PROGRAM AND ABSTRACTS FOR 2019 ANNUAL MEETING OF THE SOCIETY FOR GLYCOBIOLOGY

November 2–5, 2019
Phoenix, AZ, USA

SFG MEETING 2019

DAY 1: Saturday, Nov 2, 2019

8:00 AM – 6:00 PM	**Registration** Ballroom Foyer
9:00 AM – 5:00 PM	**Satellite 1: Tools in Glycoscience**
Session chair:	**Richard Cummings** (Harvard Medical School) & Robert Woods (CCRC) Maricopa Room
9:00AM – 1:00 PM	**Satellite 2: Glyco in Biotechnology**
Session chair:	**Parastoo Azadi** (University of Georgia, CCRC) Pima Room
12:00 PM – 4:00 PM	**Board of Directors Meeting** (by invitation only) Gila Room
5:30 PM – 7:15 PM	**Opening Meyer and Kornfeld Awards Lectures** North Ballroom
5:30 PM – 5:45 PM	**Conference Opening Remarks**
5:45 PM – 6:30 PM	**Karl Meyer Award Lecture:** Robert Linhardt (Rensselaer Polytechnic Institute)
6:30 PM – 7:15 PM	**Rosalind Kornfeld Award Lecture:** Nancy Dahms (Medical College of Wisconsin)
7:30 PM – 9:30 PM	**Welcome Reception & Exhibits** Pueblo Ballroom

DAY 2: Sunday, Nov 3, 2019

7:30 AM – 2:00 PM	**Registration** Ballroom Foyer
7:30 AM – 8:30 AM	**Continental Breakfast** Ballroom Foyer

8:30 AM – 10:10 AM Session 1: Glycans and Evolution

Session chair:	**Christopher West** (University of Georgia, BCMB) North Ballroom
8:30 AM – 8:55 AM	**Pascal Gagneux** (UC San Diego) *The Runaway Self" – Changing while remaining the same.*
8:55 AM – 9:20 AM	**Marco Sardiello** (Baylor College of Medicine) *A Stepwise Mechanism for ER-to-Golgi Transport of Lysosomal Enzymes.*
9:20 AM – 9:45 AM	**Jerry Eichler** (Ben Gurion University of the Negev, Israel) *N-glycosylation in Archaea: Extremely creative.*
9:45 AM – 9:50 AM	**Poster talks: Rahil Taujale** (University of Georgia) *Understanding the sequence-structure-function relationships through a comprehensive evolutionary analysis of GT-A fold glycosyltransferases. #4/B001*
9:50 AM – 9:55 AM	**Poster talks: Alan John** (University of Melbourne) *Deciphering the molecular functions of tryptophan C-mannosylation. #5/B002*
9:55 AM – 10:00 AM	**Poster talks: Ishita Chandel** (Texas A&M University) *Functional players of Protein O-mannosyltransferases 1/2 –mediated regulation of sensory neuron connectivity in Drosophila. #6/B003*
10:00 AM – 10:05 AM	**Poster talks: Camilo Perez** (University of Basel, Biozentrum) *Structure and mechanism of a pH sensing lipoteichoic-acid-anchor flippase. #7/B004*
10:05 AM – 10:10 AM	**Poster talks: Sudeshna Saha** (University of California San Diego) *Exploring Evolutionary Origins of Human-Specific CD33/Siglec-3 Alleles that Protect against Late Onset Alzheimer's Disease: Prior Selection by Uniquely Human Pathogens?*
10:10 AM – 10:30 AM	**Coffee Break** Ballroom Foyer

10:30 AM – 12:10 PM Session 2: Glycobiology of the Microbiome

Session chair:	**Christine Szymanski** (University of Georgia, MB) North Ballroom
10:30 AM – 10:55 AM	**David Gerlach** (University of Tübingen, Germany) *Staphylococcus aureus remodels surface glycopolmers to shape colonization and invasion capacities.*
10:55 AM – 11:20 AM	**Thierry Hennet** (University of Zurich, Switzerland) *Prebiotic action of dietary and mucosal carbohydrates on the gut microbiota.*
11:20 AM – 11:45 AM	**Katharina Ribbeck** (Massachusetts Institute of Technology) *Mucin glycans attenuate microbial virulence.*
11:45 AM – 11:50 AM	**Poster talks: Atossa C. Ghorashi** (University of Texas Southwestern Medical Center) *Characterizing the role of host fucose in cholera toxin action.*
11:50 AM – 11:55 AM	**Poster talks: Anna Blenda and Nourine Kamili** (USC School of Medicine Greenville and Emory University School of Medicine) *Distinct antimicrobial properties of the N- and C- terminal domains of the human protein galectin-9.*
11:55 AM – 12:00 PM	**Poster talks: Joanna Coker** (University of California San Diego) *Bacterial community manipulation through glycan-lectin interactions.*
12:00 PM – 12:05 PM	**Poster talks: Sun-Mi Choi** (University of California San Diego) *Staphylococcus Aureus Exacerbates Epithelial Barrier Dysfunction in Chronic Rhinosinusitis.*
12:05 PM – 12:10 PM	**Poster talks: Mathias Braun** (University of Natural Resources and Life Sciences, Vienna) *A conserved glycosyltransferase from the general protein O-glycosylation pathway of Bacteroidetes.*
12:10 PM – 12:15 PM	**Poster talks: Sohyoung Lee** (Cornell University) *Host adaptations of the Salmonella Typhi typhoid toxin and its orthologue from a nontyphoidal Salmonella*
12:15 PM – 1:30 PM	**Lunch on your own**
12:15 PM – 1:30 PM	**Glycobiology Editorial Board Meeting** (by invitation only) Salons 7 & 8
1:30 PM – 4:00 PM	**Poster Session I and Exhibits** Pueblo Ballroom

4:00 PM – 5:45 PM Session 3: Glycotechnology, a translational perspective

Session chair:	**Parastoo Azadi** (University of Georgia CCRC) North Ballroom
4:00 PM – 4:25 PM	**Max Crispin** (University of Southampton) *Glycosylation of enveloped viruses: structure and immunogen design.*
4:25 PM – 4:50 PM	**Laurence Mulard** (Institut Pasteur, France) *Synthetic oligosaccharide-based conjugate vaccines against shigellosis: from concept and design to first-in-human study.*
4:50 PM – 5:15 PM	**Fredrik Zetterberg** (Galecto Biotech, Denmark) *Translational aspects of drug discovery and development: TD139 case story, a small molecule Galectin 3 inhibitor in phase 2b trials against IPF.*
5:15 PM – 5:20 PM	**Poster talks: Stacy A. Malaker** (Stanford University) *Enzyme toolkit for selective enrichment and analysis of mucin-domain glycoproteins*
5:20 PM – 5:25 PM	**Poster talks: Uriel Ortega-Rodriguez** (University of Texas at El Paso) *Structural characterization of T. cruzi Epimastigote Glycosylphosphatidylinositol-Mucin sialoglycans*
5:25 PM – 5:30 PM	**Poster talks: Sriram Neelamegham** (State University of New York- Buffalo) *Tuning metabolic decoy efficacy by modifying the linkage between carbohydrate and aglycone*

5:30 PM – 5:35 PM	**Poster talks: Manuela Mally** (LimmaTech Biologics AG, Switzerland) *CustomGlycan: A novel platform for production of therapeutics*
5:35 PM – 5:40 PM	**Poster talks: Jonathon E. Mohl** (University of Texas El Paso) *Identification and design of transferase specific mucin-type O-glycosylation peptides using ISOGlyP's selective peptide function*
5:40 PM – 5:45 PM	**Poster talks: Akshi Singla** (Texas A&M University) *Glycolipid-based targeted drug delivery system against multidrug resistant Pseudomonas aeruginosa*
5:45 PM – 6:45 PM	**Innovator Award Lecture** North Ballroom Gerald Hart (Complex Carbohydrate Research Center, UGA)

DAY 3: Monday, Nov 4, 2019

8:00 AM – 2:00 PM	**Registration** Ballroom Foyer
7:30 AM – 8:30 AM	**Continental Breakfast** Ballroom Foyer

8:30 AM – 10:15 AM Session 4: Systems Biology approaches to Glycobiology

Session chair:	**Thierry Hennet** (University of Zurich) North Ballroom
8:30 AM – 8:55 AM	**Morten Thaysen-Andersen** (Macquarie University) *Uncovering New Aspects of Neutrophil Glycobiology using Glyco(proteo)mics.*
8:55 AM – 9:20 AM	**Nathan E. Lewis** (UC San Diego) *The cellular impact of glycoengineering.*
9:20 AM – 9:45 AM	**Ryan Weiss** (UC San Diego, Esko-Laboratory) *Genome-wide Regulation of Heparan Sulfate Assembly.*
9:45 AM – 9:50 AM	**Poster talks: Ronghu Wu** (Georgia Institute of Technology) *Mass Spectrometry-Based Chemical and Enzymatic Methods for Global Analysis of Protein Glycosylation in Complex Biological Samples*
9:50 AM – 9:55 AM	**Poster talks: Benjamin L. Schulz** (The University of Queensland) *SWATH glycoproteomics to interrogate post-translational modification dynamics in yeast and sparkling wine*
9:55 AM – 10:00 AM	**Poster talks: Yusen Zhou** (University at Buffalo, SUNY) *Integrating Mass Spectrometry and RNA-Seq data for Glycosylation Pathway Generation and Simulation*
10:00 AM – 10:05 AM	**Poster talks: Steve M. Fernandes** (Johns Hopkins University School of Medicine) *Comparative sialoglycan microarray analyses of selected human and mouse Siglecs*
10:05 AM – 10:10 AM	**Poster talks: Kiyoko F. Aoki-Kinoshita** (Soka University) *The GlyCosmos Portal as a part of the GlySpace Alliance: towards an international glyco-data science collaboration environment*
10:10 AM – 10:15 AM	**Shu Zhang** (Zhongshan Hospital, Fudan University) – *N-glycopeptide signatures of IgA2 in serum from patients with hepatitis B virus-related liver diseases.*
10:15 AM – 10:30 AM	**Coffee Break** Ballroom Foyer

10:30 AM – 12:10 PM Session 5: Regulatory functions of glycans

Session chair:	**Lance Wells** (University of Georgia, BCMB) North Ballroom

10:30 AM – 10:55 AM	**Jürgen Lassak** (Ludwig-Maximilians University Munich, Germany) *An odd couple? – Arginine and rhamnose form a novel glycoconjugate to rescue bacterial translation.*
10:55 AM – 11:20 AM	**John Allan Hanover** (National Institutes of Health) *Critical roles for O-GlcNAc in Metabolic Signaling, Stem Cell Biology, and DNA damage repair.*
11:20 AM – 11:45 AM	**Natasha E. Zachara** (Johns Hopkins School of Medicine) *Decoding the role of intracellular glycosylation in cytoprotection and disease.*
11:45 AM – 11:50 AM	**Poster talks: Yanzhuang Wang** (University of Michigan) *GRASP55 senses energy and nutrient deprivation through O-GlcNAcylation to promote autophagosome-lysosome function.*
11:50 AM – 11:55 AM	**Poster talks: Adam J. Kanack** (Medical College of Wisconsin) *Platelet and myeloid cell phenotypes in a rat model of Fabry disease*
11:55 AM – 12:00 PM	**Poster talks: Brett E. Crawford** (BioMarin Pharmaceutical) *Discovery of novel ceramide galactosyltransferase inhibitors and their therapeutic application to Krabbe disease*
12:00 PM – 12:05 PM	**Poster talks: Seung Yeop Han** (Baylor College of Medicine) *A Conserved Role for N-Glycanase 1 in Regulating Energy Metabolism through AMPK signaling*
12:05 PM – 12:10 PM	**Poster talks: Angelica M. Gomes Ueltschy** (Cleveland Clinic) *Glucose homeostasis is regulated by hyaluronan synthases 1 and 3*
12:10 PM – 1:30 PM	**Lunch on your own**
1:30 PM – 4:00 PM	**Poster Session II and Exhibits** Pueblo Ballroom
4:00 PM – 4:45 PM	**Society Business Meeting** North Ballroom
4:45 PM – 6:15 PM	**MCP and Significant Achievement Award Lecture** North Ballroom **Manfred Wuhrer** (Leiden University Medical Center) – MCP Award Lecture **Jochen Zimmer** (University of Virginia) – Significant Achievement Award Lecture
6:15 PM – 7:00 PM	**Break**
7:00 PM – 11:00 PM	**Banquet** North Ballroom

DAY 4: Tuesday Nov 5, 2019

8:00 AM – 12:00 PM	**Registration** Ballroom Foyer
7:30 AM – 8:30 AM	**Continental Breakfast**

8:30 AM – 10:10 AM Session 6: Glycobiology of Mammalian development and Stem cells

Session chair:	**Jeffrey D. Esko** (UC San Diego) North Ballroom
8:30 AM – 8:55 AM	**Jamey Marth** (UC Santa Barbara) *Glycoprotein aging and turnover in the pathogenesis of disease*
8:55 AM – 9:20 AM	**Pamela Stanley** (Albert Einstein College of Medicine) *An Inhibitor of N-glycan Maturation in Mouse Germ Cells*
9:20 AM – 9:45 AM	**Hans Wandall** (University of Copenhagen) *Contextualized Functions of Glycans in Tissue Formation*
9:45 AM – 9:50 AM	**Poster talks: Ilhan Akan** (National Institute of Health) *Oga mutants reveal epigenetic, transcriptional and metabolic factors effecting life span and body size in Drosophila*

9:50 AM – 9:55 AM	**Poster talks: Frank Leon** (University of Nebraska Medical Center) *Role of Immature CD44 O-glycosylation and its Activation of Targets Responsible for Stemness Properties of Pancreatic Cancer*
9:55 AM – 10:00 AM	**Poster talks: Vladislav Panin** (Texas A&M University) *Role of sialylation in the control of cardiac functions in Drosophila*
10:00 AM – 10:05 AM	**Poster talks: Yang Yang** (Georgetown University) *SULF2 overexpression affects survival and modulates sulfation of heparan sulfate proteoglycans in Squamous Cell Carcinoma of the Head and Neck*
10:05 AM – 10:10 AM	**Poster talks: Yan Wang** (Cleveland Clinic) *Enhanced myofibroblast differentiation in Hyaluronan Synthase1/3 double knockout mice is independent of hyaluronan and mediated by a TGFβR/p38MAPK/MRTF pathway.*
10:10 AM – 10:30 AM	**Coffee Break** Ballroom Foyer

10:30 AM – 11:45 PM Session 7: Glycobiology of the Immune System

Session chair:	**Richard Cummings** (Harvard Medical School) North Ballroom
10:30 AM – 10:55 AM	**Jim Paulson** (Scripps Research, La Jolla) *Siglecs as checkpoints in immune cell responses*
10:55 AM – 11:20 AM	**Avery Posey** (University of Pennsylvania) *Reprogramming T cells to target glycopeptide epitopes and glycolipids for effective cancer therapy*
11:20 AM – 11:25 AM	**Poster talks: Marija Pezer** (Genos Glycoscience Research Laboratory) *Immunoglobulin G glycosylation changes in diseases and aging*
11:25 AM – 11:30 AM	**Poster talks: Damien Restagno** (University California Santa Barbara) *Glycoprotein Aging with Increased Mannose Exposure Linked to Cardiovascular Disease through the Macrophage Mannose Receptor (Mrc1)*
11:30 AM – 11:35 AM	**Poster talks: Jiaxuan Chen** (Beth Israel Deaconess Medical Center) *Resident and Elicited Macrophages Differ in Expression of their Glycomes and Lectins*
11:35 AM – 11:40 AM	**Poster talks: Vivianne I. Otto** (ETH Zurich) *The particular glycomes of lymph node lymphatic endothelia and their role in localization and activation of Siglec-1+ subcapsular sinus macrophages*
11:40 AM – 11:45 AM	**Poster talks: Melissa M. Lee-Sundlov** (Blood Research Institute, Versiti) *Megakaryocyte O-glycan sialylation regulates platelet production through interferon-secreting plasmacytoid dendritic cells*
11:45 PM – 1:30 PM	**Lunch on your own**

01:30 PM – 3:10 PM Session 8: Glycobiology of Cancer

Session chair:	**Pamela Stanley** (Albert Einstein College of Medicine) North Ballroom
1:30 PM – 1:55 PM	**Robert Sackstein** (Florida International University) *E-Selectin Ligands in Human Leukemogenesis*
1:55 PM – 2:20 PM	**Susan Bellis** (UAB School of Medicine, Birmingham) *ST6Gal-I sialyltransferase promotes pancreatic cancer progression through imparting a cancer stem cell phenotype*
2:20 PM – 2:45 PM	**Heinz Läubli** (University of Basel, Dep of Biomedicine) *Targeting the sialoglycan–Siglec axis augments antitumor immunity allowing effective PD-1 and CTLA-4 blockade*

2:45 PM – 2:50 PM	**Poster talks: Yasuyuki Matsumoto** (Beth Israel Deaconess Medical Center- Harvard Medical Center) *Identification of Novel Glycoproteins with Defined anti-Tn IgG and IgM; Applications as Tumor Diagnostic Biomarkers*
2:50 PM – 2:55 PM	**Poster talks: Rachel A. Willand-Charnley** (South Dakota State University) *Modulation of Siglec Binding Via SIAE and CASD1-An Immune Evasion Pathway for Breast and Colon Cancers*
2:55 PM – 3:00 PM	**Poster talks: Su-Ryun Kim** (Food and Drug Administration) *The Role of Core 3 β3-N-Acetylglucosaminyltransferase in Colorectal Cancer*
3:00 PM – 3:05 PM	**Poster talks: Kathrin Stavenhagen** (Beth Israel Deaconess Medical Center-Harvard Medical Center) *Endogenous Ligands Of The Mannose Receptor C-Type Lectin Domain In Cancer And Control Tissue*
3:05 PM – 3:10 PM	**Poster talks: Ryan N. Porell** (University of California San Diego) *Reprogramming the Tumor Microenvironment with Macrophage-Targeted Glycopolymers*
3:10 PM – 3:15 PM	**Closing Remarks**

SFG 2019 POSTER PROGRAM

Poster #: B051 (presented @ PS2) **"Assembly of chondroitin sulfate glycosaminoglycan-containing hypothalamic perineuronal nets contributes to the sustained antidiabetic effect of FGF1 action in the brain";** Kimberly M. Alonge[1], Zaman Mirzadeh[2], Jarrad M. Scarlett[1,3], Jenny M. Brown[1], Marie A. Bentsen[1,4], Aric F. Logsdon[5,6], William A. Banks[5,6], Gregory J. Morton[1], Thomas N. Wight[7], Miklos Guttman[8] and Michael W. Schwartz[1]; *[1]University of Washington Medicine Diabetes Institute, Department of Medicine, Seattle, WA; [2]Department of Neurosurgery, Barrow Neurological Institute, Phoenix, AZ; [3]Department of Pediatric Gastroenterology and Hepatology, Seattle Children's Hospital, Seattle, WA; [4]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; [5]Department of Geriatric Research Education and Clinical Center (GRECC), Veterans Affairs Puget Sound Health Care System, University of Washington, Seattle, WA; [6]Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA; [7]Matrix Biology Program, Benaroya Research Institute, Seattle, WA; [8]Department of Medicinal Chemistry, University of Washington, Seattle, WA* 74

Poster #: B052 (presented @ PS1) **"Hemolytic anti ABO antibodies (hemolysins) in transplant patients have a broader IgM and restricted IgG glycan recognition repertoire";** Waseem Q. Anani[1,2], Anna P. Schmidt[5], Greg A. Denomme[3,5] and Hoffmeister M. Karin[5,4]; *[1]Medical Sciences Institute, Versiti; [2]Department of Pathology, Medical College of Wisconsin; [3]Diagnostic Laboratory, Versiti; [4]Department of Biochemistry, Medical College of Wisconsin; [5]Blood Research Institute, Versiti* 75

Poster #: B053 (presented @ PS2) **"The glycosyltransferase ST^{6}Gal-I confers resistance against natural killer cell mediated cytotoxicity";** Katherine E. Ankenbauer, Andrew T. Holdbrooks, Amanda F. Swindall and Susan L. Bellis; *Department of Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham* 76

Poster #: B022 (presented @ PS1) **"The GlyCosmos Portal as a part of the GlySpace Alliance: towards an international glyco-data science collaboration environment";** Kiyoko F. Aoki-Kinoshita[1], Frederique Lisacek[2], Raja Mazumder[3] and William S. York[4]; *[1]Soka University; [2]Swiss Institute of Bioinformatics; [3]George Washington University; [4]CCRC, University of Georgia* 34

Poster #: B054 (presented @ PS1) **"Collaboration, Service and Trainings at the Complex Carbohydrate Research Center";** Stephanie A. Archer-Hartmann, Christian Heiss, Artur Muszynski, Zhirui Wang, Jiri Vlach, Ian Black, Asif Shajahan, Sara Porfirio, Nitin Supekar, Anne Gleinich, En Tzu Lu, John Tang and Parastoo Azadi; *Complex Carbohydrate Research Center, UGA, Athens, GA* 77

Poster #: B055 (presented @ PS2) **"Understanding the glycoconjugate receptors for cholera toxin: searching for alternative receptors";** Stephanie A. Archer-Hartmann[1], Han Wu[2], Atossa Ghorashi[2], Ian Black[1], John Tang[1], Jennifer Kohler[2] and Parastoo Azadi[1]; *[1]Complex Carbohydrate Research Center, The University of Georgia, Athens, GA, USA; [2]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, USA* 78

Poster #: B056 (presented @ PS1) **"GlyThyra: An accessible and high-throughput mass spectrometry-based N-glycomics platform";** Christopher Ashwood[1,2] and Rebekah L. Gundry[1,2]; *[1]University of Nebraska Medical Center, CardiOmics Program; [2]Medical College of Wisconsin, Department of Biochemistry* 79

Poster #: B057 (presented @ PS2) **"Sickle Cell Trait and Sickle Cell Disease Change the Profile of Plasma Glycan-Binding Proteins";** Heather E. Ashwood[1], Waseem Q. Anani[2], Anna P. Schmidt[1] and Karin M. Hoffmeister[1],3; *[1]Blood Research Institute, Versiti; [2]Medical Sciences Institute, Versiti; [3]Department of Biochemistry, Medical College of Wisconsin*..... 80

Poster #: B058 (presented @ PS1) **"Altered Glycosidase Activities at Physiological pH in the Pathogenesis of Sepsis";** Benjamin S. Haslund-Gourley[1,2], Peter V. Aziz[1,2,3], Douglas M. Heithoff[1,3], Julia S. Westman[1,2], Damien Restagno[1,2], Benjamin J. Lewis[1,2], Jeffrey C. Fried[4], Mai-Britt Ilse[5], Torben Lübke[5] and Jamey D. Marth[1,2,3]; *[1]Center for Nanomedicine; [2]Sanford Burnham Prebys Medical Discovery Institute; [3]Department of Molecular, Cellular, and Developmental Biology, University of California-Santa Barbara, Santa Barbara, California 93106; [4]Department of Pulmonary and Critical Care Medicine, Santa Barbara Cottage Hospital, Santa Barbara, California 93105, USA; [5]Department of Chemistry, Bielefeld University, Bielefeld D-33615, Germany* 81

Poster #: B036 (presented @ PS1) **"Resident and Elicited Macrophages Differ in Expression of their Glycomes and Lectins";** Jiaxuan Chen[1], Diane D. Park[1], Matthew R. Kudelka[1], Nan Jia[1], Carolyn A. Haller[1], Revanth Kosaraju[1], Melian Galizzi[2], Alison V. Nairn[2], Richard D. Cummings[1] and Elliot L. Chaikof[1]; *[1]Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School; [2]Complex Carbohydrate Research Center, University of Georgia;*.....56

Poster #: B009 (presented @ PS2) **"Staphylococcus Aureus Exacerbates Epithelial Barrier Dysfunction in Chronic Rhinosinusitis";** Sun-Mi Choi[1], Sandra Christiansen[1], Taylor Doherty[1], Adam DeConde[3] and Victor Nizet[2]; *[1]Department of Medicine, University of California San Diego, CA; [2]Department of Pediatrics, University of California San Diego, CA; [3]Department of Surgery, University of California San Diego* 15

Poster #: B008 (presented @ PS1) **"Bacterial community manipulation through glycan-lectin interactions";** Joanna Coker[1], Austen L. Michalak[2], Amber Hauw[3] and Karsten Zengler[4]; *[1]Biomedical Sciences Graduate Program, University of California San Diego; [2]Department of Chemistry and Biochemistry, University of California San Diego; [3]Division of Biological Sciences, University of California San Diego; [4]Department of Pediatrics, University of California San Diego* 14

Poster #: B067 (presented @ PS2) **"EndoS and EndoS-like active and inactive endoglycosidases as a framework to study antibody glycosylation in vitro and in vivo";** Mattias Collin; *Infection Medicine, Clinical Sciences, Lund University, Lund, Sweden;* 90

Poster #: B025 (presented @ PS2) **"Discovery of novel ceramide galactosyltransferase inhibitors and their therapeutic application to Krabbe disease";** Michael Babcock[1], Christina Mikulka[2], Bing Wang[1], Sanjay Chandriani[1], Sundeep Chandra[1], Yue Xu[1], Katherine Webster[1], Ying Feng[1], Alex Giaramita[1], Bryan K. Yip[1], Joseph Elsbernd[1], Melanie Lo[1], Qi Chao[1], Josh Woloszynek[1], Jerry Shen[1], Shripad Bhagwat[1], Mark Sands[2] and Brett E. Crawford[1]; *[1]BioMarin Pharmaceutical; [2]Department of Medicine, Washington University School of Medicine, St. Louis MO* 40

Poster #: B068 (presented @ PS1) **"Acidosis, Zinc, and HMGB[1] in Sepsis: A Common Connection Involving Sialoglycan Recognition";** Chirag Dhar[1,2], Shoib S. Siddiqui[1,2], Venkatasubramaniam Sundaramurthy[1,2], Aniruddha Sasmal[1,2], Hai Yu[3], Esther Bandala-Sanchez[4,5], Leonard C. Harrison[4,5], Xi Chen[3], Ding Xu[6] and Ajit Varki[1,2]; *[1]Departments of Medicine and Cellular and Molecular Medicine, University of California, San Diego; [2]Glycobiology Research and Training Center, University of California, San Diego; [3]Department of Chemistry, University of California, Davis; [4]The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; [5]Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia; [6]Department of Oral Biology, School of Dental Medicine, University at Buffalo, The State University of New York* 91

Poster #: B069 (presented @ PS2) **"Structures of DPAGT[1] give insights into glycosylation disorders and advance antibiotic development against TB";** Yin Yao Dong[2,3], Hua Wang[1], Ashley CW Pike[3], Stephen A. Cochrane[4], Sadra Hamedzadeh[1], Filip J. Wyszynski[1], Simon R. Bushell[3], Sylvain F. Royer[1], David A. Widdick[5], Andaleeb Sajid[6], Helena I. Boshoff[6], Yumi Park[6], Ricardo Lucas[1], Wei-Min Liu[1], Seung S. Lee[1], Takuya Machida[1], Leanne Minall[1], Shahid Mehmood[7], Katsiaryna Belaya[2], Wei-Wei Liu[2], Amy Chu[3], Leela Shreshtha[3], Shubhashish MM Mukhopadhyay[3], Claire Strain-Damerell[3], Rod Chalk[3], Nicola A. Burgess-Brown[3], Mervyn J. Bibb[5], Clifton E. Barry[6], Carol V. Robinson[7], David Beeson[2], Benjamin G. Davis[1] and Elisabeth P. Carpenter[3]; *[1]Chemistry Research laboratory/University of Oxford; [2]Nuffield Department of Clinical Neuroscience/University of Oxford; [3]Structural Genomics Consortium/University of Oxford; [4]School of Chemistry and Chemical Engineering/ Queen's University; [5]Department of Molecular Microbiology/John Innes Centre; [6]Tuberculosis Research Section/National Institute of Allergy and Infectious Diseases; [7]Department of Chemistry/University of Oxford* 92

Poster #: B070 (presented @ PS1) **"Toward a Genome-Wide CRISPR Screen to Elucidate the Unconventional Mechanism of Galectin Secretion";** Justin Donnelly, Simon Wisnovski, Roarke Kamber, Mike Bassik and Carolyn Bertozzi; *Stanford University* 93

Poster #: B071 (presented @ PS2) **"Mammalian lectin arrays for screening interaction of microbes with the innate immune system";** Sabine AF Jégouzo[1], Angela Holder[2], Dirk Werling[2], Maureen E. Taylor[1] and Kurt Drickamer[1]; *[1]Imperial College London; [2]Royal Veterinary College* 94

Poster #: B072 (presented @ PS1) **"Trypanosoma cruzi trypomastigote glycosylphosphatidylinositol-anchored mucins and an α-Gal-containing neoglycoprotein as Chagas disease biomarker candidates";** Igor L. Estevao[1], Uriel Ortega-Rodriguez[1], Alba Montoya[2], Luis Izquierdo[3], Julio Padilla[3], Maria-Jesús Pinazo[3], Joaquim Gascon[3], Katja Michael[2] and Igor C. Almeida[1]; *[1]Department of Biological Sciences, Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, USA; [2]Department of Chemistry and Biochemistry, Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, USA; [3]ISGlobal, Barcelona Institute for Global Health Hospital Clínic-Universitat de Barcelona, Barcelona, Spain*.....95

Poster #: B021 (presented @ PS2) **"Comparative sialoglycan microarray analyses of selected human and mouse Siglecs";** Steve M. Fernandes[1], Steven Arbitman[1], Ryan McBride[2], Corwin Nycholat[2], James C. Paulson[2] and Ronald L. Schnaar[1]; *[1]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA.* 33

Poster #: B073 (presented @ PS2) **"Unique Mannose Binding Epitopes Dictated by Innate Immune Receptors and Immunoglobulins from Healthy Individuals and Patients with Common Variable Immunodeficiency";** Chao Gao[1], Tanya McKitrick[1], Alyssa McQuillan[1], Barbara Eckmair[2], Kathrin Stavenhagen[1], Akul Y. Mehta[1], Lenette Lu[3], Galit Alter[3], Peter Jandus[4], Mark B. Jones[1], Stephan von Gunten[5], Jamie Heimburg-Molinaro[1] and Richard D. Cummings[1]; *[1]Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA; [2]Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria; [3]Ragon Institute of MGH, MIT, and Harvard, Boston, USA; [4]Department of Internal Medicine, University Hospital and Faculty of Medicine, Switzerland; [5]Institute of Pharmacology, University of Bern, Switzerland* 96

