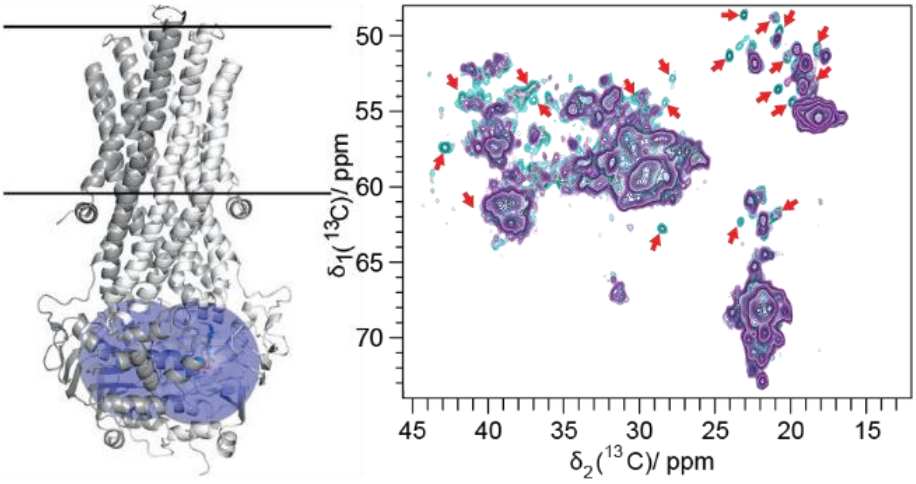

Parallel Lecture

IL08

Magic-angle-spinning NMR of pivotal protein-lipid interactions implicated in programmed cell death.

Patrick C. Van Der Wel[*1]

[1]Department of Structural Biology, University of Pittsburgh, Pittsburgh, United States

Abstract: Mitochondrial apoptosis plays a critical role in various kinds of human disease, including neurodegenerative disorders and also certain cancers, often associated with an increased level of reactive oxygen species (ROS). The protein cytochrome c (cyt-c) catalyzes ROS-mediated peroxidation of mitochondrial lipids, yielding lipid-derived signaling molecules that act as triggers of apoptosis. This pro-apoptotic function has been traced to specific interactions with the lipid cardiolipin, yielding a cardiolipin/cyt-c protein complex. We reconstituted its characteristic lipid-modifying activity in vitro, as part of integrated structural and functional studies of the dynamic and structural features of this pivotal protein-lipid complex. Enabled by 1D, 2D, and 3D magic-angle-spinning (MAS) NMR studies of the protein as well as the lipids, we have gained unique insights into the membrane-bound protein that enhance our understanding of the early mitochondrial events driving this lethal cellular process. Tailored variable-temperature ssNMR experiments proved to be essential for optimal ssNMR spectral quality of the surface-bound protein, while also generating invaluable insights into the global and local dynamics of both the protein and the membrane. Similar findings may apply more broadly to MAS ssNMR studies of hydrated protein samples, with and without lipid membranes. A brief discussion of dynamics-based filtering (or dynamics-based spectral editing; DYSE) as a ssNMR approach to tackle these kinds of dynamically complex samples will also be provided.

Parallel Lecture

IL09

Modelling the heterogeneous structure of a protein-RNA complex with distance distribution restraints

Gunnar Jeschke[*1], Christoph Gmeiner[1], Maxim Yulikov[1], Alexander Leitner[2], Georg Dorn[2], Emil Dedic[2], Frédéric H.-T. Allain[2]
[1]Department of Chemistry and Applied Biosciences, [2]Department of Biology, ETH Zurich, Zurich, Switzerland

Abstract: Combination of rigidity and flexibility in the same macromolecule or complex underlies function of proteins and nucleic acids. Established techniques are good for determining the structure of rigid domains or rigidified systems at atomic resolution, but often fail in characterizing the extent of conformation disorder. Moreover, they may even fail in arriving at a structural model in cases where such disorder is substantial. Both these problems can potentially be solved with distance distribution restraints between spin labels which encode the extent of disorder at residue pair level. We currently develop the RigiFlex approach that models a conformation ensemble on the basis of rigid domains that conform to the Anfinsen dogma and flexible linkers. RigiFlex is a tool for integrative structural biology that can combine restraints from different techniques, such as EPR, NMR, small-angle scattering, and mass spectrometry resolved cross-linking.
Here we explain the features of RigiFlex that enable an exhaustive search of restraint-fulfilling rigid-body arrangements. We discuss application of the approach to the complex between the human polypyrimidine tract binding protein 1 (PTBP1) with an internal ribosome entry site (IRES) of the mRNA of the encephalomyocarditis virus. To that end, we have measured about 50 distance distribution restraints between the four RNA recognition motifs (RRMs) of PTBP1, between sites in RRMs and in flexible linkers, between sites in RRMs and the IRES RNA, and between spin-labelled nucleotides within the RNA. Structure of the rigid RRMs was determined by NMR and the binding motifs of the RNA to the protein by either NMR or by the cross-linking tandem mass spectrometry approach CLIR-MS/MS [1]. Additional restraints were obtained by small angle neutron and x-ray scattering and protein cross-linking. We explain how RigiFlex can be used for assessing which additional distance distribution restraints may narrow the conformational ensemble and how we deal with the lack of restraints on the RNA structure. Finally, we discuss the problem that in a conformationally heterogeneous ensemble, some restraints may be fulfilled only by a subset of conformations.

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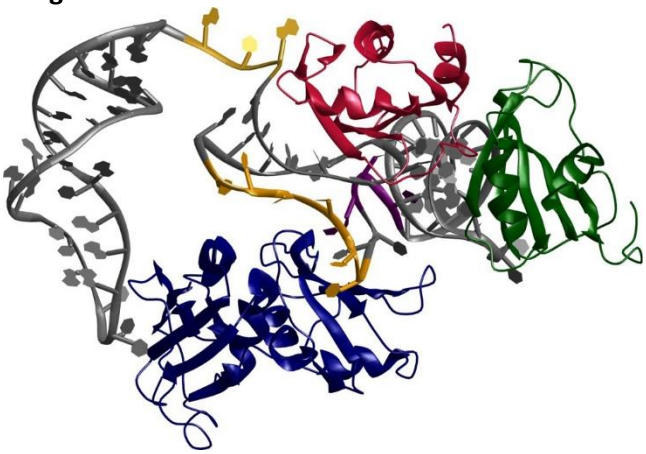

Parallel Lecture
IL10
Magnetic resonance with quantum microwaves
Patrice Bertet[*1]
[1]SPEC, CEA Saclay, Gif-sur-Yvette, France

Abstract: We report EPR spectroscopy measurements of small ensembles of donors in silicon at X-band and millikelvin temperatures, using the tools offered by circuit Quantum Electrodynamics, namely high quality factor superconducting micro-resonators and Josephson parametric amplifiers that operate at the quantum limit [1]. In this regime, the quantum nature of the microwave field plays a role. The spin detection sensitivity is strongly enhanced [2,3] (up to a value of 10spin/\sqrtHz). The coupling of the spin with the micro-resonator becomes strong enough that spin relaxation is dominated by spontaneous emission through the cavity [4], the so-called Purcell effect.
In this talk I will present measurements performed with this quantum spectrometer showing ESEEM of the donors caused by a small number (approx. 10000) of residual 29Si nuclear spins [5] in a sample that was isotopically enriched in 28Si. This enabled us to measure this residual concentration and independently confirm the level of isotopic enrichment. I will also show that the Purcell effect can be used as a new method for electron spin hyperpolarization, by reducing the temperature of the intra-resonator microwave field with which the electron spins thermalize.
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[3] S. Probst et al., Appl. Phys. Lett. 111, 202604 (2017)
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Parallel Lecture

IL11

State of the Art of the Compressed Gels Technology to Measure High Quality Anisotropic NMR Parameters

Roberto R. Gil[*] [1]

[1]Chemistry Department, Carnegie Mellon University, Pittsburgh, United States

Abstract: The development of the application of Residual Dipolar Couplings (RDCs) to the structural analysis of natural and synthetic small molecules has matured enough in the recent years to perform this task is an almost straightforward way. In addition to RDC data, ^{13}C Residual Chemical Shift Anisotropy was recently added to toolbox of NMR in anisotropic media. In the last 10 years, our group has concentrated its efforts in the development of the application of aligning gels compatibles with $CDCl_3$, DMSO and MeOD to the analysis of small molecules. In 2010, we have proposed a fast and tuneable alignment method by reversible compression/relaxation of PMMA Gels compatible with $CDCl_3$,[1] which it was further extended to gels compatible with DMSO and MeOD. This method made it possible to have access to RDCs and RCSAs within hours. The high quality of the gels in combination with pulse programs designed by the group of Teodor Parella from Universitat Autònoma de Barcelona (Barcelona, Spain) led us to collect highly accurate RDCs ($^1D_{CH}$, $^{2,3}D_{CH}$and $^2D_{HH}$).[2] E.g. we have obtained a quality factor *Q* as low as 0.028 for a structure of strychnine generated by DFT and RDCs collected in compressed PMMA gel swollen in $CDCl_3$. We will also show very accurate one- and two-bond ^{13}C-^{13}C RDCs at natural abundance collected in PMMA gels and with impressive power to discriminate configuration. Our group has also developed in a ^2H 1D imaging experiment to not only monitoring the spatial homogeneity of alignment but also the mechanical properties of flexible gels. We have also recently developed, in collaboration with Armando Navarro-Vázquez from Universidade de Pernambuco (Recife, Brazil), a Computer Assisted 3D Structure Elucidation protocol (CASE-3D) that uses combinations of RDCs, NOE-derived distances, ^1H and ^{13}C Chemical Shifts and *J* coupling constants to determine configuration/conformation from molecular constitution in automated way. [3,4] Compressed gels technology is slowly becoming a preferred method for collecting RDCs and RCSAs due to the fact that the sample can be fast and easily diffused inside the gel, as well as it can be easily recovered. In the current presentation we will show applications of compressed gels technology and the CASE-3D protocol to the structure elucidation of complex natural and synthetic small molecules.

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Parallel Lecture

IL12

Monitoring photochromic processes and photoinduced reactions via in-situ irradiation NMR-spectroscopy

Jonas Kind[1], Christina M. Thiele[*1]

[1]Clemens-Schöpf-Institut für Organische Chemie und Biochemie, Technische Universität Darmstadt, Darmstadt, Germany

Abstract: NMR spectroscopy is widely used for (in-situ) reaction characterisation and reaction monitoring e.g. to identify intermediate species or to quantify side product formation. This is also applicable to photochromic processes and photoinduced reactions if NMR samples are irradiated inside the magnet. The recent LED setup published by the Gschwind group allows an easy and generally applicable access to in-situ irradiation NMR-spectroscopy.[1] Recent results on the investigation of a three-fold photochromic switch[2] as well as on two photoinduced reactions will be shown. Furthermore, we discuss benefits of in-situ irradiation NMR using LEDs and silica waveguides as well as unexpected pitfalls of this technique – especially as far as reaction monitoring is concerned.
Firstly, we show results on a dye-sensitized seleno-mediated photo-catalytic lactonization of an alkenoic acid in the presence of oxygen [3]. Production of the lactone occures via an isolatable seleno-substitued lactone, which yields the final product after photo-catalytic elimination. Here we present data of an initial rate approach to identify the rate-limiting step in the catalytic cycle. [4]
Secondly, we show kinetic data of a reaction cascade yielding a substituted poly-phenylene-vinylene (PPV). The reactive quinobimethane is produced in-situ from the premonomer by adding potassium tert-butoxide below the threshold temperature of the thermal polymerization reaction. Polymerization of the quinobimethane can be induced by irradiation of the reaction mixture with UV light [5,6].

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Parallel Lecture
IL13
Ideal Pulses - Perfect Results?
Matthias Ernst[*1]
[1]Physical Chemistry, ETH Zürich, Zürich, Switzerland

Abstract: Pulse transients in NMR experiments lead to a deviation of the experimental B_1 field experienced by the spins compared to the ideal behavior programmed on the spectrometer [1]. The differences between the real and the ideal spin trajectories lead to performance degradation in many experiments [2]. Using pulse transient compensation [3], ideal non-rectangular (shaped) pulses can be generated that lead to well defined and predictable spin trajectories.

I will discuss the sensitivity of various resonant (e.g., heteronuclear and homonuclear recoupling) [4] and non-resonant (e.g., heteronuclear and homonuclear decoupling) pulse sequences in solid-state NMR under MAS to transient effects. Floquet theory can be used to understand the consequences such pulse imperfections have on the performance of different sequences and how the sequences have to be modified in order to work well with shaped transient-compensated pulses. I will also compare the transient compensation to other methods like more complex phase cycles that can be used to reduce the performance degradation caused by pulse transients. Experimental considerations and instrumental requirements of implementing different solutions will be discussed.

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Parallel Lecture

IL14

Utilizing phase modulated saturation pulses to obtain distance and relaxation of quadrupolar spins subjected to extensively large quadrupolar frequencies

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Abstract: The majority of atoms in the periodic table have a spin larger than one-half hence their spectra are influenced by the nuclear quadrupolar interaction, the frequency of which can be very large, up to several MHz. Those and other nuclei are commonly also of low abundance. Excitation of such nuclei under magic-angle spinning for the purpose of direct or indirect detection requires therefore special excitation methodologies. In order to measure efficiently accurate distances to spins that experience extensively large interactions (CSA or quadrupolar), and in order to measure their longitudinal relaxation times (and not practical recovery times), the pulses must result in a uniform effect over the crystallites in the solid powder.

We will demonstrate how phase-modulated pulses, which combine adiabatic and nutation segments, can efficiently generate macroscopic saturation of the broad powder width. When such pulses are incorporated into REDOR-type experiments, the dipolar recovery curve can be fit with an analytical function without the requirement for scaling and therefore an accurate (±0.1Å) distance can be obtained. Notable examples are distance measurements between ^{31}P and the two equally-abundant bromine isotopes (^{79}Br, ^{81}Br) experiencing a quadrupolar frequency of ~5 MHz, and distances between ^{13}C and bismuth-209, a spin-9/2 nucleus with a quadrupolar frequency of over 10 MHz.

Owing to the efficient saturation, we can perform saturation recovery experiments that are not affected by any coherent magnetization recovery from the satellites, hence provide a reliable measure of T_1.

Relevant literature:

Nimerovsky et al. JMR 244, 107, 2014; JCP 146, 124202, 2017. Makrinich et al. SSNMR 84, 196, 2017; SSNMR, in press, 2018.

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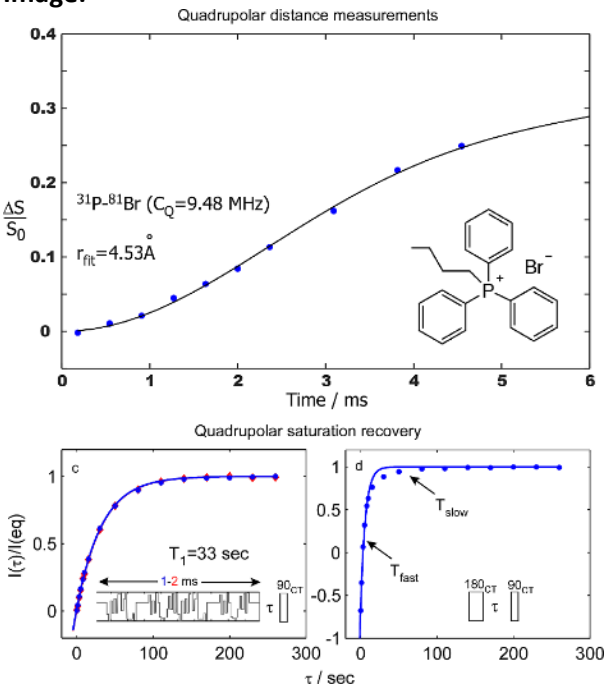

Parallel Lecture

IL15

In vivo MRI tracking of single cells labelled with iron oxide nano particles

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Abstract: Non-invasive imaging of defined population of cells or even of individual cells is gaining increasing attention in basic biomedical research and also in the context of cellular therapies in patients. MRI is the method of choice for clinical imaging, but suffers from limited molecular sensitivity if thermal polarization at patient temperature is used. Therefore, paramagnetic agents are used to enhance contrast in the images and make distinct cells detectable, by exploiting either paramagnetic relaxation enhancement (PRE) or bulk susceptibility effects of superparamagnetic nano particles.

Different strategies to label cells include ^{19}F-containing compounds, molecules with highly shifted ^{1}H-resonances such as Tm-DOTMA, or super paramagnetic iron oxide nano particles (ION). For both ^{19}F and ^{1}H PRE can be exploited to make small cellular populations detectable, if FID instead of the common echo detection for image acquisition is used[1,2].

Detection and tracking of individual cells in vivo is possible if ION-labeling is employed[3,4] (Figure, showing individual immune cells in the mouse brain detected at 9.4 T). Tracking of cells requires sufficient temporal resolution to perform time-lapse MRI. We have shown that it is feasible to detect single cells in sequential acquisitions with an effective temporal resolution of one minute for the whole mouse brain. Simulations of image contrast showed that cells with velocities of roughly 1 µm/s can be detected, while faster moving cells did not give rise to sufficient image contrast. This velocity range is perfectly suitable to differentiate between slowly patrolling immune cells in healthy mice from faster moving cell after an immune response has been triggered. In vivo MRI cell tracking thus provides diagnostic criteria to detect onset of immune response with high sensitivity.

Quantification of ION-labelled cells, however, remains dificult. For this purpose, ^{57}Fe-ION can be employed and MRI combined with elemental mass spectrometric imaging by LA-ICP-MS. This approach allows to unambiguously identify labelled cells, follow their migration, assess biodistribution of the administered iron and study metabolization of ION.

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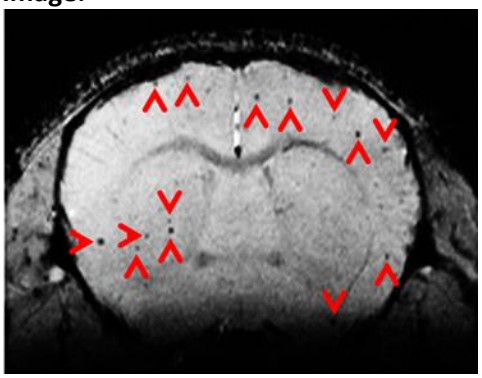

Parallel Lecture

IL16

Quadrupole enhanced proton relaxation: Proof of principle for a novel extrinsic MRI contrast mechanism

Hermann Scharfetter[*1], Rupert Kargl[2], Christian Gösweiner[1], Evrim Umut[3], Carina Sampl[4], Roland Fischer[5], Stefan Spirk[4], Andreas Petrovic[1], Karin Stana-Kleinschek[2], Danuta Kruk[3]

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Abstract: Introduction: MRI is a powerful medical imaging tool due to its non-invasive character, high versatility and excellent soft tissue contrasts. However, concerning molecular imaging, MRI still suffers from low sensitivity and requires signal amplification strategies. Specifically tailored contrast agents (CAs) with the possibility to switch on and off the contrast either by extrinsic stimuli or by binding to targets could significantly enhance MRI for this purpose. In this context we present first data on a novel extrinsic contrast mechanism based on T_1 shortening by quadrupolar relaxation enhancement (QRE). In contrast to well-established paramagnetic relaxation enhancement, QRE relies on dipole-dipole (DD) interaction between water protons and high-spin quadrupolar nuclei (QN). One condition for QRE is a high probability for quantum-mechanical transitions of the QN nuclear spin at f_L or $2f_L$ (f_L = ^{1}H Larmor frequency). This can cause narrow peaks of efficient QRE with center frequencies depending both on the magnetic flux density B_0 and the electric field gradient (EFG) close to the QN. MRI contrasts could thus be switched on and off e.g. by B_0 shifts and/or EFG modulations induced by structural changes of chemical bonds due to interaction with the biological environment. Additional prerequisites are slow tumbling of the ^{1}H-QN-system, sufficient water exchange rate and strong DD coupling between the two nuclei due to close approach. Then the magnetization can be transferred from ^{1}H via the QN to the lattice by the QN's quadrupolar relaxation. Due to several advantageous properties we selected Bi-Aryl compounds containing ^{209}Bi as QN and show QRE in such systems.

Methods: Several organo-Bi-compounds with favourable quadrupolar transition frequencies were pre-selected by zero-field nuclear quadrupole resonance spectroscopy. Two of the most promising model systems containing Tris-(2-Orthomethoxy-Phenyl)Bismuthane were further investigated experimentally for potential QRE: (1) solid powder (2) polymer-coated nanoparticles dispersed in water. Nuclear magnetic relaxation dispersion profiles were acquired by 1H NMR relaxometry measurements in the B_0 range 0.5 – 3T and at various temperatures.

Results: QRE peaks could be observed at several frequencies, also close to the 1H Larmor frequencies of typical MRI scanners (1.5 and 3T) in both solid powders as well as in the aqueous dispersion. In the first case the signals stem from intrinsic protons of the Bi-compound, in the second case from bulk protons.

Discussion: To our knowledge we have successfully demonstrated for the first time QRE in two selected organobismuth compounds. The QRE peak frequencies of the solids are in agreement with quantum mechanical predictions. The confirmation of QRE peaks in the liquids, though still weak due to suboptimal particle design, can be interpreted as an important proof of principle, stimulating further research for the development of QRE based T_1 CAs for MRI.

Parallel Lecture
IL17
Field-cycling NMR of porous materials at zero and ultralow magnetic fields
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Abstract: In this presentation, I will discuss measurements of NMR relaxation at magnetic field strengths in the range 0.1 nT < B < 10 mT, and their potential to improve our understanding of molecular interactions between porous material surfaces and an imbibed fluid phase.

The dependence of NMR relaxation rates on the ambient magnetic field is due to the fundamentally non-white spectral density profile of molecular motion. Simple and well-known theoretical models predict that the largest dispersion of NMR relaxation rates occurs at fields where the inverse of the nuclear Larmor frequency is similar to the correlation time of the molecular motion. In the case of porous materials, relaxation dispersion at Larmor frequencies <100 kHz provides an opportunity to examine relatively slow dynamic phenomena of inter-molecular association and adsorption, for which correlation times are on the order of $10^{-4} – 10^{-6}$ s. This has become highly applied in studies of liquid confined within porous rocks and catalytic support materials to probe aspects of molecular aggregation and surface affinity in situ [1], as well as complement information on the Brownian motions that can be provided by NMR measurements of diffusion[2].

We have measured NMR relaxation of the fluid component in porous materials at ultralow-field inside an 8-inch outer diameter magnetic shield. Common laboratory solvents were absorbed into porous oxide catalyst support materials and the NMR signals were detected with a hot-vapor alkali magnetometer. The NMR detection field ranges from 1 uT where inhomogeneous line broadening due to the porous solid matrix is negligible, down to zero field where specific chemicals in the porous material matrix are identified via heteronuclear J coupling spectroscopy[3]. Overall the instrument is compact enough to fit on a laboratory benchtop, inexpensive to set up and easy to operate. In the talk I will discuss present capabilities and applications.

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Parallel Lecture

IL18

Ultrafast Laplace NMR using a single-sided magnet

Jared King[1], Alfredo Fallorina [1], Justin Yu[1], Vanessa Lee [1], Tyler Meldrum[1], Guannan Zhang[2], Christian Hilty[2], Otto Mankinen[3], Susanna Ahola[3], Vladimir Zhivonitko[3], Ville-Veikko Telkki[* 3]

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Abstract: Relaxation and diffusion NMR experiments provide versatile information about dynamics and structure of porous media, polymers, proteins, etc. Since the relaxation and diffusion data consist of exponentially decaying components, the processing requires the Laplace inversion in order to determine diffusion coefficient and relaxation time distributions. Consequently, these methods can be referred to as Laplace NMR (LNMR). [1]

Like in traditional NMR spectroscopy, the resolution and information content of LNMR can be increased by the multidimensional approach [1]. However, long experiment time restricts the applicability of the multidimensional methods. As a solution for this problem, we are developing a broad range of ultrafast, single-scan multidimensional LNMR experiments [2-5], based on the principles of continuous spatial encoding that have been recently successfully applied in ultrafast multidimensional NMR spectroscopy [6]. The method shortens the experimental time by one to three orders of magnitude as compared to the conventional method, offering unprecedented opportunities to study fast molecular processes in real time. Furthermore, the ultrafast approach enables using hyperpolarized substances to boost sensitivity by several orders of magnitude in the multidimensional LNMR experiments [3], which is not feasible in the case of traditional methods requiring extensive repetition of the experiments.

In the presentation, the principles of various ultrafast multidimensional LNMR experiments are explained. We have demonstrated that the ultrafast approach is feasible also with low-field, single-sided instruments, which are portable and much cheaper than the high-field spectrometers [7]. When combined with hyperpolarization, even single-scan experiments become feasible at low fields, offering great prospects for mobile NMR analysis [8].

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Parallel Lecture

IL19

Evolution of Protein Dynamics - Time Travel into the Past and Future

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Abstract: The essential role of protein dynamics for enzyme catalysis has become more generally accepted in part due to a number of dynamic NMR studies on different enzymes. Since evolution is driven by organismal fitness hence the function of proteins, we are asking the question of how enzymatic efficiency has evolved.

First I will address the question thermoadaptation. As a direct manifestation of molecular kinetic energy, temperature is a fundamental evolutionary driver for chemical reactions. However, it is currently not understood how the natural evolution of catalytic efficiency responds to dramatic changes in environmental temperatures. Using Ancestral Sequence Reconstruction (ASR) we resurrect and biophysically characterize the oldest common ancestral kinase and enzymes along the evolutionary path to modern kinases. Strikingly, enzymes coped with an inherent drop in catalytic speed caused as the earth cooled down over 3.5 billion years by accelerating protein dynamics and adapting thermostability by unexpected mechanisms, as characterized by NMR. Tracing the evolution of enzyme activity and stability from the hot-start towards modern hyperthermophilic, mesophilic and psychrophilic organisms illustrates active pressure versus passive drift in evolution on a molecular level (1).

Second we will answer the question of the molecular mechanism underlying improved catalytic enzymatic function via directed evolution. Intriguingly we find that increased turnover in evolved enzymes is solely due to faster functional conformational changes. In a more general sense, the presented results highlight the power of combining NMR CEST and dispersion experiments with room-temperature x-ray crystallography for unraveling molecular mechanism at atomic resolution.

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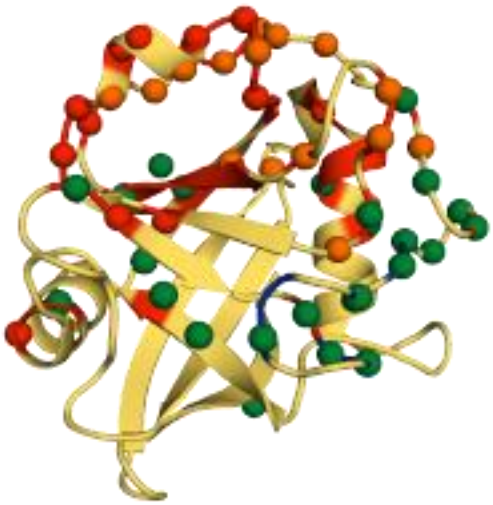

Parallel Lecture
IL20
Millisecond motions in allostery and phosphoryl transfer
Joseph Patrick Loria[* 1], Danica Cui[1], Victor Beaumont[1]
[1]Chemistry, Yale University, New Haven, United States

Abstract: Protein tyrosine phosphatases are critical enzymes that regulate numerous metabolic pathways. Specifically, PTP1B and VHR are signal transduction modulators of insulin and leptin and extracellular signaling and c-Jun N-terminal kinases, respectively. The catalytic function of these enzymes is initiated by the closure of the active site acid loop upon substrate binding, where a critical aspartic acid in the catalytic loop drives the cleavage and hydrolysis of the phosphotyrosine substrate to yield the dephosphorylated product. We probed the conformational equilibrium landscape of the catalytic loops in PTP1B and VHR by characterizing a series of alanine point mutations along the loop sequence with NMR spectroscopy, X-ray crystallography, steady-state, and transient kinetic analysis. In a series of 2D NMR spectra of these mutants, we identified residues comprising a novel allosteric network whose chemical shifts align in a linear manner on a trajectory tracking an open (apo) – to – closed (substrate bound) acid-loop transition. Crystal structures of these mutants in the apo form demonstrate a range of loop conformations ranging from open, to 50% closure to 100% closure. These data suggest that modifying the flexibility of the catalytic loop alters the conformational equilibrium landscape of the phosphatases locally (at the loop) and globally. Additionally, our kinetic studies of the dephosphorylation reaction for the acid-loop mutants found that the loop conformational equilibrium and rate of catalysis are highly correlated. This suggests that the rate of catalysis in tyrosine phosphatases is governed by the kinetics of acid-loop closure. These results provide insight into the evolutionary differences between structural homologs of tyrosine phosphatases, and how acid-loop primary sequence and backbone flexibility can affect global structure and function.

Parallel Lecture

IL21

Investigating Disorder in A2B2O7 Ceramics Using NMR Spectroscopy and First-Principles Calculations

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Abstract: NMR spectroscopy provides an element-specific probe of local structure in the solid state, without any requirement for long-range order. While techniques such as magic-angle spinning (MAS) can improve resolution in many cases, for disordered systems we typically find a distribution of NMR parameters and a corresponding broadening in the NMR spectrum, hindering analysis. Here we exploit two approaches for studying disorder in $A_2B_2O_7$ ceramics – materials that are of particular interest for the encapsulation of radioactive waste.

The first approach exploits first-principles calculations to predict NMR parameters and aid spectral interpretation of ^{89}Y and ^{119}Sn NMR spectra of pyrochlore ceramics. We compare a simple cluster-based method[1,2] (using $Y_2(Ti.Sn)_2O_7$ as a model system) to a more comprehensive approach using site occupancy disorder (SOD).[3] This code enables *all* symmetry unique atomic arrangements in a material to be determined. The full spectrum can then be simulated, with the contribution of each arrangement weighted by the corresponding configurational degeneracy (also determined by SOD) and, if necessary, by a Boltzmann weighting. We also demonstrate the importance of the protocol chosen for geometry optimisation and demonstrate some potential pitfalls when comparing DFT calculations to experiment.

In a second approach, we turn to ^{17}O NMR spectroscopy to characterise $A_2B_2O_7$ pyrochlore, defect fluorite and layered-perovskite phases. We show that considerable care must be taken both in choosing the conditions for ^{17}O enrichment and the experimental acquisition parameters if the necessary quantitative measurements are to be obtained for more complex systems.[4] We combine experiment with DFT calculations to enable spectral assignment, and facilitate the interpretation of complex and overlapped spectral lineshapes. We demonstrate that, although challenging, ^{17}O NMR has the potential to be a powerful probe of local structure and disorder in oxide ceramics.

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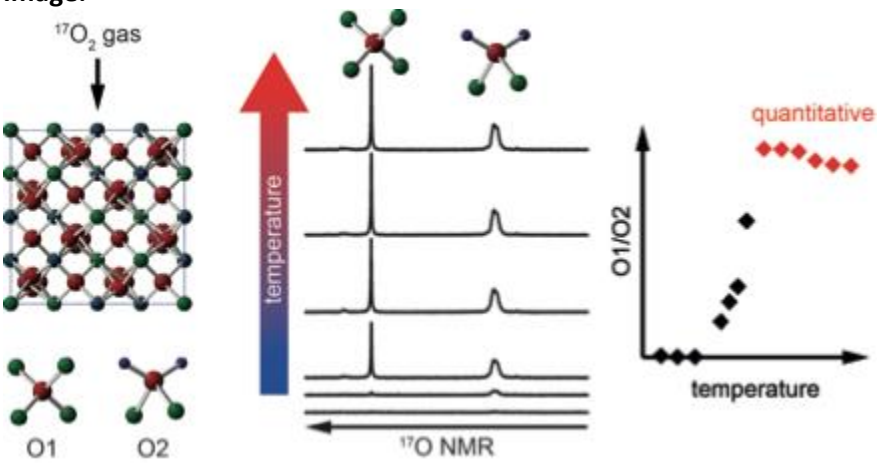

Parallel Lecture

IL22

Unprecedented details on the structure of chemisorbed CO2 species in mesoporous solid sorbents enabled by NMR and computer modeling

Luís Mafra* [1], Tomaž Čendak[1], Lisa Sequeira[1], Mariana Sardo[1], Moisés L. Pinto[2], José R. Gomes[1]

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Abstract: Employing amine-functionalized solid sorbents for CO_2 capture and sequestration/utilization is a promising alternative to reduce greenhouse gases over the use of liquid amine scrubbers, which are corrosive and present high cost during regeneration. Although emphasis has been given to the synthesis of new materials with improved CO_2 adsorption capacities, very few studies have focused on the molecular level understanding of surface CO_2-amine interactions. Herein, we present new insights into the structure of chemisorbed CO_2 species formed in functionalized SBA-15 with primary, secondary, tertiary and mixed primary-secondary amines loaded with ^{13}C-labeled CO_2, under controlled CO_2, partial pressure. The amorphous nature of this materials present an additional challenge in their characterization, which demands for the use of complementary computer modeling approaches to support the results obtained from various ssNMR experiments, as it will be showcased.[1-3]

To date, several aspects remain unclear, for example, the intermolecular interactions between neighboring amine groups and the role of these interactions in the stabilization of the formed CO2 species. To obtain further insight on the CO2 structure, this presentation shows a combination of multiple 1D, 2D ^{1}H-^{29}Si/^{13}C HETCOR and $^{13}C\{^{14}N\}$ recoupling experiments in as-prepared and H/D exchanged SBA-15, aiming at unraveling the interactions of CO2-adducts formed,[1] either in the presence of pure CO_2 gas or CO_2, /H_2O, binary mixtures. CO_2, species are engaged in complex hydrogen bonding networks involving amine amine and amine silanol interactions, where different populations of CO_2, species such as carbamate, carbamic acid, alkylammonium carbamate as well as very dilute species - moisture-sensitive/moisture-induced CO_2, species (*e.g.,* bicarbonate) are distinguished.

A second aspect is addressed in this talk. It has been recognized that amine surface density, plays an important role in intermolecular interactions driving CO_2 adsorption, however controlling amine density for the purpose of NMR studies is not straightforward. Herein, an alternative experimental protocol that relies on the high sensitivity of ^{13}C chemical shift anisotropy (CSA) tensors to proton-transfer is presented, allowing distinguishing between ionic/charged and neutral CO_2 species formed, which has been a longstanding problem. Smart control of surface amine-amine distances, during the functionalization step and the ability to perform variable-CO_2 pressure MAS NMR experiments under tightly controlled atmospheric conditions, was crucial to take advantage of the ^{13}C CSA tensor analysis. This approach enabled the formation of either "isolated" or "paired" carbamate/carbamic acid species, providing a first experimental NMR proof towards the identification of both aggregation states.[2]

Acknowledgments

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Parallel Lecture

IL23

Hyperpolarized 129Xe Lung MRI and Biosensors

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Abstract: Computed tomography (CT) is a popular and routine imaging modality to evaluate lung diseases, and has shown a substantial value for clinics. However, the use of CT in longitudinal studies is limited because of exposure to ionizing radiation. Magnetic resonance imaging (MRI) does not use ionizing radiation and therefore can be used repeatedly in longitudinal studies. Unfortunately, MRI of the lung airways is not possible due to the exceedingly low density of nuclear spins present in the air space

With the technique of spin-exchange optical pumping (SEOP), the spin polarization of hyperpolarized xenon can be enhanced four or five orders of magnitude, which makes feasible to obtain the gas phase signal, like in the lung's airspace morphology. We built a new designed 129Xe hyperpolarizer with a two-body optical cell, which can produce hyperpolarized ^{129}Xe with ~30% polarization for natural abundance xenon[1-2]. It enables us to image lung with hyperpolarized natural abundance xenon, showing a great potential for applying in clinics. By using hyperpolarized ^{129}Xe ADC or ADK MRI, the microstructure of smoke-induced lung can be well visualized and evaluated [3-4]. Being a trace element in the atmosphere, xenon is soluble in water, blood and tissues. Therefore, dissolved phase xenon MRI could provide rich information related to the gas-exchange function of the lung. The morphological and physiological parameters of the radiation-induced lung injury (RILI), chronic obstructive pulmonary diseases (COPD) and emphysema can be non-radioactively and non-invasively obtained in vivo using hyperpolarized ^{129}Xe diffusion, CSSR and CEST MRI [5-8], which could not be comprehensively achieved by the currently other imaging modalities. It demonstrates that such a new imaging technology is able to evaluate the microstructure and function of the lung, which paves a new way for the pulmonary disease research. Furthermore, different xenon biosensors were designed and developed to specifically detect ions, biothiols and H2S in living cell [9-12], showing the potential great applications of hyperpolarized xenon MRI in biomedicine.

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Parallel Lecture
IL24
Diffusion-weighted MRS to probe brain cell structure in vivo
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Abstract: Diffusion-weighted NMR spectroscopy (DW-MRS) offers the unique ability to non-invasively quantify the diffusion of endogenous brain metabolites *in vivo*. In contrast to water molecules, which are ubiquitous in biological tissues, most brain metabolites are confined in the intracellular space. Their diffusion properties are thus expected to mostly depend on intracellular parameters such as cytosol viscosity, molecular crowding, size and shape of the cell… Furthermore, some metabolites are thought to exhibit preferential cellular compartmentation, with N-acetyl-aspartate (NAA) and glutamate (Glu) residing essentially in neurons, while myo-inositol (Ins) and choline compounds (tCho) are thought to be preferentially compartmentalized in glial cells.
Cellular specificity has been the main motivation driving methodological research and applications of DW-MRS *in vivo* over the last 25 years. Alterations of metabolite diffusion have been reported in brain diseases, illustrating the potential of the method. However, the origin of these variations remains unclear. The basic reason is that many potential factors may affect apparent diffusion, such as cytosol viscosity but also subcellular compartmentation or cell size, the relative contribution of these various factors being not clearly understood.
To try to elucidate what governs metabolite diffusion, our group has engaged in the exploration of brain metabolite diffusion over very different diffusion times t_d, to probe the intracellular space over very different spatial scales. Measurements of the apparent diffusion coefficient (ADC) as a function of t_d reveal rapidly decreasing ADC as t_d is increased, followed by a relatively stable plateau for long t_d, which is very clear signature of metabolites being “freely” diffusing along long and thin fibers. Intracellular viscosity is found to be not much larger than pure water viscosity. Moreover, these measurements rule out any significant contribution of active transport or compartmentation in subcellular structure.
This framework of long and thin cylinders can also lay the foundation for new models to extract cell-specific morphological parameters, e.g. as recently proposed for data at ultra-long t_d, where fibers are now modeled with some branching and finite length. It appears that reconstructed compartments of neuronal (NAA, Glu) and glial (Ins, tCho) metabolites are found to be different, with reconstructed glial cells being smaller and less complex than neurons, consistently with histology.
Finally, we evaluate the potential of some of these approaches to quantitatively assess morphological variations under “pathological” conditions, in a mouse model of astrocytic activation. Increased fiber diameter and fiber length are measured for myo-inositol compartment by DW-MRS, consistently with astrocytic hypertrophy as quantitatively assessed by confocal microscopy.
In conclusion, DW-MRS has progressively emerged as a tool to specifically probe cellular alterations in various neurological disorders. In parallel, the basic understanding of the main features governing metabolite diffusion has progressed, allowing more relevant interpretation of DW-MRS under normal and pathological conditions, opening new possibilities for microstructure quantification.

Parallel Lecture

IL25

Structural Investigations on Bacillus Subtilis Biofilms and Experiences with 110 kHz spinning as well as Dynamic Nuclear Polarisation (DNP)

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Abstract: Microorganisms form surface-attached communities, termed biofilms, which can serve as protection against host immune reactions or antibiotics. Bacillus subtilis biofilms contain TasA as major proteinaceous component in addition to exopolysaccharides. In stark contrast to the initially unfolded biofilm proteins of other bacteria, TasA is a soluble, stably folded monomer, whose structure we have determined by X-ray crystallography. Subsequently, we characterized in vitro different oligomeric forms of TasA by NMR, EM, X-ray diffraction, and analytical ultracentrifugation (AUC) experiments. However, by magic-angle spinning (MAS) NMR on live biofilms, a swift structural change toward only one of these forms, consisting of homogeneous and protease-resistant, β-sheet-rich fibrils, was observed in vivo.

Aiming at the design of an allosteric modulator of the neonatal Fc receptor (FcRn)-Immunoglobulin G (IgG) interaction, a new methodology including NMR fragment screening, X-ray crystallography, and magic-angle-spinning (MAS) NMR at 100 kHz after sedimentation was developed, exploiting very fast spinning of the nondeuterated soluble 42 kDa receptor construct to obtain resolved proton-detected 2D and 3D NMR spectra. A small molecule is presented that binds into a conserved cavity of the heterodimeric, extracellular domain composed of an α-chain and β2-microglobulin (β2m) (373 residues). Proton-detected MAS NMR experiments on fully protonated [13C,15N]-labeled FcRnECD yielded ligand-induced chemical-shift perturbations for residues in the binding pocket and allosteric changes close to the interface of the two receptor heterodimers present in the asymmetric unit as well as potentially in the albumin interaction site.

In future, a major factor facilitating investigations on large protein complexes and native samples will be dynamic nuclear polarisation (DNP), which was introduced to increase signal-to-noise by one or two orders of magnitude. In order to improve the quality of DNP spectra and to obtain maximum signal-to-noise, new radicals were synthesized and employed in measurements of protein samples around 190K. Enhancements in the range of 15-20 were observed in this temperature range while acceptable spectral resolution is observed. A comparison of signal-to-noise per unit time and enhancements of several radicals is presented, showing deviations concerning radical performance when proton T_1 of the compared samples is affected differently by the radical.

Parallel Lecture

IL26

De novo atomic structure, dynamic allostery and enzymatic mechanisms of a 0.5 MDa protease from an integrated ssNMR/cryo-EM approach

Diego Gauto[1], Pavel Macek[1], Hugo Fraga[1], Astrid Sivertsen[1], Rime Kerfah[2], Leandro Estrozi[3], Gregory Effantin[3], Charles Schwieters[4], Elena Schmidt[5], Peter Güntert[5], Remy Sounier[6], Adrien Favier[1], Guy Schoehn[3], Alessandro Barducci[7], Jerome Boisbouvier[1], Paul Schanda[* 1]

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Abstract: Understanding the function of proteins requires the characterization of the structure, dynamics and interactions of proteins, and linking these data to their activity.
We demonstrate here that an integrated structural biology approach in which solid-state NMR data are complemented with cryo-EM, microsecond-long MD simulations and functional assays is able to provide important functional insight into a very large molecular assembly, the 0.5 MDa large TET2 aminopeptidase enzyme.

By combining cryo-EM with solid-state NMR in a novel calculation approach we solved the structure of this 12 x 39 kDa-large protein to a precision of ca. 1 Å, including a hitherto unobservable although functionally important loop region. We studied the dynamics of this loop and provide direct evidence from NMR, EM and functional assays for its importance in the enzymatic mechanism. We furthermore reveal that in addition to this central loop motion, an extended part of the structure of TET2 undergoes microsecond mobility, connecting the entry pore to the active site of this large enzyme.

Time permitting, we will show a specific isotope labelling approach which provides detailed insight into the motion of aromatic residues in TET2 and give quantitative access to ring flips and ring axis motions over a wide range of time scales.

Parallel Lecture
IL27
Spatially encoded diffusion-ordered NMR spectroscopy
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Abstract: Diffusion-ordered NMR spectroscopy (DOSY) is a powerful approach for the analysis of solution mixtures.[1] DOSY correlates NMR signals with the translational diffusion coefficients of the corresponding molecules, and provides a virtual separation of NMR spectra. While classic DOSY experiments require a series of consecutive scans with incremented diffusion-encoding gradients, spatially encoded (SPEN) DOSY relies on a spatial parallelisation of the diffusion dimension to collect a complete data set in a single scan of less than 1 second.[2-4]

This presentation will describe several developments that aim at improving the accuracy of spatially encoded (SPEN) DOSY experiments, and making them suitable for the analysis of samples that evolve in time. For example, we have developed pulse sequences that mitigate the effect of sample convection on apparent diffusion coefficients, which is necessary for experiments in low-viscosity solvents. We have also shown that SPEN DOSY can be used to collect diffusion data in a single scan from hyperpolarized substrates prepared by dissolution dynamic nuclear polarisation (D-DNP).[5] These developments rely in part on numerical simulations that simultaneously describe spin and spatial degrees of freedom.[6] Combined with an analytical description, numerical simulations of the spatial encoding scheme were notably used to develop a more accurate model of the SPEN DOSY data.[4]
Together these developments offer a new opportunity to use the power of diffusion NMR for the real-time analysis of mixtures.

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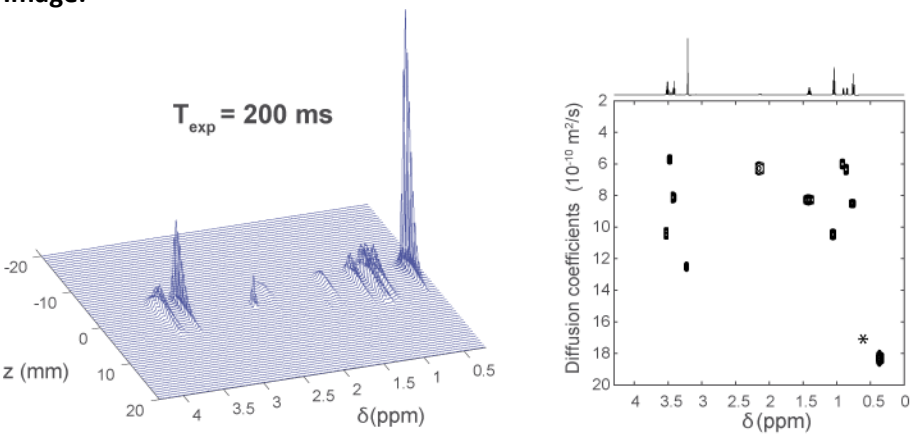
Image:



SPEN DOSY: the diffusion information is encoded spatially and the experiment lasts less than 1 s.

Parallel Lecture
IL28
Divide and rule: new NMR methods for the analysis of mixtures
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Abstract: NMR is a powerful and versatile tool for analysing mixtures in solution due to its ability to identify and determine structures of unknowns without physical separation, offering big savings in time, money and effort. The method most widely used in mixture analysis is 1D ^{1}H NMR, however almost all ^{1}H spectra suffer from low resolution due to signal overlap, which severely complicates spectral analysis. Classical strategies for alleviating overlap include using nuclei with wider shift ranges, spectral editing (e.g. using selective TOCSY), and/or multidimensional NMR techniques that disperse resonances in additional dimensions. More recently, approaches using pure shift methods, in which signal overlap is reduced by suppressing the effects of homonuclear couplings, have been proposed for obtaining ultrahigh resolution spectra and hence facilitating analysis. Such methods are very efficient for the analysis of simple mixtures but their use in complex mixtures is still limited, and further developments are needed.

Here several novel NMR approaches to solving some of the most challenging problems encountered in mixture analysis will be shown, and different strategies set out for extracting the signals of individual species from mixtures and determining chemical structures. The methods proposed use one or more of the following approaches:
- Virtual separation of components according to their different diffusion or relaxation behavior (REST[1])
- Factorization of a complex ^{1}H spectrum into subspectra for individual spin systems of components by homo- or heteronuclear (FESTA[2]) spectral editing, to allow extraction of structural information.
- Spectral simplification to facilitate analysis by combining previous approaches with pure shift NMR methodology (e.g. PSYCHE-iDOSY[3],1D selective PSYCHE-TOCSY[4]).

The usefulness of these new experiments will be illustrated across a wide range of applications, such as the analysis of peppermint oil, the identification of sugars present in lager beers, and structural analysis of a mixture of fluorinated pharmaceuticals. In addition, a new free and open-source NMR software package that includes most of the currently available tools for the univariate and multivariate analysis of mixtures – the GNAT[5] – will be introduced.

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Parallel Lecture
IL29
Magnet Technology Suitable for 30 T NMR & 20 T Human MRI
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Abstract: Superconducting (SC) magnet technology suitable for both NMR and MRI is undergoing dramatic change at present for a variety of reasons. Dramatic increases in field available for condensed matter physics have been seen in recent years with a 32 T magnet being tested at the NHMFL in Tallahassee. Higher fields for NMR are expected to emerge in coming years. Preliminary coil designs and technological options for NMR magnets up to 30 T and & human MRI magnets up to 20 T will be discussed.
NMR: The highest field available routinely for high resolution NMR today is 23.5 T (1.0 GHz) which pushes the Low-Temperature Superconductors (LTS), NbTi and Nb_3Sn, to their limit. In 1986 the High-Temperature Superconducting (HTS) materials were discovered. There followed a 20-year period of development of a number of HTS materials in a number of formats, none of which were suitable for ultra-high-field (UHF) magnets.
In 2007 a new form of REBCO (Rare-Earth-Barium-Copper-Oxide) tape was produced by SuperPower. Unlike previous HTS materials, this product is fully processed at the factory and is very strong and stiff. This was the start of a 10-year period of development of HTS conductors suitable for UHF magnets and the development of the first such magnets. In 2013 a reinforced version of Bi2223 tape became available from Sumitomo and a reinforced version of Bi22212 was demonstrated in 2016. The first UHF SC magnets using a HTS section are now operational: 24 T in Sendai, Japan, using Bi2223 and also 24 T in Daejeon Korea using REBCO. The NHMFL has tested a 32 T SC system for condensed matter physics was tested to full field in Dec. 2017.
MRI: Most clinical work is limited to 3 T. A few years ago 7 T MRI became a commercial clinical product. The highest field available today for human head MRI research is 9.4T while an 11.7 T system has been delivered to Neurospin. MRI magnets suitable for small animals exist at fields up to 21 T, and images from these systems demonstrate dramatic improvements in resolution and/or data-acquisition time via application of higher fields and justify a serious consideration of pursuing 20 T for human-head imaging.
Historically, the field available for human MRI has been primarily limited by safety regulations which are regularly being relaxed.
All of today's head and whole-body MRI magnets rely on NbTi superconductors and most rely on copper for structural reinforcement. Going beyond 11.7 T will require use of Nb_3Sn. The NHMFL has built two magnets with field/bore combinations of 14.2T/60cm and 13T/50cm using Nb_3Sn cables reinforced with steel. Conceptual designs of 600 MHz (14.1 T) and 20 T NMR magnets using both LTS and steel reinforcement have been developed. Preliminary design calculations suggest that a 20 T head MRI system incorporating HTS materials would be of comparable size to the 11.74 T Iseult magnet presently being installed at Neurospin. This surprising results is due to 1) using a 68 cm bore instead of 90 cm, 2) the higher current density of HTS materials at 20 T compared to NbTi as 12 T, and3) using steel to support the Lorenz forces instead of Cu.

Parallel Lecture

IL30

Combining solution flow and micro-detection (and sometimes hyperpolarization) for enhanced NMR

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Abstract: There are numerous cases where NMR is not easily amenable to the study of liquid samples.
The recent advent of hyperpolarized species has enabled lowering of the detection threshold, however at the price of a trickier handling of these out-of-equilibrium magnetizations.
In the aim of facilitating introduction of laser-polarized xenon into liquid samples, we designed small automated devices composed of fluidics and miniaturized NMR cells both fitted inside the narrow bore of an NMR magnet. In our setup, a gas flow driven by a programmable syringe pump actuates a micro bubble-pump which leads to circulation of the liquid sample. NMR experiments performed with the 3D-printed devices reveal high and homogeneous dissolution of hyperpolarized xenon in water.[1]
Another application of the mini bubble pump deals with the study of slowly-relaxing nuclei. By a simple circulation of the liquid sample in a closed-loop with controlled flow rate, the NMR sensitivity per time unit for these nuclei can be increased, as the interscan delay has no more to be linked with the longitudinal relaxation time. NMR velocimetry experiments have led to a precise characterization of the sample flow, largely improved by operating in stopped-flow mode. The versatility of our system has also a great interest for the continuous monitoring of chemical reactions and particularly reactions involving modification of quaternary carbons.[2]
The first versions of our device were designed to be plugged onto the body of a micro-imaging probe head: this enabled us to perform both spectroscopy and imaging experiments, but lacked versatility and limited us to use one type of commercial probe head. Recently we have proposed a wireless version of the device, by developing NMR inserts inductively-coupled to the commercial detection systems. In addition to their ease of use, these devices offer numerous advantages, since the sensitivity gain afforded by the sample flow and the micro-detection is completed by the properties of the host probe head.[3]

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Image:

Parallel Lecture

IL31

Surface-Mediated Hyperpolarization of Water and other Neat Liquids from Parahydrogen

Evan Zhao[1], Raghu Maligal-Ganesh[2], Yong Du[1], Tommy Zhao[1], Wenyu Huang[2], Clifford R. Bowers[*1]
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Abstract: In the 1980s, the PASADENA (parahydrogen and synthesis allows dramatically enhanced nuclear alignment) effect was introduced, where the singlet proton spin order of p-H_2 is revealed by symmetry-breaking chemistry.[1,2] Since then, scores of different substrates have been hyperpolarized using parahydrogen. However, despite three decades of intensive research, hyperpolarization of water from parahydrogen had not been achieved until only last year.[3] Here, the discovery of surface-mediated parahydrogen-induced alignment of the protons in liquid water as well as liquid methanol and ethanol will be presented.[4] As seen in the Figure, the hyperpolarization of the solvent protons is induced simply by the bubbling of parahydrogen through a suspension of insoluble Pt_3Sn intermetallic nanoparticles (iNPs) encapsulated within a protective mesoporous silica shell (Pt_3Sn@mSiO_2). In this effect, dubbed SWAMP (surface waters are magnetized by parahydrogen), conversion of singlet spin order into proton magnetization is mediated by symmetry-breaking interactions on the surface of the iNPs. The hydroxy protons exhibit stimulated emission NMR signals relative to those of Boltzmann polarized water. Hyperpolarization of the nonexchangeable methyl or methylene protons is also observed. Pt@mSiO_2 and PtSn@mSiO_2 catalysts were also tested but produced no detectable SWAMP signals. Apparently, the surface structure of the Pt_3Sn@mSiO_2 iNPs (see Figure) optimizes the balance between facile dissociative adsorption of H_2 and restriction of H ad-atom surface diffusion due to the presence of Sn, which likely interacts with the solvent oxygen atom. By restricting diffusion, the spin-spin coupling in the H ad-atom pair is prolonged. The spin-dynamics was investigated by performing the parahydrogen bubbling at a series of four conveniently accessible magnetic fields: near-zero field, Earth's field (~50 mT), the fringe of the NMR magnet (3 mT), and high field (9.4 T). Enhanced net emission NMR signals were observed only at 50 mT and 3 mT, but bubbling at near-zero field or 9.4 T produced no discernable SWAMP signals. Consistent with a simple density matrix model, the lack of a SWAMP effect at near-zero field suggests that symmetry-breaking Zeeman interactions are required, whereas its absence at high field indicates the importance of strong spin-spin coupling. A key advantage of the heterogeneous Pt_3Sn@mSiO_2 catalyst is its insolubility, which allows it to be quickly and completely separated from the hyperpolarized water without any leaching. SWAMP can generate NMR-observable hyperpolarization of liquids that are free of free radicals, catalyst residues, or other additives at low magnetic field. This could enable low-field MRI without superconducting magnets, which could provide wider access to inexpensive MRI.

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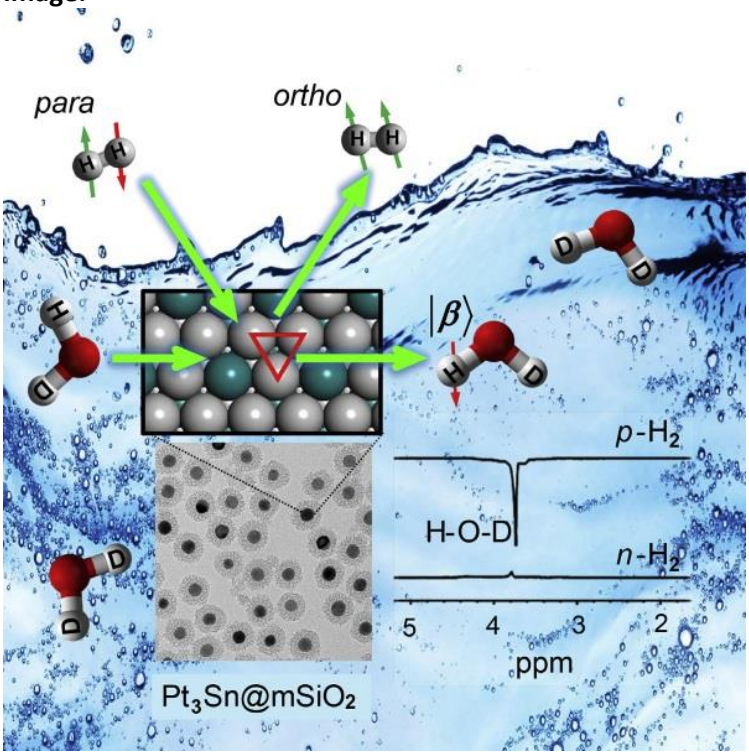
Image:



Parallel Lecture

IL32

Novel mechanisms of polarization propagation under MAS DNP

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Abstract: The active or passive propagation or spreading of enhanced nuclear polarization is of utmost importance in MAS DNP. In a typical experiment, a diamagnetic sample is doped with a paramagnetic polarizing agent which will transfer the large electron polarization to surrounding (core) nuclei. This polarization will then propagate due to spin-diffusion before it is actively transferred from ^{1}H to a low-γ nucleus in an indirect DNP experiment, or is directly read-out on the low-γ nucleus in a direct DNP experiment.

At the same time, the core nuclei are subject to enhanced paramagnetic relaxation and hyperfine shifts. This results in the appearance of a spin-diffusion barrier, limiting the efficiency of accumulation and spreading of enhanced nuclear polarization.

We will show in theory and experiment that hyperfine coupling of ^{13}C to the electron spin of the polarizing agent can be a driving element in homonuclear spin diffusion under MAS. The resulting hyperfine-driven spin diffusion allows for an additional pathway of enhanced nuclear polarization to cross the spin-diffusion barrier, leading to an increase in effective ^{13}C DNP enhancement of the bulk nuclear signals.

Furthermore, it is commonly expected that DNP of the ^{1}H and ^{13}C nuclear bulks evolves independent of each other, and that no cross-talk is present between different spin-baths under MAS DNP. However, it has recently been shown that heteronuclear cross-relaxation can result in incoherent transfer between ^{1}H and ^{13}C polarization due to temperature-activated processes. We will provide experimental evidence for methyl-reorientation in protein side-chains leading to selective transfer in direct ^{13}C DNP experiments. This effect also allows to probe selectively for ligand-host interactions as is demonstrated on proteins as well as nucleic acids.

Parallel Lecture
IL33
Ensembles of structures revealed by NMR
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Abstract: A multitude of structural states is inherent to biomolecules. One of the major challenges in structural biology is a comprehensive description of the entire structural landscape and the exchange dynamics between structural states at atomic resolution. Whereas NMR relaxation provides important aspects of local dynamics, exciting progress is currently being achieved in formulating more comprehensive descriptions of the structural landscape and the dynamics of a protein. In particular, direct methods to infer atom coordinates of slow-scale motions and to detect concerted motion are much sought-after. We have replaced the standard procedure for structure determination by an approach that generates multi-state ensembles using tight averaged distance restraints derived from exact NOEs (eNOEs) [1].

The exact measurements of 1H-1H nuclear Overhauser enhancements (eNOEs) can be converted into distances with an experimental random error of only ≈ 0.1 Å. Such eNOEs retrieve a wealth of information, which is sacrificed in routine structure determination. The collection of a dense net of eNOEs traversing a macromolecule serves as an excellent probe towards a more complete representation of structure and dynamic as demonstrated here for four proteins GB3, cyclophilin A, PDZ2 and WW domain.

The ensemble description of the prototypical enzyme cyclophilin reveals the presence of an open and a closed state, which are indicative of large scale correlated motion. In the open state, the catalytic site is preorganized for catalysis. This suggests that the mechanism of action is conformational sampling, while the ligand-binding loop appears to act through an induced fit mechanism. Overall, more than 60-70% of the side chain conformations appear to be correlated. [2]

By applying the multi-state ensemble approach to the WW domain of Pin1 free and in the presence of two ligands, the nature of allostery between two ligand binding sites was determined to be of complex nature including both conformational selection as well as decorrelation.

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Parallel Lecture

IL34

Disordered proteins function and dysfunction: being closer to physiological conditions matters.

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Abstract: Disordered proteins have essential functions in cell signalling. They act mainly through interactions that are most often modulated by post-translational modifications (PTMs) (1,2). We have reported novel, multiple phosphorylation mechanisms on oncogenic proteins established by NMR-friendly kinases, which permit NMR monitoring at 298K and pH7 and 310K; all these parameters can affect drastically specificity/efficiency of modifying enzymes. These conditions make the standard 1H/15N NMR experiments useless for most of the IDPs because of very fast water-amide proton exchange. 13C-direct detection circumvents this drawback, but its sensitivity is often limiting. Here, we will present our improvements of 2D Ca/CO experiments to enable PTMs monitoring in physiological concentrations (7.5 and T>303K). We will show results obtained on important transcription factors and oncogenic proteins. We also show that recording NMR spectra in these physiological conditions allows to observe new behaviors of lipid binding proteins such as alpha-synuclein, which opens new avenues for in-cell measurements. (1) Theillet FX et al. J Biomol NMR, 2011. (2) Theillet FX et al. Chem Rev, 2014. (3) Theillet FX et al. Nat Protoc, 2012. (4) Mylona A, Theillet FX et al. Science, 2016.

Parallel Lecture

IL35

Fundamental physics with unusual magnetic resonance

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Abstract: I will describe the work of our group and collaborators, based on NMR, to test fundamental symmetries of nature and to search for dark matter. Some of this work is described on our web pages: https://budker.uni-mainz.de/ and http://budker.berkeley.edu/, for example:

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The generaral principles of the zero-and ultralow-field (ZULF) NMR are outlined in the paper:

John W. Blanchard and Dmitry Budker, Zero- to Ultralow-Field NMR, eMagRes **5**(3), 2016.

Parallel Lecture

IL36

Electron spins help to polarise, excite and detect individual nuclear spins

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Abstract:

A typical NMR experiment consists of the steps relaxation in a large static magnetic field, excitation and detection of an ensemble of nuclear spins. Here, we report on experiments with individual nuclear spins. To achieve sufficient sensitivity, we have to optimise each of these steps: the preparation must bring the nuclear spin into the chosen state (e.g. aligned with the magnetic field) with close to unit efficiency and the detection process must generate an observable signal with minimal signal averaging. These goals can be achieved in the nitrogen-vacancy (NV) center of diamond, with the help of the resident electron spin. The polarisation process occurs through laser pulses that polarise the electron spin and subsequent microwave pulses that transfer the polarisation to the nuclear spin. Excitation of the nuclear spin can be achieved with radio-frequency pulses, but also indirectly, via microwave pulses driving the electronic spin. Detection occurs by transferring one component of the nuclear spin to the electron and subsequently detecting the electron spin polarisation by counting the photons emitted during a laser pulse. The characteristic properties of these spins include large enhancement of the gyromagnetic ratio. Applications are found in quantum information or as sensors of magnetic and electric fields as well as temperature. While the focus is on single spins, the same techniques can also be used to generate and detect highly enhanced polarisation of 13C nuclear spins in diamond.

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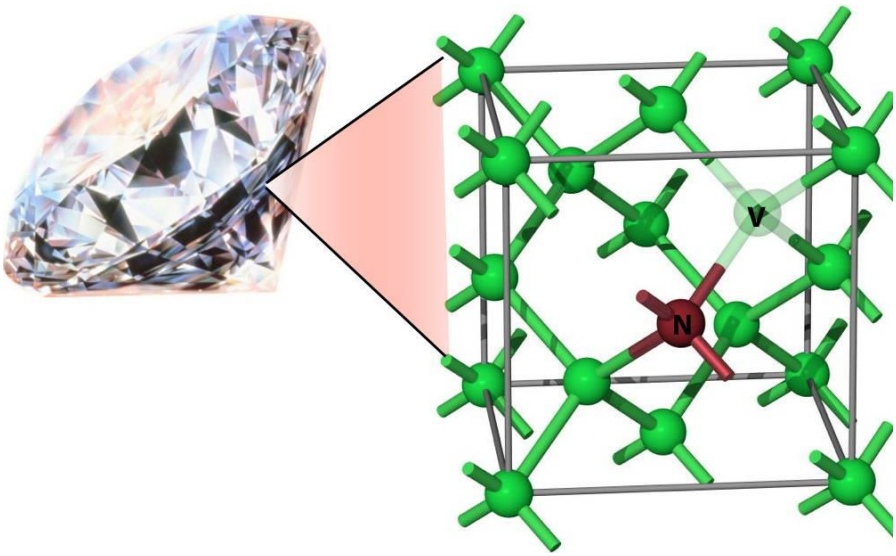
Image:



Parallel Lecture

IL37

Carbon Capture and MOFs: A Role for SSNMR

Jeffrey Reimer[*1]

[1]Chem & BioM Engr, UC Berkeley, Berkeley, United States

Abstract:

Amine-modified metal-organic frameworks (MOFs) are ideal catalysts for the reversible reaction of CO2 with amines. This chemistry has been abundantly exploited in the absorption of CO2 in aqueous solutions. NMR studies of the addition of CO2 to amine-modified MOFs, however, reveal a few surprises regarding the relationship between adsorption isotherms and the detailed chemical mechanism of amine-CO2

reactions. Surprisingly, NMR shows us how to control the mechanism by which adsorption occurs and thereby develop materials that are most practical for flue gas remediation.

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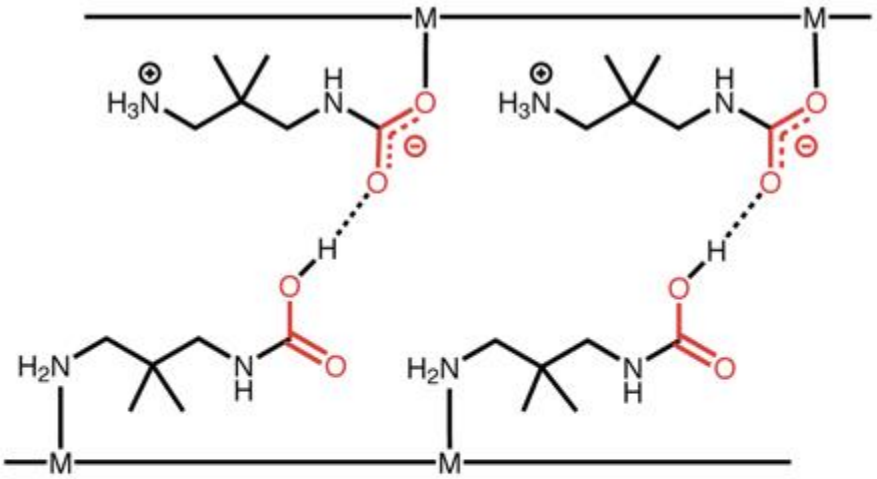

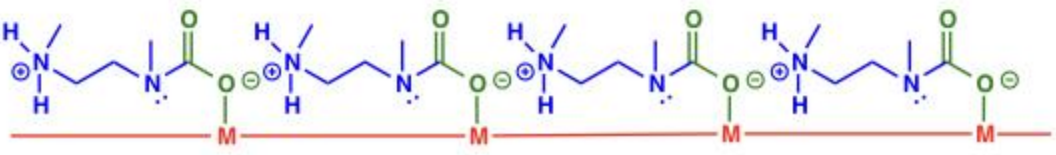

Parallel Lecture

IL38

From crystalline to amorphous Ca-phosphate biomaterials: structural insights using multinuclear solid state NMR approaches

Christian Bonhomme[1], Christel Gervais[1], Jérémy Soulié[2], Pierre Gras[2], Laetitia Mayen[2], Christele Combes[2], Thomas-Xavier Métro[3], Danielle Laurencin[* 4]

[1]LCMCP, Sorbonne Université, Paris, [2]CIRIMAT, ENSIACET, Toulouse, [3]IBMM, [4]ICGM, CNRS, Montpellier, France

Abstract: Calcium phosphate minerals are abundantly present in living organisms, where they are found in tissues like bone and teeth, as well as pathological calcifications.[1] They can be crystalline or amorphous, and involve a wide variety of compositions, depending on (i) the nature of the phosphate anions (ortho- or pyro-phosphates) and their protonation state, (ii) the Ca/P ratio, (iii) the hydration level (number of water molecules in close interaction), and (iv) the nature and number of other ionic substituents (e.g. carbonate, sodium, magnesium…).

In view of preparing new materials for bone-substitution, or understanding the formation of healthy (or pathological) minerals in vivo, a wide diversity of synthetic calcium phosphate biomaterials have also been synthesized. While crystalline phases related to hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) are the most widely studied, due to their similarity to bone mineral (especially when they are prepared in a nanocrystalline form), other amorphous phases like hydrated calcium (pyro)phosphates and calcium phosphate glasses have more recently been looked into.[2] Just like for the natural minerals, there are still a number of questions regarding the structure of these synthetic materials.

In this presentation, it will be shown in what way multinuclear solid state NMR techniques, in conjunction with computational modeling, can provide insight into the structure of a variety of natural and synthetic Ca-phosphate compounds. Particular emphasis will be placed on ^{43}Ca and ^{17}O NMR techniques, showing in particular some of our latest developments to try to tackle sensitivity issues for these poorly receptive quadrupolar nuclei, including new labeling schemes for the preparation of ^{17}O-enriched compounds using mechanochemistry, and natural abundance ^{43}Ca NMR studies performed at ultra-high magnetic field (up to 36 T) [2,3].

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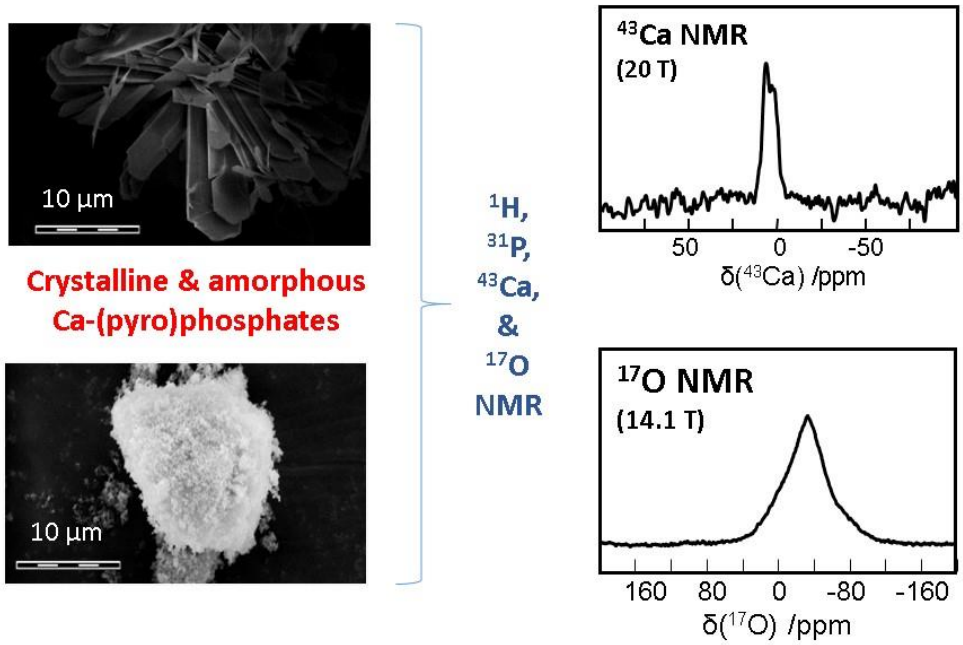
[3] (a) T.-X. Métro, C. Gervais, A. Martinez, C. Bonhomme, D. Laurencin, *Angew. Chem.* **2017**, *56*, 6803; (b) Unpublished results (obtained at the NHMFL, with Z. Gan, I. Hung and X. Wang).

Image:


10 µm
Crystalline & amorphous
Ca-(pyro)phosphates
10 µm
^{1}H,
^{31}P,
^{43}Ca,
&
^{17}O
NMR
^{43}Ca NMR
(20 T)
50
0
-50
δ(^{43}Ca) /ppm
^{17}O NMR
(14.1 T)
160
80
0
-80
-160
δ(^{17}O) /ppm

Parallel Lecture

IL39

HUMAN AND IN VITRO METABOLOMICS IN HEALTH RESEARCH

Ana Gil[*1]

[1]Chemistry and CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal

Abstract: This presentation is composed of two parts, one regarding the more common use of metabolomics to search for disease biomarkers in biofluids, and a second part relating to selected *in vitro* applications that may, in time, also impact positively on human health.

Increasingly, biofluid metabolomics has been developing towards non-invasive biological samples, such as urine and saliva. The former biofluid is particularly interesting due to its exquisite complexity and sensitivity to general health status of the human organism. However, these same features also lead to added challenges related to the contribution of individual and population phenotype (including the effects of diet, lifestyle, geographic characteristics, etc) to the sought disease signature or biomarker. In this talk, urine metabolomic studies focusing premature birth, exposome effects on pregnancy or age-related diseases will be presented, while considering the effects of phenotype variability, either within single cohorts, or between different national or trans-national cohorts.

Following a bottom-up approach, *in vitro* metabolomic strategies may provide valuable levels of biological information (e.g. regarding drugs or environmental contaminants), which may eventually translate into tissue or biofluid biomarkers of potential clinical use. This will be exemplified by an *in vitro* metabolomics study of the use of the biomaterial poly-L-lactic acid (PLLA) and its piezoelectric properties in tissue regeneration approaches. Ours results show that cell metabolomics may provide new and detailed information on the response of cells and tissues to biomaterials employed as implants. Such metabolic features may in time translate into biomarkers of biomaterial performance, for both *in vitro* and *in vivo* applications.

Parallel Lecture
IL40
Isotopomic 13C NMR
Serge Akoka[*1]
[1]CEISAM, Université de Nantes, Nantes, France

Abstract: Nuclear Magnetic Resonance (NMR) spectrometry has been extensively used for analysing complex mixtures. Two approaches are currently used: the determination of the isotopic profile of major components and the determination of the metabolic profile. Concerning the isotopic profiling, NMR spectrometry is the only analytical technique which allows the quantification of each isotopomer of a given molecule. Isotopic ^{2}H NMR spectrometry is recognized as a powerful technique for authentication and traceability of numerous products [1]. However, it requires long measurement time and large molecules could not be studied because of the complexity of the NMR spectrum. These drawbacks can be overcame by using ^{13}C-NMR [2] and polarisation transfer sequences which induce a dramatic reduction in the experimental time without deterioration in short time or long time stability [3]. The results obtained show that this strategy can be used effectively to determine the ^{13}C distribution within a given molecule in a very short analytical time and to accurately compare differences in the isotopic distribution between different samples of the given molecule, even for molecule as large as Docetaxel. Furthermore, in the isotopomic approach, metabolomic profiling and carbon isotopic fingerprinting are simultaneously obtained from ^{13}C-NMR [4]. In cases where this approach could be limited by peak overlaps, we have shown that it can be extended to a higher dimensionality: the precision required for isotopic analysis can be reached using an optimized ^{1}H–^{13}C HSQC sequence with an experimental time of only 22 min [5].

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Parallel Lecture

IL41

Spin-dependent Processes in Organic Solar Cell Materials

Felix Kraffert[1], Ula Yasin[1, 2], Jan Behrends[* 1, 2]

[1]Berlin Joint EPR Lab - Fachbereich Physik, Freie Universität Berlin, [2]Berlin Joint EPR Lab - Institut für Nanospektroskopie, Helmholtz-Zentrum Berlin, Berlin, Germany

Abstract: Photocurrent generation in solar cells based on organic semiconductors involves localised states which, if occupied by single electrons or holes, are paramagnetic and can be detected by electron paramagnetic resonance (EPR) spectroscopy. EPR techniques can thus provide valuable insight into excitation transfer pathways in organic solar cell absorber materials. However, these measurements are usually performed on "model systems", and the conclusions drawn from such experiments may not necessarily be valid under true solar cell operating conditions.

Here we report on the development of a setup that allows for simultaneous detection of transient EPR as well as transient electrically detected magnetic resonance (trEDMR) signals from fully-processed and encapsulated solar cells. Combining both techniques provides a direct link between photoinduced triplet excitons, charge transfer states and free charge carriers as well as their influence on the photocurrent generated by organic photovoltaic devices. Our results obtained from solar cells based on poly(3-hexylthiophene) and a fullerene-based electron acceptor show that the resonant signals observed in low-temperature (T = 80 K) trEDMR spectra can be attributed to positive polarons in the polymer as well as negative polarons in the fullerene phase, indicating that both centres are involved in spin-dependent processes that directly influence the photocurrent [1]. We will further show how electrically detected hyperfine sublevel correlation (ED-HYSCORE) spectroscopy can help to identify current-influencing paramagnetic states in organic tandem solar cells [2].

The second part of the talk will be devoted to triplet excitons, which are encountered in various materials used in high-efficiency organic solar cells. Triplet excitons can, on the one hand, be involved in loss mechanisms and reduce the yield of separated charge carriers. On the other hand, they play an important role in processes such as singlet fission that provide the possibility of building solar cells with quantum efficiencies exceeding 100 %. Using transient EPR spectroscopy we study intermediate paramagnetic states generated upon fission of one singlet exciton into two separated triplet excitons. Particular emphasis will be given to strongly exchange-coupled triplet pairs in organic molecules that form quintet states [3].

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Parallel Lecture

IL42

Combining DEER, ODNP, X-ray and MD simulations to unveil the mechanics of a heterodimeric ABC transporter

Enrica Bordignon[* 1], M. Hadi Timachi[1], Cedric A.J. Hutter[2], Hendrik Göddeke[1], Svetlana Kucher[1], Laura Galazzo[1], Sukhendu Nandi[1], Lars V. Schäfer[1], Markus A. Seeger[2]

[1]Chemistry and Biochemistry, Ruhr University Bochum, Bochum, Germany, [2]Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

Abstract: ATP binding and hydrolysis in the nucleotide-binding domains of ABC transporters are chemo-mechanically coupled to conformational changes in the transmembrane domains, leading to substrate translocation. Here we show that the combination of biochemical assays, site-directed mutagenesis, X-ray crystallography, EPR and MD simulations allow to deepen the understanding of the molecular details of the large-scale movements underlying the functional working cycle of the heterodimeric ABC exporter TM287/288.

Using site-directed spin labelling and DEER we unravelled an unexpected conformational equilibrium of the protein during nucleotide cycle, and nucleotide-dependent features which are specific for exporters carrying non-symmetric nucleotide binding sites[1]. By performing unbiased multi-microsecond MD simulations in an explicit membrane/water environment we could also show for the first time how in response to ATP binding, TM287/288 undergoes spontaneous conformational transitions from the inward-facing (IF) state (the only state crystallized up to now) via an occluded (Occ) intermediate to an outward-facing (OF) state[2]. The OF structural model was experimentally validated by Double Electron Electron Resonance (DEER, also known as PELDOR) performed on transporters spin labeled with nitroxide probes in detergents as well as in liposomes. The MD study highlighted the role of water cavities responsible for the net transport of substrate through the protein, which were experimentally investigated by X-band room temperature Overhauser Dynamic Nuclear Polarization (ODNP) techniques probing site-specific water accessibility during the cycle. The OF structure was finally solved by x-ray (unpublished) using a synthetic nanobody, which causes strong inhibition of ATP hydrolysis. Comparison between the crystallized and MD-obtained models will be presented. To monitor the effects of this nanobody on the conformational cycle of TM287/288, we spin labeled it with a gadolinium probe, to follow on the same sample, both the movements of the transporter and the binding of the sybody. To add another spin on this story, we also engineered nitroxide probes on putative substrates of the transporter, to address via EPR their translocation mechanism.

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ORAL COMMUNICATIONS

Bioliquids

O01

MECANISTIC VIEW OF THE OPIOID mu-RECEPTOR ACTIVATION

Helene Demene[*1], Remy Sounier[2], Toon LAEREMANS[3], Jan Steyaert[3], Aashish Manglik[4], Brian kobilka[4], Sebastien Granier[2]
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Abstract: Opioid receptors (OR), members of the G protein-coupled receptor (GPCR) superfamily, constitute the major and the most effective target for the treatment of pain[1]. The use of opioid drugs acting at these receptors is however a leading cause of death by overdose in Europe and North America. Both beneficial and adverse effects of illicit opioid drugs (opium, heroin) as well as approved therapeutics (morphine and codeine) are mediated by the activation of the mu-opioid receptor (μOR).

We recently described the structure of an inactive and active conformation of the μOR[2],[3] bound to a G protein mimetic nanobody. It provided important information regarding the binding site of small morphinan antagonists and agonists, and demonstrated the key molecular determinants for ligand binding and activation process common to other GPCRs. However, much remains to be learned about the mechanisms by which different agonists can induce distinct levels of Gi protein activation and/or arrestin recruitment upon activation of μOR. Pharmacological and biophysical studies suggest that this versatility can be achieved through the structural plasticity of GPCRs[4].

In this work, we analyze the conformational landscape of the μOR in distinct pharmacological conditions using liquid-state NMR spectroscopy by monitoring signals from methyl-labelled lysines and methionines. We also investigate the structure and dynamics changes upon binding to different ligands ranging from agonist to antagonists, as well as upon binding the effector Gs protein and a mimetic nanobody thereof. Our results show that there is very weak allosteric coupling between the agonist binding pocket and G protein coupling interface (transmembrane TM 5 and 6). Furthermore, the analysis provides clues on the successive structural events leading to the full active conformation of moR [5]. We know extend this approach to biased ligands, that are able to elicit G-protein activation without arrestin activation, and to partial ligands, that promot only partial activation of the G-protein[6]. A better knowledge of the structural basis of all activation pathways for opioid drug efficacy may lead to new therapeutic approaches with limited side effects.

References:

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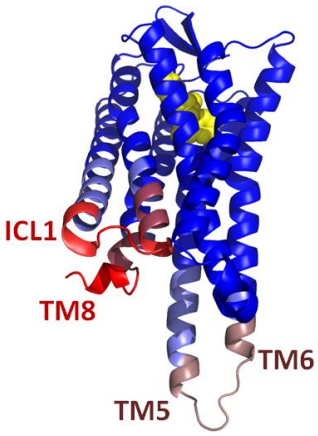
[6] in preparation.

Image:

GPCR agonist pre-activation



Bioliquids

O02

Chaperone–client-interactions: From basic principles to roles in health and disease

Björn M. Burmann[*1]

[1]Department of Chemistry and Molecular Biology, Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden

Abstract: Molecular chaperones are essential for maintaining a functional proteome in the cells. Nevertheless, central functional aspects of chaperones are still not well understood at the atomic level, including how chaperones recognize their clients, and in which conformational states clients are bound. I will describe recent research efforts to understand such aspects, employing high-resolution NMR spectroscopy as the main method.

Initial work on the periplasmic holdase Skp with bound outer membrane proteins provided the first atomic-level description of a natural full-length chaperone–client complex [1, 2]. Subsequent work combining NMR spectroscopy with single-molecule force spectroscopy showed how periplasmic chaperones shape individual client folding trajectories, funneling the client polypeptide towards the native structure [3]. Then, a characterization of the dimeric state of the chaperone Trigger Factor revealed how the dynamic high-affinity dimer is formed and provides the foundation into how it is able to quickly engage with a diversity of client proteins [4].

We then utilize our mechanistic insights to investigate the functional role of chaperones in Parkinson's disease. Parkinson's is one of the most common neurodegenerative disorders, pathologically manifested by intracellular accumulation of aggregates of the intrinsically disordered protein α-Synuclein. Systematic investigations on an array of chaperones identified a general chaperone interaction motive at the α-Synuclein amino-terminus. The dominant role of chaperone interactions for cytosolic α-Synuclein was validated with *in-cell* mass-spectrometry and NMR spectroscopy and the functional basis for the effects of several known post-translational modifications of α-Synuclein could thus be reconstituted *in vitro*. The data reveal how molecular chaperones control the state and function of α-Synuclein *in vivo* and how the disturbance of these interactions leads to progress of pathologically relevant aggregates.

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Bioliquids

O03

Capturing Protein Dynamics with Unprecedented Speed by High Power Relaxation Measurements

Supriya Pratihar[*1], G. J. Reddy[2], Stefan Becker[1], B. L. de Groot[1], Donghan Lee[3], Christian Griesinger[1]

[1]Max Planck Institute for Biophysical Chemistry, Gottingen, Germany, [2]CSIR-Indian Institute of Chemical Technology, Hyderabad, India, [3]University of Louisville, Kentucky, United States

Abstract: Protein dynamics occurring on a wide range of timescales play a crucial role in governing protein function. While structural ensembles are well characterized by molecular dynamics, the kinetics of their interconversion is still difficult to assess computationally. NMR spectroscopy measures dynamics in proteins with atomic resolution and accesses time scales from ps to seconds. A time range of four orders of magnitude, from the rotational correlation time (T_c; single digit ns) to around 40 µs was inaccessible for kinetic investigations by NMR until recently[1, 2, 3, 5]. The faster limit of the accessible timescale is defined by the amplitude of applied radiofrequency power. Recently, in cryo-probes using Helmholtz coils we could apply 40 kHz RF power on 1H, which pushed the detectable limit to ~2.5 µs. For the first time, this method, performed under super-cooled conditions, enabled us to detect a functionally relevant global motion in the first β-turn of protein GB3, exhibiting the potential link of the observed supra-τ_c motion with molecular recognition.[1]
A further increase of the RF field is possible by using solid state NMR probes, which apply RF via a solenoid coil to samples in capillaries with 0.7 mm outer diameter. This allows raising the RF field to 300 kHz making kinetics available down to 300 ns. Kinetic investigation of supra-τ_c motion in ubiquitin revealed a correlated motion that involves the whole protein including a prominent peptide flip motion between D52-G53. The peptide flip occurs at 308K at 5 µs.[4] Abrogating the peptide flip by E24A or G53A mutation quenches the correlated motion in the whole protein and changes binding affinities to other proteins. We have applied this high-power RD technique to an intrinsically disordered protein, p53. p53 is partially folded and adopts an α-helix (aa 18-26) with a population of 30% in the wt and 60% in P27A mutant. With high-power RD, a folding process could be observed at 263 K with a rate of 4 µs for the WT. Surprisingly, the same folding process was slowed down on average 80-fold in the P27A mutant.

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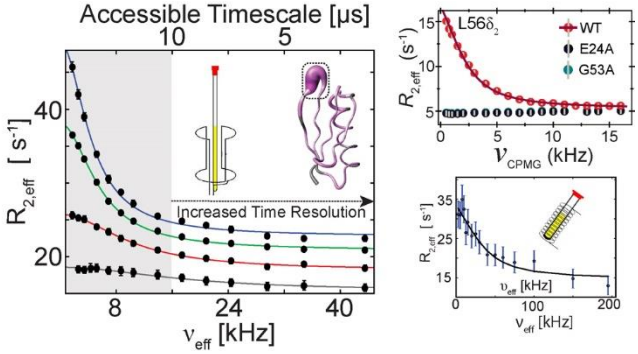
Image:



Hyperpolarization

O04

Hyperpolarized water to visualize disordered, well-folded and lowly-populated states in proteins

Or Szekely[*1], Gregory Olsen[1], Rina Rosenzweig[2], Lucio Frydman[1]

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Abstract: A number of high-field dynamic nuclear polarization (DNP) methods were developed in recent years to improve the sensitivity of solution NMR; foremost among these is the dissolution DNP (dDNP) approach.[1] Ex-situ dDNP of hyperpolarized water ("HyperW") holds great potential in protein NMR studies: when injecting this solvent into an NMR tube containing a protein that can readily exchange its amide protons, amide-based hyperpolarized protein NMR studies become feasible.[2],[3] 2D ^{1}H-^{15}N correlations are collected with high sensitivity due to rapid exchange which transfers magnetization from hyperpolarized water to the amide groups, and the long-lived (≈1-2 min) signal enhancements that can be achieved thanks to the long relaxation times T_1 of water protons. HyperW can be particularly useful for studies of intrinsically disordered proteins or domains, which exhibit fast amide exchange.[4],[5],[6] With optimizations and resolution improvements[6] of enhanced 2D protein NMR spectra at hand, one can use the HyperW method in the structure and dynamics study of a variety of proteins. So far these have mainly focused on intrinsically disordered peptides and proteins;[2]-[6] here, we extend HyperW and compare its applicability to three types of proteins: PhoA4, a fully unfolded protein; fully folded barstar; and a drkN-SH3 domain which exists in equilibrium between a folded and an unfolded state. For the unstructured PhoA4, the sensitivity enhancements were very high, thanks to the fast amide exchange experienced by residues throughout the sequence. For the barstar protein an "exchange filter" behavior was observed, whereby higher enhancements were measured for surface exposed yet fully folded residues (Fig. 1). For the drkN-SH3 domain, HyperW HMQC managed to bring the lowly-populated, usually invisible folded state to light (Fig. 2) with the aid of a 3-site exchange magnetization transfer. This demonstrates the many strengths of the HyperW method: it can serve for sensitizing the NMR of disordered proteins; it can also be applied to study fully folded proteins and probe the latter's solvent accessibility; and it can be used to visualize what are normally "invisible" protein states.

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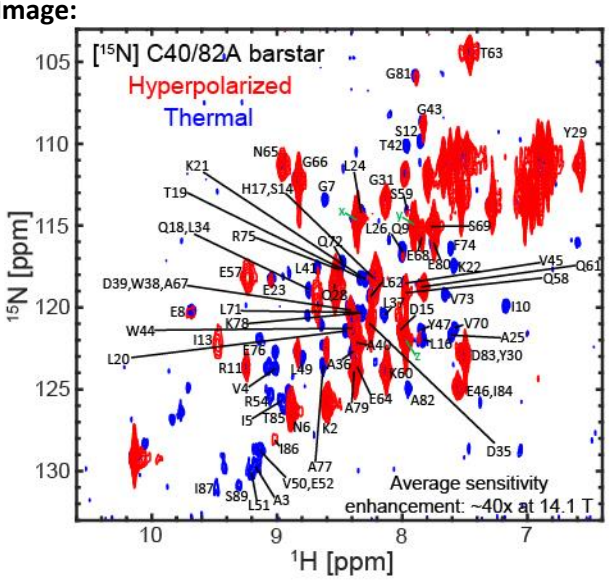

Fig. 1. 2D HMQC spectra measured at 600MHz on a barstar C40/82A double mutant upon injection of hyperpolarized water (red, 2 scans / 64 t1 points) and at thermal equilibrium (blue, SOFAST acquisition, 64 scans/ 64 t1 points).

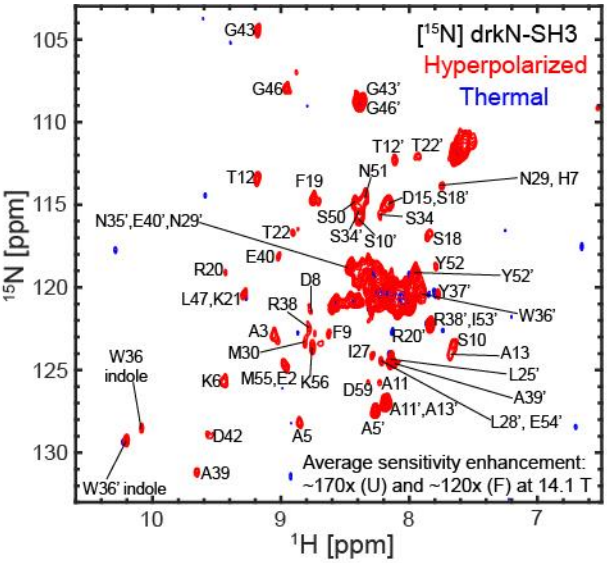

Fig. 2. 2D HMQC spectra measured at 600MHz on drkN-SH3 upon injection of hyperpolarized water (red, 2 scans / 64 t1 points) and at thermal equilibrium (blue, SOFAST acquisition, 512 scans/ 64 t1 points).

Hyperpolarization

O05

Radical polarization and liquid-state dynamic nuclear polarization (DNP) with photo-excited fullerene nitroxide derivatives

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Abstract: Dynamic Nuclear Polarization (DNP) has been established as a method to enhance NMR signals in solution and solid state by transferring the polarization from unpaired electron to nuclear spins. Herein, we report on radical polarization and Overhauser type DNP (ODNP) performance of nitroxide radicals functionalized by photo-excitable fullerene nitroxide (FN) derivatives in liquids. In a previous study, we observed a large-electron spin saturation factor of FN radical by conventional DNP with microwave at 0.34 T/9.8 GHz. [1] In this study, pulse laser excitation of the FN derivatives leads to a transient nitroxide radical polarization, which is at least one order of magnitude larger than Boltzmann equilibrium. Besides, the lifetime of radical polarization increases with the size of the FN derivative and correlates with the electronic spin-lattice relaxation time (T_{1e}). Small Overhauser ^{1}H-NMR signal enhancements of the toluene solvent were observed under steady-state illumination of FN derivatives. [2] The build-up curve of the DNP enhancements follows the expected kinetics of ODNP by nuclear spin-lattice relaxation. Unlike conventional microwave DNP, the observed optical-driven DNP enhancements of toluene are positive, because the photo-induced, positive radical polarization ($P_{exc} > P_0$) generates a negative DNP saturation factor ($s_{eff} = (S_0 - \langle S_z \rangle)/S_0$).

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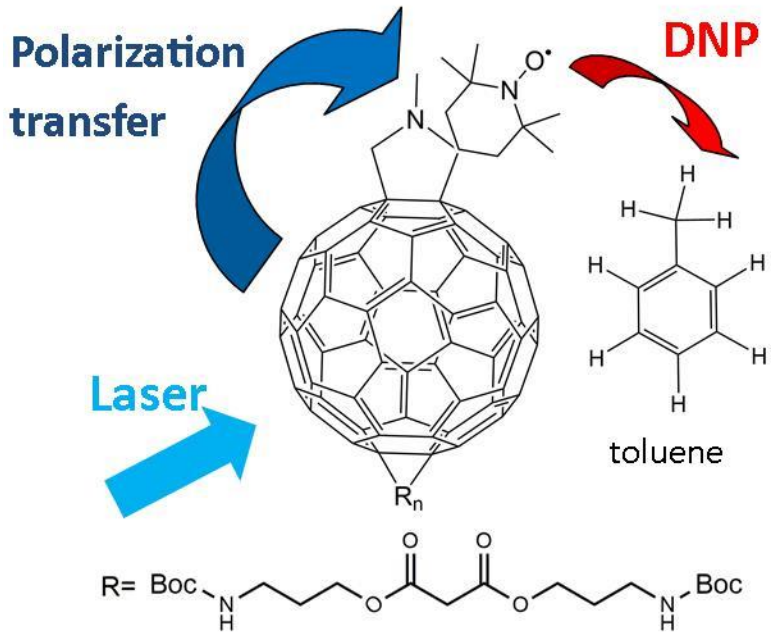

Hyperpolarization

O06

Direct hyperpolarization of 15N2 singlet states in terminal diazirines (R-CH15N2) enables long lived hyperpolarization and sensitive proton detection.

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Abstract: For applications of hyperpolarized compounds, it is attractive to have slow relaxation to allow for complex experimental procedures and to probe slow processes. Particularly slow relaxation can be found in singlet states on pairs of heteronuclei such as $^{13}C_2$ or $^{15}N_2$ [1,2]. A special opportunity presents itself in direct hyperpolarization of heteronuclear singlet states using SABRE (Signal Amplification By Reversible Exchange): Singlet order can be transferred directly from parahydrogen to targeted heteronuclear spin pairs. For example, $^{15}N_2$ in diazirines, one of the smallest 3 membered heterocycles, supports SABRE induced singlet order with decay time constants of above 20 minutes.[1]

A remaining challenge in these experiments is that direct detection of ^{15}N signals is significantly less sensitive than detection on ^{1}H. Addressing this problem we designed terminal diazirines (R-CH$^{15}N_2$, see figure, part A) and show that hyperpolarization transfer from ^{15}N to ^{1}H enables sensitive detection of relaxation protected states on the more sensitive ^{1}H channel. Importantly and counter intuition, the close proximity of the proton to the $^{15}N_2$ pair does not significantly reduce the singlet relaxation decay constant T_S.

Typically, when a proton is brought close to a spin pair, T_S becomes significantly shorter because the dipolar coupling induces fast relaxation. This is not the case in a terminal diazirine because of the high symmetry: The dipolar interaction of the protons with each individual ^{15}N spin is identical. Therefore, the singlet state commutes with the dipolar Hamiltonian and remains protected from this, otherwise detrimental, relaxation channel. We observe singlet decay time constants T_S of up to 17.5 min in terminal diazirines (This is a surprisingly small reduction from 20 min T_S in non-terminal diazirines).

The T_S measurements and results from hyperpolarization transfer are illustrated in parts B and C of the Figure. First, it is possible to selectively hyperpolarize either magnetization or singlet order and measure their lifetimes (T_1 and T_S) independently. (Polarization levels of ~2% are typical) Next, hyperpolarization transfer efficiencies of up to 70% were observed on ^{1}H.

In conclusion, we have combined long-lived hyperpolarization induced by SABRE on ^{15}N spin pairs with sensitive detection on ^{1}H promising hyperpolarized experiments that exceed current approaches in signal lifetimes and sensitivity. Importantly, the employed terminal diazirines can replace methyl groups in biomolecules without significantly altering steric burden and therefore represent a highly promising molecular tag for hyperpolarized experiments.

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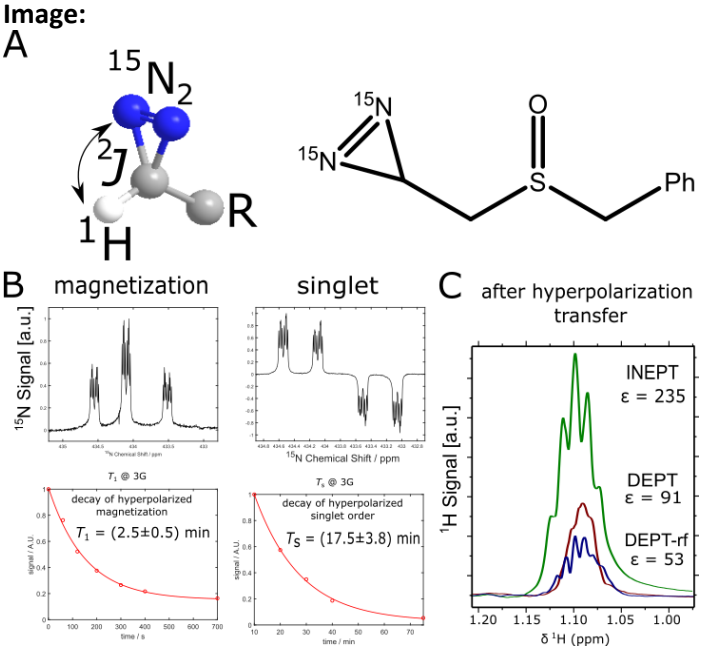
A
15N2
2J
1H
R
15N
15N
O
S
Ph
B
magnetization
singlet
15N Signal [a.u.]
15N Chemical Shift / ppm
T1 @ 3G
decay of hyperpolarized magnetization
T1 = (2.5±0.5) min
signal / A.U.
time / s
Ts @ 3G
decay of hyperpolarized singlet order
TS = (17.5±3.8) min
time / min
C
after hyperpolarization transfer
1H Signal [a.u.]
INEPT
ε = 235
DEPT
ε = 91
DEPT-rf
ε = 53
1.20 1.15 1.10 1.05 1.00
δ 1H (ppm)

Computation

O07

Capturing the Dynamics of Intrinsically Disordered Proteins Using NMR Relaxation and Molecular Dynamics Simulations

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Abstract: The function of Intrinsically Disordered Proteins (IDPs) is encoded both in their conformational heterogeneity and dynamics. Spin relaxation reports on the timescales and amplitudes of ps-ns motions, although conventional model-free analysis is often not sufficiently insightful in the case of IDPs because measured relaxation rates are averaged over the entire ensemble of conformations accessed by the protein.

We introduce the measurement of ^{15}N relaxation rates over a large range of temperatures (268-298K) on three constructs of different length of the N_{tail} domain of the nucleoprotein of Sendai virus. This extensive dataset, acquired at four different magnetic fields, provides a wealth of information on dynamics that is modelled using a novel procedure based on an Arrhenius-type relationship. Our results allow us to assign the physical origin of different dynamic modes in this archetypal IDP [1].

Molecular Dynamics (MD) simulations are, in principle, the perfect complement to NMR relaxation data, provided that a) suitable *sampling* schemes permit to characterize the vast phase space of IDPs in a computationally efficient manner and b) accurate *force fields* provide a sound description of the rugosity of the free-energy landscape and, consequently, of the motions. We develop an original approach termed ABSURD (Average Block Selection Using Relaxation Data, [2]) in which an ensemble of short trajectories, initiated from conformers that are distant in the energy landscape, are used to adequately sample the conformational space accessible to IDPs, effectively mimicking the averaging process intrinsic to ensemble techniques such as NMR. In addition, ABSURD uses a subset of measured relaxation data to improve the accuracy of the simulated dynamics. In particular, we show that rates reporting on the spectral density function evaluated at zero frequency (e.g., R_2) are a sensitive probe of systematic errors in force fields. The method systematically improves agreement with independent experimental relaxation data, irrespective of the targeted rates, suggesting interdependence of motions occurring on timescales varying over three orders of magnitude.

Finally, combining MD simulation and NMR, we introduce a framework in which distinct motions are attributed to local librations, backbone dihedral angle dynamics and longer-range tumbling of one or more peptide planes [3]. This model provides unique insight into the segmental organization of dynamics in IDPs. We show that spin relaxation and its temperature dependence are exquisitely sensitive to intrasegment dynamics. Our MD simulations illustrate the complexity of intrasegment dynamics and its crucial coupling with the solvent.

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Computation

O08

TReNDS - software for reaction monitoring with time-resolved non-uniform sampling

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Abstract: NMR spectroscopy, used routinely for structure elucidation, has also become a widely applied tool for process and reaction monitoring. However, the most informative of NMR methods - correlation experiments - are often useless in this kind of applications. The traditional sampling of a multidimensional FID is usually time-consuming, and thus the reaction-monitoring toolbox was practically limited to 1D experiments (with rare exceptions, e.g. single-scan [1] or fast-sampling experiments [2]). Recently, the technique of time-resolved non-uniform sampling (TR-NUS) has been proposed, which allows to use standard multidimensional pulse sequences preserving the temporal resolution close to that achievable in 1D experiments [3,4,5,6]. However, the method existed only as a prototype and did not allow on-the-fly processing during the reaction. Here, we present TReNDS (Time-Resolved N-Dimensional Spectroscopy) - an open-source program for processing and display of TR-NUS data, combined with acquisition macros for spectrometers. The program is written in Python and exploits the CS module of the mddnmr package [7]. The pre- and post-processing of the NUS signal is performed using the nmrglue library [8]. The package also has an effective peak-tracking module allowing on-the-fly analysis of the reaction progress. TReNDS is compatible with Bruker, Agilent, and Magritek spectrometers, and therefore opens up an avenue for applications of TR-NUS in a variety of scientific and industrial problems.

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Computation

O09

QUANTUM-CHEMICAL COMPUTATION OF pNMR PARAMETERS

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Abstract: Quantum-chemical methods for the assignment, prediction and analysis of paramagnetic nuclear magnetic resonance (pNMR) parameters have been a subject of much interest lately, both due to the increasing importance of pNMR experiments in bio- and materials sciences, as well as the improved predictive power of such calculations. I review the classic Kurland-McGarvey theory of the pNMR chemical shift [1] and our reformulation thereof [2], which describes the shielding tensor using electron paramagnetic resonance (EPR) parameters and as resulting from essentially two kinds of physical effects: (a) magnetic couplings by the Zeeman and hyperfine operators between the zero-field split states belonging to the ground multiplet and (b) thermal occupations of these states.

Combination of this theory with a practical, mixed *ab initio*/density-functional theory computational protocol leads to useful accuracy from the point of view of experimental pNMR activities on transition-metal systems [3,4]. A point-like dipole approximation of the "long-distance terms" contained in the theory allows for *ab initio* investigation of the chemical shifts in an entire metalloprotein [5]. Scalar relativistic effects can also be introduced for improved predictions on 4*d* and 5*d* systems, as well as lanthanides [6,7]. Recently, the Kurland-McGarvey -like method was introduced also for the paramagnetic enhancement of the indirect spin-spin coupling tensor in open-shell systems [8].

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Biosolids

O10

Selective Enlightenment of Interaction Surfaces in Biological Systems by DNP-enhanced NMR

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Abstract: X-ray crystallography, cryo-electron microscopy (cryo-EM) and nuclear magnetic resonance (NMR) are the main tools used in structural biology to solve the structure of proteins at atomic resolution. Beyond solving structural questions, it is also crucial to address interaction processes. This often remains a challenge, both in terms of selectivity and sensitivity for the aforementioned techniques.

Here we present a novel approach, dubbed SelDNP that enables the selective enlightenment of residues from and close to the binding site of proteins using DNP-enhanced solid-state NMR. DNP under MAS has rapidly developed in the last years to increase the overall sensitivity of NMR experiments and has been demonstrated in various applications. Its use for biomolecular systems is however often limited by the necessity to run experiments at cryogenic temperatures (usually 100 K), which can induce line broadening and loss of resolution. In this context, **SelDNP is a powerful site-directed approach, which relies on the use of ligand-functionalized polarizing agents. It is demonstrated on the binding site of the galactophilic lectin LecA. The well resolved SelDNP spectra contain resonances from the binding site only and enabled the *de novo* assignment of more than 20 residues from the binding interface of LecA.** This approach allows resolving the main limitation of DNP-enhanced NMR for biomolecular applications, i.e. the lack of resolution often encountered at low temperature, while providing a new tool to address selectively binding interfaces.

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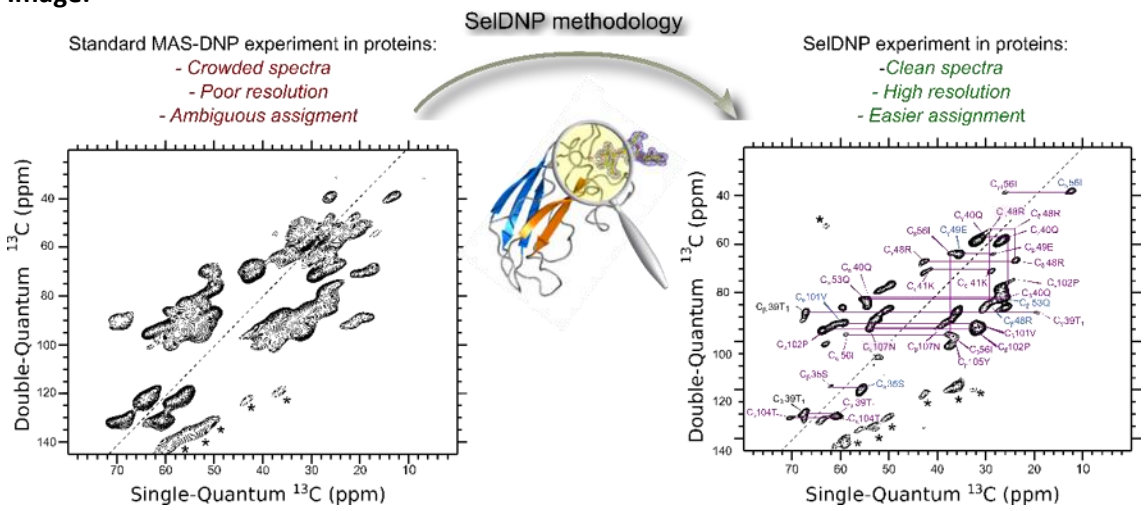

Biosolids

O11

A single NaK channel conformation is not enough for non-selective ion conduction

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Abstract:

NaK and other non-selective channels are able to conduct both sodium (Na^+) and potassium (K^+) with equally high efficiency. Although NaK has a similar SF sequence (TVGDG) as K^+ selective channels, high-resolution crystal structures of NaK revealed the conservation of only the S3 and S4 ion binding sites, with the upper part of the SF becoming a vestibule [1]. No structural rearrangement was observed in the crystal structures of NaK with Na^+, K^+, or rubidium (Rb^+), and the different ions were therefore proposed to permeate through the same SF conformation with different preferential binding sites [2]. In contrast to previous crystallographic results, we show that the selectivity filter (SF) of NaK in native-like lipid membranes adopts two distinct conformations that are stabilized by either Na^+ or K^+ ions. The atomic differences of these conformations are resolved by solid-state NMR (ssNMR) spectroscopy and molecular dynamics (MD) simulations. Besides the canonical K^+ permeation pathway, we identify a side entry ion-conduction pathway for Na^+ permeation unique to NaK. Moreover, under otherwise identical conditions ssNMR spectra of the K^+ selective NaK mutant (NaK2K) reveal only a single conformational state. Therefore, we propose that structural plasticity within the SF and the selection of these conformations by different ions are key molecular determinants for highly efficient conduction of different ions in non-selective cation channels [3].

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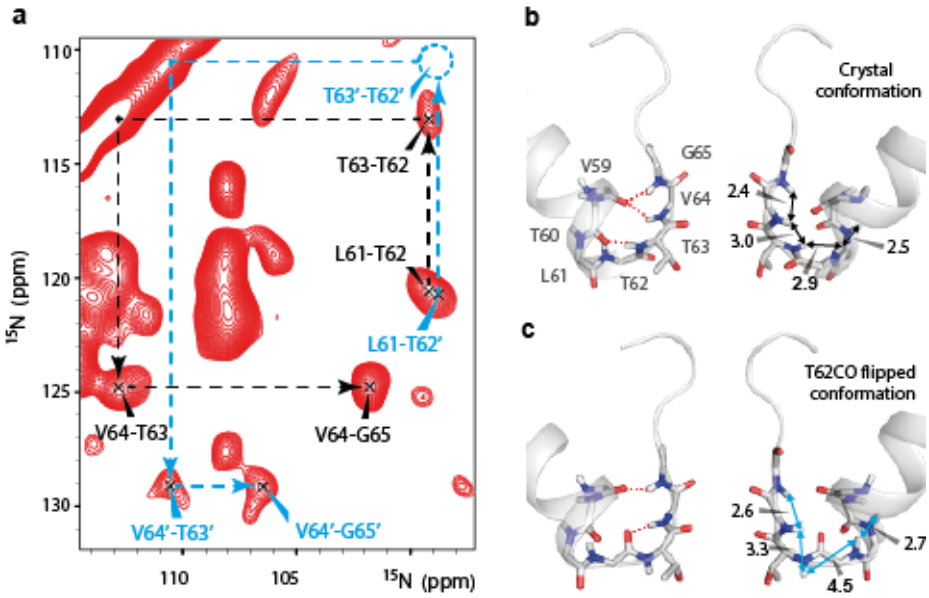

Biosolids

O12

NMR Spectroscopy at 1.5 GHz Using a 35.2 T Hybrid Magnet with a view toward Science in High Temperature Superconducting Magnets

Timothy Cross[* 1], Joana Paulino[2], Eduard Chekmenev[3], Xiaoling Wang[2], Ivan Hung[2], William Brey[2], Ilya Litvak[2], Jeffrey Schiano[4], Peter Gor'kov[2], Zhehong Gan[2], Myunngi Yi[5]

[1]NMR & MRI User Program & Dept. of Chemistry & Biochemistry, [2]NMR & MRI User Program, National High Magnetic Field Lab, Florida State University, Tallahassee, [3]Department of Chemistry, Wayne State University, Detroit, [4]Schoolof Electrical Engineering and Computer Science, Pennsylvania State University, University Park, United States, [5]Dept. of Biomedical Engineering, Pukyong National Univ. , Busan, Korea, Republic Of

Abstract: Solid state NMR spectroscopy is being performed at the MagLab on a 35.2 T magnet having a superconducting outer shell and an inner resistive set of coils. This magnet is powered with 12.5 MWatts of DC power and cooled with 700 g/min of supercritical LHe and 1800 gal/min of deionized water at 283K. A series connection between a superconducting outer shell and an inner resistive set of coils essentially eliminates noise associated with multiples of 60 Hz. Noise at a frequency less than a few Hz is taken care of by a modified Bruker Lock. Suppression of the middle range of noise has recently been demonstrated from low frequencies to well above 60 Hz using a Cascade Compensation System (CCS) developed by Jeff Schiano at Penn State Univ. with his engineering group in collaboration with Bill Brey and his team at the Magnet Lab. The CCS uses an inductive pickup coil for fast field fluctuations, and a field estimator based on NMR signals for slow field variations. Together, they form a two-loop cascade that has now been shown to reduce fluctuations much more significantly than the Bruker lock system. While we currently have stability of ~0.2 ppm for a few seconds, and ~0.5 ppm over a few hours, with the CCS we anticipate having better than 0.1 ppm for hours.

Three NHMFL probes are available for initial users of the magnet: 1) an oriented sample HX double resonance probe with a 4x4mm square coil or a 4 mm solenoid used to observe bicelles or glass slide aligned membrane proteins. 2) A single resonance probe with a 3.2 mm MAS stator used to observe a broad range of quadrupolar nuclei in materials science and biological systems with an the initial focus on ^{17}O. 3) a triple resonance HXY 2.0 mm MAS probe for characterizing protein samples. A triple resonance fast MAS (100 kHz) probe for ^{1}H detection at 1500 MHz is under development.

Scientifically, initial excitement has surrounded the observation of quadrupolar nuclei, in particular, ^{17}O resonances –sites of much biological chemistry. While some ^{17}O spectroscopy has been achieved in superconducting fields, the ^{17}O sensitivity at 35.2 Tesla is often an order of magnitude greater than in a superconducting magnet and the linewidths are often much narrower. To illustrate that new insights into the chemistry of biological systems can be observed with ^{17}O spectroscopy, spectra of single site ^{17}O labeled gramicidin in liquid crystalline lipid bilayers have been recorded. While this monovalent cation selective channel has been characterized at high resolution by multiple technologies and labs, it has always been observed to be a symmetric dimer in the absence of ions until 35.2 T ^{17}O spectroscopy showed that the symmetry is broken. Possible explanations will be discussed along with other initial scientific ventures on the Series Connected Hybrid.

EPR/ESR

O13

High resolution structure determination of molybdenum cofactors in enzymes through EPR, HYSCORE and DFT studies

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Abstract: Mononuclear molybdo-enzymes are found in virtually all living organisms. In Prokaryotes, most of these enzymes harbour a large Mo-bis pyranopterin guanosine dinucleotide cofactor and catalyse a wide diversity of redox reactions involved in major biogeochemical cycles of nitrogen, sulfur, carbon [1]. In spite of the similarity of their Mo-bisPGD cofactor, these enzymes are able to use a broad diversity of substrates, but the molecular factors which trigger their reactivity remain largely unknown. During catalysis, the molybdenum ion cycles between the +IV and +VI redox states, the intermediate Mo(V) state being EPR-active (S=1/2). Several Mo(V) species have been identified, but in spite of numerous crystallographic and spectroscopic studies, their structure and catalytic relevance is still strongly debated [1, 2].
To address these questions, we use various kind of bacterial nitrate reductases as model Mo-enzymes. By combining site-directed mutagenesis with EPR and DFT calculations, we brought new insights on the structure of the various spectroscopically detected Mo species and on their role in the catalytic process. Notably, by using specific isotope enrichment of the enzymes (^{98}Mo, ^{15}N) [3] and high resolution EPR techniques (ESEEM, HYSCORE), the nuclear environment of the Mo ion could be studied in details, providing an accurate view of the Mo ion environment [4]. The results emphasize the role of the second coordination sphere of the Mo ion in the stabilization of catalytic intermediates and provide some clues for the design of new bioinspired catalysts.

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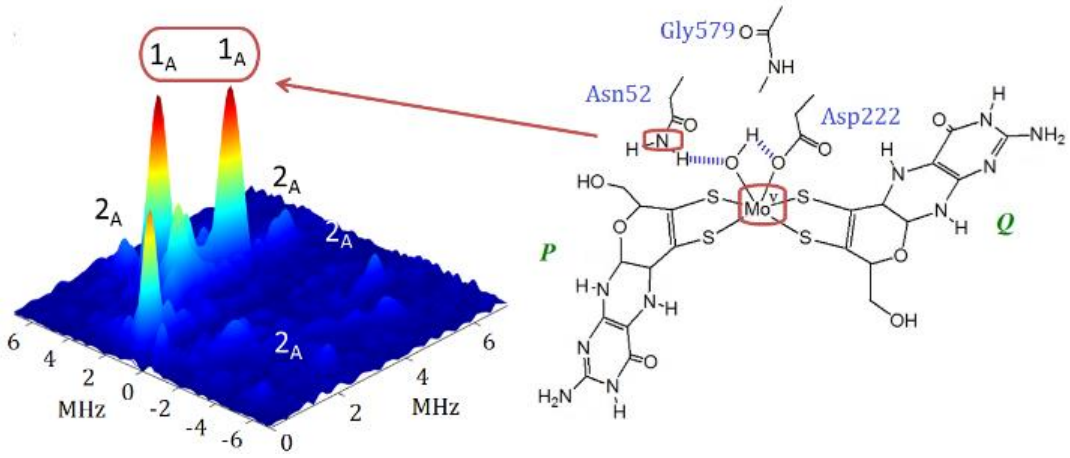

EPR/ESR
O14
The CHEESY Way to Hyperfine Spectroscopy

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Abstract: Hyperfine couplings to surrounding nuclei can give valuable information about the spatial and electronic structure of paramagnetic centers, such as transition metal complexes or organic radicals.
We introduce a new hyperfine technique based on spectral hole burning and detection with a chirp echo: Chirp Echo Epr SpectroscopY (CHEESY)-detected NMR [1]. A long pulse with high nominal turning angle (HTA pulse) creates side holes by driving forbidden transitions, similar to solid effect DNP. The positions of the holes encode the nuclear frequencies, from which the hyperfine couplings can be deduced. The complete hole pattern in the inhomogeneous EPR spectrum is detected in a single shot, leading to a multiplex advantage. The necessary bandwidth for large couplings is provided by the use of chirp pulses, created by an arbitrary waveform generator (AWG) [2]. We discuss similarities and differences to related sequences, such as electron double resonance (ELDOR)-detected NMR [3] and FT EPR-detected NMR [4].
Additionally, we introduce a 2D technique which uses a selective π pulse before the HTA pulse to provide HYSCORE-type correlations. Q band spectra are shown for Ti(iii) complexes where couplings to directly bonded ^{13}C nuclei could not be detected by other techniques. This type of correlation could also be achieved on conventional spectrometers, as it replaces one of the two HTA pulses of the published 2D ELDOR-detected NMR [5] by a selective π pulse.
We expect CHEESY-detected NMR to be generally applicable to systems with medium to large hyperfine couplings. It will further motivate the implementation of coherent ultra-wide band detection, especially at higher fields and frequencies.

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EPR/ESR

O15

Combined ESR and NMR study of antisymmetric exchange in quantum spin liquids

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Abstract: Ground state of QSLs exhibits large sensitivity even to weak perturbations. The antisymmetric exchange, the Dzyaloshinskii-Moriya interaction (DMI), present in all spin systems without space-inversion symmetry, could result in a phase transition from the quantum critical phase to an antiferromagnetic phase already at moderate magnetic fields. This transition and the emerging critical state is best tracked via detailed Electron Spin Resonance (ESR) studies. Using combination of multi-frequency ESR in the 1-500 GHz frequency range, Nuclear Magnetic Resonance (^{1}H-NMR), and muon spin rotation (μSR) techniques, we studied the influence of the DM interaction and local disorder in two-dimensional organic QSL candidates.

In the triangular lattice QSL, κ-$(ET)_2Ag_2(CN)_3$ (J'/J=0.94, J=175 K), ambient-pressure ESR measurements found a sizable static staggered moment of 6×10^{-3} μ_B (at 1.5 K and 15 T), stemming from the DMI (DM/J=0.5 %) due to proximity of the spiral-ordered phase. Strikingly, the effect of DMI under moderate pressures is completely suppressed while moving away from spiral ordering.

In a new quasi-one-dimensional QSL candidate, $(EDT\text{-}TTF\text{-}CONH_2)_2^+BABCO^-$, a weak Mott insulator with a distorted triangular lattice (J'/J=3, J=360 K), our combined ESR and μSR study confirmed the absence of magnetic ordering down to 20 mK. This remarkable observation is partially attributed to a unique structural motif of the $(EDT\text{-}TTF\text{-}CONH_2)_2^+BABCO^-$ salt. Here, the $(EDT\text{-}TTF\text{-}CONH_2)_2^+$ conducting layers are separated by the highly disordered $BABCO^-$ molecular rotors, as found by our ^{1}H-NMR studies. Despite presence of a sizeable DM interaction, the magnetic field dependence of the ESR linewidth does not show its effect. Instead, linear dependence of the linewidth reveals fast spin fluctuations; this is supported by longitudinal-field μSR measurements that reveal diffusive two-dimensional spin excitations. The quenching of the effect of the DM interaction is explained by the strong local disorder introduced by the anion layer.

Despite the magnitude of the DMI is 2 to 3 orders of magnitude weaker than the symmetric exchange, it can substantially alter the phase diagram of QSLs. Our work gives a novel explanation to field-induced phase transitions, and it demonstrates that high-frequency ESR is a powerful technique to study the spin dynamics of QSLs.

Small Molecules and Pharmaceutical

O16

General approach to access long-range 1H-1H residual dipolar couplings

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Abstract: Residual dipolar couplings (RDCs) are a powerful means for conformational and configurational analyses [1, 2]. For small molecules, ^{1}H-^{13}C one-bond couplings are mostly exploited. However, the quality of, for instance, configurational analysis greatly depends on the number of RDCs that sample independent orientations, which may not always be provided by one-bond couplings alone. Ways to obtain long-range RDCs are thus in high demand. Long-range ^{1}H-^{1}H RDCs would be an obvious choice, but are very challenging to access. The main reason is that, in alignment media such as PBLG, the high abundance of ^{1}H-^{1}H RDCs results in broad multiplets that overlap and from which individual splittings are not resolvable. Here, we propose a general approach to obtain ^{1}H-^{1}H RDCs.

We demonstrate that the recent PSYCHEDELIC experiment [3] gives access to long-range $^{n}T_{HH}$ couplings at a resolution close to the natural linewidth. PSYCHEDELIC delivers a 2D J-resolved spectrum with absorption mode lineshapes and with only ^{1}H-^{1}H couplings to one or more chosen spins, thus allowing coupling measurement as simple doublets at pure shift resolution and resolving both the issues of spectral overlap and multiplet complexity. The experiment is based on PSYCHE [4], which allows broadband homodecoupling at good sensitivity and tolerance to strong coupling. The tolerance to strong coupling can be further enhanced by introducing frequency-swept 180° pulses applied during gradients [5]. Although PSYCHEDELIC delivers the magnitude of the ^{1}H-^{1}H coupling, it offers no sign information, which is crucial for RDC interpretation. In isotropic samples, E.COSY or similar experiments can provide relative sign information, but in weakly aligned samples the overflow of ^{1}H-^{1}H couplings complicate cross-peak analysis. Solutions based on P.E.COSY or z-COSY using similar principles as PSYCHEDELIC to provide relative sign information of only selected couplings will be introduced.

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Small Molecules and Pharmaceutical

O17

Very Fast MAS Solid State NMR in Service of Pharma - Methods for Controlling of Preparation and Structure Elucidation of Pharmaceutical Cocrystals.

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Abstract: The design of the new drug formulations is frequently oriented on the preparation of the systems with exactly intended physicochemical properties. For solid medicines the effort is mainly put on obtaining crystals with good solubility, and hence bioavailability. One of the most interesting solutions allowing for the modification of physicochemical properties of a solid drugs is co-crystals formation. At room temperature co-crystals are solids composed from two or more components in a certain stoichiometric ratio, bonded in one crystal lattice. Their intrinsic properties, such as solubility, bioavailability, melting temperature, photoluminescence and thermal stability, are all different from those of starting substances, which makes them particularly attractive for many branches of industry, especially pharmaceutical industry. A key issue in co-crystal engineering is a search for appropriate coformers, i.e. substances able to form with a certain drug molecule co-crystals with the desired physicochemical properties.

In this communication we present the power of relatively new solid state NMR techniques, called Very Fast MAS in controlling of preparation and structure elucidation of pharmaceutical co-crystals.[1,2] The applicability of ^{1}H-^{13}C invHETCOR, ^{1}H-^{15}N invHETCOR as well ^{1}H-^{1}H NOESY and ^{1}H-^{1}H SQ-DQ BaBA correlations recorded with spinning rate 60 kHz will be presented. The special attention will be paid for defining of supporting role of VF MAS in controlling of formation of cocrystals obtained by means of mechanochemistry.[3] The advantages of complementary approach will be presented for case of Apremilast, a novel anti-inflammatory drug used in psoriasis (Ps) and psoriatic arthritis (PsA) treatment. p-philic molecular recognition in the solid state as a driving force for mechanochemical formation of Apremilast solvates and cocrystals will be discussed.[3]

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Small Molecules and Pharmaceutical

O18

Small molecule mixture analysis by heteronuclear NMR under spin diffusion conditions in viscous DMSO-water solvent

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Abstract: Mixture analysis by NMR has caught considerable attention for more than twenty years, however only a few solutions have been proposed, such as LC-NMR hyphenation, diffusion ordered experiments (DOSY), multi-quantum NMR spectroscopy combined or not with broadband homonuclear decoupling, sparse sampling and pure shift data acquisition. The recent use of viscous solvents has provided an interesting alternative approach to the study of mixtures by lowering the molecular tumbling rate in solution. [1] As a result, the molecules display a negative nOe regime and their resonances can be grouped according to their ability to exchange magnetization through intramolecular spin diffusion. The 2D ^{1}H-^{1}H NOESY spectrum of a mixture shows correlations between all the ^{1}H resonances of each analyte when recorded in spin diffusion conditions, thus giving access to the individual ^{1}H NMR spectra of the mixture components.
We have reported for the first time that the DMSO-d_6/H_2O binary solvent turned out to be the easiest to use and most efficient viscous solvent reported so far for the resolution of both polar and moderately apolar components within complex mixtures, taking advantage of NMR spin diffusion. [2] Using DMSO-d_6/H_2O as viscous binary solvent presents valuable advantages compared to other viscous solvents in terms of NMR sample tube preparation, of choice of the range of analysis temperature and of easy main field locking and shimming. The component individualization within a Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr mixture in DMSO-d_6/H_2O (7:3 v/v) was achieved at 268 K by selective 1D, 2D ^{1}H-^{1}H NOESY (see Figure 1) and ^{1}H-^{15}N HSQC experiments. ^{15}N nuclei were considered as chemical shift markers that improved the spectrum readability. The heteronuclear NMR spin diffusion approach was also exemplified by ^{19}F NMR of compound 2'αFTp3'αFT in DMSO-d_6/H_2O (8:2 v/v) at 238 K by means of 1D selective, 2D ^{1}H-^{1}H NOESY and ^{1}H-^{19}F HOESY experiments.

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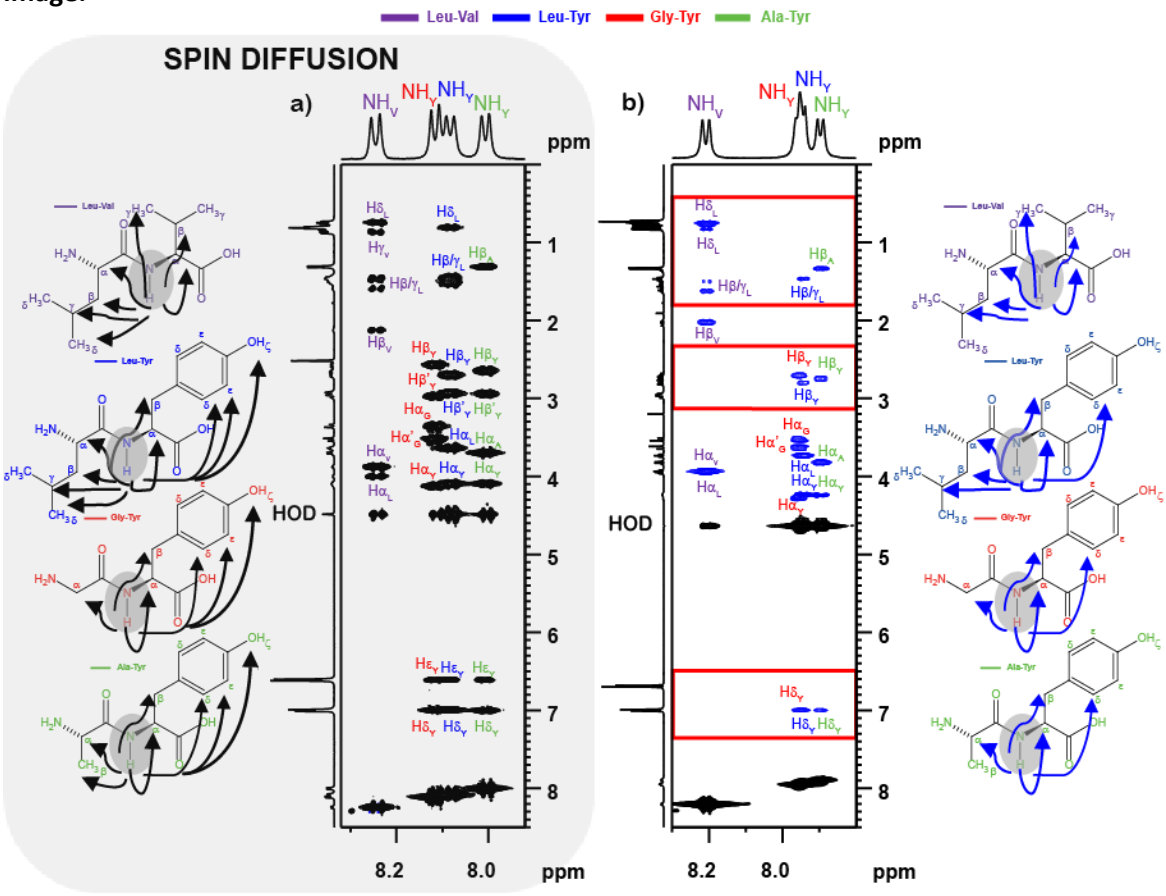

Figure 1. Amide proton region of band-selective detection 2D NOESY spectra of dipeptide test mixture: Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr at 500 MHz **a)** dissolved in DMSO-*d6*/H_2O (7:3 v/v) at 268 K, mixing time (t_m) = 1 s

→ **SPIN DIFFUSION is observed.**

Vertical slices in the 2D spectrum constitute the set of the separated 1D spectra of the dipeptides.

b) Amide proton region of 2D NOESY spectrum of dipeptide test mixture dissolved in water at 298 K, t_m = 1 s. The red frames correspond to spectral regions of interest in which water as solvent has a major effect on the number and sign of observable NOESY cross peaks.

Solid-state NMR methods

O19

Looped, PROjected-SpectroscopY (L-PROSY) Applied to Solid-State Proton-Detected HETCOR: A New Method for Enhancing Solid-State 2D Heteronuclear Correlation NMR Spectra

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Abstract: Recent work in our group has focused on developing a new family of sensitivity-enhanced NMR experiments employing so-called Looped, PROjected-SpectroscopY (L-PROSY).[1,2] At the heart of these techniques lie three key features. First: the excitation and labeling of a magnetically-dilute spin pool (^{13}C at natural abundance) whose evolution under shifts and couplings is of interest. Second: the coherent (by isotropic homonuclear mixing) or incoherent (by relaxation or chemical exchange) driven transfer of this frequency-labeled information to a magnetically-abundant spin pool of much easier detection (^{12}C-bound protons or H_2O protons). Third: the repetition of these encoding/transfer elements multiple times without detection, so as to impart onto the magnetically-abundant, sensitive spin pool the desired frequency-labeled polarization to the largest possible degree. As the overall modulation increases with subsequent repetitions in a Zeno-like fashion[3] up to a point dictated by the T_1 of the sensitive (*e.g.*, ^{1}H) spins, the homogenous excitation of this magnetically-abundant spin pool reveals the frequency-labeled modulation with a large enhancement in the SNR. Effects of this kind have been used in *in vivo* experiments,[4,5] and we have recently exemplified them in 2D homo- and heteronuclear solution NMR spectroscopy. Herein, we extend the L-PROSY modality to ultra-fast magic-angle spinning solid-state NMR, and apply it to enhance the sensitivity of organic solids. The pulse sequence assayed is shown in Fig. 1a. First, cross polarization (CP) generates ^{13}C polarization that evolves under chemical shifts and high-power ^{1}H decoupling for an incremented time t_1. Back CP then relays the ^{13}C frequency-labeled polarization to proximate ^{1}H spins. Radio-frequency-driven recoupling (RFDR)[6] is used to re-introduce the homonuclear ^{1}H-^{1}H dipolar coupling into the effective Hamiltonian, thereby achieving efficient isotropic mixing and extending the ^{13}C frequency labeling onto distant ^{12}C-bound ^{1}H spins (*i.e.*, the magnetically-abundant spin pool) via spin diffusion. These two steps are repeated N_{loops} times per scan, which stamps the overall ^{13}C frequency modulation onto nearby and distant protons. A final excitation pulse homogeneously excites this ^{13}C-modulated bulk proton polarization (that would otherwise be phase-cycled away) revealing a substantially enhanced ^{13}C signal. Preliminary results show significant reductions in the overall acquisition time, by at least an order of magnitude, when combining L-PROSY with conventional ^{1}H-detected HETCOR[7] NMR experiments (Figs. 1c, 1d). Numerical simulations (Fig. 2b) also confirm that theoretical enhancements in the SNR are proportional to half of the number of ^{1}H spins that constitute the magnetically-abundant spin pool. The underlying spin physics, experimental considerations, limitations, and the extension of these L-PROSY-enhanced SSNMR techniques to other dilute heteronuclei, will be discussed.

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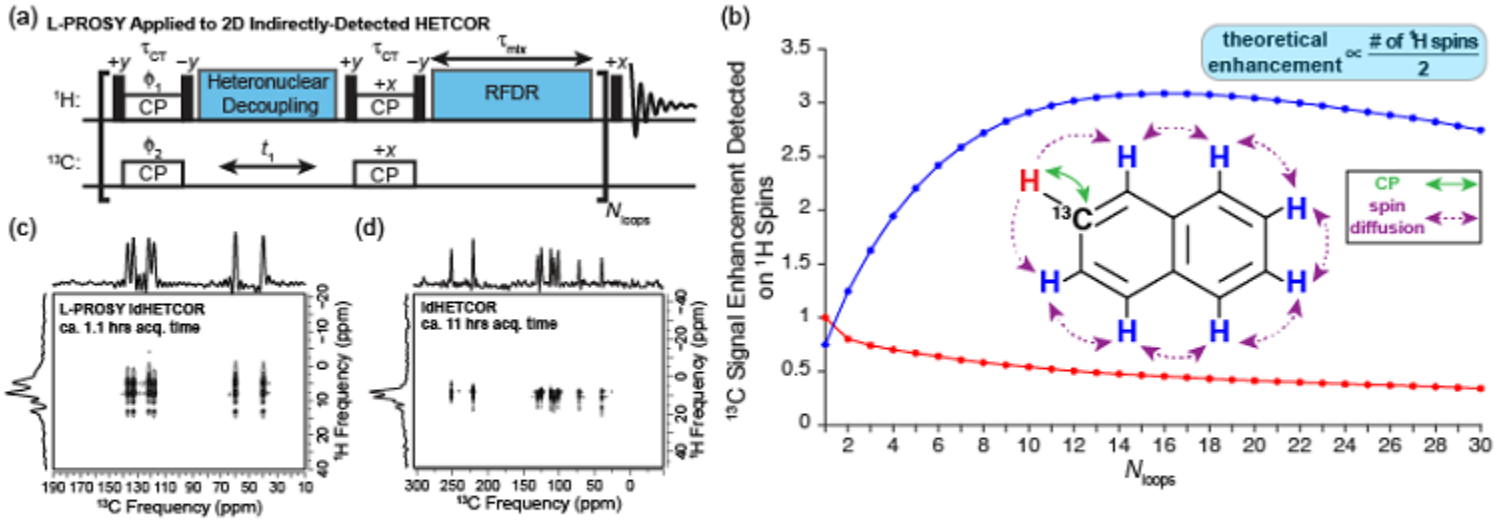

Figure 1. (a) Schematic representation of the L-PROSY idHETCOR pulse sequence. (b) Numerical simulations showing the ^{13}C signal enhancement in the magnetically-abundant spin pool (blue protons) vs. the ^{13}C signal detected in the magnetically-dilute spin pool (red proton) as a function of Nloops. Experimental (c) L-PROSY idHETCOR and (d) conventional idHETCOR spectra of naturally abundant L-tyrosine HCl acquired at 40 kHz MAS.

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Solid-state NMR methods

O20

In situ DNP NMR investigation of metastable polymorphs of glycine at low temperatures

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Abstract: Polymorphism - *i.e.* the ability of a chemical compound to crystallize in different forms - affects almost 50% of all the organic compounds referenced in the Cambridge Structural Database. It can have huge economic and practical consequences for industrial applications in fields like pharmacy and energy because different polymorphs display different physicochemical properties. If, on the one hand, it offers great opportunities for tuning the performance of the organic material, on the other hand, manufacture or storage-induced, unexpected, polymorph transitions can compromise the end-use of the solid product. These transformations often imply the formation of metastable forms, which are receiving growing attention because they can offer new crystal forms with improved properties. However, because metastable forms are generally transient, their detection and accurate structural analysis remains challenging. Several *in situ* techniques have been applied to this scope on a large number of materials. However, limitations both in terms of resolution and sensitivity exist.

Solid state NMR (SSNMR) is a powerful technique for the structural elucidation of functional organic solids. It offers an alternative and complementary spectroscopic approach to diffraction techniques in the investigation of the structure of solids because of its inherent molecular-level, atom-specific, response, and because it does not require periodicity. Some of us have recently demonstrated that SSNMR can be extremely powerful for *in situ* monitoring of polymorph transformation at room temperature [1]. This technique can provide access to both the liquid and the solid phase, but the inherently limited time resolution typically prevents the acquisition of 2D experiments.

In the attempt of achieving a better understanding of polymorphism, we present a new approach for *in situ* investigation of metastable polymorphs using SSNMR and DNP SSNMR at cryogenic temperatures [2]. As a model sample, we chose to investigate glycine, a compound often used as a reference in crystal structure studies because of its rich polymorphism and known behavior. *In situ* solid-state NMR is here exploited to monitor the structural evolution of a glycine/water glass phase formed on flash cooling an aqueous solution of glycine, with a range of modern 1D and 2D solid-state NMR methods applied to elucidate structural properties of the solid phases present. Our *in situ* NMR results allowed to reveal the formation of intermediate, transient crystalline phases of glycine and to investigate their structure

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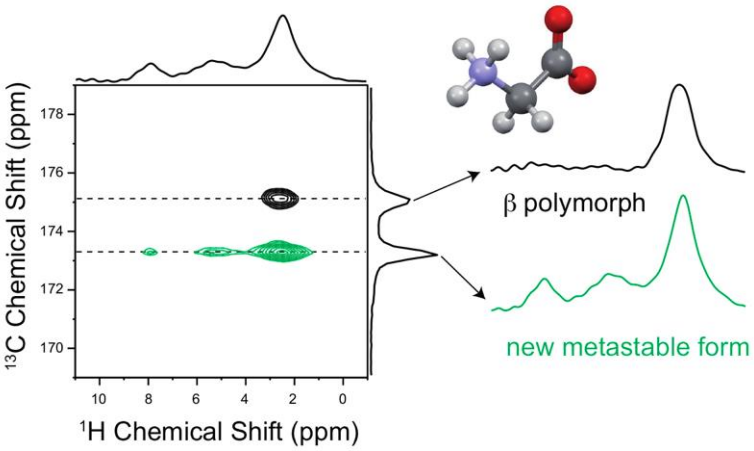
178
176
174
172
170
^{13}C Chemical Shift (ppm)
10
8
6
4
2
0
^{1}H Chemical Shift (ppm)
β polymorph
new metastable form

Solid-state NMR methods

O21

Understanding Hydrogen bonding structure by electron and NMR nano-crystallography

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Abstract: We will present a combined approach of electron diffraction (ED) and solid-state NMR to reveal the hydrogen bonding network in organic molecules from nano- to micro-crystalline samples. When large (typically > 10-100 um) single crystals are available, single crystal X-ray diffraction (XRD) easily solves the crystalline structures. If the sample is pure powder crystalline, powder XRD can be used instead. On the other hand, ED potentially solves the crystalline structure from the nano- to micro-crystalline samples, using diffraction tomography, and continuous rotation methods. One of the stumbling block of ED application to organic samples is radiation damage, however, the recent development of high-sensitivity camera paves a way for structure determination of organic crystals at a very low dose.

It is still a challenging task to locate hydrogen positions by ED. Moreover, discrimination of carbon, nitrogen and oxygen atoms is usually difficult. Yet, solid-state NMR can provide complemental information to reconstruct the structure including hydrogen positions, since NMR can directly detect ^{13}C, ^{14}N, ^{15}N, and ^{1}H.

As a proof of concept, first we have solved the crystalline structure of orthorhombic L-histidine whose structure is already known. The 3D atomic resolution structure was determined by rotation ED method. This yielded twelve possible configurations due to ambiguity of carbon and nitrogen atoms and lack of hydrogen in the structure. $^{1}H/^{14}N$ correlation solid-state NMR clearly showed the orthorhombic L-histidine is Zwitterion, and ruled out four configurations among them. The comparison of experimental ^{1}H solid-state NMR spectrum and calculated spectra by the first principle quantum computation exclusively identified one configuration. Thus, we obtained the intra- and inter-molecular hydrogen network in the crystalline structure. This approach has also been applied to cimetidine form B whose structure is unknown to our knowledge. Then, we have successfully solved the crystal structure and revealed hydrogen bonding in the structure.

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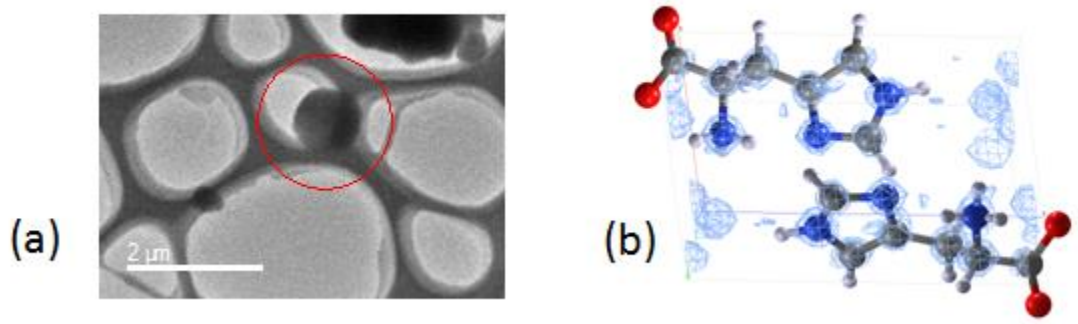

MRI and in vivo

O22

Advances in single-scan time-encoding magnetic resonance imaging

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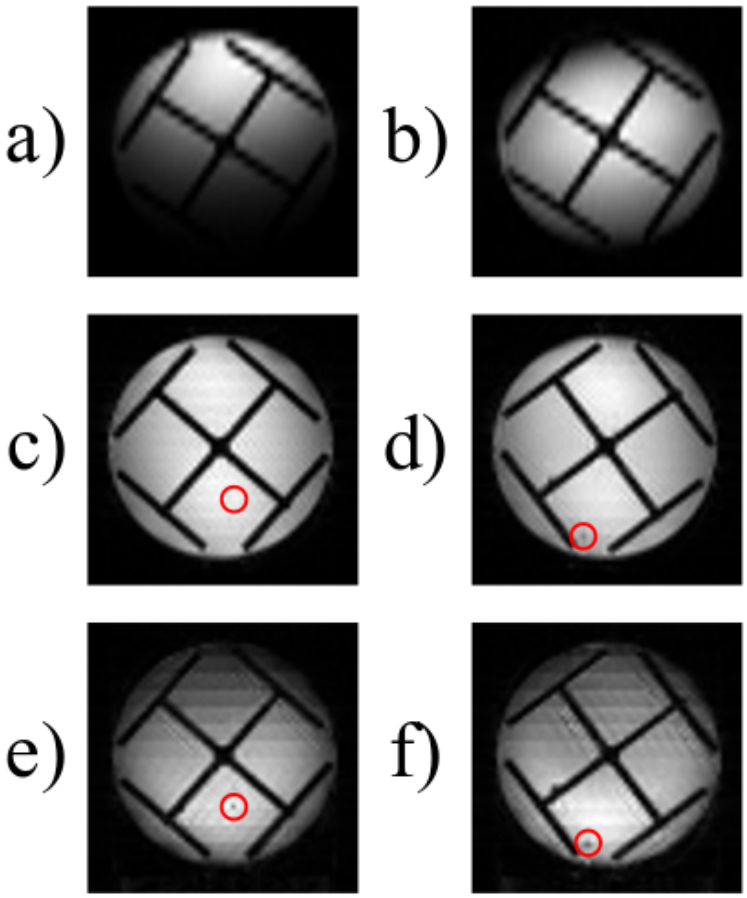
Abstract:

Time-encoding magnetic resonance imaging (MRI) is a single-scan method that uses traditional *k*-encoding only in one direction. In the orthogonal "time-encoding" direction, a string of echoes appears in an order that depends on the position of the corresponding spin packets[1–5]. In one variant of time-encoding[2], this is achieved by using a series of selective pulses and appropriate gradients in both *k*-encoding and time-encoding directions. This is similar to line-scanning techniques that use traditional *k*-encoding in one direction only, while the second direction is retrieved like in other line-scanning techniques, i.e., through a series of parallel strips (also known as "lines" or "spin packets") that are excited selectively. One advantage of doing so is that like other line-scanning techniques, time-encoding is less sensitive to motion, and can be particularly useful to study a dynamic system, e.g., for cardiac imaging[5]. Although time-encoding offers some advantages over traditional single-scan Fourier methods such as echo planar imaging (EPI), the original time-encoding sequence also has some drawbacks that limit its applications.

In this work, we show how one can improve several aspects of the original time-encoding sequence. By using an additional gradient pulse one can change the order in which the echoes appear, leading to identical echo times for all echoes, and hence to a uniform signal attenuation due to transverse relaxation and a reduction in average signal attenuation due to diffusion. Figures a and b show how this modification improved the image quality.

By rearranging positive and negative gradients one can reduce the switching rate of the gradients. Furthermore, we show how one can implement time-encoding sequences in an interleaved fashion in order to reduce signal attenuation due to transverse relaxation and diffusion, while increasing the spatial resolution. Finally, suitable sequences lead to strings of echoes that are uniformly affected by inhomogeneous T_2^{inh} effects. As depicted in Figures (c)-(e), different areas in the image are differently weighted if one does not use this modification, resulting in non-faithful images.

Image:



(a) Image of a phantom of a cross-shaped piece of plastic immersed in water obtained with the original time-encoding sequence, with different echo times for different echoes. (b) A time-encoding image obtained with the modified sequence, with identical echo times for all echoes. Both images have been recorded with a matrix size of 64 in the (horizontal) k-encoding direction, and 32 in the (vertical) time-encoding direction, a FOV of 27.0 × 27.0 mm, and a slice thickness of 1.0 mm. The average echo time was TE = 22.6 ms in (a), while TE = 22.6 ms for all echoes in (b). In both cases a readout bandwidth of 125 kHz, a total scan time 39.0 ms, and a Gaussian pulse with a duration of 256 μs and a flip angle of 90° were used for spin-packet selection. (c)-(f) Four different images of the same phantom with a thin glass fibre, the tip of which is positioned within the thickness of the selected slice at the coordinates marked by red circles. Images (c) and (d) were obtained using a non-modified sequence, and those in (e) and (f) using a modified sequence. The glass fibre can be seen in (d), (e) and (f), however, in (c) it cannot be seen since T_2^{inh} effects are refocused. Note that in (e) and (f) T_2^{inh} effects are partially refocused to the same extent in all echoes across the entire object. These four images were obtained with a matrix size 64 × 64 with four-fold interleaving, and TE = 14.4 ms. The lengths of the excitation pulses were 256 μs in all cases.

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MRI and in vivo

O23

2D Selective Excitation with Resilience to Large B0 Inhomogeneities

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Abstract:

Purpose

Spatially-selective pulses are widely used in MRI. When large B_0 inhomogeneity (ΔB_0) is present, spatial selectivity becomes distorted and displaced. This problem is most severe when using low bandwidth RF pulses. Here, it is shown that by segmenting multidimensional frequency-modulated pulses, the pulse bandwidth can be substantially increased to yield 2D spatial selectivity that is robust to large ΔB_0. Here, this method is used in 3D MRI to achieve simultaneous high bandwidth excitation and readout for high tolerance to ΔB_0.

Methods

The spiral k-space trajectory of the 2D HS1 pulse in [1] was modified to traverse a Cartesian trajectory. This 2D pulse was then segmented to traverse one line of k_x-space per excitation, with 36 segments (Fig). Each segment has a fixed k_y value determined by blipping the gradient after the pulse and is frequency-swept along k_x, yielding extremely high bandwidth in both directions. As a result of the HS1 amplitude modulation in k_y, each segment yields a different flip angle. Alternatively, to keep constant T_1-weighting, a fixed flip angle can be used for each segment. In experiments, a 3D gradient echo sequence was used with one line of k_x transmitted per excitation, with a full readout for each of the 36 excitation segments. To keep imaging time reasonable, an isotropic resolution of 2x2x2 mm^3 was used with a FOV of 256x128x128 mm^3. Data was scaled in post-processing to obtain proper relative weighting of the pulse segments. Spatial distortion was evaluated in the two phase-encoded dimensions which are not affected by ΔB_0.

Results

Experiments were performed on phantoms and human brain. One experiment was performed on a well shimmed sample and one was performed with a linear shim fixed at 5 kHz/12 cm for the duration of the experiment. There was little visible difference in the quality of the images or in the shape of the excited region.

Discussion

In contrast to the original spiral-sampled pulse, this new 2D pulse can be driven adiabatically. By using the same flip angle for each pulse segment, T_1-weighting of each excitation remains constant. However, after summing the acquired signals with unequal weighting, SNR is lower than the maximum possible without segmenting. For the 2D HS1 pulse used here, this processing yields only 66.97% of the maximum SNR; but when using constant flip angle and applying equal weighting, the selected region is ill-defined and extends beyond the zoomed field-of-view. To increase the SNR of segmented implementations, higher order 2D HSn pulses can be employed which more evenly distribute energy in k-space. For example, with a fully segmented 2D HS4 pulse, the SNR is 87.35% of the maximum SNR.

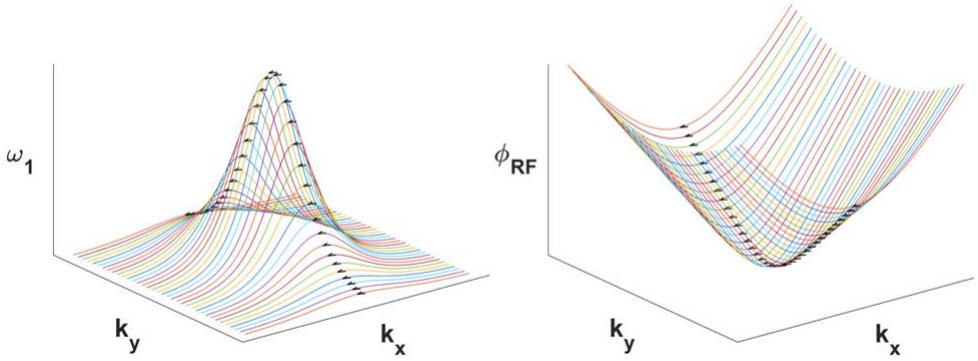
Conclusion

The proof of concept for a high bandwidth, adiabatic 2D excitation and readout technique with exceptional resilience to B_0 inhomogeneity has been demonstrated. Future work will increase the SNR and resolution of the sequence.

Funding: NIH U01 EB025153

1) Jang et al, Magn Reson Med 2017

Image:



MRI and in vivo

O24

Characterizing the microscopic heterogeneity of the living human brain with spatially resolved 5D diffusion-relaxation distributions

JP de Almeida Martins[1, 2], C Tax[3], F Szczepankiewicz[2], G Eklund[2], K Bryskhe[2], DK Jones[3], C-F Westin[4], D Topgaard[* 1]
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Abstract: Brain tissue is a heterogeneous anisotropic material where each millimeter-scale MRI voxel contains water in multiple environments with different cellular structures and chemical compositions. The sensitivity of MRI observables such as the relaxation rate R_2 and the diffusion tensor **D** allow us to detect changes in the voxel-averaged set of microenvironments. Albeit sensitive, conventional diffusion and relaxation MRI techniques are not specific, and to interpret the acquired signal in terms of microscopic tissue properties remains a challenging problem.

We introduce a novel experimental protocol to characterize the heterogeneity of the living human brain with spatially resolved 5D diffusion-relaxation (**D**-R_2) distributions. Signal is acquired with a diffusion-weighted EPI sequence modified for tensor-valued diffusion encoding [1] and variable echo times, giving a 5D acquisition space with dimensions directly corresponding to the ones of the distribution. Such acquisition protocol establishes correlations between the transverse relaxation rate R_2, axial and radial diffusivities, D_A and D_R, and the diffusion tensor orientation (ϑ,φ), which in turn allow the model-free voxel-wise estimation of $P(R_2,D_A,D_R,\vartheta,\varphi)$ distributions [2]. Figure 1a displays the recovered distributions for voxels containing white matter (WM), gray matter (GM), and cerebrospinal fluid (CSF), as well as the binary combinations WM+GM, WM+CSF, and GM+CSF. The distributions are represented as 3D logarithmic plots of the isotropic diffusivity $D_{iso} = (D_A+2D_R)/3$, axial-radial ratio D_A/D_R, and R_2. The area and color of each point report on the weight and orientation of the corresponding solution. Binning the **D**-R_2 space allows us to naturally separate the signal contributions from distinct microscopic environments. The microstructure of the various tissues can then be characterized via bin-resolved parameter maps calculated from the distributions (see Figure 1b, where $D_\Delta = (D_A-D_R)/3D_{iso}$ is a normalized measure of anisotropy).

Achieving high specificity while relying on few assumptions, the presented protocol shows great promise in studies in which there is no prior information on the microstructure of investigated tissue.

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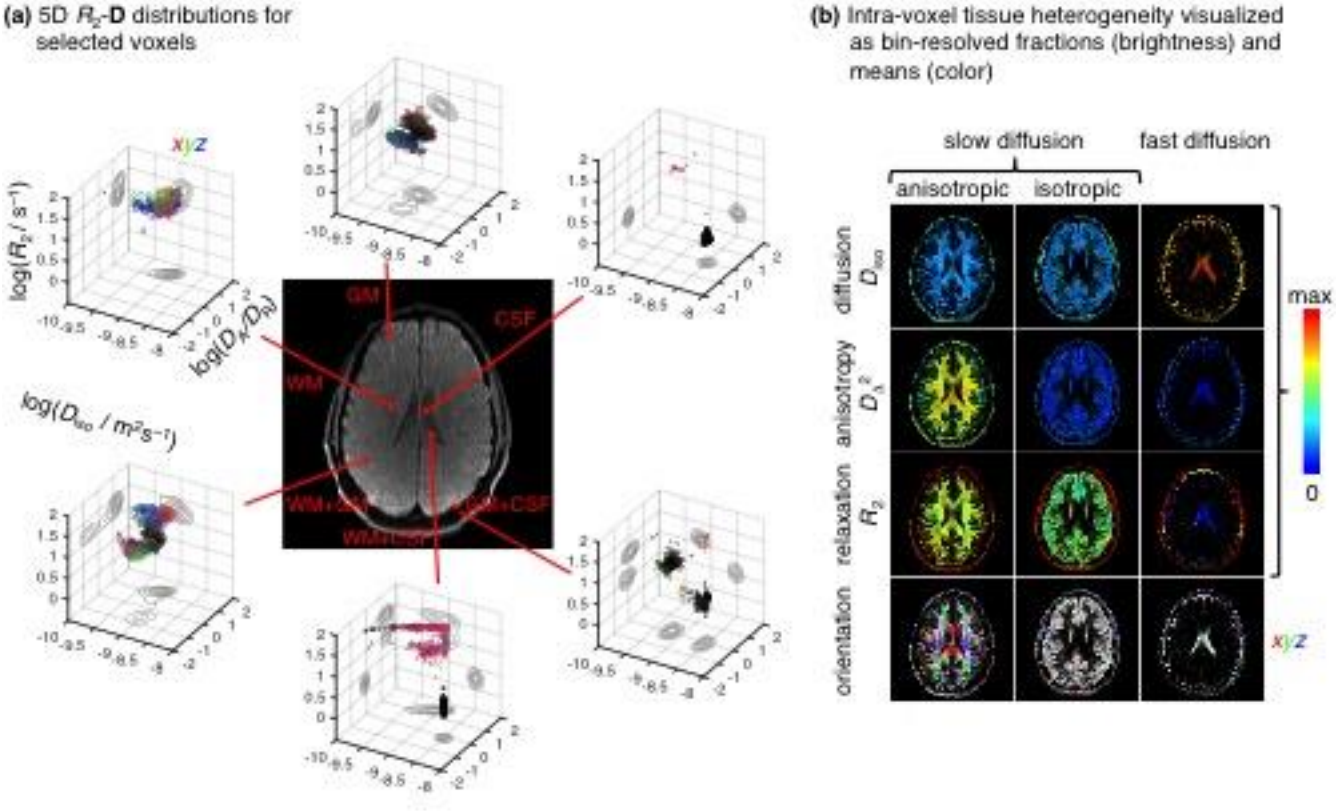
Image:



Disclosure of Interest: J. de Almeida Martins Conflict with: Employee Random Walk Imaging, C. Tax: None Declared, F. Szczepankiewicz Conflict with: Employee Random Walk Imaging, G. Eklund Conflict with: Employee Random Walk Imaging, K. Bryskhe Conflict with: Owner Random Walk Imaging, D. Jones: None Declared, C.-F. Westin: None Declared, D. Topgaard Conflict with: Owner Random Walk Imaging

Benchtop and lowfield

O25

Low-field RheoNMR as a new tool for the study of soft materials

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Abstract: Hyphenated set-ups of NMR and rheology have received great attention in the past 20 years because such combinations lead to correlated information on flow and molecular properties of soft matter, e.g. polymers, liquid crystals or colloids. Additionally, flow profiles can be applied to modify the state of a sample and measure its implications on molecular properties and the resulting flow behavior. Most RheoNMR designs have been realized for high-field NMR spectrometers using shear flow cells without a quantification of the rheological parameters. Here, we present a low-field RheoNMR set-up based on a portable 30 MHz NMR unit that was integrated into a high end commercial strain-controlled rheometer [1]. This combination can be employed to conduct a full rheological characterization (G', G'', $|\eta^*|$, FT-Rheology: $I_{3/1}$, Q_0) while monitoring molecular dynamics in-situ via ^{1}H TD-NMR for temperatures from -15 to +210 °C. Possible applications include the measurement of quantitative composition in crystallizing soft matter (fats, polymers, etc.) and multiphase systems during the application of shear protocols [2, 3]. To display the possibilities of this new technique, studies on the quiescent and flow-induced crystallization of polyolefins are presented.

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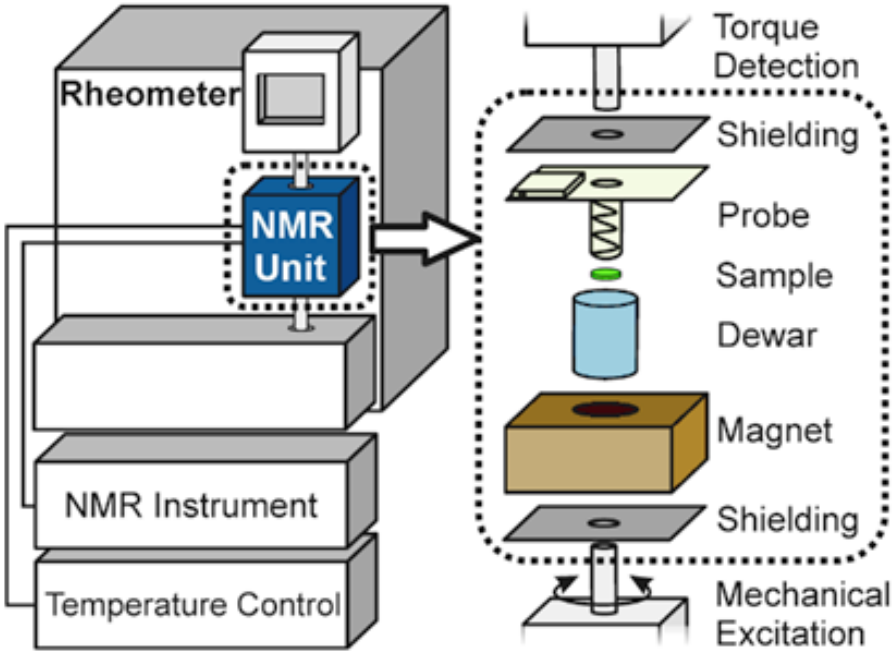
Image:



Benchtop and lowfield

O26

31P NMR for lipid analysis with a low-field benchtop spectrometer

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[1]iNANO, Aarhus University, Aarhus, Denmark

Abstract: High-field (HF) NMR is an emerging powerful tool for **lipidomics studies**. The high analytical reproducibility, together with the non-destructive character warrant the use of NMR-based lipidomics in several applications from disease diagnostic to food science.[1] However, HF NMR is not generally regarded as a routine analytical tool due to both economic and practical reasons. In this context, recent **low-field (LF) benchtop spectrometers** – based on 1-2 T permanent magnets - have met the need for easy accessible NMR spectroscopy. Recently, LF NMR spectroscopy have been applied in lipidomics studies in food science.[2] These promising applications are mainly based on ^{1}H NMR spectra of highly concentrated triglycerides in different matrices, whose resulting spectra are congested and suffer from peak-overlaps. Usually, this resolution loss is alleviated thanks to chemometric tools and/or 2D experiments to obtain significant and reliable insights.

While heteronuclear NMR is widely used in HF lipidomics studies, this type of experiments has not been exploited in LF NMR for lipidomics applications despite the ability of the modern benchtop spectrometers to probe different hetero-nuclei. In particular, ^{31}P provides interesting features. In addition to its high chemical shift dispersion and its 100 % natural abundance, ^{31}P NMR enables the analysis of phosphorus lipids such as phospholipids (PL) and ammonium phosphatides. **We present here the first study in ^{31}P NMR at LF for the analysis of phosphorus lipids in mixtures** and discuss the capabilities of a 1T-benchtop spectrometer in terms of **limit of detection, identification and quantification**. First, the performance of a one-pulse ^{31}P NMR experiment with proton-decoupling is presented and discussed. Thereafter, we highlight the further improvements provided by the implementation of a **^{1}H-^{31}P heteronuclear cross-polarization (Het-CP)**. Besides a 2-3 times SNR enhancement, this Het-CP based on a WALTZ-16 block can easily be turned into a **^{1}H-^{31}P 2D TOCSY experiment**, which correlates all the protons involved in the polar head group of the phospholipids to the phosphorus nucleus. The resulting traces along the ^{1}H-dimension exhibit different fingerprints enabling indentification of **several classes of phospholipids**. This assignment method is particularly relevant since ^{31}P chemical shift values can vary with minor differences in the experimental parameters.[3] Finally, we present the potential of Het-CP, in combination with an external calibration, **as a quantitative tool**.

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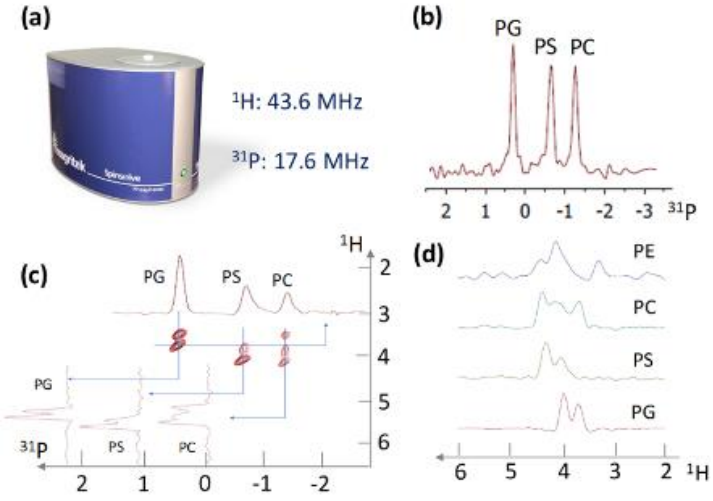
Image:



(a) picture of a LF spectrometer (Spinsolve, Magritek) with ^{1}H, ^{19}F, and ^{31}P detection. **(b)** ^{31}P NMR spectrum with proton decoupling recorded in 2 h on a mixture of three PL (25 mM). **(c)** 2D ^{1}H-^{31}P TOCSY on the same mixture recorded in 20 h. **(d)** Series of ^{1}H-traces of different PL extracted from 2D TOCSY spectra. All the axes are scale in ppm.

Benchtop and lowfield

O27

Details of polymer chain dynamics as probed by low-resolution NMR

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Abstract: Low-resolution (possibly low-field) proton NMR provides a rich toolbox with various methods for the characterization of elastomeric materials with regards to their structure and dynamics. Relying on the orientation dependence of proton-proton dipolar couplings and the changes with time following the motions of the chains, one can estimate the cross-link density, its distribution throughout the sample, and the timescales of the complex macromolecular dynamics [1].

Here, we present applications of a new approach to the analysis of multiple-quantum NMR data [2] in order to extract – robustly and with little assumptions – details on the shape of the segmental orientation autocorrelation function in the range of µs-ms, i.e., the range typically covered by T_2 and T_{1r} relaxation times. While the approach is of general use to study dynamics in all kinds of (e.g. biological) materials, it here provides valuable insights into the motions of polymer chains. Specifically, we highlight an application to permanent as well as supramolecular networks of poly(isobutylene). Supramolecular networks were obtained by lateral modification of the polymer backbone with ionic liquids, which leads to the formation of nm-sized ionic clusters acting as "stickers". These were found to enhance mechanical properties and also impart self-healing behavior [3,4]. Using our approach, we could observe changes in the chain dynamics arising from thermal activation of the stickers, which leads to non-trivial "sticky" dynamics [5] and complex rheological behavior.

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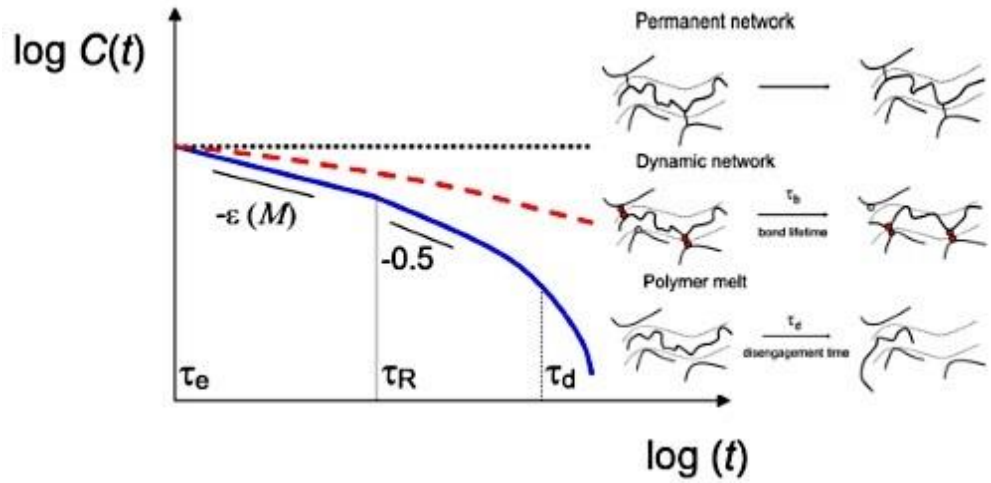
Image:



Bioliquids

O28

Quantifying ribosome–nascent chain interactions and intracellular quinary structure using adaptively sampled measurements of cross-correlated relaxation in methyl spin systems

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[1]Department of Structural and Molecular Biology, University College London, London, [2]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

Abstract: NMR measurements of nuclear spin relaxation provide extraordinarily powerful probes of polypeptide dynamics across a range of timescales. In particular, measurements of cross-correlated relaxation processes provide a valuable description of 'pure' dynamics within a spin system, free from contributions due to chemical exchange. However, such measurements have typically been associated with low experimental sensitivity. Here, we describe a sensitivity-optimised pulse sequence for the measurement of cross-correlated relaxation in perdeuterated and selectively $^{13}CH_3$ methyl-labelled spin systems. We also describe the application of optimal design theory to implement an 'on-the-fly' adaptive sampling scheme, calculated in real time during acquisition, to maximise the accuracy of the measured rate constants.

We have applied these new methods to study the dynamics of translationally-arrested ribosome–nascent chain complexes comprising the FLN5 filamin domain (Cabrita et al. (2016) Nat. Struct. Mol. Biol.). In particular, cross-correlated relaxation may be used to quantify interactions between the domain and the associated ribosome surface, and so deduce the impact of such interactions on the co-translational folding process. We will also describe the application of our new method to in-cell NMR spectroscopy, where measurements of cross-correlated relaxation provide a sensitive probe of quinary structure interactions.

Bioliquids

O29

Combinatorial Selective Labeling in Studies of Membrane Proteins with Limited Stability: Mapping of Binding Interface of Spider Toxin with Voltage-Gated Sodium Channel

Zakhar O. Shenkarev[*] [1, 2], Alexander Paramonov[2], Antonina Berkut[1], Dmitrii Kulbatskii[2], Ekaterina Lyukmanova[1, 2], Alexander Vassilevski[2], Mikhail Myshkin[1, 2]

[1]Department of Biological and Medical Physics, Moscow Institute of Physics and Technology (State University), [2]Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation

Abstract: Voltage-gated Na^+ (Na_V) channels contain domains that have discrete functionalities. The central pore domain allows current flow and provides ion selectivity, whereas peripherally located four voltage-sensing domains (VSD-I/IV) are needed for voltage-dependent gating. Certain mutations trigger a leak current through VSDs leading to various diseases. For example, hypokalemic periodic paralysis (HypoPP) type 2 is caused by mutations in the S4 voltage-sensing segments of VSDs in the skeletal muscle channel $Na_V1.4$. The gating modifier toxin Hm-3 (crab spider *Heriaeus melloteei*) inhibits leak currents through such mutant channels and represents useful hit for HypoPP therapy [1].

To study molecular basis of Hm-3 interaction with the VSD-I of $Na_V1.4$ channel, we expressed the isolated domain (~150 a.a., four transmembrane helices, S1-S4) in the cell-free system. Mixed micelles of zwitterionic detergents (DPC/LDAO 1:1) provided optimal conditions for NMR study. The limited stability of the VSD-I samples in this milieu (half-life time of ~24 h at 45 °C) prevents usage of classical assignment approach based on the 3D triple-resonance (^1H-^{13}C-^{15}N) experiments. Therefore we used combinatorial selective labeling (CSL) and extracted sequence-specific information from the 2D TROSY and HNCO spectra measured for several selectively ^{13}C,^{15}N-labeled samples. To collect maximum information from as few NMR samples as possible we developed new deterministic *CombLabel* algorithm that calculates price-optimal labeling schemes using a protein sequence and stock of available labeled amino acids as input. CSL provided straightforward assignment for ~50% of VSD-I backbone resonances. That permitted to characterize the secondary structure and backbone dynamics of VSD-I in micellar environment. The tertiary structure of the domain was characterized by paramagnetic relaxation enhancement data.

NMR data show that Hm-3 partitions into micelles through a hydrophobic cluster formed by aromatic residues and reveal complex formation with VSD-I through electrostatic and hydrophobic interactions with the S3b helix and the S3–S4 extracellular loop. Two different hydrophobic interfaces on the Hm-3 surface are responsible for the interactions with the micelle and VSD-I. The model of the Hm-3/VSD-I complex was built using protein-protein docking guided by NMR restrains. Our data identify VSD-I as a novel specific binding site for neurotoxins on sodium channels [1]. To best of our knowledge our report is the first NMR study of structural interactions between toxins and Na_V channels.

Work was funded by the Russian Science Foundation (#16-14-10338).

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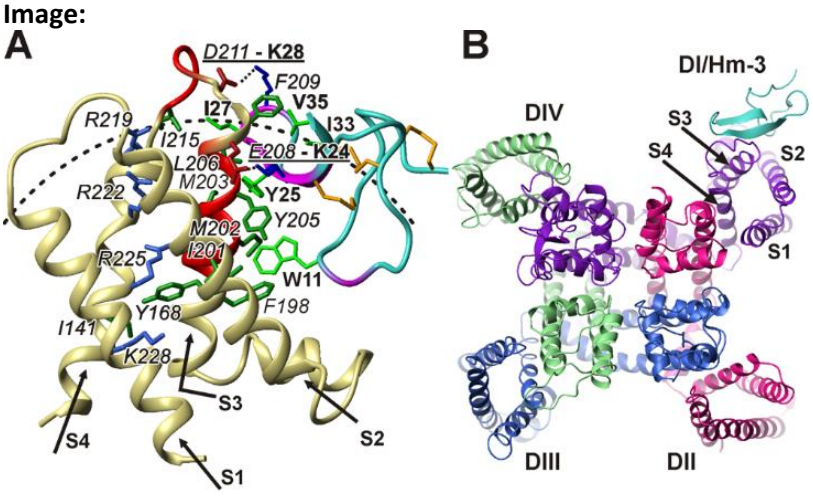
Image:


A
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F209
V35
I27
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E208 - K24
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I201
R225
W11
F198
Y168
I141
K228
S3
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DI/Hm-3
DIV
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S4
S2
S1
DIII
DII

Bioliquids

O30

NMR for following BRCA2 phosphorylation during mitosis: characterization of breast cancer variants.

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[1]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, UMR9198, Gif-sur-Yvette, [2]Paris Sud University, Paris Saclay University, [3]Genotoxic Stress and Cancer, Institut Curie, PSL Research University, CNRS, UMR 3348, Orsay, France

Abstract:

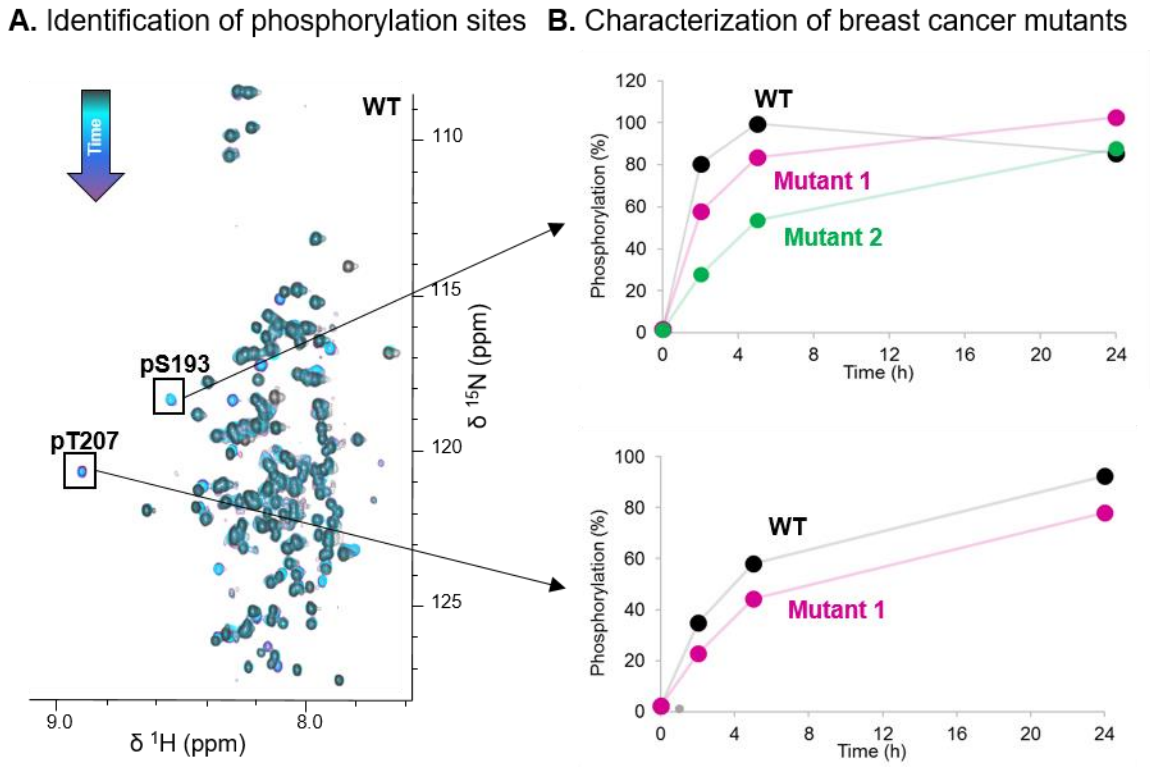
Members of the polo-like kinase (PLK) family are crucial regulators of the cell cycle progression and the DNA damage response (Zitouni et al., *Nat Rev Mol Cell Biol* 2014). PLKs undergo major changes in abundance and localization at different stages of the cell life. PLK1 is the most ancestral and best-conserved member of the family. Expression of PLK1 is tightly regulated in time, being low in interphase and high in mitosis. It phosphorylates a large set of proteins critical for mitotic entry, G2/M checkpoint, spindle assembly, mitotic exit and cytokinesis.

BReast CAncer susceptibility 2 (BRCA2) is a DNA repair protein that is phosphorylated by Plk1 during mitosis. Part of these events seems to be involved in the regulation of its DNA repair function (Lee et al., *Oncogene* 2004). However, its intrinsically disordered N-terminal region from aa 1 to aa 284, also hyperphosphorylated by PLK1 during mitosis, is associated with other biological processes as modulation of the mitotic progression via the spindle assembly checkpoint and cytokinesis (Lin et al., *J Biol Chem* 2003; Mondal et al., *Dev Cell* 2012).

Here we used liquid-state NMR spectroscopy to follow BRCA2 phosphorylation by PLK1. We first assigned the ^{1}HN and ^{15}N NMR signals of two overlapping BRCA2 fragments: BRCA2N1 from aa 48 to aa 218 and BRCA2N2 from aa 190 to aa 283. NMR chemical shift analysis confirmed that region from aa 48 to aa 283 is intrinsically disordered. Then we showed that the highly conserved region between aa 190 and 210 is rapidly phosphorylated by PLK1 at S193 and T207, and that phosphorylation at T207 creates a further docking site for PLK1.

Moreover, BRCA2 is associated with an increased risk to develop breast cancer and 10% of breast cancers are associated with a hereditary transmission. In collaboration with the team of Dr Aura Carreira, we tested the functional impact of several BRCA2 mutations found in breast cancer patients that are rare and of uncertain clinical significance (VUS). In order to identify variants with a defective PLK1-dependent phosphorylation, we followed their phosphorylation *in vitro* and in cells. We revealed that in cells region from aa 1 to region 250 is significantly less phosphorylated in the case of variants S193A, S206C and T207A. We showed that *in vitro*, PLK1 phosphorylates less efficiently S193 in BRCA2N1 and BRCA2N2 with the mutation T207A, confirming the critical functional role of this mutation. We also demonstrated by ITC that S206C and T207A impair binding of PLK1 to the BRCA2 fragment from aa 194 to aa 210. Finally, we discuss how following phosphorylation of BRCA2 fragments *in vitro*, in cell extracts and in cells and measuring the impact of mutations hindering BRCA2 phosphorylation on chromosome alignment and segregation during mitosis have contributed to decipher a new mechanism of control of mitotic events that depends on the PLK1-BRCA2 interaction.

Image:


A. Identification of phosphorylation sites
B. Characterization of breast cancer mutants
Time
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pT207
110
115
120
125
9.0
8.0
δ ¹H (ppm)
δ ¹⁵N (ppm)
Phosphorylation (%)
Time (h)
WT
Mutant 1
Mutant 2
Phosphorylation (%)
Time (h)
WT
Mutant 1

Materials

O31

Methylammonium Lead Halide Perovskites: From Stable Photovoltaic Materials to NMR Thermometers with Pb-207 Solid-State NMR Spectroscopy

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Abstract: Perovskites, a unique class of crystalline materials, were named in honour of Russian mineralogist Lev Perovski due to his initial discovery of the mineral calcium titanate ($CaTiO_3$, Pm3m) in 1839. Today, a wide range of materials are broadly defined as perovskites, displaying a high degree of tunability where they may exhibit conductive, semi-conductive or insulating properties depending on their composition. Recently, interest in a hybrid class of perovskite materials of the type ABX_3, where A = methylammonium, B = Pb, and X = Cl, Br or I have re-emerged following nearly 40 years of dormancy when it was discovered that these materials exhibit exquisite photovoltaic and/or optoelectronic properties with the added benefit of being low-cost.

Unfortunately, the stability of these materials and their decomposition products create environmental and health concerns, where leaching of toxic Pb from device failure could lead to detrimental long-term issues. As the chemical structure directs the materials' properties, an innate ability to understand their intricate short- and long-range structure is vital to engineering solids with improved photoconversion efficiency (PCE) and/or to minimizing degradation. Our research efforts have focussed on determining the decomposition pathways of these materials as well as improving the stability of methylammonium (MA) lead halide perovskites ($MAPbX_3$, X=Cl, Br and I).

The impact of humidity and thermal cycling on the atomic structure of these materials, as determined using multinuclear magnetic resonance spectroscopy including ^{13}C and ^{207}Pb NMR will be discussed.[1] Mixed-halide perovskites have recently been shown to be effective in circumventing decomposition and improving resistance to humidity and thermal cycling. Recent results from a series of mixed halide solid-solutions implementing a mechanochemical synthesis method will be described. In particular, atomic-level mixing is confirmed using multi-field one-dimensional NMR experiments as well as a two-dimensional ^{207}Pb exchange spectroscopy (EXSY) at ultrahigh fields (21.1 T) to track Pb---Pb neighbours and indirectly detect halide exchange.[2]Quantum chemical calculations were also used to assess the gamut of up to 10 unique local Pb polyhedra. Expanding beyond the structural aspects of these next-generation photovoltaic materials, a perovskite that displays nearly a 1 ppm per degree ($\delta^{207}Pb$/ppm) chemical shift dependence will be demonstrated. The cubic nature of $MAPbCl_3$exhibits this linear behaviour over a 200 K range with a nearly symmetric ^{207}Pb NMR line shape, providing easy temperature calibration and an alternative option in NMR thermometry.[3]

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Materials

O32

DNP assisted study of the interface region in composite materials

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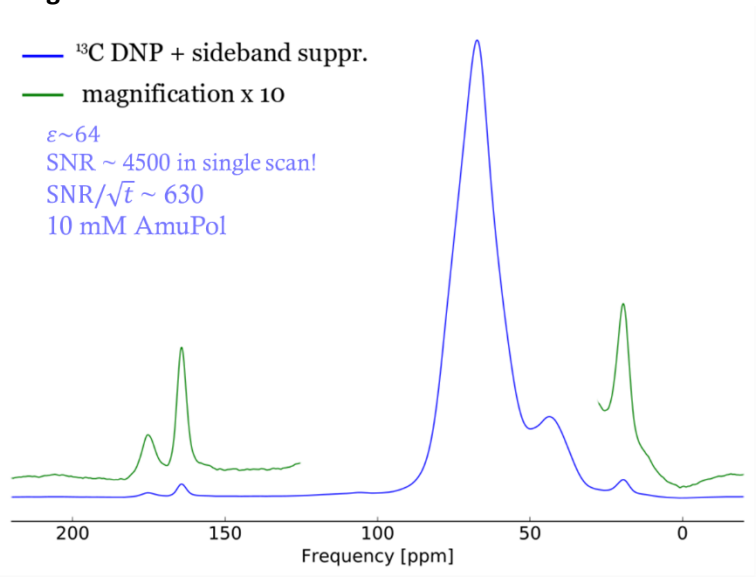
Abstract:

Polyaramid fibers have superior mechanical properties but are chemically almost inert.
To incorporate these fibers into composite materials, such as tires, an activation finish is required for improved adhesion between the fibers and the rubbery-matrix of the composite material. It is known from pullout tests of the fibers, that the performance increases dramatically by the application of an appropriate finish (+40%). Interestingly, the mechanism of this reinforcement is still debated.
In principle, the adhesion of the finish to the fiber can take place via physical interactions, such as hydrogen bonding, or via chemical bonding directly to the surface of the aramid fiber.

We study this system in two steps: first we analyze the curing process of the finish to establish the chemistry and to elucidate the topology of the polymer network at the interface. We show that although the finish on the fiber makes up only 1%>wt of the sample, even without DNP, proton spectroscopy including 2D TOCSY-like correlation experiments are feasible in a reasonable amount of time.
In order to study the finish by 13C & 15N spectroscopy we need to overcome the main challenge: low concentration of the finish (1%>wt) in combination with low natural abundance. Therefore, we selectively enhance the sensitivity at the interface by a DNP protocol geared to that region of the sample. This achieved by a matrix-free approach to incorporate stable DNP-biradicals into the interface region of the composite material.
We show that good DNP enhancements (ε>60) are obtained using this matrix-free approach, acquiring data at helium temperatures on a 600 MHz NMR spectrometer coupled to a 395 GHz gyrotron by a quasi-optical waveguide. The sensitivity for natural abundant carbon is very competitive for polymer systems and surpasses earlier reports in literature by far.
We use spectral editing techniques to overcome the overlap in the spectra caused by the slow spinning speeds inherent to the 4 mm DNP-setup. The sensitivity is very good for 13C NMR experiments at low concentrations and natural abundance and even allows for 15N NMR spectroscopy for these heterogenous polymer samples.
To exploit the high sensitivity by our matrix-free protocol (13C S/N 4500 in a single scan), we explore which protocol is best for the acquisition of 13C-13C DQ-SQ experiments for at slow spinning speeds. We will demonstrate that, 13C-13C correlation spectra are within reach for these challenging heterogeneous samples.
Last, but not least, we will discuss the implications of the chemistry we observe for the adhesion model in the composite material.

Image:


^{13}C DNP + sideband suppr.
magnification x 10
ε~64
SNR ~ 4500 in single scan!
SNR/√t ~ 630
10 mM AmuPol
200
150
100
50
0
Frequency [ppm]

Materials

O33

Bio-inspired solid-state NMR experiments to study reaction intermediates and surface species in heterogeneous catalysis

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Abstract: Over the last 2 decades, multidimensional (^{1}H,^{13}C,^{15}N) correlation experiments using dipolar and scalar-based transfer schemes have greatly expanded the scope of biological solid-state NMR[1].
In our contribution, we have adapted such methods to identify and characterize reaction intermediates and (by-)products occurring in heterogeneous catalysis using ^{13}C isotope labeled reactants.

Using 2D and 3D methods, we obtained crucial information about the initial C-C bond formation from methanol during the Methanol-to-olefin (MTO) reaction on a SAPO-34 catalyst. In addition, we provide spectroscopic evidence for the formation of surface acetate and methyl acetate, as well as dimethoxymethane during the MTO process[2].

In the case of zeolite (ZSM-5) catalyzed ethyl benzene formation from bioethanol and benzene, we furthermore have obtained detailed mechanistic insights through the direct identification of an active surface ethoxy species, a surface adsorbed zeolite-aromatic π-complex as well as the more controversial Wheland-type σ-complex. Using dedicated ssNMR experiments we can also distinguish rigid and mobile zeolite-trapped organic species, providing further evidence for distinct host-guest chemistry during catalysis[3]

References

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MRI and in vivo

O34

Single-scan synchronized diffusion and T2 mapping

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Abstract: A new simultaneous diffusion and T_2 imaging method, single-shot synchronized diffusion and T_2 mapping through overlapping-echo detachment planar imaging (DT_2M-OLED) together with corresponding reconstruction method based on deep learning, was proposed.

DT_2M-OLED sequence uses two excitation pulses (α=45°) and two echo trains to generate four echoes with two different diffusion weighting and three different T_2 weighting in a single scan. The two excitation pulses induce two echoes (Echo1 and Echo2) in the first echo train. Echo-shifting gradients are set behind each excitation pulses to shift the echoes from the k-space center. The diffusion gradients (G_d) are set between the two excitation pulses, so only Echo1 is diffusion-weighted, and have different T_2 weighting from Echo2. A refocusing pulse is applied behind the first echo train to produce another two spin echoes (Echo3 and Echo4), which have same T_2 weighting and different diffusion weighting during the second acquisition time.

Two DT_2M-OLED images are generated from inverse Fourier transformation of the original k-space signals gained by the two echo trains. A residual network is trained to reconstruct the corresponding diffusion and T_2 maps from the two DT_2M-OLED images. The training data of residual network is derived from simulated data which were randomly generated by SPROM software.

A phantom experiment was conducted on a 7T Varian MRI system to prove the validation of DT_2M-OLED. The phantom was a container with four vials inside. The container and the four vials contained different levels of glycerol and $MnCl_4{\cdot}4H_2O$, to produce a ADC range of 1.5×10^{-9} ~ 2.5×10^{-9} m^2/s and a T_2 range of 25 ~ 300 ms. Spin-echo echo planar imaging (SE-EPI) was used as a reference. For the two methods, FOV = 55 × 55 mm^2, thickness = 2 mm, TR = 3, resolution = 0.57 × 0.57 mm^2, diffusion direction is [1, 1, 1]. For SE-EPI, TE = 50/74/122 ms, b = 530/0 s mm^{-2}, total scan time is 15 s. For DM-OLED, b =530 s mm^{-2}, total scan time is 122ms. Five regions of interest (ROI) were selected for statistical analysis. In Fig. 1, the ADC and T_2 values estimated by DT_2M-OLED are quite consistent with SE-EPI counterparts. The obvious artifact in the left side of ROI3 may be due to chemical shift artifact of glycerol, because the T_2 map from SE-EPI also has obvious amplitude change in the same areas (marked by blue arrows in T_2 maps from both methods).

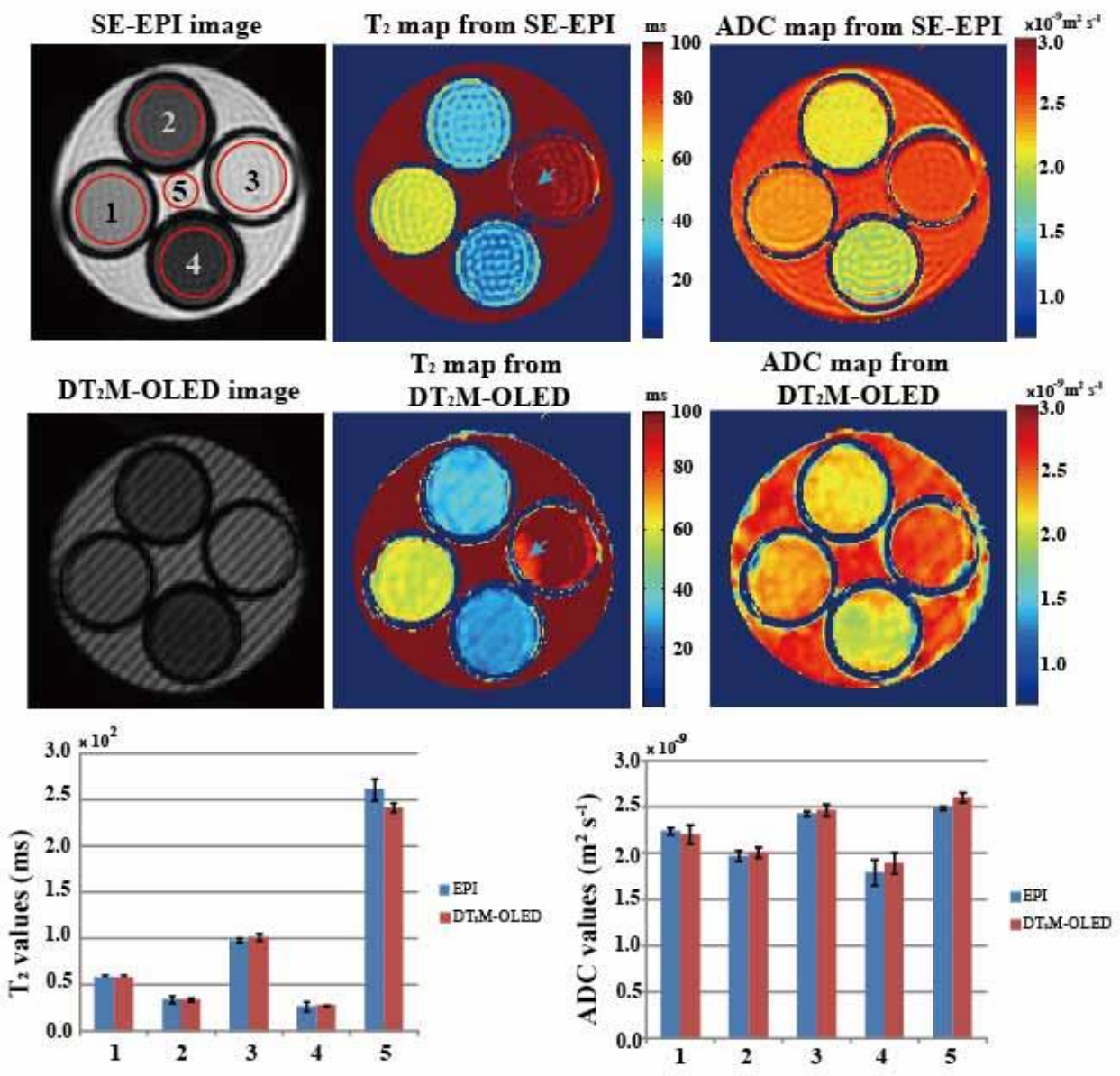
In a word, DT_2M-OLED method can simultaneously obtain reliable diffusion and T_2 mappings within a single scan. It is promising for clinical applications.

Figure 1 Results of phantom experiments.

Acknowledgements

This work was supported in part by the National Natural Science Foundation of China under Grant 11761141010 and 81671674.

Image:


SE-EPI image
T₂ map from SE-EPI
ms
100
80
60
40
20
ADC map from SE-EPI
×10⁻⁹m² s⁻¹
3.0
2.5
2.0
1.5
1.0
1
2
3
4
5
DT₂M-OLED image
T₂ map from DT₂M-OLED
ADC map from DT₂M-OLED
T₂ values (ms)
×10²
ADC values (m² s⁻¹)
×10⁻⁹
EPI
DT₂M-OLED

MRI and in vivo

O35

Improving detection limits by using a 22 T magnet and microcoils – Potential and challenges in imaging biological samples at ultra-high field strength

Julia R. Krug[*1,2], Remco van Schadewijk[3], Frank J. Vergeldt[2], Andrew G. Webb[4], Huub J.M. de Groot[3], A Alia[3,5], Henk Van As[2], Aldrik H. Velders[1]

[1]Laboratory of BioNanoTechnology, [2]Laboratory of Biophysics, Wageningen University & Research, Wageningen, [3]Solid-state NMR, Leiden Institute of Chemistry, Faculty of Science, [4]C.J.Gorter Center for High Field MRI, Department of Radiology, Leiden University Medical Center, Leiden, Netherlands, [5]Institute for Medical Physics and Biophysics, Leipzig University, Leipzig, Germany

Abstract: Magnetic Resonance applications suffer from the inherent low sensitivity of these techniques. Especially when using localized magnetic resonance spectroscopy to obtain information about metabolites present in small volumes within a tissue, the low sensitivity results in detection limits much higher than other analytical techniques. Sensitivity can be increased by increasing the magnetic field strength. The 22 T uNMR-NL spectrometer is currently one of the highest magnetic field strengths for magnetic resonance imaging worldwide. We characterized the Signal-to-Noise Ratio (SNR) and compared the performance of the 22 T to similar spectrometers operating at lower field strengths. The measured SNR increase with respect to that obtained on a 14 T system was a factor of 3 at 18 T, and a factor of 6 at 22 T using 5 mm diameter coils on each of the narrow-bore systems. The detection limit for localized spectroscopy has been found to be 10 mM of acetate with 27 nL voxel volume within 18 minutes of measurement time using the 5 mm diameter coil. These findings show the potential for imaging and localized spectroscopy at these high field strengths for biologically relevant concentrations in small volumes. Additionally, challenges with regards to susceptibility issues when imaging biological samples (e.g. plants, roots or granular biofilms) at these high field strengths are illustrated. A further approach to achieve an SNR increase is to increase the detector sensitivity by reducing the diameter of the RF coils (*1*). To this end, we have custom-built a solenoid coil with a diameter of 1.5 mm. Our results include imaging examples to characterize the highest achievable spatial and temporal resolutions using either the 5 mm birdcage or the 1.5 mm solenoid coil at 22 T.

1. S. C. Grant *et al.*, NMR spectroscopy of single neurons. *Magnetic resonance in medicine* **44**, 19-22 (2000).

MRI and in vivo

O36

Micro-NMR on 40 nanoliter droplet arrays

Ronald Kampmann[*1], Maximilian Benz[2], Marius Brehm[2], Li Zhao[1], Vlad Badilita[3], Pavel Levkin[2], Jan Korvink[3]
[1]Voxalytic GmbH, Karlsruhe, [2]Institute of Toxicology and Genetics (ITG), [3]Institute of Microstructure Technology (IMT), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

Abstract: In the present work we enable, for the first time, the chemical mapping of nanoliter droplets using nuclear magnetic resonance (NMR), by combining a droplet microarray (DMA) with micro-NMR technology. The DMA consist of a glass slide with a functional polymer coating. The layer is modified via spatial UV-click chemistry to achieve a superhydrophobic surface with wettable spots in the range of 500 µm square and a pinned droplet volume of around 40 nanoliter. Because the NMR measurement is effectively decoupled from processes such as synthesis, yield, or biological screening taking place within a droplet, miniaturization and parallelization opens the possibility to ameliorate parallel analysis of large compound libraries, which has huge potential e.g. for drug development.

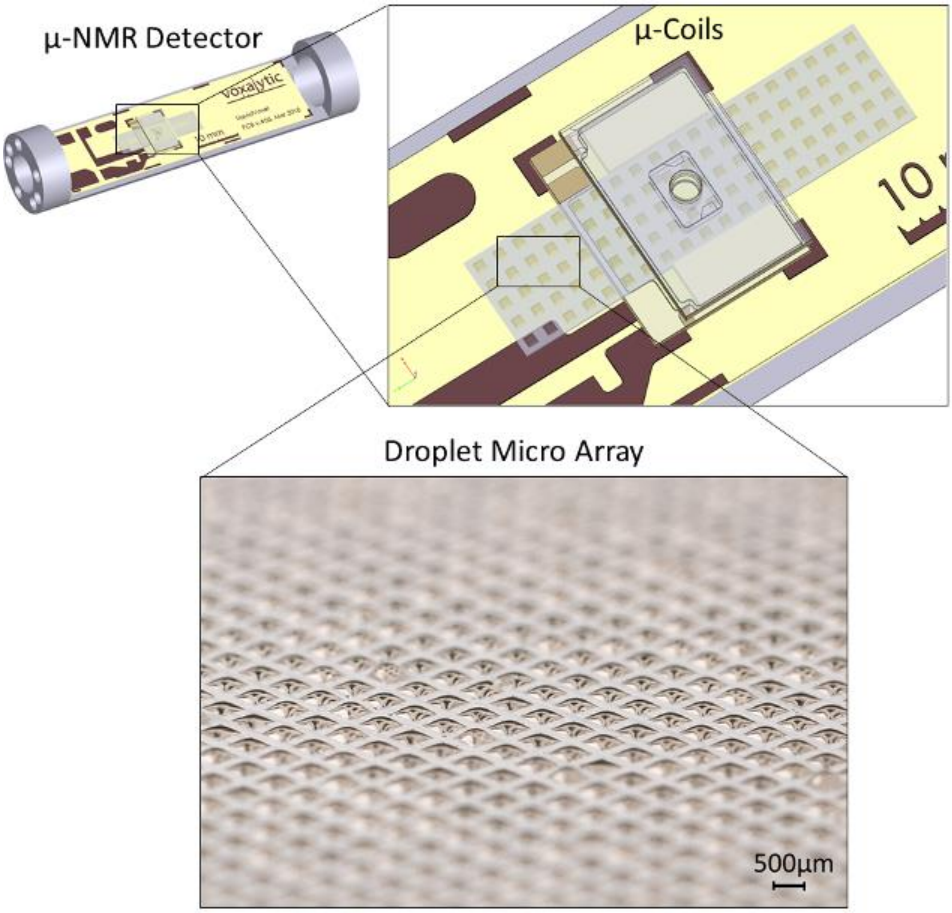
Parallelization, reduction of sample volume, and straightforward connection to analytical methods enhance the effectiveness of developing new interesting compounds for further applications while reducing the need for expensive chemicals and workload. To achieve chemical information from different substances under identical conditions, parallel monitoring of chemical composition is advantageous. Through classic liquid or solid phase combinatorial chemistry, the distinct spots are modifiable with compounds and can be tested in cell-based screens directly after synthesis.

While current technologies allow for a rapid and highly miniaturized synthesis of huge molecule libraries for biological and medical screening, the analysis of formed products in those cases is mostly done by mass spectrometry (MS) and lacks the pace of library creation. Also, additional information is desired for a reliable identification of a compound, because MS does not clarify constitution or stereoisomerisation. Although NMR is able to close this gap, classic devices lack the throughput and sensitivity offered by MS. Here we aim to address these issues.

A limited sample volume requires a special approach to boost the NMR Signal. Microcoils can be applied in order to move the detector as close as possible to the sample. By arranging microcoils in a Helmholtz configuration, flexible space is made available for sample carriers (N. Spengler et al. PLoS ONE 11(1): e0146384). Sample containers can thus be flexibly introduced in the detector to perform NMR spectroscopy and magnetic resonance imaging (MRI).

For the NMR experiment of nanodroplets, we arranged them as a microarray (4 wells simultaneously in the detection volume) built upon a 200 µm thick glass slip. Initial investigations using an 11.74 T NMR spectrometer prove the ^{1}H detectability of individual liquid sample volumes of only 40 nanoliters. Furthermore, by performing MRI encoding on spatially resolved and hence separate droplets in the array, we demonstrate the correct function of the parallelization approach.

Image:



Biosolids

O37

Structural characterization a of 29 kDa enzyme-inhibitor complex at 110 kHz

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[1]Department Chemistry, LMU Munich, Muncih, Germany

Abstract:

Proton-detection under Magic-Angle Spinning (MAS) has been revolutionizing solid-state NMR studies of micro-crystalline samples, amyloids and membrane proteins by efficient detection with improved resolution using minimal amounts of samples and ultra fast spinning (> 100 kHz). However, due to limitations in sensitivity and resolution the size of the individual monomeric units has rarely exceeded 20 kDa. Enzymes, which are often more complex and bigger in size, have not been easily accessible, even though manifold desirable information could potentially be provided by solid-state NMR studies.

Carbonic anhydrases (CA) are ubiquitous metalloenzymes that catalyze the inter-conversion between dissolved CO_2 and bicarbonate. They belong to the fastest enzymes known, reaching up to 10^6 turnovers per second for the human variant hCAII. Despite good structural understanding and various pharmacological studies, both the mechanism of turnover as well as proton shuttling in and out of the active site are poorly understood. Due to the presence of the His64 sidechain in two different conformations in crystal structures a flip is assumed to be essential for proton transport. An ordered network of hydrogen bonded water molecules between the zinc-bond water and His64 is believed to be the pathway for intramolecular proton transfer in catalysis. Solid-state NMR structure calculation and dynamics studies with a focus on hydrogen atoms is opening new perspectives to revisit the enzyme's dynamic interaction with substrate and water.[1]

Here, we report characterization of human carbonic anhydrase (29 kDa) in its complex with acetazolamide at ultra-fast MAS using proton detection. First, we assigned nearly 90% of backbone and side-chain shifts using amide-detected triple-resonance experiments, enabling characterization of overall protein properties and the active site (Fig. 1). Unambiguous assignment of aromatic sidechain resonances from uniformly labeled samples is a known, significant NMR hurdle. Here we present dedicated pulse sequences for the unambiguous detection of these resonances. In the next step, we obtained long-range distance information using time-shared ^{15}N- and ^{13}C-edited RFDR experiments[2]. Later, we complemented these distance restraints with water-accessible surface data[3] and hydrogen bond pattern data. Finally, we present an atomic-resolution model of a 29 kDa enzyme-ligand complex based on the ssNMR observations (Fig. 1C) as well as detailed information on the protein dynamics (Fig. 1B) and water interactions. Our results demonstrate that solid-state NMR studies of proteins in the 30 kDa range, in particular enzyme-ligand complexes, are well amenable for valuable mechanistic insights despite the complexity of the spin system.

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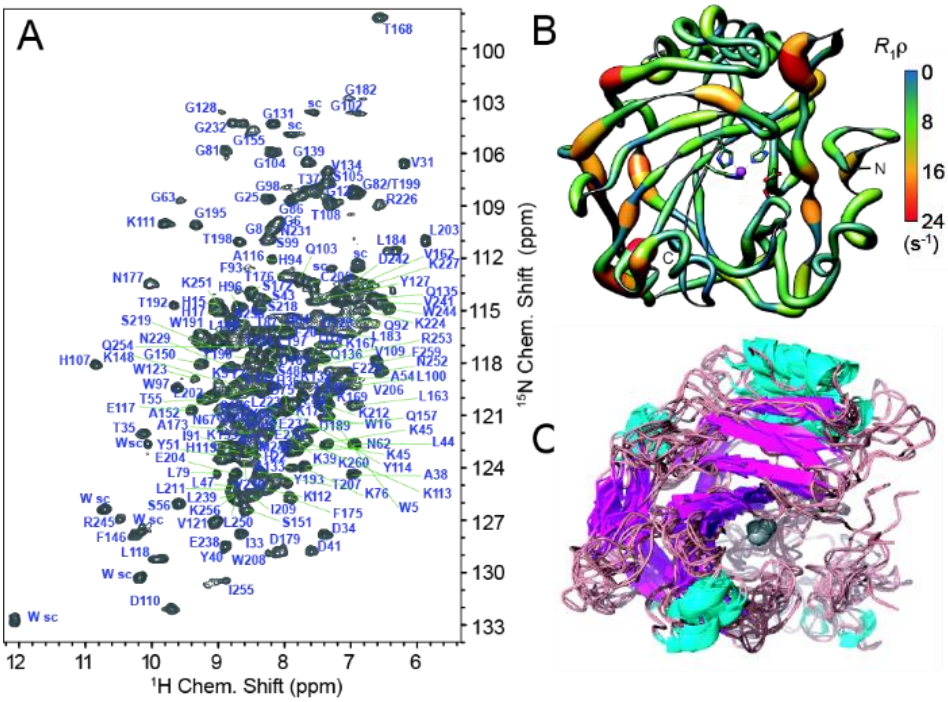
Fig. 1 *(A) Assigned ^{1}H-^{15}N correlation spectrum of [^{13}C, ^{15}N]-labeled hCAII at 110 kHz spinning speed and 800 MHz ^{1}H Larmor frequency. (B) ^{15}N $R_1\rho$ relaxation rates plotted onto a crystal structure (PDB: 2cba). (C) Preliminary NMR structure of hCAII in complex with the small-molecule inhibitor acetazolamide.*

Image:


A
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(s^{-1})
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C
^{15}N Chem. Shift (ppm)
^{1}H Chem. Shift (ppm)
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Biosolids

O38

Structural studies of Nucleosomes and Human Cytoskeleton Proteins: The power of high-sensitivity/resolution Solid-state NMR methods

Shengqi Xiang[*1], Ulric le Paige[1], Yanzhang Luo[1], Velten Horn[1], Anna Akhmanova[2], Hugo van Ingen[1], Marc Baldus[1]

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Abstract: Emerging technologies in solid-state NMR(ssNMR) spectroscopy, such as proton detection and Dynamic Nuclear Polarization (DNP), can greatly improve spectral sensitivity and resolution. Together with novel sample preparation approaches these advances provide new opportunities for ssNMR to study complex biomolecular systems such as nucleosomes and human microtubules.

[1] We demonstrate how protein structure, dynamics, and interactions of nucleosomes can be interrogated in a residue-specific manner using state-of-the-art ssNMR. Using sedimented nucleosomes, high-resolution spectra are obtained for both flexible histone tails and the non-mobile histone core. Through co-sedimentation of a nucleosome-binding peptide, we demonstrate that protein binding sites on the nucleosome surface can be determined[1]. These results provide the basis for future studies of interactions of proteins with higher-order chromatin structures, including isolated or cellular chromatin.

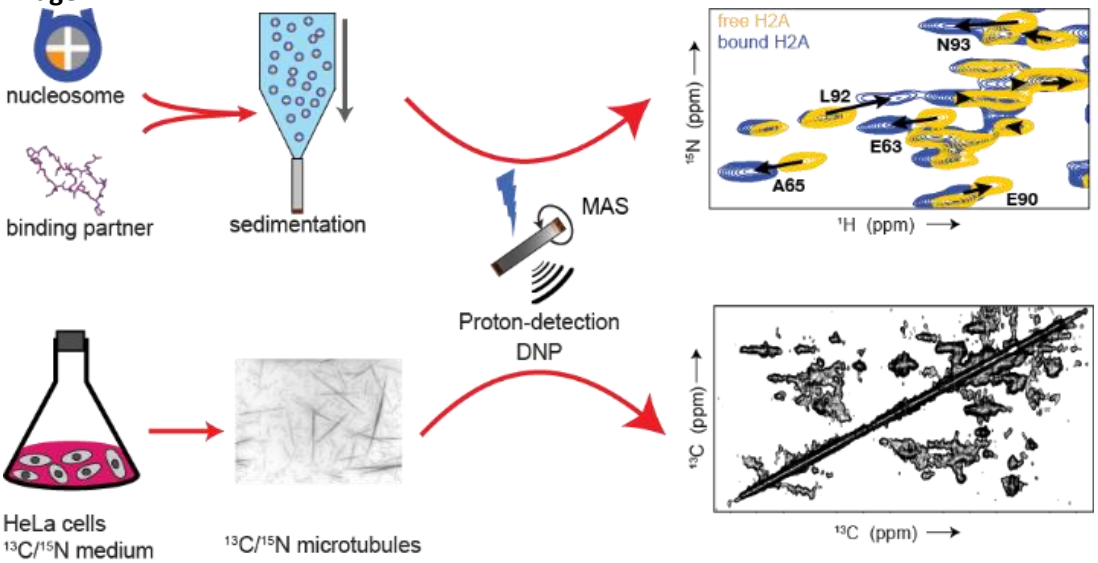
[2] In addition, we present high-resolution ^{1}H and DNP ssNMR spectra of human microtubules extracted from HeLa cells that were cultured on isotope-enriched media. Initial experiments allowed us to study the rigid cores of tubulins, as well as their flexible tails in comparison to existing X-ray and cryo-EM structures. Our results pave the way for further NMR studies on microtubules and their lattice structure as well as their post-translational modifications and interactions with other proteins. As an example, we show the use of ^{1}H ssNMR to study protein-MT interactions[2] at atomic resolution.

References:

(1) Angew.Chem.Int.Ed. 2018,57,4571 –4575

(2) Nat. Struct. Mol. Biol. 2017,24,931–943

Image:



Biosolids

O39

The hydrophobic pore of AlkL revealed in lipid bilayers and detergent micelles

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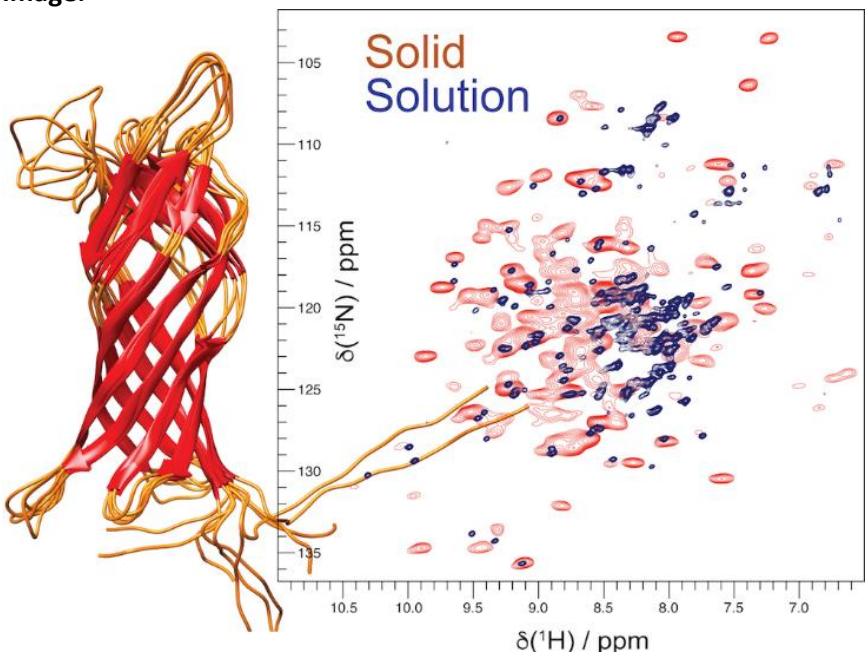
Abstract: The membrane protein AlkL is known to conduct hydrophobic molecules across the outer membrane of bacteria. Though no structure is available for AlkL, by homology it is counted into the OmpW family of proteins, usually comprised of 8-stranded beta barrels with long extracellular loops. Diverse biological roles have been proposed for OmpW proteins mainly derived from *in vivo* studies, but even after high resolution structures have been solved, no agreement on the function and mechanisms has been found. This is due to a quite commonly observed discrepancy between outer membrane protein structures elucidated by solution NMR or X-ray crystallography. For membrane proteins, solid-state NMR is ideally suited to study the protein within the context of a near-native lipid bilayer environment, ensuring a reduction in the potential for artifacts, which are sometimes encountered in detergent or contacts within a crystal. Here we used ^{1}H-detected solid-state NMR under fast (>60 kHz) magic-angle spinning (MAS) to resolve the structure of AlkL in DMPC bilayers and to probe local backbone dynamics over different timescales.

The comparison with solution NMR data on the same system in micelles revealed surprising differences in the stability of a barrel extension implicated in the function of AlkL. Slow µs dynamics was evidenced in the hydrophobic extra-membrane pore, highlighting transport-related rearrangements along the proposed pathway for hydrophobic molecules. The transport mechanism was further supported by observing localized changes in chemical shift and dynamics in the presence of a small hydrophobic model substrate.

Overall we present a membrane protein analysis in a unique completeness, setting new standards for future structural biology studies by solid-state NMR.

Image:



Small Molecules and Pharmaceutical

O40

Interaction studies with secondary-labelled hyperpolarized ligands

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Abstract: Hyperpolarization by dissolution-DNP[1] provides a way of enhancing ^{13}C MR sensitivity by more than four orders of magnitude on a wide range of small molecules. d-DNP can potentially be a game changer in numerous applications involving the observation of small molecule, such as metabolic imaging[2], metabolomics, drug discovery, or more generally analytical chemistry where NMR is often a method of choice to determine properties, structures and behaviors. However, to be truly useful in these applications, d-DNP would sometime require higher efficiency, throughput, and repeatability.

In this context, we have recently shown that by combining d-DNP with low temperature microwave-gated cross-polarization, unprecedentedly high levels of polarization could be attained in short times (60% in 8 min)[3]. We have also shown that a high level of repeatability could be afforded with our current d-DNP setup (CV=3.6%)[4].

The main problem that remains when all these issues have been tackled is the fact that d-DNP generally relies on ^{13}C spins which are of only 1.1% natural abundance, and that most NMR applications such as metabolomics studies on natural products or biological samples are difficult to label. In 2009, Wilson et al. have proposed an approach where amines groups in amino acids where labeled with [1,1-^{13}C] acetic anhydride[5], and subsequently hyperpolarized, but this approach has not been taken up by the d-DNP community since.

Here, we propose to revisit this secondary labeling approach with our recent d-DNP advances and in the context of NMR fragment based drug discovery (FBDD). ^{1}H ligand-observed approaches (STD / WaterLOGSY) is powerful for big protein (> 30 KDa) but can't be applied for small proteins. We show how ligands can be secondary labeled and hyperpolarized to probe interactions with their target proteins (66 kDa and 8 KDa). We show that the labelling is used for an ultra-rapid interaction determination, via the detection of a change in hyperpolarized T_1 of the weak ligands ^{13}C-tag. We are currently developing this new concept with the aim of decreasing by orders of magnitudes either the time or the concentrations required to detect ligands binding compare to classical approach.

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Small Molecules and Pharmaceutical

O41

The Concept of Tensorial Constraints in Molecular Dynamics: Conformation and Configuration of Flexible Molecules by RDCs without Alignment Tensor

Burkhard Luy[*1], Pavleta Tzvetkova[1], Maria Enrica Di Pietro[1], Emine Sager[1], Thomas Gloge[1], Armando Navarro-Vazquez[2], Alvar Gossert[3], Ulrich Sternberg[1]

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Abstract: Residual dipolar couplings (RDCs) and other residual anisotropic NMR parameters provide valuable structural information of high quality and quantity, bringing detailed structural models of flexible molecules in solution in reach. Corresponding data interpretation so far is based on the concept of a so-called alignment tensor, which, however, is ill-defined for flexible molecules. The concept is also only applied to a single or a small set of lowest-energy structures, ignoring the effect of vibrational averaging. Here we introduce a different approach based on time-averaged molecular dynamics with dipolar couplings as orientational restraints that can be used to solve structural problems in molecules of any size without the need of introducing an explicit or approximate molecular alignment tensor in the computations. RDC restraints are represented by their full 3D tensor in the laboratory frame. Enforced rotational averaging of each individual tensorial restraint leads to structural ensembles that best fulfil experimental data. Using one-bond RDCs, the approach has been implemented in the MD program COSMOS and extensively tested. A detailed description of the underlying theory, including the special treatment of force fields for stable and fast MD runs, and applications regarding configurational and conformational analyses of small-to-medium-sized organic molecules with different degrees of flexibility are given.

Small Molecules and Pharmaceutical

O42

Hit-target interaction study by in cell NMR

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Abstract: Detailed information on hit-target interaction is a prerequisite in rational drug design. We developed a new approach that combines NMR in whole cells (*in cell* NMR) and docking to characterize hit-target interaction at the atomic level (1). By using *in cell* NMR, we validated the target engagement of a promising antituberculosis drug (IPK317) from the imidazo (1,2-a) pyridine (IP) series identified by phenotype-based high throughput screening (2). The most advanced IP drug is currently in phase I clinical trial under a US FDA Investigational New Drug application (3). Furthermore, we identified the atoms of IPK317 interacting with the target, the *Mycobacterium tuberculosis* cytochrome *b*. NMR data and the Self-Organising Map algorithm were then used for the clustering of a large set of drug-target complex models. The selected ensemble reveals IPK317 in a transient cavity of cytochrome *b*, interacting with the residue T313, the site of a spontaneous mutation that confers bacterial resistance. Our approach makes it possible to set up a pipeline for obtaining atomic information on hit-target interactions in the cellular context (4).

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Hardware

O43

Micro-integrated Gradient System for Magnetic Resonance Microscopy

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Abstract: We report a novel micro-gradient system fabricated using newly-established micro-electro-mechanical systems (MEMS) process technology. The spatial resolution in a micro-imaging experiment is proportional to the magnetic field gradient strength available. The concept presented here exceeds the field strength of commercially available systems by a respectable factor whilst maintaining an acceptable duty cycle and low current level. Several techniques can be used to achieve a stronger gradient field strength, such as increasing the current strength, or making the coil windings more dense. Both lead to more heating, either due to more dissipation, or to the difficulty of removing the heat from the coil body. As a result, the performance is always a compromise. The present approach uses the fact that increased magnetic field gradients can be generated by bringing the gradient coils physically closer to the sample. However, heat dissipation is then a critical issue. An optimisation method was developed to design bi-planar micro-gradient coil pairs to exhibit genuine minimum power dissipation [1]; in addition, a micro-fluidic cooling system was co-integrated in the coil micromanufacturing process. Figure 1 illustrates the design of a gradient flip-chip. Figure 2 shows the major chip features of the fabricated triaxial gradient set, with one half in an exploded view. An integrated micro-fluidic cooling layer separates the two gradient coil layers on one chip. To maintain thermal stability of the sample, the active cooling system compensates the heat-up of the gradient coils, which is caused during high duty cycles. The z-gradient coil shown in Figure 4 achieves a gradient strength of 19.3 T/m for an applied constant cur-rent of 4.5 A, causing Joule heating of the assembly up to 10°C above ambient. In a preliminary investigation, the single-axis gradient was used in an MR imaging experiment (Figure 6). Line-image profiles taken from a custom-designed sample insert were recorded to analyse the linearity of the resultant gradient field.

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The authors would like to thank the ERC for founding under the grant number 290586 - NMCEL.

Image:

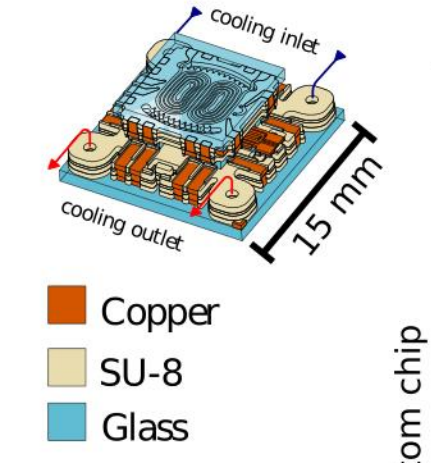

Fig. 1: Flip-chip assembly

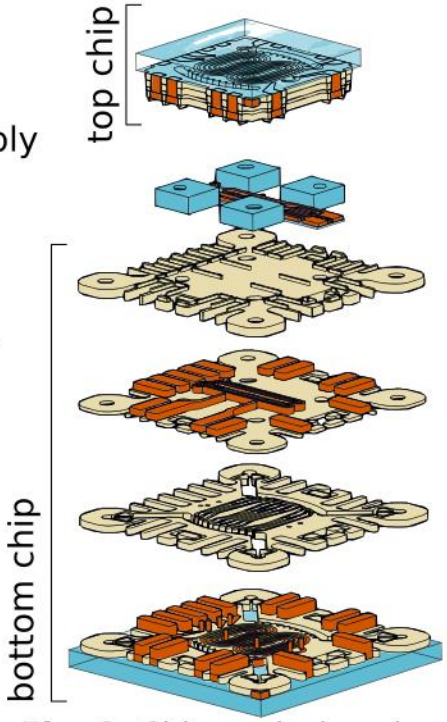

Fig. 2: Chip explosion view

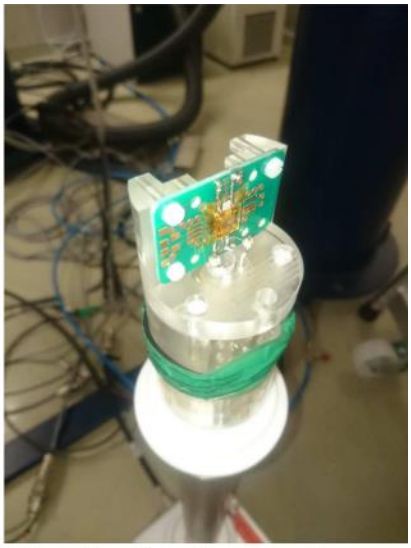

Fig. 6: Gradient coil attached to a probe base, with an integrated micro coil and tuning network for MR imaging.

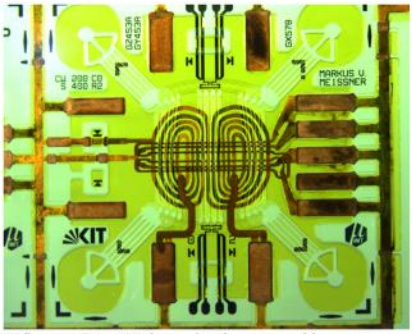

Fig. 3: Triaxial gradient

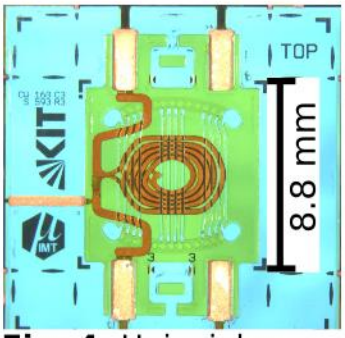

Fig. 4: Uniaxial z-grad.

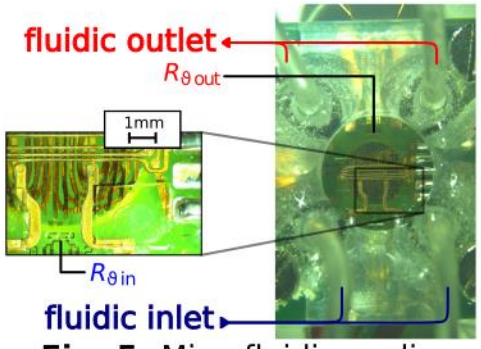

Fig. 5: Microfluidic cooling

Hardware

O44

A Fast MOSFET Switch for RF Pulse Management and Shaping in Low-Field NMR and MRI

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Abstract: TRansmit Array Spatial Encoding (TRASE) is a MRI method that achieves spatial encoding through repeated pulses of radiofrequency (RF) B1 fields from different phase-gradient transmit coils. It does not require the application of B0 gradients and is a distinct alternative to the standard MRI approach.[1] An evaluation of the benefits and limitations of TRASE MRI at low field (a few mT) is under way using hyperpolarised gas samples.[2,3] Fast switching between two or more transmit coils with negligible wait time between pulses is required to reduce the total RF encoding time. PIN diodes are often used for coil switching in MRI but bias control with RF isolation involves response times (several RF periods) which are too slow for the targeted low-frequency application. We have designed bidirectional MOSFET switches with floating and optically isolated gate control.[4] A similar device was recently reported to be suitable as Q-switch in low-field NMR.[5] Our tests have been performed with different RF power amplifiers and different loads at various frequencies (in the DC – 200 kHz range). An Apollo Tecmag console was used to manage RF pulses and TTL signals controlling the switches. RF currents were recorded using a digital oscilloscope by monitoring the voltage drop across a 1-Ω resistor inserted on the ground line at the output of the RF amplifier.

Our MOSFET switch (with components rated for 500V and 32 A or for 1.7kV and 9A) has switching times below 1 µs. It has a low ON impedance (R~1 Ω) and a frequency-dependent OFF impedance (~500 pF capacitance), yielding efficient RF current switching in resistive loads and in transmit coils (various situations will be reported). Additionally, current switching at null current and maximum voltage can be used to abruptly stop or start pulses in series-tuned RF coils, therefore avoiding the rise and fall times associated with the Q-factors (Fig. 1). RF energy can be efficiently stored in tuning capacitors for times as long as hundreds of milliseconds. Besides TRASE MRI, this energy storage approach may find applications in fast repeated spin-echo experiments.

Fig. 1: RF currents (top) driven by RF voltages (middle trace) at 83.682 kHz in two (series-tuned) transmit coils A and B (L=2.4 mH, C= 9.5 nF, Q=15). Currents are fed to the coils from a single RF amplifier through switches (TTL-controls: bottom). The first pulse (P1) builds up RF energy in coil A while performing a 2π rotation in 18 RF periods. When P1 is interrupted the capacitor C is fully charged and excitation with full RF amplitude is resumed, allowing for a rectangular π pulse in 6 periods (P2) at the end of which energy remains stored. Without energy storage in coil B, a π pulse lasts 12 periods (P3), with fast current decay thanks to the RF sign change after 9 periods.

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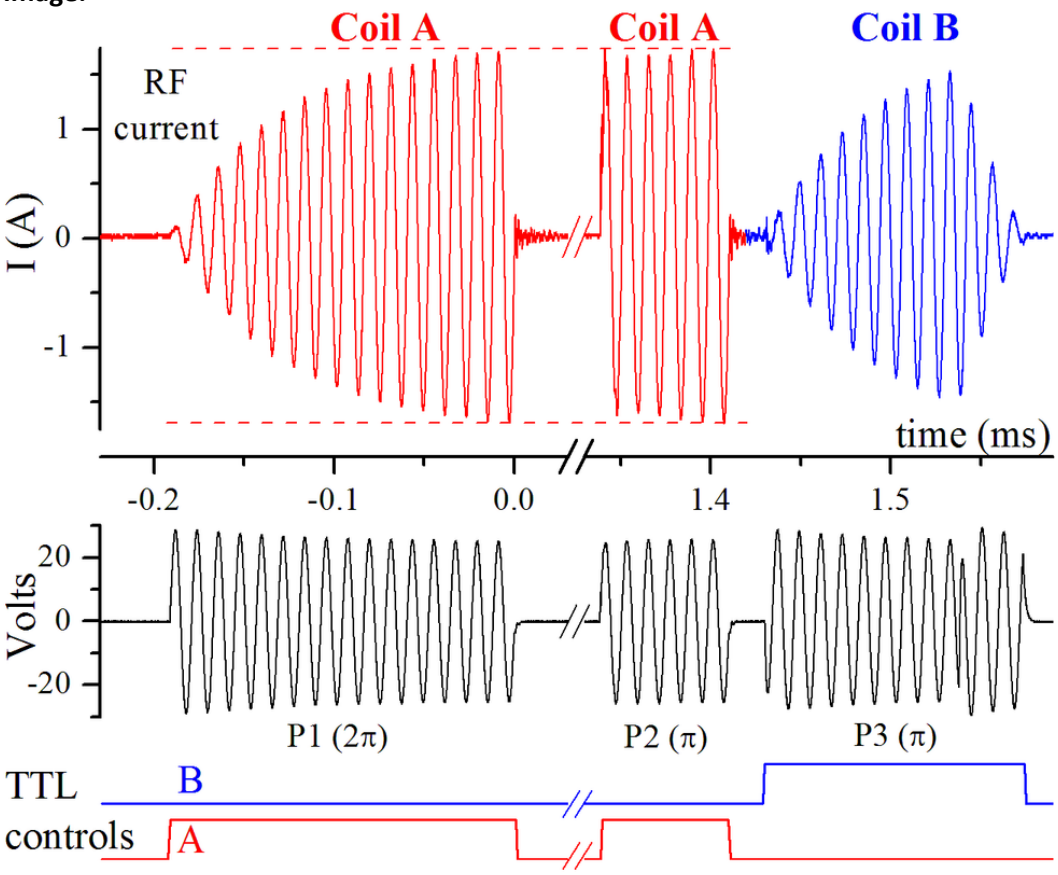
[5] JZ Zhen et al., J Magn Reson 287 (2018) 33

Image:


Coil A
Coil A
Coil B
RF
current
I (A)
1
0
-1
time (ms)
-0.2
-0.1
0.0
1.4
1.5
Volts
20
0
-20
P1 (2π)
P2 (π)
P3 (π)
TTL
controls
B
A

Hardware

O45

High performance fluid-path for dissolution-DNP

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Abstract: NMR's intrinsic lack of sensitivity can be under some circumstances overcome with hyperpolarization by dissolution dynamic nuclear polarization (d-DNP).[1] In a typical d-DNP experiment, high nuclear spin polarizations are achieved at low temperatures on frozen solutions by transferring the near-unity electron spin polarization of embedded free radicals through microwave irradiation. The sample is then melted and transferred to an NMR or MRI machine. One of the major challenges is the preservation of the hyperpolarization once the sample is melted as nuclear spin relaxation irreversibly drives it to Boltzmann's equilibrium.

We have recently proposed the use of a magnetic tunnel[2] to prevent excessive losses of polarization at low field during transfer. The group of Christian Hilty has extensively worked at speeding up this dissolution and transfer step by proposing an original design with fast valves[3] that was further improved[4] and later on equipped with high flow syringe pumps leading to flow rates up to 16mL/min[5] and most recently even 150 mL/min[6], leading to total dissolution and transfer times as short as 1.6s.

Here we propose a design based on a micro gear pump (HNP Mikrosysteme mzr-11508X1) with flow rates exceeding 1100 mL/min. These pumps can drive hyperpolarized samples at pressure exceeding 3 MPa, resulting in measured transfer times as short as 0.6 s over 5-meter-long distances.

We will present the simple design of this new system (figure 1), including a fluid path featuring ultra-fast valves (8ms switching time) and a dedicated sample injector compatible with virtually any 5mm NMR probes. Finally, we will discuss on the performances of the system and the associated hyperpolarization results.

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Image:

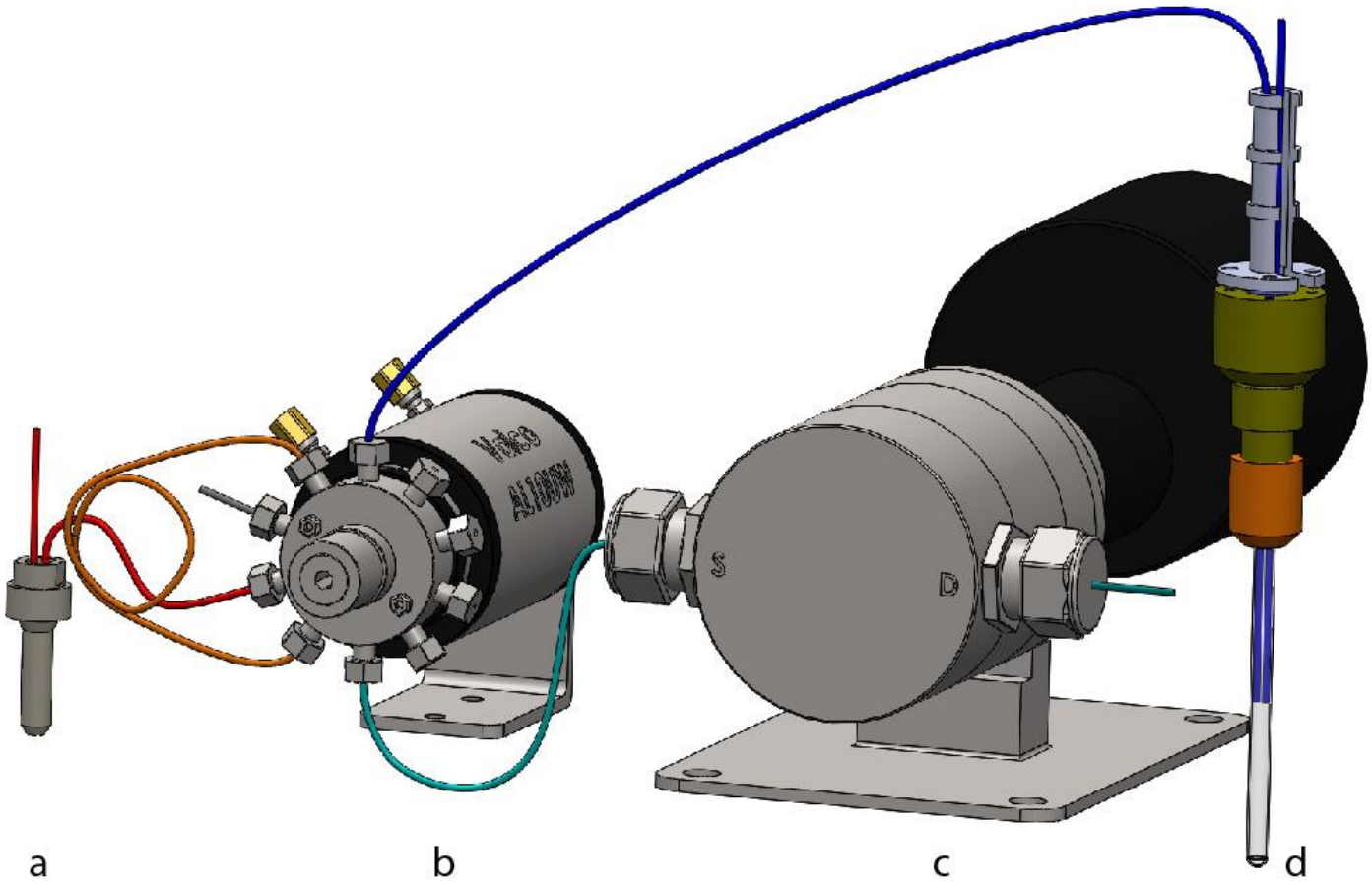

Fig. 1 - d-DNP fluid path principally composed of a) a DNP sample holder at low temperature (T = 1.5K), b) a high speed switching valve (Valco AL10UW), c) a high flow pump (HNP Mikrosysteme mzr-11508X1), and d) a sample injector to collect the hyperpolarized solution at room temperature. The hyperpolarized sample is dissolved and pushed out of the sample holder through the red tubes to the 10-way valve, and fills the orange loop. The valve is switched and the micro gear pump pushes the sample through the blue tube to the NMR tube of the sample injector.

Hyperpolarization

O46

DNP-enhanced ssNMR sensitivity: free radicals at work

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Abstract: Dynamic Nuclear Polarization (DNP) is one of the most promising approaches to overcome the sensitivity limitations of solid-state NMR, opening new possibilities and applications in materials and life sciences. The recent advances result from significant developments in DNP instrumentation, in the introduction of new methodological concepts and in the design of ever more efficient polarization sources. In a DNP experiment, the larger polarization of unpaired electrons (usually from a stable free radicals) is transferred to surrounding nuclei by microwave irradiation at or close to the EPR Larmor frequency, providing maximum theoretical signal enhancements of a factor 658 for ^{1}H. Better understanding the polarization mechanisms and improving the rational design of polarizing agents have contributed to the success of ssNMR/DNP experiments.[1-3] Signal enhancements (ε) of 50-200 are routinely obtained today at 9.4 T and 100 K, allowing the investigation (not feasible without DNP) of an ever broader range of molecular and macromolecular systems including biomolecules, hybrid materials, mesoporous silica, metal oxides, polymers, nanoparticles and microcrystals. However, the enhancement factors are still far from the predicted maximum values, notably at very high-fields. We will report our recent results on the design, synthesis and EPR characterization of new and improved polarizing agents, giving large enhancement factors (>150) at 9 and 18 T, opening new perspective in determination structure.

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Hyperpolarization

O47

PHIP on a Chip - Hyperpolarisation in microfluidic NMR

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Abstract: In this work we show that para-hydrogen induced polarisation (PHIP) can be implemented, and observed, on a single microfluidic device that integrates with a bespoke micro-NMR detector. This allows observation of molecules in mM concentrations in mL volumes. Our ultimate goal is to enable quantitative characterisation of metabolic processes in Lab-On-A-Chip cultures of cells, tissue slices, and small organisms.

Microfluidic systems operate on small volumes ranging from pico- to micro-litres. While miniaturised NMR detectors have good mass sensitivity at these size scales, their concentration sensitivity is still insufficient to observe many metabolites at physiological concentrations.

PHIP requires reaction of a suitable unsaturated substrate with parahydrogen gas. In the present work, we show that this can conveniently be achieved in a microfluidic system by allowing the hydrogen gas to permeate through a poly(dimethyl siloxane) (PDMS) membrane. In addition to demonstrating the PHIP effect, we present quantitative data on the reaction and transport kinetics, as well as the relevant spin relaxation times. The challenge here is two-fold: designing and fabricating a para-hydrogenation capable microfluidic device whilst integrating the device with a bespoke micro-NMR detector; and ensuring the hydrogenation, and transfer, of polarised product is sufficiently fast to observe PHIP.

While hyperpolarisation through membranes has been performed previously [1,2], these focused on macroscopic samples. Whilst our work integrates hyperpolarisation with a microfluidic device compatible with an optimised probe with corresponding micro-NMR detector [3]. To this end, we have simulated the flow of reaction material and subsequent H_2 depletion with the PDMS membrane (Fig. 1A); have verified the Ts of p-H_2 is significantly long enough to be transported through PDMS without significant relaxation; used an analogous hydrogenation system (Fig. 1B) to successfully observed an ALTADENA signal with enhancement ~200 (Fig. 1C)

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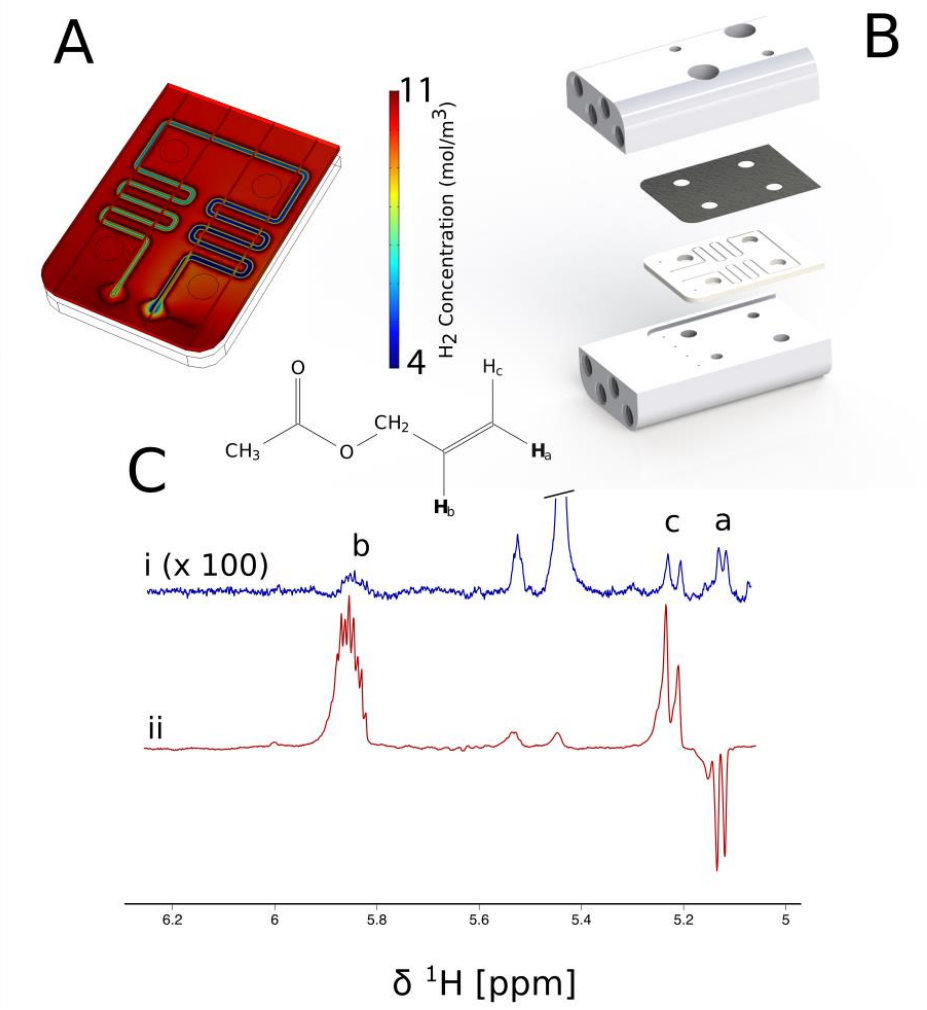
Image:


A
B
C
11
4
H_2 Concentration (mol/m^3)
O
CH_3
O
CH_2
H_c
H_a
H_b
i (x 100)
ii
b
c
a
6.2
6
5.8
5.6
5.4
5.2
5
δ ^{1}H [ppm]

Hyperpolarization
O48
Protein-Ligand Interaction Monitoring
by Dissolution-DNP and Long-Lived Deuterium Spin States

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Abstract: Protein-Ligand Interaction Monitoring
by Dissolution-DNP and Long-Lived Deuterium Spin States
Dennis Kurzbach*[a], Thomas Kress[a], Konstantin Ivanov[b], and Geoffrey Bodenhausen[a]
[a] *Laboratoire des biomolécules, LBM, UMR 7203, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris*
[b] *International Tomography Center, Siberian Branch, Russian Academy of Science (SB RAS), Institutskaya 3a, Novosibirsk 630090, Russia*

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Dissolution dynamic nuclear polarization (D-DNP) allows one to generate a long-lived population imbalance involving two or three deuterium nuclei.[1; 2] These states have lifetimes that exceed conventional deuterium Zeeman magnetization – which is governed by fast quadrupolar relaxation - by factors over 20. We demonstrate how to monitor these states via neighboring carbon nuclei and how to exploit them to monitor interactions between deuterated ligands and bio-macromolecular hosts. Even slight variations in rotational motion of the deuterated moieties lead to substantial effects on the lifetime of the long-lived states (LLS), which allows one to detect very weak interactions. This enhanced sensitivity is due to a coupling of the rotational correlation time t_R with the quadrupolar coupling constant w_Q through terms of the form $w_Q^2 t_R$ in the relevant relaxation rates. As w_Q is typically quite large, even slight changes in t_R can entail strong effects on relaxation and hence a large contrast between free and bound ligands. The latter feature, coupled with the enormous sensitivity enhancement that can be obtained by D-DNP, enables an interaction-monitoring technique characterized by high precision at low concentrations. This methodology might become of importance for screening deuterated drugs, a class of molecules that has received ample attention in the recent years because of their slow metabolic consumption, allowing reduced dosage and, hence, reduced side effects.[3]
Figure 1 shows a sketch of the strategy for interaction monitoring using long-lived deuterium spin states. In a first step a molecule hosting a deuterated moiety is hyperpolarized at 1.2 K in a field of 6.7 T. Subsequently, the sample is dissolved with a burst of superheated heavy water and transferred in less than 4 s to a conventional 400 MHz NMR spectrometer operating at 9.4 T where the hyperpolarized ligand is mixed with a solution containing a prospective binding partner. Finally, the LLS spanning the deuterons is detected indirectly by means of time-resolved ^{13}C NMR.

References
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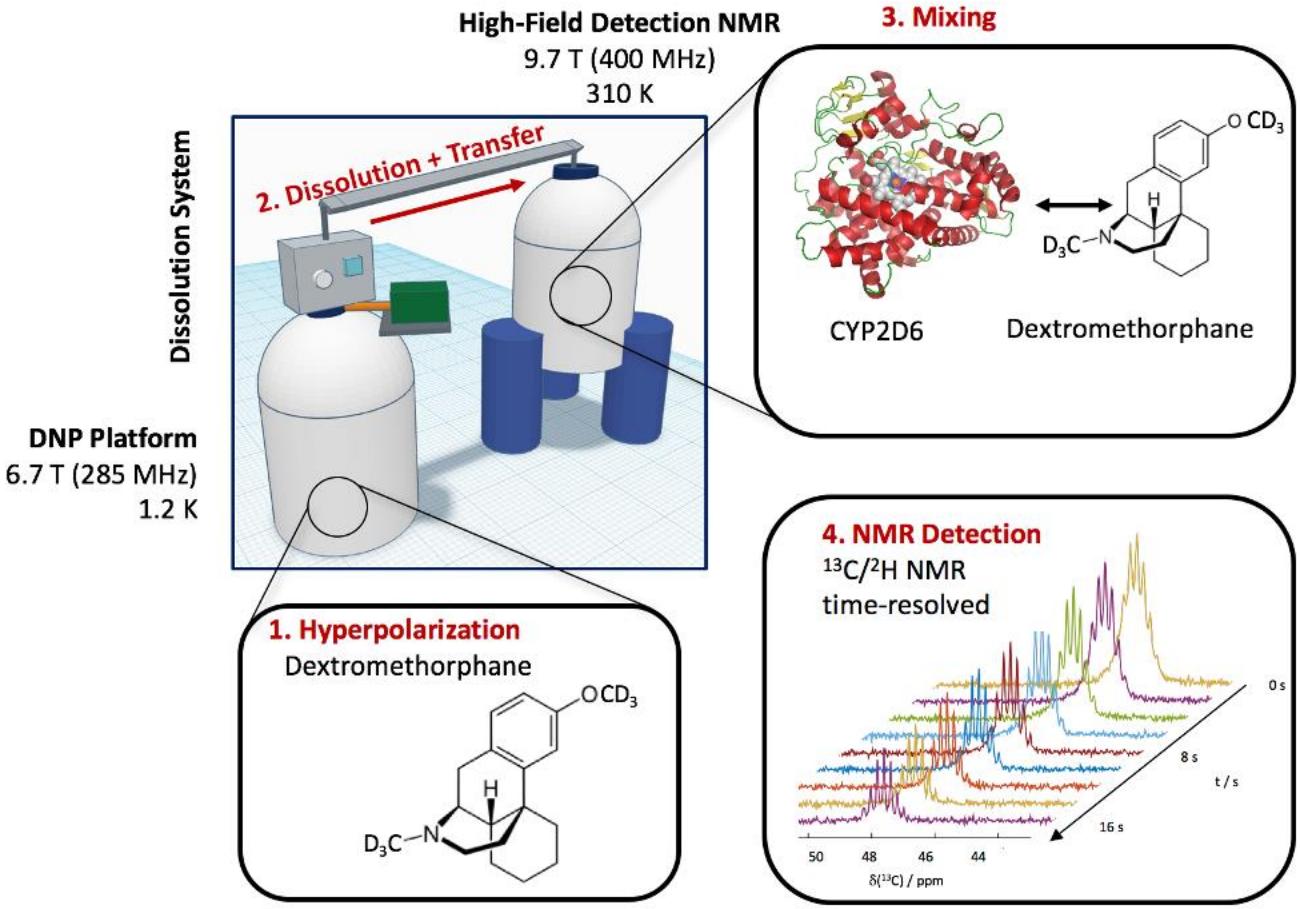
Image:


High-Field Detection NMR
9.7 T (400 MHz)
310 K
3. Mixing
Dissolution System
2. Dissolution + Transfer
DNP Platform
6.7 T (285 MHz)
1.2 K
CYP2D6
Dextromethorphane
1. Hyperpolarization
Dextromethorphane
4. NMR Detection
13C/2H NMR
time-resolved
0 s
8 s
16 s
t / s
50
48
46
44
δ(13C) / ppm

Liquid-state NMR methods

O49

Interaction of Metabolites with Macromolecules in Human Blood Plasma Investigation by High-Resolution NMR Relaxometry.

Ziqing Wang[*] [1, 2], Veronica Ghini[3], Pavel Kadeřávek[2], Morgan Kazmierczak[2], Guillaume Bouvignies[2, 4], Thorsten Marquardsen[5], Jean-Max Tyburn[6], Giacomo Parigi[3, 7], Claudio Luchinat[3, 7], Fabien Ferrage[2, 4]

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Abstract: Metabolomics is the study of all metabolites in biological samples such as bio-fluids. In biomedicine, this unique chemical fingerprint of the metabolic status of an individual is widely used to characterize diseases and in the early diagnosis and prognosis of pathologies. Conventional metabolomics approaches do not provide information of interactions between metabolites and macromolecules in biological fluids.

Here, we use high-resolution NMR relaxometry to identify the interactions of metabolites with macromolecules in biological fluids such as human blood plasma. The method is based on the measurement and analysis of longitudinal relaxation rates over three orders of magnetic field (from 20 mT to 14.1 T) with high-resolution detection at 600 MHz (*Fig.1 A*)[1]. The approach was first developed on a model sample containing a solution of bovine serum albumin (BSA) and two small molecules: 3-(Trimethylsilyl)propionic acid-D4 (TMSP) and alanine. The relaxation dispersion profiles (*Fig. 1B*) are very different for methyl protons in TMSP, which interacts with BSA and in alanine (no interaction). The analysis provides accurate estimates of known parameters (*e.g.*correlation time for the complex) and was cross-validated by chemical exchange saturation transfer (CEST)experiments.

This approach was applied to a biological fluid relevant for metabolomics studies: human blood plasma, where we found relaxation dispersion profiles with a variety of different features. The interactions of several metabolites with macromolecules were identified and the size dispersion of macromolecules in human plasma was estimated with the help of Fast Field Cycling (FFC) NMR. High-resolution relaxometry is an efficient tool for the investigation of dynamic interactions in biological fluids and provides valuable information complementary to conventional metabolomics approaches.

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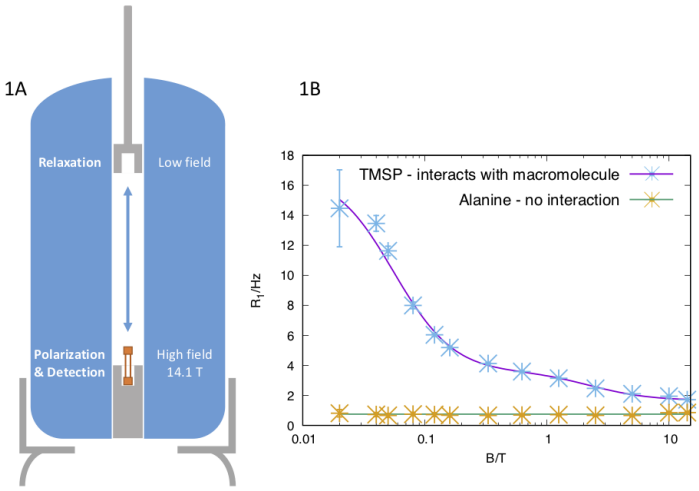

Figure 1A Schematic representation of a high-resolution relaxometry system; 1B Comparison of TSP and alanine relaxation dispersion profiles in our model sample. The TSP profile (light blue point with purple fitting curve) is characteristic of an interaction with a macromolecule, here bovine serum albumin. The flat profile of alanine (yellow points with green fitting curve) demonstrates the absence of interactions.

Liquid-state NMR methods

O50

NOAH: NMR Supersequences for Small Molecule Analysis and Structure Elucidation

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Abstract: The modern approaches to structure characterization of small molecules by NMR spectroscopy largely follow well-established protocols that are reliant on a core set of 2D correlation experiments such as HSQC, HMQC, HMBC, COSY, NOESY, TOCSY, and similar.[1] Having established themselves as the primary techniques, much focus has now turned to developing experimental methods that allow the faster collection of these data sets. Herein, we exploit the concept of tailored polarization storage by recording multiple 2D data sets nested within a single *supersequence* that requires only a single recovery delay, *d1*. In this way the data collection time may be dramatically reduced and sample throughput increased for basic NMR applications, such as structure elucidation and verification in synthetic, medicinal, and natural product chemistry.

The proposed technique is outlined schematically in Figure 1a and combines several previously introduced approaches, as discussed in detail in our recent publication.[2] Specifically tailored pulse sequences have been developed previously to reduce the duration of data acquisition by minimizing the recovery delay between repeated transients, as exemplified in the acceleration by sharing adjacent polarization (ASAP) scheme.[3] We term this concept NOAH (NMR by Ordered Acquisition using ^{1}H-detection). The NOAH experiments offer a more generalized approach to minimizing delays associated with magnetization recovery, enabling many combinations of the routinely employed 2D methods to be merged as supersequences. With the NOAH sequences, we demonstrate the nesting of up to five 2D correlation experiments (NOAH modules) including various combinations of the established methods for small-molecule characterization mentioned earlier. An example of 2D ^{15}N HMQC, ^{13}C HSQC, COSY and NOESY spectra of gramicidin S recorded in a single NOAH-4 experiment are shown in Figure 1b.

We suggest that hundreds of such combinations are possible.[2] While not all of the recently proposed 285 NOAH supersequences are equally efficient and/or practical, the existence of a large number of possible combinations highlights the need for a systematic classification of this technique, and a more complete description of their design and operation will be provided. Finally, the efficiency of the NOAH type supersequences can be further improved by combining these experiments with other fast techniques, such as non-uniform sampling (NUS), Hadamard spectroscopy, projection spectroscopy, use of multiple receivers and similar.

Figure 1. (a) Schematic representation of the NOAH super-sequences, here comprising a NOAH-5 experiment; (b-e) the spectra of gramicidin S recorded in the NOAH-4 experiment combining 2D ^{15}N HMQC (b), ^{13}C HSQC (c), COSY (d) and NOESY (e) sequences.

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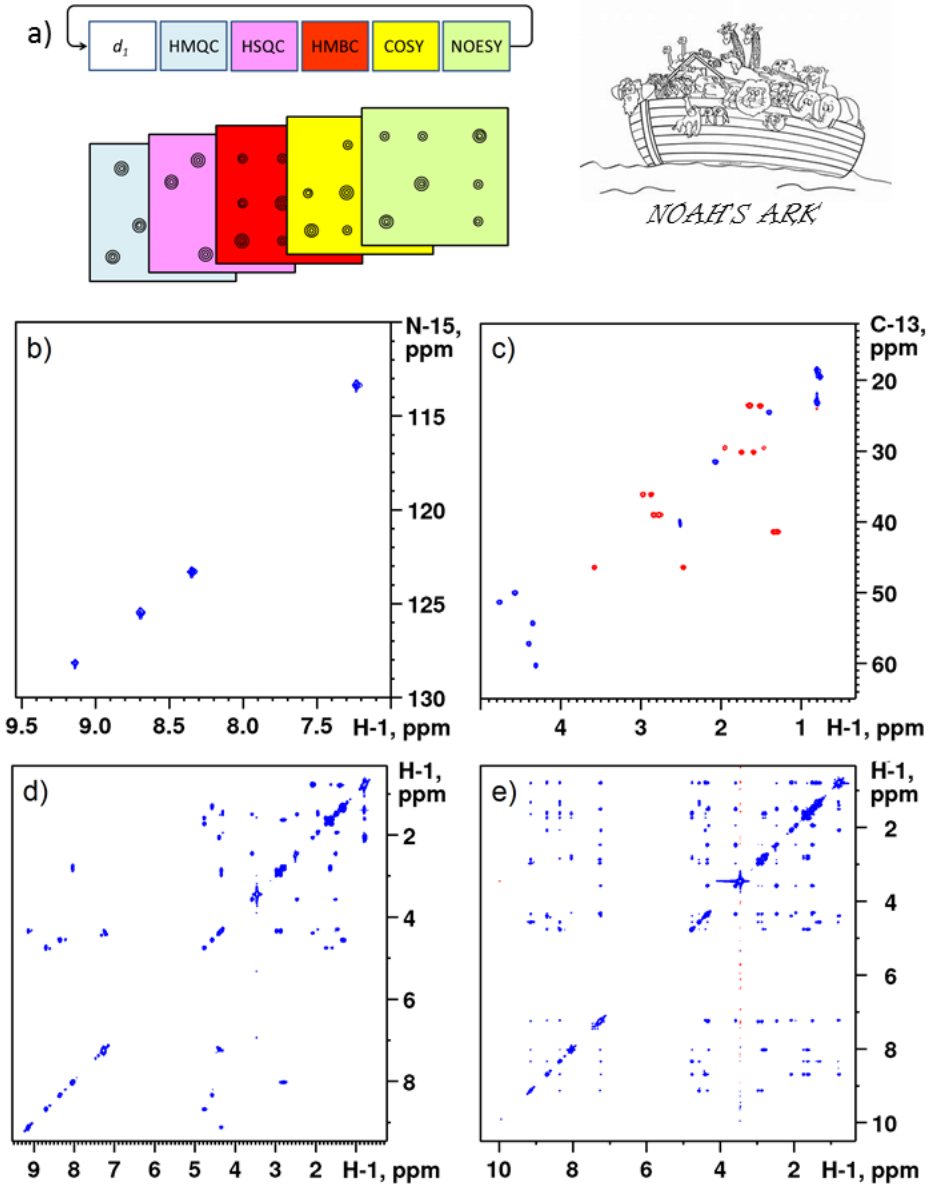

Disclosure of Interest: E. Kupce Conflict with: none, T. Claridge Conflict with: none

Liquid-state NMR methods

O51

Dramatic Decrease in CEST Measurement Times Using Multi-Site Excitation

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Abstract: Chemical exchange saturation transfer (CEST) has recently evolved into a powerful approach for studying sparsely populated, "invisible" protein states in slow exchange with a major, visible conformer. Central to the technique is the use of a weak, highly selective radio-frequency field that is applied at different frequency offsets in successive experiments, "searching" for minor state resonances. The recording of CEST profiles with enough points to ensure coverage of the entire spectrum at sufficient resolution can be time-consuming, especially for applications that require high static magnetic-fields or when small chemical shift differences between exchanging states must be quantified. Here I will show, with applications involving ^{15}N CEST, that the process can be significantly accelerated by using a multi-frequency irradiation scheme, leading in some applications to an order of magnitude savings in measurement time.

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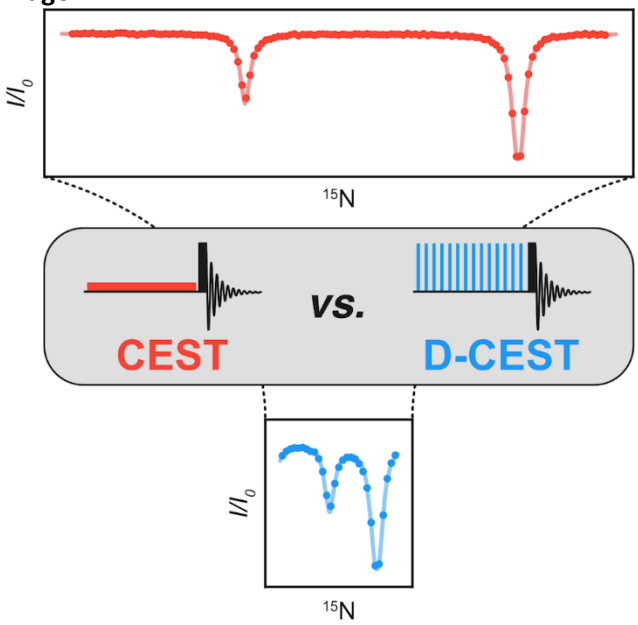

Exotica

O52

From LASER physics to the para-hydrogen pumped RASER

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Abstract: In this lecture, I will bridge the fundamental concepts from the LASER theory described by H. Haken [1] to the recently discovered para hydrogen (p-H_2) fueled RASER [2] (Radio Frequency Amplification by Stimulated Emission of Radiation). The synergetic properties of the LASER are compared with the key features of the p-H_2 pumped RASER. According to theory the equations of motion for the LASER can be derived from the slavery principle, i.e. the slowest order parameter (the light field in the resonator) enslaves the rapidly relaxing atomic degrees of freedom. Likewise, the equations of motion for the p-H_2 pumped RASER result from a set of order parameters. Here the transverse magnetization of the RASER active spin states enslave the electromagnetic modes. The consequences are striking for nuclear magnetic resonance (NMR) spectroscopy, since long lasting multi-mode RASER oscillations allow for unprecedented spectroscopic resolution down to the micro-Hertz regime. Based on the theory for multi-mode RASER operation we analyze the conditions that reveal either highly resolved *J*-coupled NMR spectra, renormalization of the entire NMR spectrum or self-organized mode locking. Certain RASER experiments involving the protons of ^{15}N pyridine or 3-picoline molecules pumped with p-H_2 via SABRE [3] (Signal Amplification By Reversible Exchange) show in the time domain either a complex RASER beat pattern, one single RASER oscillation or giant RASER pulses. The corresponding ^{1}H spectra derived by Fourier transformation consist of highly resolved lines reflecting NMR properties, one ultra-narrow line at the renormalized frequency or equidistant narrow lines (frequency comb), respectively. Numerous applications in the area of material sciences, fundamental physics and medicine involving sensitive magnetic sensors or NMR spectroscopy with ultra-high precision become feasible at low cost.

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Fig. 1: Experimental RASER setup including electromagnet system and pH$_2$ injection system mounted on the external high Q-factor probehead.

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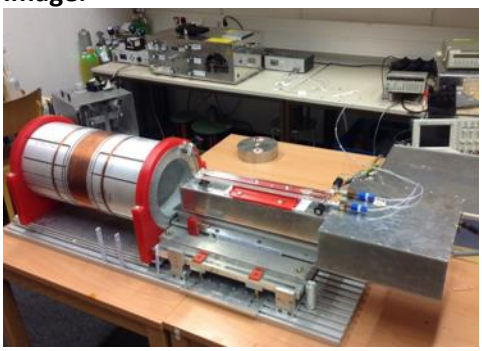

Exotica

O53

HyperStore: Towards storage and remote transport of (hyper)polarised order via supercritical fluids

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Abstract: The introduction and development of various hyperpolarisation techniques has brought important advances in magnetic resonance. In clinical MRI, for example, hyperpolarisation has enabled the *in-vivo* study of metabolic pathways which, in turn, has been used to diagnose and stage diseases or to assess the response to treatments.
Among all hyperpolarisation techniques, dissolution dynamic nuclear polarisation is the most versatile. However, it is costly, bulky and requires highly skilled personnel to be run. End-users such as hospitals and research centers need to have *in situ* access to such hardware as well as to specialized personnel.
In order to be able to separate the point-of-production from the point-of-use of this technology one has to fight against spin relaxation, an incoherent mechanism that destroys the enhancement gained through hyperpolarisation within a few minutes, in the very best cases.
Our aim is to enable the storage and remote transport of hyperpolarised material from the site of production to the site of use. We are trying to do this by combining the use of long-lived spin states, low magnetic fields and supercritical fluids. For all mechanisms but spin-rotation, the lifetime of spin states in liquid state is inversely proportional to the viscosity of the fluid in which molecules are dissolved. Therefore, it is possible to prolong storage time by storing polarisation in a low-viscosity medium. Supercritical fluids such as supercritical-CO_2 (sc-CO_2) have just the perfect specs for such purpose. sc-CO_2 has a viscosity 30 times lower than water; it is a good solvent for polar molecules and can solubilize polar ones with the addition of a co-solvent; it is very easy to dispose of by venting; can be used in conjunction to chromatography for purification purposes (a step required for *in-vivo* applications and to prolong lifetimes). A factor of 10-20 in storage time is typically gained with the use of singlet order and a further factor of 8-10 can be gained with the use of sc-CO_2 therefore storage times of 2-12h are theoretically achievable. The combination of these techniques is however complicated by the pressure/temperature requirements of the supercritical state.
In this contribution I will discuss our efforts in this direction.

Exotica

O54

3D Spin Noise Imaging with Enhanced Sensitivity

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Abstract: The work presented here demonstrates the extension of nuclear spin-noise-detected imaging [1] to 3D imaging. Spin noise detection provides a high potential for application at nano-sized samples, is implicitly free of rf-related artifacts and allows for fast recycling times owed to the independence from longitudinal magnetization recovery. Also no rf-related power restrictions need to be considered. Applying the enhancements and insights of over 10 years of spin noise research [2] the realization of this intrinsically insensitive experiment has become possible. The advancements include sliding window processing [3] of the continuously recorded time-domain data, avoiding T/R switching related "transient effects" [4], and optimal tuning of the probe tuning for spin noise detection [5]. With these improvements in the experimental setup and advanced data processing, it is now possible to record 3D nuclear spin-noise-detected images in a reasonable time frame using a state-of-the-art high resolution spectrometer. When recording conventional (rf-pulse excitation based) NMR experiments there is a trade-off between the resolution and the S/N ratio (which is determined mostly by the number of scans), which needs to be decided before starting the experiment. For spin-noise-detected experiments this trade-off can be adjusted as part of the data processing after the recording is complete. This involves novel iterative image reconstruction techniques based on the algebraic reconstruction algorithm [6]. Even molecular imaging (spatially resolved spectroscopy) becomes feasible that way.

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Materials

O55

Transition Metal Doping and Phase Segregation in Mn2+- and Co2+-Doped Lead Halide Perovskites from 133Cs and 1H NMR Relaxation Enhancement

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Abstract: Lead halide perovskites attract growing interest owing to their highly tunable optoelectronic properties. We have recently used multi-nuclear (^{1}H, ^{2}H, ^{13}C, ^{14}N, ^{133}Cs, ^{87}Rb, ^{39}K, ^{207}Pb) solid-state MAS NMR to elucidate cation reorientation dynamics, microscopic phase composition and phase segregation in various multi-cation and multi-anion lead halide perovskites, both in bulk and in thin films.[1-3] Mn^{2+} doping is a firmly established way of enhancing luminescence yield in all-inorganic $CsPbX_3$ (X=Cl, Br, I) perovskites for high performance light-emitting diodes.[4-6] Co^{2+} doping has recently been reported as a viable way of tailoring the photovoltaic properties of methylammonium lead iodide ($MAPbI_3$).[7] However, until now the atomic-level picture of lead replacement in these materials has been missing. Since incorporation of transition metals into a diamagnetic perovskite lattice should lead to paramagnetic relaxation enhancements (PREs), nuclear relaxation is expected to be a sensitive probe of transition metal substitution.[8] Here, using ^{1}H and ^{133}Cs solid-state MAS NMR relaxation measurements at 21.1 and 9.4 T we show that, contrary to current conviction, Co^{2+} does not replace Pb^{2+} in $MAPbI_3$. Similarly, we provide the first atomic-level evidence that Mn^{2+} forms $CsPb_{1-x}Mn_xX_3$ alloys and model the Mn^{2+} ion distribution inside the perovskite lattice.

Figure 1. Schematic representation of $CsPbX_3$ doped with Mn^{2+}. Each edge of an octahedron contains an X^- anion.

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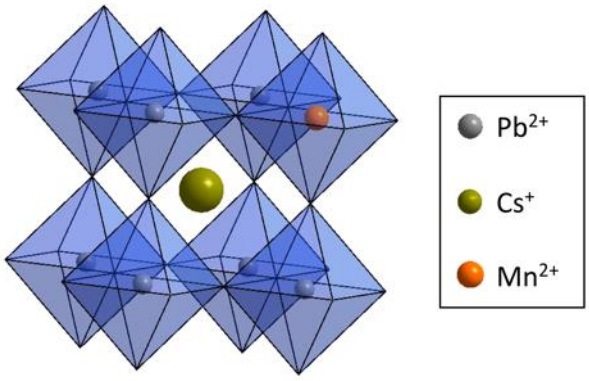

Materials

O56

Extracting the Reaction and Aging Mechanism of Lithium Ion Batteries in Real Time

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Abstract: Lithium ion batteries (LIBs) play more and more important roles in energy storage systems for a variety of applications such as mobile devices, portable tools, electric vehicles and large-scale electric power storage. Elucidating the mechanisms and kinetics of electrochemical reactions during cycling and understanding the degradation mechanisms of LIBs is crucial for a systematic improvement of performance, lifetime and safety of LIBs.

Here, we combine in-operando solid state nuclear magnetic resonance (ssNMR) with diffusion NMR to characterize the reaction and degradation mechanisms of LIBs. First, an in-operando NMR probehead and a cylindrical battery, which are suitable to for long term battery operation over several hundred cycles, were designed and prepared. Secondly, different factors, such as temperature, current rate, lithium dendrite growth, electrolyte decomposition and solid-electrolyte-interphase (SEI) layer formation, that influence the electrochemical performance of LIBs were investigated. The experimental results showed that: (i) a concentration gradient of lithium ions in the electrolyte forms when the battery voltage is above 3.5 V; (ii) at low temperatures, for example at 2° C, the capacity retention decays very fast during cycling and lithium dendrite formation occurs within 100 cycles; (iii) fast charging causes capacity loss and accelerates the process of lithium dendrite growth; (iv) lithium dendrite formation, rather than electrolyte decomposition or SEI layer effects, is the main reason for the capacity degradation of a $LiCoO_2$/graphite battery. In summary, in-operando ssNMR is demonstrated to reveal the electrochemical reaction and degradation mechanism of LIBs during long time cycling.

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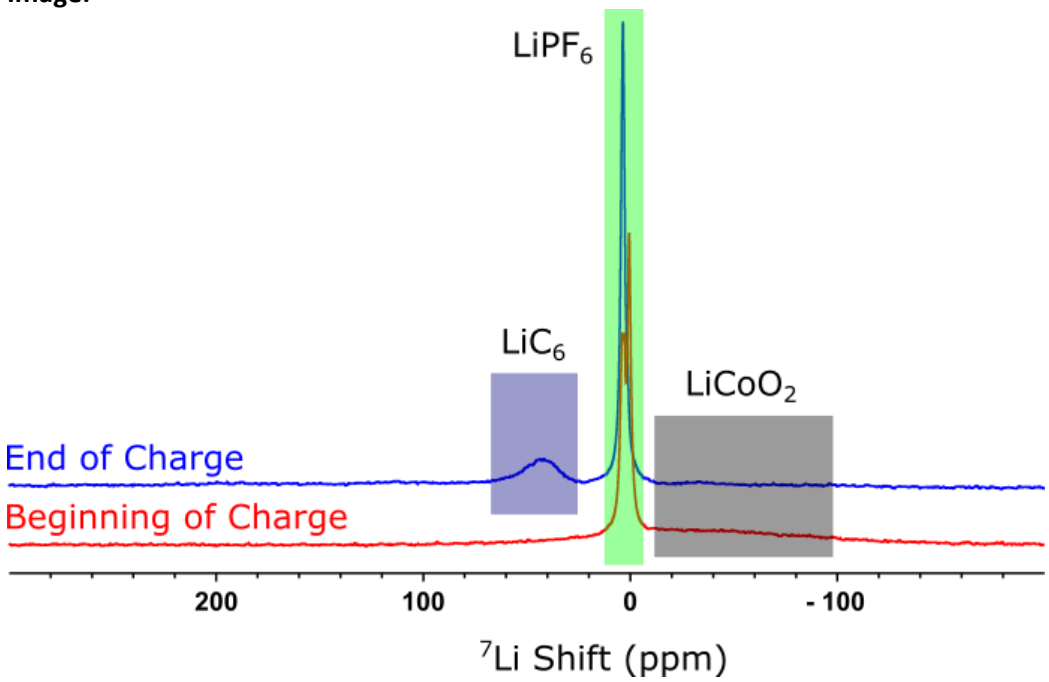

Materials

O57

Combined Solid-State NMR and Molecular Dynamics Study of the Structure of Strontium-Aluminosilicate glasses

Thibault Charpentier[*1], Kirill Okhotnikov[1], Pierre Florian[2], Frank Fayon[2], Alexey Novikov[2], Louis Hennet[2], Daniel R. Neuville[3]
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Abstract: Solid-state NMR has firmly established itself as a method of choice for providing key information for the elucidation of glass atomic-scale structure. Recently, a methodology based on the combination of DFT-NMR calculations with molecular dynamics simulations has emerged as a significant step for the improvement of the detailed interpretation of experimental NMR spectra.

Using this approach, we have investigated the structure of aluminosilicate SiO_2-Al_2O_3-SrO based glass compositions which are largely unexplored systems. Glasses on the compensation line Al_2O_3 = SrO, were studied with ^{17}O, ^{29}Si and ^{27}Al solid state NMR at high (11.7 T) and very-high (20.0 T) magnetic fields, together with neutron diffraction spectroscopy. Classical and ab-initio molecular dynamics (MD) simulations were performed and combined with calculationsof NMR parameters with the DFT-GIPAW method. Computed NMR parameters were linked to local structural features to establish relationships between experimental NMR spectra and the underlying topological disorder (in terms of chemical and geometrical disorder) . NMR fingerprints of debated units such as tricoordinated oxygen atoms could be predicted with the aims to assess their existence from experimental data.

In agreement with experimental NMR data, MD simulations predict that aluminium is predominantly tetrahedrally coordinated for all the studied compositions with a small fraction of AlO_5 units ranging from 2-5%. Variations of the ^{29}Si NMR spectra, and to a less extent of ^{27}Al spectra, could be quantitatively correlated to the Al/Si mixing. In parallel, the Al/Si connectivities were investigated using advanced NMR techniques enabling the resolution of the ^{29}Si NMR spectrum in terms of Q^n(mAl) units (i.e., Q^n connected to m Al units). Simulations of ^{17}O NMR experiments from our first-principles methodology combined to ^{17}O-^{27}Al correlation experiments allowed extractions of Al-O-Si, Al-O-Al and Si-O-Si peaks which were found to be strongly overlapping in experimental 1D and 2D ^{17}O MAS NMR spectra.

Metabolomics and other "omics"
O58
Improved NMR methods for 13C NMR based fluxomics
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Abstract: Measuring the carbon isotopic content of different metabolites is a powerful tool to quantify fluxes in living systems. Spanning the different forms of life, the approach has found applications in the study of bacteria, yeast, plants, or humans. In the latter case, the altered metabolism of cancer cells has attracted significant interest. Two main techniques used for such analysis are mass spectroscopy and NMR, and recent trends integrate both methods. Whereas the former has the main advantage of tremendous sensitivity, allowing the determination of isotope distribution of ever smaller samples, determining the exact position of the ^{13}C nucleus in a given metabolite remains challenging. NMR spectroscopy on the other hand suffers from low sensitivity, but has – at least in principle – the possibility to determine the ^{13}C content at the individual positions of a metabolite.
We introduce the pure shift method in the heteronuclear 2D J-resolved (2DJ) pulse sequence that was previously proposed for ^{13}C fluxomics. The measurement of ^{13}C incorporation in the aliphatic side chains of the branched chain amino acids (BCAA) Ile, Leu and Val, residues obtained from a bacterial culture grown on U-^{13}C labeled glucose with unlabeled Thr underscores the superior resolution of the pure shift 2DJ experiment, and allows quantitative elucidation of the biosynthetic pathway of the BCAA in the absence of protein over-expression, thereby providing novel biochemical insight.
We further explore how ^{15}N-based NMR experiments can be used in the field of fluxomics when rapid quantification is of prime importance. Borrowing from protein NMR, these novel experiments can quantify the control exerted by the first enzyme of the pentose phosphate pathway (G6PDH) on the glycolytic and pentose phosphate flux in *Escherichia coli*. Estimation of these fluxes is based on the absolute quantification of the four isotopic species for the (Cα, CO) two-carbon block of leucine. We demonstrate both a significant gain in completeness of information and of time.

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Metabolomics and other "omics"

O59

Optimisation of Diffusion-Ordered NMR Spectroscopy Methods for Use in Metabolic Profiling of Human Biofluids

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Abstract: Diffusion-Ordered Spectroscopy (DOSY) experiments have long been used in the analysis of simple mixtures and in product control (e.g. of paints and fragrances), but have yet to be optimised for high-throughput metabolic profiling in phenome centres. The analysis of human biofluid samples by NMR for the purposes of metabolic profiling is now well established [1]. The methodology so far primarily utilises 1D-^{1}H, 1D CPMG and *J*-resolved experiments to analyse changes in metabolite concentrations between clinical, epidemiological and population groups. We feel that an extra dimension may be added to this approach by the inclusion of metabolite mobility data obtained from DOSY experiments, allowing for the discovery of novel biomarkers.

Thus far, two DOSY experiments have been optimised using a variety of pooled samples and model solutions – the first, a typical DOSY pulse sequence (RD-90°-δ/2-τ-180°-δ/2-τ-90°-Δ-90°-δ/2-τ-180°-δ/2-τ-90°-ED-90°-ACQ where RD=relaxation delay, ED=eddy current delay, τ=short delay, 90°/180°= 90° or 180° pulse, δ=gradient pulse, Δ=diffusion delay, ACQ=acquisition period) suitable for analysing simple mixtures of metabolites, and the second, a relaxation-edited DOSY pulse sequence (RD-90°-τ-180°-τ-90°-δ-τ-180°-τ-δ[δ-τ-180°-τ-δ-90°-δ-τ-180°-τ-δ]-δ-τ-180°-τ-δ-90°-τ-180°-τ-45°-ACQ where [/] indicates the loop) [2] for analysing metabolite mixtures containing macromolecules such as blood plasma and serum, as shown in Figure 1. A presaturation pulse was added to the relaxation delay in order to suppress the solvent signal from the large water content inherent to biofluids. Experimental parameters (in particular, the diffusion delay and gradient pulse length) were optimised according to the sample matrix to be analysed i.e. human urine, plasma or serum samples, and the experiment length adjusted for high-throughput metabolic profiling. The effects of temperature, field strength and convection were explored to determine the optimal experimental conditions. These new DOSY parameter sets will be applied to future metabolic phenotyping projects to investigate changes in metabolic mobility associated with disorders and diseases such as liver and kidney disease, which affect the macromolecular content and viscosity of the biofluids in question.

Figure 1: PROF_PLASMA_DOSY acquired on a pooled human blood plasma sample at 310 K on a Bruker 600 MHz AVANCE III HD spectrometer. Pulse programme developed using the PROJECTED pulse sequence [2]

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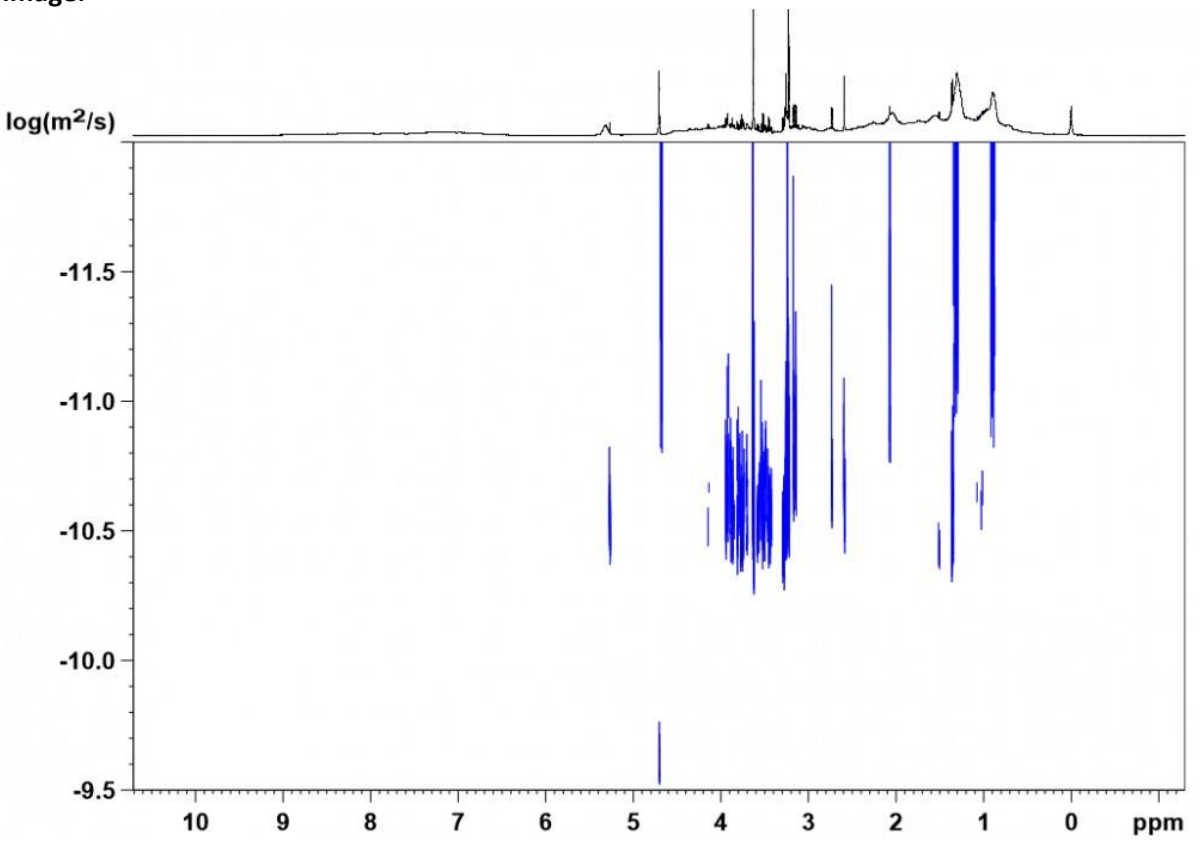

Metabolomics and other "omics"

O60

NMR-based Metabolomics for New Target Discovery and Personalized Medicine: Application to Age-Related Macular Degeneration (AMD).

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Abstract: Nuclear Magnetic Resonance (NMR) is an indispensable analytical tool for research in the biomedical and pharmaceutical fields. More recently, NMR appeared as one of the major and more powerful technological platform for metabolomics approach. Metabolomics is a growing area of the "omics" sciences and is defined as the comprehensive identification and quantification of low-molecular weight metabolites in biological samples. It provides a unique and direct vision of the functional outcome of organism's activities that could be correlated to pathologies and/or treatment administration. The links between metabolic changes, patient phenotype, physiological and/or pathological status and treatment are now well established and have opened a new area for the application of metabolomics in new target identification and in personalized medicine.

Age-related Macular Degeneration (AMD) is the leading causes of blindness among the elderly population in developed countries. 90% of all vision loss due to AMD result from the exudative form, which is characterized by choroidal neovascularization (CNV). Treatment is mainly based on regular intravitreal injection of anti-VEGF to stabilize CNV. Nevertheless, the comprehensive understanding of the pathogenesis and the evolution of this complex multi-factorial disease remain incomplete. Moreover, due to the long-term disease chronicity and to some resistance to treatment, a continuous follow-up of patients, a personalization of treatment and the discovery of new therapeutic approaches are mandatory. In order to study CNV occurrence and evolution and to get new and innovative insights into this pathology, we decided to apply a NMR-based metabolomics approach on both clinical and pre-clinical models (a murine laser-induced CNV model and patient's cohorts). This approach led us to identify some metabolites linked to CNV developments in both human and murine samples. These molecules could be considered not only as markers of the pathology but also as putative target for a new treatment of AMD. Among those, lactate emerges as a key metabolite in both settings. Mechanistically, we demonstrated that lactate, initially produced in the eyes increases at the systemic level and play a critical role in the onset of the inflammatory and angiogenic phases. For this purpose, we use a combination of NMR measurements (both 1D and 2D) and molecular biology approaches. The control of the systemic concentration of lactate by PDHK inhibition or by LDH modulation decreases significantly CNV development.

Based on a metabolomics approach, our data support the innovative concept of lactate as a putative target for a new therapeutic approach of AMD as well as a useful marker for patient's follow-up during treatment. We highlight also the utility and the efficacy of NMR for metabolomics and metabolites measurement in complex biological samples.

EPR/ESR

O61

Nanoscale zero-field electron spin resonance spectroscopy

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Abstract: Electron spin resonance (ESR) spectroscopy has broad applications in physics, chemistry and biology. Combining with site-directed spin-labeling of radicals, structural or dynamic information derived from the electron fine and hyperfine interaction can be obtained from the ESR spectra with accuracy limited by the inhomogeneous line broadening. The line broadening of powder spectra, induced by the magnetic inequivalence of otherwise identical spins, can be partly removed in high-field ESR (HF-ESR) experiment. While a complete removal can be achieved by zero-field ESR (ZF-ESR) spectroscopy, because the energy levels of a spin system are no longer orientation dependent in the absence of Zeeman term. ZF-ESR spectroscopy has been proposed for decades and shown its own benefits for investigating the electron fine and hyperfine interaction. However, the ZF-ESR method has been rarely used due to the low sensitivity and the requirement of much larger samples than conventional ESR. In this work, we present a method for deploying ZF-ESR spectroscopy at the nanoscale by using a highly sensitive quantum sensor, the nitrogen-vacancy (NV) center in diamond. By precisely tuning the energy levels of NV centers in dressed states to be resonant with target spins, we successfully measure the nanoscale ZF-ESR spectrum of a few P1 centers residing at a distance of less than 15 nanometers from the NV center, and show that the hyperfine coupling constant can be directly extracted from the spectrum. Moreover, we also show that the nanoscale ZF-ESR can perform significantly better than nanoscale ESR without loss of sensitivity. This method opens the door to practical applications of ZF-ESR spectroscopy, such as investigation of the structure and polarity information in spin-modified organic and biological systems.

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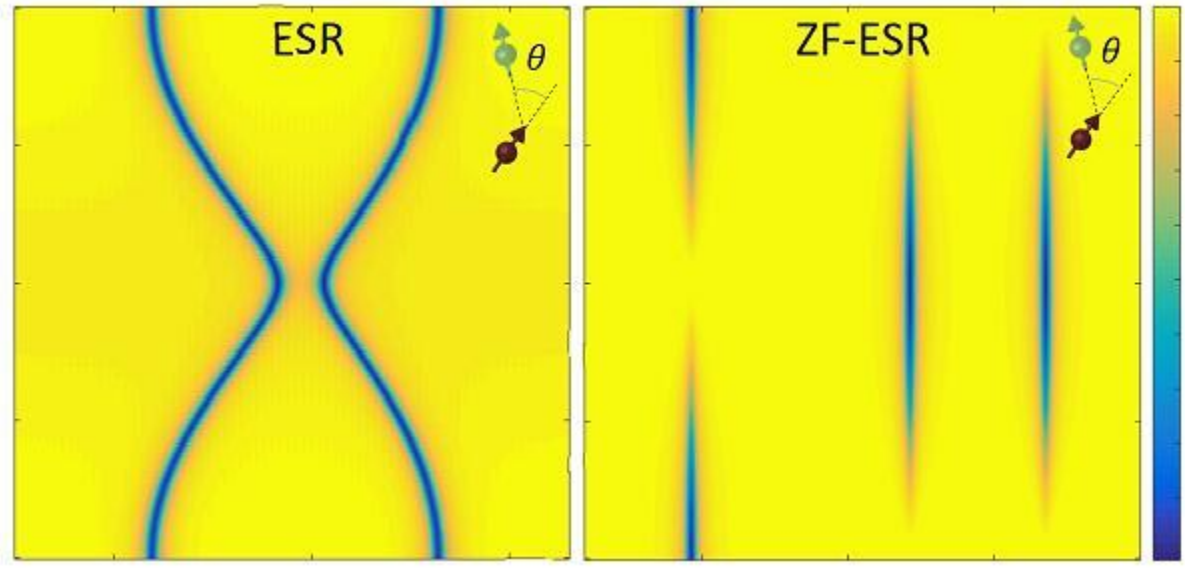

EPR/ESR

O62

Lanthanide Binding Tags as Self-Assembling Gd(III) Spin-Labels for In Vivo and In vitro Nanometric Distance Determination in Proteins

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Abstract: Nanometric distances determination by Pulse Electron Double Resonance (PELDOR or DEER) has become an important tool in structural biology. PELDOR measures the distance between two paramagnetic centers by determining the strength of their magnetic interaction. These paramagnetic centers are in general introduced *in vitro* by chemical reaction of cysteines at the protein surface with sulfur-reactive spin-labels such derivatives of nitroxide radicals or DOTA chelator. Such a labeling approach requires unique cysteines and cannot be accomplished inside cells. A different approach is to genetically encode a peptide sequence capable of coordinating a paramagnetic metal ion that can subsequently form self-assembled metal spin-labels *in vivo*. Mn(II) bound to His-Tags and Gd(III) bound to Lanthanide Binding Tags (LBT) are two examples of such self-assembled metal spin-labels (SAMSL) that have been used for *in vitro* PELDOR measurement. We have studied whether these SAMSL can be biosynthetically produced inside the cell and directly used for in-cell distance measurements. To this end, two LBTs were genetically fused to the ends of a small 3-helix-bundle (3Hx) protein and the constructs expressed in *E. coli*. A clear PELDOR response arising from Gd(III):LBT-3Hx-LBT:Gd(III) synthesized by the cells was detected on the purified protein. More importantly, the same signal was detected directly from the cells overexpressing the protein grown on media supplemented with Gd(III).

In addition to the *in vivo* studies, LBTs are also helpful for studying cysteines-rich proteins. HYL1 is a protein involved in the miRNA processing on *A. thaliana*. This protein has two independent domains connected by a flexible linker. The independent structure of each domains is known; however, structural information of the HYL1/RNA complex is yet unknown. HYL1 contains 5 cysteines and it has proven difficult to express HYL1 mutants carrying only two exposed cysteines residues due to poor protein stability. Instead, we have decided to use Gd(III):LBT SAMLS-tags. Three constructs were prepared, each of them carry two LBTs: N-Ter/C-Ter (HYL1-L12L), N-Ter/central loop (HYL1-L1L2) and central-loop/C-Term (HYL1-1L2L). PELDOR measurements were carried out on these constructs and their complexes with RNA.

The results on these systems as well as the advantages/disadvantages and possible improvements for the use of LBT-SAMLS in *in vivo* and *in vitro* nanometric distance determination will be discussed.

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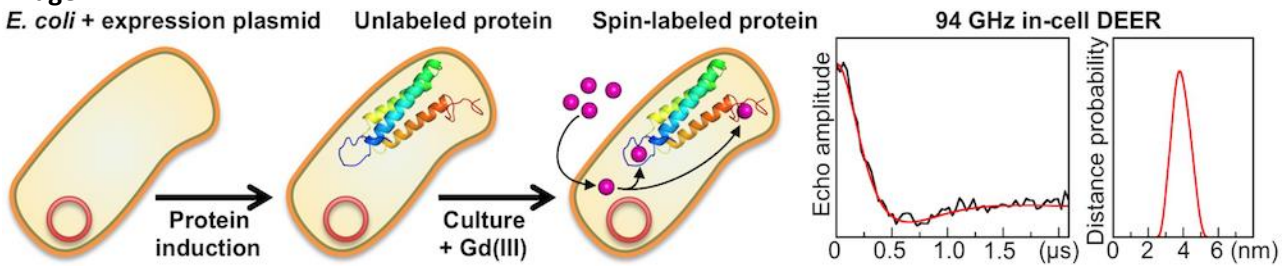

EPR/ESR

O63

Gd(III)-Gd(III) DEER measurements on proteins at W-band with an arbitrary waveform generator – advantages and pitfalls

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Abstract: The introduction of shaped pulses to pulse-EPR using an arbitrary waveform generator (AWG)[1,2] has been shown in the past years to be advantageous for numerous experiments. Recently we have demonstrated an enhancement of sensitivity using chirp pulses to obtain broadband pump pulses for DEER measurements at W-band[3]. This methodology has since been further optimized for Gd(III)-Gd(III) DEER at W-band. We found that the best results are achieved when detecting on the peak of the Gd(III) spectrum and placing 300MHz broad chirp pulses on both sides. Under these conditions the detected echo is of the largest possible intensity while due to the chirp pulses we obtain modulation depths which are close to what was obtained previously when pumping on the spectrum peak. This was applied by us to different proteins using different types of Gd(III) tags. We will show here different examples of DEER with AWG on proteins with varying gains. We will also show that the application of the broadband pulses can result in significant artefacts due to echoes from coherent pathways that do not appear when using rectangular pulses. These artefacts appear at the tail of the DEER trace and result in loss of more than 1 us of data. As a consequence, we use an alternative 4-pulse DEER sequence with different timings (see Figure) for which this artefact does not appear.

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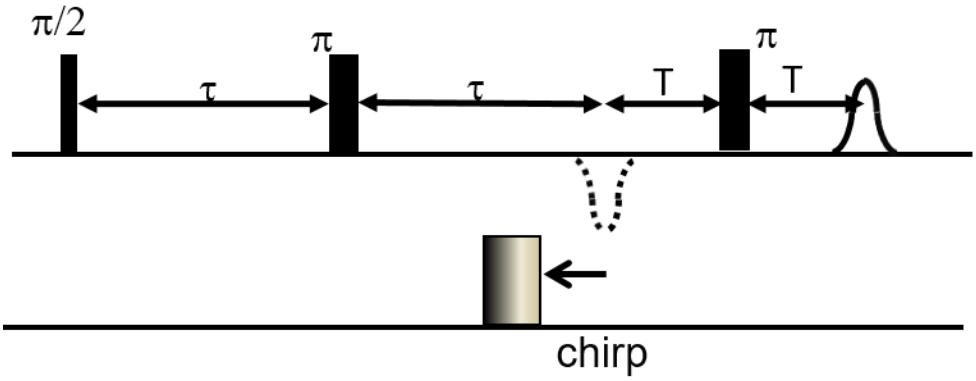

POSTER COMMUNICATIONS

Benchtop and lowfield

P001

Applications of Compact NMR to Nondestructive Testing and Reaction Monitoring

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Abstract: Compact NMR instruments have evolved from mobile stray-field sensors to benchtop spectrometers suitable for nondestructive material testing and small-molecule research, respectively [1-3]. Stray-field relaxometers detect the dynamics of small molecules in materials, for example, water in building materials like mortar [4] and wood or solvent molecules in paint layers. Tabletop spectrometers are pedominantly employed for the analysis of molecular structures and transformations of small molecules in solution. Yet also solutions of macromolecules can be analyzed and provide pertinent information for chemical analysis, quality control, authentication, reaction monitoring and process.
Recent studies with the NMR-MOUSE and the Spinsolve tabletop spectromter by the Aachen group concern the automated comparison of depth profiles through ancient frescoes in search for similarities [4] and of a hidden Giotto fresco. Furthermore, a study of the reaction kinetics for the hydrolysis of acetic anhydride at different water deuteration will be reported that reveals details of the transition state in the chemical transformation [5].

Figure 1. Applications of compact NMR. a) Nondestructive analysis of a painted wall in search for a hidden, underlying fresco in the Cathedral of St. Anthony in Padua. b) Transition-state model for the hydrolysis of acetic anhydride, which is supported by our reaction monitoring study in the fume hood at 1 T.

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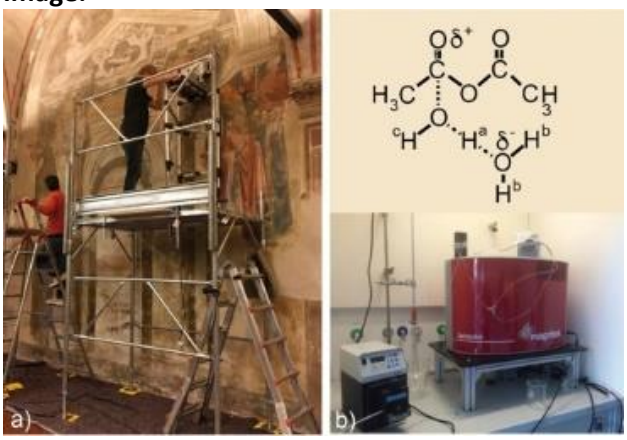

Benchtop and lowfield

P002

Diffusion-ordered spectroscopy on a benchtop spectrometer for drug analysis

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Abstract: Although high-field (HF) NMR is not considered as a routine tool for quality control due to its high purchase and maintenance costs, the ability of this technique to provide a large amount of information could make it an essential tool in pharmaceutical industries and quality control laboratories. As an alternative to this bulky and sophisticated instrumentation, a new generation of compact and cryogen-free low-field (LF) spectrometers has emerged.[1] These permanent magnets have nowadays reached a sufficient level of maturity to afford a stable and homogeneous enough static magnetic field to resolve the chemical shift information, and thus to be employed for numerous applications.

However, the low operating frequencies of such instruments lead to a drastic loss of their analytical performance, in particular due to the strong peak overlaps arising from the reduced chemical shift range. As a result, the spectroscopic dimension is not always sufficient to enable a complete characterization of complex mixtures. Introducing a diffusion dimension based on pulsed field gradient (PFG) experiments is hence an appealing solution for the analysis of drug formulations. We will show the first implementation of diffusion-ordered spectroscopy (DOSY) experiments on a benchtop spectrometer equipped with a gradient coil. Several pulse sequences were evaluated and the associated parameters were carefully optimized in the context of LF NMR. While convection-compensated pulse sequences were helpless since B_0 is horizontal on the LF spectrometer used, other optimizations of diffusion pulse sequences commonly applied at HF were found useful to enhance the quality of LF DOSY spectra. Among them, the bipolar pulse pair-stimulated echo sequence with a longitudinal eddy current delay prior to detection provided significant advantages against the effect of zero quantum coherences, which is even more detrimental at LF, as well as against the unwanted PFG effects such as eddy currents, inducing respectively phase anomalies and baseline discrepancies.

Furthermore, in complex mixtures such drugs, sampling the diffusion dimension through a linear gradient ramp could not provide reliable information on both small and large molecules[2]. Shaped gradient sampling schemes, e.g. exponential and semi-gaussian, giving a significant number of points for both fast and slow diffusing molecules were thus implemented and compared.

The advantages and limitations of the method will be discussed on a model formulation of paracetamol and a real medicine containing esomeprazole. The developments detailed here for DOSY experiments operated at 43 MHz offer an efficient tool for the initial deformulation of drug samples on a routine basis, enabling for instance the detection of counterfeit medicines.

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Benchtop and lowfield

P003

Online monitoring of the kinetic isotope effect in chemical reactions with 1H and 19F low-field NMR spectroscopy

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Abstract: The kinetic isotope effect (KIE) describes the change in the rate of a chemical reaction by substituting one of the atoms in the reactants with one of its isotopes. Investigating the KIE and its temperature dependency in reactions renders information for reconstructing chemical processes and confirming the rate-determining step [1]. However, conventional methods to study the KIE, e.g. by calorimetry, conductivity, titration, Raman spectroscopy etc., require calibration and sophisticated handling of the reaction calorimeter, and the data are obtained at irregular and sparse intervals [2]. This current study employs a compact NMR system [3] as an alternative means to determine the temperature dependency of the reaction rate and, thus, the KIE, as well as the activation energy, enthalpy, and entropy of each reaction. Here the neutral hydrolysis of acetic anhydride and ethyl trifluoroacetate was studied in H_2O, D_2O and H_2O-D_2O mixtures with ^1H and ^1H-^{19}F NMR spectroscopy. The activation energies for the hydrolysis of acetic anhydride with D_2O and H_2O were found to be 45 ± 2 kJ/mol and 40 ± 2 kJ/mol, respectively. The activation energies of ethyl trifluoroacetate hydrolysis via ^{19}F NMR spectroscopy were determined to 46.7 ± 1 kJ/mol and 54.9 ± 1 kJ/mol for the reaction with H_2O and D_2O, respectively, and via ^1H NMR spectroscopy to 48 ± 3 kJ/mol and 55.8 ± 1 kJ/mol. The differences in rate constants and activation energies for both reactions in H_2O and D_2O are due to the kinetic isotope effect, involving the breakage and formation of O-H and O-D bonds during the rate-determining step [4]. The proton inventory was studied to determine the number of protons transferred during the rate-determining step of both reactions by fitting the Gross-Butler equation to different possible transition-states of reactions for accurate determination of isotopic fractionation factors. The compact NMR system is a relevant and practical tool to unmask precise reaction pathways, by tracing the KIE in real time with densely sampled data [5], which are essential for obtaining accurate rate constants.

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Benchtop and lowfield

P004

The possibilities of benchtop (60 MHz) 1H NMR spectrometers for semiempirical determination of aromatic carbon atoms (Car) content in petroleum and its products.

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Abstract: The ecological regulation REACH (Registration, Evaluation and Authorisation of CHemicals) [1] is used more than 10 years in the EU for Car content control in petroleum products causes their increased toxicity. ^{13}C qNMR is the only direct method for measuring the relative content of C_{ar} and is implemented in method ASTM D 5292-99 for some groups of oil products that do not contain olefinic fragments. The most important disadvantage of this method is its labor intensity, requiring a multi-hour acquisition of each spectrum. We propose the modification of this method which slightly reduce the experiment, but this not solve the problem of laboriousness.

Earlier, we proposed a method [2] for semiempirical Car determination from ^{1}H qNMR spectra fragments Har, Hα,Hβ ,H γ signals integral intensities:

$C_{ar} = 7.0(\pm0.9) + 3.06(\pm0.17)H_{ar} + 0.42(\pm0.09)H_{\alpha} - 0.17(\pm0.02)H_{\gamma}$, $R^2 = 0.974$, S = 0.8, n = 102 (1)

Where α, β , γ - position of fragments of $C_{alk}H$ relative to aromatic rings.

This equation was obtained by studying of the ^{1}H qNMR spectra at frequency 600 MHz and ^{13}C at 150 MHz.

The developed Car calculation method is adapted for NMReady 60 MHz ^{1}H NMR spectrometers which sensitivity is sufficient for obtaining a quantitative spectrum of analyte in $CDCl_3$ (50%) solutions in 10-15 minutes. For 38 samples of oils, gas condensates, gasoline and diesel fuels, fuel oil were measured [3] values of the signals integral intensities (A) at frequencies of 60 and 600 MHz.

The coupling equation has the form:

$A(600) = 0.003(\pm0.005) + 1.000(\pm0.016)A(60)$ $R^2 = 0.962$ S = 4.0 n = 152 (2)

The low quality of the correlation is due to the fact that the signals for Hβ and Hγ significantly overlap in 60 MHz spectra. Using of ΣH_{β} and H_{γ} significantly improves its quality:

$A(600) = 0.0007(\pm0.0028) + 0.998(\pm0.016)A(60)$ $R^2 = 0.999$ S = 1.5 n = 114 (3)

Various forms of Cap = f (H) relations examination made it possible to make a choice of the following equation for 60 MHz ^{1}H spectra:

$C_{ap} = -0.47(\pm0.064) + 1.159(\pm0.460)H_{ap} + 1.551(\pm0.209)H_{\alpha}$ $R^2 = 0.942$, S = 1.02, n = 38 (4)

This ratio allowed for 84% of the studied samples to predict Car values with an error of less than 1%. Taking into account the labor-saving gain (several minutes instead of hours in the case of ^{13}C NMR and equipment availability), development and standardization of such approach seems appropriate, as well as wider application of low-field ^{1}H NMR spectrometers in petrochemistry.

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Benchtop and lowfield

P005

Analysis of poor-quality medicines using Low-Field Benchtop NMR Spectroscopy

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Abstract: The proliferation of counterfeit and poor-quality medicines is a major public health problem, especially in developing countries lacking adequate resources to effectively monitor their prevalence. In addition to the serious health risk they pose to populations, fake medicines constitute a major challenge for analytical laboratories to detect and characterize them.

The aim of this study was to evaluate the ability of low-field (LF) benchtop NMR as a screening method for detecting fake drugs. We focused our analyses on two classes of medicines known to be largely counterfeit, i.e. antimalarial and erectile drugs. For each class of medicines, both genuine and fake drugs were analyzed.

The first part of the study focused on the analysis of erectile drugs. Among the sixteen fake formulations studied, ten contained active pharmaceutical ingredients which did not correspond to those announced. The main actives detected were sildenafil and tadalafil. The second part of the study was dedicated to the analysis of ten fake antimalarial formulations, seven claimed to contain artesunate while the three others were presented as an association of sulphamethopyrazine and pyrimethamine. Among the seven formulations for which artesunate was the claimed API, one contained the correct API, two had no API and four a wrong API (paracetamol, dipyrone, diphenhydramine or artemisinin). Quantitative NMR experiments were also performed on some genuine and fake formulations.

This study demonstrated that the recording of a 60 MHz NMR spectrum with a benchtop spectrometer allows unveiling fake drug formulations. Indeed, the method brings enough structural clues for a rapid and conclusive screening of low-quality medicines.

Benchtop and lowfield

P006

Low field NMR relaxation studies of cement materials containing silica fume

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Abstract: Low field nuclear magnetic resonance (NMR) relaxometry techniques are powerful instruments for probing molecular dynamics inside the pores of cement-based materials [1-3]. Their advantage, as compared with the high field NMR experiments, is the reduced influence of the internal gradients on experimental data [4], which is an important issue in the case of samples containing magnetic impurities. Of course, there is the disadvantage of lower NMR signal and the lack of spectral resolution. Silica fume is introduced in cement-based materials due to its pozzolanic reactivity and was successfully used for increasing the compressive and flexural strengths of concrete materials [5]. Beside its demonstrated pozzolanic activity, it is considered that the presence of silica fume in a cement mix also reduces the size of capillary pores, consequently increases the strength of the hardened materials on this basis. In our investigations it was demonstrated that no significant change in the size of capillary pores is introduced by the presence of a moderate amount of silica fume in a cement mix. Moreover, the presence of silica fume does not accelerate the hydration dynamics. Both longitudinal and transverse relaxation measurements were used to extract these conclusions. The transverse relaxation measurements allowed us to monitor and compare the pore size evolution during the hydration process, for 28 days. The longitudinal relaxation measurements, performed at different frequencies using the Fast Field Cycling (FFC) technique [1], allowed us the determination of the transverse diffusional correlation time at the surface of cement grains at early hydration times. The results showed no influence on the transverse diffusional correlation time introduced by the addition of silica fume. It was however detected a continuous increase in time, and with the amount of silica fume, in the surface-to-volume ratio of capillary pores [3]. This continuous increase of the surface-to-volume ratio takes place even during the dormancy stage [2] and should not be mandatory associated with the increase in the pore size, it can be also due to surface roughness.

ACKNOWLEDGMENTS

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Benchtop and lowfield

P007

A new approach to interpret non-negative least squares (NNLS) relaxation results

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Abstract: The fitting of an NMR signal decay in a weighted sum of exponentials is an ill-posed problem, i.e. different sets of relaxation times and amplitudes will lead to the same least-squares distance between the model and the experimental noisy data. To reduce the number of solutions, an efficient strategy consists in adding a constraint of positivity on the amplitudes. Non-negative least squares (NNLS) algorithm is nowadays the most popular algorithm incorporating this constraint. By this manner, the algorithm returns a unique solution of positive amplitudes, the relaxation time values being *a priori* defined by the so-called decomposition basis. To obtain a smooth amplitude distribution, a Tikhonov regularization is most often performed. A critical step with this approach deals with the choice of the operator (i.e. norm-, slope- or curvature-smoothing) and then of the corresponding regularization parameter.[1] Because these parameters strongly affect the obtained distributions, the operator selection is based on both prior knowledge and T_2 distribution hypothesis followed by an optimization procedure.

In this work, we propose to analyze NNLS results without regularization to circumvent these drawbacks. Our approach is based on the analysis of NNLS outputs by cumulative distribution functions (cdf) and not by probability density functions (pdf) as it is usually done. This concept is validated in different simulations for which the true T_2 distributions are built from discrete to continuous functions. Simulation results showed that the T_2 amplitude measured at a plateau of the cdf is unbiased and (almost) independent of both the decomposition basis and the signal-to-noise ratio (see Figure). This observation allows to quantitatively interpret the NNLS inversions, especially when the true distribution is continuous. Indeed, while the pdf are largely biased and parameter-dependent for a continuous T_2 distribution, the cdf reach a plateau for the expected amplitude. In short, we suggest that NNLS by itself suffices in many situations, provided that cdf plateau can be discernable. The degrees of freedom to adjust in the method are then limited to the decomposition basis. To exemplify, this pragmatic and fruitful approach is also applied on real NMR data obtained by spectroscopy and imaging.

Zou Y.-L. et al., Pet. Sci. (2016),13:237-246

Figure: Averaged results for 1 000 simulated T_2 NMR signal decays (SNR = 1000) analyzed with the NNLS algorithm and a decomposition basis containing either 40 (blue) or 200 (red) T_2 values logarithmically spaced between 1 and 1000 ms. The black (full or dashed) lines represent the theoretical values. Representation with the pdf (top) and the cdf (bottom) for a discrete (left), continuous (middle) or mixed (right) models.

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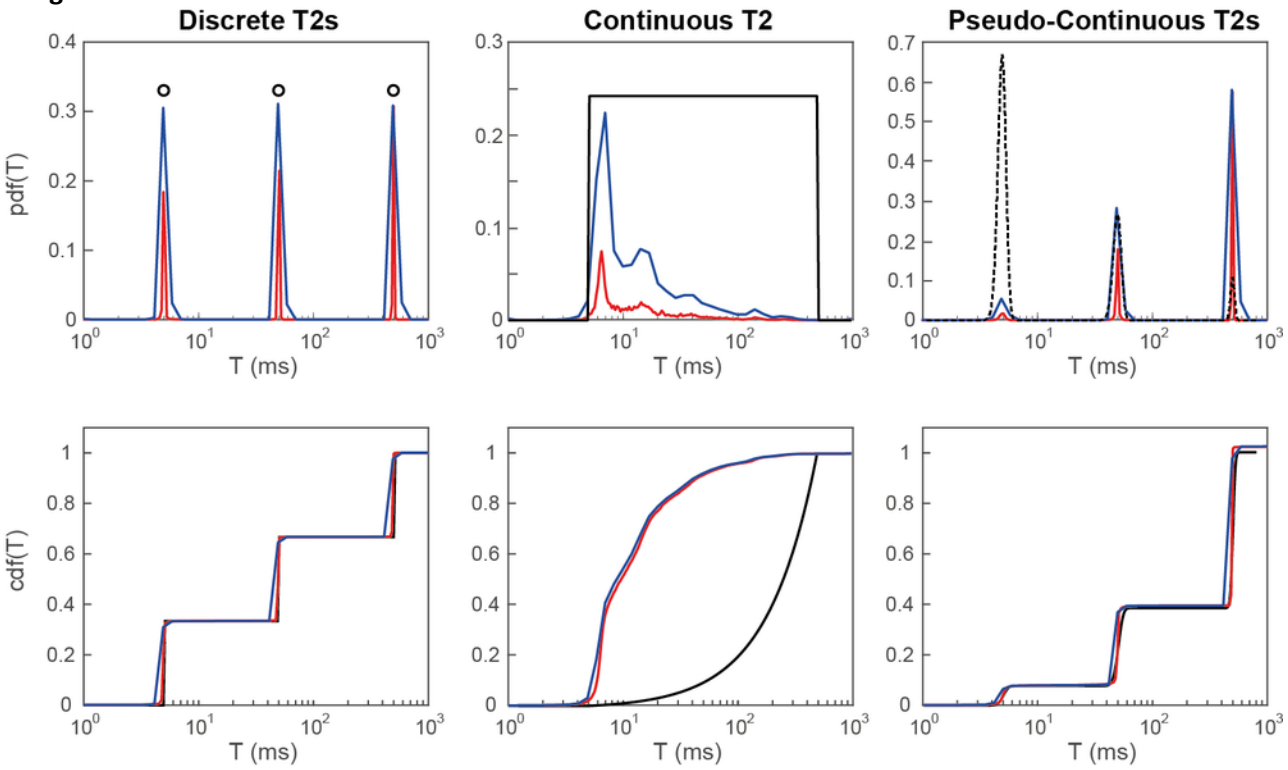
Discrete T2s
Continuous T2
Pseudo-Continuous T2s
pdf(T)
cdf(T)
T (ms)

Benchtop and lowfield

P008

Detecting Low Concentrations of Unsaturated C-C Bonds by Parahydrogen-Induced Polarization using the Benchtop NMR and Home-Built Parahydrogen Generator

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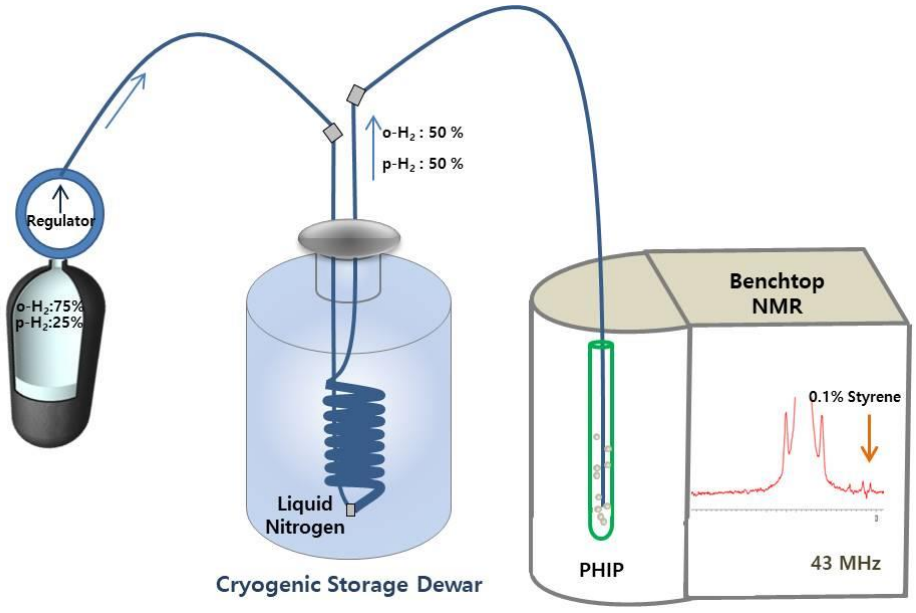
Abstract: In the developed NMR hyperpolarization techniques, Parahydrogen-Induced Polarization (PHIP) technique is widely utilized to overcome the low sensitivity of the NMR/MRI. Parahydrogen generator is essential to produce high spin order of parahydrogen molecule. Commercial parahydrogen generator is well developed with user-friendly systems. However, it has drawbacks of long preparation time (~ 2h including cooling down time of 1h) and high cost for the commercial setup. Several home-built parahydrogen generators were proposed as the alternatives for the liquid nitrogen-based parahydrogen generator which can overcome those demerits of commercial one. Especially, using liquid nitrogen is a great way of lessening the time for producing the ~ 50% parahydrogen, however, the biggest problem for designing the home-built parahydrogen generator using liquid nitrogen is the duration time that the liquid nitrogen stays in the dewar. There have been suggested several ways of overcoming those problems; storing in the other aluminum tank which will lasts days to months with singlet state, refilling the liquid nitrogen continuously on the dewar. With those suggested ways of using hyperpolarized parahydrogen longer time, we applied another simple and creative way to save much liquid nitrogen and it lasted more than 20 days in a single fueling. A principle-based experiment, identifying small concentration of unsaturated bond compound in mixture by using hyperpolarization and phase difference, was carried out by hydrogenation reaction. Lower than 1 µl styrene in 1 ml chloroform was identified by a single scan of 43 MHz Benchtop NMR after hydrogenation reaction via 50% parahydrogen. This method could be developed with great potential such as using high field NMR, higher parahydrogen concentration, and more scan time to collect data etc. Because hydrogenation reaction by parahydrogen induce reversed phase in attaching on the unsaturated C-C bond, many other unsaturated bonds in organic molecules might be used for detection. This study will not only broaden the parahydrogen-based unsaturated bond detecting study but also hyperpolariazation study on many researchers by providing the long time lasting system of home-built parahydrogen generator

Image:



Benchtop and lowfield

P009

Implementation of standard ASTM 19F method on a benchtop NMR 43 MHz in comparison with high magnetic field performance for the determination of primary hydroxyl content of polyols

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Abstract: Polyurethane chemistry is highly versatile leading to a wide variety of end-use products used in industries such as furniture, construction, electronics & appliances, automotive, footwear & packaging. For polyurethane manufacturing, polyols are used as raw materials reacting with isocyanates in presence of catalysts. Determination of the nature of end hydroxyl polyol groups; distinguishing between primary and secondary hydroxyl groups, is a critical parameter for understanding and controlling the reactivity of polyols with isocyanates. There are various analytical methods for determining the end hydroxyl group including an ASTM standard method (D4273-11). The ASTM method is based on a derivatization reaction of primary hydroxyl polyol groups with trifluoroacetic acid and subsequent hydrolysis, followed by ^{19}F NMR. In this work we focus on the implementation and validation of the ASTM method performed on a benchtop 43 MHz NMR and on the statistical comparison between the performance of high magnetic field equipment and benchtop technology.

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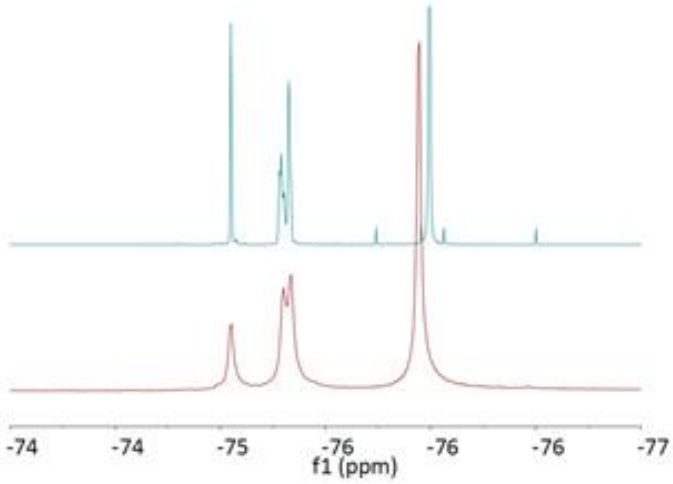

Figure 1: Example of 19F spectra acquired by a 43 MHz benchtop NMR (red) and 400 MHz NMR (green) on a polyol containing 25% primary hydroxyl groups

Benchtop and lowfield

P010

Oxygen saturation dependent effects on blood transverse relaxation at low fields

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Abstract: Blood oxygen saturation is a critical physiological parameter for patient health. The clinical importance of this parameter means that measurement of blood oxygenation is a routine part of care. Magnetic resonance provides a way to measure blood oxygenation through the paramagnetic effect of deoxy-haemoglobin, which decreases the apparent T_2 relaxation time of blood[1]. This effect has been well characterised at high fields (> 1.5 T) for use in Magnetic Resonance Imaging[2–6], and it is a contributing factor to the Blood Oxygenation Level Dependent (BOLD) contrast used in functional MRI. However there are relatively few studies of this effect at low magnetic fields, and these have only looked at extreme levels of oxygenation/deoxygenation [7,8]. To study this effect for potential application in low-field devices, we investigated how factors such as oxygenation, field strength and CPMG echo time affect the apparent T_2 of blood.

A continuous flow circuit, similar to a cardiopulmonary bypass circuit, was used to control blood parameters such as oxygen saturation and temperature before the blood sample flowed into a variable field magnet (set at fields between 5-40 MHz), where a series of CPMG experiments with echo times ranging from 1-20 ms were performed to measure the T_2. Additionally, the oxygen saturation was continually monitored by an optical sensor, for correlation with the T_2 changes. This allowed us to test the sensitivity of this effect at low fields.

These results show that at low fields, the T_2 relaxation change still follows the trends shown in the literature, with a dependence on B_0 squared, and on the fraction of deoxyhaemoglobin squared. The data shows good agreement with the Luz-Meiboom equation which is typically used to describe this effect at higher fields[2,4,5]. These experiments show that T_2 changes in blood due to different oxygenation levels are observable at low field, thus providing the opportunity to exploit this effect for blood oxygenation monitoring in future low-field mobile NMR medical devices.

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Benchtop and lowfield

P011

Detection of [1-13C]pyruvate metabolism by 1H NMR

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Abstract: An Oxford Instruments 1.4T benchtop NMR spectrometer, situated adjacent to a GE Spinlab hyperpolarizing system, was used to explore the interconversion of [1-^{13}C]pyruvate and [1-^{13}C]lactate in two renal cancer cell lines. To complement ^{13}C experiments using hyperpolarized [1-^{13}C]pyruvate, initial studies were carried out using ^{1}H NMR spectroscopy. While the cells maintained high viability during the time course of the NMR experiment, they nevertheless showed a large ^{1}H signal from lactate, visible as a doublet at 1.3 ppm arising from the methyl protons. Following addition of non-hyperpolarized [1-^{13}C]pyruvate to the cell suspensions, ^{1}H spectra revealed additional satellite signals on either side of each of the two doublet peaks, with a splitting of 4 Hz. These additional signals are attributable to the formation of [1-^{13}C]lactate, as the splitting of 4 Hz is consistent with the known ^{1}H-^{13}C spin-spin coupling interaction between the 1-^{13}C of lactate and its methyl protons. We can therefore conclude that the 1-^{13}C label of the pyruvate has been transferred to lactate via the lactate dehydrogenase reaction.

Our results demonstrate that the lactate dehydrogenase reaction can be monitored by ^{1}H NMR spectroscopy at 1.4T utilizing non-hyperpolarized [1-^{13}C]pyruvate, as well as by ^{13}C NMR spectroscopy of hyperpolarized [1-^{13}C]pyruvate. A major conclusion from this work is that a relatively low-field (1.4T), low-cost benchtop NMR spectrometer that can be positioned adjacent to a clinical hyperpolarizer offers a highly effective means of studying metabolic processes in cancer models *in vitro*. This could provide a powerful complement to clinical hyperpolarization investigations, including studies exploring pyruvate metabolism in human cancer.

Benchtop and lowfield

P012

Evaluation of pure shift NMR methods on a benchtop spectrometer

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Abstract: Nuclear magnetic resonance (NMR) is one of the most widely used analytical techniques for accessing both structural and quantitative information. However, high field NMR needs expensive superconducting magnets and requires cryogenic fluids as well as human and material maintenance costs. As an alternative, a new generation of mobile, compact and cryogen-free low-field (LF) spectrometers has emerged.[1] These permanent magnet-based systems have reached a sufficient level of maturity to provide a stable and homogeneous enough static magnetic field to resolve the chemical shift information and, as such, can deliver NMR spectra of high enough quality to generalize their use in various application areas such as chemical process monitoring [2,3] food screening, [4] or quality control of dietary supplements [5]. As expected, though, LF can give rise to highly overlapped spectra, limiting the performance of such instruments for some applications. In this context, recent developments in NMR pulse sequences have helped to overcome some of the limitations in spectral resolution. Among them, Ultrafast NMR has pushed the limits of LF NMR, thanks to the addition of a gradient coil in the spectrometer [2]. This additional gradient coil paves the way for the implementation of other advanced NMR methods, significant among them homodecoupling strategies such as *pure-shift* NMR. We have implemented different and complementary homodecoupling methods including the 1D projection of 2D J-resolved spectra [6], the Zangger-Sterk scheme [7] and the more recent PSYCHE method [8]. These methods will be evaluated in terms of resolution gain versus sensitivity losses. We will also evaluate their analytical performance (trueness and precision) in the context of quantitative NMR. Various samples of increasing complexity from pure samples to mixtures with different concentration ranges and molecular weights will be investigated. The proposed evaluation and related discussion will help users to choose the best tools depending the amount of sample available and molecular size.

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Benchtop and lowfield

P013

Potential of Benchtop Quantitative NMR for Monitoring Enzyme Catalyzed Reactions

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Abstract: Exploring the kinetics and online monitoring of chemical reactions requires integration of a continuous flow system to a spectrometer with real-time data acquisition. As an example, *Raman spectroscopy* has been commonly utilized for such purposes. Thanks to the recent progress of benchtop NMR spectrometers at moderate field strength (~up to 1T), it has become possible to monitor reaction kinetics in real time and with an acceptable resolution. Hydrolysis reactions are an important class of reactions in food industry as invert sugar, corn syrup, lactose free milk are all produced through enzyme catalyzed reactions. In this study, sucrose hydrolysis is monitored online using the invertase enzyme for invert syrup production. The quantification of monosaccharides was made challenges by the strong overlap with the non-deuterated water peak. Therefore, we used recently optimized pulse sequences for water presaturation at low field (1). Before the hydrolysis, optimization was performed on individual sugars (sucrose, glucose, and fructose) and their mixed solution. The hydrolysis reaction was conducted at with an initial sucrose concentration of 10% (w/w) both in stirring and continuous flow modes at a pH of 4.5. NMR spectroscopy experiments were conducted using a Magritek 1T system equipped with a gradient coil. In addition to spectroscopy experiments, spin-spin (T2) relaxation times of the reaction medium were measured using a CPMG sequence at another low field system working at a frequency of 20.34 MHz. Relaxation spectra were obtained using *Non-Negative least Square Analysis* (NNLS). Results from spectroscopy and relaxation measurements were analyzed to obtain a kinetic model from the experiments.

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Benchtop and lowfield

P014

Medium Resolution 1H-NMR at 62 MHz as a Chemically Sensitive Online Detector for Chromatography of Polymers (SEC–NMR)

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Abstract: The recent advances in NMR hardware at medium fields (0.5 – 2 T) with high homogeneity (FWHM ~ 0.5 Hz) enable frequency resolved experiments at a much lower spatial demand, cost and level of sophistication than previously possible. The new instruments allow new applications for NMR techniques, which were prohibited by either the high demands of high field instruments or the lack of spectral resolution at low fields. We present one of these new applications within this context: the usage of medium resolution 1H-NMR (MR-NMR) as a coupled online detector for the chromatography of polymers.

Polymers have three important molecular characteristics that determine their properties: the molecular weight distribution (MWD), the chemical composition and the topology, e.g. branching. The MWD is usually determined using size exclusion chromatography (SEC). SEC detectors commonly in use, such as refractive index detectors, do not provide information about the chemistry or topology. This information is normally gained in separate experiments where current NMR methods give detailed insights. However, when it comes to analyzing complex materials like copolymers, blends or unknown samples, chemistry and topology are often a function of molecular weight. Thus, the combined and correlated measurement of size and chemical properties is of special interest but tedious with dedicated but separate instruments and the online coupling is a good alternative.[1]

Our method consists of a 62 MHz NMR (Magritek Spinsolve 60) coupled to a SEC system as an online detector (see top of figure) to identify the analytes as they are eluting from the column. The system runs as close as possible to optimum chromatographic conditions to retain MWD resolution. This means using protonated solvents, measurement at continuous flow and very low analyte concentrations (< 0.5 g/L at the NMR detector). The inherent challenges are the low signal-to-noise and the strong solvent signals overlapping regions of interest in the analytes' spectrum and other detrimental effects. Therefore, careful optimization of the sensitivity and solvent signal reduction are most important.

We improved the signal to noise ratio by optimizing all aspects of the setup such as flow cell, SEC conditions, pulse sequences, and signal treatment by a factor of at least 10 when compared to previous work at low fields.[2] This results in a LOD in the range of 30 – 100 µg, sufficient for first applications.[3] The solvent suppression is tackled by employing state-of-the-art pulse sequences such as WET or WEFT yielding a suppression by up to a factor of 400.

As a result unique 2D correlations can be established where chemical shift is one dimension and the molecular weight the second. Thus, either molecular weight specific spectra or chemical shift specific elugrams can be generated. We show application results of typical homopolymers (PS, PMMA, PB, P2VP), their blends and copolymers. For an example of a PS-PMMA 1:1 blend see the bottom of the figure.

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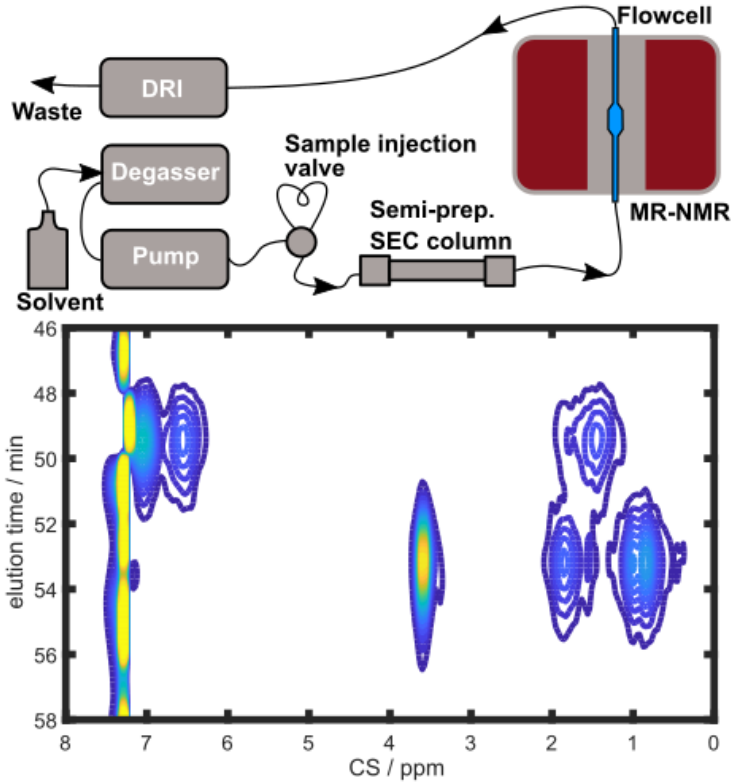
Image:


Flowcell
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Benchtop and lowfield

P015

Rapid screening of oligopeptide drugs by Benchtop NMR spectrometers

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Abstract: Oligopeptide drugs (OD) contain different sequences of several of the 20 proteinogenic amino acids (PAA). Their identification and quantitative determination is usually carried out in Pharmacopoeia analysis by HPLC using certified reference materials (CRM). The European Pharmacopoeia contains an example of application for the identification of buserelin by ^{1}H NMR spectroscopy using spectrometers with frequency of 300 MHz or higher [1]. All PAA contain known molecular fragments identified and quantified by NMR ^{1}H by their characteristic signals (e.g. aromatic, methyl, α, β-dimethylen). Measurement of their integral intensities allows not only to identify many OD, but also to determine their content in aqueous solutions. This approach was developed by us in the study of dosage forms containing OD Imunofan, Sedatin, Thymodepressin, Octreotide and Buserelin on the NMR spectrometer with frequency of 600 MHz [2]. For quantitative determination, the total intensities of signals of the same molecular fragments (for example, CH_{ar}, CH_3, etc.) are preferred. Quantitative errors in determining the content of OD in dosage forms do not exceed 1-3% and depend on their content in the drug composition and its complexity. The obtained results stimulated an attempt to adapt a such technique to Benchtop NMR spectrometer 60 MHz ("Pulsar", Oxford Instruments; "NMReady", Nanalysis) and 80 MHz ("Spinsolve", Magritek). An example of its implementation for OD Buserelin is presented in Fig.1, which contains the structural formula of this OD, the NMR ^{1}H spectrum of its aqueous solution 4.1 mg in 1 ml D_2O, parameters of registration and integral intensities of 1, 10 and 18 protons fragments -CH=, CH_{ar} and CH_3, respectively, for which, using as an internal standard TSP-d4, the content of OD in solution is determined with an error less than 1%. The applicability of the technique to other studied OD is confirmed by the use of all these Benchtop NMR spectrometers.

References:

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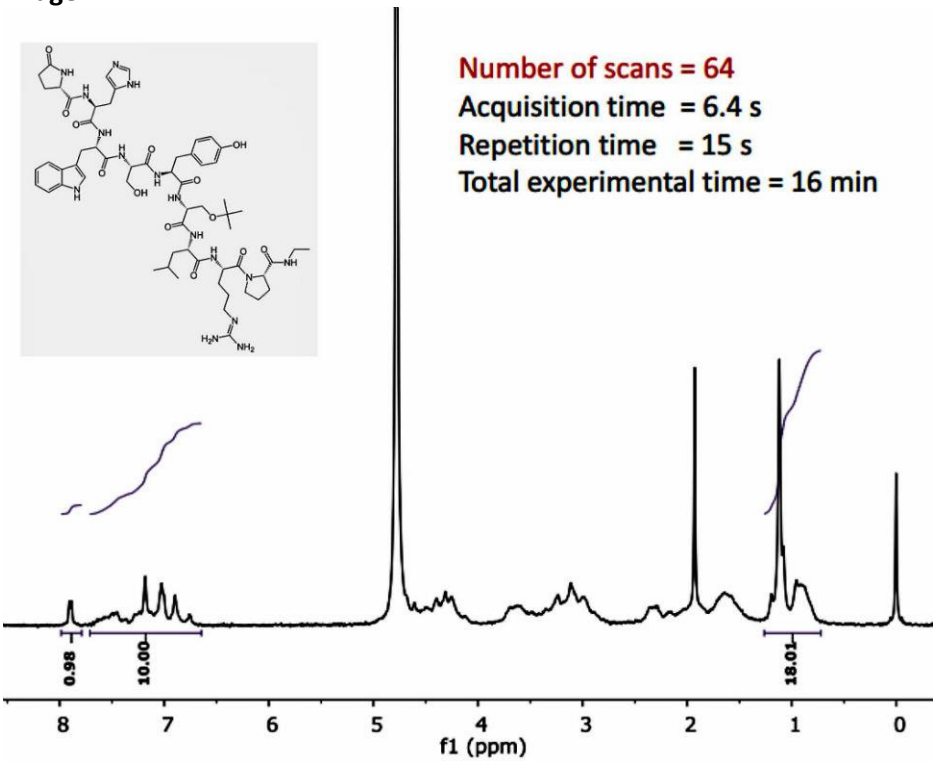

Benchtop and lowfield

P016

Water dynamics in a mesoporous bioactive glass studied by NMR relaxometry

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Abstract: Water confined in a new class of mesoporous bioactive glasses (MBGs) has been studied by NMR relaxometry technique. Two CaO-SiO_2-P_2O_5 materials have been compared: both have the same chemical composition but they have been made with different methods – standard or microfluidic techniques -, inducing different textural properties (surface areas, porous volume, mesoporous arrangement).

NMR is known for being particularly well suited for studying fluids embedded in mesostructured porous materials because, in addition of being non-invasive and relatively fast, it allows the characterization of molecules dynamics. In this study, we used NMR relaxometry, which consists in measuring spin relaxation times as a function of the measurement frequency (or equivalently of the static magnetic field B_0), in order to determine the dynamics of water molecules embedded in the MBGs. The low field domain (10kHz-10MHz proton Larmor frequency) was investigated with measurements done with a fast field-cycling relaxometer, and the so-called dispersion curves (longitudinal relaxation rate as a function of the measurement frequency) were analyzed in order to have additional insights on the motion of confined water.

The frequency dependence of the proton relaxation rate in MBGs was first studied by comparing the relaxation dispersion detected in HOD (residual proton of heavy water) with that in H2O, the proton low-frequency relaxation is thus shown to be mainly due to intramolecular dipolar interactions. Secondly, dispersion curves were recorded as a function of the filling degree and analyzed in the frame of the two-phase fast exchange model: tiny differences in the dynamical behavior of the water inside the two bioglasses (exchange timescale, hydration layers) could be evidenced.

Bioliquids

P018

Getting in the Groove:

Chaperone-assisted peptide exchange dynamics in the Major Histocompatibility Complex

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Abstract: Molecular chaperones TAPBPR (TAP-binding protein related) and tapasin associate with class-I major histocompatibility complex (MHC-I) molecules to promote optimization (editing) of peptide cargo. Here, we use methyl-based, solution NMR to investigate the molecular mechanism of peptide exchange performed by the 90 kDa chaperone protein complex. We identify TAPBPR-induced conformational changes on conserved MHC-I surfaces, consistent with our independently determined X-ray structure of the complex. Conformational dynamics present in the empty MHC-I are stabilized by TAPBPR in a peptide-deficient complex, and become progressively dampened with increasing peptide occupancy. Incoming peptides are recognized by the chaperoned groove according to the global stability of the final pMHC-I product, and anneal in a native-like conformation. Our results demonstrate an inverse relationship between MHC-I occupancy by peptide and the affinity of TAPBPR for such pMHC-I molecules, where the lifetime of transiently bound peptides controls the dynamic regulation of a conformational switch, located near the TAPBPR binding site, which triggers TAPBPR release. Lastly, we discuss the role of protein dynamics in shaping chaperone specificity towards different human and murine class-I MHC alleles. These results suggest a similar mechanism for the editing function of tapasin in the peptide-loading complex.

Bioliquids

P019

Proline conformation in the TAD domain of p53

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Abstract: Intrinsically disordered proteins are highly flexible molecules with no stable secondary structure. This means that many conformers are present under physiological conditions, some of them contain transient secondary structural elements. Prolines, which are more frequent in IUP proteins than in globular ones causing the lack of structure in proteins, play role in several physiological processes, such as modulation of signaling, enzyme function and membrane binding.[1,2]
Our research focused on the transactivation domain of the well-studied tumor suppressor, p53. The ^{1}H-^{15}N HSQC spectrum contains previously unreported peak multiplications for at least 20 residues that arise from a minor conformational state. The minor peaks (with about 10 % signal intensity) appeared for residues surrounding prolines. This is characteristic for IDP proteins.[3,4] The reason is the fact, that peptide bonds involving prolines are in an equilibrium between *cis* and *trans* conformations. In the major component each proline favors the *trans*, while in the minor conformer 6 of 10 prolines adapts a *cis* conformation. Further, this conformation is maintained in complexed p53 with S100A4.
We performed a structural characterization of the minor component using solution state NMR spectroscopy. Sequential connectivities were determined on the basis of BEST-type 3D HNCO, HNCA, HNCACB, HNCOCA, HNCOCACB and HNCOCA; CCCONH; CON spectra; $^{3}J_{HNHA}$ information was derived from HNHA and Φ and Ψ torsion angles were calculated from TALOS-N server.[5]
We found structural and dynamical differences between the minor and the major component: minor form is less flexible than the major component: according to δ2D[6] the regions with *cis*-prolines have β propensity, and we were able to assign a minor form for K24-S33, which is the nascent helical region.

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Bioliquids

P020

A structural description of the nucleoskeleton-chromatin interface

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Abstract: Lamins are the main components of the nucleoskeleton. Mutations in lamins A and C or abnormalities in the processing of lamin A cause cause degenerative tissue-specific disorders and premature aging syndromes. A large number of these mutations are located in the tail region of lamin A, composed of a globular Igfold domain and a large C-terminal disordered fragment. NMR analyses revealed that mutations causing muscle diseases affect residues of the hydrophobic core of the Igfold domain, suggesting that destabilization of the globular domain is responsible for these diseases [1]. Moreover, mutations causing progeroid syndromes either impact a specific site at the surface of the Igfold domain or introduce a large deletion in the unfolded region [2]. Using a combination of NMR and X-ray crystallography analyses, we identified that the progeria-associated site at the surface of lamin Igfold domain is responsible for lamin interaction with the DNA binding protein BAF. We revealed that this complex directly interacts with the emerin protein anchored at the nuclear membrane [3], and we suggested that it also binds to DNA (Figure 1). Finally, we showed using a combination of NMR and negative-staining EM that the lamin Igfold domain can also directly interact with self-assembled emerin [3,4]. We will discuss how post-translational modifications (phosphorylation during cell cycle or after a mechanical stress) can regulate these interactions at the periphery of the nucleus.

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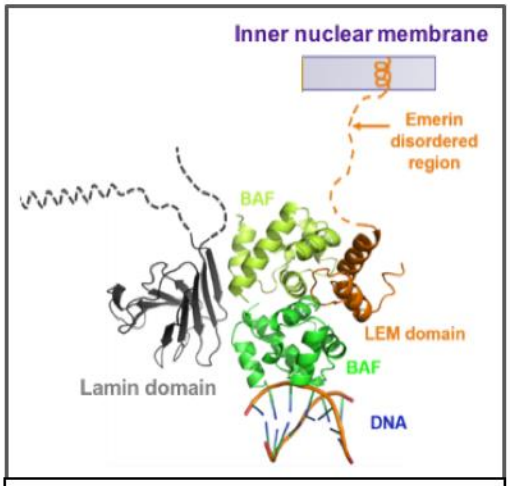

Figure 1. A model of the complex between the lamin A/C immunoglobulin-like domain (in grey), the emerin LEM-domain (in orange) and the DNA-bound dimeric protein BAF (in green) [5].

Bioliquids

P021

Structural studies of the antimicrobial peptide Brevinin-1BYa and its analogue

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Abstract: Brevinin-1BYa (FLPILASLAAKFGPKLFCLVTKKC), first isolated from skin secretions of the foothill yellow-legged frog *Rana boylii*, is particularly effective against *C. albicans*, and is also active against *E. coli* and *S. aureus*. The structures of brevinin-1BYa and its less-potent analogue [C18S,C24S]brevinin-1BYa were investigated in various solution and membrane-mimicking environments by ^{1}H-NMR spectroscopy and molecular modelling. Brevinin-1BYa possesses two cysteine residues, one at the C-terminal end of the peptide and the other 6 residues prior to it, joined by a disulphide bridge. The peptide does not appear to possess a secondary structure in aqueous solution. In a 33% 2,2,2-trifluoroethanol (TFE-d_3)-H_2O solvent mixture, as well as in membrane-mimicking sodium dodecyl sulphate micelles, and dodecylphosphocholine micelles, however, the structure is characterised by a helix-hinge-helix motif, with a hinge located at the Gly^{13}/Pro^{14} residues, and the two α-helices extending from Ile^{4} to Lys^{11} and from Lys^{15} to Thr^{21}. Positional studies of both the native peptide and its serine analogue in sodium dodecyl sulphate micelles using 5-doxyl labelled stearic acid and manganese chloride paramagnetic probes show that the N-terminal helical segment of the peptide lies parallel to the micellar surface, with the residues of the hydrophobic side of the amphipathic helix facing towards the micelle core and the hydrophilic residues pointing outwards, with similar results obtained for the C-terminal segment of the native peptide.

Bioliquids

P022

NMR characterization of the C3 domain from Streptococcus mutans adhesin P1

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Abstract: The *S. mutans* adhesin P1 is a modular protein of 185 kDa, which is secreted and attaches to the cell wall, and is a key virulence factor under-pinning the role of this gram-positive bacterium in dental caries. Adhesin P1 consists of a 38-residue signal sequence, an uncharacterized N-terminal region, three alanine-rich repeats (A1–3), a central domain containing a so-called variable (V) region, three proline-rich repeats (P1–3), a C-terminal region consisting of three domains (C1–3), an LPxTG sortase-recognition motif, wall- and membrane-spanning regions (Fig1). Structure of various regions of Adhesin P1 in soluble form have been studied by X-ray crystallography [1]. Recent publication highlight the specific binding of the C123 domain, naturally found in saliva, to the intact adhesin P1 on the cell surface [2, 3, 4]. These results reveal that the C123 domain forms amyloid fibrils, plays a vital role in cellular adhesion, and is involved in biofilm formation. Identifying how the C123 domain specifically interacts with P1 at the atomic level is essential for understanding the virulence properties of *S. mutans*. However, with 519 residues, the C123 domain is too large to easily achieve high-resolution data for NMR analysis. Here, we focus on the NMR characterization of the C3 domain, which is 171 amino acids in length. The goals are to obtain functional and structural data on C3 in monomeric form using solution NMR and its fibrillar amyloid form using solid state NMR. Preliminary NMR data highlight that the C3 domain of adhesin P1 is a folded protein (Fig 2) which is able to interact specifically with the A3VP1 domain of adhesin P1. NMR titrations of A3VP1 with ^{15}N-enriched C3 indicate that several C3 ^{15}N-^{1}H resonances shift significantly with ligand addition (Fig 2). The partial C3 NMR assignment enables us to define the interaction surface.

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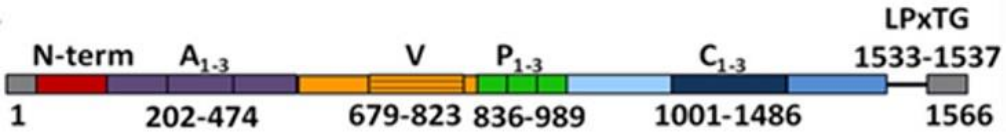

Fig 1: *S. mutans* Adhesin P1 protein is a globular protein of 185 kDa.

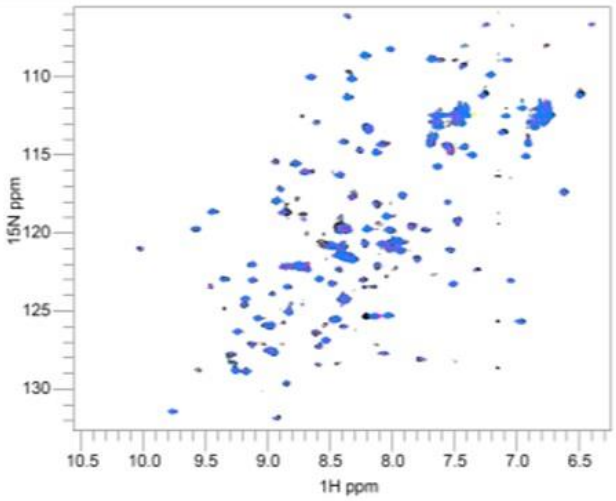

Fig 2: The **C3 domain of adhesion P1 is able to interact with A3VP1.** Chemical shift perturbation experiments (600 MHz) for C3 in the presence of different concentrations of A3VP1. ^{1}H-^{15}N HSQC of C3 alone (black), C3/A3VP1 complex at a 1:0.5 C3/A3VP1 concentration ratio (blue), and C3/A3VP1 complex 1:0.75 C3/A3VP1 concentration ratio (pink).

Bioliquids

P023

Optimization of glycine-specifically labelled samples for solution protein NMR

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Abstract: Solution NMR of proteins suffers from two major drawbacks: low sensitivity and resolution. These limitations preclude the detailed structural studies of large molecular systems (>30 kDa). However, previous works have demonstrated that the use of TROSY (Transverse Relaxation Optimized Spectroscopy) experiments on highly-deuterated proteins allow significant improvements both to sensitivity and resolution.[1]NMR signals have been obtained in the liquid state for biomolecular complexes up to 1 MDa using methyl specific labelling procedures.[2] Compared with TROSY pulse schemes dedicated to other chemical groups, the CH_2-TROSY experiments have been largely underexploited so far, despite the large fraction of methylene-type hydrogens in biomolecules and the significant improvements demonstrated both in sensitivity and resolution.[3]We think this is due, at least partly, to the fact that any procedure has yet been described that enables the specific labelling of CH_2 groups in a perdeuterated background.

In the present work we describe a protein expression protocol that allows specific ^{1}H-labelling of glycines while other residues are fully deuterated. We expressed the H23 protein, a nucleotide-binding domain of a Heavy Metal ATPase, using a cell-free approach that enables us to simultaneously reduce the amount of isotopes used and inhibit biological processes responsible for amino acid scrambling. Additionally, proton exchange with the solvent can be catalyzed by transaminase, lowering the deuteration levels of non-Gly amino acids.[4]Slow-relaxing components that are selected in the TROSY experiments are very sensitive to the residual proton levels.

In this presentation we assess the efficiency of the cell-free approach for the production of specifically ^{1}H,^{15}N,^{13}C-Gly H23, using various cocktails of enzyme inhibitors, different S30 extracts and isotope sources (isogrow®, celtone® or separate amino acids) and using H_2O or D_2O as culture solvent (Figure 1). Amino acid scrambling and residual protonation have been quantified using NMR experiments and mass spectrometry. The most efficient conditions will be applied to the Pin1 protein and CH_2-TROSY spectra will be recorded to demonstrate the versatility and usefulness of this approach.

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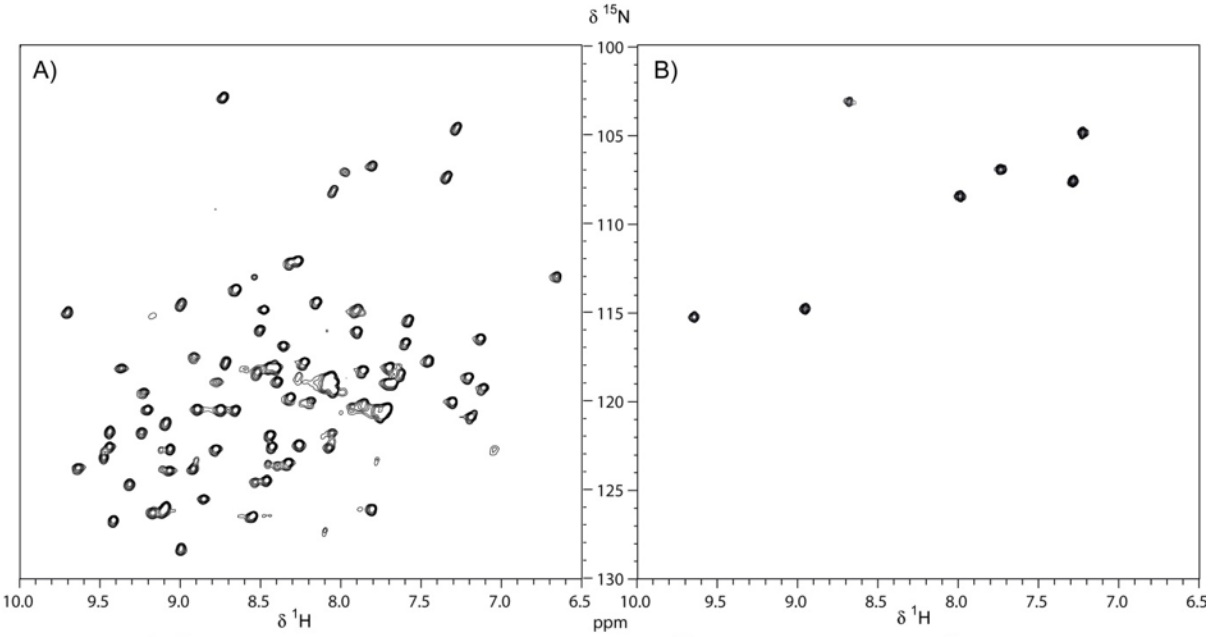

Figure 1: ^{1}H-^{15}N HSQC spectra of cell-free expressed H23 protein. A) uniformly ^{15}N-labelled sample. B) Specifically ^{15}N-Gly labelled sample (TCI cryoprobe at 500 MHz, 25°C, 200 µM, tris buffer 100mM pH 9, NaCl 20 mM).

Bioliquids

P024

The pH-dependence of ligand binding in human ileal bile acid-binding protein. Exploring the role of histidine protonation.

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Abstract: Besides aiding digestion, bile salts are important signal molecules exhibiting a regulatory role in metabolic processes. Human ileal bile acid-binding protein (I-BABP) is an intracellular carrier of bile salts in the epithelial cells of the distal small intestine and has a key role in the enterohepatic circulation of bile salts. Large conformational rearrangements accompanying ligand binding in the GH- and CD-turn regions hosting the three histidines of the protein prompted us to investigate the effect of pH on the binding process and internal dynamics of human I-BABP. Macroscopic thermodynamic and kinetic data obtained from calorimetric and stopped-flow fluorescence measurements are complemented by NMR relaxation dispersion analysis. While the thermodynamics of binding at pH=7.2 and 5.8 is similar, the rate of association is markedly increased at lower pH. Importantly, residues exhibiting an increased sensitivity to pH in their backbone ^{15}N chemical shift near the pK_a of histidines, undergo a global conformational exchange on the fast end of the millisecond timescale indicating a direct connection between slow internal fluctuations and the protonation equilibrium of histidines. Slow motions become diminished and heterogeneous at pH ~5. Fluctuations have previously been shown to cease upon ligand binding and for a subset of residues, ^{15}N chemical shift differences between the ground and the excited state are in good agreement with the chemical shift difference between the *apo* and the *holo* forms. Our results shed new light on the role of histidine protonation in a conformation selection mechanism of ligand entry in human I-BABP, a protein with an enclosed binding cavity.

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Bioliquids

P025

RRM2 of CELF1 protein from Plasmodium falciparum preferentially binds to UG repeats RNA

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Abstract: ***RRM2 of CELF1 protein from Plasmodium falciparum preferentially binds to UG repeats RNA***

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Malaria causes high mortality (445,000 casualties) despite continued efforts for its eradication. *Plasmodium falciparum* is the prime cause of lethal malaria in human. In spite of having a small genome, it exemplifies a vast proteome, which leads to its widespread resistance against various anti-malarial drugs. The process of alternative splicing is mostly responsible for this proteomic diversity.

CELF (CUGBP Elav like family member) proteins play an active role in pre-mRNA alternative splicing, translational regulation, de-adenylation, mRNA stability and RNA editing among various eukaryotes. *Pf*CELF (*P. falciparum* CELF) has three RNA recognition motifs (RRMs), of which second and third are separated by a WW domain. To understand the molecular basis of RNA recognition by *Pf*CELF we have undertaken extensive structural and biochemical studies. We have completed the sequence-specific NMR resonance assignment of RRM2 of *Pf*CELF and the initial model generated using torsion angles derived from chemical shift shows it of having a characteristic *β-α-β-β-α-β* fold. Our calorimetric studies and NMR titration show that *Pf*CELF-RRM2 has a strong affinity towards UG repeats RNA that possibly interact in a canonical manner.

Bioliquids

P026

MODULATION BY PHOSPHORYLATION OF TAU PROTEIN INTERACTION WITH PROTEIN PARTNERS

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Abstract: In the course of our molecular investigation of Tau functions and dysfunctions in AD, nuclear magnetic resonance (NMR) spectroscopy is used to identify the multiple phosphorylations of Tau and to characterize Tau interactions with its molecular partners. These tasks remain challenging due to Tau highly dynamical character and its 80 Ser/Thr residues, potential sites of phosphorylation that can be combined to give a multiphosphorylated Tau, leading to a very complex regulation of Tau interactions. It has proven crucial to identify phosphorylation sites to be able to link specific phosphorylations with structural or functional modifications. Functional aspects include the regulation by Tau phosphorylation of both interaction of Tau with protein partners and aggregation.

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Bioliquids

P027

NMR study of the structure and dynamics of the intrinsically disordered tail of ErbB2, phosphorylation and interaction

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Abstract: ErbB2/HER2/neu is a member of the ErbB family of receptor tyrosine kinases. These receptors are located upstream of major signaling pathways, controlling cell proliferation, cell migration and apoptosis. ErbB2 is the only member of the family for which no ligand is needed for efficient signaling. Its overexpression is correlated with the occurrence of several types of cancer, and especially poor prognosis in breast cancer. Signal transduction is triggered by homo- or hetero-dimerization with ErbB proteins, leading to activation of the kinase domain and phosphorylation of the C-terminal end of the protein, which we showed to be an intrinsically disordered region (IDR). This tail, which we call CtErbB2, is the hub for the interactions that will determine cell fate.

CtErbB2 is a 268-residue, proline-rich IDR. We determined the structural and dynamic features of unphosphorylated CtErbB2 using high-field NMR. It was shown to retain very little residual structure and to be locally particularly extended. However, a N-to-C terminal contact was observed, potentially modulating the accessibility of certain sites to partners.

In addition to phosphorylatable tyrosines prone to interact with SH2 or PTB domains, CtErbB2 contains PxxP motifs that could favor interaction with SH3-containing proteins. Grb2, a central protein in Ras-dependent pathways and cell proliferation, contains both SH2 and SH3 domains and could therefore be subject to particular interaction mechanisms. We determined the solution organization of Grb2 domains, and then studied the interaction between unphosphorylated CtErbB2 and Grb2, as well as between Grb2 and phosphorylated peptides of ErbB2, showing that the SH3 domains indeed modulate the interaction.

Strategies to obtain the phosphorylated form of CtErbB2 are also tested, to have a more global idea of the interdependence of phosphorylation events, behavior of the modified protein, and role of phosphorylation in regulating interactions.

Bioliquids

P028

RNA@HD - High definition structure and dynamics of RNAs

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Abstract:

MicroRNAs (miRNAs) target and downregulate different mRNAs, and thereby affect many biological processes. In addition, they are involved in various human diseases such as cardiac diseases, Alzheimer's, diabetes or cancer making them prime drug targets and candidates for molecular diagnostics. However, this opportunity can only be fully exploited by a better comprehension of how and when each mRNA is targeted by a miRNA, a fact currently still poorly understood. Besides regulatory RNAs, synthetic antisense oligonucleotides (AONs), which also bind segments of mRNAs and lead to their downregulation, are currently tested as cancer drugs. Progress has however been slow due to unspecific off-target-effects, difficulty to quantify the cellular uptake and again, the lack of understanding the targeting mechanism of AONs.

NMR is the method of choice to obtain high-resolution information of biomolecules and therefore to shine a light on this targeting process. Here we present new and improved solution state NMR methods for assignment and relaxation dispersion measurements of labeled and unlabeled nucleic acids. To demonstrate the scope of the NMR methods we are investigating the molecular structure and dynamics of a miRNA as well as an AON cancer drug candidate. Other RNAs and/or proteins, which are relevant for the biological functionality, are identified and complexes of the nucleotide of interest with those RNAs/proteins are studied. Based on the obtained NMR data we intend to characterize the mechanism of the mRNA targeting process in unprecedented detail.

Bioliquids

P030

Structural Characterization of Dynamics in Loop Regions of c-di-AMP-Binding Proteins

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Abstract: Secondary messengers are intracellular messengers generated at the receptor of the cell surface and act in response to an extracellular signal, the primary messenger. Cyclic di-adenylate Monophosphate(c-di-AMP) is a classical secondary messenger produced by the adenylatecyclase after activation of G-protein-coupled receptors. c-di-AMP has been found in bacterial processes for signal transduction in movement and production of viral factors. Despite the efforts in identifying interactions of cyclic di-nucleotides with corresponding protein binding partners for therapeutic intervention, the cellular cascades for the regulation of c-di-AMP in the viral bacteria *Staphylococcus aureus* are not yet completely identified. The c-di-AMP-binding protein, PII-like signal transduction protein A (PstA) is homotrimeric in nature with each monomer comprising a compact core of two alpha-helices and four beta strands connected by the loops. The T- and B-loops accommodate ligand binding sites and ATP-binding sites, respectively. In absence of c-di-AMP, both loops are invisible as reported in several published crystal structures of PstA, but upon ligand binding, the T-loop adopts a stable conformation. However, the B-loop remains invisible in both apo- and bound forms assuming salt bridging between both loops is responsible for the decrease in flexibility. Using state-of-the art liquid-state and solid-state NMR methods, we have expanded the structural insights of the trimeric complex, investigating ligand binding and associated changes in structure and loop dynamics. We first obtained full chemical shift assignments of the trimer in its free and bound forms using (^{2}H, ^{15}N, ^{13}C) triple-labeled protein. We then determined site-specific protein dynamics on various timescales using^{15}N-derived relaxation parameters. The binding and relaxation experiments confirm the peculiar loosening of the B-loop upon c-di-AMP binding and its preferential propensity to secondary structural elements despite the flexibility. With the aid of proton-based solid-state NMR relaxation,[2,3] we are now characterizing the intermediate-timescale conformational dynamics that governs the structural stability of B- and T- loops in the trimer using^{1}H, ^{15}N, ^{13}C-labeled protein at110 kHz MAS. With the progress of structural calculation, we aim to highlight the interaction model by which the B-loop detaches from the trimeric core in presence of c-di-AMP and initiates binding to partner proteins. In addition to altered structural properties in the loops, the description of how dynamics are modulated by c-di-AMP binding may be key to explaining cooperative effects of the trimer and effects on downstream signaling interaction partners.

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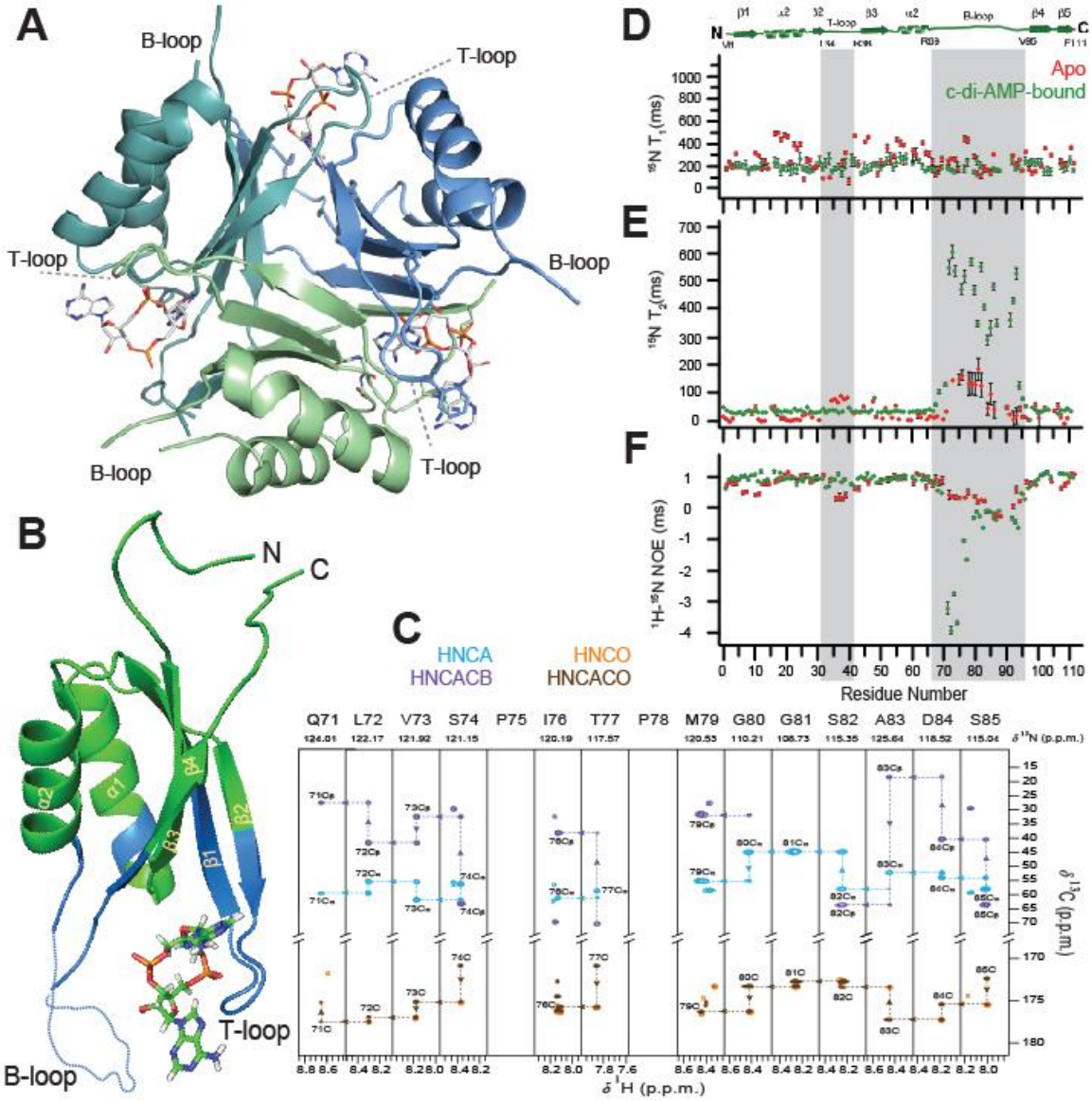
Figure 1- (A) Crystal Structure of PstA Trimer. (B) Ligand binding sites are highlighted in blue on the monomeric unit of PstA. (C) NMR Backbone Sequential Assignment of the B-loop. Comparison of (D) T_1, (E) $T_2$and (F) ^{15}N Heteronuclear NOE Relaxation Experiments for apo (red) and c-di-AMP-bound (green) PstA trimer.

Image:


A
B-loop
T-loop
T-loop
B-loop
B-loop
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B
N
C
α2
α1
β4
β3
β1
β2
T-loop
B-loop
C
HNCA
HNCACB
HNCO
HNCACO
Q71 L72 V73 S74 P75 I76 T77 P78 M79 G80 G81 S82 A83 D84 S85
δ 15N (p.p.m.)
δ 13C (p.p.m.)
δ 1H (p.p.m.)
D
N
C
T-loop
B-loop
Apo
c-di-AMP-bound
15N T1 (ms)
E
15N T2 (ms)
F
1H-15N NOE (ms)
Residue Number

Bioliquids

P031

Weak Specific Interactions of Proteins Assessed by NMR

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Abstract: Weak interactions of proteins are often ascribed to non-specific contacts of surface residues. In particular calorimetric methods of fluorescence based assays are considered meaningful only if a threshold of micromolar dissociation constants is surpassed. We have been studying a broad range of protein interactions, where NMR methods could clearly show that interactions considered too weak for being “specific” show the highly selective involvement of regions, which in the static structures are characterized as intrinsically disordered. First, this was observed by group selective saturation transfer difference experiments (gs-STD) [1] for the bacterial protein acpP [2] interacting with a synthetic inhibitory peptide. Second, the assembly of the oxygen evolving complex in higher plants involves three proteins (PsbP, PsbQ and PsbO) [3,4] and depends on interactions of their disordered regions, as was assessed by CEST experiments. Finally, in the human protein STIM1, which is involved in Calcium-channel activation, weak interactions, which are hardly affecting the chemical shifts but cause highly residue-specific changes in relaxation parameters, cooperatively trigger conformational transitions and oligomer formation.[5] In spite of the vastly different sources and functions of these proteins, a general picture emerges: Cooperative protein interactions often involve weak specific contacts, which occur in a conformational state of low population and thus, individually, cause only minor perturbations in chemical shifts. Site specific NMR relaxation parameters are sensitive to these weak interactions and can be used to discriminate between selective and non-selective interactions.