Poster #: B074 (presented @ PS1) **"Lectenz®: A Novel Class of High-Specificity Affinity Reagents for Detection and Purification of Glycoconjugates";** Christian Gerner-Smidt[1], Sheng-Cheng Wu[1], Lu Meng[1], Robert J. Woods[2] and Loretta Yang[1]; *[1]Lectenz Bio; [2]CCRC, University of Georgia*.....97

Poster #: B006 (presented @ PS1) **"Characterizing the role of host fucose in cholera toxin action";** Atossa C. Ghorashi and Jennifer J. Kohler; *University of Texas Southwestern Medical Center*.....12

Poster #: B075 (presented @ PS2) **"Analysis of PD1/PD-L1 Glycoforms by LC-MS/MS and Reactivity of the Immune Checkpoint Proteins with Therapeutic Antibodies";** Radoslav Goldman[1], Oliver C. Grant[2], Robert J. Woods[2], Miloslav Sanda[1], Zuzana Brnakova-Kennedy[1] and Julius Benicky[1]; *[1]Georgetown University, Department of Oncology and Clinical and Translational Glycoscience Research Center, Washington DC 20057; [2]Complex Carbohydrate Research Center, Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia, GA 30602-4712* 98

Poster #: B027 (presented @ PS2) **"Glucose homeostasis is regulated by hyaluronan synthases 1 and 3";** Angelica M. Gomes, Steven Shaffer, Rebecca C. Schugar, Jonathan M. Brown, Vincent C. Hascall and Mark A. Aronica; *Cleveland Clinic* 42

Poster #: B076 (presented @ PS1) **"Siglec ligands in mouse and human brain";** Anabel Gonzalez Gil, Steven Arbitman, Steve M. Fernandes, T. August Li, Karan Patel and Ronald L. Schnaar; *Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD*.....99

Poster #: B077 (presented @ PS2) **"Oral-supplemented 2-fucosyllactose attenuates spontaneous colitis in Il10-/- mice";** Thomas Grabinger[1], Jesus F. Glaus Garzon[1], Martin Hausmann[2], Annelies Geirnaert[3], Christophe Lacroix[3] and Thierry Hennet[1]; *[1]Institute of Physiology, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland; [2]Department of Gastroenterology and Hepatology, University Hospital Zurich, University of Zurich Raemistrasse 100, 8091 Zurich, Switzerland; [3]Laboratory of Food Biotechnology, Department of Health Sciences and Technology, ETH Zurich, Schmelzbergstrasse 7, 8092 Zürich, Switzerland* 100

Poster #: B026 (presented @ PS1) **"A Conserved Role for N-Glycanase 1 in Regulating Energy Metabolism through AMPK signaling";** Seung Yeop Han[1], Ashutosh Pandey[1], Antonio Galeone[1,2], Tereza Moore[3], Tina M. Cowan[3] and Hamed Jafar-Nejad[1]; *[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [2]Department of Bioscience, University of Milan, Milan, Italy (current address); [3]Department of Pathology, Stanford University, Stanford, CA* 41

Poster #: B091 (presented @ PS2) **"Increased antibody response to fucosylated oligosaccharides in inflammatory bowel disease";** Katharina Kappler[1], Yi Lasanajak[2], David F. Smith[2] and Thierry Hennet[1]; *[1]Institute of Physiology, University of Zurich, Zurich, Switzerland; [2]Emory Comprehensive Glycomics Core, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, U.S.A.* 114

Poster #: B092 (presented @ PS1) **"A Structural Approach to Broadening Glycosyltransferase Binding Specificity";** Benjamin P. Kellman and Nathan E. Lewis; *UC San Diego;*....115

Poster #: B094 (presented @ PS1) **"Aberrant serum glycans as survival prognostics for the haematological cancer, multiple myeloma";** Michelle Kilcoyne[1], Marie LeBerre[1,2], Marta Utratna[1], Lokesh Joshi[2] and Michael O'Dwyer[3]; *[1]Carbohydrate Signalling Group, Discipline of Microbiology, National University of Ireland Galway, Galway, Ireland ; [2]Glycoscience Group, National Centre for Biomedical Engineering Science, National University of Ireland Galway, Galway, Ireland; [3]Department of Medicine, National University of Ireland Galway, Galway, Ireland* 117

Poster #: B095 (presented @ PS2) **"Genetic Alteration of Heparan Sulfate Enhances Antigen Presentation on Dendritic Cells";** So Young Kim[1,2] and Mark M. Fuster[1,2,3]; *[1]Department of Medicine, Division of Pulmonary and Critical Care, University of California San Diego; [2]VA San Diego Healthcare System, Medical and Research Sections; [3]Glycobiology Research and Training Center, University of California San Diego* 118

Poster #: B041 (presented @ PS2) **"The Role of Core 3 β3-N-Acetylglucosaminyltransferase in Colorectal Cancer";** Su-Ryun Kim, Guozhang Zou and Tongzhong Ju; *Office of Biotechnology Products (OBP), Center for Drug Evaluation and Research (CDER), Food and Drug Administration, Silver Spring, MD 20993*....64

Poster #: B096 (presented @ PS1) **"A Fast, Reliable O-Glycan Analysis Workflow";** Jason Koch and Hua Yuan; *Zoetis*.....119

Poster #: B097 (presented @ PS2) **"Unraveling functions of novel protein O-mannosyltransferases using Drosophila as a model organism";** Melissa A. Koff[1], Adnan Halim[2] and Vlad Panin[1]; *[1]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, USA; [2]Department of Cellular and Molecular Medicine, Faculty of Health Sciences, Copenhagen Center for Glycomics, University of Copenhagen, DK-2200 Copenhagen, Denmark* 120

Poster #: B098 (presented @ PS1) **"An anti-Tn Antibody Microarray Platform for Early Cancer Detection";** Matthew R. Kudelka[1,2], Wei Gu[1,2], Yasuyuki Matsumoto[2], Richard H. Barnes II[2], Robert Kardish[2], Jamie Heimburg-Molinaro[2], Sylvain Lehoux[2], Junwei Zeng[2], Cynthia Cohen[3], Brian S. Robinson[3], Kinjal Shah[3], Elliot L. Chaikof[2], Sean R. Stowell[3] and Richard D. Cummings[2]; *[1]Weill Cornell Medicine; [2]Beth Israel Deaconess Medical Center/Harvard Medical School; [3]Emory University School of Medicine* 121

Poster #: B078 (presented @ PS1) **"Regulation and fine-tuning of cadherin O-linked mannose glycosylation by the TMTC1-4 enzyme family";** Ida SB Larsen, Yoshiki Narimatsu, Hiren J. Joshi, Sergey Vakhrushev, Henrik Clausen and Adnan Halim; *Department of Cellular and Molecular Medicine, Faculty of Health Sciences, Copenhagen Center for Glycomics, University of Copenhagen, DK-2200 Copenhagen, Denmark;* 101

Poster #: B099 (presented @ PS2) **"Lectin microarray-based investigation of protein glycosylation in murine and human biological fluids in response to diet and AGEs";** Marie Le Berre; *National University of Ireland Galway;*....122

Poster #: B011 (presented @ PS2) **"Host adaptations of the Salmonella Typhi typhoid toxin and its orthologue from a nontyphoidal Salmonella";** Sohyoung Lee[1], Yi-An Yang[1], Shawn K. Milano[2], Tri Nguyen[1], Ji Hyun Sim[1], Andrew J. Thompson[3], Eric C. Hillpot[2], Gyeongshik Yoo[1], James C. Paulson[3] and Jeongmin Song[1]; *[1]Department of Microbiology and Immunology, Cornell University College of Veterinary Medicine, Ithaca, New York 14853, USA; [2]Department of Molecular Medicine, Cornell University College of Veterinary Medicine, Ithaca, New York 14853, USA; [3]Department of Molecular Medicine, The Scripps Research Institute, La Jolla, California 92121, USA* 17

Poster #: B121 (presented @ PS2) **"Molecular basis for FGF23 site specific glycosylation by GalNAc-T3";** Earnest James Paul Daniel[1], Matilde de las Rivas[2], Ramon Hurtado-Guerrero[2] and Thomas Gerken[1]; *[1]Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106; [2]BIFI, University of Zaragoza, BIFI-IQFR (CSIC) Joint Unit, Edificio I+D, Zaragoza, Spain* 144

Poster #: B004 (presented @ PS2) **"Structure and mechanism of a pH sensing lipoteichoic-acid-anchor flippase";** Bing Zhang[1], Xue Liu[2], Elisabeth Lambert[1], Guillaume Mas[1], Sebastian Hiller[1], Jan-Willem Veening[2] and Camilo Perez[1]; *[1]Biozentrum, University of Basel; [2]University of Lausanne* 7

Poster #: B033 (presented @ PS2) **"Immunoglobulin G glycosylation changes in diseases and aging";** Marija Pezer[1], Frano Vuckovic[1] and Gordan Lauc[1,2]; *[1]Genos Glycoscience Research Laboratory, Zagreb, Croatia; [2]University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia* 53

Poster #: B122 (presented @ PS1) **"TRAP Complex Facilitates N-linked Glycosylation Biosynthetic Process During ER-stress";** Chatchai Phoomak[1], Wei Cui[1], Thomas J. Hayman[1], Lance Wells[2], Richard Steet[3] and Joseph N. Contessa[1]; *[1]Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT; [2]Complex Carbohydrate Research Center, University of Georgia, Athens, GA; [3]Greenwood Genetic Center, Greenwood, SC* 145

Poster #: B043 (presented @ PS2) **"Reprogramming the Tumor Microenvironment with Macrophage-Targeted Glycopolymers";** Ryan N. Porell, Daniel Honigfort and Kamil Godula; *Department of Chemistry and Biochemistry, University of California, San Diego* 66

Poster #: B123 (presented @ PS2) **"Udderly fascinating: relationships between breast milk composition and child development";** Sara Porfirio[1], Stephanie Archer-Hartmann[1], Kathryn Lockwood[1], G. Brett Moreau[2], Girija Ramakrishnan[2], Rashidul Haque[3], William A. Petri, Jr.2 and Parastoo Azadi[1]; *[1]Complex Carbohydrate Research Center, The University of Georgia, Athens, GA, USA; [2]Dept. of Medicine/Infectious Diseases, University of Virginia, Charlottesville, VA, USA; [3]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh* 146

Poster #: B124 (presented @ PS1) **"Glycan Engineering reveals that matriglycan alone recapitulates dystroglycan functions ranging from Laminin binding to Lassa Virus infection";** M. Osman Sheikh[1], Chantelle J. Capicciotti[1,7], Lin Liu[1], Jeremy L. Praissman[1], Daniel G. Mead[2], Melinda A. Brindley[2], Kevin P. Campbell[3], Kelley W. Moremen[1,4], Lance Wells[1,4] and Geert-Jan Boons[1,5,6]; *[1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA; [2]College of V eterinary Medicine, University of Georgia, Athens, GA, USA; [3]Howard Hughes Medical Institute, Department of Molecular Physiology and Biophysics and Neurology, University of Iowa, Iowa City, IA, USA; [4]Department of Biochemistry & Molecular Biology, UGA, Athens, GA, USA; [5]Department of Chemistry, University of Georgia, Athens, GA, USA; [6]Department of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, and Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, The Netherlands; [7]Department of Chemistry, Queen's University, Kingston, Ontario, CA* 147

Poster #: B125 (presented @ PS2) **"Catalytic deficiency of O-GlcNAc transferase leads to X-linked intellectual disability";** Veronica M. Pravata[1], Villo Muha[1], Mehmet Gundogdu[1], Andrew T. Ferenbach[1], Poonam S. Kakade[2], Vasudha Vandadi[1], Ariane C. Wilmes[1], Vladimir S. Borodkin[1], Shelagh Joss[3], Marios P. Stavridis[2] and Daan M.F. van Aalten[1]; *[1]Division of Gene Regulation and Expression, School of Life Sciences, University of Dundee, DD[1] [5]EH Dundee, United Kingdom; [2]Division of Cell and Developmental Biology, School of Life Sciences, University of Dundee, DD[1] [5]EH Dundee, United Kingdom; [3]West of Scotland Genetic Service, Queen Elizabeth University Hospital, G[51] [4]TF Glasgow, United Kingdom* 148

Poster #: B126 (presented @ PS1) **"The function of Golgi alpha-mannosidase II in somatosensory dendrite patterning";** Maisha Rahman, Carlos A. Diaz-Balzac, Hannes E. Bülow; *Albert Einstein College of Medicine* 149

Poster #: B127 (presented @ PS2) **"REGULATION OF CLATHRIN-MEDIATED ENDOCYTOSIS BY O-LINKED β-N-ACETYLGLUCOSAMINE MODIFICATIONS";** Sadia Rahmani[1], Costin N. Antonescu[1,2] and Warren W. Wakarchuk[1,3]; *[1]Department of Chemistry and Biology, Ryerson University, Toronto, ON M5B 2K3; [2]Keenan Research Centre for Biomedical Science of St. Michael's Hospital, Toronto, ON M5B 1W8; [3]Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2G2* 150

(1) "The Runaway Self" – Changing while remaining the same

Pascal Gagneux
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Mammals are highly sialylated. These long-lived species have complex body plans with many different cell types. They are obligate sexual reproducers that have evolved long internal gestation with placentation and maternal feeding of the young with glycan and sialic acid rich milk. Mammals have evolved to utilize sialic acids as self-associated molecular patterns (SAMPs) and several innate immune receptors appear to carry out permanent surveillance. Appropriate sialylation patterns inhibit the complement system by engaging Factor H and prevent immune cell activation via a collection of inhibitory Siglec molecules on immune cells. The human glycocalyx lacks the sialic acid N-glycolylneuraminic acid (Neu5Gc) and has a higher abundance of its precursor N-acetylneuraminic acid (Neu5Ac). Most humans also have circulating antibodies specific for the non-human sialic acid Neu5Gc. This situation is akin to a human, "species-specific histo-blood group". Recently, several additional mammalian species have been found to have radically changed their sialome by loss of Neu5Gc. All cases involve loss-of-function mutations in the gene encoding the sialic acid-modifying enzyme CMAH. Unlike most other glycan antigens, Neu5Gc apparently cannot be synthesized by microbes, raising the question about the origin of anti-Neu5Gc antibodies in humans. Dietary exposure and presentation on bacteria coating themselves with Neu5Gc from the diet are distinct possibilities, especially given the low rates of breastfeeding, early weaning and use of bovine milk based infant formula and red meat containing baby food. The majority of the non-human species that lack Neu5Gc do not consume diets rich in Neu5Gc, making it unlikely that they are immunized against this sialic acid. A notable exception are mustelids that consume on small Neu5Gc rich prey. Levels of anti-Neu5Gc antibodies in non-human species have not been studied. Evolutionary scenarios for the repeated, independent fixation of CMAH loss-of-function mutations likely included strong selection by parasites, especially enveloped viruses, stochastic effects of genetic drift, and directional selection via female immunity to paternal Neu5Gc. Radical changes in sialome patterns would not only jeopardize innate surveillance for SAMPs but also reproductive compatibility where SAMPs are instrumental for the success of sperm and the embryo. Such radical sialome changes may necessitate the reconfiguration of innate immune lectins that use sialic acid containing SAMPs to regulate their immune status. Interestingly, many of these sialic acid-binding receptors appear to evolve rapidly, both by changes in coding sequence, expression patterns, and by copy number in the genome.

(2) A Stepwise Mechanism for ER-to-Golgi Transport of Lysosomal Enzymes

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Organelle biogenesis requires proper transport of proteins from their site of synthesis to their target subcellular compartment. Lysosomal enzymes are synthesized in the endoplasmic reticulum (ER) and traffic through the Golgi complex before being transferred to the endolysosomal system. The mechanism by which lysosomal enzymes are transferred from the ER to the Golgi, however, is poorly characterized. We have now identified the ER-associated membrane protein CLN8 as a factor that is required for ER-to-Golgi transfer of lysosomal enzymes. CLN8 is a transmembrane protein whose loss of function leads to the lysosomal storage disorder, neuronal ceroid lipofuscinosis 8 (a type of Batten disease). We show that CLN8 interacts with two thirds of lysosomal soluble enzymes at the ER and promotes their COPII-mediated transfer to the Golgi. ER-to-Golgi trafficking of CLN8 requires interaction with the COPII and COPI machineries via specific export and retrieval signals localized in the cytosolic carboxy terminus of CLN8. Analysis of mutant mice shows that CLN8 deficiency leads to a depletion of soluble enzymes in the lysosome due to a defect in the enzymes' anterograde trafficking. Enzymes that are not trafficked properly are then cleared by ER-associated degradation. Thus, CLN8 loss of function causes a defect in the biogenesis of functional lysosomes. Binding to lysosomal enzymes requires the second luminal loop of CLN8 and is abolished by some disease-causing mutations within this region. Our data establish an unanticipated example of an ER receptor serving the biogenesis of an organelle and indicate that impaired transport of lysosomal enzymes underlies Batten disease caused by mutations in CLN8.

(3) N-glycosylation in Archaea: Extremely creative

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Since first having been described some 40 years ago, Archaea have provided insight into a variety of basic biological questions relevant to all forms of life. At the same time, Archaea have devised unique solutions to common biological problems, often reflecting the extremophilic lifestyle of members of this domain. N-glycosylation offers numerous examples of such Archaea-specific strategies, as revealed by our studies of *Haloferax volcanii*, originally isolated from the Dead Sea, as well as by others working with additional species. In delineating archaeal pathways used for assembling and attaching N-linked glycans, aspects of these processes seemingly exclusive to Archaea have been identified. Likewise, many of the roles assumed by N-glycosylation in Archaea, including those related to structure/function and cell physiology, appear

to be particular to these organisms. Indeed, the unparalleled diversity of archaeal N-glycan composition and architecture, as well as the broad range of niches in which Archaea exist, suggests that continued efforts will uncover additional aspects of this post-translational modification distinct to this domain of life. In my presentation, examples of how N-glycosylation in Archaea can be distinguished from what occurs in Bacteria and/or Eukarya will be discussed.

(4) Understanding the sequence-structure-function relationships through a comprehensive evolutionary analysis of GT-A fold glycosyltransferases

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Glycosyltransferases (GTs), through their roles in transfer of sugars to different substrates by the formation of a glycosidic bond, are involved in many important biological functions such as protein folding and biosynthesis of polysaccharides, glycolipids and glycoproteins. To facilitate the non-template driven biosynthesis of one of the most diverse biological macromolecules in complex carbohydrates, GTs themselves are incredibly diverse in sequence and mechanism of action. However, the evolutionary basis for their functional diversity is not well understood.

Here, we map the evolutionary relationship connecting various GT-A fold families using an evolutionary systems approach. We propose a phylogenetic framework that reveals novel relationships between evolutionarily divergent families and highlights underlying similarities that have not been appreciated before. We quantify the evolutionary constraints shared by families within clades and show that they reflect shared mechanisms of action. Our analysis serves as a framework for investigating the functional roles of understudied and novel GT-A families and can also be extended to other GT folds.

(5) Deciphering the molecular functions of tryptophan C-mannosylation

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Tryptophan C-mannosylation is an unusual type of protein glycosylation whereby an α-D-mannose is covalently attached to a tryptophan side chain with the formation of a carbon-carbon bond. This protein modification is predominantly found on type I cytokine receptors and thrombospondin repeat domains, though it is found on many other proteins, including RNase 2, hyaluronidases and siglecs. The first gene to encode a Tryptophan C-mannosyltransferase was discovered in *Caenorhabditis elegans*, which has catalysed the discovery and characterisation of homologues from humans and apicomplexan parasites.

The study of tryptophan C-mannosylation has been hampered by a dearth of tools for producing proteins with the modification, detecting the modification, and for inhibiting protein modification. To address this issue, we have engineered a simple microbial expression system to produce a wide variety of human proteins with and without tryptophan mannosylation. These proteins enabled us to assess the impact of C-mannosylation on protein fold and function, which provided insights into the significance of tryptophan mannosylation. Using these proteins, we have generated and validated monoclonal antibodies (mAbs) specific for tryptophan mannosylation and obtained structural insights into how these mAbs recognise C-mannosyl tryptophan.

These antibodies are being employed to discover novel endogenously C-mannosylated proteins in a wide variety of organisms. With these antibodies and our microbial expression system, we have also been able to generate the first inhibitors of tryptophan C-mannosyltransferases by applying a rational inhibitor design approach to the creation of substrate-mimics. These inhibitors work in cell culture and represent an excellent starting point for the development of chemical probes with more drug-like properties to explore in certain disease indications.

(6) Functional players of Protein O-mannosyltransferases 1/2 –mediated regulation of sensory neuron connectivity in Drosophila

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Protein O-mannosyltransferases 1 and 2 (POMT1/2) are best-studied enzymes that modify Ser/Thr residues of proteins with O-mannose. POMTs, along with Dystroglycan, their well-characterized substrate, are highly conserved in animals, from Drosophila to humans. . Deficiencies in POMT1/2 underlie congenital muscular dystrophies, indicating their roles in development and physiology of muscles and the nervous system. However, the pathological mechanisms causing the disease and diverse roles of POMT1/2 in these conditions remain not well understood. Therefore, we use *Drosophila* as a model system to uncover genetic and molecular pathways that underlie POMT1/2 functions. We have discovered that

POMT1/2 mutant embryos display abnormalities in their body posture and muscle contractions. These phenotypes could be rescued by expressing POMT1/2 in the nervous system, including sensory neurons. POMT1/2 mutant embryos were found to have defects in sensory axons that terminate in the brain. These results established that POMT1/2 regulate sensory neuronal connectivity and thus body posture. Interestingly, Dystroglycan mutants did not show defects in sensory axon terminals or body posture, suggesting that other POMT substrates are involved in regulating neuronal communication in *Drosophila*. To uncover these substrates, we used candidate gene approach and focused on Receptor Protein Tyrosine Phosphatases (RPTPs) that are known to function in axon targeting and can be modified with O-mannose in mammals. We screened mutants of different RPTP genes for sensory axon defects. We also analyzed alleles of different RPTPs in combinations with POMT1/2 mutations. Our experiments isolated one of Drosophila RPTPs as a candidate for a POMT1/2 functional target. That RPTP is potentially highly glycosylated and may bear O-mannose glycans that affect its function. Our current studies involve biochemical approaches to uncover possible O-mannosylation of the RPTP protein, including mass spectrometric analysis of its glycan modifications. To this end, we are using a transgenic construct to express and purify a tagged RPTP protein from Drosophila genetic strains with different levels of POMT activity. These experiments will elucidate novel players of the POMT-mediated pathway that regulate neural functions. Our data are expected to shed new light on pathogenic mechanisms of muscular dystrophy and neurological abnormalities associated with POMT defects. This project was supported by grants from NIH (NS099409) and CONACYT-TAMU (2012-037S) to VP.

(7) Structure and mechanism of a pH sensing lipoteichoic-acid-anchor flippase

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Lipoteichoic acids (LTA) are essential cell wall components in the human pathogen *Staphylococcus aureus*. Their biosynthesis pathway involves translocation across the plasma membrane of a lipid-linked-disaccharide that serves as an anchor for the LTA backbone-polymer. Lipid-linked-disaccharide transmembrane transport is mediated by the flippase LtaA. Here we present the structure of *S. aureus* LtaA that shows a central cavity consisting of a hydrophilic and a hydrophobic pocket each harbored by a half-transporter domain, a feature not observed before in transporters of the major facilitator superfamily. We show that LtaA works as a proton-coupled antiporter-flippase that by sensing environmental pH, contributes to *S. aureus* survival under physiological acidic conditions. Furthermore, we found that LtaA flipping activity is inhibited by a headgroup-derived disaccharide with high selectivity. Our results describe unprecedent mechanistic basis of proton-coupled flipping-antiport and give basis for the development of novel strategies to counteract *S. aureus* infections.

(8) Exploring Evolutionary Origins of Human-Specific CD33/Siglec-3 Alleles that Protect against Late Onset Alzheimer's Disease: Prior Selection by Uniquely Human Pathogens?

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Genome-wide association studies defined SNPs in the *CD33* gene associated with protection against Late Onset Alzheimer's Disease (LOAD). CD33 is the founding member of CD33-related subset of Sialic acid (Sia)-binding Ig-like lectins (Siglecs), which modulate vertebrate innate immunity by recognizing endogenous sialoglycans as "self-associated molecular patterns" (SAMPs). LOAD-protective CD33 allele increases the ratio of an alternately spliced form CD33m(D2-CD33), which differs from ancestral full-length CD33(CD33M) in lacking the amino terminal V-set domain containing the Sia-binding site. Genomes of chimpanzees and other related "great apes" do not harbor the protective SNPs, and express minimal amounts of CD33m—indicating a derived hominin-lineage evolution of this allele, which seems to have been co-opted to protect the cognition of elderly human caregivers who increase survival of helpless human young (The Grandmother Hypothesis). But what selection forces generated the allele that was eventually protective against LOAD? We found that unlike great ape sequences that show minimal changes over >10 million years, human CD33 show 10 lineage-specific mutations in Sia-binding V-set domain (3 polymorphic), most of which were also found in Neanderthal and Denisovan genomes sharing a more recent common ancestor with humans. Certain human commensals and pathogens have evolved sialylated molecular mimics of SAMPs that dampen immune responses by engaging Siglecs. Since Sia ligand binding is key to CD33-mediated signaling, the complete loss of Sia binding by CD33m suggests that V-set domain initially underwent evolutionary selection to evade such molecular mimicry. Our recent studies of human and chimp CD33 showed selective binding of the former to uniquely human pathogen *Neisseria gonorrhoeae* which uses host CMP-Sia to sialylate its LOS, evading

immune attack. Given the negative impact of gonorrhea on human fertility, we reasoned that multiple evolutionary changes of human CD33 V-set domain were driven by this pathogen and/or other human-specific sialylated pathogens of fetuses or neonates, such as Group B *Streptococcus* (GBS). Indeed, some common GBS strains also bound selectively to human but not chimp CD33. We identified amino acid 21 (modelled near Sia binding pocket) of human CD33 V-set domain that allows selective binding of sialylated pathogens. An evolutionary scenario consistent with all findings is that CD33 Sia-binding was first upregulated in human ancestors for unknown reasons, providing an opportunity for emergence of sialylated pathogens, which then drove selection in hominin lineage towards diverse allelic variations of CD33, eventually impacting signaling pathways associated with CD33, including processes linked with AD. As CD33 expression in microglia is linked to beta amyloid (Aβ) accumulation in AD progression, we plan to explore the role of human-specific changes on Aβ clearance and determine their sialoglycan binding profile.

(9) Staphylococcus aureus remodels surface glycopolmers to shape colonization and invasion capacities

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Staphylococcus aureus is a major pathogens causing high numbers of invasive, often difficult-to-treat infections. *S. aureus* is a members of the normal human microbiota of the nasal cavity with up to 30% of the human population being colonized. Wall teichoic acids (WTA), glycopolymers at the staphylococcal cell surface play crucial roles for the colonization and infection capacities of staphylococcal pathogens. WTA has a characteristic species and often strain-specific structure. However, our recent studies indicate that *S. aureus* can vary WTA structure in subtle ways that have profound impacts on the capacities of the bacteria to interact with antibodies or bacteriophages that govern the balance between commensal and pathogen lifestyles. WTA-biosynthetic genes on mobile genetic elements can be exchanged between different clones and species thereby contributing to the ongoing evolution of staphylococcal pathogens.

(10) Prebiotic action of dietary and mucosal carbohydrates on the gut microbiota

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Dietary complex carbohydrates remain undigested through the gastrointestinal tract due to the limited specificity of host-derived carbohydrate hydrolases. By contrast, gut bacteria that are mainly located in the colon express a broad range of glycolytic enzymes able to cleave oligo- and polysaccharides. Dietary and endogenous carbohydrates therefore represent a constant source of nutrients to the gut microbiota. In addition to their nutritional value, liberated monosaccharides can also be used as building blocks in the biosynthesis of bacterial cell wall glycans, thereby influencing interactions between the gut microbiota and host mucosal immunity. Lactose and milk oligosaccharides are the first source of carbohydrates ingested postnatally by mammals. The structural diversity of milk oligosaccharides makes it difficult to determine the impact of specific structures on the gut microbiota and host gastrointestinal physiology. We found that oral supplementation with single milk oligosaccharides affected the course of colitis in interleukin-10 null mice. The uptake of oligosaccharide 2-fucosyllactose promoted the outgrowth of the gram-positive bacterium *Ruminococcus gnavus*, which was paralleled with decreased intestinal inflammation and permeability. Intestinal inflammation in interleukin-10 null mice was also accompanied by increased release of monosaccharides from mucosal glycans. Tracking of the monosaccharide N-azidoacetylglucosamine in colonic bacteria revealed the preferential incorporation of this carbohydrate by members of the *Bacteroidaceae* family in interleukin-10 null mice. Specific *Bacteroides* speciesalso efficiently incorporated N-azidoacetylmannosamine and N-azidoacetylsialic acid into their surface glycoconjugates. The variable availability of monosaccharides to *Bacteroides* also influenced their ability to activate and polarize $CD11c^+$ dendritic cells. These results underline the importance to assess not just the composition of the gut microbiota, but also the dynamic changes in bacterial glycoconjugate expression when addressing associations between specific bacterial taxa and intestinal inflammatory diseases.

(12) Characterizing the role of host fucose in cholera toxin action

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Cholera is a diarrheal disease caused by the bacteria *Vibrio cholerae*. The etiological agent of disease is an AB5 toxin comprised of a catalytic A subunit and a cell surface receptor binding B subunit (CTB). For decades, the binding and internalization of cholera toxin into host cells has been attributed solely to the ganglioside GM1; however, we now know that genetic disruption of GM1 synthesis in mice does not abolish cholera toxin activity. Furthermore, levels of GM1 in the human intestine are significantly lower than previously hypothesized—below levels needed for accurate quantification. Our lab has established that fucosylated glycoconjugates also function in host cell intoxication by cholera toxin. Specifically, treatment of intestinal cells with a metabolic inhibitor of fucosylation significantly decreases cholera toxin binding and activity. Human variation in fucosylated glycans

observed in the ABO blood groups has been known to impact severity of cholera symptoms, further supporting a role for fucose in cholera toxin activity. Here, we will address two major questions in the field: what does cholera toxin bind in the physiological context of infection, and how does variation in fucose-containing structures among humans impact toxin activity. To address the former question, we have initiated a genome-wide CRISPR knockout screen to identify mediators of CTB binding in a human intestinal cell line. To address the latter question, we have constructed human intestinal cell lines modeling three ABO blood types. Using flow cytometry to measure binding and internalization of CTB to each of these cell lines, we have generated preliminary evidence suggesting that blood group B cells internalize CTB less efficiently than the A and O blood group cells. Interestingly, binding of CTB does not vary significantly between the blood groups. We hypothesize that differences in receptor expression between the blood group cell lines may be masked by consistent expression of decoy receptors.

(13) Distinct antimicrobial properties of the N- and C-terminal domains of the human protein galectin-9

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Escherichia coli (strain O86:B7), *Klebsiella pneumoniae* (strain KP01), and *Providencia alcalifaciens* (strain PAO5) are gram negative bacteria known to alter their membrane lipopolysaccharides (LPS) to resemble mammalian cell membrane proteins and sugars such as blood group antigens. Previously, several human galectins, including tandem repeat galectin-4 and galectin-8, were found to play a key role in the innate immune response to these self-like antigens through binding to the terminal β-galactoside sugar of LPS and subsequent bacteria killing. However, each galectin domain displays unique binding and killing ability. Human galectin-9 (Gal-9), which consists of distinct N- and C-terminal carbohydrate binding domains, targets KPO1 and PAO5 through both the N- and C-terminal domains. However, only the N-terminal domain targets O86:B7, while the C-terminal domain fails to kill this bacterial strain. This contrast in antimicrobial activity indicates that variation in binding affinity may be responsible for the disparate killing activity of each domain. Binding analysis by flow cytometry demonstrated that Gal-9N and C-domains each bind KPO1, PAO5, as well as O86:B7, while failing to bind to the corresponding negative controls that lack self-like LPS, KPO4, PAO19, and the mutant O86:B7 Waal-, respectively. However, despite binding of both domains to O86:B7, dosing experiments demonstrated dose-dependent antimicrobial activity of both Gal-9 domains to KPO1 and PAO5, but of only Gal-9N to O86:B7. Indeed, Gal-9C failed to efficiently kill O86:B7 at any concentration tested. Assessment of differences in relative binding affinity to microbial antigen by binding of each domain of Gal-9 to immobilized bacteria revealed preferred binding to O86:B7 by the Gal-9N domain compared with Gal-9C. Thus, while binding is required for galectin killing of bacterial targets, variations in binding affinity of galectin to target bacteria may add an additional level of complexity to antimicrobial activity of galectin family members towards various bacterial strains.

(14) Bacterial community manipulation through glycan-lectin interactions

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Atopic dermatitis (AD) is a complex disease involving a decrease in skin barrier function, immune system processes, and the skin microbiome. Skin swab samples from patients with AD show a characteristic overgrowth of *Staphylococcus aureus*. Controlled manipulation of *S. aureus* abundance in the skin microbiome is required to quantitatively study the role of *S. aureus* in AD disease processes. Here, we developed a novel method to deplete or enrich *S. aureus* from bacterial communities. Incubation of glycan-coated beads with stained bacteria allows binding between bacterial lectins and glycans on the beads. Binding between bacteria and different glycans can be assessed qualitatively and quantitatively through flow cytometry, as demonstrated by characterization of binding between *Escherichia coli* and mannose-coated beads. This method will enable novel studies of *S. aureus* in AD skin microbiota and has broader applications for microbial ecology studies in general.

(15) Staphylococcus Aureus Exacerbates Epithelial Barrier Dysfunction in Chronic Rhinosinusitis

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Rationale:

Sinusitis, inflammation or infection of the sinuses, impacts 12.5% (approximately 30 million) of adults each year in the United States. Furthermore, greater than 20% of antibiotic prescriptions in adults are for sinusitis. Patients with chronic symptoms (chronic rhinosinusitis, CRS) despite medical care undergo surgical procedures, however more than 30% will experience poor surgical outcomes. The most common pathogen isolated from sinusitis is *Staphylococcus aureus*(SA).

SA is the leading cause of skin infections presenting to the emergency department. More than 30% of the U.S. population is colonized with SA, 1-3% are colonized with methicillin resistant *Staphylococcus aureus*(MRSA) strains. It colonizes mucocutaneous epithelia of the nasopharynx and can lead severe life-threatening conditions such as sepsis, meningitis, endocarditis, and pneumonia in immunocompromised hosts.

The causative role and mechanism of MRSA leading to chronic inflammation seen in CRS has yet to be elucidated. Nasal epithelium provides the first line of defense in differentiating between pathogenic and commensal bacteria. Increased permeability of epithelium, decreased antimicrobial production, and dysregulated mucin secretion are features of CRS. Regenerating islet-derived protein 3 gamma (Reg3g) is an antimicrobial peptide with c-type lectin domain with activity against MRSA. Previous studies showed its production by lung epithelium and role in clearance of MRSA in mice. Reg3g has not been studied in human airway. Furthermore, although different glycosylation variants of Muc5B were detected during acute airway inflammation of asthma, no investigation has analyzed the quality of mucin produced by airway epithelium during acute exposure to SA.

Methods:

Nasal and sinus epithelial cells were obtained from human volunteers, expanded *in vitro*culture and stimulated with MRSA. Epithelial barrier integrity was measured by migration of FITC-dextran from apical to basolateral layer of air liquid interphase (ALI), RNA expression was analyzed at different time points with RT-qPCR and protein levels were measured by western blot analysis.

Results:

Airway epithelium express Muc5AC, Muc5B, and Reg3g upon exposure to MRSA. SA alpha toxin is sufficient to induce Reg3g expression. Early alpha toxin induction of Reg3g expression was attenuated in CRS. Airway epithelium from CRS subjects showed impaired epithelial barrier integrity compared to normal airway epithelium when exposed to MRSA with increased levels of FITC-dextran and colony forming units of SA that is able to traverse to the basolateral layer.

Conclusions:

Nasal epithelium produces antimicrobial peptide Reg3g and mucin when exposed to *Staphylococcus aureus*. We propose that MRSA colonization in CRS leads to persistent inflammation resulting in excess secretion of altered glycosylated forms of mucin.

(16) A conserved glycosyltransferase from the general protein O-glycosylation pathway of Bacteroidetes

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The *Bacteroidetes* phylum of bacteria employs a general protein *O*-glycosylation system wherein a D(S,T)(A,I,L,M,T,V) motif is targeted. Exemplarily, *Bacteroides fragilis, Tannerella forsythia* and *Pedobacter heparinus* from that phylum have been found to glycosylate several of their proteins at multiple sites in a species-specific manner. Several glycan biosynthetic enzymes have been identified and found to be clustered in a locus for glycosylation (lfg). Despite compositional and structural differences of the three *Bacteroidetes* glycans, a conserved glycosyltransferases (cGT) is encoded in the respective lfgs.

Initial evidence of the role of the cGT came from *T. forsythia*, where it was shown to transfer a branching fucose to the innermost reducing-end galactose of a complex dekasaccharide of known structure. Upon knocking-out of the corresponding Tanf_01305 gene, a heavily truncated version of the glycan was produced. Surprisingly, the mutant glycan did not lack immediate downstream constituents but all residues branching off from the fucose residue of a linear fucose-glucuronic acid-galactose backbone. The glycosylation pattern of this mutant could be restored upon provision of Tanf_01305 as well as orthologous genes from *B. fragilis* (Bf_4306) and *P. heparinus* (Phep_4048), respectively, indicating interchangeability of these genes.

To learn more about the specificity of the cGT, it was sought to elucidate the *B. fragilis* protein *O*-glycan structure. Glycopeptides were prepared and β-eliminated glycans were subjected to MS and NMR analyses. Initial data MS indicate an *m/z* of the full glycan of 1571.8 Da, and ^{1}H-NMR experiments revealed a heterooligomer of nine sugar residues, including hexoses, deoxyhexoses, *N*-acetylhexoses and hexuronic acids.

This study shed light on a *Bacteroidetes* cGT, which predictably has an important signalling function in the *O*-glycan biosynthetic pathway. The characterization of this interesting cGT is ongoing in our laboratory.

This work is supported by the Austrian Science Fund, P24317-B22 and I2875-B22 (to C.S.) and the Doctoral Program "BioToP – Biomolecular Technology of Proteins" W1224.

(17) Host adaptations of the Salmonella Typhi typhoid toxin and its orthologue from a nontyphoidal Salmonella

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Typhoidal and nontyphoidal Salmonellae (NTS) cause typhoid fever and gastroenteritis respectively in humans. Typhoid toxin from typhoidal *Salmonella* contributes to typhoid disease progression and chronic infection, but little is known about its orthologue from NTS. We found that these two Salmonella toxins do not cause similar toxicities *in vivo*. The altered virulence outcomes are due to the altered glycan binding preferences of these two toxins, which are correlated to the glycan expression profiles of target host cells at the primary infection or intoxication sites of their associated bacteria. The co-crystal structures of the glycan-receptor binding PltB subunits of the toxins bound to specific glycan receptor moieties explain how PltBs of these two toxins display markedly different glycan binding preferences and contribute to the subsequent virulence outcomes. We also revealed that administration of either Javiana toxin or *S.* Javiana into an animal model provides cross-reactive protection for typhoid-related illnesses and mortality.

(18) Glycosylation of enveloped viruses: structure and immunogen design

Max Crispin

School of Biological Sciences, University of Southampton, Southampton, UK

The attachment and fusion proteins of enveloped viruses are often heavily glycosylated. Viruses utilize host enzymes to glycosylate their proteins during biosynthesis and yet the resulting glycans can differ substantially from those typically presented by host glycoproteins. In this talk, I will discuss how our understanding of viral glycoproteins have been greatly aided by the integration of glycoproteomics with X-ray crystallography and cryo-electron microscopy. Factors shaping viral glycosylation will be explored with an emphasis on human viruses displaying Class I fusion architectures such as HIV-1, Lassa and SARS viruses. I will also discuss how glycosylation can inform anti-viral vaccines design and therapies.

(19) Synthetic oligosaccharide-based conjugate vaccines against shigellosis: from concept and design to first-in-human study

Laurence A. Mulard

Institut Pasteur

Shigellosis, or bacillary dysentery, caused by the enteroinvasive bacteria Shigella, remains one of the top diarrheal diseases in children under five years of age in low- and middle-income countries. Natural infection induces short term protection against reinfection. Species and serotype diversity added to geographical distribution strongly support the development of a multivalent S. flexneri vaccine. Lattice-type conjugate vaccines based on Shigella detoxified lipopolysaccharide were shown to have a good safety profile in adults and children. Efficacy was demonstrated in recipients older than three. However, vaccine-induced protection was insufficient in the 1-3 year olds. Instead, in the search for a highly immunogenic Shigella vaccine able to generate protective immunity in young children living in low- and middle-income countries, we have engaged into the development of immunogens consisting of synthetic fragments of selected putative S. flexneri O-antigens (O-Ags) covalently linked via single point attachment to protein carriers.

A multidisciplinary strategy interfacing medicinal chemistry and structure-based vaccinology was implemented. "Protective" epitopes were identified by use of a panel of synthetic O-Ag fragments. Glycoconjugates encompassing the most promising oligosaccharides were evaluated for their immunogenicity in mice. A conjugate derived from a synthetic hapten corresponding to three basic repeating units of the O-Ag from S. flexneri 2a, the most prevalent Shigella serotype, was designed accordingly. This vaccine candidate, named SF2a-TT15, has been shown to induce anti-S. flexneri 2a bactericidal antibodies. A GMP batch of SF2a-TT15 was produced and a first-in-human, single-blinded, dose escalation, placebo-controlled study was conducted.

With the first rationally designed synthetic oligosaccharide conjugate vaccine candidate in hand, this presentation primarily provides an overview of our strategy for a broad coverage S. flexneri vaccine. Hapten selection, vaccine design, safety and immunogenicity data following first use in human are discussed.

In addition, the presentation highlights our recent investigation on chemo-enzymatic strategies as an substitute to chemical synthesis to produce relevant oligosaccharides for use as components of a broad coverage S. flexneri conjugate vaccine.

Shigellosis, or bacillary dysentery, caused by the enteroinvasive bacteria Shigella, remains one of the top diarrheal diseases in children under five. Species/serotype diversity and geographical distribution strongly support the need for a multivalent S. flexneri vaccine. In the search for a highly immunogenic Shigella vaccine able to generate protective immunity in young children living in low and middle income countries, we have engaged into the development of immunogens consisting of synthetic fragments of selected putative S. flexneri O-antigens (O-Ags) covalently linked via single point attachment to protein carriers.

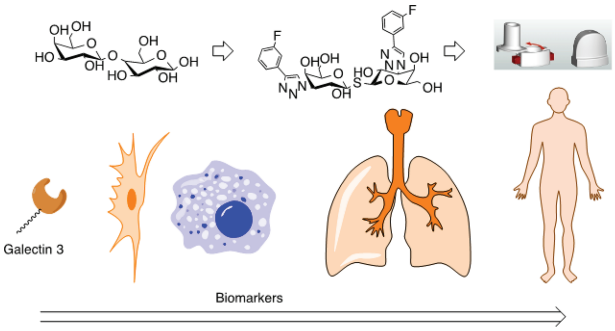
A multidisciplinary strategy interfacing medicinal chemistry and structure-based vaccinology was implemented. "Protective" epitopes were identified by use of a panel of synthetic O-Ag fragments. Protein conjugates of the most promising oligosaccharides were then evaluated for their immunogenicity in mice. A conjugate encompassing a synthetic hapten corresponding to three basic repeating units of the O-Ag from S. flexneri 2a, the most prevalent Shigella serotype, was designed accordingly. This vaccine candidate, named SF2a-TT15, has been shown to induce anti-S. flexneri 2a bactericidal antibodies. A GMP batch of SF2a-TT15 was produced and a first-in-human, single-blinded, dose escalation, placebo-controlled study was conducted.

With the first rationally designed synthetic oligosaccharide conjugate vaccine candidate in hand, this presentation primarily provides an overview of our strategy for a broad coverage S. flexneri vaccine. Emphasis is on hapten selection, vaccine design, safety and immunogenicity data following first use in human. In addition, the presentation highlights progress on the way to a S. flexneri bivalent glycovaccine candidate.

(20) Translational aspects of drug discovery and development: TD139 case story, a small molecule Galectin 3 inhibitor in phase 2b trials against IPF

Fredrik Zetterberg
Galecto Biotech

The goal of every drug discovery/development program finding new treatments for a given disease is indeed obvious, but what about the route to get there? What do you aim for and what is the critical path getting there? Is a mechanism enough?



This lecture will cover translational aspects of drug discovery and development as exemplified by TD139, a selective small molecule galectin-3 inhibitor in development (phase 2b) as a treatment for idiopathic pulmonary fibrosis.

(21) Enzyme toolkit for selective enrichment and analysis of mucin-domain glycoproteins

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Mucin domains are densely O-glycosylated modular protein domains that are found in a wide variety of cell surface and secreted proteins. Mucin-domain glycoproteins are known to be key players in a host of human diseases, especially cancer, wherein mucin expression and glycosylation patterns are altered. Mucin biology has been difficult to study at the molecular level in part because methods to manipulate and structurally characterize mucin domains are lacking. One major issue is that these domains are resistant to degradation by trypsin, meaning the majority of their sequence space is often left unanalyzed. Selective mucin degradation or enrichment, especially in a sequence- and glycan-specific manner, can facilitate study of these proteins by mass spectrometry.

Previously, we expressed and characterized a bacterial mucinase, StcE, and used its unique properties to improve sequence coverage, glycosite mapping, and glycoform analysis of recombinant human mucins by mass spectrometry. To expand on this work, we expressed and characterized several other bacterial mucinases to generate a mucin-selective enzymatic toolkit. Their activities were confirmed using a panel of O-glycoproteins by mass spectrometry. We manually validated peptide sequences from MS/MS spectra to elucidate all cleaved peptides present in the mucinase-digested samples but not in the control samples, revealing that each enzyme has a slightly different cleavage motif. Interestingly, all of the enzymes rely on a combination of peptide sequence and glycosylation status. Together with StcE, we have characterized a total of five bacterial mucinases capable of digesting mucins into peptides amenable for mass spectrometric analysis.

Further, given the enzymes' selectivity for mucin-domain glycoproteins, we reasoned that they could be employed to purify mucins from protein mixtures. Thus, inactivated mucinases were conjugated to aldehyde beads using reductive amidation. Using the enzyme-conjugated beads, we demonstrate that we can selectively enrich for mucin-domain glycoproteins from lysate and crude cancer patient ascites fluid. We are thus attempting to define the "mucinome", as a comprehensive list of mucin-domain glycoproteins does not exist. Future experiments will be devoted to isolation, digestion, and characterization of mucins from human cancer patient ascites fluid, with the ultimate goal of identifying diagnostic and/or prognostic markers of disease states.

(22) Structural characterization of T. cruzi Epimastigote Glycosylphosphatidylinositol-Mucin sialoglycans

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Trypanosoma cruzi, the causative agent of Chagas disease (CD), currently affects 6-7 million people across the world.

Currently, only two drugs, benznidazole and nifurtimox, are available for treatment of CD and they are highly toxic and less effective in the chronic stage of the disease. *T. cruzi* contains a complex cell surface consisting of several classes of glycoconjugates anchored by a glycosylphosphatidylinositol (GPI) post-translational modification. One of these GPI-anchored glycoconjugates are the GPI-mucins, which exist abundantly and serve important protective roles in both vector- and mammal-dwelling developmental stages of the parasite. *T. cruzi* GPI-mucins are heavily modified with *O*-linked oligosaccharides which are separated into core 1 (Gal$p\beta\rightarrow$4GlcNAcα) and core 2 (Gal$f\beta\rightarrow$4GlcNAcα) the latter restricted to *T. cruzi* genotype TcI, and one strain of TcVI, Tulahuen strain. *T. cruzi* utilizes a specialized *trans*-sialidase enzyme to sialylate terminal β-galacopyranose residues creating protective surface coat that prevents lysis from vector- and mammalian defense mechanisms. Here, we show mass spectrometric analysis of *T. cruzi* epimastigote GPI-mucins *O*-glycans obtained from DM28c strain (TcI) as we develop techniques to effectively lay the ground work for glycomic analysis across *T. cruzi* life stages. We identified 14 *O*-glycan structures consisting of heavily branched and sialylated glycans in DM28c eGPI-mucins, 11 previously unreported in this strain.

(23) Tuning metabolic decoy efficacy by modifying the linkage between carbohydrate and aglycone

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Metabolic decoys are synthetic analogs of naturally occurring biosynthetic acceptors. These compounds divert cellular biosynthetic pathways by acting as artificial substrates that usurp the activity of natural enzymes. While O-linked glycosides are common, they are only partially effective even at millimolar concentrations. In contrast, we report that N-acetylglucosamine (GlcNAc) incorporated into various thioglycosides robustly truncate cell surface N- and O-linked glycan biosynthesis at 10-100 μM concentrations. The >10-fold greater inhibition is in part due to the resistance of thioglycosides to hydrolysis by intracellular hexosaminidases. The thioglycosides reduce β-galactose incorporation into lactosamine chains, cell surface sialyl Lewis-X expression, and leukocyte rolling on selectin substrates including inflamed endothelial cells under fluid shear. Treatment of granulocytes with thioglycosides prior to infusion into mouse inhibited neutrophil homing to sites of acute inflammation and bone marrow by ~80%-90%. Overall, modifying the linkage between carbohydrate and aglycone enables a straightforward strategy to enhance/tune decoy efficacy. In one example, this study demonstrates that thioglycosides are an easy to synthesize class of efficient metabolic inhibitors or decoys. Due to their enhanced efficacy such compounds may be applied to reduce N-/O-linked glycan biosynthesis in cells and reduce inflammatory leukocyte accumulation *in vivo*.

(24) CustomGlycan: A novel platform for production of therapeutics

Manuela Mally, Amirreza Faridmoayer
LimmaTech Biologics AG, Switzerland

LimmaTech Biologics pursues active R&D programs applying its long-standing expertise to create improved biotherapeutics with a novel technology. We have engineered a unicellular organism with unique human N-glycosylation features to produce a wide range of recombinant glycoproteins with highly homogenous custom-made glycans. Using this approach, we have generated different cell lines encompassing arrays of different homogeneous glycoforms to exploit the full biological potential of glycosylation functions in human therapy. The CustomGlycan Platform is an enabling technology for the production of different classes of glycoproteins such as hormones, enzymes and monoclonal antibodies. We are currently focusing on producing glyco-optimized monoclonal antibodies to improve functionality and efficacy. In several proof of concept studies, we show glycoengineering of anti-TNF alpha and anti-CD20 antibodies as function-optimized therapeutic modalities. Besides the improved product characteristics, the CustomGlycan Platform provides simplified manufacturing that enables faster and lower cost production, whilst avoiding inherent mammalian cell production issues such as micro- and macroheterogeneity of glycans, endogenous viruses, complex media, and time consuming complex upstream processing.

Using our novel CustomGlycan Platform, we are developing a novel glycoengineered monoclonal anti-TNF alpha antibody to overcome shortcomings in the treatment of patients with inflammatory bowel disease (IBD). Current monoclonal antibody therapy in IBD patients suffers from relatively low efficacy and high immunogenicity. Driving the technology forward with mode of action studies, we show the effects of the glyco-optimized therapeutic candidate.

(25) Identification and design of transferase specific mucin-type O-glycosylation peptides using ISOGlyP's selective peptide function

Jonathon E. Mohl[1], Thomas Gerken[2], Ming-Ying Leung[1]

[1]*The University of Texas at El Paso*
[2]*Case Western Reserve University*

Mucin-type O-glycosylation is a highly complex process involving numerous proteins and biological functions within humans. Changes in mRNA expression levels of proteins involved in mucin-type O-glycosylation have been associated

with various cancers and developmental processes. There are at least 20 human isoforms of polypeptide GalNAc-transferases (ppGalNAc-Ts) that catalyze the addition of GalNAc to proteins. There is a large amount of redundancy in the various ppGalNAc-Ts site preferences but they do have sites that are specific to a given ppGalNAc-T.

ISOGlyP (Isoform Specific O-Glycosylation Prediction) is a user friendly, web-based mucin-type O-glycosylation prediction resource (ISOGlyP.utep.edu) that takes into account the differences in amino acid patterns recognized by 11 different ppGalNAc-Ts. Unlike other prediction programs that use databases to train the program, ISOGlyP uses experimental random peptide studies to determine isoform-specific amino acid preferences for regions around the Threonine or Serine site. Using the amino acids sequence, the likelihood of a site being glycosylated is reported as an enhancement value product (EVP) value. Sites with an EVP greater than one are considered as being likely to be glycosylated with the larger values deemed as more likely to be glycosylated first. The results are returned as a table containing all the calculated EVP values that the user can scroll through to identify sites that are more likely to be glycosylated.

ISOGlyP users may have a need to quickly identify or create peptides that are uniquely glycosylated by a given set of transferases allowing them to design experiments to test various sequence modifications surrounding glycosylation sites of interest. This work describes how the core ISOGlyP program was expanded to be able to identify those glycosylation sites that are glycosylated by a subset of transferases from a list of fasta sequences. The selective peptide function capitalizes on ISOGlyP's ability to calculate isoform specific EVPs of a site to identify particular sites that are uniquely glycosylated by a desired set of Selected-For transferases to the exclusion of other Selected-Against transferases. The results page shows the peptides along with the EVPs of the "Selected-For" and the "Selected-Against" transferases that pass both selected cutoffs. Additionally, the selective peptide function allows for peptides to be generated that are unique to a desired list of transferases by either randomly generating a sequence of up to 11 amino acids or by modifying a sequence at select locations using a predefined sequence. This work also highlights examples of the various features within the Selective Peptide function and show compiled run analysis, such as runtime, peptides returned, etc, for the different features.

The inclusion of the Selective Peptide functionality to the ISOGlyP.utep.edu site will continue to make the web interface valuable to its users from around the world.

(26) Glycolipid-based targeted drug delivery system against multidrug resistant Pseudomonas aeruginosa

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Pseudomonas aeruginosa (PA) is one of the three most critical pathogens in need for new antibiotics because of the increased resistance against multiple antibiotics. While research is being done on development of new drugs, these drugs could be toxic. Hence, an effective targeted drug delivery system is required to enhance drug efficacy and reduce drug toxicity. Inspired by the mechanism of PA adhesion to host cells, we have developed an innovative drug delivery system which uses glycan ligands on host cell surfaces to target PA. We discovered a new glycan ligand, lactosylceramide (LacCer) that could significantly improve the targeting efficiency. Interestingly, LacCer is a low-affinity ligand of PA. When liposomal drug carriers were decorated with LacCer along, no improvement of drug retention was observed. However, by integrating LacCer with the other known PA ligand, Gb3, on the surface of liposomal drug carriers, we could observe LacCer contributed to the PA binding, leading to an increased liposomal retention rate. This is because the two-dimensional ligand mobility on the liposome surface enhances LacCer binding rate. Glycolipids on a liposome can self-organize to reach multivalent interactions with different receptors on a bacterium. After the first ligand attachment, because of the reduced dimension of diffusion, the reaction rates of the subsequent bindings are at least 10^4 times higher than the first binding event. Thus, low-affinity ligands, such as LacCer, can also contribute to the bacterial attachment significantly. We observed up to 400% improvement of retention rate *in vitro*. In a thigh infected mouse model, the retention rate increased up to 200% in the thigh and 1300% in the blood. Furthermore, we evaluated the antimicrobial activity of a common antibiotic, ciprofloxacin, in three delivery systems, a) free ciprofloxacin, b) ciprofloxacin encapsulated targeted liposomes (liposomes containing both Gb3 and LacCer as targeting ligands), and c) ciprofloxacin encapsulated non-targeted liposomes (liposomes composed of only phospholipids) against PA *in vivo*. We found that the mice treated with targeted liposomes had 83% and 33% higher survival rate compared to the mice treated with free ciprofloxacin and non-targeted liposomes respectively. Our *in vitro* and *in vivo* observations indicate that this glycan-based liposomal drug carriers have significant potential as treatment against the PA bacteria. We envisage that the techniques used in this study can be used to design drug carriers for treatment against other multi drug resistant pathogens too.

(27) Uncovering New Aspects of Neutrophil Glycobiology using Glyco(proteo)mics

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Neutrophils, an abundant type of granulocytes in blood circulation, are first responders to threats posed by microbial pathogens and harmful auto-immune reactions. Despite being essential for our innate immune system, the glycobiology of neutrophils remains incompletely understood. We have over the past decade developed powerful glycomics and glycoproteomics technologies which we have employed to obtain previously unattainable insight into the peculiar and granule-specific *N*-glycoproteome of human neutrophils. Notably, these enabling glycoanalytical technologies have facilitated the discovery of heavily truncated paucimannosidic- ($Man_{1-3}GlcNAc_2Fuc_{0-1}$) and chitobiose core-type ($GlcNAc_{1-2}Fuc_{0-1}$) immune-active *N*-glycoproteins including neutrophil elastase, cathepsin G and myeloperoxidase residing in the primary granules of resting neutrophils. With a focus on detailing the structure, biosynthesis and functions of the unusual glycoprotein repertoire observed in neutrophil-mediated immune processes and diseases including pathogen infection, cystic fibrosis, sepsis and cancer, this talk will discuss how the emerging 'omics technologies have improved our understanding of the peculiar neutrophil glycobiology.

(28) The cellular impact of glycoengineering

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Protein glycosylation is fundamentally important to most biological processes and it is often important to regulate it in biopharmaceutical development. Thus, substantial efforts have been taken to engineer glycosylation of a variety of biologics. However, the diversity and complexity of glycosylation make it difficult to control the glycan structures and unravel how engineering efforts impact the host cells.

To enable rational glycoengineering and elucidate how such strategies impact the host cell, we have comprehensively studied the impact of glycoengineering on more than 180 CHO cell clones, wherein each has single or multiple glycosyltransferase genes knocked out. First, the clones were all glycoprofiled, and we developed a novel computational platform to rapidly study the changes in glycosylation across all mutants. Second, we quantified the impact of different glycosyltransferase knockouts on the bioprocessing phenotypes of the CHO cells (e.g., cell size, growth, viability, and metabolism). Finally, we conducted a large-scale RNA-Seq study of the clones to study the molecular basis of the phenotypic changes.

Here we identified dominant glycosyltransferses in CHO cells, and studied instances wherein the cells differentially expressed isozymes in response to a knockout. Furthermore, we identified groups of glycosyltransferases whose deletion had a more severe impact on cell glycoprofiles and phenotype. We also found specific molecular pathways were perturbed when different glycosyltransferase families were perturbed. Through this effort we are gaining a more comprehensive view of the impact of glycoengineering on biopharmaceuticals and the host cells producing the recombinant protein drugs.

(29) Genome-wide Regulation of Heparan Sulfate Assembly

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Heparan sulfate proteoglycans (HSPGs) are expressed on virtually all animal cells and are involved in many important biological processes. Each HSPG consists of a core protein with one or more covalently attached linear heparan sulfate (HS) chains composed of alternating glucosamine and uronic acids that are heterogeneously *N*- and *O*-sulfated. The arrangement and orientation of the sulfated sugar residues of HS specify the location of distinct ligand binding sites on the cell surface, and these modifications can vary temporally during development and spatially across tissues. While most of the enzymes involved in HS biosynthesis have been studied extensively, much less information exists regarding the specific mechanisms that give rise to the variable composition

and binding properties of HS. A genome-wide CRISPR/Cas9-mediated screen was developed to uncover novel genes other than those encoding known HS biosynthetic enzymes. A lentiviral single guide RNA (sgRNA) library was utilized to knock down gene expression across the entire genome in a human malignant melanoma cell line. Subsequently, high-throughput screens were adapted to identify sgRNAs that induce resistance to cytotoxins whose action depends on HSPGs and decrease binding of HS-dependent ligands to the cell surface. Among the resulting targets, we identified well-known HS biosynthesis enzymes as well as candidate genes with a previously unrecognized link to HS biosynthesis. The top overlapping hits from the screens were then characterized based on gene function and validated to regulate HS assembly. Overall, these studies demonstrate novel approaches to uncovering regulatory factors and provide a better understanding of the genetic regulation of HS biosynthesis. Furthermore, these studies lead us to new methods to manipulate heparan sulfate and its activities in other cellular processes that go awry in human diseases.