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Acknowledgments: This work was supported by the Austrian Research Promotion Agency (FFG) project 841325, the Austrian Science Fund (FWF) program W 1250 “NanoCell”, the Austrian Academic Exchange Service (ÖAD) and the European Union, project "RERI-uasb", EFRE RU2-EU-124/100-2010 (ETC Austria-Czech Republic 2007-2013, project M00146)

Bioliquids

P032

A multimodal NMR investigation of lignocellulosic biomass heterogeneity

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Abstract: Over the last 10 years, research efforts have been dedicated to identifying the factors that influence lignocellulose enzymatic deconstruction to develop alternatives to fossil carbon resources.
This study focused on the characterization of resistance factors to enzymatic deconstruction, which may be attributed to the heterogeneity of plant biomass. To take into account this parameter, dry fractionation was applied to maize biomass. Separation process was first based on particle size by air classification, and then on sample composition by electrostatic separation. Six fractions were obtained and characterized by different NMR approaches.
Solid-state NMR $^{1}H/^{13}C$ CP/MAS was used to determine cellulose, hemicellulose, lignin proportions and characterize cellulose crystallinity. Variable contact time (VCT) CPMAS experiment was recorded to assess the ordering level of the six particle fractions through relaxation parameters as T_{1rho}. Furthermore, the porosity distribution of these fractions was evaluated by low field T_2 measurements at different water content to take into account the swelling process.
Only slight differences were observed in CP/MAS between the fractions. VCT-CPMAS highlighted a linear correlation between the T_{1rho} of hemicellulose's methyl and the particle size demonstrating that the larger the particles, the greater the molecular structuration. Moreover, the porosity depends on the size and the electrostatic separation of the particles.
In conclusion; the different NMR modalities used are complementary tools to characterize the lignocellulosic biomass heterogeneity, which is a critical point to develop new enzymatic processes for lignocellulosic biomass deconstruction.

Bioliquids

P033

Structure and Dynamics of NADPH Oxidase Organizer 1/NADPH Oxidase Activator Complex by NMR Spectroscopy

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Abstract:

NADPH oxidase or NOX (nicotinamide adenine dinucleotide phosphate-oxidase) is a family of membrane-bound enzyme complexes primarily responsible for the single-electron reduction of oxygen molecules to produce ROS (reactive oxygen species) and it is responsible for host defense against microbial infections by two regulatory cytosolic proteins that form a hetero-dimer, Noxo1 (NOX organizer 1) and Noxa1 (NOX activator 1). The interaction between Noxa1 and Noxo1 is critical for activating NOX1. We determined solution structure of Noxa1 SH3 and inter-molecular interaction between the Noxa1 and Noxo1 by pull-down assay and NMR spectroscopy. Data from biochemical assay and NMR on Noxo1/Noxa1 interaction showed that Noxa1 SH3 is responsible for interaction with Noxo1 C-terminal tail harboring proline rich region (PRR domain). Sequence comparison of the C-terminal tail of Noxa1 and p47phox revealed the presence of additional polybasic region in Noxo1 C-terminal tail, which may be crucial for the protein interaction explaining the weaker binding between Noxa1-Noxo1 as compared to p67phox and p47phox which lack the polybasic region. Our results will provide a detailed structural mechanism for inter-molecular interaction between Noxa1 and Noxo1 at the molecular level, providing insights into their cytoplasmic activity-mediated functioning as well as regulatory role of C-terminal tail of Noxo1 in the NOX1 signaling. I will also discuss ROS effects on Noxo1/Noxa1 interaction related to the single-electron reduction of oxygen molecules.

Bioliquids

P034

Heme interaction of the intrinsically disordered N-terminal peptide segment of human cystathionine-β-synthase

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Abstract: Heme is an ubiquitous molecule in living organisms. Not limited to its role as prosthetic group, heme in the last decades emerges as versatile signaling or regulatory molecule for many receptors, transcription factors and enzymes [1]. However, very limited information is available at the structural level on how heme interacts with proteins and regulates their function.

We used a combinatorial library approach to identify peptides with heme binding capacities. Some of the peptide sequences reflect part of the primary structures of proteins for which we subsequently could establish a heme interaction by applying a number of biophysical and spectroscopic techniques [2-3]. In addition, nuclear magnetic resonance spectroscopy is employed to reveal the nature of transient interactions between protein and heme. Human cystathionine-β-synthase (CBS) is one of the proteins we are currently focusing on. CBS takes part in the transsulfuration pathway to convert toxic homocysteine to nontoxic cysteine. Mutations in CBS are responsible for the inherited disease called homocystinuria, which triggers a series of severe pathological conditions.

Specifically we studied the N-terminal intrinsically disordered region (amino acids 1-40), which contains one of the heme-binding motifs with the amino acid combination cysteine-proline (CP). This site constitutes for approximately 30% activity of the enzyme, which turned out to be required for achieving the full efficacy of the CBS enzyme for sulphur processing and for prevention of pathological conditions. Hence, this second heme binding site might be an attractive target for drug intervention to prevent pathological conditions without losing the complete biological functions of CBS [4]. NMR, biophysical and in vitro assay results will be presented which confirm the interaction of the CBS(1-40) with heme and reveal the second heme-interacting site to be independent from the canonical heme binding site.

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Bioliquids

P035

Structural characterization and immersion properties of the micelle-immersed FATC domain of the protein kinase Ataxia Telangiectasia Mutated (ATM) by NMR

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Abstract: Ataxia telangiectasia mutated (ATM) is a large homodimeric serine/threonine protein kinase involved in DNA damage repair [1]. ATM belongs to a family of six phosphatidylinositol-3-kinase-related protein kinase (PIKKs), which regulate various stress responses and metabolic control [2]. The N-terminal PIKK region is less evolutionarily conserved and mainly composed of α-helical HEAT repeats. The following FAT, kinase and FATC domains are highly evolutionarily conserved in eukaryotes. The C-terminal FATC domain is only about 33 residue long but is indispensable for PIKK kinase activity. The C-terminal half of FATC domain is hydrophobic because it contains many aliphatic and aromatic residues including two tryptophans, which are known for their affinity towards membranes. In line with this, previous studies showed that the FATC domains of all PIKKs can interact with membrane mimetics albeit with different preferences for membrane properties such as charge, curvature and packing density [3-6]. This suggested a general role of the FATC domain as membrane anchor [3, 4]. Here we report the characterization of the structure and backbone dynamics of the micelle-immersed state of the ATM FATC domain. The micelle immersed state of the ATM FATC domain contains two flexibly-linked α-helices and a C-terminal helical turn. To understand the role of specific residues in the membrane mimetic binding affinity, we replaced one or two aromatic residues (Phe/Trp) with alanine and monitored the interaction of the respective mutants with different membrane mimetics by 2D ^{1}H-^{15}N HSQC spectroscopy. To obtain more information about the immersion depth, 2D ^{1}H-^{15}N HSQC spectra of the ATM FATC domain in the presence of DPC micelles containing about 1-4 mM spin labelled stearic acids (either 5-SASL or 16-SASL) were analyzed. Overall the data provides insights into how the FATC may contribute to the observed localization of ATM at cellular membrane compartments [7].

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Bioliquids

P036

Heme regulates proinflammatory IL-36 cytokines

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Abstract: Cytokines of the interleukin-1 superfamily are known to regulate immune and inflammatory responses. The recently discovered members interleukin (IL)-36α, β and γ, are discussed to be involved in the pathogenesis of rheumatoid arthritis, psoriasis and pulmonary diseases. Activation of NF-kB via MAP kinases is mediated by binding of IL-36 proteins to their receptor IL-36R and leads to the stimulation of other proinflammatory cytokines such as IL-6 and IL-8 that are involved in inflammatory and apoptotic events. Knowledge of the regulation of the IL-36 cytokines is crucial for therapy development, especially when unusual and so-far unknown interactions are detected. We identified labile (regulatory) heme as a potential physiological regulator of IL-36α, as well as IL-36β and IL-36γ. Regulatory heme binding was demonstrated by advanced spectroscopic methods and structural analysis revealing an in-depth insight into the IL-36α-heme complex based on the NMR solution structure of IL-36α. Structural analysis of IL-36α revealed high N-terminal structural flexibility and gave insight into the requirement of N-terminal truncation for full biological activity. Proinflammatory signaling, e.g. induction of IL-6 and IL-8, mediated by the IL-36 cytokines, is significantly reduced upon heme binding. With this study we provide further insight into the role of labile heme as an effector molecule and its potential role in regulating the function of a human protein critically affecting the pathophysiology of IL-36-related inflammatory diseases.

Bioliquids

P037

NMR metabolomics on a chip: adherent vs. spheroid cell culture

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Abstract: Integration of nuclear magnetic resonance (NMR) with lab on a chip (LoC) technology provides a unique opportunity to study metabolic processes in cultured cells, cell aggregates, and tissue samples in situ.[1] In this work, we used a LoC cell culture device compatible with a custom-made transmission-line NMR detector to compare the metabolic activity of MCF7 cells adherent to a surface (2d) as opposed to cells aggregated into a spheroid (3d). A single device design, made of PMMA was used for 2d as well as 3d cell culture with of an equal number of cells. Both the devices were monitored for 48 hours to study the glucose consumption and lactate production. Microfluidic devices were made with a cell culture chamber of 2.5 µl volume. MCF-7 cell suspensions (1000 cells/ µl) were introduced into the devices after pre-coating with either Fibronectin (2d) or Pluronic (3d) to create 2d and 3d cell cultures, respectively. Cells either attach to the surface or aggregate to form a spheroid within 4 hours incubating the seeded chip [Figure (A)]. The devices were then sealed with optical adhesive film and put into a custom-made detector to obtain ^{1}H NMR spectra using a 600 MHz Varian NMR system. NMR spectra were taken at 4, 8, 24, 32, and 48 hours. Blank samples without cells were used as controls for both 2d and 3d pre-treatment. Figure (B) shows the time-lapse spectra of 2d and 3d cell cultures. Peaks were assigned for lactate (1.32 ppm), glucose (3.5 ppm), and the HEPES buffer (2.9 ppm). A higher rate of lactate production was observed in 2d compared to 3d cell culture. Shifts in the signals from the HEPES buffer, indicative of progressing acidification of the growth medium, were visible within 8 hours for 2d and within 24 hours in 3d cell culture. Almost all the glucose was consumed within 24 hours of 2d cell culture. However, 3d cell cultures consumed significantly less glucose and produced less lactate during the 48 hours culture time. The present cell culture model could be helpful towards metabolomics study of tissue culture on a chip or the ex-vivo study of drugs and toxicity testing.

Keywords: NMR, metabolomics, 3d cell culture

Reference:

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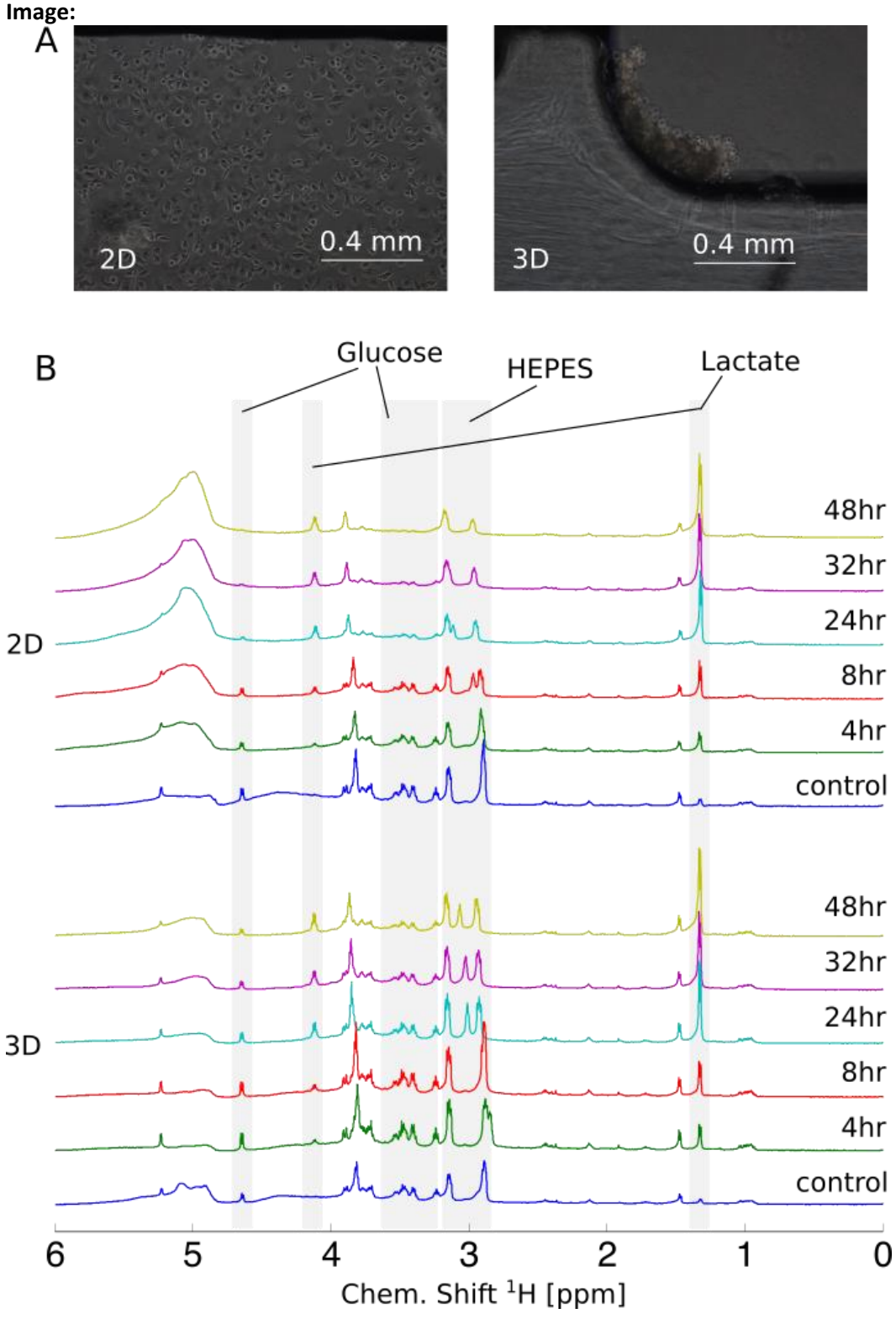
Image: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Bioliquids

P039

Insights on membrane translocation properties of homeodomains from NMR conformational studies in bicelles

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Abstract: Homeoproteins constitute a major class of transcription factors active throughout development and during adulthood. They were initially discovered in the fruit fly due to homeotic mutations. They are all characterized by a conserved 60 residue, three helix domain called homeodomain. In addition to interacting with DNA, homeodomain can also translocate across biological membranes by unconventional mechanisms that are not well characterized. Structure-function relationship studies on this domain have shown a critical role in internalization of the conserved Trp-48 as well as basic residues (Arg, Lys) located in the third helix. To date, most biophysical studies of this translocation process have been conducted on small peptides derived from the third helix of the homeodomain but rarely in a more relevant context of the full homeodomain.

We have analysed the conformation of Engrailed 2 homeodomain (En2HD) using bicelles as a membrane mimetic environment. NMR studies of En2HD show that a conformational transition is driven by the presence of anionic lipids leading to homeodomain unfolding. Remarkably, despite the loss of the native three-dimensional structure, near-native helical secondary structures are maintained in anionic membrane environments. The three helical segments adopt parallel orientations to the membrane surface. Residual Dipolar Couplings were measured in stretched polyacrylamide gels as an alignment medium to investigate in detail En2HD conformational space. Altogether, NMR spectroscopic data in combination with molecular dynamics simulations suggest that electrostatic interactions with membrane may be determinant not only in providing membrane affinity but also in inducing a conformational change in homeodomain structure enabling optimal interactions of basic residues with lipids and membrane anchoring of Trp-48.

Bioliquids

P040

Molecular recognition dynamics in ubiquitin establishes its binding to SH3 as conformational selection

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Abstract: Molecular recognition depends on conformational dynamics of the protein, i.e. the transitions between conformational states that are found in the complex with the binding partner and those that are not binding competent. The critical question is whether the conformational change to the binding competent conformation takes place prior to binding (conformational selection) or after the binding (induced fit). In literature this question is answered by studying the kinetics of chemical relaxation with varying ligand concentration. Although this is a 50 year old question [1, 2], this topic has been intensely discussed in the recent literature [3-5]. Recent NMR methods have provided tools to characterize kinetics of conformational interconversion [6]. Indeed, NMR has several powerful methods to measure kinetics, however the methods are effective in narrow timescale windows [7]. We have recently described the new Extreme CPMG (ECPMG) experiment that can measure kinetics in a much broader range of timescale [7]. With this experiment we can measure kinetics with varying concentration of ligand. This opens up the possibility of investigating the protein-protein interaction in unprecedented detail.

Ubiquitin is a model protein, which is a hub of cellular networks. It has been implied that ubiquitin recognizes the different partners by conformational selection [8]. The free ubiquitin exists as an ensemble in solution that encompasses the structures of ubiquitin in complex [8], however the question of conformational selection or induced fit is about kinetics. Till now there was no kinetic evidence for the structural interpretation of conformational selection. With the new Extreme-CPMG experiments and a novel analysis based on theoretical work [9] we establish that ubiquitin binds SH3 by conformational selection in a very comprehensive demonstration by measuring kinetics in a previously inaccessible regime. [9]. The theoretical expressions and the ECPMG experiment will allow us to apply this new methodology to a wide range of recognition processes.

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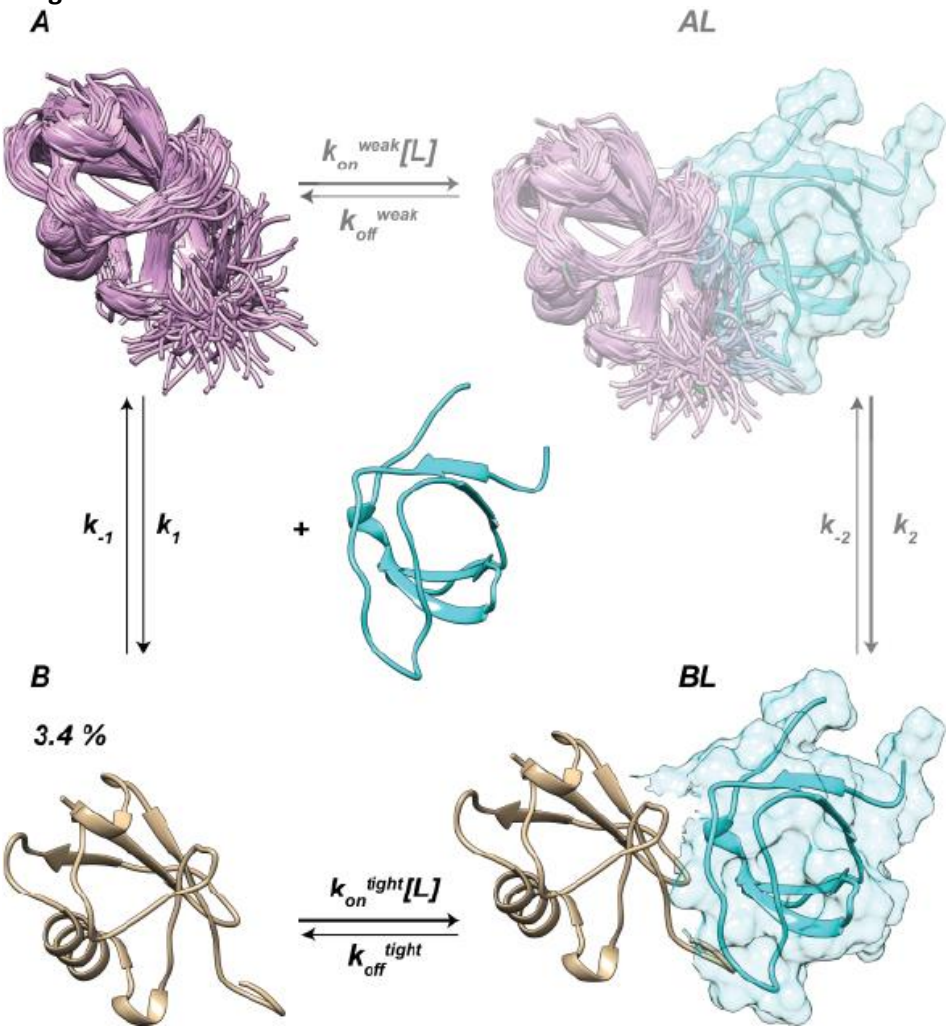
Image: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$k_{on}^{tight}[L]$
k_{off}^{tight}

Bioliquids

P041

New protein-DNA complexes in archaea: a small monomeric protein induces a sharp V-turn DNA structure

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Abstract: In archaea the two major modes of DNA packaging are wrapping by histone proteins or bending by architectural nonhistone proteins. MC1 (Methanogen Chromosomal protein 1) is a small basic monomeric nucleoid-associated protein (NAP) which is structurally unrelated to other NAPs. Like most proteins that strongly bend DNA, MC1 is known to bind in the minor groove. The first model of a complex in which MC1 binds to the concave side of a strongly bent 15 basepairs DNA was obtained by two complementary docking approaches.

This model allowed us to calculate the expected protein-DNA contacts and helped us to unambiguously identify 50 intermolecular distance restraints derived from NOEs. However, the use of NOEs restraints is generally not sufficient to determine global structural features such as bending in nucleic acid. RDCs constraints, known to improve the precision and accuracy of both the local and global structures of the double helix were measured on both the DNA and the protein. Their addition in the calculation was an absolute necessity to resolve the 3D structure of the complex.

We report here the 3D solution structure of a new DNA-protein complex formed by MC1 and a strongly distorted 15 base pairs DNA. While the protein just needs to adapt slightly its conformation, the DNA undergoes a dramatic curvature and an impressive torsional stress due to several kinks caused by the binding of MC1 to its concave side, and thus adopts a sharp V-turn structure. On longer DNAs, MC1 stabilizes multiple V-turn conformations in a flexible and dynamic manner. It participates to the genome organization of some *Euryarchaea* species through an atypical compaction mechanism. It is also involved in DNA transcription and cellular division through unknown mechanisms. The V-turn conformations of the MC1-DNA complexes open new opportunities to studying and understanding the different roles of MC1 in *Euryarchaea*.

Bioliquids

P042

Site-specific description of intrinsically disordered protein dynamics from multi-temperature modelling of NMR relaxation

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Abstract: While intrinsically disordered proteins (IDPs) consititute about half of the human proteome, a complete understanding of their functional behavior remains enigmatic. As proteins of this class do not possess a stable, three-dimensional structure, but rather sample flat energy landscapes, information about their function is inherently linked to their dynamics occurring on multiple timescales. Here, we present a recently developed approach [1] based on model-free analysis of ^{15}N relaxation rates, coupled with an Arrhenius type relationship to characterize temperature-dependent ps to ns dynamics of the intrinsically disordered domain of the cancer-associated mitogen-activated protein kinase kinase (MKK4), which was recently characterized structurally using chemical shifts and residual dipolar couplings [2]. In agreement with our previous observations, the obtained results point towards existence of three dominant dynamic modes on relaxation active timescales associated with fast librational motion, conformational sampling of backbone dihedral angles and slower chain-like segmental dynamics [1]. In addition, the temperature-dependent analysis, in the range of 273.1 to 288.1 K, allowed us to derive activation energies of the two latter modes at single-residue resolution revealing the existence of sequence dependent features of the dynamics in the unfolded chain. Altogether, the study presents a novel method for understanding the dynamic behaviour of IDPs underlying their function and/or malfunction in a myriad of diseases.

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Bioliquids

P044

Aspergillus fumigatus hydrophobin functional amyloids

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Abstract: Hydrophobins are fungal proteins characterised by a conserved amphipathic profile and an idiosyncratic pattern of eight cysteine residues involved in four disulphide bridges. Their functions are due to their remarkable physicochemical properties. Hydrophobins are secreted in a soluble form that self-assembles at hydrophobic/hydrophilic or air/water interfaces to form **amphipathic layers** showing the hallmarks of protein amyloids. Hydrophobin assemblies are thus **functional amyloids** used by fungi to breach the air/water barrier and develop aerial hyphae, to prevent water-logging, to cover aerial hyphae and spores rendering them hydrophobic thus facilitating aerial growth, spore dispersal and resistance to desiccation, to participate in the extracellular matrix or to form a protective layer during fruiting body development. Hydrophobins can also participate in host-fungi interactions.
Aspergillus fumigatus is the most important airborne fungal pathogen, causing over 200 thousands deaths per year among immunocompromised people. Its spores, which are the infectious form of the mould, are covered by an amyloid-fibre layer with rodlet morphology formed by a hydrophobin called RodA. This rodlet coat renders the spores inert relative to the innate and adaptive human immune systems. We have solved the solution structure of RodA, studied its self-assembly *in vitro*, performed a mutational analysis to highlight the regions involved in the formation of the amyloid core of the rodlets and on their lateral association to form layers, correlated the kinetics of rodlet formation *in vitro* with their rate of appearance on the spores and analysed the relationship between the structure of RodA and its immunological properties. We have also studied two close homologues of RodA, named RodB and RodC, which form functional amyloids on *A. fumigatus* conidial cell wall.

Bioliquids

P045

Blood is thicker than water: The hormone hIAPP forms toxic oligomers in plasma involving type 2 diabetes and cardiovascular diseases

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Abstract: Human Islet Amyloid Polypeptide (hIAPP) and Insulin are fundamental hormones that control the glucose metabolisms in mammalians. During Type 2 diabetes (T2D) hIAPP produces β-cell destruction. Since this strong amyloidogenic polypeptide circulates freely in blood plasma, the questions how it behaves in blood, which molecules can cause structural or kinetic changes on it and how these interactions can be responsible for their accumulation in other organs have been raised. In our study, these questions have been addressed showing for the first time that diabetic blood plasma has the capacity to stabilize hIAPP oligomers. Additionally, we identified that low-density lipoprotein (LDL) and sugars can stabilize oligomers in the same way. These oligomers show increased β-cell toxicity and hemolytic activity in a concentration-dependent manner. We characterized these conformations using a broad number of biophysical experiments including high-resolution NMR spectroscopy and demonstrated that a higher degree of the structure of hIAPP is produced as a result of these interactions and these changes generate intrinsic fluorescence on the peptide. Our results provide insights towards a better understanding of off- and on-pathway oligomer formation in a native environment suggesting a link between metabolic and cardiovascular diseases due to the possibility that hIAPP could play a role as a nucleation prone system in other parts of the body. Our study opens a new viewpoint in which a broad number of researchers can find new perspectives for the development of therapeutics using NMR.

Bioliquids

P046

Toward the three-dimensional structure of pore-forming amyloid-β oligomers by solution NMR

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Abstract: Alzheimer's disease (AD) pathogenesis is associated to the production and deposition of the amyloid-β peptide (Aβ). Due to the pivotal role of Aβ in the pathobiology of AD, great efforts have been made to determine the exact form/s of the Aβ peptide responsible for neuronal dysfunction to then develop efficient therapeutic strategies targeting it/them. Hence, the determination of the different Aβ molecular assemblies and the mechanisms by which they underlie its pathological effects are of great interest.

Mature Aβ fibrils have long been considered the cause of AD. However, in the last decades many studies have demonstrated that fibril formation is preceded by various intermediates such as soluble oligomers and protofibril forms, which are suggested by experimental and clinical findings to be particularly important for the pathogenesis of AD.[1],[2]

We are working with the hypothesis that pore-forming Aβ oligomers can be formed in a membrane environment.[3] We have recently worked with the two major Aβ variants (Aβ40 and Aβ42) and handled them as membrane proteins.[4] By working under detergent micelle conditions, intended to mimic the hydrophobic environment of the membrane, we found that Aβ42, the Aβ variant most strongly linked to AD, assembled into a stable oligomer that adopted a specific structure in the form of a β-barrel arrangement. This oligomer also incorporated into membranes as pores, a feature linked to neurotoxicity. Based on the properties of this oligomer, we named it β-barrel pore-forming Aβ42 oligomer (βPFO). On the contrary, when we incubated Aβ40, the Aβ variant most abundantly produced, under the same conditions, it aggregated into fibrils.

Here we present our progress toward the determination of the three-dimensional structure of βPFO using solution NMR. Having access to the 3D structure of this oligomer will be crucial to assess the druggability of βPFO and thus to design molecules that target the formation of this type of oligomer and/or blockage the pores it forms.

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Bioliquids

P047

NMR study of human Lypd6 and Lypd6b proteins: differences and common features with other Ly-6/uPAR proteins acting on nicotinic acetylcholine receptors

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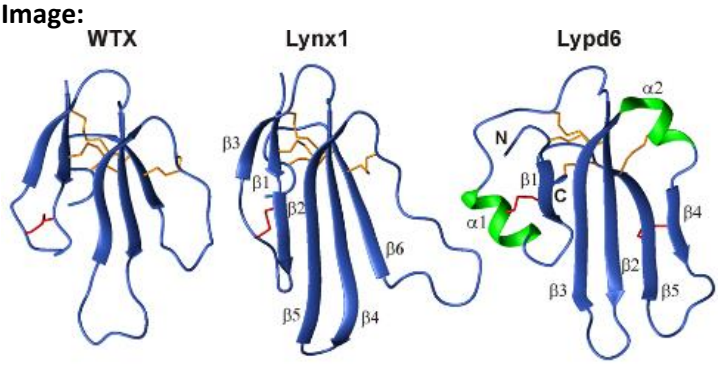
Abstract: Several endogenous ligands of nicotinic acetylcholine receptors (nAChRs) belonging to the Ly-6/uPAR family were discovered in higher animals. These proteins share structural homology with 'three-finger' snake α-neurotoxins, specific inhibitors of nAChRs. Some of these proteins (e.g. Lynx1, Lypd6) are membrane-tethered via GPI-anchor and co-localize with nAChRs modulating their functions in the brain. Others (SLURPs) are secreted and act as autocrine/paracrine hormones in epithelium. Lypd6 and Lypd6b are expressed in the brain and unlike to other human Ly6/uPAR proteins have additional long *N*- and *C*-terminal sequences flanking the 'three-finger' LU-domain. C-terminal sequences contain sites for GPI-anchor attachment. Lypd6 increases the amplitude of nicotine-induced calcium currents in mouse trigeminal ganglion neurons. Lypd6 of *D. rerio* fish is involved in the regulation of Wnt/β-cathenin signaling pathway, and the knockdown of Lypd6 leads to the impairment of embryonic development. Expression of Lypd6b in *X. laevis* oocytes increases the sensitivity of nAChRs to acetylcholine and their desensitization rate.

In the present work the water-soluble LU-domains of human Lypd6 and Lypd6b have been expressed in *E. coli* cytoplasmic inclusion bodies with subsequent solubilization and refolding, as well as LU-domain of Lypd6 with the additional *N*-terminal sequence. Analysis of recombinant N-Lypd6, Lypd6 и Lypd6b by NMR revealed that N-Lypd6 is likely to be unfolded. Spatial structure and intermolecular dynamics of Lypd6 and Lypd6b were studied using ^{13}C,^{15}N-labeled proteins. According to obtained NMR data, the proteins adopts typical 'three-finger' fold and possess three loops (I, II, III) protruding from the β-structural core ('head'). In contrast to other Ly6/uPAR proteins, the Lypd6 and Lypd6b contain only one β-sheet formed by five strands and involving residues form all three loops. The loops I and III are stabilized by additional disulfide bonds and accommodate two 'unexpected' α-helical elements. *C*-terminal regions (87-95) of Lypd6 and Lypd6b demonstrate conformational heterogeneity in solution possibly connected with *cis-trans* isomerization of Leu85-Pro86 bond going with characteristic time ~0.1 s. The sites of exchange motions were also observed in the β-strands of the molecules. ^{15}N-relaxation data revealed significant ps-ns mobility in the unstructured *C*-terminal region of both proteins and in the Lypd6b loop II. The overall rotation correlation time determined for Lypd6 and Lypd6b (~5.5 ns at 30 °C) confirmed monomeric state of proteins in solution.

Obtained spatial structure of Lypd6 permitted to model its interactions with the α4β2 neuronal nAChR. Protein-protein docking and molecular dynamics revealed tight contacts between the loop I of Lypd6 and the entrance to the agonist binding site that is located between the α4 and β2 subunits of the receptor.

The work was supported by the Russian Science Foundation (project № 16-14-00102).

Image:



Bioliquids

P048

Fluorine NMR study of the interaction between polyprolines and a SH3 domain

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Abstract: SH3 are small protein domains that recognize poly-proline helices in PP2 conformations. They are involved in numerous regulatory functions in eukaryotes. We have investigated such a regulatory mechanism in the case of Retinoic Acid nuclear hormone receptor, whose gene transactivation function involves its interaction with a SH3 domain of a co-regulator: the vinexin b. The affinity of this interaction is modulated by the phosphorylation of a serine residue by a specific kinase, cdk7. To investigate the molecular basis of modulation, we used polyproline helices harbouring different types of fluorinated prolines. The combination of the NMR properties of fluorine with the conformational perturbations resulting from its introduction in the proline ring allows to establish interesting correlations between the conformational properties of fluorinated prolines and their SH3 binding properties. We used an approach based on model peptides to study these correlations. The insertion of modified prolines was performed at sequence positions that avoided a direct interaction between the fluorine atom and the protein surface. The affinity between several peptides and SH3 were measured using both classical chemical shift perturbation methods, based on 1H-15N HSQC as well as the fluorine chemical shifts. These measurements were performed on the same samples thanks to the performance of a QCIF cryoprobe enabling both the resonances of the ligand and the receptor to be observed at the same time. Fluorine line shape analysis performed throughout the peptide-protein titration allowed kinetic parameters of the interaction to be obtained in addition to equilibrium concentrations.

Bioliquids

P049

Further strategies for NMR assignments and probing transient interactions with heme of intrinsically disordered proteins

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Abstract: Intrinsically disordered proteins and intrinsically disordered protein regions attached to folded protein domains (IDPs) have been found to be involved in critical biological functions[1,2].

In contrast to covalent binding, as in many metalloproteins, IDPs have been shown to be involved in functionally important transient interactions. In many cases, heme binding motifs or heme regulatory motifs with special amino acid combinations ($CXXHX_{16/18}H$ and CP) in small sequence stretches have been found to be responsible for the heme association with moderate binding constants[3,4]. Different spectroscopic studies have shown the possibility for IDPs to undergo conformational changes upon heme binding[5,6].

NMR spectroscopy is one of the few spectroscopic techniques allowing the investigation of IDPs and potential IDP-ligand interactions at an atomic scale.

Nevertheless, sequence specific resonance assignment is a key step in NMR based investigation of biomolecular systems. In the study of IDPs, although a large number of resonance assignment strategies have been reported, it is clear that there is no unique experiment that can be used for resonance assignment in all experimental situations. The applicability of the most often employed [^{1}H,^{15}N]-HSQC approach for resonance assignment or for mapping protein-ligand interactions in IDPs can be limited under physiological conditions (e.g. temperature or pH). From alternative strategies involving $^{1}H^{\alpha}$ and ^{13}C direct detection it is apparent that dispersion of cross-correlation peaks observed in spectra such as $^{15}N–^{13}C'$ and $^{13}C'–^{1}H$ is typically better in IDPs. Hence, multidimensional experiments based on such heteronuclear correlations are expected to bear considerable impact on to the study of IDPs. In continuing our previous work, where the application of band-selective ^{15}N-$^{13}C^{\alpha}$ heteronuclear Hartmann-Hahn (HEHAHA) mixing sequence was used to achieve transfer of ^{15}N magnetisation to adjacent ^{15}N nuclei for resonance assignment[7,8], we demonstrate here that the ^{15}N-$^{13}C^{\alpha}$HEHAHA can also be applied to transfer $^{13}C^{\alpha}$ magnetisation to neighbouring $^{13}C^{\alpha}$ without relaxation losses. This is shown by the acquisition of 3D (HA)CA[CAN HEHAHA](CA)CON data of α-synuclein. The possibility to obtain correlation spectra arising from the simultaneous transfer of ^{15}N and $^{13}C^{\alpha}$ magnetisations in one-shot is also demonstrated. In addition, we show that the HCBCACON experimental protocol provides an alternative approach for probing transient interactions of heme interacting IDPs (Figure 1). Heme binding studies were carried out with a GB1 fusion peptide sample of intrinsically disordered N-terminal segment of human cystathionine-β-synthase.

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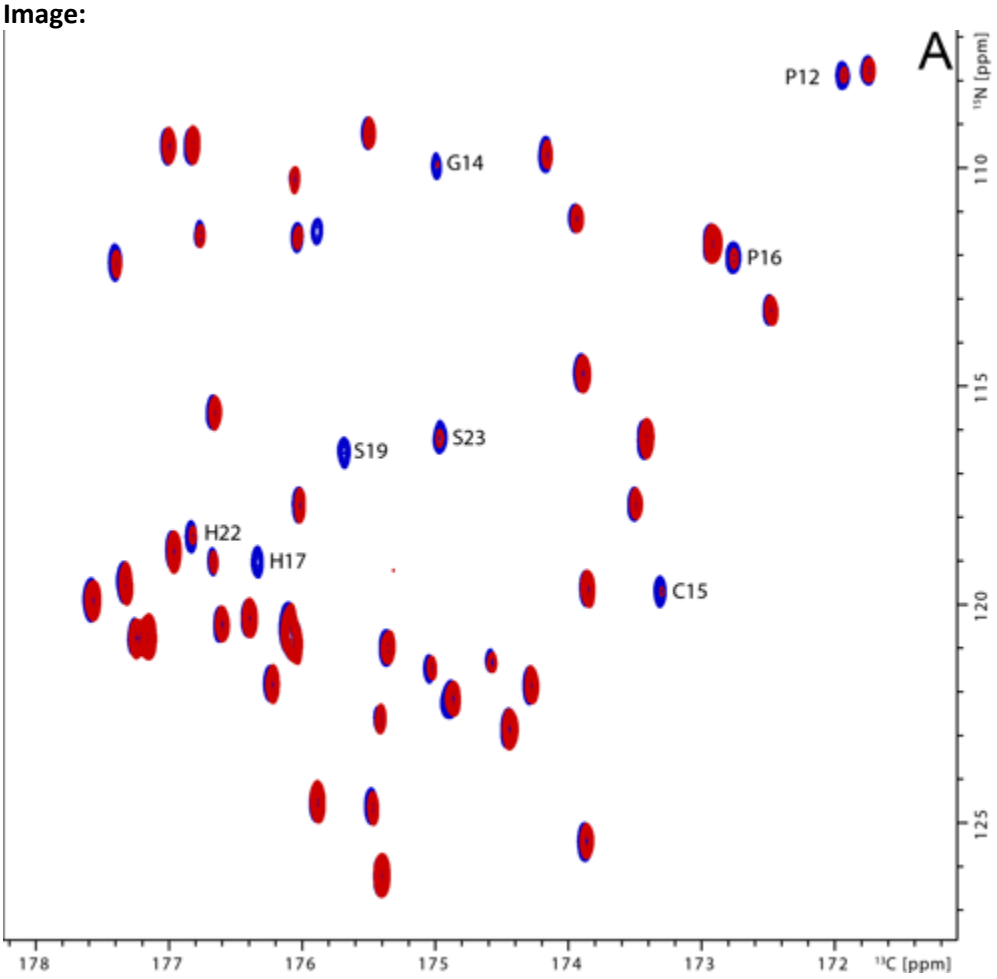
A
P12
G14
P16
S19
S23
H22
H17
C15
178
177
176
175
174
173
172
^{13}C [ppm]
110
115
120
125
^{15}N [ppm]

Bioliquids

P050

Solution structure of Helicobacter pylori HypA from 4D NMR experiments

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Abstract:

Helicobacter pylori HypA (*Hp*HypA) is Ni metallochaperone necessary for [Ni,Fe]-hydrogenase maturation and for the insertion of Ni(II) in the active site of urease. *Hp*HypA contains a tight-binding structural Zn(II) site as well as a unique lower affinity Ni(II) binding site. The resonance assignment and solution structure of apo- *Hp*HypA, containing Zn(II) but devoid of Ni(II), was determined using 2D, 3D and 4D NMR data.

Distance restraint were extracted from 4D NOESY experiments: 4D [1H,1H]-NOESY experiments recorded in the ^{15}N,^{15}N-resolved, $^{13}C_{ali}$,^{15}N-resolved, $^{13}C_{ali}$,$^{13}C_{ali}$-resolved, $^{13}C_{ali}$,$^{13}C_{aro}$-resolved and $^{13}C_{aro}$,$^{13}C_{aro}$-resolved varieties were recorded using a Bruker AVANCE 800 MHz spectrometer equipped with 5 mm TCI-HCN z-gradient cryo-probes. All 4D experiments utilized sparse sampling of indirectly sampled dimensions and were processed using the Signal Separation Algorithm, implemented in the cleaner4d software [1]. The spectra were inspected and manually peak-picked using the UCSF Sparky software [2], and the sidechain resonances were manually assigned using the sequence-specific backbone assignment.

The structure was determined with an approach (named YARIA) that combines the iterative protocol for NOE assignment implemented in ARIA software [3] with the YASARA molecular dynamics software [4].

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Bioliquids

P051

Temperature dependence of ps to ns motions in protein side chains

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Abstract: Protein side chain motions play a critical role in protein function: these motions allow the local structure to adapt to binding partners and are essential for protein thermodynamics (Frederick *et al.* 2007). Many studies have been performed to quantify motions of side chains containing methyl groups, based in particular on deuterium relaxation rates. Additional insight can be obtained from the temperature dependence of relaxation rates, which provides information about protein thermodynamics (Massi *et al.* 2004) as well as the activation energies of motions (Lewandowski *et al.* 2015). We have recently developed a new approach to determine ps-ns motions of side chain methyl groups using a combination of high-resolution relaxometry and molecular dynamics simulations (Cousin *et al.* - submitted), that we applied to study of the isoleucine side chains (δ_1 methyl groups) of ubiquitin.

Here, we extend this approach to other residues containing methyl groups and that can thus serve as probes of local motion, with the $^{13}C^2H_2{}^1H$ labelling of Leu^{proS} and Val^{proS} methyl groups that allows us to probe hydrophobic moieties located in the core as well as on a surface patch of the protein. We have measured high-field ^{13}C relaxation rates at temperatures ranging from 5.4 ºC to 35 ºC. High frequency motions are quite variable, as shown by the different temperature dependence of heteronuclear steady-state nuclear Overhauser effects (Figure 1). Rotating-frame relaxation rates $R_{1\rho}$ confirm that several side chains undergo a slow exchange process (especially V17, L50, and L56). This shows that high-field relaxation rates alone cannot be used to analyse ps-ns motions of side-chain methyl groups. The investigation of these motions in the low nanosecond range with the use of high-resolution relaxometry is underway and will be interpreted with long molecular dynamics simulations. This work will provide a full picture of nanosecond motions throughout the hydrophobic core of ubiquitin.

Figure 1: Steady-state NOE relaxation rates of 13C2H21H labelled methyl groups of Ubiquitin. Data were acquired at 18.8 T (5.4 °C, 13 °C, 25 °C, 35 °C) and at 14.1 T (25 °C, 35 °C).

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Image:

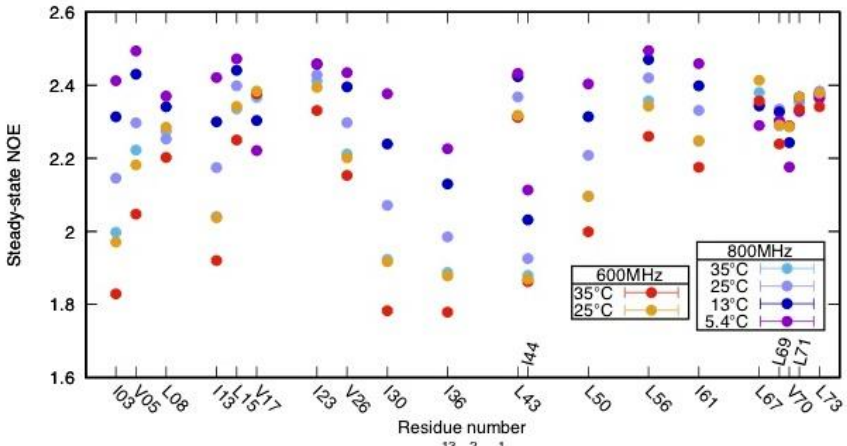

Figure 1: Steady-state NOE relaxation rates of $^{13}C^{2}H_{2}{}^{1}H$ labelled methyl groups of Ubiquitin. Data were acquired at 18.8T (5.4°C, 13°C, 25°C, 35°C) and at 14.1T (25°C, 35°C).

Bioliquids

P052

Structural evidence of a phosphoinositide-binding site in the Rgd1-RhoGAP domain.

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Abstract: Phosphoinositide lipids recruit proteins to the plasma membrane involved in the regulation of cytoskeleton organization and in signalling pathways that control cell polarity and growth. Among those, Rgd1p is a yeast GTPase-activating protein (GAP) specific for Rho3p and Rho4p GTPases, which control actin polymerization and stress signalling pathways [1]. Phosphoinositides not only bind Rgd1p, but also stimulate its GAP activity on the membrane-anchored form of Rho4p [2]. Both F-BAR (F-BAR FCH, and BAR) and RhoGAP domains of Rgd1p are involved in lipid interactions. A specific interaction has been observed between phosphoinositides and the Rgd1p-RhoGAP domain [3]. Its doubly labelled 15N13C form has been overexpressed in E.coli and purified.
This allowed us to assign the backbone resonances and the secondary structure [4]. We report the X-ray structure of the Rgd1p-RhoGAP domain, identify by NMR spectroscopy and confirm by docking simulations, a new but cryptic phosphoinositide-binding site, comprising contiguous A1, A1' and B helices [5]. The addition of helix A1', unusual among RhoGAP domains, seems to be crucial for lipid interactions. Such a site was totally unexpected inside a RhoGAP domain, as it was not predicted from either the protein sequence or its three-dimensional structure. Phosphoinositide-binding sites in RhoGAP domains have been reported to correspond to polybasic regions, which are located at the unstructured flexible termini of proteins. Solid-state NMR spectroscopy experiments confirm the membrane interaction of the Rgd1p-RhoGAP domain upon the addition of PtdIns(4,5)P_2 and indicate a slight membrane destabilization in the presence of the two partners.

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Bioliquids

P053

Stabilization of an Amphipol-reconstituted Amyloid-β Oligomer

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Abstract: We have previously reported on the preparation and initial characterisation of an Aβ42 oligomer that inserts into lipid bilayers as a well-defined pore with characteristics indicative of a β-barrel structure. We have termed this species "*β-barrel Pore-Forming* Aβ42 Oligomer" ($\beta PFO_{A\beta 42}$) and, due to its stability and homogeneity, it represents an opportunity to characterise a specific oligomer-form of potential relevance to the progression of Alzheimer's disease. However, the detergent micelle conditions required for the preparation of $\beta PFO_{A\beta 42}$ are incompatible with many *in vitro* and *in vivo* tests wherein dilution of the detergent leads to oligomer destabilisation. Herein, we describe the process of exchanging DPC micelles for amphipols (APols); amphipathic polymers designed to stabilize membrane proteins in aqueous solution. APols bind in an irreversible, albeit non-covalent, manner to the hydrophobic surface of membrane proteins maintaining their structure in spite of dilution. We tested the stability of $\beta PFO_{A\beta 42}$:DPC following exchange by three different APols with distinct physical-chemical properties. Data from SEC, SDS-PAGE and NMR spectroscopy showed that the $\beta PFO_{A\beta 42}$/DPC complex can only be trapped in non-ionic APols (NAPols), which then preserve its oligomeric structure and stability even after extreme dilution. Furthermore, Thioflavin T fluorescence measurements and transmission electron microscopy confirm that the sample is devoid of Aβ42 fibrils and homogeneous in nature. Based on these findings, this work constitutes a first step towards the *in vivo* validation of $\beta PFO_{A\beta 42}$ in AD.

Bioliquids

P054

NMR structure of the enzymatically active domain of human Aprataxin - a quite challenging DNA repair protein requesting the full range of NMR tools

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Abstract: The enzyme Aprataxin resolves AMP-DNA intermediates that are formed during abortive DNA single strand break repair. The human full-length protein consists of 356 amino acids harbouring an FHA domain mediating interactions with other proteins, and the enyzmatically active HIT-ZnF domain. Whereas the FHA domain is replaced by other functional domains in non-vertebrates and absent in lower eukaryotes, the HIT-ZnF is highly conserved among the APTX orthologous. Mutations inside the catalytically active domain are responsible for a destinct subtype of an autosomal recessive cerebellar ataxia called ataxia with oculomotor apraxia type 1 (AOA1). Besides it's unique function during DNA repair, the protein has attracted attention as possible drug target, as lower levels of APTX in patients suffering from certain colon cancers seems to be benefical for chemotherapy with topoisomerase inhibitors - a situation in which single strand breaks are activly induced.

Although the expression and purification protocol of the 22kDa HIT-ZnF domain could be set up straight forward, the collection of suitable NMR data was hampered by the aggregation propensity at concentrations above 100 µM as well as by an quite unfavourable relaxation behavoir. We will present data on the way towards an high resolution NMR structure including the use of an stabilizing cholin-based buffer, extensive deuteration, selective Methyl-labeling and Residual Diploar Couplings (RDCs). In addition we will discuss the application of state-of-the-art experiments for backbone and methyl-group assignment.

Bioliquids

P055

Structure and Dynamics of the HNOX domain of the human soluble Guanylate Cyclase

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Abstract: The human soluble guanylate cyclase is a heterodimer that catalyzes the production of the secondary messanger cGMP upon sensing of NO. Binding of NO onto the heme of the HNOX domain, allostericaly trigers the increase of catalytic conversion of GTP to cGMP with an as-of-yet unknown mechanism.
Here we present the solution NMR structural and dynamical characterization of the human β1 H-NOX bound to the heme mimetic cinaciguat. It is shown that this complex has a solution structure similar to the ones determined previously for bacterial homologues. However, it appears to be very flexible in the µs to ms timescale in a manner that is consistent with the hypothesis that cinaciguat modulates the dynamics of H-NOX.

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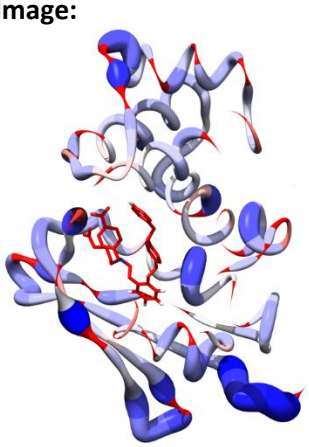

Bioliquids

P056

Transient Chaperone-Substrate Interactions Visualized using NMR Spectroscopy, X-Ray Crystallography and Coarse Grain Molecular Modeling

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Abstract: Chaperones are key elements in the regulation of protein folding, aggregation prevention and cellular stress response. Despite their critical biological function, the mechanisms by which chaperones interact with their biological substrates remain particularly elusive, in part due to the important role played by conformational disorder[1].
Here we describe the interaction between Spy, a recently discovered chaperone, and Im7, an *in vivo* substrate[2]. Spy is a small ATP-independent periplasmic chaperone. Its minimalistic character makes it an ideal candidate to define the fundamental properties required for chaperone activity. A possible role of Spy conformational dynamics in its chaperone activity was suggested by the fact that often Spy mutants with increased flexibility also present improved chaperone activity [3].
To unravel how Spy interacts with its substrate Im7, we developed two parallel approaches, combining coarse grain modeling with either NMR spectroscopy or X-ray crystallography. In the first approach, NMR data were used to create NMR-informed molecular models that were applied to characterize at the residue-specific level the two partners either individually or in interaction[4]. In the second approach, by classical crystallographic methods only Spy structure could be solved due to the high degree of Im7 dynamics in the complex. To overcome this problem, we implemented a sample and select approach, inspired from NMR methodologies to build a conformational ensemble of Im7 in interaction with Spy, using the residual electron density complemented with multiple site-specific iodine labeling[5,6]. The two approaches provide a coherent and convergent picture of a chaperone-substrate interaction allowing for unprecedented description of the mechanism by which chaperone proteins act.

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Bioliquids

P057

Dynamical studies of the Mef2D ß-domain by NMR and computational methods

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Abstract: The MEF2 transcription factors play crucial roles in muscle cell myogenesis and morphogenesis[1]. Bioinformatical analysis indicates that the alternatively spliced b-domain of MEF2D does not fold into a well-defined structure and also remains to be conformationally heterogeneous in its bound state. To study the role of protein dynamics in the biological function, a series of 15-37 AA variants containing the MEF2D ß-domain were designed. These peptide constructs differ in their predicted degree of fuzziness, i.e. dynamics in their bound state.

The dynamics of the free peptides has been studied using ^{15}N relaxation measurements (T_1, T_2, heteronuclear NOE), from which spectral density functions were calculated. Random Coil Indexes (RCI) were determined from the chemical shifts (CA, CB, CO, N, HA) to derive model-free order parameters [3]. NMR-ensembles of the peptide variants were generated on the basis of NOE distance restraints using torsion-angle dynamics. Each minimized conformation has been subjected to molecular dynamics simulations (MD) to explore the conformational space. Residue fluctuations, order parameters of the backbone amide bond vectors and the propensity of secondary structure elements were determined from the 300 ns long MD trajectories, which were in reasonable agreement with the predicted parameters. Detailed analysis of the dynamical and structural properties of the peptides will give insights into the altered interaction patterns of the MEF2D ß-domain corresponding to altered biological activities.

Acknowledgements: Financial support from grants from the EU co-financed by the European Regional Development Fund under projects GINOP-2.3.3-15-2016-00004, GINOP-2.3.2-15-2016-00044 and GINOP-2.3.2-15-2016-00008 is gratefully acknowledged.

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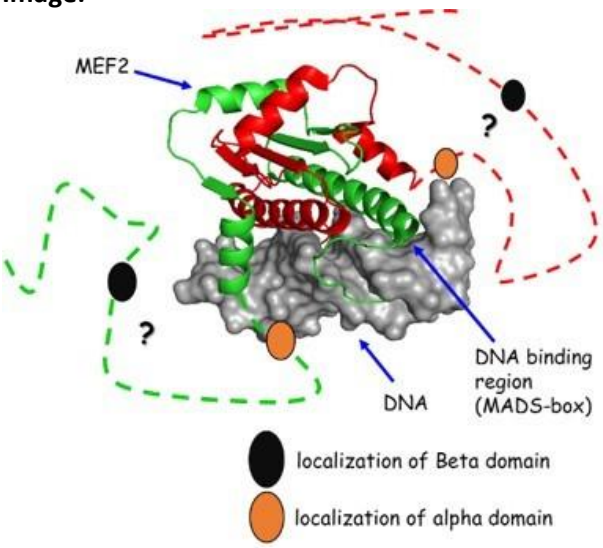
NMR *structure of MEF2-DNA complex (PDB:1c7u)[2], Mef2D is displayed by green and red cartoon, scattered lines indicate regions with missing electron density. The localization of the ß-domain is represented by black circles.*

Image:



Bioliquids

P058

Recognition of the Magnaporthe oryzae effectors AVR-Pia and AVR1-CO39 by the decoy domain of the rice NLR immune receptor RGA5

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Abstract: Plants have evolved a complex immunesystem to protect themselves against phytopathogens. A major class of plant immune receptors called nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) is ubiquitous in plants and is widely used for crop disease protection,making these proteins critical contributors to global food security (Cesari, 2017). NLRs are important receptors in plant immunity that allow recognition of pathogen effectors. The rice NLR RGA5 recognizes the Magnaporthe oryzae effectors AVR-Pia and AVR1-CO39 through direct interaction (Cesari et al., 2013). Here, we gained detailed insights into the molecular and structural bases these interaction and the role of the RATX1 decoy domain of RGA5. We previously solve the NMR structures of AVR-Pia and AVR1-CO39 and we have demonstrated that they belong to the MAX-effector new family (de Guillen et al., 2015). NMR titration combined with in vitro and in vivo protein-protein interaction analyses identified the AVR-Pia and AVR1-CO39 interaction surface that binds to the RATX1 domain (Ortiz et al., 2017). Structure-informed AVR-Pia and AVR1-CO39 mutants showed that, although these effectors associates with additional sites in RGA5, binding to the RATX1 domain is necessary for pathogen recognition, but can be of moderate affinity. Therefore, RGA5-mediated resistance is highly resilient to mutations in the effector. We propose a model that explains such robust recognition as a consequence and an advantage of the combination of integrated decoy domains with additional independent effector-NLR interactions.

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Biosolids

P059

Solid-state NMR study of the microalga Chlamydomonas reinhardtii and its constituents.

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Abstract: *Chlamydomonas reinhardtii* is a popular model unicellular microalga used for the study of functions as varied as photosynthesis, cell-division, or, more recently, mitochondrial function [1]. This micro-organism could also potentially be used as an environmental sensor by monitoring how different parts – the glycoprotein-rich cell-wall in particular - are affected by the external medium. Solid-State NMR is non-destructive and can in certain cases be applied to the *in vivo* study of micro-organisms[2]. We thus explore how SS-NMR might be employed to study this organism *in vivo*.
In a first step, we have studied the intact algae fully ^{13}C labelled. Whole cell-spectra are simplified by applying different polarization transfer excitations (ultrashort CP, NOE and INEPT) which differentially excite rigid, semi-rigid and mobile species in the algae [3]. Resolution is further enhanced by dipolar (DARR or PDSD) or J-mediated 2D polarization transfers [4]. This approach allowed us to discriminate and characterize the highly crystalline starch reserves *in situ* and to assign the highly mobile and abundant galactolipid headgroups. The complex cell wall of this microalga appears to have considerable dynamic complexity since both rigid and mobile saccharides are found in the cell wall glycoproteins.
In order to improve their assignment and characterization, it appears to be necessary to isolate parts of the microalgae. Due to the importance of starch - the main energy storage molecule in the plant kingdom and most used polysaccharide in the food industry - we concentrate on its detailed characterization after extraction from *C. reinhardtii* and compare it to the *in situ* case.

In addition to providing an extensive characterization of a whole microalga, the NMR strategies described herein might prove valuable in the study of other micro-organisms *in vivo*.

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Biosolids

P060

Investigation of alpha-synuclein aggregates by cryogenic DNP enhanced NMR

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Abstract: Protein misfolding is the primary reason for the Alzheimer's, Parkinson's and type II diabetes diseases.[1] Growing evidence suggests, that oligomers of these proteins, formed during the aggregation process, constitute the major toxic species.[2] In our and collaborating groups a series of 3,5-diphenyl-pyrazole derivatives have been designed and synthesized to interfere with the processes associated with neurodegenerative diseases. Among these compounds, anle138b showed the highest efficacy in mouse models of taupathies, Parkinson's-, Alzheimer's- and Prion Disease.[3-5] Understanding the nature of the particular proteins and their folding/fibrillization patterns including their interactions with proposed drugs at an atomic level is paramount. In this work, we study alpha-synuclein, an intrinsically disordered protein associated with Parkinson's disease, and investigate interaction with the small molecule anle138b by cryogenic DNP enhanced NMR. 100K temperatures for DNP allow us to study the oligomers of the protein alpha-synuclein in association with relevant low concentrations of anle138b.

Fig. 1: Solid-State-NMR-spectra of ^{15}N-labelled α-synuclein oligomers in the presence of ^{13}C-labelled anle138b and phospholipids.

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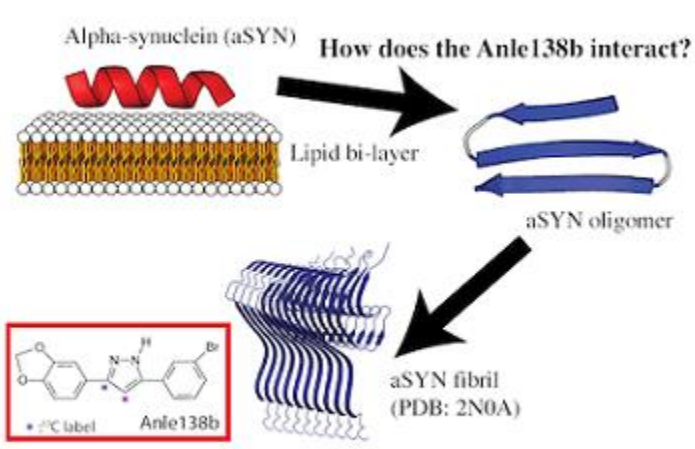
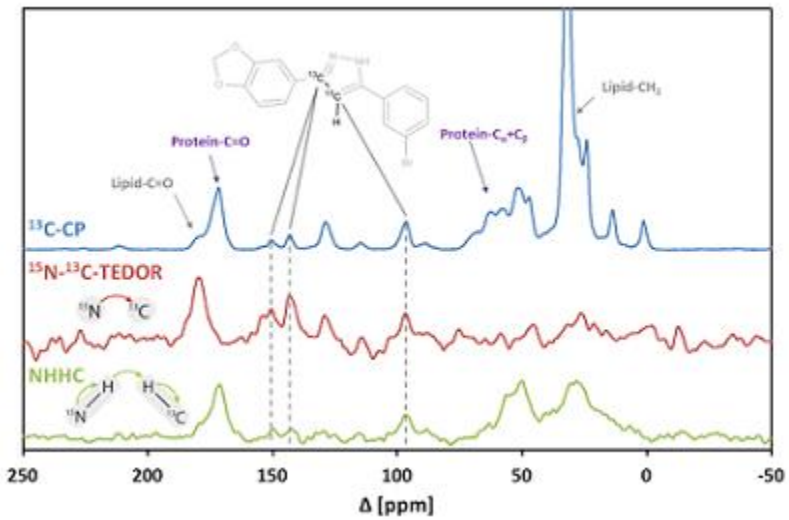
Image:





Biosolids

P061

Overall protein rocking in crystals as observed by the near-rotary-resonance 15N NMR relaxation in the rotating frame

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Abstract: The NMR relaxation in the rotating frame is a powerful tool for studying molecular motions in the microsecond time scale. Under MAS, the relaxation rate $R_{1\rho}$ due to the heteronuclear dipolar and CSA relaxation mechanism is determined by the spectral density functions $J(\omega_1 \pm \omega_{mas})$ and $J(\omega_1 \pm 2\omega_{mas})$, where $\omega_1/2\pi$ and $\omega_{mas}/2\pi$ are the spin-lock and MAS frequencies. If the difference between ω_1 and ω_{mas} is small, then one may experimentally sample very slow motions in solids. This makes $R_{1\rho}$ experiments under MAS very attractive for studying slow molecular dynamics in various molecular systems. At the same time, conducting measurements and analysis of the relaxation data in the vicinity of the rotary-resonance condition are associated with several methodological challenges that should be properly handled. In this work these issues are considered in detail by the example of the ^{15}N relaxation in solid protein samples. Among them are the signal amplitude as a function of the difference between ω_1 and ω_{mas}, "dead time" in the initial part of the relaxation decay caused by transient spin-dynamic oscillations, measurements under HORROR condition and proper treatment of the multi-exponential relaxation decays. We formulate several practical recommendations for optimal experimental setup and correct analysis of the data.

The ^{15}N $R_{1\rho}$ measurements are very well suited for a quantitative investigation of a specific type of molecular dynamics reported recently – overall rocking motion of proteins within a crystal lattice. We have performed multiple $R_{1\rho}$ measurements at a wide range of differences between ω_1 and ω_{mas} and various temperatures for a set of four microcrystalline protein samples - GB1, SH3 domain, MPD-ubiquitin and PEG-ubiquitin (the two last ones are different crystal forms of the same protein). The quantitative analysis of the data made possible quite accurate determination of the amplitude and time scale of the rocking motion in these samples. We found that the correlation time of the rocking motion is almost the same for all samples (30-50 μs), however, the amplitudes are very different. The absolute values of the order parameter of the rocking motion are rather high; for different samples it varies from 0.987 to 0.9995. This corresponds to the angular amplitude of few degrees. The comparison of the results for different samples reveals a correlation between the rocking motion amplitude and the density of protein packing in a crystal lattice. The results suggest that the rocking motion is not diffusive but likely jump-like dynamic process.

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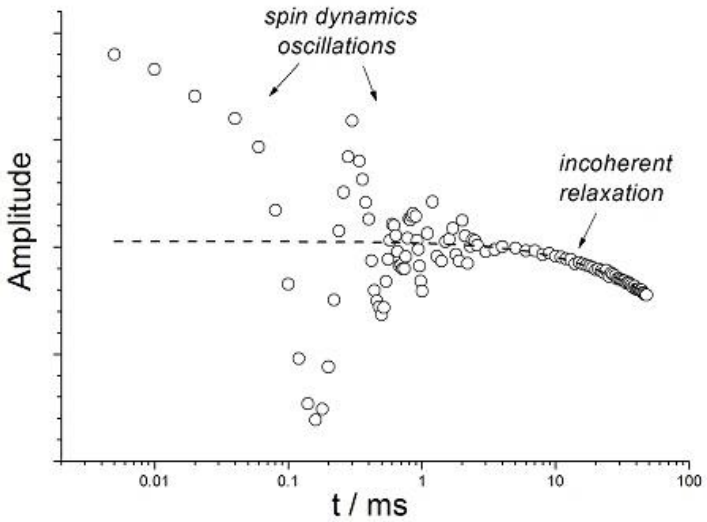

Biosolids

P062

Role of the C-terminal region specific to Lamin A encoded by exons 11 and 12 of LMNA

Florian Celli[*1], Camille Samson[2], Ambre Petitalot[1], François-Xavier Theillet[3], Sophie Zinn-Justin[1]
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Abstract: Lamins are the main components of the nucleoskeleton. They are primarily located at the nuclear envelope, where they interact with inner nuclear membrane proteins, chromatin-associated proteins and cell signaling modulators. The LMNA gene codes for prelamin A and lamin C. The C-terminal region of prelamin A is the target of several maturation events. Indeed, the protein is farnesylated, cleaved, carboxymethylated and cleaved again; losing eventually its farnesyl group. A mutant of this protein, lacking 50 amino acids, is responsible for the Hutchinson-Gilford Progeria Syndrome (Eriksson et al., *Nature* 2003). In this mutant, called progerin, the final cleavage site is absent and the protein stays constitutively farnesylated.

We here report a structural analysis of the C-terminal region of prelamin A encoded by exons 11 and 12 of *LMNA*. We show that this region is mostly unstructured and contains a conserved patch forming a residual secondary structure that could be a binding site for partners (Celli et al., *Biomol NMR Assign* 2018). We also studied the structure of the C-terminal region of farnesylated progerin. We will describe the structural consequences of farnesylation on lamin A tail region. We will also report molecular details on the ability of prelamin A and progerin tails to interact with partners at the interface between nuclear envelope and chromatin.

Biosolids

P063

Localizing conformational hinges by NMR: where do Hepatitis B Virus core proteins adapt for capsid assembly?

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Abstract: The hepatitis B virus (HBV) icosahedral capsid is assembled from 240 chemically identical core protein molecules and, structurally, comprises four sets with 60 molecules each in four symmetrically nonequivalent environments (A, B, C and D, *panel A*). 'A' subunits form pentamers while 'B', 'C' and 'D' subunits form hexamers.

Interestingly, this asymmetry is reflected in solid-state NMR spectra of the capsids in which peak splitting is observed for a subset of residues[1]. Where four distinct signals are observed (*panel B*), they can be attributed to the four distinct molecules in the asymmetric unit. When two sets of peaks are observed, it suggests that at least one monomer is significantly distinct from the others. In total, detectable peak splitting was observed for almost 20 % of the HBV core protein (*represented in spheres in C and D panels*). We show that structural differences between quasi-equivalent core proteins occur in strategic positions near the interfaces between the dimers, while no distortions are found in the helices that form the spikes of the capsid.

We compare this information to dihedral angle variations from available 3D structures and also to computational predictions of "dynamic" domains and molecular hinges (DynDom program)[2]. We find that although, at the given resolution, dihedral angles variations directly obtained from the X-ray structures are not precise enough to be interpreted, the chemical-shift information from NMR correlates, and interestingly goes beyond, information from bioinformatics approaches. The residues for which NMR peaks split directly point to regions involved in the response to constraints due to capsid assembly.

Our studies reveal the high sensitivity with which NMR can detect the residues allowing the subtle conformational adaptations needed in capsid formation, and are important for understanding the formation and modulation of protein assemblies in general.

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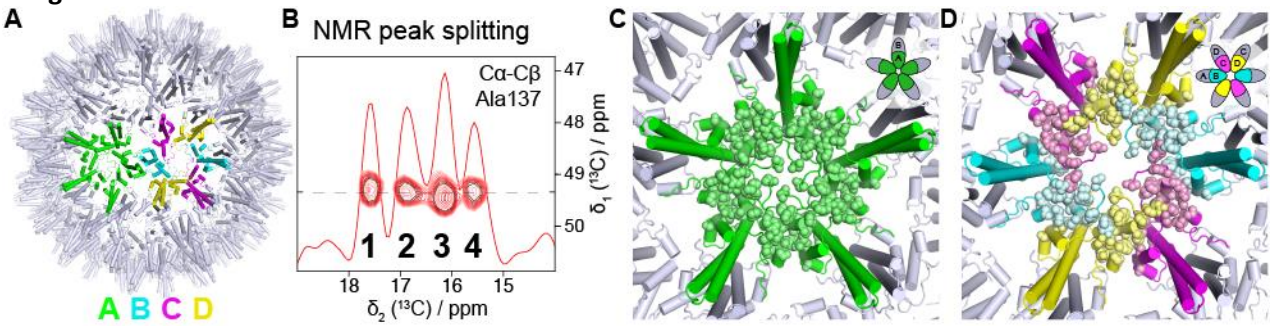
Image:



Biosolids

P064

Exploring the conformation flexibility of the apo mouse TSPO reconstituted in proteoliposomes by solid-state NMR

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Abstract: Structure determination of a membrane protein in its natural lipidic environment still remains a major challenge[1] despite recent developments of X-ray crystallography and cryo-electron microscopy (cryoEM) which lead to the high-resolution structure of a couple of membrane proteins[2]. CryoEM does not need crystals and is mostly suitable for membrane protein complexes of high molecular weight. The remarkable advantage of solid-state NMR (ssNMR) spectroscopy over other structural methods is the access not only to the atomic structure of a membrane protein in lipid bilayers but also to its dynamic behavior[3]. ssNMR has not yet become a widespread method for structural studies of membrane proteins due to various limitations. Among them, sample preparation for ssNMR remains one of the difficult steps[4] and is essential for obtaining high-quality ssNMR spectra for these challenging systems.

Translocator protein (TSPO), a mitochondrial membrane protein, is involved in numerous cellular functions, including steroidogenesis, stress regulation, neuroinflammation and apoptosis[5]. The versatility of cellular functions suggests that this protein could present various distinct conformations and their probability is directly related to the dynamics of the biological environment. The protein interacts with various ligands and the 3D structure of the mouse TSPO (mTSPO) complexed with the (*R*)-enantiomer of the PK11195 ligand in detergent has been determined by high-resolution solution NMR[6]. If the (*R*)-PK11195 ligand drives the TSPO into a rigid five-helix fold, the ligand-free TSPO in different detergents lacks full tertiary contacts indicating extensive molecular motions[7]. However, little is known about the conformational flexibility of the apo protein in natural lipids. We present herein magic-angle ssNMR studies of apo mTSPO reconstituted in proteoliposomes which reveals less conformational flexibility than in detergent. We will briefly overview the protocol which lead to a homogenous and stable sample, and hence high-quality ssNMR spectra for the apo mTSPO membrane protein. Chemical shift correlation spectra at 11.7 T and 10 kHz spinning frequency has been employed to identify a series of amino acid types. The effect of temperature on ^{13}C CPMAS, ^{13}C – ^{13}C DARR and NCO spectra have been explored.

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Acknowledgements: We would like to thank B. Rigaud (UPMC, Paris, France), D. Cailleu (UPJV, Amiens, France) and V. Decker (Bruker, Germany).

Biosolids

P065

Molecular determinants of spontaneous mode shifts of K+ channels in membranes

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Abstract: The highly-conserved selectivity filter of K^+ channels regulates the flux of ions across the cell membrane. Gating at the selectivity filter is orchestrated by a surrounding hydrogen-bond network (Figure A). Point mutations in this network, representative for eukaryotic K^+ channels (Figure B), lock the K^+ channel KcsA into different, intrinsically occurring and otherwise spontaneously shifting gating modes with unknown molecular correlates.[1] This lack of knowledge severely limits our understanding of spontaneous mode shifts and, most importantly, of the eukaryotic selectivity filter.

Here, we use modern proton-detected solid-state Nuclear Magnetic Resonance (ssNMR) spectroscopy for an extensive study in native-like lipid membranes and at physiological temperatures to compare the selectivity filter in WT KcsA and the three mutant channels that are best representative of spontaneous mode shifts and the hydrogen-bond network in eukaryotic channels (Figure C). In sharp contrast to crystallographic studies[1,2], we show that substitutions at residue E71 cause stark conformational changes in the selectivity filter. Strikingly, extensive site-resolved ssNMR relaxation studies reveal that these local perturbations correlate with marked changes in the selectivity filter dynamics on functionally relevant timescales. Furthermore, using high-resolution ssNMR spectroscopy in combination with proton/deuterium exchange, we demonstrate that spontaneous mode shifts go hand in hand with the increase and decrease of the size of the functionally critical water cavity behind the selectivity filter.[3] Altogether, these results provide a new perspective on the molecular origins and the triggers of modal gating in K^+ channels suggesting that a similar behaviour applies to the selectivity filter of eukaryotic K^+ channels. Thereby, our study provides a critical novel perspective of the highly conserved selectivity filter of K^+ channels in membranes.

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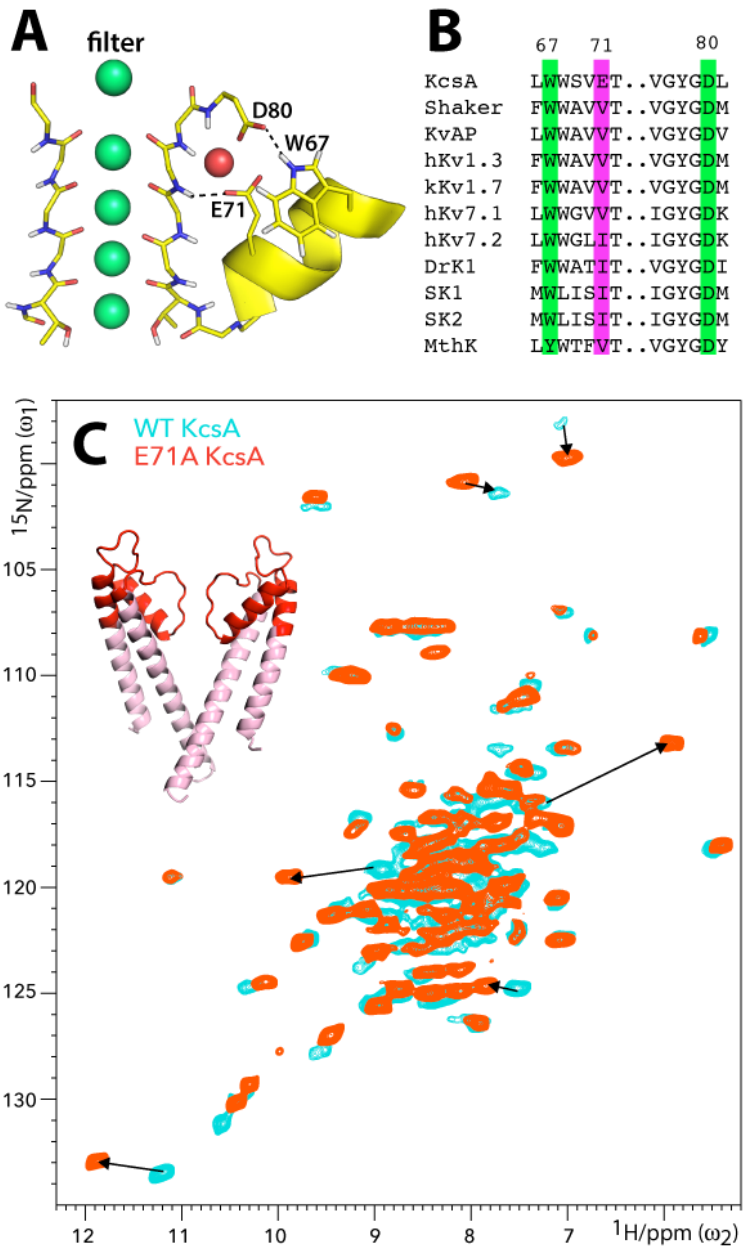
A
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E71
B
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Shaker FWWAVVT..VGYGDM
KvAP LWWAVVT..VGYGDV
hKv1.3 FWWAVVT..VGYGDM
kKv1.7 FWWAVVT..VGYGDM
hKv7.1 LWWGVVT..IGYGDK
hKv7.2 LWWGLIT..IGYGDK
DrK1 FWWATIT..VGYGDI
SK1 MWLISIT..IGYGDM
SK2 MWLISIT..IGYGDM
MthK LYWTFVT..VGYGDY
C
WT KcsA
E71A KcsA
15N/ppm (ω1)
105
110
115
120
125
130
12
11
10
9
8
7
1H/ppm (ω2)

Biosolids

P066

Conformational dynamics in the bacterial β-barrel transporter FhaC investigated by solid-state NMR

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Abstract: The Two-Partner Secretion (TPS) pathway in Gram-negative bacteria is dedicated to the export of large proteins serving notably as virulence factors. TpsB transporters are transmembrane β-barrel proteins secreting their substrates across the outer membrane. They belong to the ubiquitous Omp85 superfamily mediating protein insertion into or translocation across membranes. In the whooping cough agent *Bordetella pertussis*, the TpsB transporter FhaC mediates secretion of the adhesin FHA. Its resting-state crystal structure composed of a 16-stranded b-barrel preceded by two periplasmic POTRA domains is known, but its mechanism of protein transport has so far remained elusive[1].

FhaC is characterized by considerable dynamics. EPR, mutagenesis and accessibility studies have shown that crucial elements of its structure populate a dynamic equilibrium already in the absence of FHA. Notably, helix H1 and loop L6, which block the translocation pore in the resting state, may be displaced from the pore[2,3]. Such movements are likely implicated in the transport mechanism.

Our aim is to characterize the conformational dynamics of FhaC in molecular detail and to elucidate its role in transport. We study FhaC using ^{1}H-detected solid-state NMR experiments on perdeuterated, Ile-d_1 methyl-labeled samples embedded in lipid bilayers. We report resonance assignment of methyl signals in this 61.5 kDa protein, spectral signatures of mutants known to affect the open-close equilibrium of the protein, as well as experiments probing dynamics on the µs to ms time scale.

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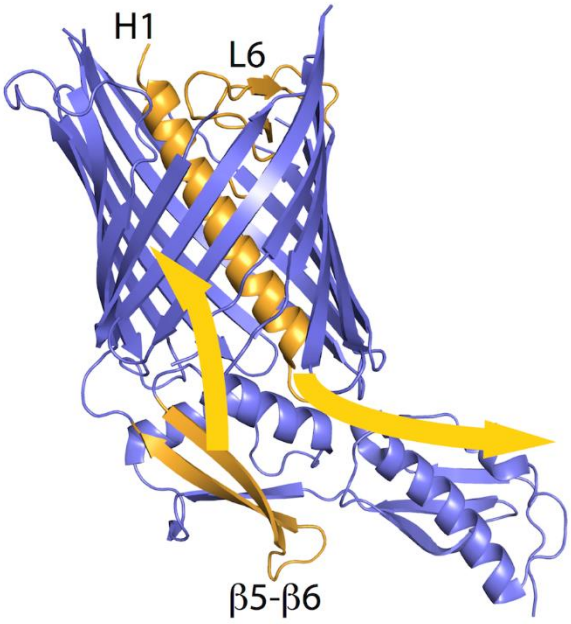

Biosolids

P067

Following a DnaB helicase during DNA translocation: The special role of the ATP-hydrolysis transition state

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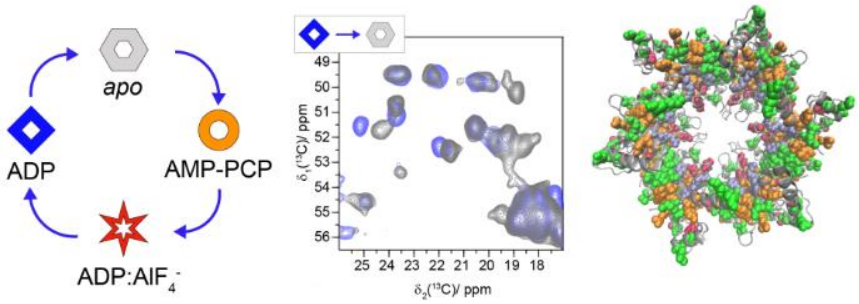
Abstract: Solid-state NMR is a versatile tool to investigate catalytic reactions of motor proteins which couple ATP hydrolysis to mechanical work, e.g. movement of a protein along a DNA strand. The various steps of ATP hydrolysis can be trapped for NMR by employing ATP-analogues which mimic the stages of ATP hydrolysis. We use this strategy for studying the (double-) hexameric DnaB helicase from *Helicobacter pylori* with a molecular weight of 672 kDa. Doing so, we were able to estimate structural and dynamic changes associated with three reactions, namely ATP hydrolysis in the motor domain of such proteins as well as DNA binding and DNA translocation, the latter two being essential for the role of the helicase in DNA replication.

$^{31}P,^{1}H$ cross-polarization experiments were used to monitor the binding of nucleotides (ATP-analogues and DNA) to the helicase, while $^{31}P,^{13}C$ and $^{31}P,^{15}N$ dipolar-coupling based polarization transfer experiments allow to identify protein residues involved in DNA binding. $^{15}N,^{13}C$ correlation experiments highlight the non-covalent coordination of lysine and arginine sidechains to DNA. In particular, lysine K373 located in one of the DNA binding loops coordinates the DNA in the pre-hydrolytic and transition state of ATP hydrolysis, but not in the post-hydrolytic, ADP-bound state. We thus believe that interactions between K373 and the DNA help to guide the DNA through the inner channel of the protein during translocation. ^{13}C chemical-shift perturbations were used to follow the changes in protein conformations for all of our reaction coordinates. We illustrate that the protein is structurally highly flexible during such reactions.

DNA binding to the DnaB:ATP-analogue complexes requires significant conformational rearrangements, except for the transition state mimicked by ADP:AlF_4^-. In this case, the helicase is already pre-organized in the absence of DNA and DNA binding solely rigidifies the inner core of the protein, mainly the DNA binding loops which are flexible and thus unobserved in NMR experiments in the absence of DNA. This agrees also with our macroscopic observation of the by far highest binding affinity of the DnaB:ADP:AlF_4^- complex for DNA identified by fluorescence anisotropy measurements. Thanks to the flexibility of NMR sample preparation, where sedimentation allows to obtain a sample from every complex that can be produced stably and homogeneously, a sufficiently dense sampling of the different reaction coordinates has been possible and opens a new avenue for the investigation of different classes of motor proteins. In particular, new insights into the coupling of ATP hydrolysis and DNA translocation were obtained.

Image:



Biosolids

P068

Box C/D snoRNPs: solid-state NMR fingerprint of an early-stage 50 kDa assembly intermediate

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Abstract: Many cellular functions rely on stable protein-only or protein-RNA complexes. Deciphering their assembly mechanism is a key question in cell biology.

We here focus on box C/D snoRNPs (Ribo Nucleo Proteins), involved in ribosome biogenesis and present in archae, yeast and human. Despite their relatively simple composition – one snoRNA and 4 core proteins – these particles don't self-assemble and their formation requires a large number of other proteins, called assembly factors.

We use solid-state NMR to get atomic-scale information on the early steps of *Saccharomyces cerevisiae* box C/D snoRNP assembly pathway, through the study of a protein complex composed of Snu13p core protein and Rsa1p:Hit1p assembly factors.

Spectra of uniformly ^{13}C,^{15}N labeled Snu13p co-sedimented with unlabeled Rsa1p:Hit1p show good linewidths.

We present first solid-state ^{13}C and ^{15}N assignments of the 126-residue protein Snu13p in the context of the 50 kDa complex. They are compared with solution chemical shifts of both the isolated protein and the protein in the presence of a 22-residue peptide from Rsa1p.

As expected, the overall conformation of Snu13p is retained in the complex, but some residues experience significant chemical shift perturbations, an indicator of local conformational changes upon interaction with Rsa1p and Hit1p.

Besides structural and conformational details, we could obtain the spectroscopic fingerprint of this complex, setting the basis for further studies, which will involve other partners of Snu13p:Rsa1p:Hit1p, e.g. snoRNA.

Biosolids

P069

PELDOR on Trimeric Betaine Symporter BetP

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Abstract: PELDOR (pulsed electron electron double resonance [1]) is a magnetic resonance meth-od for distance, orientation, and dynamic measurements of two or more paramag-netic centers in macromolecules like proteins, RNA, or DNA as well as polymers. Here we apply this method to analyze the different states of the trimeric betaine symporter BetP [2-3]. This symporter does activate at osmotic stress and transports betaine and sodium through the membrane. BetP cycles through several states dur-ing the transport. From the periplasmic open via an occluded to a cytoplasmic open state. By PELDOR and site-directed spin labeling we probe the changes at different conditions as well as the occurring of different states.

We carried out molecular simulations of structures of the BetP monomer to interpret the PELDOR data, using the enhanced-sampling methodology EBMetaD [4], whereby the dynamics of the protein are minimally biased to reproduce the experimental da-ta. We illustrate how integrative simulations can aid interpretation of ambiguous structural and spectroscopic data on the BetP membrane protein.

This work is financially supported by DFG-CRC 807, BMRZ and Goethe University.

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Biosolids

P070

Interactions of amyloid peptide AS71-82 with model membranes: structural and morphological study via FTIR and ssNMR

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Abstract: α-Synuclein (AS) is an amyloid protein involved in Parkinson's disease. In pathological cases, aggregates of this protein form in the dopaminergic neuronal network, leading to its progressive degeneration accompanied with a dramatic decrease in dopamine levels. Under physiological conditions, AS is disordered in solution or weakly bound to neuronal membranes, via the formation of α-helices. The triggers and steps underlying the formation of insoluble β-sheet rich fibrils are still unclear. In our work, we focus on a central 12 amino acids segment of AS in the amyloidogenic part of the protein that is believed to be responsible for the fibrillization of the whole protein: AS_{71-82}. Interactions between AS and neuronal membranes are thought to be the starting point of the fibrillization process, triggering the pathogenic amyloid cascade. In order to investigate and probe the mechanisms responsible for this fibrillization, model membranes composed of different ratios of zwitterionic (PC) and anionic (PG) phospholipids were used in our work. Infrared spectroscopy allowed the identification of irreversible changes in the β-sheet structure of AS_{71-82} upon the gel→fluid phase transition of the lipids, underlining the critical role of peptide/membrane interactions. Furthermore, the ^{31}P solid-state NMR study of phospholipid polar headgroups is arguably a powerful method to probe the interactions between peptides and model membranes. Recently, a 2D pulse sequence named PROCSA (phosphorus recoupling of chemical shift anisotropy) was developed in order to study model membranes composed of a mixture of phospholipids[1]. This MAS (magic-angle spinning) pulse sequence gives rise to spectra with isotropic chemical shifts in the direct dimension and the powder spectra of each phospholipid in the indirect dimension. This feature is the main advantage of PROCSA when compared to standard static ^{31}P spectra where the powder spectra of all phospholipids are superimposed and hard to separate. Eventually, PROCSA provides insights into the peptide/membrane interactions with structural and dynamical information on the preferentially interacting phospholipid in the mixture composing the membrane.

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Biosolids

P071

Towards solid-state NMR Structural Characterization of a 390 kDa Ribonucleoprotein.

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Abstract: Composed of both RNA and protein, ribonucleoprotein(RNP) is biologically important molecular assembly that plays vital roles in many essential biological functions including DNA replication, regulation of gene expression and regulation of RNA metabolism. For example, the box C/D RNP enzyme methylates ribosomal RNA at the 2'-O-ribose, a vital post-transcriptional modification for both pre-rRNA processing and ribosome assembly. This enzyme has been extensively studied using both solution NMR in our laboratory and X-ray crystallography elsewhere. However, detailed information of the methylation process is lacking due to the absence of atomic details of the exact interaction of the methyl-transferase and the target RNA.

In the past few decades, advances in solid-state NMR expanded the range of biomolecules amenable to structural studies, including membrane and fibril proteins. However, these studies are hampered by the limited number of structural restraints that can be derived from the weak dipolar couplings among several nuclei. For instance, the low gyromagnetic ratios of these nuclei place a fundamental limit on the range of these restraints (approximately 5-10 Å). In addition, the quality and quantity of these restraints is limited by the low intensities of the resonances associated with them in multidimensional correlation spectra. The introduction of paramagnetic centres can dramatically improve both the length scale as well as the number of restraints accessible from ssNMR spectra that can be detected as electron-nucleus distance-dependent paramagnetic relaxation enhancements (PREs) or pseudocontact shifts (PCSs).

In our lab, we are developing ssNMR strategies to study RNP complexes. Recently we have demonstrated that ssNMR yields high-resolution structure of RNA in context of RNP. We are currently extending these strategies to explore the protein-RNA interfaces in RNP complexes. Namely, we incorporated paramagnetic tags to the protein and utilised the hyperfine dipolar coupling of these tags to the nuclear spins on RNA to extract long-range restraints to define the protein-RNA interface. I am going to present our most recent results of ssNMR-based structure of the complex consisting of the 26mer box C/D RNA and the protein L7Ae, based on combined site-directed spin-labeling of the protein and selective nucleotide-labeling of the RNA. In addition, I will present our preliminary structural studies of both the RNA binding protein (L7Ae) and the methyl transferase (fibrillarin) as well as the guide RNA in the context of the 390kDa RNP box C/D enzyme.

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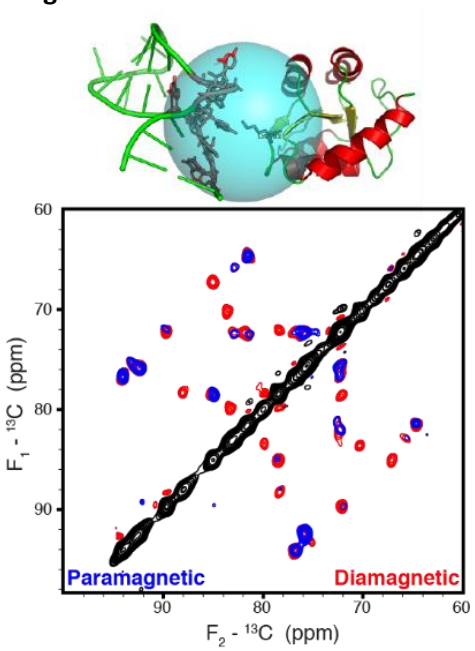
60
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80
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F1 - 13C (ppm)
Paramagnetic
Diamagnetic
90
80
70
60
F2 - 13C (ppm)

Biosolids

P072

High Resolution Structures of Metallated Host Defense Peptides that Oxidize Bacterial Lipid Bilayers and Activate Host Cell Chemotaxis

Myriam Cotten[*1], Alexander Greenwood[1], Yawei Xiong[1], Steven Paredes[1], Qiaoyue Kuang[1], Dana Moore[1], Jessica Hill[1], Ji Ming Wang[2], Riqiang Fu[3]

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Abstract: This research features mechanistic studies of host defense peptides (HDPs) from the piscidin family. Not only do HDPs perform direct bacterial killing but they also have immunomodulatory properties. We previously showed that the two isoforms piscidin 1 (p1) and piscidin 3 (p3) form helical structures bound to model membranes. We also demonstrated that they find Cu^{2+} in bacterial cells and coordinate it via a conserved amino terminal copper and nickel binding (ATCUN, XXH) motif. Redox cycling of Cu^{2+} allows the peptides to form radicals that enhance bacterial death by oxidizing unsaturated fatty acids (UFAs) and nicking DNA. We discovered that while p1 is more membrane active, p3 is more disruptive to DNA and particularly effective on drug-resistant biofilms and persister cells.

Given the relevance of these findings to the need of developing new therapeutics to fight drug-resistant bacteria, we have pursued mechanistic studies on metallated p1 and p3. Our working hypothesis is that the metallated peptides strategically place their ATCUN motif near the double bonds of UFAs to enhance the formation of oxidized lipids, which render the bilayer more susceptible to the membrane disruptive effects of the peptides. We report therein the structures of metallated p1 and p3 bound to UFA-containing model membranes. We also show that the peptides are chemotactic.

For the structural studies, we used optimized sample conditions to make oriented-sample (OS) solid-state (OS) NMR preparations containing ^{15}N-backbone labeled peptides. Metallation for the NMR samples was achieved using Ni^{2+}, which is diamagnetic bound to polypeptides, allowing us to detect signals from residues in the vicinity of the metal-binding site. We performed two-dimensional $^{15}N/^{1}H$ HETCOR experiments to obtain correlations between the ^{15}N anisotropic chemical shifts and ^{15}N-^{1}H dipolar couplings in each peptide plane. These structural restraints were used to calculate the structures in XPLOR-NIH with the implicit membrane potential eefx. **Fig. 1** shows the structure of p1-Ni^{2+} (A: side view; B: metal binding motif). The four expected metal coordination sites includes the backbone nitrogens (blue) at positions 1, 2, and 3, as well as the histidine side chain at position 3. We also observe that the histidine side chain at position 4 and the phenylalanine at position 2 are also surrounding Ni^{2+}.

Several HDPs activate immune cells through formyl peptide receptors (FPRs), which are important GPCRs implicated in the immune response and inflammatory diseases. We found that p1 and p3 activate chemotaxis through FPRs, and that Cu^{2+} decreases chemotaxis.

To our knowledge, these studies provide the first high-resolution structures of ATCUN-HDPs bound simultaneously to a metal and biologically relevant bilayers. This is also the first report that piscidin activates chemotaxis in a copper-dependent fashion, giving additional significance to our NMR structural investigations of the metallated peptides interacting with cell membrane mimics.

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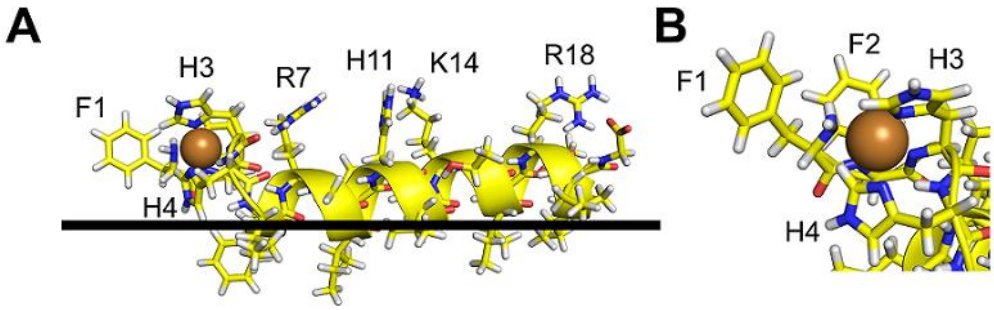

Biosolids

P073

Two-dimensional J-resolved 1H MRS measurements on inhomogeneous tissues

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Abstract: As a noninvasive tool, magnetic resonance spectroscopy (MRS) can provide valuable molecule-level information, such as chemical shifts, *J* coupling, and multiplet patterns for investigating chemical composition of biological tissues. By separating chemical shifts and *J* couplings into two different frequency dimensions, conventional localized two-dimensional (2D) *J*-resolved MRS (*J*PRESS) can provide a better analysis for complicated and overlapped MR spectra in *in vivo* study. However, the *J*PRESS method is sensitive to field inhomogeneity caused by macroscopic magnetic susceptibility variations within biological samples.

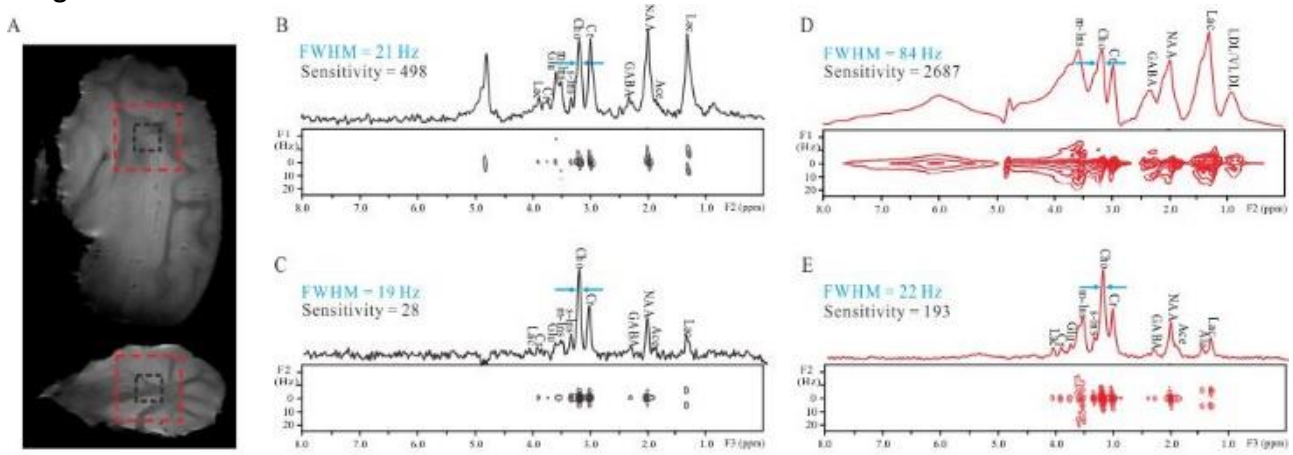
In this study, a MRS method based on intermolecular double-quantum coherences (iDQC) combined with optimal echo signal sampling scheme, named sensitivity-enhanced localized iDQC-based *J*-resolved spectroscopy (SEL-iDQC*J*) was proposed for high-resolution 2D *J*-resolved spectroscopy measurement of biological samples. Since the indirect evolution period is carefully designed for desired iDQC signal, the echo center is fixed in the sampling window during the SEL-iDQC*J* acquisition. The acquisition time can then be shortened to just cover the echo signal. Therefore, the signal-to-noise ratio of the spectrum could be maximally retained.

The 2D *J*-resolved spectra of an intact pig brain tissue acquired using conventional *J*PRESS method and the SEL-iDQC*J* method are shown in Figure 1. The fast spin-echo images of the brain tissue are presented in Figure 1A. The field inhomogeneity is directly dependent on the investigated voxel size of biological tissues. For a relatively small voxel (6 × 6 × 6 mm^3), a homogeneous field can be guaranteed and high-resolution 2D *J*-resolved spectra can be obtained with conventional *J*PRESS method (Figure 1B) and the SEL-iDQC*J* method (Figure 1C). When a relatively large voxel (15 × 15 × 15 mm^3) is selected and the magnetic field is not shimmed, the field homogeneity decreases remarkably due to the magnetic susceptibility gradients within the brain tissue. The spectral quality of the 2D *J*PRESS spectrum is degraded (Figure 1D), whereas the spectral quality of the SEL-iDQC*J* spectrum is not influenced (Figure 1E). Therefore, SEL-iDQC*J* method presents an effective way for high-resolution 2D *J*-resolved ^{1}H MRS measurements of biological samples, and may be a promising tool for *in vivo* MRS studies.

Figure 1 *2D J-resolved spectra of an intact pig brain tissue acquired with conventional JPRESS method and SEL-iDQCJ method.*

Acknowledgements
This work was supported in part by the National Natural Science Foundation of China under Grant 11474236.

Image:


A
B
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Sensitivity = 498
C
FWHM = 19 Hz
Sensitivity = 28
D
FWHM = 84 Hz
Sensitivity = 2687
E
FWHM = 22 Hz
Sensitivity = 193

Biosolids

P074

Towards the Structural Investigation of HCV Membrane Protein NS4B in Proteoliposomes by Proton-Detected Solid-State NMR at 110 kHz MAS

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Abstract: The hepatitis C virus (HCV) is an enveloped RNA virus causing acute and chronic hepatitis. Approximately 70 million people worldwide are infected with HCV and about 400,000 HCV positive patients die every year due to liver cancer and cirrhosis. The nonstructural protein 4B (NS4B) is 261 amino acid residues long (27 kDa) oligomeric membrane protein, which consists of eight putative α-helices. The NS4B plays a crucial role in host cell membrane rearrangements required for viral genome replication and virion assembly. It was also shown to be a suitable target for antiviral inhibitors in preclinical studies. The exact mode of action of NS4B is still unknown and structure analysis is a pre-requisite to understand its molecular mechanism.

The NS4B protein was expressed in a wheat germ cell-free system to overcome its high cytotoxicity in *E. coli*. The translation was performed in a presence of detergent to obtain soluble protein, without the necessity of precipitate solubilization or protein refolding step, followed by one-step purification on the Strep-Tactin column. The deuterated or fully protonated uniformly labelled protein was then reconstituted into proteoliposomes by detergent removal with methyl-β-cyclodextrin.

The proton-detected ^{1}H-^{15}N correlation spectra were recorded at 110 kHz MAS frequency and 850 MHz proton Larmor frequency. Various lipid compositions, types of detergent, lipid to protein ratios (LPR) as well as types and amounts of cyclodextrin were tested by two-dimensional ^{1}H-^{15}N correlation spectra to yield spectra with optimal line widths and signal to noise ratio. The NS4B reconstituted into egg yolk phosphatidylcholine-cholesterol mixture at LPR 2 provided well-resolved two-dimensional spectra, with the ^{1}H line widths of selected isolated peaks as narrow as 90 Hz, without any apodization function, for both deuterated as well as fully protonated sample. Despite relatively short relaxation properties with a ^{1}H T2' of 2.7 ms for the deuterated sample, well-resolved hCANH and hCONH three-dimensional spectra with median signal to noise ratio of 6 were recorded. Approximately 170 cross-peaks out of 297 expected were visible and about 70 isolated peaks could be connected between the hCANH and hCONH spectra, allowing future spectral backbone resonance assignment and structural investigation.

Biosolids

P075

Elucidating structure and dynamics of extracellular matrix collagen using solid state NMR

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Abstract: Collagens are the most abundant components of the extracellular matrix (ECM). Due to their diverse structures and compositions collagens serve many functions, providing structural and mechanical support for surrounding cells, and playing important roles in cell-to-cell communication [1]. Nonetheless, despite being at first glance a simple protein formed by three homologous polypeptide chains of repeating three-amino-acid triads trimerized into a triple helix, it is a highly versatile and complex system. With over 3000 amino acids per triple helix, it is insoluble and does not crystallize. Due to its complexity and size, and in spite of technological advances, there is still poor understanding of collagen structure, flexibility, and dynamics at the atomic level [2]. Furthermore, our knowledge of undesirable structural changes within the extracellular matrix, such as non-enzymatic glycation reactions with reducing sugars, is very limited. Glycation-modified ECM leads to abnormal cell behavior and widespread cell necrosis, potentially causing numerous complications, e.g. in diabetic patients[3]. Solid state NMR is a powerful probe to study these structural changes.

The work presented here focuses on ^{15}N NMR spectral assignments in synthetic collagen model peptides, U-^{13}C,^{15}N labelled collagen and mouse bone. As nitrogen is a component of peptide bonds, ^{15}N relaxation is a sensitive probe of collagen protein backbone dynamics. Interpretation is assisted by selectively labelled amino acids in model collagen peptides which allow characterization of sequence and neighbor effects on ^{15}N relaxation. Further, selective deuterium labelling provides additional information on side-chain and backbone dynamics. Additionally, the effects of the glycation chemistry on ECM flexibility is addressed here, focusing on amino acids that are not directly involved in the glycation chemistry, which are effectively inert reporters of changes in collagen backbone and sidechain conformations and dynamics upon glycation. Overall, NMR is a powerful tool for detailed characterization of normal and glycation processes crucial to collagen ordering and mechanical properties.

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Biosolids
P076
Digging into the core: Studies of the yeast prion protein via DNP-NMR

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Abstract: The yeast prion protein, Sup35, adopts different conformations due to varying environmental conditions within a cell. Indeed, recent work on Sup35NM fibers assembled in cellular lysates demonstrate that the biological environment can and does have a dramatic effect on protein structure. I seek to harness sensitivity gains from DNP-NMR to determine structures of Sup35 in cellular environments. However, a major limitation for many samples using DNP NMR is spectral resolution. In this study, I have segmentally isotopically labeled a small portion of the Sup35NM amyloid core. I will use this labeling scheme to characterize the amyloid core of Strong and Weak [PSI+] phenotypes for the Sup35NM both in vitro and in cellular environments. These results will be used to identify environmentally robust and environmentally sensitive regions of the Sup35NM amyloid core.
In conjunction, I have optimized the maximum sensitivity and specificity for DNP NMR on cellular matrices. Using segmental labeling decreases the number of spin labels in a system, setting a high bar for experimental sensitivity. Traditionally, purified protein samples use a matrix of 60:30:10, d8-glycerol:D_2O:H_2O, or “DNP Juice”. A simple way to increase sensitivity is by increasing the amount of sample. I found that in the presence of the cellular matrix maintain high DNP enhancements with lower cryoprotectant concentrations. Also, working at physiological cellular protein concentrations creates a need for specificity from natural abundance in the biological environment. Increasing the deuteration of the cellular matrix increases the specificity at least two-fold. These conditions provided enough sensitivity and specificity to visualize the segmentally labeled Sup35NM amyloid core via DNP NMR.

Biosolids

P077

NMRlib 2.1: User-friendly liquid and solid pulse sequence tools for Bruker NMR spectrometers

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Abstract: NMRlib 2.1: User-friendly liquid and solid pulse sequence tools for Bruker NMR spectrometers

We present an updated version (2.1) of our pulse-sequence-tool software NMRlib that allows easy setup, running, and sharing of complex NMR experiments on Bruker spectrometers. Each experiment consists in a combination of python setup scripts and a Bruker pulse-sequence program. In particular, shaped pulse parameters are computed on the fly within the pulse sequence from user defined input values (in ppm) for the desired excitation band, that makes the experimental setup independent from the magnetic field strength. The different experiments are accessible via a graphical user interface (GUI) powered by Java swing classes included in the Bruker acquisition software TopSpin. The GUI provides a convenient way of personalizing and classifying the experiment library.

A new experiment is set-up by simply clicking on the corresponding button of the NMRlib GUI. In some cases, pop-up windows will open that allow the user to define the most important parameters for the experiment, or choose among different options, e.g. constant time and conventional frequency editing.

This new release provides a set of solid state MAS experiments combined with tools performing automatic calibration of hard pulse lengths and cross-polarization transfer parameters.

Interestingly, NMRlib also provides a tool that allows to save any interesting experiment as a NMRlib-type python script, and add a corresponding button in the NMRlib GUI. NMRlib is compatible with TopSpin versions 3.2 and 3.5. The software is freely available for academic users from the IBS web page (http://www.ibs.fr/science-213/scientific-output/software/pulse-sequence-tools/)

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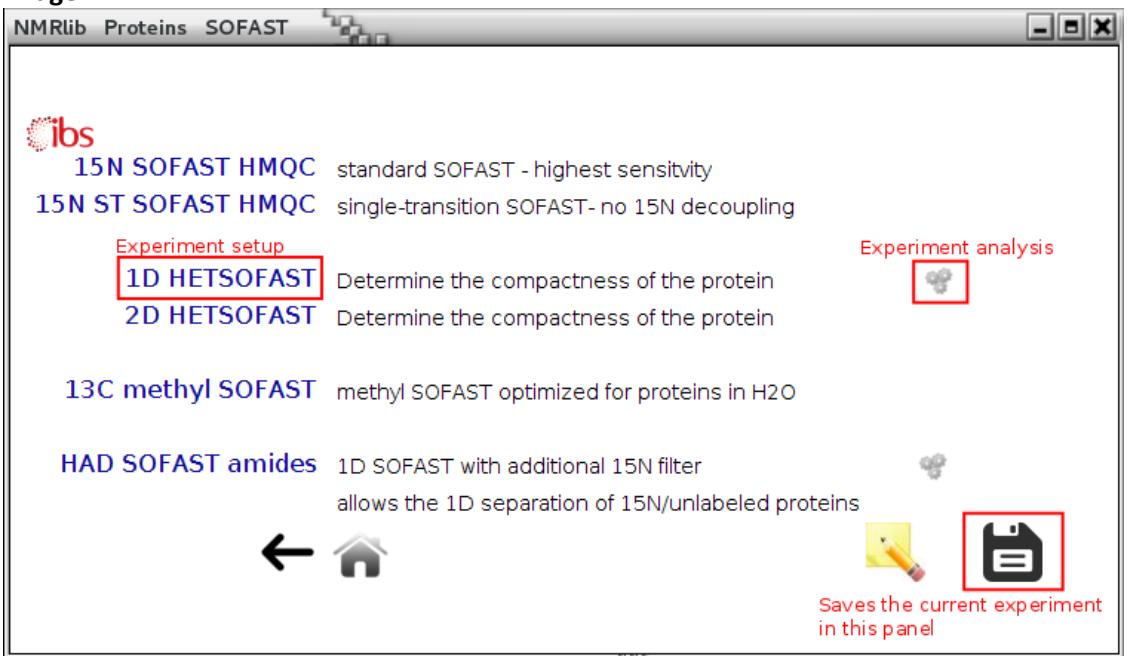

Biosolids

P078

Nuclear Magnetic Resonance investigation of cell penetrating peptides of the LAH4 family in fibrils and in interaction with nucleic acid and model membranes.

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Abstract: LAH4 is a synthetic cationic amphipathic histidine-rich peptide of 26 amino acids (KKALL ALAL**H H**LA**H**L AL**H**LA LALKKA), first designed to mimic natural antimicrobial peptide like magainin for structural studies. It has served as a basis for the design of a whole family of peptide that not only have high antimicrobial and cell penetrating but also DNA transfection activies, and are potent siRNA delivery vehicles, making them very attractive for potential medical application. The delivery of cargo by these peptides is complex, involving many steps, which we investigated on a structural and biophysical level

The core of the LAH4 peptide consists of alanines and leucines with four histidines interspersed in such a manner, that in membrane environments the peptides form amphipathic helices where all four histidines are localized on one face, two lysines at each terminus assure good solubility of the peptides in aqueous environments and serve as membrane interfacial anchors or as nucleic acid contact site

Its membrane-associated structure and topology was studied in detail, it has a pH dependent membrane orientation: Oriented ^{15}N solid-state NMR indicates that at neutral pH, when the histidine are not protonated, the peptide adopts a transmembrane topology whereas at low pH, when they are protonated, the overall charge and hydrophobic moment of the peptide considerably change and it adopt an in-plane orientation. LAH4 peptides have a strong tendency to adopt alpha-helical structure in various environments.

Several biophysical studies have been conducted in our lab, in this presentation we will show how solution NMR can be used to study the peptide structure in aqueous and in membrane mimicking environment, and to probe the initial contact for fibrils formation and interaction with nucleic acid, whereas static solid state NMR can provide orientation and dynamical informations when peptides interact with model membranes, and finally MAS NMR can be used to study the structure of the supramolecular aggregates that form when peptides self-assembly and when the peptides interact with nucleic acid.

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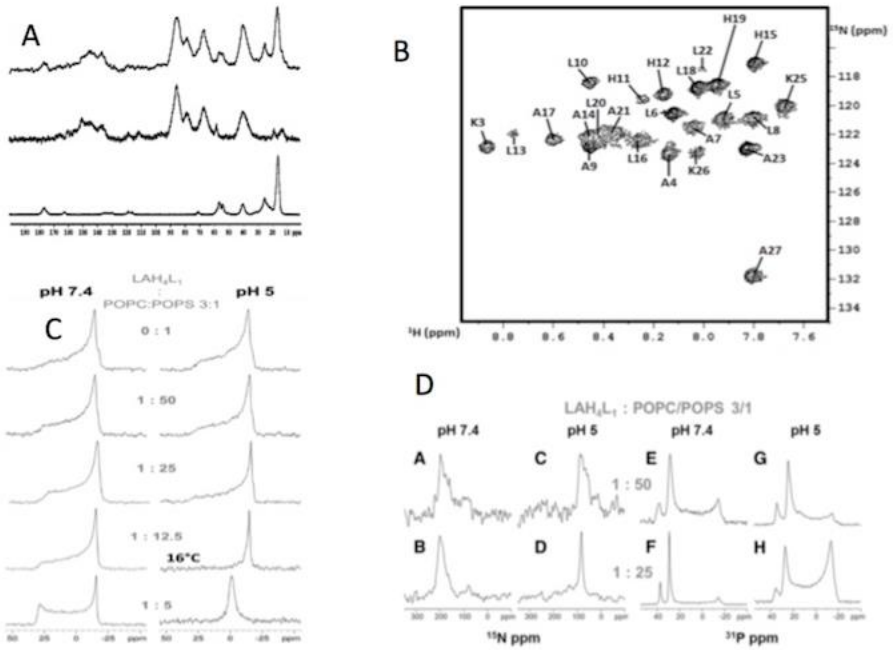

A : ^{13}C MAS spectra of LAH4 in different supramolecular assembly, **B** $^{1}H/^{15}N$ HSQC of LAH4 in DPC micelle **C** 31P unoriented static NMR of lipide vesicles in presence of various quantities of LAH4, **D** ^{15}N and ^{31}P spectra of LAH4 in oriented membranes.

Biosolids

P079

Selective Proton-Proton Distance Restraints by Ultrafast MAS of HIV-1 Capsid Assemblies

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Abstract:

Structural analysis of HIV-1 CA capsid tubular assemblies by ^{1}H-detected magic angle spinning (MAS) NMR is presented. Atomic-resolution structures of HIV-1 CA protein have been determined by solution NMR[1], and X-ray crystallography in various crystal contexts.[2,3] A model of the entire capsid (216 hexamers and 12 pentamers) has been built from an integrated cryo-EM/solution NMR/MD approach[4], and, most recently, the cryo-EM model of immature capsid including pentameric subunits has been determined at 8.8 Å resolution.[5] However, a high-resolution all-atom structure of the capsid is still lacking. Although potentially powerful, multidimensional ^{13}C- and ^{15}N-resolved MAS experiments were found insufficient for providing key long-range ^{13}C-^{13}C restraints that define the mutual orientations of N- and C-terminal domains of CA.

We have performed a number of 2D and 3D ^{1}H-detected experiments at 23.5 T (1 GHz) and 18.8 T (800 MHz) with ultrafast spinning (MAS ≥ 100 kHz) to identify ^{1}H resonances and ^{1}H-^{1}H distances in fully protonated, uniformly ^{13}C,^{15}N-labelled CA assemblies. Assignments are near complete for both backbone and side-chain ^{1}H resonances. To record ^{1}H-^{1}H distance restraints we acquired a suite of (H)CHH, (H)C(HH)CH, (H)C(HH)NH, (H)N(HH)CH, and (H)N(HH)NH experiments with broadband dipolar ^{1}H-^{1}H recoupling (RFDR) to detect correlations between aliphatic and amide protons. These experiments yield unique distance restraints, particularly between methyl protons, that are anticipated to be critical in the CA structure calculations.

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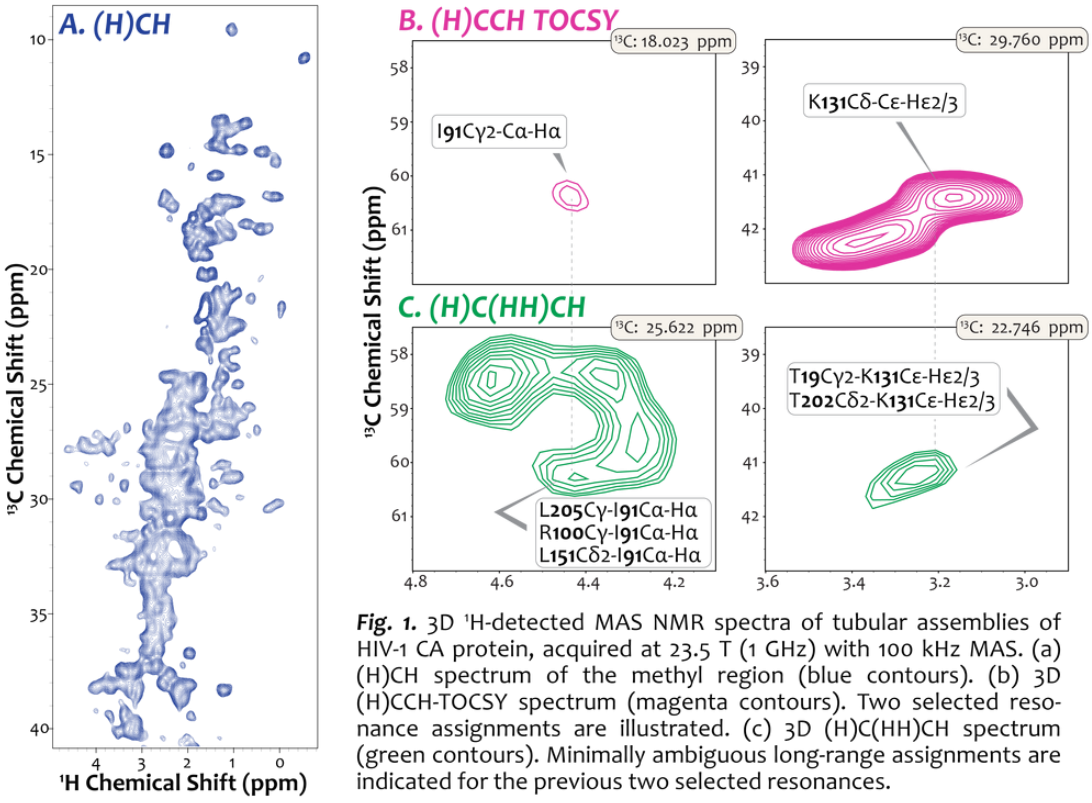

Fig. 1. 3D ¹H-detected MAS NMR spectra of tubular assemblies of HIV-1 CA protein, acquired at 23.5 T (1 GHz) with 100 kHz MAS. (a) (H)CH spectrum of the methyl region (blue contours). (b) 3D (H)CCH-TOCSY spectrum (magenta contours). Two selected resonance assignments are illustrated. (c) 3D (H)C(HH)CH spectrum (green contours). Minimally ambiguous long-range assignments are indicated for the previous two selected resonances.

Biosolids

P080

Structural studies of Amyloid Beta interaction using ssNMR-DNP

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Structural studies of Amyloid Beta interaction using ssNMR-DNP

Cellular membrane disruption induced by the aggregation of amyloid beta (Aβ) peptide is considered a main mechanism responsible for neuronal death in Alzheimer's disease. However, the molecular basis of this toxicity, in particular the interaction of Aβ and its aggregates with a cell membrane, remains unclear[1]. Solid-sate NMR (ssNMR) is a very well suited technique for studies of the molecular basis of Aβ peptide interactions with cell membranes, but low sensitivity limits such studies due to a large fraction of the sample volume being taken up by lipids. Dynamic Nuclear Polarisation (DNP) allows increasing the signals in ssNMR experiments, thus enabling measurements with lower amounts of protein material[2]. In this work we explore the feasibility of structural ssNMR-DNP studies of Aβ(1-40) peptide at low concentration interacting with biomimetic lipid bilayers.

Aβ(1-40) peptide, uniformly labelled at specific amino acids, was pre-incorporated into buffered multilamellar vesicles consisting of the phospholipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG) in a 3:1 ratio. AMUPol polarizing agent and glycerol cryoprotectant were added to the sample to facilitate low temperature DNP. Experiments were carried out on an AVANCE III 600 MHz (^{1}H lamor frequency) spectrometer with a 395 GHz gyrotron microwave source.

^{13}C signals were enhanced by a factor of 68 due to DNP as observed in ^{1}H-^{13}C cross-polarisation experiments (figure 1). Two-dimensional ^{13}C-^{13}C (DARR) and ^{15}N-^{13}C (NCA, NCACX) correlation experiments were possible in under 3 hours at lipid-to-peptide ratio (L:P) of 20:1. In order to reduce the contribution of signals from natural abundance ^{13}C of lipids we used double quantum (DQ) experiments (figure 1). POST-C7 double-quantum single-quantum (DQSQ) correlation was shown to be feasible at L:P of 100:1 (70 nmol peptide) and 200:1 (35 nmol peptide) with well resolved cross-peaks in under 10 hours and under 20 hours, respectively. Secondary chemical shifts at the uniformly labelled amino acids in the Aβ(1-40) sequence agree with β-sheet conformation at these positions. This demonstrates that DNP enhanced ssNMR can be used to probe structures of Aβ(1-40) which exist at low concentrations in neuronal cell membranes, which will lead to a better understanding of the mechanism of cell disruption.

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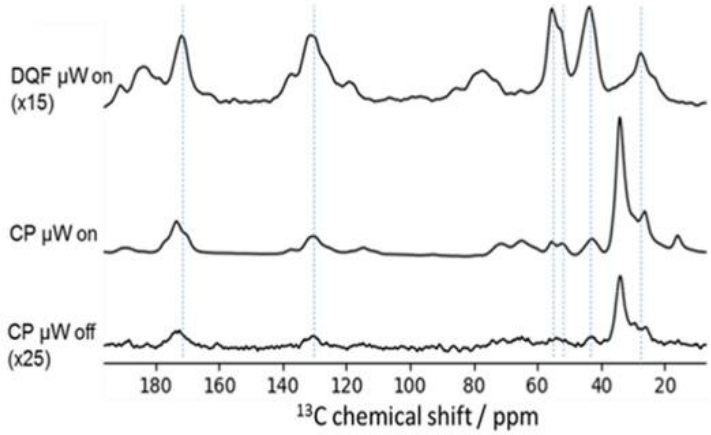

Figure 1. Cross polarisation (CP) show DNP enhancement of 68 by comparing spectra without (bottom) and with (middle) microwave irradiation. Lipid signals are minimized by applying double-quantum filtering (DQF) (top). Sample consists of Aβ(1-40) pre-incorporated into POPC/POPG lipids at L:P of 20:1. Protein peaks are shown by dashed lines.

Biosolids

P081

Towards structural studies of self-assembled subviral particles: combining cell-free expression with 110 kHz MAS NMR

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Abstract: Viral membrane proteins are important targets to fight infection. Their structure determination however remains a challenge, mainly because of two issues. First, sample preparation has to provide an environment suited for the production of the protein in its lipid-inserted native form. Second, the need for structural methods that allow for protein analysis in this membrane environment. Cell-free protein synthesis and solid-state NMR are promising approaches to solve these two issues. The first because of its high potential for native expression of complex membrane proteins[1], as it is an open in vitro system, and the second for its ability to give structural insights of membrane proteins in lipids[2]. While the two approaches were impossible to combine only a few years ago due to the notoriously low yields obtained with cell-free protein expression, the recent development of fast MAS using small rotors asking for less than a milligram of sample provided the missing link between them.

We showed[3] that milligram amounts of the small envelope protein (DHBs S) of the duck hepatitis B virus (DHBV) can be produced using wheat germ cell-free expression, and that the protein self-assembles into 30 nm subviral particles (Fig. 1). 2D proton-detected NMR spectra recorded at 110 kHz magic angle spinning on less than 500 µg protein show a number of isolated peaks with linewidths between 120 and 190 Hz (Fig. 2) comparable to model membrane proteins. This paves the way for structural studies of this protein homologous to a potential drug target in HBV infection.

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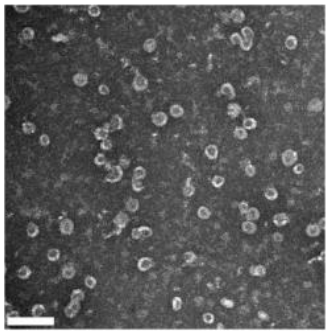

Fig 1. Transmission electron microscopy image obtained on a DHBs S sample produced using cell-free expression. The sample was stained with phosphotungstic acid and imaged at 100 kV. Scale bar 100 nm.

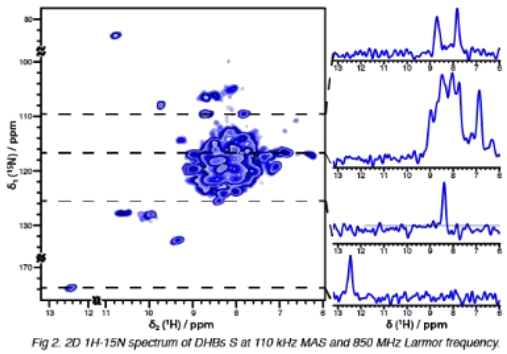

Fig 2. 2D 1H-15N spectrum of DHBs S at 110 kHz MAS and 850 MHz Larmor frequency.

Biosolids

P082

Is protein deuteration beneficial for structural and dynamics studies by solid-state NMR above 100 kHz magic-angle spinning?

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Abstract: Proton dilution by perdeuteration is one of the most efficient ways to achieve narrow ^{1}H linewidths in biomolecular magic-angle spinning (MAS) NMR. This is typically achieved by expressing a protein in a deuterated medium, and then re-protonating the amide groups by exchange in aqueous buffers with controlled H_2O/D_2O ratios during the purification stage. This approach, however, prevents the observation of ^{1}H in the side-chains, and is not viable for samples that do not unfold reversibly. Fast MAS at high magnetic field is a key tool to narrow ^{1}H linewidths, allowing to record spectra with resolved ^{1}H lines from samples with progressively higher ^{1}H contents. This opens up the possibility to efficiently assign side-chain ^{1}H resonances and record ^{1}H-^{1}H contacts defining a protein fold. Here, we report the ^{1}H linewidths and coherence lifetimes on a microcrystalline protein, a viral nucleocapsid and a membrane protein at variable spinning rates up to 111 kHz at high field. We show that fast MAS rates conjugate the availability of resolved ^{1}H-^{15}N (backbone) and ^{1}H-^{13}C (side-chains) correlations with high sensitivity. The particular advantage of extended coherence lifetimes is exploited in new multiple transfer experiments yielding resolved and unambiguous resonance correlations along protein backbone. By investigating samples with different ^{1}H/^{2}H levels, we evaluate the relative importance of homogeneous and inhomogeneous contributions to the observed linewidths, and estimate the future resolution gains obtainable with even faster MAS rates for different sample preparations.

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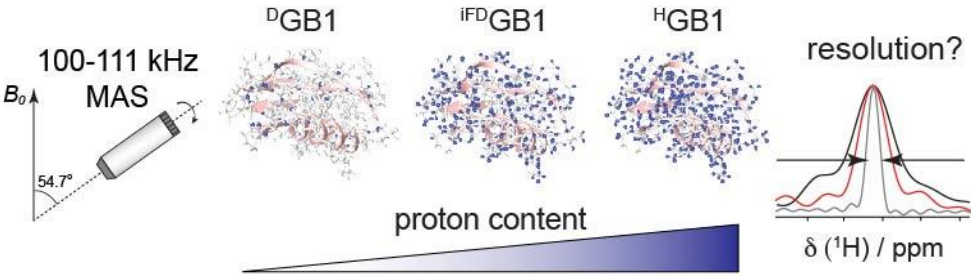

Biosolids

P083

Structural investigations of non-selective NaK channel by proton detected MAS NMR.

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Abstract: Proton detection, ultra-fast MAS rates and deuteration can significantly improve resolution and sensitivity of solid-state NMR spectra [1]. In this work we employ these techniques to study the non-selective channel NaK in a native-like lipid environment. Our recent research [2] shows that the selectivity filter of NaK adopts two conformations that are stabilized by either Na+ or K+ ions. Peak doubling can be seen for the selectivity filter residues dependent on the ion species and 1H-1H distance measurements confirmed the structural differences between the conformations proposed by MD simulations. We employ 40 kHz MAS rates to achieve high resolution proton detected spectra of fully protonated and per-deuterated, proton back-exchanged NaK. The high quality spectra yield the assignment [3] of most of the backbone resonances. In 1H detected 3D spectra we observe splitting of many 15N and 1H resonances originating from two different conformations. These chemical shift differences were not accessible before. Significant simplification of the proton network in the deuterated sample allows us to characterize with high precision proton-proton proximities within the protein and between the protein and different types of water molecules. Inter-spin contacts were obtained by 3D spectra employing spin-diffusion and RFDR for proton-proton magnetization transfers. The presented approach paves the way for characterization of the structural plasticity in the selectivity filter.

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Biosolids

P084

Invisible states in crystals: molecular bases of D76N beta-2 microglobulin pathological aggregation propensity

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Abstract: Spontaneous aggregation of folded and soluble native proteins in vivo is still a poorly understood process. A prototypic example is the D76N mutant of beta-2 microglobulin (β2m) that displays an aggressive aggregation propensity both *in vivo* and *in vitro*.

Here we investigate the intrinsic dynamics of β2m in crystals by solid-state NMR and provide crucial answers on protein stability and aggregation propensity, unveiling the effects of the D76N mutation. Notably, using a combination of ultra-fast (>60 kHz) spinning rates with 100% amide re-protonation in a perdeuterated background and high magnetic fields, we acquire sensitive ^{1}H-detected correlations allowing resonance assignment of both native and D76N β2m in microcrystalline form. The resolution of 2D ^{1}H,^{15}N-HSQC spectra allowed us to investigate site-specifically the backbone dynamics of the two proteins on different timescales. ^{15}N R_1 and $R_{1\rho}$ relaxation rates, sensitive to ps-ns motions were recorded and analyzed as a probe of the flexibility of the backbone. In addition, ^{15}N $R_{1\rho}$ relaxation dispersion experiments revealed the presence of low-populated disordered substates exchanging in a ms timescale with the native states within the crystals.

Taken together with X-ray crystallography and molecular dynamics simulations, these data highlight the destabilization of the outer strands of D76N β2m, which accounts for the increased aggregation propensity.

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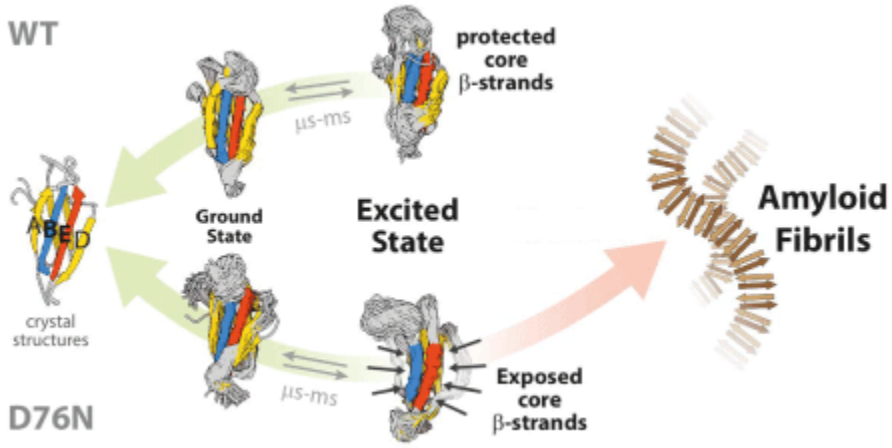
WT
protected core β-strands
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Ground State
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Amyloid Fibrils
A B E D
crystal structures
D76N
μs-ms
Exposed core β-strands

Biosolids

P085

Structural and Functional Studies of a Heptahelical Membrane Protein in Native-like and in Synthetic Environments at 100 kHz MAS

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Abstract: Nanostructured silica-surfactant materials hosting high concentrations of the light-driven proton-pump proteorhodopsin are promising for solar-to-electrochemical energy conversion. We report an atomic-level investigation of the factors that influence the structure, dynamics, and functional activity of this transmembrane protein incorporated into synthetic silica membranes. Proton-detected solid-state NMR spectra of perdeuterated proteorhodopsin reconstituted in native-like and in artificial membranes were conducted under conditions of fast (60-100 kHz) magic-angle-sample spinning, at high magnetic fields (800-1000 MHz). The narrow proton lines, which reflect the exceptional uniformity of the biomaterial, allow for the acquisition of triple resonance experiments using small sample quantities and short acquisition times that enable the backbone resonance assignment of the protein. The comparison of spectra of proteorhodopsin embedded in different environments demonstrates the retention of its native-like structure in synthetic host materials and reveals crucial influences of the silica nanochannel environments on hydrophobic protein regions. These atomic-level insights are related to the light-activated functional behaviors of proteorhodopsin molecules and establish the efficacy of the synthetic nanostructured silica host for maintaining functionally active membrane protein properties in robust synthetic environments. The analyses are expected to provide important information for designing new materials that harness membrane protein properties under abiotic conditions.

Computation

P086

Paramagnetic Relaxation Enhancement – Forgotten Anisotropy

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Abstract: Paramagnetic relaxation enhancement (PRE), widely employed in structural biology and magnetic resonance imaging, is often assumed to be isotropic in space, *i.e*. nuclear relaxation rates depend only on the distances to a paramagnetic center ($R_{1,2} \sim 1/r^6$). Here, we show that not only the distance but also the orientation of the nucleus in the molecular frame affects its PRE. Detailed theoretical analysis and systematic study of proton relaxation rates in a series of isostructural lanthanide complexes reveals significant angular dependence in both Curie and dipolar relaxation mechanisms due to large zero-field splitting and the anisotropy of the magnetic susceptibility tensor, and these effects are distinct from cross-correlations.

The analysis is based on combinatorial assignment and fitting of 31 paramagnetically shifted NMR signals of 8-coordinate lanthanide complexes (Ln=Tb, Dy, Ho, Er, Tm, Yb) of a 1,4,7,10-tetraazacyclododecane derivative with a coordinated pyridyl group and triphosphinate donors.[1-2] Magnetic susceptibility tensor extracted from pseudocontact shifts was shown to deviate from the periodic trends predicted by Bleaney. Remarkably large magnetic anisotropy of these molecules caused by strong ligand field also made the relaxation anisotropic in space. The proton relaxation measured at 1 Tesla was found to be dominated by dipolar mechanism. As predicted by Sharp in the limit where zero-field splitting (ZFS) is much larger than Zeeman splitting, the nuclear relaxation depends not only on the distance to paramagnetic center but also on the orientation of the nuclei in the ZFS frame. [3] We have also demonstrated that magnetic susceptibility anisotropy makes the first-rank Curie relaxation as important as the second rank, and both contributions are anisotropic in space as predicted by Fiat and Vega.[4]

With the increasing use of strongly magnetically anisotropic lanthanide tags in biological and medical application in NMR, the long forgotten anisotropy of the paramagnetic relaxation enhancement have to be taken on board.

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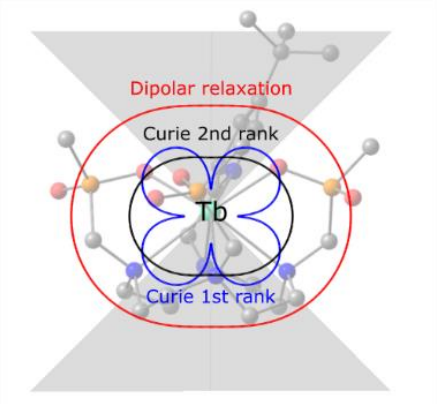

Computation

P087

What motions am I measuring? : Characterizing protein dynamics with NMR 'detector' analysis and MD simulation

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Abstract: NMR is a powerful tool for characterization of protein dynamics, having an array of relaxation experiments sensitive to different timescales of motion. However, these experiments contain information primarily about the motion of individual bonds in the molecule, for example, backbone H–N motion. Determining how this local motion results from larger, multi-residue motions can be a challenging problem from experimental data alone. Molecular dynamics simulation (MD) can in principle be used in conjunction with NMR to elucidate the relationship between the local measured motions and the overall protein behavior.

Joint analysis of NMR and MD data is, however, not trivial. It was recently found that the classic "model-free" approach[1,2] to NMR data analysis yields biased model parameters,[3] making direct comparison of the resulting amplitudes and time scales to MD simulation difficult. Therefore, we have developed the 'detector' approach to NMR dynamics analysis, which avoids the modeling problems caused by model-free analysis,[4] and as a result can be used for quantitative and timescale-specific validation of MD trajectories. In the detector analysis, one obtains several detector "responses" for each bond motion measured. Each response quantifies the motion occurring over a range of correlation times, with that range defined by the detector's sensitivity. The response yields an unbiased, but less precise, characterization of dynamics than the model-free approach. We then use MD to recover a more precise description of the motion, and to further determine how the bond-specific detector responses are correlated with the larger, multi-residue motions. We demonstrate how to filter such correlations so that we only obtain motion on the range of correlation times specific to the detector sensitivity. This method is demonstrated on HET-s(218-289) fibrils, where we successfully validate an MD trajectory with detectors. Then, using timescale-specific correlations, we identify the motions in the MD trajectory that lead to our experimental detector responses. We identify both local motion and motions occurring over multiple fibril layers, and find a direct connection between the number of atoms involved in a motion and the timescale on which that motion occurs, yielding insight into the overall complexity of motion in proteins.

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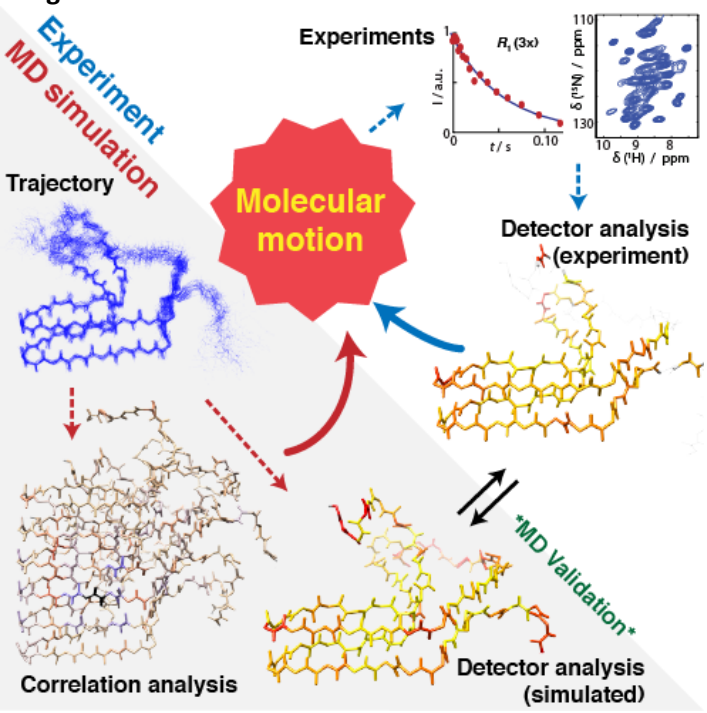
Experiment
MD simulation
Experiments
R_1 (3x)
I / a.u.
t / s
0.10
δ (¹⁵N) / ppm
δ (¹H) / ppm
Trajectory
Molecular motion
Detector analysis (experiment)
MD Validation
Correlation analysis
Detector analysis (simulated)

Computation

P088

Evolution of tripartite entangled states in a decohering environment and their experimental protection using dynamical decoupling

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Abstract: We embarked upon the task of experimental protection of different classes of tripartite entangled states, namely,
the maximally entangled Greenberger-Horne-Zeilinger (GHZ) and W states and the tripartite entangled state called the W $\bar{W}$ state, using dynamical decoupling. The states were created on a three-qubit NMR quantum information processor and allowed to evolve in the naturally noisy NMR environment. Tripartite entanglement was monitored at each time instant during state evolution, using negativity as an entanglement measure. It was found that the W state is most robust while the GHZ-type states are most fragile against the natural decoherence present in the NMR system. The W WBAR state, which is in the GHZ class yet stores entanglement in a manner akin to the W state, surprisingly turned out to be more robust than the GHZ state. The experimental data were best modeled by considering the main noise channel to be an uncorrelated phase damping channel acting independently on each qubit, along with a generalized amplitude damping channel. Using dynamical decoupling, we were able to achieve a significant protection of entanglement for GHZ states. There was a marginal improvement in the state fidelity for the W state (which is already robust against natural system decoherence), while the W WBAR state showed a significant improvement in fidelity and protection against decoherence.

Computation

P089

NMR Meets Machine Learning:
Chemical Shift Predictions in Solids in Less than a Minute

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Abstract: Chemical shift based NMR crystallography is proving to be a powerful method for determining the atomic-level structure of powdered crystalline or amorphous solids. The approach consists in the combination of solid-state NMR measurements (typically ^{1}H chemical shifts) and computational methods, and is now widely used for both structure validation and *de novo* crystal structure determination. The major bottleneck of this method lies in its high computational cost associated with the required density functional theory (DFT) chemical shift calculations. This prevents both efficient high throughput screening of potential crystalline structures and the application to larger and more complex crystals.
In the past few years, machine learning (ML) methods have shown to be a powerful tool to bridge the gap between the need for high accuracy calculations and limited computational power in many areas of chemical and physical science. Here we propose a machine learning framework to predict chemical shifts in solids, based on Gaussian Process Regression (GPR) using local SOAP fingerprints. The combination of these two methods has been recently shown to achieve DFT accuracy for energy and force calculations, while been systematic and generally applicable to many different systems.
We train this model on DFT calculated chemical shifts of a set of 2000 molecular crystals chosen to be as diverse as possible. We then demonstrate the prediction performance on a set of 500 randomly chosen crystal structures not included in the training set (we obtain R^2 coefficients between the chemical shifts calculated with DFT and with ML of 0.97 for ^{1}H, 0.99 for ^{13}C, 0.99 for ^{15}N, and 0.99 for ^{17}O, corresponding to RMSEs of 0.49 ppm for ^{1}H, 4.5 ppm for ^{13}C, 13.3 ppm for ^{15}N, and 17.7 ppm for ^{17}O). We show that the model can be used in an NMR crystallography protocol in combination with CSP to correctly determine the structure of cocaine and AZD8329. We also show that it is possible to calculate the NMR spectrum of very large molecular crystals (>1000 atoms) within a few CPU minutes, in contrast with the CPU years required by DFT. The program used to calculate shifts using this protocol is called ShiftML, available as an online server at http://shiftml.epfl.ch.

Computation

P090

First-principles computations of NMR shifts for extended paramagnetic solids: significant effects beyond the contact shifts

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Abstract: Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for studying the structural and electronic properties of paramagnetic solids. However, the interpretation of paramagnetic NMR spectra is often challenging as a result of the interactions of unpaired electrons with the nuclear spins of interest. Recently, we reported a novel protocol to compute and analyze NMR chemical shifts for extended paramagnetic solids, accounting comprehensively for Fermi-contact (FC), pseudo-contact (PC), and orbital shifts, and applied to the important lithium ion battery cathode materials such as $LiFePO_4$ or $LiCoPO_4$.[1] This approach combines periodic DFT computation of hyperfine and orbital-shielding tensors with an incremental cluster model[2] for g- and zero-field-splitting (ZFS) D-tensors. The hyperfine tensors were computed with hybrid DFT functionals using the highly efficient Gaussian-augmented plane-wave implementation of the CP2K code. The incremental cluster model allows the computation of g- and ZFS D-tensors by ab initio complete active space self-consistent field and N-electron valence-state perturbation theory methods. Application of this protocol shows that the ^{7}Li shifts in the high-voltage cathode material $LiCoPO_4$ are dominated by spin-orbit-induced PC contributions, in contrast to previous assumptions, changing fundamentally interpretations of the shifts in terms of covalency. PC contributions are smaller for the ^{7}Li shifts of the related $LiFePO_4$, where FC and orbital shifts dominate. We will also compare results with the related materials $LiMPO_4$(M = Mn, Ni). The ^{31}P shifts of all materials finally are almost pure FC shifts. Nevertheless, large ZFS contributions can cause non-Curie temperature dependences for both ^{7}Li and ^{31}P shifts. Similar protocols can be applied to the computation of pNMR shifts for clusters with multiple paramagnetic centers. Using such a procedure, ^{1}H and ^{13}C shifts have been computed for derivatives of the porous Cr-MIL-101 solid, which contain Cr_3O clusters with magnetically coupled metal centers within the metal-organic frameworks.[3] A combination of experimental and computational methods has been used to explore the competitive small-ligand binding to these MOFs. The developments described pave the way towards a more-widespread computational treatment of NMR shifts for paramagnetic materials.

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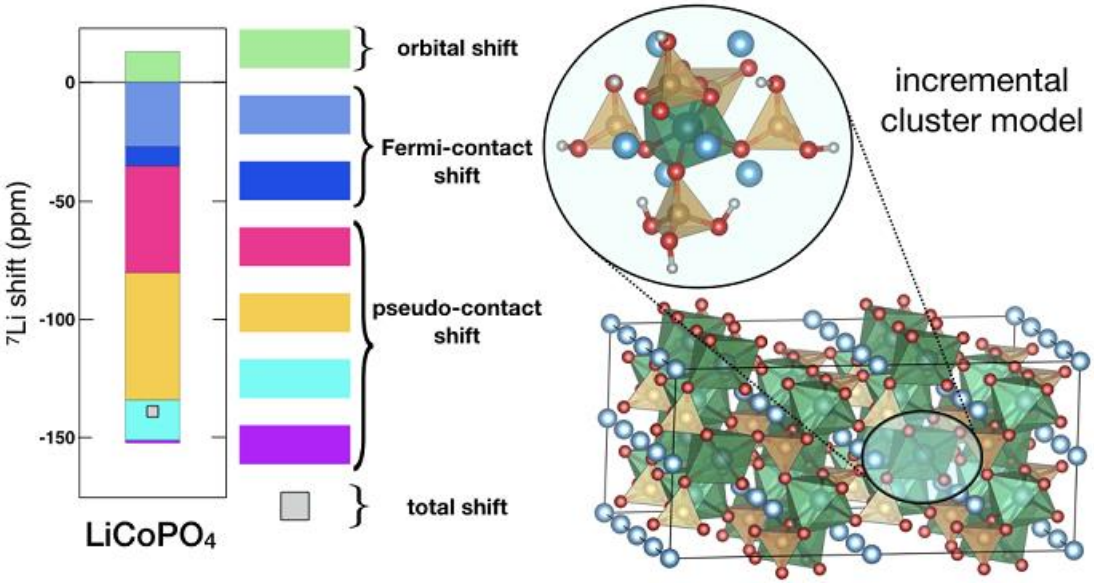
orbital shift
Fermi-contact shift
pseudo-contact shift
total shift
0
-50
-100
-150
7Li shift (ppm)
LiCoPO4
incremental cluster model

Computation

P091

Aluminum and Gallium Distribution in the Lu3(Al5-xGax)O12 Multicomponent Garnet Scintillators Investigated by the Solid-State NMR and DFT Calculations

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Abstract: $Lu_3Al_{5-x}Ga_xO_{12}$ garnets doped with Ce belong to the group of functional materials used as, e.g., infrared laser medium, blue-to-yellow downconverter phosphor in white light emitting diodes, or very fast and efficient scintillators with high light yields. Tailoring of the band gap, and the energy position of the localized Ce^{3+} levels within the gap, improves the scintillation performance and in such regard the distribution of aluminum and gallium atoms over the tetrahedral and octahedral sites in the garnet structure is important.

The mixed $Lu_3Al_{5-x}Ga_xO_{12}$ crystals were studied by means of the ^{27}Al and ^{71}Ga MAS NMR together with the single crystal ^{71}Ga NMR, and the experimental study was complemented by electronic structure calculations based on the density functional theory. The relevant spectral parameters (chemical shifts and quadrupole frequencies) were determined from the experiment and well reproduced in the DFT model structures for various Ga contents and Al/Ga distributions over the crystal sites. In particular, it was found that quadrupole interactions of both ^{27}Al and ^{71}Ga in tetrahedral sites essentially do not change with Ga content in the solid solutions, while on the contrary, quadrupole parameters of both nuclei substantially increase in the octahedral sites with increasing Ga concentration.

Our experiments and calculations both show a non-uniform distribution of Al and Ga in the garnet structure: Ga strongly prefers to occupy the tetrahedral sites regardless of Ga concentration, despite Ga having larger ionic radius than Al and the tetrahedra having significantly smaller volume than the octahedra. We explain the Ga occupation preference by different character of the chemical bonds of Al and Ga in the tetrahedral and octahedral environments, and by involvement of the Ga 3d electronic shell in the interactions. Additionally, we describe the structure relaxation upon Al substitution by Ga as being realized mainly through deformation of the octahedra, leaving the tetrahedra rotated but relatively unaltered.

Computation

P092

Diffusional attenuation in Zangger-Sterk iDOSY experiments

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Abstract: Diffusion-ordered spectroscopy (DOSY) is a method widely used for mixture analysis, but the quality of results is critically limited by resolution in the NMR dimension. [1] The use of an active spin refocusing element such as the Zangger-Sterk element, a shaped soft 180° pulse in the presence of a weak constant field gradient, allows the acquisition of "pure shift" DOSY spectra with much higher resolution both in the NMR and in the diffusion domain. The highest sensitivity in such experiments is obtained when internal ("iDOSY") diffusion encoding is used, with field gradient pulses either side of the radiofrequency selective pulse. [1] However, the slow evolution of coherence during the selective pulse complicates the relationship between diffusional attenuation, sequence timing, and gradient pulse amplitude, and the normal Stejskal-Tanner equation overestimates the attenuation.

Numerical simulations, using the Fokker-Planck formalism [2] in the Spinach software, [3] show that for typical experimental parameters the diffusional attenuation is still well represented by a Gaussian function, but one that is shifted so that the minimum diffusional attenuation occurs at a non-zero amplitude of diffusion-encoding gradient pulse. This is because the weak field gradient applied during the soft pulse acts to refocus the effect of the diffusion-encoding gradient pulses. The gradient shift can be calculated analytically for the limiting case where the soft pulse is replaced by a hard 180° pulse at its midpoint; the numerical calculations show that the effect of using different shapes of soft pulse is to scale down this limiting gradient shift by a constant factor that depends on the pulse shape used. For small gradient shifts the width of the Gaussian attenuation curve is almost exactly the same as in a conventional experiment, but for large shifts the change in the width of the Gaussian is significant .

The practical consequence is that under the experimental conditions appropriate for small molecules, the pure shift iDOSY method should allow good diffusion coefficient measurements to be made if either the gradient shift is calculated and corrected for in a conventional two-parameter Stejskal-Tanner fit, or a three-parameter fit is used.

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Computation
P093
A new, direct method for the analysis of high-resolution relaxometry

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Abstract: High-resolution relaxometry is a powerful technique to characterize molecular motions with atomic resolution. It combines relaxometry, where spectral density functions are sampled on a broad range of frequencies, and high-resolution NMR so that relaxometry profiles can be obtained in a site-specific manner in a large molecule or a complex fluid. High-resolution relaxometry experiments are performed on a commercial high-field spectrometer equipped with a sample shuttle apparatus. The sample is at high field for polarization and detection while it is transferred to chosen positions of the stray field for relaxation at low field. When the sample is outside the probe, the control of cross-relaxation pathways becomes impossible since no radiofrequency pulse can be applied. Thus, focusing on longitudinal relaxation, we expect systematic deviations of the experimental decays from monoexponential ones. These deviations have been taken into account, so far, with an iterative algorithm (ICARUS) [1] which computes at each step correction factors applied to the apparent rates of the experimental decays. Here, we introduce an alternative and direct approach for the analysis, which does not require the use of correction factors and is more general in principle.
To that end, we simulate the evolution of the spin system throughout the experiment. The Master equation is integrated at each step of the trajectory: from the high-field magnetic center to the low-field position, where the sample remains for a variable delay before moving back to the high-field position. The relaxation matrix is computed at each time step of the simulation with selected models of the spectral density functions. For a given set of parameters to be optimized, we calculate a model function as the simulated intensity computed for each relaxation delay at each magnetic field. Then, we fit the parameters of the model for all relaxation delays at all magnetic fields simultaneously. This approach was implemented with a python-based software.
This method was applied to the description of relaxation in a methyl group from 3-(trimethylsilyl)propionic acid-D4 (TSP) exchanging between a free state and a complex with the protein bovine serum albumin (BSA). We have considered the intra-methyl group relaxation of TSP with all dipole-dipole cross-correlations, chemical exchange between the free and the bound forms and an external dipole-dipole interaction with an effective proton of BSA. The figure shows sample results for a given fit of correlation times and exchange rate constants. This approach should be applicable to the analysis of high-resolution relaxometry in more complex systems.
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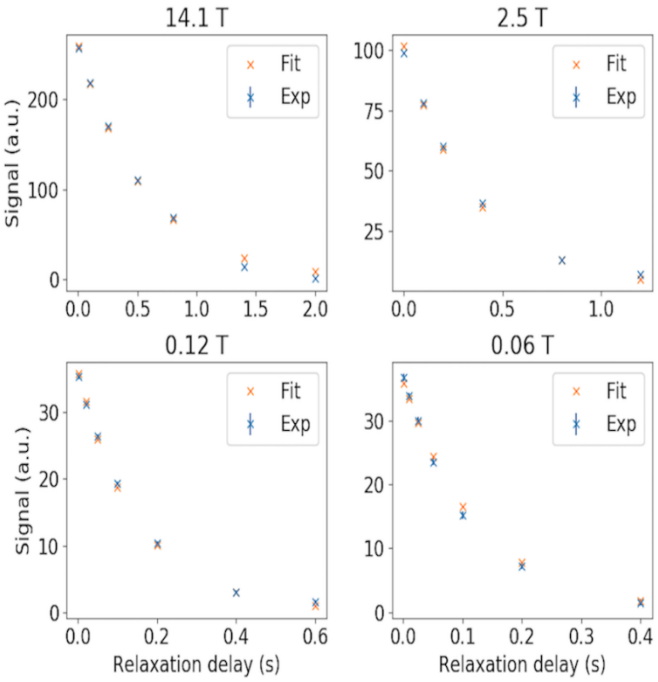

Computation

P094

Mapping contact shifts in paramagnetic coordination compounds and metal-organic frameworks by density-functional theory

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Abstract: The promise of reliable NMR spectroscopy of paramagnetic solids continues to tantalize chemists [1,2]. With increased spinning rates and recent improvements in homogeneous excitation bandwidths, many of the experimental challenges that once hindered effective paramagnetic NMR have been met. Still lacking is a comprehensive and tractable understanding of contact shifts in typical inorganic compounds and materials. We use natural bond orbital analysis in density functional theory to generate spin-density maps which serve as straightforward visual guides for peak assignments in typical coordination compounds. Combined with a general understanding of the delocalization and polarization spin-transfer pathways, this approach performs very well to rationalize ^{13}C and ^{1}H shifts in a series of transition-metal complexes with acetylacetonate and oxalate ligands, leading to reliable peak assignments with minimal dependence on the choice of basis set or exchange-correlation functional. Along with the enhanced ^{1}H MAS NMR spectral resolution deriving from large contact shifts, peak assignments based on this strategy are used to facilitate the identification of various $Mn(acac)_3$ polymorphs in mixtures. The predictive power of this method is tested by application to a series of phosphonate-based metal-organic frameworks (MOFs), providing key structural information about linker-node connectivities in poorly crystalline MOFs, where x-ray diffraction is ineffective. Treating extended frameworks as suitably sized discrete "molecular" clusters appears to reproduce the electronic features governing contact shifts, providing a widely accessible approach to the calculation of paramagnetic peak positions.

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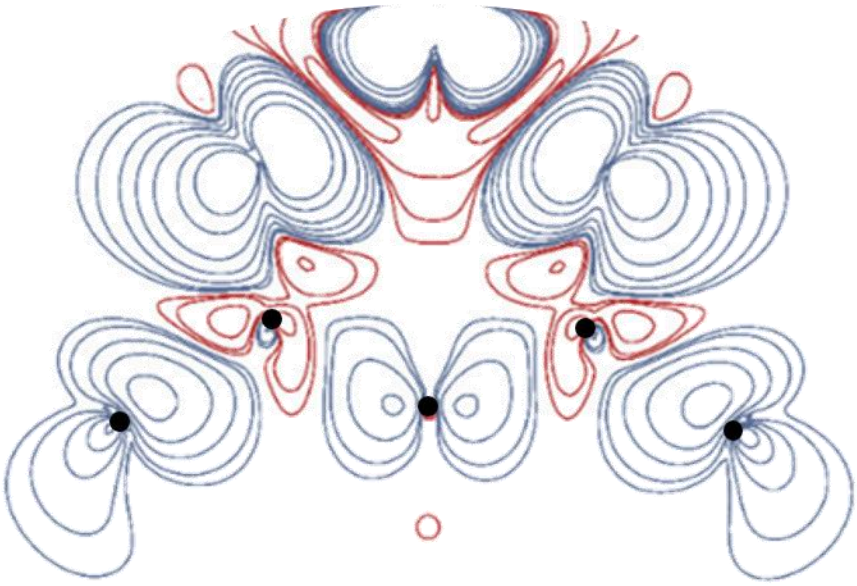

Computation

P095

Recent improvement in the SPIKE processing program

Lionel Chiron[1], Laura Duciel[1], Afef Cherni[2], Laure Margueritte[3], Fabrice Gray[4], Christian Rolando[4], Marc-André Delsuc[* 1, 2]
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Abstract: SPIKE (**S**pectrometry **P**rocessing **I**nnovative **KE**rnel)(1) is an open-source Python package dedicated to Fourier spectroscopies. It provides basic functionalities such as apodisation, a complete set of Fourier transforms, phasing, advanced baseline correction, peak-picking and peak-fitting, etc. It currently allow to process 1D and 2D NMR, 1D and 2D FT-ICR MS, and 1D Orbitrap experiments. It is optimized for the processing of very large datasets required in 2D FT Ionic Cyclotronic Resonance (FTICR) mass spectrometry(2). Organized in an open manner, the processing procedures are defined independently of the spectroscopy, it also presents a plugin architecture which allows to extend its capabilities. Recent usage of this package in various contexts are presented with examples of how information is handled:

Implementation of a DOSY analysis package, available locally and on-line, with parallelisation on a MPI cluster(3).

Unattended automatic 1D and 2D NMR processing pipeline for discovery of natural substance(4), including full processing and calibration, bucketing and statistical analysis.

We have recently implemented NUS processing algorithms for the analysis of 2D-FTICR(5), and the same algorithms can be used for NMR datasets. A new algorithm has been recently developed, which allows a fast processing of large data-sets, and is particularly robust in low SNR conditions. As an example, we show the processing of a 8k x 8k ^{13}C-HSQC of a protein in natural abundance acquired in 1h 30 with a subsampling below 10%.

SPIKE is organized as a processing library, and its natural interface is a python program. Examples of such programs are shown. However it lives perfectly in jupyter, the scientific python interface, where an easy interactivity is permitted, with all the graphics are integrated. However this still requires some minimum programming capabilities from the user. In consequence, we are currently developing a simpler graphic user interface, with an emphasis on the ease of use. Beta-version of this interface will be presented and discussed.

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Computation

P096

Insight into the factors influencing NMR parameters in crystalline compounds of the KF-YF3 binary system

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Abstract: High resolution solid state NMR opens the possibility to study complex materials, whereas first-principles calculations of NMR parameters (shielding and electric field gradient (EFG) tensors with their orientation in the cell) can be now applied to periodic compounds, providing a set of NMR parameter values for each of the atoms. Thus, *ab initio* calculations can help to interpret NMR spectra exhibited by complex materials, to assign NMR lines to structural environments, and even to enlighten the environmental factors influencing the NMR parameters for a given nucleus.

Crystalline compounds from the KF-YF_3 binary system present a wide variety of YF_n and KF_m polyhedra, and all atoms have an NMR-active isotope allowing to probe with solid-state NMR spectroscopy the wide variety of cationic and anionic environments encountered. Multinuclear solid state NMR and *ab initio* calculations have been applied to γ-K_3YF_6, K_2YF_5, KYF_4, β-KY_2F_7 and α-KY_3F_{10}. The ^{19}F, ^{89}Y and high field ^{39}K NMR spectra have been recorded and the measured NMR parameters are compared to those calculated using CASTEP [1,2] code. When unambiguous, NMR lines have been assigned to crystallographic sites, leading to the establishment of linear correlations between experimental isotropic chemical shifts, δ_{iso}, and calculated isotropic shieldings, σ_{iso}, for the three nuclei. Theoretical ^{19}F and ^{39}K NMR spectra have then been modelled for KYF_4 (24 F sites, 6 K sites) and β-KY_2F_7, (19 F sites, 4 K sites) using the linear correlations to predict δ_{iso}. Overall, good agreements are achieved as evidenced in Fig. 1 for the ^{39}K NMR spectrum of KYF_4, indicating the accuracy of both the *ab initio* calculations and the linear correlations.

Both the ^{89}Y and ^{39}K δ_{iso} values roughly increase when the coordination numbers decrease, *i.e.* when the average cation-F bond lengths decrease. Moreover, the ^{89}Y δ_{iso} values increase linearly with the number of K atoms present in the second coordination sphere, in agreement with the shielding effect of the potassium atom over that of yttrium. For ^{19}F, the combination of ^{19}F δ_{iso} and δ_{csa} values allows to distinguish four kinds of F environments: connecting three YF_n polyhedra, bridging by edge or by corner two YF_n polyhedra and non-bridging.

This work illustrates the great interest of first principles calculations for the investigation of links between NMR parameters and nucleus environment.

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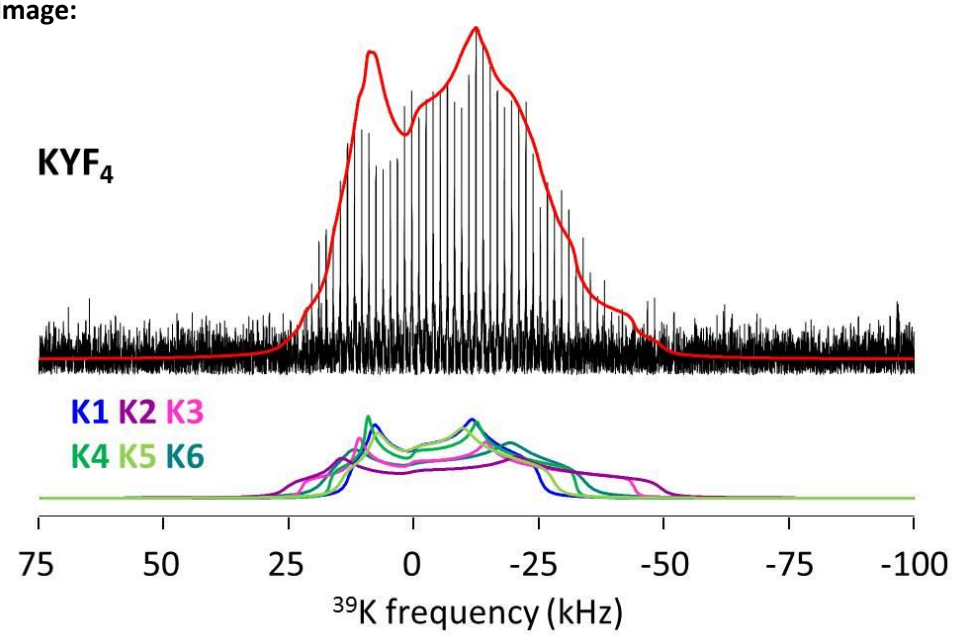

Fig. 1. ^{39}K experimental (black dots) and theoretical (red lines) static high field (17.6 T) NMR spectra of KYF_4. The six individual contributions are shown below.

Computation

P097

Kinetics of multiple-contact cross-polarization under magic-angle spinning : classical and non-classical spin coupling models

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Abstract: Hartmann-Hahn (HH) cross-polarization (CP) is employed in virtually every solid-state NMR experiment to enhance the magnetization of rare spins S with low gyromagnetic ratios such as ^{13}C and ^{15}N from abundant spins I with higher gyromagnetic ratios (e.g., ^{1}H) under static and magic-angle spinning (MAS) experimental conditions. However, in principle, reliable relative peak intensities within a CP spectrum cannot be compared without a careful study of the kinetics of CP [1]. One of the major problems arises from the competition between CP and spin-lattice relaxation in the rotating frame ($T_{1\rho}$). Methods for obtaining « quantitative » CPMAS spectra have nevertheless been proposed. Among these, the multiple-contact CP (MC-CP) scheme has been recently shown to provide quantitative ^{13}C NMR spectra of complex organic materials [2] as well as an enhancement of the *I-S* magnetization transfer when compared to HHCP and adiabatic passage through the HH condition (APHH-CP) [3].

The kinetics of HHCP and MC-CP are usually treated by a simplified thermodynamic theory, the so-called classical model denoted *I-S*, which assumes that the rate of the spin diffusion process among the *I* spins is faster than the magnitude of the coupling interaction between the two spin systems. This assumption leads to a simple exponential behavior of CP kinetics and permits the calculation of a cross-relaxation rate $1/T_{IS}$ (Markovian approximation). However, as spin diffusion is reduced by MAS, heteronuclear interactions often lead to coherent energy transfer so that the kinetics of CP has a non-exponential character, i.e., memory (or non-Markovian) effects become important. In this case, the non-classical *I-I*-S* model is more appropriate. The system is then treated as a tightly coupled $S\text{-}I_N^*$ cluster immersed in a spin-bath consisting of the remaining I spins.

In this work, we derive analytical solutions for HHCP and MC-CP kinetics under MAS using the *I-I*-S* model and considering spin-lattice relaxation. Deviations and convergences of the classical and non-classical spin coupling models are examined. Moreover, the two different processes of repolarization of the *I** spins in MC-CP, namely spin diffusion and T_1 relaxation, are clearly disentangled. All these theoretical results are verified in practice for the particular case of L-alanine.

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Computation

P098

A flow-based programming design for NMR simulations

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Abstract: In the past two decades, numerous software tools have been developed to expand the range of simulations or the ease of interacting with a graphical user interface. The most frequently used software packages include DMFit (1), SIMPSON (2,3), SpinEvolution (4), SpinDynamica (5), and SPINACH (6). Of these, DMFit distinguishes itself by being focused on the ease of interaction with the data through a graphical user interface. The other programs use more advanced inputs to model the effect of pulse sequences and other features. In addition to these general-purpose software packages, many tools exist to perform dedicated simulations of e.g. dynamics (7)or rapid simulation of protein NMR spectra (8).

A common feature of almost all NMR simulations is that they are part of a larger work flow. Focusing only on the extraction of nuclear spin interaction parameters from experimental spectra, the spectra need be processed correctly, relevant regions must be selected, the appropriate models must be chosen, and the parameters optimised to minimise the RMS deviation between the simulated and experimental spectra. In some cases, it is desirable to perform optimisations of multiple spectra simulaneously, e.g. to exploit different magnetic field dependencies of different interactions.

In this work, we propose a flow-based programming (FBP) design to setup such simulations, since the modular and customisable nature of FBP allows a versatile configuration of virtually all kinds of simulations. By defining all steps of the simulations as modules, following our JavaScript-based approach for spectrum visualization (9), which may be connected in various ways, we can establish even very sophisticated simulations very easily. The figure of the abstract shows the simple FBP layout of (a) an overlay plot of two spectra from a compressed file, (b) a more complex simulation of an experimental spectrum involving several models, and (c) a two-spectrum optimization (c).

The FBP layout interfaces seamlessly with SIMPSON and other existing packages and allows easy customation of even complex NMR simulations. With its modular approach, we provide an interface that is easy to use and very simple for developers of NMR algorithms.

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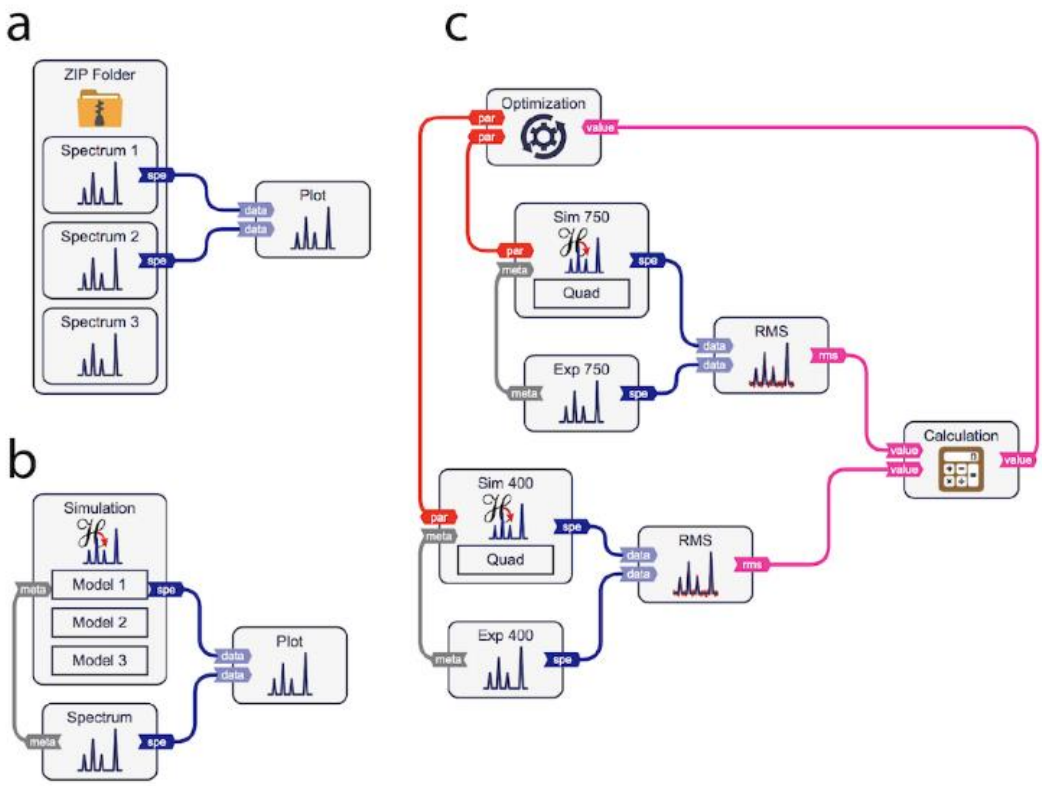
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value
Sim 750
Quad
RMS
rms
Exp 750
Calculation
Sim 400
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Computation

P099

Broadband excitation pulses with a defined phase profile, designed using optimal control theory.

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Abstract: Optimal control has found success in designing shaped pulses for use in magnetic resonance systems, particularly through design of broadband pulses. Similarly, chirped pulses are used to excite a broadband offset range through a linear offset frequency sweep. It has been shown that this is a good approximation to a linear frequency sweep but will still result in some undesirable phase dispersion.

Work presented here uses optimal control theory to refine a chirped pulse to give a desired quadratic phase dispersion with respect to offset frequency. Point-to-point and universal rotation optimisations are investigated in the amplitude-frequency representation of control pulses.

Computation

P100

NOMAD - NMR Online Management And Datastore

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Abstract: In last few decades NMR spectroscopy became a routine tool to investigate the properties of organic molecules for many chemists and biochemists. In order to cope with high demand for NMR experiments, research institutions can possess several NMR spectrometers that are often placed in centralized NMR facility that operates fully or partially in open access environment. Furthermore, modern NMR instruments are usually equipped with sophisticated automation that enables 24/7 operation and hence facilitates high throughput and utilization. The NMR laboratories set up in this way can run thousands of experiments every month and provide service to hundreds of researches. The software for automation control provided by instrument vendor usually offers rather elaborate functionality that works well only for one isolated instrument with rather small user base and does not address data management and traffic control issues that can arise in modern high throughput NMR labs.

We present here a working prototype of web-based system that provides a resilient searchable data-store and centralized control of automation for 6 Bruker instruments of various vintage in 24/7 open access environment that serves currently about 600 users.

Recent development has been focused on improving of tools for data management and sharing. The NMR spectra can be quickly inspected on NOMAD platform using a web browser-based viewer which provides basic tools for analysis of 1D NMR spectra such as peak picking and integration. Furthermore, the infrastructure has been improved in order to allow easier installation of NOMAD system at other NMR labs. In a long term perspective, this effort could allow to create collaborative decentralized database for sharing of NMR data between labs and institutions.

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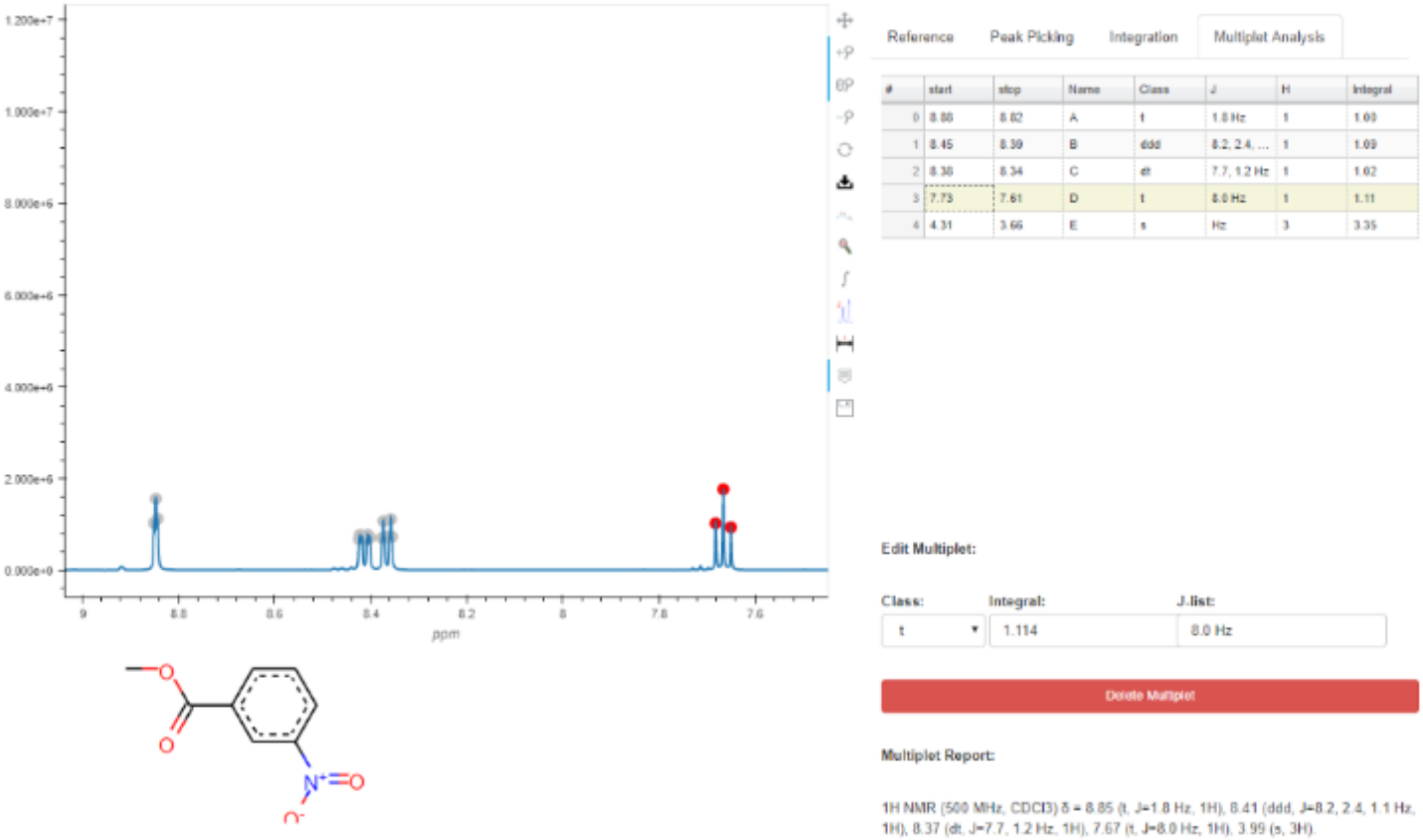

Computation

P101

An accurate method for J-coupling based conformational analysis of five-membered rings: study of β- and γ-fluorinated prolines

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Abstract: Fluorinated prolines (FPros) can be exploited in two ways in proteins or peptides [1]. First, they can modulate proline's *cis-trans* isomerization and its five-membered ring conformation (pucker) due to the stereoelectronic effect of fluorine. Second, they can serve as ^{19}F NMR reporters to probe intermolecular interactions. For this, the conformational bias to proline should be minimal, which can be achieved by judiciously incorporating two fluorines with opposing effects, such as the (3*S*,4*R*)-F_2Pro variant recently reported by us [2]. However, for both applications, it is necessary to gain insight into the preorganizing effect of fluorine and the (residual) conformational bias to proline, both for model compounds and within the actual peptide sequence of interest.

For experimental five-membered ring analysis, current procedures use Karplus relations [3] that connect vicinal scalar couplings to dihedral angles. Unfortunately, the approximate and general nature of state-of-the-art Karplus relations severely limits accuracy, especially in the case of $^3J_{HF}$ couplings, which are particularly important for doubly fluorinated FPro's. Here, we present an alternative means to translate experimental *J*-couplings into a probability distribution of five-membered ring conformation (pucker) based on an *ab initio* full energy landscape analysis of FPro model compounds.

In a first step, 901 ring conformations were generated, evenly spaced in the pseudorotation coordinate space [4]. Next, a geometry optimization was performed for each coordinate while constraining the ring pucker. This was done using DFT in Gaussian16, on Ac-FPro-OMe and Ac-FPro-NMe_2 model compounds of all β- and/or γ- fluorination patterns. From the resulting energies, a very detailed probability distribution of conformations was obtained. Experimentally measured ^1H-^1H and ^1H-^{19}F scalar couplings from the same model compounds (using PSYCHEDELIC [5]) satisfyingly matched the Boltzmann weighted average of the computed scalar couplings, validating the calculations. The calculated couplings as a function of ring pucker can then be directly compared with experimental coupling data from FPro residues incorporated in peptides, providing a means to accurately evaluate ring pucker in different contexts. Alternatively, we also developed parameterized relations that directly translate the scalar couplings into pseudorotation coordinates rather than individual torsion angles, allowing for through-space effects across the ring to be taken into account. This approach can in principle be applied to any five-membered ring compound.

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Computation

P102

Peaks Pattern Recognition System applied to NMR Spectroscopy

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Abstract: One of the most exhausting task in natural products is the molecular elucidation. Thought the interpretation of spectra from many techniques, such as NMR and mass spectroscopy, it is possible to identify the structure of a molecule. However, the whole procedure can take days, weeks or even months. Taking in consideration, the necessity of molecular elucidation with the drawbacks of the nowadays pattern recognition systems, it was developed a new software based on a patented methodology that will help the elucidation of new or known compounds even if the molecule is not presented in the software database. The patented algorithm, calculate the similarity between two spectra based on the mathematical distance of all peaks from two different samples. By arranging the result information from all spectra presented in a database into a square matrix, a similarity matrix is generated. Finally, by applying computational and statistical analysis in this final matrix, it is possible to group the substances in multivariate spaces, clusters and networks according to their structures based in one or more analysis information.bvThe software, has more than 20k command lines. Around 17k analysis data, from predict and experimental NMR analysis divided into HSQC, ^{1}H, ^{13}C, COSY, and HMBC, are inserted in the software. These analysis are from four online free database, and from one database created from the UNESP-Institute of Chemistry experimental data during 10 years of NMR studies. All the software, with all files and database, has only 66 MB and need 500MB of RAM to run. In the future, it is possible to incorporate analysis from any kind of detector, once this algorithm can be applied to any analysis that generate peaks* making the result more robust and reliable. *i.e. NMR, Mass spectroscopy, IV, UV, LC, and etc…

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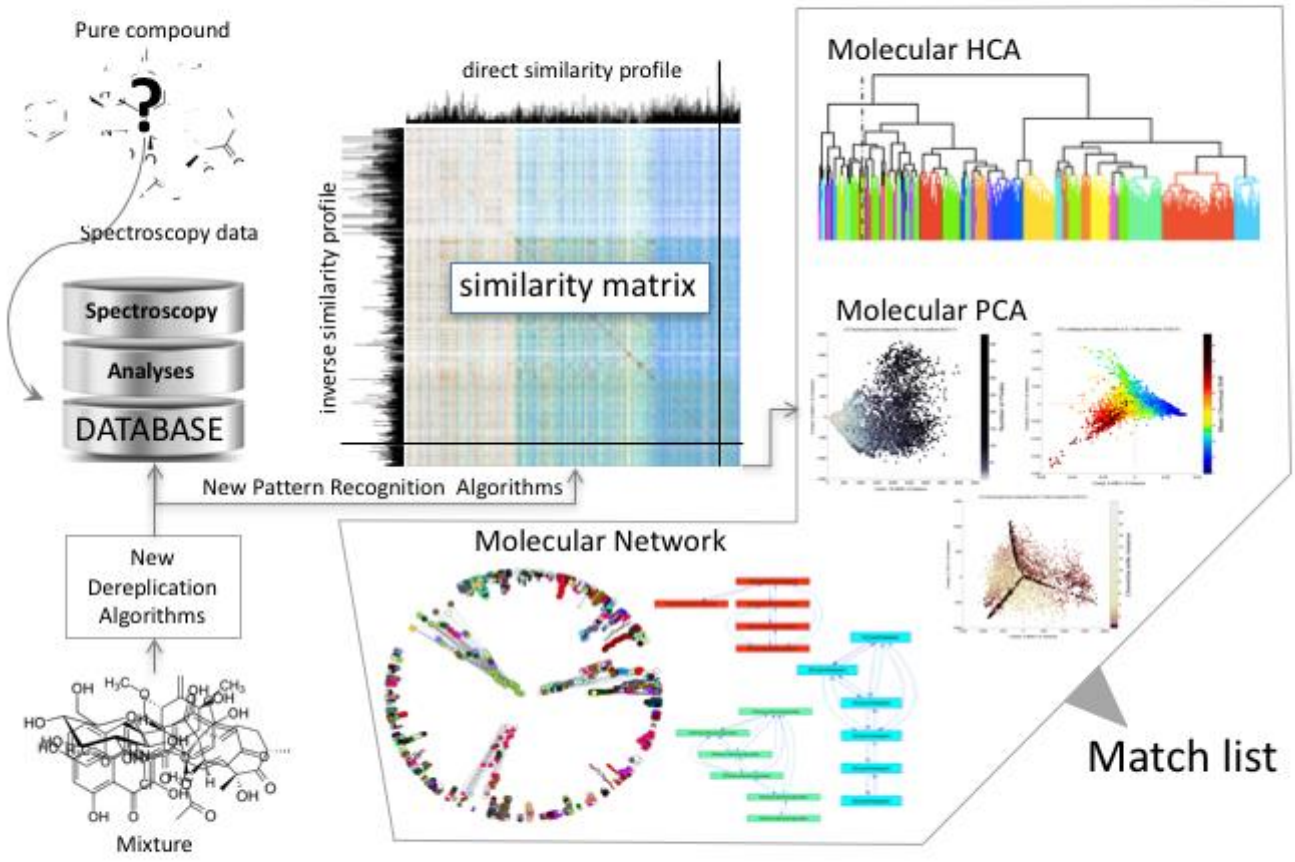
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Molecular PCA
New Pattern Recognition Algorithms
Molecular Network
New Dereplication Algorithms
Mixture
Match list

Computation

P103

Understanding detailed mechanisms of Quadrupole Relaxation Enhancement for novel MRI contrast agents

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Abstract: Quadrupole Relaxation Enhancement (QRE) means a frequency specific enhancement of proton relaxation (often referred to as quadrupole peaks) originating from a dipole-dipole coupling between protons and neighbouring quadrupole nuclei (for instance ^{14}N, ^{209}Bi) [1,2]. The quadrupole peaks appear at magnetic fields at which the ^{1}H frequency (or twice the ^{1}H frequency in the case of double- quantum spin transitions) matches one of the transition frequencies of the quadrupole nucleus, provided they are not diminished by fast molecular dynamics or/and fast quadrupole relaxation. Their positions are determined by the quadrupole parameters. This implies that the QRE is a fingerprint of electric field gradient tensor at the position of the quadrupole nucleus; the positions of the quadrupole peaks are highly sensitive to subtle changes in molecular arrangement caused, for instance, by pathological changes in tissues. In contrary to QRE, Paramagnetic Relaxation Enhancement (PRE) routinely exploited in Magnetic Resonance Imaging (MRI) to increase the contrast is observed in the whole frequency (magnetic field) range as a result of a strong proton–electron dipole–dipole coupling. Thus, comparing QRE and PRE one can expect that the first effect can lead to a new generation of MRI contrast agents that can be switched on and off in response to the local electric field gradient – in consequence, the contrast can be changed e.g. by chemical interaction with the surrounding tissues.