(30) Mass Spectrometry-Based Chemical and Enzymatic Methods for Global Analysis of Protein Glycosylation in Complex Biological Samples

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Proteins and sugars are highly abundant in mammalian cells and the interactions between them are ubiquitous. When glycans are covalently bound to proteins, it is called protein glycosylation which is one of the most common protein modifications in biological systems. Glycosylation often determines protein folding, trafficking and stability. Moreover, it regulates nearly every extracellular activity, including cell-cell and cell-matrix interactions and cell immune response. Currently mass spectrometry (MS)-based proteomics provides a unique opportunity to analyze proteins and their modifications on a large scale. However, global and site-specific analysis of glycoproteins is extraordinarily challenging because of the low abundance of many glycoproteins, the heterogeneity of glycans and the complexity of biological samples. In order to globally analyse protein glycosylation in complex biological sample, effective enrichment methods are imperative. We have developed effective MS-based chemical and enzymatic methods to globally and site-specifically analyze glycoproteins [1-4]. For instance, boronic acid can form reversible covalent interactions with glycans, which is universal and holds great potential to enrich glycopeptides/proteins. However, the interactions between boronic acid and glycan are relatively weak. Therefore, they are not effective to capture low-abundance glycoproteins. Several steps have been taken in our lab to make the methods more effective, including testing many boronic acid derivatives, enriching glycopeptides under different conditions and benefiting from the synergistic interactions between boronic acid and glycan. For glycoproteins, beside the protein part, glycans also contain much valuable information. We have integrated chemical and enzymatic reactions to analyze glycoproteins with a particular and important glycan. Considering the importance of glycoproteins, the newly developed methods will have extensive applications in the biological and biomedical research fields.

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(31) SWATH glycoproteomics to interrogate post-translational modification dynamics in yeast and sparkling wine

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Glycosylation is a critical post-translational modification that influences the folding and function of half of the cellular proteome. We have developed data-independent acquisition SWATH/DIA glycoproteomic workflows and used these to understand the causes and consequences of changes in glycosylation. In tandem mass spectrometry, glycopeptides have largely predictable fragmentation profiles, and we have used this property to develop DIALib, software that generates theoretical fragmentation spectra of glycopeptides in a sample, to enable unbiased SWATH glycoproteomics. We have used these analytical workflows together with yeast genetics to investigate the regulation of protein biosynthesis and post-translational modification. We have also translated these approaches to industrial settings, shedding light on the complexity of the glycoproteome of sparkling wine.

(32) Integrating Mass Spectrometry and RNA-Seq data for Glycosylation Pathway Generation and Simulation

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The glycome constitutes the entire complement of glycans and glyco-conjugates that compose a cell, tissue or organism. With the advent of new high-throughput technologies, vast

amounts of data related to the glycome are being collected at the structure level using mass spectrometry (MS) and at the transcript level using next-generation sequencing (NGS). However, the relation between the transcriptome and the glycome remains unclear since there is no streamlined computational method to integrate knowledge from these two different experimental modalities. To address this limitation, we developed an online glycoEnzyme database called GlycoEnzDB. This resource contains a custom, curated database for 150+ human glycosyltransferases and glycosidases. When coupled to a previously developed Glycosylation Network Analysis Toolbox (GNAT, [1, 2]), GlycoEnzDB enables the automated creation of *in silico* glycosylation networks that describe O-linked glycosylation, N-glycosylation, glycolipid and glycosaminoglycan biosynthesis in any arbitrary system. Using MS Glycomics profiling data for a variety of cell lines (various human leukocyte subtypes) and tissue (from different human organs) that are available from the CFG database (Consortium of Functional Glyomics DB), this tool kit enables both the creation of relatively simple single compartment glycosylation network models, and also multi-compartment systems that more closely mimic the Golgi cisternae. Fitting of experimental data to predict enzyme distribution and reaction rate parameters in each compartment is then feasible using constraint based flux analysis. Such analysis demonstrates that human dendritic cells, natural killer cells and erythrocytes have abundant high-mannose structures relative to B-cells and eosinophils which are rich in bi-antennary structures, and monocytes which have more tetra-antennary structures. Unlike human primary cells, the distribution of predict glycan profile is similar among different human tissues, with high levels of complex bi-antennary glycans. Once baseline models are established for a wild-type/control cell system, perturbations can be performed based on RNA-Seq to derive the modified distribution of glycan profiles in different cells or tissue. Such an analysis strategy that couples GlycoEnzDB with GNAT allows the integrated use of MS and RNA-Seq to develop computational predictions that can be further tested in the wet-lab.

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(33) Comparative sialoglycan microarray analyses of selected human and mouse Siglecs

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Siglecs (sialic acid binding immunoglobulin-type lectins) are membrane proteins expressed primarily on the surfaces of overlapping sets of immune cells. They bind to sialoglycan ligands in *cis* or *trans* mode to regulate immune system function. Soluble Siglec chimeras have been used to identify natural and synthetic Siglec ligands. In the current study, a matched set of Siglec-Fc chimeras including human (h) Siglec-3, -6, -8, and -10 and mouse (m) Siglec-3, and -F were created in the same plasmid, transiently expressed in HEK293 cells, treated with *V. cholerae* sialidase to remove endogenous sialoglycans, and purified. Binding specificities of the matched Siglec-Fc chimeras were determined using two glycan array screening platforms: a limited glycolipid plate array and a more extensive covalently printed sialoglycan microarray (Yu et al, Glycobiology 27:657, 2017 & Gonzalez-Gil et al, Glycobiology 28:786, 2018). Siglec-8 had the narrowest binding specificity, binding exclusively to 6'Su-SLacNAc (Neu5Acα2-3[6S]Galβ1-4GlcNAc and its fucosylated analog 6'Su-SLe^x (Neu5Acα2-3[6S]Galβ1-4(Fucα1-3)GlcNAc). hSiglec-3 bound to the same two glycans and (less robustly) to select branched di- to tetrasialylated structures including gangliosides GQ1bα>GT1b>GD1a. Unlike hSiglec-3-Fc, mSiglec-3-Fc failed to bind any glycan on either array. Mouse Siglec-F, reported to be a functional paralog of human Siglec-8, bound to 6'Su-sialyl LacNac and 6'Su-SLe^x (like Siglec-8) but also to select bi-antennary core-N-linked structures and terminally sialylated gangliosides (GD1a>GT1b=GQ1bα). Siglec-10 bound robustly (and equally) to the same three terminally sialylated gangliosides, and to synthetic branched structures on the printed array that mimic the ganglioside sialic acid spacing. Siglec-6, in contrast, bound to long extended terminally sialylated branched poly-LacNAc structures. The combination of arrays provides additional insights into the overlapping specificities of some of the "CD33-related" mouse and human Siglecs. We also used the same matched set of expressed Siglec-Fc chimeras to validate commercial anti-Siglec antibody binding to native and paraformaldehyde-fixed Siglecs. Supp. by NIH grant AI136443.

(34) The GlyCosmos Portal as a part of the GlySpace Alliance: towards an international glyco-data science collaboration environment

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The Glycoscience Portal GlyCosmos (https://glycosmos.org) has been developed with the support of JST/NBDC. GlyCosmos utilizes Semantic Web technologies to integrate

heterogeneous datasets relevant to the glycosciences, provides user-friendly Web interfaces and datasource access via SPARQL endpoints and application programming interfaces (APIs). It is composed of a Repositories section and a Data Resources section. In the Repositories section, the glycan structure repository GlyTouCan and the glycomics mass spectrometry repository GlycoPOST are available. In the Data Resources sections, currently, glycan-related data from Reactome, UniProt, Lipid Maps have been processed and made available. GlycoNAVI, ACGG-DB and GlycoEpitope are also accessible, and all data is organized according to data type, such as genes, proteins, lipids, diseases, pathways and glycomes. GlyCosmos has been acknowledged as the official portal of the Japanese Society for Carbohydrate Research. Moreover, updates to GlyCosmos are scheduled to be released every four months, ensuring that the data is up-to-date and new resources are continuously being added.

GlyCosmos, together with GlyGen (https://glygen.org) and Glycomics@ExPASy (https://www.expasy.org/glycomics), have formed the GlySpace Alliance (http://glyspace.org), whose members are all nationally funded projects aimed at providing integrated Web access to glycoscience data. In order to formalize data sharing standards and quality control, the Alliance meets annually to discuss areas where further development can be made, to provide validated information to the community. Each portal site provides their own unique user interfaces focused on different aspects of data. Some of this data are common with other Alliance members, and so each member ensures that no discrepancies exist. If inconsistencies are found, meetings are held to reexamine the data and ensure that they are accurate. Provenance is also an important factor, not only for quality assurance, but also for giving proper credit where it is due. Therefore, all data must be properly recorded with evidence, provenance and quality checks.

We have come to a stage where the Internet is flooded with information, and quality control is close to nonexistent. The GlySpace Alliance aims to provide the community with data in which they can be confident. Conversely, users are encouraged to send feedback to the Alliance if any discrepancies are found. Such feedback will be considered by the Alliance to improve the data being provided. This cycle of communication is crucial to ensure high-quality data is maintained in this international glyco-data science collaboration environment.

(35) An odd couple? – Arginine and rhamnose form a novel glycoconjugate to rescue bacterial translation

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We discovered a new form of N-linked glycosylation that activates the polyproline specific bacterial translation elongation factor EF-P. Reportedly N-linked glycosylation occurs almost exclusively at asparagine residues. In stark contrast, EF-P is rhamnosylated at an arginine. However, the underlying molecular mechanism remains enigmatic. Here we describe the structure and function of the EF-P arginine glycosyltransferase EarP. Using NMR spectroscopy we were able to show that EarP is an inverting glycosyltransferase that catalyzes the formation of α-rhamnosylarginine from TDP-β-L-rhamnose. We solved the crystal structure of EarP from Pseudomonas putida and found the enzyme to be composed of two opposing Rossmann domains, thus constituting a GT-B glycosyltransferase. The activated nucleotide sugar donor is located within a highly conserved pocket of the C-domain and EarP recognizes EF-P via its KOW-like N-domain. Based on our structural data combined with an in vitro / in vivo enzyme characterization, we propose a mechanism of inverting arginine glycosylation. As EarP is essential for pathogenicity in P. aeruginosa these findings provide the basis for targeted inhibitor design.

(36) Critical roles for O-GlcNAc in Metabolic Signaling, Stem Cell Biology, and DNA damage repair

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O-GlcNAcylation has emerged as a central regulator of signaling, metabolism, stress, and epigenetics. Proteomics efforts suggest that the enzymes mediating O-GlcNAc addition and removal, (O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA)), form complexes with other signaling molecules and can influence protein stability, nuclear transport, and protein-protein interactions. These important functions have been linked to the pathophysiology of human disease including diabetes, obesity, neurodegeneration and cancer. We have exploited genetic tools in C. elegans, Drosophila, and the mouse to begin to dissect the essential roles of O-GlcNAcylation in development, physiology and disease. The enzymes of O-GlcNAc cycling are components of chromatin complexes with important roles in regulating differentiation and development. Both enzymes are essential for mammalian development with knockouts exhibiting embryonic (OGT) or perinatal (OGA) lethality. Also emerging from these studies is the recognition that highly proliferative cells including self-renewing adult stem cells are highly dependent upon O-GlcNAc cycling. Our studies using both C. elegans genetics and mouse transcriptomics/metabolomics revealed that blocked O-GlcNAc cycling interferes selectively with mitochondrial function and the regulation of fat and carbohydrate metabolism by the PPAR transcriptional coactivators. The b-oxidation of fatty acids in mitochondria and peroxisomes was dramatically altered when O-GlcNAc cycling was interrupted. In addition, the dependence of adult stem cells on glycolysis renders those cells uniquely dependent upon O-GlcNAcylation. The increased flux of glucose uptake and utilization in metabolically active proliferating cells can

lead to oxidative damage. We identified a highly conserved pathway in which O-GlcNAc acts as a central regulatory module to control stem cell proliferation and DNA damage repair pathways in response to oxidative stress. These findings highlight the importance of hexosamine signaling in normal tissue homeostasis and nutrient sensitive stem cell-driven diseases like cancer. We have shown that blocked O-GlcNAc cycling also interferes with neurogenesis and influences neurodegenerative damage to neurons. We have begun to develop O-GlcNAc-specific imaging tools (PET ligands and biorthogonal probes) for diagnostic applications. Thus, our genetic findings define disease-relevant roles for the enzymes of O-GlcNAc cycling in metabolic signaling, stem cell biology, and DNA damage repair.

(37) Decoding the role of intracellular glycosylation in cytoprotection and disease

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In response to injury, cells and tissues remodel their cellular environment to repair damaged structures and if necessary to initiate apoptosis. This process, known as the cellular stress response, includes robust and dynamic changes in the modification of intracellular proteins by monosaccharides of O-linked β-N-acetylglucosamine (O-GlcNAc). Acute enhancment of O-GlcNAcylation reduces apoptosis and necrosis in both *invivo* and *invitro* models of injury that include cardiac ischemia reperfusion injury. Here, we report our efforts to identify the oxidative stress-signaling network regulated by O-GlcNAc. Proteins from numerous functional groups were identified including chaperones, transcriptional regulators, mRNA processing proteins, kinases, and methyltransferases. Our studies have focused on protein arginine methyltransferase 1 (PRMT1), which displays reduced O-GlcNAcylation during oxidative stress. PRMT1 activity is associated with reduced longevity, as well as enhanced sensitivity to oxidative stress, in part by inhibiting AKT signaling. Deletion of the O-GlcNAc transferase (OGT), the enzyme that catalyzes the addition of O-GlcNAc, leads to reduced AKT signaling and augmented levels of asymmetric dimethylation, the product of PRMT1 enzymatic activity. Both *in vitro* and *in vivo* data suggest that OGT binds to and inhibits PRMT1 resulting in enhanced survival. Inhibition of PRMT1 does not appear to require OGTs enzymatic activities, suggesting an additional modality by which OGT regulates cellular function to promote survival.

(38) GRASP55 senses energy and nutrient deprivation through O-GlcNAcylation to promote autophagosome-lysosome fusion

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GRASP55 senses energy and nutrient deprivation through O-GlcNAcylation to promote autophagosome-lysosome fusion

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The Golgi apparatus is the central hub for protein trafficking and glycosylation in the secretory pathway. Previously, we have provided evidence that Golgi cisternal stacking, mediated by the *trans*-oligomerization of the Golgi stacking proteins GRASP55 and GRASP65 (*1, 2*), is required for accurate N-glycosylation (*3*). In addition, our studies also indicate that the Golgi adjusts its structure and function in response to different physiological and pathological conditions (*4*). Here, we explored the Golgi response to energy and nutrient deprivation, and report that GRASP55, the Golgi stacking protein located in *medial*- and *trans*-Golgi cisternae, is O-GlcNAcylated by the O-GlcNAc transferase OGT in growth condition. Glucose deprivation does not significantly disrupt the Golgi structure, but reduces GRASP55 O-GlcNAcylation (*5*). De-O-GlcNAcylated GRASP55 forms puncta outside of the Golgi area, which colocalize with autophagosomes and late endosome/lysosomes. GRASP55 depletion reduces autophagic flow and results in autophagosome accumulation. Biochemically, GRASP55 interacts with LC3-II on the autophagosomes and LAMP2 in late endosome/lysosomes, and functions as a bridge between LC3-II and LAMP2 for autophagosome and lysosome fusion; this function is negatively regulated by GRASP55 O-GlcNAcylation. Similar to glucose starvation, amino acid starvation also causes relocalization of GRASP55 from the Golgi to the autophagosome-lysosome interface, where it recruits the UVRAG PtdIns3K complex to facilitates autophagosome-lysosome fusion (*6*). Therefore, in addition to its role in Golgi stack formation and N-glycosylation, GRASP55 also functions as an energy and nutrient sensor in the Golgi and regulates autophagosome maturation.

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(39) Platelet and myeloid cell phenotypes in a rat model of Fabry disease

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Lysosomal storage diseases (LSDs) are a class of approximately 70 genetic disorders and are among the most common genetic disorders in newborns. Frequently caused by mutations in lysosomal enzymes, LSDs are typically progressive, impact multiple organ systems and significantly decrease

quality of life and lifespan. Of LSDs, Fabry disease is most common and is caused by mutations in the X-linked gene GLA which encodes the acid hydrolase a-galactosidase A (a-GalA). Fabry disease is a member of a larger class of sphingolipidoses whereby patients accumulate glycosphingolipids, specifically the a-GalA substrates globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Patients with Fabry disease typically experience pain crises and ocular abnormalities early in life and develop GI disorders and kidney dysfunction during adolescence. As adults, Fabry patients experience progression of renal insufficiency and often suffer serious cardiovascular or cerebrovascular events that contribute to the significant decrease in lifespan observed for Fabry patients. Currently, the mechanism underlying the increased risk of stroke and myocardial infarction in patients with Fabry disease is incompletely understood.

Recently, we developed a rat model of Fabry disease that closely recapitulates human disease phenotypes including the pain, cardiac and ocular symptoms observed in patients. Using our rat model of Fabry disease, we are investigating the underlying mechanisms of cardiovascular disease that occurs in patients. We observed that a-GalA deficient rats have an altered hematopoietic bone marrow environment that correlates with changes to circulating blood cells and a prothrombotic platelet phenotype. Bone marrow changes observed using immunohistology and electron microscopy included the accumulation of both lipid and terminal a-galactosyl containing molecules consistent with the build-up of the a-GalA substrates Gb3 and lyso-Gb3. Additionally, there was a significant increase in the presence of myeloid-derived white blood cells in the bone marrow. Using flow cytometry to examine changes to circulating blood cells, leukocyte abundance was increased by 58%, with neutrophils, lymphocytes, and monocytes increased by (2.3-, 1.4-, and 2.4-fold, respectively). Circulating leukocytes also had increased levels of the platelet and endothelial adhesion protein PECAM-1. Consistent with a prothrombotic phenotype, platelets from a-GalA deficient animals were increased by 20% overall and functionally had an increased responsiveness to the agonists thrombin and ADP that significantly increased both platelet activation (77%) and platelet aggregation (17.5%). Studies are ongoing to define how the accumulation of the a-GalA substrates Gb3 and lyso-Gb3 contribute to a prothrombotic phenotype in order to improve the understanding and treatment strategies for the cardio- and cerebrovascular pathology frequently present in patients with Fabry disease.

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(40) Discovery of novel ceramide galactosyltransferase inhibitors and their therapeutic application to Krabbe disease

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Galactosylceramides and their sulfated derivatives (sulfatide, 3-O-sulfogalactosylceramide) are known to be important components of myelin throughout the central and peripheral nervous systems. Investigation of their biological roles has been limited to genetic manipulation of their synthesis through the key biosynthetic steps catalyzed by ceramide galactosyltransferase (CGT/UGT8) and cerebroside sulfotransferase (CST). Here we describe the identification and characterization of potent and selective small molecule inhibitors of CGT and describe the relationship between CGT inhibition and the resulting changes in galactosylceramide and sulfatide synthesis in vivo. We discovered that CGT inhibitors can be used to selectively reduce the synthesis of the non-hydroxylated galactosylceramides over their hydroxylated counterparts while higher doses that yield more complete inhibition that can block the synthesis of all galactosylceramides.

Krabbe Disease (KD) and Metachromatic Leukodystrophy (MLD) are lysosomal leukodystrophies that are caused by deficiencies in enzymes required for the lysosomal recycling of sulfatide (MLD, deficiency in the 3-O sulfatase - ARSA) and galactosylceramides (Krabbe, deficiency in the galactosyceramidase - GALC). Substrate Reduction Therapy (SRT) has been proposed as a potential therapeutic option to treat these devastating diseases; however, the lack of potent and selective inhibitors has prevented meaningful progress. Using our newly discovered CGT inhibitors, we explored their therapeutic potential in the mouse models of KD and MLD. CGT inhibitors were able to increase lifespan and improve behavioral measures in the KD mouse; however, the window between therapeutic benefit and toxicity appears to be narrow. Further studies are needed to determine if combination therapies and alternative doing schedules could improve the therapeutic window. These drug-like CNS penetrant small molecule inhibitors of CGT will be powerful tools to further understand the biological roles of galactosylceramides and sulfatides in vivo.

(41) A Conserved Role for N-Glycanase 1 in Regulating Energy Metabolism through AMPK signaling

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Mutations in human *N*-glycanase 1 (*NGLY1*) cause a rare developmental disorder with global developmental delay and a host of other phenotypes, including neuropathy, movement disorder, and chronic constipation. NGLY1 is a cytoplasmic deglycosylation enzyme capable of removing *N*-linked glycans from misfolded *N*-glycoproteins undergoing ER-associated degradation. However, the molecular basis for the phenotypes observed in NGLY1 deficiency patients is not well understood, and no mechanism-based therapy exists for this disease. We have previously shown that the *Drosophila* homolog of NGLY1 (Pngl) regulates BMP signaling in a tissue-specific manner. We also reported that *Drosophila Pngl* mutant larvae show a severe failure in gut clearance that cannot be explained by the loss of BMP signaling. Here, we report that the gut clearance phenotype in these animals is associated with a significant decrease in gut peristalsis. It has previously been reported that mutations in *Drosophila AMP-activated protein kinase α*(*AMPKα*) result in a similar gut clearance defect associated with decreased gut peristalsis. Accordingly, we examined whether decreased AMPK signaling can explain the food accumulation and lethality in *Pngl* mutants. The expression of AMPK*α* is decreased in *Pngl* mutant midguts, and genetic restoration of AMPK levels in mesoderm significantly rescues the gut clearance and peristalsis and also the lethality of *Pngl* mutants. Importantly, *Ngly1* null mouse embryonic fibroblasts (MEFs) also showed decreased AMPK activation. AMPK is a sensor of cellular energy status and maintains cellular metabolic homeostasis. Both *Pngl* mutant fly guts and *Ngly1* mutant MEFs show impaired energy metabolism (altered redox state and ATP levels), which can be rescued by restoring AMPK level. NGLY1 has been shown to activate a transcription factor called NRF1 (official name: NFE2L1), which is a key regulator of proteasome and mitochondrial homeostasis. Experiments in *Drosophila* and MEFs indicate that the AMPK defects caused by *Ngly1* deficiency are independent of NGLY1's role in regulating NRF1 activation and proteasomal gene expression. Importantly, fibroblasts from *NGLY1* deficiency patients show decreased mitochondrial respiration compared to control fibroblasts, which can be rescued upon treatment with an AMPK agonist. Collectively, our data establish an evolutionarily-conserved, NRF1-independent role for AMPK signaling downstream of NGLY1. The activity of AMPK can be regulated pharmacologically. Therefore, our data suggest that enhancement of AMPK signaling can serve as a potential therapeutic approach in *NGLY1* deficiency patients.

(42) Glucose homeostasis is regulated by hyaluronan synthases 1 and 3

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Glucose metabolism consists of highly regulated reactions to ensure cell energy demands as well as cellular homeostasis. Hyaluronan, an abundant extracellular matrix glycosaminoglycan, is synthesized by 3 hyaluronan synthases, Has (1, 2, 3), from cytosolic activated sugar precursors (UDP-GlcNAc and UDP-GlcUA), which connects hyaluronan with glucose metabolism. Moreover, hyaluronan matrix accumulation has been observed during diabetic pathologies. In order to understand how hyaluronan synthase function impacts glucose metabolism we exposed Has1/3$^{-/-}$and WT mice to either low or high fat diet (LFD or HFD) for 20 weeks. Has1/3$^{-/-}$male mice showed similar increase in weight gain compared to control mice when challenged with HFD and LFD. Further, EchoMRI^{TM} analysis showed that fat and lean mass were similar in both groups. To study the effects of Has1/3 deletion on systemic glucose homeostasis, we performed i.p. glucose tolerance tests (GTT) and insulin tolerance tests (ITT) on fasted animals. Has1/3$^{-/-}$male mice displayed higher blood glucose levels during the GTT compared to WT mice in LFD and HFD. In addition, Has1/3$^{-/-}$male mice fed LFD were slightly more insulin resistant as compared to control mice during the ITT. To further evaluate the metabolic profile of Has1/3$^{-/-}$ mice, indirect calorimetry analyses were employed. While VO_2 and VCO_2 measurements were similar at all temperatures analyzed [thermoneutrality (30°C), room temperature (22°C), or under cold stress (4°C)], the respiratory exchange ratios were lower in Has1/3$^{-/-}$ mice fed LFD compared to control mice only at 30°C, which may suggest a preference for using fat as a fuel source at thermoneutrality. Additionally, we found lower plasma levels of adiponectin and leptin in Has1/3$^{-/-}$mice fed HFD as compared to control mice. Finally, liver gene expression analyses showed lower levels of glycogen synthase 2, aldolase C and pyruvate dehydrogenase kinase 1in Has1/3$^{-/-}$mice fed HFD as compared to control mice. Overall, our results suggest that hyaluronan synthases 1 and 3 regulate glucose homeostasis, which may help understand the correlation between diabetes and hyaluronan accumulation.

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(44) An Inhibitor of N-glycan Maturation in Mouse Germ Cells

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MGAT4D has the domain structure of a Golgi glycosyltransferase and has been assigned to the *Mgat4* gene family on the basis of its sequence. When transfected into mammalian cells however, it causes inhibition of MGAT1. MGAT1 is the GlcNAc-transferase that initiates the synthesis of complex and hybrid N-glycans. MGAT4D forms heterodimers with MGAT1, but not with other GlcNAc-transferases, in the Golgi. We have identified two amino acids in MGAT4D that are each necessary for its ability to inhibit MGAT1. In the

mouse, *Mgat4d* transcripts are detected only in spermatocytes and spermatids of the testis. We have investigated biological functions by deleting *Mgat4d* in male germ cells and in the whole mouse. We predicted that deletion in germ cells should have consequences for spermatogenesis and fertility, since conditional knockout of *Mgat1* in spermatogonia causes a block in spermatogenesis and halts the production of sperm. We also investigated misexpression of the transgene *Mgat4d-L-Myc* under the control of germ cell specific promoters: *Stra8* in spermatogonia, *Ldhc* in spermatocytes and *Prm1* in round/elongated spermatids. In these mice, we predicted inhibition of spermatogenesis due to over-expression or mis-expression of *Mgat4d*. We found that global KO or removing *Mgat4d* from spermatogonia (cKO) did not disrupt male fertility nor alter testis morphology. Similarly, each of the transgenic males exhibited unperturbed spermatogenesis and were fertile. Strikingly, however, inactivation of the *Mgat4d* gene increased the sensitivity of male germ cells to mild testicular heat stress. By contrast, the targeted over-expression of *Mgat4d* in 7-month C57BL/6J mouse germ cells increased their resistance to the same heat stress treatment compared to wild type males. Microarray analysis of C57BL/6J mice observed 8 hr after mild heat stress, when morphological damage to germ cells is not observed, has identified gene expression differences that shed light on potential functions of MGAT4D in male germ cells.

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(46) Oga mutants reveal epigenetic, transcriptional and metabolic factors effecting life span and body size in Drosophila

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The reversible O-GlcNAc modification of serine or threonine residues of intracellular proteins is involved in many cellular events from signaling cascades to transcriptional regulation. O-GlcNAcylation is a conserved nutrient-dependent process governed by *O*-GlcNAc Transferase (OGT), that adds O- GlcNAc , and O-GlcNAcase (OGA), which removes it in a manner that's protein and context dependent. Cataloging the *O*-GlcNAc proteome serves a great purpose in understanding the biology of this modification. *O*-GlcNAc is essential for repression gene expression and activation of gene expression through modulating the functions of Polycomb and Trithorax and Compass complexes, respectively.The important role of *O*-GlcNAc on adult life and health have been largely unexplored, mainly due the lack of available model systems. In this study, we identified the *O*-GlcNAcylated proteins in adult *Drosophila melanogaster* using recently developed *oga* knockout flies that are viable and fertile. The adult *O*-GlcNAc proteome revealed that *O*-GlcNAc modification is important for proteins related to cell and organismal growth, development, differentiation, and epigenetics. Phenotypically, *oga* mutant flies are larger and have shorter life span compared to wild type flies, suggesting increased *O*-GlcNAc results in increased growth due to increased cell number. The proteomics screen identified many *O*-GlcNAcylated proteins that could potentially have a role in increased growth and decreased longevity, including HCF, SIN3A, LOLA, KISMET, ATX2, SHOT, and FOXO. Our results suggest that *O*-GlcNAc alters the function of many pathways from transcription, epigenetic modification and signaling pathways that regulate growth rate and affect longevity. Therefore, our findings highlight the importance of O-GlcNAc on growth and life span in adult *Drosophila*.

(47) Role of Immature CD44 O-glycosylation and its Activation of Targets Responsible for Stemness Properties of Pancreatic Cancer

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Pancreatic cancer (PC) is an aggressive malignancy that is expected to be the second leading cause of cancer death by 2030. Accumulating evidence suggests truncated O-glycans is largely observed in PC and correlates with poor prognosis and reduced patient survival. Recent reports show that loss of C1GALT1 (Core 1 β1,3-Galactosyltransferase 1) expression in PC results in the upregulation of genes associated with aggressive PC and metastasis. To study the impact of this immature O-glycophenotype in pancreatic cancer stem cells (CSCs), we utilized the CD44 glycoprotein, a widely used marker of CSCs. This highly O-glycosylated protein constitutes several isoforms with diverse cellular activity that is essential in cell-cell recognition and interaction with the extracellular matrix, however, little has been shown how glycosylation variation of CD44 alters the characteristics of pancreatic CSCs. Thus, we hypothesize truncation of O-glycosylated structures on CD44 imparts activation of downstream targets responsible for self-renewal and maintenance of pancreatic CSCs. To investigate the mechanism by which truncated o-glycosylation of CD44 imparts activation of stem-like properties in PDAC, we utilized human and animal models to genetically delete C1GALT1. Truncated o-glycosylation in PC cells exhibited enhanced expression of

markers responsible for self-renewal and maintenance (e.g. Nanog) and characteristic properties as measured by FACS-based side population analyses and tumor sphere formation. To determine a potential mechanism by which CD44 truncation is maintaining CSC characteristics, we genetically deleted CD44 in existing PC cells with a loss of C1GALT1. We observed a loss of CD44 significantly reduced expression of Nanog and phosphorylated p65, a major component in the Nf-κB signaling pathway. These results provide a novel mechanism by which truncated o-glycosylation of CD44 is responsible for maintaining CSC properties through activation of Nf-κB to activate Nanog for self-renewal and maintenance. Taken together, these studies will provide therapeutic approaches to specifically target pancreatic CSCs and potentially reveal their involvement in the sequence of events in metastasis.

(48) Role of sialylation in the control of cardiac functions in Drosophila

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Previous studies have demonstrated that sialylation is required for the regulation of cardiac functions in mammals. Aberrations in that post-translational protein modification cause cardiac arrhythmias and defects in heart physiology. Voltage-gated ion channels and some other membrane and extracellular matrix sialylated glycoproteins are among the molecules known to be important for mammalian heart regulation. However, functional mechanisms that underlie the effect of sialylation on heart physiology remain poorly understood. *Drosophila* heart has emerged as a powerful tool to study evolutionarily conserved pathways involved in human heart development and physiology. The *Drosophila* model provides a number of important advantages for studying the role of sialylation in heart regulation, such as decreased genetic redundancy, amenability to genetic manipulations, simplified glycosylation, and available approaches for detailed analyses of heart functions. Using that model, we analyzed the effect of mutations in the sialylation pathway genes, including sialyltransferase (DSiaT) and CMP-sialic acid synthase (CSAS), on heart functions. Our results revealed that adult mutants have cardiac abnormalities, including a reduced heart rate, commonly referred to as bradycardia. Our experiments focused further on elucidating pathological mechanisms underlying this heart phenotype. Using a rescue approach with tightly controlled transgenic expression, we found that sialylation is required in the nervous system for normal heart physiology. *Drosophila* adult heart innervation is known to develop during pupal stages, while the larval heart does not show a prominent innervation that could contribute to its regulation. Thus, we analyzed the function of larval hearts and did not find any phenotype in *DSiaT* mutants, which was consistent with the scenario that sialylation affects adult heart functions by modulating neurons that control the heart. Our current experiments concentrate on elucidating cellular mechanisms that underlie the sialylation-mediated control of cardiac functions. We expect that our study will shed light on the analogous mechanism involved in cardiac regulation in mammals and may contribute to developing new therapeutic approaches for heart abnormalities. This project was supported by NIH/NS075534, NS099409 and CONACYT 2012-037(S) grants to VP.

(49) SULF2 overexpression affects survival and modulates sulfation of heparan sulfate proteoglycans in Squamous Cell Carcinoma of the Head and Neck

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Sulfation pattern of heparan sulfate proteoglycans (HSPGs), consisting of four different sulfation modifications, regulates growth factor/receptors signaling pathways via specific interactions with the sulfate groups. 6-O-sulfate is the most impactful among the sulfate modifications and is highly regulated by the activity of dedicated endosulfatases. Specifically, human endosulfatase 2 (SULF2) removes 6-O-sulfate from HS chains, modulates affinity of carrier HSPGs to their ligands, and thereby influences activity of the downstream signaling pathway. Here we explore the effect of SULF2 expression on the HSPG sulfation in tumor and its relationship to clinical outcomes of patients with head and neck squamous cell carcinoma (HNSCC).

We found significant overexpression of SULF2 mRNA and protein in tumor compared to adjacent benign tissues in our study of RNA-seq data from the TCGA database and IHC staining of patient tumor samples, respectively. We found that the SULF2 mRNA in HNSCC tumors differs by location and etiology as mRNA associated with human papillomavirus infection is 2-fold lower than in tumor caused by tobacco and alcohol consumption. Remarkably, high SULF2 mRNA is significantly correlated to poor overall survival of HNSCC patient (hazard ratio=1.49, $p<0.01$) after adjusting for age, gender, tumor stage and tumor location. SULF2 protein expression shows a similar survival trend but did not

reach statistical significance (hazard ratio=1.65, p=0.198) in this sample set of limited size (n=124) and semi-quantitative nature of the IHC staining. LC-MS/MS analysis of the composition of heparan sulfate chains extracted from HNSCC tumor samples revealed that tumors negative for SULF2 carry higher content of unmodified disaccharide units, the fraction of nascent HSPG backbone chains, compared to the positive SULF2 tissues. N-deacetylation/sulfation of the glucosamine units is the initial step necessary for all downstream modifications by setting up the basic N-sulfation domains. We found that total amount of N-sulfated disaccharides in SULF2-positive tumors is 4-times higher than in SULF2-negative tumors (22% vs 4%). Absolute quantity of combined 2-O-sulfate and 6-O-sulfate is the same between tumor samples with different SULF2 expression. However, the percent of trisulfated disaccharide IdoA(2S)-GlcNS(6S), the preferred substrate of SULF2, is markedly lower in high-SULF2 tumors, which corroborates the sulfation-editing effect of SULF2 in HNSCC tumors. Besides, our TCGA data analysis identified synthetic enzymes and carrier proteins of HSPG with both significant tumoral overexpression and prognostic impact in HNSCC, including EXTL2/3, EXT1/2, SDC2-4 and AGRN, which may cooperate to generate altered HSPG in HNSCC. At present, we are completing proteomic analyses of tumor samples to develop a comprehensive view of the HSPG synthetic pathway in the HNSCC tissues.

(50) Enhanced myofibroblast differentiation in Hyaluronan Synthase1/3 double knockout mice is independent of hyaluronan and mediated by a TGFβR/p38MAPK/MRTF pathway.

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During skin wound healing, fibroblasts differentiate into myofibroblasts that synthesize an extracellular matrix and assist in wound closure, scar formation and tissue remodeling. Neoexpression of α-smooth muscle actin (α-SMA) is an established marker for myofibroblast differentiation, and is driven by signaling mediated by TGFβ receptor (TGFβR). Hyaluronan (HA), and its synthesizing enzymes, Hyaluronan Synthases(HAS1, 2, 3), are also thought to participatein this process. To further understand how HA, HAS, and TGFβR pathways interact in regulating myofibroblast differentiation, primary mouse skin fibroblasts were isolated from either wild type (WT) or HAS1/3 double knockout (HAS1/3-/-) mice and cultured *in vitro*. Compared to the WT counterpart, we found that HAS1/3-/- fibroblasts had a higher level of HAS2 gene expression, made a larger pericellular HA coat, had a higher level of TGFβ activity and upregulated gene expression of α-SMA. Surprisingly, removal of extracellular HA by hyaluronidase or inhibition of HA synthesis by 4-MU treatment or Has2 RNAi had no effect on α-SMA levels. The abundance of CD44 protein, the primary receptor for HA on cell surfaces, was also increased in HAS1/3-/- fibroblasts. Paradoxically, CD44 knockdown by RNA interference (RNAi) resulted in increased α-SMA expression and enhanced formation of α-SMA-containing stress fibers. Exploration of mechanisms contributing to the upregulated α-SMA expression in Has1/3-/- fibroblasts, using either RNAi or a chemical inhibitor approach, revealed a requirement for non-canonical TGFβR signaling through p38MAPK, but no role for Smad2, which mediates the canonical pathway of TGFβR signaling. In addition, Myocardin-related Transcription Factor (MRTF), a known regulator of α-SMA transcription in other contractile cell types, showed an increased binding to its transcriptional cofactor, Serum Response Factor, in Has1/3-/- fibroblasts. MRTF knockdown suppressed α-SMA expression at baseline and abrogated the increased expression of α-SMA in Has1/3-/- fibroblasts, suggesting that MRTF is required for upregulated gene expression of α-SMA in Has1/3-/- fibroblasts. Finally, pretreatment with a p38MAPK inhibitor reversed nuclear MRTF accumulation after rTGF-β1 treatment, revealing a crucial role for p38MAPK in regulating MRTF nuclear accumulation. Thus, increased α-SMA gene expression in Has1/3-/- fibroblasts is dependent upon MRTF nuclear accumulation that is regulated by a TGFβR/p38MAPK pathway. These findings provide novel insights in how HA and hyaluronan synthases may regulate pro-fibrotic signaling and myofibroblast differentiation during skin wound healing.

(51) Siglecs as checkpoints in immune cell responses

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The sialic acid-binding immunoglobulin-type lectin family of cell adhesion receptors called Siglecs comprise 14 members in humans and 9 members in mice that are predominately expressed on white blood cells of the immune system. Many siglecs carry regulatory motifs that act as checkpoint inhibitors that regulate immune responses, and help the immune system distinguish between self and non-self. We have developed a liposomal nanoparticle platform that exploits the inhibitory functions of siglecs for suppression of antigen induced immune cell activation. Key to this platform are synthetic ligands that bind with high avidity and high specificity for a single Siglec. The tolerogenic nanoparticles display an antigen and a ligand of an inhibitory Siglec expressed on the cells that recognize the antigen. When an immune cell receptor recognizes the antigen, the Siglecs are recruited to the immunological synapse, suppressing the immune response and effectively inducing tolerance to the antigen. The ligand decorated antigenic particles

show potential for suppression of antigen-mediated immune responses of B cells and mast cells in autoimmune and allergic disease, respectively. (Supported by NIH grants AI050143, AI136443, AI132790, HL107151).

(52) Reprogramming T cells to target glycopeptide epitopes and glycolipids for effective cancer therapy.

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T lymphocytes (or T cells) exhibit rapid proliferation and cytotoxicity after immune recognition of foreign or non-self antigens. Cancerous cells can often display mutated peptides that can be recognized as non-self by the immune system, but a frequent mechanism of tumor escape is loss of the antigen display complexes. Chimeric antigen receptor (CAR) technology allows T cells to be genetically engineered with a novel immune receptor (as well as additional pro-inflammatory or effector molecules) that redirect T cell specificity based on the targets of monoclonal antibodies. This genetic engineering allows the manufacturing of billions of T cells specific for one tumor-associated antigen.

We have designed a CAR-T cell therapy that targets the glycopeptide epitope Tn-MUC1 and demonstrated anti-tumor efficacy against a variety of tumor histotypes, including T cell lymphoma, pancreatic cancer, breast cancer, prostate cancer, and other antigens. Additionally, we have improved upon the activity of the CAR-T cell platform by engineering additional glycoediting machinery into T cells, such as a re-engineered cell surface neuraminidase, in order to induce cooperative killing from non-engineered innate immune cells through decreased sialoglycan-mediated inhibition. CAR-T cells can also be generated with specificity for glycolipids, such as SSEA-4. Using SSEA-4 CAR-T cells and CRISPR/Cas9-edited tumor cells, we were able to define the specificity of the CAR-T cells for SSEA-4 and demonstrate that SSEA-4 is expressed in many differentiated cell types of adult and murine origin.

(53) Immunoglobulin G glycosylation changes in diseases and aging

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Background

Immunoglobulin G (IgG) is one of the key players in various immune response pathways, involved in the protection against invading pathogens but also inducing inflammation and tissue destruction in autoimmune disorders and other diseases with an inflammatory component. Each IgG molecule contains two biantennary N-glycans covalently attached to the conserved N-glycosylation sites at Asn-297 on each of its heavy chains. IgG glycans are of key importance for structural stabilization of the Fc region as well as for the IgG effector functions, affecting IgG binding affinity for FcγRs and other receptors, thus participating in the fine-tuning of the molecule's biological activity. Polyclonal IgG glycosylation varies markedly in different physiological and pathological states (age; gender, pregnancy, menopause; infectious, inflammatory and autoimmune diseases, cancers). However, changes observed in different diseases often show similar patterns (e.g. decreased galactosylation and sialylation), which diminishes specific biomarker potential of the IgG glycome when it comes to individual diseases.

Aim

The aim of this study was to compare IgG glycome in all large studies performed in our lab with the purpose of identifying possible shared and disease-specific biomarkers in the IgG glycome.

Materials and methods

We compiled the data from 19 different cohorts comprising over 15.000 subjects and examined the changes of IgG glycome with age and in people suffering from different diseases: systemic lupus erythematosus, ulcerous colitis, Crohn's disease, rheumatoid arthritis, Hashimoto's thyroiditis, allergic sensitization, type 2 diabetes and colorectal carcinoma. Since the IgG glycomics data have been obtained using different analytical methods: hydrophilic interaction ultraperformance liquid chromatography (HILIC UPLC) and liquid chromatography mass spectrometry (LC MS), the analysis was performed on major derived traits: agalactosylation, digalactosylation, sialylation, fucosylation and the presence of bisecting *N*-acetylglucosamine.

Results

The changes in IgG glycome composition in many different diseases resemble the changes that occur with aging: most notably, a decrease in the abundance of galactosylated and sialylated structures was observed, reflecting the loss of immunosuppressive potential of IgG. Systemic lupus erythematosus and type 2 diabetes patients, in addition, display an increase in the abundance of structures with bisecting *N*-acetylglucosamine, also seen in aging.

Conclusion

IgG glycome seems to be an indicator of common processes underlying various diseases and aging. The fact that the alterations in IgG glycosylation patterns that are known to significantly affect IgG's inflammatory capacity are similar across different diseases and aging points to IgG glycome as one of the mechanisms contributing to the aging process and disease development through modulation of inflammation.

(54) Glycoprotein Aging with Increased Mannose Exposure Linked to Cardiovascular Disease through the Macrophage Mannose Receptor (Mrc1)

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N-glycosylation is common to the vast majority of secretory proteins that are found at the cell surface or secreted. We have previously discovered that as secreted glycoproteins age in the blood circulation their glycan linkages are progressively hydrolyzed from the terminal position inward in what appears to be primarily a stepwise exo-glycosidic process starting with neuraminidases and followed by a series of glycosidases that results in the exposure of different potential lectin ligands. We have also published that this mechanism includes cell surface glycoproteins of the intestinal tract. This intrinsic mechanism of glycoprotein aging is common to all plasma glycoproteins and appears to determine the half-lives of glycoproteins in circulation in the limited cases where it has been studied - specifically two circulating alkaline-phosphatase enzymes. The presence of lectins that recognize further aged glycoproteins includes the mannose receptors, which would detect mannose ligands exposed following loss of sialic acid, galactose, and N-acetylglucosamine linkages. Herein we have investigated the identities of glycoproteins modulated by mannose exposure with increasing age, and those are further regulated in their clearance and function by the endogenous macrophage mannose receptor (Mrc1). Mrc1 is found on macrophages and specialized cells in most organs as well as the vasculature and has a broad spectrum of both glycan and protein ligands due to its complex domain-repeat structure composed of three distinct binding sites. A cysteine rich domain and a fibronectin like domain allow the binding of sulfated glycoproteins and collagens respectively. The lectin domain of Mrc1 can bind and endocytose mannosylated particles ranging from pathogens and allergens to mannosylated glycoproteins. Our studies reveal that the Mrc1 receptor has a lectin function that controls the half-lives of specific glycoproteins in blood circulation, including those that have a regulatory role in cardiovascular and inflammatory processes.

(55) Quantification of intact N-glycopeptides in hepatitis B virus-related liver diseases

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Aberrant N-glycosylation is implicated in the development and progression of cancer, such as cell signalling and communication, tumor cell dissociation and invasion. Characterizing intact N-glycopeptides including glycosylation site and site-specific glycan enable better understanding of the molecular pathogenesis of liver damage and cancer. Herein, accurate quantification of intact N-glycopeptides from a band with 40-55 kDa molecular weight in the SDS-PAGE was investigated in hepatitis B virus (HBV)-related liver diseases. Using $^{18}O/^{16}O$ C-terminal labeling method, we achieved intact N-glycopeptide quantification of serum from patients with HBV-related hepatocellular carcinoma (HCC) and liver cirrhosis (LC). Furthermore, we applied Multiple Reaction Monitoring (MRM) to quantify intact N-glycopeptide relative to the protein content, especially in healthy donor-HBV-LC-HCC cascade. Finally, we found two intact glycopeptides (TPLTA$\underline{N}^{205}$ITK (H5N5S1F1) and (H5N4S2F1)) of IgA_2 significantly elevated in LC compared with HCC, and profound increased from HBV infection. In addition, the variation in the abundance of two intact glycopeptides was not caused by its protein concentration. Combination of an $^{18}O/^{16}O$ labeling N-glycopeptide method and MRM improved the quantitative power and increased the understanding of their functional impact of the observed changes.

Keywords: Glycopeptide; 40 kDa-band; HBV-related liver diseases; Mass spectrometry; IgA_2

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(56) Resident and Elicited Macrophages Differ in Expression of their Glycomes and Lectins

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The pleiotropic function of macrophages in immune defense, tissue repair and maintenance of tissue homeostasis is supported by heterogeneity in macrophage subpopulations that differ both in ontogeny and polarization. The glycans and lectins essential for macrophage function are not well characterized. Here we employed mass spectrometry, metabolic labeling, flow cytometry, and quantitative PCR array to analyze the expression of glycans, glycogenes, and lectins in murine peritoneal resident macrophages, derived from embryonic progenitors, and elicited/polarized macrophages, derived from bone marrow hematopoietic cells. Our analysis revealed that the macrophage glycome primarily reflects developmental origin and to a lesser degree, cellular polarization. Resident macrophages were characterized by a simple O-glycome, primarily consisting of core 1 structures, while elicited macrophages also expressed core 2 O-glycans and more highly branched and extended complex type N-glycans. This result was consistent with the observed expression pattern of correlate glycosyltransferases. Strikingly, we observed that macrophages express a large number of lectins, with 139 lectin genes detected in both resident and elicited cells. Differential expression was observed in 49 lectin genes, including galectins, siglecs, and C-type lectins. Notably, resident macrophages have a higher NeuGc to NeuAc ratio and a higher level of C-type lectin pattern-recognition receptors. Our results provide the first comprehensive analysis of resident and elicited macrophages, establishing a framework for exploration of specific glycan and lectin functions in a variety of host tissues.

(57) The particular glycomes of lymph node lymphatic endothelia and their role in localization and activation of Siglec-1+ subcapsular sinus macrophages

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The composition and functional roles of the dense glycan coats that cover vascular endothelia are largely unknown. We have investigated the glycomes of different vascular endothelia *in vitro* and *in vivo*. When analyzing the vasculature of mouse lymph nodes, we found that glycans terminating in α2,3-linked sialic acid were present at uniquely high densities on the lymphatic endothelium of the subcapsular sinus (SCS). Upon closer investigation, we saw that it was only the lymphatic endothelial cells (LEC) forming the floor of the SCS that were strongly stained by the plant lectin MAL-II. Binding was abolished by sialidase, suggesting that it was due to Siaα2,3-Galβ1,3-GalNAc which may be present on *O*-glycans or on glycolipids. Heavily sialylated glycans are known to be bound by the sialic acid–binding immunoglobulin-like lectins (siglecs) that are expressed by various leukocyte subtypes. Siglec-1 is the largest member of the siglec family, comprising 17 Ig-like domains. Interestingly, there is a subpopulation of Siglec-1$^+$ macrophages which resides on the floor of the SCS. The so-called SCS macrophages (SSM) are intimately associated with the LEC composing the floor of the SCS. Together, these cells form a sieve for the incoming lymph and the SSM capture and transfer particulate antigens to the underlying B cell follicles. We thus asked whether the presence of the SSM in this particular localization may be related to the α2,3-linked sialic acid on the SCS floor LEC. Indeed, we found that Siglec-1 bound to sialic acid displayed by primary lymph node LEC. The relative abundance of Siglec-1$^+$ SSM was significantly reduced in the lymph nodes of mice expressing a mutated version of Siglec-1 that does not bind sialic acid. The numbers of other macrophage subtypes such as the Siglec-1$^+$ medullary sinus macrophages remained the same. Interestingly, the SSM of Siglec-1 mutant mice were less proliferative than those of wild-type mice, which is in accordance with their reduced numbers. When looking at the inflammatory activation state, we found that the SSM of Siglec-1-mutant mice had a more anti-inflammatory phenotype than the SSM of wild-type mice. Taken together, our results suggest that the peculiar and very local, heavily sialylated glycophenotype of SCS floor LEC may be recognized by macrophage Siglec-1 *in vivo*. Such interaction may contribute to the presence and maintenance of the SSM in the lymph node SCS and favor differentiation into a pro-inflammatory phenotype under steady state conditions.

(58) Megakaryocyte O-glycan sialylation regulates platelet production through interferon-secreting plasmacytoid dendritic cells

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Circulating platelet count, an indicator of health and disease, has been linked with platelet glycosylation. Loss of sialic acid during platelet circulation leads to their recognition by the hepatic receptors/lectins, specifically the Ashwell-Morell receptor and the macrophage lectin $\alpha M\beta 2$ integrin, prompting their clearance. The role of lectins and glycans in regulation of bone marrow (BM) platelet production, or thrombopoiesis, is understudied.

We investigated the role for BM plasmacytoid dendritic cells, major producers of Type 1 interferon (IFN-I), in regulating thrombopoiesis of aberrantly sialylated megakaryocytes (MKs). The TF-antigen is a cryptic disaccharide on O-glycans usually covered by a sialic acid moiety added by the sialyltransferase ST3Gal1. In cancer, the increased presence of TF-antigen is immunogenic, even immunomodulatory. To investigate the role of the TF-antigen in thrombopoiesis, we generated ST3Gal1$^{MK\text{-}KO}$ mice (PF4-Cre) that display increased TF-antigen specifically in MKs.

ST3Gal1$^{MK\text{-}KO}$ mice developed significant thrombocytopenia, but had normal platelet half-life, suggesting that the TF-antigen affected BM thrombopoiesis. *In vitro* MK maturation and proplatelet production from primary ST3Gal1$^{MK\text{-}KO}$ mouse BM cells were normal, pointing to extrinsic factors in the BM environment affecting thrombopoiesis. Immunofluorescence stain of the ST3Gal1$^{MK\text{-}KO}$ BM revealed proplatelet structures positive for platelet marker GPIbα co-localizing with TF-antigen being infiltrated by mononuclear cells resembling lymphocytes.

Platelet counts of ST3Gal1$^{MK\text{-}KO}$ mice were restored to wild-type levels by crossing ST3Gal1$^{MK\text{-}KO}$ mice with Jak3^{KO} mice that have impaired of lymphoid cell development. BM immunostaining showed infiltration of ST3Gal1$^{MK\text{-}KO}$ MKs by immune cells, marked by CD4. A comparison of bulk RNAseq between ST3Gal1$^{MK\text{-}KO}$ and wild-type control CD4+ cells within the BM confirmed a population bias for interferon-releasing plasmacytoid dendritic cells, a cell type regulated by unique sialic acid binding lectins (Siglecs). Inhibition of IFN-I activity, by a blocking receptor antibody, recovered platelet counts in ST3Gal1$^{MK\text{-}KO}$ mice to wild-type levels. Together, the data shows that recognition of MK O-glycan sialylation by plasmacytoid dendritic cells, likely through inhibitory Siglecs G and H, regulates thrombopoiesis through IFN-I secretion.

(59) E-Selectin Ligands in Human Leukemogenesis

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Though typically not considered "metastatic", Acute Myeloid Leukemias (AMLs) and Acute Lymphoblastic Leukemias (ALLs) are highly metastatic cancers. These malignancies begin within a discrete marrow microenvironment and rapidly disseminate via the vasculature to occupy the entire systemic marrow compartment. Despite increasing understanding of the genetic features (i.e., genomics) predisposing to leukemias, essentially nothing is known about cell surface carbohydrate modifications (i.e., glycomics) that confer and propagate this malignant phenotype. Migration to bone marrow is critically dependent on E-selectin receptor/ligand interactions.The marrow microvascular endothelium is one of only two sites where E-selectin is constitutively expressed, and circulating cells that express high amounts of E-selectin ligands are therefore programmed to home to the marrow. E-selectin binds to a canonical tetrasaccharide motif known as sialylated Lewis X (sLeX: CD15s). Importantly, studies in our laboratory indicate that E-selectin is expressed on marrow microvessels, both luminally and abluminally. Consistent with this finding, there is increasing evidence that E-selectin expression on marrow microvessels serves a fundamental role in creation of hematopoietic growth-promoting microenvironments, collectively known as "vascular niches". Studies from our laboratory have also revealed that human hematopoietic stem/progenitor cells (HSPCs) express a variety of E-selectin ligands, that expression of sLeX is tightly regulated in a stage- and lineage-specific fashion during human hematopoiesis, and that malignant hematopoietic progenitors ("leukemic blasts") abundantly express E-selectin ligands. Accordingly, we have undertaken studies to characterize the E-selectin binding activity and the specific glycoprotein scaffolds that display sLeX on native HSPCs and on leukemic blasts. To this end, we are integrating hemodynamic fluid shear assays together with flow cytometry and Western blots analyses of HSPCs and of primary blasts obtained from patient blood and marrow. Collectively, our results unveil a striking diversity in the expression of E-selectin ligands, with discrete subsets of leukemia favoring distinct patterns of E-selectin ligand expression. Our findings are providing novel insights on the molecular effectors of leukemia-endothelial interactions, highlighting the role of cell surface glycosylations in the pathobiology of hematologic malignancy.

(60) ST6Gal-I sialyltransferase promotes pancreatic cancer progression through imparting a cancer stem cell phenotype

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Cancer cells upregulate distinct glycosyltransferases and their cognate glycan structures, however the functional role of glycans in tumor cell behavior is not fully understood. One prevalent tumor-associated glycosyltransferase is the ST6Gal-

I sialyltransferase, which adds α2-6 linked sialic acids to select N-glycosylated receptors. Through modulating receptor activity, ST6Gal-I imparts cancer stem cell (CSC) characteristics including tumor-initating potential, cell invasiveness and resistance to cytotoxic stimuli such as chemotherapy drugs and hypoxia. ST6Gal-I is overexpressed in many cancers including pancreatic ductal adenocarcinoma (PDAC) and animal studies by our group have confirmed ST6Gal-I's tumor-driver function. Forced expression of ST6Gal-I in human PDAC cells impels tumor growth and metastasis in orthotopic xenograft models, and likewise, ST6Gal-I promotes carcinogenesis in genetically-engineered mouse (GEM) models. More specifically, we developed a pancreas-specific ST6Gal-I knock-in mouse and crossed this line to the well-known "KC" PDAC model, which harbors oncogenic K-ras in the pancreas. Mice with both ST6Gal-I knock-in and K-ras expression ("KSC" mice) exhibit greatly accelerated PDAC initation, metastatic progression, and mortality, when compared with KC mice (with Kras alone). Given ST6Gal-I's role in conferring CSC features, we hypothesized that ST6Gal-I would contribute to PDAC initiation. One of the earliest events in PDAC development is acinar to ductal metaplasia (ADM), where pancreatic acinar cells transdifferentiate into stem-like, ductal cells which are primed for neoplastic transformation. Immunohistochemical (IHC) staining of the GEM models revealed that ductal markers are abnormally expressed in the non-malignant acinar cells of mice with ST6Gal-I knock-in, suggesting that ADM had occurred. Moreover, using organoids derived from the GEM models, we determined that ST6Gal-I activity increases the expression of ductal and stem cell genes, while suppressing hallmark acinar genes. ST6Gal-I activity also potentiates the formation and overall viability of organoid cultures, consistent with promoting stem cell properties. These results were mirrored by studies using the ADM cell model, 266-6 cells. Ectopic expression of ST6Gal-I in 266-6 cells induces an upregulation in ductal and stem genes, but downregulation in acinar genes, while the inverse was noted in 266-6 cells with ST6Gal-I knockdown. Taken together, these data support a role for ST6Gal-I in ADM and PDAC pathogenesis, and further implicate ST6Gal-I as a promising therapeutic target.

(61) Targeting the sialoglycan–Siglec axis augments antitumor immunity allowing effective PD-1 and CTLA-4 blockade

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Escape of tumor cells from immune control is a hallmark of cancer. Recent evidence has suggested that interactions between sialoglycans and inhibitory Siglec receptors mediate immune evasion and this glyco-immune checkpoint can be therapeutically targeted to improve anti-cancer immunity. We efficiently disrupted the sialoglycan–Siglec pathway by utilizing an antibody-sialidase construct targeted to the tumor-associated antigens. Tumor-specific desialylation induced remodeling of the tumor microenvironment, skewing the polarization of macrophages towards the anti-tumorigenic M1 phenotype, enhancing both dendritic cell activation and the subsequent rapid T cell infiltration. Desialylation inhibited tumor growth and showed synergy with classical ICIs,including anti-PD-1 and anti-CTLA-4 antibodies. We further demonstrated that the effect of the tumor-targeted sialidase was dependent on Siglec-E. Analysis of TCGA gene expression data from human cancers lead to the identification of a sialylation signature correlating with a reduced survival in patients with clear cell renal carcinoma and squamous cell carcinoma of the lung. Taken together, our results demonstrate that systemic targeting of the sialoglycan–Siglec axis is a new immunotherapeutic modality that targets both the innate and adaptive immune systems. Potential synergism with ICI therapy argues for the future development of combination therapies.