Among several high spin quadrupole nuclei we consider ^{209}Bi as a very promising candidate for QRE based contrast agents. The first step towards exploiting the potential of ^{209}Bi compounds is to reveal the quantum-mechanical mechanism of QRE for S = 9/2 including multi-quantum spin transitions. For this purpose four compounds showing considerably different QRE pattern have been selected: tris(2-methoxyphenyl)bismuthane, tris(2,6- dimethoxyphenyl)bismuthane [3], triphenylbismuth dichloride and phenylbismuth dichloride in solid form. ^{1}H spin-lattice relaxation experiments have been accompanied by Nuclear Quadrupole Resonance experiments to independently determine the quadrupole parameters and measure the quadrupole relaxation for individual ^{209}Bi transitions. Thorough quantum-mechanical calculations have been performed to attribute the observed QRE patterns to specific single- and double-quantum transitions of the participating spins. ^{209}Bi relaxation processes in these compounds have been discussed in connection to the presence of the quadrupole peaks and the overall ^{1}H spin-lattice relaxation.

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EPR/ESR

P104

ESR characterization of a natural tridymite

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Abstract: Tridymite is a high–temperature and low-pressure SiO_2 polymorph that is present as crystal in near volcanic natures. In this study, the natural tridymite samples obtained from Lanzarote, Canary Island, were characterized by using Electron Spin Resonance (ESR) spectroscopy using different spectrometer conditions. ESR spectroscopy is a powerful and unique technique that is used directly to identify electronic structure and geometry of paramagnetic centers by determining ESR parameters hyperfine coupling constants (hfccs) and spectroscopic splitting value (g). According to results, it is concluded that Tridymite has Fe and Mn impurities in addition to radiation sensitive E' center in its structure. The metal impurities are an indication that the mineral is of volcanic origin. Furthermore, the presence of E' center is an indication that this mineral can be used in dosimetry and dating studies.

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EPR/ESR

P105

Pulse dipolar EPR uncovers the structural arrangement of hnRNP subunits

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Abstract:

The contribution of pulse electron paramagnetic resonance (EPR) to structural biology becomes more important and demanded in recent years. The method relies on the site-directed spin labelling (SDSL) and on distance measurements, most often, based on the double electron-electron resonance (DEER) experiment. This experiment is capable of routinely accessing distances in a range from 1.5 nm up to 10 nm. No limitations in biomolecule size, as well as information on the whole structural ensemble makes DEER an indispensable technique for studying protein-RNA complexes. Furthermore, long-range EPR distance restraints can be combined with other data, e.g. from nuclear magnetic resonance (NMR), electron microscopy (EM) or small-angle scattering techniques (SAS), for integrative structural modelling to understand large biological systems down to an atomic resolution.

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are studied intensively in disease research because of their role in gene expression regulation or participation in mRNA metabolism[1]. Here, using pulsed dipolar EPR spectroscopy, we studied the structural arrangements of two hnRNPs in complexes with RNAs. The first example, hnRNP1 or Polypyrimidine Tract Binding Protein1 (PTBP1) consists of four RNA Recognition Motifs (RRMs), and plays an important role in alternative splicing and in the 5'cap-independent translation initiation.[2] PTBP1 is able to form an 85 kDa complex with 5'-near RNA stem loops (SLs) of the Internal Ribosomal Entry Site (IRES) of the EncephaloMyoCarditis Virus (EMCV).[2] RRM/RNA subcomplexes were studied recently, giving first insights to the structural arrangements in this protein/RNA complex.[3] By measuring inter-domain distance distributions of doubly spin-labelled PTBP1 in complex with native EMCV-IRES we are able to determine the arrangement of RRMs, and demonstrate the importance of one RRM binding to a flexible RNA loop for the whole complex arrangement.

The second protein, hnRNPC1, consists of different building blocks: an RRM, a basic leucine zipper-like motif (bZLM), a zipper domain and an unstructured C-terminal domain.[4] The zipper domain facilitates formation of the protein tetramer. By measuring distance distributions for the hnRNPC1 with singly-spin-labelled RRM or zipper domain, in RNA-bound and RNA-unbound fashion, we aim to differentiate between tetrameric and dimeric protein form, and between parallel and anti-parallel arrangement of hnRNPC1 monomers. Obtained distance information can then be used for integrative structural modelling, combined with data derived from cryo-EM, NMR and SAXS.

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EPR/ESR

P106

Two-Dimensional Distance Correlation Maps from Pulsed Triple Electron Resonance (TRIER) on Model Compounds and Proteins

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Abstract: Recently we introduced the pulsed triple electron resonance (TRIER) experiment [1] as a complement to double electron-electron resonance (DEER). DEER is well known for its ability to provide distance distributions from doubly labeled proteins. However, if the investigated biomolecule exists in more than one conformation, a problem with the one-dimensional DEER data arises: Peaks in the distance distribution cannot be unambiguously assigned to one conformation. By adding a third spin label, the TRIER sequence allows for correlation of dipolar frequencies that stem from the same molecule. This information can be used to solve the assignment problem.

As compared to our first publication [1], we have improved the sequence to increase sensitivity. Initially we were only able to obtain two-dimension TRIER spectra in frequency domain. With an improved data processing we can now present the first two-dimensional distance correlation maps. This was made possible by two-dimensional approximate Pake transformation of the two-dimensional time domain data and is applicable not only to model compounds. We were also able to obtain distance correlation maps of triply labeled (Fig. 1). The data we obtained through TRIER were in good agreement with DEER and simulated inter spin distances.

Though TRIER still faces some challenges, we expect such maps to facilitate the interpretation of sets of DEER data and to give more insight into the structure of complex proteins. As TRIER requires pulses with three different excitation windows that must not overlap, we aim to extend our scope to compounds with two different types of spin labels such as the combination of nitroxide and gadolinium labels.

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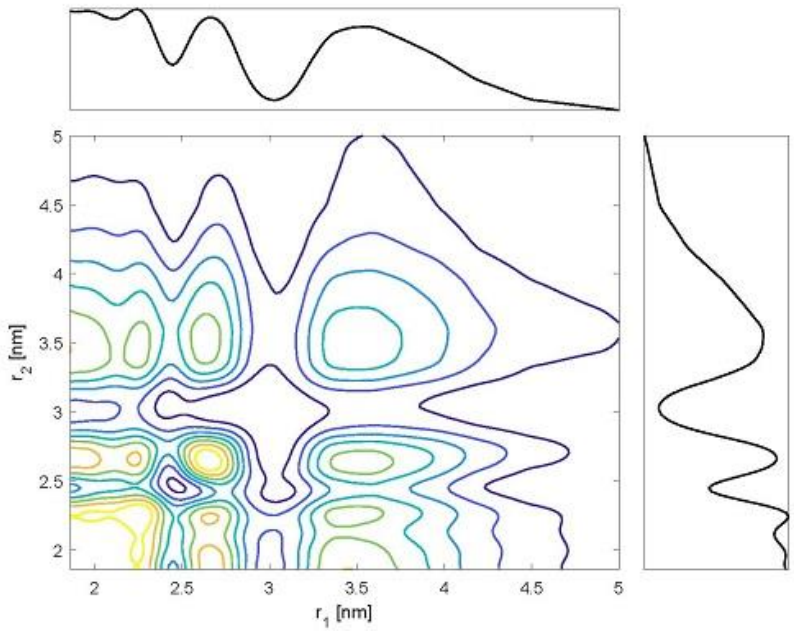
Image:



EPR/ESR

P107

Multi-extreme THz ESR -the high-sensitive membrane ESR and the high pressure ESR

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Abstract: Recent developments and applications of our multi-extreme THz ESR in Kobe will be shown. Our multi-extreme THz ESR can cover the frequency region between 0.03 and 7 THz [1], the temperature region between 1.8 and 300 K [1], the magnetic field region up to 55 T [1], and the pressure region up to 1.5 GPa [2] simultaneously. Recently we have developed the hybrid-type pressure cell, which consists of the NiCrAl alloy inner cell, the Cu-Be alloy outer cell and the ceramic piston parts, and achieved 2.7 GPa [3]. Moreover, the developments of our micro-cantilever ESR [4] has also reached 1.1 THz [5]. Especially, I will mainly focus on the recent developments of the torque magnetometry [6] and ESR [7] measurements using a commercially available membrane-type surface stress sensor, and the discovery of the first-order pressure-induced transition at 1.85 GPa in the Shastry-Sutherland Model Compound $SrCu_2(BO_3)_2$ using our high-pressure THz ESR [8].

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EPR/ESR

P108

Monitoring the effect of phosphorylation on the positioning of the cardiac specific troponin N-terminus with respect to regulatory N-domain by conventional and pulsed (DEER) EPR

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Abstract: Troponin (Tn) is the molecular switch of striated muscle contraction. It is a heterotrimeric protein consisting of a Ca^{2+} binding subunit (TnC), an inhibitory subunit (TnI), and a thin filament anchoring subunit (TnT). Despite the vast amount of x-ray and NMR structural data available for Tn, defining the molecular details of the conformational changes triggered by Ca^{2+} binding is still needed, and experimentally remains a challenge. Additionally, isoform differences between the Tn subunits may play a more significant role in switching muscle between the relaxed and active states than traditionally believed. In particular, for the cardiac isoform, the phosphorylation of the cardiac specific TnI N-terminus (a unique 31-residue region) is proposed to play a key regulatory role by binding to the TnC subunit. However, detailed molecular models of this interaction are lacking.

We used site directed spin labeling and EPR to track the movement and interaction of the TnI N-terminus with respect to the TnC regulatory N-domain, in response to Ca^{2+} binding and phosphorylation. The effects of phosphorylation were mimicked through the introduction of aspartic acid residues in the TnI N-terminus (S23D, S24D). The interspin interactions between the spin labeled pairs of (i) TnI39 to TnC35, and (ii) TnI39 to TnC84, were monitored by both Continuous wave (CW) and Double Electron-Electron Resonance (DEER) pulsed EPR spectroscopy in the intact soluble Tn ternary complex. Under conditions of high Ca^{2+}, the population of 'short' distances with narrow distance distributions (26 ± 8 Å) increased for both TnC35/TnI39 and TnC84/TnI39 interspin pairs to 30% and 66% compared to the low Ca^{2+} state values of 17% and 24%, respectively. These results suggest that Ca^{2+} binding to the N-domain of TnC stabilizes the positioning of the TnI N-terminus with respect to TnC. Upon phosphorylation, and under conditions of high Ca^{2+}, the population of the short (27 ± 8 Å) TnC35/TnI39 interspin distance significantly increased to 53%, while that of TnC84/TnI39 decreased to 29%. Consideration of the population changes for a second 'longer' DEER detected distance further confirmed the movement of the TnI39 residue towards TnC35 but away from TnC84 upon phosphorylation. Thus, phosphorylation of Tn triggers the TnI N-terminus to 'swing around' the N-domain, moving closer to residue 35 which is in close proximity to the regulatory Ca^{2+} binding site. Under conditions of phosphorylation and low Ca^{2+}, the population of the short (30 ± 14 Å) TnC35/TnI39 interspin distance decreased to 42%, and that of TnC84/TnI39 increased to 42%, supporting the release of the TnI N-terminus from TnC N-domain back to the prior position. Together, these phosphorylation triggered changes in the positioning of the TnI N-terminus with respect to the N-domain of TnC could help explain the decrease in Ca^{2+} binding affinity, and the increased rate and decreased duration of relaxation observed in cardiac muscle.

EPR/ESR

P109

Hyperfine EPR spectroscopy of nitroxides in DNP-water-glycerol mixtures reveals clustering of radicals

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Abstract:

Dynamic nuclear polarization (DNP) is a powerful technique to overcome the main shortcoming of regular NMR, which is its relatively low sensitivity. The essential idea is to take advantage of the high polarization of electron spins and transfer it to nuclear spins. To this end, samples are supplemented with a stable free radical often denoted as polarizing agent (PA). Nitroxide radicals like TEMPOL, which are extensively used in electron paramagnetic resonance (EPR) spectroscopy, are very popular for DNP applications as they can lead to remarkable proton polarization levels, frequently exceeding 80% at temperatures close to 1 K [1]. However, at the current stage, the origin of their performance is not entirely clear. To shed light on this, we performed pulsed EPR spectroscopy on prototypical DNP samples, i.e., hyperfine sub-level correlation (HYSCORE) and double electron-electron resonance (DEER) spectroscopy [2].

In the present work, typical DNP solvent mixtures consisting of 50% glycerol-d_8, 40% D_2O, 10% H_2O with varying concentrations of TEMPOL (1 to 50 mM) were investigated. The data unveil an unexpected inhomogeneous distribution of PAs as evidenced by intermolecular couplings between the unpaired electron of the PAs and the ^{14}N nuclei of neighboring molecules (Fig. 1). The formation of dimers or multimers in solution, which are trapped through rapid vitrification near the glass transition temperature prior to use for DNP, may favor the cross effect (CE), which involves triple spin-flips of two electrons and one nucleus, thus explaining - at least in part - the good performance of TEMPOL in DNP of protons at temperatures close to 1 K.

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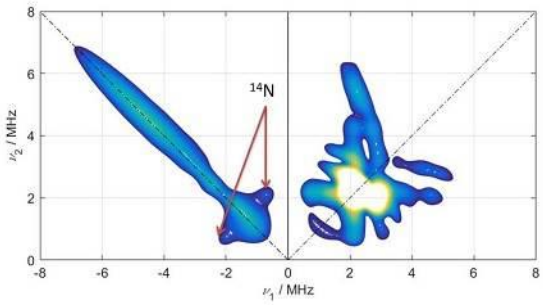

Figure 1- HYSCORE spectra of a sample with 25 mM TEMPOL at B_0 = 3421 G with τ = 200 ns. The red arrows indicate evidence of intermolecular ^{14}N hyperfine couplings.

EPR/ESR

P110

Photonic Band-Gap Resonators for mm-Wave EPR of Microliter-Volume Liquid Aqueous Samples

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Abstract: High field/high frequency (HF) EPR continues a steady growth. This growth is supported by a number of state-of-the-art continuous wave (CW) and pulsed spectrometers operating at 94, 130, 263-275 GHz and even higher resonant frequencies. While significant progress has been achieved with regard to high-field EPR instrumentation, studies of liquid aqueous biological samples are still facing substantial technical difficulties stemming from high dielectric mmW losses associated with non-resonant absorption by water molecules. The strong absorbance of mmW by water (13-36 dB/mm) also limits the penetration depth to just fractions of mm or even less, thus making fabrication of suitable sample containers rather challenging. Even without the mmW penetration problem, the optimal volume for single mode mmW resonators does not exceed *ca.* 0.1 µl at 95 GHz due to the decreasing dimensions of such structures that scale down with the mmW wavelength. Here we describe a radically new line of high Q-factor mmW resonators that are based on one-dimensional photonic band-gap (PBG) structures (see Fig.1), which alleviate some of the abovementioned problems. The mmW resonant structure is based on creating a defect in a 1D photonic crystal split by a metal mirror in the middle of the defect. A sample (either liquid or solid) is located on the top of the metallic mirror, corresponding to the E=0 node, and the position of the metal mirror is adjusted for the frequency tuning. The dielectric layers are composed of $\lambda/4$ ceramic discs with alternating dielectric constants. A resonator prototype has been built from an 8-layer dielectric structure consisting of alternating $\lambda/4$ discs of YTZP and alumina and tested at 94.3 GHz. The experimental Q-factor of an empty resonator was ≈520. The Q-factor decreased slightly to ≈450 when loaded with a water-containing nanoporous disc of 50 µm in thickness. Experimental single-scan room temperature 94.3 GHz EPR spectra of 1 µM of aqueous solution of nitroxide Tempone obtained with 100 kHz magnetic field modulation of 0.8 G in amplitude, time constant of 1 s, and 21 mW of incident power, demonstrated signal-to-noise ration of ca. 100. Detailed HFSS simulations have shown that the resonator structure could be further optimized by properly choosing the thickness of aqueous sample and employing metallized surfaces. The PBG resonator design is readily scalable to higher mmW frequencies and incorporates significantly larger sample volumes than previously achieved with either Fabri-Perot or cylindrical resonators. Supported by the National Institutes of Health 1R21EB024110.

Figure 1. **A**. Cartoon representation of a nanoporous anodic aluminum oxide (AAO) membrane employed as a flat holder for aqueous solutions and as a mechanical template for lipid alignment. **B**. Distribution of B_1 mmW field in 1D PBG resonators. **C**. Experimental single-scan, room-temperature 94.3 GHz EPR spectra of spin-labeled gramicidin A in aligned lipid bilayers with and without KCl present.

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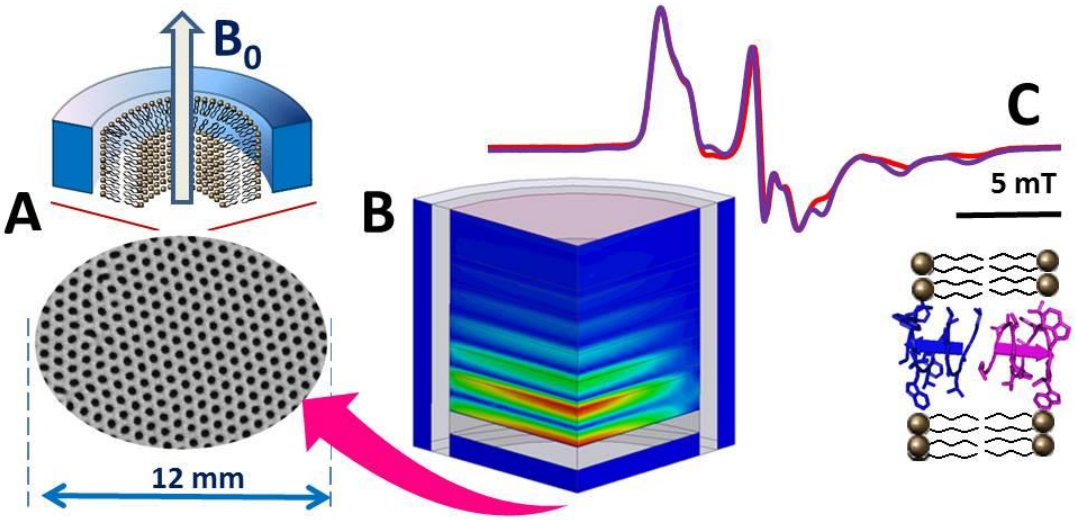

EPR/ESR

P111

Pulse EPR and ENDOR study of manganese doped [(CH3)2NH2][Zn(HCOO)3] hybrid perovskite

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Abstract: Recently, the dimethylammonium metal-formate frameworks $[(CH_3)_2NH_2][M(HCOO)_3]$ (M is divalent transition metal ion) attracted attention of the scientific community due to the indications of the multiferroic behavior [1]. In these compounds, the metal-oxygen octahedra are linked by the formate linkers into pseudocuboid cavities, each containing a single $(CH_3)_2NH_2^+$ cation. This compound exhibits a structural phase transition at 163 K due to the ordering of these cations. The ordered phase shows indications of the ferroelectric behavior, while compounds with the paramagnetic transition metal ions exhibit magnetic order at low temperature.

Here, we use pulse EPR and ENDOR spectroscopy to investigate the phase transition as well as the ordered phase of the manganese doped $[(CH_3)_2NH_2][Zn(HCOO)_3]$ hybrid framework [2]. The echo-detected field sweep Mn^{2+} EPR spectrum of the disordered phase reveals a significant EPR transition-dependent relaxation. The ^1H ENDOR pattern indicates several protons in the vicinity of the Mn^{2+} center in agreement with the density functional theory calculations. A multifrequency (X, Q and W-band) electron spin echo envelope modulation (ESEEM) technique reveals a peculiar signal which is unaffected by the external magnetic field. The temperature dependence of the longitudinal relaxation time indicates a coupling between the Mn^{2+} electron spins and a hard optical phonon mode, which undergoes a damping at the phase transition point. The temperature dependent measurements of the phase memory time reveal methyl group motion in the ordered phase.

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EPR/ESR

P112

DEER: RELOADed and under new ROOPh

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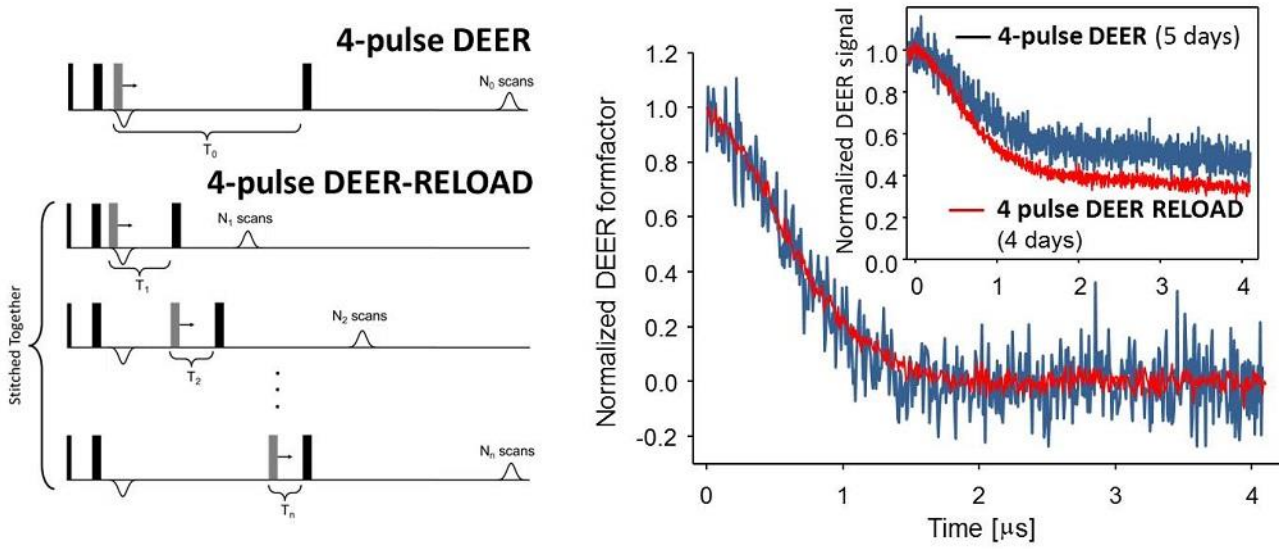
Abstract: Double electron-electron resonance (DEER) remains the main workhorse method for measurements of nm-scale distances between electronic spins in biological and other systems even though other variants of EPR pulsed dipolar spectroscopy (PDS) have been developed. While the recent progress in both DEER instrumentation and pulse sequence development has been significant, there are at least two issues that require a further attention. One is the presence of a significant unmodulated fraction in the detected signal that arises from an incomplete inversion of the coupled spins by the pump pulse. Such a fraction has been perceived as one of the major sources of error for the reconstructed distance distributions. Another is suboptimal sensitivity of pulsed EPR related to relatively fast phase memory loss for nitroxides and other electronic spins in biologically relevant systems that limits the distance range accessible by DEER.

Here we describe some of the approaches to alleviate these long-standing problems. Firstly, we describe an alternative detection scheme – a Refocused Out-Of-Phase DEER (ROOPh-DEER) – to acquire only the modulated fraction of the dipolar DEER signal. Under optimal experimental conditions the out-of-phase magnetization components of 4-pulse DEER cancel each other and are not observed. In ROOPh-DEER these components are refocused by an additional pump pulse while the in-phase component containing an unmodulated background is filtered out by a pulse at the observed frequency applied right at the position of the refocused echo. Experimental implementation of the ROOPh-DEER detection scheme requires at least three additional pulses as was demonstrated on an example of a 7-pulse sequence. The application of ROOPh DEER 7-pulse sequence to a model biradical yielded the interspin distance of 1.94±0.07 nm identical to the one obtained with the conventional 4-pulse DEER, however, without the unmodulated background present in the latter signal.

Secondly, we describe a novel albeit simple approach to improve DEER sensitivity/shorten data acquisition time by utilizing RELaxation Optimized Acquisition (Length) Distribution (DEER-RELOAD). In DEER-RELOAD the experimental trace is "stitched" from several segments acquired in the same DEER experiment but each with different separation of the observer pulses optimal for the segment. By testing a 4-pulse DEER-RELOAD variant with model membrane protein systems we demonstrate that splitting of the acquired DEER trace into two or three segments improves signal-to-noise ratio (SNR) by a factor of 2-3, thus, cutting the required data acquisition time by 4- to 9-fold. Criteria for optimization of such segmented data acquisition are also described. This research was supported by U.S. DOE Contract DE-FG02-02ER15354 to A.I.S. (DEER experiments and DEER trace modeling).

Image:



EPR/ESR

P113

Probing the structure of Titanium(III) Alkyl Species and their role in Ethylene Polymerization by EPR spectroscopy

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Abstract: Despite more than 50 years of research, the nature of active sites of heterogeneous Ti-based Ziegler-Natta catalysts remains elusive. Although the use of co-catalysts, such as $Al(C_2H_5)_3$, undoubtedly leads to reduction of some Ti sites, it is unclear whether Ti(III) alkyl complexes (with d^1 electron configuration) can be efficient in olefin polymerization [1].

In the present work, we describe the synthesis of well-defined silica- and alumina-supported β-diiminato Ti(III) alkyl species obtained via Surface Organometallic Chemistry (SOMC) [2] upon grafting of the molecular complex (Nacnac)Ti($CH_2tBu)_2$ (Nacnac = [Ar]NC(Me)CHC(Me)N[Ar], Ar = 2,6-$(CHMe_2)_2C_6H_3$), **1**. Molecular and supported species were characterized using a combination of HYSCORE spectroscopy [3] and DFT computations. Based on X-ray crystallography and DFT-optimized structures for complex **1**, and on computational cluster models of the supported species, the ^{14}N hyperfine and quadrupole tensors and ^1H hyperfine tensors were calculated. The HYSCORE spectra, simulated using these calculated parameters, are in a very good agreement with corresponding experimental spectra, supporting the formation of the well-defined Ti(III) sites of general formula [(≡SiO)Ti(Nacnac)(CH_2Bu)] and [(Al_SO)Ti(Nacnac)(CH_2tBu)] for silica- and alumina-supported species, respectively.

Molecular and supported species are active towards ethylene polymerization, with the highest catalytic activity of $3.6\cdot10^4$ g PE/(mol·h) for the alumina-supported system. These are then, to the best of our knowledge, the first examples of well-defined neutral Ti(III) alkyl species competent to initiate ethylene polymerization. This data indicates that Ti(III) may play a crucial role in the activity observed for industrial Ziegler-Natta catalysts.

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EPR/ESR

P114

Isolation of a new radical species in a pyrylium-based photopolymerization detected by cw-ESR and pulsed Electron Nuclear Double Resonance spectroscopy

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Abstract: Light-induced polymerization reactions are used in many industrial applications.[1] However, controlling the polymerization rate and chain growth remains an important challenge in polymer research to enhance the complexity of polymer architectures that can be designed. Pyrylium derivatives are promising candidates for homogeneous metal-free photocatalysis, which can be efficiently excited with visible light.

In a previous study, a pyrylium photocatalyst was employed to obtain size-tunable isobutylvinyl ether (IBVE) polymers with good dispersity by controlling the irradiation time of the reaction.[2] Each step of the photocatalytic cycle was successfully elucidated and various intermediates involved in the mechanism were detected. Specifically, during the photoinduced electron transfer between the pyrylium catalyst and IBVE, the creation of a donor-acceptor complex was identified by UV-Vis absorption, and by quenching of the pyrylium fluorescence.

In this new study, we wish to investigate further the formation of this "donor-acceptor" radical in order to quantify the impact of this intermediate of the photocatalytic efficiency on the polymerization reaction. We carried out an analysis of the different photoinduced radicals by X-band cw-ESR and Q-band Pulse Electron Nuclear Double Resonance (ENDOR). The change in the hyperfine splitting features was revealed and quantified by Mims ENDOR in liquid-state at 220 K. Assisted by Density Functional Theory simulation, these new hyperfine coupling constants were chemically attributed to the creation of a donor-acceptor [pyrylium-IBVE]⍰ radical.

References

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Image:

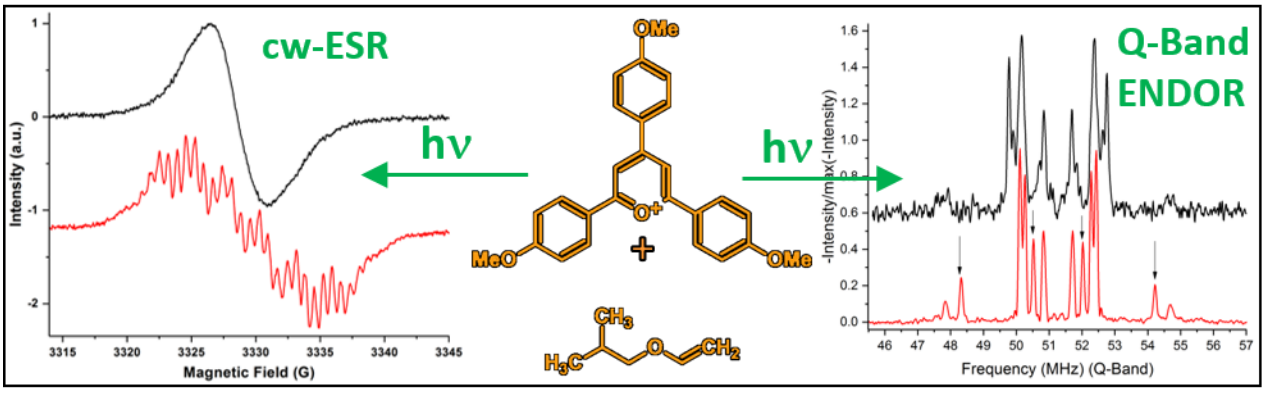

Figure 1: cw-ESR spectra and Q-Band pulsed ENDOR spectra obtained for the two systems "2,4,6-tris(4-methoxyphenyl)pyrylium tetrafluoroborate + tetrahydrofuran" (black curves) and "2,4,6-tris(4-methoxyphenyl)pyrylium tetrafluoroborate +isobutylvinylether" (red curves)

EPR/ESR

P115

Dipolar and hyperfine spectroscopic studies of the Hsp90 chaperone

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Abstract: Heat shock protein of 90 kDa (Hsp90) is an important ATP-dependent molecular chaperone (ATPase) evolutionary conserved from *E. coli* to humans and one of the most abundant proteins in the cytosol. Hsp90 is a protein folding chaperone that prevents misfolding and aggregation of client proteins participating in cancer. The protein is dimeric with each protomer consisting of three domains: the amino N, middle M and carboxyl C. During the catalytic ATP cycle Hsp90 adopts a variety of conformations that facilitate its function. It is postulated that the chaperone is a promising target for anti-cancer drugs *via* inhibition of its ATPase activity.[1] Despite recent progress in mapping the structural dynamics of full size Hsp90 by FRET [2], keys aspects of the ATPase-coupled mechanism remain enigmatic. Here, we study the ATP binding site of yeast Hsp90 using W-band hyperfine spectroscopic methods, while monitoring in parallel the Hsp90 conformations at various stages of the ATP cycle using electron-electron resonance (DEER or PELDOR) distance measurements. Specifically, we produced wild type (WT) Hsp90 and substituted the hydrolysis essential Mg^{2+} cofactor, which together with ATP binds to the N-domain of each protomer, with paramagnetic Mn^{2+} and ascertained that the ATPase activity does not change upon substitution of Mg^{2+} with Mn^{2+}. Next, we performed Mn^{2+}-Mn^{2+} DEER. All measurements exhibited low modulation depths due to the weak binding of the nucleotides with Hsp90 with the post hydrolysis (ADP) state yielding the deepest dipolar oscillations in accordance with literature where the Hsp90-ADP complex has the smallest dissociation constant K_d.[3] Importantly, the use of chirp pulses generated by the AWG unit [4] implemented in our spectrometer has allowed us to reliably resolve DEER dipolar oscillations. We performed DEER in presence of ATP, ADP, AMP-PNP and in presence of ATP+VO_4^{3-} ions which are known to trap the hydrolysis before release of the *γ*-phosphate. The distances between the catalytic centers were found to be similar among the different nucleotide samples and within the expected range as found by X-ray in other Hsp90 homologues.[3] Additionally, we studied the interaction of the Mn^{2+} ions with the phosphates of the respective nucleotide under the same sample conditions (buffer, concentration) as in DEER measurements. Here, we employed electron nuclear double resonance (ENDOR) spectroscopy and we were able to observe the ATP hydrolysis from ^{31}P ENDOR signals. We could also observe the interaction of Mn^{2+} with coupled nuclei (^{1}H, ^{31}P, ^{14}N, ^{15}N) from the nucleotide and the protein with ELDOR (electron-electron double resonance) detected NMR (EDNMR). ^{15}N labelled nucleotide was used to distinguish signals originating from protein and nucleotide.

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EPR/ESR

P116

Exploring Hybrid Perovskites by EPR Spectroscopy

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Abstract: Organometal halide perovskites, also known as hybrid perovskites, are an exciting class of semiconductors which recently attracted worldwide interest in the research community, thanks to their huge potential for optoelectronic applications, first of all in the field of photovoltaics. Hybrid perovskites have the composition ABX_3, where typically $A = CH_3NH_3^+$, $B = Pb^{2+}$, $X = Br^-$, I^-, and the interplay between the organic and inorganic constituents results in an interesting combination of properties. While several different characterization techniques have been employed so far to investigate them, and despite the great deal of information accessible by EPR spectroscopy, only a few EPR studies are available in the literature [1,2]. Part of the reason for this is the low amount of paramagnetic species in hybrid perovskites (which are intrinsically diamagnetic), so that different approaches must be followed in order to successfully apply EPR to these systems: here we present the results of two such approaches, illustrating the ability of EPR to address a broad range of issues in these materials.

In the first case, we aimed at introducing a paramagnetic probe (vanadyl ion VO^{2+}) into the perovskite structure to gain insight into the dynamics of the organic cation. EPR measurements on $CH_3NH_3PbBr_3$ single crystals precipitated by different methods pointed out that the conditions of synthesis determine the way in which the dopant gets incorporated. In one case, evidence of a direct involvement of the dopant in the photophysical processes of the host was also observed.

A second investigation focused on the interaction of hybrid perovskites coupled with other materials. We examined laser-ablated $CH_3NH_3PbI_3$ nanoparticles decorated with carbon residues that are formed during the synthesis [3]. EPR measurements, supported by other characterization techniques, established a correlation between the nature of the residues and their ability to sustain charge transfer with the perovskite. This basic process was associated to the observed inhibition of so-called electrical hysteresis, which is a detrimental effect for the performance of the final devices [4].

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EPR/ESR

P117

Integration of DEER data in Maximum Occurrence analysis

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Abstract: Calculating the expected experimental observables (e.g.: NMR, EPR, SAXS,…) from a given conformation, or ensemble thereof, is an affordable task. On the contrary finding the relative population of different conformers that compose the natural ensemble from the averaged experimental observables is an ill-posed inverse problem that admits an infinite number of solutions. Among the several approaches that have been provided over the years to address this problem,[1,2] we have proposed the Maximum Occurrence approach [3]. Maximum Occurrence is defined as the largest amount of time that the system can spend in a given conformation and still be compatible with the experimental observables. We here demonstrate how the inclusion of DEER data [4] can assist in constraining the maximum occurrence and identify those conformations that can be sampled longer consistently with the experimental data.

Acknowledgements: the authors thank Gottfried Otting for many helpful discussions. This work has been supported by the EC contracts #317127 (pNMR), # 653706 (iNext) and #675858 (West-Life) and the support and the use of resources of Instruct-ERIC.

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EPR/ESR

P118

Electrostatic Effects at Lipid Interfaces in Nano-Bio Hybrid Systems as Reported by Spin-Labeling EPR

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Abstract: Interfacing biological and artificial systems at the nano-scale level is essential for developing novel living-nonliving biotechnology platforms for applications in biology and medicine as well as for designing of biosensors. Despite an impressive progress achieved in creating new bio-nano hybrid systems, the needs remain high to understand the influence of a nanostructured support and nanoconfinement on structure and properties of the membrane-protein interface.

In this work we report on spin-labeling EPR studies to assess effects of solid inorganic interface, specifically, silica support, on 1) the phospholipid membrane surface electrostatic potential and 2) effective pKa of the membrane-burred peptide ionisable sidechains. Novel EPR active pH-sensitive lipids IMTSL-PE and IKMTSL-PE [1-3] were employed to measure the phospholipid membrane surface potential. The change in the protonation state of the label was directly observed by CW EPR allowing for determination of the effective pKa of the probe. We have shown that by forming POPC or POPC/POPG mixed bilayers on the surfaces of silica nanoparticles the absolute value of the negative electric potential at the membrane surface could be increased significantly. The potential of mixed bilayer was observed to be more sensitive to the silica support, suggesting a different mechanism of the mixed bilayer response to the nanostructured surface. Only single protonation transition was observed for EPR pH-sensitive probe, thus, suggesting that both leaflets of the silica-supported phospholipid bilayers have the same electrostatic surface potential. Addition of cholesterol to phospholipid bilayers did not diminished the bilayer response to silica.

Effects of the silica support on the dynamics of lipids and transmembrane peptide have been also investigated. Specifically, a model transmembrane α-helical WALP peptide was covalently-modified with cysteine-specific pH-sensitive nitroxides and incorporated into bilayers of various compositions. We have shown that the effective pKa of the probe positioned in a site-directed manner at the interface of the peptide and bilayer increases by more than 2 pH units upon replacing zwitterionic PC with anionic PG lipids. We have also investigated the effect of placing a phospholipid bilayer with the integrated transmembrane α-helical WALP peptide on the surface of silica nanoparticles on the peptide dynamics and the effective pKa of the probe. The silica support caused shift in the pKa of the probe consistent with the negative charge on the silica surface but induced a peptide transition upon the probe protonation not observed in liposomes. Supported by NSF 1508607 to TIS.

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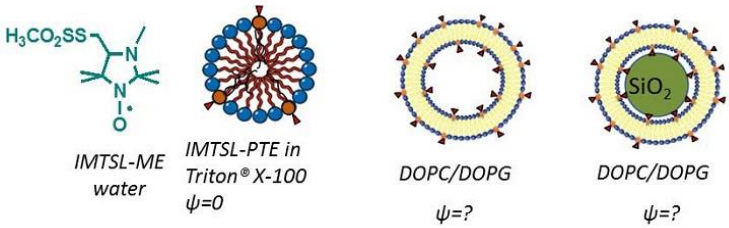
Image:

$$pK_a^i = pK_a^0 + \Delta pK_a^{pol} + \Delta pK_a^{el} \text{ or } \Delta pK_a^{Silica}$$



EPR/ESR

P119

Enhanced catalytic activity via an artificial heme protein: EPR characterization of the catalytic intermediate

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Abstract: Metalloenzymes catalyze a broad range of chemical reactions. Their catalytic activities depend on the detailed electronic properties of the metal active site, while non-canonical ligands can be exploited to fine-tune this catalytic metal-coordination sphere beyond nature's capability.

Here, we characterize a modified myoglobin equipped with the non-canonical amino acid N_δ-methylhistidine (NMH) as an axial ligand, called Mb*(NMH) [1]. The NMH coordination enhances catalytic activity of this heme protein in the abiological cyclopropanation reaction modelled by styrene and ethyl diazoacetate (EDA), even under aerobic and non-reducing conditions (Figure), in contrast to the wild type protein.

We study the underlying catalytic mechanism by reacting Mb*(NMH) with excess of EDA. Continuous wave (CW) EPR measurements characterize Mb*(NMH) as a high-spin Fe(III) species dominated by axial symmetry. Upon addition of EDA, the reactive intermediate forms quantitatively as a low-spin Fe(III) species of rhombic symmetry, featuring a direct ^{13}C-EDA coordination as evident from pulsed EPR experiments.

This reaction route through an Fe(III) species is contrary to previously proposed Fe(II) intermediates for a number of heme proteins, in particular myoglobin [2, 3]. Moreover, the subtle change in the nature of the axial ligand from a natural histidine to NMH coordination leads to a remarkable increase in catalytic activity. We investigate this intriguing relationship by probing the electronic environment of the Fe(III)-intermediate by EPR techniques in combination with isotope labelling.

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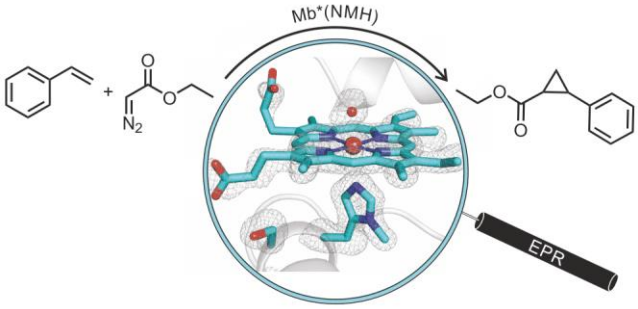

EPR/ESR

P120

Surface-supported low-coordinated Ti(III) alkyl species identified by Pulsed EPR Spectroscopy and DFT calculations

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Abstract: Low-valent Titanium(III) alkyl species have been proposed as possible key reaction intermediates in important industrial processes, such as Ziegler-Natta heterogeneous catalysis [1], an ethylene polymerization reaction which in spite of its widespread application remains poorly understood. Previous studies on Ti-based Ziegler Natta catalysts have shown multiple Ti(III) sites in active catalysts, including surface-exposed sites suggested to be active centers for catalysis [2]. Accurate structural characterization in this complex mixture of different Ti centers remains challenging.

Here, we address this problem via synthesis and characterization of well-defined, soluble, low-valent Ti(III) alkyl model complexes. These allow us to benchmark the spectroscopic toolkit of modern pulsed EPR-based hyperfine spectroscopy. Starting from a combination of the pulsed EPR experiment HYSCORE [3] and density functional theory (DFT) calculations on structures obtained by X-ray crystallography, we demonstrate the identification of nuclei, e.g. ^{1}H or ^{13}C, with couplings indicative of the Ti(III) environment for model complexes, such as $(ArO)_2Ti[CH(TMS)_2]$ (Fig. 1). The experimental hyperfine couplings are found to be in good agreement with DFT predictions and hence are shown to serve as diagnostic features of the structure of the molecular Ti(III) complexes. Comparing these to hyperfine couplings obtained from surface-bound Ti(III) species that are catalytically active in ethylene polymerization allows to characterize the local structure in an environment that is barely accessible by other techniques. This way potential intermediates can be identified that are responsible for catalytic activity.

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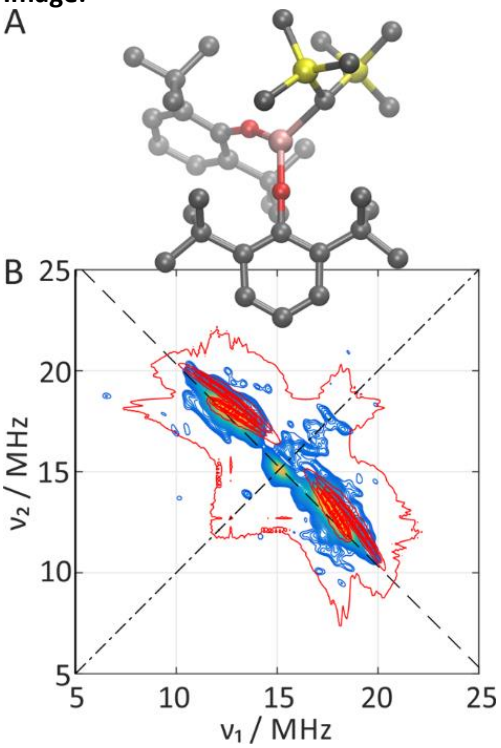

EPR/ESR

P121

EPR spectroscopy for the investigation of photophysical and morphological properties of polymeric thin films

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Abstract: Conjugated polymers are among the most studied materials for energy conversion in the field of Organic Photovoltaics (OPV). They can reach over 10% power conversion efficiency (PCE) and offer various advantages on inorganic photovoltaics like their light-weight and flexibility, a low cost, the ease of production through roll-to-roll printing methods. The main interests in polymer photovoltaic research are related to the characterization of the photoinduced processes and the morphological properties of the materials.

Electron paramagnetic resonance has established itself as a powerful tool to unravel both photophysical and morphological properties of these emerging materials[1-3]. Paramagnetic species such as polymer radicals, spin-correlated radical pairs and triplet states are involved in the photophysics of OPV blends and they are selectively detected by EPR methods, in contrast to optical spectroscopies that often give ambiguous results. Furthermore, EPR can give useful information on the average molecular orientation in the polymeric thin films[4-5], that are often quite amorphous materials, thus hardly accessible by diffraction techniques. This kind of information is highly significant considering the directionality of charge transport in these materials[6].

Some cases of small-bandgap conjugated polymers thin films will be presented, focusing on the EPR characterization of photophysical and orientational features. The techniques vary from Light-Induced CW EPR, to Time-Resolved EPR and Pulse methods, in order to fully exploit the potentialities of Electron Paramagnetic Resonance.

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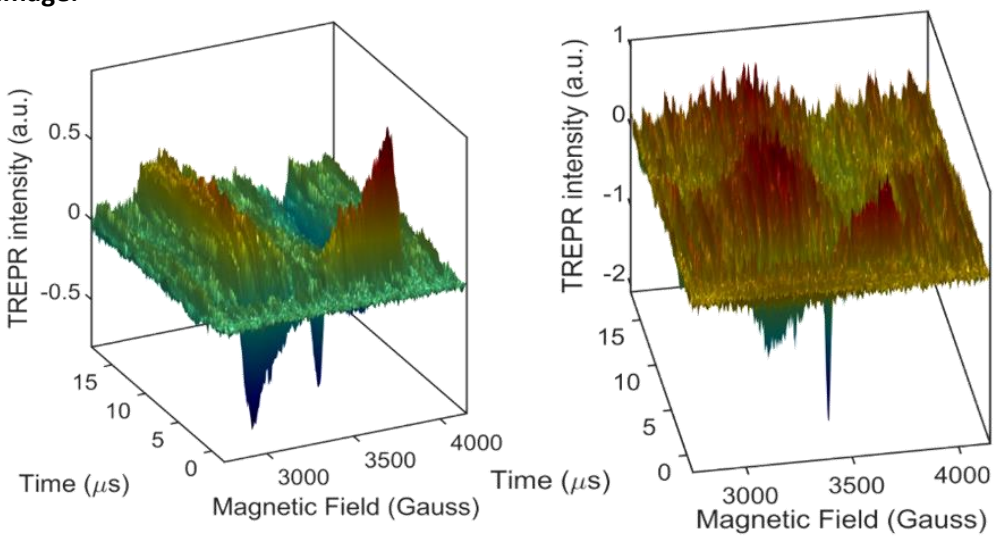

Figure. TR-EPR spectra detected upon variation of the relative orientation of the magnetic field and the film plane, in a spin-coated film.

EPR/ESR

P122

Accurate and Direct Determination of Distance Distributions for Pulsed Dipolar ESR by Singular Value Decomposition

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Abstract: Pulsed Dipolar Spectroscopy (PDS) methods, such as Double Electron Electron Resonance and Double Quantum Coherence, are powerful methods for studying the structure and function of biological systems. In PDS, a dipolar signal is acquired from the interaction between a pair of spin labels, from which the distance distribution between them, P(r) may be obtained. The P(r) can be in the range of 1 to 10 nm.

Problem and Current Approaches: However, due to the ill-posed nature of the inversion of the dipolar signal to yield the P(r), one must resort to regularization or model fitting methods to obtain reasonable results. The method of Tikhonov regularization (TIKR) is commonly used, but it relies heavily on the choice of regularization parameter (λ) that yields a compromise between good resolution and stability of the P(r). However, this procedure is still vulnerable to the appearance of spurious peaks and negative values for P(r), as well as effects of noise in the dipolar signal. Model fitting methods, on the other hand, require *a priori* model functions to estimate P(r), which may not accurately represent the actual distance distributions. This is especially true if the P(r) is multimodal.

New Method: We developed a new and objective approach based on singular value decomposition (SVD) that yields an optimum approximate solution, obviating the need for regularization.[1] Instead of solving for the complete distance distribution all at once, the method finds the optimal distribution value at each distance or distance range by determining each of their different singular value cut-offs. We show that each distance converges to its accurate value at different steps providing the singular value cut-off using the Piccard condition. In addition, all regularization methods are compromises, so they suffer from some regions of the P(r) not converging, while others have become unstable—both of which are sources for spurious peaks. The new method ensures optimal convergence at all distance ranges, while preventing a premature or unstable solution at some or all distance ranges.

Results: We tested the new SVD method on several model and experimental dipolar signals with unimodal and multimodal distributions. The method yields high resolution P(r) without any spurious peaks or negative P(r)'s and consistently performs better than TIKR.

Conclusion: The new method can successfully reconstruct multimodal distributions, both overlapping and independent, with varying distribution widths. It can be applied directly to a dipolar signal with sufficiently high SNR (say >100) or the dipolar signal can first be denoised using our recently developed WavPDS[2] method based on wavelet transforms[3].

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EPR/ESR

P123

Crude Oil's Trace Metal Analysis by Electron Paramagnetic Resonance Spectroscopy

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Abstract: Due to the unique magnetic characteristics of rare elements in crude oil, the research work focuses on their magnetic resonance properties and their distribution in different crude oil samples. Among the naturally existing paramagnetic species, the major one is vanadyl-porphyrin complex, which reveals a specific hyperfine structure (HFS) in its EPR spectrum. The performed analysis covers a comprehensive investigation of vanadium(IV)-ions in different solvents, at variable temperature, as well as an exploration of selected crude oil samples using EPR spectroscopy. Three types of Arabian light crude oils have been tested for their vanadium content, namely, Arabian Light (AL), Arabian Extra Light (AXL), and Arabian Super Light (ASL). The results show three different splitting of 'rhombic symmetry' of the vanadyl-porphyrin unit, which was confirmed by investigating the anisotropic feature of a standard vanadium-oxide-porphyrin material in toluene at the boiling point of liquid nitrogen. Moreover, a quantitative determination of the crude oil's vanadium content was investigated compared to the standard complex solution within the range of 0.1– 20 ppm (see Figure below). These results are more accurate than the ICP-MS results of a fully digested crude oil samples. The EPR-quantitative data indicated that the obtained results were comparable to the reported values and are found to be 13.94 ppm in AL, 1.87 ppm in AXL, and < 0.1 ppm in ASL crude oils.

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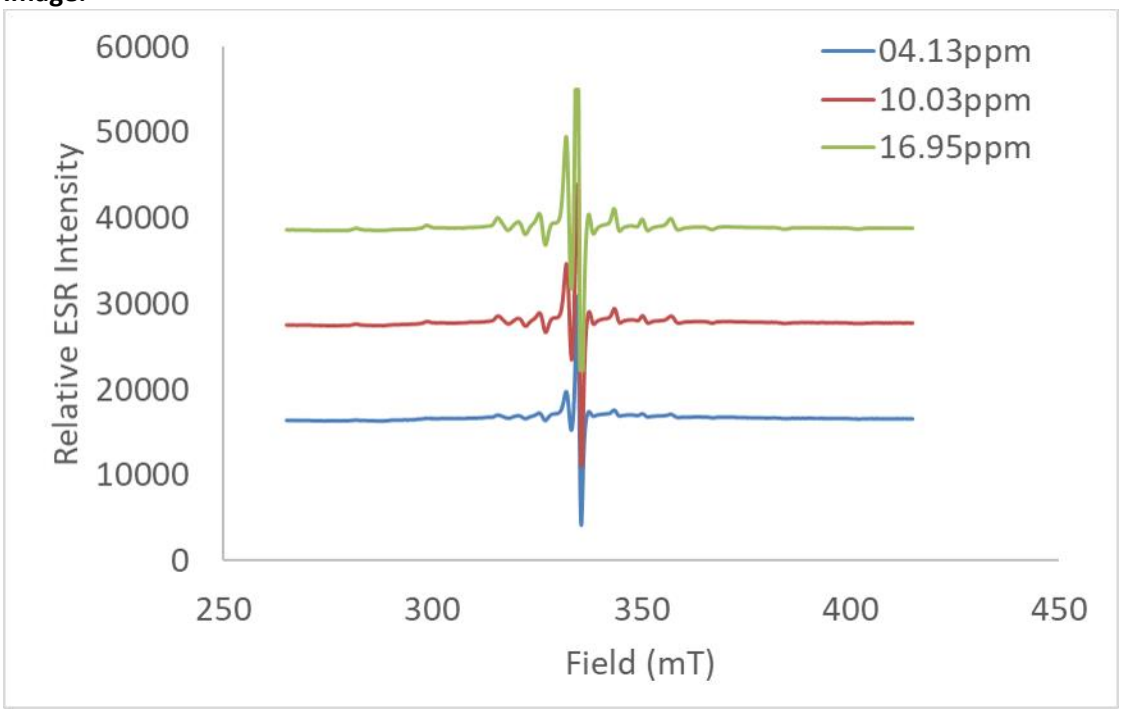

EPR/ESR

P124

Multi-frequency rapid-scan HFEPR

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Abstract: The development of the rapid scan technique was historically connected to the problem of the enhancement of the signal-to-noise ratio in NMR [1,2], but it did not find wide application in NMR or EPR due to the rapid development of high power radio-frequency and microwave sources for pulse methods. However, the past decade is marked by the intense development of solid-state THz instruments, which has made it possible to perform EPR spectroscopy at very high frequencies and fields [3-5]. Unfortunately, the output power of such tunable THz sources is not sufficient for the implementation of pulse methods. Consequently, the rapid scan is the only affordable technique for multi-frequency investigation of spin dynamics at THz frequencies. To our best knowledge, this EPR technique was demonstrated at frequencies up to 94 GHz only [6].

Here we present results of the first successful implementation of multi-frequency rapid-scan EPR in the (sub)millimetre frequency range. The experiments were performed using a home built HFEPR spectrometer operated in induction mode [4]. The spectrometer does not require any resonator, and therefore we are able to use frequency sweeps instead of magnetic field sweeps as it was done previously in the majority of experiments [7-9]. The main advantages of the frequency domain are the extremely high sweep rates (thousands of THz/s) and absence of eddy currents in the sample holder and/or resonator.

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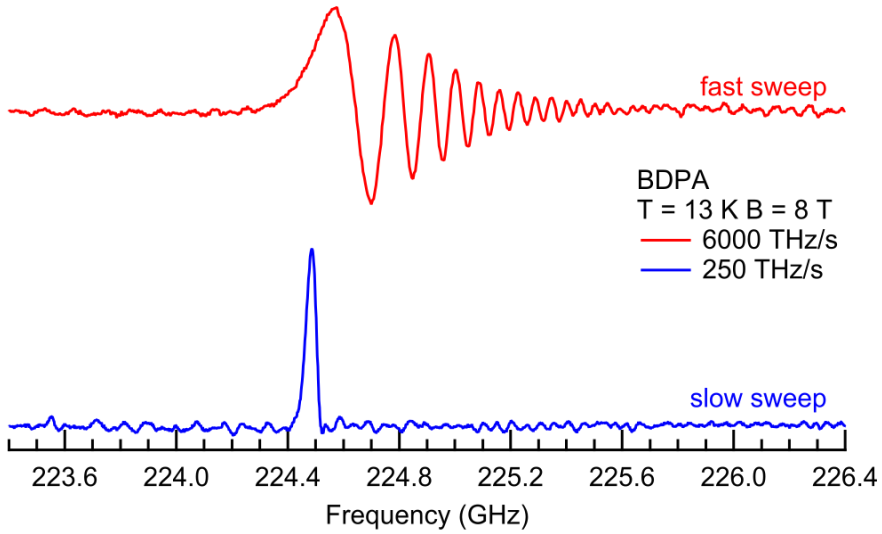

Spectrum of BDPA (dissolved in toluene) at low (blue) and high (red) sweep rates

EPR/ESR

P125

Putidaredoxin serves as the effector role by interacting with the C helix of P450cam

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Abstract: Cytochrome P450 is a heme-contained monooxygenase superfamily, which catalyzes oxidation of various substrates by molecular O_2. P450cam (CYP101A1) from *Pseudomonas putida* is a model enzyme of P450, with a strict specificity of the substrate (camphor) and reductase (putidaredoxin, pdx). Pdx not only transfers two distinct electrons to P450cam, but also shifts the camphor bound P450cam from the closed to the open structure. [1] Two possible mechanisms of pdx's effector role were purposed: pdx induces a structural perturbation of the heme in P450cam, or the hydrophobic interaction between C helix of P450cam ($P450cam_{Chelix}$) and C terminus of pdx alters the hydrogen bond network in P450cam.

In this study, the conformation of P450cam upon pdx binding is elucidated by Double Electron-Electron Resonance (DEER) and Molecular Dynamics (MD) simulations. DEER is a pulsed EPR spectroscopic method to measure the distance between radicals. A bifunctional spin label (BSL), with limited rotamer freedom, is employed to probe the delicate motions on $P450cam_{Chelix}$. DEER spectra reveal that $P450cam_{Chelix}$ undergoes a subtle conformation change upon pdx binding. In addition, MD simulations indicate this conformation change is due to the interaction between the C-terminus of pdx and $P450cam_{Chelix}$. Besides, we show that two mutants of pdx that lack either an aromatic group on the C-terminus or the salt-bridge to P450cam, cannot regulate the P450cam substrate access conformation. With the combination of DEER spectroscopy and MD simulation, the mechanism of the effector role for pdx to modulate P450cam conformation is clarified, which is important to understand the catalytic metabolism of other members in the P450 superfamily.

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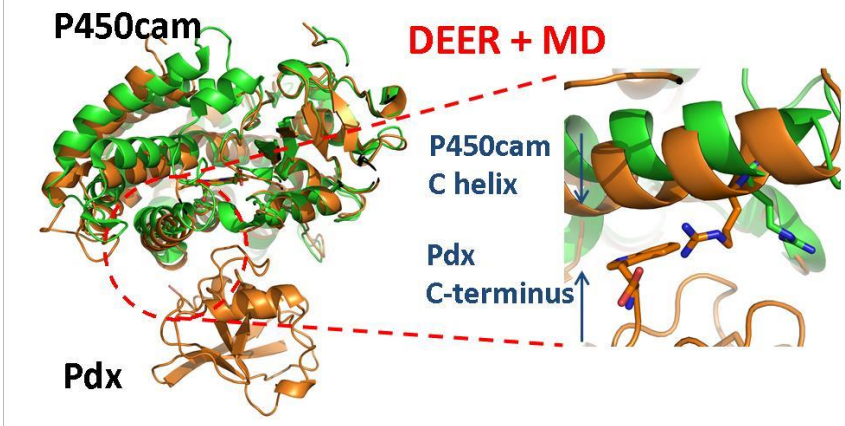

EPR/ESR

P126

Study of electron Spectral Diffusion process under DNP conditions by ELDOR Spectroscopy while considering the Solid Effect

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Abstract: Recent studies have shown that electron Spectral Diffusion (eSD) influences strongly the Cross Effect (CE) DNP mechanism at low temperatures and high radical concentration under static conditions[1]. During the CE process, the nuclei coupled to an electron spin pair at a CE condition are hyperpolarized directly by microwave (MW) irradiation of one of these electrons or indirectly by an off-resonance irradiation which creates a polarization difference between the two electrons via the eSD. This process can result in a partial depolarization of all electrons in the sample, which can be studied using ELDOR (electron-electron double resonance) spectroscopy. In addition to the eSD process, electron depolarization can also occur as a result of the Solid Effect (SE) DNP process. The SE induced polarization depletions become evident in ELDOR spectra for radical concentrations that are below the usual concentration used for DNP. For example when TEMPOL radicals are used as a polarizers, the SE of both ^{14}N and ^{1}H nuclei contribute to the SE depolarizations manifested in the ELDOR spectrum.

To improve our understanding of the eSD and the SE depolarization processes by taking into account the spin dynamics of the spin system under MW irradiation and relaxation conditions, we measured and simulated ELDOR spectra of TEMPOL radicals dissolved at 0.5 and 10 mM concentrations in solid solutions of 50/50 wt% mixture of DMSO/H_2O at 20 K and at W-band frequency (94.8GHz). In addition we carried out T_{1e} measurements to determine the electron-spin lattice relaxation times along the EPR spectra. Initially we analyzed the ELDOR spectra of the 0.5mM TEMPOL sample. Here the eSD process is minimal and ELDOR frequency profiles are mainly determined by the SE depolarizations. To analyze these data we carried out quantum mechanical calculations based on the complete spin Hamiltonian describing the interaction of an unpaired electron with ^{14}N and ^{1}H nuclei, taking also into account the microwave irradiation and relaxation using the Liouville master equation. Then, we analyzed the 10 mM TEMPOL spectra by including in the analysis the polarization exchange rate equations (the eSD model), which describe the eSD process in the electron spin system by introducing a single eSD exchange parameter, Λ^{eSD}. The experimental ELDOR spectra could be simulated rather well but with a L^{eSD} parameter that is a function of the detection frequency, showing higher values at frequencies that correspond to the positions of high g_{zz} values where also long T_{1e} values are detected. This suggests that the Λ^{eSD} parameter can become anisotropic at low TEMPOL concentrations.

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EPR/ESR

P127

Conformational study of an Archaeal Photoreceptor/Transducer Complex from Natronomonas pharaonis assembled in lipid nanoparticles using the EPR spectroscopy

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Abstract: The transmembrane protein signaling complex *Np*SRII/*Np*HtrII plays a key role in negative phototaxis of the halophilic archaeon *Natronomonas pharaonis*[1]. Photon absorption induces transient structural changes in the photoreceptor sensory rhodopsin II (*Np*SRII),[2] which are conducted to the transducer *Np*HtrII. The subsequent signal propagates along the cytoplasmic part of the transducer *Np*HtrII to the intracellular signaling pathway[3] that modulates the rotation of the flagellum. The aim of this work was to study conformation and dynamics of *Np*SRII/*Np*HtrII after assembly in cell-membrane mimicking nanoparticles, namely, styrene maleic acid lipoprotein particles (SMALPs) (Figure 1) and lipid nanodiscs. We used the continuous wave (cw) electron paramagnetic resonance (EPR) spectroscopy to analyze the protein dynamics through tracking the residual motion of spin labeled side chains.[4] As revealed by the cw EPR, the dynamics of the nitroxide spin label was not affected significantly by incorporation into nanoparticles. The distance distributions between spin labels were determined by pulse EPR experiments and compared with *in silico* spin-labeling rotamer analyses based on available X-ray crystallographic data. The acquired pulse EPR data indicate that the SMA-engaged *Np*SRII/*Np*HtrII complex had a 2:2 stoichiometry. The measured distance distribution corresponds to the "V"-shaped conformation of the two sensory rhodopsin molecules, as found in crystal structures[5, 6]. The presented data indicate the integrity of the *Np*SRII/*Np*HtrII complex upon its reconstitution in SMA/lipid particles.

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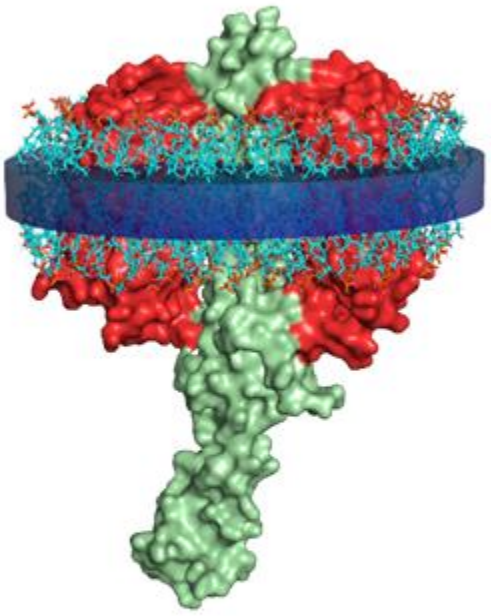

Figure 1. Schematic SRII/HtrII complex in SMALPs.

EPR/ESR

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Flexible viologen cyclophanes : Substituent and odd/even effect on intramolecular interactions

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Abstract: Stimulus-responsive molecular species are of primary importance in modern host–guest chemistry in the era of molecular machines. Among the numerous redox-active molecules, viologens have been selected for decades as building blocks with switchable properties due to their multiple and accessible redox states. In addition, viologen derivatives form p-dimers in their radical cation states at low temperature and in aqueous or confined media. The present work is intended to explore the physical and chemical properties of two original different families of viologen cyclophanes recently synthesized (Figure 1). Comparative spectro-electrochemical studies of these macrocycles show that the development of intramolecular interactions in aqueous solution depends on the length of the bridges. This dependence is confirmed by EPR spectroscopy and DFT studies of the magnetic coupling in the diradical dication species. The anti-ferromagnetic or ferromagnetic nature of the coupling depend, respectively, on the odd or even number of methylene groups in the spacer[1].

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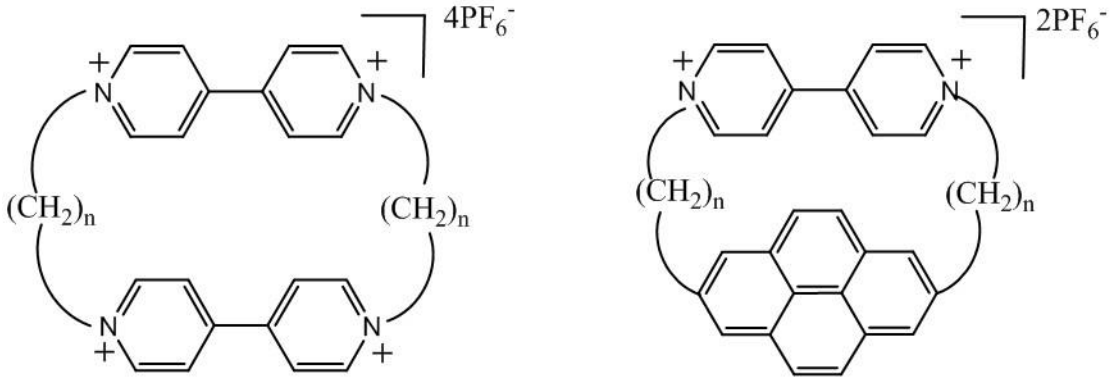

Figure 1

EPR/ESR

P129

Performance of 1H-ENDOR at 263 GHz/9.4 Tesla

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Abstract: Electron-nuclear double resonance (ENDOR) is a method to detect magnetic nuclei in vicinity of a paramagnetic center that plays a crucial role in a wide number of applications. ENDOR is mainly performed at medium EPR microwave frequencies (≈ 1.2 T/ 35 GHz). However, at higher frequencies (≥ 90 GHz), the technique delivers several advantages. Among the most important ones, the hyperfine spectra can be analyzed in a high-field approximation and microwave pulses excite only a small fraction of molecular orientations, which leads to enhanced resolution and facilitates determination of hyperfine tensor orientations.

Here, we present the performance of ^{1}H-ENDOR at 263 GHz EPR frequency and a corresponding 9.4 T magnetic field using a commercially available quasi-optical EPR/ENDOR spectrometer (Bruker E780). We employ protonated BDPA as well as a protein sample from *E.coli* RNR containing a stable Y□ tyrosyl radical as different model systems to test ENDOR efficiency and orientational selectivity. Proton ENDOR spectra were recorded with different pulse techniques, i.e. Davies-, Mims- and CP-ENDOR [1] and compared. They show that all methods work at this frequency and demonstrate high S/N. However, their performance strongly depends on sample temperature and pulse optimization.

Experiments on a 200 mM Y_{122}□-*E. coli* RNR protein sample were performed in H_2O and D_2O at 5 K, a regime of almost 100 % electron spin polarization. The high spin polarization is manifested in a slight asymmetry of intensities of hyperfine transitions due to a selective population of spin manifolds and relaxation effects. Spectral simulations for a set of five coupled protons based on parameters reported at lower frequency (140 GHz, [2]) demonstrated that the high orientational selectivity at 263 GHz leads to unprecedented resolution. In contrast to previous experiments [2], the hyperfine tensors of all internal proton couplings could be unambiguously resolved.

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Exotica

P130

On the way to NMR at Mega-Bar Pressures: Observation of Hydrogen Bond Symmetrisation in High Pressure Ices

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Abstract: The last 15 years have seen an astonishing boost in NMR sensitivity and accessible pressure ranges [1] owing to developments in implementing NMR in diamond anvil cells (DACs) for high pressure experiments. Recently, with application of electro-magnetic lenses, so called Lenz lenses, in toroidal diamond indenter cells, pressures of up to 72 GPa with NMR spin sensitivites of about 10^{11} spins/$Hz^{1/2}$ could be realised [2]. In the first part of this contribution, a refined NMR resonator structure will be introduced. This structure employs a pair of double stage Lenz lenses driven by a Helmholtz coil within a standard DAC, with only 100 pl sample volume available prior to compression. With this set-up, pressures close to a mega-bar (1 Mbar = 100 GPa) could be achieved [3]. The second part focuses on the recently observed hydrogen bond symmetrisation in high pressure ices [4].

It is a long-standing paradigm that hydrogen bond symmetrisation in H-bonded systems is triggered by pressure induced nuclear quantum effects (NQEs). While theory clearly predicts that quantum-mechanical tunnelling of protons within hydrogen bonded systems is present for pressures between 20 GPa and the H-bond symmetrisation transition at about 70 to 80 GPa, clear experimental evidence is mostly absent. Here, first ^{1}H-NMR studies of high pressure ice in a range from 8 to 90 GPa demonstrate that NQEs govern the behaviour of the hydrogen bonded protons in ice VII already at significantly lower pressures than previously believed. A pronounced tunnelling mode was found to be stable up to the highest pressure, well into the stability field of ice X where NQEs are believed to be absent due to the unimodal probability distribution of the protons in a fully symmetrised H-bond network. Two distinct transitions in the shift data could be attributed to the step-wise symmetrisation of the H-bond network, i.e. High Barrier H-Bonds --> Low Barrier H-Bonds --> symmetric H-bond. This behaviour might be ubiquitous in other H-bonded systems, like hydrous minerals, which are also thought to exhibit pronounced proton conductivity.

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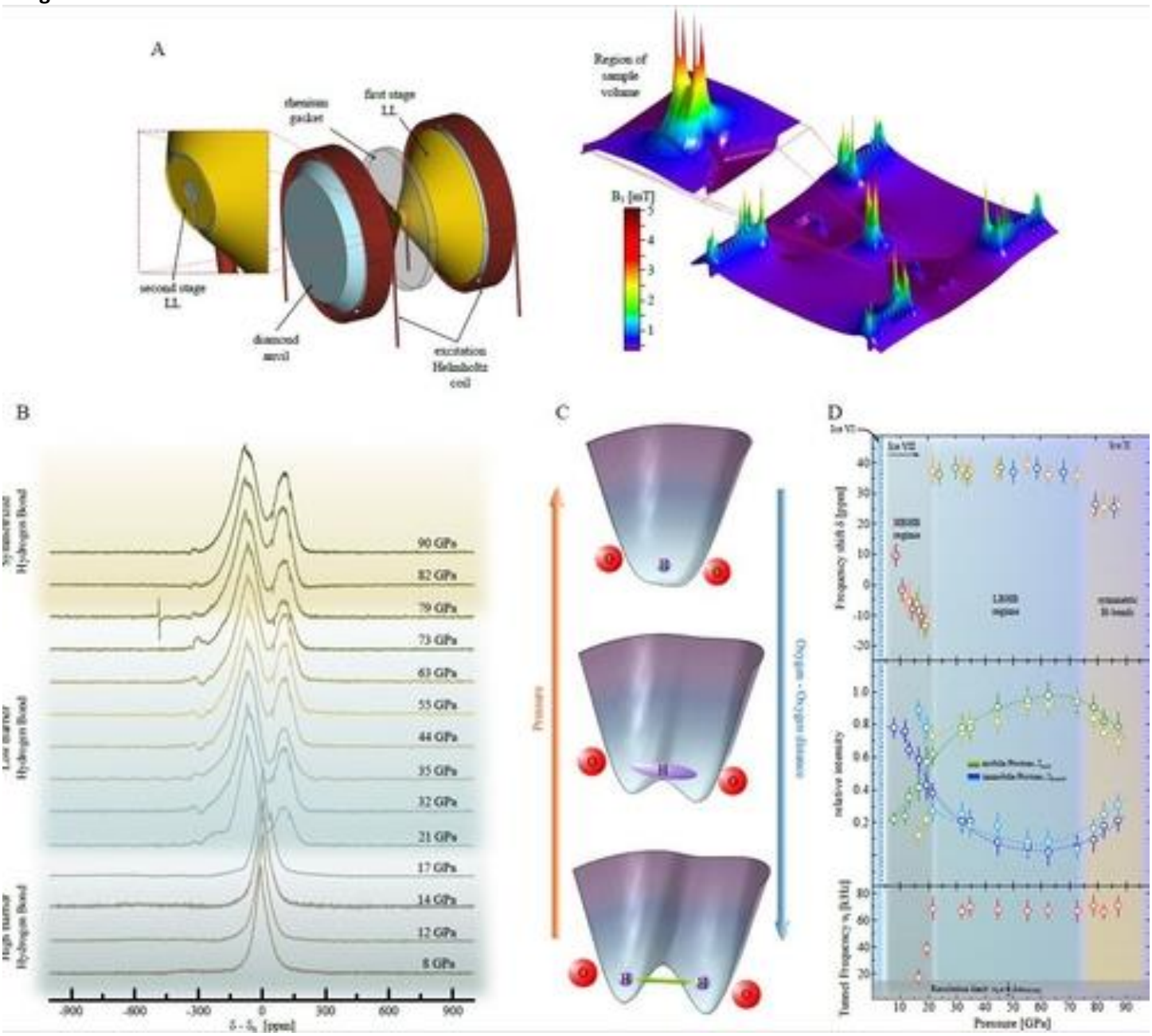
A
first stage LL
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diamond anvil
excitation Helmholtz coil
Region of sample volume
B
90 GPa
82 GPa
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73 GPa
63 GPa
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35 GPa
32 GPa
21 GPa
17 GPa
14 GPa
12 GPa
8 GPa
C
Pressure
Oxygen - Oxygen distance
D
Pressure [GPa]

Exotica

P131

Exploring the folding pathway of the multi-modular Titin protein with high-pressure NMR.

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Abstract: Titin is a giant, multi-modular muscle protein that spans half of the sarcomere in vertebrate striated muscle. The titin molecule is divided into two segments for which the function differs. While the A-band segment, anchored to the myosin thick filaments in the sarcomere, is largely invariant amongst different splicing forms, the I-band region of titin shows some variations[1]. Since these variations correlate with the differences in passive tension, the I-band region of titin has attracted a lot of attention for the past decades. It is known to be involved in the generation of muscle elasticity and passive muscle tension, but the molecular basis of this phenomenon is not yet fully understood, even though partial unfolding of its constitutive modules has been invoked[2]. We have used high pressure NMR[3] to monitor the folding pathway of titin I91 module and to track possible folding intermediates that might be formed and have some role in muscle passive elasticity. Owing to the residue-specific analysis allowed by high-pressure multidimensional NMR, we were able to experimentally decipher the molecular events leading to the unfolding of titin I91 module without the help MD or protein engineering.

We have also characterized the unfolding parameters (steady-state and kinetics) of I91 module, alone or in tandem. The unfolding of these two constructs appeared rather similar, even though a slight stabilizing effect has been detected for I91 tandem, as well as a slight increase in the unfolding rate constant.

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Figure: The structure of I91 titin single-module adopts the classical Ig frame fold: a sandwich of two ß-sheets, each consisting of 4 **ß**-strands, packed against each other. The A'G parallel ß-sheet is colored in red. The analysis of the residue-specific unfolding maps allowed us to establish the contact probabilities (P_{ij}) at a given pressure (red ticks stand for $P_{ij}<0.5$ at 600 bar). It appears as the Achilles heel of the structure: its disruption corresponds to the earliest event preceding I91 complete unfolding.

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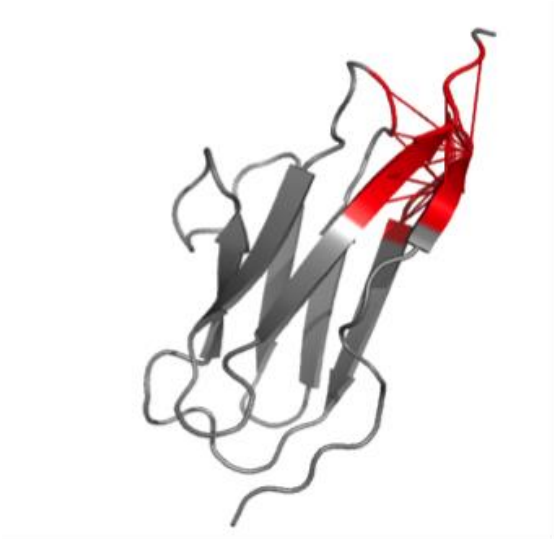

Exotica
P132
Analog quantum simulator of a four-mode Bose-Einstein Condensation using coupled quadrupolar nuclei
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Abstract: Ultra-cold many particle systems are efficiently described in terms of creation and annihilation operators, by using the Gross-Pitaevskii equation or the Bogoliubov transformation [1-3]. But in special cases, such as a two-mode Bose-Einstein condensate (BEC), there is another formalism to describe those many particle systems, it is the Schwinger representation, which is used to describe a many-body Hamiltonian of ultra-cold particles into an angular momentum formalism [4,5]. The set of angular momentum operators, which obeys Schwinger's representation, preserves their commutation rules and properties of Lie algebras [6].

On the other hand, there are some sets of spin angular momentum operators, also called Isospin operators (notation introduced by Heisenberg), which satisfy analogous properties of angular momentum operators [6]. Those Isospin operators were explored and discussed in the context of quadrupolar nuclei systems, and implemented experimentally using the technique of Nuclear Magnetic Resonance (NMR) in samples of lyotropic liquid crystals [7-9].

Here, we focus our attention about the theoretical description, and we discuss an extension of the formalism from the two-mode to the four-mode BEC, their consequences and restrictions. The theoretical spin model used to achieve this analog quantum simulator is a spin system of two quadrupolar nuclei, each one of different nuclear specie, and between them coupled by a spin-spin dipolar interaction.

The authors acknowledge financial support from brazilian agencies: CAPES, CNPq, the National Institute for Science and Technology of Quantum Information (INCT-QI) and Fundação-Araucária.

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Exotica

P133

Development of an in-operando NMR setup for carbon dioxide electrolysis at silver electrodes

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Abstract: The electrolytic reduction of carbon dioxide (CO_2) is performed in aqueous media at silver electrodes to obtain carbon monoxide (CO). It is assumed that the reduction step takes place at the silver surface by formation of an adsorbed $CO_2^{\cdot-}$ radical. Still, confirmative results as well as a detailed knowledge about the reaction mechanism are missing to date [1]. CO is a versatile reagent and is commonly involved in a variety of processes to gain energy-rich compounds and fuels. With the transition towards renewable energy production, there is an increasing interest in the industrial application of the CO_2 electrolysis for energy storage and conversion. The industrial implementation requires an increase in efficiency, which can be provided by a deeper understanding of the reaction steps taking place.

NMR is widely used to study reaction kinetics and mechanics. Spectral investigations allow real time tracking of reactant concentrations. Even if reactions cannot be assessed directly, NMR offers indirect methods to obtain information about the system, e.g. relaxometry which can provide information about reaction dynamics. Since the electrolytic CO_2 reduction can only be studied during operation, NMR in-operando techniques are required. The prerequisite is a setup which allows operation of the electrolysis inside the NMR magnet. The combination of in-operando NMR and CO_2 electrolysis imposes several challenges: the metal electrodes disturb the magnetic field homogeneity which leads to line broadening. NMR on conductive materials distorts the radiofrequency pulses, and thus causes distortions in pulse sensitive experiments. Furthermore, standard electrolysis cells do not fit inside the probe head and therefore need to be tailored for in-operando methods.

To overcome these challenges, a twofold approach is presented in this contribution. On one hand, an in-operando NMR electrolysis setup has been developed, which is compatible with standard liquid state probe heads. The in-operando electrolysis cell is set up inside a standard 5 mm NMR tube consisting of a three-electrode setup, following the approach of Ziegs et al. [2].

On the other hand, ^{13}C NMR studies have been performed on ^{13}C enriched CO_2 and equilibrium species. The ^{13}C NMR relaxometry experiments confirm a dynamic chemical equilibrium between solved carbon dioxide and the bicarbonate electrolyte indicated by decrease in T_2. Exchange experiments show that the exchange is fast enough to resupply the electrolysis with CO_2 at low current densities.

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Exotica

P134

NMR implementation of the quantum Battle of the Sexes game

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Abstract: In the last twenty years, Game theory had an extension to improve some tasks in the context of Quantum Information Processing. This transition explores the superposition principle of strategies, and the concept of entanglement. Both quantum tools enable better payoffs for players and avoid paradigms that some games under the classical regime have. There are some emblematic games to discuss the mathematical definitions at the quantum regime, such as the Prisoners' Dilemma, Battle of the Sexes, the PQ penny flipover [1], among many others [2]. From those, the Prisoners' Dilemma [3] was the most studied and also implemented experimentally by NMR using a two qubit system represented by two Hydrogens of a cytosine sample [4].

Another game that reveals the quantum advantages is the Battle of the Sexes was proposed by L. Marinatto and T. Weber [5]. Both authors developed other point of view of a quantum protocol, but with restrictions for player's choices. In order to put in evidence the versatility of the protocol, other discussions were developed, such as to propose another scheme to quantize the game [6], new procedures to find more than two Nash equilibria [7], as well as arguing that the protocol proposed by Marinatto-Weber is a reduced version of the Prisonner's Dilemma protocol [8]. Many of those discussions coincide that the players win the same payoff, but they do not point the commom strategy for both players that solve the dilemma. Perhaps, the most important comment was done by S. C. Benjamin [8], about a relation between strategies performed by both players, in order to find a maximum gain for both. He stated that the strategy operator of player B must be equivalent to the conjugate strategy operator of player A, that is, U_B = U_A^*.

From the arguments discussed above, our study was devoted to search an answer to Benjamin's comment. From that knowledge, we find many strategies such that the players win the same payoff. In particular, there are two strategies that allow the players win the game. In addition, we must perform an experimental verification via the NMR technique, using a two qubit system represented by the Hydrogen and Carbon nuclei of a enriched Chloroform sample.

The authors acknowledge financial support from Brazilian agencies: FAPESP, CAPES, CNPq, the National Institute for Science and Technology of Quantum Information (INCT-QI) and Fundação-Araucária.

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Exotica

P135

An 17O NMR Study of Diamagnetic and Paramagnetic Lanthanide-tris(oxydiacetate) Complexes in Aqueous Solution.

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Abstract: ^{17}O-enriched complexes between oxydiacetate (ODA) ligand and several diamagnetic and paramagnetic lanthanide-(III) metal ions (Ln) were investigated by solution-state ^{17}O NMR spectroscopy to get information on their isostructurality. Such research is complicated by the limited stability constants (the presence of free ligand or 1:1 and 2:1 species cannot be avoided) and by chemical exchange between free and bound ligands.

The bound-state signals of chelating (O_{in}) and nonchelating (O_{out}) oxygen atoms of the carboxylate groups were observed for all the samples investigated, with the exception of $[Yb(ODA)_3]^{-3}$ whose O_{in} signal is superimposed with the signal of the solvent (water).

The chemical shift of O_{out} signals are barely affected by the Ln ion, and show values similar to those already obtained for diamagnetic complexes. In contrast, O_{in} signals display very different chemical shift values, ranging between about 700 and -2300 ppm.

The data indicate that ^{17}O line width is dominated by quadrupole relaxation and also by chemical exchange in the case of Pr and Nd complexes. The measured O_{in} chemical shift values were analysed using the Reilley method. The Lanthanide Induced Shifts were dissected into Fermi contact and pseudocontact contributions: the dipolar contribution is negligible for the majority of the studied systems and the hyperfine coupling constant was estimated (A/ħ=0.6 MHz). The obtained data were perfectly fitted using a single line. No evidence of structural change along the Ln series was detected.

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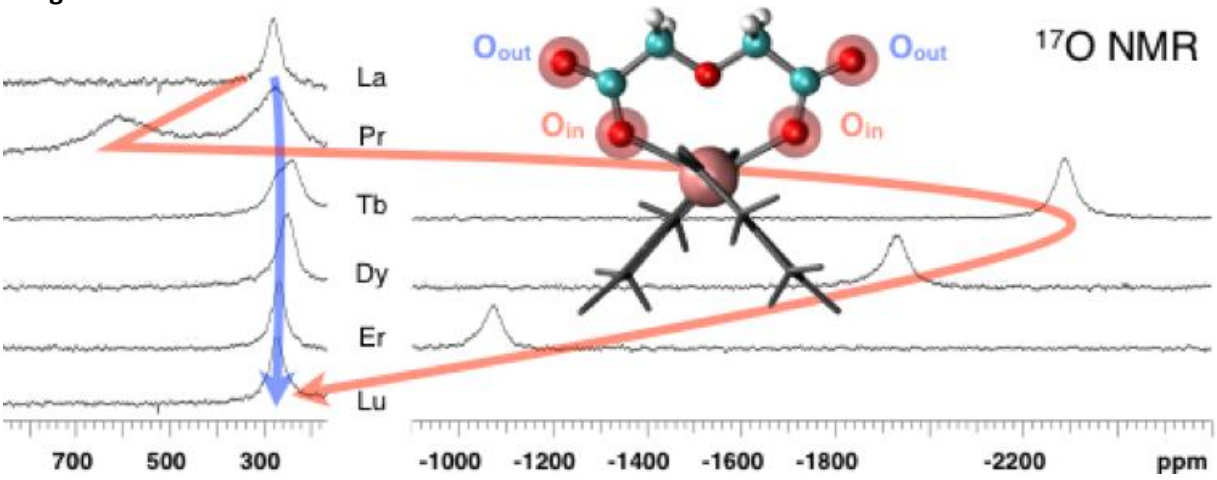

Exotica

P136

Solution-state NMR of transition metals with half-integer spins

Xavier Trivelli[*1], Frédéric Capet[2], Stéphane Cordier[3], Jérôme Cuny[4], Laurent Delevoye[2], Simon Desset[2], Franck Dumeignil[2], Régis Gautier[3], Régis M. Gauvin[2], Laurent Le Pollès[3], Yann Molard[3], Yohann Morin[2], Duc Hanh Nguyen[2], Thi Tuong Nguyen[3], Jean-Francois Paul[2], Sébastien Paul[2], Frédérique Pourpoint[2], Julien Trébosc[2], Tom Vancompernolle[2], Lei Zhang[2]

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Abstract: We characterized some clusters and organometallic catalysts by solution-state NMR. Quadrupolar nuclei such as Scandium-45 [1] and Manganese-55 [2] have been studied by monodimensional experiments on organometallic complexes. Molybdenum-95 NMR measurements coupled with nuclear shielding and chemical shift DFT calculations have been investigated on $[Mo_6X_{14}]^{2-}$ clusters [3]. Very-low frequency nucleus iron-57 has been studied by bidimensional NMR in several organometallic hydrides [4].

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Acknowledgements:

French national NMR facility IR-RMN-THC (FR CNRS 3050), Chevreul (FR CNRS 2638), French *Ministère de l'Enseignement Supérieur et de la Recherche*, *Régions Nord-Pas de Calais et Bretagne*, FEDER, *Institut pour la Transition Energétique* (ITE) PIVERT, French *Agence Nationale de la Recherche* (ANR), Supercomputing facility of Toulouse III University (CALMIP).

Exotica

P137

Using SABRE hyperpolarization to increase the sensitivity in nuclear magneto-optic spectroscopy

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Abstract: Nuclear magneto-optic spectroscopy (NMOS) is a young field of research studying effects based on interaction between nuclear spin magnetization and the light radiation mediated by the molecular electron cloud. The simultaneous influence of optical excitations and localized magnetic fields from aligned nuclear spins give rise to intrinsic, molecule-specific optical responses, such as linear or circular birefringence. While NMOS phenomena are closely related to NMR observables, such as chemical shift and dipolar coupling, the presence of perturbation by the light also brings in new information about the molecular structure and offers a way to enhance the chemical resolution between different nuclei by using the optical properties of the sample.

The most well-documented NMOS effect is the so-called nuclear spin-induced optical rotation (NSOR). Several experimental studies of NSOR have been reported [1-4], but the efforts have so-far dealt mostly with pure substances, such as neat solvents. The NSOR of samples of substantially lower, chemically more relevant, concentrations was so-far not extensively studied.

In this contribution, we present a new combination of NSOR instrumentation with a solution-based continuous hyperpolarization technique. The setup, based on the SABRE method, allows observation of NSOR of samples in sub-molar concentrations with the signal quality comparable to that of neat liquids investigated in previous studies. The presented approach marks a shift in NSOR from measurements of pure substances towards solution-based NSOR spectroscopy, bringing it closer to chemically relevant conditions and significantly widening the pool of viable samples. Such option opens up the field to more versatile studies of these intriguing new phenomena.

In addition, new theoretical results will be shortly discussed, showing how the influence of the radiation field of the light allows NMOS effects to reflect detailed features of the molecular structure in a manner inaccessible to the conventional NMR, such as the shape of the electron density upon electronic excitation. Such insight could prove useful in design and studies of molecules with a specifically tailored optical properties.

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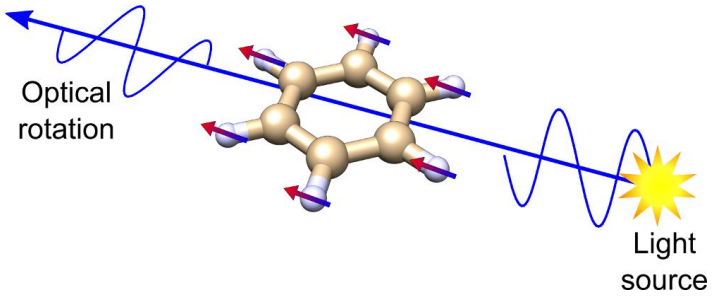

Exotica

P138

Breaking the quantum adiabatic speed-limit by jumping along geodesics

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Abstract: Because of their intrinsic robustness, adiabatic evolutions find a broad range of applications in quantum state engineering, quantum computing, and quantum simulation. According to the original form of the quantum adiabatic theorem, for a process to remain adiabatic, its rate of change at all times must be much smaller than the smallest energy gap of the Hamiltonian.

On the other hand, in order to avoid perturbations from the environment high rates of change are desirable. This tension can impose severe limitations on the practical use of adiabatic methods. Current approaches to obtain adiabatic evolution within system coherence times are shortcuts to adiabaticity by using intricate schemes of additional counterdiabatic driving fields which however can be exceedingly challenging to implement experimentally.

Here, we experimentally verify a recently derived adiabatic condition, which is based on the dynamical phases instead of energy gaps, by using a nitrogen-vacancy (NV) centre in diamond. We demonstrate experimentally that, surprisingly, energy gaps may vanish while maintaining adiabatic evolution, a result that challenges traditional views. This shows that dynamic phases are more fundamental than energy gaps in quantum adiabatic evolution, the key insight that allows us to overcome the limits on evolution times imposed by the traditional form of the adiabatic theorem and to achieve, within experimental uncertainties, unit fidelity quantum adiabatic processes in finite time.

The results provide a deeper understanding on quantum adiabatic processes, as well as promising strategies and directions in the control of quantum systems.

Exotica

P139

Multidimensional NMR for Studying Relaxation Correlation and Exchange in Articular Cartilage with Time Domain Analysis

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Abstract: Articular cartilage is the thin, load bearing tissue that lines joint surfaces. Nuclear magnetic resonance (NMR) transverse relaxation and diffusion data in articular cartilage has been shown to be multi-exponential and correlated to the health of the tissue [1, 2]. The observed relaxation rates are dependent on experimental parameters such as solvent, data acquisition methods, data analysis methods, and alignment to the magnetic field [8]. The aim of this study is to characterize the effect of diffusive exchange on observable relaxation and diffusion rates in cartilage by utilizing T_1-T_2, D-T_2 correlation methods, and T_2-T_2 exchange methods [3].

Using two site T_2-T_2 exchange experiments in combination with time domain data processing [4-7], the exchange rate was determined for porcine cartilage based on a single mixing time. Characteristic exchange times are on the order of 1 s which is commensurate with T_1 and much slower than T_2. This causes the T_1 relaxation times of the two sites to be averaged by exchange, thus reducing T_1 to only one observable value while the T_2 relaxation times are not affected. This is consistent with the intermediate exchange regime [4]. Using the time domain fitting results of the T_2-T_2 data [4] and analytical modelling [5], the T_1-T_2 behaviour of the individual sites is predicted. Diffusive exchange leads to an exchange peak in the T_1-T_2 correlation experiment [3]. Moreover, using D-T_2 correlations experiments one can estimate average fluid displacements which occurs over the period of the exchange time. These displacements are found to be in the order of ~100 µm which is consistent with diffusion between distinct oriented fiber domains featuring different T_2 [8].

The approach used here allows for a meaningful interpretation of one- and multidimensional NMR relaxation and diffusion data obtained in articular porcine cartilage. Time domain analysis allows for the reliable quantification of NMR relaxation behavior in the presence of diffusive fluid exchange between two environments.

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Exotica

P140

Field-cycling NMR experiments in an ultra-wide magnetic field range

Alexey Kiryutin[*1], Ivan Zhukov[1,2], Alexandra Yurkovskaya[1], Yuri Grishin[3], Hans-Martin Vieth[1,4], Konstantin Ivanov[1]
[1]Department of Magnetic Phenomena, International Tomography Center SB RAS, [2]Physics Department, Novosibirsk State University, [3]Group of Experimental Methods and Instruments, Voevodsky Institute of Chemical Kinetics and Combustion SB RAS, Novosibirsk, Russian Federation, [4]Department of Physics, Free University of Berlin, Berlin, Germany

Abstract: We present a novel, low-cost experimental setup as an add-on to commercial NMR spectrometers, which can be used for fast field-cycling NMR experiments over a field range extending over more than 9 decades, from approximately 5 nT to 9.4 T. The method makes use of a hybrid technique: the high field range is covered by positioning the sample in the inhomogeneous stray field of the NMR spectrometer magnet. For fields below 2 mT a magnetic shield is mounted on top of the superconducting magnet. Inside the shield the magnetic field is controlled by a specially designed coil system, allowing shimming and adjusting the magnetic field down to 5 nT. As demonstrated here, the method is suitable for various NMR experiments, such as relaxation measurements, studies of long-lived spin order and coherent polarization transfer [1]. Additional to the thermal pre-polarization we have implemented the possibility to illuminate the sample by an LED source and to bubble the solution by parahydrogen gas, which allows us to perform CIDNP and SABRE/PHIP experiments. Non-adiabatic field variation to ultralow field range gives the opportunity to excite heteronuclear zero quantum coherencies which can be converted to J-spectra by Fourier transformation (see figure). The developed add-on is easily mounted in a few minutes on the NMR spectrometer and allows using standard probes without modification.
The setup was tested on the small molecule of methylpropiolate. T_1-relaxation of four isotopomers was measured in the full range of magnetic field. The high field part (1 to 9 Tesla) of the relaxation dispersion curve allowed us to determine chemical shift anisotropy parameters; the low field part (below 1 mT) demonstrates influence of strong coupling between ^{1}H and ^{13}C nuclei leading to a common T_1. For the isotopomer with the strongest coupling between C1 and H1 spins (**H**-**C**≡ bond, J=258.5 Hz) a long-lived singlet state between the heteronuclei is found at ultralow fields.
This work has been supported by the Russian Science Foundation (grant no. 14-13-01053). The authors acknowledge FASO Russia (project 0333-2017-0002) for financial support of the NMR facility at ITC.

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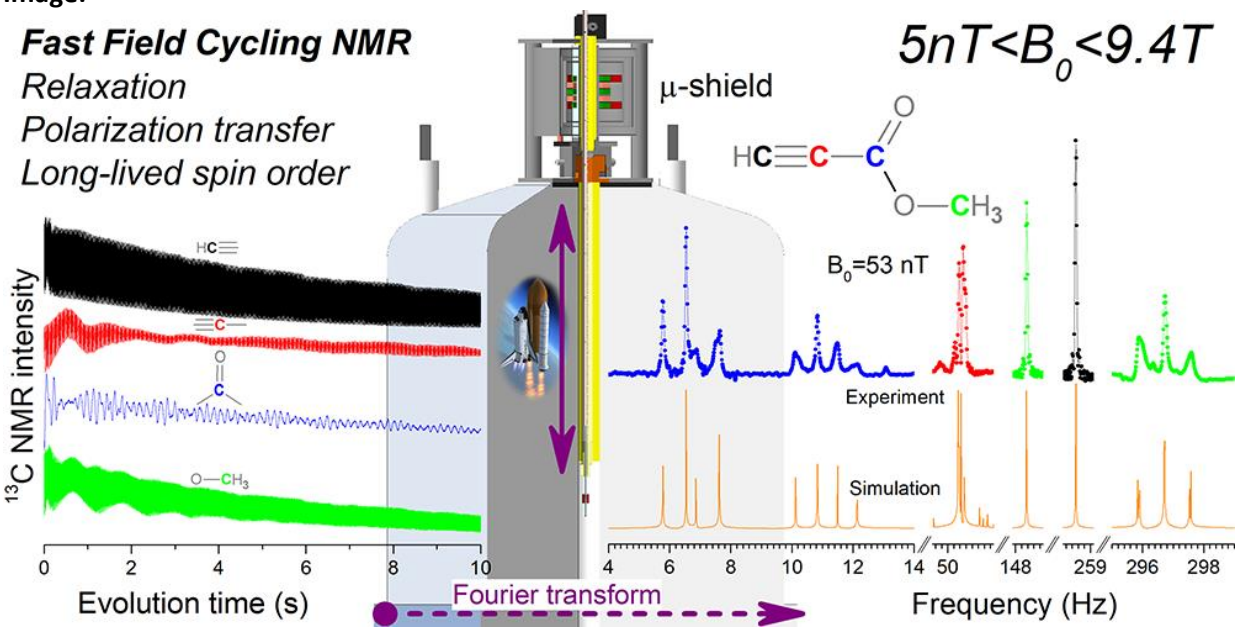

Exotica

P141

A solid-state NMR maser: nonlinear dynamics of DNP-hyperpolarized, dipolar broadened protons of frozen water.

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Abstract: Dynamic nuclear polarization (DNP) allows one to achieve high polarization of nuclear spins through their couplings to low-abundance paramagnetic centers under microwave irradiation.[1] In the past decade or so, a series of technological improvements has led to an expanding range of applications in liquid and solid state NMR. Hyperpolarised samples can be dissolved and transferred to a standard high-resolution NMR spectrometer, allowing for the observation of small molecules in solution, in cells or in vivo, with sensitivity enhancements that can reach 4 to 5 orders of magnitude, at room temperature. Since the seminal paper [2], a rich literature has been published.

In the set-up used in our laboratory, the nuclear spin bearing molecules are dissolved in an aqueous solution containing TEMPOL (4-hydroxy-2,2,6,6 tetramethylpiperidine-1-oxyl) as a stable radical that plays the role of a paramagnetic impurity, and is placed in a cryostat at 1.2 K inside a 6.7 T magnet, where microwave irradiation saturates part of the electron spin resonance spectrum.

The resulting large nuclear magnetisation can lead to well-known radiation damping effects. Radiation damping is due to the back action of the NMR circuit onto the precessing magnetization through mutual coupling, and is known to give rise to unconventional behaviour in liquid state NMR. However, this is most unexpected in solids with dipolar coupled nuclear spins, where the dipolar linewidth is on the order of tens of kHz.

Several surprising observations are reported in solid samples at 1.2 K. On the one hand, a series of maser pulses were observed on a ~ 100 ms time scale. This stands in harsh contrast with the decay of the conventional FID of the thermally polarized spins without DNP that typically lasts only a few hundred microseconds. This is indeed a puzzling observation, for a nonlinear behaviour, determined by radiation damping is not expected for such wide dipolar-broadened NMR lines. These results will be discussed, and can be qualitatively interpreted using a simple Maxwell-Bloch-Provotorov dynamical model.[3,4]. Furthermore, revivals of the NMR signal occurring after intervals of several tens of seconds, associated with irregular free induction signals, were also recorded and analysed along the lines of dynamical system theory.

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Exotica

P142

Probing sodium management in cells in physiological shear fields: A pilot study on red blood cells

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Abstract: The sodium ion (Na^+) is pivotal as it is an essential, abundant ion in our bodies with approximately 85% of total Na^+ found in the circulation. Na^+ is also asymmetrically distributed across the plasma membrane of most mammalian cells and is actively extruded from the cell through specific membrane-bound transporters. With respect to disease, Na^+ is a major factor in the onset and progression of hypertension but also influences other diseases including cancer and autoimmune conditions. For example, a cancer cell's ability to metastasise, a poorly understood phenomenon, is linked to the mechanics of cancer cell membranes and their robust adaptation to higher shear rates experienced by these cells travelling in the bloodstream. Red blood cells have been reported to experience similar effects.

We have applied bulk rheology, ^{23}Na single quantum (with shift reagent (Tm-DOTP)) and multiple quantum filtered (MQF) rheo-NMR methods to probe sodium management across the human RBC membrane in health under physiological shear rates and to further correlate the findings with mechanical properties of RBC membrane. We also varied hematocrit concentration to mimic natural mechano-sensing occurring as one descends with the branching in the human vascular system. During persistent shear fields (7.8 s^{-1},n=3) intracellular Na^+ $[Na^+]_i$ increases at room temperature, for 58% and 70% hematocrit. The effect is attenuated for the 90% hematocrit. Both trends were elevated at 37˚C (body temperature) as expected from basic physiology. The wealth of obtained results suggests that shear promotes increased Na^+ permeability and softening of the human RBC cell membrane in healthy cases, and may be informative in understanding pathologies where there is reduced perfusion such as chronic kidney disease (CKD) and diabetes.

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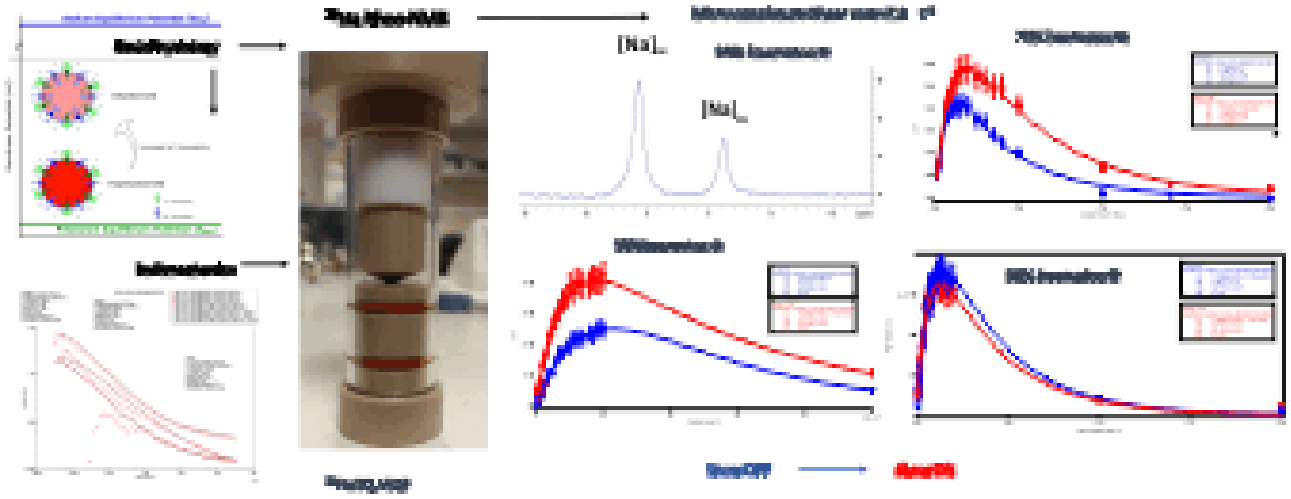

Exotica

P143

NMR of small-molecule endofullerenes

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Abstract: Small-molecule endofullerenes are complexes in which single small molecules such as H_2, H_2O and HF are completely enclosed in symmetric carbon cages, such as C_{60}. The encapsulated molecules are protected from the environment by the cage and retain complete rotational freedom down to cryogenic temperatures.

When an endofullerene is dissolved in an anisotropic solvent the encapsulated molecule is partially aligned. This alignment is evidenced by residual dipolar coupling (RDC) of the nuclei in the encapsulated molecule [1]. We studied alignment of ^{17}O enriched $H_2O@C_{60}$ in a nematic liquid crystal N-(4-Methoxybenzylidene)-4-butylaniline (MBBA). The ^{1}H and ^{17}O spectra show ^{1}H-^{1}H and ^{1}H-^{17}O RDC as well as residual quadrupolar coupling (RQC) of the ^{17}O nucleus. The observed residual couplings indicate that the molecule is preferentially oriented with its plane perpendicular to the liquid crystal director.

The H_2O molecule has two distinct spin isomers: ortho and para with total spins of ^{1}H nuclei 1 and 0 respectively. At room temperature the ortho-to-para ratio in $H_2O@C_{60}$ is 3:1, but at low temperatures the equilibrium changes to essentially pure para water. Samples of $H_2{}^{16}O@C_{60}$ and $H_2{}^{17}O@C_{60}$ solutions were thermalised at 4.2K to induce conversion into the para isomer. Then the samples were rapidly transferred into a high-resolution NMR magnet and dissolved in room temperature solvent. The conversion of excess para water to ortho leads to slow increase of ^{1}H signal for $H_2{}^{16}O@C_{60}$. In $H_2{}^{17}O@C_{60}$ the conversion gives rise to an antiphase pattern in the ^{1}H spectrum which is attributed to quantum-rotor-induced polarization. We estimate time constants for the para-to-ortho conversion at room temperature as 30 ± 4s for $H_2{}^{16}O@C_{60}$ and 16 ± 3 s for $H_2{}^{17}O@C_{60}$ [2].

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Disclosure of Interest: K. Kouril Conflict with: Director of Hyperspin Scientific Ltd, B. Meier Conflict with: Director of Hyperspin Scientific Ltd, C. Bengs: None Declared, H. Kourilova Conflict with: Director of Hyperspin Scientific Ltd, S. J. Elliott: None Declared, S. Alom: None Declared, R. J. Whitby: None Declared, M. H. Levitt: None Declared

Exotica
P144
New constraints on ultralight dark matter from ultralow-field nuclear magnetic resonance
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Abstract:
The nature of dark matter, the invisible substance that makes up over 80\% of the matter in the universe, remains one of the most intriguing mysteries of modern physics.
Elucidating the nature of dark matter will profoundly impact our understanding of cosmology, astrophysics, and particle physics, providing insights into the evolution of the Universe and potentially uncovering new physical laws and fundamental forces beyond the Standard Model.

The nature of dark matter, the invisible substance that makes up over 80% of the matter in the universe, remains one of the most intriguing mysteries of modern physics. Elucidating the nature of dark matter will profoundly impact our understanding of cosmology, astrophysics, and particle physics, providing insights into the evolution of the Universe and potentially uncovering new physical laws and fundamental forces beyond the Standard Model.
Recent theoretical work considering possible couplings between dark matter and Standard Model particles indicates that direct detection of ultralight bosonic dark matter may be possible via nuclear magnetic resonance (NMR) spectroscopy [1]. The Cosmic Axion Spin Precession Experiment (CASPEr) is a multifaceted international research program using NMR techniques to search for ultralight dark matter based on dark-matter-driven spin precession.
We will present new results from the CASPEr collaboration based on zero- to ultralow-field (ZULF) NMR measurements. We will briefly review the physical principles enabling the detection of dark-matter via ZULF NMR and introduce the "broadband" measurement scheme used for low-frequency detection [2]. We will describe the current ZULF NMR apparatus and discuss how post-processing phase cycling can be used to enable coherent averaging of NMR signatures arising from external sources of unknown frequency, such as dark-matter-induced modulations (see figure). Finally, we will consider how hyperpolarization methods such as signal amplification by reversible exchange of parahydrogen (SABRE) [3] can be implemented in the future to dramatically extend the reach of these experiments.
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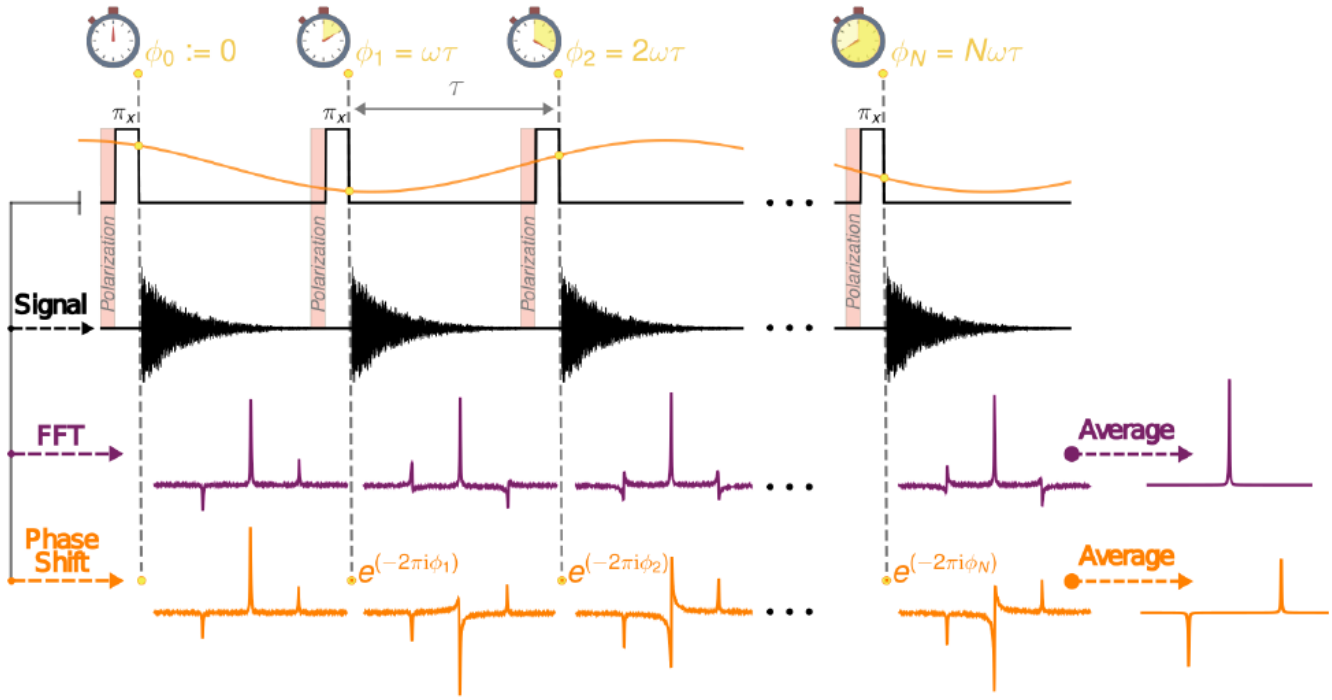
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Exotica

P144b

Magic Angle micro-MRI of biomaterials at very high field

Vincent Sarou-Kanian[*1], Maxime Yon[1], Dominique Massiot[1], Ulrich Scheler[2], Franck Fayon[1]
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Abstract: Magnetic Resonance Imaging is a powerful tool which offers high resolution spatial localization of mobile species in soft tissues providing insight of their internal structure. However, the application of MRI in rigid solids remains challenging as they usually exhibit short transverse relaxation time which prohibits the use of spin echo MRI sequences and strong line broadening which decreases the sensitivity and limits the resolution obtained with frequency encoding.
Magic Angle Spinning (MAS), which averages anisotropic interactions through a macroscopic rotation of the sample, allows obtaining narrow resonances in the solid-state. We show the potentiality of combining MAS and MRI[1-3] to carry out multi-nuclei (^{1}H, ^{31}P) 3D micro-imaging in rigid solid, at very high magnetic field (17.6 T) with greatly improved SNR and spatial resolution when compared to static conditions. These will be exemplified on biomaterials with spatial resolutions ranging from 100 to 300 µm, at MAS spinning frequencies up to 10 000 Hz using classical MRI spin-echo or Zero Echo Time (ZTE) sequences.
In biomaterials, very high magnetic field and moderate MAS spinning rate provide high spectral resolution and enable the use of various frequency selective excitation schemes for ^{1}H chemically selective imaging. This allows to record for the first time a 3D image probing the spatial distribution of apatitic hydroxyl protons inside a mouse tooth (≈2x2 mm) with a nominal isotropic resolution nearing 200 µm.
Moreover, Cross-Polarization (CP) can be used to enhance contrast and to further obtain spatially localized chemical characterization of bones and related materials. An example of these new contrast possibilities is performed in ^{31}P with a Zero Echo Time MRI sequence on the upper part of a mouse femur bone. We show that the CP contact time can be tailored to enhance the trabecular or the cortical bone. Indeed, at short contact time (1.5 ms) protons of the hydroxyl groups and of adsorbed water molecules both contribute to the ^{1}H-^{31}P magnetization transfer which enhance signal of the trabecular bone while at longer contact time only the hydroxyl group remains and give a more efficient transfer in the rigid part of the bone.

References:

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2. U. Scheler, B. Blumich and H.W. Spiess, *Solid State Nuclear Magn. Res*., **2** 105-110 (1993)
3. D.E. Demco and B. Blumich, *Concepts in Magn. Res.,* **12** 269-288 (2000).
4. M. Yon, V. Sarou-Kanian, U. Scheler, J-M. Bouler, B. Bujoli, D. Massiot, F. Fayon, *Scient. Rep.,* **7** 8224 (2017)

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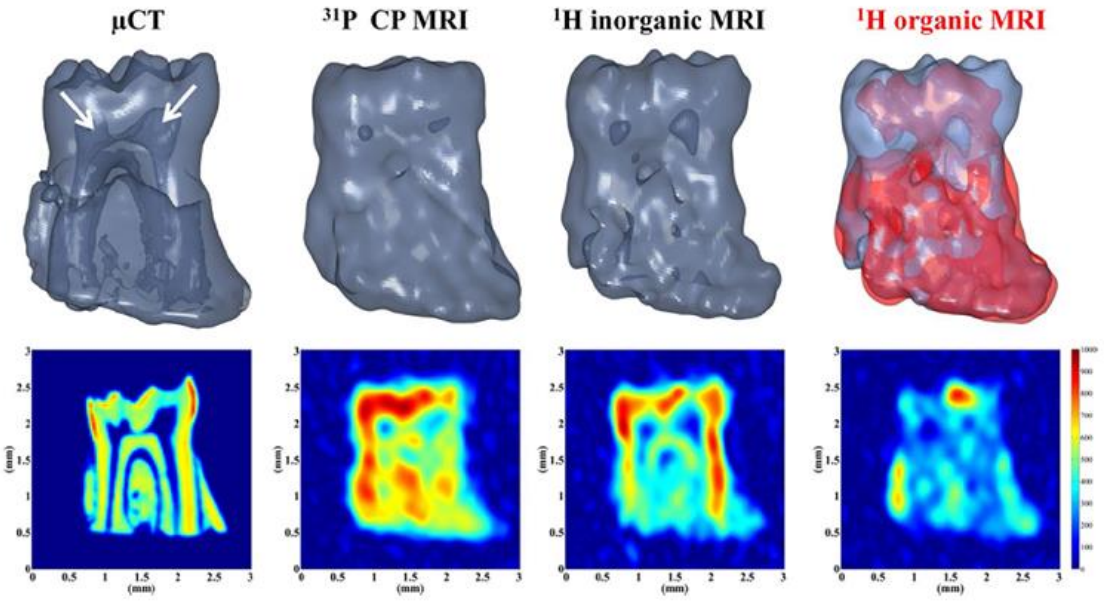

Hardware

P145

A Matlab toolbox enables the use of a digital lock-in amplifier as a wide-band spectrometer

Mazin Jouda[*1], Jan Korvink[1]

[1]Institute of Microstructure Technology IMT, Karlsruhe Institute of Technology KIT, Eggenstein, Germany

Abstract: High performance NMR is never possible without the cooperation of several high quality software and hardware components. These include advanced pulse sequences and post-processing routines as examples of software, and stable magnets, high-sensitivity detectors, strong gradients, and high-order shims as examples of hardware. Yet at the centre of the NMR experiment, the signal processing spectrometer is the critical element that is necessary to control and orchestrate the components precisely. In many commercial systems, the spectrometer electronics, despite of being of uttermost quality, often lack one or more important features that facilitate flexible experimentation. For instance, some spectrometers are narrow-band, limiting their efficiency to homo-nuclear experiments. Others do not support the automatic tuning and matching of RF detectors, while in many cases, it is not possible to simultaneously excite and read out the signals from two or more nuclei. Moreover, most systems lack a flexible hardware interface, especially when the user wants to perform non-standard experiments. In this contribution we report on a Matlab software package that unleashes the power of a high performance digital lock-in amplifier to perform as a generalised ultra wideband spectrometer. The system is capable of acquiring broadband NMR signals up to 600 MHz, transforming them simultaneously with multiple frequency demodulators, and filter the resulting signals down to sub-Hertz bandwidths. The combination of our user-friendly toolbox and the lock-in amplifier results in a highly portable wideband NMR spectrometer capable of performing simple and advanced experiments in magnetic fields ranging from 0-14 T, with state-of-the-art performance, and can therefore potentially replace, complement, and even outperform dedicated NMR systems should one intend to explore detection schemes that necessitate hardware modifications and the need to go beyond the standard system's capabilities.

Acknowledgements: The financial support from the European Union (H2020-FETOPEN-1-2016-2017-737043-TISuMR) is greatly acknowledged.

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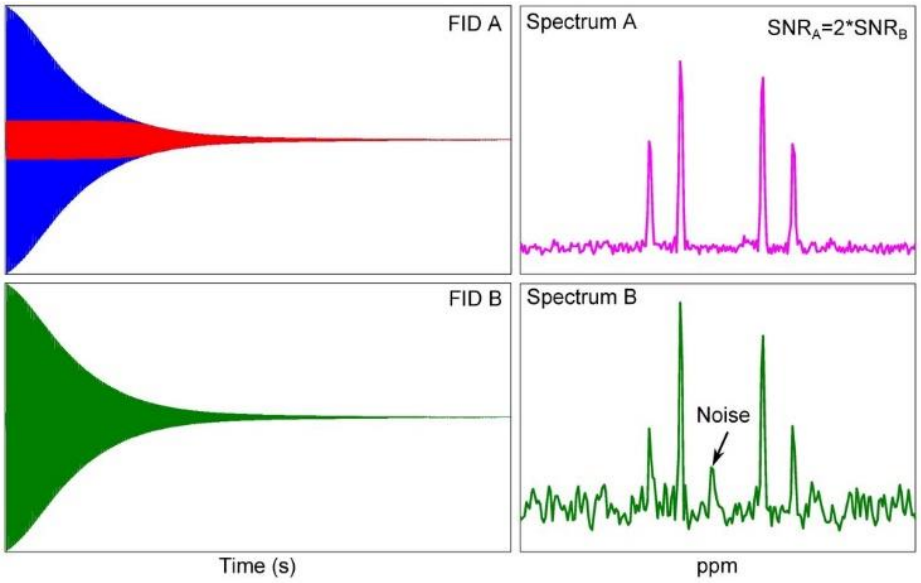

SNR enhancement due to FID segmentation and multiple ADC acquisition. A technique that is possible with the Matlab toolbox

Hardware

P146

Magneto-resistive sensors for detection of magnetic resonance at micron scale

Andrin Doll[*1], Aurélie Solignac[1], Elodie Paul[1], Myriam Pannetier-Lecoeur[1], Claude Fermon[1]
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Abstract: Magneto-resistive sensors feature an outstanding sensitivity to radio-frequency magnetic fields down to the picotesla range combined with a micrometric sensor footprint. Thanks to these two features, these sensors are used in hard disk drives for reading back the magnetically encoded data since two decades. In the context of magnetic resonance, the small size and high sensitivity render magneto-resisitive sensors as promising candidates for detection at micron scale [1]. In particular, dipole fields emerging from the precessing sample magnetization cause a change in the resistance of the sensor in proximity to the sample. Accordingly, spin precession of microscopic samples can be detected by a radio-frequency resistance measurement.

Our research is primarily dedicated to nuclear magnetic resonance of ^{1}H proton spins in water that precess at 30 MHz in an external field of 0.7 T. Our reference sensor is a rectangular 12x100 µm^2 spin valve exhibiting giant magneto-resistance. By immersing this sensor into the sample, ^{1}H precession can be detected with a spectral linewidth of 1.1 ppm at an integrated amplitude on the order of 100 pT.

A critical aspect of this approach is the external field required for polarization of the sample spins. With respect to the giant magneto-resistance sensor, this strong out-of-plane field imposes an unusual configuration for field sensing. In particular, the ferromagnetic layers inside the sensor are influenced by the external field, so that magneto-transport and magneto-static properties of the sensor are altered. The consequences of these effects for the proposed application in magnetic resonance are discussed and corrective actions taken. As an example, we are investigating to which extent the ferromagnetic layers of the sensor affect the magnetic field homogeneity in proximity of the sensor. In order to obtain experimental insight, micro-shims that alter the magneto-static properties at micron scale have been designed around the sensor.

[1] *Guitard et al*, Appl. Phys. Lett. 21, 2016, 212405

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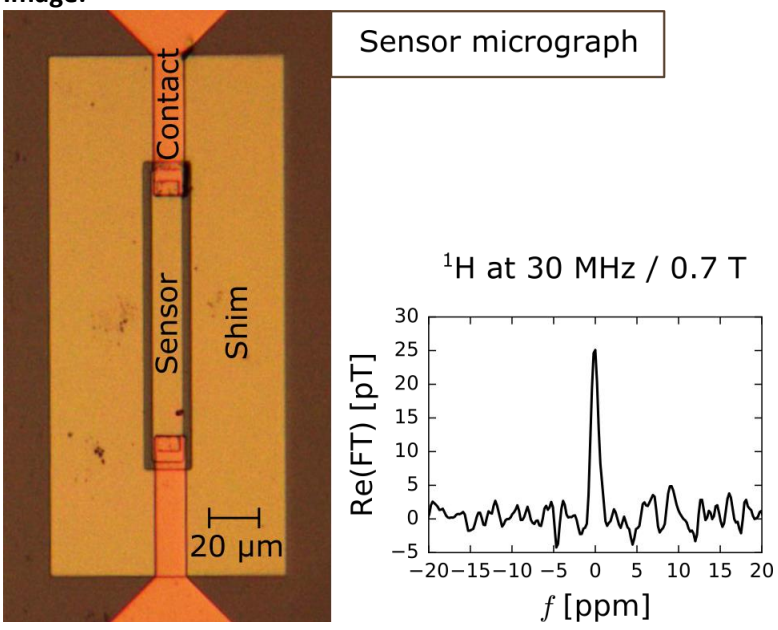

Hardware

P147

Electronics tools for a mobile NMR (MBNMR) spectrometer

Alain Louis-Joseph[*1], Denis Coupvent-Desgraviers[1], Daniel Paget[1]

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Abstract: Nuclear Magnetic Resonance (NMR) spectroscopy is a technique widely used nowadays to characterize molecules. Numerous compact[1] NMR spectrometers have been designed for easy measurement of proton NMR spectra. High sensitivity and resolution can be reached even with low field spectrometers, thanks to great improvements in electronic hardware, which opens up a wide field of methodologies and of analytical quantification and relaxation applications. Recently we presented a portable NMR spectrometer fabricated in our laboratory[2], confined to a single rack unit, connected to a *PC* via one USB port, easy to install and with a small footprint. In this presentation, we detail the key elements required to build a homemade NMR spectrometer with a sensitivity and resolution adapted to NMR for broadband applications.

There are many reasons for a research laboratory to develop and have its own compact NMR spectrometer. First of all, this mobile NMR (MBNMR) unit can be used as an additional channel to a high resolution commercial spectrometer with a high field cryogenic magnet, or used on a table with a low field permanent and cryogen free magnet technology : avoiding the need for weekly and expensive cryogenic services. The MBNMR is naturaly dedicated to education and quantification of species, which do not require high resolution. For education, the hardware of the compact NMR unit is nonetheless open and accessible to students for the study of the basic functions of an NMR spectrometer. MBNMR, may be coupled with scientific experiments, for example when nuclear spins are dynamically polarized by coupling with spin-polarized electrons[3]. In our laboratory we want to explore nuclear spin dynamics and electron-nuclear spin couplings in polarized microluminescence experiment[4] and to measure by optically-detected NMR the spatial profile of nuclear magnetization. The compact nature and versatility of a MBNMR device requires the use of powerful electronics. Minimizing energy consumption is a crucial problem for portable units. Much of the power consumed is that due to the necessary radio frequency amplifiers. We introduce stochastic[5] NMR technique which offers advantages in terms of a compromise between resolution and sensitivity with very low RF power.

We describe the hardware[6] and software features to achieve these objectives. From the sample to the data processing, we review the aspects of the instrumentation to build an operational and efficient MBNMR spectrometer.

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[4] I. Favorskiy, D. Vu, E. Peytavit, S. Arscott, D. Paget and A. C. H. Rowe , Rev. Sci. Inst. **81**, 103902 (2010)

[5] Ernst R. R. and Primas H., Helv. Phys. Acta., vol. 36, 583-600 (1963)

[6] LNA : Low Noise Amplifier, DDS : Direct Digital Synthesis, FPGA : Field Programmable Gate Area

Hardware

P148

Droplet NMR: An Integration of Digital Microfluidics (DMF) and High Resolution NMR

Bing Wu[* 1, 2], Ian Swyer[3], Sebastian von der Ecken[3], Ronald Soong[1], Michael Fey[4], Armin Beck[5], Franck Vincent[5], Dieter Gross[6], Oliver Gruschke[6], Nicolas Freytag[7], Till Kuehn[7], Martine Monette[8], Henry Stronks[8], Werner Maas[9], Aaron Wheeler[3], André Simpson[1]

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Abstract: High Resolution Nuclear magnetic resonance (NMR) spectroscopy is one of the most informative spectroscopic techniques capable of providing detailed insights into the molecular structure and dynamics of even the most disordered systems. However, compared to other spectroscopies, NMR suffers from low mass sensitivity which hampers trace analysis. To overcome this limitation, the size of the receiver coil can be reduced, producing microcoils (namely, coils with sub-mm diameter) capable of leveraging the increase of magnetic flux density induced by a unit current (and therefore the voltage induced in the coil by nuclear spins) compared to that of resistive noise, leading to improved signal-to-noise ratios. Although microcoils can improve sensitivity, sample handling of small volume becomes extremely challenging. Digital microfluidics (DMF) involves the movement of droplets (from low nL up to 5 µL) on an open surface of electrodes and can be used in various applications including, liquid-liquid extraction, liquid solid-extraction, in chip chromatography, binding assays to name a few. A combination of DMF and microcoil NMR would be an ideal solution for micro-volume sample handling in combination with small scale NMR analysis.

In this study, we introduce the first digital microfluidic (DMF) system capable of interfacing droplets of analyte with microcoils within a high-field NMR spectrometer. Droplets can be moved inside the spectrometer to perform a range of routine DMF protocols, while also permitting the acquisitions of several 1D and 2D NMR experiments. Meanwhile, a series of rapid polymerization reactions were measured in real time on this interphase, which implies a great potential of this device for *in-situ* reaction monitoring. Hence, we propose that the combination of DMF and NMR will be a useful new tool for a wide range of applications in chemical analysis.

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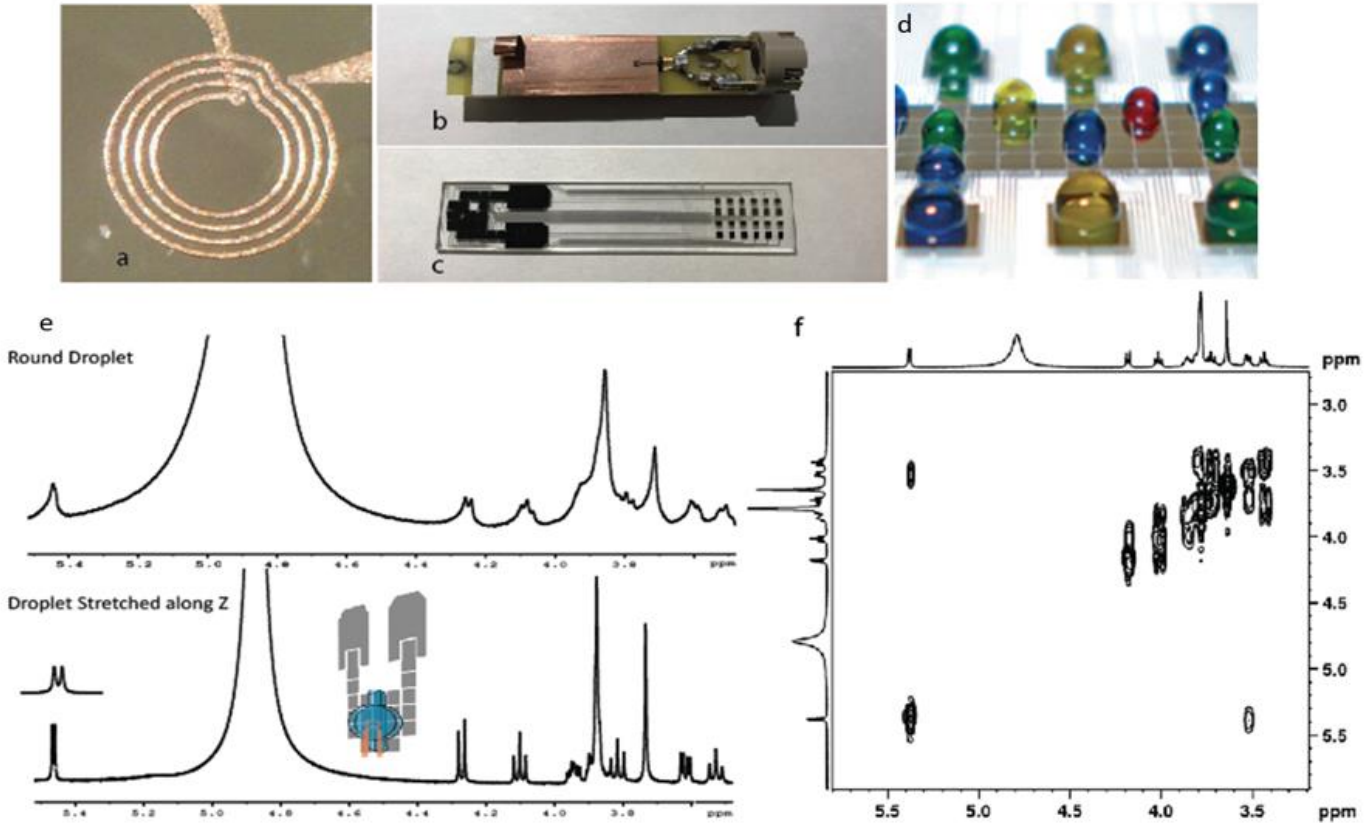
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4.5
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ppm

Hardware

P149

Modelling, characterization, and performance analysis of non-tuned spiral micro-coils for multi-nuclei NMR

Hossein Davoodi[*1], Jan G. Korvink[1], Neil MacKinnon[1], Vlad Badilita[1]

[1]Institute of Microstructure Technology (IMT), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

Abstract: Although broadband spiral coils have demonstrated their applicability for multi-nuclei NMR spectroscopy [1], as of yet this type of detectors have not been sufficiently characterized, consequently preventing them from being widely adopted by the community. The aim of this work is to fill this gap by presenting a comprehensive performance analysis of non-tuned spiral micro-coils supported by simulations, electrical and MR experiments.

For detectors meant to operate in a wide frequency range, any form of tuning and matching should be avoided, while tuned coils benefit from the signal and noise passive-amplification at their resonance frequency. This amplification prevents any further distortion of the signal in posterior processing blocks as far as the input-referred noise level of those blocks is lower than the boosted noise level. The matching also adjusts the impedance of the coil at the resonant frequency to 50 Ω, which improves the power transfer efficiency between the coil and the electronics.

In a non-tuned coil, signal amplitude should be maximized while the total noise is minimized. The higher signal amplitude by the coil corresponds to a higher inductance, and therefore a lower self-resonant frequency (SRF). So, the distribution of the generated field at the sample region and the impedance of the coil are strongly frequency-dependent. Besides, power transfer efficiency between electronics and coil, and hence the overall SNR of the system is more sensitive to any perturbation introduced by the surrounding environment, while in a tuned coil it is usually compensated by wobbling, such as the presence of any other hardware in the magnet bore, sample loading, as well as the physical properties of the cable, e.g., length and propagation constant.

In this work, we have analytically investigated the working principle of non-tuned coils, i.e., their impedance, power transfer efficiency, and magnetic field distribution, while the optimized geometrical parameters of the coil are obtained using COMSOL simulations for a certain bandwidth. As depicted in Figure a, the coil exhibiting the optimal overall SNR and impedance has 22 turns, the spacing between the turns and track thickness is 20 µm, and the inner diameter is 250 µm. This coil was fabricated using micro-fabrication techniques discussed elsewhere [2]. The electrical measurements performed on this coil are in good agreement with simulations (see Figure b), both predicting the first SRF of the coil within the working bandwidth. The B_1 field distribution was investigated in MRI experiments using a Bruker 11.7 T wide-bore magnet and a comparison with simulation is presented in Figure c. Figure d demonstrates proof of principle broadband operation of the coil at the Larmor frequencies for ^{1}H (500 MHz), ^{23}Na (123 MHz), and ^{31}P (202 MHz).

[1] R. M. Fratila, *et al.*, **Nature communications**, 2014.

[2] V. Badilita, *et al.*, **Lab on a Chip**, 2010.

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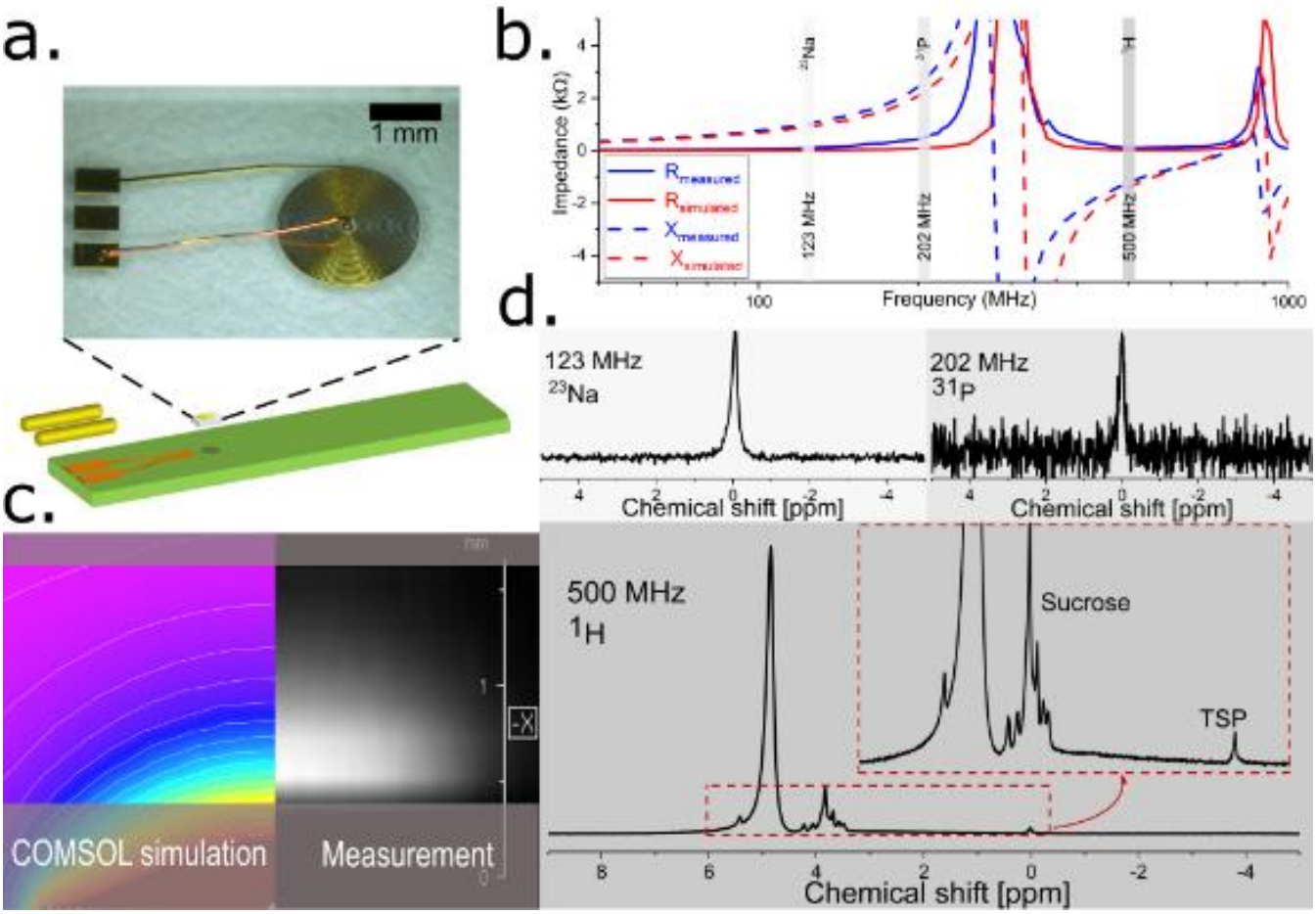
a.
1 mm
b.
Impedance (kΩ)
Frequency (MHz)
100
1000
123 MHz
202 MHz
500 MHz
R measured
R simulated
X measured
X simulated
c.
COMSOL simulation
Measurement
d.
123 MHz
23Na
202 MHz
31P
Chemical shift [ppm]
500 MHz
1H
Sucrose
TSP
Chemical shift [ppm]

Hardware

P150

Rapid scan ESR with 1 GG/s inside a portable spectrometer

Benedikt Schlecker[1], Anh Chu[1], Burkhard Luy[2], Klaus Lips[3], Jens Anders[*1]

[1]University of Stuttgart, Stuttgart, [2]Karlsruhe Institute of Technology, Karlsruhe, [3]Berlin Joint EPR Lab, Helmholtz Zentrum Berlin für Materialien und Energie, Berlin, Germany

Abstract: Recently, miniaturized CMOS-integrated LC-tank oscillators have become popular both as a very good candidate to improve the limit of detection in inductively detected electron spin resonance (ESR) experiments[1,2] and to realize battery-operated, portable spectrometers. This is because they conveniently provide both the B_1-field required to excite the spin ensemble and they can simultaneously detect the magnetic resonance signal as a change in their oscillation frequency, cf. Fig. 1. The technique has then been extended to the use of voltage-controlled oscillators (VCOs)[3] and arrays of VCOs[4], which offer linear control over their oscillation frequency by a tuning voltage V_{TUNE}, cf. Fig. 1. Here, affects both the LC resonant frequency and the oscillation frequency in the same way, allowing for frequency sweeps, where the resonator is always critically coupled to the B_1-frequency[3]. Therefore, VCO-based detectors are ideally suited for frequency-sweep ESR experiments with large sweep ranges and yet constant detection sensitivity[3]. In the proposed talk, we will focus on the possibility of performing rapid scan experiments using VCO-based detectors with unprecedented scan rates. These improved scan rates are enabled by the large bandwidth of the varactor diode and the almost instantaneous change of the oscillation frequency in response to a change in the tank capacitance, where the corresponding bandwidth is in the range of the oscillation frequency. To demonstrate the excellent performance of VCO-based rapid scan experiments, we will show data obtained from a single VCO-based detector achieving scan rates of and larger inside our portable setup[3]. Furthermore, we will show rapid scan data from our recently presented injection locked VCO array, which achieves a concentration sensitivity of around As a further extension to the state-of-the-art, our portable setup contains an open-loop compensation of the VCO nonlinearity. In the proposed scheme, the VCOs nonlinearity is determined prior to each scan by incorporating the VCO into a phase-locked loop (PLL) and using the recorded calibration data to perform a predistortion of the tuning voltage to obtain a very close to ideal linear frequency sweep. Since the proposed VCO-based technique also scales to much lower frequencies with correspondingly larger sensitive volumes, it is also a very interesting candidate for rapid scan NMR of samples with very wide spectra, which are hard to be recorded with standard pulsed techniques.

[1] J. Anders et al., J. Magn. Res., 217, p. 19-26, 2012.
[2] G. Boero et al., J. Magn. Res., 278, p. 113–121, 2017
[3] J. Handwerker et al., ISSCC 2016 Digest of Technical Papers, p. 476-478, 2016
[4] A. Chu et al., ISSCC 2018 Digest of Technical Papers, p. 354-356, 2018.

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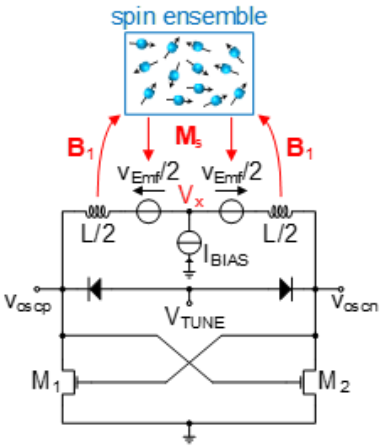

Fig. 1 Schematic of an LC-tank VCO including a model for the coupling with the spin for rapid scan experiments.

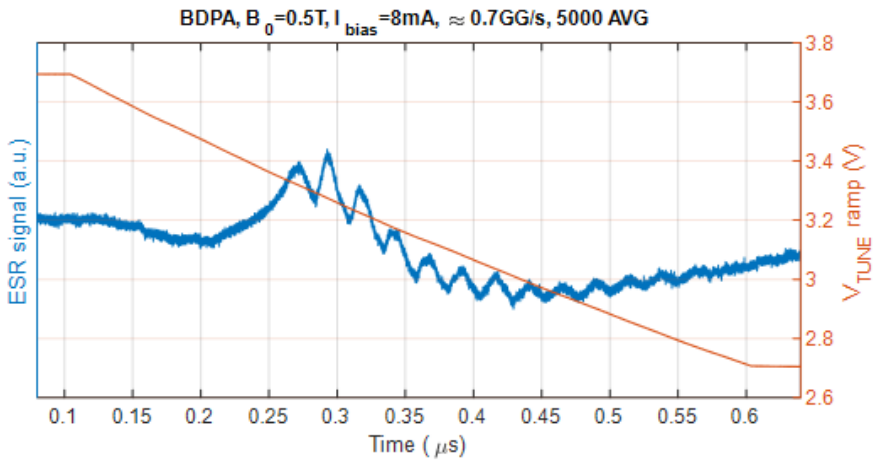

Fig. 2 Rapid scan ESR signal of a small BDPA sample at a scan rate of 0.7 GG/s recorded with a portable ESR spectrometer. The ramp of the tuning voltage V_{TUNE} displays a curvature that compensates the varactor nonlinearity.

Hardware

P151

High-performance modular probe assemblies for microfluidic nuclear magnetic resonance spectroscopy

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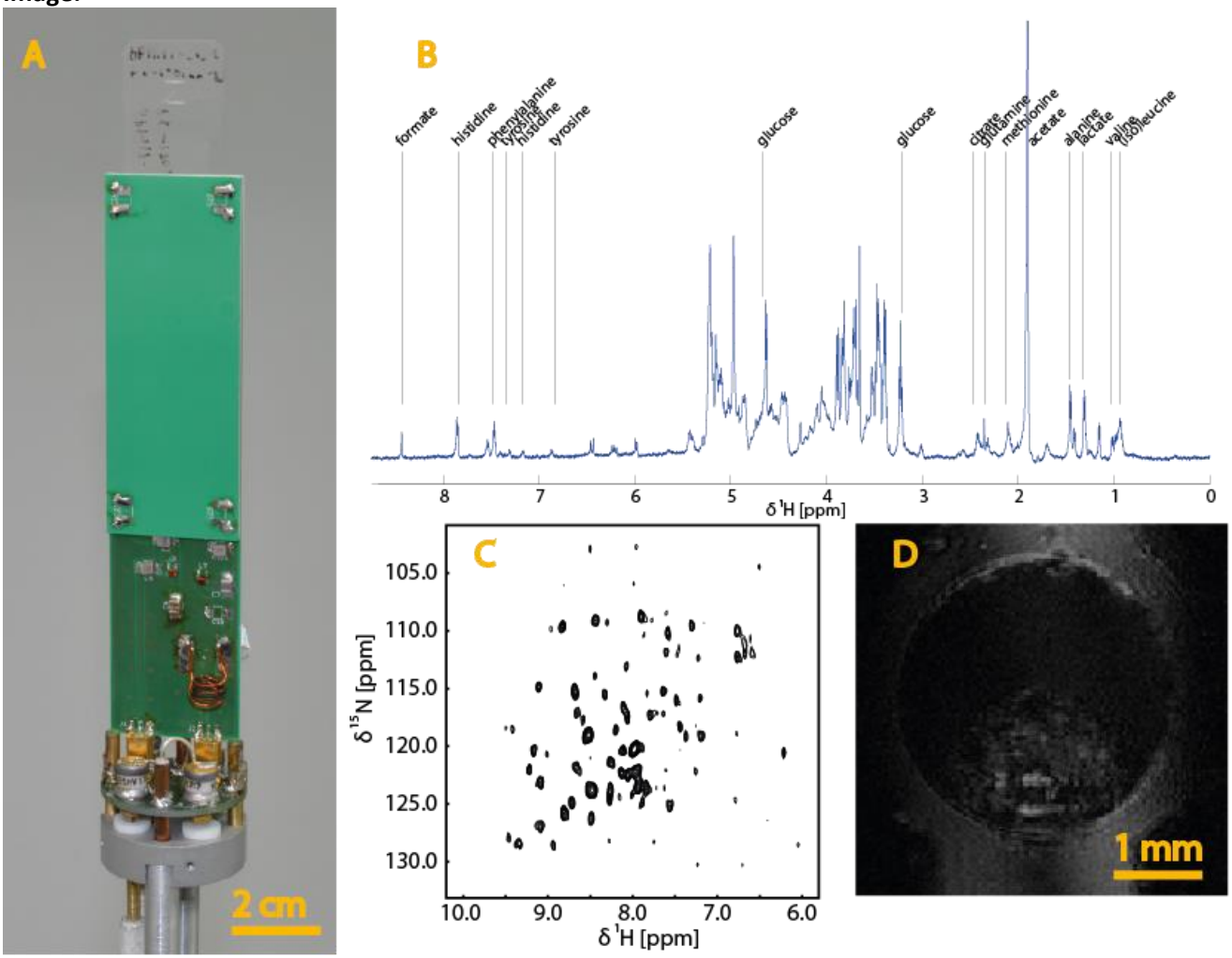
Abstract: Symbiosis of microfluidics with NMR (Nuclear Magnetic Resonance) spectroscopy provides a powerful platform combining the benefits of both the techniques. One of the key advantages of microfluidic devices is their flexibility, which allows implementation of a wide range of experimental conditions. We present a novel modular design for an NMR probe assembly that accommodates microfluidic devices without constraining their design beyond their overall shape. The main advantages of this new probe design are its high sensitivity, high resolution, and its modular design. The NMR detector (A) is based on a transmission line design [1], and fabricated from easily accessible PCB materials. The NMR detector including the tuning and matching circuit of the probe is modular. This allows the usage of a single probe base with many detectors. Although a single detector can be used under several experimental conditions, different detectors can be optimised for specific requirements like B_0 field, mass sensitivity, concentration sensitivity, sample volume, single or dual channel etc. The sample is loaded in an exchangeable microfluidic device. The microfluidic device itself can be made from low-cost plastic materials by inexpensive rapid prototyping techniques. The fluidic network on the device can be designed freely, as long as the outline shape (approximately 90 mm x 20 mm) and the thickness (1 mm) are maintained. NMR spectra with sensitivity around 1 nMol/sqrt(Hz) and a resolution of better than 0.01 ppm from 2 µl of fluid have been recorded. Generic 1D (B, DMEM cell growth media, 256 scans) and 2D (C, 1 mM ^{15}N ubiquitin, HSQC) NMR experiments have been successfully performed at different magnetic fields. A micro MR (magnetic-resonance) image (D, gradient echo image) of a mouse liver tissue slice has been recorded as a proof of principle for micro imaging.

[1] Finch, G., Yilmaz, A., & Utz, M. (2016). An optimised detector for in-situ high-resolution NMR in microfluidic devices. Journal of Magnetic Resonance, 262, 73-80.

Image: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histidine
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(Ile)leucine
8
7
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1
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δ ¹H [ppm]
C
105.0
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120.0
125.0
130.0
δ ¹⁵N [ppm]
10.0
9.0
8.0
7.0
6.0
δ ¹H [ppm]
D
1 mm

Hardware

P152

CMOS transceivers for compact architectures and nL to sub-nL high performance NMR micro-sensors

Marco Grisi[*1], Giovanni Boero[1]

[1]Microengineering Institute, EPFL, Lausanne, Switzerland

Abstract: CMOS technologies allow for the implementation of miniaturized electronics and they can be used to realize pulsed NMR probes that are compact and versatile. In one implementation single-chip CMOS transceivers are interfaced with external (i.e., off-chip) excitation-detection resonators and proved to be valuable tools for the manufacturing of compact instrumentation. Such configuration delivered state-of-art performance in probes with sample volumes ranging from a few \textmu L down to 100 nL, and was used to demonstrate high-resolution multi-nuclear measurements [1] and broadband magnetometry that also includes quadrature detection capabilities [2]. As a result, CMOS chips interfaced with external resonators simplify the construction of compact NMR probes, reduce their costs, and enable novel architectures. Ultra-compact NMR probes, where multilayer micro-coils are co-integrated on the same chip with the transceiver electronics, represent an innovative and excellent use of CMOS technologies. This type of probe allows for an exceptional degree of versatility and state-of-art performance for the analysis of microscopic samples, i.e. having volumes ranging from 10 nL down to 100 pL. By using an ultra-compact probe having a sensing region of about 200 pL and a spin sensitivity of $1.5x10^{13}$ spins/$Hz^{1/2}$ at 7 T, we demonstrated direct reading of endogenous compounds in sub-nL eggs of microorganisms [3]. More recently, the combination with cutting-edge microfabrication techniques also allowed for spectroscopy of sub-sections of intact C. elegans worms [4]. In this poster we focus on the advantages of CMOS ultra-compact architectures with respect to alternatives by comparing performance and geometrical features, indicating the path towards high-sensitivity CMOS-based micro-sensors that will enable NMR on samples that are currently out of reach, such as large unicellular microorganisms, micro-tissues derived from stem-cells, and even mammalian embryos (humans included).

[1] M. Grisi, G. Gualco and G. Boero, *Review of Scientific Instruments*, 2015, **86**, 044703. [2] M. Grisi and G. Boero *58th Experimental Nuclear Magnetic Resonance Conference*, ENC 2017. [3] M. Grisi, B. Volpe, R. Guidetti, N. Harris and G. Boero, *Scientific Reports*, 2017. [4] E. Montinaro, M. Grisi, M. C. Letizia, L. Petho, M. A. M. Gijs, R. Guidetti, J. Michler, J. Brugger, and G. Boero *arXiv:1707.05500*, 2017.

Hardware

P153

Synchronizing MR measurements on fluidic plugs using integrated impedance spectroscopy

Omar Nassar[*1], Mazin Jouda[1], Nan Wang[1], Michael Rapp[1], Jan G. Korvink[1], Dario Mager[1], Neil MacKinnon[1]
[1]Institute of Microstructure Technology, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

Abstract: Microfluidic technologies combined with micro-NMR have become increasingly popular given their promise of increased sample throughput and mass-sensitivity. A common challenge is the relatively large dead volume in the tubing feeding the micro-NMR detector, which may reach 1 - 2 meters if sample injection is done outside of the magnet. To circumvent this issue, concentrated sample plugs may be dispersed within an immiscible fluid. This two-phase flow eliminates the dead volume, but requires continuous online measurement to ensure the desired sample completely fills the NMR detection volume. In this contribution, a capillary flow system featuring an integrated impedance spectroscopy sensor for sample position measurement is demonstrated. Knowledge of the sample position is used to trigger NMR acquisition, maximizing the time spent collecting the desired signal.

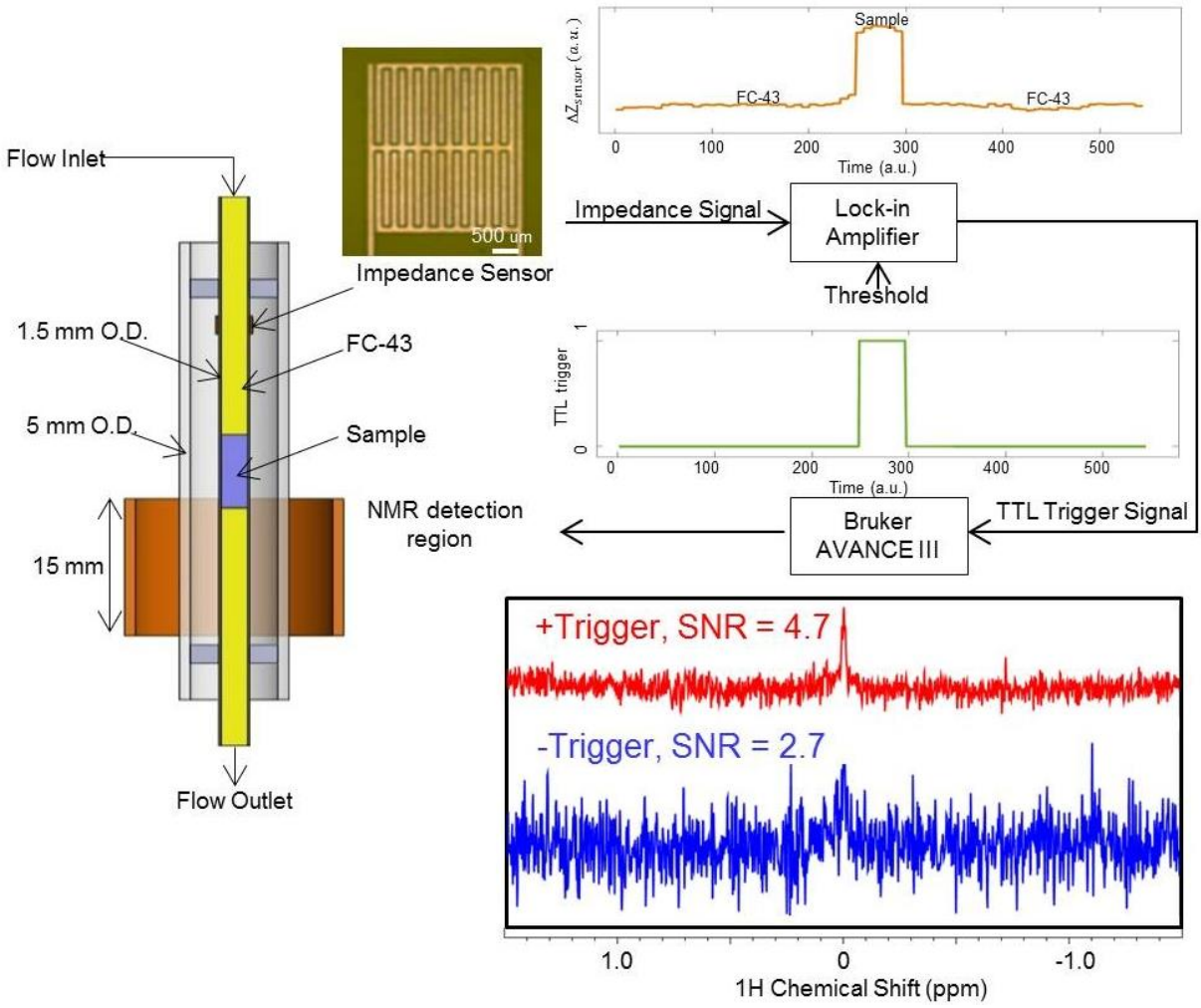
Interdigitated electrodes were fabricated on a 25 µm Kapton foil laminated on one side with a 9 µm copper layer. The electrodes were rolled around a 1.8 mm (O.D.) glass capillary as recently described (Wang, N., *et al.*, J Micromech Microeng 28 (2018) 025003). The electrodes covered a length of 2.5 mm along the capillary. The capillary was inserted into a 5 mm saddle coil and positioned so that the electrodes were 4 cm upstream of the detector. Inlet and outlet tubing was connected to the capillary so that sample flow could be controlled from outside of the magnet. The inlet tube was filled with a single aqueous plug (~ 20 cm long) bordered by FC-43, and the flow was controlled by a syringe pump at a flow rate of 1.0 cm s^{-1} running continuously until the aqueous plug left the probe. The electrodes were driven by a Zurich Instruments UHFLI amplifier at a frequency of 540 MHz and the impedance was measured to sense the position of the water plug. NMR spectra were acquired at a ^{1}H Larmor frequency of 500 MHz using a Bruker AVANCE III spectrometer. A single-pulse experiment was used, with data acquisition controlled by a trigger signal delivered to the spectrometer by the UHFLI amplifier.

Impedance measurements could robustly distinguish the water and oil phases (Figure 1). Importantly, driving the electrodes had no visible deleterious effect on the ^{1}H NMR spectrum. It was observed that the SNR was improved by 75% when implementing the automated triggered data acquisition, even with a relatively long sample plug.

The first step towards micro-coil integration with impedance-based sensing of flow has been demonstrated. With this technology we believe high-throughput, automated sample measurement will be possible in a dual phase, droplet-based approach where each droplet could be a different sample. Future developments will include: optimization of impedance sensor dimensions, increasing sensor functionality to include flow velocity measurement, and integration with a micro-NMR saddle coil.

Acknowledgement: the authors gratefully acknowledge the DAAD support under the program DAAD GERLS 2016.

Image:


Flow Inlet
500 um
Impedance Sensor
1.5 mm O.D.
FC-43
5 mm O.D.
Sample
NMR detection region
15 mm
Flow Outlet
Sample
FC-43
FC-43
Time (a.u.)
Impedance Signal
Lock-in Amplifier
Threshold
TTL trigger
Time (a.u.)
Bruker AVANCE III
TTL Trigger Signal
+Trigger, SNR = 4.7
-Trigger, SNR = 2.7
1H Chemical Shift (ppm)

Hardware
P154
Shim-on-Chip
A novel shim system for stripline NMR detectors

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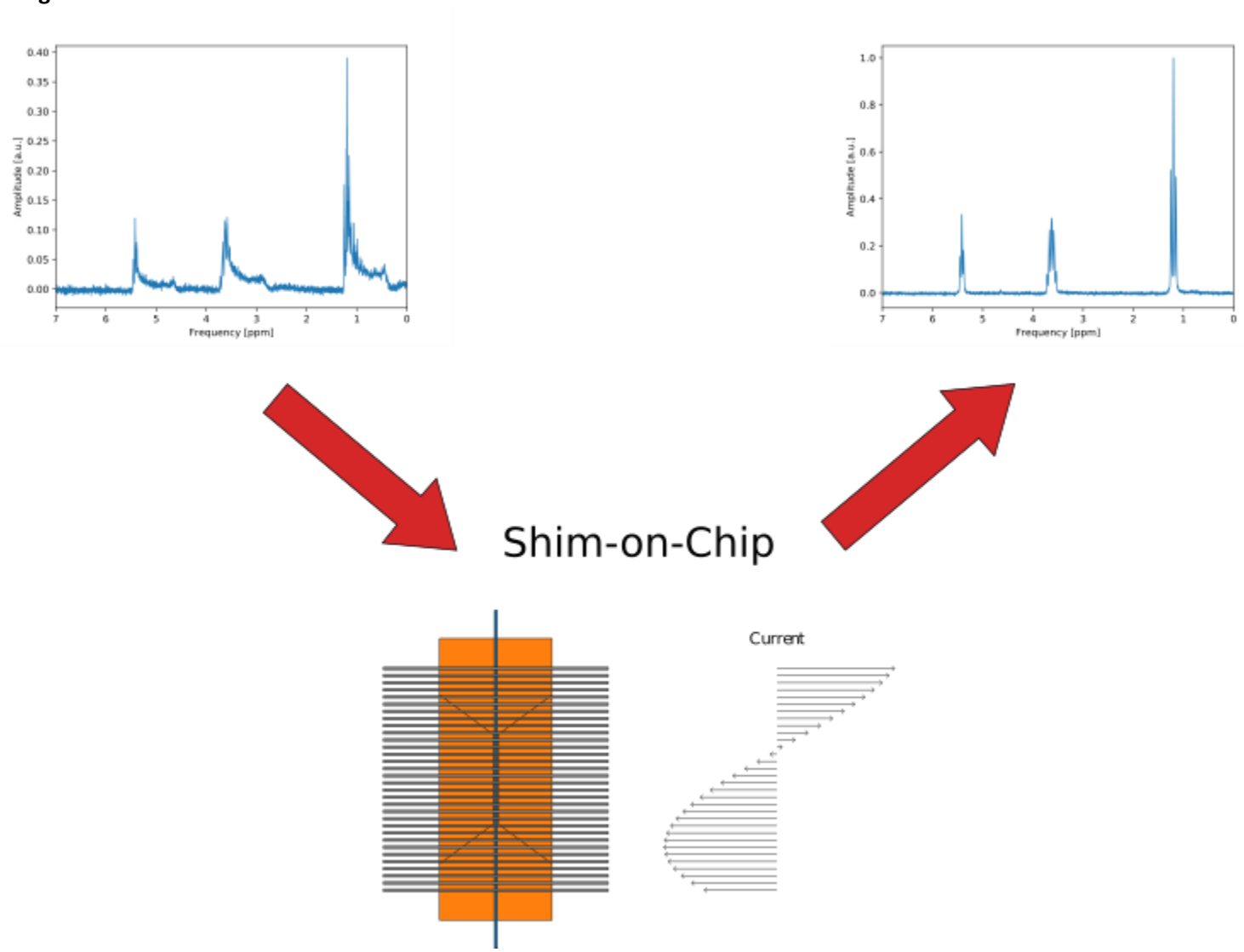
Abstract: We have shown that our stripline "microcoil" is a versatile tool for Nuclear Magnetic Resonance experiments. Such a stripline is designed to acquire spectra of sub-microliter samples with high sensitivity. Since the sample is inside a fused silica capillary it is well suited for in-flow experiments. In addition, the capillaries are able to withstand high pressures, making hyphenation with superfluidic chromatography relatively easy. Finally, tapered striplines can be used to generate a well-defined rf-gradients, which can be used for imaging experiments or measure diffusion.
In this contribution, we present a novel shimming approach for stripline NMR, which we call Shim-on-Chip. Conventionally shimming is done by applying currents to a set of shim coils located in between the probe and the magnet. Due to the small sample size shimming microliter samples using regular shim coils is complicated. Here we demonstrate the use of a series of parallel wires placed perpendicular to B_0 as a Shim-on-Chip shim system. This is achieved by placing a ribbon flat cable horizontally over the stripline detector. The current through each wire of the ribbon cable can be controlled independently employing a 16 channel DAC. This makes for a simple, cheap and easy to construct alternative to regular shim systems. The Shim-on-Chip is, nevertheless, quite flexible in creating a magnetic field which matches the inhomogeneity of the magnet in 1 dimension. The sample geometry in a stripline detector is well suited for this type of shimming since its length (8 mm) is much larger than its width (100 µm to 250 µm). With this Shim-on-Chip system we have reached linewidths of 2:2 Hz (at 50 %) and 27 Hz (at 0:55 %) on a 144 MHz NMR spectrometer without any other room temperature shims.
As the Shim-on-Chip is located inside the NMR probe, it is always centred on the NMR sample, because of this the shims have an intuitive effect on the lineshape. Therefore, manual shimming is simpler when compared to a regular shim system, as it is difficult to position a microliter sample in the exact center of the shim coils. We demonstrate the use of a Shim-on-Chip method in a 400 MHz Rapid-Melt DNP system. Decent linewidths are achieved even for a sample which is located off-center inside the NMR magnet.

Image:


Amplitude [a.u.]
Frequency [ppm]
Amplitude [a.u.]
Frequency [ppm]
Shim-on-Chip
Current

Hardware

P155

Loop-gap resonator of enlarged bandwidth for pulsed EPR applications

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[1]Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt, Frankfurt am Main, Germany

Abstract: Recently Q-band pulsed EPR spectroscopy has strongly advanced its performance by the introduction of high power microwave amplifiers and the use of shaped pulses. In pulsed EPR applications, a resonator Q-value has to be low enough to achieve sufficient bandwidth for short microwave pulses and to reduce the ring-down time after the pulses. However, a low Q-factor reduces the detection sensitivity as well as the conversion efficiency of the microwave input power to the magnetic field strength at the sample position. Therefore, the resonator Q-value has to be optimized for the given microwave input power and specific application [1]. We designed a 3-loop/2-gap resonator by simulating it with CST Microwave Studio for such applications, and tested its performance in comparison with a standard Bruker D2 Q-band resonator by accomplishing broadband SIFTER experiments on a nitroxide model compound [2].

1. J. Hyde, W. Froncisz, in: A.J. Hoff (Ed) *Advanced EPR: Applications in biology and biochemistry* (Elsevier, Amsterdam, 1989).

2. V. Denysenkov, P. van Oss, T. Prisner, (2017) Q-band loop-gap resonator for EPR applications with broadband-shaped pulses, *Appl. Magn. Reson.* 48, 1263-1272.

Hardware

P156

High-Sensitive Solid-State NMR Probe: Application of High-Temperature Superconductor for RF Coil

Shota Kato[*1], Techit Tritrakarn[1], Tetsuji Okamura[1], Masato Takahashi[2], Kotaro Irie[3], Haruki Hoshi[3], Atsushi Saito[3], Kazuhiko Yamada[4]

[1]Tokyo Institute of Technology, [2]RIKEN, Yokohama, [3]Yamagata University, Yonezawa, [4]Kochi University, Nangoku, Japan

Abstract: Solid-state NMR is one of effective methods widely applied in materials science, especially for amorphous state. However, quadrupolar nuclei, sometimes playing important roles in materials, make very wide and weak NMR spectra due to quadrupolar interaction. Although a lot of NMR techniques have been developed to overcome this kind of problems, some of them such as rubber vulcanization mechanism with sulfur are left untouched. Thus, we have developed a totally different NMR measurement method [1] and NMR probe to promote materials science. The probe with High-Temperature Superconductor (HTS) RF coils has been developed.

HTS RF coils are expected to have higher Quality Factor (Q), which leads higher sensitivity, than conventional copper RF coils due to HTS's much less surface resistance [2]. The factors that decrease Q such as eddy current loss and dielectric loss should be avoided in the probe, because Q is defined as a value that total stored magnetic energy of RF coils divided by total energy loss. The materials and shape of HTS support structure in very narrow bore with 32 mm were carefully considered and designed using electromagnetic field simulators.

Our HTS RF coil probe has been achieved very high Q, 16000 at 9 K, 36 times larger than copper RF coils of the same shape, at the same temperature. This corresponds to sensitivity increase of 6 times. The first NMR measurement using a standard sample, KBr powder was performed. The sensitivity enhancement by a factor of 6.6 compared to copper RF coils at 9 K has been achieved. This result shows that the sensitivity of HTS RF coils is 11.3 times larger than that of copper RF coils in room temperature.

[1] K. Yamada, K. Kitagawa, M. Takahashi, Field-swept ^{33}S NMR study of elemental sulfur, Chemical Physics Letters 618 20-23 (2015)

[2] K. Koshita, K. Kitajima, T. Yamada, M. Takahashi, H. Maeda, A. Saito, and S. Ohshima, Development of HTS Pickup Coils for 700-MHz NMR: Resonance Frequency Tuning Using a Sapphire Plate, IEEE Trans. Appl. Supercond., Vol. 26, No. 3, pp. 1500104, (2016)

Hardware

P157

High-pressure sample cell for liquid state NMR applications

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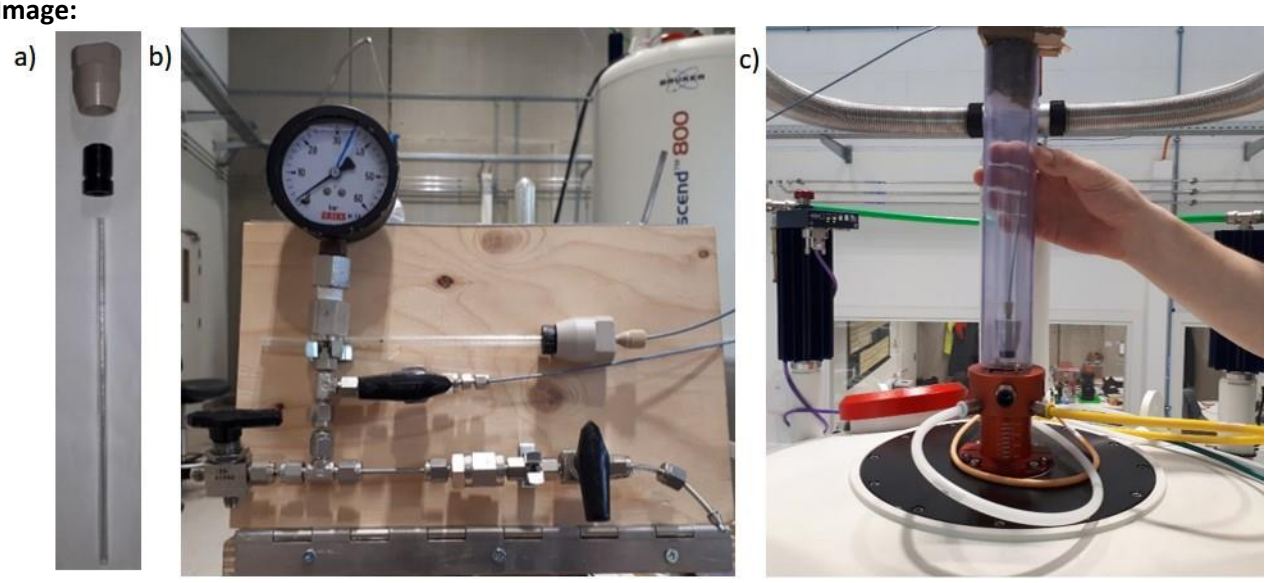
Abstract: High-pressure liquid state NMR experiments allows to study the dynamic structure of liquids, supercritical fluids, polymers and disordered solids[1] Despite its versatility, the number of high-pressure NMR studies remains limited, owing to the complexity and hazards of high-pressure NMR equipment. A safe, low-cost sample environment for high-pressure liquid state NMR (up to 300 bars), compatible with standard 5 mm commercial probe heads was developed. The novel setup is based on a 5 mm sapphire tube (Al_2O_3 single crystal structure) which is capable of withstanding pressures up to 400 bar and temperatures > 1800 K while having almost zero background in NMR experiments.[2] The tube is connected to a PEEK (polyetheretherketone) HPLC column (Figure 1a), providing *in situ* access to the sample environment with high-pressure gasses and liquids via a 5 m long PEEK connection (0.25 mm ID, 0.8 mm OD) to an external flexible pressure regulating system (Figure 1b). Safety measures have been implemented to prevent any direct exposure to the pressurized cell during filling, transport or insertion into the probe (Figure 1c). The system bears the promise of making high-pressure liquid state NMR affordable and more accessible.

Figure 1. a) Deconstructed view of the high-pressure NMR tube: PEEK HPLC column + sapphire tube. **b)** Pressure regulating system, allowing both *in situ* and/or *ex situ* pressurization of the sapphire tube. Because of the 5 m PEEK tubing, the sapphire tube can be inserted into the spectrometer like any other liquid state NMR tube. **c)** Protective Plexiglas casing preventing direct exposure to the pressurized tube.

1. Jonas, J. in *High Pressure NMR.* (ed Jiri Jonas) 85-128 (Springer Berlin Heidelberg).
2. Bai, S., Taylor, C. M., Mayne, C. L., Pugmire, R. J. & Grant, D. M. A new high pressure sapphire nuclear magnetic resonance cell. *Review of Scientific Instruments* **67**, 240-243.

Image:



Hardware

P158

Depth profiles acquired in the gradient of a high field magnet

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[1]Institut des Molécules et Matériaux du Mans (IMMM), Le Mans Université, Le Mans, France, [2]Math and Science Department, Pratt Institute, Brooklyn NY, United States

Abstract: Low-field unilateral NMR instruments for spatial depth profiling are today sold as commercial products for the study of material properties and degradation, typically at the surface until a depth of some hundred micrometers. The technique is non-invasive due to the open geometry of the instrument. A drawback is the relatively low sensitivity because of the low spin polarization, which also often limits the achievable resolution in solids.

In practice, there are many applications where samples are taken; in these cases the technique does not necessarily need to be non-invasive. The fringe field region of high field magnets in principle offers a field gradient of some thousand G/cm, with the advantage of a theoretical improvement of sensitivity by one to two orders of magnitude compared to a typical low field magnet. In this work, we investigate the feasibility of acquiring depth profiles in the fringe field of a 7 T wide bore magnet by relatively simple hardware.

So far a resolution of some micrometers has been achieved. The factors limiting the resolution such as the gradient properties, relaxation, echo times, and sample orientation will be discussed. The advantages and drawbacks of various acquisition schemes between slice selection and frequency encoding with broadband adiabatic pulses are studied, in particular with respect of the properties of the material and the goal of the experiments. Acquisition by CPMG trains with hard or adiabatic pulses are used for signal enhancement and the determination of relaxation rates as contrast criterion. For experiments with long acquisition time, drift compensation is essential.

We will demonstrate the technique on test samples, a sample of paint layers and we will show the direct observation of molecular diffusion in liquids.

Image:

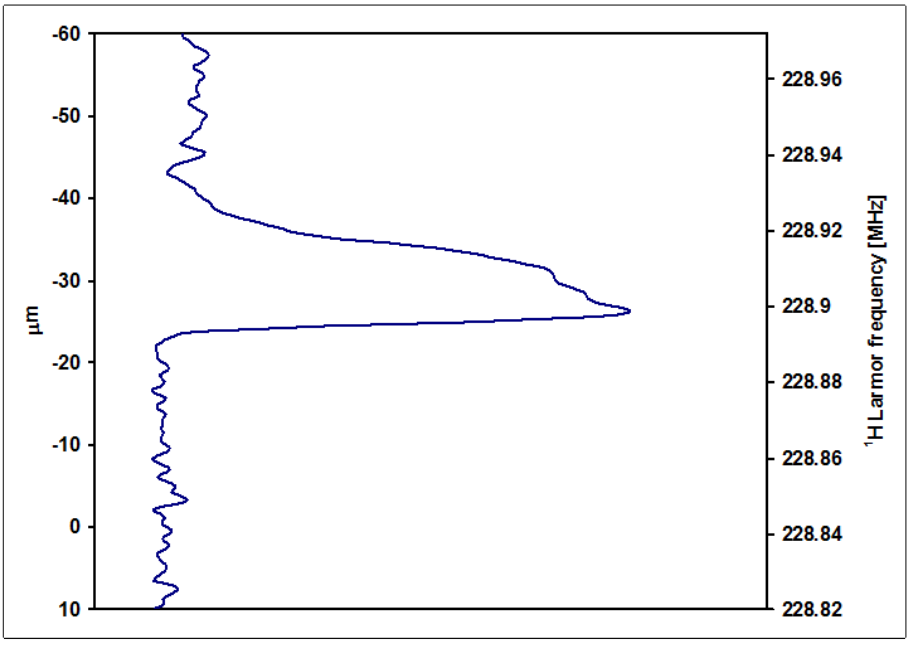

Image of a grease layer of about 10 µm thickness between glass slides

Hardware

P159

Paving the way to centrifugal microfluidics in MR

Stefan Hotz[1], Erwin Fuhrer[1], Anna Zakhurdaeva[*1], Mazin Jouda[1], Dario Mager[1], Jan Korvink[1]
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Abstract: Motivation

We are interested in MR studies of living precision-cut tissue slices. Viability of these slices is ensured by a constant nutrition supply and the removal of excreta, functions which can be provided by a custom-designed centrifugal microfluidic system. The rotation of the sample needed for the microfluidics will also average out inhomogeneities of the B_0. It allows to narrow down spectral lines and hence achieve better resolution. At sufficient speed, magic angle spinning (MAS), carried out at several kHz around a 54.74° tilted axis with the respect to the B_0 field, can also average out dipolar interactions. Hence the spinning will fulfil a double function.

Electromagnetic spinning mechanisms are out of bounds inside the magnet bore, therefore the need for non-standard solutions arises. The suggested concept is based on pneumatics and implemented by using rapid prototyping fabrication methods. Simplicity of fabrication makes this technique a potential engineering novelty and opens up new possibilities for MRI and NMR studies of in vitro samples. The aim of this contribution is to prove the feasibility of spinning a fluidic lab inside the magnet, and its effect on NMR signal quality.

Methods

A pneumatically-driven MR compatible spin-stand was designed and manufactured (Figure A). The turbine and housing were fabricated by UV polymerising 3D printing out of an acrylic polymer (MiiCraft, Taiwan). A rigid and MR compatible fibre glass rod was used as rotation shaft. The PZT ceramic ball bearings were chosen for their diamagnetic properties. A sample container was 3D printed out of PLA using fused deposition modelling (Ultimaker 2+, NL). The sample container was filled with 13 ml water and mounted on top of the spinner. The setup was placed in an unshimmed 1 Tesla ICON imaging system from Bruker, and the spectra were recorded using a lock-in amplifier from Zürich Instruments. Measurements were performed at different spinning frequencies with the sample container rotating around an axis perpendicular to the B_0 field. The stationary resonant detector coil was placed close to the spinning sample container and concentric with the rotation axis.

Results

The data obtained from the experiment indicates that rotation of the sample enhances the obtained signal (Figure B). Spinning frequency of 30 Hz enhanced the linewidth of the obtained spectrum by a factor of 44%. Higher spinning frequency of 60 Hz showed similar linewidth improvement as the 30 Hz frequency compared with the 0 Hz case.

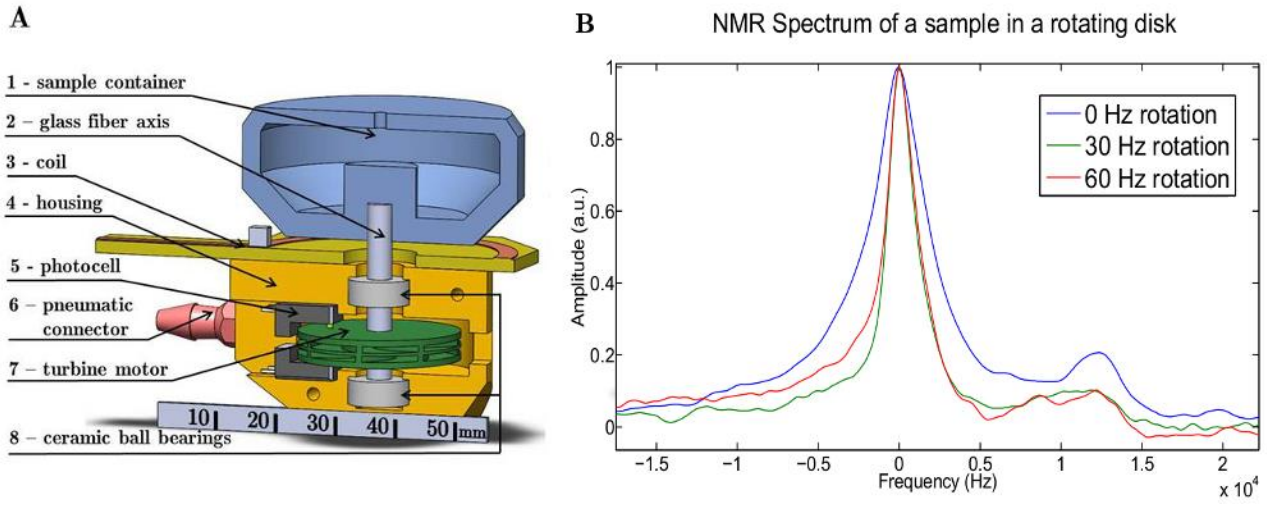
Conclusions & Outlook

The results show that controlled spinning of big samples inside the magnet is possible. As expected, the rotation of the sample increases the signal obtainable from the probe through improvement of the line shape. Tests with additional frequencies will be conducted. Future experiments will also include sample spinning at a range of angles with the respect to the magnetic field.

Acknowledgements

This work was funded by H2020-FET Open TISuMR (#737043).

Image:


A
1 - sample container
2 – glass fiber axis
3 - coil
4 - housing
5 - photocell
6 – pneumatic connector
7 – turbine motor
8 – ceramic ball bearings
10 20 30 40 50 mm
B
NMR Spectrum of a sample in a rotating disk
0 Hz rotation
30 Hz rotation
60 Hz rotation
Amplitude (a.u.)
Frequency (Hz)
x 10^4

Hyperpolarization

P160

Theoretical treatment of pulsed Overhauser DNP for multicomponent EPR spectra

Egor Nasibulov[*1,2], Alexey Kiryutin[1,2], Alexandra Yurkovskaya[1,2], Hans-Martin Vieth[1,3], Konstantin Ivanov[1,2]

[1]International Tomography Center, [2]Novosibirsk State University, Novosibirsk, Russian Federation, [3]Freie Universität Berlin, Berlin, Germany

Abstract: DNP is a powerful method to create non-thermal polarization of nuclear spins, thereby enhancing their NMR signals. The DNP effect is due to transfer of the electron spin polarization to nuclear spins in the presence of MW-pumping; the NMR enhancement is proportional to the ratio of the magnetogyric ratios of electron γ_e and nucleus γ_n. In the case of Overhauser-type DNP (DNP in liquids) the NMR signal enhancement is $\varepsilon=1+\xi f s \gamma_e/\gamma_n$ with ξ, f and s being the coupling factor, leakage factor and saturation factor, respectively. It is well-known that MW-pumping, required for reaching maximal values, can cause substantial heating of the sample. For this reason, using pulsed techniques of pumping instead of cw-pumping is of great interest [1].

Here Overhauser-type DNP formed by a periodic sequence of EPR-pulses is discussed. Earlier [2] the case of a single ideal pulse per period was discussed and an elegant general expression for the NMR enhancement has been obtained. The expression for the enhancement was shown to be similar to that known for cw-pumping with the saturation factor re-defined as the deviation of the electron spin magnetization from its equilibrium value averaged over the cycle of the pulse sequence.

In this work a general theoretical approach to pulsed Overhauser-type DNP is presented. The theory can treat pulsed irradiation of EPR transitions for an arbitrary periodic pulse sequence. The NMR enhancement is analyzed as a function of the EPR-pulse length for ideal pulses and pulses with a finite rise-time. It is shown that one can achieve the maximal theoretically allowed NMR enhancement for pulsed pumping even when the duty cycle of pumping is low. Characteristic oscillations of the DNP enhancement are found when the pulse length is stepwise increased, originating from the coherent motion of the electron spins driven by the pulses. The dependence of the DNP effect on the duty cycle, pulse length and electron spin relaxation times has been studied in detail. Once the lines in the EPR spectrum are inhomogeneously broadened, higher DNP effects are expected in the pulsed pumping mode than in the cw-mode for the same total power of microwave irradiation.

In multicomponent EPR spectra pulsed pumping allows one exciting even spectral components, which are far from resonance with offsets exceeding B_1; thus the enhancement is higher than for cw-pumping with same B_1. The contribution from off-resonant components and its dependence on the pulse sequence parameters were analyzed. Heisenberg exchange can increase the contribution from off-resonant components thus increasing the total NMR enhancement. Experimental low-field DNP data are in good agreement with this theoretical approach.