(62) Identification of Novel Glycoproteins with Defined anti-Tn IgG and IgM; Applications as Tumor Diagnostic Biomarkers

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The Tn antigen is a neoantigen abnormally expressed in many human carcinomas and expression correlates with metastasis and poor survival. To explore its biomarker potential, new antibodies are needed that specifically recognize this antigen in tumors. Here we generated two recombinant antibodies to the Tn antigen, Remab6 as a chimeric human IgG1 antibody and ReBaGs6 as a murine IgM antibody, and characterized their specificities using multiple biochemical and biological approaches. Both Remab6 and ReBaGs6 recognize clustered Tn structures, but most importantly do not recognize glycoforms of human IgA1 that contain potential cross-reactive Tn antigen structures. In flow cytometry and immunofluorescence analyses, Remab6 recognizes human cancer cell lines expressing the Tn antigen, but not their Tn-negative counterparts. In immunohistochemistry (IHC), Remab6 stains many human cancers in tissue array format but rarely stains normal tissues and then mostly intracellularly. We used these antibodies in pull-down immunoprecipitation to identify several unique Tn containing glycoproteins in Tn-positive Colo205 cells, demonstrating their utility for glycoproteomics in biomarker studies. Thus, recombinant Remab6 and ReBaGs6 are useful for biochemical characterization of cancer cells and IHC of tumors, and represent promising tools for Tn biomarker discovery independently of recognition of IgA1. [This work was supported by

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(63) Modulation of Siglec Binding Via SIAE and CASD1-An Immune Evasion Pathway for Breast and Colon Cancers

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Cancers are very adept at finding immune evasion pathways. One interesting, but poorly studied escape route involves modification of sugar residues (glycans) on cancer's cell surface. All cells wear a "sugar coat," the glycocalyx, that covers the lipids and proteins on the cell's surface. Alterations to the composition and structures of glycans in the glycocalyx are indicators of aggressive cancers, affect tumor biology, and the anti-cancer immune response. For example, excessive addition of the glycan Sialic acid (Sia) to proteins, lipids and carbohydrates, and alteration of the Sia structure contribute to cancer progression, including increased metastasis, and immune evasion by the host immunity by imitating healthy cells. These alterations in Sia may contribute to immune evasion, by Sia interactions with inhibitory receptors on immune cells, called Siglecs. Although the Sia-Siglec interaction is suspected as an immune evasion pathway of cancer cells, how the enzymatic activity of Sialic Acid Acetyl Esterase (SIAE) and CASD1 play a role, aren't well understood. SIAE is a serine esterase that removes the acetyl group on C9 and C4, while CASD1 put acetyl groups onto the C9 position. We demonstrate that SIAE modulates cancer associated Siglec binding and plays a role in the Sia-Siglec immune evasion of breast and colon cancers cells. Furthermore, we demonstrate that the presence of these genes also modulates the level of susceptibility and cytotoxicity of certain breast and colon cancer to chemotherapeutics. Important new therapeutic avenues are currently being investigated by our lab as a result of understanding this new mechanistic insight.

(64) The Role of Core 3 β3-N-Acetylglucosaminyltransferase in Colorectal Cancer

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O-GalNAc glycosylation, also known as mucin-type O-glycosylation (O-glycosylation) which is characterized by a-GalNAc linked to Serine, Threonine or Tyrosine residues in proteins is one major type of protein glycosylations. The O-glycans on glycoproteins play important roles in many biological processes. The common O-glycans are either Core-1, Galb1-3GalNAc-a-R or Core-3, GlcNAcb1-3GalNAc-a-R based structures. Core-1 O-glycans are the most predominant ones found in all animal cells, while Core-3 O-glycans appear to be restricted to proteins from epithelial cells of gastrointestinal tract. Notably, the Core-3 O-glycans were reported to play significant suppressive roles in colorectal tumor biology. But the mechanisms underlying Core-3 O-glycans' tumor suppression are not well understood. Core-3 N-acetylglucosaminyltransferase gene (*C3GnT*, *b3GnT6*) encodes the enzyme responsible for the initiation of Core-3 O-glycans. It is not known how *b3GnT6* in intestinal epithelial cells is transcriptionally regulated, and what biochemical properties the enzyme possesses. Furthermore, existing cell lines do not express *b3GnT6*, and how the *b3GnT6* is suppressed in colorectal cancer remains elusive. Herein, we firstly established colorectal tumor cell lines with the expression of *b3GnT6* and performed characterizations. Notably, ectopic expression of *b3GnT6* eliminated the expression of Tn antigens in the *Cosmc*-deficient cells and led to synthesize Core-3 O-glycans as evidenced by CORA (Cellular O-glycome Reporter/Amplification). Our ongoing studies will address the mechanisms for how the suppression of *b3GnT6* and loss of core-3 O-glycans lead to the progression and metastasis of human colorectal carcinoma. Overall, this study will lead to our better understanding of important role of *b3GnT6* in colon cancer, and the development of potential therapeutics.

(65) ENDOGENOUS LIGANDS OF THE MANNOSE RECEPTOR C-TYPE LECTIN DOMAIN IN CANCER AND CONTROL TISSUE

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The mannose receptor (MR) is an endocytic lectin receptor expressed on the surface of macrophages, dendritic cells, and some endothelial cells. It plays an important, but poorly understood role in recognition of 'self' and 'non-self' in homeostasis and immunity. Several lectin receptors have also been shown to promote immune suppression towards the glycans of the tumor microenvironment. The MR is a pattern recognition receptor containing three types of extracellular binding domains. One of these domains, the C-type lectin-like domain (CTLD), is known to bind glycoconjugates containing the terminal sugar residues mannose, fucose or N-acetylglucosamine, resulting in interaction with ligands of microbial origin, as well as internalization of some endogenous glycoproteins. However, detailed structural information about the MR glycan ligands is limited. To fully understand the MR role in homeostasis and immunity, targeted studies are needed to identify the structural properties of these ligands. To determine the glycan and glycoprotein ligands of the MR CTLD, we used a hybrid protein consisting

of CTLDs 4-7 of the murine MR coupled to the Fc-part of IgG (MR-Fc). With this construct we evaluated glycan binding on glycan microarrays, including the Consortium for Functional Glycomics (CFG), oligomannose, mannose-6-phosphate, and microbial glycan microarrays. While all microarrays identified mannose-containing glycans as the main binders of the CTLD, our recently developed oligomannose array allowed identification of specific oligomannose epitopes recognized by the MR-Fc. We then screened a variety of cancer and control tissues for endogenous ligands of MR-Fc using immunohistochemistry of tissue microarrays, and complemented those findings with binding studies to cancer cell lines. The MR-Fc exhibits tissue- and cell-specific binding to intracellular and cell surface ligands. To further identify specific glycoprotein ligands in cancer cell lines, the MR-Fc was used in immunoprecipitation studies, followed by subsequent glycoproteomics and proteomics analysis. While these glycoproteins included many lysosomal enzymes, we also identified cell surface glycoprotein ligands. The enriched glycoproteins almost exclusively carry oligomannose glycans, resembling the results of the glycan microarrays. The identification of MR ligands in cancer cells will facilitate further studies to understand the role of this receptor, and innate immune receptors in general, in cancer cell recognition and evasion and might reveal a new set of therapeutic targets.

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(66) Reprogramming the Tumor Microenvironment with Macrophage-Targeted Glycopolymers

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Cancer cells demonstrate a unique ability to evade and manipulate immune cells such as tumor-associated macrophages (TAMs) into assisting their proliferation and metastasis. Several tumor-proliferative growth factors, secreted by TAMs, are known to bind heparan sulfate (HS) proteoglycans, which act as reservoirs for growth factors in the extracellular matrix. Heparanase enzymes are over-expressed in the tumor microenvironment and are thought to release bound growth factors and unravel the compact extracellular matrix that slows migration of cancerous cells. In line with these observations, exogenously added heparin limits tumor progression and metastasis, perhaps in part by sequestering growth factors. To better understand this inhibitory role of HS, we synthesized HS proteoglycan mimetic glycopolymers consisting of a polyethylene glycol polymer scaffold decorated with HS disaccharides, which retained growth factor binding but is resistant to degradation by heparin lyases. Along with HS-disaccharides, mannose monosaccharides are appended to our polymer scaffold, which target the polymers to the lysosomes of TAMs through the macrophage mannose receptor (MMR). The MMR is a common route for targeting and delivering cargo to macrophages and has been shown to activate macrophages, stimulate the production of pro-inflammatory molecules, and repolarize the macrophages to an anti-tumor phenotype. Our approach is to use heparanase-insensitive HS-mannose-glycopolymers to sequester growth factors in the tumor microenvironment, traffic these bound growth factors to the lysosome for degradation, while activating tumor-associated macrophages towards an anti-tumor phenotype. This research investigates a unique approach towards reprogramming the tumor microenvironment and explores heparan sulfates involvement in cancer proliferation. Supported by NHLBI K12 career development in glycosciences program K12HL141956.

(67) ABNORMAL HYALURONAN IN INTRACELLULAR COMPARTMENTS OF MONOCYTES/MACROPHAGES UNDER HYPERGLYCEMIC STRESS

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Our previous studies showed that rat kidney mesangial cells (RMCs) dividing under hyperglycemic stress synthesize hyaluronan (HA) abnormally in intracellular compartments. This initiates a large stress response that makes a unique HA matrix outside the cells after completing division that recruit inflammatory cells. Dialogue between macrophages recruited into the glomeruli and the damaged rat mesangial cells leads to diabetic nephropathy, fibrosis and proteinurea that is inhibited in the heparin treated uncontrolled diabetic rat. Our results show that murine monocyte progenitor cells, derived from femur bone marrow, and U937 cells, a human leukemic cell line used to study monocyte/macrophage biology, that divide in hyperglycemia have HA in intracellular compartments that is inhibited by heparin. Both cell types expressed higher levels of pro-inflammatory markers – inducible nitric oxide synthase (iNOS), Cluster Differentiation 80 (CD80), Interferon Regulatory Factor 8 (IRF8), and Tumor Necrosis Factor-a(TNFa) – when cultured *in vitro* under hyperglycemic stress, which is inhibited by heparin. Furthermore, this abnormal intracellular HA was also observed in peripheral blood monocytes derived from monocyte progenitor cells in bone marrow during uncontrolled high levels of glucose from three different diabetic mouse models – streptozotocin treated, high fat fed and $Ins2^{Akita}$ – compared to control. Moreover, peripheral blood monocytes in human subjects of poorly controlled high blood glucose levels with a HbA1c > 7, also had intracellular HA.

(68) O-GlcNAc cycling disrupts hematopoietic stem cell homeostasis and alters T cell activation

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Human immunity requires exquisite regulation of proliferation and differentiation that ultimately allows our bodies to combat infection. To do this, both hematopoietic stem cells (HSCs) and activated T cells undergo substantial metabolic reprogramming utilizing glucose and glutamine to support their energy and growth requirements. Further, differentiated T cell subsets require distinct metabolic programs. How nutrients are utilized by the cell to support an effective immune response remains a critical question. Hexosamine signaling, ultimately contributing to the post-translational modification *O*-GlcNAc, has been implicated as an essential signal for proper T cell activation. Here, we used conditional targeted deletions of the enzyme that removes *O*-GlcNAc, *O*-GlcNAcase (OGA), to determine the impacts of blocked O-GlcNAc cycling on immune function. *Oga* deletion in mouse HSCs resulted in greatly diminished progenitor pools, impaired stem cell self-renewal and transcriptional deregulation of key genes involved in nutrient transport. Further, early T cell development was particularly sensitive to *Oga* deletion. When *Oga* was deleted specifically in mouse lymphocytes, there was an increase in late activation markers after T cell stimulation, suggestive of T cell exhaustion. Together, these data indicated that *O*-GlcNAc cycling plays a critical role in supporting immune homeostasis by contributing both to HSC maintenance and T cell activation.

(69) Probing the role of β-O-GlcNAc-ylation in T-cells using metabolic glycan engineering

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Post-translational modifications (PTM) of proteins, such as phosphorylation, methylation, and acetylation, provide fine spatiotemporal control of biological processes including signalling, gene expression, cell cycle, and development. Recently, there has been a growing interest on another PTM that is commonly found in nuclear and cytoplasmic proteins, namely, β-O-GlcNAc-ylation. Similar to phosphorylation and acetylation, β-O-GlcNAc-ylation occurs abundantly, in a dynamic and inducible manner, on Ser and Thr residues in the vicinity of Pro rich regions and does not have predictable consensus sequence (Torres CR, Hart GW, J. Biol. Chem. 1984). β-O-GlcNAc-ylation is mediated by the action of UDP-N-acetyl-D-glucosamine:peptide β-N-acetyl-D-glucosaminyl transferase (OGT) on nuclear and cytoplasmic proteins and is removed by the action of protein O-GlcNAc-ase (OGA). Unlike kinases which are coded by ∼500 genes in Homo sapiens, both OGT and OGA are coded by single genes. Both OGT and OGA act on multiple protein substrates in a cell-state dependent manner. β-O-GlcNAc-ylation is governed by the flux through the hexosamine biosynthetic pathway, intracellular glucose levels, and metabolism. Several well-studied transcription factors such as p53 and NF-kB are known to undergo β-O-GlcNAc-ylation in addition to other PTMs. The functional, regulatory, and modulatory roles of β-O-GlcNAc-ylation in cellular processes are only beginning to be unravelled.

We were interested to study β-O-GlcNAc-ylation of proteins on a global level as well as on FoxP3, which is the master transcription factor in regulatory T cells (Treg). Tregs play varied roles in immune homeostasis, auto immunity, and cancer immunity. Also the role of differential expression of transcription factors, including Foxp3 and its post-translational modifications, in enabling cellular plasticity is only beginning to be investigated. Recent studies have shown that β-O-GlcNAc modification on Foxp3 is essential for lineage stability of Treg. The goal of our study is to comprehensively characterize the multiple PTMs present on Foxp3 in Jurkat (human T-lymphoma) cells using mass spectrometry. We employed the metabolic glycan engineering methodology, using both peracetyl 2-azido-2-deoxy-D-glucopyranose (Ac4-2-N3-Glc) and peracetyl 2-azidoacetylamino-2-deoxy-D-galactopyranose (Ac4GalNAz). Cells were cultured under varying glucose concentrations to study metabolic incorporation. Immunoprecipitation of Foxp3 from Jurkat cells followed by bio-orthogonal ligation confirmed the presence of β-O-GlcNAc-ylation. Additionally FLAG-tagged Foxp3 was expressed in HEK293T cells using lentiviral transduction and purified. Tryptic digestion followed by nano-LC-ESI-MS/MS analysis revealed Foxp3 peptides that were modified with both phosphorylation and β-O-GlcNAc in a competitive manner. Results of our investigation will be discussed in this presentation.

(70) Many Apparently Non-Selective GAG – Protein Systems May Exhibit Interesting Selectivity Features: The Case of Human Neutrophil Elastase

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Glycosaminoglycans (GAGs) are complex, linear, carbohydrate molecules that are found essentially on all animal cell surfaces and modulate a host of biological processes. GAGs are highly negatively charged, which induces binding to hundreds of proteins. This is the reason why the vast majority of GAG – Protein Systems are thought to be non-specific. Yet, recent studies point to the existence of several

specific or selective GAG-protein systems wherein non-ionic interactions and distinct structural features, such as chain length and/or position of sulfate groups, play key roles.

Human neutrophil elastase (HNE), an arginine-rich serine protease implicated in a number of inflammatory diseases, is known to bind to and be inhibited by a number of different GAGs. The interaction between GAGs and HNE is thought to be completely non-specific. In fact, the nature of GAG binding to HNE remains largely unclear. To determine the GAG-binding site of HNE and also assess non-specificity or selectivity features of GAG – HNE system, we undertook detailed computational, biophysical and biochemical characterization studies.

First, we employed an in-house computational method known as the combinatorial virtual library screening (CVLS) algorithm to identify promising GAG sequences that could inhibit the protease. Per our computational analysis, the interaction between GAGs and HNE was found to be allosteric with the binding site comprising Arg36, Arg65, Arg75, Arg76, Arg80 and Arg149 residues. Interestingly, the computational analysis pointed to several non-ionic residues, in addition to these charged residues, as being important for binding. Most importantly, the studies predicted that GAG sequences longer than a hexasaccharide would be required for tight binding to HNE. The studies also indicated that a particular tetrasaccharide sequence was the 'selectivity' element in GAG recognition of HNE.

To validate our computational results, we employed biochemical studies involving chromogenic substrate hydrolysis assay, salt dependence of binding, and fluorescence binding titrations. The results show that GAGs inhibit HNE via an allosteric mechanism with octasaccharide sequences being at least 6-fold more potent than corresponding hexasaccharide sequences. Additionally, GAG sequences with similar number of sulfates but different sulfation patterns were found to display significantly different HNE inhibitory potency, which indicates that distinct GAG structures drive inhibition of this protease.

Overall, our work shows that even for cases where GAGs appear to bind to proteins with relative non-specificity, 'specific' interactions can be invoked. Thus, it is important to recognize that seemingly non-specific systems may possess elements of selectivity or specificity. Further, our work has advanced the concept of structurally-defined GAG oligosaccharides as inhibitors of HNE.

(71) Oxidized-Desialylated Low Density Lipoprotein Inhibits the Antitumor Functions of Human Lymphokine Activated Killer Cells

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Cancer cells often become able to evade the immune system, which is otherwise capable of destroying them. Lymphokine activated killer (LAK) cells display increased cytotoxicity against cancer cells, and they have been evaluated as a potential cancer therapeutic. LAK cells are composed of NK cells ($CD3^{-}CD56^{+}$) and NKT ($CD3^{+}CD56^{+}$) cells. Prior studies have shown that native low-density lipoprotein (LDL) reduces T cell antitumor functions. It is known that desialylation of LDL is an early event in the development of atherosclerosis that leads to LDL oxidation, and that when LDL is oxidized by a different route it inhibits LAK cell cytotoxicity. Hence, we asked whether oxidized-desialylated LDL affects the functionality of lymphokine activated killer cells in vitro. LAK cells were preincubated with native and oxidized-desialylated LDL for 72 hours, and LAK cell cytotoxicity was evaluated in a 4-hour killing assay with K562 cells by flow cytometry. Results revealed that LDL is a human serum factor that when it becomes both desialylated and oxidized, inhibits LAK cell antitumor activity. The cytotoxicity assays showed that oxidization alone is not enough to inhibit LAK cell cytotoxicity, and only a slight inhibition is observed when LDL is only desialylated. The reduced cytotoxicity of LAK cells was further corroborated by a reduction in the number of CD56 positive cells. pHrodo Green LDL was used to measure the rate at which LAK cells take up both native and oxidized-desialylated LDL for a period of 72 hours. It was observed that during the first two hours native LDL is taken up faster than oxidized-desialylated LDL. However, after 16, 32 and 72 hours, oxidized-desialylated LDL is taken to a significantly greater extent than native LDL. In summary, these results suggest that oxidization and desialylation facilitate LDL uptake by LAK cells and inhibit their antitumor functions.

(72) Shortening heparan sulfate chains prolongs survival and reduces parenchymal plaques in fibrillar prion disease

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Prion diseases are fatal neurodegenerative disorders caused by the misfolding of the physiologically expressed prion

protein into an insoluble form that accumulates as amorphous aggregates (subfibrillar prions) or amyloid fibers (fibrillar prions). Cofactors are essential to drive recombinant prion protein assembly into pathogenic conformers. Heparin, heparan sulfate (HS), and other glycosaminoglycans promote prion aggregation and fibrillation *in vitro*, however the cofactors that facilitate prion pathogenesis *in vivo* are largely unknown. Here we determine how HS chain length impacts the progression and cell targeting of prion strains *in vivo*. Exostosin-1 haploinsufficient ($Ext1^{+/-}$) mice, which produce shortened HS chains, show a prolonged survival and redistribute prion plaques from the brain parenchyma to blood vessels when infected with fibrillar prions, but only modestly slowed subfibrillar prion disease. Remarkably, the fibrillar, plaque-forming prions were largely composed of ADAM10-cleaved prion protein lacking a glycosylphosphatidylinositol (GPI)-anchor, indicating these prions were mobile and assembled extracellularly. By analyzing the prion-bound HS using liquid chromatography mass spectrometry (LC-MS), we identified the disaccharide signature of HS differentially bound to fibrillar compared to subfibrillar prions, and found approximately 20-fold more HS bound to the fibrils. Finally, LC-MS of prion-bound HS from human patients with familial and sporadic CJD also showed distinct HS signatures and higher HS levels associated with fibrillar prions. This study provides the first *in vivo* evidence of an endogenous cofactor that accelerates prion disease progression and enhances parenchymal deposition of ADAM10-cleaved, mobile prions.

(73) Carbohydrate microArray Analysis and Reporting Tool: CarbArrayART

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Since their inception in 2002, microarrays of sequence-defined glycans [1] have become essential tools in biology and medicine, particularly to study glycan-binding specificities of viruses, bacteria, antibodies and lectins. Currently, there are up to 900 glycan probes in the largest glycan microarray facilities [2,3,4], and the number is increasing.

Beyond data storage and display as charts, it is desirable to be able to sort and filter, according to structural features of glycans bound or not bound by particular recognition systems. Unique software tools with such functions were developed in the Glycosciences Laboratory by Mark Stoll [5]. These have been the mainstay of data storage, presentation and reporting at Imperial College (160 published and over 8000 internal data sets which can be retrieved at will). However, these software tools were developed in stages using Microsoft Office and Visual Studio, and as such are not readily distributable.

CarbArrayART (Carbohydrate microArray Analysis and Reporting Tool) has been developed to address the need to have distributable software for microarray data. This software tool capitalizes on GRITS Toolbox [6] which was originally developed for processing, interpreting and archiving glycomic mass spectrometry data. CarbArrayART utilizes the functionalities provided by GRITS Toolbox for storing glycan structures and metadata such as project information, sample description and experimental details.

The main features of CarbArrayART are: (i) Storage of carbohydrate microarray data from different array formats including scan data and array-specific metadata such as glycan probe lists, array geometry, information on glycan-binding samples and experimental protocols. (ii) Presentation of data as tables and charts that can be automatically generated with filtering and sorting capabilities as desired. (iii) Reporting of microarray data in Word, PDF and Excel formats, together with metadata that are compliant with MIRAGE (Minimum Information Required for a Glycomics Experiment) [7].

At the time of writing this abstract, we are finalizing the user's guide for CarbArrayART version 1.0. The software will be released after beta testing.

New features will be developed in CarbArrayART for uploading and downloading data to and from the glycan microarray repository which is under construction within the GlyGen project (https://www.glygen.org/), and for handling compositions and binding signals of glycan fractions in Beam Search [8] and Shotgun arrays [9].

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(74) Assembly of chondroitin sulfate glycosaminoglycan-containing hypothalamic perineuronal nets contributes to the sustained antidiabetic effect of FGF1 action in the brain

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The central nervous system (CNS) plays a key role to promote homeostatic control of blood glucose levels, and defective hormonal and nutrient-sensing neurocircuits in the arcuate nucleus-median eminence (ARC-ME) of the mediobasal hypothalamus (MBH) are implicated in the pathogenesis of type 2 diabetes (T2D). This perspective is buttressed by our recent finding that sustained diabetes remission can be induced by a single bilateral injection of fibroblast growth factor 1 (FGF1) targeting the ARC-ME in Zucker Diabetic Fatty (ZDF) rats. RNA sequencing analysis of the MBH in T2D mice after central administration of FGF1 reveals changes in differentially expressed genes involved in extracellular matrix (ECM) remodeling, neural plasticity, and perineuronal nets (PNNs). PNNs are latticelike ECM specializations (composed of chondroitin sulfate (CS) glycosaminoglycan (GAG) chains attached to core proteins) that enmesh, and thereby regulate, network interactions of key neurons in defined circuits. *Wisteria floribunda agglutinin* (WFA) labeling CS-GAG chains comprising the PNN matrices show aggrecan as the main CSPG found in human and rat hypothalamic PNNs. Furthermore, hyperglycemic ZDF rats exhibit a significant loss in hypothalamic PNN honeycomb structures compared to age-matched normoglycemic WT controls. PNN matrix stability and function are determined by the composition of specific CS isomers that constitute the PNN matrix. Using our recently published LC-MS/MS + MRM methodology to measure the relative percentages of chondroitin sulfate isomers from hypothalamic fixed brain tissue, we report an increase in Δ4S-CS and decreases in Δ6S- and Δ4S6S-CS isomers within the MBH of ZDF rats that are suggestive of destabilized PNN structures and associated ECM scarring. The effect of centrally-administered FGF1 to lower blood glucose in ZDF rats was also accompanied by both a normalization in CS composition within the MBH and increased assembly of PNN structures in the ARC-ME. To determine whether these changes in PNN matrices are required for sustained normalization of blood glucose induced by central FGF1 treatment, the CS-GAG digesting enzyme Chondroitinase ABC (ChABC) was bilaterally microinjected into the ARC-ME in ZDF rats in conjunction with intracerebroventricular (icv) FGF1 administration. ChABC digestion of hypothalamic PNNs markedly shortens the duration blood glucose normalization induced by icv FGF1 and identifies ARC-ME PNNs as key targets for the effect of FGF1 to induce sustained diabetes remission. We hypothesize that this effect is achieved by enhancing the stability of hypothalamic PNNs, which in turn may help to normalize ARC-ME neural network interactions involved in the homeostatic control of blood glucose.

(75) Hemolytic anti ABO antibodies (hemolysins) in transplant patients have a broader IgM and restricted IgG glycan recognition repertoire

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Hemolysins are clinically important antibodies that destroy red blood cells (RBCs), but their specific target on RBCs remains unclear. Naturally occurring A, B, and O antibodies are thought to be clinically inconsequential, but some individuals can produce hemolytic ABO antibodies (hemolysins) that actively destroy RBCs in the intravascular and extravascular system. These potent antibodies are especially important for transplant patients, since hemolysins directed against the ABO blood group can preclude incompatible organ transplantation and decrease graft survival. ABO isoagglutinin titer and blood group testing are currently used to identify a hemolysin. However, these approaches are not standardized and do not accurately predict pathologic hemolysin activity, putting transplant patients at risk. We hypothesized that specific ABO glycan chains, which are not detected by clinical testing, contribute to the potency of hemolysins.

Serum from healthy donors and pre-transplant renal, hepatic, and cardiac recipients were screened for hemolytic ABO antibodies (hemolysin) using clinical serologic techniques. The A, B, and H antigen specificity of non-hemolysin and hemolysin antibodies were analyzed with a microarray containing types I-VI and di/tri-saccharide A, B, and H antigens. Twenty non-hemolysin donors and 20 hemolysin renal, cardiac, and hepatic transplant recipients were analyzed. Transplant recipients with IgM hemolysin antibodies had a wide repertoire and more avidly bound all A, B, and H antigens types I-VI, compared to controls. No pattern in IgG antibodies were observed. In contrast, healthy donor IgG avidly bound only specific H antigen types I, II, and IV, potentially representing autoantibody reactivity. No single IgM antibody predominated in the control group . When IgM and IgG antibodies were evaluated individually between

groups, no statistically significant differences were identified between cohorts. However, when IgM and IgG antibody data were analyzed together, the healthy and disease groups were easily discernible. Surprisingly, although often clinically used, ABO isoagglutinin titer and blood group were not statistically significant factors in determining hemolysin activity. Instead, our data show that only the combination of ABO IgG and IgM antibodies can clearly delineate the two cohorts H antigen reactivity was the strongest predictor of a non-hemolytic ABO antibody compared with the broad IgM binding to all A, B, and H antigens in patients with hemolysins. This data suggest that using the specific ABO blood group microarrays, transplant patient hemolysin antibodies can be predicted before transplant and acted upon to prevent ABO-mediated organ rejection.

(76) The glycosyltransferase ST6Gal-I confers resistance against natural killer cell mediated cytotoxicity

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ST6Gal-I, a glycosyltransferase that adds α2-6-linked sialic acids to N-glycosylated proteins, is upregulated in multiple cancers and correlates with decreased patient survival. Previous studies by our group and others have demonstrated that sialylation of select cell surface receptors by ST6Gal-I modulates receptor function, leading to alterations in downstream signaling. Additionally, our lab has shown that high ST6Gal-I activity confers a cancer stem cell phenotype and promotes several of the hallmarks of cancer, including increased cell invasiveness, dysregulated energetics, resistance to apoptosis, and acquisition of metastatic potential. However, the role of ST6Gal-I in tumor escape from the immune system has received limited attention. In the present study, we investigated ST6Gal-I's contribution to immune evasion by modulating ST6Gal-I expression in ovarian or pancreatic cancer cells, and then co-culturing cells with the NK-92 natural killer (NK) cell line. We find that ST6Gal-I overexpression protects cancer cells against NK-mediated cell death, as measured by cell morphology, caspase 3/7 activity, and annexin/PI staining. Contrarily, ST6Gal-I knockdown sensitizes cancer cells to the action of NK cells. Furthermore, using SNA precipitation, we have identified that the two death receptors, Fas and TNFR1, are targets of ST6Gal-I mediated sialylation. By immunoblotting and examining caspase 3/7 activity, we find that sialylation of these two receptors leads to a decrease in apoptotic signaling. Additionally, we have shown that ST6Gal-I-mediated sialylation of the Fas and TNFR1 prevents receptor internalization as measured by flow cytometric analysis and immunofluorescent imaging. In turn, receptor sialylation promotes the survival of tumor cells exposed to FasL and TNF-α, both of which are secreted by NK cells in order to kill their targets. Based upon these data, we hypothesize that tumor cells with high levels of ST6Gal-I evade NK-mediated cytotoxicity through the sialylation of Fas and TNFR1 and the activation of pro-survival pathways.

(77) Collaboration, Service and Trainings at the Complex Carbohydrate Research Center

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For more than 30 years, the Complex Carbohydrate Research Center (CCRC) has collaborated with universities, federal agencies, and industry from the US and other countries on detailed structural characterization of glycoconjugates. We have expertise in a full range of analytical methodologies needed to conduct comprehensive research in glycobiology. We are equipped with the state-of-the-art instrumentation including a Thermo Orbitrap-Fusion MS, Velos Orbitrap-Elite MS, LTQ-Orbitrap MS, AB SCIEX TOF/TOF 5800 MALDI-MS, Bruker MALDI-TOF MS, SPR, multiple analytical and preparative separations options, and GC-MS. The CCRC's NMR facility offers outstanding resources for high-field NMR spectroscopy including 900 MHz, 800 MHz, three 600 MHz and a 500 MHz spectrometer. These spectrometers are equipped with variety of probes including a HRMAS probe for solid state analysis and several H-C/H-N cryoprobes and HCN cryoprobes dedicated to biomolecular NMR applications.

Collaborative projects with the CCRC can be very diverse and can include: Glycomics and glycoproteomics, isolation and analysis of Glycosaminoglycans (GAGs) and GAG-derived products, polysaccharides and Lipopolysaccharides (LPS), peptidoglycans, and glycolipids. Our scientists have particular expertise in specialized method development for isolation and characterization of novel glycoconjugates, depending on individual projects.

The CCRC offers yearly training courses on **techniques for structural characterization glycans of glycoproteins, glycolipids, polysaccharides, and GAGs, as well as courses on mass spectrometry techniques and software analysis.** Training participants will perform hands-on experiments and analysis by NMR, ESI-MS/MS, MALDI-MS, GC/MS, HPLC-UV and fluorescent detection, HPAEC-PAD, lectin blotting, and thin layer chromatography. The hands-on experience is further enhanced by lectures from faculty on various fields of glycobiology.

(78) Understanding the glycoconjugate receptors for cholera toxin: searching for alternative receptors

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The disease of cholera is caused by the secreted protein complex cholera toxin (CT) produced in the gram-negative *Vibrio cholerae*, resulting in acute diarrhea. While most patients survive with treatment of intravenous and oral fluids, the mortality rate can reach 50% if the patient is left untreated. The World Health Organization (WHO) estimates that more than 100 000 people die every year from cholera infection, with young children (under 5) being the most susceptible.

CT is long believed to have the binding partner ganglioside GM1a as the main functional receptor, allowing the toxin to enter cells. GM1a binds with high affinity to the B-subunit to CT, and studies focusing on the exogenous addition of GM1a to the ileum in multiple species significantly increases the response to CT. However, GM1a was found only in very small amounts in human intestine, leading to the question of whether it is present in large enough quantities to mediate a response that is lethal in humans.

Recent studies have indicated that CT has a second glycan binding site with affinity for fucosylated glycoconjugates. Lewis X structures were found to bind the CT in the secondary glycan binding site, though with a significantly weaker affinity than that of GM1a. Despite much lower affinity, fucosylated glycoconjugates can potentially play an important role in host cell intoxication by CT if they are present at high abundance and/or if they function in concert with high-affinity receptors like GM1a.

While previous results provided evidence for direct binding of CT to fucosylated glycoproteins, fucosylated glycolipids may also be targets for CT binding. Indeed, fucosyl-GM1 has been previously implicated as a CT receptor. Here we describe the profiling of additional potential CT receptors found in colon epithelial cell lines. Glycolipids isolated from T84 cells were initially assayed by thin layer chromatography to assess lipid type. Lipids of interest were then permethylated and analyzed by DI-MS/MS to sequence the glycan structure, and compositional analysis by GC-MS was used to confirm monosaccharide identity. The results suggest a number of different glycosphingolipid and glycolipid structures, including those that may also correspond to the same fucosylated Lewis X structure. The identification of novel CT receptors could help explain individual variation in CT sensitivity and may suggest new therapeutic strategies to interfere with CT action.

(79) GlyThyra: An accessible and high-throughput mass spectrometry-based N-glycomics platform

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Protein glycosylation plays a role in a wide range of biological functions and disease processes, from protein folding, cell adhesion and signalling, to pathogen recognition and immune responses. However, our current view of protein glycosylation is incomplete because current technologies offer limited throughput and accuracy. To address these challenges, we developed a new analytical platform, GlyThyra, integrating new preparation and analysis workflows. The semi-automated sample preparation workflow exploits solid-phase PNGase-F digestion with reduced handling time. To ensure accuracy, we developed a released glycan quantitation assay to enable normalized on-column loading, improving quality control over current protein normalization approaches. The data analysis burden is reduced by using an *N*-glycan library of over 300 structures covering all major biosynthetic classes.

GlyThyra was compared to a widely used manual-intensive glycomics method for the analysis of monoclonal antibodies, plasma, and human primary tissue. Repeatability, throughput, and total glycan yield were assessed for each sample type. Our new fluorescent glycan quantitation method significantly reduces LC-MS variation for both the classic and newly developed methods and is accurate over 3 orders of magnitude with a limit of detection of 12 pmol. Automated glycan assignment is informed by three dimensions of LC-MS values, high-resolution MS1, MS2 and normalized retention time, enabling over 95% of structures to be automatically assigned correctly and the remaining 5% of peaks highlighted for manual inspection. Overall, GlyThyra enables complete *N*-glycan release, LC-MS acquisition and data analysis to be completed within 24 hours, compared to >3 days with the classic method. Moreover, as two of the samples are NIST standard materials, we expect these glycan libraries will be useful references for future QC efforts. Overcoming throughput and accuracy challenges by generating glycomic data with improved precision, the GlyThyra platform will facilitate future translational glycomics across a broad range of sample and disease types.

(80) Sickle Cell Trait and Sickle Cell Disease Change the Profile of Plasma Glycan-Binding Proteins

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Sickle cell disease (SCD) is caused by the homozygous inheritance of a V6E mutation in the hemoglobin HbS beta chain which causes the polymerization of deoxy sickle hemoglobin and results in sickled cells. SCD is associated with anemia, episodes of pain, vision problems, and frequent infections. These infections are due in part to the infarction of the spleen, chronic inflammation, and translocation of gut bacteria.

The pan selectin inhibitor Rivipansel is currently in Phase 3 of clinical trials and is used in SCD during vasoocclusive crisis to ameliorate the diseases side effects. Data from these trials shows that glycan-selectin interactions play a role in in SCD. We hypothesized that the activity of plasma proteins is inherently different, including glycan recognition patterns of plasma proteins, in people with SCD and sickle cell trait (heterozygous inheritance). Glycan recognition of plasma proteins was therefore explored using microarray technology.

Plasma samples from 80 healthy donors (20 samples each from type O, A, B, and AB donors) were collected with additional samples from patients with SCD and sickle cell trait (18 and 13 type O samples, and 5 and 7 type A samples from SCD and trait patients respectively). Each sample was diluted, biotinylated, and applied to a RayBiotech Glycan Array containing 100 different glycans ranging in structure from monosaccharides, disaccharides, and blood groups, to sialylated, milk, and natural oligosaccharides. Each sample was analyzed for fluorescent signals using a GenePix microarray scanner and averaged data was normalized using a quantile approach with R.

Data analysis showed that three main glycan structures were bound differently between healthy donors of different blood groups. These were Sialyl Lewis X and SF-G (differing in blood groups containing the A antigen), and B antigen tetrasaccharide (minimally bound by type AB plasma). These results point to blood group specific antibodies in plasma, which was expected. When looking at differences between healthy samples and those from people with SCD and sickle cell trait, only type O and A plasma was analyzed. There were a number of glycans that were bound with an increased affinity by blood group O SCD samples. These were mainly aminoglycosides and fucosylated oligosaccharides including Lewis A. In blood group A samples, there was an increased binding of Sialyl Lewis A, Lewis A, and Lewis Y structures by SCD plasma. This suggests that there is decreased expression of the A antigen in people with SCD, which was corroborated in preliminary analysis of red blood cells by lectin array. In future studies, we will increase the donor pool and combine results with demographic data of each patient. We will also determine if glycans are differentially expressed on plasma proteins in response to SCD and sickle cell trait.

(81) Altered Glycosidase Activities at Physiological pH in the Pathogenesis of Sepsis

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Glycosidases are hydrolytic enzymes that are primarily studied in the context of intracellular catabolic pathways within the lysosome. Reductions in circulating glycosidase activities have been linked to lysosomal storage diseases and are typically detected in the blood acidified to mimic lysosomal pH. There are also instances of lysosomal storage diseases linked to increased glycosidase activities in blood circulation wherein the mannose-6-phosphate-dependent trafficking is rendered dysfunctional. In addition, changes in circulating glycosidase activities have been associated with other syndromes including cancer, arthritis, alcohol abuse, sepsis, and colitis. We recently discovered that glycosidases present in multiple cell types and the sera and plasma are involved in the aging of secreted and cell surface glycoproteins. The exo-glycosidase activities of endogenous circulating glycosidases generate the stepwise loss of glycan linkages over time as glycoproteins age in circulation, sequentially exposing underlying glycan linkages starting with the removal of the terminally-positioned sialic acids. More is known of the neuraminidases in this first step and the role of asialoglycoprotein lectin receptors that can bind and endocytose the previously underlying and cryptic galactose ligands. We have optimized the detection of glycosidases involving those with galactosidase, glucosaminidase, mannosidase, and fucosidase activities using fluorimetric substrates in blood serum and plasma at physiological pH 7.4. We found all four glycosidase activities significantly above background in the blood and plasma at normal basal levels among healthy mouse and human species. We also identify the source of these glycosidases using glycosidase-deficient mouse strains. We further present these measurements of normality and origin in comparison with measurements made during the onset and progression of sepsis caused by different pathogens in mice and humans. Our findings to be presented include the determinations of specific activities of glycosidases in response to experimental sepsis in the mouse caused by each of the five different clinically-derived bacterial pathogens, and the discovery of a specific change in glycosidase activity statistically linked to a poor outcome (death) in human sepsis patients.

(82) Functional Criticality of Glycosylation Attributes of a Therapeutic Cytokine-IgG Fc Fusion Protein.

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Glycosylation of therapeutic proteins may impact bioactivity, pharmacokinetics, immunogenicity and safety. Detailed characterization of glycosylation attributes of a recombinant cytokine-IgG Fc fusion protein produced in Chinese Hamster Ovary cells was undertaken to facilitate the assessment of the molecule's potential critical quality attributes (pCQAs). Fusion protein glycovariants with varied glycosylation patterns and total sialic acid content were generated to assess impact of glycosylation on ligand-receptor binding (*in vitro* relative potency by ELISA and/or cell-based assay) and on pharmacokinetics in a non-clinical mouse model.

The fusion protein was found to be heavily N-glycosylated but with some of the glycosylation sites being partially occupied. Glycosylation profiles were highly complex resulting from heterogeneity in the extent of branching, core fucosylation, galactosylation, antenna-truncation, LacNAc repeats, and sialylation. Glycosylation profoundly affectedbinding of the fusion protein to its receptor. Removal of N-glycans resulted in significant increase of the *in vitro* relative potency. Sialylation had a critical functional impact but other glycan features also mattered. In general, sialic acid content exhibited a clear negative correlation with the *in vitro* relative potency. Analysis of variants with intentionally varied glycosylation patterns and sialic acid content further refined correlations of glycan levels with the *in vitro* relative potency and pharmacokinetic parameters.

Overall, our results demonstrate the critical role of glycosylation in *in vitro* cytokine-IgG Fc fusion protein-receptor interactions and *in vivo* pharmacokinetics, and highlight the importance of developing a detailed understanding of glycosylation attributes and their functional criticality during the technical development of therapeutic glycoproteins. The results also highlight the usefulness of HILIC UHPLC methodology for the analysis of highly complex glycosylation patterns in biopharmaceutical industry setting.

(83) Targeting the sialoglycan/Siglec-9 immune checkpoint for cancer therapy

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The interaction of tumor-associated highly sialylated glycans (sialoglycans) with inhibitory Siglec receptors has been recently defined as a potential new immune checkpoint that could be targeted for cancer immunotherapy. In particular, interactions of tumor-associated sialoglycans with Siglec-9 on various immune cells can mediate immune evasion. Siglec-9 is expressed on myeloid and NK cells within the tumor microenvironment. Siglec-9 has also been found upregulated on tumor-specific infiltrating T cells. Therefore, agents that target the sialoglycan/Siglec-9 immune checkpoint have the potential to stimulate both the innate and adaptive anti-tumor immune response.

To test the potential of Siglec-9 blocking antibodies to increase anti-tumor immunity we generated Siglec-9 expressing NK cells and tested their killing capacity. While overexpression of Siglec-9 on NK cells led to reduced killing of tumor cells compared to control cells, addition of anti-Siglec-9 antibodies restored their killing capacity. To study the effect of Siglec-9 on T cells, we used cells from a T cell-specific Siglec-9 transgenic mouse. While Siglec-9 transgenic T cells were not responsive to stimulation with anti-CD3/anti-CD28, addition of anti-Siglec-9 antibodies completely restored activation and proliferation of these cells. The decreased functionality of T cells in Siglec-9 transgenic mice could also be seen in the 3-methyl-cholanthrene (MCA) induced tumor model. Here, appearance of MCA-induced subcutaneous sarcoma was earlier in Siglec-9 transgenic mice compared to littermate controls.

If treatment of tumor-bearing mice with anti-Siglec-9 antibodies can delay or prevent tumor growth alone or in combination with immune checkpoint inhibitors like PD-1/PD-L1 is under investigation.

(84) Targeting Neurodegeneration in Gaucher Disease

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The lysosomal storage disorder Gaucher disease (GD), caused by mutations in the GBA1 gene, has been linked to an enhanced risk of Parkinson's disease. Traditional intravenous delivery of recombinant GBA protein can alleviate non-neuronal GD symptoms, but the blood-brain barrier presents an obstacle to treatment of neurodegeneration. To address this problem, we are preparing recombinant GBA for conjugation to GNeo (Guanidinylated Neomycin), a small molecule previously demonstrated to deliver biomolecules, including enzymes, to the lysosome. We plan to utilize a recently published intranasal delivery system to investigate α-synuclein clearance in Gaucher mouse models with and without GBA-GNeo treatment. Alongside this work, we are testing enzymatic activity in Gaucher cell lysates with and without treatment as well as monitoring conjugate delivery into cultured rat neurons. Overall, we hope to reduce protein aggregate formation by replacing dysfunctional mutant protein with our GBA-GNeo conjugate, thus taking a first

step in developing a treatment for human patients with neuronopathic GD.

(85) Diagnostic Peak Search for Glycomics and Glycoproteomics

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Here we describe two additions to a popular proteomics / glycoproteomics search engine (Byonic): (1) detached glycan search (in effect replacing the peptide part of a glycopeptide by water or a reducing end label), and (2) diagnostic peak search within MS/MS data sets of glycopeptides or detached glycans. Diagnostic peak search finds all spectra containing sufficiently many peaks at key m/z values, for example, all spectra with at least one intense peak within +/- 0.01 Da of 204.087, 274.092, or 366.110. Spectra can be indexed by key m/z values for ultra-fast searching, and users can set the intensity rank threshold, m/z tolerances, and m/z values. Diagnostic peak search can be used to find unusual PTMs and monosaccharides, test for glycosylation in ordinary proteomics samples, and check the completeness of glycan derivitizations. Unusual PTMs and monosaccharides with reliable diagnostic peaks include phospho-GlcNAc, acetyl-NeuAc, phosphomannose, non-standard sialic acids, phosphoryl choline, ADP-ribose, etc. Less obviously, diagnostic peak search can also be used as a fast scorer to find all the glycopeptides with the same peptide part, for example, by searching for Y1 and Y2 and/or y5, y6, and y7. We will show examples of diagnostic peak searches on detached glycans and glycopeptides, including some examples with novel or at least not well-known peptide and glycan modifications.

(86) Sialyltransferase ST6Gal-I creates ligands for the Siglec receptors on immune cells and dampens the immune response during PDAC progression

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Sialyltransferase ST6Gal-I adds α2-6 sialic acids to select *N*-glycosylated cell surface receptors leading to alterations in receptor function and downstream intracellular signaling. Prior studies from our group have shown that: (1) ST6Gal-I is upregulated in multiple tumor types including Pancreatic Ductal Adenocarcinoma (PDAC); (2) oncogenic Ras signaling leads to upregulated ST6Gal-I; and (3) ST6Gal-I promotes a cancer stem cell phenotype. We generated a genetically engineered mouse model (GEMM) of PDAC with either pancreas specific knock-in of oncogenic K-Ras alone (KC mouse) or K-ras in combination with ST6Gal-I (KSC mouse). Single cell RNA sequencing studies revealed that genes involved in B cell response are repressed, whereas genes involved in B cell anergy are upregulated, in KSC mice in comparison to KC mice. KSC mice also exhibited dramatically accelerated PDAC progression and mortality compared to KC mice. This led us to hypothesize the role of ST6Gal-I activity in dampened immune response and accelerated PDAC progression. To test this hypothesis, we used another GEMM generated in the lab, wherein ST6Gal-I alone was knocked into the pancreas of the mouse (SC mouse). The sialic acids added by ST6Gal-I serve as ligands for the Sialic acid binding Immunoglobulin-like lectins (Siglec). Siglecs are lectin receptors present on immune cells and are involved in dampening an immune response due to the presence of ITIM domains. In order to determine which Siglecs bind to sialic acids added by ST6Gal-I, we used human and murine pancreatic cancer cell lines with either overexpressed or knocked down ST6Gal-I. Our flow cytometry data revealed that unlike Siglec 3, 7 and 9, Siglec 2 binds specifically to α2-6 linked sialic acid. We validated this data using pancreatic cells isolated from SC mice which corroborated our cell line results. Given that Siglec 2 is selectively expressed on B cells, we evaluated the effect of sialic acids added by ST6Gal-I on the B cell response. Murine WEHI-231 B cells were co-cultured with murine pancreatic acinar cancer cell line, 266-6, with ST6Gal-I overexpression or knock-down. From preliminary studies, we demonstrate enhanced phosphorylation of the B cell receptor and activation of the key downstream mediator, Syk, in the presence of hypersialylated 266-6 cells, suggesting that ST6Gal-I may regulate B cell response through Siglec 2. Collectively, these results reveal a potential mechanism for suppressing B cell response in an ST6Gal-I mediated hypersialylated tumor microenvironment.

(87) Carbohydrate-Carbohydrate and Carbohydrate-Lectin Interactions. Evolution of Glycan Mediated Cross-linking Interactions

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Abstract

We recently provided evidence that a family of glycan tumor antigens including Tn (GalNAcα1-Ser/Thr), STn (Neu5Acα2,6GalNAcα1Ser/Thr), LewisX and LewisY possess carbohydrate-carbohydrate interactions (1). Lectin-carbohydrate interactions are known to be involved in many biological activities including embryogenesis and oncogenesis. The ability of lectins to form homogeneous and heterogeneous cross-linked complexes with specific multivalent glycans and glycoconjugates are important in their biological activities (2-4). These cross-linking interactions appear to be mirrored in carbohydrate-carbohydrate interactions. We suggest that carbohydrate-carbohydrate interactions are a primitive form of glycan mediated cross-linking that preceded lectin-carbohydrate cross-linking interactions.

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(88) Manipulating PrP glycan structure to understand toxic signaling pathways driving prion-induced neurodegeneration.

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Prion proteins cause an infectious and rapidly progressive neurodegenerative disease characterized by an exponential increase in prion aggregates as well as spongiform encephalopathy, dystrophic neurites, and neuronal death. These processes depend on the neuronal expression of prion protein (PrP^C), which exists on the outer leaflet of the cell membrane as a glycosylphosphatidylinositol (GPI)-anchored glycoprotein containing two variably occupied N-linked glycosylation sites on its carboxy terminus. Previous work has shown that glycan modifications may impact PrP aggregation and neuronal toxicity. To further investigate the role of glycans in prion-induced neurotoxicity, we have engineered a new knockin mouse model (Prnp93N) that expresses PrP with an additional glycan on the amino terminus of PrP. This mouse spontaneously develops neurodegeneration characterized by spongiform encephalopathy in the CA3 region of the hippocampus and dystrophic neurites. In contrast to other murine models of prion disease, this neurodegenerative pathology develops in the absence of PrP aggregates or infectivity, as shown by RT-QuIC, ThT fluorescence, and inoculation of wild-type mice with 93N brain homogenates. Therefore, this model provides the opportunity to investigate the neurotoxic role of PrP^C, uncoupled from its aggregation. We show that although the extra glycan at position 93N does not affect PrP^C expression, stability, or turnover in cells, the brains of 93N mice display alterations in autophagy signaling proteins and myelin-associated proteins. We are currently further defining the N-linked glycans on PrP^C, with the goal of understanding how glycans mediate the neurotoxic functions of prion protein. These studies hold relevance not only to diseases of prion aggregation, but also to neurodegenerative diseases characterized by protein aggregation, including Alzheimer's, Parkinson's, and Huntington's Disease.

(89) IL-22-DEPENDENT REGULATION OF a1-3-FUCOSYLATION AND B3GNT7 GENE EXPRESSION

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Interleukin (IL) - 22 is an intestinal epithelial cytokine that plays a critical role in maintenance of intestinal epithelial homeostasis by inducing genes necessary for intestinal epithelial cell proliferation, tissue regeneration, tight junction fortification and production of antimicrobials. The influence of IL-22 on intestinal homeostasis is dynamic and requires cross-talk between commensal bacteria and the mucosal immune system. Interestingly, recent studies have identified **fucose** as a critical biological element in the maintenance of this dual-natured interaction between commensal bacteria and IL-22.

In the mammalian gastrointestinal tract, glycans containing fucose have emerged as key regulators of host-commensal symbiosis. For example, commensals can stimulate fucosylation in the host and, conversely, host fucosylation can control microbial colonization. Interestingly, fucosylated glycans can serve as a carbon source and provide a nutritional advantage to beneficial microflora. Recent efforts to characterize the molecular mechanisms that regulate intestinal fucosylation show commensal-dependent regulation of IL-22 production and subsequent induction of *Fut2* via the IL-22Ra1-STAT3 pathway, leading to IL-22-dependent increase of intestinal epithelial a1-2-fucosylation in mice. However, whether IL-22 promotes intestinal fucosylation in humans in a similar mechanism as in mice remains to be explored.

Using recombinant human (rh) IL-22, a blocking antibody to IL-22Ra1 and a pharmacological inhibitor of STAT3, we first examined the effects of IL-22 on human intestinal epithelial cell fucosylation, and characterized the fucosylated glycan epitopes and scaffolds that display them. In follow-up experiments, we used RNA-seq and qRT-PCR analysis to identify glycosylation-specific genes modulated by rhIL-22.

We show that exposure of cultured human intestinal epithelial cells (IEC) to 10 ng/mL rhIL-22 led to enhanced binding of LTL and had no effect on UEA I binding. Further analysis showed an IL-22-dependent increase of the Le^x antigen expression and this modification

was primarily found on mucin/mucin-like glycoproteins. Additionally, gene expression analysis showed an IL-22-dependent increase of *B3GNT7*, which encodes a β-1-3-N-acetylglucosaminyltransferase that can synthesize poly-N-acetyllactosamine (LacNAc) chains, but had minimal effect on FUT gene expression and genes involved in GDP-fucose synthesis in both Caco-2 BBe1 and T84 human IECs. Interestingly, we also observed a commensal-dependent regulation of *b3gnt7* in mouse colonic epithelium. Furthermore, *b3gnt7* expression in mice was *Stat3*-dependent.

In conclusion, we demonstrate that rhIL-22 regulates a1-3-fucosylation and *B3GNT7* gene expression via the IL-22-IL-22Ra1-STAT3 pathway in IECs.

(90) EndoS and EndoS-like active and inactive endoglycosidases as a framework to study antibody glycosylation in vitro and in vivo

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The endoglycosidase EndoS from the strictly human pathogen *Streptococcus pyogenes* represents a growing family of enzymes in related bacteria that hydrolyses the chitobiose core of the conserved N-linked glycans in IgG-Fc. This activity can be used to assess the contribution of Fc glycans in immunological processes *in vivo* as well as to specifically release Fc glycans *in vitro* directly from complex samples for subsequent identification. The activity has also proven successful in treating antibody mediated autoimmunity. Furthermore, engineered inactive variants of these enzymes can be used to affinity purify IgG from complex samples under mild conditions and glycosynthase mutants of them can be used to tailor Fc glycans. Here will be presented a number of examples of how EndoS and EndoS-like enzymes can be used as direct experimental treatment of autoimmunity, to modify antibody-based drugs, for assessing glycan dependency in immunological processes, to unformly add payloads to IgG, and finally as tools to purify IgG with desired properties based on the glycoform and species specificity of the enzymes.

(91) Acidosis, Zinc, and HMGB1 in Sepsis: A Common Connection Involving Sialoglycan Recognition

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Cells in the human body exist in a wide pH range (from 1.5 in the stomach to 8.0 in the urinary tract), but blood pH is tightly regulated in a narrow range of 7.35-7.45. Low blood pH, or lactic acidosis, is an occurrence and poor prognosticator in sepsis. We attempted to mimic sepsis by adding low millimolar concentrations of lactic acid to anticoagulated whole blood. Addition of lactic acid first caused a rise in blood pH before further addition caused a fall to pH 7.2, mimicking a pattern often seen in patients with sepsis. Apart from lactic acidosis, low zinc levels and release of HMGB1 from activated/necrotic cells are also indicators of poor prognosis in sepsis. Surprisingly, we observed that HMGB1 added to hirudin-anticoagulated whole blood at physiological pH did not bind to leukocytes that are known to carry receptor sites. However, when lactic acid was added to lower whole blood pH mimicking sepsis conditions, binding of HMGB1 to leukocytes occurred. Additionally, neutrophils were activated by HMGB1 only at reduced pH. These findings imply the presence of natural inhibitor(s) of HMGB1 that prevent its interaction with receptors at normal pH. Independent studies have shown that glycoproteins such as CD52 or CD24 presenting high levels of sialic acids can engage HMGB1 in a sialic acid-dependent manner. We noted that the buffer used in such studies included millimolar concentrations of manganese, a feature likely carried over from unrelated work on binding of nuclear HMGB1 to DNA. Testing micromolar concentrations of many divalent cations we found that only zinc supported robust binding with sialylated glycoproteins. Further characterization of HMGB1 as a sialic acid-binding lectin suggested that optimal binding takes place at physiological blood pH and is markedly reduced when pH is adjusted with lactic acid to levels found in sepsis. Glycan array studies further confirmed binding of HMGB1 with multiple sialylated glycans, again dependent on zinc and normal blood pH. The hypothesis arising from all these findings is that HMGB1-mediated hyper-activation of innate immunity in the bloodstream during sepsis requires lowering of blood pH and that addition of micromolar amounts of zinc might partially protect against this effect. We suggest that the potent inflammatory effects of HMGB1 are normally kept in check via sequestration by plasma sialylated glycoproteins at physiological pH and zinc levels, and triggered when pH and zinc levels fall in the late stages of sepsis. Notably, the "acute phase response" to inflammation results in high production of heavily sialylated molecules such as alpha-1-acid glycoprotein from the liver and endothelium, which may then act as a negative feedback loop. Current clinical trials

that are independently studying zinc supplementation or pH normalization may be more successful if these approaches are combined with HMGB1 inhibition and supplemented by infusions of heavily sialylated molecules like CD52 or CD24.

(92) Structures of DPAGT1 give insights into glycosylation disorders and advance antibiotic development against TB

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Dolichyl-phosphate N-acetylglucosamine-phosphotransferase (DPAGT1, also known as GPT and ALG7) catalyses the first step of the lipid linked oligosaccharide synthesis pathway of N-glycosylation. Missense mutations in DPAGT1 can lead to the devastating multisystem disorder congenital disease of glycosylation (CDG), or the much milder congenital myasthenic syndromes (CMS). Structural and biochemical studies of DPAGT1 will be presented that gave insights into its enzymatic mechanism, and helped explain why different missense mutations led to different disease phenotypes. Tunicamycin is an effective naturally occurring antibiotic, which unfortunately is also highly toxic to humans due to its potent inhibition of DPAGT1. A co-structure of DPAGT1 with tunicamycin helped develop novel analogues that no longer inhibited DPAGT1, but remain potent at killing *Mycobacterium tuberculosis*.

(93) Toward a Genome-Wide CRISPR Screen to Elucidate the Unconventional Mechanism of Galectin Secretion

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Galectins are a family of mammalian glycoside-binding proteins (lectins) that are involved in diverse intracellular and extracellular functions, such as RNA splicing, immunomodulation, and caspase activation, to name a few. Their increased expression is correlated with the aggressiveness of various epithelial cancers, and clinical trials are underway targeting galectin-1 and galectin-3 in cancer and immunological indications. Despite these advances, the specific mechanisms by which galectins promote tumorigenesis remain largely unelucidated.

A major barrier to studying galectin biology is that the mechanism by which these cytosolic proteins are secreted remains unknown. Our group has recently shown that the balance of intra- and extracellular galectin-1 is regulated at least in part by the cell's glycocalyx, where the availability of galectin binding sites modulates a dynamic equilibrium between both compartments. Furthermore, this dynamic partitioning equilibrium has been shown to have functional relevance: in mammary epithelium, nuclear galectin-1 promotes invasive, migratory behavior, while it is primarily extracellular in quiescent, nonmigrating cells. However, conflicting data exist on the details of this transduction process that remain unresolved. Its dependence on galectins' galactoside-binding ability, whether galectins are released directly across the plasma membrane or via exosomes, and the role of posttranslational modifications such as phosphorylation or proteolysis in its regulation, for example, all necessitate further investigation.

Here we describe the design and implementation of a genome-wide CRISPR screen to systematically identify genes associated with plasma membrane permeability to galectin-1 and galectin-3. This screen will be accomplished by hijacking the process to deliver a fluorescent signal to cells capable of taking up galectins by adding purified, recombinant fluorescent galectins to cells transduced with a whole-genome CRISPR knock-out guide library, generously supplied by Prof. Mike Bassik's lab. Cells will then be sorted using fluorescence-activated cell sorting and gRNAs will be sequenced to identify associated genes, which will offer clues as to the mechanistic details and regulatory processes underlying this aspect of galectin biology.

Galectins 1 and 3 were selected because both have been shown to be unconventionally secreted, active in the nucleus, and related to cancer and immune regulation. Both have been targeted in the clinic. They also represent two of the three classes of galectins – galectin-1 is prototypical, while galectin-3 is chimeric. Examining both family members will be indicative of whether different galectins follow different secretion and uptake pathways. By working to demystify these pathways, we hope to support current clinical efforts targeting galectins and outline new opportunities for targeted relocalization of galectins in research and therapeutic contexts.

(94) Mammalian lectin arrays for screening interaction of microbes with the innate immune system

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A novel lectin array format is being developed to characterize the roles of glycan-binding receptors in innate immunity by probing their interactions with pathogenic and commensal micro-organisms. The array is constructed with carbohydrate-recognition domains (CRDs) from mammalian C-type lectins modified with single-site biotin tags, thus avoiding potential confounding effects of appended Fc domains or chemical immobilization. The C-terminal biotin tag projects from the opposite site of the CRD compared to the sugar-binding site, so that CRDs displayed on a streptavidin-coated surface have a defined orientation and are accessible to the surfaces of microbes. Protocols have been defined for binding of micro-organisms that express markers such as green-fluorescent protein or are labelled with membrane-permeant or impermeant reagents.

A model array containing all of the sugar-binding C-type lectins from cow has been generated. Some key results have emerged from screening of the array: (1) Yeast, Gram positive bacteria, and Gram negative bacteria bind to distinct but overlapping sets of receptors. (2) There is redundancy in the receptors, since multiple receptors interact with each type of micro-organism. For macrophages and dendritic cells, the binding profiles of mannose receptor, langerin and DC-SIGN show extensive overlap. (3) A few receptors show relatively narrow specificity. For example, dectin-2 binds exclusively to yeast. (4) Several receptors show binding not anticipated from earlier studies. Although LSECtin is known to have affinity for truncated glycans on viruses, it also binds well to yeast and bacteria. (5) Receptor binding to Gram positive and Gram negative bacteria is targeted to common sugar structures found on their walls and outer membranes rather than to strain-specific oligosaccharides. (6) Although galactose-binding receptors are generally associated with binding of endogenous mammalian glycans, many of these receptors can interact with galactans in yeast walls.