[1] M. Alecci, D.J. Lurie, J. Magn. Reson. **1999**, *138*, 313-319

[2] E.A. Nasibulov, K.L. Ivanov, A.V. Yurkovskaya and H.-M. Vieth, Phys. Chem. Chem. Phys., **2012**, *14*, 6459-6468

This work has been supported by the Russian Foundation for Basic Research (project No. 18-33-00251)

Hyperpolarization

P161

PHIP NMR signal enhancement vs. heterogeneous catalyst structure

Dudari Burueva[* 1, 2], Kirill Kovtunov[1, 2], Igor Koptyug[1, 2]

[1]Laboratory of Magnetic Resonance Microimaging, International Tomography Center SB RAS, [2]Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russian Federation

Abstract: Parahydrogen-induced polarization (PHIP) technique is an attractive hyperpolarization technique for NMR signal enhancement. The PHIP approach exploits parahydrogen as a source of hyperpolarization – the spin order from the single state of p-H_2 is transferred to the molecule of interest through the catalytic hydrogenation over the catalyst. With regard to the promising application of hyperpolarized gases for biomedical MRI, the heterogeneous PHIP (HET-PHIP) catalysts have attracted considerable interest due to the fast, scalable and continuous production of hyperpolarized gases, which are not contaminated with the catalyst. However, the nature of heterogeneous catalyst causes itself relative low levels of polarization (1-3%) of HP molecules and achievement of the high level of pairwise hydrogen addition remains the main challenge in HET-PHIP approach.

Herein, the Pd-based catalyst with the single-site nature of Pd distribution was utilized and allowed to produce a 3400-fold NMR signal enhancement (*P*=9.3%) of propene during propyne hydrogenation with p-H_2. This high signal enhancement of HP propene along with high conversion of 20% and selectivity of 93% made it possible to obtain MR images of hyperpolarized propene selectively for the signals of CH-, CH_2- and CH_3 groups with a spatial resolution of 0.8×0.8 mm^2, while recording of MR images of thermally polarized propene under the same conditions was insufficient. The single-site nature of catalyst was confirmed by XPS and FTIR-CO and caused the large contribution of pairwise H_2 addition route. For the first time, it was shown the possibility to observe the PHIP effects using a Mo-carbide catalyst in the selective hydrogenation of propyne. It was found that the phase composition of Mo_2C catalyst dramatically affected on the catalytic activity and correlated with both levels of ortho-para conversion of H_2 and pairwise hydrogen addition. Also, the effect of the catalyst preparation of model bimetallic Pd-Au catalysts on the PHIP effects has been studied. It was shown that the composition and structure of Pd-Au affected significantly to the contribution of pairwise H_2 addition route – Au_{shell}-Pd_{core}/HOPG catalyst showed higher pairwise addition level. This behavior of bimetallic Pd-Au catalyst was explained by the formation of isolated Pd atoms confirmed by XPS.

This work is aimed to connect the composition/structure of heterogeneous catalysts to pairwise H_2 addition selectivity in HET-PHIP. It was found that the level of pairwise H_2 addition can be significantly tuned by the composition/structure of the heterogeneous catalyst. This work paves the way for the further development of HET-PHIP for NMR and MRI applications and significantly expands the applicability of PHIP approach.

Acknowledgments: This work was partially supported by SB RAS integrated research program (#0333-2018-0006/II.1.13). The authors thank RFBR (grant # 17-54-33037) for providing the funding support.

Hyperpolarization

P162

Photonic Band-Gap Resonators for Boosting Microwave Powers in DNP NMR

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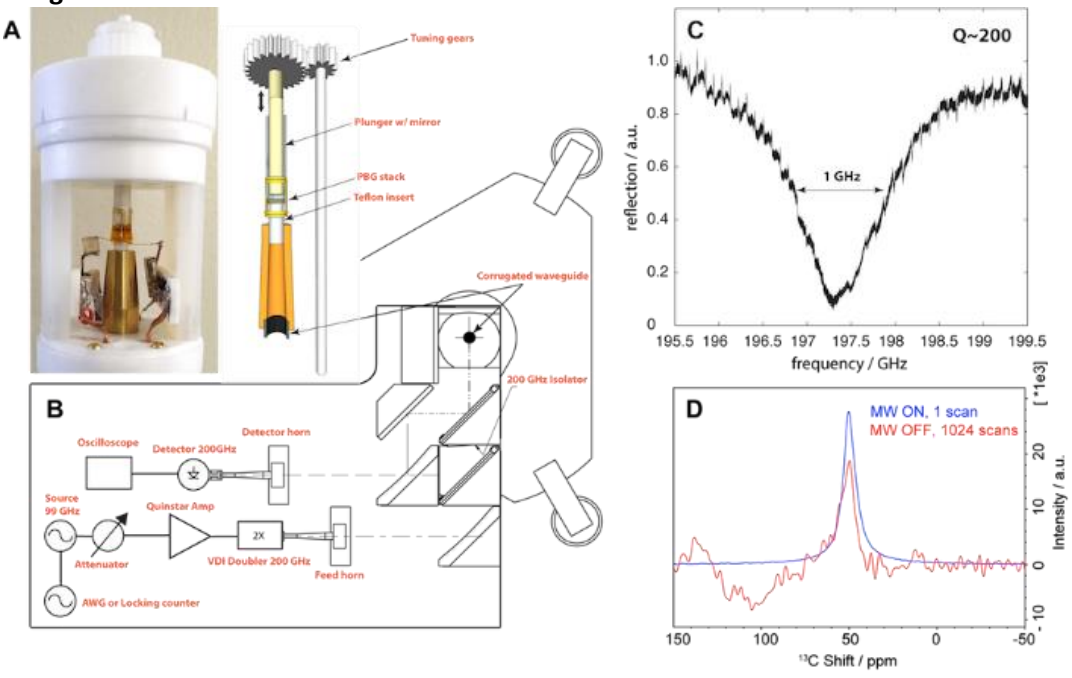
[1]Department of Chemistry, North Carolina State University, Raleigh, United States

Abstract: Achieving sufficiently high electronic B_{1e} fields over large NMR samples represents an essential prerequisite for maximizing the DNP transfer. Here we report on a room-temperature DNP apparatus which is based on a quasioptical bridge operating at 200 GHz electron frequency and an integrated microwave (MW)/radiofrequency (RF) probehead. MW is generated by solid-state devices including a broadband varactor-based W-band (90-100 GHz) source, a W-band power amplifier and an active frequency doubler yielding up to 27 dBm (0.5 W) of incident mm-wave power at ca. 192 GHz and up to 23 dBm (0.2 W) at 197.5 GHz. An integrated DNP/NMR probehead operating at 198 GHz EPR / 300 MHz ^{1}H NMR frequencies consists of a Photonic Band Gap (PBG) resonator incorporated inside a double-tuned saddle RF coil. The PBG resonator is based on creating a defect in one-dimensional photonic crystal formed by dielectric layers that is split by a thin aluminum mirror in the middle of the defect. When loaded with 0.3×3.0×3.0 mm^3 single crystal of HPHT diamond (having 3 µl volume) the resonator exhibited Q≈200 yielding increased B_{1e} fields at the sample. Significant DNP enhancements (ε≈90) of ^{13}C natural abundance NMR signal from the diamond sample at room temperature were observed at incident MW powers of as low as <100 µW and reached a record ε=1,500 at the maximum power of ca. 200 mW. Detailed power-dependence measurements in continuous and gated modes of the DNP operation have been performed. From these measurements, the power gain from using such PBG resonator vs. non-resonant sample irradiation is conservatively estimated to be at least ten-fold. The presented MW resonator/RF probehead approach may constitute a new direction in DNP for boosting MW fields at the sample even at moderate input powers.

Figure 1. **A.** Photograph and schematics for the integrated tunable MW Photonic Bandgap Resonator/RF double-tuned saddle coil probehead. **B.** Schematics of the 200 GHz quasioptical bridge with the main components as shown. **C.** Resonator probehead tuning curve exhibiting the quality factor Q~200. **D.** Record 1,500-fold DNP enhancement on a 3x3x0.3 mm^3 HPHT monocrystalline diamond at room temperature (single scan, blue) as compared to direct detection (1024 scans, red).

Image:



Hyperpolarization

P163

Following the growth of Saccharomyces Cerevisiae in standard culture conditions using hyperpolarized 129Xe NMR

Celine Boutin[*1], Estelle Leonce[1], Patrick Berthault[1], Guillaume Carret[1]
[1]IRAMIS, CEA Saclay, Gif-sur-Yvette, France

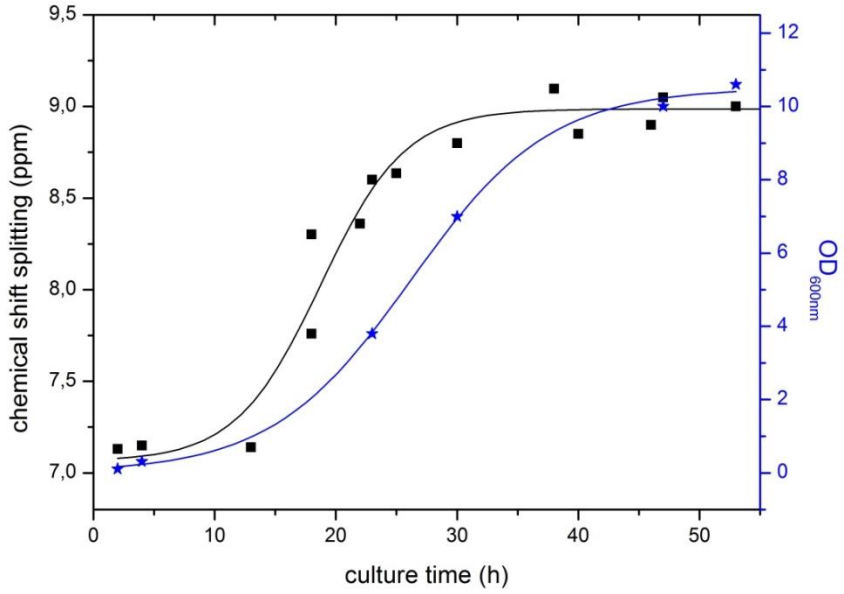
Abstract: Xenon has several interesting properties for the NMR study of biological cells: 1) its nuclear polarization can be boosted via optical pumping, which increases the detection sensitivity by several orders of magnitude. 2) It is nontoxic and soluble in biologic medium and crosses the plasma membrane while keeping its polarization. 3) It has a wide range of chemical shift which makes its NMR signature sensitive to fine cell changes. In particular with cell suspensions two distinct signals are observed on the ^{129}Xe NMR spectrum, corresponding to xenon in the bulk and xenon inside the cells.[1] This has led to promising applications, such as the discrimination of cells sensitive and resistant to chemotherapy.[2]

In this work, we studied Saccharomyces Cerevisiae cells at different times of a culture via hyperpolarized ^{129}Xe NMR (xenon was hyperpolarized using our home made optical pumping setup) and controlled the number of cells by the measure of the optical density at 600 nm. For each time stage of the yeast culture, a sample was withdrawn and after hyperpolarized xenon addition, the corresponding ^{129}Xe spectra were analyzed in a 11.7 Tesla spectrometer: the chemical shift splitting, the in-out exchange rate and the proportion of xenon inside the cells were extracted and compared with classical analyses using optical density. The evolution of the spectral signature of ^{129}Xe in S. Cerevisae is dependent of the culture time as presented in the figure. Hypotheses of explanations will be presented.

In parallel, new methodologies, compatible with narrow-bore NMR spectrometers, were developed to allow the in situ follow-up of cell cultures by NMR, using microfluidic system and micro-NMR detection. Surface treatment such as parylene coating was shown to increase biocompatibility.

[1] C. Boutin et al., Hyperpolarized 129Xe NMR signature of living biological cells, NMR Biomed. 24 (2011) 1264.
[2] France Patent 12 52922 (2012) C. Boutin, P. Berthault, Procédé de détermination de la résistance cellulaire aux médicaments.

Image:



Hyperpolarization

P164

Detection of protein-ligand interactions with deuterium long-lived states and dissolution DNP

Thomas Kress[*1], Geoffrey Bodenhausen[1], Dennis Kurzbach[1]

[1]Laboratoire des biomolécules, LBM, UMR 7203, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France

Abstract: Binding affinities in protein-ligand complexes are often weak, which makes them hard to detect and costly to study considering the low sensitivity of conventional detection techniques and the amount of material needed. This is particularly problematic in the case of deuterium-labelled drugs or drug fragments, which change the biochemical properties of drugs and could greatly extend the library of compounds available for drug design [1].

Recent work has shown that it is possible to measure dissociation constants K_D of protonated ligands with Dissolution Dynamic Nuclear Polarisation (D-DNP) coupled with NMR [2]. The main goal of the D-DNP approach is to enhance the intrinsically low sensitivity of NMR spectroscopy. Additionally, so-called long-lived states (LLS) can be generated: these persist longer than spin-lattice relaxation times, which enables time-resolved experiments. This is particularly interesting to overcome fast quadrupolar relaxation of deuterium nuclei. Long-lived states involving deuterium nuclei have favourable properties for detection of protein-ligand interactions since their lifetime depends strongly on the rotational motions of the ligands. [3]

Figure 1 Transfer of magnetisation from a long-lived state involving deuterium nuclei to carbon-13. The build-up and decay rates depend on the correlation times of overall and internal rotations.

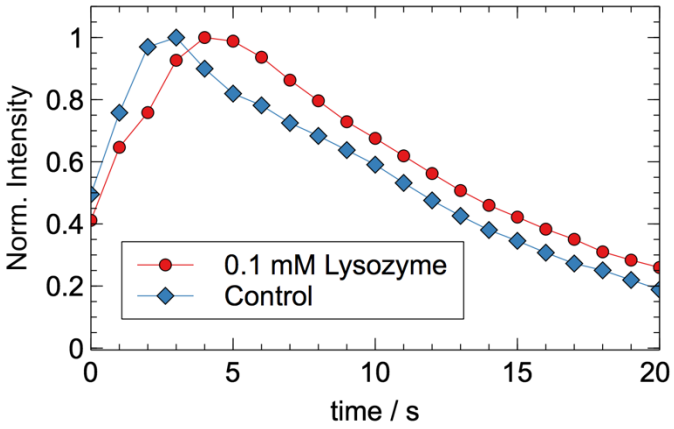
Here we show that magnetisation stored in long-lived states involving deuterium nuclei in $^{13}CD_2$ or $^{13}CD_3$ groups can be used to monitor interactions of deuterated molecules with macromolecular hosts as the transfer of polarization stored in the LLS to the attached carbon-13 proceeds with a rate depending on the rotational motion of the group and, hence, on its local confinement. As a proof of concept, we studied the interaction of deuterated glycerol with human lysozyme. In deuterated glycerol, we observed an extension of lifetime $T_{LLS}(D)/T_1(D) = 47$. Despite the binding constant which was expected to be low, changes in the correlation times of overall and internal rotations could be detected.

[1] K. Sanderson, “Big interest in heavy drugs,” Nature, vol. 458, no. 7236, pp. 269–269, Mar. 2009.

[2] Buratto, R., Mammoli, D., Chiarparin, E., Williams, G., & Bodenhausen, G. (2014). Exploring Weak Ligand-Protein Interactions by Long-Lived NMR States: Improved Contrast in Fragment-Based Drug Screening. Angewandte Chemie International Edition, 53(42), 11376–11380. https://doi.org/10.1002/anie.201404921

[3] A. Jhajharia, E. M. M. Weber, J. G. Kempf, D. Abergel, G. Bodenhausen, and D. Kurzbach, “Communication: Dissolution DNP reveals a long-lived deuterium

Image:


Norm. Intensity
time / s
0.1 mM Lysozyme
Control
0
0.2
0.4
0.6
0.8
1
0
5
10
15
20

Hyperpolarization

P165

Dynamic Nuclear Polarization to enhance 1H and 13C NMR at 1.4 K and 9.4 T

Mathieu Baudin[*] [1], Basile Vuichoud[2], Dennis Kurzbach[1], Aurélien Bornet[3], Gilles Casano[4], Olivier Ouari[4], Geoffrey Bodenhausen[1], Sami Jannin[2]

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Abstract: Dissolution Dynamic Nuclear Polarization (D-DNP) is a popular hyperpolarization method that enables one to boost both NMR and MRI signals. The method consists in the following steps: 1) the sample, for instance a metabolite such as sodium pyruvate or glucose, is dissolved in a glassy matrix (typically water and a glass-forming agent such as glycerol-d_8) containing free radicals. 2) It is polarized via DNP at low temperatures ($1.2 < T < 4.2$ K). 3) Once a satisfactory level of polarization has been achieved, the sample is quickly dissolved with water at high temperature and high pressure and then transported in a few seconds to an NMR spectrometer or MRI scanner for observation in liquid state at room temperature. The signals can be enhanced by more than four orders of magnitude [1].

In the last decade, DNP polarizers have benefited from ever-higher levels of polarization by increasing their magnetic fields, typically from 3.35 to 6.7 T. The use of nitroxide radicals such as TEMPOL allows a rapid build-up of ^{1}H polarization. Cross polarization (CP) sequences, associated with modulation of microwave frequency and microwave gating, have made it possible to obtain a high ^{13}C polarization within remarkably short times (about 30 minutes, compared to several hours for direct polarization without CP) [2]-[4].

To explore the performance of DNP at higher magnetic fields, we have developed a polarizer operating at 9.4 T using ca 75 mW microwave irradiation at 263 GHz. In order to reduce the operational costs of such a system, in particular those linked to the consumption of liquid helium, our cryogen-free 9.4 T magnet is equipped with a built-in cryostat, cooled by a helium recycling loop, which can condense gaseous helium drawn from a storage volume. The probe can operate down to 1.4 K, and is equipped with a home-built resonator that can be doubly tuned to ^{1}H and ^{13}C, thus enabling CP. The performance of high-field DNP has been optimized for a variety of nitroxide radicals, and preliminary dissolution experiments appear promising.

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[2] S. Jannin, A. Bornet, R. Melzi, and G. Bodenhausen, “High field dynamic nuclear polarization at 6.7 T: Carbon-13 polarization above 70% within 20 min,” *Chem. Phys. Lett.*, vol. 549, pp. 99–102, Oct. 2012.

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[4] A. Bornet, A. Pinon, A. Jhajharia, M. Baudin, X. Ji, L. Emsley, G. Bodenhausen, J. H. Ardenkjaer-Larsen, and S. Jannin, “Microwave-gated dynamic nuclear polarization,” *Phys. Chem. Chem. Phys.*, vol. 18, no. 44, pp. 30530–30535, Nov. 2016.

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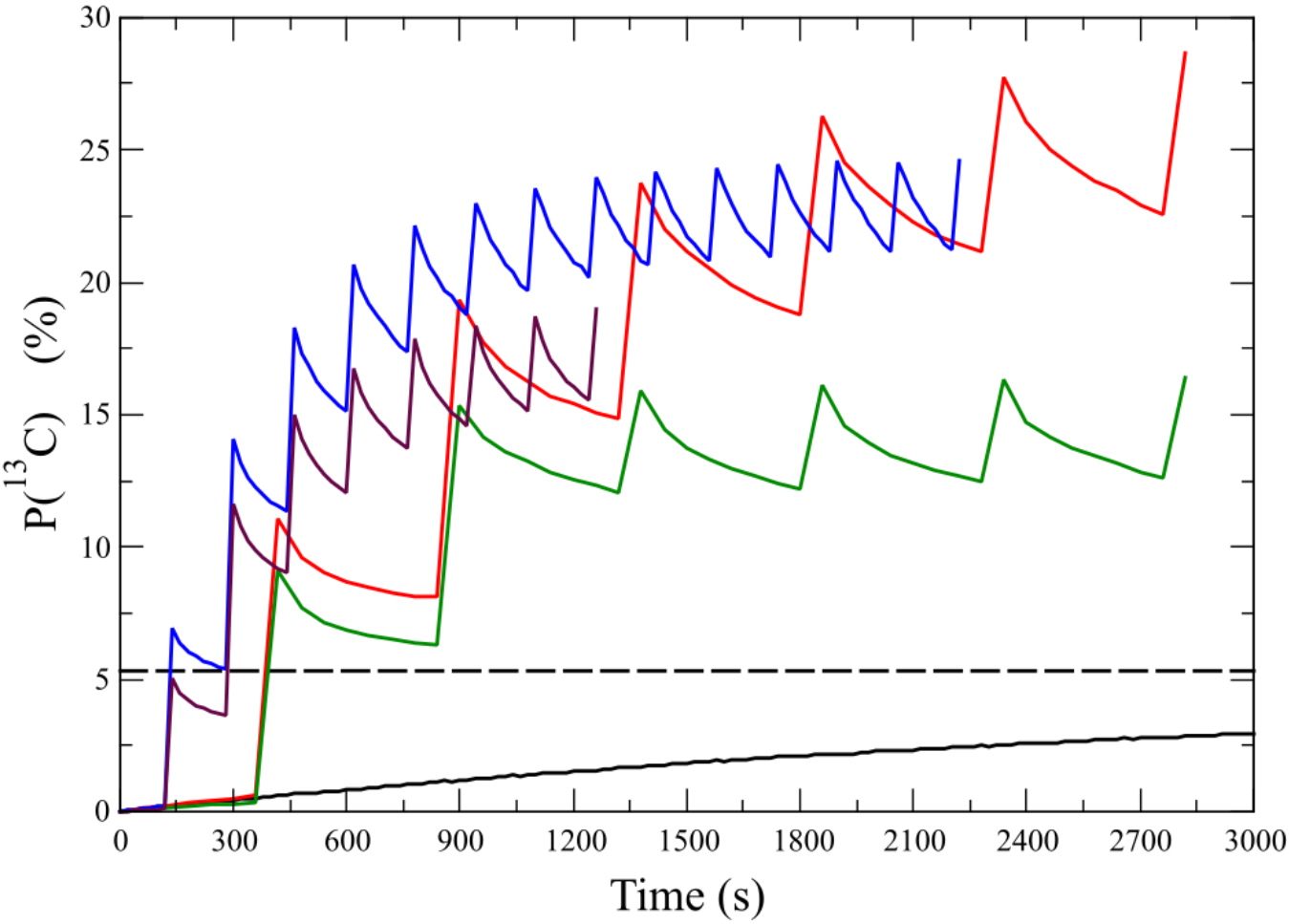
30
25
20
15
10
5
0
P(13C) (%)
0
300
600
900
1200
1500
1800
2100
2400
2700
3000
Time (s)

Hyperpolarization

P166

Transferring frozen hyperpolarized droplets for dissolution DNP

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Abstract:

Dynamic nuclear polarization (DNP) is currently the subject of many new developments in view of boosting the sensitivity of NMR. Dissolution DNP is a promising approach, where samples are cooled to low temperatures (usually between 1.2 and 4 K), and where the high polarization of electron spins is transferred to nuclear spins by microwave irradiation. After rapidly dissolving the sample, the NMR spectrum is obtained at room temperature.

In the most widely used approach, a hyperpolarized frozen sample of ca. 0.05 mL is dissolved with ca. 5 mL overheated D_2O under ca. 1 MPa, and the resulting liquid is transferred through a 'magnetic tunnel' to the NMR instrument where the signal is acquired [1]. Inevitable losses of polarization due to relaxation can be reduced by increasing the speed of transportation. If the final solution is less diluted, the signal will be stronger. These are the main motivations behind the present work. The goal is to transfer hyperpolarized solid droplets pushed by pressurized gaseous helium as fast as possible to the NMR probe, where the dissolution will occur in ca. 0.5 mL of warm solvent, i.e., in a much smaller volume of solvent than is currently used. This is inspired by similar work by Meier et al. [2]. The design of our probe has been modified to accommodate a few small frozen droplets of ca. 0.01 mL each consisting of vitrified DNP juice that can be transferred to the NMR spectrometer for detection.

[1] J. Milani, B. Vuichoud, A. Bornet, P. Miéville, R. Mottier, S. Jannin, G. Bodenhausen, A magnetic tunnel to shelter hyperpolarized fluids, Rev. Sci. Instrum., 86 (2015) 024101.

[2] B. Meier, K. Kouřil, C. Bengs, H. Kourilova, T. J. Barker, S. Elliott, S. Alom, R. Whitby, M. Levitt, Spin-isomer conversion of water at room temperature, and quantum-rotor-induced nuclear polarization, in the water-endofullerene H2O@C60, arXiv:1802.00676 (2018).

Hyperpolarization

P167

Impact of the nanoscale heterogeneity of water/glycerol mixtures in proton polarization in DNP

Emmanuelle Weber[*1,2], Giuseppe Sicoli[3], Ghislaine Frébourg[4], Daniel Abergel[1,2], Hervé Vezin[3], Geoffrey Bodenhausen[1,2], Dennis Kurzbach[1,2]

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Abstract: Dynamic Nuclear Polarisation (DNP) can help to overcome the intrinsically low sensitivity of NMR spectroscopy. This technique involves the saturation of the electron spin transitions of a free radical by microwave irradiation which eventually leads to hyperpolarization of nearby protons. To achieve high nuclei polarisation in dissolution DNP (d-DNP), a mixture of water and glycerol (1:1 v/v) containing paramagnetic agents is required. Here we focus on the most commonly used nitroxide radicals in DNP: TEMPOL, AMUPOL, and Trityl, and we combine EPR and cryo-EM experiments to demonstrate that the time-dependent nanoscopic separation of the glycerol and water phases in DNP mixtures impacts the final polarisation of protons at 4 K in a magnetic field of 6.7 T (see figure 1).[1] Discovering and controlling different parameters that influence the final maximum polarisation in DNP experiments can increase their reproducibility and help refining the models and enable a more accurate description of DNP mechanisms.[2]

Water/glycerol mixtures at room temperature feature dynamic nanophase separation, resulting in a completely homogeneous phase only after hundreds of minutes.[3] EPR experiments reveal a heterogeneous distribution of radicals in DNP samples that directly impacts electron relaxation rates. After allowing the samples to ripen for 45 min at room temperature, the increased concentration of radicals in the water-ich phase can boost the proton polarisation by ca. 20% compared to homogeneous samples.

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[3] Murata, K. & Tanaka, H., *Nat. Commun., 4: 2844, 2013.*

Image:

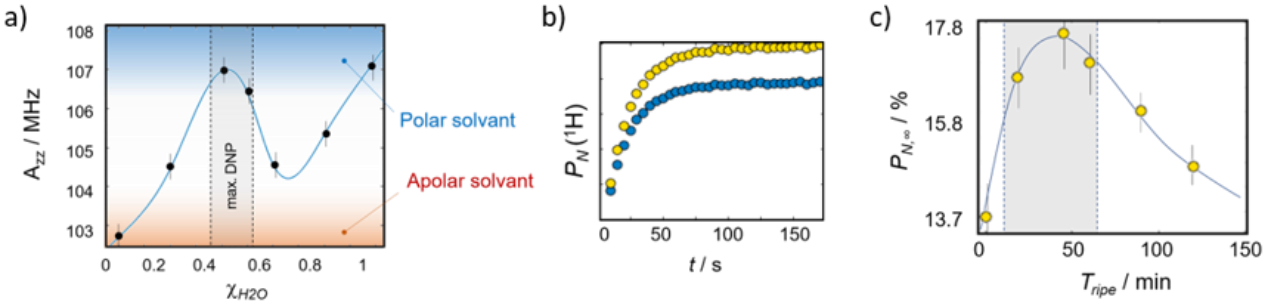

Figure 1. *a) Evolution of the z component of the hyperfine splitting (A_{zz}) of 1 mM TEMPOL on a CW X-band spectrometer at 120 K versus the fraction of water (χ_{H2O}) in the water/glycerol mixture. The samples were allowed to ripen during 45 min at room temperature prior to their vitrification in liquid nitrogen. b) DNP ^1H polarisation (P_N) build-up curves at 4 K in a 6.7 T magnet for samples composed of 50% glycerol-d_8, 40% D_2O and 10% H_2O with 50 mM TEMPOL. The samples have been vitrified in liquid helium after their ripening at room temperature for 0 min (blue) and 45 min (yellow). c) Maximum polarisation ($P_{N,\infty}$) versus the ripening time T_{ripe} of DNP samples.*

Hyperpolarization

P168

Spin order of H2 in high-field experiments with parahydrogen: riddle of the partially negative line of H2 and SABRE experiments

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[1]International Tomography Center SB RAS, ITC SB RAS, Novosibirsk, Russian Federation, [2]Medical Center Freiburg-University of Freiburg, Freiburg, [3]Technische Universität Darmstadt, Darmstadt, [4]Freie Universität Berlin, Berlin, Germany

Abstract: The ^{1}H NMR signal of dissolved molecular hydrogen enriched in parahydrogen (p-H_2) exhibits in the presence of an organometallic hydrogenation catalyst a completely unexpected, partially negative line shape (PNL) [1]. It results from a strongly enhanced two-spin order connected to the population of the T_0 level of orthohydrogen (o-H_2). This two-spin order is made visible by a slow asymmetric exchange process between free hydrogen and a transient catalyst-hydrogen complex. By Only Parahydrogen Spectroscopy (OPSY) it is possible to selectively detect the two-spin order and suppress the signal from the thermal o-H_2. The intensity of the PNL can be strongly affected by long narrow-band RF-irradiation. When the RF-frequency is in resonance with the chemical shift values of the hydrogen bound to the elusive catalyst or of the free hydrogen, a strong intensity reduction of the PNL is observed: the indirect detection of complexes with H_2 has at least three orders of magnitude higher sensitivity than the normal NMR experiment. A theoretical model is developed, which reproduces the NMR line-shape, the nutation angle dependence and the dependence on the frequency of the irradiation field of the PNL and permits the determination of parameters of the transient NMR invisible complex where singlet-triplet conversion takes place.

S-T_0 mixing strongly affects SABRE (Signal Amplification By Reversible Exchange) polarization generated at high fields. First, we are able to elucidate the previously unknown mechanism of spontaneous polarization transfer in such experiments [2]. Specifically, polarization transfer goes in three steps: (i) S-T_0 mixing in catalyst-bound H_2; (ii) formation of net polarization of H_2 due to cross-correlated relaxation; (iii) polarization transfer to the SABRE substrate, occurring due to NOE. The proposed mechanism is supported by a theoretical treatment, magnetic field-dependent studies and high-field NMR measurements with both p-H_2 and thermally polarized H_2. Second, we demonstrate that variation of the spin order of H_2 requires modification of NMR methods used in high-field SABRE [3]. Specifically, methods proposed for the initial singlet order may fail once S-T_0 mixing comes into play; however, a simple modification makes them efficient again. Hence, for efficient use of SABRE one should note that polarization formation is a complex multi-stage process: careful optimization of this process may not only deal with chemical aspects but also with the spin dynamics, including the spin dynamics of H_2.

We acknowledge the Russian Science Foundation (grant No. 14-13-01053) and DFG (Contract Bu 911/22-1) for financial support.

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[2] S. Knecht et al. *J. Magn. Reson.*, **287**, 74-81 (2018) **DOI**: 10.1016/j.jmr.2017.12.018

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Hyperpolarization

P169

Bulk nuclear hyperpolarization of inorganic solids

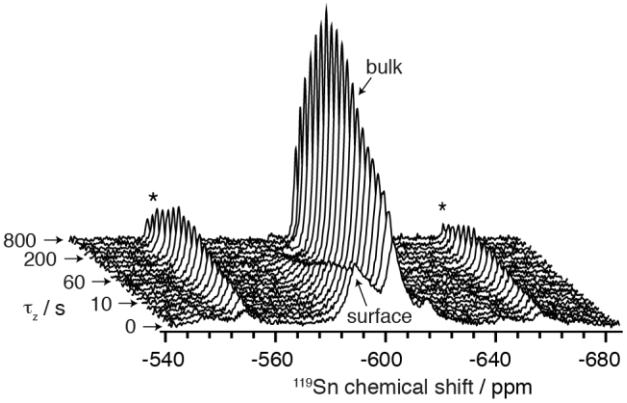
Snaedis Björgvinsdóttir[*1], Brennan Walder[1], Arthur Pinon[1], Lyndon Emsley[1]

[1]Institut des sciences et ingénierie chimiques, EPFL, Lausanne, Switzerland

Abstract: Dynamic nuclear polarization (DNP) using incipient wetness impregnation can be used to hyperpolarize bulk organic materials, given that spontaneous proton spin diffusion carries magnetization from the surface and into the particle. We report a general strategy for hyperpolarizing proton-free inorganic bulk materials, using impregnation DNP and spin diffusion among heteronuclei. Hyperpolarization is generated in a radical containing wetting phase and transferred from protons to heteronuclei at the particle surface using a pulse cooling cross-polarization method. Spin diffusion between heteronuclei is relatively slow, but when heteronuclear T_1 values are long, it can transport enough polarization from the surface and into the particle to exceed the sensitivity of conventional ssNMR. We demonstrate a factor 50 gain in overall sensitivity for the ^{119}Sn spectrum of SnO_2 using pulse cooling. The method is also shown to work for ^{31}P spectra of GaP, and for ^{113}Cd spectra of dTe.

Figure 1:DNP enhanced CP-MAS ^{119}Sn spectra of SnO_2 impregnated with 16 mM TEKPol in tetrachloroethane, showing spin diffusion among tin nuclei. The spectra are acquired as a function of polarization delay, t_z.

Image:



Hyperpolarization

P170

Only Para-hydrogen SpectroscopY (OPSY) revisited

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[1]Section for Biomedical Imaging, Molecular Imaging North Competence Center (MOIN CC), Department for Radiology and Neuroradiology, University Medical Center Kiel, Christian-Albrechts University of Kiel, Kiel, Germany

Abstract: ParaHydrogen Induced Polarization (PHIP) is a well-established hyperpolarization technique that is used to enhance the signal of ^{1}H and heteronuclei alike. The PASADENA [1] experiment is one of the simplest and the most popular PHIP experiment because hydrogenation occurs in situ at high magnetic field of NMR/MRI and no additional automatic or manual transfer of sample is required. Due to that advantages, PASADENA experiments are especially often used in analytical chemistry and catalysis. In some cases, however, the PHIP signal is weak and buried under thermal signal of reagents and solvents. To beat that obstacles different pulse sequences for selective registration of PHIP were developed (OPSY, SEPP, out of phase echo, APSOC, 45x-135x etc).

Recently, we revisited Only Para-hydrogen SpectroscopY (OPSY [2]) because the method offers the highest level of background signal suppression in single short. We have shown e.g. that in OPSY with zero quantum selection filter signal can be accidentally lost at some durations of the gradient.

We also analyzed four “equivalent” double quantum filters based on the application of two gradients and demonstrated the different performance. Only one among four analyzed sequences (see Figure) allows efficient detection of PHIP even at the magnetic field with low homogeneity. Furthermore, we have shown that OPSYd-111 robustly converts an anti-phase signal of PASADENA to in-phase (net magnetization). Net magnetization can be easily manipulated and be used in consequent NMR or MRI experiments.

Conversion of PHIP signal to net magnetization together with excellent suppression of background signal, without phase cycling, a short duration of the sequence and ease of application makes the method suitable for the analysis of PHIP at high magnetic fields even at the conditions of low magnetic field homogeneity.

[1]. C.R. Bowers, D.P. Weitekamp, *J Am Chem Soc* 1987, pp. 5541–5542.

[2]. J.A. Aguilar et al., *Chem Commun* 2007, pp. 1183-1185.

We acknowledge support by DFG (HO 4604/2-1), the Christian-Albrechts-University (CAU), the Medical Faculty, the European Regional Development Fund (ERDF) and the Zukunftsprogramm Wirtschaft of Schleswig-Holstein (122-09-053) and DAAD fellowship (A.P.).

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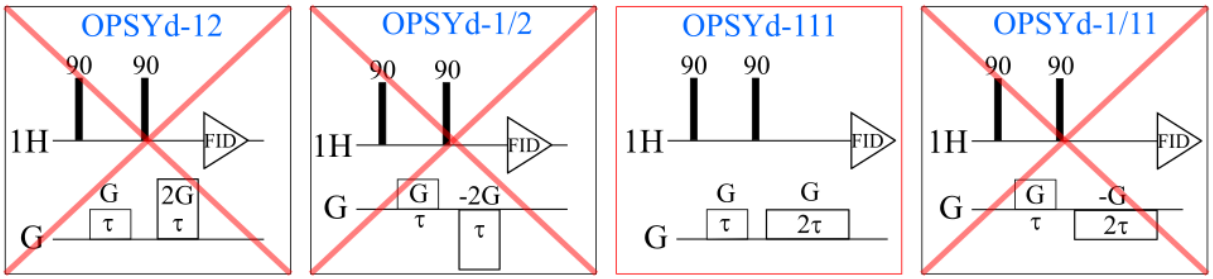

Figure. Four variants of OPSYd (OPSY with double quantum selection filter) sequences: OPSYd -12, OPSYd-1/2, OPSYd-111 and OPSYd-1/11.

Hyperpolarization

P171

HyperVac: a long distance transport system for hyperpolarized substrates

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Abstract:

Purpose

Due to the relatively short T_1 dependent lifespan of hyperpolarized substrates, the transport time and conditions between polarizer and the MRI scanner become important. Therefore, specialized transportation schemes are beneficial, especially if the hyperpolarized sample needs transport over longer distances, floor levels or even buildings. We present a low cost and robust approach to this issue, utilizing a pneumatic transport scheme.

Materials and methods

The preliminary hyperpolarized vacuum (HyperVac) transport system is comprised of 32 m of flexible PVC hose (2.5 cm OD) used in conjunction with two household vacuums with a combined power of 3050 W. This enables a pneumatic transport pathway for hyperpolarized samples between our polarizer and 9.4 T pre-clinical MRI system, see figure 1. Sleeved vacuum-pods made from 2.0 ml syringes or 4.0 ml blood collection tubes, is used to transport the samples. Transport time was measured using a stop-clock. The combined purchase cost of all HyperVac hardware was less than 400 USD.

Possible gains in polarization for the MR experiment by using pneumatic transport was simulated in Matlab (MathWorks, Natick, MA, US). Simulation was performed using T_1 equal to 71.3 s and 50.7 s for [1- ^{13}C]pyruvate and [^{13}C,$^{15}N_2$]urea, respectively.

Results

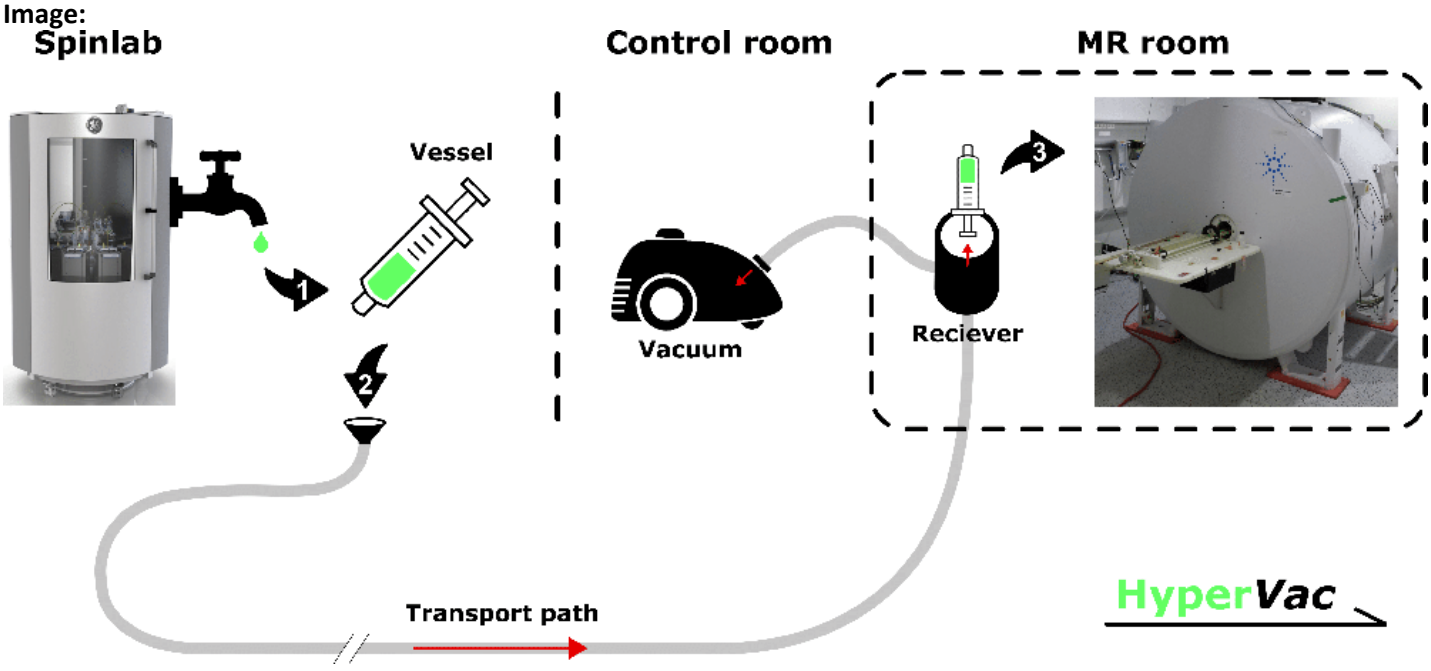
Sample transport was significantly faster when using pneumatic transport compared to running (syringe: $p < 0{,}0001$ or collection tube: $p < 0{,}0001$). Measured mean transport time / velocity for the collection tubes (4.0 ml sample, n=10) and syringes (2.0 ml sample, n=10) were 3.6 ± 0.2 s / 8.9 ± 0.4 m/s and 3.9 ± 0.2 s / 8.3 ± 0.4 m/s, respectively. The mean transport time / velocity when running (n = 30, measured ten times each on three different subjects) was 9.5 ± 0.7 s / 3.4 ± 0.3 m/s. This corresponds to a decreased transport time of up to 62% over a short distance.

Simulated relaxation curves predict polarization gains of 9.4% and 13.5% for [1-^{13}C]pyruvate and [^{13}C,$^{15}N_2$]urea, respectively.

Discussion

The potential polarization gain compared to transport by running derived from fast transport schemes can be directly translated to a gain in SNR of the acquired ^{13}C images or spectra. Therefore, transporting the sample by pneumatic transport potentially brings a benefit to hyperpolarized MR experiments. Future work include polarization tests of hyperpolarized substrates using this system. Additionally, since the relaxation of hyperpolarized samples is negatively affected by varying field strengths as well as exposure zero field regions, integration of a constant magnetic field in the transport vessel is also very relevant.

Image:


Spinlab
Control room
MR room
Vessel
1
2
3
Vacuum
Reciever
Transport path
HyperVac

Hyperpolarization

P172

1H and 13C benchtop NMR spectroscopy with SABRE hyperpolarization

Peter M. Richardson[*1], Richard John[1], Andrew Parrott[2], Olga Semenova[1], Wissam Iali[1], Alison Nordon[2], Meghan Halse[1], Simon Duckett[1]

[1]Centre for Hyperpolarisation in Magnetic Resonance, Chemistry, The University of York, York, [2]Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom

Abstract: Nuclear magnetic resonance (NMR) is an extremely powerful analytical technique with well-defined limitations, such as relatively low sensitivity, an effect which is exacerbated at the lower magnetic fields of benchtop devices. Hyperpolarisation methods can provide orders of magnitude increases in NMR signals. The combination of hyperpolarisation and benchtop NMR opens up many potential new applications, where high-sensitivity, portable and low-cost NMR solutions are desirable. For example, in industrial process monitoring the analytical capabilities of NMR would be an advantage but high-field NMR spectroscopy is non-viable.

The hyperpolarisation method used here, Signal Amplification by Reversible Exchange (SABRE), uses *para*hydrogen (p-H_2) as the source of polarisation in a catalytic process that does not require hydrogenation of the analytes of interest. Therefore it is completely reversible with the addition of fresh p-H_2.[1] While initially limited to the hyperpolarisation of analytes that interact directly with the SABRE catalyst, a new approach called SABRE-Relay [2] expands the technique to include target molecules that contain exchangeable protons. This allows for the hyperpolarisation of a much broader range of analytes and thus significantly broadens the scope of potential applications of this approach.

In this work, we explore the potential for SABRE hyperpolarised benchtop NMR to be used as a quantitative analytical technique. We demonstrate significant ^{1}H and ^{13}C SABRE enhancements for analytes detected using both benchtop and high-field NMR systems, highlighting that the observed SABRE polarisation is independent of detection field strength. The viability of working in protonated solvents on the benchtop instrument is also established. In addition, we exploit the characteristic reversible nature of SABRE to acquire hyperpolarised 2D spectra using a bespoke automated flow system that has been integrated with the benchtop NMR device [3,4].

One of the advantages of SABRE over other hyperpolarisation techniques is the relatively low cost of producing and storing p-H_2. However this cost is highly dependent on the required level of p-H_2 purity. Here we monitor the effect of p-H_2 purity on the observed signal enhancements for both the SABRE and SABRE-Relay techniques. The resulting linear relationship is modelled theoretically to determine SABRE efficiency parameters for each solvent-substrate-catalyst system that is independent of the level of p-H_2 purity and which can be used to estimate the level of p-H_2 required for a given application.

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2. Iali W et al. Sci. Adv. 4(1) (2018)
3. Lloyd LS et al. J Am Chem Soc. 134(31), 12904-7 (2012)
4. Mewis RE et al. Magn Reson Chem. 52(7), 358-69 (2014)

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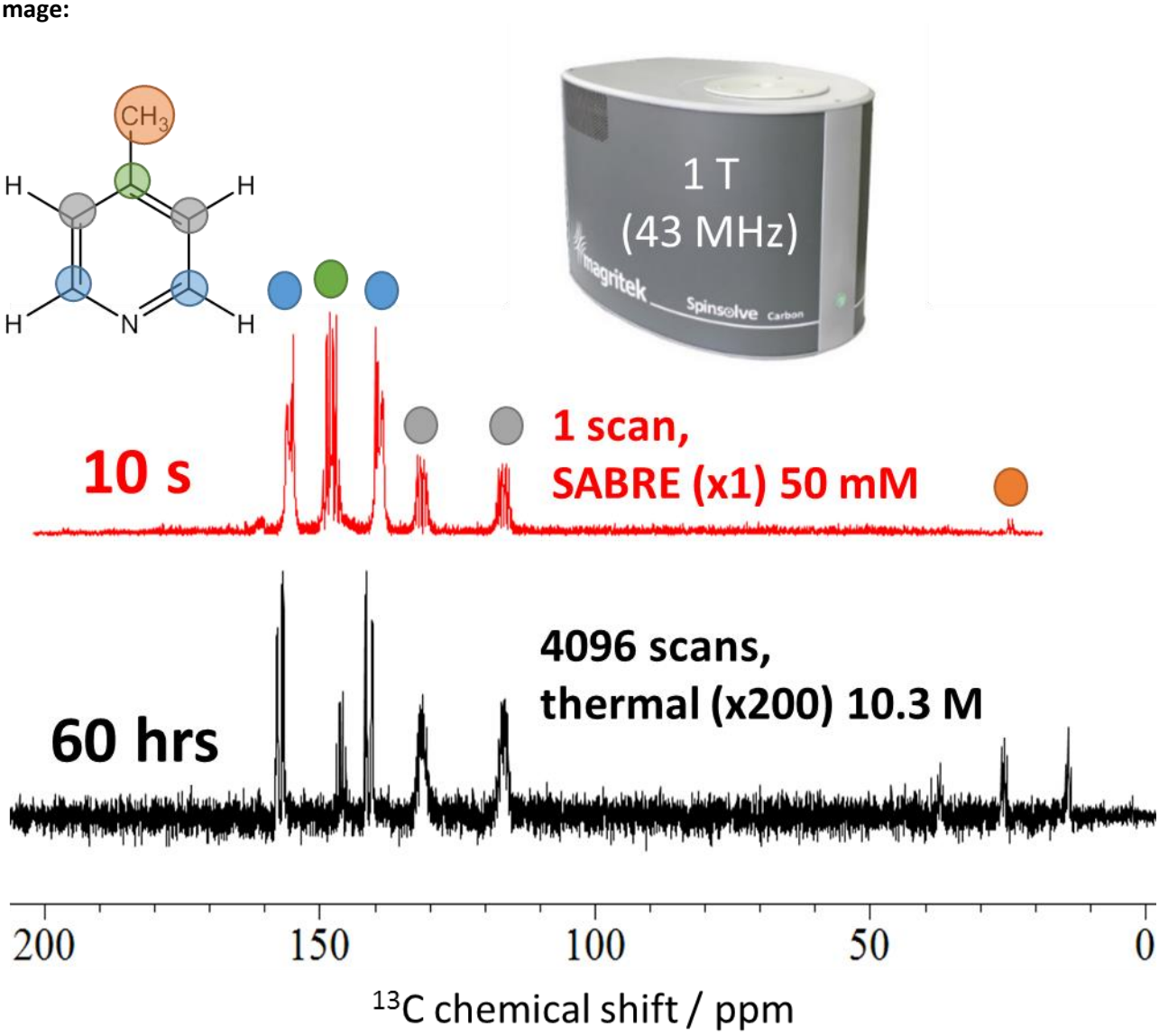
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4096 scans,
thermal (x200) 10.3 M
200
150
100
50
0
13C chemical shift / ppm

Hyperpolarization

P173

Multi-echo bSSFP imaging of the mouse liver using hyperpolaized [1-13C]pyruvate at 9.4T

Christian Ø. Mariager[*1], Kasper Faarkrog Høyer[2], Steffen Ringgaard[1], Christoffer Laustsen[1]

[1]MR Research Centre, the Department of Clinical Medicine, [2]Department of Clinical Medicine, Aarhus University, Aarhus N, Denmark

Abstract:

Purpose

We investigate the use of a fast multi-echo balanced steady state free precession (bSSFP) sequence to acquire spectroscopic images of ^{13}C-phantoms and in the mouse liver following a dose of [1-^{13}C]pyruvate, at 9.4T. Resconstruction is performed using a Dixon-type technique called IDEAL that requires prior knowledge of the present spectroscopic species.

Materials and methods

A multi-echo bSSFP sequence was implemented on a 9.4T pre-clinical MR system (Agilent, UK) equipped with a dual tuned 13C/1H volume coil, see figure 1.

The phantom experiment was performed using three cylindrical phantoms containing ^{13}C acetate, urea and bicarbonate to test the sequence and IDEAL reconstruction, see figure 1. Imaging parameters were 15 echoes with TE = 1.1 ms, ΔTE = 0.61 ms, TR = 11.3 ms, FOV = 6 x 6 cm , slice thickness = 20 mm, BW = 156 kHz, acq. matrix = 32 x 32 and flip angle = 30°. A free induction decay (FID) experiment was performed to acquire the frequencies of each species.

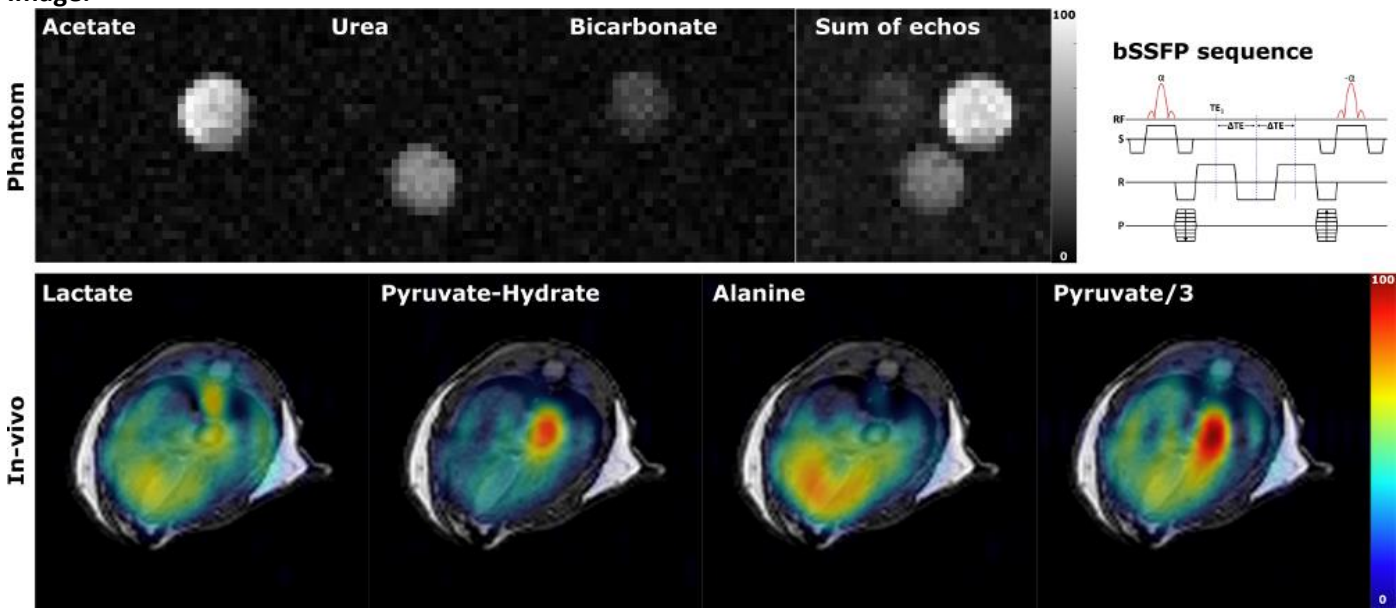
Two 12 week old mice (C57BL/6NTac) were fasted for five hours prior to hyperpolarized [1-^{13}C]pyruvate injection. Hyperpolarized ^{13}C imaging and spectroscopy was performed using the bSSFP approach interleaved with a FID acquisition. bSSFP parameters: one axial oblique slice, 9 echoes, TR=7.9 ms, TE=1.1 ms, ΔTE=0.7 ms, FOV=50×50 mm^2, matrix=24×24, flip angle=30° and 8 mm slice thickness. FID parameters: TR=206 ms, TE=0.5 ms, spectral width=10 kHz, 2048 complex points and flip angle=15°. Timing: one bSSFP or FID acquisition every two seconds.

Data processing was performed in MATLAB R2016a (The Mathworks, Natick, MA, US). Using spectral information obtained in the FID acquisition, the bSSFP data was reconstructed using an IDEAL method. Prior to the IDEAL reconstruction, a first order phase correction was applied. The shown metabolic maps are the sum of all time points for each metabolite, see figure 1.

Results and discussion

The IDEAL reconstruction of the phantom experiment show satisfactory seperation of the present spectroscopic species, with little to no artifacts, confirming the feasibility of the method. For the in-vivo experiment, the metabolic maps are well seperated with the artery only having a high signal in the pyruvate and pyruvate-hydrate maps. A small area is of signal above the artery is visible in the lactate and alanine maps which could be due to the heart moving in and out of the slice. The good metabolite seperation combined with high resolution and fast acquisition provided by multi-echo bSSFP imaging affirm the expectation that this method can be used to acquire dynamic metabolite imaging for future in-vivo HP experiments.

Image:


Phantom
Acetate
Urea
Bicarbonate
Sum of echos
100
0
bSSFP sequence
RF
S
R
P
In-vivo
Lactate
Pyruvate-Hydrate
Alanine
Pyruvate/3
100
0

Hyperpolarization

P175

Application of Rapid-Transfer Dissolution-DNP to produce long-lived hyperpolarized fumarate

James Eills[*1], George Bacanu[1], Karel Kouril[1], James Kempf[2], Ivan Sergeyev[2], Hana Kourilova[1], Benno Meier[1], Malcolm Levitt[1]
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Abstract: Hyperpolarized fumarate has been used in medical imaging to observe tumour activity in mice.[1,2] Perhaps the largest drawback to this form of imaging is the lifetime of the hyperpolarized NMR signals, which is often on the order of just tens of seconds.

We have recently shown it is possible to extend the lifetime of ^{13}C nuclear spin polarization in fumarate molecules by temporarily precipitating them out of solution.[3] This is possible because at high field the solid state T_1 is significantly longer than the solution state T_1.

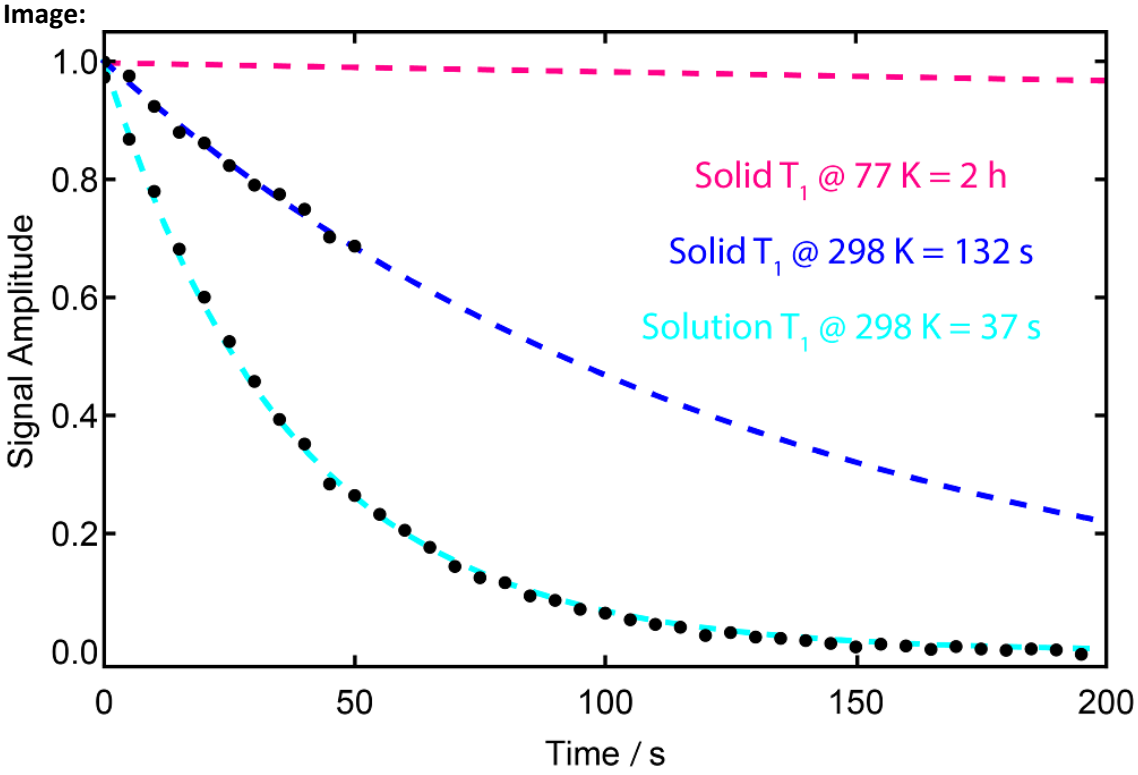
Using the new bullet-DNP technique, we demonstrate that hyperpolarized ^{13}C nuclear magnetization of fumaric acid can be stored for times dramatically exceeding the solution-state T_1. A hyperpolarized bullet of fumaric acid in DMSO doped with trityl radical was fired into 12M HCl. The fumaric acid immediately precipitated, purifying it from the toxic radicals. The solid-state signal decay was measured for 60 s with 5° flip-angle pulses. NaOH was then added to dissolve the fumarate and we continued to measure the solution-state signal decay (Fig. 1).

In non-hyperpolarized experiments, we have measured the T_1 at 2 T and liquid nitrogen temperatures to be around 2 h, which should allow for future application of this technique in transport and storage of hyperpolarized samples.

Figure 1: The hyperpolarized ^{13}C NMR signal decay for solid and solution-state fumarate at 11.7 T, measured with 5° flip-angle pulses. In pink is the experimentally measured T_1 at 2 T and liquid nitrogen temperature of the same molecule, without hyperpolarization.

1. F. Gallagher et al, Proc. Natl. Acad. Sci., **2009**, 106, 19801
2. M. R. Clatworthy et al, Proc. Natl. Acad. Sci., **2012**, 109, 13374
3. J. Eills et al, ChemPhysChem, **2018**, 19, 40

Image:


Solid T1 @ 77 K = 2 h
Solid T1 @ 298 K = 132 s
Solution T1 @ 298 K = 37 s
Signal Amplitude
Time / s
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200

Hyperpolarization

P176

Level anti-crossings and photo-CIDNP in the solid state: investigating the dependence of nuclear spin polarization on molecular orientation and magnetic field strength

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Abstract: Photochemically Induced Dynamic Nuclear Polarization (photo-CIDNP) is non-thermal polarization of nuclear spins occurring upon photochemical reactions, which have Radical Pairs (RPs) as intermediates. The photo-CIDNP effect originates from (i) electron spin-selective recombination of radical pairs and (ii) dependence of the singlet-triplet interconversion in radical pairs on the state of magnetic nuclei [1]. The photo-CIDNP effect can be investigated by using NMR methods. The benefit from photo-CIDNP compared to Boltzmann polarization is two-fold: (i) it allows to obtain considerable amplification of NMR signals, and (ii) photo-CIDNP provides access for investigating elusive radicals and radical pairs. While the mechanisms of the photo-CIDNP effect in liquids are well established and understood, photo-CIDNP mechanisms in the solid state are not yet fully understood. Difficulties in understanding the spin dynamics underpinning the photo-CIDNP effect in the solid-state are caused by the presence of anisotropic spin interactions, which result in a complex evolution of the spin system.

Recently we have shown that a theoretical approach based on Level Crossings (LC) and Level Anti-Crossings (LAC) is an efficient tool to reveal the complex spin dynamics in the case of CIDNP. In solids all features in the field dependence of CIDNP come from the LACs, because anisotropic interactions result in perturbations which turn LCs into LACs. We have interpreted known CIDNP mechanisms in terms of the LAC concept. This consideration allows one to find analytical expressions for the magnetic field range, where different CIDNP mechanisms are operative; furthermore, the LAC description gives a clear way to determine CIDNP sign rules. Experimental studies of solid-state CIDNP in photosynthetic reaction centers performed by the group of Prof. Matysik (University of Leipzig) have shown that at low magnetic fields of about 1.5 T, there is an unexpected change of the sign of the polarization [2]. We investigated orientation and magnetic field dependence of CIDNP and found a possible explanation for experimental observations: the sign change results from specific molecular orientations giving rise to particular LACs in the transient RP. The analysis of photo-CIDNP in the solid state and its magnetic field dependence thus requires studying the orientation dependence of polarization, which provides information about the contributions of different mechanisms in the total photo-CIDNP.

This work has been supported by the Russian Foundation for Basic Research (grant No. 18-33-00251, grant No. 17-03-00932).

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[2] D. Gräsing, P. Bielytskyi, I. F. Céspedes-Camacho, A. Alia, T. Marquardsen, F. Engelke, J. Matysik, Scientific Reports, 7, 12111 (2017) doi:10.1038/s41598-017-10413-y

Hyperpolarization

P177

Field swept polarization transfer in parahydrogen NMR

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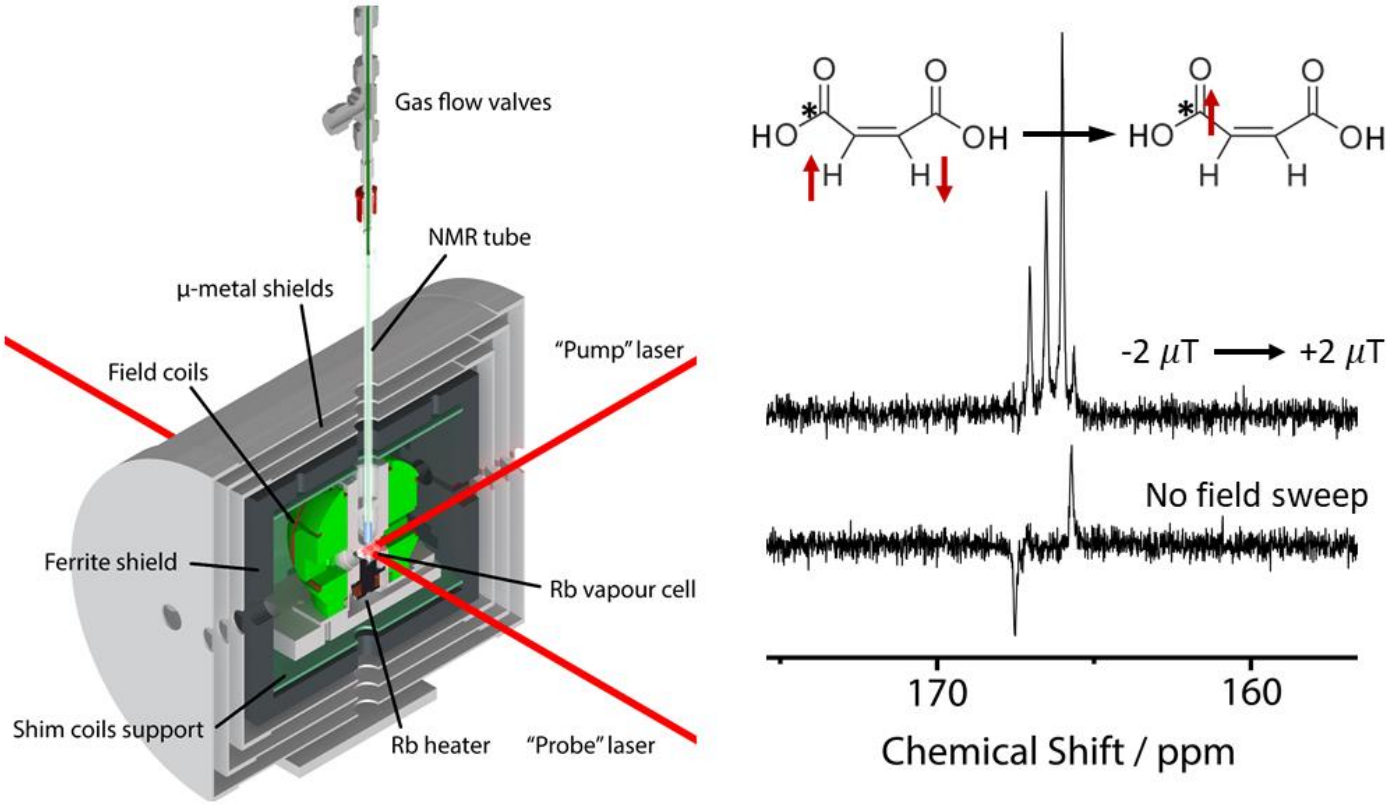
Abstract: Much work in the field of parahydrogen-enhanced NMR involves the transfer of proton polarization to heteronuclei using the molecular J-coupling network. Techniques such as magnetic field cycling[1] and 'SABRE-SHEATH'[2] have emerged for this purpose. Both techniques work by exploiting avoided state crossings at ultra-low magnetic fields.
We present a surprising new discovery: If a molecule containing a heteronuclear spin is parahydrogenated at some magnetic field to yield an AA'X spin system, and the field is adiabatically swept through the zero point and up in the opposite direction, the parahydrogen singlet order is transformed into magnetization on the heteronuclear spin.
To demonstrate this, experiments were performed in a ZULF (zero and ultralow field) NMR chamber (shown in Fig. 1), which afforded precise control over the magnetic fields applied to samples. Firstly, acetylene dicarboxylic acid was parahydrogenated to maleic acid. The field was then swept adiabatically from -2 to +2 μT to polarize the carbonyl ^{13}C spin. The sample was then shuttled into a Magritek SpinSolve high field benchtop magnet for direct ^{13}C detection. The result is shown in Fig. 2, alongside a comparison with a sample that did not undergo the field sweep.
We soon hope to employ this technique to hyperpolarize compounds in a continuous-flow manner.
Figure 1: Left: Schematic of the ZULF chamber, which is used to negate Earth's field, and apply ultralow magnetic fields to the sample, prior to detection at high field. Right: Hyperpolarized ^{13}C NMR spectra of maleic acid after performing the field sweep, and not. Without a field sweep, the net ^{13}C polarization is zero, but by using the field sweep we are regularly achieving polarizations in the order of 1%.

Image:



Hyperpolarization

P178

Radical geometry: a key toward the maximum theoretical enhancement in MAS-DNP.

Gabriele Stevanato[*1], Dominik Kubicki[1], Olivier Ouari[2], Laura Hesteban Hofer[3], Maxim Yulikov[3], Gunnar Jeschke[3], Lyndon Emsley[1]

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Abstract: Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP) is an essential tool to dramatically improve the sensitivity of numerous experiments in nuclear magnetic resonance (NMR). It allows, for example, the elucidation of the surface structure of complex materials[1] as well as the characterisation of polymorphs in organic solids[2], by transferring polarization from electron to nuclear spins. The theoretical upper limit of transferable polarization at 100K, a typical regime for MAS-DNP experiments, is about 660 for ^{1}H and even more for other low abundant nuclei such as ^{13}C, ^{15}N.

Is it possible to approach the theoretical maximum by a suitable radical[3] design?

Research conducted in the last 15 years has highlighted several crucial factors: rigidity, bulkiness and relaxation properties of the radical. Here we show, for a set of nitroxide mono- and bi-radicals, how *the design of the local geometric conformation has a dramatic impact on the DNP performance*. ***In one case, the enhancement ε calculated as the ratio of the ^{1}H signal microwave ON/OFF reaches the record value of 323.*** The value of ε translates into a reduction of the experimental time needed to perform important 2D correlation experiments proportional to ~ε^2. *In few hours one can obtain the information that would normally require years in absence of DNP.* This observation defines a *new and unaccounted for paradigm* in the radical synthesis and paves the way to the optimal design of radical species towards the maximum theoretical enhancement for MAS-DNP experiments.

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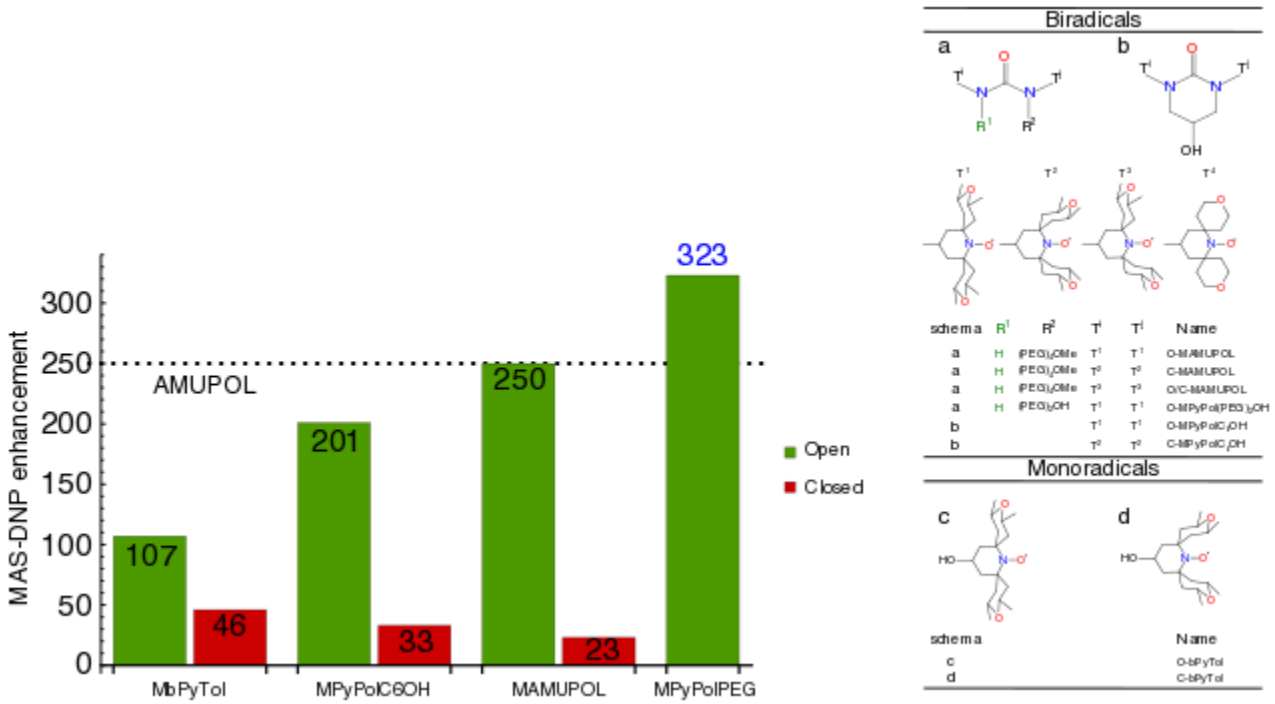
Fig.1: Mono- and bi-radicals investigated as MAS-DNP agents. The bar chart reports the ^{1}H enhancement (microwaves ON/OFF) at 100K and 9.4T under MAS (8 kHz). The solvent used is either glycerold8:D2O:H2O or DMSOd6:D2O:H2O (6:3:1). The horizontal dashed line refers to the enhancement of 10 mM AMUPOL in glycerold8:D2O:H2O (6:3:1) which is considered as a benchmark for radicals' performance in aqueous environments. The schema a), b), c), d) illustrate the molecular structures of the radicals investigated. The open conformation, with the TEMPO sidearms pointing away from the NO bond (see for example the schema c for the monoradical MbPyTol), offers always the best enhancement. The green and red bars refer to open and closed conformers respectively.*

Image:


MAS-DNP enhancement
300
250
200
150
100
50
0
AMUPOL
107
46
201
33
250
23
323
MbPyTol
MPyPolC6OH
MAMUPOL
MPyPolPEG
Open
Closed
Biradicals
a
b
OH
schema
Name
a
b
Monoradicals
c
d
HO
schema
Name
c
d

Hyperpolarization

P179

New Insights on Precession Instabilities in Highly Polarized Liquids

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Abstract: Hyperpolarisation (HP) techniques contribute not only to increase NMR sensitivity but, also, to enhance the effects of radiation damping (RD) and long-range magnetic interactions (the distant dipolar field, DDF). This leads to ill-controlled nonlinear dynamics and unusual behaviours that may be due only to DDF (precession instability, spectral clustering, multiple spin echoes) or to both RD and DDF (bizarre signals and cross-peaks, spatial-temporal chaos, multiple maser emissions).[1]

We pursue in-depth investigations of nonlinear NMR dynamics with dedicated experimental and numerical tools: low field (3 mT) NMR and MRI in laser-polarised liquid ^{3}He-^{4}He mixtures at low temperature (1 K), using an active feedback scheme to control RD; numerical simulations for arbitrary sample shapes, by time integration of nonlinear Bloch equations (which may include DDF, RD, diffusion, and applied gradients) ruling the evolution of magnetic moments on a cubic 3D lattice (up to 10^6 moments and 10^7 sites available per unit cell).

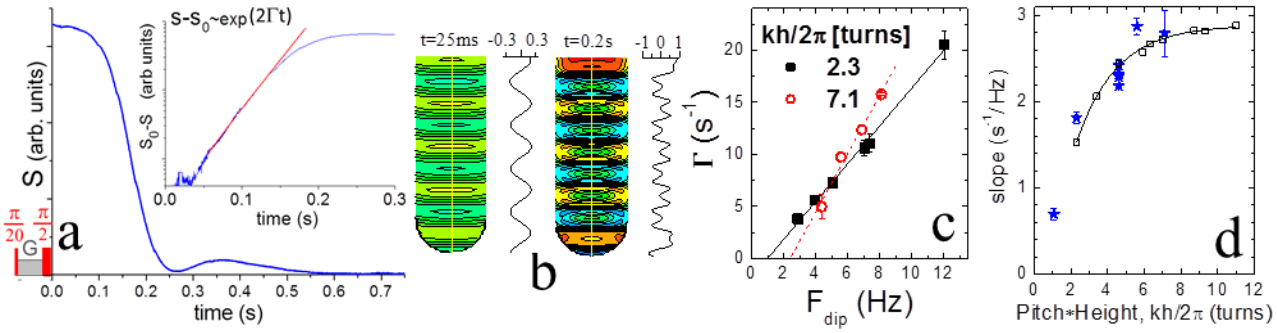
We report on a detailed study of precession instability without RD. MRI-based techniques have been used to induce and probe the evolution of periodic distributions of magnetisation.[2] Numerical simulations provide new insight on the observed parametric amplification of the imprinted pattern and the generation of spatial harmonics. In particular, we are now able to quantitatively account for the growth rates associated with the precession instabilities. These rates increase linearly with bulk magnetisation, M_0, and strongly depend on the spatial wave vector, k, of the seed pattern (Fig.1). The influence of sample shape (data not shown) is significant, as noticed in prior work on multiple spin echoes.[3]

Good understanding of precession instability provides the corner stone for further investigation of nonlinear dynamics under the joint influence of DDF and RD, relevant both for NMR with HP techniques and spectroscopy in very high field.

Fig. 1 - a: Experimental FID signal, S(t), following preparation of a small periodic pattern ($\pi/20$ flip and B_0-gradient pulse, yielding a seed modulation with wave vector $k/2\pi=5$ cm^{-1}) and $\pi/2$ flip in dilute HP liquid He mixture (4-mm inner diam. tube; fixed sample height, h=10 mm); inset: semi-log plot of signal departure from initial value, S_0, yielding the growth rate, Γ. b: Numerical data (2D maps and 1D profiles along tube axis; transverse magnetisation, quadrature component) illustrate seed growth and development of harmonics. c: Plots of growth rates versus with dipolar frequency parameter, $F_{dip}=\mu_0\gamma M_0/2\pi$. Negative offsets correspond to diffusion-induced damping rates $-Dk^2$ (the diffusion coefficient D slightly depends on ^{3}He concentration in the mixture). d: Comparison of experimental (blue stars) and numerical (open squares; solid line is a guide for the eye) slopes $d\Gamma/dF_{dip}$.

[1] H. Desvaux, Prog. Nucl. Magn. Reson. Spectrosc. 70, 50 (2013).
[2] V.V. Kuzmin et al, Euromar 2013
[3] S. Morgan et al, Eur. Phys. J. D. 67 (2013) 29.

Image:



Hyperpolarization

P180

In vivo imaging of the intra- and extracellular redox status in rat stomach with indomethacin-induced gastric ulcers using Overhauser-enhanced magnetic resonance imaging

Keiji Yasukawa[*1], Ryota Shigemi[2], Tomomi Kanbe[2], Yusaku Mutsumoto[2], Fumiko Oda[2], Hideo Utsumi[2]
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Abstract: Repeated use of non-steroidal anti-inflammatory drugs can induce changes in the redox status including production of reactive oxygen species (ROS), but the specific details of these changes remain unknown. Overhauser-enhanced magnetic resonance imaging (OMRI) has been used *in vivo* to monitor the redox status in several diseases and map tissue oxygen concentrations. We monitored the intra- and extracellular redox status in the stomach of rats with indomethacin-induced gastric ulcers using OMRI and investigated the relationship with gastric mucosal damage. To show the effectiveness of OMRI imaging in live animals, we used 0.2-T MRI after the OMRI measurement and superimposed with OMRI. The average image intensities in the fiducial markers were plotted against the probe concentration, showing a good correlation up to 2 mmol/L (R^2 = 0.987), which is sufficient sensitivity for *in vivo* stomach measurements with OMRI. One hour after oral administration of indomethacin (30 mg/kg), OMRI measurements in the stomach were performed following nitroxyl probe administration. OMRI imaging with the membrane-permeable nitroxyl probe, 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPOL), demonstrated a redox change toward oxidation, which was reversed by a membrane-permeable antioxidant. Conversely, imaging with the impermeable probe, 4-trimethylammonium-2,2,6,6-tetramethyl-piperidine-1-oxyl (CAT-1), demonstrated little redox change, which is also consistent with our previously report using *in vivo* ESR. Redox imbalance imaging of a live rat stomach with indomethacin-induced gastric ulcers was produced by dual imaging of ^{15}N-labeled TEMPOL and ^{14}N-labeled CAT-1, in addition to imaging with another membrane-permeable ^{15}N-labeled probe, 3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine-1-oxyl (MC-PROXYL), and ^{14}N-labeled CAT-1. Pretreatment with MC-PROXYL suppressed gastric mucosal damage, whereas pretreatment with CAT-1 did not suppress ulcer formation. OMRI combined with a dual probe is a less invasive imaging technique for evaluation of intracellular ROS production contributing to the formation of gastric ulcers in the stomach of indomethacin-treated rats, which cannot be done with other methods.

Hyperpolarization

P181

Quantification in para-hydrogen induced hyperpolarized NMR

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Abstract: NMR detection of dilute components in complex mixtures suffers from signal overlap and low sensitivity. Nuclear spin hyperpolarization methods (e.g. Dynamic Nuclear Polarization (DNP), Para-Hydrogen Induced Polarization (PHIP), etc.) can be used to address these issues. Signal Amplification by Reversible Exchange (SABRE) is a variant of PHIP in which the NMR signals of small molecules in solution are enhanced without any chemical modification[1]. In SABRE *p*-H_2 and analytes reversibly associate to a mediating metal complex, providing a transient scalar coupling network through which the spin order of *p*-H_2 can be transferred to the nuclear spins of the substrate, resulting in greatly enhanced NMR signals. Although the linear dependence of SABRE/PHIP signal integrals on the concentration does not generally hold, it can be restored by addition of a large excess of co-substrate[2], as previously demonstrated using standard addition for NMR quantification in combination with PHIP/SABRE[3]. However, standard addition is a laborious and time-consuming method when handling multiple substrates and samples.

Alternatively, we are exploring the possibility to quantify NMR hyperpolarized spectra by reference to an internal standard. The hyperpolarized signal of this reference compound, added to the mixture under investigation at known concentration, could be used for the quantitative determination of other analytes in the PHIP/SABRE spectrum. Beside the considerable time reduction and simplification of the quantification step, this approach would provide the additional benefit of correcting for fluctuations in the experimental conditions (e.g. slight differences in *p*-H_2 enrichment or in the concentration of the metal complex and co-substrate, etc.). After a calibration step, it would be possible to quantify dilute compounds in complex mixtures by PHIP in one single measurement.

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Hyperpolarization

P182

TR CIDNP study of photosensitized thymine and thymidine oxidation

Natalya Fishman[*1,2], Olga Morozova[1,2], Alexandra Yurkovskaya[1,2]

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Abstract: At present, there is great interest in model studies of the processes of one-electron oxidation of DNA bases, since it results in the formation of a nucleobase radical that is subsequently consumed in chemical reactions that often lead to mutations. While direct absorption of UV light by DNA duplex results mainly in the formation of pyrimidine dimers, an interaction of UV light with DNA bases in the presence of photosensitizers leads to the formation of short-lived nucleotide radicals, including thymine radicals. We found only limited examples of the detection of radicals derived from thymine and thymidine in solution by means of EPR and transient absorption techniques. In present work, we used chemically induced dynamic nuclear polarization with time resolution (TR-CIDNP) for studying the photoinduced oxidation of thymine, T, and its nucleoside thymidine, dT, in the expectation that the information obtained can help to provide a basis for understanding photosensitized formation of the short-lived pirimidine radicals in DNA. The term CIDNP means non-equilibrium nuclear spin state populations produced in chemical reactions that involve radical pair intermediates. CIDNP effects depend on magnetic parameters of the radicals (g-factors and HFI constants) and appear as the spectral lines with anomalous intensities and phases in the NMR spectra of diamagnetic products of radical reactions. So, the structures of paramagnetic particles can be reliably established. We chose two common-used photosensitizers for the radical generation: 2,2'-dipyridyl (DP) and water-soluble derivative of benzophenone, namely 3,3',4,4'-tetracarboxy benzophenone (TCBP). A complementary analysis of the pH-dependences of geminate CIDNP and CIDNP kinetics obtained for the photoreactions of T and dT with TCBP (DP) at wide pH range enabled us to get information on transient radical intermediates of T and dT, and to establish detailed mechanisms for the reactions studied. It was found that the reaction of triplet excited DP with T in acidic and neutral solutions or with dT in neutral solution proceeds via the mechanism of hydrogen atom transfer and leads to the formation of (5m-H) methyl thymine radical. From the analysis of the geminate CIDNP spectra obtained in the reaction of T with triplet excited TCBP, the HFI constants of methyl protons of thymine radicals in acidic, neutral and basic solutions were estimated, and it was shown, that the structure of thymine radicals depends on pH. It was found that in the photoreaction of TCBP with T in acidic and neutral solutions or with dT in acidic solution at the geminate stage of reaction a thymine (thymidine) radical cation is formed, which rapidly deprotonates to form a neutral (N3-H) thymine (thymidine) radical. It has also been shown that the neutral thymine radical can be effectively reduced in the reactions with aromatic amino acids and purine nucleotides.

This work was supported by RFBR (project No. 17-03-00656).

Hyperpolarization
P183
Parahydrogen polarization transfer beyond direct J-couplings
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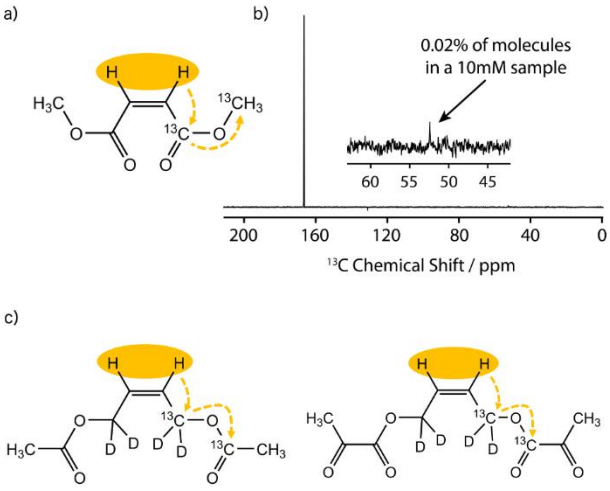
Abstract: In this work, we use radiofrequency polarization transfer techniques to transfer the spin order of a parahydrogen proton pair to a ^{13}C site without a direct J-coupling to the protons. This is achieved by using a 'bridging' nucleus in the molecule. Our overall goal is to produce hyperpolarized 1-^{13}C-pyruvate, which is in demand for in vivo medical imaging.[1]

We demonstrate the technique on methyl (methyl-^{13}C) maleate-4-^{13}C (Figure 1a), where the central proton pair comes from a molecule of parahydrogen. The S2hM sequence[2] is used to transfer proton polarization to the carbonyl ^{13}C site, followed by a homonuclear polarization transfer from the carbonyl ^{13}C to the methyl ^{13}C. In Figure 1b we show the result of applying this sequence to parahydrogenated dimethyl maleate in full natural abundance at 10 mM. The small signal doesn't resemble a classical hyperpolarized NMR peak, but that signal originates from the ~2 uM molecules with two ^{13}C spins, from one scan. This feat was possible because the methyl ^{13}C was polarized to ~2%.

We are now employing this technique on the molecules shown in Figure 1c, and working towards generating hyperpolarized acetate and pyruvate with high polarization levels.

[1] S. J. Nelson et al, Sci. Transl. Med., 2013, 5, 198ra108
[2] J. Eills et al, J. Magn. Reson., 2017, 264, 163

Figure 1: a) Transfer of parahydrogen singlet order into methyl ^{13}C magnetization. The overall efficiency of this transfer is around 50%. b) Hyperpolarized ^{13}C NMR spectrum of 10 mM dimethyl maleate in full natural abundance, at 11.7 T after parahydrogenating the precursor and performing the transfer sequence. The signal at 52.5 ppm originates from the ~2 uM molecules with two ^{13}C spins in the positions of interest, from one scan. c) The molecules we are currently investigating, with the aim to produce hyperpolarized acetate and pyruvate.
Image:



Disclosure of Interest: J. Eills: None Declared, H. Kourilova Conflict with: Director of HyperSpin Scientific Ltd, L. Brown: None Declared, F. Al-Saffar: None Declared, M. Levitt: None Declared

Hyperpolarization

P184

Carbenes - A new group of molecules for the metal free activation of Parahydrogen?

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Abstract: Parahydrogen Induced Polarisation (PHIP) is a well-established method for boosting the sensitivity of magnetic resonance for a broad range of applications, e.g. imaging applications and catalytic studies.[1]

The observation of the enhanced NMR signals requires a break in the symmetry of the H_2-molecule, usually accomplished by the catalytic addition of para H_2 to a substrate of interest (hydrogenative PHIP). For these reactions, metal-based complexes and nano-particles are used, enabling high yields in the hydrogenation-reaction.[2] However, as the interaction of para H_2 with a metal centre contributes to the relaxation of the singlet order, and to extend applicability of the technique, there has been growing interest in the investigation of metal-free substrates for the activation of para H_2.

To this end, the so-called frustrated (sterically separated) Lewis pairs (FLP) offer great potential for the design of metal-free catalysts, being able to split small molecules such as H_2 and NH_3.[3] The applicability to PHIP experiments has already been demonstrated for different FLP-type compounds, including molecular tweezers like ansa-aminoboranes[4,5] and triphosphabenzene.[6]

Cyclic (alkyl)amino carbenes (CAAC) are another promising group of molecules since they possess a lone pair of electrons together with an accessible vacant orbital (Fig. 1), and, hence, mimicking a transition metal centre.[7] The addition of H_2 to these carbene centres has already been demonstrated by Frey *et al*. in 2007.[7] However, to our knowledge, there are no studies involving the utilisation of PHIP techniques.

Here, we report preliminary investigation on the use of CAACs for PHIP experiments, including their preparation and reactivity towards H_2. These studies could provide further insight to the mechanism of H_2 splitting at a carbene centre with an electronic singlet state configuration. Furthermore, the characterisation of the spin dynamics in the hyperpolarised reaction product would greatly support the development of a new route for the metal-free activation of para H_2.

Fig. 1: Metal-free addition of H_2 to a cyclic (alkyl)amino carbene (CACC, left) and schematic representation of the electronic structure of a CAAC-type carbene (right).

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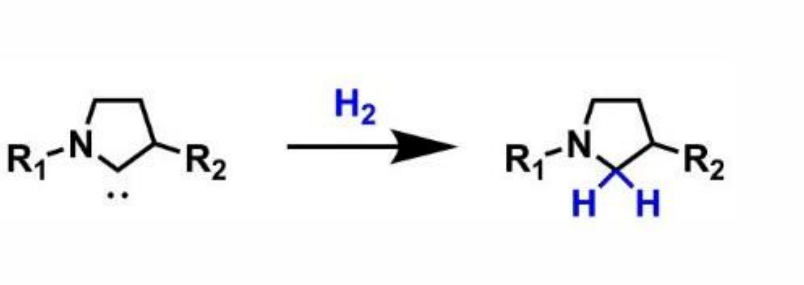

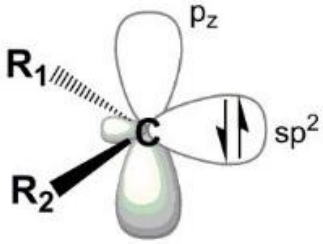

Hyperpolarization

P185

Mechanistic Hypothesis for Surface-Mediated Hyperpolarization of Liquid Water from Parahydrogen on PtSn Intermetallic Nanoparticles

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Abstract: Recently we reported the discovery of surface-mediated parahydrogen-induced alignment of the protons in liquid water, methanol and ethanol. The hyperpolarization was induced simply by the bubbling of para-enriched hydrogen through a suspension of insoluble Pt_3Sn intermetallic nanoparticles (iNPs) encapsulated within a protective mesoporous silica shell ($Pt_3Sn@mSiO_2$). In this effect, dubbed SWAMP (surface waters are magnetized by parahydrogen), conversion of singlet spin order into water proton magnetization is mediated by symmetry-breaking interactions and intermolecular spin couplings on the surface of the iNPs. The hydroxy protons exhibit stimulated emission NMR signals relative to those of Boltzmann polarized water. The nonexchangeable methyl or methylene protons also showed hyperpolarization, an observation which is key to the interpretation of the molecular mechanism and spin-dynamics of the process. $Pt@mSiO_2$ and $PtSn@mSiO_2$ catalysts were also tested but produced no detectable SWAMP signals. Apparently, the surface structure of the $Pt_3Sn@mSiO_2$ iNPs (see Figure) optimizes the balance between facile dissociative adsorption of H_2 and restriction of H ad-atom surface diffusion due to the presence of Sn, which likely interacts with the solvent oxygen atom. By restricting diffusion, the spin-spin coupling and spin correlation in the H ad-atom pair is prolonged. It addition, the NMR spectra acquired after repeated bubbling of hydrogen gas showed evidence for H/D exchange: i.e. $H_2 + D_2O \rightarrow HD + HDO$. In principle, this single H transfer could serve as the mechanism for the water hyperpolarization provided that the spin-spin coupling is retained in the H ad-atom pair formed upon dissociative adsorption into magnetically inequivalent sites. Alternatively, the polarization transfer can occur via spin-exchange mediated by the intermolecular dipolar interactions (a new kind of SPINOE effect). This poster will present the results of systematic studies as a a function of reaction conditions and materials properties to differentiate between the various mechanistic hypotheses to account for the SWAMP effect.

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Supported by National Science Foundation (NSF) grant CHE-1507230.

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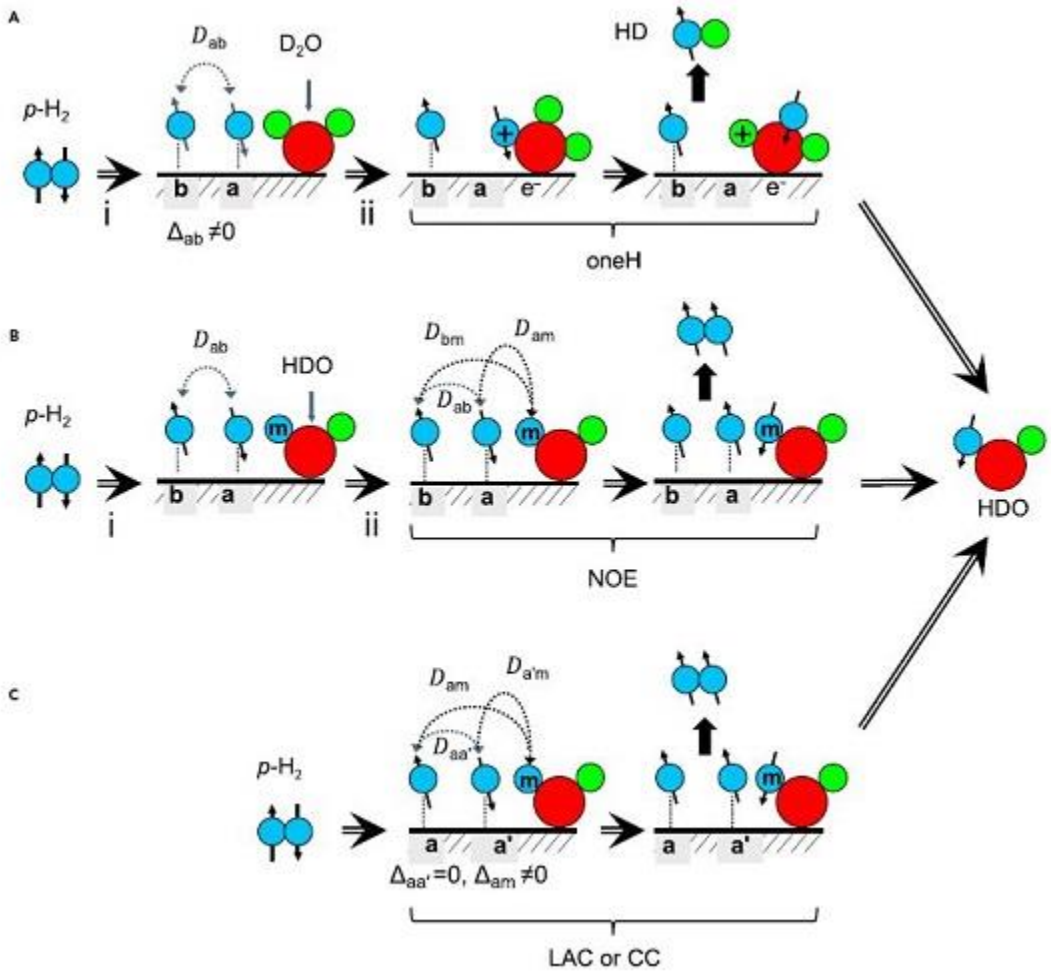
A
D_{ab}
D_2O
HD
p-H2
b
a
i
$\Delta_{ab} \neq 0$
ii
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oneH
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D_{ab}
HDO
D_{bm}
D_{am}
m
NOE
HDO
C
D_{am}
$D_{a'm}$
$D_{aa'}$
a
a'
$\Delta_{aa'}=0, \Delta_{am} \neq 0$
LAC or CC

Hyperpolarization

P186

BDPA-Nitroxide Biradicals for Efficient Cross Effect DNP at Magnetic Fields up to 21.1 T

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Abstract: Dynamic Nuclear Polarization (DNP) has been shown to be an important tool to overcome the sensitivity limitations of solid-state magic angle spinning (MAS) NMR, opening new possibilities and applications in the fields of materials and life sciences. Today, the most efficient polarizing agents are binitroxides such as AMUPol[1] or TEKPol,[2,3] featuring the Cross Effect as polarization transfer mechanism. However, the sensitivity gain from these binitroxide radicals has been shown to dramatically decrease with increasing MAS frequencies and magnetic fields.[4-6] Recently Mathies *et al.* introduced a new class of water-soluble biradicals, TEMtriPols, consisting of a hybrid between a broad EPR lineshape nitroxide moiety and a narrow EPR lineshape trityl radical.[7]

Using a similar concept, we recently introduced a new series of hybrid biradicals employing nitroxides chemically tethered to BDPA (α,γ-bisdiphenylene-β-phenylallyl). The dependence of the DNP enhancement on the length of the linker and the electron spin parameters (T_{ir}, T_M, exchange coupling) will be discussed. We will show that the best radical in this series, HyTEK2, yields ^{1}H enhancements of up to 185 at 18.8 T and 40 kHz MAS rate in a bulk frozen solution of tetra-chloroethane, which significantly outperforms current binitroxides. The DNP enhancement factor of BDPA-nitroxide radicals increases with increasing MAS rates as similarly reported for the Overhauser Effect DNP with BDPA,[9] this can be explained using a source-sink spin diffusion model. Finally, we report that HyTEK2 exhibits over 60-fold signal enhancement at a magnetic field of 21.1 T.

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Hyperpolarization

P187

Ultrasensitive beta-NMR to study interactions of metal ions with biomolecules

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Abstract: In chemistry and biochemistry classical NMR is currently the most versatile and powerful spectroscopic technique for characterization of molecular structure and dynamics in solution. However, the low sensitivity leads to relatively large amounts of sample, imposing constraints on the systems that may be explored. In addition, not all elements are easily accessible, as the most abundant isotopes display no or poor response.

Our project aims at studying the interaction of essential metal ions with different biomolecules using the ultrasensitive b-NMR technique [1], which until now has been used only for nuclear structure and material science in solid samples. Because in b-NMR the resonances are observed as changes in beta-decay anisotropy of hyperpolarized nuclei, the approach is up to 10 orders of magnitude more sensitive than conventional NMR.

Our experimental setup [2] is located at the CERN-ISOLDE facility, where over 1000 radioactive nuclei can be produced. We use optical pumping with lasers to polarize isotopes of different metallic elements [2]. The anisotropic emission of beta radiation from such hyperpolarized nuclei is them used to detect the NMR response, leading to the extreme sensitivity of beta-NMR.

Metal isotopes which have been already used for b-NMR studies in solid samples include ^{8,9,11}Li, ^{11}Be, $^{25\text{-}28}$Na, and ^{23,29,31}Mg. First studies on ^{26}Na in liquid samples were performed by our team in December 2017. Soon to be polarized are several K, Ca, Rb, Cu and Zn isotopes [1]. Our first biological studies planned for summer 2018 concern the interaction of Na and K cations with DNA G-quadruplexes, present for example in telomers [3]. At a later stage we plan to investigate the interaction of Cu and Zn with different proteins.

This contribution with cover the principles of beta-detected NMR, the experimental setup at CERN, the first liquid b-NMR signals and the first biological results.

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Hyperpolarization

P188

Intramolecular and intermolecular electron transfer in photoreaction of flavin adenine dinucleotide with tryptophan: time-resolved and field-dependent CIDNP study at high and low magnetic fields.

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Abstract: Flavin Adenine Dinucleotide (FAD) is an important cofactor in flavoproteins, which have been proposed as magnetoreceptors in many biologically relevant reactions e.g. in migratory bird navigation or in phototropism. Upon light illumination, in the cyclic reaction of intramolecular electron transfer (ET) from Adenine to Flavin a short-lived biradical (BR) with the reduced flavin and oxidized adenine moieties primarily forms. Magnetic interaction such as hyperfine and exchange interactions are important for the magnetic field sensitivity of photoreaction of FAD. At the presence of tryptophan (Trp), an electron is transferred from Trp to the adenine cation radical moiety of the transient FAD biradical leading to the formation of a secondary pair consisting of the Flavin anion and tryptophanyl cation radicals (RP). The back electron transfer rate is sensitive to the external magnetic field strength, while the magnetic field dependence of the overall reaction yield is given by the superposition of contributions from both primary biradical and secondary radical pair stages. A central aim of our study was determination of the role of exchange interaction in FAD biradicals. Two consecutive steps in light-induced processes have to be analyzed. As observables, we use chemically induced dynamic nuclear polarization (CIDNP). CIDNP is a nuclear magnetic resonance (NMR) phenomenon, which arises in spin-selective recombination of radicals and is observed as anomalous intensities and phases of the NMR spectral lines of diamagnetic products of radical reactions. During their relaxation time, the product molecules "remember" that they originated from radicals and can be detected by conventional NMR techniques with all the advantages of NMR spectroscopy, the most important of which is its high spectral resolution. The hyperpolarization formed is sensitive to magnetic resonance parameters of the radicals such as g-factors, hyperfine coupling constants, exchange interaction, and other. For disentanglement of the contributions from intramolecular and intermolecular electron transfer, we use three systems: pure FAD, FAD plus Trp, and flavin mononucleotide (FNM) plus Trp. They proceed via intramolecular ET with biradical intermediate; combined intra- and intermolecular ET forming biradical and RP, and exclusively via intermolecular ET with only RP intermediates, respectively. From the time dependence of CIDNP we can separate the geminate processes in BRs and in free RPs, while from the magnetic field dependence we get value and sign of exchange interaction (J-coupling) in the transient BRs. These observations show that the spin dynamics in the primary FAD biradical has to be taken into account when considering the magnetoreception mechanism of the photoreaction between FAD and Trp. Thus, CIDNP is a valuable alternative technique for analyzing magnetic interactions in transient radicals and biradical, in particular when EPR and optics alone are not sensitive enough.

Hyperpolarization
P189
Sub-second dissolution-DNP at minimal dilution
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Abstract: In dissolution-DNP, a sample containing hyperpolarized molecules such as pyruvate is dissolved with a jet of hot solvent propelled by helium gas. The solute is then transferred to a target magnet where strongly enhanced signals report structural and dynamic information, such as human metabolic fluxes in tumours.[1,2]

D-DNP has great potential also in NMR spectroscopy, but substantial dilution and long transfer times often lead to disappointing results.

We are developing rapid-transfer dissolution-DNP, in which the sample is loaded into a bullet that is shot to the target magnet using pressurized helium gas, within typically 100 ms. A dissolution dock in the target magnet dissolves the sample (typically 50 uL) in 600 to 700 uL of aqueous solvent and reliably loads 5 mm NMR tubes within 700 to 800 ms. Polarization levels of several percent have been observed on 1-13C pyruvate, with a substantial potential for further improvements.

We will present a detailed description of our implementation and compare the method to conventional dissolution-DNP.

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Disclosure of Interest: K. Kouril Conflict with: Director of Hyperspin Scientific Ltd, H. Kourilova Conflict with: Director of Hyperspin Scientific Ltd, M. H. Levitt: None Declared, B. Meier Conflict with: Director of Hyperspin Scientific Ltd

Hyperpolarization

P190

Over 60% 13C polarization by pulsed Para-Hydrogen Induced Polarization and Sidearm Hydrogenation

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Abstract: Hyperpolarization has been proven to be one of the solutions to intrinsically low sensitivity of magnetic resonance (MR). Its methods like Dynamic Nuclear Polarization (DNP) opened the possibility to obtain ^{13}C hyperpolarized metabolites and contrast agents and thus to investigate their transformation in vivo, as their T_1 times are usually longer than those of hydrogen.[1] Thus, diagnostic applications of MR are broadened not only in vitro but also in vivo.

One of the limitations of DNP are the costs of polarizers and need for cryogenic temperatures, which hinders rapid development of hyperpolarized methods in medicine. Another way of hyperpolarization is the Parahydrogen Induced Polarization (PHIP) that is a chemistry-based technique; it is easier to handle, cost effective, and allows much shorter polarization times. Proton polarization on the order of unity can be achieved.[2] However, a drawback of this technique is the small number of molecules for which unsaturated precursors are available. Thus, only few biomolecules were polarized by parahydrogenation. One option to overcome this is PHIP-SAH (PHIP by means of sidearm hydrogenation).[3] Here, a metabolite is functionalized by moieties that can be hydrogenated and proton polarization stemming from parahydrogen is transferred to the biomolecule's ^{13}C nucleus. Subsequent detaching of the hydrogenated moiety, for example by hydrolysis, allows to get pure hyperpolarized metabolites. The efficiency of the polarization transfer is crucial for the whole technique. It is governed by scalar couplings and can be achieved either by field cycling or by applying pulse sequences inside an MR machine.

Here we aim for polarization transfer using the newly invented NMR pulse sequence ESOTHERIC (Efficient Spin Order Transfer to HEteronuclei via Relayed INEPT Chains).[4] By hydrogenating 1-^{13}C-vinylacetate-d6 followed by subsequent polarization transfer from two-spin longitudinal order (from parahydrogen originating protons) to the carbonyl ^{13}C moiety of acetate (in the reaction product ethyl acetate), results in 60% polarization. Transfer can also be done in two steps, first from hyperpolarized protons to the carbon of a side arm and then to carbon of interest in a metabolite. In the later case, hyperpolarization is accomplished by developing an isotopic labeling strategy for generating precursors containing a favorable nuclear spin system to add para-hydrogen and convert its two-spin longitudinal order into enhanced metabolite signals.[4] Our technique provides a fast way of generating hyperpolarized metabolites using para-hydrogen directly in a high magnetic field where all spins are weakly coupled, achieving ^{13}C signal enhancements up to 100,000-fold (B_0 = 7T) and without the need of field cycling.

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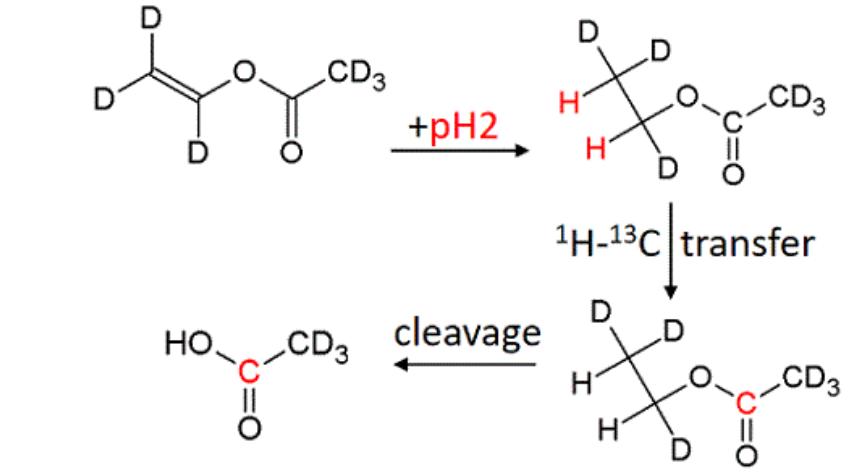
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^{1}H-^{13}C transfer
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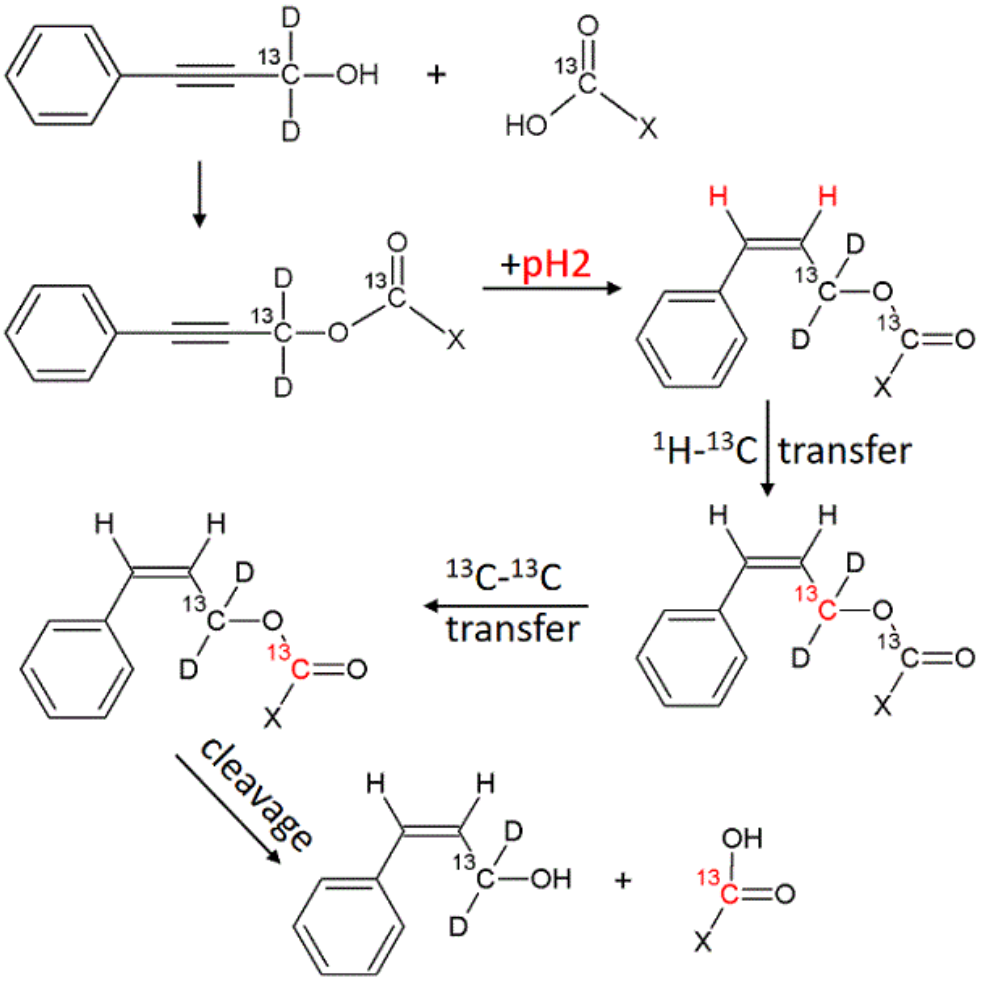
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^{13}C-^{13}C transfer
cleavage

Hyperpolarization

P191

Electron Spectral Diffusion under Static DNP condition

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Abstract: Under static DNP conditions, the steady-state electron polarisation profiles along the EPR spectra determine the steady-state nuclear polarisation. In practice, these polarisation profiles have been mapped by Electron-Electron Double Resonance (ELDOR) experiments and show substantial deviation from the shapes predicted by the Thermal Mixing (TM) model. The polarisation profiles were analyzed using a phenomenological model describing the electron spectral diffusion (the eSD model)[1] that is based on a set of coupled rate equations for the polarizations composing the EPR line shapes.

In this study, in order to substantiate the eSD model and relate it to basic principles, we performed exact spin dynamics computations on a small coupled-spin systems (11 electron spins) taking into account the total spin Hamiltonian including dipolar flip-flop term, the MW irradiation field and relaxation processes[2]. The master equation in Liouville space for the diagonal matrix elements (the populations) in the eigenstate representation is solved and steady-state energy-population plots are presented. These plots together with their EPR spectra for different conditions demonstrate for which interaction and relaxation parameter these results correspond to the Thermal Mixing (TM) model and provides a possible reason for the disagreement with the TM model in our experimental results with TEMPOL with concentration 20-40 mM and temperature range 3-20 K. Furthermore it is shown that a zero-quantum electron cross-relaxation mechanism must be added to the calculations to obtain EPR profiles that resemble our experimental results that can be simulated using the eSD model. As will be shown, the addition of the cross relaxation mechanism also enables us to reproduce with the help of the eSD model the concentration dependence of spectral diffusion observed experimentally. These calculations can also be extended to include the effects of the Solid Effect on the ELDOR spectra and can reproduce experimentally obtained DNP spectra that are derived using the indirect Cross Effect[3].

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Hyperpolarization
P192
Master equation for spin-systems far from equilibrium
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Abstract: The recent developments in Magnetic Resonance clearly indicate an increasing interest in hyperpolarization techniques. Dynamic Nuclear Polarization (DNP), quantum rotor induced polarization (QRIP) and para-hydrogen induced polarization (PHIP) allow for the study of previously inaccessible systems. Polarization levels greatly exceed thermal Zeeman polarization and the spin-systems are far from equilibrium. As a consequence relaxation processes will equilibrate the spin-system with its environment.

Within the NMR community relaxation phenomena are often described by semi-classical relaxation theories [1]. The drawback of the semi-classical approach is that it cannot account for finite temperatures of the environment. The spin-system would therefore relax towards a non-physical equilibrium state. To correct for this misbehaviour a class of thermalization techniques have been developed [2, 3].

Recently we were able to report para-to-ortho conversion of water at room temperatures by means of rapid dissolution-DNP for the first time [4]. Description of the relaxation dynamics by means of conventional thermalization procedures led to wrong results. In general conventional thermalization procedures are not well-suited for spin-systems far from equilibrium.

We now propose a new thermalization technique which faithfully describes relaxation dynamics of spin-states far from equilibrium and generates the correct thermal state. The intuition behind our approach is based on the stochastic wave function approach [5]. Theoretical considerations are complemented by SpinDynamica simulations of simple model systems.

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Hyperpolarization

P193

ESOTHERIC: A pulse sequence for transferring para-hydrogen spin order to in-phase heteronuclear magnetisation in PHIP

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Abstract: Hyperpolarization methods aim to overcome the inherent low-sensitivity of NMR and MRI by creating transient states which display signals up to 10^5 larger than those available in normal conditions at typical magnetic fields. Enhanced NMR signals are essential for applications of MRI that endeavour to monitor metabolic processes in vivo.[1]

In para-hydrogen induced polarization (PHIP) methods, the addition of para-H_2 to unsaturated bonds, mediated by a catalyst, results in a dramatic enhancement of the hydrogenating ^{1}H NMR signal.[2,3] The application of direct PHIP methods in clinical settings has been limited by the lack of precursors with unsaturated bonds for biologically relevant molecules as well as by the short lifetime of the enhanced ^{1}H polarization, which is typically few seconds. The first drawback has been partially relieved by the introduction of the PHIP-SAH technique, in which a sidearm is "para-hydrogenated" and polarization is transferred to a sidearm-bonded moiety followed by a cleavage step to release the hyperpolarized molecule of interest.[4]The useful lifespan of the enhanced signal has been extended by storing the polarization as longitudinal magnetization in heteronuclei such as ^{13}C or ^{15}N, whose T_1 is several minutes. For the successful application of PHIP to in vivo MRI, it is paramount to achieve more than 10% polarization as well as to make use of effective pulse sequences for polarization transfers.[5]

Here we present a sequence for the Efficient Spin Order Transfer to HEteronuclei via Relayed Inept Chains (ESOTHERIC). The sequence is designed to transfer para-hydrogen spin order to in-phase heteroncuclear magnetization in AMX spin systems. It is shown that the full longitudinal para-H_2 spin order is transferred when the *J*-couplings between the heteronucleus and the added proton pair are asymmetric. The transfer efficiency is higher than PH-INEPT sequences with a minimum polarization transfer at 50% for symmetrically coupled heteronuclei. The sequence allows a facile implementation, is offset independent and is robust with respect to B_1 inhomogeneity and time missets.[6,7]

In a set of PHIP experiments, high heteronuclear polarization levels have been achieved directly in the field of the magnet using the ESOTHERIC sequence, without the need of field cycling.[6,7] With an excellent reproducibility, 95% of spin order transfer efficiency has been obtained, leading to ~10% polarization in ^{15}N-labelled and ~60% polarization in ^{13}C-labelled metabolite precursors.[8,9]

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Hyperpolarization

P194

Hyperpolarized 3D NMR for biomolecular applications

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Abstract: NMR spectroscopy of biomolecules demands a large toolbox of experiments ranging from conventional 2D spectra, to relaxation measurements, to higher-dimensional correlation datasets, each of which can be indispensable for full characterization of protein structure and dynamics.

Recently, hyperpolarization techniques which could potentially boost the signal intensity and thereby accelerate acquisition of such NMR spectra for both folded and intrinsically disordered proteins (IDPs) have attracted increased attention. Here we show how dissolution DNP methodology can be extended to three-dimensional NMR, illustrated by the examples of folded ubiquitin and the IDP Osteopontin (OPN). We present HNCO and HNCA spectra acquired in just over one minute (76 s and 64 s, respectively, at 9% NUS) by immersing the proteins in hyperpolarized buffers, such that proton exchange leads to the introduction of hyperpolarized $^{1}H^{N}$ atoms into our targets. Residue-resolved enhancement factors vary between 10 and 100. The figure shows the hyperpolarized HNCO spectra and its conventional NMR counterpart.

As previously observed in 2D hyperpolarized NMR, these experiments display only a subset of the signals seen in regular NMR, reflecting the respective site-specific exchange and water-NOE rates. Our method is robust and provides access to signals that would remain undetectable by other means.

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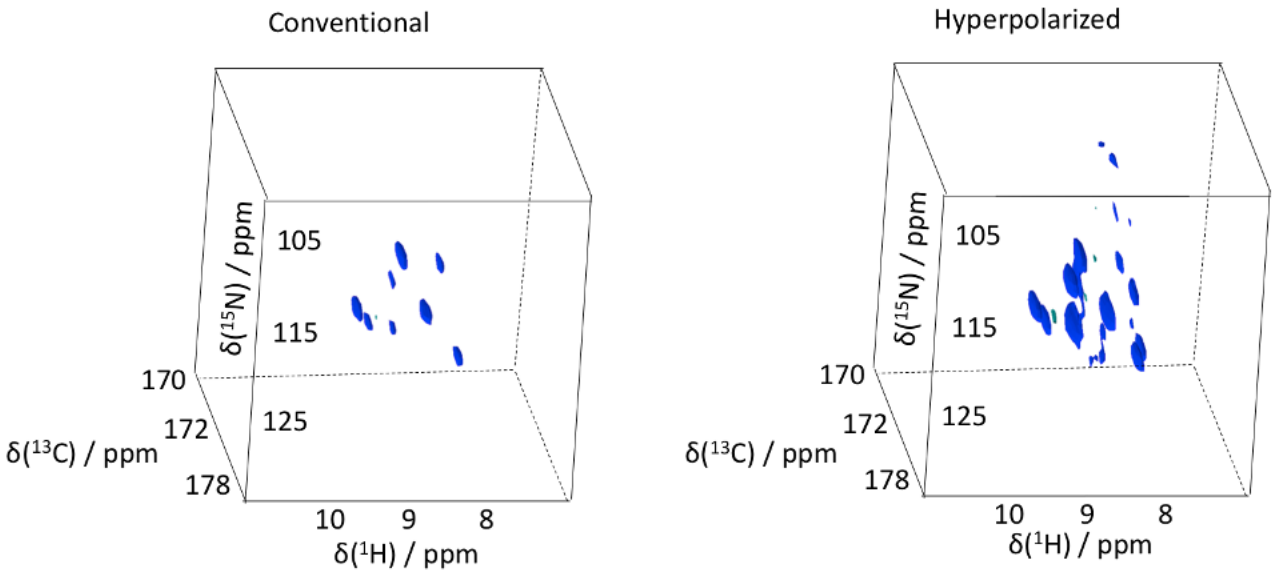
Conventional
Hyperpolarized
δ(15N) / ppm
105
115
125
170
172
178
δ(13C) / ppm
10
9
8
δ(1H) / ppm

Hyperpolarization

P195

Microwave-Gated Dissolution Dynamic Nuclear Polarization

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Abstract: Dynamic Nuclear Polarization (DNP) aims at transferring the large electron spin polarization to surrounding nuclear spins via microwave irradiation. Dissolution-DNP (d-DNP) experiments are usually performed in frozen samples doped with paramagnetic polarizing agents (PAs) where ^{13}C polarization enhancements factors as high as 10'000 are possible with respect to thermal polarization in the liquid state [1]. We have recently implemented $^{1}H \rightarrow ^{13}C$ cross-polarization (CP) during d-DNP experiment to further boost ^{13}C enhancements to factors of about 50'000 [2].

However, $^{1}H \rightarrow ^{13}C$ CP has so far been suboptimal because of the rapid proton relaxation in the rotating frame arising from the presence of PAs. We show in this work that $T_{1\rho}(^{1}H)$ can be significantly extended, and therefore CP greatly improved, by switching off the microwave irradiation briefly prior to CP. During this interruption, the electron spins relax from their partially saturated state to their highly polarized state (P_e = 99.9% at B_0 = 6.7 T and T = 1.2 K), so that paramagnetic relaxation becomes ineffective. As a result, $T_{1\rho}(^{1}H)$ is extended by several orders of magnitude and CP contact times can be extended to achieve optimum transfer.

The use of microwave gating in this context has two favourable effects; (*i*) preventing losses of proton magnetization during spin-locking and (*ii*) improving the CP transfer efficiency. Altogether, the efficiency of multiple contacts CP is greatly improved by microwave gating; polarizations as high as $P(^{13}C)$ = 65% can be achieved in acetate with an overall polarization build-up time constant as short as t_{bup} = 3 min. A record polarization $P(^{13}C)$ = 78% was even achieved in ^{13}C labelled urea [3].

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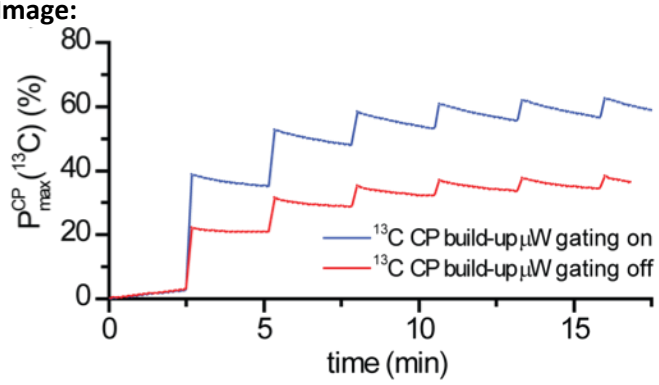

Hyperpolarization

P196

In vitro and In cell kinetic studies by Dissolution Dynamic Nuclear Polarization

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Abstract: Dissolution Dynamic Nuclear Polarisation (D-DNP) represents one of the remarkable developments in nuclear magnetic resonance (NMR) of the last decade.1 NMR signal enhancements of several orders (4-5) of magnitude have opened new avenues for a wide range of applications, ranging from in vivo imaging to in-cell observation of metabolism, or real-time in vitro studies of metabolic processes.

We have recently initiated an investigation of the kinetics of the oxidative branch of the Pentose Phosphate Pathway (PPP), one of the crucial metabolic pathways in cells. This stage, through the production of NADPH, is one of the main sources of reductive power in the cell. The PPP comprises three enzymes: Glucose-6 Phosphate dehydrogenase (G6PDH), 6-Phosphogluconolactonase (6PGL) and 6-Phosphogluconic acid dehydrogenase (6PGDH).

Experiments involving either Glucose of Glucose-6-phosphate as the primary substrate have been performed, allowing to monitor complex reaction schemes.2, 3

The reaction network involved probed by these experiments can be rather complex, and once the sensitivity issue has been overcome through increased polarization, the quantitative analysis of the reaction kinetics is one of the major issues. In particular, the spontaneous interconversion of δ- to γ-phospho-gluconolactone and their spontaneous hydrolyses into 6PGA were observed (Fig. 1) and analyzed using a first-order kinetic model, and taking into account the fast equilibrium between both G6P anomers.3

Several PPP enzyme kinetics experiments will be presented that illustrate the complexity of the task as well as the solutions that we currently propose.

Finally, following several authors (see for instance Ref. 2 and the literature cited therein), the possibility of performing such quantitative measurements in cells has been tested, and preliminary experiments of PPP kinetics on various cell models, will be presented.

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Hyperpolarization

P197

Development of narrow line UV-induced non-persistent radical for highly polarized transportable glucose solid samples

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Abstract: Nuclear Magnetic Resonance (NMR) and magnetic resonance imaging (MRI) play unique and critical roles in chemistry, biology, and clinical research where they impact directly on diagnosis. Both of these approaches would benefit from improved sensitivity and by using hyperpolarization via microwave driven dynamic nuclear polarization (DNP), the detected response can be improved by several order of magnitudes in liquid states.

Molecules containing unpaired electrons (i.e. free radicals) are vital component for any sample to be hyperpolarized by Dynamic Nuclear Polarization (DNP). Conventionally, doping the sample with persistent stable radicals such as trityl, TEMPO or BDPA have been used extensively. However, these radicals accelerate polarization decay after DNP (particularly in the solid state) and need to be filtered from the hyperpolarized solution before it can used in clinical applications due to toxicity of persistent radicals.

Photo-induced radicals, generated by UV-light irradiation of frozen solutions containing a fraction of pyruvic acid, are suitable to perform DNP on several substrates. The interesting property of such polarizing agents is their non-persistency as they recombine as diamagnetic compounds and can be quenched inside the polarizer (via thermalisation at around 200K) when the DNP sample is still solid, providing the way for hyperpolarization storage and transport of hyperpolarized samples.

Herein, we present the new and improved polarizing agents to overcome the actual limitation of low achievable ^{13}C polarization, mainly associated with the broad ESR linewidth of radical generated from photolysis of pyruvic acid. The focus of the present study is on developing narrow ESR line radicals that results in unprecedented higher ^{13}C polarization in liquid state.

To test the DNP properties of new UV-induced non-persistent radical precursors, i.e. trimethyl pyruvic acid (TMP) and its deuterated analogue (TMP_d9); samples containing 2M of fully labelled glucose in a mixture of glycerol: water 1:1 (v/v) and 10 % (v/v) of the precursors (0.75 M of radical precursor), were prepared. For each sample, 65 frozen pellets were made pouring 4.0±0.5 µL droplets of solution into a transparent quartz dewar (Wilmad-LabGlass WG-850-B-Q) filled with liquid nitrogen. The samples were irradiated for 300 s with a high power (20 W/cm2) broad-band UV source (Dymax BlueWave 75). X-band ESR measurements (Magnettech MiniScope 5000) showed the radical concentration of 20±2 mM for TMP and 45±5 mM for TMP_d9 (see Fig 1A). Each sample was then transferred to a homebuilt 6.7 T polarizer. DNP was performed at 1.1±0.1 K shining microwaves with a nominal power of 55 mW and on frequency range going from 187.8 GHz to 188.3 GHz (see Fig 1B, 1C). For both samples, the microwave output modulation (1 kHz frequency and 20 MHz amplitude) allowed to double the maximum achievable solid-state DNP enhancement. In optimized conditions, ^{13}C polarization of 45±5 % was measured for TMP_d9 in 25minutes and for the sample containing as TMP, ^{13}C polarization of 37±3 % was measured in 60 minutes. The higher polarization achieved using the new molecules are in good agreement with its sharper ESR spectrum and higher radical concentrations. By moving away the methyl groups and further deuteration reduces the hyperfine coupling between the electron and the molecule's nuclear environment, providing a DNP radical with features better than currently used trityl radicals.

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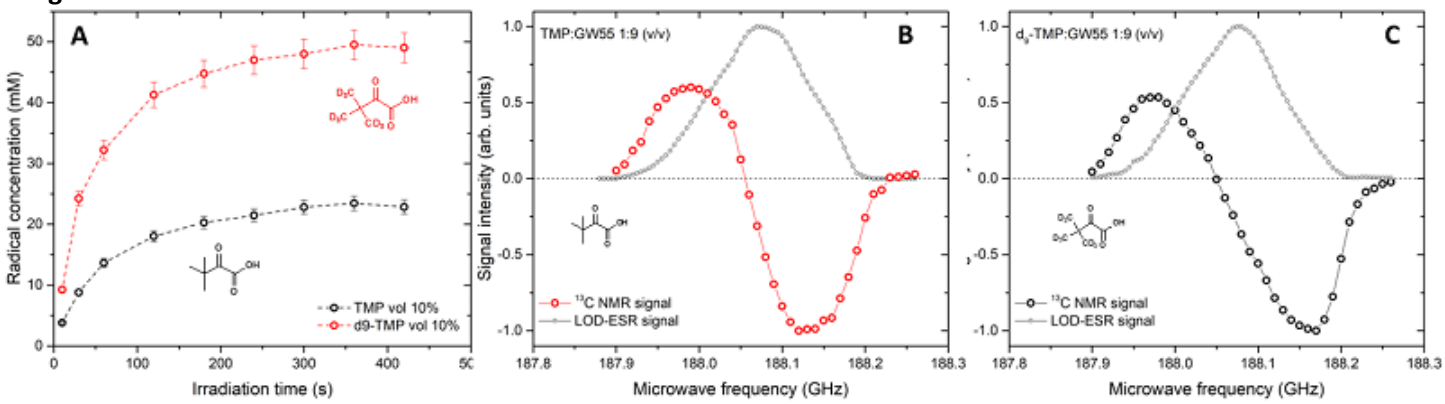

Figure 1 Radical generation as a function of the irradiation time for the sample containg TMP as precursor (black) and TMP_d_9 as precursor (red) (A); LOD ESR and ^{13}C DNP microwave sweep at 1 K and 6.7 T for the sample containg TMP as precursor (B) and TMP_d_9 as precursor (C).

Liquid-state NMR methods

P198

Enhancement of long-lived singlet-order at natural abundance in a large spin system via the bang-bang optimal control

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Abstract: Long-lived singlet-order has gained significant theoretical and experimental interest due to its wide range of applications in NMR [1]. However, it is still a big challenge to observe ^{13}C -^{13}C singlet-order at natural abundance where ^{13}C - ^{13}C pair appears with a probability of 0.011% [2]. One of the approach to enhance the singlet-order can be to use a large spin system in which singlet pair is coupled to a set of ancillary spins so that polarization transfer is feasible. In this work, we use an eleven-spin system for this purpose which includes a pair of naturally abundant, weakly-coupled (chemical shift = 2.32 ppm, J-coupling = 12.7 Hz) ^{13}C spins surrounded by nine chemically equivalent ^{1}H spins (^{13}C-^{1}H J-coupling = 2.7 Hz) of 1,4-Bis (trimethylsilyl) butadiyne. We utilize optimal control technique to achieve maximum polarization transfer from the surrounding nine ^{1}H spins directly to long-lived ^{13}C -^{13}C singlet-order. The numerical complexity of conventional optimal control techniques, which use smooth modulation of amplitude and phases of radio frequency (RF) field, scales rapidly with the size of the spin system because of the high dimensionality of the Hilbert space. Therefore, we use bang-bang optimal control technique [3] which uses a sequence of full power RF pulses with variable phases separated by variable delays. Its numerical complexity scales comparatively much slower with the size of the spin system and hence is an ideal candidate for optimal control of large spin systems. Experimentally, compared to the standard method [4] not involving polarization transfer, we find an enhancement of singlet-order by about 3.4 times. Also, since the singlet-order magnetization is contributed by the faster relaxing ^{1}H spins, the recycle delay is halved. Thus effectively we observe a reduction in the overall experimental time by a factor of 23. We also compare the enhancement of singlet-order achieved (a) by our direct polarization transfer scheme using bang-bang optimal control and (b) by INEPT polarization transfer followed by standard singlet sequence [4]. Because of its comparatively high robustness against magnetic field inhomogeneity direct polarization transfer scheme using bang-bang optimal control performs better than the later. Exploiting the enhanced sensitivity, we investigated the decay of the singlet-order under spin-lock and found it to be three to four times longer lived compared to individual spin-lattice relaxation time constants (T_1) of each carbon. Since T_1 of faster relaxing protons is smaller than the singlet-order lifetime, we also discuss the possibility of iterative polarization transfer where singlet-order stores the polarization while protons re-thermalize with the bath for further transfer of polarization.

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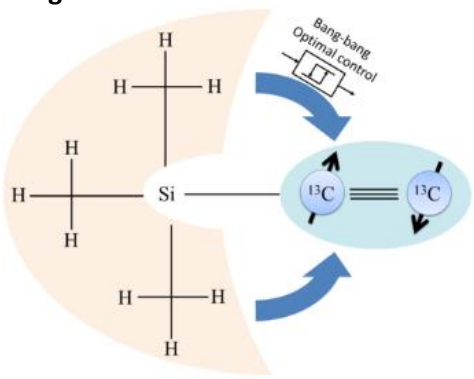

Liquid-state NMR methods

P199

Evolution of LC-NMR: hyphenation at 1.5 Tesla.

Paolo Sabatino[*1], Andreas Schweizer-Theobaldt[1], Klas Meyer[2], Juergen Kolz[2]
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Abstract: NMR spectroscopy provides unique structural information on organic molecules as well as quantitation without standards, but is often limited by its ability to differentiate components in mixtures. Therefore, a hyphenated technique such as LC-NMR is very desirable, as it would allow the simplification of ^{1}H-NMR spectrum by separating different components in the chromatographic column and the direct monitoring of compositional changes during the separation process (on-flow) as the eluents are sampled in "real-time" as flowing through NMR detection coil. However, despite the progress in this field, LC-NMR is not yet routinely used as analytical technique mainly due to the practicality issues in terms of operation.

Here we present the use of a compact benchtop Magritek Spinsolve Ultra 60 MHz NMR system for LC-NMR hyphenation. The results clearly show that the performances of the benchtop NMR have reached a level where coupling with Gel Permeation Chromatography (GPC) can be taken in serious consideration for routine applications.

We show that 60MHz benchtop NMR coupled to a GPC instrument allows monitoring shifts of bulk polymer composition directly by on-flow analysis allowing reliable quantitative analysis, which can be considerably improved when using stop-flow mode. In addition, we observed that extracolumn band broadening of benchtop NMR flow cell is low enough for peak resolution of analytical GPC. We demonstrated the applicability of the GPC-NMR for current analytical needs by characterizing several sample such as acrylate blends, gradient co-monomer acrylate, as well as surfactants blends.

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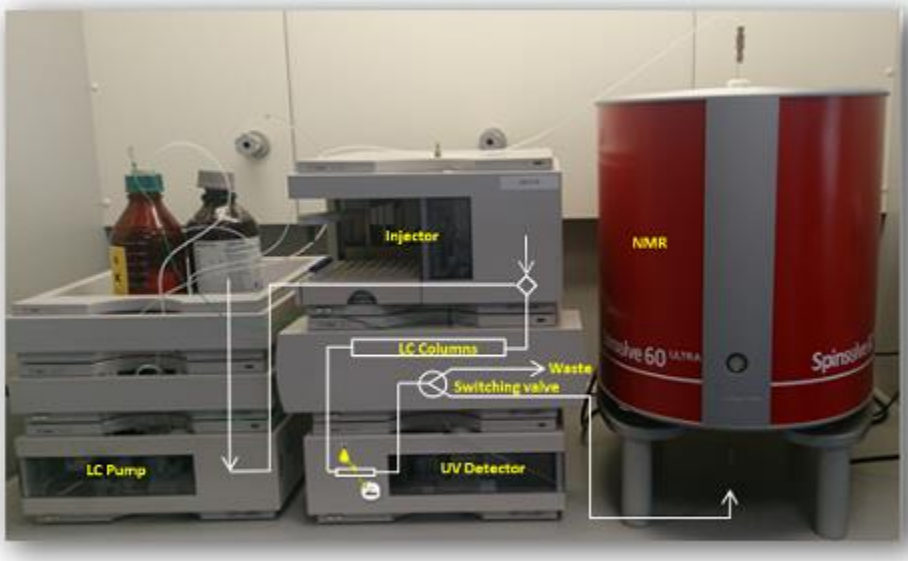

Liquid-state NMR methods

P200

Monitoring 15N chemical shifts during protein folding by pressure jump NMR

Cyril Charlier[*1], Joseph Courtney[1], Reid Alderson[1], Jinfa Ying[1], Philip Anfinrud[1], Ad Bax[1]
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Abstract: Understanding how proteins fold into stable structures without external assistance remains one of the main questions in biophysics. The ability of NMR to reveal atomic details for both folded and unfolded proteins makes it a powerful spectroscopic method to study protein folding. However, as folding of small proteins is typically a fast process (ms or sub-ms), the observation of structural changes occurring during protein folding requires the ability to rapidly change from denaturing to native conditions. For a perfect, two-state, downhill folding protein, the change in resonance frequency will occur nearly instantaneously, when the protein clears the transition state barrier, and the ensemble average observed by NMR spectroscopy will reflect mono-exponential kinetics. However, protein folding pathways can be more complex and contain meta-stable intermediates. Our group utilizes novel hardware that enables the rapid switching of hydrostatic pressure between 1 bar (native) and 2.5 kbar (denaturing) conditions as quickly as a few ms. Using this instrumentation, it is possible to follow the ^{15}N chemical shifts during the folding process through stroboscopic observation, using a pseudo-3D NMR experiment[1-2]. After preparing the spin state at high-pressure (2.5 kbar), the pressure is dropped to 1 bar and the chemical shift is allowed to evolve during a pi/2-k-pi/2 "snapshot" element with k as short as 500 ms. The snapshot element is placed at a variable position after folding is initiated, and then followed by detection of the encoded signal with an HSQC experiment. The stroboscopic measured chemical shift corresponds to its unfolded values at 1 bar when the snapshot is placed right after the pressure drop and converges towards to its folded value at the end of the folding period. We demonstrate our method for a pressure-sensitized mutant of ubiquitin[3]. For residues that can be described by a two-state folding model, the chemical shift follows a mono-exponential trajectory (Figure - G47). As observed in our previous hydrogen exchange experiments, some other residues (Figure – Q62) deviate from this two-state behavior[3] and provide evidence for a meta-stable intermediate that involves strand b5 and its preceding turn. This new technology paves the way for measuring atom-specific structural information along the folding pathway.

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Figure: Chemical shifts as a function of folding time for a typical two-state folder (G47) and a residue with more than two states (Q62).

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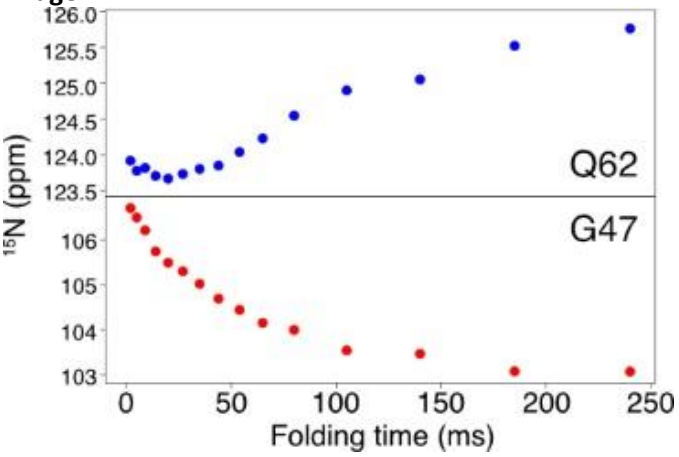

Liquid-state NMR methods

P201

Implementation of Quantitative Adiabatic-Refocused INEPT (QA-RINEPT) method to accelerate the characterization of polymer samples

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Abstract: ^{13}C-NMR is a powerful tool for the detailed characterization and structure elucidation of polymeric samples. The low natural abundance and sensitivity of the ^{13}C isotope, however, lead to very long acquisition time, therefore limiting the use of such technique.

We report here the implementation of a novel and quantitative method, QA-RINEPT, for the characterization of polyol samples. The method, based on the well-known INEPT sequence, allows the boost in sensitivity of the carbon resonances without sacrificing the quantitative aspects of the analysis. This is achieved controlling the polarization transfer mechanism and introduce a response factor to calculate precisely the sensitivity gain of the different carbon signals.

Compared to the standard single pulse quantitative experiment, the QA-RINEPT method produces up to 5.8x signals enhancement per unit of time. An in-depth statistical analysis was conducted to confirm the reliability of the QA-RINEPT method. We show that there is excellent agreement between the new and old ^{13}C-NMR methods for the quantitative determination of several important polyolefins[1] and polyols[2] properties such as the co-monomer and initiator contents as well as the ratio of primary and secondary hydroxyl groups.

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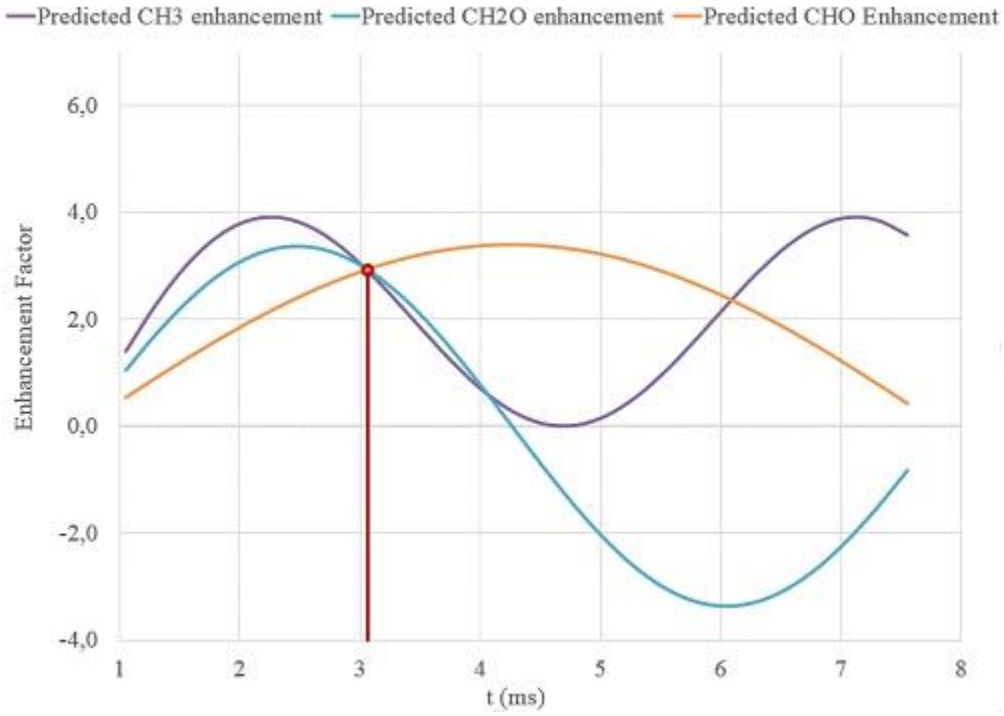

Liquid-state NMR methods

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High-Sensitivity Rheo-NMR Spectroscopy for Protein Studies

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Abstract: Shear stress can induce structural deformation of proteins, which might result in aggregate formation. Rheo-NMR spectroscopy has the potential to monitor structural changes in proteins under shear stress at the atomic level; however, existing Rheo-NMR methodologies have insufficient sensitivity to probe protein structure and dynamics. Here we present a simple and versatile approach to Rheo-NMR, which maximizes sensitivity by using a spectrometer equipped with a cryogenic probe [1] (Fig). As a result, the sensitivity of the instrument ranks highest among the Rheo-NMR spectrometers reported so far.

We applied the newly established Rheo-NMR instrument to study the fibril formation of M1-linked hexa-ubiquitin. Previously, we found that M1-linked hexa-ubiquitin forms amyloid fibrils upon the application of shear stress [2]; however, the structural changes that take place during fibril formation remain unclear. Indeed, application of shear stress inside the Rheo-NMR Couette cell resulted in the formation of amyloid-like fibrils of hexa-ubiquitin. We observed changes in the chemical shift of cross-peaks in real-time ^{1}H-^{13}C HSQC spectra. The affected cross-peaks correspond to surface-exposed residues of ubiquitin subunits in hexa-ubiquitin. We also acquired real-time profiles of fibril formation of superoxide dismutase 1 (SOD1) using Rheo-NMR in the presence of thioflavin T (ThT), which is a benzothiazole dye that exhibits fluorescence enhancement upon selective binding to amyloid fibrils [3]. As a result, we revealed the time evolution of the interaction between ThT and SOD1 fibrils during amyloid formation. From the chemical shift changes of ThT, we detected weak and strong interactions between thioflavin T and SOD1 fibrils in a time-dependent manner.

The formation of amyloid fibrils from intrinsically disordered or chemically unfolded proteins has been well studied, but the conformational conversion of folded proteins, such as hex-ubiquitin and SOD1, into fibrils remained elusive. Therefore, our results show that Rheo-NMR is a powerful tool to investigate changes in the natively folded structure in real time at the atomic level.

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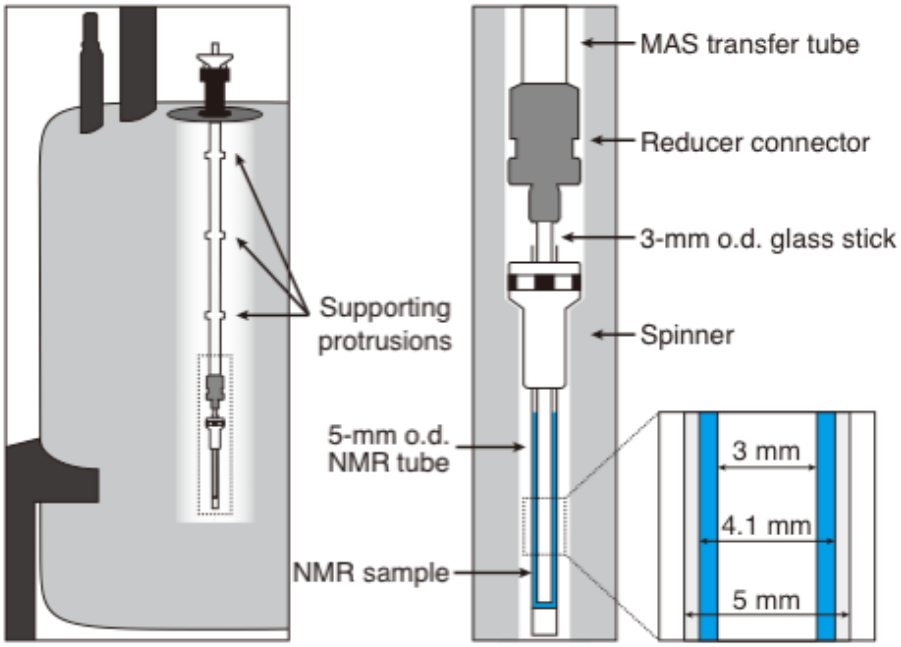
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Reducer connector
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Spinner
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Liquid-state NMR methods

P203

Elucidation of amyloid fibril formation mechanism by using novel Rheo-NMR spectroscopy

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Abstract: Abnormal protein aggregation is a common hallmark of Alzheimer's disease and other neurodegenerative disorders. Despite the growing interest in their pathogenesis, no existing method can capture aggregate nucleation and subsequent growth at atomic resolution in real time. In this study, we have recently established high-sensitivity Rheo-NMR spectroscopy that enables us to detect atomic-level structural changes of a protein during amyloid formation in real time [1]. By using the newly developed Rheo-NMR, we detected site-specific structural information on amyloidogenic proteins during their amyloid formation, gaining insight into the mechanism underlying amyloid nucleation at atomic resolution. Notably, in the formation of polyubiquitin fibrils from the native state structure, we detected the chemical shift changes of the side chains of residues located in flexible regions such as the edges of the alpha-helix and loops. These observations are consistent with our hydrogen-deuterium exchange results of polyubiquitin fibrils that the intrinsically flexible regions became highly solvent-protected in the fibril structure [2]. Thus, inter-molecular associations and secondary structure changes in the identified flexible regions can take place in the course of fibril formation.

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Liquid-state NMR methods

P204

Supersequences with Nested Acquisition using Compressive Sensing

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Abstract: NMR has been established as a leading technique for structural elucidation of unknown analytes. The information required to deduce the structure of a molecule can be obtained through a combination of different experiments such as COSY, HSQC and HMBC. This can be time consuming and there have been substantial efforts to speed up the acquisition of such experiments.

One of the approaches commonly used is the Non-Uniform Sampling of increments, which allows the acquisition of an experiment with a similar resolution in a lower amount of time. Compressive sensing processing [1] is now standard in most NMR processing software packages and this acquisition scheme is now widely used.

A different approach combines several of these pulse sequences into a nested supersequence in what has been termed as NOAH (NMR by Ordered Acquisition using ^{1}H-detection) [2]. Multiple combinations of standard pulse sequences are possible, such as an NOAH experiment combining 5 experiments: ^{1}H-^{15}N HMQC, multiplicity edited ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{1}H COSY and ^{1}H-^{1}H NOESY or a NOAH combining ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H TOCSY. Experiments such as the HSQC ^{1}H-^{13}C detect the magnetization from the protons attached to 13C and do not require the detection of protons attach to 12C, this enables to use magnetization from different proton reservoirs for the execution of several experiments without having to wait for the relaxation of the spins detected in the immediately precedent experiment. This exploitation of different magnetization reservoirs is the basis of NOAH experiments, and it means that there are restrictions into how these experiments can be combined and which version of the experiments can be used.

Here we explore the combination of NOAH with NUS, also known as SNACS (Supersequences with Nested Acquisition using Compressive Sensing). The combination of these two techniques to reduce experimental time allows the acquisition of structural information in a very short amount of time. This has been demonstrated with an experiment of less than 2 minutes, which acquires multiplicity edited ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H COSY at once under Non-Uniform Sampling. This has been implemented in JEOL ECZ spectrometers which allow the direct acquisition of 3 (or more) separate SNACS datasets without the need of scripts to untangle the acquired data. This allows using the pulse sequence in open access environments using third party software for processing.

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Liquid-state NMR methods

P205

Accurate electron–nucleus distances from paramagnetic relaxation enhancements

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Abstract: Measurements of paramagnetic relaxation enhancements (PRE) in ^{1}H-NMR spectra are an important tool to obtain long-range distance information in proteins, but quantitative interpretation is easily compromised by non-specific intermolecular PREs. Here we show that PREs generated by lanthanides with anisotropic magnetic susceptibilities offer a route to accurate calibration-free distance measurements. As these lanthanides change ^{1}H chemical shifts due to pseudocontact shifts, the relaxation rates in the paramagnetic and diamagnetic state can be measured with a single sample that simultaneously contains the protein labeled with a paramagnetic and a diamagnetic lanthanide ion. Non-specific intermolecular PREs are thus automatically subtracted when calculating the PREs as the difference in nuclear relaxation rates between paramagnetic and diamagnetic protein. Although PREs from lanthanides with anisotropic magnetic susceptibilities are complicated by additional cross-correlation effects and residual dipolar couplings (RDC) in the paramagnetic state, these effects can be controlled by the choice of lanthanide ion and experimental conditions. Using calbindin D_{9k} with erbium, we succeeded in measuring intramolecular PREs with unprecedented accuracy, resulting in distance predictions with a root-mean-square-deviation of less than 0.9 Å in the range 11 to 24 Å.

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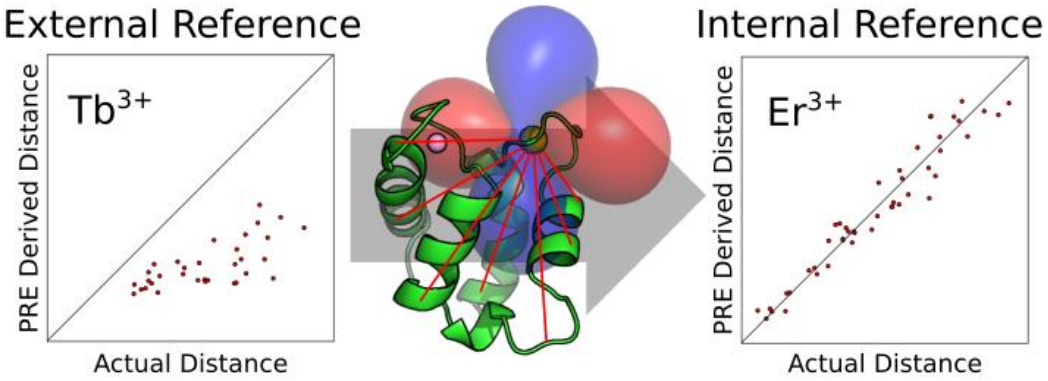

Liquid-state NMR methods

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Excitation of singlet-triplet coherences in the NMR of near-equivalent spin pairs

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Abstract: We present several approaches for efficient excitation and probing the evolution of the singlet-triplet (ST) coherences in near-equivalent spin pairs. First, we propose and compare different methods for the enhancement of the outer singlet-triplet (OST) coherences ST_+ and ST_-. They correspond to the lines of low intensity in the spectrum of an AB-type spin system. The first technique is based on selective excitation by weak RF-pulses; the second technique exploits a SLIC (Spin-Lock Induced Crossing) pulse [1]; the third technique uses a J-synchronized CPMG spin echo, which is related to the M2S (Magnetization-to-Singlet) approach of manipulating singlet spin order [2]. The latter method appears to be the most efficient and robust, enabling enhancement of the OST coherences with the efficiency close to the theoretically allowed maximum. We used here two previously studied molecules with nearly equivalent spin pairs: the 9,10-^{13}C-isotopomer of a naphthalene derivative, 1,2,3,4,5,6,8-heptakis([D3]methoxy)-7-(([D7]propan-2-yl)oxy)-naphthalene [3], and ^{15}N,^{15}N-azobenzene [4].

The coherences between the singlet and central triplet states are expected to have a lifetime up to 3 times longer than the corresponding longitudinal relaxation times T_1; they are also insensitive to field inhomogeneity. We present here two pulse sequences for efficient probing of such coherences. In the first pulse sequence, a spin-locking field is applied to the OST coherences; this suppresses the chemical shift evolution and the singlet-triplet leakage. The method is analogous to the one suggested for detection of the ST coherences in weakly coupled spin pairs [5]. The OST coherences can be further converted into the inner singlet-triplet (IST) coherence, ST_0: this idea is exploited in the second pulse sequence. The evolution of the IST coherence can be probed in absence of spin-locking. Therefore, field-cycling experiments with sample shuttling were performed to reveal the field dependence of the T_{IST} relaxation time. We observed drastic changes of the ratio T_{IST}/T_1 from about 1 (in strong fields) up to 2.4 (in weak fields), which is the evidence of a significant influence of chemical shift anisotropy on the IST relaxation. We detected remarkably long lifetime of the IST coherence of about 200 s for ^{13}C spins of the naphthalene derivative in the magnetic field of 5 mT.

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Liquid-state NMR methods

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Rapid Acquisition of High Quality Pure Shift NMR Spectra

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Abstract: Pure shift NMR experiments provide impressive resolution improvement by collapsing the multiplet structure that causes much of the signal overlap in conventional proton spectra. One drawback hitherto has been that either broad lines or long experiments have to be accepted. Here, in contrast, we demonstrate a new paradigm for fast acquisition of pure shift spectra with no extraneous line broadening. The interferogram methods [1-2] initially developed use an acquisition scheme that increases the dimensionality of the parent experiment [1-2]. Real-time acquisition [3-4] was introduced to provide faster experiments by repeating *J*-refocusing elements in a single-shot experiment. Unfortunately the price of speeding up the experiment is that some resolution is lost: relaxation and pulse imperfections cause signal loss in each successive *J*-refocusing element, broadening the pure shift lines and reducing the resolution advantage of the method [5].

Figure 1 (a) Pulse sequence for semi-real-time band-selective pure shift NMR. The selective pulses labelled a and p select active and passive spin regions respectively. Two complementary acquisitions are performed, and data chunks (in red) concatenated alternately. The ^{1}H NMR spectrum of 0.8 mM *R*- and 1.6 mM *S*-ibuprofen and 10 mM b-cyclodextrin in D_2O is shown below. The inset shows the less shielded aromatic protons with (b) conventional proton NMR, (c) real-time and (d) semi-real-time band-selective pure shift acquisition.

Here we present a novel approach to pure shift data acquisition (see Fig. 1a) that avoids this problem, allowing pure shift spectra to be measured with no extraneous broadening. This semi-real-time approach is illustrated for a band-selective homodecoupled (BASH) experiment in which all couplings to a defined chemical shift range are suppressed. Data are only acquired in the intervals between the *J*-refocusing elements, leaving gaps in the record of pure shift evolution, but these gaps are filled using a second, time-shifted, acquisition, as in the lower half of Fig. 1a. Combining the two conjugate datasets yields a complete pure shift FID, with full resolution, acquired in just two shots. Results obtained using conventional NMR and the real-time [4] and new semi-real-time BASH methods are compared in Fig. 1. The real-time spectrum (c) shows the broadening typical of the method, preventing resolution of the two peaks, but as the linewidths in the semi-real-time spectrum (d) are the same as those in the parent multiplets the peaks are well resolved.

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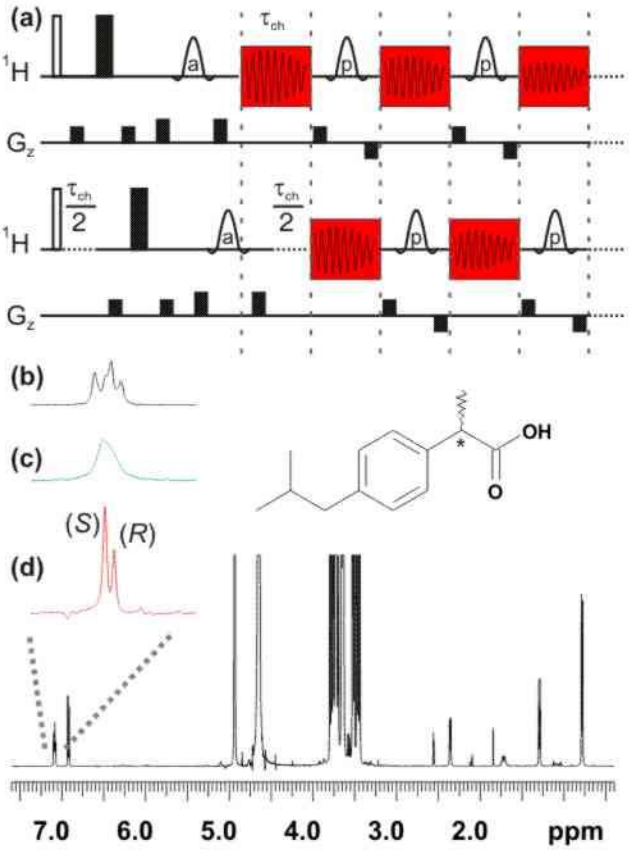
(a)
τ_{ch}
^{1}H
a
p
p
p
G_z
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$\frac{\tau_{ch}}{2}$
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p
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G_z
(b)
(c)
OH
*
O
(S)
(R)
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7.0
6.0
5.0
4.0
3.0
2.0
ppm

Liquid-state NMR methods

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Dipole-dipole interactions in liquids entrapped in confined space

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Abstract: At consideration of the spin dynamics in nano-pore materials the dipole-dipole interaction Hamiltonian is conveniently divided into two parts: intramolecular interaction, interaction between spins inside a molecule, and intermolecular interaction of spins of different molecules. We show that the dipolar intramolecular interactions have to be taken into account in nano-pores with the characteristic size less than 0.2 nm, when the random motion of water molecules can be neglected. At the same time, the contribution of the intermolecular dipolar interactions should be considered for porous materials where the size of a water-contained pore up to 400 nm.

The residual dipolar interaction, which is determined exclusively by intermolecular interaction, can play an essential role in the dynamics of a spin system of a liquid in a nano-pore with the typical size much less than 400 nm, like the spin dynamics in a solid, with an only remarkable difference: in liquids entrapped in a nano-pore the averaged dipolar coupling constant is the same for all pairs of spins. This property, in particular, can explain the anisotropy of NMR characteristics measured in experiments, such as the second moment and the transverse relaxation time for liquid entrapped in nanopores. Our results were compared with experimental date for collagen fiber sample possessing three histological zones with different degree of ordering of fibers. Using the Gaussian distribution of nano-pore directions, we have calculated the angular anisotropy of the transverse relaxation time for water between collagen fibers of the sample extracted from a pig leg. The sample was divided into the three histological zones. The good agreement with the experimental data was obtained by adjustment of few parameters, the standard deviation, averaged fiber direction, and weight factors, which characterize the ordering of fibrils, their orientations in each zone, and the relative dimensions of the corresponding zones. Thus, the value of the standard deviation obtained at the matching of the calculation to experimental results can be used as a parameter characterizing the disorder of the collagen fibers in the biological sample.

Liquid-state NMR methods

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A new molecular switch approach: Silver actuated cucurbituril translocation switches in water

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Abstract: Molecular switches are increasingly investigated because of their high potential as elementary building-blocks for the next generation of molecular machines.[1]

In this work, we show that ^{1}H-NMR is a powerfull tool to study the reversible ring translocation or uptake of a rigid and water soluble molecular switch, triggered by a block-d metal ion. Silver remotely controls cucurbit[7]uril (CB[7]) movements on a 3-station viologen-phenylene-imidazole (*V-P-I*) derivative, which could open a way to the synthesis of silver templated cucurbituril rotaxanes, catenanes and molecular machines.

By investigating the imidazole function of the molecular axle, we discovered that Ag^+ ions were able to reversibly translocate one molecule of CB[7][2] along the axle of ***V-P-I*** between stations ***V*** and ***P*** or pull a second CB[7] on the ***I*** station of the ***V-P-I***@CB[7] complex (Fig 1). ***V-P-I*** (2 Cl- counter-ions) is water soluble and CB[7] complexation on the ***V*** station was easily monitored by ^{1}H NMR in D_2O (Fig 1). Addition of $AgNO_3$ triggered ring translocation on station ***P*** as monitored by ^{1}H NMR in D_2O (Fig. 1).

To the best of our knowledge, there is no previous example of a ring molecule guided (translocated) or pulled (caught) on a rigid axle by silver cations in water. Its water solubility can open perspectives for biological applications of molecular switches, especially owing to the acid-responsive imidazole function.

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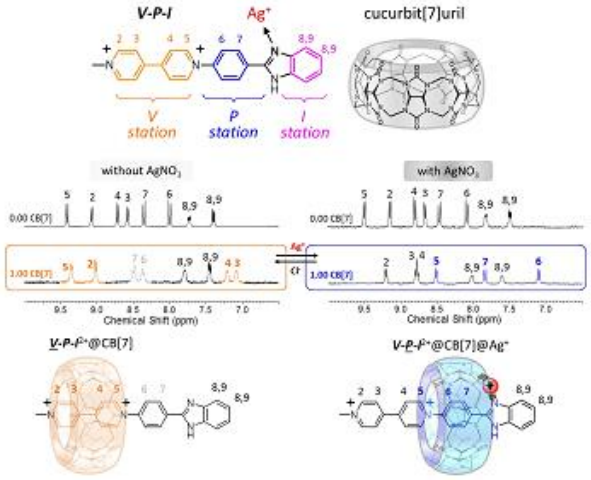

Fig. 1 Excerpts of the aromatic region of ^{1}H NMR spectra for the *V-P-I*@CB[7], without (left) and with (right) $AgNO_3$ in D_2O with proposed structures of the complexes (Ag^+ ions: red spheres).

Liquid-state NMR methods

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Spatially encoded diffusion-ordered NMR spectroscopy of reaction mixtures in organic solvents

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Abstract: The analysis of chemical mixtures is a challenging task, for which nuclear magnetic resonance spectroscopy has many advantages. Diffusion-ordered NMR spectroscopy (DOSY) is a powerful analytical method that makes it possible to separate the spectra of compounds from a mixture according to their diffusion coefficient, gives information on molecular sizes and shapes, and is also used to investigate molecular interactions [1-2]. Classic DOSY methods require several minutes of acquisition and are not compatible with the analysis of mixtures with a composition that varies on a comparable or shorter timescale. Signal intensities indeed vary depending on the chemical transformation over the DOSY experimental time, which causes a systematic error in the analysis of the diffusion decay.

Here, we show that DOSY experiments can be recorded in less than one second for the challenging case of solution mixtures in low-viscosity solvents. The proposed method relies on a spatial encoding of the diffusion dimension, for which convection-compensation and spectral-selection strategies are introduced. The effect of sample convection and spectral overlap are illustrated with a model mixture in dichloromethane. The method is illustrated with the real-time monitoring of an organic reaction, on a timescale that could not be achieved with conventional DOSY experiments. The possibility to obtain a sensible estimate of the molecular weight for a reaction intermediate is also illustrated. The proposed methodology should be useful for reaction-monitoring applications in chemical synthesis.

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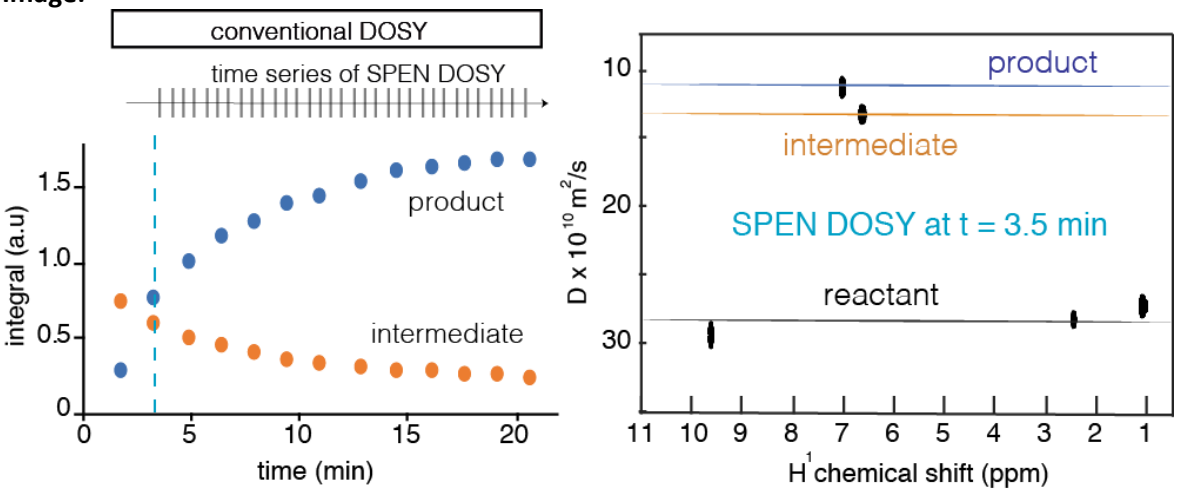

Liquid-state NMR methods

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Spatial/spectral encoding in ultrafast 2D NMR

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Abstract: A significant step towards the challenging goal of reducing the duration of nD experiments was taken in 2002 with the introduction of ultrafast NMR by Frydman and coworkers [1]. Indeed, the technique allows 2D spectra to be recorded in a single scan. Thanks to successive methodological developments, ultrafast NMR has demonstrated its ability to answer analytical problems such as reaction monitoring, quantification of analytes and analysis of complex mixtures [2]. Since ultrafast NMR relies on a spatial encoding of the spin dynamics throughout the sample by the use of pulsed field gradients, some limitations arise due to hardware capabilities. Sensitivity enhancement may be obtained by coupling with hyperpolarization [3] but even in this context, a compromise between resolution and spectral width has to be made concurrently in both dimensions of the spectra. This is an intrinsic limitation of ultrafast NMR arising from the linear encoding of the resonance frequencies.

To overcome these limitations in terms of spectral width, a controlled spectral aliasing can be done to fold spectral regions in a similar way to what exists in conventional NMR. One approach is to perform a non-linear encoding of the resonance frequencies by the use of the so called spatial/spectral (SPSP) pulses which have been first developed in the field of MRI to spatially select or suppress signals. Such spatial encoding scheme requires an a priori knowledge of NMR signal positions and is not straightforward to implement [4].

We will describe the key features of the bidimensional SPSP pulses consisting in a combination of simultaneous radiofrequency pulses and oscillating gradients. An optimal encoding is achieved with a spectral component and a spatial component to endow a specific spatial winding for each resonance frequency of interest. We will present the design of SPSP pulses and discuss the pros and cons of the associated tailored encoding scheme.

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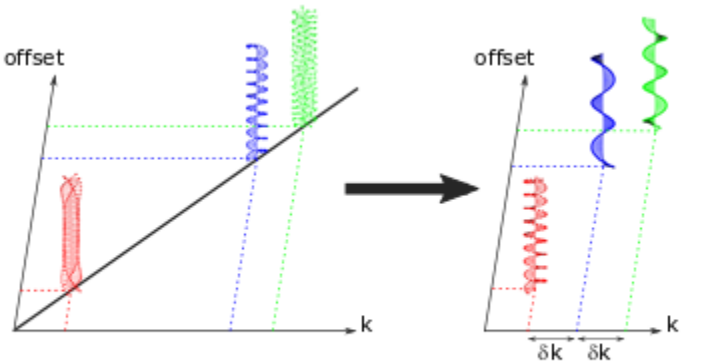

Spatial encoding of resonance frequencies in ultrafast NMR, with linear (left) or SPSP encoding (right)

Liquid-state NMR methods

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Nuclear spin dynamics in an externally applied electric field

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Abstract: In the nuclear magnetic resonance experiment, the externally applied electric field perturbs the nuclear spin dynamics. Several effects induced by that field have been predicted. Those which are linear in the electric field enable the direct discrimination between enantiomers by NMR. Moreover, if supplied by results of low-level DFT computations of relevant pseudoscalars, permit to find the absolute configuration of a molecule. The effects which are quadratic in the electric field do not depend on chirality. However, they give a useful piece of information about the molecular structure.

The influence of the electric field on the spin dynamics is found in a systematic way using the irreducible spherical tensor decomposition and then solving the approximated Liouville-von Neumann equation obtained from the Wigenstrass-Redfield-Bloch theory. The predicted effects are as follows. (*i*) In contrary to the case when the electric field is absent, the transverse relaxation rate induced by the electric field depends on the isotropic part of the nuclear shielding tensor. (*ii*) The magnetization during its return after application of the 180° pulse to the thermodynamic equilibrium has a transverse component. This component depends on the shielding tensor and the permanent electric dipole moment of the molecule. (*iii*) If the indirect spin-spin coupling tensor has an antisymmetric part, then the electric field induces chirality-dependent coherences. (*iv*) The perturbation of the interference between the chemical shift tensor anisotropy and the dipolar relaxation mechanisms by the electric field permits transitions whose measurement allow to distinguish between enantiomers. (*v*) An analogous effect is expected for the cross-correlated dipolar relaxation under the influence of the electric field (see Fig. 1). The amplitudes of the expected signals for the experiments designed in order to observe the predicted effects are a fraction of the conventionally recorded NMR signal. However, they are still potentially observable in an experiment performed using an NMR spectrometer equipped with a probe capable for generation of the oscillating electric field of the strength E=1 kV/mm.

Fig. 1 A sketch of the analysis of one of the predicted effects. The linear perturbation of the dipolar interaction between spins I_1 and I_2 by the electric field interferes with the dipolar interaction between spins I_1 and I_3 in a chiral molecule possessing the permanent electric dipole moment $\boldsymbol{\mu}^e$. The tensors of these interactions, $\mathbf{U}_{12}$ and $\mathbf{D}_{13}$, are represented as ellipsoids oriented along their eigenvectors and of semiaxes equal to absolute values of their eigenvalues, *i.e.*, the ratios of semiaxes are 0:1:1: and 1:1:2, respectively.

The author thanks Prof. A. David Buckingham for fruitful discussions and acknowledges the National Science Centre, Poland, for financial support through the SONATA grant no. 2015/19/D/ST4/00953.

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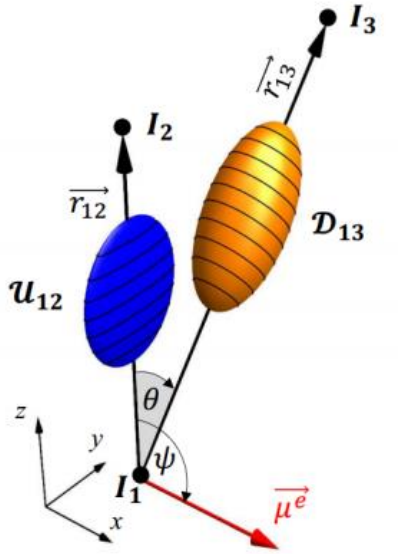
I_3
$\overrightarrow{r_{13}}$
I_2
$\overrightarrow{r_{12}}$
$\mathcal{D}_{13}$
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θ
ψ
z
y
x
I_1
$\overrightarrow{\mu^e}$

Liquid-state NMR methods

P213

Evaluation of Band-Selective HSQC and HMBC for Poly(3-hydroxyalkanoate)s Stereoregularity Determination

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Abstract: Band-selective (bs) HSQC, improving spectral resolution by restriction of the heteronuclear dimension without inducing spectral folding, has been recently used for polymer tacticity determination [1]. ^{13}C selectivity can be obtained from different schemes:

- Substitution of a broadband ^{13}C refocalisation by a selective one [2,3]
- Use of opposite sign gradients bracketing a selective ^{13}C refocalisation [4]
- Insertion of Pulsed Field Gradients Spin Echoes (PFGSE) [1,5]

In a first part we evaluated various bs-HSQC and HMBC sequences from a methodological point of view, studying selectivity, dependence to INEPT interpulse delay or relaxation delay. For HSQC sequences, the ^{13}C selectivity scheme consisting in substituting a ^{13}C broadband refocalisation by a selective one revealed itself problematic, with unwanted aliased signals. The use of opposite sign gradients bracketing a selective refocalisation was found quite sensitive to relaxation delay incorrect setting, whereas the insertion of double pulsed field gradients spin-echo (DPFGSE) gave satisfactory results.

In a second applicative part we compared tacticity determined from bs-HSQC and -HMBC experiments to the one obtained from 1D $^{13}C\{^1H\}$ on poly(3-hydroxyalkanoate)s samples. Determination of the probability of syndiotactic enchainments, P_s, by bs-HSQC is fully consistent and no precision loss was observed when decreasing acquisition time (37 min vs. 106 min for 1D $^{13}C\{^1H\}$). Bs-HMBC, although not straightforwardly applicable for tacticity determination, could provide (after a calibration step) an alternative for compounds of which only ^{13}C carbonyl signals are resolved enough for discriminating between *syndiotactic* and *isotactic* configurations.

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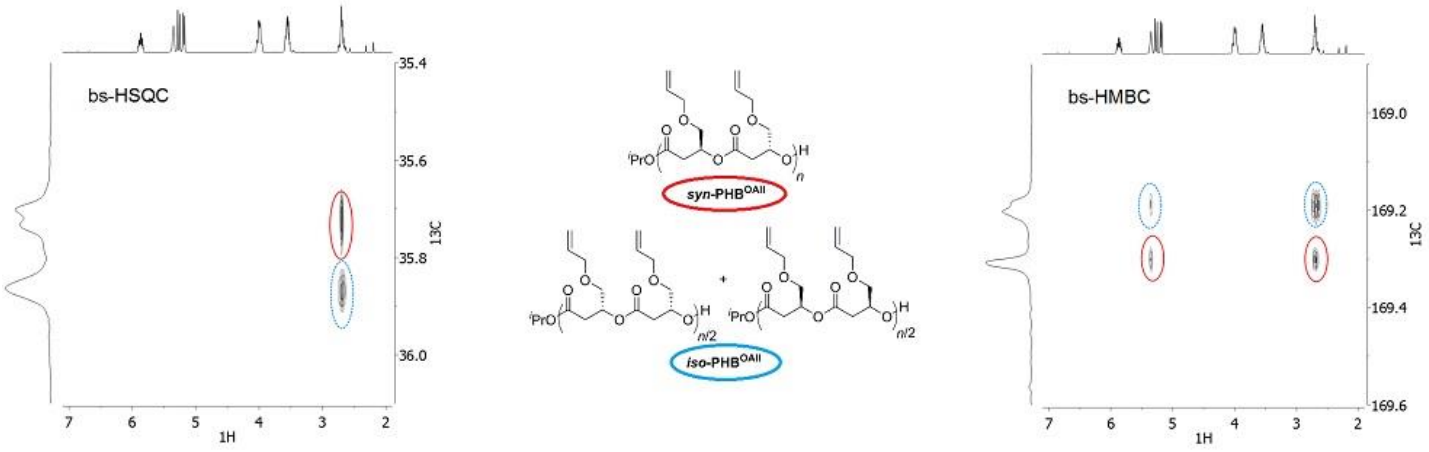
bs-HSQC
35.4
35.6
35.8
36.0
13C
7
6
5
4
3
2
1H
syn-PHBOAll
iso-PHBOAll
bs-HMBC
169.0
169.2
169.4
169.6
13C
7
6
5
4
3
2
1H

Liquid-state NMR methods

P214

Using optimal control methods with constraints to generate singlet states in NMR

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Abstract: Singlet state NMR is receiving increasing attention because of the attractive possibility to generate long-lived spin order. Such a spin order can be used for investigating slow dynamics, diffusion and transport in NMR and MRI. One of the problems associated with LLSs is to develop a general and efficient method for generating and observing such states. Specifically, for running experiments with singlet LLSs a technique is needed, which can perform the magnetization-to-singlet (M2S) conversion and the reverse S2M conversion.

Here we propose a method for optimizing the performance of the APSOC (Adiabatic-Passage Spin Order Conversion) technique, which can be exploited in NMR experiments with singlet spin states. In this technique magnetization-to-singlet conversion (and singlet-to-magnetization conversion) is performed by using adiabatically ramped RF-fields. Optimization utilizes the GRAPE (Gradient Ascent Pulse Engineering) approach, in which for a fixed search area we assume monotonicity to the envelope of the RF-field. Such an approach allows one to achieve much better performance for APSOC; consequently, the efficiency of magnetization-to-singlet conversion is greatly improved as compared to simple model RF-ramps, e.g., linear ramps. We also demonstrate that the optimization method is reasonably robust to possible inaccuracies in determining NMR parameters of the spin system under study and also in setting the RF-field parameters. The present approach can be exploited in other NMR and EPR applications using adiabatic switching of spin Hamiltonians.

This work has been supported by the Russian Science foundation (grant No. 14-13-01053).

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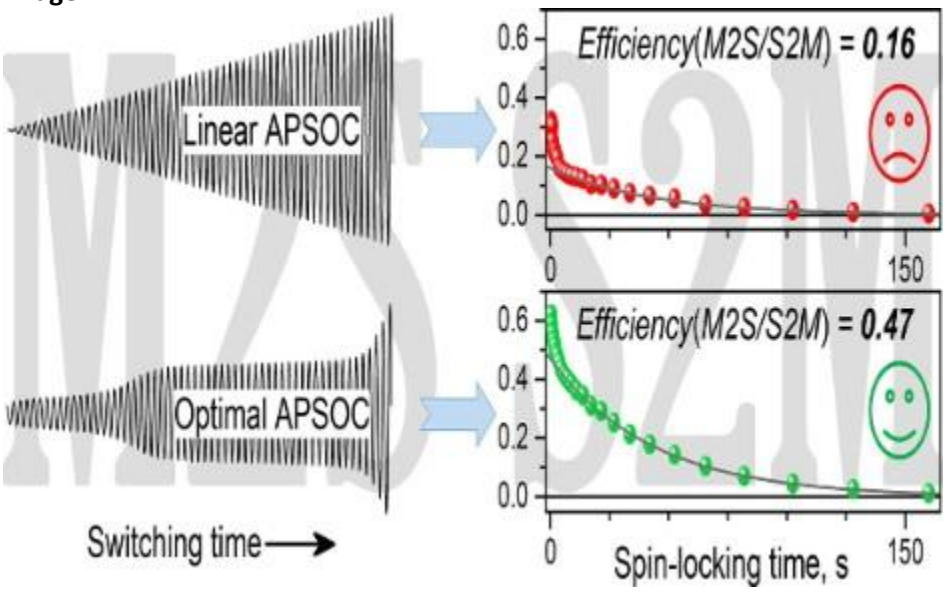

Liquid-state NMR methods

P215

Ultrafast maximum-quantum NMR spectroscopy for the analysis of aromatic mixtures

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Abstract: Maximum-quantum (MaxQ) NMR experiments have been introduced to overcome issues related to peak overlap and high spectral density in the NMR spectra of aromatic mixtures [1]. In MaxQ NMR, spin systems are separated on the basis of the highest-quantum coherence that they can form. MaxQ experiments are however time consuming and methods have been introduced to accelerate them [2].

In this work, we demonstrate the ultrafast, single-scan acquisition of MaxQ NMR spectra, using a spatial encoding of the multiple-quantum dimension. So far, the spatial encoding methodology has been applied only for the encoding of single and double-quantum spectra [3], and here we show that it can be extended to higher coherence orders, to yield a massive reduction of the acquisition time of multi-quantum spectra. The validity of the method is shown in Figure 1, in which comparable UF and conventional 4Q NMR spectra were obtained from an aromatic mixture of three compounds with different MaxQ orders.

We have developed an experimental and theoretical strategy to determine the best experimental conditions with an optimal compromise between sensitivity and resolution in UF MaxQ NMR experiments. We have also written a numerical spin simulation code, based on the Spinach library [4], that allows a quick identification and assignment of the specific coherence orders accelerating and simplifying quantitative analysis of complex aromatic mixtures.

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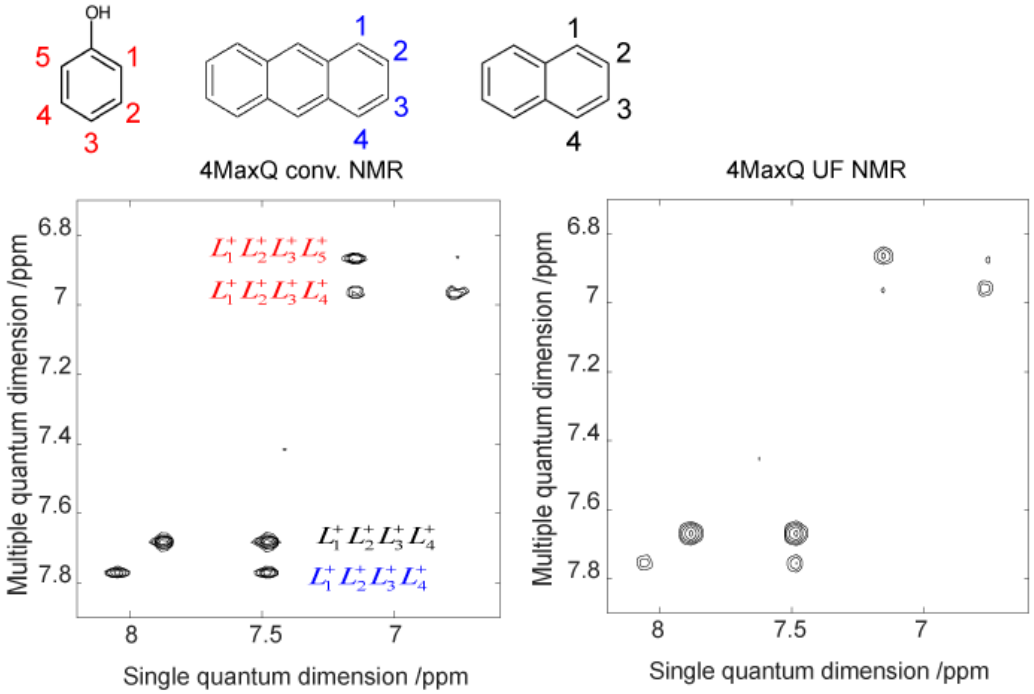

Figure 1: Comparison between conventional and UF 4Q NMR spectra of phenol (MaxQ equal to 5), anthracene and naphthalene (MaxQ equal to 4), represented with a reduced chemical shift scale, for the indirect dimension, the inserts show the assignment of the 4Q belonging to the three compounds.

Liquid-state NMR methods

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Acceleration of 3D DOSY NMR with a spatial encoding of the chemical shift

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Abstract: Diffusion-ordered NMR spectroscopy (DOSY) is a powerful method for the analysis of mixtures. With DOSY, NMR signals are separated according to the translational diffusion coefficient of the associated compounds. The DOSY principle can be applied to a variety of nD NMR experiments, by incorporating a pair of incremented diffusion-encoding magnetic-field gradients (1). This results in a (n+1)D DOSY display, where the nD spectra of each component are separated. DOSY is most commonly used to separate 1D spectra, but its application to 2D spectra can reduce signal overlap, simplify the analysis and bring additional structural information. However 3D DOSY experiments have the major drawback of a long duration, needed to construct the diffusion and the indirect dimensions.