These results provide a unique overview of the interactions between mammalian glycan-binding proteins in the innate immune system and different classes of potential pathogens.

(95) Trypanosoma cruzi trypomastigote glycosylphosphatidylinositol-anchored mucins and an α-Gal-containing neoglycoprotein as Chagas disease biomarker candidates

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Trypanosoma cruzi is the causative agent of Chagas disease (CD). It is a neglected tropical disease that kills or permanently disables thousands of people annually. About 6-8 million people are estimated to be infected worldwide. Due to a marked increase in population migration from endemic countries, CD is becoming a considerable burden to Europe, Australia, Japan, and the U.S.A.. Despite their toxicity, the two current drugs for CD, benznidazole (BZN) and nifurtimox (NFX), have medium-to-high efficacy in the chronic stage of the disease. Conventional serology (CS) techniques (i.e., enzyme-linked immunosorbent assay-ELISA, indirect immunofluorescence-IIF, and indirect hemagglutination-IHA), using parasite lysates (ELISA) or fixed parasites (IIF and IHA), have high sensitivity and good specificity and are largely used for CD diagnosis. However, these methods fail in performing an effective and fast clinical follow-up of chronic CD (CCD) patients following chemotherapy with BZN or NFZ, especially in adult patients. Therefore, negative seroconversion in treated patients, as measured by the conventional serology, may take 10-20 years to occur. This is one of the reasons that less than 1% of CCD patients undergo treatment. Identification of biomarkers (BMKs) for CCD diagnosis and early response to current and novel chemotherapies has become of paramount importance because of lack of dependable, specific, and validated BMKs in the clinical settings. The cell surface of infective *T. cruzi* trypomastigote form is covered by highly immunogenic glycosylphosphatidylinositol-anchored (GPI) mucin-like glycoproteins (tGPI-mucins). CCD patients have high levels of protective antibodies (Ch anti-α-Gal Abs) against the terminal nonreducing α-Gal glycotopes expressed on tGPI-mucins (or TcMUCII mucins). A major and immunodominant glycotope expressed on tGPI-mucins, the trisaccharide Galα(1,3)Galβ(1,4)GlcNAcα (Galα3LNa), which is the only tGPI-mucin *O*-glycan that has been fully characterized to date, is strongly recognized by Ch anti-α-Gal Abs, but only weakly by anti-α-Gal Abs from healthy individuals (normal human serum (NHS) anti-α-Gal Abs). These Ch anti-α-Gal Abs correlate with parasitological cure following chemotherapy with BZN, thus negative seroconversion for those antibodies could be considered a reliable criteria of cure in contrast to PCR assay, which is currently the leading test for assessing the response to treatment in a short period of time in patients with chronic infection. In this project, we validated the synthetic glycotope Galα3LNα covalently conjugated to a carrier protein (bovine serum albumin, BSA), giving rise to a neoglycoprotein (NGP) named NGP24b (Galα3LNα-BSA), as a reliable surrogate BMK for the tGPI-mucins for early assessment of treatment outcomes.

(96) Unique Mannose Binding Epitopes Dictated by Innate Immune Receptors and Immunoglobulins from Healthy

Individuals and Patients with Common Variable Immunodeficiency

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Background

Oligo- and high-mannose type *N*-glycans and mannans are present in both prokaryotic and eukaryotic cells. They are ligands for key components of both innate and adaptive immune responses, such as innate immune receptors and immunoglobulins. Despite the essential roles of these proteins in homeostasis, pathogen recognition and clearance, and immune cell trafficking revealed by extensive studies, their fine-binding specificities to mannose-containing glycans have been elusive due to a lack of a comprehensive collection of defined oligomannose glycan standards.

Methods

We generated an oligomannose array using 84 sequence-defined synthetic glycans, including 67 linear and branched oligomannose variants and 12 high mannose-type *N*-glycan fragments. This was complemented with an array of Asn-linked naturally occurring *N*-glycans featuring Man4 to Man9 purified from bovine RNase B. With these tools, we analyzed the detailed binding specificities of a group of recombinant innate immune receptors, including human DC-SIGN, L-SIGN, mannose receptor and Dectin-2, as well as preparations of pooled human IgG, IgM and IgA, and commercial intravenous immunoglobulin G (IVIG) that is used clinically. Sets of normal human sera and sera from patients affected by common variable immunodeficiency (CVID) were also tested to compare with the pooled immunoglobulins.

Results

Human DC-SIGN, L-SIGN, mannose receptor and Dectin-2 all strongly bound to oligo- and high-mannose glycans with distinct specificities. Surprisingly, purified human IgG, IgM, IgA and IVIG also showed strong binding with structural preferences to unique oligomannose epitopes. These binding features were recapitulated by the sera of healthy donors, whose IgG and IgM responses to oligomannose were clearly associated with gender and age. Patients with CVID showed altered IgG and IgM responses compared to the healthy controls. In particular, the activities and binding specificities of the IgMs of the CVID patients resembled those of the IgG-specific responses in healthy individuals, which highlights the compensatory effect of the IgMs in those patients with the absence of the IgG-specific antigenic responses to those vital oligomannose epitopes.

Conclusions

Our results demonstrate that key lectins and antibodies in the human innate and adaptive immune system have characteristic yet complementary binding preferences towards features of oligomannose structures. This suggests the presence of a collaborative recognition of pathogen-derived glycans in both systems. The knowledge of their binding specificities is instructive for the design and development of glycan mimetics that selectively antagonize mannose-binding proteins, as well as developing new therapeutic options for CVID.

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(97) Lectenz®: A Novel Class of High-Specificity Affinity Reagents for Detection and Purification of Glycoconjugates

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Using computationally-guided directed evolution, Lectenz Bio has been developing novel proteins that target glycan epitopes to which existing reagents either do not exist or are sub-optimal. These reagents have high specificity and affinity in comparison to established carbohydrate detection reagents such as antibodies and lectins. Currently, two sialic acid-specific Lectenz® (SiaFind™) engineered from a catalytically inactivated sialidase enzyme have been commercialized. They have been validated in a variety of sialylation detection and enrichment assays, such as BioLayer Interferometry analysis, glycan array assays, affinity chromatography, Western blotting, and immunohistochemistry. These applications demonstrate the simplicity, robustness, and accuracy of SiaFind™ reagents in glycoscience research. Supported by NIH grants R44GM113351 and R44OD024964.

(98) Analysis of PD1/PD-L1 Glycoforms by LC-MS/MS and Reactivity of the Immune Checkpoint Proteins with Therapeutic Antibodies

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PD-1/PD-L1 immune checkpoint blockade is a promising therapeutic strategy in multiple cancer diseases. PD1 and PD-L1 are each N-glycosylated at four sequons and recent literature suggests that the N-glycosylation affects their interaction. However, little is known about the distribution of the site-specific glycoforms of these important proteins or about the impact of the glycoforms on the interaction with therapeutic or diagnostic antibodies. We therefore optimized LC-MS/MS methods for the analysis of the site-specific glycoforms of the PD1/PD-L1 proteins and analyzed the impact of the glycans on the recognition of the proteins by clinically relevant antibodies.

Our results show that both PD1 and PD-L1 carry high mannose and complex N-glycans at all four sequons. The distribution of the glycoforms as well as site occupancy vary substantially between different cell types. Mouse myeloma NS0 cells express proteins with >95% occupancy at all sequons while site occupancy in the HEK293 cells ranges at individual sequons from 1% to 100% and breast cancer cell lines show intermediate degree of occupancy. We have detected up to 90 N-glycoforms at the four sequons by our optimized LC-MS/MS workflows. Majority of the structures represent complex glycans, including polyLacNAc and LacdiNAc structures in the human cell lines and Gal alpha(1,3) Gal epitopes in the mouse cell line; however, some sequons carry primarily high mannose glycoforms. Molecular modeling of the N-glycans on the interacting proteins suggests both glycan-glycan and glycan-protein interactions that may impact the PD1/PD-L1 interaction. Reactivity of the proteins with some antibodies is glycan dependent and binding of several clinically relevant antibodies to the immune checkpoint proteins is altered by removal of the glycans. In summary, N-glycosylation substantially modifies the surface of the PD1 and PD-L1 proteins and is likely to affect their interaction as well as interaction of these immune checkpoint glycoproteins with clinically relevant antibodies.

(99) Siglec ligands in mouse and human brain

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Siglecs, sialic acid binding Ig-like lectins, are regulatory molecules expressed selectively on subsets of immune cells. Upon engagement with their complementary sialoglycan ligands, Siglecs may down regulate immune response via their intracellular ITIM domains, making them appealing drug targets for anti-inflammatory therapeutics. Microglia, resident macrophages of the brain, express their own unique set of Siglecs which differentiate them from other immune cells, including peripheral macrophages. In the brain, microglia mediate inflammatory responses and clear cellular debris through phagocytosis. Neurological disorders, especially proteinopathies such as Alzheimer's disease and Parkinson's disease, may be impacted positively or negatively by microglial function and dysfunction. Understanding the role of microglial Siglecs and their ligands in regulating microglial cells may provide therapeutic targets for central nervous system disorders. Transcriptomic analysis of purified human microglia (Galatro et al, Nature Neurosci, 20:1162, 2017) revealed expression of several Siglecs among them Siglec-8, -10, -9, -14, -11 and -3. In mice, microglia express Siglec-F, -E, and -H (Matcovitch-Natan et al, Science 353:789, 2016). To identify Siglec sialoglycan ligands, human cerebral cortex and mouse brain were extracted in guanidinium hydrochloride, the extracts dialyzed against urea buffer, treated with or without sialidase, resolved on composite agarose-acrylamide gels and blotted unto PVDF membranes. Upon probing for ligands using Fc-tagged Siglecs, a single large molecular weight species (~1 MDa) was bound in a sialic acid dependent manner by hSiglec-3, -8 and mouse Siglec-F in both human and mouse brain extracts. Glycan and protein characteristics of the Siglec target in mammalian brains may provide insights into molecules that regulate microglial activation in health and disease. Supp. by NIH grants AG062342 and HL141952 (to AGG).

(100) Oral-supplemented 2-fucosyllactose attenuates spontaneous colitis in Il10-/- mice

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The number of diagnosed inflammatory bowel disease in Western countries patients drastically increased within the past 50 years. Considering the fundamental etiologic role of intestinal dysbiosis in gut inflammation, restoring microbial homeostasis is gaining importance for disease therapy. Milk oligosaccharides have been shown to positively impact the establishment of microbiota homeostasis through their prebiotic effect in the first phase of life. Remaining intact after passage through stomach and small intestine, milk oligosaccharides can potentially influence the composition of the gut microbiota after ingestion as a dietary supplement. However, positive effects on microbial homeostasis in the context of intestinal inflammation have not been addressed in

the post-weaning period. By oral supplementation with four major milk oligosaccharides in *Il10*$^{-/-}$ mice, we found that 2-fucosyllactose (2FL) was specifically capable of significantly alleviating spontaneous colitis after weaning. Signs of colitis, such as inflammation marker expression, colon shortening, histological scoring and intestinal epithelium permeability were drastically decreased after 2FL supplementation. Microbiota analysis revealed that oral supplementation with 2FL led to a pronounced expansion of the *Lachnospiraceae* member *Ruminococcus gnavus*, which was paralleled by increased cecal concentrations of propionate. Consistently, *R. gnavus* cultures could grow efficiently with 2FL as sole carbohydrate source. Decreased pro-inflammatory potential of *R. gnavus* was shown in a 2FL-independent manner by reconstitution of antibiotic-treated *Il10*$^{-/-}$ mice, as well as with *in vitro* stimulation of dendritic cells with fixed cultures of *R. gnavus*. By establishing the significance of milk oligosaccharides regulating the gut microbiota in a post-weaning period, our study affirms the critical contributions of specific carbohydrates, in particular 2FL, for sustaining intestinal homeostasis in an inflammatory setting as observed in *Il10*$^{-/-}$mice. This might open novel therapeutic options for the treatment of inflammatory conditions of the intestine, in which the expansion of *R. gnavus* sets a positive microbial environment at the cost of pro-inflammatory gram-negative bacteria, thereby lowering intestinal inflammation.

(101) Regulation and fine-tuning of cadherin O-linked mannose glycosylation by the TMTC1-4 enzyme family

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Adhesion molecules and receptors mediate a multitude of cellular functions and play important roles in tissue development and maintenance; however, the understanding of these processes is far from complete. We recently described the existence and biosynthetic regulation of a novel type of O-linked mannose glycosylation (O-Man) on cadherins, which belong to a major class of mammalian adhesion molecules involved in morphogenesis and homeostasis of tissues. The biosynthetic machinery responsible for O-Man on cadherins is composed of a family of four proteins known as Protein O-mannosyl-transferase TMTC1-4 (TMTC1-4). Deficiencies and mutations in the *TMTC1-4* genes are associated with neurological disorders, including intellectual disabilities, hearing loss and severe brain malformations (cobblestone lissencephaly), indicating that O-Man glycans on cadherins fulfill important biological roles through molecular mechanisms that are not fully understood.

In this study, we aimed to characterize the TMTC1-4 isoenzymes to understand their individual substrate specificities on a proteome-wide scale in human cell lines. HEK293 cells with triple-KO of TMTC1-4 isoenzymes in combination were engineered by CRISPR/Cas9, thus producing four individual cell lines with only one isoenzyme intact in each. The O-Man glycosylation capacity in each cell line was subsequently analyzed by our established Concanavalin A - lectin weak affinity chromatography (ConA-LWAC) enrichment and differential O-glycoproteomics workflow on an Orbitrap Fusion Tribrid mass spectrometer. We found that TMTC3 directs O-Man specifically to G-strands on extracellular cadherin (EC) domains on all cadherin superfamily members identified in our analyses, which includes classical-, non-classical and proto-cadherins. In contrast, TMTC2 was found to direct O-Man to B-strands located on the opposite side of EC-domains in the cadherin superfamily. TMTC1 and TMTC4 were found to have limited O-Man capacities in HEK293 and only a few cadherin superfamily members were identified as specific substrates for these two isoenzymes. Finally, we analyzed the glycosylation capacity of the TMTC3 H67D mutant, which is responsible for the cobblestone lissencephaly phenotype, and observed a complete loss of O-Man on G-strands on all cadherin superfamily members, indicating that this single mutation renders the TMTC3 isoenzyme inactive.

In conclusion, our results demonstrate that individual TMTC isoenzymes have specificity for B- or G-strands on EC-domains, suggesting that cadherin functions may be fine-tuned by differential O-Man glycosylation on specific EC-domains and β-strands. These findings advance the understanding of molecular mechanisms in cadherin-dependent interaction networks and their involvement in neurological diseases.

(102) N-glycome inheritance from cells to extracellular vesicles in B16 melanomas

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Tumor cells secrete extracellular vesicles (EVs) to promote tumor growth and metastasis. The lack of structural information of glycans expressed on tumor-derived EVs has hampered our understanding on glycan functions of the EVs in tumor progression. Here, we investigated the correlation between metastatic behaviors of tumor cells and asparagine-linked glycosylation (N-glycosylation) of tumor-derived EVs. Three mouse melanoma B16 variants with distinct metastatic potentials (B16-F1, B16-F10 and B16-BL6) show similar gene expression levels and enzymatic activities of glycosyltransferases involved in N-glycosylation. All melanoma variants and EVs have

nearly identical profiles of de-sialylated N-glycans. The major de-sialylated N-glycan structures of cells and EVs are core-fucosylated, tetra-antennary N-glycans with a β1,6-*N*-acetylglucosamine branch. A few N-glycans are extended by *N*-acetyllactosamine repeats. Sialylation of these N-glycans may generate cell-type-specific N-glycomes on EVs. Taken together, melanoma-derived EVs show high expression of tumor-associated N-glycans, and the core structure profile is inherited during multiple selection cycles of B16 melanomas and from tumor cells to EVs.

(103) Relationship between modified heparin-derived oligosaccharide non-polar surface areas and electrospray ionization response

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The detection and structural analysis of native carbohydrates by mass spectrometry is often hindered by poor electrospray ionization (ESI) efficiency. Native carbohydrates, especially highly sulfated glycosaminoglycans (e.g., heparin), are inherently hydrophilic biomolecules that preferentially reside in the core of electrospray droplets rather than on the charged surface where ionization into the gas-phase occurs. Various chemical modification strategies including reducing end tagging, permethylation, and alkylamine ion-pairing agents are available to tailor carbohydrate hydrophobicity thereby improving ESI responses as well as orthogonal detection (e.g., UV-vis and fluorescence) and chromatographic performance. We report a systematic investigation of the effects of chemically modified heparin-derived carbohydrates (disaccharides and dp4-dp12) and their resulting non-polar surface areas on ESI response.

(104) Role of Galectin-1 and Galectin-3 expression in acute lymphoblastic leukemia protection

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B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is a type of cancer that originates in the bone marrow. Galectin-1 and Galectin-3 are related proteins encoded by distinct genes, with diverse contributions to inflammation and carcinogenesis. Interestingly, expression of these Galectins is highly increased in bone marrow of BCP-ALL patients after induction chemotherapy, and high expression levels correlate with poor clinical outcome. *In vitro*, bone marrow stromal cells support the proliferation of BCP-ALL cells and protect them against the deleterious effects of drug treatment. Bone marrow stromal cells contain high levels of endogenous and cell surface Galectin-1 and Galectin-3. BCP-ALL also express Galectin-1 and Galectin-3 endogenously, at varying levels, depending on the individual BCP-ALL sample. Moreover, we have previously shown that when BCP-ALL cells develop loss of responsiveness to drug treatment (drug resistance) in co-culture with stromal cells, endogenous Galectin-3 synthesis is induced in the BCP-ALL cells, providing a survival advantage. Thus, Galectin-1 and Galectin-3 are produced in complex ways and may have multiple effects on drug resistance. In addition, based on the finding that mouse *lgals1 x lgals3* double null mutant BCP-ALL cells grow very poorly compared to wild type controls and are easily eradicated by nilotinib or vincristine monotherapy, Galectin-1 and Galectin-3 may have partly overlapping functions. We used Cas9/CRISPR to knock out Galectin-1 and Galectin-3 individually in OP9 murine stromal cells to investigate their contribution to drug resistance. We found that neither stromal-produced Galectin-1 nor Galectin-3 is needed for steady-state BCP-ALL proliferation or viability. However, efficient BCP-ALL cell adhesion and migration towards stromal cells are significantly promoted by stromal Galectin-3. The motor protein non-muscle myosin IIA was identified as a novel binding partner for Galectin-3 in BCP-ALL cells that could regulate some of these activities. Importantly, loss of stromal Galectin-3 production sensitized BCP-ALL cells to conventional chemotherapy. These results show that Galectins can support BCP-ALL cells in multiple ways, such as stimulating migration and adhesion to protective stromal cells, and by direct intracellular effects. Because of this, inhibition of Galectin activities using specific inhibitors would be useful to sensitize BCP-ALL cells to treatment with conventional chemotherapy.

(105) Induction of peripheral lymph node addressin in human nasal mucosa with eosinophilic chronic rhinosinusitis

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Eosinophilic chronic rhinosinusitis (ECRS) is characterised by formation of nasal polyps with prominent eosinophilic infiltration; however, how eosinophils are recruited in this pathological setting remains unclear. In the present study, we carried out quantitative immunohistochemical analysis of nasal polyps associated with ECRS ($n = 30$) and non-ECRS

($n = 30$) to evaluate expression of an L-selectin ligand peripheral lymph node addressin (PNAd) on vascular endothelial cells. We found that PNAd was induced primarily on the luminal surface of venular vessels present in nasal mucosa in both ECRS and non-ECRS, while the number of PNAd-expressing vessels in ECRS significantly exceeded that seen in non-ECRS. Moreover, the number of eosinophils attached to the luminal surface of PNAd-expressing vessels in ECRS was significantly greater than that in non-ECRS, while the number of neutrophils and lymphocytes attached did not differ significantly between conditions. Furthermore, eosinophils, which express cell surface L-selectin, adhered to PNAd-expressing Chinese hamster ovary (CHO) cells in a calcium-dependent manner, and that adhesion was significantly inhibited by pretreatment of eosinophils with DREG-56, an anti-human L-selectin monoclonal antibody. These findings combined suggest that interaction between L-selectin and PNAd plays at least a partial role in eosinophil recruitment in human nasal mucosa with ECRS.

(106) Synthetic galectin-3 oligomers to understand the role of carbohydrate-recognition domain multivalency in extrinsic pro-apoptotic signaling

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Galectin-3 is a carbohydrate-binding protein that can activate or inhibit apoptosis signaling depending on whether it is present in the extra- or intracellular space. Extracellular galectin-3 activates pro-apoptotic signaling by forming multivalent oligomers upon binding to cell surface glycans. Understanding galectin-3 valency-function relationships is central to deciphering its role as a modulator of cell behavior in healthy and pathological processes; however, studying these relationships is challenged by the dynamic, glycan-dependent assembly of galectin-3 oligomers. Here we present synthetic galectin-3 oligomers to probe relationships between carbohydrate-recognition domain (CRD) valency and pro-apoptotic signaling. Synthetic galectin-3 oligomers with defined CRD valency were created by fusing peptides that form α-helical coiled coils of different strand numbers (e.g., 2-6) to the N-terminus of galectin-3 ("$(Gal3)_n$", where n = 2-6). $(Gal3)_{2\text{-}6}$ did not mediate Jurkat T cell agglutination, in contrast to native galectin-3, but did induce Jurkat T cell death to an extent that increased with CRD valency. However, synthetic oligomers required longer times to induce cell death than native galectin-3. Consistent with this, native galectin-3 upregulated caspase-8 activity, as well as caspase-3/-7 activity, to a greater extent than synthetic galectin-3 oligomers. These results suggest that native galectin-3 within the extracellular space activates caspases more efficiently than fixed-valency oligomers, leading to earlier induction of apoptosis. We envision this platform will establish key structural features that define the roles of galectin-3 activity as an extracellular signaling molecule, thereby identifying new opportunities for therapeutic intervention at the interface of galectin-glycan recognition.

(107) Characterization of specific cell-surface heparan sulfate-protein interactions

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Heparan sulfate (HS) is a linear polysaccharide that is widely distributed on the cell surface, where it exists as proteoglycan component. The HS chain is initially assembled as a simple 1→4-linked copolymer of *N*-acetyl-α-D-glucosamine and β-D-glucuronic acid (GlcA), but the seemingly regulated but non-template driven modifications cause extensive micro-heterogeneity along the sugar backbone comprised of around 50 to 200 disaccharide units. These modifications, which include *N*-deacetylation, *N*-sulfonation, GlcA 5-C epimerization forming α-L-iduronic acid, and multiple *O*-sulfonations, are implemented by several enzyme isoforms of varying specificities and are always incomplete, accounting to theoretically 48 disaccharide variations. The myriad of functional group patterns decorating the sugar backbone allowed HS to encode a high density of structural information. Such array of modifications is responsible for mediating or modulating protein activity. Keen interests are focused in deciphering the molecular level details of the HS–protein interactions because they may present therapeutic opportunities. Here, chemical synthesis of HS oligosaccharides in conducting structure–activity relationship studies will be presented.

(108) Databases for 3D-Structure of Lectins and Prediction Tools

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The UniLectin interactive database is a web platform dedicated to the classification and curation of 3D-structures of lectins (UniLectin3D module) with more than 2000 entries, and information on structure, bibliography, carbohydrate ligands, and integration with other databases of glycosciences. The new developments aim at the prediction of occurrence of lectin sequences in genomes.

The module PropLec allows for identification of β-propeller lectins. These proteins are formed by repeats of short domains, raising questions about evolutionary duplication. However, these repeats are difficult to detect in translated genomes and seldom correctly annotated in sequence databases. To address these issues, a database has been developped based on the blade signature of the five types of β-propellers using 3D-structural data. The data reveals a widespread distribution of β-propeller lectins across species. Prediction also emphasizes multiple architectures and led to uncover a novel β-propeller assembly scenario. This was confirmed by producing and characterizing a predicted protein coded in the genome of Kordia zhangzhouensis.

New modules are under development for the identification of β-trefoil lectins in genomes and extension to all classes of lectins will be proposed in the future.

(109) Capturing and detection of pharmaceutical glycoproteins by anti-glycan binding tools

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It has been revealed that the oligosaccharide structure of a glycoprotein pharmaceutical is closely correlated with the medicinal benefits. For instance, effector activities of IgG "ADCC, CDC activities" and blood stability of EPO are well-known. Therefore, it is a very important subject to select the oligosaccharide structure exhibiting a high effect of functional mechanism.

To solve this problem, we have developed a high-performance lectin tool for capturing glycoproteins. This tool has different specificities depending on the type of lectin through the recognition for a limited part of oligosaccharide structure on glycoprotein, and dissociate the glycoprotein with a small inhibitive sugar. So far, pharmaceutical antibodies remodeled with structure-defined oligosaccharide and containing non-human type of glycan epitopes have been successfully captured in our system.

Animal cells are usually used as a host cell and animal source materials also may be present as contaminants in the manufacture of biopharmaceuticals. Contamination with non-human type of glycan epitopes has been considered as a problem that may cause anaphylactic symptoms and reduce the antibody effect. Alfa-Gal epitope (Galα1-3Gal) and *N*-glycolylneuraminic acid (Neu5Gc) are known as non-human type of glycan epitopes. We have developed a unique detection tool that specifically detects the non-human type of glycan epitopes because they must be identified. This tool indicates high specificity and it revealed that some biopharmaceuticals contain the exogenous epitopes.

For the development of the anti-glycan binding tools, it is very important to prepare a structure-defined oligosaccharide for evaluating the binding specificities. In this case, chemically synthesized oligosaccharides would be valuable responding to that we have established a systematic chemical synthesis technology for functional oligosaccharides based on industrial production.

(110) Evidence for reverse migration of 9-O-acetyl esters to 8- and 7-carbon positions of sialic acids

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Migration of *O*-acetyl esters within polyhydroxy groups on the linear C7-C8-C9 side chain of sialic acids (Sias) such as *N*-acetylneuraminic acid (Neu5Ac) under physiological conditions is well known. However, migration in mono-*O*-acetyl-Neu5Ac is assumed to be unidirectional from C7 (in Neu5,7Ac$_2$) to C9 (the primary hydroxyl group, giving Neu5,9Ac$_2$) without a Neu5,8Ac$_2$ intermediate. However early literature refers to a compound eventually found to be Neu5,9Ac$_2$ as "8-*O*-acetyl-sialic acid", based on the surprising resistance to mild periodate oxidation of glycosidically-bound Neu5,9Ac$_2$ (which should allow cleave the C-C bond between C7- and C8 hydroxyls). This resistance was assumed to be due to cis-trans conformation and/or internal hydrogen bonds. Here we use an improved method of low temperature 1,2-diamino-4,5-methylenedioxybenzene dihydrochloride (DMB) derivatization to show evidence of *O*-acetyl group migration between C8 and C9-positions and back migration from C9 to C7-position. In a free sialic acid sample containing about 40% of Neu5,8Ac$_2$ and 60% of Neu5,9Ac$_2$, more than 25% of 8-*O*-acetyl group migrated to C9-OH at pH 7.0 after 2 hour-preincubation at 37 °C. Reverse migration of 9-*O*-acetyl groups to the C8-OH (7%) and C7-OH (2%) of Neu5Ac was also observed using another free sialic acid sample. Migration in both directions seemed to reach an equilibrium mixture containing 10% of Neu5,8Ac$_2$. No significant migration occurred during the low temperature DMB derivatization. Of more biological importance, 9-*O*-acetyl-group in a Neu5,9Ac$_2$–containing sialoside migrated towards the C8-OH (3-7%) and C7-OH (1-8%) with pH increases towards 8.0, as confirmed by DMB-HPLC and NMR. Ongoing interconversion between Neu5,9Ac$_2$ and Neu5,8Ac$_2$ was further suggested by resistance to mild periodate oxidation, with the resulting product loss detected on HPLC. Furthermore, the synthetic analog 9-*N*-acetyl-Neu5Ac, in which the *N*-acetyl group does not migrate was much more sensitive to periodate. Notably, a sialoglycan microarray study on esterase-inactivated HE proteins of porcine torovirus (which preferably bind 9-*O*-acetyl Sias), showed much stronger binding intensity to sialosides containing 9-*N*-acetyl-Neu5Ac than those containing Neu5,9Ac$_2$. Taken together, data suggest that *O*-acetyl group migration in Sias may be bidirectional between C7-, C8- and C9-positions. Further investigation would be necessary to understand whether ongoing dynamic migration take place after reaching the "equilibrium" state. Dynamic *O*-acetyl group shifting should impact on biological recognition involving Sias, such as viral infections, and may also be finely regulated by pH changes. The observation of reverse migration of acetyl group from 9-*O* towards 8-*O* and 7-*O* might also change our understanding of the biosynthesis of di- or tri-*O*-acetyl Sias, which was previously hypothesized to

occur by adding an acetyl group to the 7-O-position followed migration from C7- to the C9-position.

(111) The human lung glycome reveals novel glycan ligands for respiratory pathogens

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Glycans, an essential class of macromolecule, are involved in infectious diseases by acting as adhesion molecules at the site of infection. Due to direct contact with the environment, human lungs are frequently subjected to infection by a variety of pathogens including viruses, bacteria and fungi. The viral protein, hemagglutinin, of influenza A viruses recognizes sialylated glycans that are expressed on the surface of human airway. As a general paradigm, human strains mainly bind α2,6-sialylated glycans while avian strains preferentially bind α2,3-sialylated glycans. Despite much effort to understand the initial virus-host recognition event, however, little is known about the natural repertoire of glycans present in the human respiratory system.

In the present work, we extracted N-, O- and glycosphingolipid-glycans from a healthy human lung and characterized their structural features via Matrix Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF-MS). A huge structural diversity was observed for N-glycans with a bi-antennary, di-sialylated glycan being the most abundant structure. Most species were core-fucosylated on the reducing end while on the non-reducing end, sialylation and fucosylation were detected on poly-N-acetyllactosamine units with variable lengths. The O-glycome of human lung was dominated by the