Here we show that 3D DOSY experiments can be accelerated dramatically with a spatial encoding of the indirect chemical-shift dimension. Ultrafast (uf) NMR, based on spatial encoding, is a very effective method to reduce the duration of 2D experiments, from minutes or tens of minutes to less than one second. Spatial encoding is used here to decrease the experiment time of 3D DOSY from several hours minimum to less than 10 minutes. The approach is illustrated on two types of homonuclear ^{1}H-^{1}H 2D spectra separation, with a single-quantum (COSY) or double-quantum (DQS) indirect dimension.

We further compare several ways to incorporate the diffusion-encoding gradients (2) in uf 2D pulse sequences, accounting for possible interferences between the diffusion-encoding and spatial-encoding steps. The effect on non-linear magnetic-field gradients on the accuracy of the measured diffusion coefficient is also analysed, Both the DOSY-ufDQS and the DOSY-ufCOSY experiment give a good separation of spectra (see Fig.1 for an example), with a sensitivity advantage for the ufCOSY version.

The proposed fast 3D DOSY method should be useful for the analysis of compounds that evolve in time or of a large number of samples.

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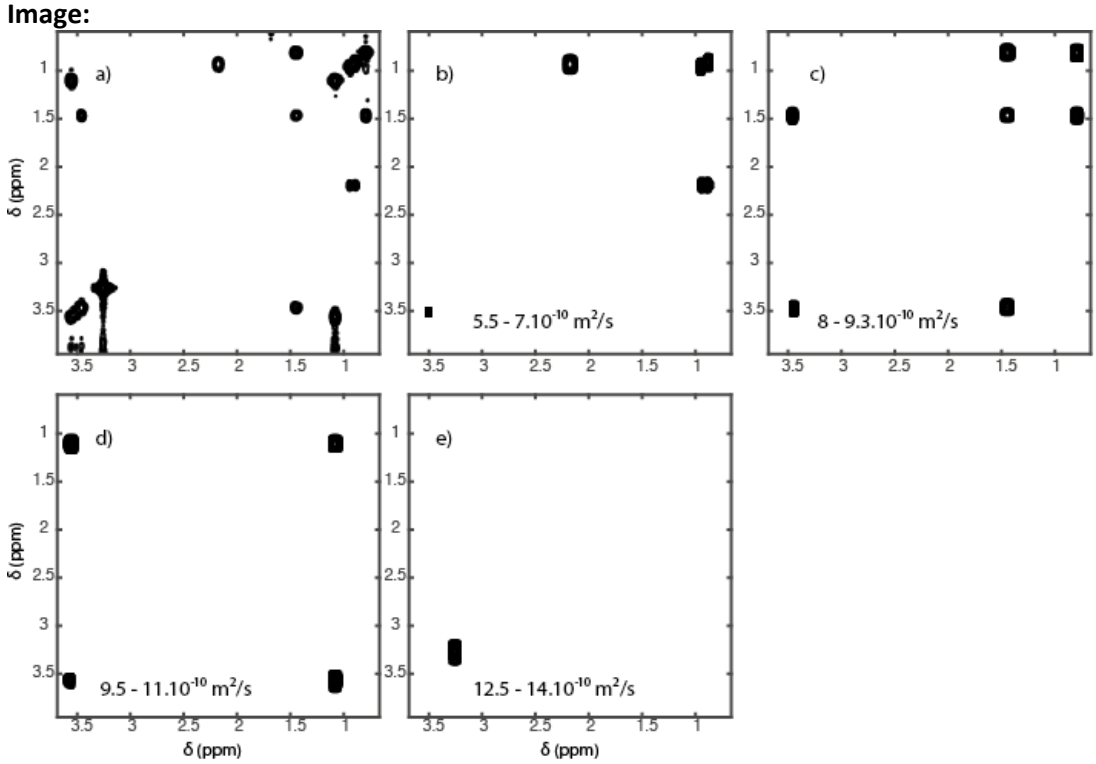

Fig. 1: 3D-DOSY-ufCOSY on model mixture. a) ufCOSY spectrum obtained with the weakest diffusion gradient; b-e) slices extracted from 3D spectra; b): valine; c) propanol; d) ethanol; e) methanol. The selected range of D in shown on each figure..

Liquid-state NMR methods

P217

A high-resolution SERF experiment for accurate extraction of scalar coupling constants

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Abstract: NMR spectroscopy serves as a powerful tool for molecular structure elucidation. Scalar coupling is widely used in molecular structure analysis. Many methods have been proposed for accurate measurement of scalar coupling constants. The SERF (selective refocusing) experiment can measure the scalar coupling constant between two selected protons[1]. Recently, many methods have been proposed to facilitate the extraction of scalar coupling constants[2,3]. However, the extraction of scalar coupling constants still suffers from low resolution in some cases such as when measuring small coupling values or under inhomogeneous field.

Here, we present a high-resolution SERF experiment for more accurate extractions of *J* values (see the pulse sequence in Fig. 1a). In our experiment, we use doubly selective 180° pulses which acts on the detected proton and another proton coupled to it, thus retaining the scalar coupling between these two protons. We place the RF carrier frequency at the resonant frequency of the detected proton, in a similar way as in the SHARPER experiment[4]. As a result, we can obtain a signal with zero chemical shift modulation. The repeating "180°-acquisition" elements on the detected proton yield a series of signals with consistent chemical shift modulation. Moreover, the effect of filed inhomogeneity is also eliminated through consecutive refocusing.

Experimental results on a sample of propylene carbonate in $CDCl_3$ are shown in Fig. 2. The conventional proton 1D spectrum and local expansions of proton 2 and 3b are shown in Fig. 2. Experimental results of measuring $J^{3b,2}$ and $J^{2,1}$ by using sequence in Fig. 1a are shown in Fig. 2b and 2c. It should be noted that the sequence in Fig. 1a leads to upscaled *J* values which result from extra *J* evolution during the doubly selective 180° pulses. The upscaling factor of *J* values can be calculated by taking account of the length of the acquisition window (Δ) and the delay time (T_d), as well as the multiple quantum effects associated with the doubly selective pulses[5]. Through the combination of datasets from the sequence in Fig .1a and its time-shift variant in Fig. 1b, original *J* values can be obtained. For further enhanced resolution, the pseudo 2D version in Fig. 1c can be applied.

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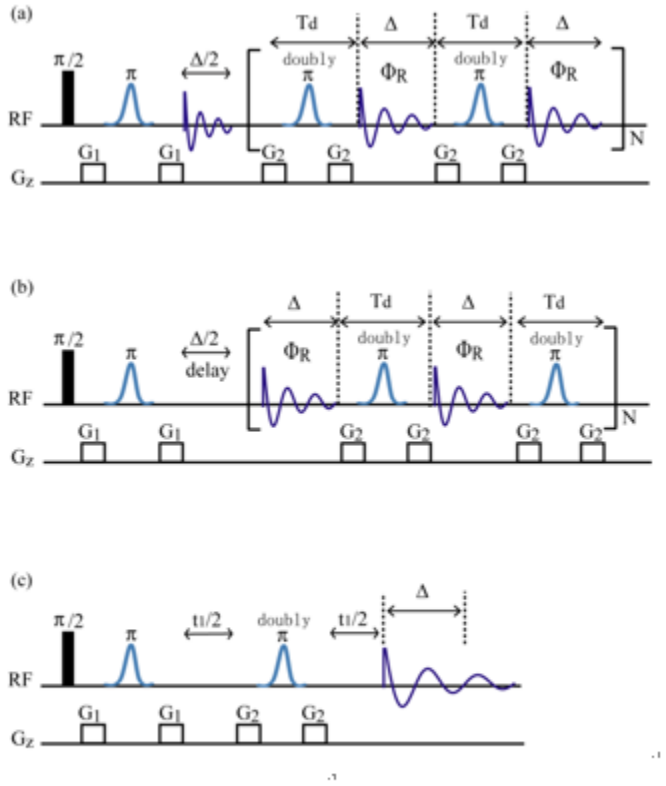

Fig. 1 (a) The pulse sequence for the high resolution SERF experiment. (b) Time shift variant of a. (c) The pseudo 2D version.

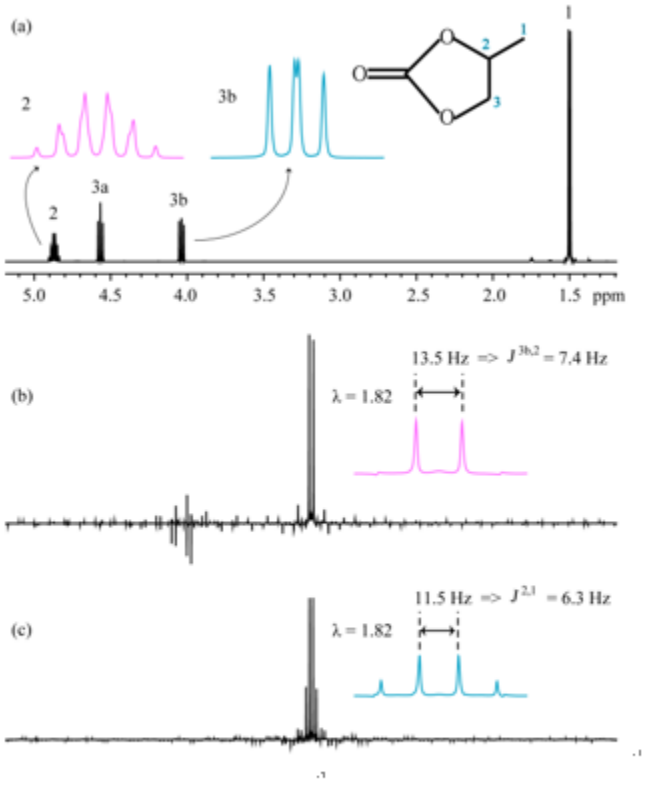

Fig. 2 (a) Conventional ^{1}H 1D spectrum of propylene carbonate in $CDCl_3$ (b) and (c) High-resolution SERF spectra for measuring $J^{3b,2}$ and $J^{2,1}$, respectively. The upscaling factor is denoted by λ.

Liquid-state NMR methods

P218

Ultrafast Laplace NMR: Towards Chemical Exchange

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Abstract: NMR relaxation and diffusion measurements provide versatile information about dynamics and structures of e.g. porous materials, and reveal interactions of nuclei within their microscopic environment. Since relaxation and diffusion data comprise exponentially decaying components, the processing requires a Laplace inversion in order to extract the diffusion coefficient and relaxation time distributions. Thus, these methods are referred to as Laplace NMR (LNMR). [1]

Multidimensional approach increases the chemical resolution of an NMR experiment. Multidimensional and even some 1D experiments are really time consuming, since the experiment needs to be repeated several times with varying evolution delay or gradient strength to gain proper multidimensional data. This restricts the applicability of multidimensional LNMR methods and is considered general problem of multidimensional NMR. Also in many cases it prevents the use of hyperpolarized substances for signal amplification. Problem of a long experimental time can be tackled by introducing spatial encoding of two-dimensional data, as was originally done in ultrafast NMR spectroscopy [2,3] and later in ultrafast LNMR [4-6]. The price to pay is reduced sensitivity. However, the single-scan approach enables the use of hyperpolarization methods (e.g. PHIP, DNP [3] and SEOP [4]), which provide much higher sensitivity boost than the loss due to spatial encoding.

In this presentation we describe the concept of multidimensional ultrafast LNMR, and introduce the recent progress towards chemical exchange. We demonstrate that ultrafast LNMR methods can utilize modern hyperpolarization methods to increase sensitivity to study many interesting systems such as dynamics of porous medium. The ultrafast LNMR methods are applicable by mobile NMR instruments, thus widening the application range even further. [4-7]

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Liquid-state NMR methods

P219

Investigation of effect of thermal denaturation on eye lens crystallin proteins using NMR spectroscopy.

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Abstract: Crystallins are the major vision-related (i.e. refractive) proteins found in the eye lens. The mammalian lens consist of three classes of structural proteins, i.e α-, β- and γ-crystallins. The former also acts as chaperone. Commonly, proteins are subject to a continuous degradation and replacement process, but the eye lens proteins have to remain stable and soluble for a lifetime. Heat shock or other stressors can cause aggregation and lead to cataract, thus the major chaperone function is to prevent aggregation. The conventionally used techniques to study aggregation include observations by optical techniques applied mostly to dilute solutions. Here we demonstrate the use of ^{1}H NMR relaxometry as an alternative to study the aggregation kinetics of crystallin proteins in highly concentrated protein solutions. From the rather different relaxation times of the globular aggregates and unfolded proteins we were able to quantitatively characterize the protein native and aggregated state. We have studied the thermal denaturation and aggregation kinetics of γB-crystallin and α-crystallin and our data demonstrates the qualitative changes associated with these proteins during thermal degradation.

Liquid-state NMR methods

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Quantification of Spectroscopically Resolved Glycerol and Water Exchange in Human Aquaporins with Diffusion Exchange Methods

Sarah Mailhiot[*1], Sofia de Mare[2], Karin Lindkvist-Petersson[2], Daniel Topgaard[1]
[1]Physical Chemistry, [2]Biomedical Center, Lund University, Lund, Sweden

Abstract: Aquaporins are cellular membrane proteins that transport water. They are critical for maintaining a stable osmotic pressure within the cell. A subset of aquaporin proteins also transport non-charged molecules such as ammonia, glycerol, and carbon dioxide. NMR is well suited for measuring membrane permeability. Previously, it has been shown that NMR diffusion exchange methods (1-3) can be used to measure the exchange rate of water across the cellular membrane, but for membrane proteins that co-transport molecules more information is needed. This work expands on previous filter exchange spectroscopy (FEXSY) methods (2,3). Specifically, we present a method for the simultaneous measurement of glycerol and water transport in yeast cells with and without human aquaporins. Using this method, the exchange rate of spectrally resolved water and glycerol is measured simultaneously.

The method proposed here uses a double pulsed gradient stimulated echo (PGStE) with variable mixing and echo times, t_m and t_e respectively, and records the free induction decay. The double PGStE is used to accommodate samples with short T_2 relaxation times. The variable echo spacing combines FEXSY with a D-T_2measurement (3). A two-step phase cycle is used. The experiment time is 4 minutes. Finally, the free induction decay and consequently the chemical spectra is acquired. Using spectral resolution and peak selection, the exchange of water and glycerol is measured. The pulse program and a data set are shown below. The data set is from a sample of yeast cells expressing aquaporin in culture medium and 15% glycerol. An exchange life time of 0.2 and 2.0 seconds is measured for water and glycerol, respectively. The advantage of this method is the ability to measure exchange with spectral resolution in a time efficient manner.

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Figure- (a)The pulse program is composed of an initial diffusion encoding period (red), a mixing period with mixing time $t_m$where molecules diffuse between the intracellular and extracellular spaces (green), diffusion encoding where the gradient amplitude is varied (blue), and a variable echo time t_e. A thin radio frequency (RF) line represents a 90^0pulse and a thick line represents a 180^0pulse. The free induction decay is acquired. (b) A data set for one echo time is displayed. The signal decay is shown for water at 4.53 ppm and glycerol at 2.9 ppm (c). The top line in the signal decay is obtained without a diffusion filter and the lines below use a diffusion filer and short to long mixing times from top to bottom. An exchange life time of 0.2 and 2.0 seconds is measured for water and glycerol, respectively.

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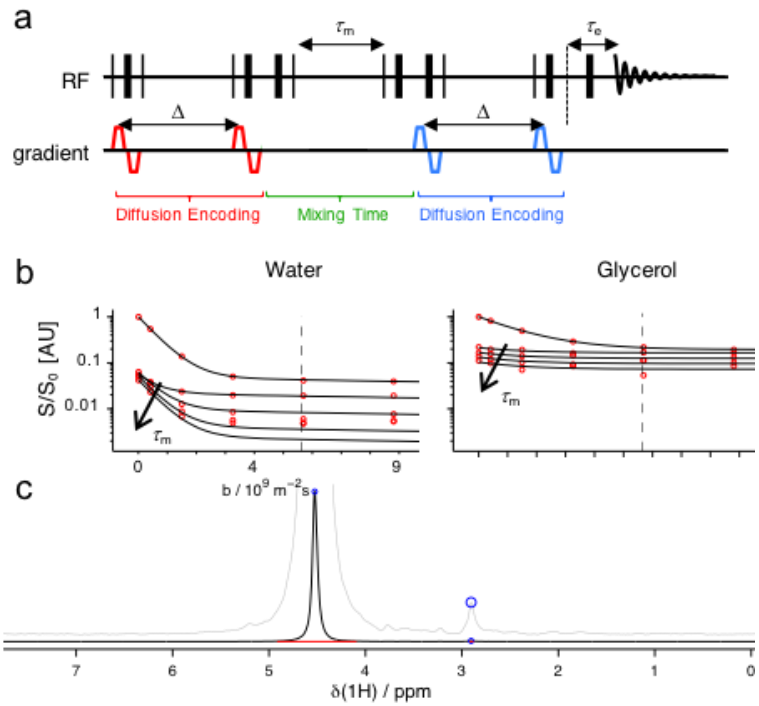

Disclosure of Interest: S. Mailhiot: None Declared, S. de Mare: None Declared, K. Lindkvist-Petersson: None Declared, D. Topgaard Conflict with: Author holds patents related to this technology.

Liquid-state NMR methods

P221

Diffusion Measurements by Ultrafast Laplace NMR

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Abstract: Diffusion NMR measurements provide important information about dynamics of nuclear spins under investigation, and therefore are useful for characterization of different materials and substances. The adoption of multidimensional (MD) methodology in such experiments allows one correlating diffusion data to spectroscopic information and various spin dynamics parameters like T_1 and T_2 relaxation times.[1] These approaches substantially increase the available information content, but implemented with conventional methodologies they require very long experimental acquisitions.

The family of diffusion/relaxation experiments constitute the so-called Laplace NMR methods since mathematical Laplace inversion procedure is used to extract diffusion, T_1 and T_2 distributions from the measured NMR signals. This is in the full analogy like Fourier transformation is used to extract frequency distributions in traditional NMR spectroscopy. The application of NMR imaging principles to perform spatial encoding of spectroscopic information allowed creation of ultrafast approaches in NMR spectroscopy.[2] The similar principles were employed to create T_1-T_2 [3] and D-T_2 [4] correlation ultrafast Laplace NMR methods. In addition to thermally polarized samples, the latter method was applied in several demonstrations with hyperpolarized (HP) ones, since it provides the single-shot acquisition, essentially required with the HP samples.

In this communication, we discuss different diffusion encoding approaches based on the use of the spatial encoding and the utilization of multi-echo methods to be used as building blocks in the ultrafast Laplace NMR methods. Analytical and numerical theoretical treatments of the expected diffusion encoding patterns will be presented. We also will provide experimental validations of the discussed approaches, including experiments with HP samples.

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Liquid-state NMR methods

P223

Filling gaps in FID signals: when, how and what for?

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Abstract: There are a number of tasks in NMR that are enabled by introducing gaps into the FID acquisition. These gaps can be used to introduce additional pulses: for example, to refocus couplings, heteronuclear or homonuclear. They can also reduce the power demands of long experiments by switching off broadband decoupling.

If such an FID with gaps is directly Fourier transformed, the gaps introduce strong artifacts into the spectrum. To avoid this one can interpolate the missing points prior to the Fourier transform. This interpolation can be achieved with compressed sensing (CS) algorithms, normally applied in NMR when reconstructing indirect dimensions in non-uniform sampling (NUS) experiments. Here, these algorithms are applied for reconstructing the direct dimension in 1D or multidimensional experiments.

The technique is called EXtended ACquisition Time (EXACT). It has been applied in a conventional HSQC experiment [1] as well as in ultra-fast ("ASAP") experiments (HSQC and HMBC) [2] to partially replace broadband decoupling during the acquisition with echo-type pulses within gaps and in pure-shift NMR to improve resolution in,[1] or accelerate,[3] homodecoupling experiments.

However, the CS theory states that a "non-coherent" (irregular) sampling is required for reliable reconstruction, whereas in the EXACT case the sampling is highly "coherent" (regular). This leads to regular and high artifacts in the spectrum concentrated in the regions near the peaks, contrary to the case of NUS, where the artifacts are low but occupy the whole spectral domain. The EXACT type of artifacts is a more challenging case for the reconstruction.

In this work, we discuss the challenges arising when reconstructing EXACT data, in particular when there is coincidental overlap of large artifacts with real peaks; modifications of the sampling scheme (sizes of gaps and acquired FID chunks) in the context of the reconstruction efficiency; and propose adaptations of the reconstruction algorithms to minimize these effects.

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Liquid-state NMR methods

P224

Determining isoleucine rotamer populations form chemical shift.

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Abstract: Understanding protein dynamics is vital for describing biological processes and diseased states. Nuclear magnetic resonance (NMR) is an ideal tool to study protein dynamics as it reports on a dynamic ensemble in solution. In particular many experiments allow us to measure relaxation parameters which can inform us about dynamics processes and minor states[1]. This has led to the development of many established tools that relate chemical shift to the protein backbone conformation. However it is often side-chains that mediate biological functions such as ligand binding or allosteric communication. Furthering our understanding of the relationship between side-chain conformation and chemical shift will provide a powerful tool to describe side-chains in biologically important events.

In this study we develop a method to determine the isoleucine side-chain rotamer distribution from carbon chemical shifts. As part of this study we carried out Density Functional Theory calculations to characterize the relationship between chemical shift and side-chain conformation (example shown in Figure 1). These surfaces show that all the side-chain carbon atoms show significant chemical shift variations depending on their rotamer conformation. Importantly, these calculations show that each of the populated rotameric states has a unique 'chemical shift profile'.

Unlike previous methods[2,3] we define each rotamer by both χ1 and χ2 angles providing a near complete description of the side-chain. This gives a more detailed description of the side-chain dynamics than what is available using scalar-couplings. The readily available nature of chemical shifts allows this method to be applied in situations where long-range scalar-couplings are impossible to obtain. To demonstrate the method's utility isoleucine rotamer distributions were determined from chemical shifts for the DsbB–DsbA complex, a 41 kDa membrane protein, and the L24A FF domain's 'invisible' folding intermediate that exchanges with the ground state.

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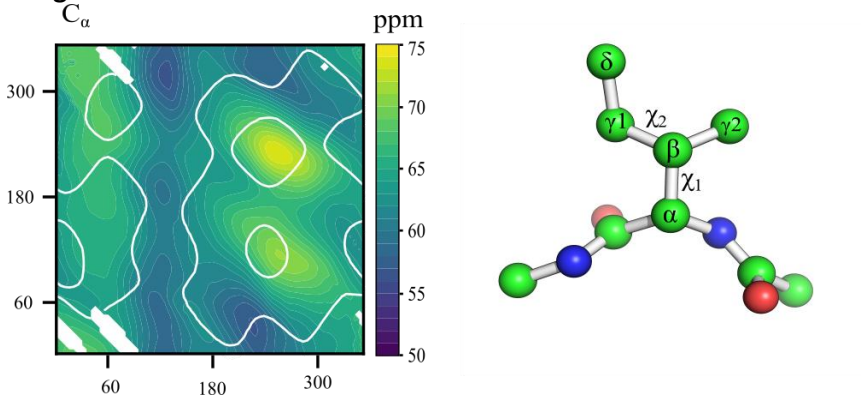
Figure 1: Dependence between chemical shift and conformation. Left: Cα chemical shift surface from DFT calculations carried out using a B3LYP/EPR-III basis set. The White contours show the regions with a population greater than 10^{-4}. White stripes show regions where the structure optimisation did not converge. Right: Ace-Ile-NMe used for the DFT calculations.

Image:



Liquid-state NMR methods

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13C selective real-time decoupled HSQC for pure shift HαCα correlations – application for small molecules and proteins

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Abstract: Biomolecular NMR assignments are mostly based on HN detection, though several factors can contribute to the deterioration of $^{1}H,^{15}N$-HSQC spectra. Moreover, especially in the case of disordered proteins the serious signal overlap, the mobile exchangeable NH proton environments and the non-detectability of Pro residues can cause serious hurdles. In this respect $^{1}H,^{13}C$ correlation can be of help regardless of pH, temperature, and Pro residues are also present in the spectra. Thus, we introduce an optimized $^{1}H,^{13}C$-HSQC sequence for achieving spectral simplification for protein characterization and mixture analysis approaches. In this context a novel band-selective BIRD filter element is introduced for real time $^{1}H,^{1}H$ homonuclear decoupling with gains in both sensitivity and spectral resolution. The measurement is performed in aqueous solution, with very weak presaturation of the water signal and the application of gradients during selective BIRD elements to avoid radiation damping.

The experiment allows acquisition of 2D HαCα correlation spectra with high resolution in the indirect dimension in short time - already from one scan - and further speeding up is possible by the the NUS approach. Fine tuning of the backtransfer allows detection of $-CH_2-$ environments (Gly residues) in a separate experiment. The application of this novel pulse sequence is demonstrated on several samples, including small molecules, an intrinsically disordered protein and a folded protein.

Liquid-state NMR methods

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Optimization of the CPMG sequence for the analysis of small molecules in paint matrices

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Abstract: NMR detection (and quantitation) of small molecules in low concentration in presence of higher-molecular weight materials (e.g. metabolites in plasma and proteins) is thwarted by overlapping signals from the matrix and dynamic range difficulties. Several NMR methods have been developed to circumvent these problems and applied to the study of biological systems. These methods exploit the differences in the diffusion rates and relaxation times existing between molecules: in general small molecules diffuse faster and have a substantially longer relaxation time than molecular entities with a higher molecular weight [1].

Our goal is to extend the use of these techniques, which are regularly employed in biofluids studies, to the analysis by ^{1}H NMR of paint formulations containing low amounts of small biocides (insecticidal paints). Insecticidal paints are one of the most recommended forms of vector control that the World Health Organization promotes for reducing the spreading of mosquito-borne diseases like Malaria, Zika virus and yellow fever [2]; among the active ingredients WHO-recommended we chose to focus on pyrethroids, which are the safest insecticide for public health use from both the human and environmental point of view [3,4]. However, there is a fundamental lack of understanding on the influence of the paint matrix components on the insecticidal effectiveness.

In this work we show our initial results obtained by exploring the differences in spin-spin relaxation time (T2) between small and larger molecular entities that are commonly part of paint formulations. We used the spin-echo CPMG (Carr-Purcell-Meiboom-Gill) sequence for attenuating or eliminating signals belonging to molecules associated to the paint matrix without significantly affecting the resonances of the small molecules present. The optimization of the CPMG sequence involved the analysis of materials with a range of molecular weight spanning from 300 g/mol to 600 kg/mol. The aim was to find sequence parameters that would balance the desirable loss of intensity from the signals related to the large molecules without seriously compromising the signal to noise ratio for the small molecule.

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Liquid-state NMR methods

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Nuclear-spin comagnetometer based on a liquid of identical molecules

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Abstract: Atomic comagnetometers typically consist of overlapping ensembles of at least two different species of atomic spins [1]. It is the ratio of the spin-precession frequency of the different species under the influence of a bias magnetic field that is measured. The ratio is relatively insensitive to the changes in magnetic field but retains sensitivity to Zeeman-like nonmagnetic spin interactions. Therefore, atomic comagnetometers are widely used in fundamental physics experiments and searches for anomalous spin-dependent interactions [2].

However, in fundamental physics experiments using comagnetometers based on overlapping ensembles of different species, one of the systematic effects reducing accuracy is due to uncontrolled magnetic field gradients [3]. Here we describe a comagnetometer based on the nuclear spins within an ensemble of identical molecules. In this single-species comagnetometer, different nuclear spins are probed within the same molecule. By taking advantage of the techniques of ultralow-field nuclear magnetic resonance and sensitive atomic magnetometry [4], the *J*-coupling (indirect spin-spin coupling) spectrum of a liquid-state ensemble of acetonitrile-2-^{13}C molecule can be measured with sub-mHz precision in an ultralow-field with a single scan. Under the influence of a bias field, the *J*-coupling resonance lines at different frequencies split into separate peaks (show in Fig. 1). The frequency separation between the split peaks for each *J*-coupling resonance lines has distinct linear coefficients with respect to the magnetic field. Measurements of these splittings can be employed as a comagnetometer.

We experimentally demonstrate that in the presence of a temperature gradient, such a comagnetometer is insensitive to first-order magnetic field gradients (shown in Fig. 2). Our single-species comagnetometer is shown to be capable of measuring the hypothetical spin-dependent gravitational energy of nuclei at the 10^{-17} eV level, comparable to the most stringent existing constraints. Combined with techniques for enhancing the signal such as parahydrogen-induced polarization [5], this method of comagnetometry offers the potential to improve constraints on spin-gravity coupling of nucleons by several orders of magnitude.

Figure 1: Schematics of the liquid-state nuclear spin comagnetometer (left) and the experimentally measured *J*-coupling spectrum of acetonitrile-2-^{13}C in a 80 nT bias field along *z* (right). The related transitions used for comagnetometry are shown with solid red arrows.

Figure 2: Comparison of the normalized frequency ratios between a single-species comagnetometer and two dual-species reference comagnetometers.

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Image:

Figure 1

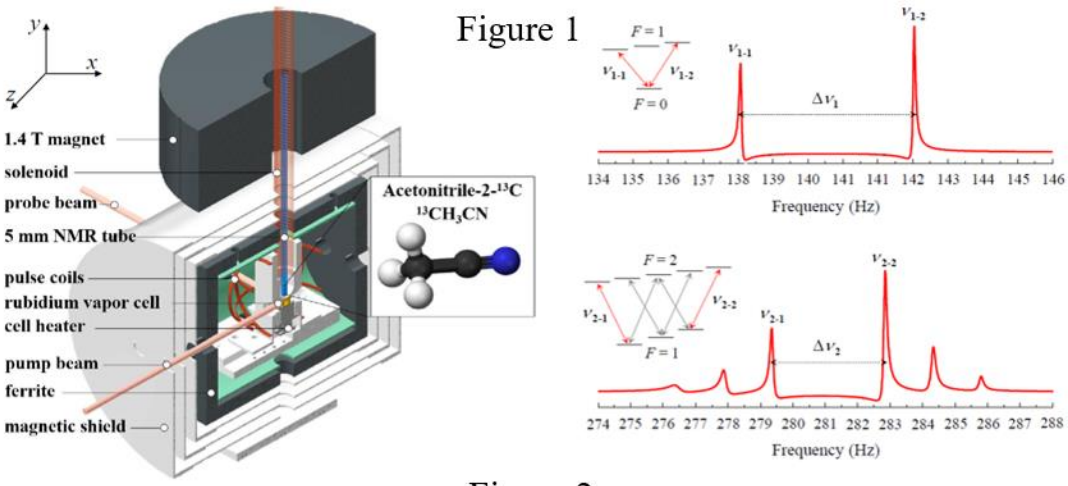
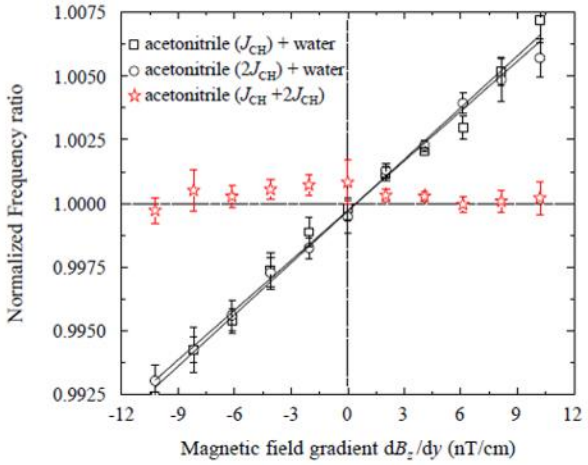


Figure 2



Liquid-state NMR methods

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Field-Cycling Long-Lived State NMR of 15N2 Spin Pairs

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Abstract: The applications of nuclear magnetic resonance spectroscopy and imaging are limited by the short lifetimes of conventional magnetization in solution. Systems containing homonuclear spin-1/2 pairs are immune to in pair dipole-dipole relaxation, with other symmetric decay mechanisms strongly attenuated. Long-lived states therefore provide an opportunity to alleviate this limitation.

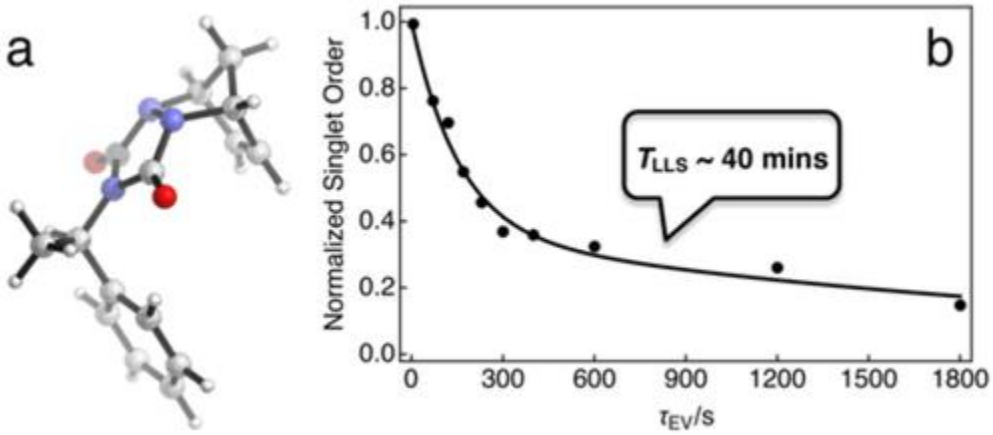
A number of molecular structures exhibiting large ratios of the long-lived state lifetime T_{LLS} to the spin-lattice relaxation time T_1 have previously been demonstrated. An asymmetric cis-fumarate diester supports a proton long-lived state of ~10 minutes and a T_{LLS}/T_1 ratio of ~50 at high field. A ~77 minute long-lived state is provided by a ^{13}C labelled naphthalene derivative in room temperature solution, and a ^{15}N labelled diazirine spin pair exhibits a long-lived state of ~23 minutes at low field. The long-lived state of ^{15}N-nitrous oxide has been recorded utilizing field-cycling equipment and surpasses 26 minutes in solution.

We present a ^{15}N-labelled molecular system ($^{15}N_2$-I, *Fig. 1a*) showcasing a long-lived state lifetime exceeding ~40 minutes in room temperature solution (*Fig. 1b*). Although the spin-lattice and long-lived state relaxation times are relatively short at high field, impressive relaxation times are unveiled at low field. A relatively large relaxation time ratio $T_{LLS}/T_1 \approx 21$ is observed. Experiments at low field make use of a dedicated two-field NMR spectrometer with sample shuttling capabilities. The relaxation mechanisms of the long-lived state are discussed.

These results are encouraging for the future construction of core molecular units which may support long-lived states, and demonstrate that $^{15}N_2$ systems house a suitable target spin pair. We are currently investigating other molecular candidates of this kind. We anticipate that similar spin systems may be easily hyperpolarized, or functionalized for various applications.

Fig. 1 *(a) Molecular structure of $^{15}N_2$-I displaying the preferred 3D geometry in solution. (b) Experimental relaxation curve for the ^{15}N long-lived state of 1M $^{15}N_2$-I in degassed CD_2Cl_2 solution acquired at 0.33 T and 25°C with 2 transients per data point. The decay curve has a multi-exponential form, and was fit with a bi-exponential decay function.*

Image:


a
b
Normalized Singlet Order
1.0
0.8
0.6
0.4
0.2
0.0
0
300
600
900
1200
1500
1800
τ_{EV}/s
T_{LLS} ~ 40 mins

Liquid-state NMR methods

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Interaction of Huntingtin Exon-1 peptides with lipid-based micellar nanoparticles probed by solution NMR and Q-band pulsed EPR

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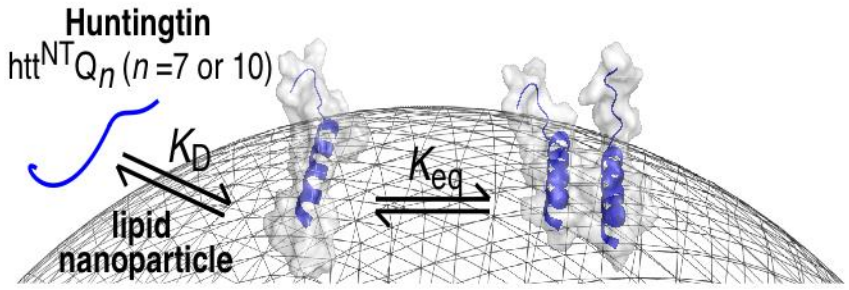
Abstract: Huntington's disease is a fatal neurodegenerative disease arising from the presence of 36 or more CAG repeats within exon 1 of the Huntingtin (htt) gene, resulting in expansion of the polyQ domain that lies immediately downstream of the 16-residue N-terminal amphiphilic sequence (htt^{NT}) of the huntingtin protein. The presence of a long polyQ stretch results in the rapid formation of polymorphic fibrils, the rate of which is modulated by the presence of flanking regions (htt^{NT} and the proline rich domain C-terminal to the polyQ sequence). Although the length of the polyQ domain is directly related to the severity of disease, the aggregation schemes of mutant exon 1 can be further complicated by additional factors including binding to surfaces (including lipid membranes) and the presence of reactive oxygen species (ROS). Recent atomic force microscopic (AFM) studies have emphasized the role of htt^{NT} in modulating the membrane-associated oligomerization on supported lipid bilayers. Nevertheless, the molecular details of how membrane association can possibly impact polyglutamine nucleation as well as the existence of transient oligomeric membrane-associated states involved in the early-stages of amyloid fibril formation are currently missing. In this paper, we have characterized the interaction of two such peptides, $htt^{NT}Q_7$ and $htt^{NT}Q_{10}$ comprising the N-terminal amphiphilic domain of huntingtin followed by 7 and 10 glutamine repeats, respectively, with lipid micelles as membrane-mimic using NMR chemical exchange saturation transfer (CEST), circular dichroism and pulsed Q-band EPR. Exchange between free and micelle-bound $htt^{NT}Q_n$ peptides occurs on the millisecond time scale with a K_d ~ 0.5-1 mM. Upon binding micelles, residues 1-15 adopt a helical conformation. A structure of the bound monomer unit is calculated from the backbone chemical shifts of the micelle-bound state obtained from CEST. Pulsed Q-band EPR shows that a monomer-dimer equilibrium exists on the surface of the micelles and that the two helices of the dimer adopt a parallel orientation, thereby bringing two disordered polyQ tails into close proximity which may promote aggregation upon dissociation from the micelle surface through high local concentration effects. Interestingly, oxidation of Met_7 to a sulfoxide due the presence of ROS reduces the binding affinity ~3-4 fold, increases the length of the helix by a further two residues and inhibits aggregation.

Image:



Liquid-state NMR methods

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Homonuclear ADAPT: A general preparation route to long-lived nuclear singlet order

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Abstract: We introduce a simple strategy to access and readout long-lived nuclear singlet order based on the alternate repetition of hard pulses and delays. We demonstrate the general applicability of this method by accessing long-lived singlet order in spin systems characterized by diverse regimes of chemical inequivalence. The pulse sequence is compared against commonly used long-lived state preparation procedures (Fig. 1). Experimental demonstrations are presented for the cases of a ^{13}C labelled naphthalene derivative (strongly-coupled) and the dipeptide alanine-glycine (weakly-coupled) in room temperature solution.

We show that the pulse sequence is highly efficient and rapidly interconverts transverse magnetization and long-lived singlet order under strong-coupling conditions. The pulse sequence is easy to implement and flexible with respect to the use of small or large hard pulse flip angles. The pulsed method is therefore additionally robust to the choice of excitation and reconversion sequence parameters.

The inter-delay between hard pulses and the number of repetitions is simple to optimize, and can be predicted in the strong-coupling limit or simulated based on prior knowledge of the spin pair J-coupling and chemical shift difference. An analytical expression for the inter-delay and the number of loops has been derived under strong-coupling conditions, and theoretical predictions are in good agreement with experimentally determined parameters. A custom-made *SpinDynamica*-based simulation package is presented which allows numerical calculation of the inter-delay between hard pulses and the number of loops for a pair of spins experiencing any regime of chemical inequivalence.

As the same strategy has recently been implemented to achieve heteronuclear polarization transfer in PHIP and SABRE experiments, we rename the sequence homonuclear ADAPT.

Fig. 1 Experimental relaxation curves for: (a) ^{13}C long-lived singlet order of a 0.2 M $^{13}C_2$-naphthalene derivative in degassed tert-butanol-d_{10} solvent; and (b) ^{1}H long-lived singlet order of 0.1 M alanine-glycine in degassed D_2O solution. Data points were acquired at 16.45 T and 25°C with 2 transients. The ADAPT subscript denotes hard pulse flip angle. (a) Red curve: $ADAPT_{18}$; blue curve: SLIC: black curve: M2SS2M. (b) Blue curve: $ADAPT_{90}$; black curve: Sarkar.

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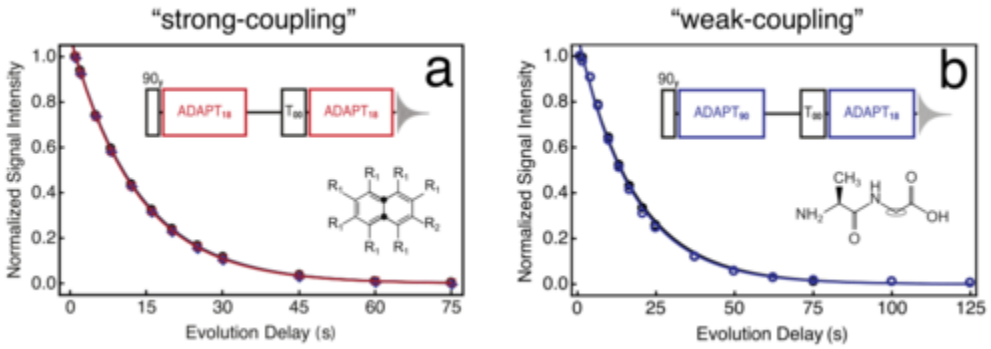

Liquid-state NMR methods

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Singlet-Assisted Diffusion NMR (SAD-NMR) to measuring tortuosity in 3D scaffolds for tissue engineering

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Abstract: Diffusion-NMR is a well-established means of characterizing the structure and fluid transport properties of porous media. Morphological parameters, such as the pore-size distribution, porosity and tortuosity of the pore space can all be determined from measuring the restricted diffusion coefficient at "long" measurement times; i.e. when the displacement of the diffusing particles exceeds the characteristic length scale of the sample. In traditional MR diffusion experiments, the longitudinal relaxation time, T_1, sets an upper limit on the measurement time (a few seconds) and, consequently the length scales that can be investigated (~ 50 - 100 µm). Singlet-assisted diffusion NMR (SAD-NMR) exploits long-lived singlet states to supersede this limit, allowing diffusion to be measured over several minutes [1]. SAD-NMR has recently been used to determine compartment sizes of up to 2 mm in diffusion-diffraction experiments [2] and measure the tortuosity in random packings of mono-sized spheres > 500 µm in diameter, with subsequent pore spaces > 150 µm [2].

We present the application of SAD-NMR to the measurement of tortuosity in tissue engineering scaffolds. These are three dimensional, porous structures that support and guide cell growth for the regeneration of biological tissue, with pores sizes of 50 – 500 um. The pore space in these structures changes with cell proliferation. The ability to non-invasively measure parameters such as the tortuosity of the scaffold is essential for understanding the dynamic nature of nutrient and waste transport within the structure. This information can then be used to inform the design of next-generation scaffold architecture for more optimized tissue growth. 3D-printed, melt-electrospun polycaprolactone scaffolds with a cross-hatch architecture were fabricated, seeded with fibroblast cells, and cultivated in well plates for up to ten days. At several timepoints following initial seeding (3, 5, 7 and 10 days) a subset of scaffolds was removed from the culture medium and fixed, halting the growth process, but preserving the biological tissue structure within the scaffold. The SAD-NMR technique was used to measure the anisotropic diffusion tensor after 90s diffusion time in the fixed scaffolds. We found a decrease in the restricted diffusion coefficient in the seeded scaffolds, compared to the controls, dependent on the timepoint at which the scaffold was fixed. This is consistent with expectations of increased tortuosity as the cells infiltrate the available pore space. Additionally, we found the diffusion anisotropy decreased at longer timepoints, consistent with a more random and spherical-pore space resulting from tissue growth.

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Liquid-state NMR methods

P232

Water suppression for pure shift NMR data: towards cleaner, more sensitive proton NMR spectra of biofluids and other aqueous complex mixtures

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Abstract: Pure shift NMR methods, which have been developed to a high level in recent years for simplifying the appearance of ^{1}H NMR spectra through the elimination of J-coupling patterns, have yielded tantalizing promises of greater resolution in NMR data of complex mixtures of small molecules.[i]-,[ii],[iii] This is particularly relevant to biofluid analysis and the research discipline of metabolic phenotyping. At least three challenges exist by way of ensuring that such data can be routinely delivered: a) robust water suppression; b) adequate signal sensitivity; c) reliable, clean, artefact-free, reproducible data. Of the pure shift methods developed, the 2D interferogram/data reconstruction approach results in fewer artefacts compared with direct data acquisition methods. However, the interferogram approach gives rise to issues when dominating protonated solvent signals are involved, water being a particular example in the context of biofluid analysis. Here we report some exploratory excursions into the use of different approaches to assess the water signal suppression problem in the context of pure shift data acquisitions. Test results are reported from single component solutions, constructed mixtures and natural complex mixtures of small molecules. The data suggest that bracketing with different water suppression modules either side of typical pulse sequences used to acquire pure shift data results in reasonable quality outputs. Speculation is made about further improvements that may be made that could assist with both sensitivity issues and artefact reduction whilst delivering quality, useable data with clean solvent elimination.

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Liquid-state NMR methods

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Models for Polarization Recovery During ASAP and ALSOFAST Experiments

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Abstract: The ASAP method is a scheme to accelerate heteronuclear experiments like the HSQC at natural isotope abundance level. It was originally derived in the ASAP-HMQC and recently received renewed attention in the ASAP-HSQC. Sharing the polarization of active protons with the surrounding reservoir can result in seemingly instant polarization recovery and therefore enormous gains in sensitivity, but can also lead to a slight reduction of polarization and spectral intensity, depending on sample and setup. To our knowledge, no attempt to describe its available polarization has been undertaken.

Deriving from the well-known Ernst angle model, we present mathematical models to describe the polarization over the course of ALSOFAST and ASAP type experiments. The models can be used to visualize the initial scans of an experiment and even more importantly, show the polarization and achievable signal intensity in the steady state of an experiment. Together with the theoretical model, corresponding experimental data on the steady state was obtained by a special saturation recovery type pulse sequence and examples are shown for different spin environments. The results show good agreement between theory and experiment.

The models show that the ASAP experiment allows higher effective excitation than the ALSOFAST experiment in many cases. However, as expected, the number of coupled spins plays an important role for sensitivity. Together with the relaxation times it determines the optimal experiment for given experiment durations. The models help selecting the right experiment and allow accelerated setup, but also are a step towards quantitative interpretation of ASAP and ALSOFAST experiments. The models show that the ASAP experiment allows higher effective excitation than the ALSOFAST experiment in many cases. However, as expected, the number of coupled spins plays an important role for sensitivity. Together with the relaxation times it determines the optimal experiment for given experiment durations. The models help selecting the right experiment and allow accelerated setup, but also are a step towards quantitative interpretation of ASAP and ALSOFAST experiments.

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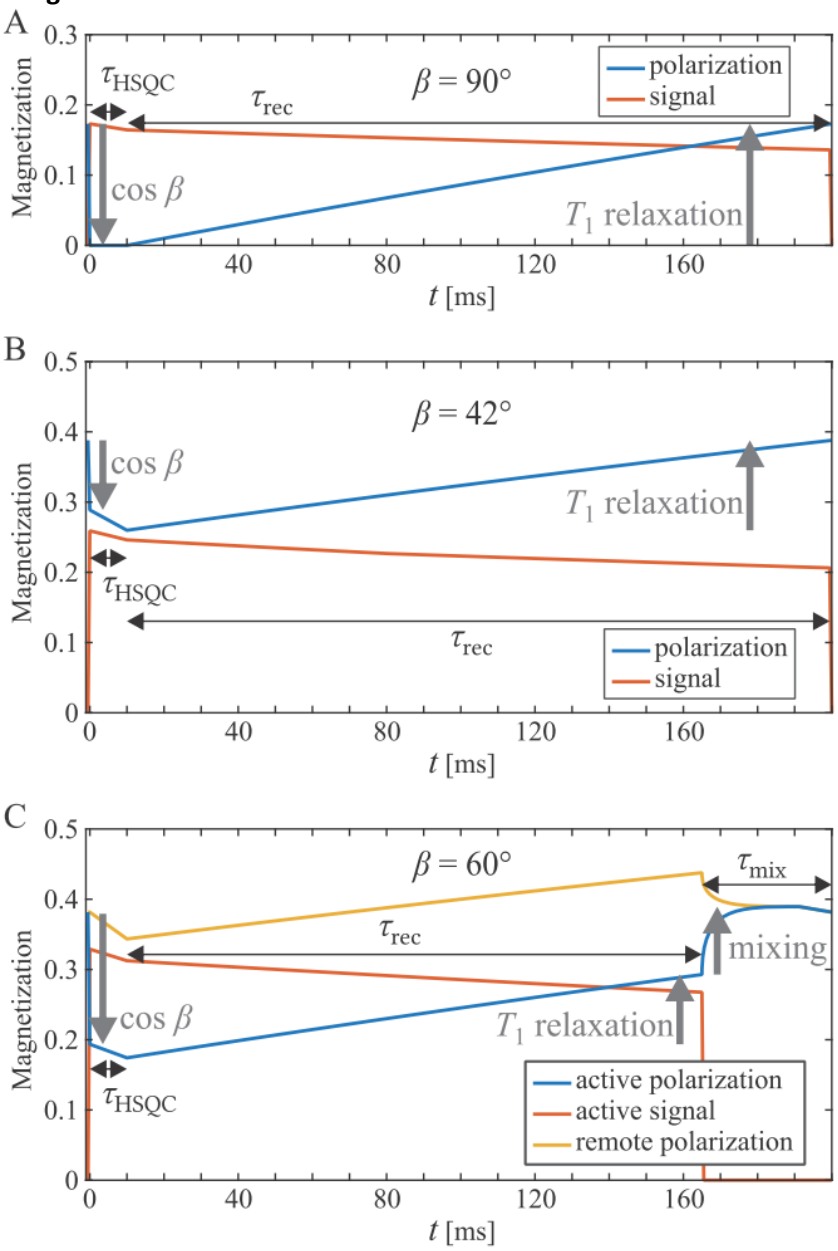
A
Magnetization
t [ms]
τHSQC
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τHSQC
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polarization
signal
C
β = 60°
τmix
τrec
mixing
cos β
T1 relaxation
τHSQC
active polarization
active signal
remote polarization

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Looped, PROjected SpectroscopY (L-PROSY) as a sensitivity-enhancing approach for detecting HSQC-TOCSY cross-peaks at natural abundance

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Abstract: 2D HSQC-TOCSY is a widely used experiment involving the establishment of correlations between heteronuclei such as ^{13}C or ^{15}N, and all protons partaking of a common coupled network with the heteronuclei-bonded ^{1}Hs.[1] It follows that a majority of protons in these experiments, those bonded to ^{12}C or ^{14}N, are only passively used, and act as "recipients" of the polarization starting from the heteronuclear pair. In parallel, methods have been developed (for other purposes) that exploit non-hetero-bound protons; most notably the ASAP experiment that relies on homonuclear isotropic mixing to transfer magnetization from the unused proton reservoir to the ^{13}C bound protons.[2] **The present study explores an alternative possibility to use proton reservoirs, for enhancing signals in complex experiments such as HSQC-TOCSY or HSQC-NOESY.** These gains arise from the recently introduced Looped, PROjected SpectroscopY (L-PROSY, as in "leprosy") approach, which enhances spin-diffusive processes by looping them in multiple projective measurements.[3] To extend L-PROSY's ideas to HSQC-TOCSY correlations we incorporate into them cross-polarization hetero-transfers and a homonuclear isotropic mixing stage, leading to the scheme illustrated in Figure 1a. This sequence involves looping a block that begins and ends with the ^{1}H reservoir along the longitudinal, z-state. At the same time, however, it amplitude-modulates the full ^{1}H network connected by the isotropic mixing process, with the ^{13}C frequencies encoded over t_1. More importantly, when compared to the conventional 2D HSQC-TOCSY experiment where all the polarization that is eventually detected initiates from the ^{13}C-bound protons, the L-PROSY scheme repeatedly shares the ^{13}C modulation with the full proton pool, and subsequently repolarizes back ^{13}C-coupled protons. After multiple repetitions of this (e.g., Figure 1b) the result is a Zeno-like process,[4] whereby the modulation imparted on the full proton spin pool is significantly enhanced. Figure 1c demonstrates this for two model compounds, for which ≈300% signal enhancements vis-à-vis conventional experiments are observed. The precise extent of L-PROSY's sensitivity improvement will depend on multiple parameters including the number of protons in the spin-coupled networks of the molecule, their J-coupling values, as well as the proton T_1 relaxation dictating the maximum plateau that can be reached by these values. These parameters were examined in Bloch-McConnell simulations and experimental enhancements match the predicted values. Further applications of this method to improve HSQC-TOCSY correlations in biomolecules such as amino acids, peptides and sugars, will be examined and presented.

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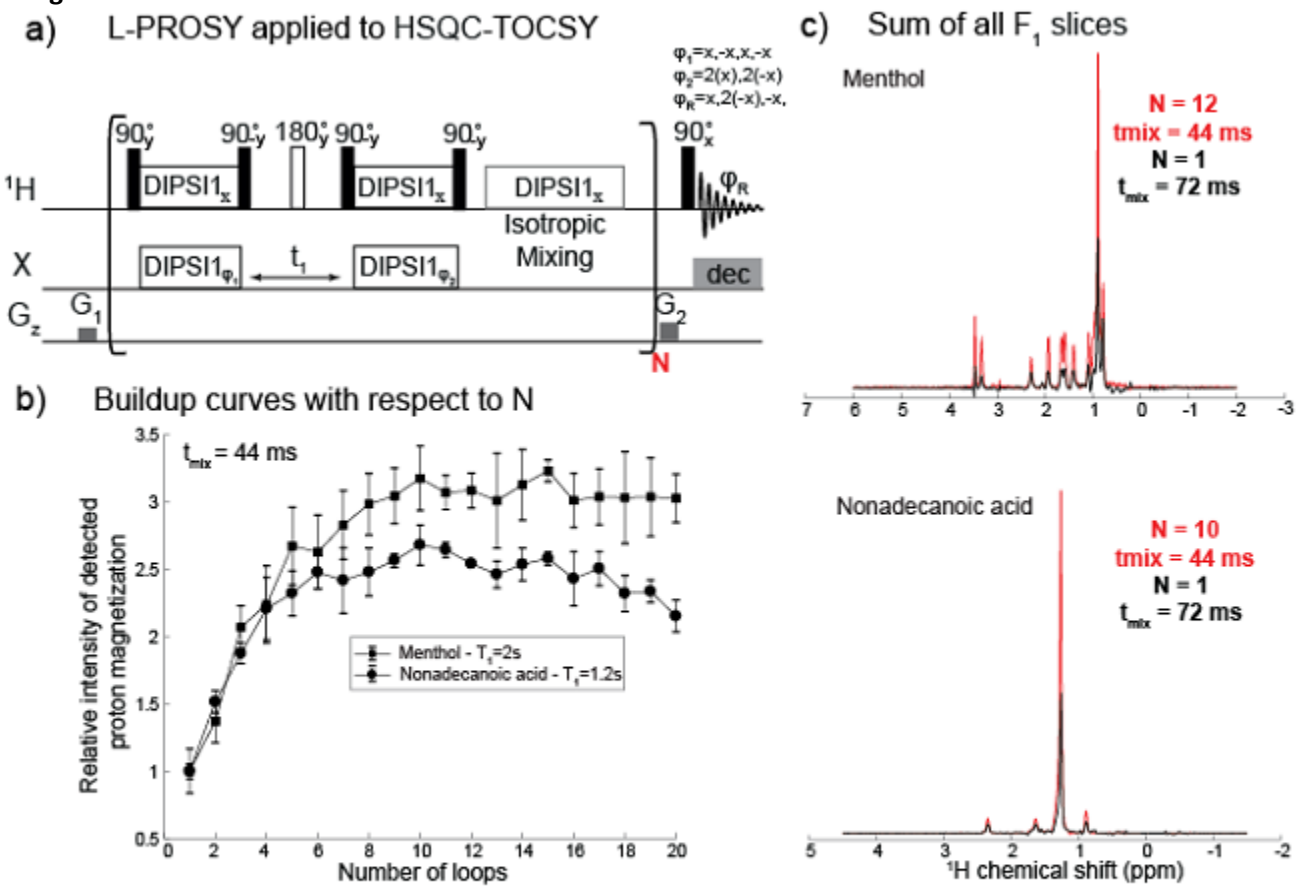

Figure 1. a) L-PROSY scheme as adapted to HSQC-TOCSY. b) Buildup curves with respect to the number of loops for Menthol and Nonadecanoic acid (1 corresponding to the unlooped, conventional experiment). c) Comparisons of the sum of F1 traces from conventional and from L-PROSY HSQC-TOCSY experiments demonstrating the enhancements for total ^{13}C-selected resonances.

1) Lerner L.; Bax A, *J. Magn. Reson.*, **1986**, *69*, 375; 2a) Kupče, E.; Freeman, R. *Magn. Reson. Chem.* **2007**, *45* (1), 2.; 2b) Schulze-Sünninghausen, D.; Becker, J.; Luy, B. *J. Am. Chem. Soc.* **2014**, *136* (4), 1242; 2c) Suryaprakash, L.; Suryaprakash, *N. Chem. Commun.* **2014**, *50* (62), 8550; 3) Novakovic M., Cousin S., Frydman L., L-PROSY abstract nr 15. In: 59th ENC; Orlando, Florida; 4) Bretschneider C. O., Alvarez G. A., Kurizki G. and Frydman L., *Phys. Rev. Lett.*, **2012**, *108*, 1.

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NMR relaxation rates and diffusion processes in aqueous solutions of Gd (III) ion doped upconversion nanoparticles

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Abstract: Upconverting nanoparticles (UCNPs), especially Gd-containing, attract an increasing interest as magnetic and optical bimodal nanoplatform. It has been widely known that the coating layers enhance their fluorescent intensities. However, the main challenge remains: how to optimize the UCNP's structure and size in order to achieve the most efficient relaxivity without loss of their optical properties. The relaxation mechanism in such paramagnetic systems is usually explained by three sub-systems: the water molecules that bind directly to the gadolinium ion forming the first coordination shell, water molecules with protons that make up the long lived second coordination shell and water molecules that move in the bulk.

Only in the first sub-system spin-electron interaction takes place. This is why processes around the surface of the NPs is at the first importance. If surfactant molecules are used, water is slowed down, hence diffusion must also decelerate. This is the reason why diffusion measurements can give fundamental details of such system when effectiveness of magnetic resonance imaging (MRI) contrast agent is concerned. The goal of our work is to measure the longitudinal spin relaxation rates in aqueous solutions of chosen Gd-containing nanoparticles, as well as to analyze the diffusion processes of water molecules at the NP's surface and in the bulk.

Two aqueous solutions of different sized *β*-$NaGdF_4$ NPs (12 nm core and 26 nm core-shell) were prepared. Both NPs were coated with TWEEN80 to improve the solubility of NPs. NMR experiments (T_1 and diffusion measurements) were performed at 9.4 T magnetic field. The experimental results were compared with those obtained with commercial contrast agent GD-DO3A-butrol (*Gadovist*).

In vitro relaxivity measurements have shown that the ionic relaxivity increased with decreasing NPs size (Fig. 1). It clearly indicates the role of surface Gd^{3+} ions in the relaxivity enhancement. Additionally, the diffusion experiments have revealed two components (Fig. 2) having different diffusion rates that could be attributed to water molecules in the solvation layers and in the bulk.

The obtained results are promising for further research and modeling of different Gd-doped nanostructures. They should help predicting the major factors that influent the rates of the longitudinal relaxation and finding the most optimal structure of UCNPs for magnetic and upconversion fluorescent bimodal imaging.

Image:

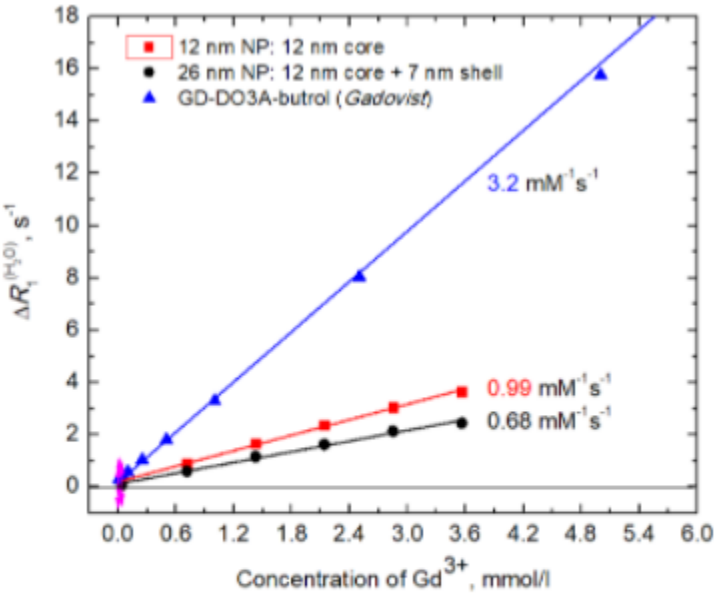

Figure 1. Longitudinal relaxation rates in aqueous solutions of NPs as a function of concentration of Gd^{3+} ions at a temperature of 37°C.

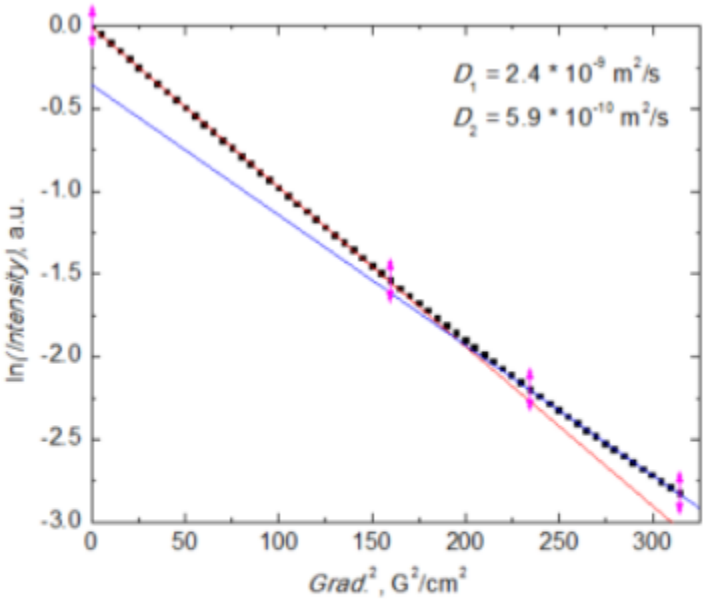

Figure 2. Signal decay of aqueous solution of NP versus squared gradient strength. Two separate diffusion coefficients (D_1 and D_2) were calculated by fitting.

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SCoT: Swept coherence transfer for quantitative 2D NMR

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Abstract: Quantitative Nuclear Magnetic Resonance (qNMR) Spectroscopy has become very popular topic in recent years, mainly due to growing interest in metabolomics and related sciences. However, the qNMR is rather limited to one dimensional (1D) spectra and offered resolution is becoming not sufficient. The use of 2D NMR techniques would definitely solve the peak overlap problem, but there are more factors affecting quantitativeness.

The main problem in a case of heteronuclear experiments is uneven coherence transfer from excited/detected ^{1}H nuclei to other nuclei (typically ^{13}C). The Insensitive Nuclei Enhancement by Polarization Transfer (INEPT) employs J-couplings to transfer coherence between pairs of nuclei and the optimal transfer occurs when Δ (INEPT delay) is equal to 1/2J. The mismatch of Δ and 1/2J will result in a peak intensity loss. This is very pronounced in one of the most popular experiments – ^{13}C Heteronuclear Single-Quantum Correlation (HSQC), where $^{1}J_{CH}$ can take a broad range of values.

In this study, we propose a Swept Coherence Transfer (SCoT) method to optimize coherence transfer efficiency for each coupled pair of heteronuclei in a sample. The method is based on co-incrementation of Δ with non-uniform sampling of evolution time. Data reconstruction is performed on overlapping data subsets referred to as moving frame processing. The result is a stack of 2D NMR spectra which are optimized for linearly increasing J-coupling (J_{av}) values. The peak intensities in third pseudo-dimension (J_{av}) reflect polarization transfer curve, therefore peak maximum can be easily found. Moreover, the obtained curve provides better insight into experimental imperfections, which could disturb peak intensities, e.g. pulse miss-calibration.

SCoT method was successfully applied in ^{13}C HSQC experiment and ^{13}C HSQC multiplicity edited experiment. In the second example, Δ and δ (multiplicity - edit delay) were co-incremented simultaneously with non-uniform sampling of evolution time. This allowed us to find peaks with maximum intensity and correct phase in indirect dimension at the same time. We also prove that such experiments can be acquired in a time corresponding to less than two conventional HSQC spectra and provide reliable results.

The analysis workflow for SCoT method applied to quantitative 2D NMR is shown in the Figure below.

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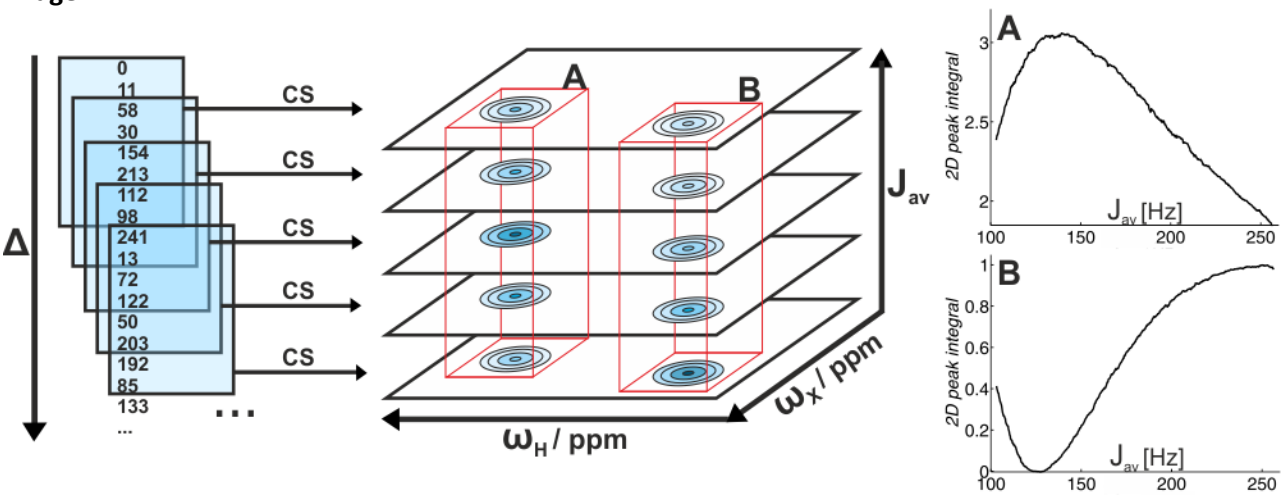

Liquid-state NMR methods

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Semi-automated NMR assignment of small to medium-sized molecules with novel edited variants of HSQC-CLIP-COSY experiment

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Abstract: The recently introduced CLIP-COSY[1] experiment providing homonuclear correlation spectrum with high quality clean in-phase multiplets expedites the assignment of scalar coupled proton spin network and so, the structure elucidation of small and medium-sized molecules. However, the resolution of COSY spectra is limited by the inherently small chemical shift dispersion of proton resonances.

To utilize the resolving power of heteronucleus offered by its superior chemical shift range, we have devised an HSQC-variant of the CLIP-COSY experiment.[2] Herein, we demonstrate that the performance of the original HSQC-CLIP-COSY experiment can be further boosted with incorporation of heteronuclear spin echo block(s) at appropriate position of the pulse sequence. These novel experiments allow editing of X-nuclei according to even or odd multiplicity and/or editing of direct HSQC correlations vs. CLIP-COSY peaks. The different phases imposed on the direct and relay peaks facilitate to track out the connectivity network of protonated carbons, making the assignment of both ^{1}H and ^{13}C resonances of small to medium-sized molecules possible from a single spectrum. Besides, utilizing the enhanced resolution of HSQC-CLIP-COSY spectra recorded with the non-uniform sampling (NUS) scheme, signal cancellation of accidentally overlapping peaks with opposite phase can be eliminated or significantly reduced.

The potential of these experiments will be illustrated on the alkaloid strychnine and on an oligo-peptide and -saccharide.

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[2] T. Gyöngyösi, I. Timári, J. Haller, M. R. M. Koos, B. Luy, K. E. Kövér, *ChemPlusChem* **2018**, *83*, 53 – 60.

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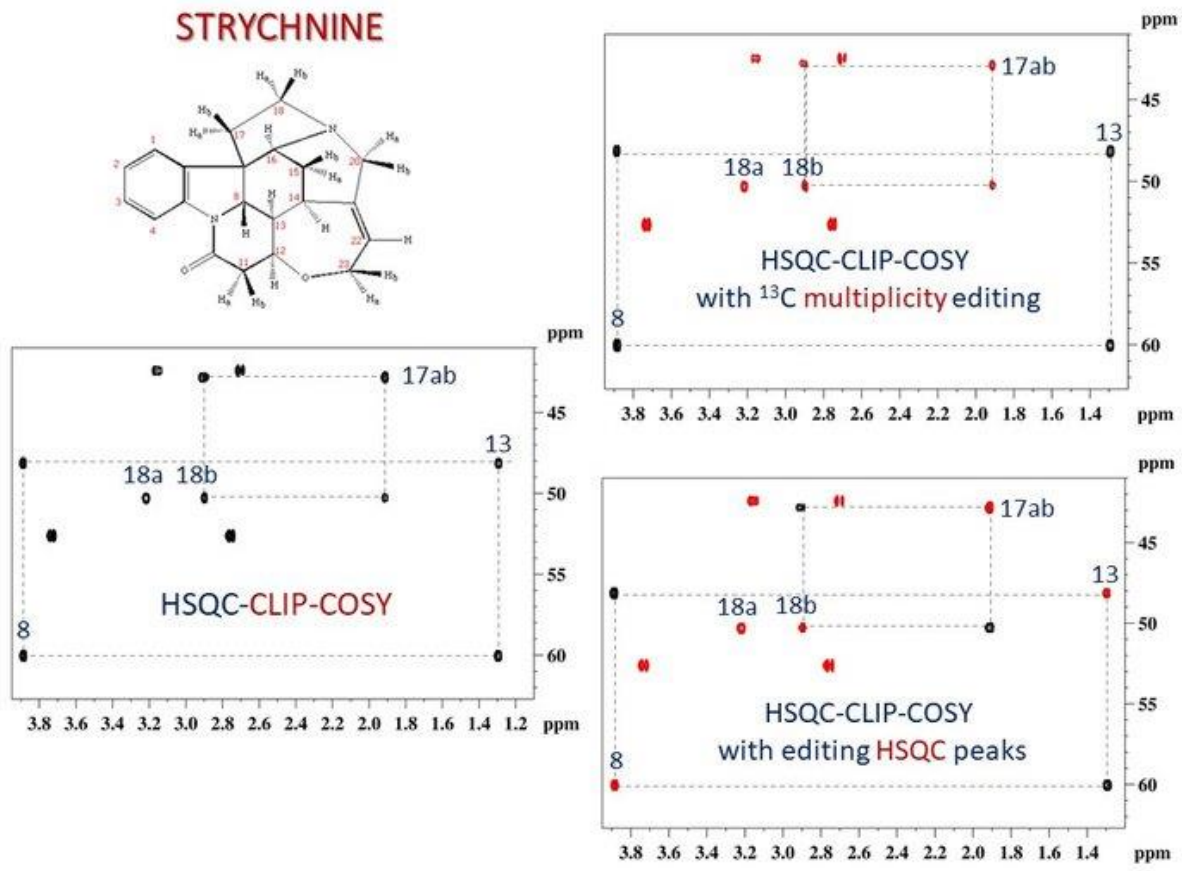
STRYCHNINE
17ab
13
18a 18b
8
HSQC-CLIP-COSY
HSQC-CLIP-COSY
with ^{13}C multiplicity editing
HSQC-CLIP-COSY
with editing HSQC peaks
ppm
3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2

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Intrinsic anisotropy parameters of the paramagnetic susceptibility tensors of a complete (La to Lu) series of lanthanide chelating tags and a comparison to the protein derived tensors by PCS and RDC experiments.

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Abstract: Lanthanide induced pseudo contact shift (PCS) NMR has been used increasingly in the last two decades as an advanced methodology for studying interactions, dynamics and structures of proteins. The long range nature of PCS of up to 70 Å, their high precision and the fact that they can easily be obtained by two-dimensional (TROSY-) HSQC type NMR experiments even on large protein complexes makes same very versatile tools in structural biology. A major challenge in applying PCS analysis is the need for rigid and high affinity chelators that can fix the lanthanide ions to the protein under investigation. Various strategies have been applied and one of the most promising approaches uses lanthanide chelating tags based on the DOTA-chelator that is tethered by one or two attachment points to the protein. Many different designs have been reported, but the properties of the different tags vary dramatically and only empirical studies are available to assess the performance of the tags. Crucial parameters are the two anisotropy parameters $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$ of the paramagnetic susceptibility tensor of the tag as they determine the size of the observed PCS and, hence, the distance range of useful applications. Up to now, only the effective anisotropy parameters have been determined by fitting the observed PCS on a protein, which does not account for the local mobility of the tag relative to the protein.

In the study presented here, we have for the first time analysed the intrinsic tensors of a complete series of 13 lanthanide tags from lanthanum to lutetium (omitting radioactive promethium and isotropic gadolinium ions) by assigning the ^{1}H and partially also the ^{13}C spectra of the lanthanide chelating tags. The challenge of analysing 1H spectra with a spectral width exceeding 1400 ppm and peaks with a line width of more than 5000 Hz was achieved by partial ^{13}C labelling of the tags and an iterative assignment strategy using DFT calculations and tensor fitting with combinatorial methods, together with old fashioned double-resonance experiments as modern two-dimensional NMR methods failed. It is noteworthy, that in contrast to current theoretical models we have clearly observed that contact shifts also play a role, even for proton spins remote from the lanthanide ion.

The resulting tensors are suprisingly large and comparison with the tensors obtained from PCS of the tags when conjugated to a ubiquitin mutant as well as the corresponding RDC alignment tensors reveal that there is still plenty of room for improvment of the mode of attachment to the proteins. We hope that this data can serve as an upper boundary for the development of new lanthanide chelating tags.

Figure 1: ^{1}H-NMR spectrum of the Dy-tag

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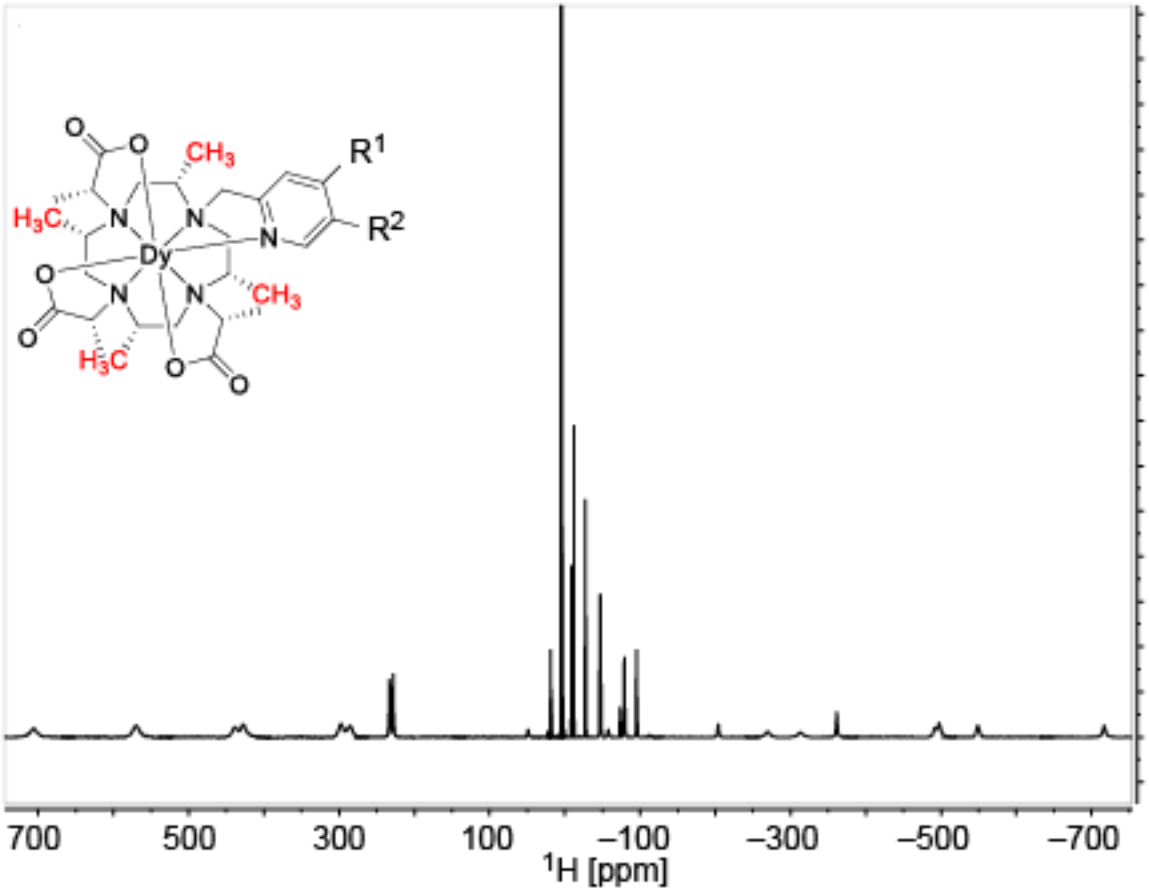
O
O
CH3
R1
H3C
N
N
R2
Dy
N
O
N
CH3
O
H3C
O
O
700
500
300
100
−100
−300
−500
−700
1H [ppm]

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Looped, PRojected SpectroscopY (L-PROSY /lep.rə.si/): A sensitivity-enhanced experiment for detecting backbone/sidechain NOE cross-peaks in proteins

Mihajlo Novakovic[*1], Michael Jaroszewicz[1], Samuel Cousin [1], Rina Rosenzweig[2], Lucio Frydman[1]

[1]Department of Chemical and Biological Physics, [2]Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel

Abstract: Nuclear Overhauser Effect (NOE),1 a result of dipole-dipole cross-relaxation phenomena2, lies at the center of structural determinations by NMR.3 Since cross-relaxation depends strongly on the spatial distance, NOESY4 relies on these effects in order to define inter-site connectivities and molecular geometries under physiologically-relevant conditions. Relatively low sensitivity, as off-diagonal cross-peaks carrying the structurally relevant information only involve a small fraction of the total magnetization, was always one of the NOESY's known weaknesses. In this study we introduce a simple and effective approach dubbed looped PROSY, capable of enhancing the NOE cross-peaks between distinct groups in proteins (e.g., amide and aliphatic resonances) by sizable factors. The enhancement is based on repeating a selective NOE's Ramsey perturbation multiple times, along the lines illustrated in red in Figure 1a. The evolution of amide and aliphatic spins in a small protein following a single such perturbation is shown with the black-colored curves in the figure: under the effects of cross-relaxation the aliphatic protons at the beginning build up promisingly, leading to a cross-peak accounting for ≈13% of the initial amide magnetization, but soon after they start decaying due to the auto-relaxation. By contrast, if the Ramsey projection is looped multiple times using shorter mixing times, destructive auto-relaxation is frozen, the initial buildup slope is reset, and a monotonic increase of the cross-peaks is achieved leading to an enhancement of 2-10x in the observable cross-peaks (red curves in figure 1a). This Zeno-like measurement5 also helps preserve the magnetization of the amides. To exploit this principle, we incorporated L-PROSY into two novel experiments: 15N-1H HMQC-NOESY and 15N-filtered 2D NOESY. Figure 1b illustrates the latter's sequence, and figure 1c demonstrates that the cross-peaks extracted from the corresponding 2D 15N-filtered experiment, possess enhancements that match well the theoretical predictions. In general, the protein correlation times and the amide-water chemical exchange rate will dictate the overall SNR improvement achieved; these parametric dependencies were examined, and L-PROSY's enhancements were confirmed on many different proteins. In particular, L-PROSY cross-peak enhancements ≥5x can be observed for unstructured proteins, where chemical exchange with the solvent averages out NOE cross-peaks in conventional experiments, while it boosts L-PROSY effect by replenishing faster the amide's magnetizations within each loop. In summary, L-PROSY is a Zeno-derived experiment capable of prolonging initial, effective NOE buildup with no penalty: cross-peaks are enhanced and remain structurally valuable. Additional applications of these concepts to be used for small molecules as well as to involve other transfer mechanisms (TOCSY, ROESY, spin-diffusion) will be explored and presented.

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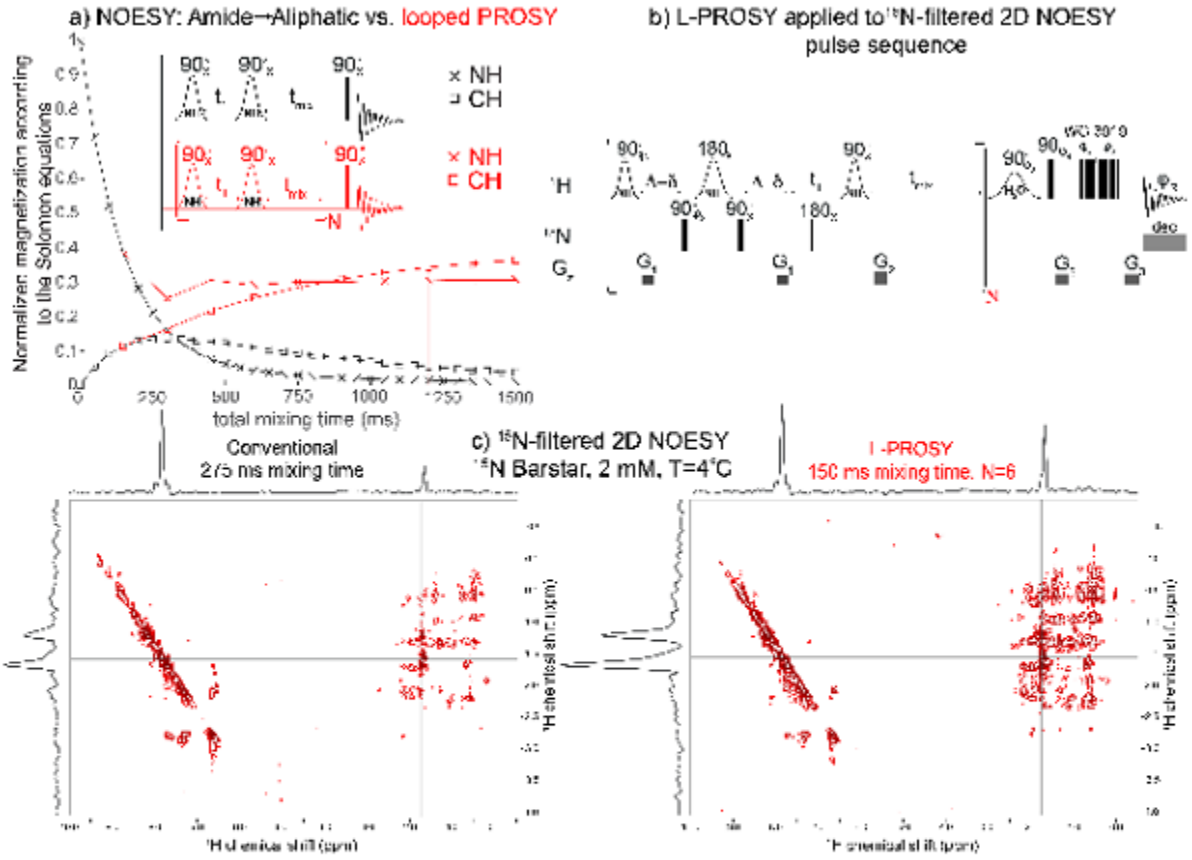

Figure 1 a) Simulations based on Solomon's equations proving the efficiency of looped PROSY over NOESY. b) L-PROSY ^{15}N-filtered NOESY pulse sequence. c) ^{15}N-filtered NOESY 2D spectra with extracted projections to demonstrate ≥2-fold enhancements of L-PROSY NOESY. Data collected on an iNova^3 console at 11.7 T and 4 °C using an HCN probe.

[1]J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect", Academic Press, New York, 1971; [2]I. Solomon, Phys. Rev., 1955, 99, 559; [3]J. Cavanagh, "Protein NMR spectroscopy", Academic Press, 2007; [4]A. Kumar, R. R. Ernst and K. Wüthrich, Top. Catal., 1980, 95, 1; [5]A. Bax and M. A. Weiss, J. Magn. Reson. 97, 71, 571; [6]C. O. Bretschneider, G. A. Alvarez,G. Kurizki and L. Frydman, Phys. Rev. Lett., 2012, 108, 1;

Acknowledgement: This work was supported by the Kimmel Institute of Magnetic Resonance (Weizmann Institute), the French Foreign Service, and the EU Horizon 2020 programme (MC Grant 642773).

Materials

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Water Dynamics in Perfluorinated Ionomer Membranes: Hydration-Level Dependent Characterization of Local Dynamics and Long-Range Motion

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Abstract: Perfluorinated sulfonic-acid (PFSA) membranes are polymers known for their high ionic conductivity and mechanical stability. The most popular PFSA membrane is Nafion, which was developed by DuPont in the 1970s. Since then, it has been used in electrochemical applications, like low-temperature fuel cells and chlorine-alkali electrolysis. For the given application, the understanding of local molecular environments and the nano-morphological structure plays a crucial role for improvement of the performance and electrochemical properties.[1]

In this work, we have characterized Nafion 117 membranes at different stages of hydration via temperature-dependent ^{1}H T_1 relaxation and ^{1}H PFG NMR experiments. The molecular environment in close vicinity of the sulfonic acid group was analyzed using an extended BPP model.[2] This model yields information about the activation energy of the local water dynamics, its motional correlation time, and internuclear distances between the two involved protons. Furthermore, motions on the millisecond time scale were investigated by temperature-dependent ^{1}H diffusion measurements. On the basis of T_1 data, we show that the formation of the first hydration shell around the sulfonic acid group occurs in the presence of four water molecules, while the second hydration shell is formed in the presence of eight water molecules. At higher hydration levels, the amount of free water molecules is increased, resulting in larger pore diameters. Furthermore, the freezing of water at hydration level >8 water molecules was observed, while at lower hydration levels (< 9 water molecules) the freezing process is inhibited. The diffusion NMR data also indicated a three-region behavior. At low hydration levels, where up to four water molecules reside in the first hydration shell of the sulfonic acid group, the ^{1}H diffusion coefficients are strongly reduced compared to that of free water. This is attributed to the strong hydrogen bonding and electrostatic interaction between the SO_3^-/H_3O^+. At higher hydration levels, the influence of the sulfonic acid group is reduced, resulting in fast diffusion similar to free water. Finally, the change in the proton transport mechanism from vehicular mechanism towards the Grotthus mechanism can be identified with increasing hydration level.

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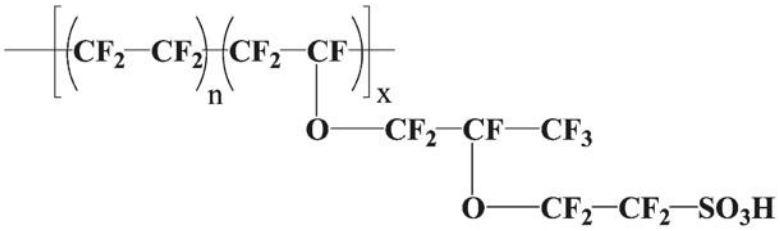

Materials

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13C/19F dipolar/J correlation solid-state NMR studies on fluorine-graphite intercalation compound

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Abstract: As an electrode material of fluoride shuttle battery, fluorine-graphite intercalation compound, C_xF, is promising because it shows fluoride ion conductivity and also shows a high theoretical capacity of 865 mAh/g at $x = 1$. In this work, $^{13}C/^{19}F$ high-resolution solid-state NMR was applied to examine local structures of a stage-1 fluorine-graphite intercalation compound ($C_{2.8}F$). Four ^{19}F (F1~F4) and two ^{13}C signals (C1 and C2) unraveled by high magnetic field (14 T) and fast magic-angle spinning (<35 kHz) were examined by various two-dimensional correlation experiments. In addition to "through space" ^{13}C-^{19}F and ^{19}F-^{19}F dipolar correlation, which reveals distance proximities among $^{13}C/\ ^{19}F$ spins, we examined feasibility of applying the *J* interaction for examination of "through bond" correlation. These experiments led assignment of two of the four F signals to F directly covalent bonded to sp^3 carbon (F2 and F3) and an interleaving domain for the local structure of the minor C2-F3 group among the major domain composed of C2-F2 and sp^2 carbon (C1). The other two ^{19}F signals (F1 and F4) were assigned to as CF_2 and F ions, respectively. To appreciate the s-covalency of the C-F bond, whose bond length (about 0.140 nm) is slightly longer than that in $(CF)_n$ and $(C_2F)_n$ (0.136 nm), we obtained the one-bond spin-spin coupling constant (J_{CF}). The observed non-zero one-bond *J* coupling value (193 ± 4 Hz) for C2-F is a spectroscopic evidence for the C-F bond being the s bond. Further, the similar J_{CF} = 197 Hz for C-F in poly(carbon fluoride) confirmed that the so-called "semi-ionic/semi-covalent" C-F bond in $C_{2.8}F$ is actually a standard covalent C-F bond.

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Materials

P242

New local structure detected in alkali activated fly ash

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Abstract: During the last two decades, alkali-activated materials have received considerable attention for their competitive mechanical properties over cement binders and for the increasing stress for greenhouse gas reduction. Metakaolin (calcined kaolinitic clay), ground granulated blast furnace slags and fly ashes are the mostly used precursors. The alkali-activation of metakaolin (with Na or K silicates) was shown to form a three-dimensional network where SiO_4 and AlO_4 tetrahedra (Q_4 and q_4 species, respectively) are fully condensed (the so-called geopolymer).[1,2] In contrast, for Ca-containing precursor like slags, calcium aluminium-silicate hydrates (C-A-S-H) are formed and can compete with the 3D geopolymeric network.[3,4] C-A-S-H phases are built of chains of SiO4 and AlO4 tetrahedra (Q_2 and q_2 species) possibly cross-linked (Q_3 and q_3 species).

^{29}Si and ^{27}Al NMR solid-state NMR was proved to be a very useful and efficient technique in such amorphous or poorly crystallized compounds due to its ability to discriminate Q_n and q_n species ($0 \leq n \leq 4$).

In the present study, we compare the alkali activation of 4 fly ashes by solid-state NMR. Two of them have low Ca content and show the formation of 3D network after activation. In contrast, the Ca-containing fly ashes show the coexistence of both 3D network and C-A-S-H phases. For activated slags, it was already observed that the 3D network can turn into C-A-S-H phase over time. Our data suggest the possibility for Q_2 and q_4 species to belong to the same phase in contradiction with the structure of both 3D network and C-A-S-H. Such coexistence, implying the creation of local structures in which Q_2 silicate chains are linked by q_4 Al species, might be viewed as a transitional phase involved in the 3D network to C-A-S-H transformation.

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Materials

P243

Solid-State 7Li NMR characterization of metallic lithium deposition on the graphite electrode in Li-ion batteries

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Abstract:

Lithium-ion batteries (LiB) are present in our day-to-day lives through lots of applications, from mobile phones to electric vehicles. Despite their common use, numerous mechanisms are still poorly understood and they can reduce significantly the lifetime of the battery.

Lithium plating is one of those phenomena in LiB[1]. Metallic lithium is deposited on the negative electrode, especially for graphite. High charging rates and low temperatures usually encourage its deposition. Lithium plating on the surface of the electrode implies a loss of exchanged lithium ions between the electrodes, which is equivalent to a loss of battery capacity and which means a loss of driving range in the case of electric vehicles. In addition, it could lead to a shortcircuit of the battery in the case of dendrite growth, which might result in electrolyte combustion.

The working voltage for the graphite electrode is close to 0 V (vs Li^+/Li^0) at full lithiation, which is favourable for lithium plating. Overpotential can locally be observed in the negative electrode and lead to metallic lithium deposition on the electrode instead of lithium insertion. Li dendrites and/or mossy structures on a lithium electrode have already been observed by *in situ* ^{7}Li NMR spectroscopy[2,3]. Graphite lithiation has also been observed before by *in situ* ^{7}Li NMR[4,5]. ^{7}Li NMR studies also detected lithium deposition on graphite in abusive conditions[6,7].

We study lithium deposition and the variety of onset conditions on graphite as close as possible to the commercial conditions. We work with a full electrochemical cell with NMC 622 and graphite electrodes and a standard electrolyte solution. We follow charge protocols in agreement with industry on an electrochemical cell[8] compatible with *in situ* ^{7}Li NMR spectroscopy and developed at the CEMHTI laboratory.

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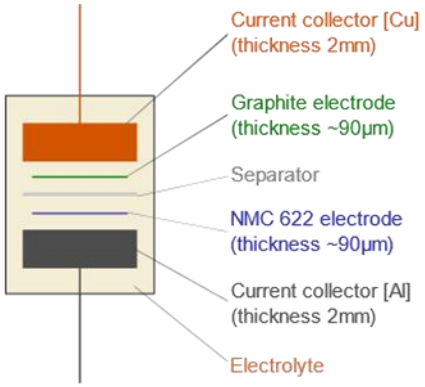
Current collector [Cu]
(thickness 2mm)
Graphite electrode
(thickness ~90µm)
Separator
NMC 622 electrode
(thickness ~90µm)
Current collector [Al]
(thickness 2mm)
Electrolyte

Materials

P244

REMOTE DETECTION NMR IMAGING OF CHEMICAL REACTIONS AND ADSORPTION PHENOMENA

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Abstract: Remote detection (RD) [1,2] is a novel tool in the already versatile toolbox of NMR spectroscopy. In the RD setup, the encoding of NMR or MRI information is physically separated from the detection of the signal. The studied sample itself is stationary and only the information encoded into the magnetization of a carrier fluid is moving with the fluid flow from the encoding region to a detector. This leads in many cases to a significant sensitivity enhancement when compared to traditional NMR setup as the separation allows optimized filling-factor and smaller size of the detection coil. In addition, the setup gives highly useful time-of-flight (TOF) information.

In this study, we have proven the unique usefulness of the RD NMR in characterization of microfluidic chemical reactions and adsorption phenomena. As a result, three topics are presented:

Firstly, we developed the new concept of remote detection exchange (RD-EXSY) NMR spectroscopy for more accurate reaction imaging in microfluidic flow systems.[3] The experiment can reveal active regions, reaction pathways and intermediate reaction products inside a microfluidic reactor. Additionally, we illustrated that direct spatial resolution can be added to RD-EXSY efficiently while applying the principles of Hadamard spectroscopy, making the experiment time much shorter than in traditional approach.

The second topic deals with the development and characterization of novel type of microfluidic hydrogenation reactors. In the project, atomic layer deposition (ALD) method was used for the first time to deposit both catalyst nanoparticles and support material on the surface of wall-coated microreactors. Continuous flow hydrogenation reaction was studied by means of RD NMR. Reaction yield, mass transport phenomena and the activity of the catalyst surface were determined from the data.[4]

Thirdly, we invented a new RD NMR based method for efficient, in situ quantification of adsorption of multicomponent gases flowing through porous materials.[5] Traditionally adsorption isotherms are determined in static conditions for a single gas only. However, we have shown that TOF images of a single RD NMR experiment make it possible to determine the amount of adsorbed gas (n_{ua}) of each gas component in the gas mixture.

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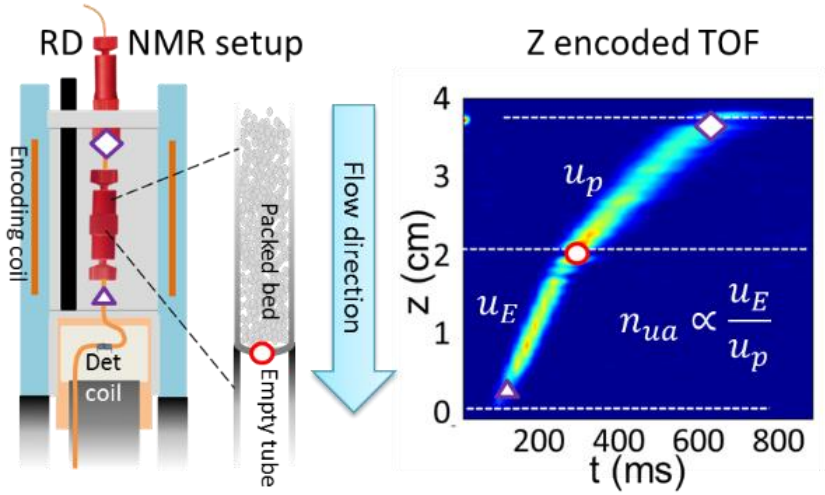
RD NMR setup
Z encoded TOF
Encoding coil
Det coil
Packed bed
Empty tube
Flow direction
u_p
u_E
$n_{ua} \propto \frac{u_E}{u_p}$
z (cm)
t (ms)
0
1
2
3
4
200
400
600
800

Materials

P245

1H NMR determination of curcumin partition in dual confinement

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Abstract: Curcumin is a molecule of high interest for its strong therapeutic potential, especially thanks to its anti-oxydant and anti-inflamatory properties. Confining curcumin in biocompatible solid lipid nanoparticles (SLN) is a possible way to overcome challenges in its oral delivery, such as poor solubility and rapid degradation in water. We therefore present a study of a novel drug delivery system[1], where SLN stability is increased by encapsulation in a porous ordered silica shell (SBA-15). The resulting material exhibits a structure of mesopores filled with surfactant micelles (P123), linking macropores containing the lipid cores (stearic acid or cetyl palmitate).

Our main objective is to understand the lipid-dependent curcumin release profiles[2], which are influenced by host-guest interactions, the guest's structure and dynamics, and the drug partitioning inside this complex system. The low curcumin concentration and the system heterogeneity raise two important challenges as sensitivity and resolution are strongly limited. Thus, we rely on modern solid-state NMR techniques to perform our study. Firstly, sensitivity and resolution issues are addressed by performing ^{1}H NMR at ultra high-field (23.5 T) and fast MAS (60 kHz). Then, we improve resolution by a combination of filtered 2D homonuclear correlation experiments (Hahn echo, dipolar zero (RFDR) and double (BABA) quanta,…). Finally intermolecular contacts are validated by the comparison of blank and loaded SLN spectra. Surprisingly, spectra do not display correlations between curcumin and lipids carriers, but only between curcumin and surfactant P123. To fully understand temperature effects on the structure of the drug and its carriers, we combine NMR and Differential Scanning Calorimetry techniques. From DSC curves, we observe additional phase transitions inside the hybrid material compared to its separated bulk constituents. NMR allows their assignment and demonstrates the simultaneous existence of two different lipid phases with separate fusion temperatures. As a consequence, optimum work temperature is a crucial parameter in our study. Indeed, as temperature is varied, compromises have to be met between high resolution and the presence of dipolar couplings that provide the structural information.

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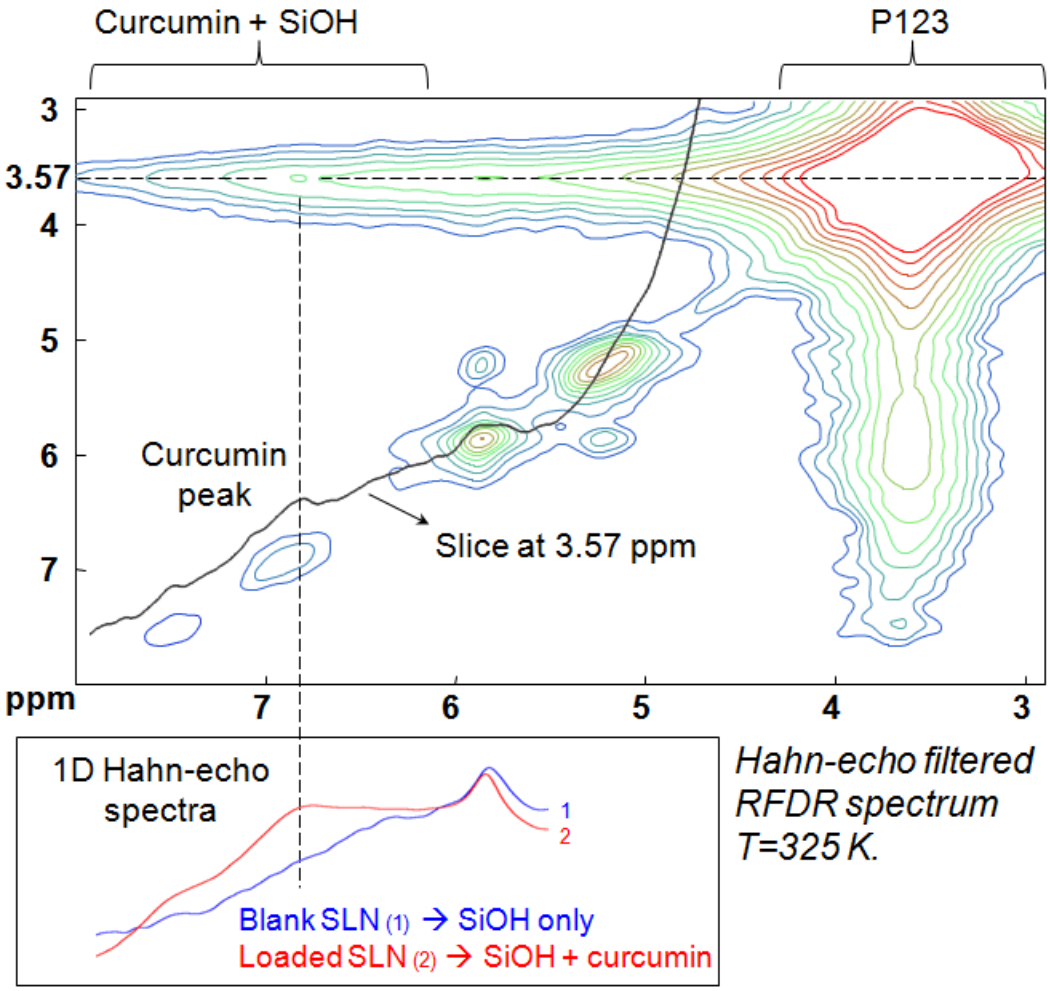
Curcumin + SiOH
P123
3
3.57
4
5
6
7
Curcumin peak
Slice at 3.57 ppm
ppm
7
6
5
4
3
1D Hahn-echo spectra
1
2
Blank SLN (1) → SiOH only
Loaded SLN (2) → SiOH + curcumin
Hahn-echo filtered RFDR spectrum T=325 K.

Materials

P246

Algorithm for the model-free inversion of 6D diffusion-relaxation correlation data

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Abstract: Porous materials as diverse as rocks, polymer gels, or dairy products all possess a range of microscopic environments characterized by distinct NMR relaxation rates, R_1 and R_2, and self-diffusion coefficients, D. The different environments can be non-invasively resolved by multidimensional NMR techniques in which relaxation- and diffusion-encoding blocks are combined to establish correlations between the relevant observables. Such techniques quantify the underlying pore heterogeneity with joint distributions of NMR parameters, related to the signal data by an inverse Laplace transformation.

Classical multidimensional Laplace protocols all use a single gradient direction to encode for diffusion, a methodology that convolves the effects of pore anisotropy and orientation. Dispersion in any of those quantities thus results in broad diffusion distributions whose complex shapes cannot be retrieved with Laplace inversion. This means that the microscopic morphology of heterogeneous anisotropic materials, such as the brain tissue, cannot be captured cleanly by conventional Laplace NMR methods. To overcome this limitation, we introduced several diffusion-encoding schemes that separate the effects of pore size, shape, and orientation into four different observables: isotropic diffusivity D_{iso}, normalized diffusion anisotropy D_Δ, and orientation of the diffusion tensor (ϑ,φ) [1]. Inserting those acquisition schemes into a Laplace framework, we devised a correlation protocol wherein the underlying heterogeneity is described by a 6D probability function $P(R_1,R_2,D_{iso},D_\Delta,\vartheta,\varphi)$ [2]. While the high-dimensionality of our approach allows characterization of the microstructure at an unprecedented level of resolution, it also complicates the inversion of the integral transform that relates the acquired signal and $P(R_1,R_2,D_{iso},D_\Delta,\vartheta,\varphi)$. When using the analysis tools of lower-dimensional Laplace methods to invert a 6D distribution one is met with memory demands incompatible with the specifications of personal computers. Here, we present a novel model-free inversion approach wherein our 12D correlation space is explored through a directed iterative approach (See Figure). Following the works of Prange and Song [3], we avoid common regularization procedures and instead use a Monte Carlo approach to retrieve an ensemble of plausible distributions. By exploring the variability between solutions we derive error metrics and define confidence intervals. The feasibility and performance of our algorithm is demonstrated with both simulations and an in vivo dataset from a human brain.

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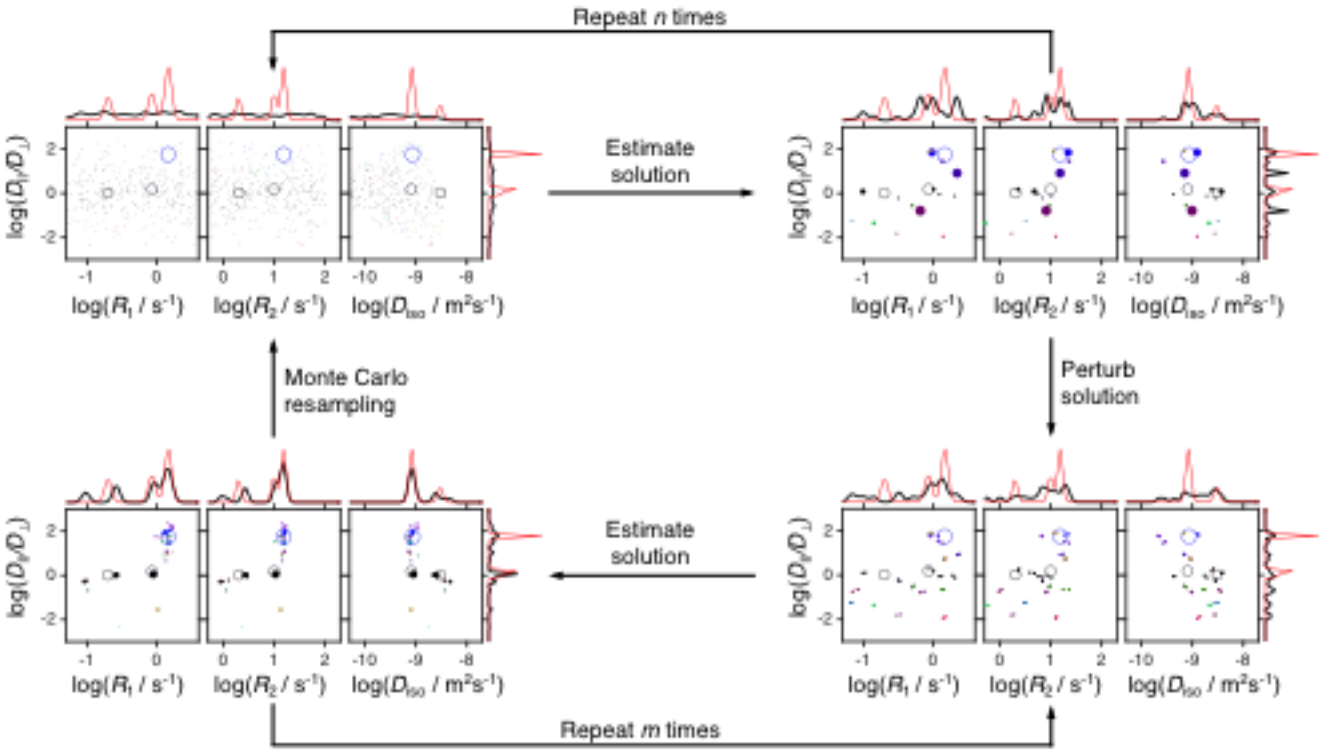
Image:



Disclosure of Interest: J. P. de Almeida Martins Conflict with: The company Random Walk Imaging AB (Lund, Sweden), where J. P. de Almeida Martins is an employee, holds patents related to the described MRI methods., S. E. Mailhiot: None Declared, D. Topgaard Conflict with: The company Random Walk Imaging AB (Lund, Sweden), where D. Topgaard is a co-owner, holds patents related to the described MRI methods.

Materials

P247

Spin probe reorientation in relation to free volume and relaxation dynamics: cis-1,4-poly(isoprene)

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Abstract: The structural-dynamic state of amorphous systems is characterized commonly by conventional techniques, such as diffraction, scattering and relaxation, e.g., light scattering, dielectric spectroscopy. In the case of molecular, i.e. *spin probes* atomistic, i.e. *ortho-positronium* (*o-Ps*) probes, electron spin resonance (ESR) or positron annihilation lifetime spectroscopy (PALS) techniques are used respectively. Our recent combined ESR and PALS studies on a series of *amorphous organics* with various intermolecular vdW- vs. H-bonding interactions[1] and on three *cis-1,4-poly(isoprenes)* (*1,4-PIP*) of different molecular weight[2] revealed some interesting correlations that imply the common origin of the various effects in the corresponding ESR and PALS responses.

We compare the spin probe reorientation of *2,2,6,6-tetramethyl-piperidinyl-1-oxyl (TEMPO)* and the free volume in *1,4-PIP 10k* via ESR and PALS to the electric dipole relaxation dynamics of the pure medium from broadband dielectric spectroscopy (BDS)[3] and to density fluctuation from the light scattering (LS). The spectral simulation of the *1,4-PIP10k/TEMPO* spin system revealed five regions of different mobility of *TEMPO* over a wide temperature range from 100 K up to 350 K, i.e. three slight decreases within slow and fast regime at $\mathbf{T_{x1}^{slow}=T_{Xi}^{fast}}$, $\mathbf{T_{x2}^{slow}=T_{x1}^{fast}=T_g^{DSC}}$, the slow to fast motion regime transition at $\mathbf{T_c}$ and the two fast regimes at $\mathbf{T_{x2}^{fast}}$. These findings are in good agreement with the characteristic bend effects in the PALS response at $\mathbf{T_g^{PALS}}$, $\mathbf{T_{b1}^{L}}$ and $\mathbf{T_{b2}^{L}}$. Finally, the structural relaxation times above $\mathbf{T_g^{DSC}}$ is describable in terms of the power law or mode coupling theory (MCT)[4] and the two order parameter (TOP) model[5] with the mutual relationships between the respective characteristic temperatures: $\mathbf{T_c \approx T_{b1}^{L} \approx T_c^{MCT} \approx T_c^{TOP}}$ and $\mathbf{T_{x2}^{fast} \approx T_{b2}^{L} \approx T_A^{TOP}}$ indicating the possible physical origins behind the structural-dynamic changes as detected by both the external probes.

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Materials

P248

Characterization of polyolefins functionalization and copolymerization using 1D and 2D NMR

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Abstract: Polyolefins are virtually ubiquitous in our everyday life, playing a key role in applications going from biomedical to automotive, building and construction, household appliances, consumer food packaging etc. However, a limitation of polyolefins is their apolar character and thus lack of chemical functionality, which results in low adhesion with other materials such as inorganic fillers, metals or other polar polymers. To overcome this problem in applications like blending, steel pipe coating, painting and bonding in automotive industry, polyolefins have to be functionalized.

Among the polyolefins functionalization methods, the only solution that provides the control over the molecular weight, polydispersity, stereoregularity but also concentration and distribution of the functional groups along the polymer chain is the in-reactor functionalization technique using dedicated catalytic systems.

In this context, we have been investigating concepts aiming at producing different types of functionalized polyolefins via catalytic routes, including here also the copolymerization of ethylene or propylene with “in-situ-pacified” ω-hydroxyl-functionalized α-olefins. We have successfully developed an NMR analytical toolbox that has proven to be very powerful in characterizing the generated materials and in confirming and evaluating the type and degree of functionalization. Random and chain-end functionalization of different polyolefins and the subsequent copolymerization with various polyesters have been studied via liquid-state NMR by using a 10mm high-temperature DUAL cryoprobe. Hydroxyl, carboxyl, unsaturated and saturated aliphatic chain ends structures have been identified and quantified in different atactic and isotactic polypropylenes via ^{1}H and ^{13}C NMR. In a following step, with the purpose of probing the formation of a desired propylene-polyester block copolymer, 2D homo- and hetero-nuclear NMR experiments combined with multi-peak suppression aiming to minimize the main polymeric peaks and to increase the measurements sensitivity have been successfully employed. The spin system characteristic for the expected reaction product has been identified and the correlation of the propylene functional group with the corresponding carbonyl signal of the polyester block has been proven. Furthermore, the assignment of signals from the polyester end-groups has been also done.

Materials

P249

Nuclear Singlet Multimers - Nano-structured Stimuli Responsive Materials

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Abstract: Nuclear singlet states are NMR "silent states" which can be populated and detected indirectly.[1,2] Such a state can be created in two spin 1/2 nuclei coupled antiparallel to each other and form a total spin of 0. The outstanding property of a nuclear singlet state is its immunity to symmetric relaxation mechanisms such as the mutual dipole-dipole relaxation, which is one of the reasons why equilibration between a singlet and triplet state (T_S) can exceed the longitudinal relaxation time T_1.[1-7] This property has for example been used to store polarization for long times and is of particular interest in connection with hyperpolarization in the context of the development of bioprobes.[3-5] Other applications include improvement in sensitivity in drug binding studies in systems displaying long T_S and as a filter for specific signals.[6,7] Here, we are introducing stimuli responsive materials that can change their singlet state lifetime upon an external trigger. We have investigated thermo-responsive dipeptides that organize into amyloid structures and form a hydrogel around 305 K. When the temperature is above 305 K, the dipeptides are dissolved and possess a long-lived nuclear singlet state $T_S > T_1$. When the temperature is below 305K , a gel forms in which T_1 is reduced by a factor of two and a singlet state cannot be accessed anymore. Such reproducible behavior resembles a nuclear singlet on/off switch with respect to the temperature. We have subsequently investigated the potential of designing nanosystems that can be utilized as stimulus responsive probes with potential applications in medical diagnostics. We succeeded in developing materials that are about 50 kDa in weight and contain more than 100 proton pairs per molecule with small dispersions in chemical shift. In such materials, long-lived singlet states can be populated at the same time allowing for concentrations of 10 µM to be detected in a single scan. Furthermore, we have succeeded in attaching chemical groups to the nuclear singlet multimers that influence T_S compared to the starting structure and have little to no effect on T_1. Upon a stimulus, the chemical group is released which is expressed by a change in the detected T_S. To prove the versatility of the designed materials, we performed experiments in biological buffer solutions in H_2O, in a gel that has been applied to simulate the viscosity of brain matter and in the presence of atmospheric oxygen. In all of the aformentioned conditions we have found long-lived nuclear singlet states. Giving the versatility of the designed materials and the possibility of probing them in small concentrations, we foresee their application as novel biosensors.

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Materials

P250

Solid State NMR and its applications in material science

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Abstract: In the last thirty years, NMR spectroscopists have endeavored to make Solid-State NMR (SSNMR) a very powerful technique to probe molecular structure of solid samples which are either insoluble in common NMR solvents or degrades in solution. In SABIC, SSNMR demonstrated its usefulness in providing structural understanding of various aspects of material science. 400 MHz SSNMR spectrometer applies Magic Angle Spinning (MAS) up to 10 KHz to average out dipolar interactions, also it uses the wide spread chemical shift range of heteronuclei like 13C, 27Al and 29Si towards providing a detailed microscopic information about the structures of solid materials. Additionally for NMR insensitive nuclei like carbon, signal intensity can be further enhanced using polarization transfer from proton.
In this presentation, we will highlight three such case studies using one-dimensional MAS SSNMR techniques:
1. Structural stability of zeolite based ZSM-5 catalyst using one pulse 27Al NMR to ensure that there is no disruptive change of collapse in frame work structure as the catalytic activity is significantly depends on the framework. The dealumination information of catalyst before, during, and after is important in catalyst preparation.
2. Study of coke deposited on used catalyst to identify the type or nature of coke species. Further, to know the root cause of coke formation, which leads to catalyst deactivation subsequently to assess the catalyst re-generation process to removal of coke.
3. To study the sequential understanding of chemical structural changes of polymer fiber during thermal oxidative stabilization. This will enable to assess the extent of stabilization and product quality.

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Order and intermolecular interactions in complex zeolites: Organic Structure Directing Agents as NMR probes

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Abstract: Zeolites' properties and performances (e.g. in heterogeneous catalysis) can be enhanced by optimizing their nanoscale organization through: the precise distribution of active tetrahedral T_d sites, the control of defects and/or crystal dimensionality. These are often challenging tasks that necessitate new and complementary characterization methods. In this context, our approach employs as an information source the Organic Structure Directing Agents (OSDA) - typically quaternary ammonium cations - used to build the zeolitic materials with a given topology, and further removed to liberate the porosity. Thanks to a combination of experimental data from multinuclear solid-state NMR and theoretical data from DFT quantum calculations (crystal models), we report here how the study of OSDA@zeolites provides new insights on the interactions between inorganic and organic species at the origin of the formation of crystals, and the local structure of the zeolites.

In the case of all-silica zeolites, the ^{14}N quadrupolar parameters of tetraalkylammonium OSDA obtained from a direct ^{14}N NMR approach[1] allow to discriminate between locally ordered and disordered silicalite-1.[2] Furthermore, the combination of experimental and calculated ^{14}N quadrupolar parameters leads to new means of understanding the effects of intermolecular interactions (hydrogen and tetrel bonding) in the stability of octadecasil zeolites (AST).[3]

^{14}N NMR is also used to investigate the nanoscale disorder promoted by Al/Si substitution in the topologically complex and industrially important ZSM-5 zeolites. The ^{14}N spinning sideband patterns are shown to evolve with the Si/Al ratio. Spectrum modeling allows to estimate the local disorder due to Al site distributions and related chemical variations. Results obtained with DFT periodic calculations augmented with a London dispersion term (DFT-D) highlight the influence of CNC angle variations on the ^{14}N quadrupolar coupling constant distributions.[4]

Besides, we report a new and complete identification of the Al site distribution in ZSM-5 by studying the spectroscopic response of $Al(OSi)_4$ units and using a self-consistent combination of solid-state NMR correlations (^{29}Si–^{27}Al and ^{1}H–^{27}Al *D*-HMQC) and DFT-D methods (Fig. 1). To unravel the driving forces behind specific Al sitting positions, our approach focuses on ZSM-5 containing its more efficient OSDA, tetrapropylammonium.[5]

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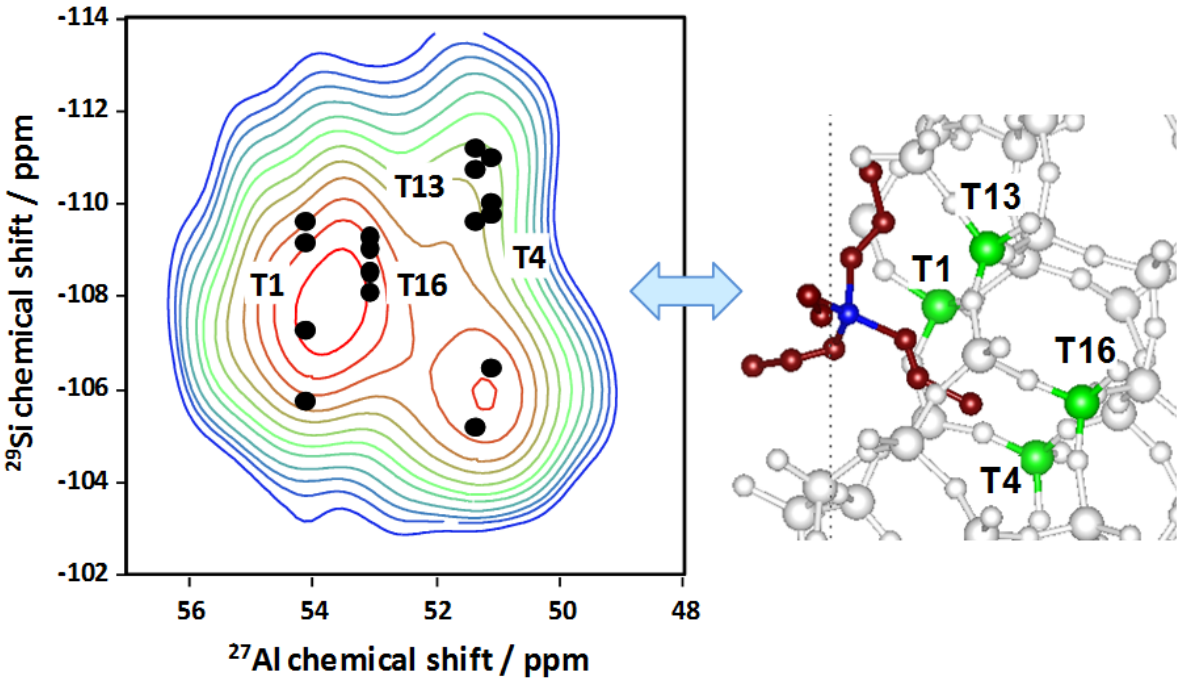

Fig. 1. Identification and study of the Al site distribution in topologically complex ZSM-5 zeolite using solid-state NMR and DFT calculation methods.

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NMR characterization of SABIC Specialties polymers

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Abstract: SABIC is a global leader in diversified chemicals headquartered in Riyadh, Saudi Arabia. We manufacture on a global scale in the Americas, Europe, Middle East and Asia Pacific, making distinctly different kinds of products: chemicals, commodity and high performance plastics, agri-nutrients and metals.

We support our customers by identifying and developing opportunities in key end markets such as construction, medical devices, packaging, agri-nutrients, electrical and electronics, transportation and clean energy. The SABIC materials portfolio consist of a wide variety of polymers. Examples include Lexan™ polycarbonate resins and its copolymers, Noryl™ polyphenylene ether and Ultem™ polyetherimides resins. These are used in the construction, automotive and transportation industries, as well as the electrical and electronics and medical devices industries. In order to understand and predict structure–property relation of our polymeric materials, having insights in the chemical structure of these materials is of the utmost importance. A general methodology for the molecular characterization of SABIC polymers by High Field NMR is presented in this poster.

The composition of our (co)polymers can be determined by (a combination) of ^{1}H and ^{13}C NMR [1]. For determination of the blockiness of our copolymers ^{13}C NMR is employed and for the determination of block-length ^{1}H or ^{29}Si NMR is used. Quantification of functional end-groups is done by ^{31}P NMR and ^{19}F NMR [2,3]. Also additives can be studied in detail by NMR. ^{1}H NMR can be used to identify and quantify a vast range of additives. Furthermore oxidation and hydrolyses mechanisms of e.g. phosphites can be determined.

Literature:

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Materials

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Solid-state NMR of a Scandium Metal-Organic Framework : insights into the formation mechanism

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Abstract:

NOTT-400 is a metal-organic framework (MOF) based on binuclear [Sc_2 (μ_2-OH) ($O_2CR)_4$] building blocks connected to each other through biphenyl-3, 3', 5, 5'-tetracarboxylic acid (H_4BPTC). NOTT-400 crystallizes in the chiral tetragonal space group $I4_122$ with each scandium center octahedrally coordinated to six O-donors. These six oxygen atoms are four from four different carboxylate groups of $[BPTC]^{4-}$ ligands and two from μ_2-OH groups that bridge two Sc (III) centers.[1] This robust MOF (which is stable up to 500 °C) shows high H_2 storage capacity, high water sorption at low relative humidity (RH), allowing an increase in CO_2 capture from 4.2 wt% (under anhydrous conditions) to 10.2 wt% at 20% RH and 30 °C.[2] Although the structure and properties of this MOF have been extensively studied, little is known about its formation mechanism.

In the present contribution, we therefore employ a combination of ^{17}O-labelling (using labeled water) and Magic-Angle Spinning (MAS) *J*-based ^{45}Sc{^{17}O} double-resonance high-field (18.8 T) solid-state NMR spectroscopy to get insights into the formation mechanism of this MOF. Further ^{13}C, ^{17}O and ^{45}Sc ssNMR data are recorded and analyzed, providing details about the adsorption sites of selected guest molecules in NOTT-400.

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Domain size measurements and characterization of the interphase region by spin diffusion experiments

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Abstract: Composite materials are commonly used in very diverse areas, from medicine to transport. These materials are usually made from a polymer matrix, the main constituent, reinforced with another material in order, for example, to improve the matrix' mechanical properties. Mechanical properties of composite materials mainly depend on those of its constituents, but also on the composite's microstructure itself: quantity, shape, size of the particles. The interphase region, where adhesion between the constituents can be observed, is a region of major importance that might strongly influence the mechanical properties of the material. Studying the proportion of each constituent in this interphase region, and their interactions, might lead researchers towards a better understanding of the macroscopic physical properties. NMR spectroscopy is used to characterize many materials; its sensitivity to molecular environment and its sensitivity at the nanometer scale makes it a very interesting analytical technique to study the material structuration at a length scale hardly visible with other techniques such as Scanning Electron Microscopy (SEM). The spin diffusion experiment allows one to determine domain sizes thanks to the differences in proton mobility of the different components within the composite material. Goldman and Shen [1] first started this kind of experiment, followed by other researchers who studied overall block copolymers or polymer blends. Landfester and Spiess [2], followed by Sun et al [3], suggested a variant of the experiment, which allows one to observe the interphase between the components in a semi-quantitative way. Our work is about characterizing, thanks to these spin diffusion techniques, a composite with very little polymer matrix (around 5%), and an explosive charge as the main constituent. The aim is to measure the different sizes of the domains, the matrix structuration within the composite, and the proportion of polymer immobilized in the interphase. The results are compared with SEM results which are complementary.

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Materials

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The Restacked-inhibited Porous Graphene Free-standing Film Cathode for Lithium-Air Battery

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Abstract:

The graphene-based electrode of lithium-air battery is investigated in order to understand the effect of the inhibition of restack graphene layers. The solid-state NMR and BET study shows that non-stacked-reduced graphene oxide has higher porosity and close interaction to electrolyte, indicating a higher capacity of molecular stacking disorder than chemically or thermally reduced graphene oxide film. In addition, ^{1}H MAS and ^{7}Li MAS NMR spectra of electrolyte could explain not only the pore structure but also pore filling properties; with increasing the electrolyte ratio and at variable tempearature. We demonstrate the anti-solvent precipitation process for graphene oxide can change the intrinsic defective and corrugated of graphene and these structural characters give to minimize the restack of graphene (scheme 1). These small change in graphene oxide synthesis lead to large changes packing arrangement in the graphene and thereby the improved capacity in lithium-air battery. The porosity of cathodes in lithium-air battery plays a key factor in increasing their oxygen exchange rate, electrochemical capacity and longer life. This study can be applied for the design of new solution-processed graphene and for the development of lithium-air battery cathode.

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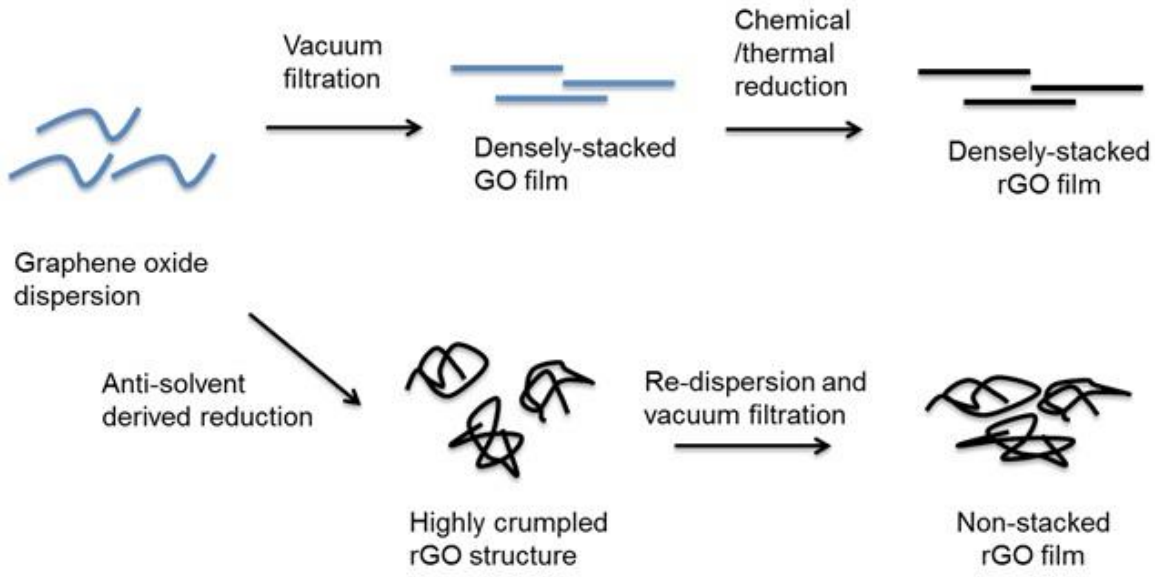

Scheme 1. Schematic illustration for fabricating non-stacked rGO film

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Counterintuitive design of non-structured-HYbrid Polarizing Solids for Dynamic Nuclear Polarization

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Abstract: Dissolution Dynamic Nuclear Polarization (d-DNP) [1] has become a technique of choice for enhancing nuclear spin polarization as it offers a way to overcome the sensitivity limitations in NMR or MRI. One of the most promising applications of d-DNP concerns metabolic imaging by MRI, where hyperpolarized solutions need to be pure and free of any polarizing agents (PAs). For this purpose, we have recently introduced a new generations of polarizing solids. They consist in mesoporous filterable materials functionalized with PAs, that enables to obtain contaminant-free hyperpolarized solutions. These materials, called HYPSOs (HYbrid Polarizing SOlids), are mesoporous hybrid silica matrices prepared by sol-gel process. Using this process, it is possible to play with and optimize different features of these materials: 1) their texture (porous volume and pore-diameters) for a maximal impregnation of the solution of interest and to optimize the spin diffusion process during DNP, 2) their particle size to facilitate their filtration and 3) their radicals concentration to optimize the DNP.[2]

So far, several generations of HYPSOs have emerged using two main strategies: a) one-pot synthesis of a functional silica material containing surface azido groups and b) the post-functionalization of pure silica solids by grafting of the (3-azidopropyl)triethoxysilane precursor. The PA is then introduced directly on the azido function by a click reaction. The first and the second generations of HYPSO differ in their porous structure. For example, HYPSO 2 is based on a SBA-15 silica framework showing a 2D hexagonal arrangement of stacked channels (diameter of ca. 6-8 nm) and HYPSO 3 is characterized by the presence of spherical cavities (6 nm diameter) interconnected in a 3D cubic arrangement by micro-tunnels.

Unlike earlier generations, HYPSO 5 was obtained by coating with an uniform silica layer containing TEMPO radicals, commercially available mesoporous silica spheres (pore diameter of ca. 6 nm; size of the particles around 250 μm) that have a highly interconnected and un-structured 3D structure. These new unstructured materials turned out to beat-up the previous generations of HYPSOs, with a remarkable polarization efficiency of P(^{1}H)=71% and P(^{13}C)=51% for a sample containing 3 M [1-^{13}C] sodium acetate in D_2O.

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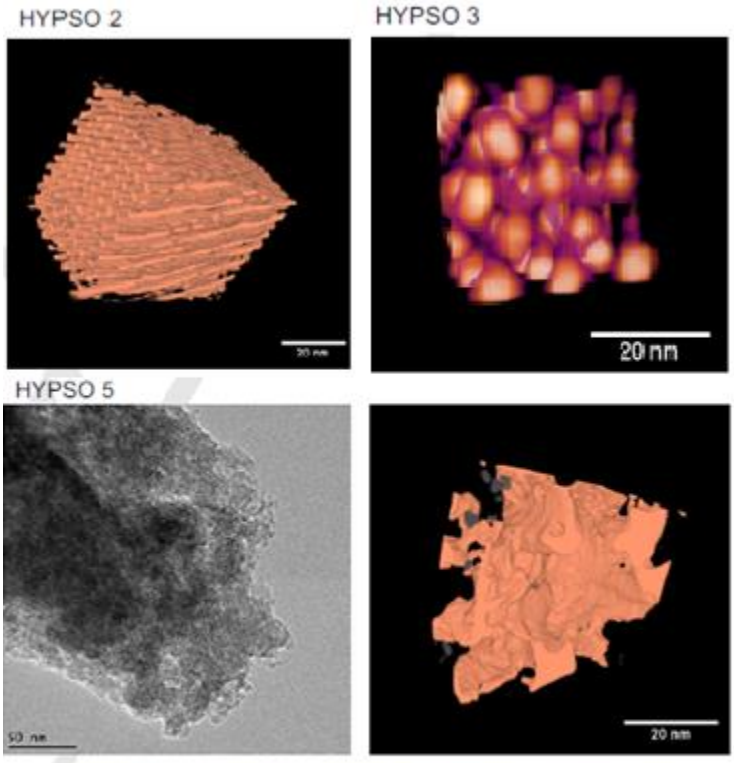

Figure 1. Electron Tomography pictures of HYPSO 2, 3 and Transmission Electron Microscopy (TEM) and Electron Tomography pictures of HYPSO 5. ***M. Cavaillès, A. bornet, X. Jaurand, B. Vuichoud, D. Baudouin, M. Baudin, L. Veyre, G. Baudenhausen, J.-N. Dumez, S. Jannin, et al., Angew. Chemie Int. Ed. 2018, DOI 10.1002/anie.201801009***

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Low resolution 1D and 2D T2 relaxation methods in High Field NMR for porous materials

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Abstract: CASC4DE, builds innovative hardware-enhanced capabilities and software to accelerate your research and extract hidden insights from your data flow. Here, we present a package of tools for low resolution relaxation measurements in High Field NMR. ***T2 ILT package*** including 1D T2 experiment and different 2D experiments as T2-Exch-T2, T1-T2 and Diff-T2.

Diffusion, nuclear spin–lattice (T1) and spin–spin (T2) relaxation times describe the mobility of the different populations of water present in your sample, providing information about the dynamics and the structure at different time and distance scales. Two-dimensional Exchange experiment allows to study the communication between the different water populations, giving information about the reticulation of a porous material, by the presence of off-diagonal peaks. Diffusion-relaxation correlation experiment (Diff-T2) can distinguish different physical environments of a water population in a sample. [1,2]

T2 ILT package allows you, firstly through the 1D analysis, the study of the different water populations present in the samples/materials. Secondly, the implementation of the 2D sequences on Bruker High-Resolution instruments, allows to obtain deep knowledge of your system structuration in different fields as petroleum, food samples, cement, cosmetic gels, biological samples, polymers, and porous media in general.

The measurement of one dimensional T2 experiment at low resolution in a High Field NMR spectrometer is performed by a one-scan CPMG sequence. This measurement contains a very large number of acquisition points for each scan, but any chemical shift information is lost since only one point is measured by echo. The intensity of the echoes evolves according to the sum of the T2 evolution of each species present in the sample. This very large number of points allows an easy setup of an ILT analysis (ILT: Inverse Laplace Transform).
The different 2D relaxometry sequences (T2-Exch-T2, T1-T2 and Diff-T2 sequences) have been written in-house with a dedicated software (based on Python and HTML tools) to read and process them.

T2 ILT-1D and -2D packages rely on solving a NNLS problem with a Tikhonov regularization term. Contrarily to the 1D problem the 2D one needs to be simplified. This is done using fast random projections instead of SVD.

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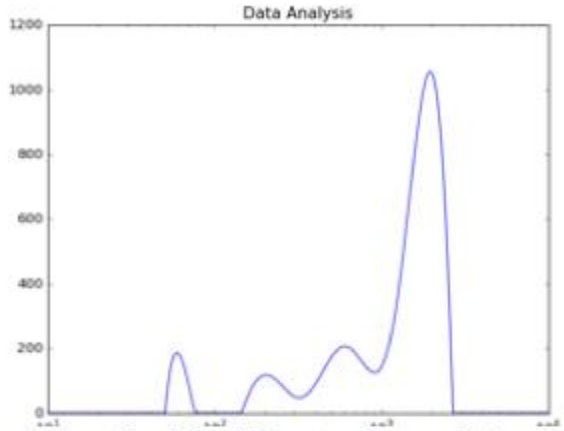

Figure 1 T_2 profile of the different water population present in a sample composed of 1mm glass balls an H_2O, measured on a 600MHz machine in a few seconds.

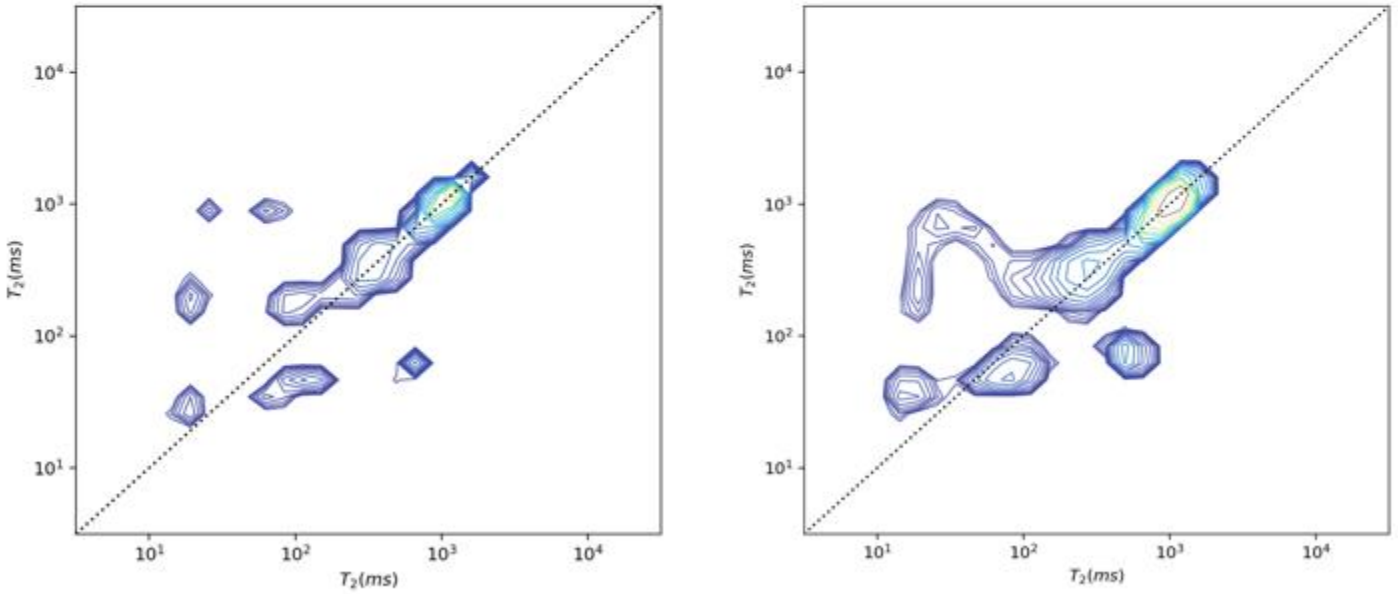

Figure 2 T2-T2 spectra obtained with a sample composed of 1mm glass balls and H2O on a 600MHz instrument. The two spectra show the effect of the unique processing parameter, indicating the confidence in the data-set.

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Porous Polarizing Polymers for dissolution-DNP

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Abstract: Hyperpolarization by dissolution dynamic nuclear polarization[1] has emerged in the last decade as a powerful tool for boosting the sensitivity of magnetic resonance imaging and spectroscopy by orders of magnitude. This has for instance brought metabolic imaging to reality.[2] Unfortunately, hyperpolarization's lifetimes in all molecules hardly exceed a minute. This means that hyperpolarization needs to be produced 'on-site', which is for many reasons and in many cases unpractical. We have recently introduced a new concept for producing long-lasting transportable hyperpolarized molecules formulated in the form of micro-powders[3].

Here we aim at generalizing this concept to virtually any soluble molecule by introducing a new generation of hyperpolarizing matrices made up of porous polymers containing stable radicals. These materials are designed to achieve two purposes: enabling efficient hyperpolarization of a broad range of systems such as neat endogenous tracers, mixtures of metabolites, or amino acids, and extending the hyperpolarization lifetime of these molecules over hours.

We will present the design and synthesis of these new porous polarizing matrices based on straightforward and very versatile epoxy-based chemistry. We will show how the morphology of these materials (macro and meso porosity) can be tuned and how this affects the efficiency and lifetime of hyperpolarization. Finally, we will present DNP results obtained on the sole matrices, with absolute polarization values as high as $P(^1H) > 50\%$ on the very first generation of these new materials. More interestingly, we have impregnated these polymers with solutions of [1-^{13}C]urea and have been able i) to observe spontaneous ^{1}H spin diffusion from the polymers to the frozen solutions and ii) to generate ^{13}C hyperpolarization via cross-polarization that was persistent for more than 7 hours.

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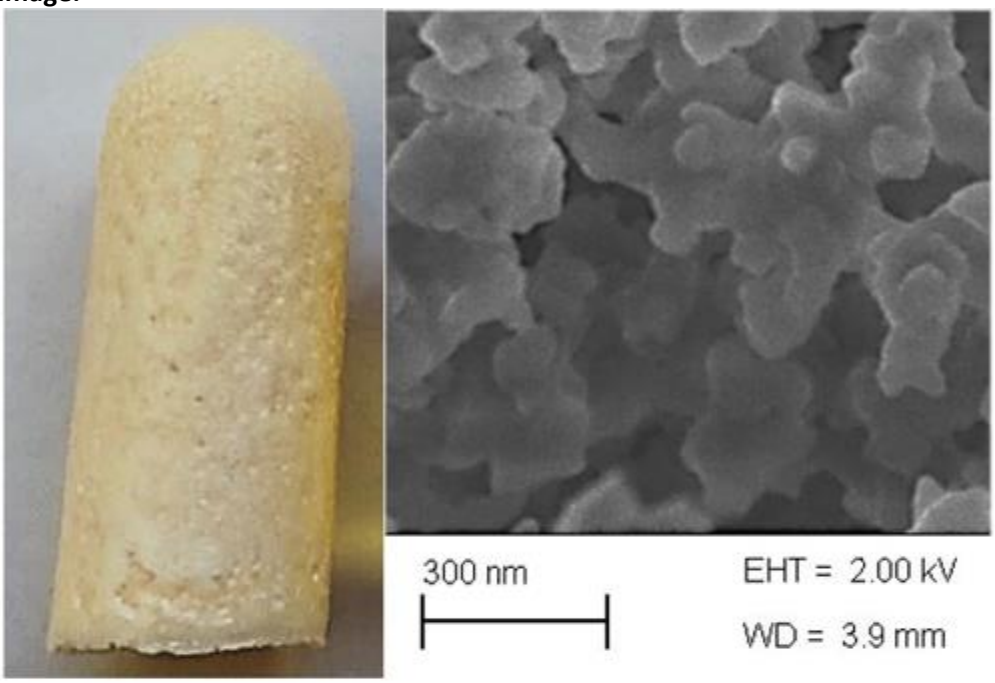

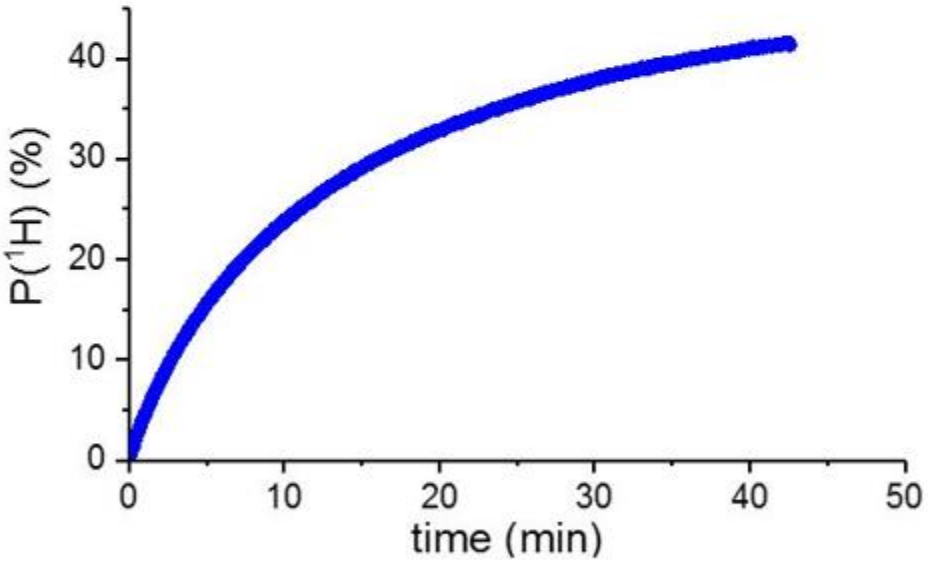

Fig1. Porous Polarizing Polymers morphology (top) and ^{1}H DNP build-up curve at 6.7 T and 1.2 K with c.a. 87mW frequency modulated microwave irradiation at 188.7 GHz.

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Xenon NMR to probe structure and dynamics of ionic liquids

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Abstract: Ionic liquids (ILs) are a wide and interesting class of materials whose smart properties are being exploited for several industrial applications. Typically, ILs are composed of asymmetric and flexible organic cations and bulky anions: these factors prevent close packing resulting in low melting points, commonly below 100°C. Additionally, the large number of combinations of anions and cations allows for a virtually infinite collections of novel liquids. A fundamental knowledge of the molecular structure, type of intermolecular interactions, as well as ions' dynamic behaviour is a mandatory pre-requisite to obtain ILs tailored for given application or to improve their performance.

^{129}Xe NMR spectroscopy has been recently employed to probe the local structure of pure ionic liquids[1,2]. The experimental results were complemented by MD simulations addressing the problem of the dependence of xenon chemical shift on the cage structure of the IL[3]. More recently, ^{129}Xe NMR has been used to explore the spatial arrangement and free volume of mixtures of ILs containing the a common imidazolium cation combined with different anions[4].

In this context, we present here the preliminary results on mixtures of alkylimidazolium based ILs with different alkyl chain length sharing a common anion. The results provide further insights in the assessment of the solution properties achievable by selected modulation of the structure of the mixture components.

In particular, novel experimental data on Xe diffusion by PFGSE NMR spectroscopy in two representative imidazolium-based ILs are the object of the present communication. The results indicate that the xenon diffusion coefficient is larger than those of the IL individual ions. Insights in the diffusion mechanism of IL dissolved xenon will be discussed.

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Materials

P260

Methanol diffusion in MOFs : a combined PFG-NMR, X-ray diffraction and MD simulations approach.

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Abstract: Metal-Organic-Frameworks (MOFs) are widely studied coordination compounds because of their large range of crystalline topologies, their porosity and their important variety of applications such as molecular storage and separation, purification, catalysis or as drug delivery system [1]. Numerous works deal with MOFs structure characterisation or their adsorption properties, but the diffusion of guest molecules inside the porous host architecture still needs to be investigated [2-5]. The study of this diffusion is crucial to understand the dynamic of the storage process or the selectivity process that should depend on sizes of guest molecule, porous architecture or host-guest interactions.

In this work, we study the diffusion of methanol molecules through porous system regarding several parameters such as solid-state flexibility of MOFs (flexible MIL-53(Al) vs rigid UiO-66(Zr)), the presence of amine-functionalized linkers (MIL-53(Al) vs NH_2-MIL-53(Al)), the structural transition between narrow-pore or large-pore forms. Our approach consists to carry out pulse field gradient NMR experiments (PFG-NMR) to measure methanol self-diffusion coefficient according to the sample temperature that allows to determine the associate activation energy. In addition, powder X-Ray diffraction experiments are performed on studied MOFs to characterise the structural transition and its dependence on temperature and the absorbed amount. All results thus obtained by PFG-NMR and X-ray diffraction are compared to the molecular dynamics simulations in order to get a better molecular understanding of the dynamic of confined methanol into a nanoporous system and the impact of MOFs flexibility on the diffusion process.

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Materials

P261

Insights on the surface structure of moisture-induced gas species chemisorbed in mesoporous materials using variable-pressure ssNMR and computer modelling

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Abstract: Increasing CO_2 concentration from anthropogenic sources in the atmosphere is one of the main causes of global climate change and post-combustion CO_2 capture from the flue gas is one of the key solutions to reduce greenhouse gases. CO_2 capture using solid adsorbents,[1] has received extensive attention due to their good sorption capacity, stability, ease of handling and reusability. Although emphasis has been given to the synthesis of new materials with improved CO_2 adsorption capacities, few studies have focused on the surface CO_2–amine interactions at the molecular level. FTIR and solid-state NMR provide structural insight, especially the local environment of chemical moieties present in chemisorption products, even in amorphous and surface-bound sites. In particular, solid-state NMR is a powerful technique, able to selectively study the local intermolecular interactions and to discriminate between similar chemical species. Recently,[2] the intermolecular interactions involving chemisorbed CO_2 species inside amine-modified silicas by the combination of solid-state NMR and computer modelling was presented as well as a detailed study on smart control of amine surface density and detection of proton-transfer through NMR chemical shift anisotropy.[3]

To shed light on the nature of moisture-induced chemisorbed CO_2 species, this work combines a solid-state NMR and computational study of tertiary amine-grafted SBA-15 (3-(diethylamino)propyl]trimethoxysilane) loaded with ^{13}C-labeled CO_2 ($^{13}CO_2$) and water vapour at variable pressures, under tightly controlled atmosphere, in an attempt to simulate real flue gas streams. Differentiating moisture-induced CO_2 species (e.g., bicarbonate) from more abundant CO_2 species (e.g., carbamic acid and carbamates) is particularly difficult, since the latter often dominate the FTIR or NMR spectra.

A combined ^{13}C solid-state NMR and computational study is presented to access all the variables involving the formation of bicarbonate and other residual species. To exploit this idea, distinct variable-pressure NMR experiments were performed in which the solid sorbent is exposed: i) to water vapour followed by $^{13}CO_2$, ii) to CO_2 followed by water vapour and iii) simultaneously to $^{13}CO_2$ and water to better understand CO_2 speciation on tertiary amines in the presence of moisture. We show how these experimental conditions may lead to very different populations of the distinct chemisorbed CO_2 species that may form in amine-modified mesoporous silicas.

Acknowledgments

The work was financed by FCT through project PTDC/QEQ-QAN/6373/2014 and developed in the scope of POCI-01-0145-FEDER-007679 UID/CTM/50011/2013 (CICECO), financed by national funds through the FCT/MEC and cofinanced by FEDER under the PT2020 Partnership Agreement. The authors are also grateful to the Portuguese NMR Network (RNRMN).

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Materials

P262

Solid-State NMR Spectroscopy Proves the Presence of Pentacoordinated Sc Sites in MIL-100(Sc)

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Abstract: Metal Organic Frameworks (MOF) offer a large range of potential applications such as gas storage or catalysis[1] since they have adjustable architectures and porosity. Amongst the wide variety of MOFs, the Sc-based MIL-100 combines high surface area, remarkable thermal stability and high Lewis acidity.[2] However, the Lewis acid sites in this MOF have never been detected by solid-state NMR spectroscopy. In this work, we observe for the first time using ^{45}Sc solid-state NMR experiments the formation of pentacoordinated scandium environments (ScO_5) during thermal activation of another Sc-based MOF: $[Sc_3O(BTB)_2(H_2O)_3](OH)(H_2O)_5(DMF)$, with H_3BTB = 1,3,5-tris(4-carboxyphenyl)benzene, called Sc_3BTB_2.[3] This assignment was supported by calculation of the ^{45}Sc NMR parameters using Density-Functional Theory (DFT). Furthermore, the appearance of ScO_5 NMR signal in ^{45}Sc NMR spectra goes along with the decrease of the water signal in ^{1}H spectra. This observation suggests that the ScO_5 sites are produced from ScO_6 ones by the removal of an aqua ligand. In addition, we also observed using ^{1}H and ^{45}Sc NMR experiments the formation of ScO_5 sites in Sc-based MIL-100 upon thermal activation. The presence of these sites explains the Lewis acidity of thermally activated Sc-based MIL-100. Moreover the use of advanced NMR experiments including MQMAS (Multiple Quantum Magic Angle Spinning) and ^{45}Sc-{^{1}H} *D*-HMQC (Dipolar Heteronuclear Multiple-Quantum Coherence) provided additional insights into the structural modifications produced by the thermal activation. Finally ^{13}C-^{45}Sc distances were probed in MIL-100 using S-RESPDOR experiment. This experiment indicates a shrinkage of the structure at high temperature.

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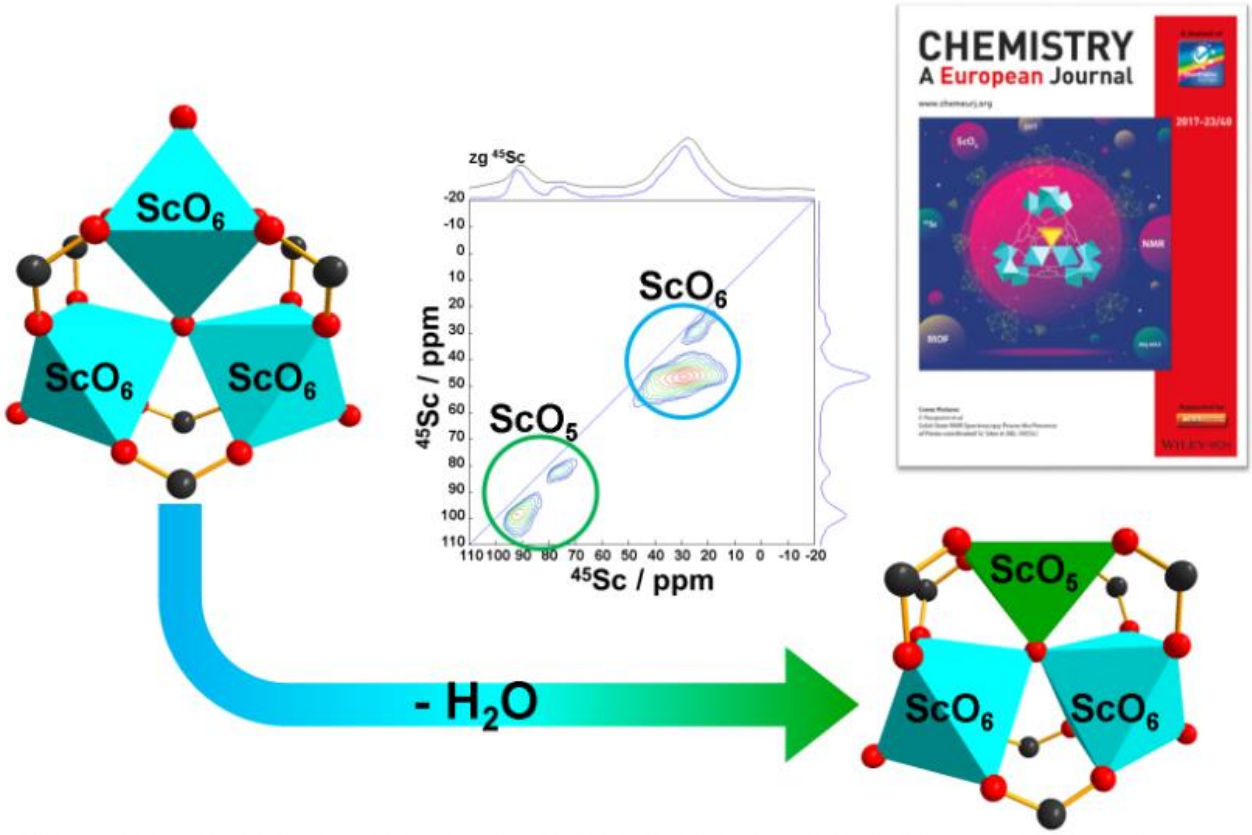

^{45}Sc MQMAS 2D spectrum highlighting the presence of the two scandium's coordinations in a scandium-based MOF.

Materials

P263

Characterization of porous structures of cements and shales by NMR spectroscopy

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Abstract: Cement is a powdery substance that acts as a binder when mixed with water, sand and gravel for the production of mortar and concrete [1]. Natural shales are much abundant sedimentary rocks that have become very important as unconventional sources of energy, i.e., methane gas [2]. The microporous structures of cement play important role for its strength, transport properties and durability. On the other hand, the micro and mesoporous structures of natural shales not only provide opportunities for the adsorption and preservation of gas but also play difficulties for its extraction.

NMR spectroscopy is sensitive, noninvasive technique for the investigation of chemical sites and dynamical processes at atomic and molecular scale. The relaxation, diffusion, NMR cryoporometry (NMRC) and magnetic resonance imaging (MRI) measurements of fluids adsorbed in porous materials reveal detailed information about pore size distribution, morphology, transport and adsorption process, while two dimensional (2D) exchange spectroscopy (EXSY) and T_2-T_2 relaxation exchange experiments show the chemical exchange between different sites [3].

In the present work, we use ^{129}Xe NMR to characterize cement and shale samples. We compare BASF and Portland cement samples having water to cement ratios of 0.3 and 0.5 and shales with controlled Pyrite (FeS_2) content. ^{129}Xe spin-echo NMR spectra of hydrated cements show two signals, one arising from large pores inside the material and free gas, and the other weaker signal from xenon adsorbed in meso-pores. The chemical shift of meso-pore signals arising from Portland samples is higher than that of BASF sample which means that Portland sample has smaller pore sizes than BASF. We also detemined chemical exchange rate between free gas and pore gas in cement samples by using EXSY experiments.

The NMR spectra of 1, 3 and 5 % pyrite content shale samples show one free gas signal and a broad peak from gas adsorbed in meso-pores. In the case of the lowest pyrite content shale, the mesopore signal is split into two peaks. We determined a qualitative estimate pore size distribution by adding acetonitrile liquid in shale samples and carrying out NMRC. In addition, we investigated chemical exchange between bulk and confined acetonitrile by 2D T_2-T_2 relaxation exchange experiments, and determined the exchange rates.

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Materials

P264

Ultra-fast Molecular Rotor Dynamics and their Regulation in Nanoporous Architectures

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Abstract: Nanoporous materials are excellent candidates for the fabrication of molecular rotors in the solid state and promise access to the control of rotary motion by chemical and physical stimuli.[1] The combination of remarkable porosity with fast rotor dynamics was discovered in molecular crystals, covalent organic frameworks and metal-organic frameworks (MOFs) by ^{2}H spin-echo NMR spectroscopy and spin lattice T_1 relaxation times.[2-4]
A microporous MOF engineered to contain in its scaffold rod-like ligands [1,4-bis(1H-pyrazol-4-ylethynyl)benzene] (BPEB) showed extremely rapid 180° flip reorientation of the central *p*-phenylene unit with rotational rates of 10^{11} Hz at 150 K. Molecular rotors are exposed to the crystalline channels, which absorb CO_2 and I_2 even at low pressure. Interestingly, dynamics could be tuned by gas absorption/desorption, showing a remarkable change of material dynamics, which, in turn, produces a modulated NMR response. Crystal-pore accessibility of the MOF allowed the CO_2 molecules to enter the cavities and control the molecular rotor spinning speed down to 10^5 Hz at 150 K (Figure). This strategy enabled the regulation of rotary motion by gas diffusion in the channels and the determination of the energetics of rotary dynamics in the presence of CO_2, enlarging perspectives in the field of sensors and gas detection.
Moreover, the insertion of dipoles onto molecular rotors in mesoporous organosilica architectures permits to obtain fast molecular rotors containing dynamic C-F dipoles. The dipolar rotors show not only rapid dynamics of the aromatic rings (10^9 Hz at 325 K) in the solid state NMR experiments, but also dielectric response typical of a fast dipole-reorientation under the stimuli of an applied electric field.[5]
Regular arrays of dipolar molecular rotors can be also mounted on crystal surfaces in an unusual way, exploiting the formation of *surface inclusion compounds*. Guest molecule is comprised of a rotator, a stopper and a shaft: 2D ^{1}H-^{13}C HETCOR NMR spectroscopy identified the moieties (shafts) which are inserted into the bulk crystal, although they represent a minor part of the nanostructured material.[6]

Acknowledgements: Cariplo Foundation, INSTM Consortium, Lombardy Region and PRIN 2016 are acknowledged for financial support.

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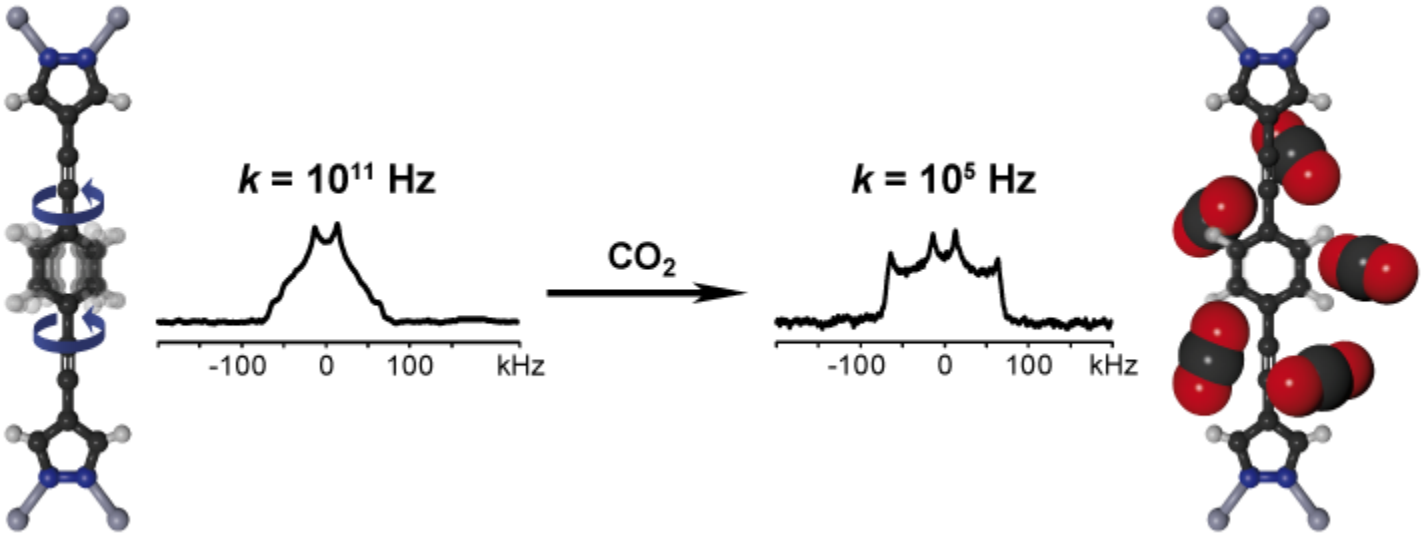

Materials

P265

NMR Spin Diffusion and Cryoporometry Study of Microheterogeneity in PEG-Based Polyacrylate Gel

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Abstract: PEG-based polyacrylate material due to its biocompatibility has various applications in the field like biomedical coating and tissue engineering. In order to satisfy the demand for this type of materials, UV-curing among all the manufacturing techniques is the most common approach used by people in industry to produce these polyacrylates in a large scale. However, the rather uncontrollable radical polymerization often results in a heterogeneous product with complicated morphological properties. Meanwhile, the addition of monoacrylates into the formulation, like those found in most commercial polyacrylate products, further convolutes the morphological and mechanical understanding of their physical properties in terms of its microstructural interpretation. These randomly distributed microstructures traditionally are probed by atomic force microscopy (AFM), electron microscopy (EM) and small angle x-ray diffraction (SAXS) analyses. However, these approaches are often instrument demanding, and also need special sample preparation procedure. On the other hand, low field NMR spin diffusion analyses has also been frequently used to analyze phase separation in polymers, while low field NMR cryoprometry has also displayed a great sensitivity on probing the pore/mesh size distribution in composite materials.

In this study, we exploit the possibility of using low field NMR spin diffusion and NMR cryoporometry to probe the 'nanogel' formation during the UV curing procedure. As concluded by previous SAXS study on similar systems, a three–phase-system was formed during the curing stage, and this three-phase system would gradually evolve into a two-phase system when the curing reaction completed. The correlation between domain size and mesh size were also discussed. Meanwhile, the effect of solvent content, side chain as well as side chain structure's impact on this multiphase system were also studied. Finally, these results were further correlated with in-situ synchrotron SAXS-WAXS analyses about these systems.

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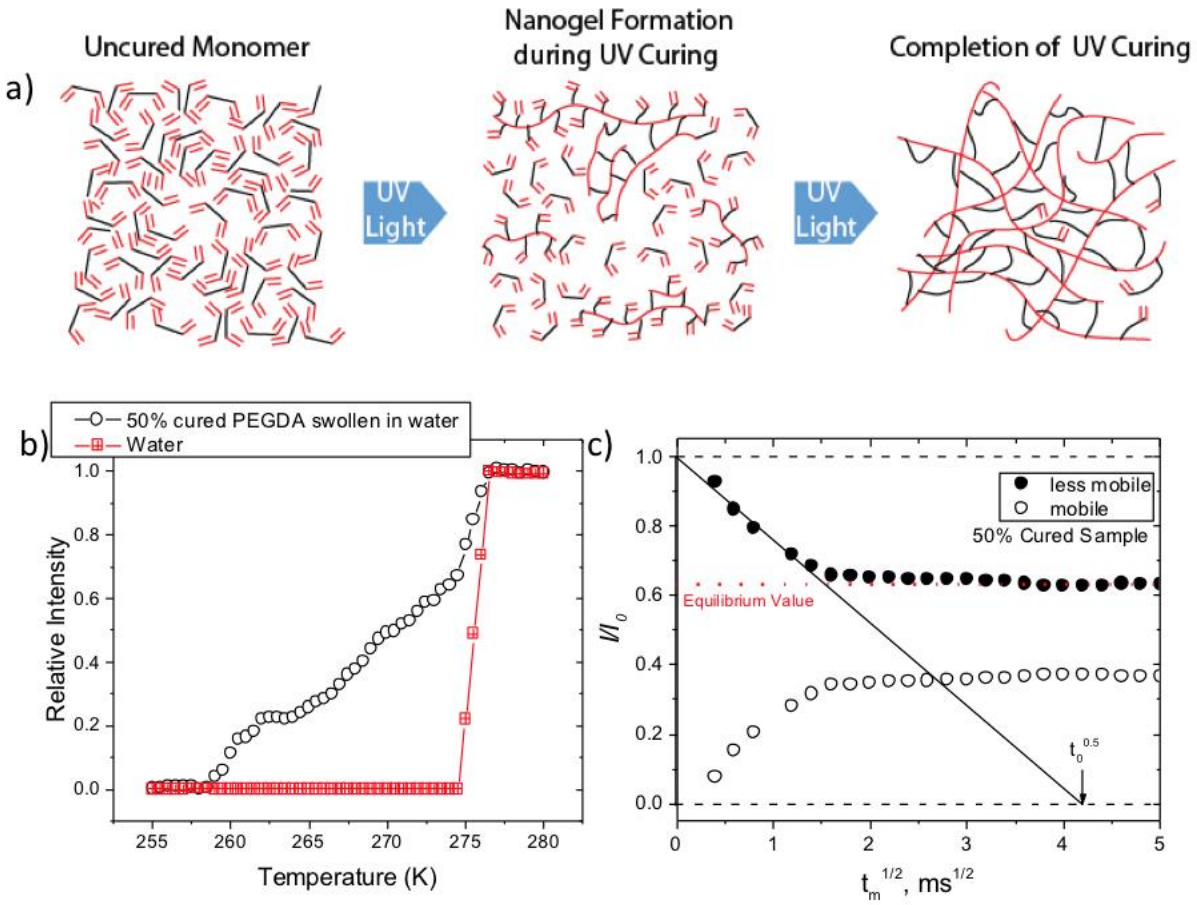
a)
Uncured Monomer
UV Light
Nanogel Formation during UV Curing
UV Light
Completion of UV Curing
b)
50% cured PEGDA swollen in water
Water
Relative Intensity
Temperature (K)
c)
less mobile
mobile
50% Cured Sample
Equilibrium Value
I/I_0
$t_0^{0.5}$
$t_m^{1/2}$, $ms^{1/2}$

Materials

P266

Characterization of inorganic phosphors doped with paramagnetic lanthanide ions by solid-state NMR and first principles calculations

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Abstract: When doped with low concentrations (~1%) of different lanthanide ions (Ln), yttrium aluminium garnet (YAG) exhibits a wide variety of luminescent properties, ranging from phosphor behaviour to laser activity. The properties of these YAG:Ln materials depend intimately on the nature of the Ln ion(s), and how these ions are distributed throughout the YAG lattice. Therefore in order to fully understand this diverse behaviour we need to link it to the local structure of the Ln ions[1].

Solid-state NMR is the method of choice for studying local structure in materials. However paramagnetic systems have traditionally resisted NMR characterization since the paramagnetic metal ions result in signals with large paramagnetic shifts, shift anisotropies, short relaxation times, and line broadening due to bulk magnetic susceptibility (BMS) effects[2]. Even if we are able to obtain a spectrum, it is difficult to interpret due to the complicated nature of the paramagnetic shift, which contains contributions from the different parts of the hyperfine interaction. However, these effects provide a direct probe of the electronic structure in these compounds.

Calculations of the paramagnetic shifts due to Ln ions are complicated by the large spin-orbit coupling effects. Until very recently calculations of these shifts have been based on the simplified models of Golding and Halton[3], and Bleaney[4]. Whilst these have been very successful advancing the field[5,6], they often fail to account for all the important effects of the Ln electronic structure[7].

We have studied a series of YAG:Ln materials using a combination of ^{27}Al solid-state NMR and first principles calculations. Using state-of-the-art methods in paramagnetic NMR we obtained spectra in which the local ^{27}Al environments are clearly resolved due to the shift from the Ln ions. These spectra were then assigned using quantum chemical methods[8,9]for calculating the paramagnetic shifts due to Ln ions in the presence of strong spin-orbit coupling. We then used these data to elucidate the distribution of the Ln ions in the lattice.

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Materials

P267

Local Structure Analysis of Proton Conducting Imidazolium Sebacate Crystal Using Solid-State NMR

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Abstract: The imidazolium salts of dicarboxylic acids are known to show relatively high proton conductivity even though these are organic crystals [1]. Imidazole and dicarboxylic acid are connected by a hydrogen bond and form a two-dimensional network in the crystal of these salts. Proton conductivity is caused by continuous proton transport in the hydrogen network between imidazolium ion and the carboxyl group and the reorientational motion of imidazolium ion is considered to play an important role for the proton conduction mechanism [2,3]. In these crystals, imidazolium sebacate crystal (ImSEB) exhibits the highest proton conductivity. In the present work, the temperature dependence of proton conductivity of powder samples was observed. The local structure and dynamics of imidazolium ions in ImSEB crystal were investigated using solid-state ^{2}H and ^{13}C NMR in order to elucidate the mechanism of proton conduction. The motion of imidazolium ion was analyzed by ^{2}H broadline spectrum, quadrupolar Carr–Purcell–Meiboom–Gill (QCPMG) spectrum and spin-lattice relaxation time (T_1). Two samples, quickly crystallized sample and slowly crystallized sample from solvent were prepared. The proton conductivities of bulk and grain boundary in polycrystalline samples were discussed. We found that although the proton conduction in single crystal is dominated by 180° flip of imidazolium ion, the fast isotropic rotation of imidazolium ion is intimately relevant to the proton conduction in grain boundary. The proton conduction of grain boundary is considered to play an important role in the high proton conductivity of ImSE at high temperatures.

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Materials

P268

NMR molecular dynamics study of water interaction with functional groups of Hydrothermal carbon (HTC) materials

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Abstract: Hydrothermal carbon (HTC) derived from biomass is a class of low cost, environmentally-friendly functional materials with many potential applications such as catalysts, absorber, electrode, etc. In particular, nitrogen-doped carbon materials (N-HTC)[1)] are beginning to play an important role in energy conversion and storage technologies like water splitting and fuel cell applications. Understanding the molecular dynamics of the water interaction with HTC material surfaces is important when applying (N)-HTC as an electrode for water splitting. In this work, N-HTCs were specifically synthesized by glucose and urotropine as precursors. Three different synthesis temperatures and three different molar ratio of precursors were used to distinguish the effect of the type and amount of N-functional groups and the synthesis temperature on water interaction. After saturation with water, (N)-HTCs were analyzed by diffusion NMR and relaxation NMR. Results show that N-functional groups are more important than the pore size distribution in affecting water mobility in N-HTC materials. Furthermore, the degree of water interaction can be controlled by adjusting the synthesis temperature and the ratio of precursors. Water motion was more restricted in N-HTC than N-free HTC, thereby suggesting that N-HTC forms more H-bond interactions with water molecules via the N-functional groups. Variable temperature NMR experiments up to 55 °C revealed that a 2D surface diffusion mechanism was the major force responsible for water motion in N-HTC than exchange with bulk water. In addition, even though HTC has greater incorporation of oxygen-containing functional groups than conventional carbon materials, the oxygenated functionalities in HTC materials play little role in the interaction with water.

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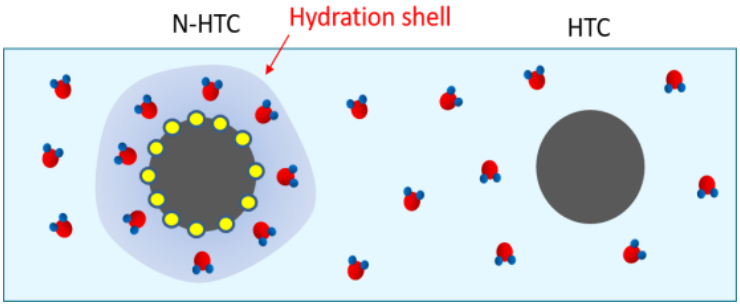
Image:



Materials

P269

Contrasting organic-inorganic interfaces of quantum dots: a multinuclear MAS-DNP approach

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Abstract: The surface-ligand interface is crucial in the control of the characteristics and functions of nanocrystals (NCs) and quantum dots (QDs). Herein, methods to determine the structure at the interfaces of organic ligand-coated ZnO NCs with sub-Ångström resolution will be presented. The studied ZnO NCs have been derived by a traditional sol-gel process as well as a recently developed one-pot self-supporting organometallic (OSSOM) procedure. The vastly different surface-ligand interfaces of the ZnO NCs, prepared via these different synthetic routes, has been determined using advanced characterization through magic angle spinning dynamic nuclear polarization (MAS-DNP-)enhanced solid-state nuclear magnetic resonance (ssNMR). It will be shown that multidimensional ^{1}H, ^{13}C, ^{15}N, ^{17}O, ^{31}P, and ^{67}Zn ssNMR can provide a highly detailed description of the surface, giving the distinct bridging ligand coordination modes, distances between ligands, and surface morphologies. For ^{17}O ssNMR spectroscopy, various straightforward isotopic labelling strategies will be presented. The ssNMR analysis shows that the OSSOM approach produces strong and stable NCs with a full surface coverage of securely-anchored and highly-ordered ligands that are resistant to ligand exchange processes. Conversely, the more traditional sol-gel approach provides a limited presence of random but stable *cold spots* with the majority of native ligands having their surface-supporting roles taken by foreign solvent molecules, resulting in an ordered *swollen shell* of ligand and solvent molecules surrounding the NCs.

This study highlights the power of MAS-DNP-enhanced ssNMR for detailed surface analysis and the superiority of the OSSOM approach for the preparation of high-quality quantum-sized ZnO crystals. This result is of large consequence considering the vast potential uses of ZnO NCs, such as in optoelectronic devices and for biomedical applications. Moreover, the presented methods have broad potential to enable the elucidation of ligand binding modes and corresponding surface structures for a wide range of nanosystems.

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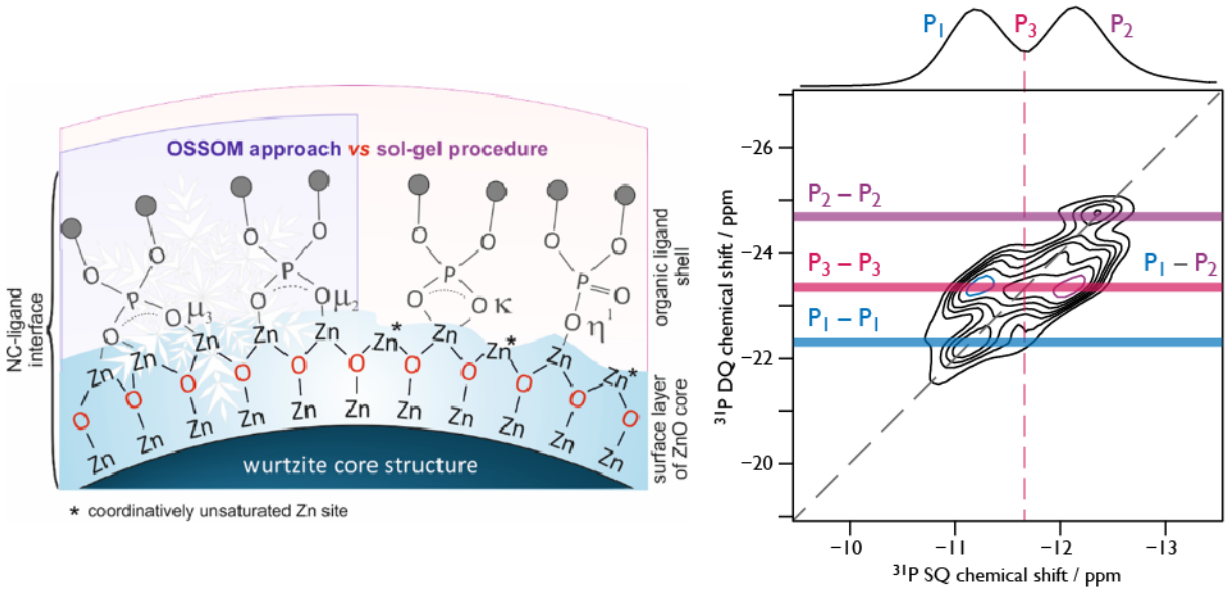

Materials

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Magnetic Field Effects Dynamics and Phase State of Some Protic Ionic Liquids in Confinement

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Abstract: Self-diffusion and NMR relaxation of ethylammonium and propylammonium cations were studied in protic ionic liquids ethylammonium nitrate and propylammonium nitrate confined between polar glass plates separated by a few um distance and exposed to an external magnetic field of 9.4 T. The diffusion coefficient and the transverse relaxation rates of –NH3 protons were increased immediately after placing the samples to the magnetic field by factors up to ~2 and ~22, respectively, in comparison with the bulk values [1,2]. The process can be reversed by removing the sample from the magnetic field. Forth and back processes occur at longer than 24 hours. Because the observed characteristic times of the change far exceed the times of molecular processes in studied ionic liquids, we suggested that this phenomenon is related to reversible phase transformations occurring in the confined ionic liquids. The process can be described well by the Avrami equation, which is typical for autocatalytic (particularly, nucleation controlled) processes. The transition can be stopped by freezing the sample. Cooling and heating investigations showed differences in the freezing and melting behavior of the sample depending on whether it had been exposed to the magnetic field. After exposure to the magnetic field, the sample demonstrated decrease in the ^{1}H NMR signal of residual water. ^{1}H NMR spectroscopy with presaturation demonstrates that the most probable mechanism of the decrease water signal is the adsorption of water on polar surface of glass plates.

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Materials

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Anionic environments of the cationic vacancies in titanium oxy-hydroxy-fluorides revealed by 19F MAS NMR

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Abstract: This communication focuses on the significant contribution of ^{19}F MAS NMR to the characterization of titanium oxy-hydroxy-fluorides featuring cationic vacancies (◊): anatase type $Ti_{1-x-y}◊_{x+y}O_{2-4(x+y)}F_{4x}(OH)_{4y}$ [1] for which the content of vacancies, related to the substitution rate of the divalent O^{2-} anions by monovalent F^- and OH^- anions, can be controlled by the synthesis temperature [2,3], hexagonal tungsten-bronze (HTB) $Ti_{0.95}◊_{0.05}O_{0.80}(F,OH)_{2.20}$ [4] and cubic $Ti_{0.90}◊_{0.10}O_{0.60}(OH)_{0.74}F_{1.66}$ [5].

In addition to the quantification of the F content, ^{19}F MAS NMR reveals the different F environments: Ti_3-F, $Ti_2◊$-F and $Ti◊_2$-F in the anatase type phases and Ti_2-F and Ti◊-F in the two other phases (Figure 1). In the anatase type phases, the proportions of the F environments point toward a preferential localization of the F atoms close to vacancies and enable to estimate, inter alia, the average F coordination numbers and the proportions of each anion in the vicinity of Ti and vacancy.

Finally, due to the vacancies that act as intercalation sites, the anatase type phases not only reversibly intercalate Li^+ ions [1,3] but also higher valence Mg^{2+} and Al^{3+} ions [6,7]. ^{19}F MAS NMR of lithiated, magnesiated and aluminisiated samples shows that Li^+, Mg^{2+} and Al^{3+} ions are inserted preferentially and firstly in di-vacancy systems.

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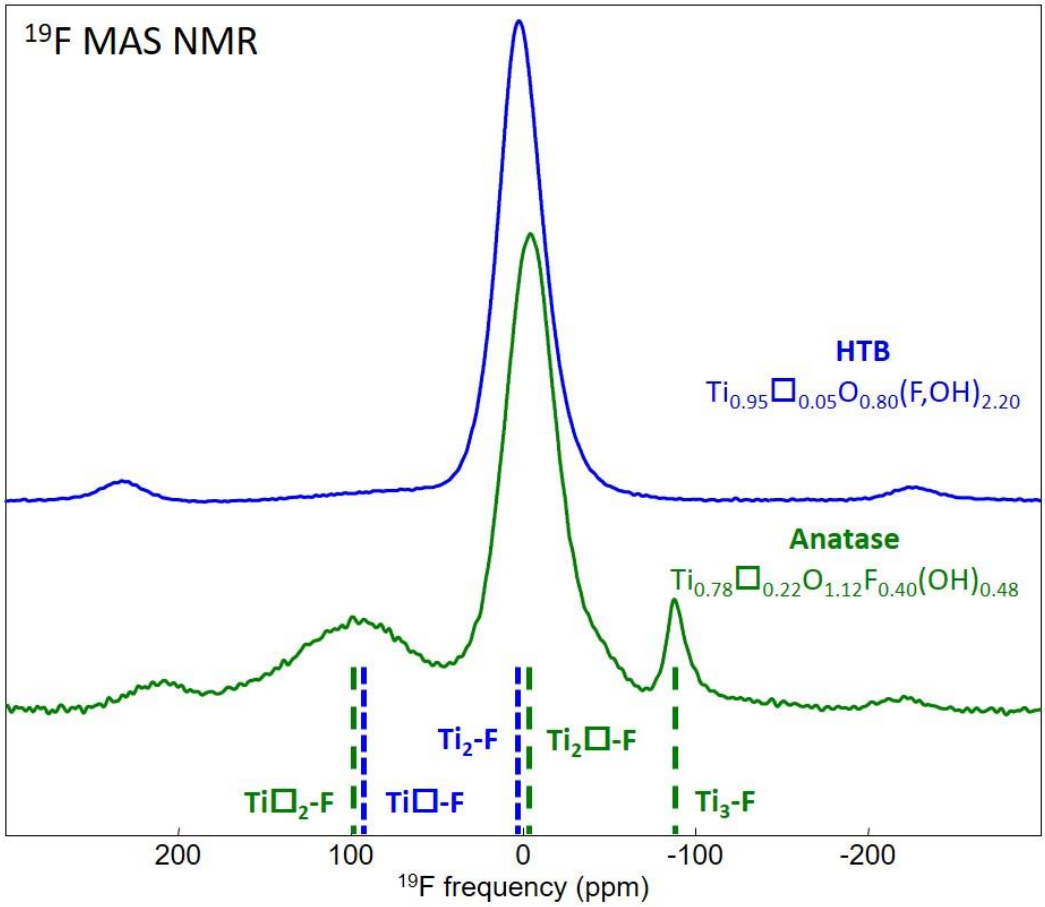

Figure 1. ^{19}F MAS (64 and 60 kHz) solid state NMR spectra of HTB and anatase phases. The blue dashed lines indicate the two main lines assigned to Ti_2–F and Ti□–F environments in the HTB phase and the green dashed lines indicate the three main lines assigned to Ti_3–F, Ti_2□–F and Ti$□_2$–F environments in the anatase phase.

Materials

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Resolving segmental mobility in starch phase transitions using polarization transfer solid-state NMR

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Abstract:

Most starch-based staple food requires heating while being immersed in water to become digestible for humans. Starch granules contain, apart from other minor constituents, two different types of polysaccharides whose chains are arranged in an amorphous or crystalline manner. On a molecular level, starch with a water content of more than 30 wt% undergoes a phase transition called gelatinization, which has been extensively studied using DSC and diffraction reporting contradictory results.

In contrast to these methods, we resolve the segmental mobility of polysaccharide chains from polarization transfer solid-state NMR by comparing CP and INEPT signal intensities. The segmental mobility, manifested through the correlation time τ_c, provides a direct measure of gelatinization, melting and recrystallization. For correlation times longer than milliseconds, solely CP signals are observed while for nanoseconds INEPT signals predominate [1].

Starch with two different water contents, 50 (wc50) and 60 (wc60) wt%, was analyzed at a MAS rate of 5 kHz. The results for wc50 are shown in Figure 1a, displaying a decline in CP signal intensities along with a growth in INEPT signal intensities for an increase in temperature. CP signals arise from starch chains with correlations times longer than milliseconds attributed to non-gelatinized chain segments, while INEPT signals belong to gelatinized ones. The CP line shape pattern at 101 ppm remains although its intensity diminishes completely at 90 °C. This implies that crystalline starch chains turn into mobile ones simultaneously as the amorphous ones.

The gelatinization temperature of starch is ca. 60 °C and varies with the water content. Figure 1b displays the CP and INEPT integrals for the peak at 61 ppm with temperature and the cross over point is at ca. 60 °C for wc60 and above 70 °C for wc50. Interestingly, we observed a smooth transition independently of the water content. Further measurements need to be recorded to complete the phase diagram of starch.

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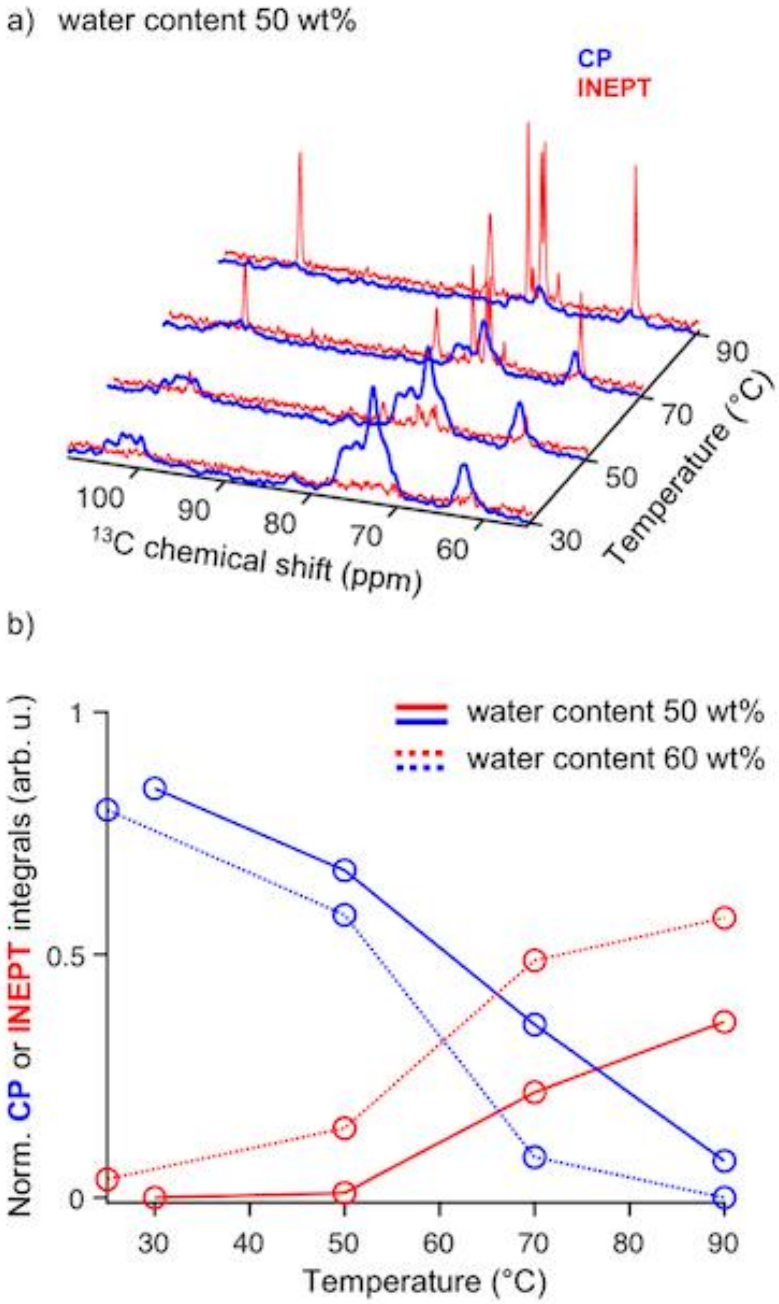

Figure 1. Stacked ^{13}C CP (blue) and INEPT (red) spectra as a function of temperature for starch with 50 wt% water (a). Normalized CP and INEPT integrals for the carbon atom resonating at 61 ppm are shown in (b) for four different temperatures for starch with 50 wt% water and 60 wt% water (dashed).

Materials

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Solid-State NMR of Li2S-P2S5 Solid Electrolytes doped with Nitrogen

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Abstract: Recently, it was reported for Li_2S-P_2S_5 glass and glass-ceramic solid electrolytes, that nitrogen doping enhances the chemical stability as well as the Li^+ conductivity.[1] However, the structure of the compounds and the Li^+ conductive pathway have not been determined, thus it is not clear how nitrogen doping contributes to the enhancement. In this study, we performed solid-state NMR to analyze the local structure of the Li_2S-P_2S_5 Li^+ electrolyte system.

Glass samples of (75-1.5*x*)Li_2S-25P_2S_5-*x*Li_3N (*x* = 0, 10, 20) were prepared by ball-milling, and a glass-ceramic sample was obtained by heating a glass sample with *x* = 10 at 300°C. Solid-state NMR measurements for ^7Li, ^{15}N, and ^{31}P were conducted with a Varian 3.2 mm T3 probe in a magnetic field of 9.4 T at room temperature. The samples were packed into a sealed rotor in a glove box filled with Ar and spun at 20 kHz at the magic angle.

Figure 1 shows the ^{31}P MAS NMR spectra of the (75-1.5*x*)Li_2S·25P_2S_5·*x*Li_3N glassy samples with *x* = 0, 10, and 20. From the spectrum of the *x* = 0 glass, i.e. the pure 75Li_2S·25P_2S_5 glass, the main peak at 83 ppm and 106 ppm can be assigned to a PS_4^{3-} unit and a $P_2S_6^{2-}$ unit respectively due to the previous report.[2] If this assignment is correct for the *x* = 0 glass where the composition ratio of S to P is 4, S must be compensated by another unit baring excess sulfur in thiophosphate compounds such as Li_7PS_6. [m1] By fitting the spectrum with Gaussian functions, a peak, which would come from Li_7PS_6, was found embedded in the left side of the main peak at 83 ppm, and the total composition rate of S to P was confirmed to be ca. 4. Hence the assignment is correct. For the glass samples substituted oxygen with nitrogen, a new peak at 65 ppm appeared. Even though the peak position resembles that of a PS_3O unit, the area fraction of the peak increased with the amount of nitrogen dopant linearly. Moreover, It is expected that replacement of sulfur by oxygen is less than 2% for all samples since the integration of the ^{31}P NMR signals of *x* = 0 glass sample is less than 2%. Thus we conclude that the peak at 65 ppm is attributed to phosphorus binding to nitrogen, and the nitrogen contributes to the enhancement of the chemical stability and Li^+ conductivity. On the glass-ceramic sample, the details will be reported in the conference.

This work was supported by JSPS Grant-in-Aid for Scientific Research on Innovative Areas “Mixed anion” (Grant Number 16H06440).

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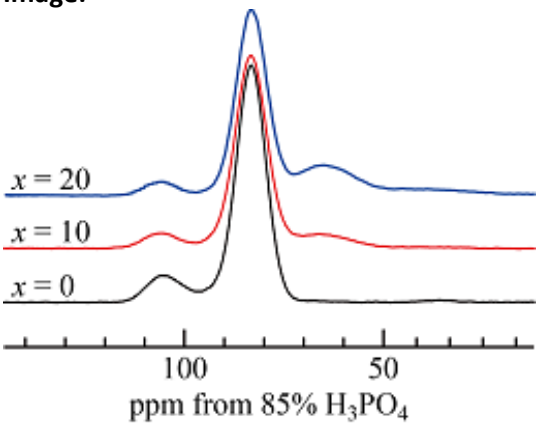

Materials

P277

Application of Solid State NMR in Catalysts and Energy Materials

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Abstract: To develop new materials with desired properties for the new energy systems and green chemical process, fundamental understanding of the materials is necessary. Solid state NMR spectroscopy is an ideal tool to get structural and dynamic information of materials even when long-range order is lacking. In this presentation, we will present solid state NMR study on the interfacial structure of mesoporous silicates and the reaction mechanisms of Li-S battery.

1) The confinement of liquids in porous media greatly influences their physical properties. Topology, size and surface polarity of the pores play a critical role in the confinement effects, however, knowledge concerning the guest-pore interface structure is still lacking. Especially, water molecules restricted in the pores are interesting in the scope of catalysis and energy storage. We will use periodic mesoporous organosilica (PMO) with various organic bridge groups to investigate the influence of the surface polarity on the melting behavior and molecular mobility of confined waters. By using 2D HETCOR NMR, spatial arrangement of water inside the pores and the interfacial structure at the guest-pore wall will be directly probed. A modulated and a uniform pore filling mode are proposed for different types of PMOs.

2) Sulfur-copolymers are promising cathode materials in Li-S batteries. However, the redox mechanisms of these materials are not well known owing to the difficulty in characterizing amorphous structures and individual ionic species in both solid and solution phases. We use solid-state NMR techniques to explore the structural evolution of the prototype S-copolymer cathodes, (poly(S-co-DIB)) during cycling. We demonstrate that polysulfides with different chain lengths can be distinguished by ^{13}C and ^{7}Li NMR spectroscopy, revealing that the structure of the copolymers can be tuned in terms of polysulfide chain lengths and resulting reaction pathways during electrochemical cycling. The mechanisms for the improved cyclability of S-copolymer cathodes will be illustrated.

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Materials

P278

Applications of 1H-13C multiple-contact cross-polarization in graphene oxide

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Abstract: Since its isolation in 2004, graphene has emerged as a fascinating nanomaterial with unique physical properties. Intensive research is ongoing to investigate applications of graphene and one of its derivative, graphene oxide (GO), in many fields, including the development of nanoelectronic devices, nanocomposite materials, as well as in biotechnology and nanomedicine. In this context, GO is a useful platform for the design of graphene-based hybrid materials. Indeed, the derivatization of the oxygenated functions of GO is a versatile and effective method to prepare chemically functionalized graphene for a wide range of applications. However, the precise atomic structure of GO remains uncertain and needs to be fully elucidated. High-resolution solid-state NMR appears to be one of the most appropriate analytical technique to elucidate the chemical structure of GO [1].
Hartmann-Hahn (HH) cross-polarization (CP) is the most widely used solid-state NMR technique to enhance the magnetization of a rare spin system with a low gyromagnetic ratio. Furthermore, as the kinetics of CP depends on the heteronuclear dipolar interactions, it also plays a major role in the determination of the molecular structure and dynamics. This is the reason why CP experiments are also used without an obvious gain in signal intensity.
In this work, ^{1}H-^{13}C HHCP and multiple-contact CP (MC-CP) [2-4] experiments are performed in GO. HHCP is found to be inefficient in GO due to very fast ^{1}H $T_{1\rho}$ spin-lattice relaxation. By contrast, the MC-CP technique which alleviates most of the magnetization loss by $T_{1\rho}$ relaxation leads to a much larger polarization transfer efficiency. Indeed, the measuring time of a 2D heteronuclear correlation spectrum is found to be reduced by an order of magnitude. Moreover, a detailed analysis of the HHCP and MC-CP kinetics indicates the existence of at least two different kinds of C-OH and epoxide functional groups, the major fraction of these groups being in the unusual “slow CP regime” in which the rate of ^{1}H $T_{1\rho}$ relaxation is fast compared to the rate of cross polarization.

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Materials

P279

WELL-DEFINED OLIGO- AND POLYSACCHARIDES AS IDEAL PROBES FOR STRUCTURAL STUDIES

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Abstract: Polysaccharides are the most abundant organic materials in nature, yet correlations between their three-dimensional structure and macroscopic properties have not been established. Automated glycan assembly enables the preparation of well-defined oligo- and polysaccharides resembling natural as well as unnatural structures. These synthetic glycans are ideal probes for the fundamental study of polysaccharides. According to molecular modeling simulations and NMR analysis, different classes of polysaccharides adopt fundamentally different conformations that are drastically altered by single-site substitutions. Larger synthetic polysaccharides are obtained via a "LEGO"-like approach as a first step toward the production of tailor-made carbohydrate-based materials.

Materials

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Expanding the experimental range of Dynamic Nuclear Polarization Surface Enhanced NMR Spectroscopy

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Abstract: In chemistry when the system under investigation is located at a surface, as in many interesting functional materials today, the problem of structure determination is largely unsolved, in particular when long range atomic order is absent. Such samples are encountered with increasing frequency, particularly in the area of energy and catalysis. Solid-state Nuclear Magnetic Resonance (NMR) spectroscopy is a method of choice to characterize materials at the molecular level. For instance, the exact 3D structure of the active site of heterogeneous catalysts is often not known. In batteries, the interphase between the redox-active (conductive) electrode and the electrolyte is often not entirely described at the molecular level. The detection limit of NMR is usually too low to allow the study of many modern materials. Today, the sensitivity gain provided by Dynamic Nuclear Polarization (DNP) under Magic Angle Spinning (MAS) the NMR signals of surfaces to be greatly enhanced and it enables the atomic level characterization of functional materials that were not accessible before.(1-5)

In the catalysis context, we have previously shown that ^{15}N, ^{13}C DNP SENS methods combined with EXAFS permits the three dimensional structure elucidation of the catalytic site of Pt-carbene immobilized on silica.(7) Here, we show that ^{195}Pt (I=1/2, ν_L= 85.987 MHz, 33 % n.a.) BRAINCP/WURST CPMG DNP enhanced NMR spectra can be used to measure the NMR spectrum of a platinum complex immobilized onto a silica surface under static condition.(6) We demonstrate how ^{195}Pt chemical shift anisotropy enables refinements of the 3D structure of the catalytic site.

In the energy context, it has been recently shown that the solid electrolyte interphase (SEI) formed on a silicon anode in a lithium ions battery can be characterized with the help of DNP NMR.(8) Here we report preliminary results of DNP enhanced NMR characterization of the decomposition products of sodium ions battery materials where DNP NMR is use to analyze the composition of the SEI on hard carbon anodes.

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Materials

P281

NMR investigation of water interfacial transport and diffusion through polymer membranes.

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Abstract: Mass transfers through polymeric membranes represent central phenomena in a variety of processes and technologies such as water separation and treatment, chemical reactors, low temperature fuel cells or electrolysers. The experimental determination of the membrane's properties in terms of liquid transport and diffusion (most often water) is usually performed using a pervaporation-like technique where the substance first adsorbs at the feed side of the membrane, transports by diffusion across the membrane and desorbs at the permeate side into vapor phase. However the precise measurement of the evolution of the mass transport coefficient with the liquid content in the membrane requires more advances techniques and NMR imaging has been shown to be an effective one [1-2]. The geometry of such membranes makes them essentially 2D objects and the investigation of the through-plane fluid transfer is only possible provided that adapted setups and NMR coils are developed.
In the present study we use a surface coil and a 1D NMR imaging sequence to record water concentration profiles through the plane of polymer membranes used in different applications. The boundary conditions at the membrane surfaces are varied from dry air to liquid water. The determination of the water concentrations and of the NMR transverse relaxation times at the interface with the humid gas makes it possible to calculate the interfacial mass transfer coefficients. The analysis of the concentration profiles, together with water flux measurements, give access to the water content dependence of the water diffusion coefficient. This procedure is applied to Nafion®, a perfluorinated membrane used in proton exchange membrane fuel cells, electrolysers and chlor-alkali production in order to quantify the relative role of diffusion and interfacial transport. We demonstrate that the transfer of water at the membrane interface is the limiting factor at low water content in this material, contrarily to the case of polymamide (nylon) where diffusion is always limiting. The same procedure is applied to membranes in cellulose acetate used in industrial filtration processes.

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Materials

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31P NMR applied to vanadium phosphate catalysts: crucial influence of 51V decoupling strategies

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Abstract: Vanadophosphates are industrial catalysts used for the production of maleic anhydride from butane oxidation. This catalytic reaction is enhanced by a V^{4+}/V^{5+} redox balance [1], between the vanadyl pyrophosphate $(VO)_2P_2O_7$ (the main solid industrially used) and $VOPO_4$ phases. Seven $VOPO_4$ polymorphs are reported: α_I-, α_{II}-, β-, γ-, δ-, ε- and ω-$VOPO_4$. These phases are generally poorly crystallized and present various degrees of local ordering making their structural studies complex. We used an "NMR crystallography" approach, combining XRD, solid-state NMR and DFT calculation of NMR parameters, in order to improve the structural description of these materials.

During the course of this project, it rapidly appeared that ^{31}P NMR spectral resolution was severely limited by heteronuclear ^{31}P-^{51}V scalar couplings. This interaction hinders a clear separation of the various $VOPO_4$ polymorphs by ^{31}P NMR spectroscopy. Different decoupling strategies based on either CW or multi-pulse (MP) decoupling schemes [2] have been applied in the present study of VPO systems. In a second stage, we investigated the scalar interaction MP decoupling of half-integer quadrupolar nuclei from ½ spin nuclei, in a more general way, i.e. quadrupolar systems experiencing various anisotropic couplings. We worked on a rationalization of decoupling strategies, combining SIMPSON calculations and NMR experiments. In many cases, a good control of the influence of the MP decoupling schemes (pulse lengths, delays, asynchronisation…) with regard to the characteristics of the spin system (spin quantum number, C_Q, dipolar and J coupling constants) gave rise to spectacular gains in spectral resolution. This work on efficient decoupling strategies allowed us to contribute significantly to the structural characterization of the $VOPO_4$ phases.

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Materials

P283

Solid state NMR study of plasticization process in polymers

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Abstract: Plasticizers modify the mechanical properties of polymeric materials without altering their fundamental chemical character. The crystalline structures of polymeric materials are stabilized by second-order bonds, which can be destroyed by plasticizers. Numerous strategies have been developed to modify the macroscopic mechanical properties, but the plasticization mechanisms can be very different. During external plasticization only secondary interactions exist between the plasticizer and the polymer, while internal plasticizers are covalently bound to plasticized material. External plasticizers can migrate in the polymer, which can lead to recrystallization of the material and a loss of elasticity. Our results on three different systems are presented.

Chitosan, a widely used natural polymer, was softened by liquid phase plasticizers (glycerol and PEG400). The plasticizing efficiencies were found to be similar in mechanical tests, but the changes in the three-dimensional H-bonded structure monitored by solid-state NMR spectroscopy were different. The analysis of cross-polarization build-up curves and FSLG HETCOR experiments demonstrated that glycerol decreases the mobility of the acetamide groups, while PEG 400 increases it. Furthermore, while glycerol molecules are immobilized in chitosan films, PEG 400 remains mobile. Overall, PEG 400 acts as an external plasticizer, while glycerol acts as an internal plasticizer.

Poly(vinyl alcohol) were plasticized by liquid (polysorbate 80) and solid (hydroxypropyl-cyclodextrin) additives to gain electrospun nanofibrous systems. Both systems are properly softened according to both mechanical and NMR results. NMR measurements predicted high mobility of polysorbate 80 component in the fibers. This higher diffusivity resulted in fast ageing and partial smashing of fibrous structure. An active pharmaceutical agent (metoclopramide) was added to the system during the electrospinning process. Metoclopramide has a stable amorphous structure in the drug delivery system due to secondary interactions with the components of the fiber.

Covalently bound plasticizers are enduring. In our case PEG was branched onto PVC by a click reaction. Both polymers have glassy state at room temperature and they are immiscible with each other. Depending on the branching density and the length of the branched polymer, crystalline to very soft polymers were gained. Analysis of cross-polarization curves helped to understand structure-properties relationships.

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Materials

P284

Fractionation and DOSY NMR as Analytical Tools: From Model Polymers to a Technical Lignin

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Abstract: One key challenge hindering lignin's valorization is its structural complexity. Artificial lignin like materials provide a stepping-stone between the simplicity of model compounds and complexity of lignin itself. Here we report an optimized synthesis of an all G β-O-4 polymer **1** designed to model softwood lignin. After acetylation, the polymer **Ac-1(V)** was fractionated using a protocol that involved only volatile organic solvents, and which left no insoluble residue. Using DOSY NMR in combination with GPC, it was revealed that this fractionated material behaved like a flexible linear polymer in solution (average $\alpha > 0.5$). Acetylated Kraft lignin was subsequently processed using the same fractionation protocol. By comparison with the model polymer, we propose that acetylated Kraft lignin is composed of two classes of material that exhibit contrasting physical properties. One is comparable to the acetylated all G β-O-4 polymer **Ac-1** and the second has a significantly different macromolecular structure.

Materials

P285

1H and Hyperpolarised 129Xe NMR study of zeolite recrystallisation during hierarchisation with alkaline media.

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Abstract: Zeolites are microporous catalysts used in a large number of industrial applications. The presence of micropores is a characteristic that has made the success of these materials, because it entails a molecular selectivity, which makes it possible to control the size of the reagent, the state of transition or of the product. However, restricted molecular diffusion within the micropores makes access to active sites difficult. This affects the conversion and deactivation of the catalyst. Many research has been devoted to the creation of mesopores through zeolite particles, while retaining as much as possible the original micropores where the catalytic sites are. Such zeolites with multimodal porosity have really proved their effectiveness. Many strategies exist to obtain these hierarchical zeolites, but post-synthesis alkaline treatment has proven to be one of the most commercially viable techniques. This is due to the direct nature of the procedure, low organic footprint and excellent performance in various reactions. The post-synthetic alkaline treatment for synthesising hierarchical zeolites is often accompanied by tetraalkylammonium ions. Indeed, the use of pore-directing agents (PDA) has proved indispensable to avoid significant amorphisation, during the creation of mesopores.
In previous work aimed at understanding the mesoporous network after an alkaline treatment in the presence of organic additives, conventional bulk characterisation techniques led to the conclusion that the dissolved zeolite did not undergo any kind of recrystallisation. Here, we demonstrate using the data obtained from double-quantum 1H NMR and hyperpolarized 129Xe NMR spectroscopy that such recrystallisation actually occurs, which leads to the formation of a very thin coating of the mesopore walls. This demonstration is carried out on a zeolite beta (BEA) treated in the presence of TPA+ in an alkaline solution. The formation of a small number of nanoscale crystals or embryonic phases of zeolite silicalite-1 (MFI) is demonstrated, as well as their homogeneous dispersion on the mesoporous surface of the zeolite beta. We believe that these results may explain why a homogeneous distribution of mesopore size is obtained when organic pore-directing agents are used in the zeolite hierarchization process performed in an alkaline medium.

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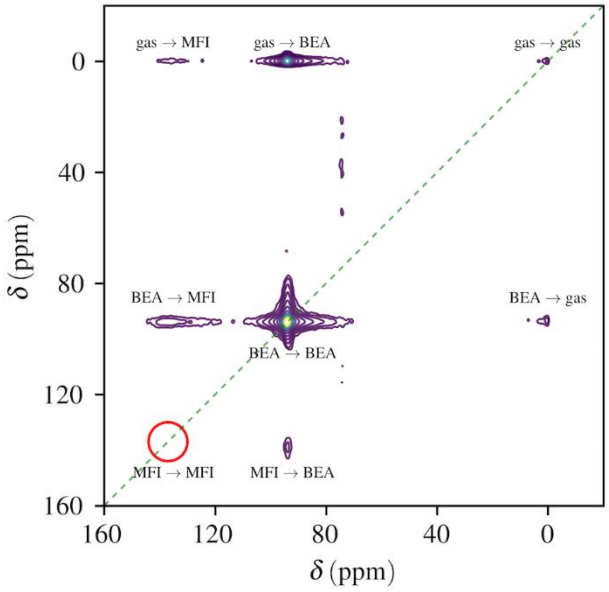

Materials

P286

Addressing functional behavior of heterogeneous catalysts using parahydrogen

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Abstract: In contrast to the studies of materials morphology, which usually require the use of solid-state NMR, the function of many fluid-containing (e.g., porous) materials can be addressed using liquid-phase NMR instrumentation and techniques. One vitally important class of such materials are heterogeneous catalysts used in various chemical transformations performed on the industrial scale, including petroleum refining, numerous chemical syntheses, and automotive emission control. Heterogeneous catalysts often comprise catalytically active centers residing on the extended surface of a porous support material. Understanding the functional behavior of these active centers is key to the rational transition to a more efficient and environment-friendly chemical industry, and NMR-based techniques provide a useful toolkit to address this major challenge. In particular, catalysts that can chemically activate H_2 are used in a broad range of chemical transformations essential for fuel production, such as hydrogenation and dehydrogenation, hydrogenolysis, hydrodesulfurization, hydrodenitrogenation, etc. An important advance in the functional NMR studies of such catalysts was made in 2007-2008 upon demonstration that the use of parahydrogen in heterogeneously catalyzed liquid and gas phase hydrogenations of alkenes can lead to a pronounced NMR signal enhancement. This finding opens the way for the utilization of parahydrogen-based NMR and MRI for the exploration of this broad and important class of functional porous materials. Parahydrogen-induced NMR signal enhancement is the simplest and technically least demanding approach particularly suited for the studies of hydrogenation catalyst performance as signal enhancement is produced by the chemical transformation catalyzed by the material under study. Importantly, parahydrogen is used here as a probe of the reaction mechanism rather than for the sake of signal enhancement itself, in particular, to explore the ways to design catalysts with an enhanced ability not only to yield a certain reaction product, but to do so through the implementation of a desired reaction pathway, namely the pairwise (as opposed to random) addition of H_2-derived H atoms to a substrate. To achieve this, we explored various ways of optimizing the behavior of supported metal catalysts (variation of substrates and reaction conditions, support, metal type and particle size and shape, metal oxidation state, metal surface modification with ligands and deposits, etc.). The studies were then translated to the next level, namely the exploration of the localized active centers purposefully designed on the surface of a porous support, such as single-metal-atom, single-site and/or molecularly defined heterogeneous catalysts, as well as to other reaction types (oligomerization, hydrodesulfurization, etc.). Recent examples of the use of NMR/MRI and parahydrogen to probe the functional behavior of such catalysts will be presented.

Materials

P287

Novel 129Xe NMR approaches for the investigation of topical materials

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Abstract: Xenon is commonly used as an internal probe due to its inert nature and easily polarizable large electron cloud. Here we utilize various experimental ^{129}Xe NMR techniques combined with the state-of-the-art quantum chemical calculations to investigate interesting and topical materials.

Porous materials contain network of variable sized channels with large total surface area. They provide a wide range of important applications, such as storage place, molecular separation and catalysis.[1,2] One increasingly studied class are porous organic cages which provide flexibility to the structure due to non-covalent packing. A solid organic cage, CC3-R, has shown a remarkably high capability in the separation of larger rare gases, like Xe, Kr and Rn. The material may also provide means to capture gases from air without cryogenic methods.[3] Study of ^{129}Xe absorption by CC3-R give detailed information about the dynamics of Xe between the cage and window cavities of the material (Fig. 1A).[4]

Metal-organic polyhedras (MOPs) are supramolecular inorganic structures that are build up via self-assembly of ligands to metal centers. This build up an inner phase with well-defined volume which can be altered by selection of ligands. These structures are widely used as sensors for organic analytes, in molecular recognition, cancer drug delivery, catalysis, gas adsorption and separation.[5] We showed that Fe_4L_6 metallosupramolecular cage (Fig. 1B)[6] encapsulate inert ^{129}Xe gas indicating its potential use *e.g.* in biosensor applications.[7]

The methane gas coming from shale rock is becoming an increasingly important, unconventional source of energy in the world.[8] One of the most important characteristics of shale is the pore structure of the material as this not only facilitates the adsorption and preservation of the gases, but also poses significant difficulties in the gas extraction. Cement is a binder used in construction in the production of mortar and concrete.[9] Pores have a dominating effect on the strength of cement, and they influence the transport properties, permeability and durability. In our experiments a very broad distribution of ^{129}Xe resonances were observed, which implies that the adsorbed xenon interacts strongly with paramagnetic impurities shales (Fig. 1D). In cement samples (Fig. 1C) pore size, heat of absorption and exchange rate were determined for differently hydrated cements.[10]

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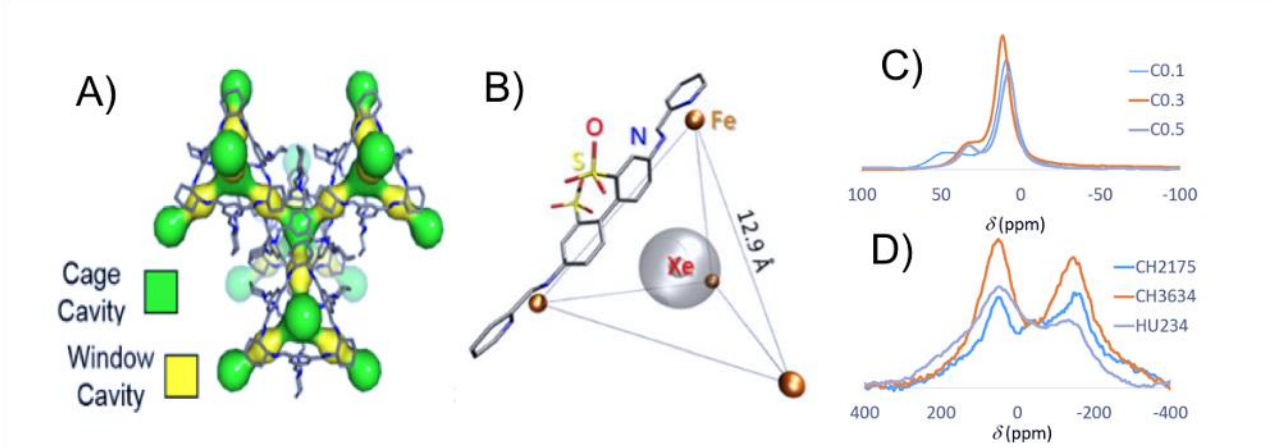

***Fig. 1**. A) 3D crystal cavity structure of CC3 material. The cage and window cavities are illustrated by green and yellow, respectively. B) ^{129}Xe encapsulated by Fe_4L_6 cage). ^{129}Xe spin echo spectra of C) hydrated white cement and D) shale samples.*

Materials

P288

89Y solid state NMR for a description of the local ordering in cristallized mixed-lanthanide polyoxometallates

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Abstract: We are studying lanthanide-containing polyoxometallates (POM). These compounds are interesting mainly for their luminescent properties. The luminescent properties of these compounds can be finely tuned by controlling the ratio of different rare earth (yttrium, lutecium and magnetic rare earth between samarium and lutecium) occupying the three inequivalent rare earth positions in the unit cell. A precise control of the luminescent properties requires a control of the rare earth magnetic interactions at a very local scale [1].

The luminescent properties of the compounds are governed by the interactions between luminescent sites and in the end [O1] by the Ln^{III}-to-Ln^{III} energy transfers. These interactions can occur within a hexanuclear entity and/or between adjacent POM entities. Two different mechanisms exist to allow this energy transfer (namely the Dexter and Förster mechanisms). The predominance of one of these mechanisms relies on the distances between the magnetic centres with a critical distance in the 10 Å range.

In term of structural characterization, X-ray powder diffraction provides information about the crystalline form of the compound and a description of the solid solutions by the evolution of the unit cell parameters when changing the rare earth ratios. However, it doesn't provide any local information about the non periodic ordering of the rare earth at a length scale of 10 Å. In this context, the use of a local probe such as ^{89}Y NMR is particularly relevant. ^{89}Y is spin ½ nucleus with a small gyromagnetic ratio (Larmor frequency 29.4 MHz at 14T).

We carried out studies on diamagnetic compounds (CPMAS), first on Y pure compounds and then on a series of compounds in the Y-Lu solid solution. Spectra interpretations were carried out using DFT calculation of NMR parameters (GIPAW as implemented in the Castep code). We also demonstrated the possibility to record two-dimensional ^{89}Y experiments in order to extract both isotropic and anisotropic chemical shifts. Finally, we carried out experiments on magnetic containing rare earth materials (Y POM doped to a few % by Gd and Yb ions) to evaluated the local ordering of the doped compounds.

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Materials

P289

Surface Structure Determination of Heterogeneous Catalysts by DNP SENS

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Abstract: Establishing fine relationships between the structure and the activity of a catalyst is essential in order to develop systems with improved catalytic efficiency. In heterogeneous catalysis, this task is extremely complex as the catalytically active surface sites are usually present at low concentration, and often incorporated on an amorphous support. Solid-state NMR spectroscopy combined with magic angle spinning (MAS) is in principle a method of choice to investigate the structure of heterogeneous catalysts. Its intrinsically low sensitivity however limits a broad applicability.

Dynamic Nuclear Polarization (DNP) is one of the most promising approaches to overcome the sensitivity limitations of solid-state NMR, and has recently emerged as a powerful technique to amplify the NMR signals of species located at surfaces.[1] We have recently demonstrated that the three-dimensional (3D) structure of a model organometallic platinum complex anchored on an amorphous silica could be fully determined by combining DNP Surface enhanced NMR spectroscopy (DNP SENS) with EXAFS data and DFT modelling.[1] Here we present the application of this multi-method approach to determine the surface structure of a hydrogenation catalyst containing iridium (Ir) N-heterocyclic carbene (NHC) complexes isolated on a silica surface.[2,3] In this case, the surface sites are a mixture of different chemical species due to the incomplete conversion of the NHC pre-catalyst to the silver NHC intermediate and the final iridium-NHC complex, making structure determination more challenging. We will first report the determination of the complete structure of the Ir pre-catalyst using ^{29}Si-{^{13}C} and ^{29}Si-{^{15}N} DNP SENS REDOR experiments. The difference with the structure of the Pt pre-catalyst determined previously[1] will be discussed. Then, we will show how using frequency selective ^{29}Si-{^{13}C} REDOR measurements[4] the structure of the silver and iridium complexes could be selectively probed. we envision this approach to be promising to characterize many modern materials that show a mixture of active and spectators sites on the surface .[2]

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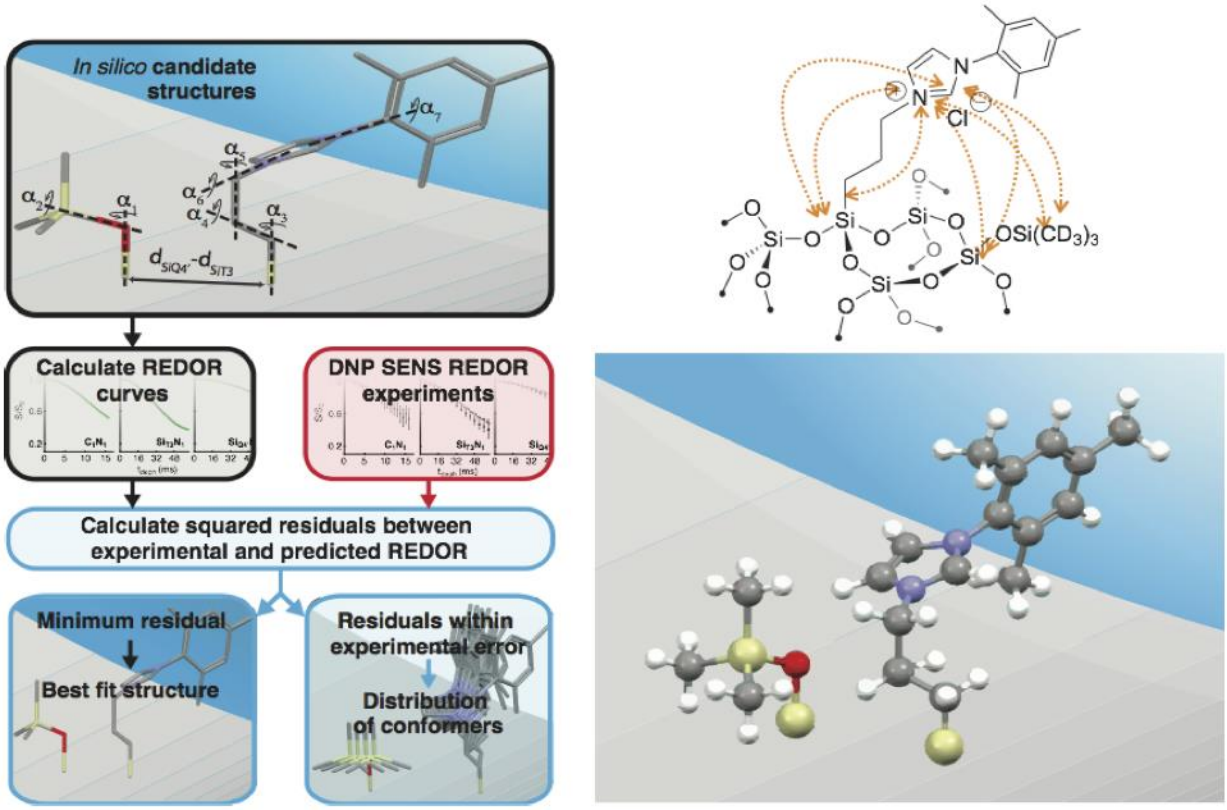

Figure 1 : left : Structure determination protocol for surface ligands. Top right: distance constraints measured on the NHC organic ligand. Bottom right: 3D structure in best agreement with the experimental data.

Materials

P290

Correlation of Solid-state NMR Parameters: A New Method to Distinguish Members of a Class of Materials. The Curious case of Transition Alumina Phases

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Abstract: Transition aluminas are intermediate phases generated during the thermal de-hydroxylation of aluminium hydroxide minerals and represent a family of materials with numerous industrial and research applications. They exhibit a high-surface-area and both Brønsted and Lewis type acidity, all interesting properties for applications in adsorption and catalysis. Typically, these phase are applied as dessicant, adsorbent, catalyst, catalyst support and as ceramics' precursor.[1]
The transformation sequences from (oxy)hydroxide up to alpha-alumina are highly influenced by starting material, crystallite size, relative humidity, presence of alkalinity, heating rate, pressure and bed depth.[2–6] While the structures of the initial hydroxides (Gibbsite, Bayerite) and oxyhydroxides (Boehmite, Diaspore) are highly ordered, the transition aluminas are more complex.
For some time they were considered as highly disordered or even completely amorphous.[6] Clear assignment of structural models to the different phases severely was, and still is hampered by the occurrence of complex phase assemblies along the calcination path in combination with their typical micro and nanocrystalline nature. Whereas the latter is a direct consequence of their formation by the release of structural water and consequent, densification, it results in broadening of the X-ray diffraction lines, rendering PXRD patterns very difficult or impossible to analyse.
^{27}Al quadrupolar interactions of ^{27}Al nuclei are highly sensitive to their local chemical environment, rendering advanced solid-state ^{27}Al NMR a unique spectroscopic tool to fingerprint and identify different Al containing phases. It allows discriminating the structural details of isomorphic Al oxides and aluminosilicates, revealing the crystal structure of transition alumina phases (α, χ, κ, θ, γ, δ, η, ρ) and their precursors.
This work attempts to compile a comprehensive library of ^{27}Al solid-state NMR studies covering all the transition alumina phases. The correlation of ^{27}Al NMR parameters isotropic chemical shift (d_{iso}), quadrupole coupling constant (C_Q) and asymmetry parameter (h_Q), as shown in the 3D ellipsoid plots below, provided extra resolution to the data, enabling the spectroscopist to do unambiguous assignments.

Figure 1. Evolution of transition alumina structures (above). Below are the correlation plots of ^{27}Al NMR parameters (d_{iso}, C_Q and h_Q) of four-, five- and the six Al-O coordinations in transition alumina phases (α, χ, κ, θ, γ, δ, η, ρ) and their precursors (Bayerite (Ba), Boehmite (Bo) Gibbsite (G) and Diaspore (D)).

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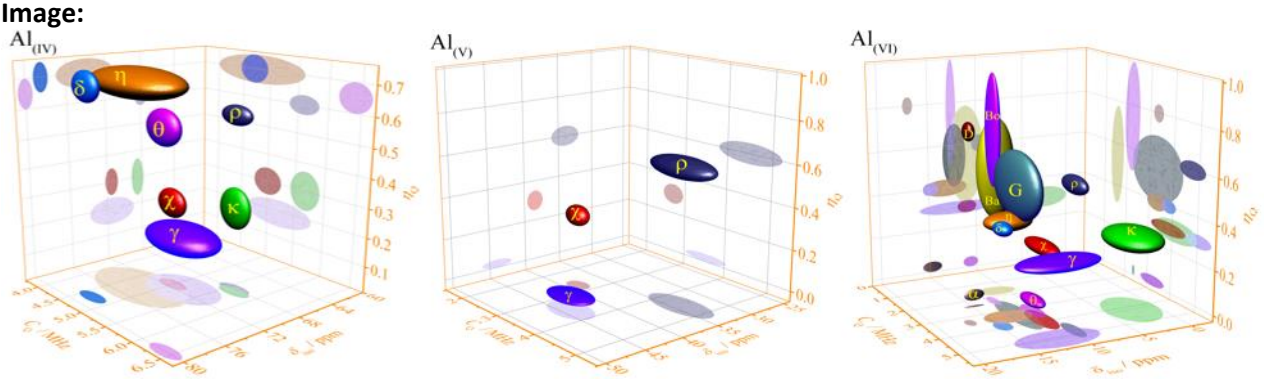

Materials

P291

ssNMR Observation of Competitive Molecular Adsorption Governing Chemical Reaction in Zeolite Micropores

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Abstract: The molecular-sized pores of zeolite catalysts provide spatial restrictions that allow for shape-selective catalysis. As most zeolites have several pore types with different geometries and properties, reaction selectivity is dependent on the pore geometry around the active site. Selectivity can be improved by restricting the access of reactants to specific zeolite pores. Competitive adsorption on zeolites contacted with complex molecular mixtures in liquid phase is poorly understood, as well as the use of this competition to achieve selectivity in heterogeneous catalysis. This contribution demonstrates ssNMR approaches to detect and characterise competitive adsorption under conditions relevant to catalysis. Using two showcase liquid state reactions heterogeneously catalysed by two different zeolites, etherification of *β*-citronellene with ethanol on zeolite beta and acid-catalyzed furfuryl alcohol oligomerization on ZSM-5, we demonstrate the principle of exploiting NMR detected competitive adsorption to guide reaction selectivity.

Combining of ^{13}C Hahn-echo and ^{1}H–^{13}C cross-polarization ssNMR discriminates between molecules freely moving in liquid phase outside zeolite beta and molecules adsorbed inside zeolite pores and in pore mouths.[1] In the absence of ethanol, *β*-citronellene molecules enter zeolite pores and react to isomers. Preferential adsorption of ethanol, excludes *β*-citronellene from the zeolite pores, restricting the etherification reaction between *β*-citronelle from the external solution and ethanol from inside the pore to the zeolite poremouths. The competitive adsorption of ethanol prevents the undesired side reaction of β-citronellene isomerization inside zeolite pores.

Figure 1 - left: $^{13}C\{^{1}H\}$ solid-state MAS NMR spectra acquired with a Hahn-echo (in red) and CP (in black): (a) ^{13}C liquid-state NMR spectrum; (b) dehydrated zeolite beta mixed with β-citronellene; (c) dehydrated zeolite beta mixed with β-citronellene and ethanol (molar ratio of 1:10).

Figure 1 - right: Schematic representation of the intergrowth structure of a ZSM-5 crystal and the relative pore orientations, combined with CLS micrographs of furfuryl alcohol oligomers accumulated within the crystals using water (bottom) or 1,4-dioxane (top) as a solvent.

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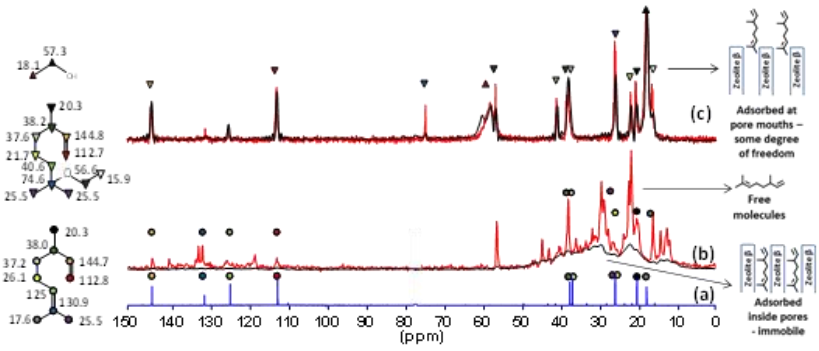
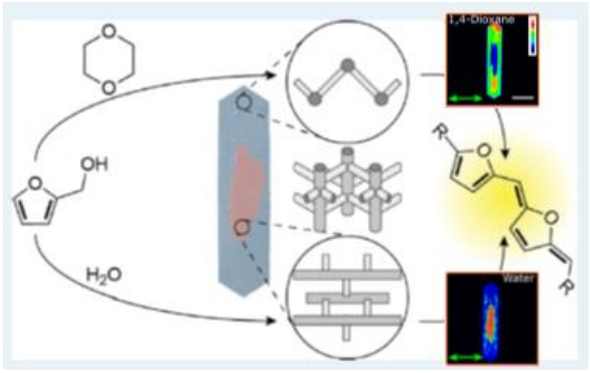

(c)
Adsorbed at pore mouths – some degree of freedom
Free molecules
(b)
(a)
Adsorbed inside pores - immobile
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
(ppm)


OH
H2O
1,4-Dioxane
Water
R
R

Materials

P292

Optimization of the Pulsed Field Gradient NMR approach for the determination of molecular weight distribution of industrial copolymers

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Abstract: Molecular Weight (Mwt) distribution affects the physical and mechanical properties of polymers. For example, in decorative paint applications, it defines the film forming properties of the paint, as well as its durability, chemical resistance and flexibility when dry.

Mwt distribution is commonly determined by the use of Gel Permeation Chromatography (GPC). The technique separates polymers based on differences in their hydrodynamic volumes and the entropic interactions of the polymer chains with the stationary and mobile phase. GPC chromatograms may, however, be incorrect for complex copolymers due to additional enthalpic interactions of the polymer chains with the GPC column. A polymer's architecture may also affect the separation. Mwt determination in multi-polymer mixtures is even more challenging due to potential signal overlapping.

In recent years, Pulsed Field Gradient Nuclear Magnetic Resonance (PFG NMR) has been proposed as an alternative approach to determine Mwt distribution of polymers[1]. Unlike GPC, PFG NMR provides simultaneous information on polymer size as well as its chemical composition. PFG NMR was developed to study the mobility of molecules and measures distribution of polymer self-diffusion coefficients D, from which weight average molecular weight (Mw) can be determined using the scaling law: $D = K\,Mw^{-\alpha}$ where K and α are scaling parameters specific for each polymer system. So far, the applicability of PFG NMR for Mwt distribution studies has been, demonstrated mainly for low molecular weight (<10 000g/mol) homogenous polymers, however[2].

In this study, PFG NMR is applied to study Mwt distribution of industrial polymers of average molecular weights varying between 100 000 g/mol and 2 000 000 g/mol. Transitional mobility of such long polymer chains is complex and may be affected by chain entanglement. Parameters such as solvent system, polymer concentration and temperature are optimized as well as pulse sequences and applied delays, to minimize relaxation and convection artefacts. Good correlation to the scaling law has been obtained within the entire Mwt range studied. The applicability of the method is also confirmed for copolymers (2 and more different monomer units) and copolymer mixtures and the obtained results compared with Mwt distribution data from GPC.

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Materials

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Elucidation of degradation behavior for thermally aged urea-urethane with various solid state NMR experiments

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Abstract: Elucidation of degradation behavior for thermally aged urea-urethane with various solid state NMR experiments

Kaori Numata, Atsushi Asano, Yasumoto Nakazawa,

1. Introduction

The measurements of Nuclear Magnetic Resonance spectra are useful to elucidate polymer degradation behavior. On the other hand, spectra in Solid State NMR are broad and usually difficult to assign. In this study, We elucidated the degradation behavior of thermally aged urea-urethane by measuring ^{13}C CP/MAS spectra at various contact times, ^{13}C DP/MAS spectra with different recycle delays, and ^{1}H spin-spin relaxation time:T_2 in low field.

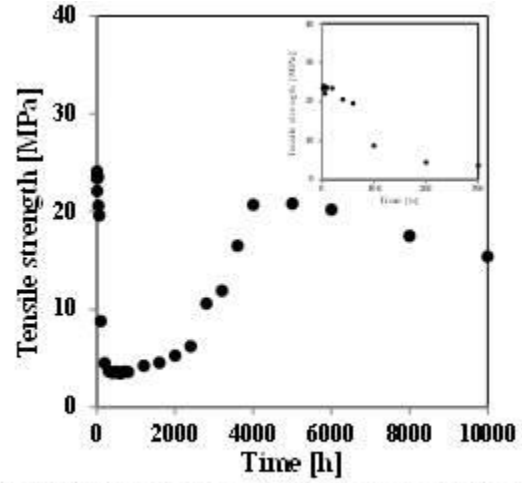
2. Experimental

The urea-urethane consists of poly tetramethylene ether glycol PTMG, 4,4'-diphenylmethane diisocyanate, 4,4'-Bis(sec-butylamino) diphenylmethane and diethyltoluenediamine. Test pieces were thermally aged at 100°C up to 10000 h. After that, we measured tensile strength (TS), elongation at break, ^{13}C NMR spectra and T_2. The ^{13}C NMR spectra were obtained on a JEOL ECA II spectrometer with a ^{1}H frequency of 700 MHz and a ^{13}C frequency of 175 MHz. The spinning speed was set at 15 kHz. The recycle delays were set to 2.7 s for CP/MAS experiments and 10 s and 80 s for DP/MAS. The CP contact times of 0.2, 1.0, 4.0, 8.0 ms were used for each CP experiment. T_2 measurements were performed on a minispec 20 at a ^{1}H frequency of 20 MHz and 23°C.

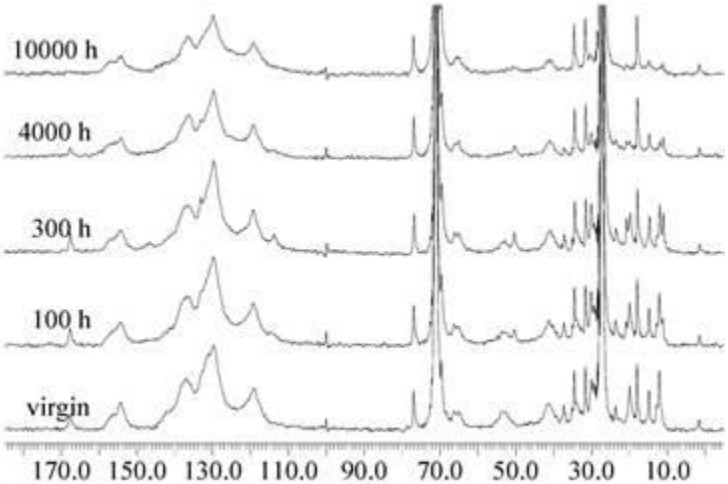
3. Results and discussion

All the results indicated that the degradation proceeded in three stages. TS rapidly decreased in the first stage, increased in the second stage, and then gradually decreased again in the final stage. Elongation decreased and increased following TS in the first and second stage. In the final stage, elongation decreased followed by TS. In all the NMR spectra, the shapes of the peaks at 27 and 71 ppm, which were assigned to CH_2 of PTMG, were stable. Therefore, PTMG unit did not degrade while thermal aging. The results of various NMR measurements clearly showed the degradation behavior: the thermal dissociation of urea bond in the first stage, the bleed out of dissociation products in the second stage, and rebonding in the second and final stages.

Image:



Tensile strength of thermally aged urea-urethane at 100° C.



DP/MAS Spectra (recycle delays of 80 s)

Materials

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Novel Ionic Liquids with Homo- and Heteroleptic Orthoborate Anions

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Abstract: Novel non-halogenated phosphonium ionic liquids (ILs) with homoleptic and heteroleptic catechol-based orthoborate anions were prepared and characterised using ^{11}B, ^{13}C, ^{31}P, ^{1}H NMR, FTIR, Raman spectroscopy, viscosity, conductivity and CV measurements. ILs with heteroleptic anions (Het-ILs) have lower melting points and lower viscosities as compared to the ILs with the homoleptic anions. Het-ILs have an excellent electrochemical stability and solubility in polar and non-polar hydrocarbon solvents. Non-halogenated boron based ILs have already been shown to provide outstanding structure dependent lubrication properties for a range of engineering materials.[1-5] Novel Het-BILs reported in this work can potentially be used in a wide range of applications, such as non-flammable electrolytes, in defoliation of graphite into single-layered graphene, as media for recrystallization of pharmaceutically active compounds, in lubrication, to mention a few.

Het-BILs in this study are highly hydrophobic, have high thermal stability and wide electrochemical windows. The most intriguing results in this study are the effects of heterolepticity of orthoborate anions on the IL viscosity. Therefore, tuning of various physico-chemical properties of BILs is possible by increasing the asymmetry in anions using the concept of heterolepticity. A simple mixing of ILs, as suggested in numerous previous reports,[6] does not deliver the same result. This our novel synthetic approach will provide a new horizon to develop further interest towards non-halogenated ILs and their applications and will hopefully assist in solving the "anion crisis" in the research field of ILs: With *n* preselected ligands for the synthesis of orthoborate anions only *n* homoleptic orthoborate anions can be synthesised, while $(n^2-n)/2$ different heteroleptic orthoborate anions could be prepared. We already screened at least 35 different interesting organic ligands for the synthesis of already known and yet unknown orthoborate anions, thus, opening a gateway for 35 different homoleptic and 595 heteroleptic orthoborate anions to be combined with a range of different classes of cations (phosphonium, ammonium, imidazolium, pyrrolidinium, etc.) The synthetic work to cover the whole this matrix of Het-ILs is currently in progress.

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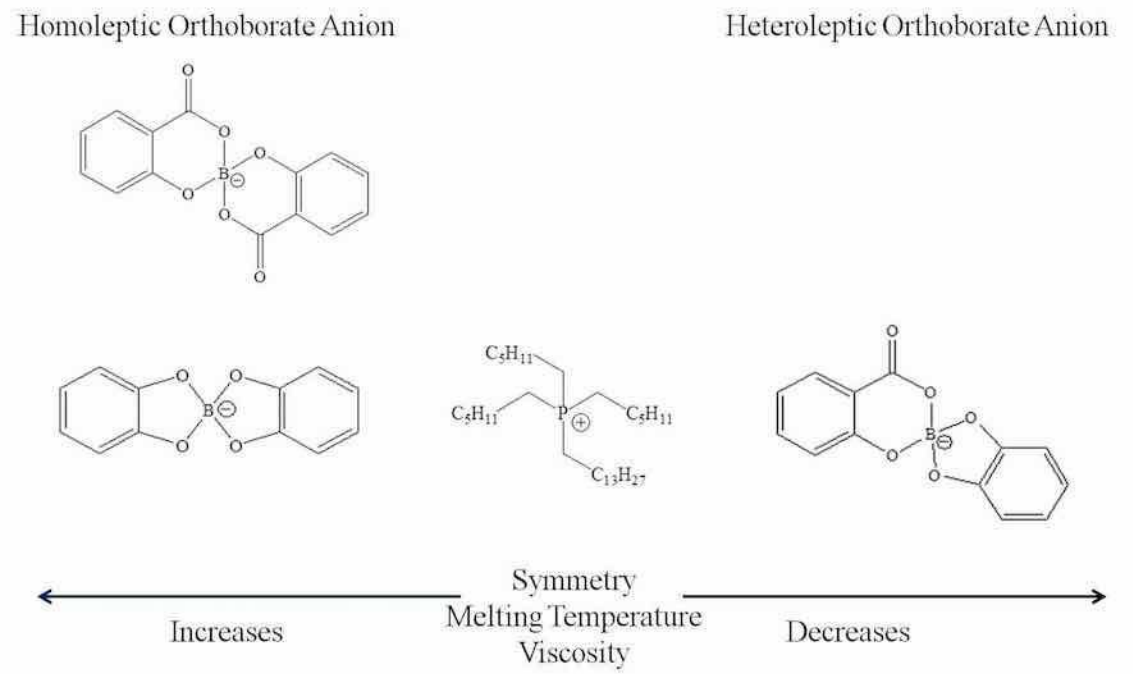
Image:


Homoleptic Orthoborate Anion
Heteroleptic Orthoborate Anion
Symmetry
Melting Temperature
Viscosity
Increases
Decreases

Materials

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Characterization and Quantification of acid sites on γ-alumina and γ-alumina impregnated with metals using 31P NMR and Triple-Quantum Magic Angle Spinning 27Al NMR

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Abstract: Aluminium oxide is widely used as a solid support for acid catalyst for a variety of reactions including dehydration, isomerization and alkylation. γ-Al_2O_3 is used as a support for aqueous-phase transformation of cellulose in the study of conversion over heterogeneous catalyst, also in refinement processes such as hydroprocessing and other. Therefore, the understanding of the acid properties of these materials is very important in a wide range of applications. Surface acidity in γ -Alumina is characterized by the chemical nature, being recognized Brönsted or Lewis acid sites, their concentrations, and distributions/locations, which are directly related to catalytic performance: activity and product selectivity.

In this work was performed the adsorption of trimethylphosphine (TMP) on a variety of a different kind of γ-alumina samples (with and without metal oxides like Ni and Mo: Boehmite, Mo/γ- Al_2O_3, NiMo/ γ- Al_2O_3 and NiMoP/γ- Al_2O_3). This probe molecule was adsorbed on dehydrated samples, which were thermal activated at 723K on a humidity free enviroment-. After the treatment and the probe molecule absorption the samples were introduced into an NMR rotor, sealed and transferred to the spectrometer.

The aim of the present work is to get information about the changes that occur at the surface of γ-alumina after impregnation with metals like molybdate, nickel or phosphate. The interaction between TMP-acid site was studied with ^{31}P MAS NMR spectroscopy, allowing to distingue the different coordinations, concentrations, distributions of the acid sites. The ^{27}Al MQMAS NMR experiments was used to increase the resolution of the spectra and to get information about the distribution of the aluminum sites and have a better understanding and resolve the overlapping signals on the samples observed in e.g. ^{27}Al NMR MAS. With the above experiments, we could obtain information about the bulk and surface structure of the samples and be able to characterize and understand the acid surface of these acid solids.

The authors want to thank the financial support of Ecopetrol S.A. and Colciencias contract 403-2013 and Vicerrectoria de Investigación y Extensión-UIS grant number 8821.

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Materials

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Noninvasive Relaxometry Evidence of Linear Pore Size Depend-ence of Water Diffusion in Nanoconfinement

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Abstract: We propose an original experimental method based on NMR at variable magnetic fields experiments (NMRD) and a theoretical analysis of the data that allows probing the spatial dependence of the diffusion coefficient of liquids specifically at proximity to pore surfaces. One of the key results found from these experiments is the linear relationship between average parallel diffusion coefficients and pore radii[1]. Another result is the robustness of the frequency scaling[1, 2, 3, 4] of the master curve approach able to take into account the complexity of the water dynamics at pore surface for samples of different geometries. This approach has proven useful for evaluating the efficiency of the coupling between liquid layers within nanopore by extracting gradients of diffusion coefficients. The application of this method to water confined in synthesized calibrated nanopores like MCM-41 for cylindrical geometry[5] and SEOS for spherical geometry[6] has been successful to deal with several dynamical processes on pore surface for different materials. This shows the ability of the proposed method to discriminate between the influence of the geometrical confinement on intrapore dynamics and the chemistry of the interface induced by different synthesis of the materials. For instance, the frequency selectivity of NMRD profiles has been able to separate the different couplings coming from the spatial heterogeneities on the pore surfaces[1, 2] . This frequency selectivity of NMRD has also allowed discriminating several steps of a complex dynamics process composed by both loops in water and surface diffusion during adsorption events[7]. Based on our experimental and theoretical results, we believe that the proposed noninvasive method allows exploring the interplay between molecular and continuous description of fluid dynamics relevant in physical and biological confinements.

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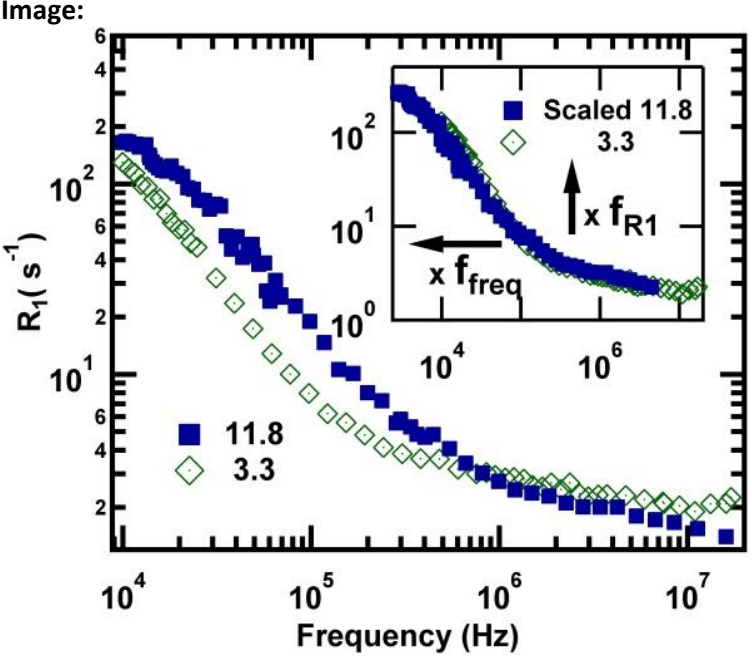
Figure 1: ^{1}H NMRD profiles of MCM-41 saturated samples with 3.3 nm (diamond) and 11.8 nm (square) pore diameters (main figure). In inset, the master curve obtained by scaling both relaxation rate and frequency by the factors f_{R1} = 1.6 and f_{freq} = 3.3/11.8, respectively is superposed to the MCM-41 of 11.8 nm pore diameter.

Image:



Metabolomics and other "omics"

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Metabolic characterization of a new rodent model of the neuropsychiatric disorders associated with Parkinson's disease

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Abstract:

Purpose

Parkinson's disease (PD) is the most common neurodegenerative disorder after Alzheimer's disease, affecting almost 1% of the population beyond the age of 60. Currently, the diagnosis of the disease relies on the expression of the well-known motor symptoms (akinesia, rigidity, and tremor) which appear when 70 to 80 % of the dopaminergic neurons of the substantia nigra pars compacta are lost. This late diagnosis limits the possibility to develop curative treatments that remain symptomatic so far. Thus, detect the disease earlier represent the key step to resolve this therapeutic issue. For a long time considered as a purely motor disease, PD is nevertheless associated with neuropsychiatric disorders which can be expressed during the early stages of the disease but also later on. Among them, depression, anxiety and apathy are the most frequently observed.

In this context, the goal of this study was to find specific molecular markers for both these neuropsychiatric symptoms and the early phases of PD. For that aim, we used proton NMR-based metabolomics on serum samples of a rodent model allowing the investigation of different phases of PD.

Methods

- Animal model: It is based on specific, partial, bilateral 6-OHDA-induced lesion in dopaminergic neurons. For each rats (n=53), before and 3 weeks after the surgery, motor functions were assessed using stepping test, and motivation and depression-like behaviors using operant sucrose self-administration test, and 200 µl of fasting blood sampled in the caudal vein. At the end of the experiment the brain was extracted in order to check the striatal dopaminergic denervation by histological examination.

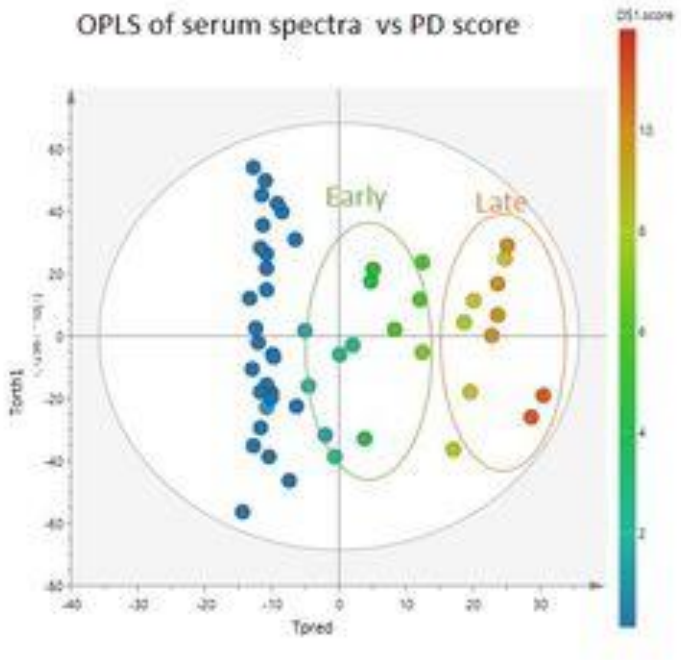
- NMR experiments: the serum samples were analyzed on a 950MHz Bruker Avance III spectrometer (IBS Grenoble), using a CPMG pulse sequence. The spectra were preprocessed and bucketed (0.001 ppm buckets) using the NMRprocflow open source software (http://nmrprocflow.org) and submitted to multivariate statistics (SIMCA v14). OPLS (orthogonal partial least square) analysis were conducted to investigate if behavioral data can be predicted from spectroscopic data.

Results

We obtained in our animal cohort a gradation in the symptoms, from neuropsychiatric symptoms alone, to the expression of neuropsychiatric associated with motor symptoms. This gradation is well in line with PD progression from early to late phases of the disease. To evaluate it more precisely, a scale was built which integrates behavioral performances (neuropsychiatric and motor) and the striatal dopaminergic denervation. OPLS analysis were then conducted using serum spectra and showed a good correlation between metabolic profiles and the score's gradation, i.e. with the different phases of PD. The energetic pathway seems to be increased with the progression of the disease, while some amino acids, like alanine and leucine are also dysregulated.

New experiments are in progress to validate our data and to further explore the metabolic pathway.

Image:



Metabolomics and other "omics"

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NMR based metabolomics: a breath of fresh air for ARDS patients on life support

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Abstract: Acute respiratory distress syndrome (ARDS) referring to patients on life support system is a clinical enigma for intensivists as the etiology is heterogeneous and remains obscure. Although advent of many rescues therapies and ventilator support has improved critical care management but the escalating morbidity and mortality calls for differential diagnosis. NMR based metabolomics provides a dynamic and global evidence based approach for ARDS that culminates from wide spectrum of illness thereby confounding early manifestation and prognosis predictors. In our study performed in collaboration with tertiary care hospital, we have established mini bronchoalveolar lavage fluid (mBALF) taken from proximal alveoli to capture the localized changes in the lung compartment and serum biofluid to snapshot the systemic changes of ARDS lung pathophysiology [1]. Firstly, a comprehensive set of 50 metabolic predictors in mBALF fluid were identified and characterized by 1D and 2D NMR approaches representing the lung milieu in diseased state [2]. Furthermore, six potential biomarker candidates (proline, lysine/arginine, taurine, threonine and glutamate) in mBALF fluid were determined by chemometrics as predictors of progression in different ARDS substages [3]. Advancing from our pilot study, we validated the ARDS clinical spectrum in large cohort of 200 diseased and control samples of both serum and mBALF. The following study resulted in determining the prognostic classifiers in circulating serum biofluid of mild, moderate and severe ARDS patients in terms of metabolic profile by statistical validation. Contribution of aromatic amino acids, alanine with notable contribution from 3- hydroxybutyrate and lipid determined by univariate and multivariate analysis elucidated the progression trajectory of ARDS severity. The mortality predictors identified in terms of significant metabolic changes and contributions in the survivor and non-survivor subjects illustrated the mortality trend and disease outcome. The metabolic fingerprint due to pulmonary and extrapulmonary changes in both fluids were targeted which highlighted the different respiratory mechanics of direct and indirect insult to lung parenchyma in terms of acetoacetate, glucose and branched chain amino acids contributions. An all-inclusive sketch of biomarkers in both mBALF and serum represents the underlying complex manifestation and mechanism of ARDS. Thus, NMR based metabolomics proves to be a complete canvas for portraying ARDS systems biology.

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Faster, Quantitative, Highly Resolved and More Sensitive approach for metabolomics

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Abstract: A better understanding of metabolism requires a detailed and quantitative analysis of complex small molecules biological samples such as extracts or biofluids. In this context, NMR is one of the most used analytical techniques and makes it possible to quantify complex mixtures with an excellent precision and trueness. In the context of metabolic studies, complex mixtures are usually hard to decipher on a 1D spectrum due to ubiquitous peak overlaps. Therefore, 2D NMR is a nice alternative for improving the spectral resolution along two dimensions. For highly overcrowded spectra, the ultimate solution can be to record heteronuclear ^{1}H-^{13}C 2D maps to benefit from the larger spectral window of ^{13}C. However, these experiments suffer from a low sensitivity per unit of time, which makes it unsuitable for high-throughput targeted metabolomics. In order to push the current limitations of this approach, we recently proposed a new method based on an "intrinsically" quantitative ^{1}H-^{13}C technique called QUIPU HSQC [1]: with a simple internal reference of accurately known concentration, it is possible with only one 2D map to access the concentration of all detectable metabolites. Thus, **metabolites in the range of 100 µM within leaf extracts were quantified thanks to sensitivity gains of 2 to 3 for ^{1}H-^{13}C correlations in comparison with the conventional version and for the same experiment time**. This technique has also been applied with success to better understand the photosynthetic and photorespiratory cycles by detecting sugar phosphates as biomarkers of these processes [2]. However, this approach is time consuming and has recently been improved through the Quick QUIPU HSQC or Q-QUIPU HSQC [3] combining spectral aliasing [4], NUS [5] and variable repetition times [6] methods. **The time necessary to obtain quantitative data is divided by 6 to 9 by keeping a good precision (from 1 to 6%) and trueness (from 4 to 8%)** with a high spectral resolution. The analytical performance of this new advanced tool dedicated to quantitative metabolomics is successfully shown on breast cancer cells and herbal dietary supplements embedding millimolar to submillimolar metabolites.

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Metabolomics and other "omics"

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Optimized slice-selective 1H NMR experiments combined with highly quantitative 13C NMR to perform 13C isotopomics at natural abundance

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Abstract: Isotope ratio monitoring by NMR spectrometry (irm-NMR) provides the complete ^{2}H or ^{13}C intramolecular position-specific composition of a molecule at natural abundance, a strategy belonging to the recent concept of **isotopomics**. Irm-^{2}H has been developed in the 1980s, but the development of irm-^{13}C NMR is much more recent. Indeed, this analytical tool requires **an accuracy of 1‰** (against 1% for irm-^{2}H NMR) due to the very small deviation range of ^{13}C natural abundance values. Currently, measuring position-specific natural abundance ^{13}C values relies on the combination of ^{13}C acquisitions with isotope ratio mass spectrometry (irm-MS) as a reference. This approach suffers from several drawbacks: i) it requires two different analytical techniques on the same sample, ii) it suffers from the low sensitivity of ^{13}C, (iii) it requires to observe the signal of all the carbons of the analyzed compound, which is not always possible and thus prevents the use of multipulse sequences such as INEPT or HSQC, the isotopic information given by quaternary carbons is lost.

To overcome these constraints, an internal reference method has been developed.(1,2) This new NMR strategy consists in (i) quantifying *in situ* the concentration ratio of both the analyte and the internal reference by quantitative ^{1}H NMR and then (ii) calculating the position-specific natural abundance deviations thanks to quantitative ^{13}C NMR. It thus requires to **perform both ^{1}H NMR and ^{13}C NMR quantitative analysis on the same sample with a very high accuracy**. This is very challenging because highly concentrated samples need to be used (to reach a sufficient SNR in ^{13}CNMR), which forms an obstacle to quantitative ^{1}H NMR due to radiation damping issues.(3) This has led to the development of a specific pulse sequence to perform highly accurate quantitative ^{1}H NMR on concentrated samples using **spatially-encoded experiments**. A pulse sequence named DWET was first described in 2015 by our group(1), which combines a spatially-encoded excitation of a thin slice of the sample after **saturation of external regions**. However it appeared unsuited to samples with short T1 values (which often occurs in isotopic analysis where relaxing agents are used). In this context, we suggest two variants of the DWET called Multi-WET and Profiled-WET, developed and optimized to reach the same accuracy of 1‰ with a better immunity towards T1 variations. With this strategy, the isotopic profile of vanillin has been recorded with a 1‰ accuracy thanks to ^{13}C NMR analysis whose experiment time reached 2 h 09 min instead of 4 h 49 min.(2) Then, we applied this strategy to a ^{13}C isotopic study where the INEPT pulse sequence was used instead of the inverse-gated decoupling one. The method was applied to the authentication of ibuprofen samples originating from different regions and/or manufacturing processes.

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Metabolomics and other "omics"
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Metabolomic spectra pre-conditioning using PcBc
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Abstract:

Metabolomic spectra are particularly difficult to properly phase and to correct their baseline roll. This is principally due to the presence of very large number of heavily overlapping peaks which are therefore difficult to phase and, in addition, leave only limited (or no) gaps between groups of peaks where the baseline can be visually or programmatically assessed.

The latter aspect makes inapplicable, or ill applicable, many traditional phase and baseline correction algorithms. Due to the coupling between the phases ph0 and ph1 and the baseline corrections coefficients, this makes the preconditioning of these spectra problematic and, when done manually, extremely subjective.

In addition, typical metabolomic studies call for the evaluation of large numbers of spectra. To speed up the pre-processing and at the same time guarantee consistency and objectivity of the results (impossible to guarantee when done manually), it is highly desirable to apply an automatic phase and baseline correction method.

We present here preliminary results obtained by the application to metabolomic spectra of a novel [1] automatic method, named PcBc, which allows a simultaneous phase and baseline correction using both the in-phase and out-of-phase parts of a spectrum.

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Metabolomics and other "omics"

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A Fast 2D 1H NMR lipidomics workflow for chemical food safety issues

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Abstract: Lipidomics is a widely recognized approach, with applications in fields of research such as disease diagnostic, food quality/authencity[1,2] and food safety[3]. Although NMR has been reported for lipidomics[2], it is still minor compared to its Mass Spectrometry counterpart[1]. 1D proton NMR suffers from considerable overlap between lipid signals and even though heteronuclear NMR offers a better resolution, it comes at the price of a limited sensitivity. To resolve these issues, **an appealing solution is the use of fast 2D ^{1}H strategies**, allowing a better resolution, as the signals are spread along a second dimension, while keeping a satisfying sensitivity. Based on these considerations, **we developed a complete NMR workflow for serum lipidomics fingerprinting, using fast NMR approaches**.

First we will describe the development of the sample preparation protocol, and the assessment of its repeatability. Extraction replicates have been performed and analyzed by 1D ^{1}H, highlighting the **robustness** of our protocol.

Next we will compare the different fingerprinting strategies implemented in the workflow, which uses 1D and **fast 2D in an automated way**, thanks to an auto-sampler. In particular, Non-Uniform Sampling ZQF-TOCSY and ultrafast COSY schemes have been carried out, as their benefits have already been highlighted for metabolomics[4]. 2D spectra provide well-separated signals, facilitating their integration, while maintaining acquisition times compatible with high-throughput lipidomics.

The workflow was subsequently applied to a real food safety research question. The lipid profile disruptions in serum of b-agonist diet-fed pigs were characterized. To our knowledge, it is **the first time that fast 2D NMR methods are used in an automated way** and in a high-throughput analytical chemistry study. The results obtained with the presented workflow highlight the ability of these methods to **discriminate sample groups** and shows their ability to **resolve ambiguities** in result interpretation and thus their **potential for food safety** applications.

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Discovery of early prediction biomarkers of liquid egg product alteration during their production process using combined metabolomics approaches (NMR and MS)

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Abstract: Liquid egg product is a complex biological matrix, mainly constituted of proteins, lipoproteins, lipids and many micronutrient (vitamins, minerals). However, once broken and mixed, the liquid egg product lose much of its preservation properties, thus becoming for some bacteria an excellent growth medium. This uncontrolled bacterial development induce, beside the involved sanitary risks, biochemical alteration that strongly modify the product organoleptic properties.The objectives of this study are to explore the involved biochemical mechanisms using combined metabolomics approaches (untargeted MS and NMR profiling) in order to describe and understand those mechanisms that were until then not well characterized and to identify, in fine, early prediction biomarkers of these microbiological alteration (Altovop project, funded by region Bretagne and Pays-de-la-Loire).

Kinetics studies of this alterations were realized on sterile liquid egg products prepared in laboratory, samples were inoculated with two model strains which are characteristic of two specific alteration type (1 and 2). The multi-omics analysis of metabolic profiles obtained by MS and NMR showed that the metabotypes were alteration type dependent, for temporally proximate samples as long as for contrasted phenotypes. The observed differences led to the identification of groups of compounds (metabolic signatures) specifics of each kinetic study analysis points for the two types of alteration. Specific individual prediction biomarkers, which are currently in the process of structural elucidation, are showing the potential to detect in an early stage the liquid egg products microbiological alteration. They could, then, allow a better quality control process on production chains in this industry.

Keywords: metabolic fingerprint, food and feed, NMR, MS, early prediction biomarkers, alteration, liquid egg products

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Development of high-precision quantitative 15N NMR using modified polarization transfer INEPT for "isotopomics" studies

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Abstract: Since 1981, the direct access to Site specific Natural Isotope Fractionation by Nuclear Magnetic Resonance (SNIF-NMR™) was immediately recognized as a powerful technique to authenticate the origin of natural or synthetic products, now named isotope ratio measurement by Nuclear Magnetic Resonance (irm-NMR).

Although now an exploitable technique, further improvements in irm-^{13}C NMR can be envisaged, to bring improved sensitivity and accuracy. Recent technological developments using multi-pulses ^{13}C NMR sequence, such as **"Insensitive Nuclei Enhanced by Polarization Transfer"** (INEPT) showed which is possible to obtain, after subsequent modifications (adiabatic pulses) the required precision (0.1%). The irm-^{13}C INEPT ^{1}J has already been applied in different domains [1,2] and be actually successfully applied using long range scalar coupling ^{n}J still with high precision (0.15%) in order to open access of all ^{13}C/^{12}C ratios (including those from quaternary carbons) on the molecule of interest.

For the first time, the know-how of irm-^{13}C INEPT ^{n}J was transferred on irm-^{15}N INEPT ^{n}J. A proof of concept was carried out with an AVANCE HD 700 MHz BRUKER -equipped with a cryo-probe- on a small molecule: 1-methylimidazole (2 nitrogens coupled with $^{n}J_{1H\text{-}15N}$ long range). The isotopic fractionation was measured in order to simulate the position-specific fractionation during distillation process on 1-methylimidazole, only few % of mass fraction was collected without traces of water.

The first results showed an accuracy of 0.15% and two different isotopic profiles between commercial and distillate 1-methylimidazole obtained by irm-^{15}N INEPT ^{n}J. It is the first time that ^{15}N provides high-precision measurement and isotopic fingerprint at naturel abundance. Furthermore, this method will be extend on forensic studies to provide a unique means to allow detailed detection and tracing of a given explosive, such as trinitrotoluene (TNT). By determining a multiplicity of variables (site-specific ^{15}N/^{14}N ratios) from different samples using the same protocols, isotopomics approach can be considered in order to study the different isotopic profiles.

Funding and acknowledgement to "Agence Nationale de la Recherche" ANR through the FRIIME project.

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Multiscale NMR analysis of entire microalgae cells towards in-vivo profiling of lipids

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Abstract: Microalgae are microscopic unicellular photosynthetic organisms proposing highly uncharacterized chemodiversity and a great ability to produce valuable sustainable products. They are used in a wide range of applications, from fish farming to the production of specific compounds such as pigments or bioactive products. Only a minor proportion of the millions of species spread in the environment are currently investigated, constituting therefore a high potential research subject.

However, the characterization of the microalgae intracellular compounds often requires one or several extraction step(s) associated with diverse analytical techniques that provide insights on its compositional information. The complexity of the matrix and of its chemical diversity constitutes a strong bottleneck often limiting its deep chemical characterization.

In this context, we propose to assess the potential of Nuclear Magnetic Resonance (NMR) for the profiling of the microalgae lipids. As biological model, we have investigated *Parachlorella kessleri*, a microalgae known to accumulate large quantity of lipids (up to 40 % of dry weight) under specific cultural conditions[1]. This preliminary investigation was carried out on different samples (extracts or entire cells) and at different magnetic fields: High Field (HF) NMR which provides the best sensitivity and resolution, but also Low Field (LF) NMR which has strong potential for the online analysis of microalgae lipids. Indeed, recent improvements in LF NMR pulse sequences, especially in solvent suppression[2] and Ultra Fast (UF) NMR[3] have pushed the limits of LF NMR, thanks to the addition of a gradient coil in the spectrometer.

We present here the results we have obtained on the evolution of the lipidic NMR profiles acquired on extracts and on entire cells. This will constitute the first step to assess the potential and the limitations of NMR to monitor in vivo lipid accumulation. In the next future we will focus our efforts on the quantification of the different lipid classes and other relevant compounds, both with HF and LF NMR.

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13C isotopomic study of glycerol-ester lipids and cholesterol using an adiabatic INEPT sequence

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Abstract: The price of food products is closely related to the origin of their raw materials. That makes the food industry a fertile ground for fraud and adulteration. In order to protect the costumer from the use of misleading labels, strong analytical techniques should be used. Therefore, NMR emerged, almost 40 years ago, as a useful analytical tool for food authentication through metabolomic analysis and position-specific isotopic measurements of molecules, such as lipids.

Lipids are complex mixtures of molecules included in a wide range of food matrices. Their isotopomic profile (isotopic composition and metabolomic profile) is influenced by the geographical and botanical or animal origin of the matrix, weather conditions and, in the case of products of animal origin, the animal's nutrition. In a previous work, Lebanese olive oils were classified according to their sub-regional origins by developing and using analytical methods based on ^{1}H [1] and ^{13}C [2] NMR of triglycerides. The aim of the present work is to apply these NMR methodologies on other lipid fractions, such as cholesterol and phospholipids, in order to avail of complete isotopomic profile of the lipid fraction. 27 isotopic variables can be calculated from the spectra of cholesterol due to the presence of 27 carbon sites. On the other hand, the analysis of phospholipids enable to distinguish between *sn-1* and *sn-3* carbons of the glycerol backbone due to the presence of a phosphate moiety, esterified by a hydrophilic head group, attached to the hydroxyl group of the *sn-3* carbon. In this respect, egg yolk was chosen as a model food matrix in this study, being easy to handle and rich in triglycerides, phospholipids, and cholesterol. The extraction procedure was established in a way to avoid isotopic fractionation and to permit a precision in the range of per mil (‰) required for isotopic ^{13}C NMR.

Egg samples from different Lebanese traditional and industrial farms were collected, and triglycerides, phospholipids, and cholesterol were isolated. The pulse sequence (adiabatic INEPT sequence [3]) and other acquisition and processing parameters of ^{13}C NMR spectra were optimized in order to reach the desired high precision. ^{13}C NMR spectra were recorded on a 500 MHz spectrometer, and peak areas were obtained from curve fitting, carried out in accordance with a Lorentzian-Gaussian mathematical model using at five parameters for each peak: position, height, linewidth, phase and Gaussian-to-Lorentzian ratio. The determined peak areas were used as descriptors in the construction of multivariate models for the classification of egg samples according to their origin or to the corresponding farming system.

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Metabolomics and other "omics"

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Metabolism in haematological cancer cells.

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Abstract: Cancer cells exhibit an altered metabolism compared to most other cells, utilising nutrients in a different manner to produce large amounts of biomass. This has first been observed by Otto Warburg's in 1924 who found that tumour cell metabolism is biased towards aerobic glycolysis. In recent years other metabolic mechanisms and their specific regulation in cancer have been investigated. However, it is not clear how metabolism varies across haematological cancers.

We used NMR to study the metabolism of acute myeloid leukemia, chronic myeloid leukemia, Burkitt's lymphoma, diffuse large B cell lymphoma cells and multiple myeloma to learn specific metabolic regulation and how metabolism could potentially be targeted. For this, we measured metabolite levels in cell extracts and media for 18 cell lines. Most cell lines show a specific metabolic pattern, although some exhibits unexpected metabolic effects. Comparison of different blood cancers suggests that Burkitt's lymphoma and diffuse large B cell lymphoma cells have a glycolytic phenotype and multiple myeloma has a distinct metabolic phenotype with low production of lactate and large uptake of branched chain amino acids. In addition to these findings, a chronic myeloid cell line showed a high level of creatine compared to other cells, suggesting that chronic myeloid leukemia cells may use the creatine as an energy source.

Overall we observe a wide variation in metabolic phenotypes which we are further characterizing by tracer-based metabolic fluxes using newly developed NMR methods.

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Improved discrimination of olive oil samples using reference lineshape adjustment and deconvolution of 1H NMR spectra.

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Abstract: ^{1}H NMR is commonly known as a non-destructive and fast analytical tool and is often used for metabolites profiling. Lipid profiling provides powerful information used in different domains such as medicine, health, and food technology. However, the analysis of 1D proton spectra of a complex mixture, such as lipids from natural extracts, is hampered by the small spectral width leading to a great number of overlapped signals. On the other hand, despite the fact that a good shimming prior to the acquisition may ensure a better spectral resolution, the sample heterogeneity can induce field inhomogeneities leading to further lineshape broadening and distortions. The processing of such spectra is therefore challenging in order to overcome these problems.

A wide number of studies focused on the improvement of the ^{1}H NMR spectra decovolution by developing specific software such as LipSpin [1], PepsNMR [2], and ICA [3]. We present in this work the deconvolution of ^{1}H NMR spectra of olive oils after their correction by means of reference deconvolution. This technique extracts a well-resolved singlet signal and compares it to that theoretically predicted in order to construct an apodization function aiming to correct the full experimental spectrum. It has been known and used since many years [4, 5] but was only recently implemented in TopSpin software (TopSpin from Bruker Biospin, Rheinstetten, Germany).

Variables obtained by such a deconvolution of ^{1}H NMR spectra of authentic Lebanese olive oil samples were used as predictors in multivariate statistical analyses in order to classify them according to the altitude of the olive field or the color of the olive drupes. Improved classifications were observed by using this methodology on TopSpin instead of classical spectral integration [6] or deconvolution without reference lineshape adjustment.

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Metabolomics and other "omics"

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Metabolomic study of diabetes mellitus associated with pancreatic cancer

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Abstract: Pancreatic carcinoma is the worst prognosis tumour, the 5-year survival rate is only 5%.[1] Early diagnosis is not easy, because early symptomatology (weakness, nausea, abdominal pain, loss of appetite or weight loss) is not specific and can have many causes. More than 85% of cases are pancreatic ductal adenocarcinoma, which is usually associated with uncontrolled proliferation, high metastasis and resistance to treatment. For now, the only treatment method is radical surgical resection of the pancreas.[2] The relationship of diabetes mellitus (DM) and pancreatic cancer is problematic. More than 2/3rd of the cases of cancer are diagnosed with DM. DM appears prior to pancreatic cancer and may convert to pancreatogenic diabetes (T3cDM) within a period of three years. T3cDM suddenly develops in older age and has characteristic features (absence of obesity, frequent infection, instability of the internal environment).[3,4]

In our recent study, ^{1}H-NMR based metabolomics was employed to plasma samples of patients with pancreatic cancer, patients with diabetes mellitus and healthy subjects. The aim of the study was to find differences between patients with pancreatic cancer and patients with diabetes mellitus type 2 (more than 5 years after diagnosis of DM and therefore with a lower risk of developing pancreatic cancer) and compare these groups with a group of patients with potential pancreatogenic diabetes (less than 3 years after diagnosis of DM) using multivariate statistical analysis (PCA, LDA, etc.) on their metabolomic profiles. This is a possible way to identify individuals with potential high risk of developing pancreatic cancer associated with diabetes. Group of healthy subjects was used as a control study group. Possible biomarkers of pancreatic cancer (for instance 3-hydroxybutyrate and others) were also identified.

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Acknowledgement

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Metabolomics and other "omics"

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Detection, characterisation and quantification of fluorinated pollutants by 19F NMR

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Abstract: Fluorine is a light and common element, but there is nearly no fluorine metabolism in any living organism. Because of the extreme chemical resistance of fluorinated molecules its use in man-made products is common, and fluorine is present in perfluorinated polymers, in nearly 50% of phytosanitary products and in many pharmaceutical drugs, including 3 in the top ten best sellers, Prozac (anti-depressive), Ciflox (antibiotics) and Nifluril (anti-inflammatory). Therefore, fluorinated compounds appear in many emergent environmental pollutants. For instance, perfluorooctanesulfonic acid (PFOS) a fluorosurfactant used in many water repellent products and known as endocrine disruptor, and accumulate in the environment as a Persistent Organic Pollutant (POP). This is a major public health issue, while fluorinated molecules are difficult to detect by classical means.

We have developed a method able to detect, characterize and quantify fluorinated molecules in effluents, based on 19F NMR spectroscopy. In contrast to the standard GC/MS and LC/MS-MS approaches, this is a non-targeted analysis with no need of solid phase extraction nor of reference compounds.

Fluorine is an ideal spin, with 100% abundance, high resonance frequency, and no background. ^{19}F spectroscopy sensitivity is better than hydrogen and NMR is a non-destructive method that allows to measure the sample on its native state without any physical and chemical modification. Calibrated on trifluoroethanol (TFE) we obtain a limit of quantification below 30µg/L and a limit of detection below 10µg/L (corresponding to 150nM in TFE).

We present the analysis procedure we have developed, and explore its robustness against sample conditions, with several examples from soil, food, or effluents.

In addition, in order to ease the analysis and deepen its analytical power, we present different statistical tools and machine learning approaches that helps the assignment and allow to determine rapidly the class of pollutant, fulling insuring the non-targeted approach.

Image:

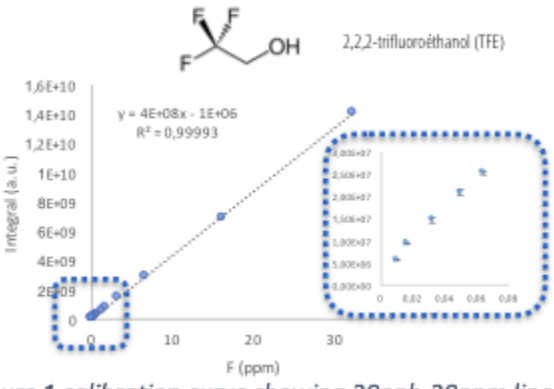

Figure 1 calibration curve showing 30ppb-30ppm linearity

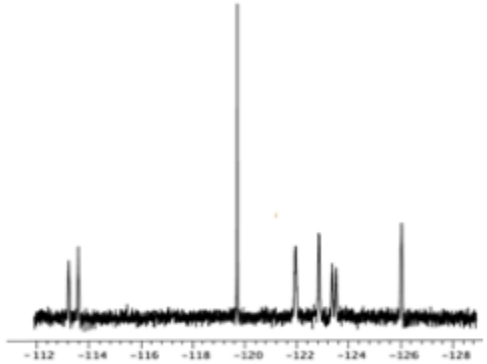

Figure 2 spectrum of a polluted river water, with per-fluorinated pollutants (sample provided by ANSES)

Metabolomics and other "omics"

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A systems approach using OSMAC and 1HqNMR - Improvement of Bioactive Metabolite Production in Microbial Cultures

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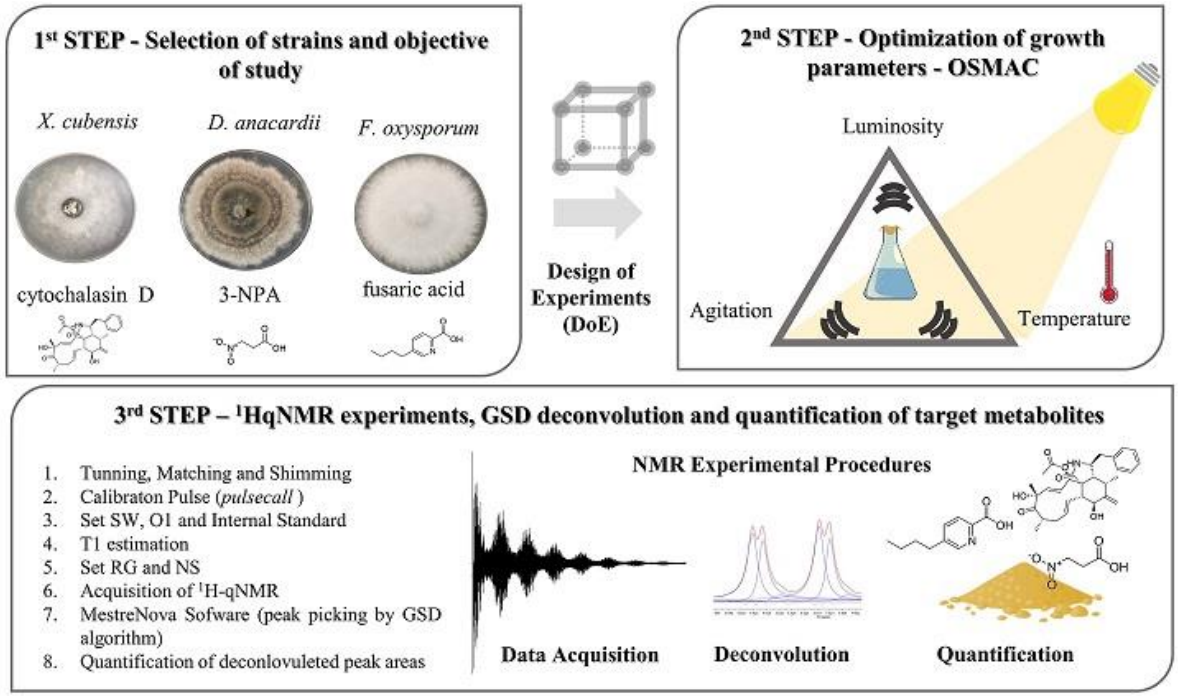
Abstract: In microbial natural product research, bioactive secondary metabolites are usually screened through single cultivars in the absence of biotic and abiotic interactions, resulting in significantly poorer chemical profiles when compared to the encountered under natural conditions. The interactions between microbes and abiotic factors has been successfully used to activate different biosynthetic pathways, including those encoded by cryptic genes for production of bioactive compounds. Among the methods used to understand the chemophysiological conditions associated with the activation of biosynthetic pathways, the One Strain Many Compounds (OSMAC) approach has successfully provided an increase in the number and diversity of metabolites available from a microbial source, including the production of *de novo* metabolites as well as the up-regulation of previously known compounds. Identifying and quantifying these induced metabolites in microbial matrixes is extremely challenging due to the variety of chemical and physical properties, generating a large, complex and multivariate data. In this context, the aim of the present work was to apply a strategy for the fast optimization of bioactive secondary metabolite production from in different fungi families (Nectriaceae, Xylariaceae and Diaporthaceae) by integration of OSMAC with deconvolution-based ^{1}HNMR quantification in raw extract. Results showed a significant increase in the production of bioactive compounds fusaric acid (*Fusarium oxysporum*), cytochalasin D (*Xylaria cubensis*) and 3-nitropropionic (*Diaporthe anacardii*) in 20, 95 and 45-fold, respectively. In addition, our global spectral deconvolution-based (GSD) ^{1}HqNMR strategy was reproducible for all species, presenting results that surpassed other proton quantitative NMR methods in selectivity, precision and accuracy. For complex microbial matrix studies, the OSMAC-GSD-^{1}HqNMR strategy showed unique advantages in both the metabolite optimization and bioactive compounds in a simple and rapid procedure.

Image:



Metabolomics and other "omics"

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Metabolomic application in fish nutrition: profiling novel aquaculture feeds and assessing their impact on rainbow trout plasma

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Abstract: Aquaculture needs high quality feeds to support the growing global demand for fish. Over the last two decades, the development of sustainable feeds based on plant feedstuffs has strongly reduced the use of marine resources. However, full plant-based feeds still reduce growth performances in carnivorous species such as rainbow trout. Thus, alternative feedstuffs are required to complement plant-based feeds.
In this study case, we have implemented an NMR-based metabolomic workflow to reveal the differences in soluble compounds of plant-based feeds complemented with selected feedstuffs insect, micro-algae and yeast and demonstrated their effect on plasma.
Fish have been fed ad libitum for three months nine experimental diets: a control plant-based diet (PB) and eight diets containing 5%, 10% or 15% of either insect extract (INS), micro-algae (SPI) or yeast fraction (YST) as complement of PB. Fish blood was collected 48 h after feeding. Ethanolic extracts of feeds were prepared and analysed by NMR using CPMG experiments on a Bruker 500 MHz spectrometer. Plasma were analysed using the same approach. Spectra were processed using NMRprocflow[1] webtool and data were analysed using BioStatFlow application (www.biostatflow.org).
Specific compounds related to feedstuff origin and process were highlighted by NMR-based metabolomics approach. In the INS feeds, glycerol was the most discriminant compound compared to plant-based feed and it should be the witness the insect meal process itself. In SPI feeds, 3-hydroxybutyrate was the most discriminant compound compared to plant-based feed and it should be issued from the micro-algae metabolism. YST feeds was similar from plant-based feed due to low soluble content of the yeast fraction. Plasma NMR spectra showed specificities in fish fed each alternative feed, and trends in selected signal were related to the graduate incorporation of the novel ingredients in feeds.
This NMR-based metabolomic approach led to the identification of unsuspected compounds in alternative aquaculture feeds and highlighted the impact of feeds on fish metabolome. Further hypotheses could be drawn when the identification work of plasma metabolites will be completed.

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NMR based metabolomics approach to cancer cells induced hypoxia condition

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Abstract: Hypoxia is a condition that is manifested by a low supply of oxygen. Tissue hypoxia is an effect of oxygen deficiency that affects the deterioration of biological functions of the cell. To the characteristic features of solid tumours belong hypoxia, limited amount of nutrients and low pH value. Research on cancer cells shows that hypoxia promotes tumour growth, but is not conducive to apoptosis. To some extent, hypoxia is associated with a more aggressiveness phenotype of cancer cells. A characteristic feature in changing the metabolism of cancer cells is to increase the glycolysis process, which leads to increased lactate production. The key element that allows the adaptation of cancer cells to the conditions of reduced oxygen levels is Hypoxia Inducible Factor - 1α (HIF-1α).

The main goal of this study is to determine metabolites changes in cancer cells in normoxia and hypoxia condition by Nuclear Magnetic Resonance (1H NMR) method together with applied chemometrics and statistical analysis. Therefore in this project we examined whether cobalt chloride hexahydrate induce HIF-1α and changes in profiles (levels) of metabolites in the cultured cancer cells and media. Two cancer two cell lines were incubated 24h with 200μM cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), known hypoxia inducible agent and without, in normoxia conditions.

The performed studies showed that these two states differentiate between each other up-regulation and down-regulation of certain set of metabolites (eg. lactate, pyruvate, glutamate), which accompanied hypoxic induced condition.

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Primary metabolism investigation of fleshy fruit species using 1H-NMR profiling

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Abstract: Ten fleshy fruit species, differing for fruit growth dynamics, climacteric status, maturation duration, phloem-transported sugars and starch storage level, were studied during their development. We focused on primary metabolism which provides energy and biosynthetic precursors to support fruit growth and ripening, and is essential for fruit quality.
The major polar metabolites were identified in the edible part of the fruit in nine species (grapevine, peach, apple, strawberry, kiwifruit, clementine, pepper, eggplant and cucumber) besides tomato, using 1D ^{1}H-NMR (single pulse and multiple selective irradiation experiments), 1D ^{13}C-NMR as well as 2D homo- and hetero-nuclear NMR experiments, in-house plant reference compound database and spiking experiments. This revealed common or species-specific patterns for 6 soluble sugars, 8 organic acids, 21 amino compounds and 3 other metabolites. The major common compounds and starch were quantified in all species across at least 9 stages of development using high-throughput microplate spectrophotometric targeted biochemical measurements. For the latter compounds, compositional differences between species were already noticed at the first stages of development and exacerbated afterwards. Tomato, eggplant and kiwifruit were studied into more details for at least eight stages of development and for a range of metabolites quantified by means of ^{1}H-NMR metabolomic profiling of polar extracts and NMRProcFlow[1] tool. Similarities and differences between these species along development were visualized using multivariate and univariate analyses, including ANOVA-PCA.
The quantitative compositional data have been complemented with IC-QTRAP-MS/MS targeted analyses of phosphorylated compounds and will be combined with phenotypic data. They will also contribute to the parameterization and validation of fruit metabolic models in order to highlight essential regulation points.

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Importance selection for fish growth factor utilizing NMR metabolomics

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Abstract: Fish are one of the most important sources of protein for human beings. Despite a large increase in consumption of fish, volume of catches are decline due to such as over fishing. Thus, expansion of aquaculture is expected to technological progress such as improvement of fish culture feed and growth assessment. Especially, the feed development and growth assessment technologies suited for cultured fish are considered to be a key subject for study. We have previously reported some analytical advances for evaluation of fish and environmental variations by chemical and microbial profiling [1-3].

In this study, we aimed at discovery of metabolic and microbial biomarkers associated with growth stage and variation based on feed. Metabolic profiles of fish muscles and gut contents were obtained from a NMRspectrometer in combination with spectral annotations using database. And Gut microbial community profiles of the fish were obtained from a MiSeq sequencer. The metabolic and microbial data were matrixed and evaluated by multivariate statistical analysis such as principal components analysis (PCA) or machine learning analysis.

Muscle tissue profiles of fish growth were evaluated by PCA, and, variation in the growth and/or feeds was remarkably exhibited. Amino acids and nucleic acids showed particularly growth dependence. Using machine learning, the integrated analysis based on the metabolic and microbial profiles of muscle and gut contents allows the characterization of growth variation. Growth model based on the integrated NMR and microbiota profiles was computed by machine learning. A high accuracy of the prediction model was confirmed at younger stage. Importance in Growth model was non-linearly evaluated by metabolites and microorganisms that is associated with growth and/or feeds. We performed evaluation about integration of heterogeneous data using NMR profiles and microbiota. Evaluation of growth factor using integrated models suggested the possibility to produce healthier and tastier fishes. We will provide these details with the results of the integrated data analysis at the conference.

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Analysis of fishery products and aquatic ecosystems by high- and low-field NMR

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Abstract: Ecosystem services are important for human life as well as sustainability of biological diversity. As the economic growth, the homeostasis degradation of environments and ecosystems, such as enormous consumption and pollution, is a serious issue toward future sustainability of human society. Along with the development of analytical instruments and data science technology in recent years, it has become possible to search for important factors by extracting features of molecular complexity of biological samples and environmental samples[1)]. The analytical approach targeting such a wide range of molecules needs to develop a database with scalable web environment[2)]. Environmental homeostasis can be evaluated by data mining of analytical parameters from natural samples, such as environmental water[3)]. Firstly, I introduce ecoinformatics approach for integrated analysis of natural fishes, sediments and water[4)]. Moreover, I will demonstrate that the fishery products, such as fish muscles can be also evaluated metabolic profiles of analytical big-data[5)]. Namely, quality control is essential in modern industry, including fishery and food industries. In these cases, benchtop NMR might potentially innovate the quality control process, identifying storage-based and fermentation-originating metabolic changes[6)]. As we have demonstrated previously, SENSI approach can overcome weak S/N ratios and enhance peak separation for the benchtop NMR data[7,8)]. Moreover, NMR has the tremendous advantages of reproducibility and inter-institution convertibility because the observed parameters such as chemical shifts and J values, are physical quantities; in addition, it is possible to refer to standard data measured by high-field NMR.

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Relaxation profiling of intact fish muscles based on computational machine learning

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Abstract: NMR-based metabolic profiling is one of the powerful techniques for characterizing and evaluating complex metabolic mixtures derived from biological and environmental systems. NMR measurements enable to obtain not only metabolic information but also physical properties such as biomolecular interactions between biological macromolecules and water that exists on the periphery although the applicability of physical properties to NMR-based metabolic profiling remains largely unexplored. From this point of view, we focus on advancement of NMR-based metabolic profiling that enables to obtain both metabolic information and physical properties concurrently in biological and environmental systems. To this end, we have currently developed several machine learning algorithms including deep learning and evaluated its applicability to metabolomics studies [1-4]. In this study, we have applied several machine learning approaches to analysis of biomolecular interactions between proteins and water included in fish muscles based on T_2 relaxation time in order to extract features of fish species and habitats. The machine learning approaches such as random forest and support vector machine based on not only ^{1}H NMR spectra but also T_2 relaxation curves showed a relatively good performance for geographical and species discriminations of fish, suggesting that T_2 relaxation time in combination with machine learning approach is applicable as a method to determine a geographical provenance in analogy with conventional metabolomics approaches. In addition, even benchtop and lowfield NMR machine enabled to obtain almost the same quality of T_2 relaxation curves and classification accuracies compared with a typical NMR machine used in metabolomics studies. Therefore, the metabolic profiling based on T_2 relaxation time in combination with machine learning approach should be a useful tool for product evaluations on site using benchtop and portable NMR machine.

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Metabolomics and other "omics"

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NMR aerosolomics

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Abstract: Atmospheric aerosols are a small but very important part of the Earth's atmosphere. The proportion of inorganic and organic compounds in aerosol particles seems to be equal on average.[1,2] While the inorganic composition of aerosols seems to be well explored, knowledge about the organic part is still very limited. It is well known that the major part of organic aerosol compounds is represented by polar, water—soluble organic compounds (WSOC).[1] So far GC-MS is the most frequently used method for WSOC analysis. GC-MS is a very sensitive technique; furthermore, it exploits huge spectra libraries accumulated over decades. Therefore, its role in the determination of aerosol composition is indisputable. Primarily owing to GC-MS, about 150 organic compounds have been identified in aerosol particles. NMR spectroscopy for the purpose of aerosol chemistry was "discovered" only recently[3] as it is rather insensitive method. Nevertheless, NMR has undergone rapid development and sensitivity gain of late. Moreover, it is fully quantitative method and no sample derivatization is needed. So far, the use of NMR spectroscopy has been limited to so called Functional group analysis.[4] In this analysis the whole NMR spectrum is divided into parts and subsequently integrated according to functional groups.

Here we propose to employ the metabolomic approach for the complex evaluation of aerosol composition. In NMR aerosolomics the assignment of dominant signals is based on precise chemical shift of the compound which enables identification of organic compounds in given aerosol sample and the original aerosol source. For this purpose, a comprehensive library of high—res ^{1}H NMR spectra of organic compounds that are known to be present in aerosol particles is essential. Originally, NMR aerosolomics was exploiting the original metabolomic library. The database of the ChenomX NMR Suite program[5] contains about 70 compounds that have also been found in aerosol samples according to the literature (the total number is estimated to be 150 compounds). We were able to identify more than 30 compounds in every analyzed sample. Up to now, 50 new compounds attributed to aerosol have been added to the database; the largest gap was in aromatic carboxylic acids (12), compounds containing sulphur (11) and amines (8). Subsequently, the score of identified compounds in real spectra jumped to over 50. Additionally, about 30 new organic compounds (mainly hydroxy carboxylic acids) were found in aerosol samples. These compounds were present in the original ChenomX library and had not been found in aerosol samples so far. This finding has to be confirmed by other techniques prior to publishing. The obtained results clearly show that NMR metabolomics is very powerful methods and can be implemented also in the analysis of organic compounds contained in aerosols.

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Monitoring of cancer cell metabolism at intervals by combined NMR and MS methods.

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Abstract: The cancer cells are widely used model of tumour metabolism that allow in vitro testing of many treatments such as: gene editing, drugs, nanoparticles, signalling molecules etc.. The investigation of cancer cells metabolism, by analytical chemistry methods (NMR and MS), allow monitoring metabolite levels and thus gives insight into cellular metabolism and its perturbations caused by external factors. However, cell growth and differentiation are dynamic processes, and thus may exhibit different metabolic profiles over time. As a result, analysis of a single metabolic profile may lead to different conclusions than time series monitoring of growing cells [1]. Therefore, multiple time dependent measurements of cell extracts and media composition could provide better understanding of cancer cells metabolism.
The major goal of this study was monitoring of metabolite changes during 80 hours of cell cultivation at 4-hour intervals. Cell and media samples were split and analysed by both NMR and UPLC-MS. Combined metabolic profiles allowed us to detect three type of trends in monitored metabolites. The first group consist of compounds that were increasing their levels over time, the second group included metabolites with opposing trend. Finally, the third set of metabolites showed uneven behaviour suggesting anabolic-catabolic switch. The metabolites continuously released to the medium can be considered as the potential biomarkers candidates' characteristic for given cell line. This study may be useful for future analysis of cancer cells response to drugs and potentially incorporated into metabolic flux analysis models of cancer cell metabolism.

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Metabolomics and other "omics"

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Can we really see a difference?

Metabolomics studies of human serum samples for breast cancer diagnostics

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Abstract: The incidence for breast cancer is increasing year by year worldwide. At the same time the development of medicine, new diagnostic methods and social awareness in most developed countries leads to significant decreasing in mortality. Metabolomics nowadays is well established method in omics science and increasingly used and trusted in clinical. Opportunities and possibilities of uses it as an additional diagnostics or basic step in fast identification of disease increasing with development of MS/NMR techniques. The diagnostics prospects of metabolomics could be alternative for more invasive biopsies and allow for process of making decision based on different type of biological material – serum, urine – which collection is more comfortable for patients. In the case of breast cancer, studies related to metabolites changes have been developed since 1993, but mainly are based on tissue samples. Since 2010, the research started investigating possibility to utilize the biological material obtained in a less invasive way. Therefore, could the selected metabolites be universal and applicable. The conducted study on a cohort of 322 serum samples, including 181 volunteers for healthy control group and 181 different breast cancer patients were investigated with proton nuclear magnetic resonance spectroscopy. The univariate and multivariate data analysis allows to verify the possibility of determining metabolites that could potentially be important in serum-based detection presence of tumor. The results led to changes in level of histidine, acetoacetate, choline, tyrosine, 3-hydroxybutyrate, alanine, citrate and lactate.

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NMR-based metabolomics workflow for characterization and effect assessment of alternative plant-based diets on plasma and microbiote in rainbow trout

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Abstract: Development of sustainable diets for aquaculture is crucial due to the decreasing availability of ingredients of marine origin. Nevertheless, alternative diets based on plant-based feedstuffs have still numerous negative impacts on fish nutrition. In this work, NMR-based metabolomic approaches were developed and tested on plasma of rainbow trout, *Oncorhynchus mykiss,* fed from the first feeding and up to 14 months with plant-based or fish-based diets and a commercial-like diet as a control. At the end of the experiment, fish were sampled 48 h after the last meal. Blood samples were collected and the corresponding plasmas prepared. The extraction of soluble compounds of the three diets was done. Plasma samples and polar extracts of diets were analysed using a Bruker 500 MHz spectrometer. Plasma CPMG and zgpr ^{1}H-NMR profiles were processed separately with NMRProcFlow[1] tool (chemical shift calibration, baseline correction, peak realignment and non-uniform bucketing, signal-to-noise ratio determination). Each spectral region of interest or bucket was determined either with intelligent bucketing or variable-size bucketing. CPMG and zgpr bucket variables were combined and used for statistical analyses with BioStatFlow (www.biostatflow.org). Biomarkers of diets were detected in plasma and indicated that the metabolism was deeply affected in trout fed plant ingredients. Several plasma biomarkers were also found in the NMR profiles of diets. A correlative approach with selected plasma NMR buckets and the intestinal microbiota (the composition of the active bacterial community associated with intestinal mucosa was determined by pyrosequencing of reverse transcripts of partial 16S ribosomal RNA) was also performed and some links appeared between the relative prevalence of intestinal bacteria and plasma NMR metabolomics [2]. Similar approaches are currently being used for trout fed with plant-based diet supplemented with alternative feedstuffs, such as insect extract, micro-algae or yeast extract.

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Fast 2D NMR methods and multiblock omics modeling for lavender variety selection

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Abstract: Lavender (*Lavandula angustifolia*) is industrially used for its aromas and fragrance. This plant is mainly cultivated in Mediterranean climate, and its essential oil exhibits various neurological-related properties [1]. In this study, the comprehensive characterization of 22 samples of lavender essential oil produced in Hauts-de-France (North of France) was carried out using complementary analytical approaches. Specially, a multiblock data modeling was used, [2] integrating heterogeneous signals from conventional methods like GC-MS and ^{1}H NMR, but also more advanced high-throughput 2D NMR pulse sequences. These included J-resolved spectroscopy and ultrafast COSY [3], as well as very high-resolution NUS-HSQC experiments [4]. The consensus OPLS-DA strategy [2] was applied to classify the oils according to their variety and stage of harvest. Our results show that some lavender essential oil produced in Hauts-de-France, exhibits resemblance to commercial lavender essential oil. They also highlight the relevance of applying recently developed fast 2D NMR methods in the context of metabolomic profiling.

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NMRProcFlow: A graphical and interactive tool dedicated to 1D spectra processing for NMR-based metabolomics.

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Abstract: Although metabolomics by 1D NMR spectroscopy has become a common approach, multiple challenges in spectra and data processing remain to be solved. Unlike techniques coupled with mass spectrometry such as LC-MS, GC-MS or CE-MS,, 1D NMR spectroscopy has only one dimension on which we can rely, and apart from very well-mastered and very reproducible use-cases, the implementation of 1D NMR spectra processing workflows within a Virtual Research Environment (VRE) and operating automatically in order to be widely used by non-expert users has not yet reached full maturity. Indeed, the expert eye is often required and even crucial to disentangle the intertwined peaks and the best way is to proceed interactively with a 1D NMR spectra viewer.

To fulfill this need, we have been developing NMRProcFlow[1], an interactive 1D NMR spectra processing (^{1}H & ^{13}C) dedicated to metabolomics. It has been built by involving NMR spectroscopists eager to have a quick and easy tool that greatly helps spectra processing and that can be of used also by new-comers. For each of the two major metabolomics approaches, namely Metabolic Fingerprinting and Targeted Metabolomics, the workflow covers all steps from spectral data up to data matrix output. Moreover, the possibility of visualising the experimental factor levels within the NMR spectra set through a spectral viewer makes the tool valuable to create links between the experimental design and subsequent statistical analyses, and thus facilitates interactions between biologists and NMR spectroscopists. In addition, NMRProcFlow allows experts to build their own spectra processing workflows, in order to become models applicable to similar NMR spectra sets, i.e. stated as use-cases.

NMRProcFlow is accessible online (http://nmrprocflow.org), or alternatively, a virtual machine for local installation can be downloaded.

NMRProcFlow is now implemented as a data pre-processing, analysis and visualization tool in the PhenoMeNal[2] Application Library and also as a Galaxy Interactive Environment (IE) offering a more complex user interface (GUI) that what is offered by standard Galaxy tool integration.

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Predicting clinical benefits of combined bevacizumab and paclitaxel therapy for HER-2 negative metastatic breast cancer: a serum NMR metabolomics investigation

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Abstract: Bevacizumab combined with chemotherapy improves the response rate and prolongs progression-free survival when used as first- or second-line treatment for advanced-stage breast cancer. A major challenge is the early identification of subgroups of patients that will benefit most from this treatment in order to improve patients' management, provide a more specific administration of bevacizumab, and allow potential non-responder patients to benefit from alternative therapeutic strategies. We present a longitudinal metabolomic investigation of serum samples from patients with HER-2 negative metastatic breast cancer, and identify serum metabolic signatures of the response to association of bevacizumab and paclitaxel treatments.

Pre-treatment and on-treatment serum samples were available for 312 patients with HER-2 negative metastatic breast cancer from the French multi-centre clinical trial COMET. Patients included in this study received paclitaxel associated with bevacizumab in the first line of cancer treatment and were not previously treated with chemotherapy for metastatic disease. A series of venous blood samples were collected under fasting conditions for each patient during the COMET trial: at baseline (T0), day 8 of cycle 1 (T1), and day 1 of cycle 2 of chemotherapy (T2), and corresponding metabolic profiles were obtained using ^{1}H high-field Nuclear Magnetic Resonance (NMR) spectroscopy (600MHz).

After outliers' identification in the COMET dataset, 588 metabolic profiles corresponding to 196 patients with suitably documented metadata and available samples for the three time points were further interpreted. A supervised analysis was carried out to investigate if differences in the NMR serum metabolic profiles of patients were able to distinguish patients according to clinical benefit at different sampling times. Clear and significant O-PLS discriminations are observed only between SD (Stable Disease) versus PD (Progressive Disease) at T0, OR (Objective Response: partial or complete response to treatment) versus PD and SD versus PD at T1. According to the results, only one week after the start of chemotherapy, it is possible to highlight a metabolomic signature predictive of beneficial effect of treatment by discriminating the patients answering from those whose disease progresses. Furthermore, stratified analysis shows that significant metabolic signatures associated with clinical benefit can be distinguished at baseline and after only eight days of treatment for patients with negative hormone receptors status.

These results open up promising routes for application of metabolomics strategies to patients' management in oncology.

Metabolomics and other "omics"

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Inter laboratory comparison of a revisited protocol for 1D 1H-NMR profiling of plant samples from extract preparation to spectra processing.

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Abstract: The main objective of the present work is to minimize uncontrolled variability in plant sample preparation before NMR profiling, taking into account sample composition, specificity in terms of pH and paramagnetic ion concentrations, and spectrometer performance. Therefore, we implemented automation of routine spectrometer qualifications (shimming, temperature control) for each sample before spectra acquisition.

In this poster we report on an inter laboratory comparison of plant derived samples by ^{1}H-NMR spectroscopy across three different sites utilising instruments from two manufacturers with different probes and magnetic field strengths of 9.4 T (400 MHz, ECZ-S JEOL, double resonance ROYAL probe), 11.7 T (500 MHz, AVANCE III Bruker, double resonance broadband probe BBI) and 14.1 T (600 MHz, AVANCE III Bruker, Triple resonance probe TXI).

Comparability of the datasets from the three laboratories was exceptionally good in terms of spectral quality. The coefficient of variation of the half-width and the signal-to-noise ratio of two selected peaks was comprised between 5 and 10% depending on the spectrometers. The three collection of spectra were processed separately with NMRProcFlow[1] tool (Fourier transformation, phasing, chemical shift calibration, baseline correction, peak realignment, non-uniform bucketing, Signal-to-Noise Ratio determination). Each spectral region of interest or bucket was determined either with intelligent bucketing or variable-size bucketing. The resulting buckets were subjected to multivariate analysis with BioStatFlow (www.biostatflow.org). This proof of concept on a medium-sized collection of sixty samples of wheat kernel extracts showed that the data collected at the three different sites on instruments of different field strength and manufacturers yielded the same discrimination pattern of the biological groups.

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Metabolomics and other "omics"

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NMR based population studies

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Abstract: The metabolomics approach seems to be method of choice for screening population-based studies, which can reflects different factors influencing human health such as nutritional, pharmaceuticals, environmental and lifestyle. To evaluate the effect of disease risk occurrence the mostly used parameter is body mass. It is known that high body mass index (BMI) has great impact on the metabolism increasing the risk of civilization diseases occurrence. Next risk agent is the age, the aging processes cause many physiological and biochemical changes, which frequently leading to cardiovascular and obesity associated diseases. Both aging and BMI have influence on quantitative composition of metabolites and state of health of organism. Metabolomics, as the interdisciplinary science join analytical chemistry methods - NMR and MS with statistical and chemometrics analysis to quantifying the metabolite levels and perform multivariate analysis of investigated objects.

The main goal of this study was metabolomics screening of plasma from the group of 90 blood donors by 1H NMR method. The performed analysis showed the differences between donor's type (frequent or rare donators) showing changes in their metabolism. Additionally the donors, as representatives of population, were analyzed by the age, sex and BMI. The OPLS-DA models together with the set of metabolites were elaborated being responsible for all comparisons.

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MRI and in vivo

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Impact of a fixed proton beamline on the in-beam MR image quality

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Abstract: Background: Online image guidance in radiotherapy using magnetic resonance imaging (MRI) is expected to improve the targeting precision of tumors. First systems integrating MRI and megavoltage X-ray therapy have recently found their way into clinical practice. Systems for MR-integrated proton therapy do not exist, because a number of hitherto open technological challenges remain to be solved. One of them is the mutual electromagnetic interaction between the MRI and proton therapy systems including the fringe field of magnets used for beam guidance, which may interfere with the static and dynamic fields of an MRI scanner and hence may compromise the MR image quality. The aim of this study was to assess the impact of an operating proton research beamline on the MR image quality during irradiation.

Methods: A low-field (0.22 T) open MRI scanner was enclosed by a compact Faraday cage. The scanner was positioned at the fixed research beamline of our proton therapy facility. The MR image quality was assessed with the ACR Small Phantom. The phantom was centrally positioned in the Field-of-View inside a knee coil. Images were acquired by performing T1- and T2-weighted spin echo (SE) sequences with parameter settings according to the ACR Phantom Test protocol. Additionally, T1 and T2*- weighted gradient echo (GE) scans were performed. The phantom was irradiated by a 125 MeV pencil beam (Ø12mm) at dose rates of 1 and 80 Gy/min. MR Images were acquired for six different scenarios, starting from a reference scan with beamline magnets off and beam off, followed by sequentially switching on the beamline magnets and the beam during both frequency calibration and image acquisition or during image acquisition alone. A validated software tool (MATLAB) was used to extract the ACR imaging parameters and to estimate geometric transformations from image pairs acquired for the different scenarios.

Results: Operating the beamline shows no visible geometrical image distortion. The SE and GE image quality was sufficient for the automated analysis of the ACR parameters. For all six scenarios, differences in ACR parameters were within measurement uncertainties. An increase in baseline resonance frequency from 70 to 110 Hz depending on beamline status was observed, leading to a sequence-dependent off-resonance image shift of up to 3 mm in frequency encoding direction. The shifts were shown to be inversely proportional to the gradient strengths of the sequences (0.7 to 5.7 mT/m). No visible effects of the beam were observed.

Conclusion: Simultaneous MR imaging and proton beam irradiation was shown feasible in an open low- field MRI scanner at a fixed research beamline of a proton therapy facility. No degradation of MR image quality was observed under simultaneous imaging and irradiation, but image shifts must be compensated for. Further research towards integration of MRI and proton therapy is worthwhile.

Disclosure: The work was partially supported from Ion Beam Applications SA, Louvain-la-Neuve, Belgium.

MRI and in vivo

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Evaluation of the pulmonary regurgitation by different cardiac magnetic resonance indices in the children with repaired tetralogy of Fallot

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Abstract: Background: Pulmonary regurgitation (PR) is an important determinant of outcome for post-surgery tetralogy of Fallot (TOF) patients. We aimed to discuss about whether pulmonary regurgitation volumn (PRV) would be a better index for reflecting the degree of ventricular preload than pulmonary regurgitation fraction (PRF).

Methods: Fifty-seven patients (age at CMR study, 105.404±60.018 months, 66.67% male) with repaired TOF and PR were identified. PRV, PRF, both ventricular systolic and diastolic volumes, and ejection fraction were measured by the use of cardiac magnetic resonance imaging (CMR). Right ventricle (RV) dilation was divided into three groups based on indexed right ventricular end-diastolic volume (RVEDVi) :slight 110~140 ml/m^2, moderate 141~170 ml/m^2, and severe >170 ml/m^2. The predictive capability of PRV and PRF for severe RVEDVi was compared by using multivariate linear regression analysis and receiver operating characteristic(ROC).

Results: The averaged PRVi was 27.087±18.672mL/m^2 and PRF was 32.979±16.173%. PRVi had good correlation with PRF (r=0.690, p=0.001). PRVi showed moderate correlation with RVEDVi and RVESVi (r=0.400/0.327, p=0.002/0.013), while the correlations of PRF to RVEDVi and RVESVi were not significant (r=0.105/0.081, p=0.439/0.551). PRVi was better than PRF at differentiating from mild to moderate RV dilation (p=0.044/0.433). ROC analysis showed that PRVi was better at differentiating mild-moderate RV dilation from severe type than PRF (AUC_{PRVi} = 0.705/0.601, p = 0.026).

Conclusions: PRV has better ability to predict RV overload than PRF and may become a more accurate indicator.

MRI and in vivo

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MR imaging of water sorption in starch-glycerol extrudates

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Abstract: Starch is widely used in controlled release systems because it is a high purity biocompatible and biodegradable material, which can be easily metabolized in the human body (1). The ingress of water in starch is therefore of great interest because of its relevance for the formulation of controlled release materials. Over the past decades, the magnetic resonance micro-imaging technique (MRμI) has proven to be an extremely reliable tool for measuring the water ingress during sorption and swelling of polymers used in a wide range of fields (food, pharmaceutical formulations, medical implants etc.) (2). However few studies present quantitative measurements when the sample of interest changes over time or in case of a long acquisition time. In this domain, two challenges have to be overcome: the introduction into the probe of a phantom as a reference signal and the guarantee that this signal is stable over the experiment duration while some conditions such as temperature and/or the moisture are varied. For that, we implemented a dedicated experimental set-up to generate a virtual phantom (ViP) signal in images (3). We will present its advantages and application to monitor the water sorption of a potato starch blend containing 20% glycerol that was shown to have interesting properties for biomedical applications. The relationships between the water rotational mobility (using T_2 maps) and the water transport diffusion (using proton density maps) were analyzed. The rate constants for water diffusion and the starch swelling extracted from the proton density images showed that starch-glycerol blends exhibited a Fickian diffusion (type I) behavior at first step of the water uptake (400min). This kinetic revealed that the water concentration gradient was the driving force for its diffusion into the extrudate, while glycerol was released into the surrounding aqueous phase. With time, the water intake of the starch-glycerol extrudates was slower due to the competition between the crystallization of starch and penetration of the water (4).

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MRI and in vivo

P331

Simultaneous measure of temperature and velocity in fluids using MRI

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Abstract: The Rayleigh–Bénard convection (RBC) is a buoyancy driven instability in a fluid layer confined between two horizontal walls. The RBC in Newtonian fluids has been extensively studied since more than a century. These last decades, a growing interest has emerged for non-Newtonian fluids, mainly due to the wide fields of applications (e.g. oil, cosmetic, pharmaceutics, food industries).

As it is possible to measure with the same technique various physical quantities such as temperature or velocities, MRI is an interesting tool to study the RBC. Nevertheless, if velocity measurements using MRI are common in engineering sciences [1], temperature measurements with this technique are seldom used outside the medical field.

In this study, we compare the various MRI thermometry techniques: phase mapping, diffusion coefficient measure, relaxation. The first one is the method of choice in medical studies but is not usable in our case due to the experimental conditions. On the contrary, the last method seems suitable for our study. We present the validity of this method on a model Newtonian fluid, the glycerol. We show that for a conductive regime, temperature measurements are in very good agreement with the theoretical profile. In the convective regime, when comparing the temperature and velocity fields obtained by MRI, we get an excellent agreement in terms of flow structure. Temperature uncertainties are found to be less than 1°C for all our results [1].

MRI thermometry and velocimetry [3] have also been tested on a xanthan solution, a shear-thinning fluid. As this aqueous solution has relaxation times very different from the glycerol, new problems have arisen when measuring temperature. We present a way to overcome them as well as some resulting temperature maps.

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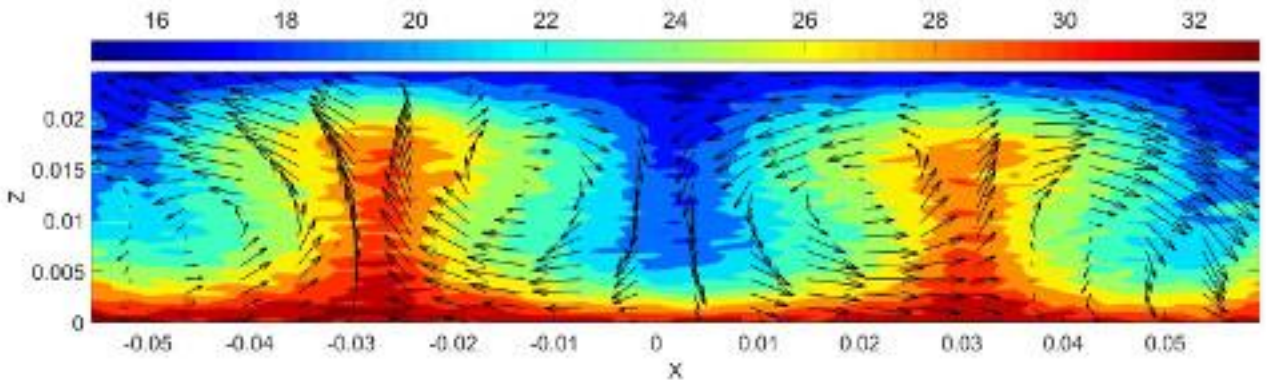
Image:



MRI and in vivo

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In Vivo Metabolism of Hyperpolarized 13C-Pyruvate in the Brain of Tumor Patients and Normal Subjects

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Abstract: Dissolution Dynamic Nuclear Polarization (dDNP)[1] is a powerful technique that can enhance ^{1}H nuclear polarization by up to 5 orders of magnitude. Its clinical potential is being investigated and successful translation to a phase I trial of [1,^{13}C]-pyruvate was realized for prostate cancer patients[2].

So far, *in vivo* dDNP experiments in the human brain have been limited[3] and have not previously investigated kinetic rate parameters.

Here, we present results of the injection of hyperpolarized (HP) [1,^{13}C]-pyruvate in 10 glioma patients and of HP [2,^{13}C]-pyruvate in 3 healthy volunteers.

A GE SpinLab[4] polarized was used for boosting the ^{13}C polarization of [1,^{13}C]- and [2,^{13}C]- pyruvate.

Signals of HP [1,^{13}C]-pyruvate, [1,^{13}C]-lactate and [1,^{13}C]-bicarbonate were detected in 22 data sets of 10 glioma patients thought an EPI-based sequence, in a 3 T MRI scanner, and demonstrated excellent SNR. Modeling of the temporal trends of these signals yielded spatially-resolved maps of the rates of conversion of pyruvate into lactate and bicarbonate (i.e., k_{PL} and k_{PB}). See Fig. 1 as an example.

HP [2,^{13}C]-pyruvate was injected in 3 healthy volunteers and a SLAB-dynamic sequence was used for detection at 3 T. Downstream reactions and conversion of [2,^{13}C]-pyruvate into [5,^{13}C]-glutamate were observed for the first time.

Kinetic k_{PL} maps can be used to track changes in the activity of LDH *in vivo* and to monitor the Warbug effect, associated with tumor presence and progression.

HP pyruvate MR molecular imaging added to a multi-parametric MRI exam demonstrates the feasibility to detect metabolic reprogramming in a tumor and may have an important clinical role in assessing response to medical treatment.

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Figure 1. *Maps of k_{PL} (top) or k_{PB} (bottom) kinetic rates, superimposed to FLAIR images (center) in a brain tumor patient. See text for details.*

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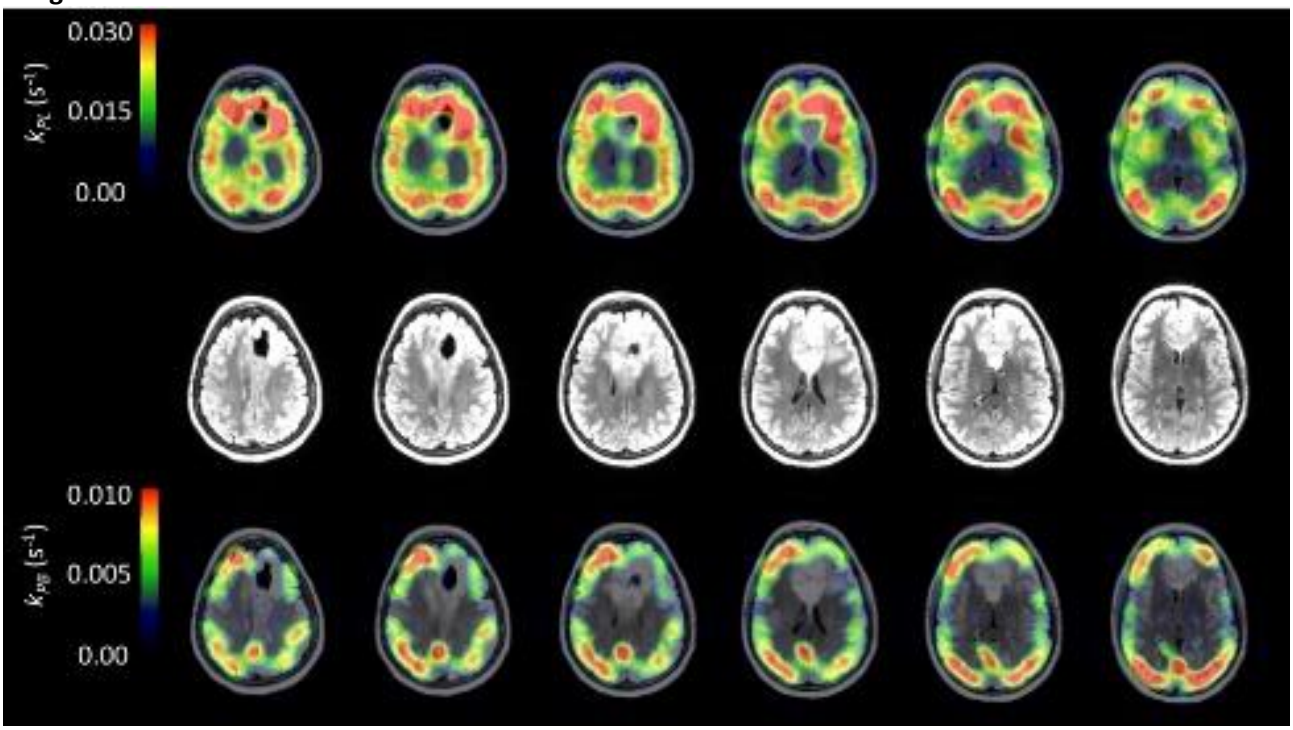

MRI and in vivo

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Fast quantitative susceptibility reconstruction via total field inversion with improved weighted L0 norm approximation

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Abstract: Analyzing susceptibility of human body quantitatively has become a new hotspot of MR imaging. Owing to its unique direct relation to actual physical tissue magnetic properties, quantitative susceptibility mapping (QSM) can efficiently provide special contrast. It can be a powerful auxiliary tool in diagnosis, especially in some brain diseases and disorders like Alzheimer's disease, Thalassemia, and Parkinson's disease.

Compared with conventional magnitude imaging, the high quality phase from gradient echo acquisition has the advantage that it can provide a better contrast in gray matter and white matter. However, the phase suffers from non-local effect resulting from the magnetization outside the region of interest (ROI). This non-local effect dramatically interferes with the features of underlying anatomical structure in phase imaging. Not only that, QSM reconstruction relies on a specific mathematical relationship with the local field, which is highly sensitive to noise and errors. So QSM must resolve the confounding non-local bias to provide a satisfactory quantitative result.

Although many kinds of methods have been proposed for QSM reconstruction, many problems remain, such as the existence of noise, residual artifacts and accumulated errors. In this work, a fast reconstruction method under the total field inversion (TFI) frame was proposed to treat the whole reconstruction problem efficiently. This method is named as fast total field inversion (fast TFI). Benefitting from the new advanced solution of regularization and some special simplifications, it may get an excellent result in 2 iterations and 120 seconds. By employing an effective model, the proposed method can well reconstruct the brain with trauma and lesion, where many different methods are helpless. We also introduced a special solution of weighted L_0 regularization, aiming at accelerating the convergence of the whole algorithm. Experimental results verify that the new method performs well with both healthy and pathological brain data with a short reconstruction time. In comparison with classical three-step QSM methods, the new method has special advantages in protecting sample details, eliminating artifacts and restoring lesion areas. Moreover, it avoids a significant volume of tests in tuning parameters. It well meets clinical needs and may find great applications.

Fig. 1 *The iDQCJRES pulse sequence. Results of a patient with cerebral infarction. (A) Amplitude image; (B) R_2^* map; (C) global field map; (D-H) QSM results reconstructed with MEDI (D), iLSQR (E), WL_1 (F), SSQSM (G), and fast TFI (H) methods.*

Acknowledgements

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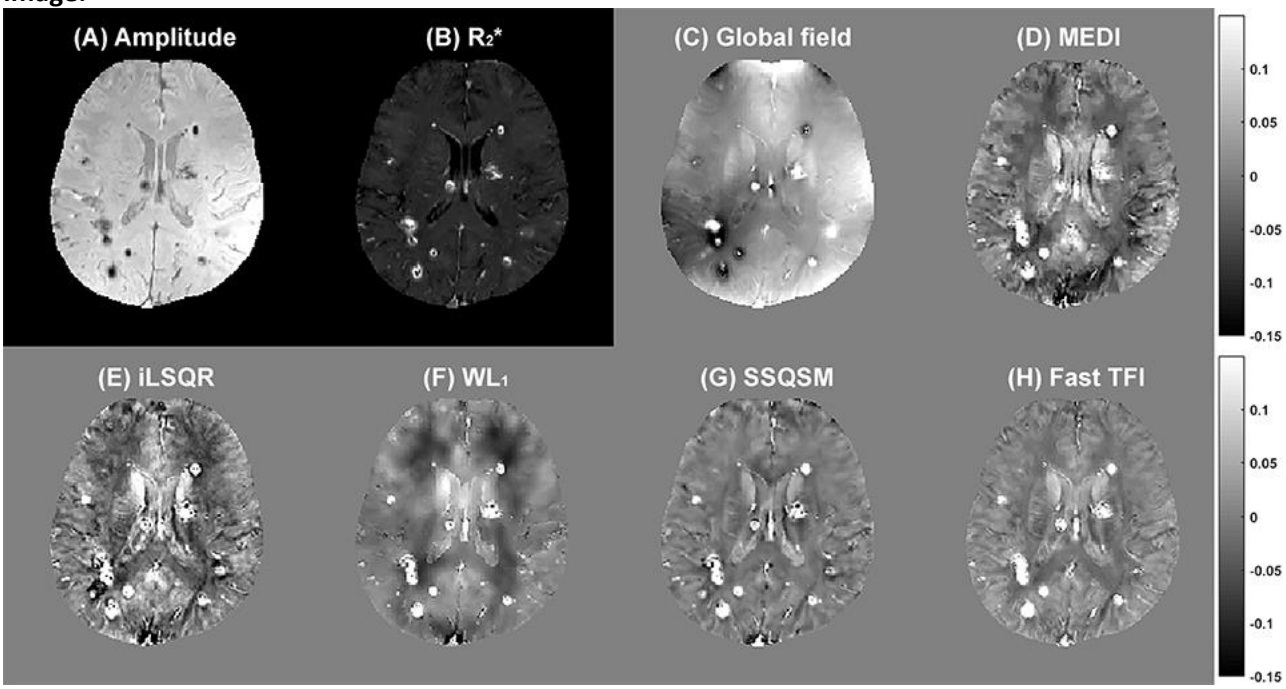
(A) Amplitude
(B) R2*
(C) Global field
(D) MEDI
(E) iLSQR
(F) WL1
(G) SSQSM
(H) Fast TFI
0.1
0.05
0
-0.05
-0.1
-0.15
0.1
0.05
0
-0.05
-0.1
-0.15

MRI and in vivo

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Interleaved SPEN experiments incorporating optimal phase control and iterative reconstruction for high-definition diffusion tensor imaging

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Abstract: Diffusion tensor MRI (DTI) probes water mobility in a non-invasive fashion, serving a variety of goals ranging from highlighting strokes and malignancies to connectomics[1,2]. Two complications arising in high-field DTI are magnetic field inhomogeneities and motions during the measurement. SPatial Encoding (SPEN)[3] is a single-shot MRI technique known for is resilience to magnetic field inhomogeneity. In addition, the encoding of the "SPEN dimension" happens in real space, freeing its reconstruction of Fourier transform's potential artifacts. Recent developments in SPEN demonstrated ways to incorporate diffusion gradients to perform DTI[4], the use of segmented acquisitions to improve image resolution[5], and multi-channel acquisitions with parallel imaging to increase resolution using sparse-sampling reconstruction (SUSPENSE)[6]. In this work, we describe a general reconstruction procedure that incorporates all these advances, plus the possibility of motion correction and phase compensations during the reconstruction of individual shots. The method also incorporates an automatic pipeline using SUSPENSE, L_1 regularization, as well as acceleration by multi-band acquisitions.

Figure 1 presents experiments recorded on a volunteer at 3T showing the application of the procedure in a restricted inner-brain field of view focusing on the pons region. Comparisons between state of the art interleaved EPI and SPEN experiments demonstrate that the latter provides superior diffusion weighted high-resolution images, particularly when the number of coil, extent of motions or regions impacted by magnetic-field inhomogeneity, preclude comparable EPI acquisitions. The method's new optimal use of multi-channel and multi-scan information, is described and illustrated in the context of relevant clinical studies.

We are grateful to Martins Otikovs, Dr. Furman-Haran (Weizmann), Dr. Lustig (UC Berkeley) and Dr. Seginer (Weizmann) for assistance. SfC thanks the FGS (Weizmann), French Embassy and dean of Weizmann for partial postdoctoral fellowships. Support came from the Israel Science Foundation (#2508/17) the EU ERC-2016-PoC grant # 751106, the Minerva foundation (#712277), the Kimmel Institute for Magnetic Resonance and the generosity of the Perlman Family Foundation.

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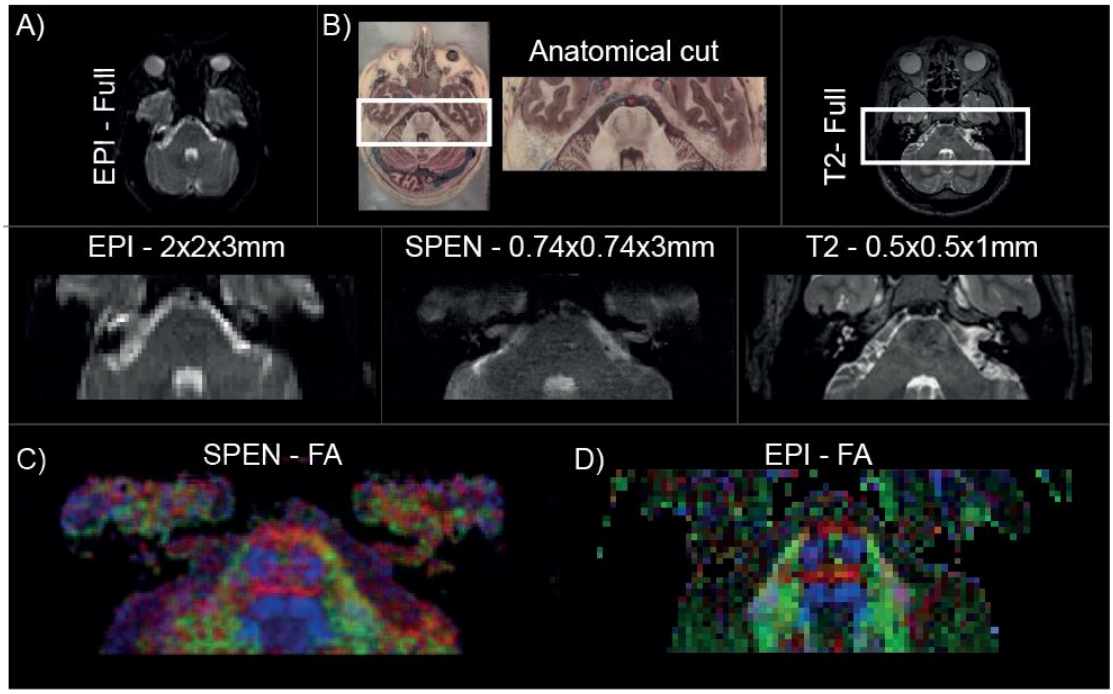
Image:



(A) Comparison between the EPI, interleaved SPEN and anatomical results arising from the pons, a restricted area located in the midbrain region. (B) Histological cut of the region (brainmaps.org). (C) Reconstructed DTI maps recorded for SPEN and EPI, with values indicating the nominal voxel resolution. (D) Representations including the conventional color map of the diffusion tensor[7].

MRI and in vivo
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Efficient quantum mechanical MRI simulation methods

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Abstract: We propose a solution to the problem that has so far prevented accurate quantum mechanical simulations of advanced MRI experiments involving complex molecules – the very large dimension of Kronecker products of 3D MRI phantom arrays and Liouville space vectors describing spin dynamics.
The solution takes advantage of the fact that the direct product structure of the time evolution generator is always known: it is a short sum of direct products of very sparse spin and spatial operators. Individually, these operators have manageable dimensions, and the action by their Kronecker product on any vector may be obtained without opening the Kronecker product, *e.g.*:
$[A\times B]v=\mathrm{vec}(AVB^{T})$
where A and B are matrices, v is a vector and V is the same vector folded into a matrix of appropriate dimensions. The same method works for direct products of three or more matrices [1].
In combination with Krylov propagation methods, this eliminates impractically large matrices from the simulation process. The performance of the proposed methods is illustrated with exact simulations of DPFGSE, PRESS, CHESS and SPENDOSY experiments on molecules involving more than ten interacting spins in three spatial dimensions with diffusion, flow, chemical kinetics, and accurate treatment of spin relaxation processes. This functionality is available in versions 2.2 and later of *Spinach* library [2].
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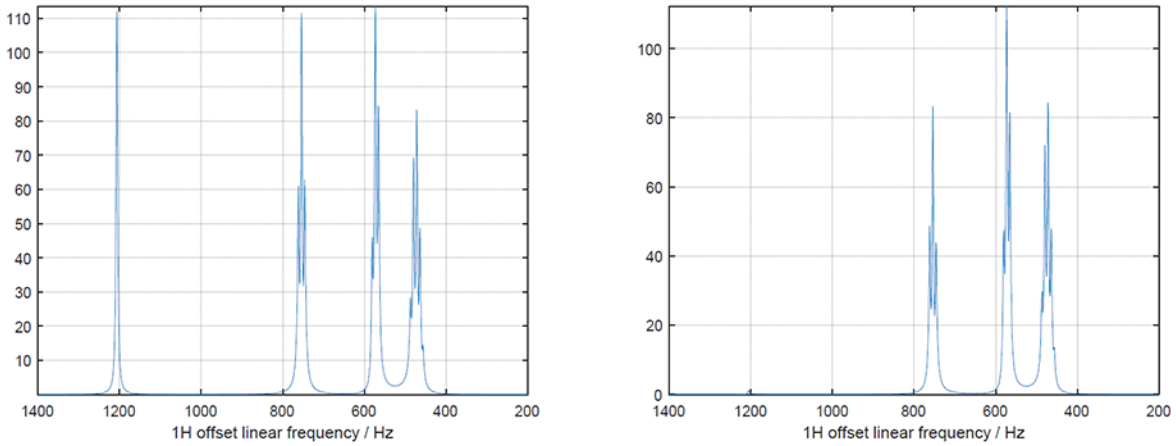

Figure 1. *An example simulation for a simple spatio-temporal NMR experiment (DPFGSE signal suppression) on a common metabolite (GABA).* ***Left:*** *DPFGSE shaped pulse off;* ***right:*** *DPFGSE shaped pulse on. The simulation is carried out in the time domain with the explicit treatment of shaped pulses and of the spatial dimension in which the gradient is applied, including diffusion and convection processes. The dimension of the complete Liouville state space in this system is $4^{7}\times$[100 spatial slices] = 1,638,400 - far too large for the conventional simulation methods.*

MRI and in vivo

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Design of colloidal phantoms for validation of in vivo diffusion MRI methods

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Abstract: Diffusion MRI allows us to non-invasively probe the microstructure of biological tissues. Such feature has motivated the development of several diffusion MRI protocols capable of quantifying microstructural changes in the living human brain. When installing or designing new microstructural contrasts, access to a stable reference object is crucial to validate parameter accuracy and system validation. To accurately determine the microstructure in tissue one needs to verify that the chosen methods are able to distinguish between phantoms with distinct microscopic pore shape.

Here, we propose three complementary colloidal phantoms providing ideal systems for validation of metrics of diffusivity, microscopic anisotropy, and compartment orientation. We have used liquid crystals to design phantoms exhibiting local diffusion tensors with normalized anisotropy D_Δ equal to the theoretical values of +1 (sticks), 0 (balls), as well as $-1/2$ (planes) [1]. The different pore shapes were obtained by exploring liquid crystalline phases in which water is restricted to compartments with at least one dimension at the nanometer scale. A schematic overview of the attained microstructures is shown in Figure 1a. The voxel-averaged diffusion tensors and microscopic diffusion tensors of the proposed phantoms were confirmed using a Bruker microimaging system. Figure 1b shows the voxel-averaged and the microscopic diffusion tensors measured for oriented- and randomly distributed crystal domains in the Plane- and Stick Phantoms [2]. These results reveal that the phantom pore shape is unaffected by orientation dispersion, and can be quantified by diffusion MRI methods.

Being produced from inexpensive and relatively harmless materials, our phantoms can be easily scaled up to the volumes required for use in a clinical scanner [3]. We then suggest our liquid crystal phantoms as the “gold standard” for diffusion MRI parameters capturing microscopic anisotropy in any of its incarnations.

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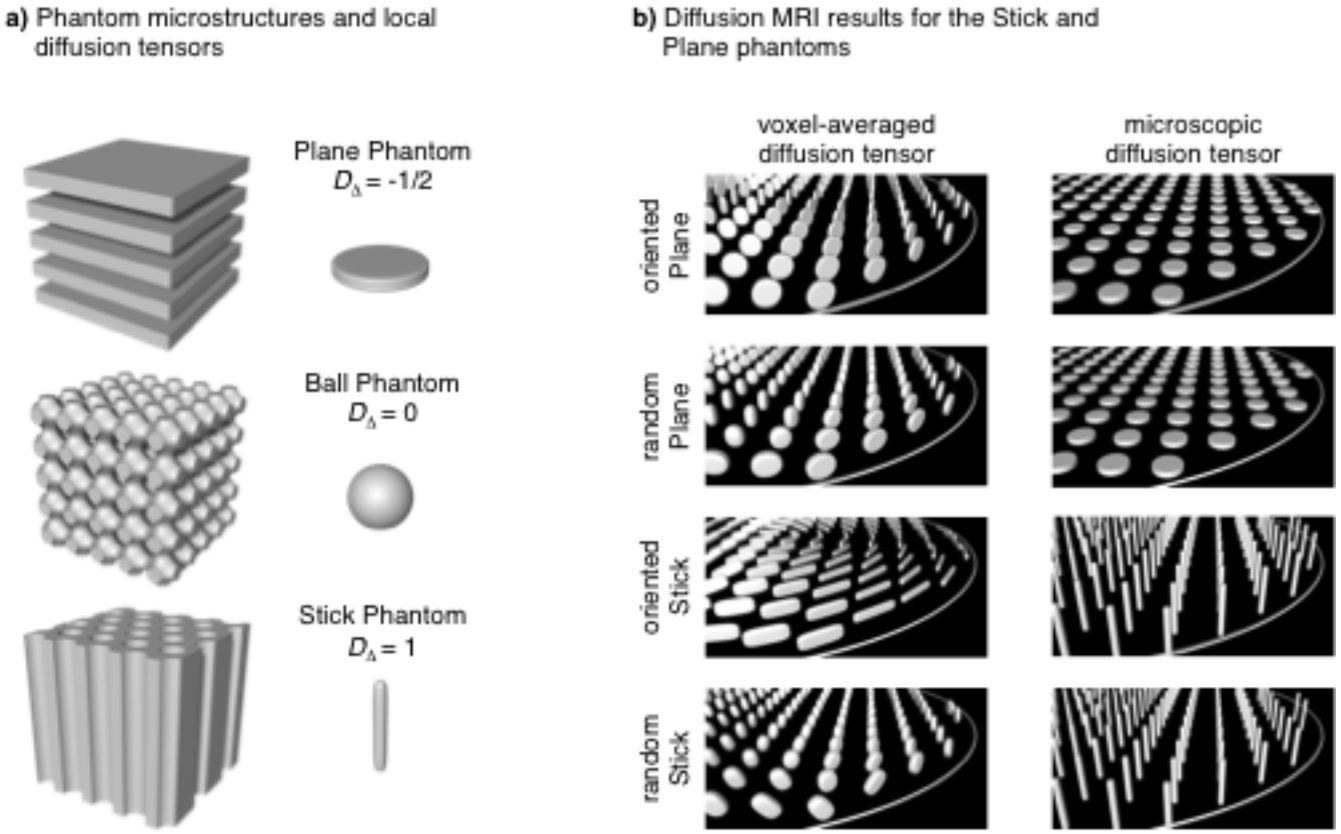

Disclosure of Interest: H. Jiang: None Declared, J. P. de Almeida Martins Conflict with: The company Random Walk Imaging AB (Lund, Sweden), where J. P. de Almeida Martins is an employee, holds patents related to the described MRI methods., J. Larsson: None Declared, D. Lundberg: None Declared, G. Eklund Conflict with: The company Random Walk Imaging AB (Lund, Sweden), where G. Eklund is an employee, holds patents related to the described MRI methods., K. Bryshke Conflict with: The company Random Walk Imaging AB (Lund, Sweden), where K. Bryshke is a co-owner, holds patents related to the described MRI methods., D. Topgaard Conflict with: The company Random Walk Imaging AB (Lund, Sweden), where D. Topgaard is a co-owner, holds patents related to the described MRI methods.

MRI and in vivo

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MANGANESE ENHANCED MRI: A METHOD IN ORDER TO VALIDATE PHYSILOGICAL MARKERS OF TINNITUS IN RODENTS

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Abstract: Tinnitus is an auditory phantom sensation (crackling, humming, whistling) experienced in absence of an external stimulus. This common disorder affects 10-15% of the population at one time or another in life. It creates discomfort sometimes associated with pain. The prevalence of tinnitus shows a worrying growth curve. The new lifestyles of developed and emerging countries (exposure to noise, urbanization, …) and aging of the population accelerate this trend and turn it a major public health problem.

The present study is designed to show physiological markers of tinnitus in rodents. One promising Magnetic Resonance Imaging (MRI) tool is used, called Manganese Enhanced MRI (MEMRI). The use of manganese chloride as an MRI contrast agent enables to follow brain neuronal activity. In fact, paramagnetic manganese ions (Mn^{2+}) accumulate in active neurons through voltage-gated calcium channels. The protocol based on MEMRI technic uses an intratympanic injection on rats with the salicylate model of tinnitus induction. Moreover, T1-weighted MRI images are collected in order to investigate the specific areas activated in presence of tinnitus or not. Two analysis methods are used: Statistical analysis by Signal to Noise Ratio (SNR) and T2 rate cartography.

Statistical analysis reveals that a SNR enhancement is observed in the main auditory areas (cochlea, cochlear nerve, cochlear nucleus, superior olivary complex) specifically in disabled rats. To extend these results, a new cartography was developed in this project using homebuilt program developed under Matlab interface. T2 rate cartography is based on high relaxation rates shown in tissues containing $MnCl_2$. The aim of this tool is to detect precisely the accumulation of $MnCl_2$. The recent works enabled to segment a particular auditory area, the contralateral inferior colliculus and to apply it to the T2 rate cartography.

Statistical analysis by SNR and T2 rate cartography are two complementary analysis methods. In fact, T2 rate cartography allows to local auditory regions with high concentration of MnCl2, and provide accurate quantitative data enables us to discriminate between heathy and tinnitus rats.

The development and quantification of T2 rate cartography open a new axis in tinnitus researches with MEMRI method. Our innovative study presents some potential applications in auditory research.

Key words: Tinnitus, Manganese enhanced MRI, T2 rate cartography

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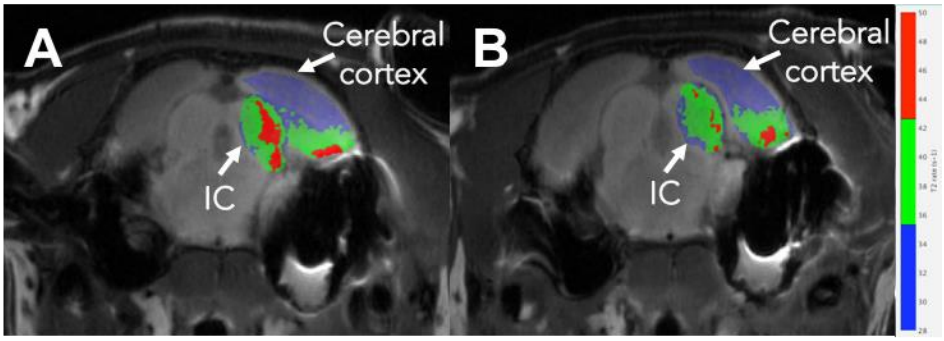

T2 rate cartography (RBG colormap) superimposed over T1-weighted MR images in particular on inferior colliculus (IC) and cerebral cortex in the ipsilateral side. MR images was acquired 24h after administration of transtympanic (TT) Mn^{2+} by injection into the left ear cavity of rat. A) A rat with salicylate induced tinnitus treatment B) A normal rat without auditory treatment.

MRI and in vivo

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Exploration of TRASE MRI at Low Magnetic Field: Potential Performance and Limitations

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Abstract: TRansmit Array Spatial Encoding (TRASE) MRI uses trains of radiofrequency (RF) π-pulses produced by "phase-gradient" coils which generate transverse B1 fields of uniform magnitude and spatially varying directions.[1-3] If coils A and B are two such coils – with phase-gradients characterized by vectors k_A and k_B – the building block of TRASE MRI sequences is the pair of π-pulses [P_A P_B] which shifts transverse magnetisation by an amount 2(k_A - k_B) in k-space and has the same action as a gradient pulse of the static field B0. We are currently exploring the benefits and limitations of TRASE MRI at low field. In the mT field range, hyperpolarisation can be employed to obtain high-SNR images but the adverse effect of concomitant B0 gradients puts a limit to the resolution achievable in standard MRI: this issue is automatically solved in TRASE. However, to accommodate for changes in B1-field orientation, concomitant RF field components necessarily exist. Moreover, oscillating RF fields are used, consisting of equal rotating and counter-rotating (CR) components. In contrast with high-field NMR for which the concomitant and CR components simply contribute to RF power deposition, both components may influence time evolution in low field since B1 magnitudes are not much smaller than B0.

We perform experiments in a 15-cm bore resistive magnet (B0: 1–5 mT), equipped with a usual (uniform-field) RF coil, standard B0-gradient coils, and additional phase-gradient coils (Fig. 1a).[4] The efficiency of RF phase-encoding is compared to that of encoding with B0-gradient pulses both in 1D and 2D imaging of water samples and sealed cells of laser-polarised ^{3}He gas. The potentially adverse effects of the RF concomitant and CR fields are systematically explored theoretically, numerically, and experimentally.[5,6] In particular, their joint effect can lead to significant image artefacts when short π-pulses are used (Fig. 1b). We have designed robust pulse sequences which are fairly immune to such effects. Generalisation of these studies to 2D and 3D gradient-free MRI is under way.

Fig1 a: low-field MRI system equipped with a spiral B1 coil[4,6] (coil S, B1 pitch k_S=0.12 rad cm^{-1} along z). A standard B1 coil, U, (not shown) surrounded coil S and generated a uniform field (k_U=0). b: Results of TRASE phase-encoding steps, chained groups of π-pulses $[P_S P_U]_x$ $[P_S P_U]_{-x}$, followed by refocussing B0-gradient pulses Gz of nominally opposite effect. Experiments were performed at 83.682 kHz (N_R=300) on doped water (a 7×7×1 cm^3 slab in the z=0 plane) using rectangular P_S pulses lasting 6 RF periods. Standard 1D spin-echo projection imaging was performed using B0-gradient pulses in the y-direction of the expected concomitant RF field gradient. In the time-domain, the echoes (the horizontal lines in the composite image) were strongly distorted and attenuated for more than 6-8 pulse groups. The corresponding 1D composite projection image displayed severe artefacts.

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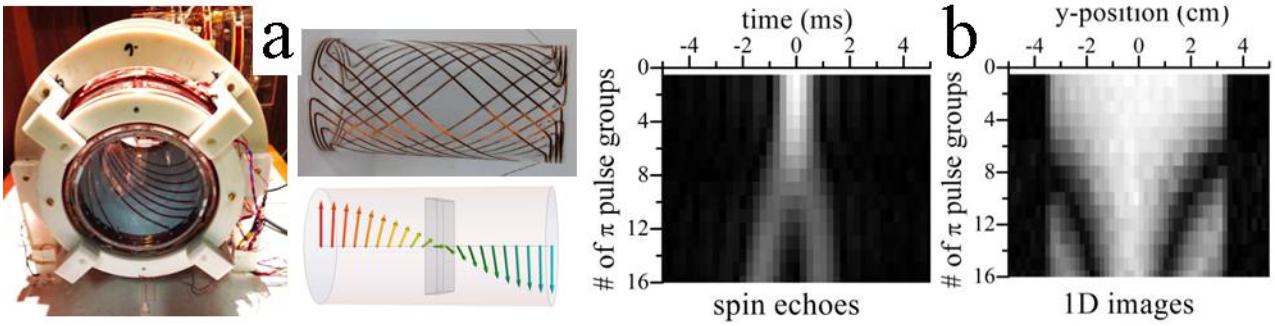

MRI and in vivo

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In vivo distortionless Diffusion Tensor Imaging of Mice Brain at 15.2 T

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Abstract: *In vivo* Diffusion Weighted and Diffusion Tensor Imaging (DWI, DTI) provide unique insights on the three-dimensional structure of healthy brains, as well as pathological. However, the spatial resolution of in vivo DWI and DTI is usually limited by the low sensitivity of the diffusion-attenuated images, and the long intrinsic acquisition times needed to acquire the multiple diffusion-weighted images necessary to compute the diffusion tensor. Strong diffusion gradients also induce high sensitivity to motion, and preclude the use of conventional multi-shot spin echo train acquisitions. Consequently, diffusion weighting usually involves single-shot imaging methods such as Echo Planar Imaging (EPI); however, those are notoriously sensitive to magnetic field distortions.

Very high magnetic fields are a valuable resource for increasing diffusion MRI's sensitivity, and thus the achievable spatial resolution[1]. This, however, enhances the susceptibility-induced magnetic field inhomogeneities, leading to image distortions along the phase dimension of EPI images. The use of high magnetic field is also associated with shortened transverse relaxation times T_2, demanding in turn the application of increasingly stronger diffusion magnetic field gradients, which increase eddy currents artifacts in the EPI images. EPI can bypass some of these drawbacks by relying on segmented acquisitions, which can yield improved resolution but also higher sensitivities to motion artifacts. Many of these issues can be solved by using SPatiotemporal Encoding (SPEN), which has been shown to provide higher immunity to magnetic field heterogeneities, eddy current and motion artifacts[2], and compatibility with segmented acquisitions[3,4]. In this work, we show that SPEN –in combination with cryoprobes and very high magnetic fields, allows performing *in vivo* 2D multislice DWI and DTI of mice brain with unprecedented spatial resolution (75*75*500 µm), and without artifacts. This development paves the way for high-resolution DTI and functional imaging at very high magnetic field.

Acknowledgments: We are grateful to Dr. Tangi Roussel, Dr. Yas Tesiram and Dr. Sascha Koehler (Bruker BioSpin) for their help with the experimental settings. The authors acknowledge the Kimmel Institute for Magnetic Resonance and the generosity of the Perlman Family Foundation for financial support.

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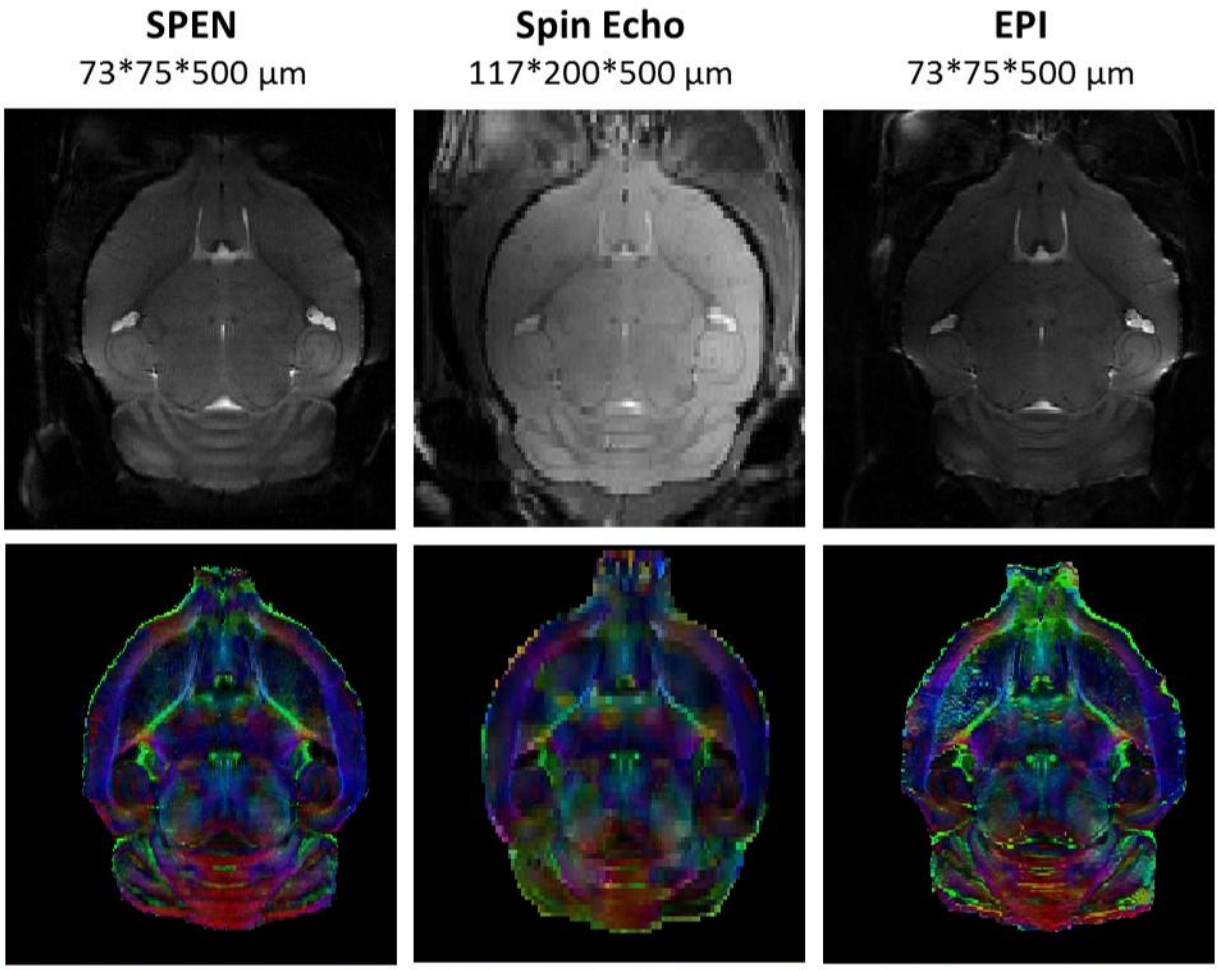

Figure 1: Non-diffusion weighted and color-coded main diffusion direction (MDD) *in vivo* brain images obtained by interleaved SPEN, conventional Spin Echo and interleaved EPI with double sampling, in identical acquisition times (1 hour 8 min) at 15.2 T

MRI and in vivo

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Validation of Interlaced Multi-Shell Imaging (iMSI) in a Clinical MRI Scanner

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Abstract: INTRODUCTION

iMSI is used to quantify diffusion characteristics in a sample. However, implementing this method on a clinical scanner is far more challenging than on small-bore research MRI scanners due to the constraints of hardware and scan time [1][2]. The purpose of this study was to evaluate iMSI in a clinical MRI scanner on both a phantom and human volunteers.

METHODS

Experiments were performed on a diffusion phantom constructed from a 3D printed ABS block wrapped with Kevlar thread (0.1mm diameter), with crossings at 45**°,** 90**°,** and 135°. Images were acquired on a Toshiba Titan 3T scanner using a 32-channel array head coil (TE/TR of 90ms/2000ms, b-values of 500, 1000, 1500 and 2000 s/mm^2, acquisition time of 3mins 4s). HARDI's were collected with a b-value of 1000 s/mm^2 with 2 averages to increase the SNR and improve reconstruction compared to a single average. The acquisition time was 4mins 29s.

Parameters for humans were TE/TR of 90 ms/13716 ms, b-values of 600, 900, 1200 and 1500 s/mm^2. The acquisition time for the iMSI dataset was 16mins 54s. For comparison, a single-shell 1-average HARDI was collected with a b-value of 1000 s/mm^2. The acquisition time was 15mins and 29s. There were 62 directions for the iMSI and 64 directions for the HARDI dataset for the phantom and human studies.

The optic chiasm was chosen as the region of interest (ROI) for analysis as the fibers from the optical nerves cross at this site; it consisted of 70-80 voxels. The ROI used on the phantom (20 voxels) centered on known crossings. iMSI data were analyzed using the approach in [3], wherein the Ensemble Average Propagator (EAP) is estimated via a sinc interpolation of the non-uniform multi-shell samples in q-space onto a BCC lattice, and then applying inverse Fourier transform. This was chosen due to the optimal spectral sphere-packing property of the BCC lattice (dual to the FCC lattice), resulting in optimal sampling [5]. HARDI's were fit with rank-4 tensors to assess the number of fiber crossings [4].

RESULTS

The iMSI method showed a significantly larger number of estimated crossings than HARDI in both the phantom (6.75±0.63 vs 3.00±0.71, n=4, $p<0.008$) and the human (32.00±3.05 vs 20.67±1.76, n=3, $p<0.033$) experiments.

CONCLUSION

Since a higher number of crossings were determined using the iMSI method, in both phantom and humans, it is inferred that this method results in superior reconstruction. A higher number of shells could be acquired for improved accuracy while combination with techniques such as multiband imaging would result in scan time reduction.

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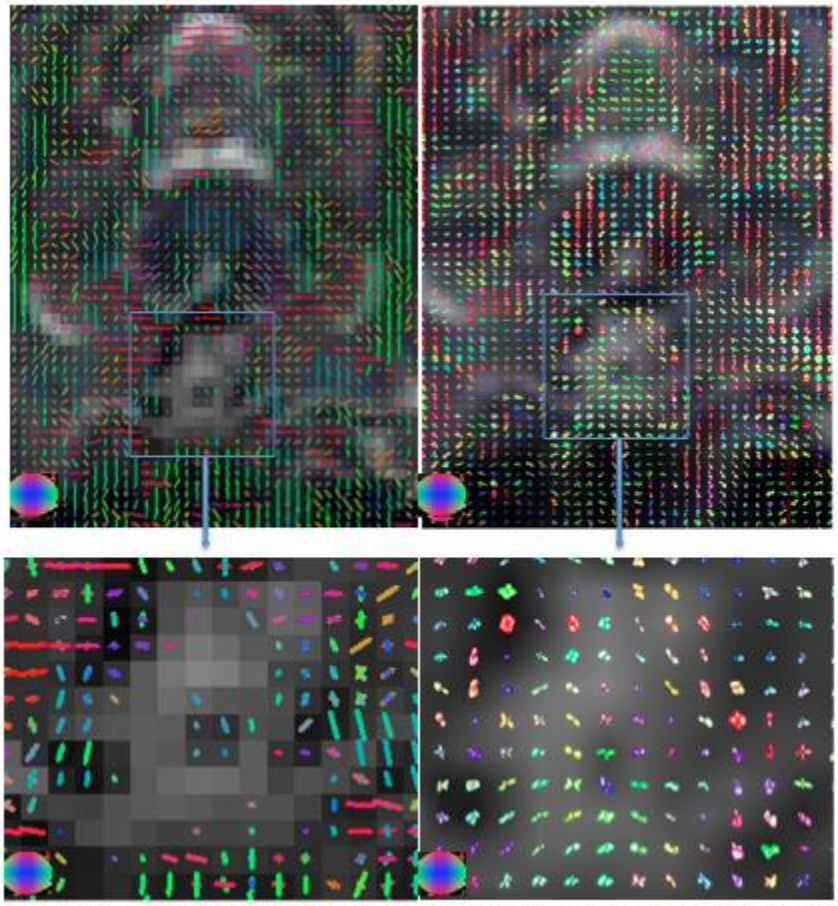

Figure 1- The images on the left (top and bottom) show the results of the iMSI experiment from the optic chiasm while the images on the right (top and bottom) are example results from the single-shell HARDI experiment. The bottom images are a zoomed-in version of the optic chiasm. The iMSI experiment displays more sensitivity to crossing fibers.

MRI and in vivo

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MRI biomarker to guide emergency therapeutic strategy during an acute ischemic stroke

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Abstract: MRI biomarker to guide emergency therapetic strategy during an acute ischemic stroke

Arterial recanalization performed shortly after acute ischaemic stroke (AIS) have demonstrated its ability to restore brain function. Besides intravenous fibrinolysis within 4.5hours, early endovascular mechanical thrombectomy (EVT) is another effective reperfusion strategy scientifically recognized since 2015, which can remove large and proximal clots rapidly. This results in higher rate of reperfusion and functional independence compared with intravenous fibrinolysis alone. However, the etiology of AIS is variable and several different kind of thrombi can caused AIS : red blood cells (RBD) dominant, fibrin dominant or mixed thrombus.

The two main cerebral arterial recanalization techniques are contact aspiration (CA) of the thrombus and stent retriever (SR) techniques. As time and number of attents are key factors of good clinical outcomes it is essential to improve recanalization prediction based on admission data, including imaging biomarkers of the thrombus in order to adapt the endovascular strategy to the occlusion.

Since 2015, the Neuroradiology Department of the Universitary Hospital of Nantes works on a radiological biomarker known as the susceptibility vessel sign (SVS) on T2* magnetic resonance imaging (MRI) scans, defined in 2004 by Rovira et al, as « a hypointense signal exceeding the diameter of the contralateral artery located at the site of the thrombus ». Its presence is associated to better clinical outcomes (Bourcier et al, 2015). SVS is seen in 50–85% of cases of hyperacute stroke, particularly in cases of a RBC dominant thrombus due to the presence of either deoxyhemoglobin, intracellular methemoglobin, or hemosiderin in the RBC.

SVS status significantly impacted the efficacy of the first-line strategy (Bourcier et al., under soumission) : compared to SR, CA achieved a lower recanalization rate when SVS was present (Risk Ratio (RR) for CA vs. SR, 0.60; 95%CI, 0.51 to 0.71; p =0.018) but not when SVS was absent (RR : 1.11; 95%CI, 0.69 to 1.77; p =0.018).

However, a binary qualitative assesment of thrombus using SVS does not reflect is complex nature. Hence, we try to develop a MRI quantitative tool : Thrombus T2* relaxation time (TT2*RT). We found that earlier and better recanalizations were signifantly associated with a shorter TT2*RT (mean SD ; range : 25,7 ;18-50ms vs 45,9 ; 35-60ms, p> 0,001) (Bourcier et al., 2017).

Thus, collaboration between clinicians and engineers are needed to optimize the radiological biomarkers of the thrombus to predict its nature and to the guide the choice of the emergy therapeutic strategy during AIS.

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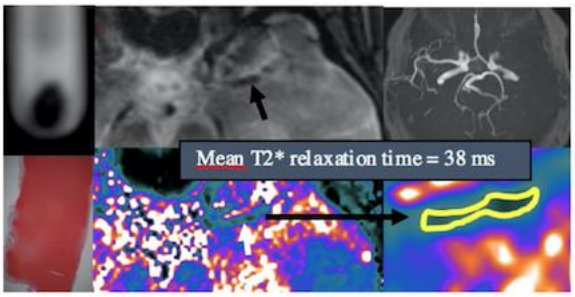
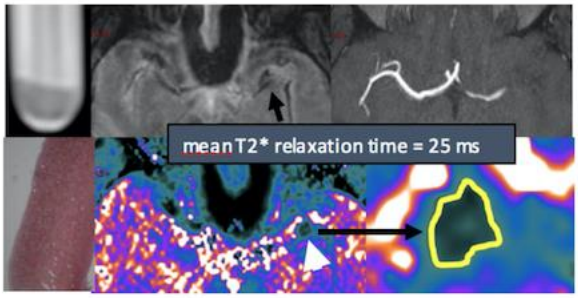


MRI of a red blood cells dominant thrombus :

a	b	c
d	e	F

a : T2* of a in vitro RBC thrombus
b : in vivo T2 star
c : time of flight
d : histology cut of in vitro RBC thrombus
e & f : T2* mapping of and RBC thrombus



MRI of a mixed thrombus :

a	b	c
d	e	f

a : T2* of a in vitro mixted thrombus
b : in vivo T2 star
c : time of flight
d : histology cut of in vitro mixed thrombus
e & f : T2* mapping of and mixed thrombus

MRI and in vivo

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Multiscale NMR analysis of apple thermal denaturation

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Abstract: Microstructure and texture evolution of foods during cooking is difficult to achieve, because conventional techniques cannot visualize the internal structure, while preserving its integrity. For this very often processed product that is apple, it is essential to understand thermal degradations. To study such phenomena, we undertook MRI, quantitative NMR and mechanical experiments at key temperatures of the cooking process.

Samples (1x1x3cm^3) were vacuum packed before water cooking at 45, 50, 53, 60 and 70°C. MRI measurements were performed on a 9.4 T Bruker Ascend 400WB instrument, with a 30mm diameter ^{1}H volume coil. We acquired 10 SE images at different TE (4.5 to 200ms), with a single echo per acquisition for preventing refocusing errors (TR=3000ms, voxel vol. 1mm^3 isotropic, duration 32min) to map T2 (hypothesis of mono exponential decrease). NMR CPMG acquisitions, $90°_x$ -[t-$180°_y$-t-(echo)]$_n$, were also carried out (t=500µs) and n=128 data points of the echo decay signal were collected. Data were fitted using non-negative least squares algorithm fed with a decomposition basis made of 200 T2 logarithmically-spaced from 1ms to 1000ms. In parallel, we performed puncture tests with a 2mm diameter flat end needle descending at 1mm/min until the strain reached 70%.

T2 maps clearly show a dramatic change in T2 values between raw (10ms) and cooked (35ms) parenchyma at a transition temperature of around 50°C (fig.). According to this finding, quantitative NMR indicate that the main T2 component, attributed to the vacuolar compartment (Hills et al., 1997), sharply decreases from 37ms to 23ms after 50°C. The resistance to a mechanical stress also displays a dramatic change, for a slightly higher cooking temperature of 53°C.

The relaxation changes observed both by CPMG and imaging at 50°C can be interpreted by the filling of pores after thermal permeabilization of the membranes. The effective T2 obtained by imaging is sensitive to internal magnetic field gradients, caused by susceptibility differences at the pore interfaces (Van As et al., 1997). It explains why this T2 is shorter than the CPMG's T2 in the porous material (before 50°C) and its dramatic increase when the pores are filled. After 50°C, some T2 differences remain due to the improper model of the T2-decay obtained by imaging. The slight shift of the transition temperature given by mechanical tests supports the hypothesis of a complex thermal denaturation of the plasmalemma, first releasing vacuolar water before being structurally affected (Bourles et al., 2009). This work emphasizes the interest of multiscale relaxation studies for studying thermal denaturation of food products. CPMG allows resolving multiple T2 compartments in the whole sample whereas imaging is sensitive to heterogeneities.

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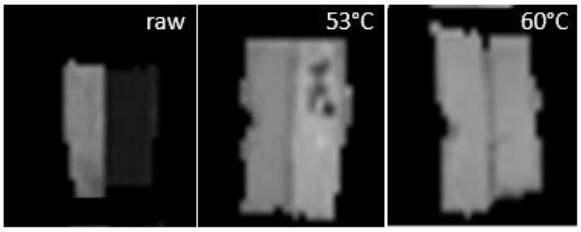
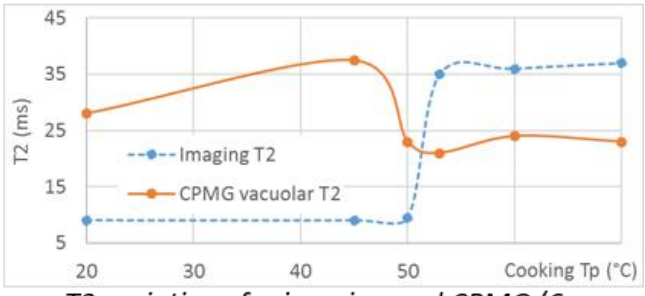


T2 maps of cooked apple samples. For 3 key temperatures: left, a reference sample cooked at 70°C and right a sample cooked at the given key temperature.



T2 variations for imaging and CPMG (6 samples per Tp). 20°C means raw sample.

MRI and in vivo

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MRI quantitative T2* mapping on thrombus to predict recanalization after endovascular treatment for acute anterior ischemic stroke.

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Abstract: Background : In anterior acute ischemic stroke (AAIS) treated with endovascular treatment (EVT), the susceptibility vessel sign (SVS+ or SVS-) is related to recanalization results (TICI 2b/3) and clinical outcome. However, a binary qualitative assessment of thrombus using SVS does not reflect its complex composition. Our aim was to assess whether a quantitative MRI marker, Thrombus-T2* relaxation time, may be assessable in clinical routine and may to predict early successful recanalization after EVT, defined as a TICI 2b/3 recanalization obtained in 2 attempts or less.

Methods : Thrombus-T2* relaxation time was prospectively obtained from consecutive AAIS patients treated by EVT (concomitant aspiration and stent retriever). Quantitative values were compared between early recanalization and late or unsuccessful recanalization.

Results : Thirty patients with AAIS were included and Thrombus-T2* relaxation time was obtained in all patients. Earlier TICI 2b/3 recanalization were obtained in 22 patients (73%) and was significantly associated with SVS+ (1/8 vs. 16/22, P=0.01) and a shorter Thombus-T2* relaxation time (mean SD, range: 257, 18-50ms vs. 45 9, 35-60ms, P<0.001).

Conclusion : A new quantitative MRI biomarker, the Thrombus-T2* relaxation time is assessable in clinical routine. In a preliminary study of 30 patients, a shorter Thombus-T2* relaxation time is related to earlier recanalization after EVT using combination of stent retriever and aspiration.

MRI and in vivo

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A Study of a MR signal reception from a model battery

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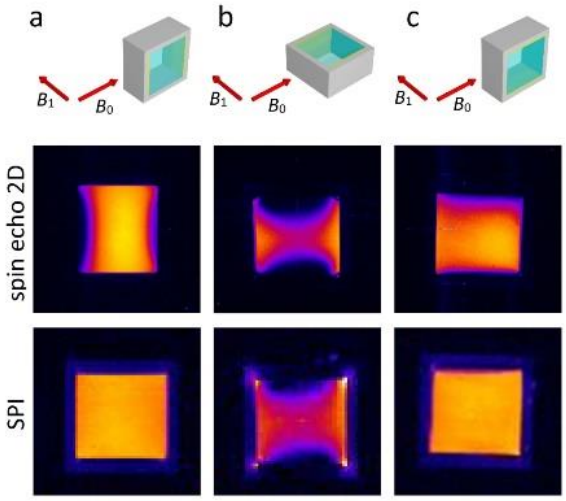
Abstract: An increasing interest in research of batteries and materials for the batteries by NMR and MRI opens a question of compatibility of these samples with NMR. Most batteries usually contain metal electrodes and as such one would expect that they are entirely inappropriate samples for liquid NMR and MRI, where high magnetic field homogeneity and long signals are required. In batteries, RF disturbances in the proximity of the metal parts, such as electrodes or lead wires are expected due to induced eddy currents. In addition to the B_1 effect, there is also a B_0 effect due to susceptibility differences between the metal parts and electrolyte and finally in MR imaging experiments eddy currents are induced in the metal parts also due to application of imaging gradients. In the study, all three effects: B_1, B_0 and the gradient effect on a signal reception were studied on a model battery.

A model battery was made in a cubic plastic container with inner dimensions of 13 x 13 x 8 mm. Two opposite side faces of the container were from the inner side covered with a 0.3 mm thick copper foil. The container was then filled with a 2 % agar gel to which 5.8% of sodium chloride and 0.08 % (weight ratios) of copper sulfate was added. When the gel hardened in the container, the battery was inserted in the magnet in various orientations and scanned with 2D and 3D spin echo MRI sequences as well as with the single point imaging (SPI) sequence. The orientations were the following: a) electrodes perpendicular to B_0, b) electrodes parallel to B_0 and perpendicular to B_1, c) electrodes parallel to B_0 and parallel to B_1. To study the effect of gradient-induced eddy currents the experiment in case a) was repeated also with a much longer delay between the gradient switching and the signal readout. The experiments were performed on a 400 MHz MRI system consisting of a Jastec superconducting wide-bore vertical magnet, Tecmag Redstone spectrometer and Bruker Micro 2.5 micro-imaging probe.

Results of the imaging experiments (Figure) showed that in case a) there was a signal voiding in the spin-echo MR images in proximity of the electrodes due to the susceptibility effects (B_0 effect) of the metal electrodes, while the artifact was not seen in the SPI images. In case b) the signal voiding was more extensive and was a consequence of RF field-induced eddy currents (B_1 effect). The effect was equally strong with both imaging methods (spin-echo and SPI). In case c) the B_1 effect was greatly reduced so that the B_0 effect dominated over it. No significant effect of gradient-induced eddy currents on the image signal was obtained.

In the study, it is shown that NMR/MRI of batteries is feasible despite metallic parts in them. The parts must be nonmagnetic and a special care must be taken for the parts to be oriented properly in order to minimize occurrence of eddy currents.

Image:



MRI and in vivo

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Localized measurement of metabolites transport by propagator STEAM-PFG

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Abstract: MRI flowmetry is the gold standard to measure flow in plants, both xylem (transpiration stream from root to leaves) and phloem (transport of photosynthetic products from source leaves to sink tissues)[1]. Up till now methods have been used that do not discriminate on chemical shift information and therefore the data reflects mainly water transport. Metabolite transport (such as sugars, the main photosynthetic products) in the phloem is of great interest, since phloem transport is still not well understood and challenging. Here we present a NMR approach to quantify the flow of sucrose in the phloem of a plant and at the same time distinguish between the flowing sucrose in the phloem elements and stationary sucrose in the surrounding tissue. The technique combines flowmetry based on the full propagator measurement by PFG with the localized spectroscopy sequence STEAM: STEAM-PFG. The sequence results in chemical shift resolved displacement or propagator distributions. The technique is demonstrated on phantoms, which show that the technique is capable of accurately measuring the diffusion coefficients of sucrose and water and can distinguish between sucrose and water in a flowing solution, measuring the predicted flow-profile and velocities. *In vivo* application of STEAM-PFG in the gel/seed region of a tomato fruit shows that even in a more complex system the diffusion of water, carbohydrates and lipids can be determined.

First results obtained in a live tomato plant at our 3T intact plant MRI system are promising. The velocities of water in both phloem and xylem measured with STEAM-PFG correspond well to flow-velocities as observed by PFG-SE-TSE imaging[1]. In addition, STEAM-PFG was successful to measure sucrose flow. The velocity of the sucrose transport in the phloem was very comparable to that of water. The method allows to study the diurnal behaviour.

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MRI and in vivo

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In-vivo spectra pre-conditioning using PcBc

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Abstract:

In vivo spectra (1H, 13C, 31P, 23Na, ...) are known to be particularly difficult to phase and to correct for baseline drifts. The reasons are multiple: apart from the usual ones encountered typical of in-vitro spectroscopy, such as post-pulse receiver transfer-function distortions (dead-time), filter delays, filter roll, an a few others, there are additional difficulties due to the following specific factors:

- Low signal-to-noise ratio (S/N),
- Unavoidably bad B1 homogeneity, involving both B1 amplitude and phase.
- Local magnetic field gradients due to mesoscopic susceptibility variations in tissues.
- Broad solid-tissue background signals.

This makes even manual pre-conditioning (phase and baseline corrections) very difficult and extremely subjective.

We present the promising results obtained with a recently developed [1] pre-conditioning method named PcBc which consists in a simultaneous phase and baseline correction using both the in-phase and the out-of-phase parts of a spectrum.
Since the method is completely automatic, it bears the promise of being also completely objective – a feature which, according to our opinion, was so far badly missing in the management of in-vivo NMR spectra.

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MRI and in vivo

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High field micro MRI velocimetry (Rheo-MRI) to obtain quantitative local flow curves of complex fluids

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Abstract: Micro MRI velocimetry (Rheo–MRI) is a non-intrusive technique that allows a direct and quantitative view on spatially resolved (local) flow behaviour of complex fluids. Of particular interest are dispersions that display shear banding, i.e. co-existing flowing and stationary regions, since their flow behaviour cannot be understood from conventional rheological measurements. Spatially resolved rheo-MRI velocity profiles are commonly obtained in the Couette geometry, which consists of concentric cylinders with a gap in between them. By measuring the applied torque on the inner cylinder the local stress can be determined, since the stress distribution over the gap is well known. Combining this information with local shear rates obtained from the rheo-MRI velocity profiles allow for mapping local stress vs. local shear rate, the so-called local flow curve. This opportunity has however not seen implementation in MRI systems operating at high magnetic field, which would be advantageous with respect to sensitivity and resolution in space and time.

Quantitative local flow curves are especially helpful to monitor and to study flow behaviour of dispersions that display time-dependent and yield stress behaviour, since their flow behaviour cannot be understood from conventional rheological measurements. Since such a local flow curve can be obtained from a snapshot rheo-MRI velocimetry experiment, it offers unique opportunities to assess transient flow behaviour of dispersions.

We constructed a set of Couette geometries with variation of gap sizes (1, 2.5 and 4 mm) that can be mounted in a conventional rheometer in a standard micro-MRI probehead of a wide bore 7 T system (^{1}H NMR frequency of 300 MHz). This allowed recording of Rheo-MRI velocity profiles in minutes with high signal to noise and a spatial resolution of 50 microns. At this high magnetic field effects of small mechanical instabilities and chemical shift dispersion became apparent and measures needed to be taken to avoid artefacts. A good match was found between global and constructed local flow curves for Newtonian (Silicon oil) and simple yield stress (Carbopol) fluids. Based on local flow curves we monitored network formation of fat crystals dispersed in oil under shear. The local yield stress as a function of time was estimated based on the Herschel–Bulkley equation. Time and position dependent local viscosity changes could be deduced from the local flow curves.

MRI and in vivo

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Exploring contrasts for identifying pancreatic tumours in orthotopic murine models

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Abstract: Despite huge strides in cancer treatment, pancreatic cancer remains with a 5-year survival rate lower than 5% – nearly unaffected for the last century and making it the fourth major cause of cancer-related deaths. [1] This dismal prognosis reflects the high metastatic index of pancreatic cancer, coupled to the paucity of early detection methods for these tumours.[2] By contrast to what happens with other malignancies, MR plays a relatively minor role in detecting this disease. This reflects the lack of contrast that pancreatic tumours show vis-à-vis surrounding tissues to typical parameters (eg, T2); it also reflects the fact that contrast agents so useful in other instances, do not extravasate out of pancreatic tumours. Recent work has shown potential correlations between diffusion MRI (DWI) and tissue fibrosis –yet these measurements tend to fail when implemented using conventional EPI techniques in mobile areas that are close to fat/air/water interfaces, like the abdomen. The goal of this work is to explore the usefulness of emerging MRI forms, and in particular of robust DWI approaches based on SPatiotemporal ENcoding (SPEN),[3] to develop new forms of contrast that can target this disease. To this end preclinical experiments were developed in mice bearing orthotopic PAN-2 tumour models, chosen due to their similarities to human counterparts. Different MR contrasts have been explored, ranging from standard T1- and T2-weighting to Diffusion-weighted experiments (DWI). These explorations showed that pancreatic tumours have lower ADC than its surroundings, and also that its diffusion tensor properties have specific orientation. Furthermore, their T1 is slightly longer than most of that in the abdominal region. These findings find parallel in previous works in the literature,[4,5] and promise to combine into a single tool capable of highlighting these malignancies (Figure 1). Dissolution DNP experiments are also conducted in parallel with these determinations, in an effort to further enhance the specificity of the diagnosis. An account of these measurements and their prospects for clinical translation, will be presented.

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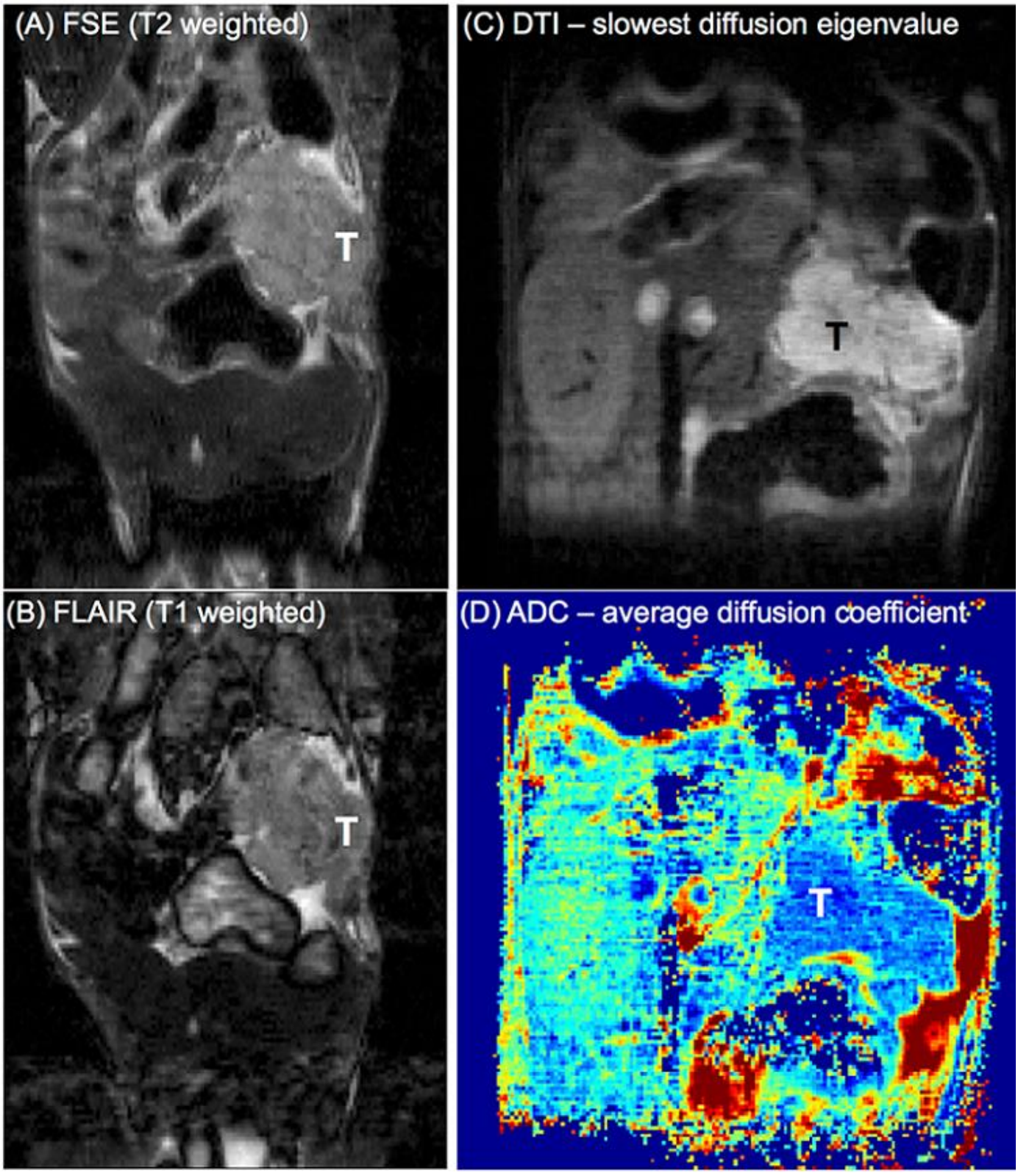
Figure 1: Images arising from orthotopic PAN-2 tumours in murine models. (A) T2-weighted Fast Spin Echo, FSE. (B) Fluid Attenuation Inversion Recovery, FLAIR with an inversion time of 470 ms, highlighting the differences in T1. (C) and (D) SPEN-based DTI and DWI experiments, pinpointing the tumour (T).

Image:


(A) FSE (T2 weighted)
T
(C) DTI – slowest diffusion eigenvalue
T
(B) FLAIR (T1 weighted)
T
(D) ADC – average diffusion coefficient
T

MRI and in vivo

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MR Relaxometry of Precision-Cut Liver Slices

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Abstract: Motivation

Liver-related diseases impose a great burden in societies, thus better understanding of their origin and development is of high interest. Better biological models, such as Precision-Cut Liver Slices (PCLS) [1], are required to study liver-related disorders like cholestasis and fibrosis. NMR spectroscopy and MR microscopy offer a non-destructive tool for studying metabolism and cholestasis progression *in situ*. Hence, protocols providing detailed relaxation parameters will allow optimized adjustments for micro-MRI to acquire morphological data of PCLS with maximised Contrast-to-noise ratio (CNR). Here, we present relaxation parameters of one chemically fixed PCLS derived from MR relaxometry measurements.

Methods

PCLS (5 mm diameter, 250-300 µm thickness and 5 mg wet weight) from mice (C57BL/6 male, weight 2530 g, 10 weeks) were prepared with a Krumdieck Tissue slicer (Alabama Research & Development, US. PCLS were fixed for 8 min. at 37 °C with a solution (4 % paraformaldehyde, 4 % sucrose in sterile phosphate-buffered saline (PBS)). Afterwards, PCLS were washed three times for 5 min. with sterile PBS at 37 °C and stored in PBS at 4 °C. For the MR measurements we anchored the slices on a PMMA holder and placed in a standard 10 mm NMR tube. MR Relaxometry experiments were conducted on a Bruker Avance III 500 MHz System using a 10 mm saddle coil. Each relaxometry measurements took 5 min with 50 µm in-plane resolution at a slice thickness of 200 µm. Based on 3D gradient echo (GRE) data a reconstruction was conducted using the software 3D slicer.

Results

In Figure 1 the sample placement of PCLS in MR sample tube and grayscale-encoded results of MR relaxometry experiments measuring T1,T2,T2* and ADC are shown. The orange arrows indicate the PCLS. The scale bars displayed in the MR images represent 2 mm. A 3D reconstruction of the boundary of the sandwiched PCLS was enabled by segmentation of the 3D GRE data. The data obtained from the MR experiments allow a proper distinction between PBS and PCLS. T_1-relaxation within the PCLS is 1100 ms compared to PBS with 2300 ms, whereas the transversal T_2-relaxation is 100 ms compared to 300 ms in PBS. T_2^* in PCLS was found to be 25 ms. The apparent diffusion coefficient (ADC) of $0.2 \cdot 10^{-3}$ mm^2/s is a factor of five smaller than in PBS. The T_1-weighted 3D GRE image of the PCLS has an isotropic resolution of 40 µm and allows a clear identification of the PCLS.

Conclusion & Outlook

The presented results provide key parameters for optimized MRI allowing maximising the contrast whilst micro-imaging PCLS. The integration of an NMR compatible perfusion stage will enable the observation of morphological progression under controlled conditions.

Acknowledgements

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References

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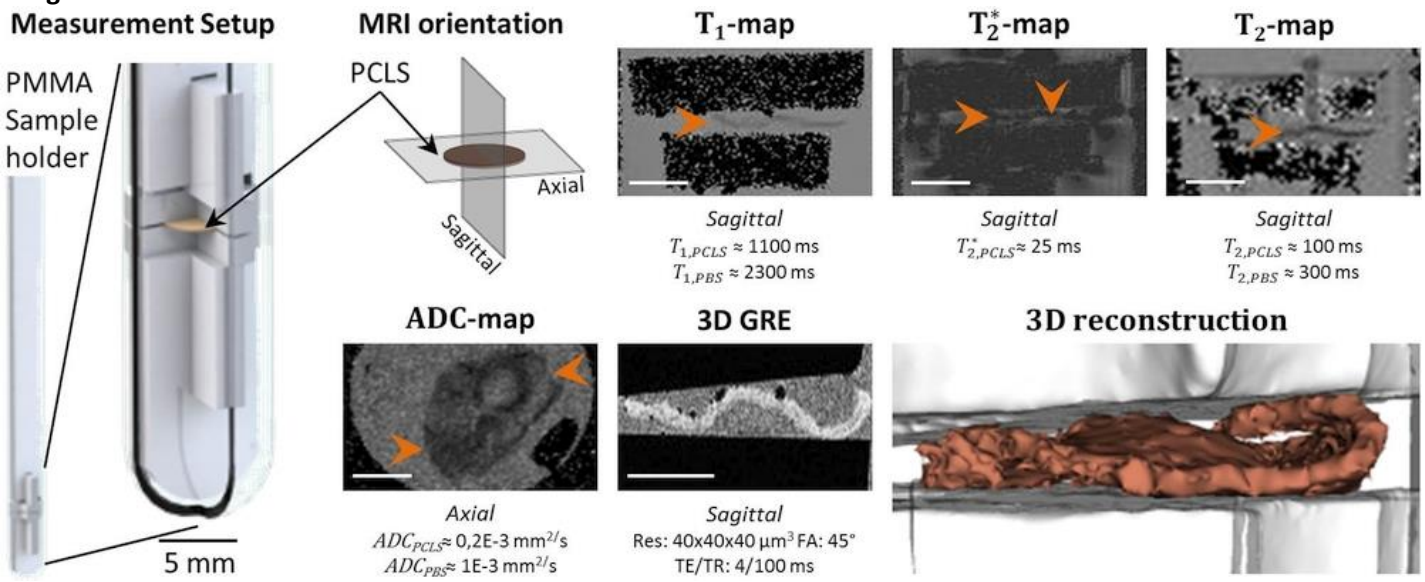
Image:


Measurement Setup
PMMA
Sample
holder
5 mm
MRI orientation
PCLS
Axial
Sagittal
T1-map
Sagittal
T1,PCLS ≈ 1100 ms
T1,PBS ≈ 2300 ms
T2*-map
Sagittal
T2*,PCLS ≈ 25 ms
T2-map
Sagittal
T2,PCLS ≈ 100 ms
T2,PBS ≈ 300 ms
ADC-map
Axial
ADCPCLS ≈ 0,2E-3 mm2/s
ADCPBS ≈ 1E-3 mm2/s
3D GRE
Sagittal
Res: 40x40x40 µm3 FA: 45°
TE/TR: 4/100 ms
3D reconstruction

MRI and in vivo

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Improving the specificity of Chemical Exchange Saturation Transfer (CEST) signals by 14N-based editing

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Abstract: Several techniques are being sought to enhance the molecular sensitivity of *in vivo* magnetic resonance. Arguably most versatile among them is CEST,[1] an experiment that makes it possible to detect certain intrinsic metabolites *in vivo* with sufficient sensitivity for their imaging. CEST amplifies the signals of labile moieties by attenuating them through a long radiofrequency saturation pulse; this saturation can then pass to the water signal through chemical exchange, thus providing an amplified response for the metabolite that contains the labile group. While capable of amplifying metabolic responses by orders of magnitude, this technique is hampered by the overlap among the many metabolites that will be simultaneously be present in tissues and contribute to the same CEST signature.[2,3] The goal of this work is to explore the possibility of improving CEST's specificity via heteronuclear editing; specifically, by modulating the signals of labile protons according to the nature of the ^{14}N sites to which they are scalar-coupled. Although ^{14}N is naturally abundant (99.6%) and presents an ample chemical shift dispersion for both amide and amine (labile) groups, it is a fast relaxing quadrupolar spin which renders most standard editing techniques difficult. Still, protons will retain information about their ^{14}N coupling via a line broadening, which although affected by scalar relaxation of the second kind[4] can be modulated by on resonance ^{14}N-decoupling.[5] This feature is exploited in a series of CEST experiments, which employ a modified sequence as shown in Fig. 1A. It is possible to understand the effects of this nitrogen-edited (NE) CEST sequence by the results in Fig. 1B, containing a model solution of lysine. Lysine possesses two labile amine sites appearing ≈3.5ppm downfield from water in this CEST (Z-) spectrum, which show a site-specific line narrowing when decoupling with a low power irradiation that addresses only one of their ^{14}N. Given the large ^{14}N chemical shift range, one can then garner specificity: the NE-CEST derived image of a sample with several metabolites can then highlight only one of them (Fig. 1C). We are currently translating this double-resonance but low power scheme to *in vivo* studies. We have also developed a frequency encoded (based on Frequency Labeled Exchange, FLEX[6]) version (NE-FLEX) in which similar effects are observed, and will be reported.

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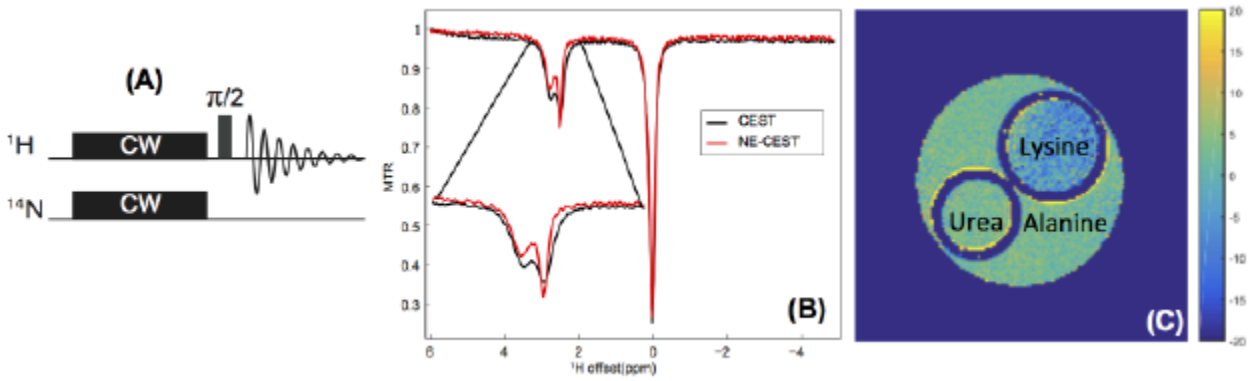
Figure 1: (A) Pulse sequence scheme for {^{14}N}^{1}H NE-CEST. (B) Experimental results comparing on- and off-resonance ^{14}N decoupling (NE-CEST and CEST) for a lysine sample, where the alpha site is being line-narrowed. (C) RARE-based imaging of the difference between on and off-resonance NE-CEST for a multi-metabolite phantom, when decoupling was set as in (B): the sole contrast arises from lysine.

Image:


(A)
π/2
¹H
CW
¹⁴N
CW
MTR
CEST
NE-CEST
(B)
¹H offset(ppm)
Lysine
Urea
Alanine
(C)

MRI and in vivo

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Visualizing biofilms on single granule bioanodes using Magnetic Resonance Imaging

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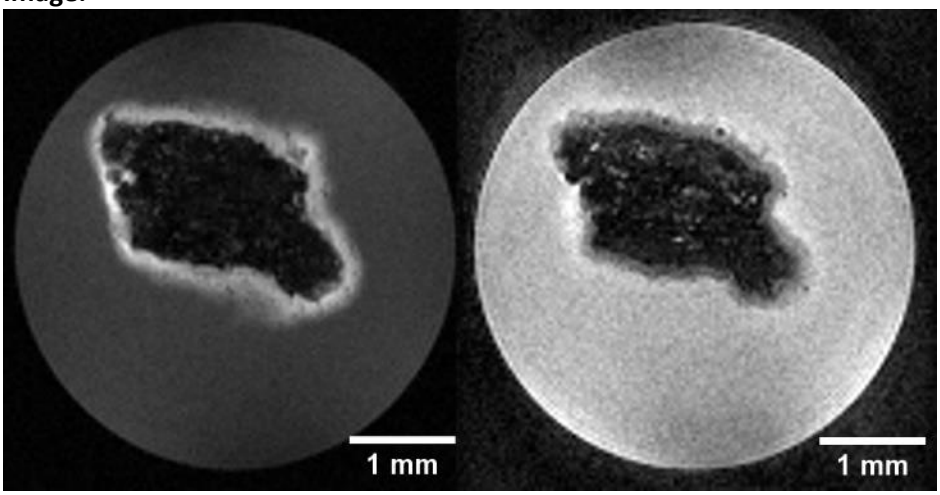
Abstract: The re-use of different waste streams is important for the transition towards a circular economy. Microbial fuel cells are a promising technology to generate electricity from wastewater. Specifically, electroactive biofilms growing on activated carbon granules are currently being investigated for electric current production when fed on wastewater. Analytical techniques to study biofilms growing on these granules (e.g. SEM and CLSM) are invasive and destructive. Furthermore, the precise biofilm growth in terms of distribution and volume cannot be determined from the currently available techniques, partially due to the irregular surface and opaqueness of the granules. Using Magnetic Resonance Imaging (MRI) we show that it is possible to non-invasively visualize the biofilm layer on an irregular surface. Image artefacts were observed during the first trials, supposedly caused by iron nanoparticles in the activated carbon support. To reduce the number of iron nanoparticles present in this support, we subjected it to an acid treatment prior to growing the biofilm on the granule. Using T_1 and T_2 contrast, the electroactive biofilm can be distinguished from bulk water in a 14 T narrow-bore spectrometer with a 5 mm saddle coil. T_1-weighted 3D-RARE scans with spatial resolutions of 28x28x28 m^3 enable us to visualize the biofilm volume and distribution over the irregular surface. Using thresholded image analysis on these 3D-scans, we can quantify the biofilm volume in different growth stages. As MRI is non-invasive and it can be conducted prior to other analyses, we were able to quantify the biofilm with a second method by using total nitrogen content analysis. With this study we show the potential of MRI for the non-invasive investigation of biofilm surfaces on granular electrodes. We conclude that MRI can be added to the existing analyses toolbox.

Figure 1: Two MSME images show the biofilm around the activated carbon granule (black). A.) In the T_1-weighted image (TE = 3.1 ms and TR = 0.5 s) and in the B.) proton density weighted image (TE = 3.1 ms and TR = 16.5 s) the signal from the biofilm differs in intensity with respect to the surrounding bulk water.

Image:



Small Molecules and Pharmaceutical

P352

Quantitative Nuclear Magnetic Resonance for the determination of Genistein in capsules

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Abstract: Quantitative Nuclear Magnetic Resonance for the determination of Genistein in capsules

Polyphenols are a group of mainly natural, but also synthetic or semisynthetic, organic chemicals characterized by the presence of large multiples of phenol structural units, which have been investigated for their potential protective effects on human health over the last decades. Numerous studies have shown that, when incorporated to the diet, they limit the development of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes, osteoporosis, hypertension and others. Polyphenols are subdivided into 2 large groups: flavonoids and non-flavonoids. Isoflavones are flavonoids in which the B ring is linked to the heterocyclic ring at the C3 instead of the C2 position. The most important isoflavones are genistein (Figure 1) and daidzein, which can occur in foods both in free and esterified forms.

FIGURE1. Chemical structure of Genistein.

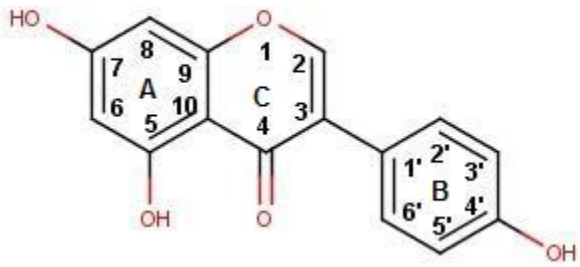
Dietary supplements containing genistein have been studied for their potential health effects in areas including prevention of breast, colon and prostate cancers, cardiovascular disease and post-menopausal ailments. Genistein acts as a chemotherapeutic agent against different types of cancer, mainly by altering apoptosis, the cell cycle, angiogenesis and inhibiting metastasis. In addition, many in vitro and in vivo studies support that genistein can be considered a promising chemo preventive agent for the treatment of different types of cancer.

Nuclear Magnetic Resonance spectroscopy provides very detailed structural information and therefore it possesses high specificity. It is a well-known analytical technique for structure elucidation of small and macro molecules. Specifically, ^{1}H NMR spectrum with chemical shift and coupling constants provides information about the quantitative relationship between intramolecular and inter-molecular resonances.

Quantitative NMR can be specific and selective when the analyzed signal does not overlap with other components in the sample solution including solvent and excipients in the formulation. Validation processes have proved that qNMR is a good analytical technique for quantitative estimation. Unlike other techniques, it has certain acquisition and processing parameters and referencing techniques that need careful consideration in order to achieve a high degree of accuracy and precision. Also, the analyst should be well acquainted with acquisition and processing parameters, referencing techniques and other analytical steps for careful optimization prior to qNMR analysis.

The aim of this study was to analyze genistein composition of commercial capsules and our in-house formulations of dietary supplements using qNMR. The method was validated according to ICH guidelines and this led us to incorporate this qNMR method to our routine analysis of genistein capsules.

Image:



Small Molecules and Pharmaceutical

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Understanding mass transport in pharmaceutical products prepared by hot-melt extrusion

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Abstract: Numerous drugs, such as Ibuprofen or Lorazepam, have low aqueous solubility (< 1 mg/mL) and thus low bioavailability (weight fraction of drug that enters the bloodstream). One formulation strategy to try to overcome this issue is to molecularly disperse the active pharmaceutical ingredient (API) into a water-soluble polymer to form an amorphous solid dispersion (ASD). ASDs lack the long-range ordering of molecules present in crystalline solids, and thus do not suffer from having to overcome the high lattice energies or poor solvation effects that are commonly found with poorly soluble crystalline APIs. There are two common methods to prepare ASDs: hot-melt extrusion (HME) and solvent evaporation. In this work, we will present data on ASDs prepared by HME.

Due to the harsh environment within an extruder (high temperature and moving mechanical parts), it is difficult to interface it to process analytical technologies and so gaps in our understanding of the HME process exist, particularly concerning the dissolution of API in molten polymers. Computational modelling of the extrusion process involves numerous process variables (e.g. screw speed, extruder architecture, dimension and degree of fill). The accuracy of any model will also depend on the determination of molecular parameters such as the API's self-diffusion coefficient. Another key challenge is ensuring that the API remains amorphous throughout the shelf-life of the product, thus ensuring the medicine performs consistently.

This poster presents the use of NMR pulsed-field gradient and MR imaging techniques to characterize the mass transport of an API (paracetamol) in a pharmaceutically relevant polymer (Kollidon®), at temperatures relevant to the extrusion process: (i) ^{1}H diffusometry (bipolar pulsed-gradient stimulated echo) data for self-diffusion coefficient measurement; and (ii) ^{1}H-density image profiles to assess transport diffusion mechanisms. Furthermore, solid-state NMR is used to probe the amorphous state of the API and the microscopic structure of the extruded product (phase separation).

Our results show that there is a significant depression in the melting point of paracetamol (from 170°C to <120°C) when it is mixed with Kollidon®, and that this depression is further enhanced in extrudate products. This indicates that paracetamol has a plasticization effect on Kollidon®. We also show that the self-diffusion coefficient of paracetamol is almost two orders of magnitude slower in Kollidon® mixtures than on its own at the extrusion temperature (170°C), which has important implications for the modelling of such systems. Preliminary studies of macroscopic mass transport allow us to quantify both advection and diffusion of the dissolved paracetamol. Solid-state NMR studies on paracetamol/Kollidon® mixtures and extrudates show evidence for the formation of an intimately mixed ASD in the HME product with domain sizes smaller than 3.5 nm.

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Suppression of 13C satellites in 1H DOSY spectra

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Abstract:

Diffusion-ordered spectroscopy (DOSY)[1] is a powerful tool for the analysis of intact mixtures. In a DOSY spectrum, signals are dispersed according to their chemical shift and scalar coupling in the spectral dimension, and according to their apparent diffusion coefficient in the diffusion dimension. Therefore, signals belonging to the same component can be distinguished based on their diffusion coefficients. However, the extraction of a diffusion coefficient for every individual signal in an NMR spectrum is not always easy. DOSY is very sensitive to spectral imperfections, and especially to signal overlap, which can lead to misleading apparent diffusion coefficients and uninterpretable DOSY spectra. Methods for avoiding or minimising signal overlap include the combination of DOSY with pure shift NMR[2], the detection of sparse nuclei[3], and the dispersion of signals in additional dimensions[4].

Here, we introduce a new NMR experiment that tackles a source of signal overlap that is particularly troublesome in mixtures that contain components with a wide range of concentrations, and hence have a wide range of signal intensities. In such high dynamic range mixtures, ^{13}C isotopomer signals from major mixture components can overlap with signals from minor components, distorting the diffusion domain of a DOSY spectrum. This is a particular problem in ^{1}H NMR, because the narrow chemical shift range and multiple ^{1}H-^{13}C splittings increase the probability of overlap.

The new method, Oneshot-*i*DISPEL, suppresses one-bond ^{13}C satellite signals in ^{1}H DOSY spectra and is based on the combination of the Oneshot[5] and DISPEL[6] experiments. The parent Oneshot experiment uses a stimulated echo and unbalanced pairs of bipolar gradient pulses for diffusion encoding; in DISPEL, a multiple-stage low-pass *J* filter[7] is combined with a perfect echo[8] and zero-quantum suppression[9] pulse sequence blocks, to allow the acquisition of phase sensitive, one-bond ^{13}C satellite-free ^{1}H spectra with no J_{HH} modulation. The combined Oneshot-*i*DISPEL experiment removes interfering ^{13}C satellites without the need for ^{13}C decoupling, avoiding problems such as signal broadening, lineshape distortions, decoupling sidebands and convection.

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Determination of the Biotechnological or Natural Origin of Vanillin by Deuterium 2D-NMR in Lyotropic Liquid Crystals : A new Analytical Tool for Fighting Against Counterfeiting ?

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Abstract:

The evaluation of the natural isotopic composition of (bio)-compounds in food products is an important and continuous challenge for the authentication/determination of their origins. From a NMR viewpoint, the ^{2}H SNIF-NMR® protocol has been developed around the quantitative ^{2}H-{^{1}H} NMR at natural abundance level in isotropic organic solvents to understand the metabolic/synthetic pathways or for authentication purposes **[1]**.

Among molecules of economical interests, an analytical challenge concerns vanillin, one of the most well-known flavouring agents used in the aroma industry. Indeed, so far, the position-specific (^{2}H/^{1}H) isotopic fractionation of vanillin measured in liquids revealed to be incomplete due to a fortuitous isochrony of two aromatic ^{2}H sites. This in turn leads to the loss of a key ^{2}H/^{1}H isotope data associated to the aromatic ring **[2]**. Despite the recent development of (^{13}C/^{12}C) isotope ratio measurements as alternative, this approach does not solve the problem **[3]**.

In this work, we exploit the analytical potential of ^{2}H 2D-NMR at natural abundance level in lyotropic liquid crystals (LLC) containing a polypeptide polymer (Poly-Benzyl-L-Glutamate) to extract the desired (^{2}H/^{1}H) isotopic information. Compared to isotropic SNIF-NMR® protocol, the anisotropic natural abundance deuterium NMR (ANAD NMR) gives access to all order-sensitive NMR interactions such as the residual quadrupolar coupling ^{2}H-RQC, $\Delta\nu_Q(^{2}H)$, specific to spin I > 1/2 **[4-5]**. In case of vanillin, the spectral discrimination of all aromatic monodeuterated isotopomers becomes possible on the basis of the anisotropic ^{2}H chemical shifts, $\delta^{aniso}(^{2}H)$ and the ^{2}H-RQCs. The use of tilted *Q*-resolved-type 2D-NMR experiments (*QUadrupole cOrrelation SpectroscopY*) separating $\delta^{aniso}(^{2}H)$ and $\Delta\nu_Q(^{2}H)$ on F_2 and F_1 dimensions, respectively,**[6]** leads for the first time to the complete experimental determination of the relative proportions (^{2}H/^{1}H) of the vanillin aromatic core.

After examining the relevant experimental conditions for optimal, reliable quantitative measurements in ANAD 2D-NMR and discussing the most important factors impacting the quality of spectra, six samples of vanillin of different biological or chemical origins have been investigated. From the analysis of their isotopic profile, we demonstrate how the determination of (^{2}H/^{1}H) fractionation on all aromatic positions provides a new tool for distinguishing between the biological or synthetic origin of the aromatic core **[7]**.

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Structural model of Mcl-1/Pyridoclax complex revealed by combining NMR, Docking Approaches and Molecular Dynamics.

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Abstract: Apoptosis, or programmed cell death, plays a protective role against tumour formation and can be regulated by either the extrinsic or the intrinsic pathway. The latter one is regulated by Bcl-2 family proteins. [1] Among them, MCL-1 and Bcl-x_L which are anti-apoptotic proteins implied in ovarian carcinoma proliferation and chemoresitance. Both proteins cooperate to protect cancer cells against apoptosis and their concomitant inhibition leads to apoptosis even in absence of chemotherapy.[2]

An emerging option is the targeting of the anti-apoptotic members MCL-1 and Bcl-x_L of Bcl-2 family. Indeed, this strategy was validated by the discovery of ABT-737 [3] whereas MCL-1 inhibition remains problematic.

In this context, Voisin-Chiret *et al*. designed and synthesized foldamers [4],[5] that inhibit specifically ML-1. Among them, Pyridoclax was designed to target the MCL-1 hydrophobic binding groove. It was demonstrated that it could interact directly and selectively with MCL-1 by SPR, and hence sensitize ovarian carcinoma cells to Bcl-x_L targeting strategies. It has been shown that Pyridoclax could inhibit MCL-1 and restore apoptosis *via* its intrinsic pathway.[6] Thus, it is of paramount importance to highlight the molecular basis of this interaction process.

Nuclear Magnetic Resonance spectroscopy (NMR) has evolved into a powerful tool for characterizing protein- ligand interactions in solution under near physiological conditions.

The aim of this study is to use a structural approach in order to study the interaction between MCL-1 and Pyridoclax.

First, NMR studies showed that Pyridoclax could mime the BH3 domain of pro-apoptotic proteins.

Then, interaction studies were conducted to map the molecular details of the ligand binding (hotspots, K_D, k_{off}).

Finally, NMR data were combined to computational methods to identify the exact binding modes of Pyridoclax in the large hydrophobic groove of MCL-1.

Overall, our study gave valuable information on MCL-1:Pyridoclax complex.

The obtained data could be useful for the design of more active compounds that modulate the biological function of MCL-1.

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A combined molecular and atomic probe characterization of aromatic hydrocarbons via ESR and PALS: Methylbenzene

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Abstract: The structural - dynamic state of *organic compounds* can be characterized by macroscopic quantities, such as enthalpy via calorimetry, by microscopic ones, such as static and dynamic density fluctuations, magnetic and electric dipole reorientations using structural diffraction, scattering, resonance or relaxation techniques, respectively or by appropriate *external* atomic- or molecular-sized probes. In our contribution, a combined probe study on one of the simplest aromatic *hydrocarbons – methylbenzene* alias *toluene (TOL)* via the rotation dynamics of nitroxide spin probe *TEMPO* by electron spin resonance (ESR) and the annihilation of *ortho-positronium (o-Ps)* by positron annihilation lifetime spectroscopy (PALS) over a wide temperature range from 10K up to 300K is presented.

The extrema line separation of the triplet spectra $2A_{zz'}$ and the correlation time τ_c of *TEMPO* in *TOL* as a function of temperature exhibit several changes which define a set of the characteristic ESR temperatures T_{X1}^{slow} = 115K, T_{X2}^{slow} = 140K and T_{X3}^{slow} ~ 170K, T_{50G} = 178K and T_{X1}^{fast} ~ 180K as well as T_{X2}^{fast} = 225K. Similarly, the *o-Ps* lifetime, τ_3, and the relative *o-Ps* intensity, I_3, as measures of free volume microstructure show up changes at $T_{b1}^{solid,\tau3,I3}$ ~ 100K, $T_{b2}^{solid,I3}$ = 160K, $T_m^{\tau3,I3}$ = 178K and $T_{b1}^{liquid,\tau3}$ = 225K in the slowly cooled sample or alternatively, at $T_{b1}^{solid,I3}$ ~ 135K in the rapidly cooled one.

The physical origin of these characteristic ESR and PALS temperatures can be interpreted by their comparison with the thermodynamic and dynamic behaviours of *TOL* as obtained by DSC or combined dynamic data from dielectric spectroscopy (DS) and nuclear magnetic resonance (NMR) as well as viscosity (VISC) from the literature, respectively. It is found that T_{X1}^{slow} is connected with the glass to liquid state transition T_g^{DSC} and T_{X2}^{slow} as well as $T_{b1}^{solid,I3}$ in the rapidly cooled sample with the onset of the *cold* crystallization at $T_{cc,i}^{DSC}$. On the other hand, $T_{b1}^{solid,I3}$ in the slowly cooled one can be related to the dynamic crossover in the local supercooled liquid region of *TOL* K from the power law fitting of the dynamics of *TOL* from the idealized and extended mode coupling theory (MCT) at $T_X = T_c^{MCT}$ = 153K and to a change in the dominance between the *solid*- and the *liquid*-like domains at the T_m^c = 156K from the two-order parameter (TOP) model of liquid state. Next, T_{X3}^{slow}, $T_m^{PALS} = T_{50G}$ and very close T_{X1}^{fast} are related to the solid to liquid phase (*melting*) transition at T_m^{DSC}. Finally, T_{X2}^{fast} ~ $T_{b1}^{liquid,\tau3}$ seems to reflect another change in the dynamics of *TOL* stemming from the super-Arrhenius to Arrhenius regime crossover at T_A^{TOP}.

Thus, both probe techniques are able to reflect consistently numerous changes in thermodynamic and dynamic properties at the corresponding ESR and PALS temperatures characterizing one of the simplest and most fragile *aromatic organic substance*.

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Design of Water-Tolerant Frustrated Lewis Pairs: Structure and Dynamics of Borane Complexes

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Abstract: Monitoring the stability of borane-water complexes, an NMR-based development of water tolerant boron/nitrogen-centered frustrated Lewis pairs (FLP) was done, resulting in I-III (see Figure). ^{1}H and ^{10}B measurements revealed ternary aqua complexes of the borane in the presence of DABCO (see Figure). Whereas adduct I remained intact in a wide temperature range, the other two complexes considerably (~50%) dissociated to free II and III. This advance allowed the reductive amination of carbonyls when using II or III as catalysts, for the reason that free borane and DABCO were available for hydrogen cleavage. Reactions were faster in the presence of III than II. 2D EXSY spectra were acquired to determine rate constants and thermodynamic activation parameters of the dissociation. While the stability of II and III adducts is similar, there is a significant difference in kinetics as the exchange rate is nearly 20 times higher for the aqua complex of III, which can be correlated with its increased steric profile [1].

II was further investigated as the probable pathway(s) of the interactions with a LB remained undiscussed. During the approach towards the initially flat (sp2) borane the base may encounter multifarious chemical environments in case of asymmetrically halogenated aryl groups. As each possible reaction route exposes the reactants to distinct steric effects, a single borane is able to display multifarious LA strengths against the same LB in the course of their interaction. In the current work ab initio computations hand in hand with NMR spectroscopy were used to model and prove the concept of multiple Lewis acidity. Hence, instead of probing different reactants this scrutiny explores the reaction pathway dependence of Lewis acidity, thus gives novel insights into the governing role of steric properties in the interactions of tri(2,6-F/Cl-aryl)boranes. Calculated structure of several stable conformers of the II-piperidine adduct were assigned using ^{19}F, ^{10}B and ^{1}H-^{19}F HOESY spectra. The distribution of the conformers quantifies LA strengths. ^{19}F EXSY spectra revealed exchange between the conformers, indicating oscillating LA strength. Based on DFT calculations the interconversion between these conformers can only happen via elongation of B-N dative bonds and/or dissociation and re-association of the adducts. As this system is catalytically active, the phenomena of chemical exchange between these conformers is an indirect detection of FLPs.

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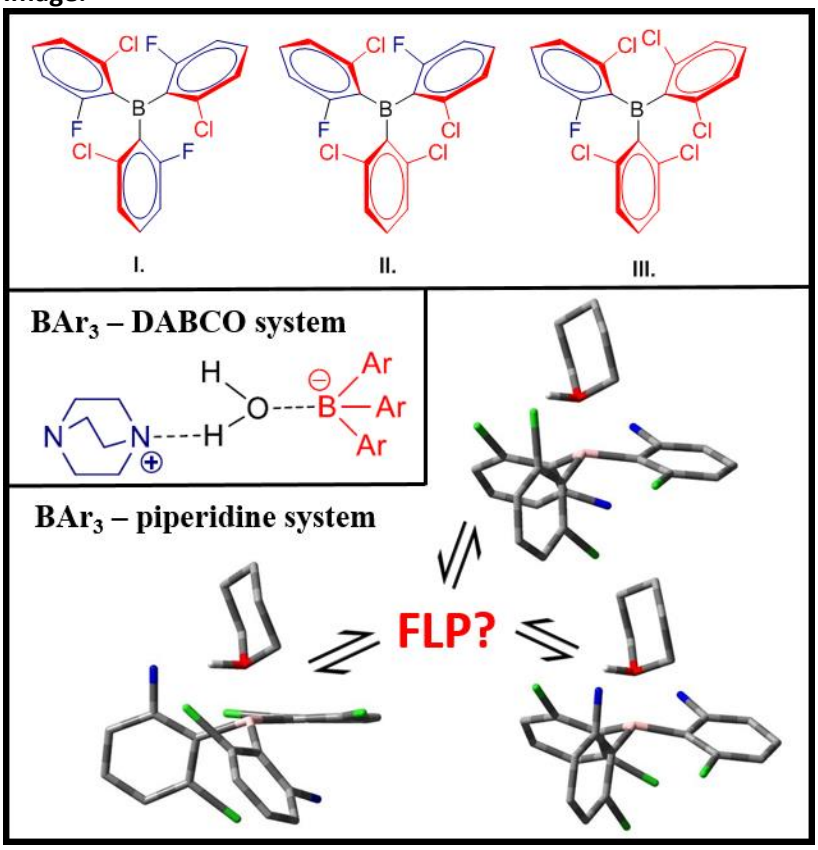
Image:



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New analysis tool for 3D diffusion NMR experiments

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Abstract: In diffusion-ordered spectroscopy (DOSY),[1] one of the most commonly used NMR experiments in the analysis of mixtures, the amplitude (or integral) of each signal from an array of 1D spectra is fitted to an appropriate variant of the Stejskal-Tanner equation,[2] and displayed as a Gaussian peak in a second dimension which represents diffusion coefficient. DOSY is commonly used to identify signals from different components in a mixture. However, signal overlap can obscure diffusion information and complicate spectral interpretation. An elegant solution to minimise signal overlap in the spectral dimension is to add a diffusion dimension to a 2D (or higher dimensionality) NMR experiment, generating a 3D (or higher) DOSY spectrum.[3] This provides a major reduction in the probability of overlap, since cross-peaks are spread out over a plane rather than along a single axis. Here we introduce a new Matlab® based toolbox, for analysing 3D diffusion NMR data, which complements the recently introduced GNAT ("General NMR Analysis Toolbox",[4] the former DOSYToolbox[5]). Within this new environment it is possible for the first time to analyse 3D diffusion data (*e.g.* 3D DOSY-HSQC, as shown in the examples below) using both univariate (**Figure 1**) and multivariate methods in a user-friendly graphical interface (GUI).

Figure 1. *3D DOSY spectrum from an Oneshot-HSQC experiment on a mixture containing the flavonoids quercetin and rutin. Peaks in the spectral dimensions F_1 and F_2 (here ^{13}C and ^{1}H respectively) are generated by conventional 2D Fourier processing, and in the diffusion domain by constructing Gaussian lineshapes with positions and widths determined by fitting the experimental signal decay to the Stejskal-Tanner equation. The estimated diffusion coefficients are 1.63×10^{-10} m^2 s^{-1} and 1.25×10^{-10} m^2 s^{-1} for quercetin and rutin, respectively.*

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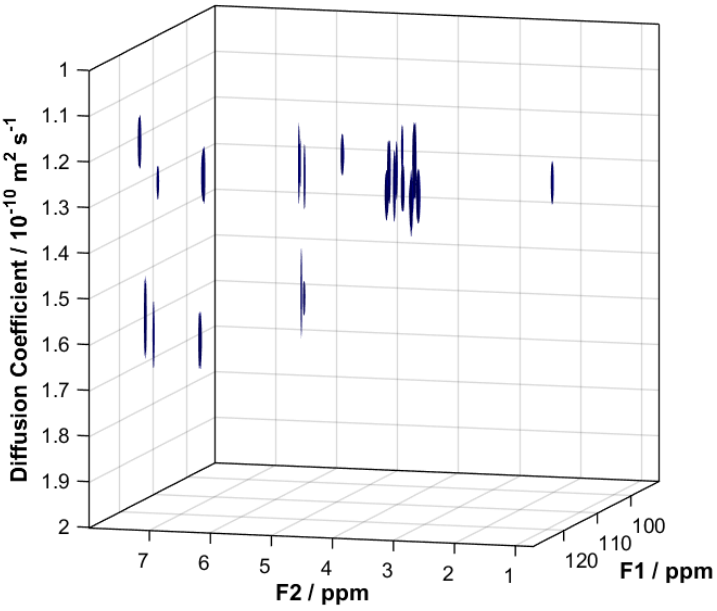
Image:


Diffusion Coefficient / 10-10 m2 s-1
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1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2
7
6
5
4
3
2
1
F2 / ppm
120
110
100
F1 / ppm

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Investigating chemical probes for protein studies using 19F NMR: A comparison of chemical shift sensitivity in tautomers

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Abstract: ^{19}F NMR is regularly used in the study of protein topology and conformational dynamics. Conjugation of this reporter to thiols allows for the study of local environmental changes in the protein and under certain conditions, identification of unique protein conformational states.

Here, we report on studies for two types of fluorinated tautomers; pyridones and beta di-ketones as potential thiol specific tags for the study of protein conformations and dynamics.

The ^{19}F compounds were tested *in vitro* for sensitivity to changes in solvent polarity, pH and temperature. A polarity series was prepared for both types of tautomers using mixtures of methanol:water ranging from relatively non-polar (19:1) to polar (0:1). The ^{19}F NMR spectra were compared to the currently used thiol specific tag, 2-bromo-N-(4(trifluoromethyl)phenyl)acetamide (BTFMA), and the commonly used tag, 3-bromo-1,1,1-trifluoro-propan-2-one (BTFA). 5-fluoro-2-hydroxypyridine (5-MFHP) demonstrated the greatest range of chemical shift as a function of solvent polarity, at 2.56 ppm. This is ideal for smaller peptides which can provide structure and conformational elucidations without significant line-broadening that is typically associated with larger proteins. 6-trifluoromethyl-2-hydroxypyridine (6-TFMHP) displayed the greatest chemical shift range of 1.61 ppm among the trifluoromethyl tags which are far more sensitive than BTFMA, which was 1.03 ppm under the same conditions and is preferable for larger and more complex macromolecules due to the higher signal intensity obtained from the CF_3 group compared to CF on 5-MFHP. The beta di-ketones, while providing more information via multiple peaks, also yields more complex spectra that would make deconvoluting the protein peaks more difficult.

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Towards NMR characterization of apelin encapsulated in liposomes

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Abstract: Cardiovascular diseases are the first cause of death in the world with 32 % for women and 27 % for men [1]. Heart failure (HF) mainly concerns people older than 50 years with a risk increasing with age. That is why it is relevant to work on new therapies against HF.

Apelin is the endogenous ligand of the G Protein Coupled Receptor (GPCR) named APJ, which plays a role in the physiological control of the cardiovascular system by activating several pathways leading to the activation of endothelial nitric oxide synthase and ions exchangers [2]. It has been shown that apelin is an important regulator of cardiovascular homeostasis by having positive inotropic effect, and causing vasodilatation of the veins and modification of the heart rate leading to a decrease of the blood pressure. Hence apelin is a potential drug for the treatment of HF [3].

The main drawback of apelin is its short half time *in vivo*. A solution could be to encapsulate this peptide in liposomes in order to control its release and therefore to decrease the number of administrations needed by the patient. Liposomes are useful Drug Delivery Systems than can be used as cargos for both lipophilic peptides, which will interact with the lipid bilayers, and hydrophilic peptides, which can be entrapped in the aqueous core.

Here we show that Nuclear Magnetic Resonance (NMR) can be used as a tool to characterize peptide-liposome complexes and to investigate the peptide-liposome interactions. First results emphasize the possibility to discriminate between the inside and the outside of the liposomes by NMR methods.

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Dynamics of choline -based ionic liquids in bulk and in carbon fibers as studied by 1H and 19F NMR

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Abstract: In recent years great interest has been attracted by room temperature ionic liquids (RTILs) due to their potential as new media for organic, catalytic and electrochemical applications[1]. Given their interesting properties, such as very low vapor pressure, non-flammability, high thermal stability and wide electrochemical window, ILs are proposed as electrolytes for batteries[2,3]. Nonetheless, traditional room temperature ionic liquids, which incorporate for example imidazolium or pyridinium cations, suffer from high toxicity, poor biodegradability and high cost.

In this study we investigate two highly biodegradable, water soluble and inexpensive choline-based ([Ch]) ionic liquids with acetate ([OAc]) and trifluoroacetate ([TFA]) as anions[4,5], by ^{1}H and ^{19}F NMR. The ILs were measured in bulk, as well as confined to carbon fibers as a "carbon–electrolyte" model system for Zn-air batteries[6]. The role of water in the dynamics of the molecules was investigated at different temperatures by Nuclear Overhauser Effect Spectroscopy (NOESY) NMR in order to probe both NOE and exchange, with the latter being found to play an important role for [Ch][TFA] confined to the carbon fibers. Additionally, the temperature dependence of the chemical shift in these ILs was found to have a non-trivial behavior, which is mainly related to cation–anion and cation/anion–water interactions[7]. Therefore, the self-diffusion coefficients have also been measured and found to correlate qualitatively with the degree of ionic pairing. Furthermore, T_1, T_2 and diffusion measurements have been performed for different loadings of [Ch][TFA] on the fibers. T_1 and diffusion coefficients were found to increase with increasing concentration, whereas T_2 was found to reflect the effects of local magnetic field gradients induced by susceptibility differences at the interface between the fibers and the IL.

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Small Molecules and Pharmaceutical

P363

A rational approach to molecularly imprinted polymers: experimental and computational evidence

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Abstract:

Molecular imprinting has become one of the most promising techniques to design biomimetic synthetic materials with molecular recognition properties similar to natural receptors in terms of affinity and selectivity[1]. Over the last two decades, molecularly imprinted polymers (MIPs) have proven their application in various fields as separation materials, biomimetic assays, recognition elements in biosensors, solid-phase extraction, etc. Despite their various applications, the vast majority of polymer synthesis protocols found in the literature use trial-and-error and/or chemical intuition to find out the recipe optimizing the affinity between monomer and template in the pre-polymerization mixture and hence the recognition properties of the final polymer. To rationalize MIP formulations, combinatorial chemistry[2] and molecular modelling[3] tools for screening the best functional monomer(s) have been proposed. However, when dealing with expensive templates and/or screening a large set of functional monomers, computational calculations appear the most appropriate.

The goal of this rational design study is to get a better understanding of monomer-template interactions, of the MIP formation and of the recognition mechanisms in MIP-ligand complexes by comparing experimental data with theoretical calculations. Two MIPs will illustrate our rational design approach: they are selective to precursors of malodorous compounds[4] and to dipicolinic acid[5]. Since monomer-template interactions are the driving forces for the selectivity of the final MIP, we characterized these interactions experimentally and *in silico*. Density functional theory (DFT) calculations were performed with the software Spartan (Wavefunction, Inc.) in vacuum and in polar solvents implicitly. The *in-silico* binding energies hence obtained were confronted to experimental data obtained by nuclear magnetic resonance and isothermal titration calorimetry. This strategy combining experimental spectroscopic and calorimetric measurements with computational chemistry calculations is a powerful tool for getting molecular insight about the interaction mechanisms responsible of the monomer-template formations and its relation to selectivity of the final MIP.

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P364

Development and Validation of a Selective NMR method for Cleaning Validation in a GMP Pharmaceutical Production Plant

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Abstract: *Zoladex*® is an injectable luteinising hormone-releasing hormone analogue that is used to treat hormone-sensitive cancers such as prostate and breast. One of the requirements of GMP includes prevention of possible contamination or cross-contamination from previous products used on the plant.

New legislation introduced by the FDA in 2014 for pharmaceutical plant cleaning validation meant that visual inspections were no longer acceptable and data from validated sources was needed. For AstraZeneca's Zoladex plant at Macclesfield there was a requirement to develop and validate analytical methods capable of detecting very low levels (~10ppm) of both drug substance (goserelin) and excipient (lactide-glycolide co-polymer) on a variety of plant components and surfaces that have been through a wash cycle.

An UPLC method capable of detecting goserelin at sub-ppm levels was available but a method for detecting the copolymer (the main component in Zoladex) was proving troublesome to develop due to the lack of chromophore.

Selective ^{1}H-NMR experiments have previously been used by AstraZeneca for trace analysis such as measuring low levels of Potential Genotoxic Impurities (PGI's) in drug products [1].

This presentation describes the challenges of using NMR for trace analysis and how a selective ^{1}H-NMR experiment (the Excitation Sculpting sequence with double pulsed field gradient spin-echo) has been tailored to develop a method to detect sub ppm levels of copolymer. Validation of the method to GMP standards is described as well as how the method was used to validate the plant cleaning process and subsequent routine use to support the plant cleaning process.

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Determination of relative configuration of small molecules using proton residual chemical shift anisotropy at microgram levels

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Abstract: Determination of 3D molecular structure remains a challenging task for natural products that are available in very minute amounts. While the constitution can be derived from proton/proton and proton/carbon correlations, *J* couplings and NOEs are used to determine the relative configuration oftentimes supported by RDCs between protons and carbons over one bond or carbon RCSAs. For compounds in the range of a few 10 µg, however, these RDC or carbon RCSAs are difficult to collect because of low sensitivity. Therefore, we introduce here the highly sensitive NMR observable ^{1}H RCSAs which, similar to RDCs or ^{13}C RCSAs, provides spatial orientation of different structural moieties within a molecule. We introduce two independent tools to robustly measure ^{1}H RCSAs using stretchable PMMA and poly-HEMA gels on several rigid and flexible molecules. ^{1}H RCSAs are also measured from Polyacetylene Liquid Crystal and their utilization in determining the 3D molecular structures of several molecules with varying complexities. It is noteworthy that polymer signals dominates and very often masks other signals when the sample concentration is around 12 micrograms. This problem is circumvented by the use of deuterated gel and it has will be been demonstrated in strychnine molecule for observation of ^{1}H RCSAs of minute quantities of the analyte. We have tested the methodology in estrone as a rigid molecule and in retrorsine, for which the RDC analysis was not possible earlier for configuration analysis. The determination of correct configuration for estrone using ^{1}H RCSA has been presented in Figure 1. The SVD-fitted ^{1}H RCSA data analysis provided lowest *Q* and Q_{CSA} factors of 0.276 and 0.319 while for 13-*epi*-estrone, they are 0.396 and 0.419, respectively; while the ^{1}H RCSAs of retrorsine, provided Q(QCSA) factors of 0.326 (0.408) and 0.406 (0.438) for the RRRS and RRRR configurations. It may be noted here that these two configurations are also the closest ones when ^{13}C RCSA analysis was performed with Q factors 0.184 and 0.216, respectively. Note that the Q factor difference and ratio is larger, i.e. discrimination between the configurations is more obvious using ^{1}H RCSA.

In summation, we reported that the ^{1}H RCSAs is very powerful parameters as they complement J-couplings and NOEs, without the necessity to use another anisotropic parameter in microgram scale.

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Image:

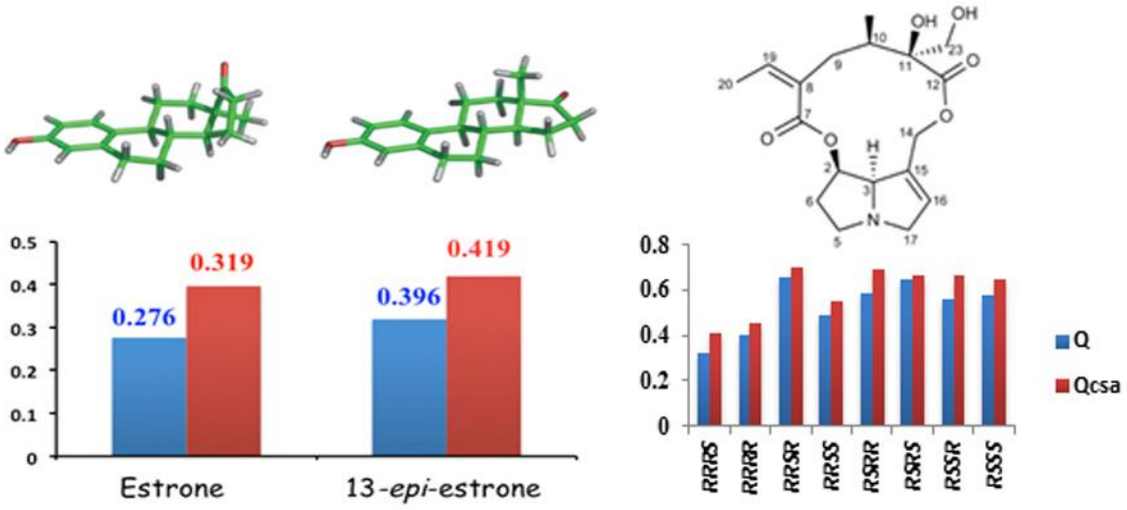

Figure 1. Discrimination of estrone and retrorsine configurations using ^{1}H RCSAs

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NMR protocol of R&D of innovative pharmaceutics for targeted oncological chemotherapy

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Abstract: Targeted chemotherapy is on a frontier of research in pharma, it is a key element of personalized medicine. It requires that a drug **binds strongly** and **site specific** to its biological target. In case of camptothecin family derivatives acting as the Top I inhibitors this site is defined as a nick in one strand of a duplex DNA in DNA/TopI complex.[1]
Using basic NMR experiments such as 1H NMR, NOESY and DOSY we have established two key elements required of compound to fulfill its biological role; **selectivity** and **strong binding** in a nick.
Thus, using NOESY it was proved that camptothecin core **is intercalating** in a nick of model nicked DNA decamer [2] in solution in the same geometry [3] as found in a crystal structure of topotecan (TPT)/nicked twentymer DNA complex. Using basic chemical know-how we have chosen the camptothecin derivative SN38 and have introduced the substituent which was supposed and proved the covalent binding [4] to nucleophiles in a nitrogen base in a nick of model DNA decamer. The formation of a bioconjugate was confirmed by 1H NMR, NOESY and DOSY. The selectivity of biding the nitrogen sites in four model nucleosides, dG,dC, dA, dT , establishing two faces of a nick, was also performed. The novel derivatives prove their innovating nature because the covalent binding inside the nick was only evidenced in this project. They were patented in US: US 9,682,992B2 and in EU, EP2 912 039 B1, validated in PL,D,F, CH, GB. These compounds proved *in vitro* their several orders of magnitude higher activity for breast, colon, blood and lung cancer cell than presently used in clinic camptothecin derivatives. Even more importantly, they are much more tolerant to normal cells, a key virtue for patients in chemotherapy.[5]
Furthermore, applying simple NMR protocol of monitoring the kinetics of solvolysis in water of novel compounds we have established their half life time, $t_{1/2}$, which is linked to a basic pharmacodynamic parameter defining the time required of a drug to reach the target in its original form. All the above NMR data give the basic set of information required for a further development of compounds in a preclinical and clinical stages.

The research was conducted in a frame of National Research Center grant no. 2017/27/B/ST4/00190

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Stereochemical elucidation of a marine diterpenoid by residual dipolar coupling based NMR analysis

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Abstract:

Residual dipolar coupling (RDC) can be measured by aligning the molecule in a weakly orienting medium. It contains rich structural information such as orientation of two nuclei in respect to the magnetic field. Since different classes of alignment media that are compatible with organic solvents were developed, great progresses have been made in the application of RDCs in the structural elucidation of organic molecules.[1,2] By using the RDC-based NMR analysis, constitution, relative and absolute configuration of several challenging natural products, such as fibrosterol sulfate A,[3] homodimericin A[4] and 4,6-diacetylhygrophorone A(12)[5] were successfully determined.

Based on the high temperature, high pressure and scarcity oxygen environment in marine, marine natural materials contain continuously prolific secondary metabolites with diverse structures and biological properties. Until now, there are 9 FDA approved drugs that directly came from, or were modified by synthesis, or even total synthesis were based on marine-sourced compounds. Furthermore, around 20 drugs that are derived from marine natural products are currently in clinical trials. Therefore, marine natural products research occupies an important position in medical research.

Diterpenoid is an important class of marine natural products. Diterpenoid of cembrane, capnosane, lobane, and perhydrophenanthrene is a class of characteristic secondary metabolites from soft corals of the genus *Sarcophyton* (order *Alcyonacea*, family *Alcyoniidae*).[6] Many novel skeletons were derived from these types of diterpenes by dimerization, cyclization or ring rearrangement, *e.g.* bissubvilides,[7] tortuosenes[8] and methyl sarcotroates,[9] with a wide range of biological activities, including cytotoxic, antibacterial, antifouling, anti-inflammatory, protein tyrosine phosphatase 1B (PTP1B) inhibitory, and immune enhancement effects.[6-9]

In the current study, we are focusing on a novel natural product named as diterpenoid sarcomililate A. It is a diterpenoid with tricyclo hexadecane core and has been isolated from the Chinese soft coral *Sarcophyton mililatenum.* Structural elucidation of sarcomililate A was challenging by conventional methods, because the molecule cannot be crystallized and is highly flexible. By means of RDC, NOE and *J*-coupling analysis, we determined the relative configuration of this novel natural project. By comparison of experimental and DFT calculated chiroptical data, the absolute configuration was established.

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A complementary MS/NMR method of component analysis and structure elucidations of new generation of synthetic narcotics – designer drugs

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Abstract:

Designer drugs are becoming dominant source of psychoactive compounds used by addicted people all over the world. It is becoming most important social problem as the price of a small amount of it is order of times more cheaper with comparison to classical drugs. On the other hand they are more dangerous because clinical effects are not thoroughly recognized and can cause unexpected death.

This situation clearly indicates and alarms that permanent recognition of drug market should be implemented in practice. It is estimated that in EU ca. 70 mln people are taking marijuana, another 12 mln take regularly or accidentally cocaine, 11 mln stay with amphetamine, but designer drugs and herbal highs are even more popular. Therefore this problem is of priority concern to European Commission.

It is therefore a crucial problem to have a means of fast recognition of a structure of compounds appearing on a market in order to put them on a list of forbidden compounds of production and distribution. It is basic property of a narcotic market that it mutates like bacteria in presence of new antibiotic, i.e. once new law regulations are imposed, the producers change the chemistry of psychotropic active compounds. A new generation of synthetic drugs, 'designer drugs', are single compounds or mixtures of synthetic compounds, characterized by psychoactive activity. Otherwise, these mixtures can contain excipients, known stimulants and plant matrix.

Here we present the method developed for recognition the components in market products and their structures. In a component analysis we use orthogonal methods LC-MS, GC-MS for total recognition of molecular masses of components in a studied sample. In NMR we apply integrated block of five modern experiments, in ^{1}H and ^{13}C resonance, in one and two dimensions; 1H, 13C, NOESY, 1H/13C HSQC and HMBC which allow, from one sample, achieve the desired goal.

We will present a research on several classes of designer drugs from cathinones,[1] and cannabinoids,[2] which were first recognized on EU marked and were reported to EMCDDA (European Centre of Monitoring Drugs and Drug Addictions).

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P369

F-19 Cross-Effect Dynamic Nuclear Polarization for MAS NMR

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Abstract: As early as the 80's, nitroxide radicals have been used for ^{19}F Overhauser dynamic nuclear polarization (DNP) to enhance solution NMR spectroscopy to study fluorinated benzenes, or small fluorinated molecules.[1] Today, with the combination of available stable biradicals and the advancement of high field DNP, it should be possible to obtain enhancements up to a factor 700 for ^{19}F DNP. Here, we report a protocol to perform solid-state ^{19}F cross-effect DNP at 9.4 T. We have identified suitable DNP matrices for ^{19}F DNP experiments, and demonstrate that using 8 mM AMUPOL in deuterated trifluoroethanol (TFE-d_3) provides signficant ^{19}F DNP enhancements (with microcrystalline KBr).[2] We believe that ^{19}F DNP will be relevant for the characterization of many modern materials containing fluorinated compounds, including pharmaceuticals. To demonstrate this potential, we show how ^{19}F and ^{19}F-^{13}C cross polarization DNP enhanced NMR spectra can be obtained for an impregnated microcrystalline sample of the pharmaceutically active agent, 5-fluorouracil, (see Figure below), and obtain enhancements in the bulk solid of about 25, which correspond to a factor 625 acceleration in signal acquisition.

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Figure 1. ^{19}F Hahn echo DNP enhanced NMR spectra of 5-fluouracil (molecule shown inset) acquired at 9.4 T impregnated with 8 mM AMUPol in TFE-d_3 with (black trace) and without (dashed trace) microwave irradiation acquired at 109 K and 12.5 kHz spinning speed. Enhancements are shown for the four crystallographically distinct fluorine sites (highlighted in purple), other resonances are the spinning sidebands.

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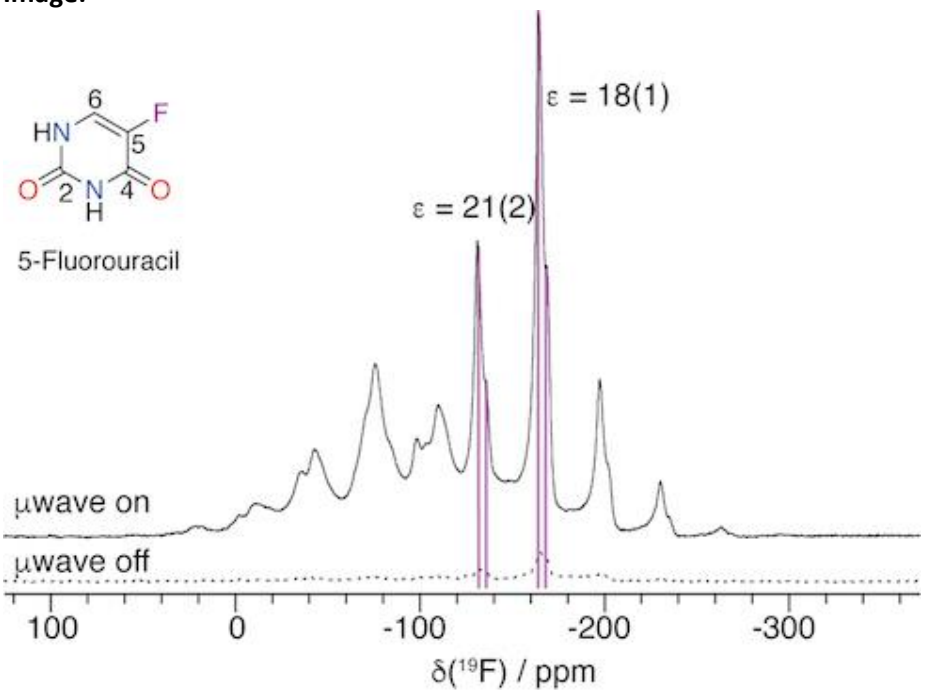

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A new approach for determination of flavonoid content in ginkgo biloba extract by 1H NMR spectroscopy

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Abstract: According to different Pharmacopoeia the identification and quantification of flavonoids and their glycosides are held by HPLC and spectrophotometry methods, which need rare and expensive standard samples unavailable for many flavonol glycosides.

The task of this work is to develop NMR ^{1}H spectroscopy method for identification and quantitative determination of flavonoids in drugs and dietary supplements based on ginkgo biloba leaf extract (*Ginkgo biloba L*.) that does not require standard samples.

Samples of drugs, dietary supplements and extract from the leaves of ginkgo biloba purchased in the Russian pharmaceutical market were studied. The measurements were performed on a JNM ECA 600 NMR spectrometer (JEOL, Japan). DMSO-d6 was chosen as the solvent, its residual proton signal was used as a comparison signal in quantitative determination.

Our new approach for determination of flavonol glycosides and their aglycones in herbal extracts by ^{1}H NMR spectroscopy is based on deshielded proton signals of hydroxyl group at position 5 (5-OH) of flavonoids, which form a strong intermolecular hydrogen bond with oxygen of neighboring carbonyl group. For different groups of flavonol glycosides these protons appear in a certain range from 11.8 to 13.5 ppm [1]. All flavonol glycosides of ginkgo biloba extracts contain 5-OH, and their main aglycons are quercetin (R=OH), kaempferol (R=H) and isoramnetin (R=OCH_3) [2]. Signals indicated by an asterisk are not typical for the extract of ginkgo biloba and belong to exogenous flavonoids.

The validation of the method was carried out according to the following characteristics: accuracy, repeatability, linearity, selectivity, quantitation limit. The accuracy of the obtained results is estimated by comparison with the results of pharmacopoeia methods - spectrophotometry (State Pharmacopoeia of Russia) and HPLC-UV (USP, EurPharm). The linearity of the method is 0.9992. The limit of quantification - 0.1 mg / ml.

Advantages of the proposed procedure are simple sample preparation, no need for reference samples, rapidity, detection of exogenous flavonoids. The developed procedure for determination of flavonol glycosides in preparations based on ginkgo biloba leaves extract satisfies the criteria of the pharmaceutical analysis methods and can be used for quality control of drugs and dietary supplements containing ginkgo biloba leaves extract. The developed approach is successfully tested on other extracts of plant origin, for example, extracts of milk thistle (*Sylibum marianum (L.) gaertn*), clover meadow (*Trifolium praténse*), leaves and flowers of hawthorn (*Crataegus laevigata*).

The publication has been prepared with the support of the “RUDN University Program 5-100”.

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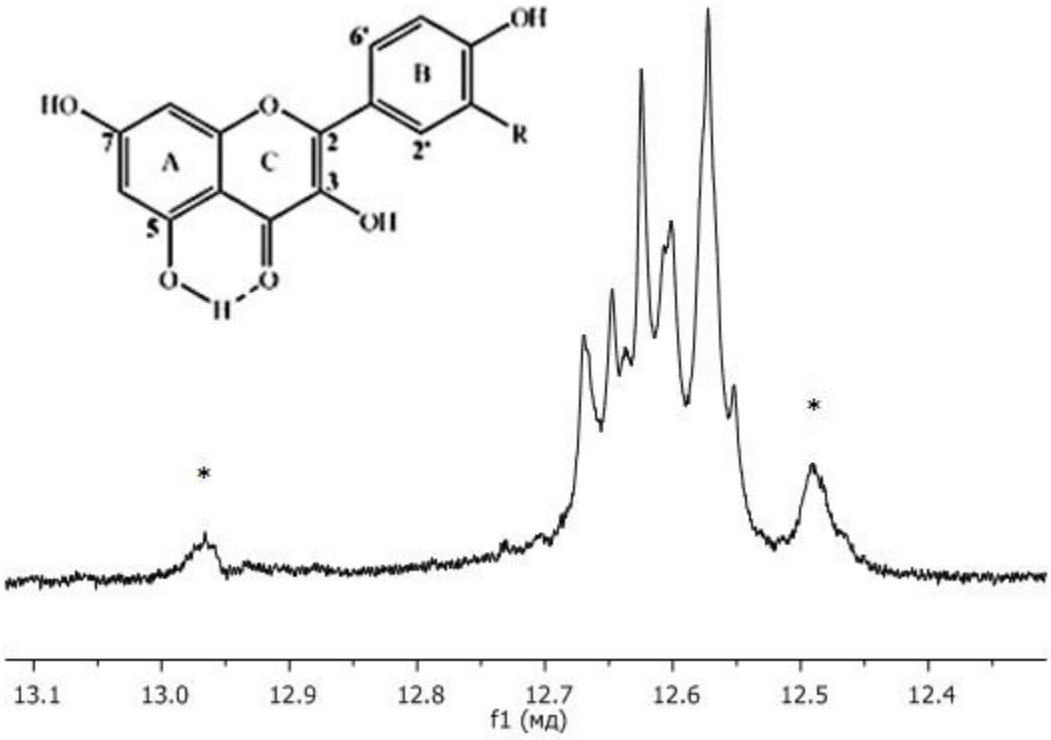
Image:



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New family of azabeta3 pseudopeptides active on multi-resistant bacteria.

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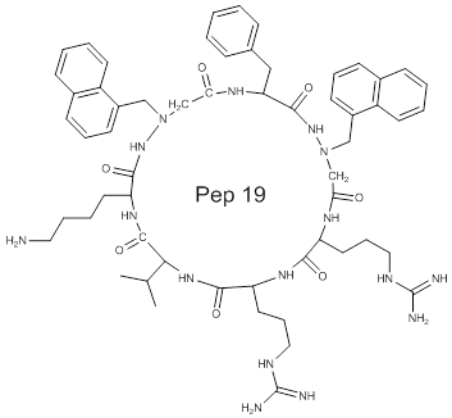
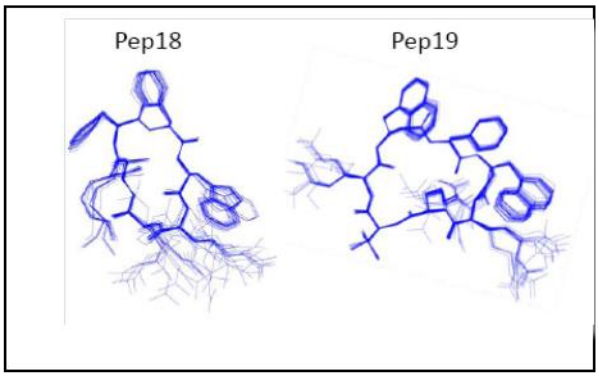
Abstract: Several cycloheptapeptides, have been synthesized and tested against multidrug resistant human bacterial pathogens (MDR). Antimicrobial activities was demonstrated on methicillin resistant Staphylocccus Aureus in two mouse infection models. The pseudopeptides incorporating azab$_3$aminoacids displayed very efficient activity with a low toxicity. Good stability as well as the absence of induced resistance even after prolonged treatment make this new family of pseudopeptides good candidate for active antimicrobial peptides.

The solution structure using NMR distance restraints have been determined for two compounds. SDS micelles was used as a membrane model, the peptides did not display stable 3D structure in water. The structures was solved using the AMBER software and the 20 structures of lowest energy.

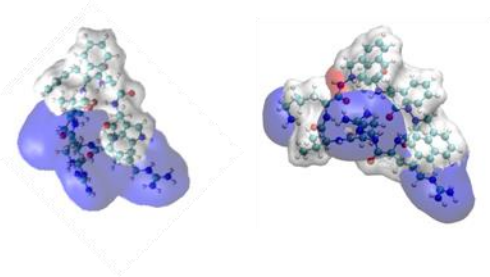
Despite having only two distinct residues, significant differences between their electrostatic potential surface maps were identified. Inactive Pep18 is amphipathic with assembled cationic charges, whereas active Pep19 has three independent cationic areas forming a tripod. Compared to inactive Pep18, Pep19 hydrophobic domain is extended, with three aromatic cycles forming a p-stacking. These differences are relevant for the association of the pseudopeptides with the membrane through electrostatic and hydrophobic interactions.

Image:





Peptide Sequence
Pep15 c(Ψ2-Nal-F- Ψ 2-Nal-RR- Ψ Hyt-K)
Pep16 c(Ψ 2-Nal-F- Ψ 2-Nal-RR- Ψ V-K)
Pep18 c(FFWRRVK)
Pep19 c(Ψ 1-Nal-F- Ψ 1-Nal-RRVK)



Small Molecules and Pharmaceutical

P372

Comparative structural analyses of urotensinergic peptides by CD spectroscopy, solution NMR and Molecular Modelling

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Abstract: UII (11 amino acids, aa) and its paralog URP (8 aa) are considered as the most potent vasoactive molecules known so far and are involved in various biological systems, particularly in the cardiovascular system and tumor cell proliferation. Both peptides are endogenous ligands of a GPCR, the UT receptor and exert common but also divergent physiological roles[1-4]. These functional differential effects could be due to differences in their 3D structures, but further experimental data are needed to confirm this point. NMR studies of several UT ligands were carried out in aqueous solutions or in the presence of SDS micelles, and showed discrepancies related to secondary structures[5-8]. Such differences suggest that the structure of UII and URP may depend on the solvent and raise the question of the bioactive structure in close proximity with membranes and GPCRs. In order to confirm this hypothesis, we performed systematic structural analyses of human UII (hUII), URP and several analogs (agonists and antagonists) using CD spectroscopy in aqueous solution (phosphate buffer) and in the presence of micelles (SDS and DPC). CD spectra indicated that the structure of all peptides was medium-dependent and that the micelle nature impacted peptide conformation. We, thus, decided to compare their 3D solution structures in a common medium and chose DPC micelles, a medium often used to mimic the eukaryotic cell membrane in NMR studies. Firstly, we determined the structures of two agonists ($hUII_{4-11}$ and URP), a super agonist (P5U) and one antagonist (Urantide). Our studies showed that although all peptides adopted a β-hairpin conformation, there was a difference in the turn nature between agonists and antagonists. Our analyses also revealed that, in agonists and the antagonist, the side-chain orientation of residues F^6, W^7 and Y^9 were different. Secondly, we showed that, contrary to URP and $hUII_{4-11}$, the conformation of hUII was dependent on concentration. This self-assembly phenomenon, which is still under investigation, may impact the interaction with the receptor and be responsible for the differential biological activities of hUII and URP.

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Small Molecules and Pharmaceutical

P373

Using Predicted C-13 NMR Spectra with Open Resources for Structure Dereplication

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Abstract: The capability to quickly and reliably separate and identify active components in natural product mixtures—identified through bio-assay and/or mass spectrometry guided fractionation—is critical to successful natural product-based drug discovery. Dereplication refers to the process of screening active compounds early in the development process in order to recognize and eliminate those compounds that have been studied in the past. This process enables scientists to proactively decrease the number of structures that will need to be fully elucidated and focuses testing on true 'unknowns'.

The question then is where a comprehensive list of all the known structures can be found. While there are several specific and/or proprietary databases for natural products these are usually limited to compounds from specific sources. There are at least two open chemicals databases (PubChem and ChemSpider) with many times more compounds than the specific ones.

The ^{13}C NMR spectrum of a compound can be considered a fingerprint since it is virtually unaffected by conditions such as pH, concentration, and solvent effects. It is also largely magnetic field independent, since there are no couplings that could cause variations in stronger or weaker fields. Searching databases of predicted ^{13}C NMR spectra for similarity to the unknown is, therefore, a very powerful and reliable strategy for structure dereplication. The search can be enhanced by including search terms such as molecular formula (expanded to cover MF ranges) and by accommodating for missing or extraneous peaks in the NMR spectrum. It is also very common to use such databases to identify structural fragments in the case of genuinely unknown structures.

In this work we explore the possibilities and limitations of using predicted ^{13}C spectra for structures from open databases. The workflow will be described together with some examples given of the results and the potential usefulness of the technique.

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Studies of Helicenes and Cyanines by Residual Dipolar Couplings

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Abstract: In the last decade, Residual Dipolar Couplings (RDCs) have become an extremely valuable tool in the structure determination of small molecules.[1] Since RDCs are highly powerful in the determination of the relative configuration of multiple or remote stereogenic centers, particularly natural products have greatly benefited from this technique. The measurement of RDCs requires the molecule of interest (= solute) to be weakly aligned, which has been achieved mainly using either stretched or compressed cross-linked polymer gels or lyotropic mesogens like PBLG. As a "by-product", valuable information about the solute alignment and hence, the polymer-solute interaction may be obtained.[2]
In this work, we investigated two highly interesting classes of molecules – helicenes and cyanines – in stretched polystyrene (PS)[3] and polybutyl acrylate (PBA),[4] and in a lyotropic polyaspartate.[5] In the case of two substituted hexahelicenes, the helical pitch could be determined with a C2…C2' distance of 4.3 Å, in agreement with previous theoretical predictions.[6] The *para*-methoxyphenyl substituent shows a clear conformational preference. Despite the highly twisted helicene structure, little enantiodiscrimination of the two helicene enantiomers was obtained by the chiral polyaspartate. In the second part of the study, we compared the alignment of cyanine dyes with different chain lenghth. As expected, the "stiffness" of the methine chain decreased with increasing length.

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P375

Structure of a cyclolipopeptide in interaction with a lipopolysaccharide

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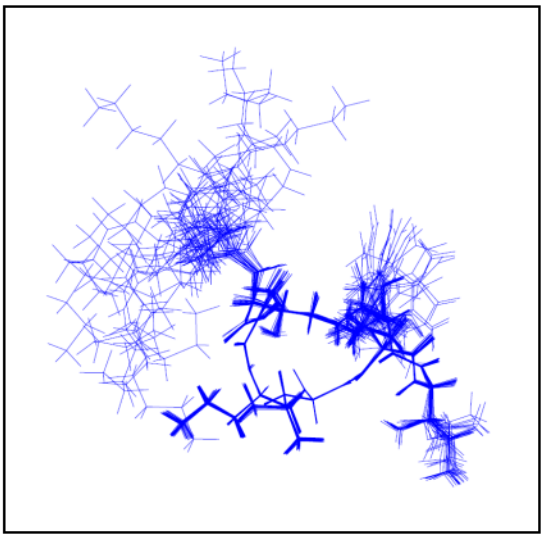
Abstract: The marine bacterium Pseudoalteromonas h*Cg*-6 was isolated from the hemolymph of an healthy oyster, Crassostrea gigas, collected in the Glenan archipelago (Defer et al, 2013). It exhibited a potent antibacterial activity against all the oyster pathogenic *Vibrio* assayed while no significant cytotoxic effect against oyster hemocyte was detected even at high concentration (Desriac et al, 2014a). The strain Pseudoalteromonas h*Cg*-6 produces a new family (8 isoforms) of cyclolipopeptides (CLP) made of a cationic heptapeptidic macrocycle connected via an amido link to various *hydrocarbon* tail (Figure). The CLP mechanism of action was deciphered using flow cytometry. The CLP provoke plasmic membrane permeabilization leading to bacterial lysis. Moreover, using a combination of biological and biophysical assay, the lipopolysaccharide (LPS)-binding affinity of CLP was highlighted (Desriac et al, 2014b).

As often encountered with small antimicrobial peptides, no defined tri-dimensional structure was observed in water. Based on the interaction with LPS, Tr-NOE method was used to determine the peptide structure in interaction with LPS. LPS easily forms micelles of high molecular weight in solution. The interaction between the peptide and LPS gives Tr-NOE to determine the tri-dimensional structure of the peptide bound to LPS. The NMR distance constraints were used to generate 100 structures of the peptide with the AMBER software. The 20 structures of lower energies were presented in the Figure below.

Image:

Pseudoalteropeptins	926	$C_{8:0}$
	940	$C_{9:0}$
	952	*cis* $C_{10:1}$ Δ^3
	954	$C_{10:0}$
	970	3'-OH $C_{10:0}$
	980	$C_{12:1}$ Δ^3
	1008	*cis* $C_{14:1}$ Δ^3

Various fatty acids side chains



Small Molecules and Pharmaceutical

P376

A data format to associate NMR-extracted data (NMReDATA) to chemical structures

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Abstract: The NMReDATA initiative addresses the problem of reporting and sharing the assignment of 1D and 2D NMR spectra of organic molecules and make the supporting spectra accessible. It involves most players of Computer-Assisted Structure Elucidation (CASE), methodology specialists, spectrometer manufacturers, database developers and members of the board of *Magnetic Resonance in Chemistry*.

The initiative introduced a file format to associate the data extracted from the "full NMR analysis" (1D ^{1}H and ^{13}C, COSY, HSQC, HMBC, *etc.*) and the structure of the identified compound.[1,2] It uses the Structure Data Format (.sdf files - compatible with .mol files) to include the assigned chemical structure. The NMR-extracted data (chemical shift, coupling and assignment) are encoded as so-called "tags", that are included in the .sdf files. These "tags" are not visible when displaying the molecules from the .sdf file, but can be accessed by specialized software and analyzed by the database during the importation of the data.

These .sdf files including NMReDATA have multiple roles. First, they make the link between the atoms of the structure and an aggregated list of signals found in the spectra (assignment). Second, they list, for each 1D and 2D spectrum, the spectral parameters of the signals (chemical shifts, couplings, integrals, 2D correlations, etc.). The format also requires to provide DOI links to the spectra making it possible to validate, reinterpret, update, complement, correct, refine, *etc.* the NMReDATA.

We call "NMR record", a compressed folder (in the .zip format) including the .sdf file (with the NMReDATA) and all the files of its associated spectra (1D and 2D spectra, FID's, acquisition and processing parameters in the manufacturer's format). The NMR records will be generated by future releases of CASE software and other edition tools and will follow manuscripts in the reviewing process. When articles are published, the NMR records are made available in open databases.

The Version 1 of the NMReDATA format was presented[2] in early 2018 in *Magnetic Resonance in Chemistry*, the first journal to request, by the end of 2018, NMReDATA for all the submitted assignment articles.

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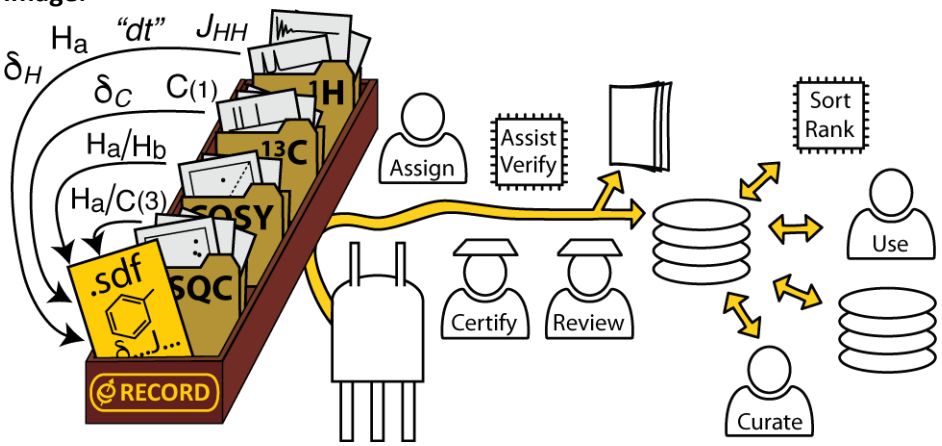

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P377

Identification of new-to-nature halogenated indigo precursors as engineered metabolites in tobacco plants

Volker Schmidts[*] [1], Sabine Fräbel[2], Bastian Wagner[2], Markus Krischke[3], Christina M. Thiele[1], Agata Staniek[2], Heribert Warzecha[2]

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Abstract: Engineering of novel metabolic pathways in plants is of broad interest in biology and biochemistry. By introducing a specific set of enzymes into the plant genes, new pathways may be established *in planta* thereby allowing the plant to act as chemists producing a tremendous variety of specialized compounds.[1]

Recently, Fräbel et al.[2] presented a new method of engineering a new pathway for the biosynthesis, halogenation and subsequent glucosylation of indican derivatives in plants devoid of a natural pathways to such substances. Indican (indolyl-β-D-glucopyranoside) is a secondary metabolite characteristic of a number of dyers plants. Its deglucosylation and subsequent oxidative dimerization leads to the blue dye indigo. Halogenated indican derivatives are commonly used as detection reagents in histochemical and molecular biology applications.

From the NMR perspective, identification of secondary metabolites in the plant extract often presents a challenge due to the low sample amount and spectral overlap in non-purified samples. We show the identification of the *new-to-nature* 5-chloroindican in a tobacco plant extract by PSYCHE-iDOSY.[3] Where classical stimulated echo DOSY analysis did not yield unambiguous results due to signal overlap, the recentyl introduced pure shift variant gives a clean separation of the indican signals from other metabolites. Unambiguous determination of the aromatic substitution pattern and the sugar moiety attached to the indolyl part by the endogenous gylcoyltransferases was carried out by classical analysis of NOE contacts and *J*-coupling patterns as well as comparison with chemically synthesized reference materials.

Our study showcases the high potential of designing new metabolic pathways in plants to develop new (bio)synthesis routes to complement established chemical synthesis. Further in depth analysis of the secondary metabolites might even lead to the discovery of new, entirely unique compounds.

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Small Molecules and Pharmaceutical

P378

The intramolecular dynamics of a 'rigid yet twisty' ferrocenyl tetraphosphine – served with some 31P-NMR delicacy

Benjamin Kovacs[*1], César A. Urbina-Blanco[2], Mark Saeys[2], Jean-Cyrille Hierso[3], José C. Martins[1]

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Abstract:

Polydentate ferrocenyl phosphines equipped with bulky functional groups are regarded as rigid ligands capable of stabilizing and/or activating a broad range of chemical compounds such as smaller complexes (being formed e.g. in transition metal-catalyzed cross coupling reactions), nanoparticles, or even larger surfaces. The fruitful rigidity of these fascinating molecular species originates from the internal steric constraints imposed by the substituents; hence, the rotational reorientation of the Cp rings around the vertical 5-fold symmetry axis is hampered, and a permanent polydentate phosphine 'cage' is created (see figure). Latter construction then provides a large variety of coordination modes for the actual substrate what is the core structural feature being responsible for the diverse applicability spectrum. If such ferrocenyl phosphine is investigated on a sufficiently long i.e. 'NMR time scale' however, its decelerated intramolecular motions might be discovered and quantitatively characterized. Indeed, selective 1D ^{31}P-{^{1}H} EXSY {Exchange Spectroscopy} pointed out the exchange of the chemically distinct phosphoruses (green and red spheres below) in the scrutinized Fc(P)$_4$^{t}Bu ligand and thus successfully demonstrated the previously unknown rotation (i.e. antiparallel twisting) of the Cp rings around the Fe centre. Series of measurements performed at different temperatures enabled the evaluation of the respective thermodynamic parameters ($\Delta S^{\#}$, $\Delta H^{\#}$, $\Delta G^{\#}$) for which the influence of the solvent was also studied – while the confrontation of the experimental and theoretical values computed by DFT methods completed the analysis of the motion.

In fact, the four ^{31}P-s of Fc(P)$_4$^{t}Bu composes an AA'BB' spin system giving rise to a puzzling second order ^{31}P NMR spectrum. Although the respective *J*-couplings had already been presented reclining upon the output of *in silico* simulations, a side track of the current work covered the full deduction of the results by the means of a quantum mechanical approach. Besides, the internal ring rotations shed new light on the 'through space' nature of the $J_{AA'}$ coupling affecting the inner phosphoruses (red spheres). That is, the interaction showed unquenchable and endured higher ring rotation rates than its actual frequency value what highlighted the intricacy of the magnetization transfer phenomena between the two nuclei.

Finally, exchange phenomena were revealed for the complexed state of the ligand as well. According to 2D ^{1}H–^{1}H EXSY spectra, in case of [Pd(II)Br$_2$-Fc(P)$_4$^{t}Bu] the familiar twisting of the cyclopentadienyl rings was complemented with the periodic transconnection of the [Pd(II)Br$_2$-] moiety between the bidentate (-PPH$_2$)$_2$ sites – perfectly illustrating the possibility for multiple coordination ways offered by polydentate phosphines.

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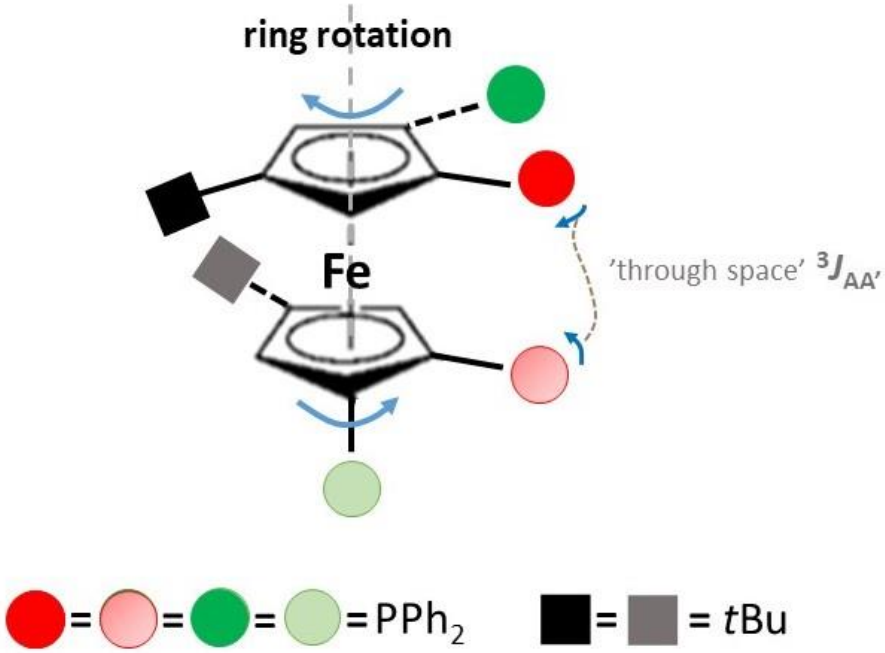

Small Molecules and Pharmaceutical

P379

Top-resolution in 2D spectra: Optimization of the acquisition parameters according to SNR, field inhomogeneity and relaxation

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Abstract: The resolution in the F1 dimension of 2D NMR experiments directly depends on the maximal t_1 evolution time t_1max. The choice of the t_1max value (or FID-Resolution) is therefore crucial when aiming to obtain spectra with highest possible information content. When t_1max is too short, the potential resolution is not fully exploited. When too long, unnecessary noise is detected due to signal relaxation. We will present how t_1max can be optimized according to: the signal-to-noise ratio of experiment (SNR), the relaxation time of the compound, the field inhomogeneity and the window function used for processing.

Once having a t_1max value at hand, one can consider then how to reach it. Typically, the t_1max in top-resolution HSQC spectra, or HMBC, of small molecule would require the acquisition of thousands of time increments. This is because the carbon dimension is quite large, especially at high fields. In order to avoid exceedingly long acquisition times, two main approaches can be used: Non-uniform sampling (NUS) and uniform undersampling, resulting to spectral aliasing.

We present here the results of an approach exploiting spectral aliasing, obtained by simply reducing the spectral window, to a finely tune value in order to obtain top-resolution spectra. The chemical shift ambiguities, introduced by spectral aliasing, can be eliminated by combining this approach with chemical shift encoding to reconstruct normal full-width top-resolution spectra.

Figure 1. (Left) HSQC spectrum recorded with an optimized carbon spectral window of 3.0641 ppm and 512 time increments corresponding to a t_1max of 0.6643s for a compound with a typical relaxation time of 0.1s (Right) Reconstructed full-width HSQC spectrum obtained using modified pulse program for chemical shift encoding with optimized acquisition parameters for top-resolution. The gain in acquisition time is 65 for an effective carbon window of 200ppm.

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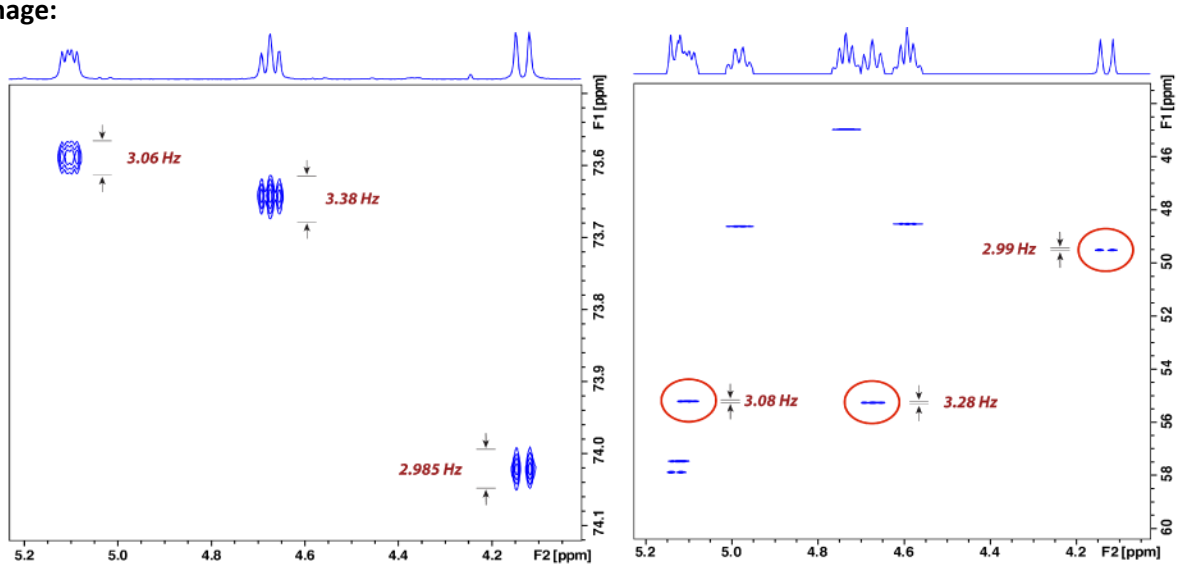

Small Molecules and Pharmaceutical

P380

Structural Revision of Terpenoids and Related Natural Products by Using CAST/CNMR System

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Abstract: The CAST/CNMR system is composed of two functions for prediction of ^{13}C NMR chemical shift values from a query chemical structure (CAST/CNMR Shift Predictor[1]) and elucidation of chemical structures from a query of ^{13}C NMR chemical shift values (CAST/CNMR Structure Elucidator[2]). These functions use a database of reported/observed ^{13}C NMR chemical shift values associated with their 3D chemical structures described using the CAST (CAnonical-representation of STreochemistry) coding method. An interface between CAST/CNMR Structure Elucidator and the Delta NMR software (JEOL) has been recently developed, where the query to the elucidator can be directly made from 1D ^{13}C NMR spectral data. The database is composed mostly of the data from peer-reviewed journals and partly of our originally assigned data. The data registration has been done after a careful check. However, even such authorized journals sometimes contain wrong signal assignments and incorrect characterization of structures[3]. Indeed, we have found incorrect data when developing the database and revised some natural products by total synthesis and by using CAST/CNMR system[4-6].

We have used the CAST/CNMR system to find and correct such wrong assignments or structures. Two functions of CAST/CNMR are complementarily used in the study of the structural revision of natural products and the evaluation of chemical shift assignments. For instance, wrong assignments of ^{13}C signals can be detected by comparing their reported data with the predicted chemical shift values. CAST/CNMR Structure Elucidator can be used to get ideas of correct structure by investigating the elucidated structures. We will report the structural revision of some terpenoids and related natural products in this paper. We have revised the structure of a clerodane type diterpenoids, solidagocanin A[7], to its stereoisomer on the decaline portion. We also have revised several prenylated phenolic compounds with the (Z)-3-hydroxy-3methylbut-1-enyl group by comparing the ^{13}C NMR data, for example, comosusols A and B[8] to be 2,2-dimethyl-8-(3-methyl-2-butenyl)-2*H*-chromen-6-ol and 2,2-dimethyl-7-(1,1-dimethylprop-2-enyl)-2*H*-chromen-6-ol, respectively.

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Small Molecules and Pharmaceutical

P381

Multiple-spectra Automatic Structure Verification (MS-ASV)

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Abstract:

Today, automatic structure verification (ASV) using 1H, 13C, and HSQC spectra and their various combinations has reached a very usable quality, even though there are frequent continuous improvements (which will no doubt continue for many decades).

When it comes to automatic structure verification using combinations of any number of available NMR spectra (MS-ASV), the problem assumes a degree of complexity comparable to that of an AI for autonomous-driving cars.

In both cases, we are talking about an AI wizard which will keep developing for many decades. Yet, we have reached a state where a first version of truly operational MS-ASV can be deployed in practice and perform better than a chemist with an average training in NMR spectroscopy. The type of spectra it covers are 1H, 13C, HSQC, COSY, and HMBC spectra, a list that will now start rapidly growing.

We have overcome a long number of challenges to achieve this result (and, yes, we are facing many more, though with a progressively decreasing impact). Some of these, the easier ones, are purely technical, like how to simulate a given 2D spectrum, or how to avoid assignment conflicts across the whole set. The hardest challenges to face are of probabilistic (fuzzy) nature. For example: if a structure appears to match four spectra and fail one, does it mean that the structure is wrong, or does it simply mean that the spectrum where it fails is of bad quality (or 'wrong' in some other way)?

In this presentation, we illustrate the current state of the MS-ASV art and discuss, to the (limited) extent to which it is feasible, some of the involved issues.

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Complementarity of decoupling elements with respect to spectral quality in 2D pure-shift homonuclear experiments

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Abstract: F1-homodecoupled 2D experiments offer a remarkable improvement in spectral resolution. We have already reported [1] a toolbox of basic experiments such as the DIAG [2] or CLIP-COSY [3] experiments, where the coupling interactions are eliminated in the indirect dimension.

In order to separate the effects of chemical shifts and homonuclear couplings in case of the 2D experiments, an intervention with a pulse sequence element is needed in the middle of the t_1 evolution time. In recent years, a lot of effort has been put to approach an ideal separation, which experimentally remains quite challenging.

One evident limitation for the separation of the interactions occurs in case of strong coupling. With this respect, some flexibility can be gained by using the BIRD filter [4] decoupling element. In this case, the insertion of the ^{13}C nucleus into a spin system alternates the J coupling interaction between the protons to be decoupled.

The second source of difficulty to obtain a high quality decoupled spectra is the necessity of using frequency-modulated selective refocusing pulses [2] or small-flip angle adiabatic pulses (PSYCHE element) [5] to improve the sensitivity of the experiments. This, in turn, leads to unwanted coherence transfers which result to artifacts in the spectra. Additional elements in a pulse sequence are thus required to average out and eliminate this surplus of magnetization.

The use of the quadrature detection and the classic Fourier Transform in both dimensions of the 2D experiments makes it easier to trace the magnetization which will contribute to artifacts in the spectra. On the other hand, the necessity to combine the echo and anti-echo coherence paths in cases where the symmetry constrains are not fulfilled may have an influence on the appearance of the artifacts.

This work aims at the description of the main artifacts observed in the spectra acquired using indirect homodecoupling. This study is facilitated by numerical simulations performed using the Spinach simulation package [6].

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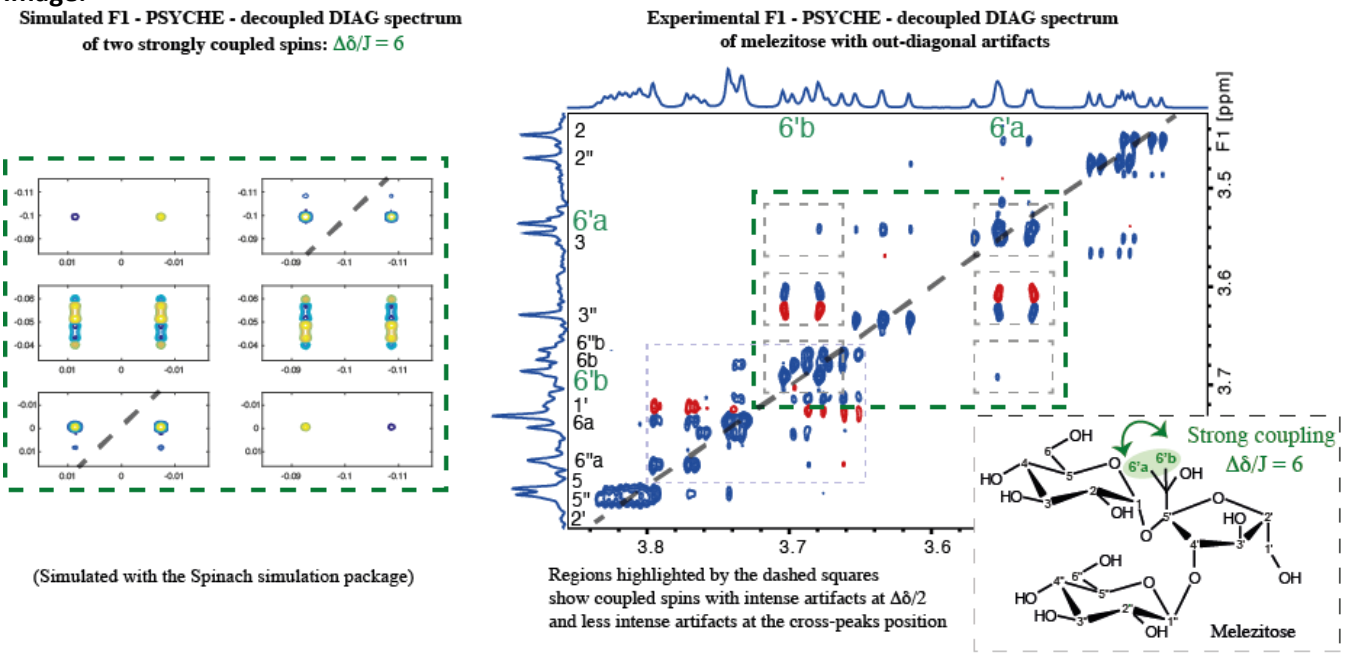
Image:


Simulated F1 - PSYCHE - decoupled DIAG spectrum
of two strongly coupled spins: Δδ/J = 6
(Simulated with the Spinach simulation package)
Experimental F1 - PSYCHE - decoupled DIAG spectrum
of melezitose with out-diagonal artifacts
6'b
6'a
F1 [ppm]
3.5
3.6
3.7
3.8
3.7
3.6
Regions highlighted by the dashed squares
show coupled spins with intense artifacts at Δδ/2
and less intense artifacts at the cross-peaks position
Strong coupling
Δδ/J = 6
Melezitose

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Label-free determination of residence times and binding energetics of fluorinated ligands with NMR

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Abstract: In recent years, fragment-based drug design became a very promising approach for the discovery of new drugs. Among other biophysical techniques, NMR methods play a pivotal role in its success. NMR monitors changes in the chemical shift or relaxation parameters of a nucleus occurring upon binding. In a drug development setting, NMR has the ability to observe ^{1}H atoms from unlabeled ligands, providing information in the most material-effective way, as ^{1}H nuclei are highly abundant and can be directly detected without the need for isotopic labelling nor immobilization of the target receptor. The most popular ligand-observed NMR experiments used to date are STD, WaterLOGSY, and T_2 edited experiments. Although these methods are qualitatively very robust and can provide quantitative estimates of the dissociation constant, rebinding events force the users to rely on different physicochemical methods to determine consistent K_D or residence time (τ_{ex}).[1]

In order to alleviate this issue, we recently presented an experiment that utilizes relaxation dispersion ($R_{1\rho}$) to determine the residence time of unmodified ligands on complex with their receptors.[2] A drawback of probing the ^{1}H nucleus is the small chemical shift changes induced by the binding due to the narrow chemical shift range, which causes difficulty in the analysis of relaxation dispersion profiles. Here, we present a new application of the eCPMG experiment[3] to the analysis of ligand binding by probing ^{19}F nuclei. The advantage of our new approach is threefold: *(1)* the eCPMG experiment is uniquely sensitive to slow and fast dissociation rates (residence time from 5 ms to 4 µs); *(2)* the large chemical shift perturbation experienced by ^{19}F nucleus upon binding eases the analysis of dispersion profiles; and *(3)* by probing an isolated spin system (in mono-fluorinated small molecules) we avoid traditional pitfalls of ^{1}H CPMG experiments (NOE and homonuclear coupling contributions to the relaxation rates).

Figure 1. Arrhenius fit of the residence time of 5-^{19}F-L-Trp bound to bovine serum albumin measured with the Fe-CPMG experiment proposed here. The temperature range was 270 to 310 K with exchange rates ranging from 20×10^{3} to 10×10^{4} s^{-1}.

The large chemical shift dispersion of ^{19}F facilitates the detection of relaxation profiles in a wide temperature range, in turn allowing us to use Arrhenius-like equations to calculate the Gibbs energy of binding from a set of Fe-CPMG experiments at different temperatures.

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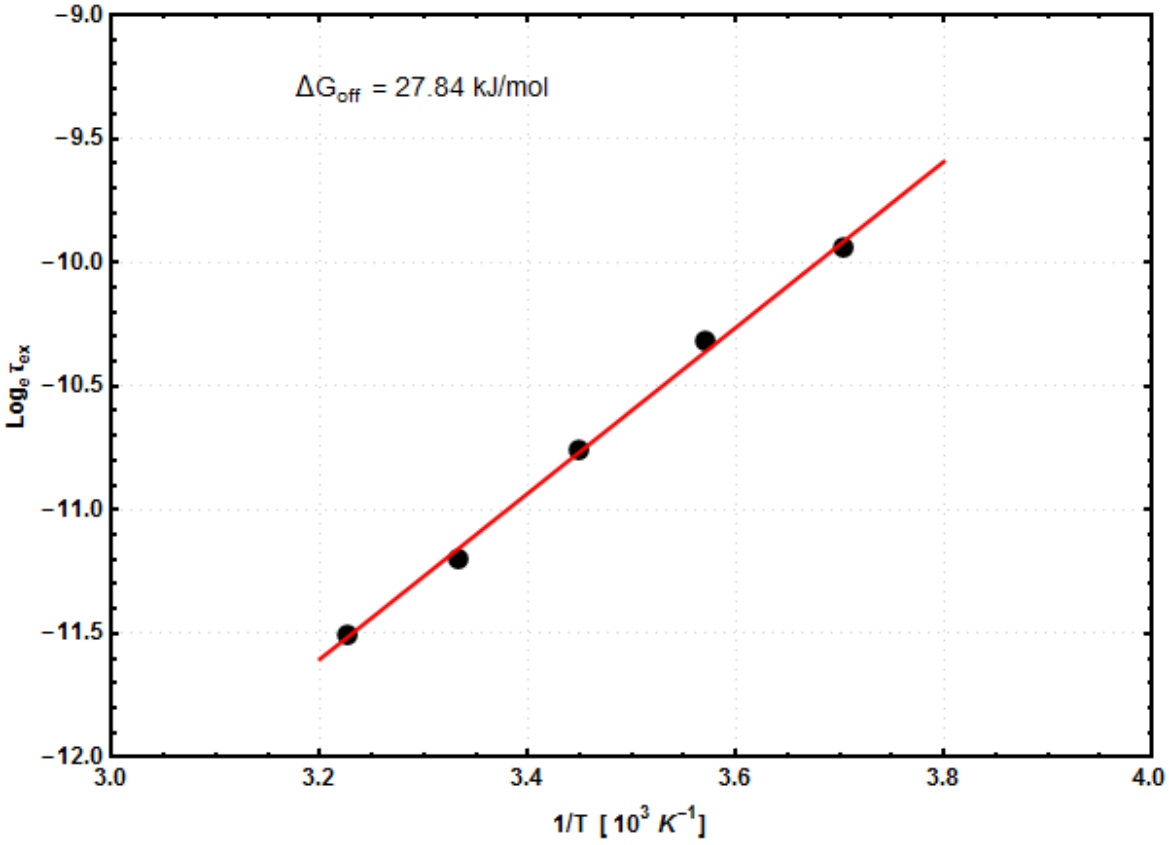
ΔG_off = 27.84 kJ/mol
Log_e τ_ex
1/T [10^3 K^-1]
-9.0
-9.5
-10.0
-10.5
-11.0
-11.5
-12.0
3.0
3.2
3.4
3.6
3.8
4.0

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NMR Structure, Dynamics and Interaction of the Proapoptotic Death Receptor 5/ TRAIL-R2 with Synthetic Ligands.

Antoine Baudin[*] [1], Anne Guichard[2], Gavin Collie[3], Mélanie Berbon[4], Antoine Loquet[4], Cameron Mackereth[5], Gilles Guichard[4], Benoit Odaert[1]

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Abstract: Tumor necrosis factor Receptor Apoptosis Inducing Ligand (TRAIL) appears as an interesting candidate for targeted cancer therapy upon binding to pro-apoptotic Death Receptors (DR). The protein induces apoptosis in cancer cells without toxicity to normal cells. However, recombinant soluble TRAIL and monoclonal antibodies against DR demonstrated insufficient efficacy in clinical trials.

We propose as an alternative to use 16-mer multivalent peptides [1] that selectively bind DR5/TRAIL-R2 with strong affinity and induce a high degree of apoptosis in BJAB lymphoma and tumorogenic BJELR cells [2,3]. The aim of the project is to decipher the mechanism of interaction between DR5 and the synthetic ligands, by solving the atomic structures of the complexes by combining NMR spectroscopy and crystallography. We over-expressed the ExtraCellular Domain of the receptor (DR5-ECD) in *E. coli* in $^{13}C^{15}N$-labeled or unlabeled forms. Three-dimensional NMR experiments allowed us to achieve a full resonance assignment and to determine secondary structure elements: the protein adopts a β-sheet structure in solution [4], similar to the one determined by crystallography in the presence of TRAIL or monoclonal antibodies anti-DR5. ^{15}N relaxation has uncovered important insights into the conformational dynamics of DR5. NMR titration studies with monomeric synthetic ligands allowed us to determine a common binding site inside the first Cysteine-Rich Domain (CRD1). This domain has been reported to play a crucial role in the Pre-Ligand Assembly Domain (PLAD) of tumor necrosis factor receptors [5]. The HADDOCK model of peptide-protein complexes reveals a different binding mechanism compared to TRAIL and antibodies. We are currently crystallizing the receptor in the presence of dimeric peptides in order to solve the three-dimensional structures of the multimeric complexes. Our results revealed a new and unexpected mode of interaction to DR5, allowing us to propose to use synthetic ligands in combination of TRAIL as an eventual bi-therapy to treat cancer.

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Opportunities offered by trifluoromethylated pseudoproline residues in the peptide design as assessed by solution state NMR investigations.

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Abstract: The design of constrained peptides is of prime importance in the development of bioactive compounds and for applications in supramolecular chemistry. By using oxazolidine-based pseudoprolines (YPro) substituted by a trifluoromethyl group, we demonstrate that several parameters of the peptide geometry can be locally tuned: *cis/trans* ratio of the amide bond and the energy barrier for this isomerization, backbone dihedral angles, puckering of the 5-membered oxazolidine ring, and presence of H-bond.[1] Solution state NMR has been extensively used to establish the structural properties of small (pseudo-)peptide molecules. In particular, CH_2-Trosy experiments [2] have been recorded to precisely measure the J-couplings on methylene groups and the presence of CF_3 groups has allowed us to record ^{19}F-based experiments for gaining some insights into the structural properties of the peptides.

Our results prove that changing the configuration of the C^{α} of the YPro or of the preceding residue is sufficient to invert the *cis*:*trans* populations while changing the nature of the side chain finely tuned the conformers ratio. Moreover, a strong correlation is found between the puckering of the oxazolidine ring and the peptide bond conformation.[3] This finding highlights the role of the trifluoromethyl group in the stabilization of the peptide bond geometry. We have taken advantage of the structural features imparted by the trifluoromethyl group in oxazolidine-based pseudoprolines to design longer peptides that mimic the collagen triple helix. A detailed structural analysis of these fluorinated collagen model peptides will be presented, and it will be shown that NMR spectroscopy can be an accurate method to monitor the triple helix formation.

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NMR characterization of N-glycans of natural glycoprotein and elucidation of their functional role

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Abstract: Immunoglobulins or antibodies mediate host-pathogen interactions by tagging unique molecule of the target cell, the antigen, and activate an immune system response. Antibodies communicate with effector cells of the immune system through interactions with membrane bound Fc receptors. These interactions induce effector responses including phagocytosis, and antibody dependent cell mediated cytotoxicity (ADCC). In the ADCC pathway, both players, Igs and Fc receptors, are complex glycoproteins. Glycosylation is key in immune response regulation.1 Glycans structural studies in antibody-receptor interactions has traditionally focused on the function of the N-linked glycans of immunoglobulins, however, recent exciting discoveries have led to renewed interest on the role of Fc receptor glycosylation and subsequent immune response modulation.2 Here we present a new strategy to enrich glycoprotein with isotope labelled nuclei and therefore to get essential structural and dynamic information aimed to the structural characterization of N-linked glycans in intact glycoprotein and to the elucidation of their functional role in immune response modulation.

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Solid-state NMR methods

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Structural defects in indium arsenide detected by static 115In, 113In and 75As one-dimensional single pulse solid-state nutation NMR spectroscopy.

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Abstract: ^{115}In (S=9/2), ^{113}In (S=9/2) and ^{75}As (S=3/2) one-dimensional single pulse solid-state nutation NMR experiments have been performed in a powdered sample of indium arsenide (InAs). Spin-3/2 and spin-9/2 signal intensities have recently been calculated by Kempgens (*Solid State Nucl. Magn. Reson.* **47**, 35-38 (2012) for spin-3/2 and *Magn. Reson. Chem.* **53**, 261-266 (2015) for spin-9/2). In this study the quadrupolar coupling constant Q_{cc} and the pulse amplitude $w_1/2p$ have been determined for ^{115}In, ^{113}In and ^{75}As in indium arsenide by a fit of their signal intensities upon rf field nutation. For ^{115}In and ^{113}In, it is found that the ratio of the quadrupolar coupling constants calculated in this work is in a relatively good agreement with the ratio of the quadrupole moments of the two nuclei. For ^{75}As, we found a quadrupolar coupling constant Q_{cc}=190 kHz and the asymmetry parameter of the electric field gradient (EFG) tensor remains undetermined. As InAs has the cubic zinc blende structure, no ^{115}In, ^{113}In or ^{75}As quadrupolar coupling constants are expected in this compound. It is concluded that the origin of the quadrupolar coupling constants detected in this work are due to structural defects in the sample as was already the case for indium phosphide (InP).

Solid-state NMR methods

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Structure and Dynamics of a Tsai-type quasicrystal and approximant from 45Sc and 67Zn NMR studies.

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Abstract: NMR is a recognized complementary method to X-rays/Neutron diffusion for structure and dynamics understanding, due to different space-time correlation function probe range, and atomic selectivity. Both benefit from Large Scale Facilities (synchrotron, high field NMR, neutron reactors …) allowing unprecedented space-time resolution. For instance, the family of icosahedral quasicrystals of Tsai type [1,2] contains binary stable i-QC with low chemical disorder that offer the opportunity of precise structural analysis [3] to reconstruct the atomic structure from a 6-dimensional superspace projection, using 1/1 and 2/1 approximant periodic structures as starting models. However, the complexity of superspace crystallography requires assumptions and approximations in order to answer to the question "where are the atoms". Thus, major questions about local structure, dynamics and possible phase transition in the quasicrystal are still to be answered. To the best of our knowledge, no NMR studies have been performed on such iQc.
In this presentation, solid-state NMR experiments of ^{45}Sc (I=7/2, 100%) and ^{67}Zn (I=5/2, 4.1%) nuclei at different fields and temperatures, in the 1/1 approximant Zn_6Sc and quasicrystal ZnScAg are reported and discussed in the light of structural studies. Both nuclei carry complementary information to probe intershell coupling and tetrahedron induced distorsions. Indeed, 12 scandium atoms constitute the second shell of a Tsai cluster, composed of a tetrahedron (4Zn), dodecahedron (20Zn), Sc icosahedron and larger shells of zinc(Fig.1). Another type of building brick is the double Friauf polyhedron, that exists only in the 2/1 approximant and iQc. We will discuss how our NMR results provide insight in the structure (double Friauf and icosahedral sites) and dynamics (frozen state in the iQc), from comparison between the 1/1 approximant and iQc.

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Fig. 1. Left : Zn tetrahedra in Sc icosahedron; (center) upper : Zn dodecahedra and Sc icosahedron (pink), lower : double Friauf structure with 2 Sc inside. (right) iQc structure.

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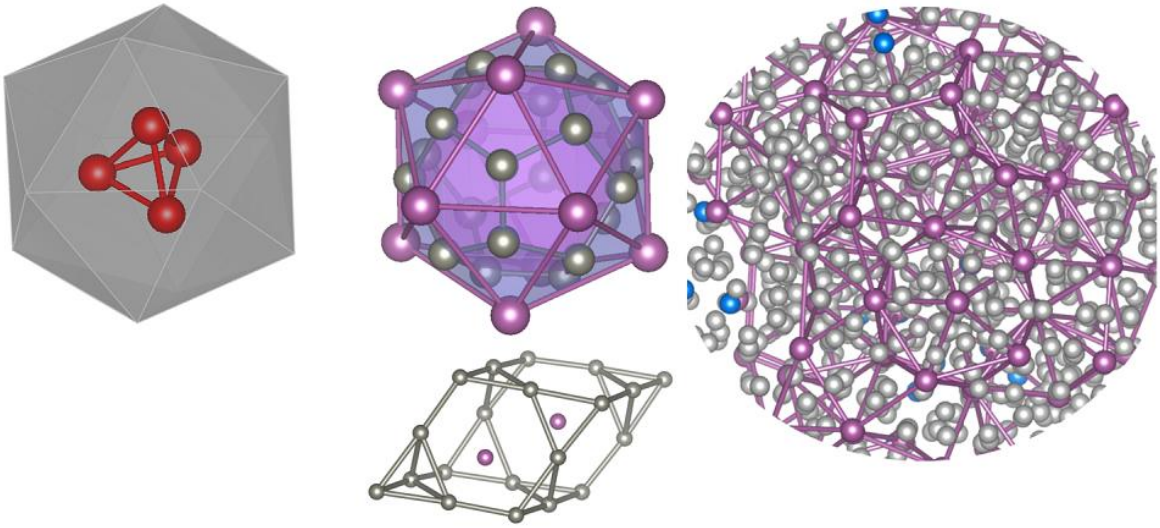

Solid-state NMR methods

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The Proton Line Width Under Fast MAS: A Fast Calculation Method

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Abstract: Understanding and disentangling the contributions to the proton line widths of multi-spin systems like proteins is still an open issue in fast magic-angle spinning solid-state NMR. Conventional line-width simulation approaches are based on direct numerical integration of the Liouville-von Neumann equation, but are limited to small spin systems (< 12 spins) by available computation power and memory. We present an alternative simulation method for coherent contributions based on the calculation of analytical expressions for second moments of an arbitrary N spin system under fast magic-angle spinning. It can easily predict coherent proton line widths for systems containing hundreds of proton spins, like fully-protonated proteins. We validate it by comparison with experimental data for microcrystalline Ubiquitin (deuterated 100% back-exchanged Ubiquitin at 110 kHz MAS, fully-protonated Ubiquitin at 125 kHz MAS) and find good qualitative and quantitative agreement between simulation and experimental data over the entire protein. Lastly, we compare the results with dynamical data from relaxation studies and estimate the contribution of incoherent effects. Application to other biologically interesting systems of known structure, like membrane proteins, virus capsids, amyloid fibrils and to materials are considered.

Fig.1: (a) Three-spin system geometry used for calculating the analytical second-moment contribution under MAS;. (b) Comparison of experimental line width obtained from site-specific T_2' measurements (blue squares) and second moment simulation (green triangles) in deuterated 100% back-exchanged Ubiquitin.

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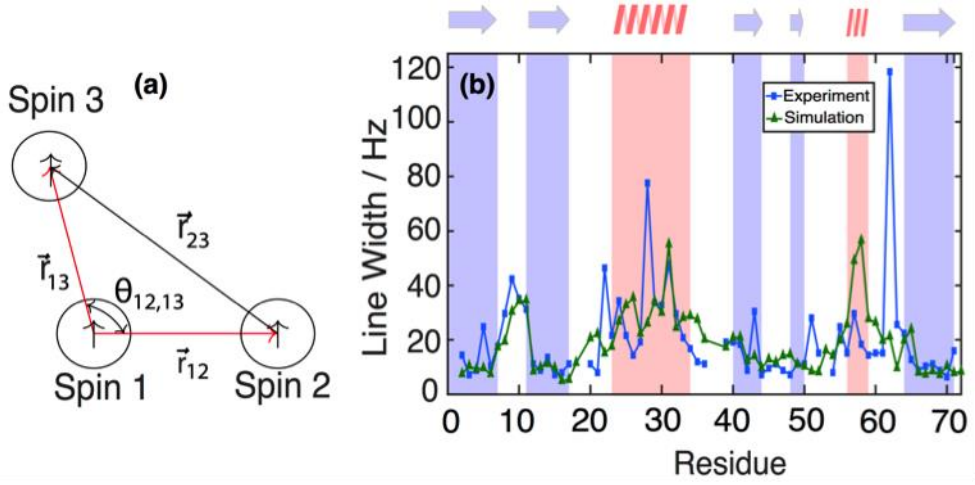

Solid-state NMR methods

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Probing local electronic structure with paramagnetic solid-state NMR

Gwendal Kervern[* 1], Nicolas Claiser[1], Pierrick Durand[1], Mamoudou Diallo[1], Laurent Le Pollès[2], Kevin Bernot[3], Cédric Carteret[4], Arnaud Di Bitetto[1]

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Abstract: Quantum calculation can predict magnetic properties which, in return, can be probed experimentally to assess fine details in the electronic structure in paramagnetic materials. However, probing magnetic properties at a molecular level is not always as simple as making macroscopic magnetic measurements. We propose to present a solid-state NMR approach to this problem. In cases where there is no chemical contact between the observed nuclei and the electronic spins, the dipolar hyperfine interaction is the only possible way of interaction between nuclear spins and electronic spins. We will show how chemical shift anisotropy (CSA) measurements in 13C and 1H spectra give us access to molecular anisotropic susceptibility in several cases of crystalline lanthanide complexes, and how it tells us about electron-spin delocalization in transition metal-based layered materials.

The first part of this study presents the use of CSA measurement in order to probe the orientational dependence of the molecular magnetic susceptibility in crystaline lanthanide complexes. Such complexes present the advantage of having a magnetism born almost exclusively by the metal center with very little, if any, electron-spin delocalization. As a consequence, the hyperfine interaction with the surrounding nuclei is mostly dipolar and modeling its effect on NMR spectra requires much less computational power than in cases where contact interaction has to be taken into account. Since the CSA is sensitive to both distance and orientation-dependent intensity of the electronic magnetic dipole, these measurements allowed us to answer relatively complex questionning about molecular magnetic susceptibility in cases where there is a discrepancy between local and crystalline symmetries.

In a second part, we will present our recent measurements in layered materials such as layered double hydroxydes. These materials are made of charged mineral hydroxyde layers that are intercalated with counter ions. Since there is no chemical bonding between the magnetic centers and interlayers ions, modeling the hyperfine interaction is again relatively costless on a calculation level. Given the fact that CSA is strongly dependent on electron-nucleus distance, we use that property to determine how much of the electronic spins was delocalized on oxygen atoms in layered hydroxydes.

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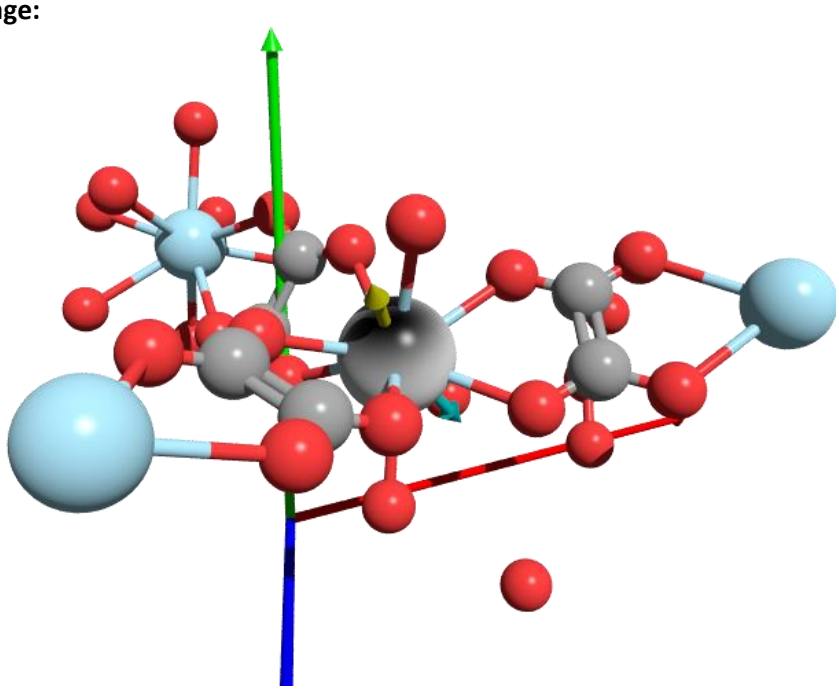

Solid-state NMR methods

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NMR resonance assignment of large fully-protonated proteins with shared-time experiments at >100 kHz MAS

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Abstract: In solution NMR, experiments for sequential backbone resonance assignment generally fail due to low sensitivity for slowly tumbling proteins (approximately MW > 25 kDa), unless extensive deuteration is employed. In contrast, high molecular weight does not impact experiments in the solid-state, but – to date – very few proteins of > 200 aa have been assigned by MAS (magic-angle spinning) NMR due to prohibitive complexity of spectra. Here we show that MAS NMR with ^{1}H-detection at ω_R > 100 kHz represents a new route to assign large proteins, with similar requirements on sample amount (< 1 mg of uniformly ^{13}C,^{15}N-labelled material) but without the need for deuteration.

In the last few years, the joint use of high magnetic fields and fast MAS probes made it feasible to resort to ^{1}H-detection and benefit from its inherently higher sensitivity (compared to the ^{13}C-detection conventionally employed in MAS NMR). We have recently demonstrated that the new generation 0.7 mm MAS probes (spinning up to 111 kHz) enables both backbone and side-chain assignment,[1] as well as structure determination[2] of fully protonated proteins with only minimal trade-offs in ^{1}H resolution compared to protocols employing deuteration.[3]

Leveraging these recent advances, here we develop a strategy to break the size limits for protein backbone resonance assignment, which requires limited acquisition time and is compatible with automatic data analysis. A key element of the approach are simultaneous experiments which (a) take an advantage of full protonation to independently polarize ^{13}Cα/C' and ^{15}N nuclei, and (b) allow multiple coherence pathways that are (c) acquired separately on amide and α-protons. Effectively, three previously proposed, mutually supportive assignment strategies are employed within the same experimental time: (1) based on detection of amide protons and correlation to either intra- or interresidue ^{13}C', ^{13}Cβ and ^{13}Cα[4] or (2) to neighboring ^{15}N nuclei[5] and (3) based on detection of alpha protons and correlation to either intra- or interresidue ^{13}C', ^{13}Cβ and ^{15}N[1]. Shared-time, single-receiver acquisition of up to 8 experiments ensures maximum consistency of the observed chemical shifts. Narrow line-widths of ^{1}H resonances due to fast MAS and of heteronuclei under low-power ^{1}H-decoupling provide high resolution to 3D spectra and allow to link resonances with low ambiguity even for proteins as large as a 371 aa maltose binding protein. We prove the data quality, redundancy and power of the recorded resonances for the sequential assignment using unbiased computational tools such as FLYA.[6]

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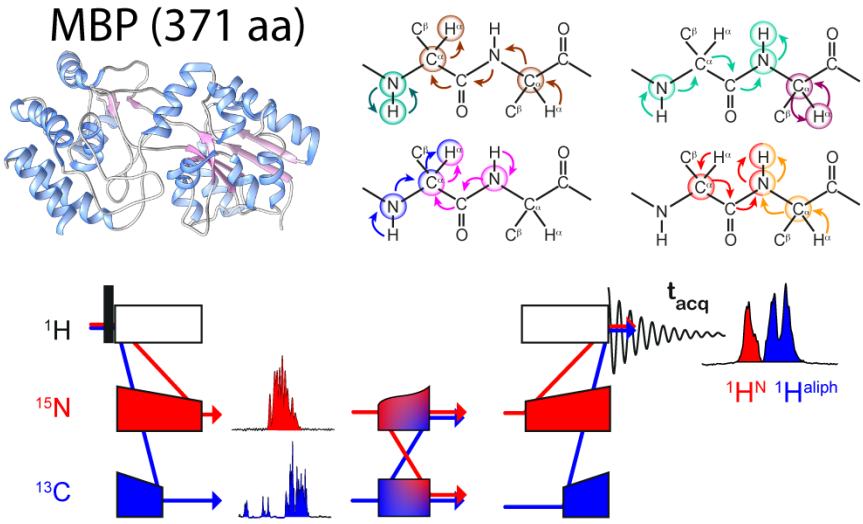
Fig. 1 *(top left) A structural model of MBP (371 aa). (top right) Scheme of the 8 coherence pathways recorded simultaneously using the blocks illustrated in the bottom panel: separate $^{1}H^{N}$ and $^{1}H\alpha$ detection, two-way ^{15}N-^{13}C cross-polarization and simultaneous ^{1}H-^{13}C and ^{1}H-^{15}N cross-polarization.*

Image:



Solid-state NMR methods

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Simple and robust study of backbone dynamics of crystalline proteins employing 1H-15N dipolar coupling dispersion

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Abstract: A few years ago, we have introduced the CPVC sequence [1] for ^{1}H-^{13}C (or ^{1}H-^{15}N) dipolar coupling measurements. However, this 2D experiment (d_C-D_{H-C}) is mainly applicable to small molecules. To overcome this limitation and extend the applicability of CPVC to more complex systems, we have developed a 3D version (d_C-d_C-D_{H-C}) with the RFDR-CPVC experiment and applied it to the dynamics of aromatic rings in GB1 and LC8 proteins [2]. This scheme is powerful, but it suffers from low sensitivity due to ^{13}C detection. One of the most promising way to overcome sensitivity problems is to detect the protons. Such approach is now extensively used in protein structure determinations. In my presentation, I will focus on the combination of 2D (d_N-D_{H-N}) CPVC with hNH and hCANH experiments to proteins to overcome sensitivity and overlapping problems. These two 3D experiments (**^{1}H-^{15}N-^{1}H-CPVC** and **hCA(N)H-CPVC)** provide unique possibilities to study the protein backbone dynamics via analysis of ^{1}H-^{15}N dipolar couplings and dipolar-order parameters (**S^2_{DD}**). Moreover, due to the two usable chemical shift editing (^{15}N and ^{13}C), it is possible to analyze the dynamics of all residues, even those with large chemical shift overlapping. I will show the benefits from paramagnetic enhancement of relaxation (PACC) [3] to increase the S/N. We have demonstrated the power of these methods on a small ^{2}H,^{13}C,^{15}N (Hn back exchanged)-GB1 protein doped with $Na_2CuEDTA$. At the end, I will present an extensive comparison of the various structures and dynamics recorded on GB1 proteins according to their conformations and paramagnetic doping.

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NMR crystallography improves confidence in two crystal structure models of beta-piroxicam

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Abstract: A structural model derived from diffraction data is often accompanied by a discrepancy value, the R-factor, which quantifies how well the model fits the diffraction pattern. The R-factor is a widely used indicator of confidence in any one structural model but a low value does not necessarily correspond to a good crystal structure and similarly a high value does not mean the model is poor. In the case of a model with a high R-factor a NMR crystallography study, involving the combination of experimental solid-state NMR and calculated NMR chemical shifts, is desirable to provide increased confidence that a model is a good description of the true crystal structure.

In this work, we present a comprehensive NMR crystallography study for b-piroxicam. Two structures deposited in the Cambridge Crystallographic Database were chosen. The first was solved from single crystal x-ray diffraction (CSD code: BIYSEH13[2]) with an R-factor of 2.4% and the second structure was solved from powder diffraction data (CSD code: BIYSEH03[3]) with an R-factor of 19%.

The R-factors alone suggest that BIYSEH13 is a better description of the crystal structure. We use NMR crystallography to show that this is not necessarily the case.

Geometry optimisation of the two structures results in convergence to structures with the same energy, with minimal atomic displacements, and good agreement with the gauge-included projector augmented wave (GIPAW)[4,5] calculated and experimentally determined NMR ^{1}H, ^{13}C and ^{15}N chemical shifts and ^{14}N quadrupolar parameters.

NMR crystallography is shown to be an effective means of comparing crystal structures with different R-factors and improving confidence in crystal structure models that might otherwise be considered sceptically based on diffraction data alone.

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7Li Diffusion in the Lithium Superionic Argyrodites as Studied by Solid-State NMR Spectroscopy

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Abstract: High Li^+ ion conductivity (σ) is one of the key factors defining the quality of solid electrolytes. Among a variety of solid electrolyte structures such as LISICON, perovskites, garnets and some others, argyrodites gained particular attention, due to their high σ-values (up to 10^{-2} S/cm) and low activation energies of the ion mobility. The commonly used method to estimate ionic conductivity is impedance spectroscopy (EIS); however, this technique provides information mostly about the total conductivity.

Solid-state ^{7}Li NMR spectroscopy is a good alternative to describe ion dynamics in a wide frequency range and to differentiate between bulk conductivity and the grain boundary contribution. In the current work we apply the set of NMR techniques to characterise a series of Li^+-based electrolytes (with chemical structure Li_6PS_5X, where X=Cl, Br, I and their mixtures) to reveal the one with the highest ionic conductivity.

Thermal motional narrowing as observed from quadrupolar spectra measured at different temperatures allowed qualitative characterisation of the ion mobility. Based on these experiments, we found that the chlorine containing solid electrolytes showed the onset of the dynamics at lower temperatures compared to iodine-based counterparts.

The temperature-dependence of T_1 relaxation times, which are sensitive to the nanosecond mobility, displays a deviation from the simple BPP-theory – low-temperature and high- temperature regimes of the characteristic curves possess different slopes. Thus, two different motional modes with distinct activation energies were visible already from T_1 analysis. The low-temperature activated process might be attributed to the very local motions between neighbouring sites, while the process with a higher activation energy found to be significant for translational diffusion of ions in the bulk. The latter process also becomes apparent in $T_{1\rho}$ analysis. Combination of the dynamical parameters with the structural data which were obtained from X-ray diffraction patterns allowed to estimate the conductivity value for each electrolyte. Electrolytes with different iodine-contents showed slower ion diffusivity. This conclusion is in line with the results of previous EIS experiments.

Self-diffusion coefficients are used to detect long-range transport of the ions and may be sensitive to the influence of the grain boundaries.

Thus, implementing the set of foregoing NMR techniques it is possible to characterise different types of ion motions in solid electrolytes, gain a deeper insight what actually influences bulk conductivity and get useful rules for further design of safer and low-cost batteries.

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Evaluation of Excitation Schemes for Use in Indirect Detection of Nitrogen-14 via Solid-State HMQC NMR Experiments

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Abstract: Nitrogen is an important element in chemical research, being commonly found in pharmaceuticals, bio-molecules and functional inorganic materials.[1] However, it is notoriously difficult to probe with solid-state NMR. ^{15}N (I = 1/2), the most commonly studied isotope, has a low gyromagnetic ratio and a very low natural abundance (NA = 0.36%), often requiring the use of expensive and difficult isotopic enrichment.[2] ^{14}N (I = 1) is quadrupolar and has an even lower gyromagnetic ratio than ^{15}N, but has a high NA = 99.64%. However, ^{14}N powder patterns are typically very broad (hundreds to thousands of kHz), making direct observation challenging.[1,3]

It has previously been shown that ^{14}N spectra can be reliably obtained through indirect detection *via* the HMQC experiment. This method exploits the transfer of coherence between single- (SQ) or double-quantum (DQ) ^{14}N coherences, and SQ coherences of suitable "spy nuclei" with spin S = 1/2, (*i.e.*, ^{1}H or ^{13}C).[4] It must be noted that SQ-SQ methods require a particularly optimised set-up to minimise the first-order quadrupole interaction (perfectly adjusted magic angle and stable spinning speed), whereas DQ-SQ ones do not. In this work, the efficiency of three ^{14}N excitation schemes (DANTE,[5] XiX,[6] and Selective Long Pulse (SLP)[7]) are compared using SIMPSON[8] simulations and either SQ-SQ or DQ-SQ ^{1}H-{^{14}N} *D*-HMQC experiments on L-histidine HCl and N-acetyl L-valine (NAV) at 18.8 T and 62.5 kHz MAS. The results obtained demonstrate that both DANTE and SLP provide a wider and more efficient ^{14}N excitation profile than XiX. Furthermore, it is shown that the SLP scheme is efficient, highly robust to offset and pulse length, and is simple to calibrate. These factors make SLP ideally suited to widespread use in solid-state NMR analyses of nitrogen-containing materials.

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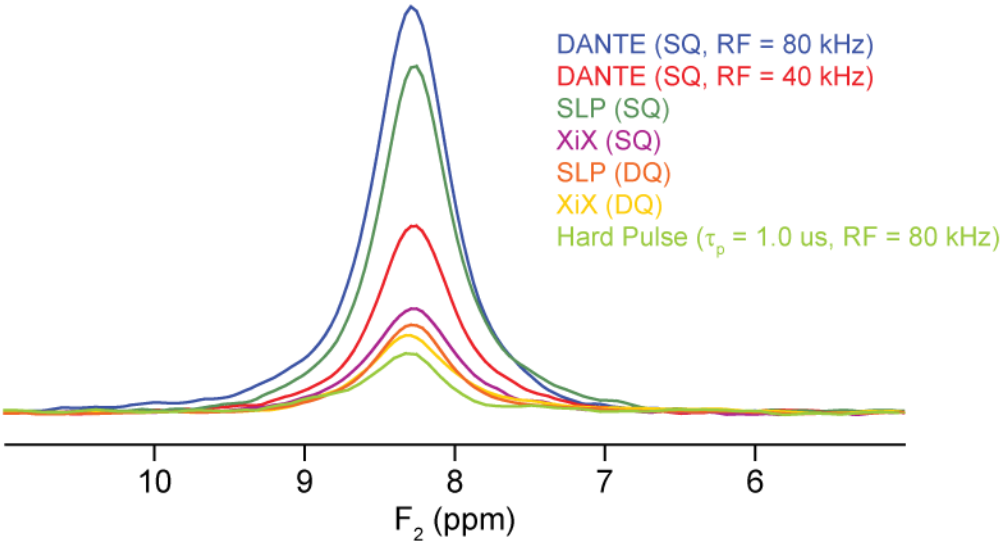

***Figure 1**. Overlaid F_2 (^1H, NH_3 resonance) slices extracted from 2D D-HMQC spectra of L-histidine HCl acquired using different ^{14}N excitation schemes.*

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NMR spectroscopic studies of diluted ionic liquids as electrolytes for EDLCs.

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Abstract: The need of resource-efficient technologies and the increased production of regenerative energy drives the development of novel energy storage devices. Electrical Double Layer Capacitors (EDLC or supercapacitors) are advantageous if high amounts of energy should be rapidly stored or delivered. Based on the principles of physisorption, they exhibit a far longer cycle lifetime than other energy storage devices. Generally, the EDLCs consist of electrodes manufactured from porous materials and electrolytes. The latter may be organic electrolytes or ionic liquids. The understanding of processes taking place in the pores of electrodes is very important for the future development of supercapacitors. In our research, we focus on the investigation of ionic liquids as promising electrolytes for modern EDLCs. Electrode-electrolyte interaction and the ion mobility inside the pores are studied by solid-state NMR spectroscopy[1].
As electrode material, commercially available YP50F and a well-defined synthetic material, OM-CDC, were chosen. In the latter, micro and mesopores are well interconnected in a hierarchical manner. 1-ethyl-3-methylimidazolium tetrafluoroborate ($EmimBF_4$) was selected as electrolyte. Using ^{11}B, ^{1}H and ^{2}H MAS NMR spectroscopy, the different electrolyte species could be studied selectively. Line shape analysis of 1D spectra and 2D EXSY NMR spectroscopy allowed to characterize the ion mobility and diffusion processes.
The samples were loaded with defined amounts of $EmimBF_4$, corresponding to the pore volume of those materials. Not all materials were able to accommodate the electrolyte completely. For sterical reasons, some $EmimBF_4$ remains outside as free bulk. Quantitative spectra evaluation thus allows to determinate the accessible internal pore volume of the carbon materials. The mobility of ions is slow. This affects the working characteristics of EDLC negatively and diminishes the amount of energy that could be stored[2]. The dilution of electrolyte with acetonitrile enhances the mobility, but also decreases the number of electrolyte ions in the pores, what also scales down the capacitance[3]. For the determination of optimal dilutions, NMR-spectroscopy as well as electrochemical characterisation were used. It was shown, that dilution up to 60% enables on the one hand the complete penetration of electrolyte in the pores and on the other hand helps to keep the overall capacitance high. The exchange rates between adsorbed species and free bulk were calculated from 2D EXSY experiments with an excess of electrolyte at the optimum dilution.

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Introducing Tritium (3H) MAS NMR in biosolids: application to crystalline diphenylalanine

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Abstract: Tritium (^{3}H) possesses the highest gyromagnetic ratio of the periodic table and its negligible natural abundance (3×10^{-16} %) ensures that no background signal is observed, in contrast to ubiquitous proton (^{1}H), so that one can work at a very low concentration (in the present work less than 0.1%) while maintaining a very good sensitivity. Despite these appealing features, only very few ^{3}H MAS NMR study has been reported so far [1], probably due to the safety issues that need to be carefully addressed for rotating a radioactive sample. We have recently developed an appropriate NMR instrumentation (using a DOTY $^{3}H/^{1}H/X$ XC4 probehead) and demonstrated the feasibility of ^{3}H MAS NMR experiments on model compounds: distance measurements up to 14Å could be reached and medium-range distances (4-8Å) could be determined with an unprecedented resolution (0.02Å) [2]. In this work, we extend these experiments to a relevant system for biological studies, the diphenylalanine peptide which has recently attracted great attention for the development of DNP-based ^{13}C-^{13}C distances measurements at natural abundance [3]. Reliable distances could be determined up to 7A so that our methodology can perfectly complement these experiments.

Regarding NMR methodologies, ^{1}H-^{3}H Cross Polarization was found to work very effectively and yielded a significant enhancement of the signal, mainly as a consequence of the much shorter relaxation time of protons. A moderate spinning rate of typically 8 kHz was found to be sufficient in obtaining narrow ^{3}H MAS NMR peaks with an excellent resolution of 0.01-0.02 ppm. Specificities of the strongly coupled ^{1}H-^{3}H nuclear spins system will be discussed, especially with regards to the choice of the pulse sequences for ^{3}H-^{3}H dipolar recoupling where HORROR was found to be the most efficient one. Indeed, it requires only a moderate ^{3}H RF field while strong ^{1}H decoupling can be applied, thus keeping the total RF power reasonable on the two channels which are very close in frequency. Our distance measurements were combined to Molecular Dynamics simulations and DFT computations of NMR shifts in the perspective to develop an integrated ^{3}H MAS NMR methodology to determine conformation of a small molecule self-assembled to form large-architecture.

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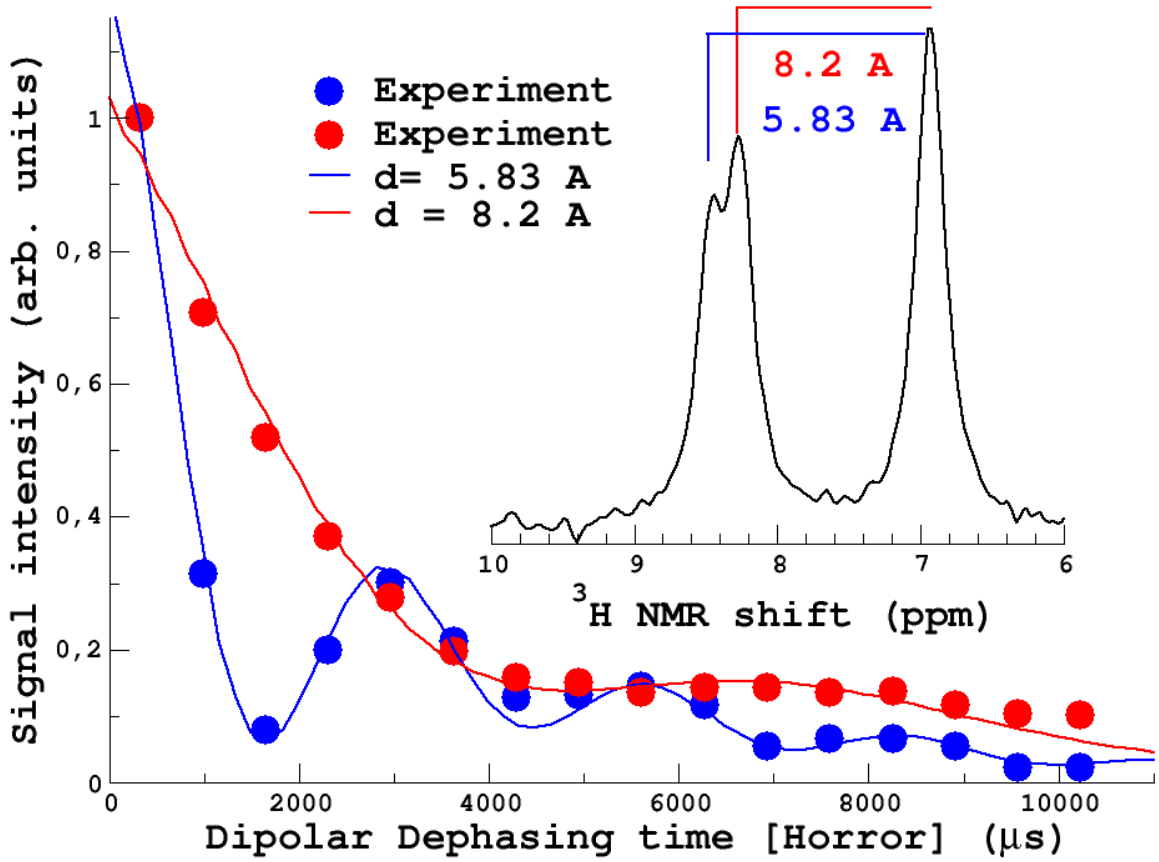
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9
8
7
6
3H NMR shift (ppm)

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Proton-detected NMR spectroscopy of nanodisc-embedded membrane proteins: MAS solid-state vs. solution-state methods

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Abstract: The structural and dynamical characterization of membrane proteins in a lipid bilayer at physiological pH and temperature and free of crystal constraints is crucial for the elucidation of a structure/dynamics – activity relationship. Towards this aim, we have explored the properties of the outer-membrane protein OmpX embedded in lipid bilayer nanodiscs using proton-detected magic angle spinning (MAS) solid-state NMR at 60 and 110 kHz. CP-based two-dimensional [^{1}H,^{15}N]-correlation spectra of sedimented OmpX in lipid bilayer nanodiscs overlay well with the corresponding solution-state NMR TROSY spectra of the same sample in solution (Figure 1).[1] Line widths as well as line intensities in solid and solution both depend critically on the sample temperature and, in particular, also on the crossing of the lipid phase-transition temperature. While for solution-state NMR temperatures above the lipid phase transition temperature are required and higher temperatures yield higher spectral intensities (because of faster overall rotational tumbling), solid-state NMR spectra can be recorded both above and below the lipid phase transition temperature, with higher intensities at lower temperatures. At 110 kHz MAS well resolved solid-state NMR spectra can be obtained also of fully protonated OmpX.

Fig. 1. Superposition of the solid-state and solution-state NMR [^{1}H,^{15}N]-correlation spectra of ^{2}H,^{13}C,^{15}N-labeled OmpX in DMPC/ DMPG lipid nanodiscs highlights their resemblance. a) The black contours are the solid-state spectrum of ca. 0.14 mg ^{2}H,^{13}C,^{15}N-labeled OmpX at 37 5 °C, while the orange contours the solution-NMR spectrum of 268 µM (equivalent to ca. 0.7 mg in the NMR active volume) ^{2}H,^{13}C,^{15}N-labeled OmpX at 45°C. The assignment was taken from [2]. **b**) Two different ribbon representations of the solution-state NMR structure of OmpX (2M06),[2] with residues assigned in the solid-state spectrum coloured in blue.

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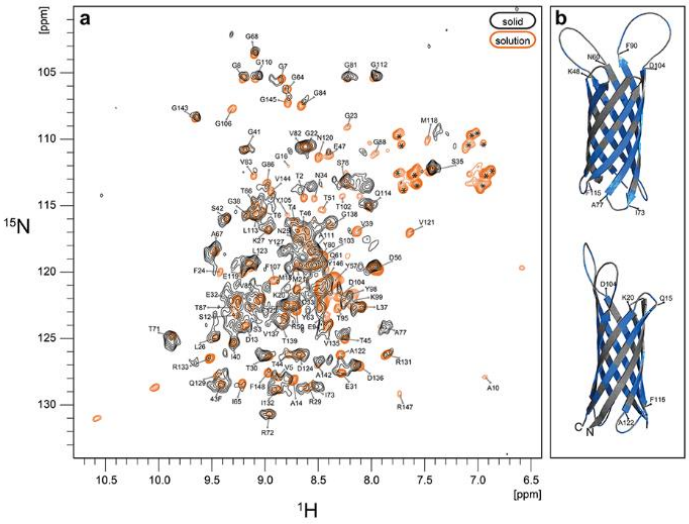

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SATURN: Satellite Transition Nutation of Half-Integer Quadrupolar Nuclei

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Abstract: Quadrupolar nuclei are abundant in many materials. Due to their asymmetric charge distribution, the electric field gradient at the nuclear site influences the NMR spectra of these nuclei, providing a powerful tool to study their local environment. In many practical applications spectral assignment is hampered by the occurrence of both the 1/2,-1/2 central transitions and the satellite transitions in a spectrum. This is particularity difficult for systems experiencing larger quadrupolar interactions combined with a certain degree of structural disorder. In these cases, line widths exceed the range where MAS is effective and therefore other techniques are needed for spectral assignment. One way to do this is by using a stepped acquisition (VOCS) experiment, and measure the whole signal manifold at different magnetic field strengths. If the observed linewidth is due to the second order quadrupolar coupling (i.e. is a central transition), it will scale inversely proportional to the magnetic field. While effective, this experiment takes excessive amounts of measurement time.

To countermand these problems, we have developed a new experiment to easily distinguish between satellite and central transitions. The experiment relies on the difference in pulse length behaviour (nutation) for the central and satellite transitions in a quadrupole system. When such a nutation experiment is measured in a 2D fashion, distinctive patterns for central or satellite transitions emerge, which can be used to identify the relative contribution of satellite/central signal in specific area of the spectrum, even in cases with strong distributions of the quadrupolar parameters. As the methods relies on RF field strength instead of MAS speed, a broader range of quadrupole coupling constants can be accessed.

In this work, we present an in-depth description of the SATURN experiment for I = 3/2 up to I = 9/2 nuclei, showing experimental data of model compounds that closely match the simulations. Finally, we show useful applications for spectra that display either very large quadrupolar interactions, such as Ziegler-Natta catalysts, but also for systems with small quadrupolar interactions such as ^{7}Li in battery materials.

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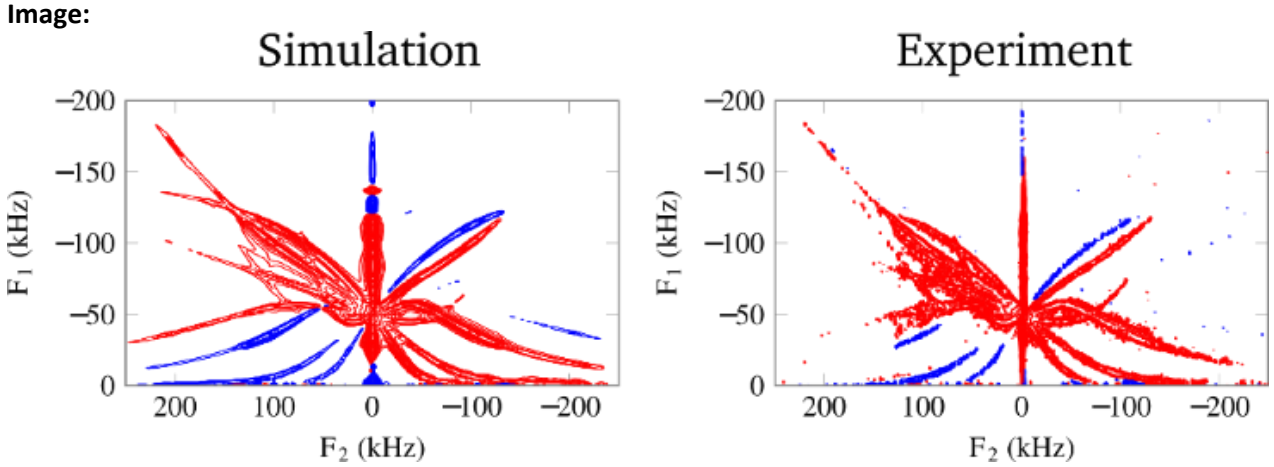

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PIETA and frequency-swept pulses: Highlight of coherence pathways and spectral distortions in WURST-CPMG experiment

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Abstract: Recording broad NMR line-shapes such as static NMR spectra of quadrupolar nuclei often represents a challenging task: the signal intensity is spread on a large frequency range leading to a low sensitivity, and the width of the spectra often exceeds the pulse bandwidth.

Among the different experimental strategies which can be applied to record such broad NMR spectra L.A. O'Dell and R.W. Schurko[1] have proposed to combine frequency-swept echoing pulses[2] with the CPMG experiment.[3] While the echo train acquisition with CPMG experiment can lead to large sensitivity enhancement, the use of frequency-swept pulses overcomes the pulse bandwidth limitation. The recommended experimental conditions are identical excitation and refocusing pulse durations and sweep rates. The radiofrequency field strength are also recommended to be equal for excitation and refocusing, and optimized experimentally by visual inspection of the Fourier transformed spectrum.

In this study, we used the PIETA method[4] combined with WURST pulses to highlight the different coherence pathways experienced by the coherences giving rise to each recorded echoes. We show that the experimental conditions which visually lead to the best NMR line-shape actually correspond to a stimulated echo train. Moreover, the formation of "Hahn" echoes (coming from $\Delta p = \pm 2$) should be avoided since the coherences coming from $p = +1$ and $p = -1$ pathways experience different phase. The presence of these types of echoes in the stimulated echo train produces strong spectral distortions.

Therefore, the PIETA experiment should lead to an easy way to select echoes from coherences which have experienced the same phase. This selection would lead to a NMR line-shape closer to the "ideal" line-shape which is needed to measure accurately NMR parameters from line-shape analysis.

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Solid-state NMR methods

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A novel labelling method to introduce alpha-protons in deuterated proteins

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Abstract: Here, we introduce a new labelling scheme to efficiently incorporate the H_{alpha} proton in an otherwise fully deuterated protein, using standard E. Coli expression. Introduction of tailored isotopic labelling strategies have become an important factor in protein NMR to overcome fundamental limitations. In solution NMR, to study large proteins (above ~30kDa) requires high deuteration levels to extend relaxation times. Similarly, in the solid state, proton detected NMR became possible though partial deuteration, most often implemented with deuteration during growth, and exchange of amide protons, which are then detected. Combined with fast magic-angle spinning (MAS) rates of ~60 kHz, this is a highly effective strategy. However, protons in the sidechain are then not detected, which has motivated a further increase in MAS rates to >100 kHz. Alternatively, tailored labelling can be applied to reintroduce side-chain protons, either using methyl precursors, synthetic amino acid sources, or combinations of 1H/2H labelled glucose and water.[1,2] [3] So far, access to alpha protons (using E. Coli expression) has come with significant drawbacks due to the mixed isotopomers that occur using carbon sources such as glucose or glycerol.

Here we introduce a method that improves the control over the labelling patterns, as compared with glucose, while maintaining compatibility with E. Coli expression.

The method is applied to challenging systems like membrane proteins in lipid bilayer preparations, providing an additional proton reporter in the backbone. We also expect the method to be useful for relaxation studies in both solids and solutions. The figure (left) demonstrates successful introduction of the alpha position for most residue types, with near complete deuteration of other aliphatic protons using ubiquitin. At right, we show an intense alpha-alpha contact. The method has the advantage that growth occurs using an H_2O medium, such that H^N is also labelled, problems with solvent exchange are avoided, and adaptation to D_2O is not required. We show an improvement in structure determination using the additional contacts available from the technique.

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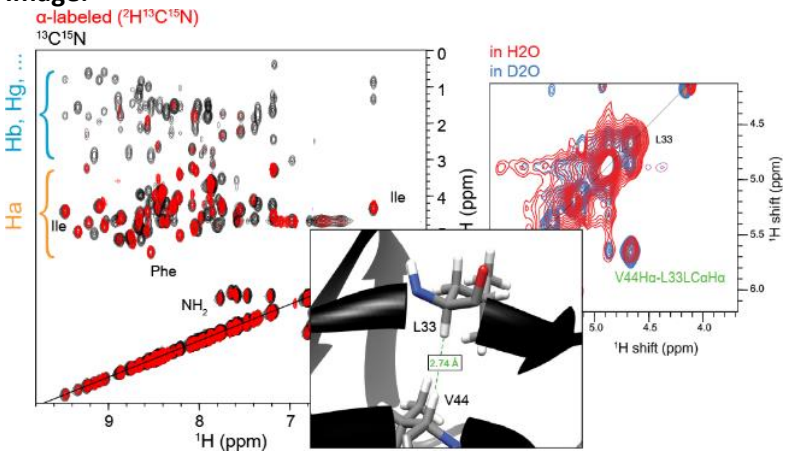
Image:



Solid-state NMR methods

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Improving solid-state NMR sensitivity using instrumentation, fast acquisition and post-processing

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Abstract: Among spectroscopies, Nuclear Magnetic Resonance (NMR) is a very precise local probe. However, its **poor sensitivity** is a major drawback, as only one nucleus over 10^5 can be detected. In solid-state NMR, the absence of fast molecular motion leads to considerable peak broadening. In such case, it is especially difficult to have sufficient signal-to-noise ratio (SNR) for quantitative results. In this work, three different innovative strategies have been implemented to improve the sensitivity limit: **instrumentation, fast acquisition and post-processing**.

First, instrumentation can be used to increase the signal strength as close as possible from the sample. For this, we focussed on **Magic Angle Coil Spinning (MACS)** [1], which is a microcoil placed inside a standard rotor, spun at Magic Angle Spinning (MAS). Such a design is especially convenient for very **small sample quantities**, around 100 µg. This corresponds for instance to the amount available when scratching a thin film deposited on a substrate. By electromagnetic coupling between the probe coil and the microcoil, it is possible to increase the SNR per unit of time by 10-100.

Secondly, fast acquisition techniques are actually under strong development. One of them, **Non-Uniform Sampling (NUS)** is based on the assumption that spectra are sparse. It is thus not necessary to respect the Nyquist-Shannon theorem during multi-dimensional spectra acquisition. Using reconstruction algorithm, data can be classically analysed. We successfully applied it to **solid-state NMR** [2], where the difficulty is that spectra are not so sparse. An improvement of 2 per unit of time on two-dimensional spectra has been obtained.

Third, when poor spectra are obtained after a week of acquisition, the only solution is to apply post-processing. We used a **denoising** algorithm named Singular Value Decomposion (SVD). Our study proved that while Lorentzian peaks are correctly denoised, Gaussian peaks need to be taken with care. Moreover, SVD is a computational intensive task. We optimised it using **performant algorithms and graphic cards** [3]. A computation time reduction of 100 and a SNR gain of 3 per unit of time have been possible.

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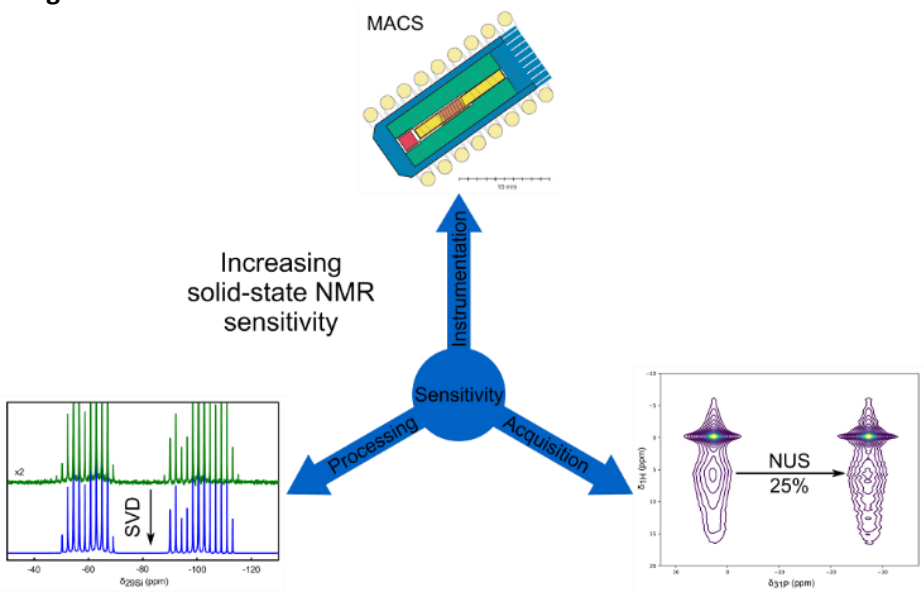
MACS
Increasing
solid-state NMR
sensitivity
Instrumentation
Sensitivity
Processing
Acquisition
SVD
NUS
25%

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Solid-state 31P NMR: a tool to characterize interactions between phosphates and titanium dioxide.

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Abstract: Titanium dioxide (TiO_2) is widely used as a white pigment in various applications such as paint, cosmetics, and also in food products, especially for the coating of chewing-gums or sweets (code number: E171). Recently, it has been shown that TiO_2 could induce the development of preneoplastic lesions in the colon of rodents [1], and it is thus necessary to perform studies in order to evidence the potential action of this compound on the biological systems.
In this context, we have initiated a systematic study of the interaction between TiO_2 (anatase form) and various phospholipids [2], and we have shown that some of them could link on the anatase surface, probably through chemical bonds. However, we could not at this stage bring evidence on the kind of chemical bonds involved. Therefore, our present work deals with the interaction between anatase and phosphate ions, in order to get a signature of the bonds between the phosphate head polar group of phospholipids and the surface of TiO_2. This work is performed through zeta potential measurements, adsorption isotherms and spectroscopic tools, such as infrared and ^{31}P solid-state NMR.
The first results show that phosphate ions are adsorbed on the TiO_2 surface in large amounts in acidic medium, this amount decreasing when pH increases. This behavior suggests that the interaction is mainly controlled by electrostatics. However, as revealed by zeta potential measurements, a strong interaction occurs even against electrostatic repulsions, leading to modification of the isoelectric point of TiO_2 in the presence of phosphates. Solid-state ^{31}P NMR and infrared spectra exhibit various distinct chemical environments for phosphorus atoms, which could correspond mainly to bidentate species adsorbed on the Ti-OH groups of the anatase surface. Work is in progress to assign each band and elucidate the structure of complexes between phosphates and surface groups of TiO_2.
This study will not only contribute to characterize the interactions between TiO_2 and phospholipids, but also to better understand the reactivity of E171 compound used in food, since on one hand its surface is partially covered by phosphate units [3], and on the other hand phosphate ions are usually present in high amount both in food products and in the digestive tract.

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Combining segmental and specific labelling with DNP NMR to study amyloid proteins

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Abstract: Self-propagating changes in the conformation of amyloidogenic proteins play vital roles in normal biology and disease. The yeast prion protein, Sup35NM, is a self-propagating amyloid. Despite intense study, there is no consensus on the organization of monomers within Sup35NM fibrils. Some studies point to a beta-helical arrangement while others suggest a parallel-in-register organization. Intermolecular contacts are often determined by experiments that probe long-range heteronuclear contacts for fibrils templated from a 1:1 mixture of ^{13}C and ^{15}N labeled monomers. However, for Sup35NM, like many large proteins, chemical shift degeneracy limits the usefulness of this approach. Segmental and specific isotopic labeling reduce degeneracy, but experiments to measure long range interactions are often too insensitive. To limit degeneracy and increase experimental sensitivity, we combined specific and segmental isotopic labeling schemes with dynamic nuclear polarization (DNP) NMR. We segmentally isotopically label not only the terminal regions of the proteins but also internal regions using pairs of split inteins with orthogonal reactivates. We specifically label tyrosines to examine the role that aromatic ring stacking in the amyloid core. Using this combination we examined an amyloid form of Sup35NM that does not have a parallel in-register structure. The combination of a small number of specific labels with DNP NMR enables testing of structural models for systems that were previously impossible due to low experimental sensitivity.

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Sensitivity enhancement in multidimensional Solid State NMR using Optimal Control optimized shaped pulses.

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Abstract: Biological solid-state NMR is an important technique for studying large protein complexes and aggregates as well as membrane proteins. Solid-state NMR experiments are, however, intrinsically insensitive in particular for more complex pulse schemes. Inhomogeneous radiofrequency profiles inherently produce a volume selectivity of the employed Hartmann-Hahn contact pulses. Critically, spinning the sample also induces time dependent changes of both RF amplitude and phase experienced by a specific portion of the sample. Here, we present a refinement of Optimal Control methodology and its application in pulse sequence design for improved dipolar recoupling in MAS solid-state NMR studies. In our simulations, we take into account the time-dependence of the RF field in the course of a MAS experiment. Using Optimal Control, we produce pulses for cross-polarization coherence transfer that display up to 100% increased signal intensity compared to that of traditional ramped amplitude pulses. In addition, we demonstrate that these pulses can be used for a range of MAS rates with little loss in efficiency. Despite being optimized for a specific spinning rate, the optimized sequences can be adapted to different MAS frequencies by time-stretching, given that rotor synchronization is maintained. Pulses optimized for high transfer efficiencies accounting for RF inhomogeneity therefore hold a great potential to drastically reduce the measurement time required for multi-dimensional solid-state NMR experiments.

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Recording 13C-15N HMQC 2D spectra in solids in 30 sec

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Abstract: Presently, nearly all the two-dimensional (2D) spectra observed in Nuclear Magnetic Resonance (NMR) are recorded with the same principle: the acquisition during a time, t_2, of a series of signals (free induction decays or FIDs) for numerous evolution times, t_1, during which the spin system is subject to a selected interaction. We propose a Hadamard encoded dipolar HMQC experiment for fast 2D correlation of abundant nuclei in solids. The D-HMQC sequence has been derived from the scalar J-HMQC scheme used with liquid samples, by introducing two symmetrical dipolar recoupling parts [1]. As a result, instead of the through-bond correlations achieved in liquids, the D-HMQC sequence allows performing through-space analyses in solids. Most of the time, dipolar interactions are much larger than scalar couplings, and therefore, D-HMQC allows using much shorter recoupling times than J-HMQC, which decreases the large losses encountered in solids. As a test sample, we have chosen the fully ^{13}C and ^{15}N labeled f-MLF tri-peptide, N-formyl-Met-Leu-Phe.

The conventional pulse sequence with ^{13}C detection, called ^{13}C-{^{15}N} D-HMQC, is shown in Fig. 1a. The multi-selective version with [H_n] Hadamard encoding [2], called ^{13}C-{^{15}N} D-HMQC-H_n in the following, is shown in Fig. 1b. It is derived from Fig.1a by fixing t_1 = 0, and by replacing the two pi/2 ^{15}N pulses with a multi-selective Gaussian pi-pulse on/off irradiation. The experiments were performed at on a 600 MHz AV-III-HD Bruker NMR spectrometer, equipped with a HCN Bruker MAS probe with a 1.9 mm rotor spinning at n_R = 13 kHz. The multi-selective Hadamard encoded excitation was generated with the WaveMaker (wvm) module available within the Topspin software.

The ^{13}C-{^{15}N} D-HMQC and D-HMQC-H_4 spectra are shown in Figure 1 (c) and (d), respectively. The conventional spectrum (left) was acquired in 22 minutes, but at least 1 hour would have been required to achieve an adequate resolution along F1. In comparison, the Hadamard spectrum, shown on the right, required only 30 sec.

The main "bottleneck" of Hadamard encoding experiments resides in the selective excitation of each indirectly detected resonance. We have analyzed this limitation in solids in more detail by recording four 2D ^{13}C-{^{15}N} D-HMQC-H_4 spectra for various t_{sel} values. As expected the optimum length for the excitation ($t_{sel,opt}$ ~ 3 *ms*) is a compromise in between the selectiveness of the excitation and the signal intensity losses.

To finish this analysis, we have recorded a series of 2D ^{13}C-{^{15}N} D-HMQC-H_4 spectra with the aim to obtain the build-up curves of the five cross-peaks versus t_{rec}. This, required recording thirteen 2D spectra, which took only *ca.* 10 min of experiment time. Obtaining this information with conventional 2D methods is typically quite time consuming.

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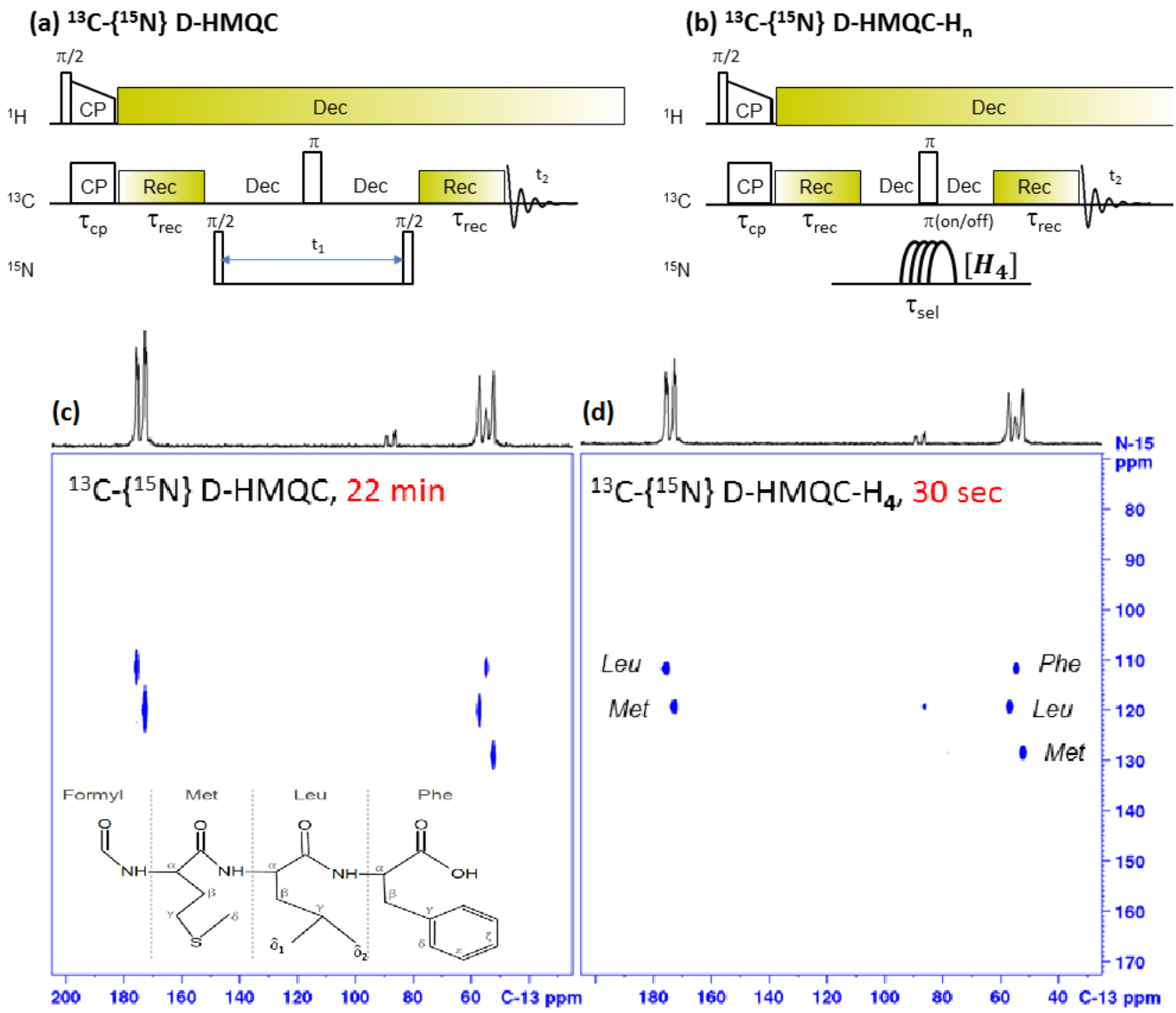
Image:



Disclosure of Interest: E. Kupce Conflict with: none, J. Trébosc Conflict with: none, B. Perrone Conflict with: none, O. Lafon: None Declared, J.-P. Amoureux: None Declared

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Structural of the active site of a paramagnetic metalloprotein by fast magic-angle spinning NMR

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Abstract: The presence of metals containing unpaired electrons represents a severe issue in solid-state NMR: signals with large shifts and shift anisotropies are spread over huge spectral windows, possess extremely short relaxation times, and are broadened by inhomogeneous susceptibility effects.

We illustrate how a new set of NMR experiments, recently developed for the study of complex paramagnetic inorganic battery materials, can be adapted to the solid-state NMR analysis of paramagnetic metalloproteins, improving the information obtainable from these systems. These experiments combine ultra-fast (60-111 kHz) magic-angle spinning, short high-powered adiabatic pulses (SHAPs), and short recoupling schemes.

Here, we apply the approach to the uniformly ^{1}H, ^{13}C,^{15}N-labeled microcrystalline metalloenzyme superoxide dismutase, which has two high-affinity binding sites for metal cations. In combination with first-principles paramagnetic NMR calculations, we are able to detect, characterize, and assign ^{1}H, ^{13}C and ^{15}N signals from residues directly coordinating the metal centers. The obtained contact shifts are extremely sensitive to the fine details of the metal ion coordination, and constitute a powerful set of restraints for the refinement of X-ray structures and the determination of the structure of the metal site at sub-atomic resolution.

The present work represents a robust approach to the NMR study of paramagnetic metalloproteins, opening a new avenue for the study of the structure and the reactivity of metal centers in complex insoluble systems.

Image: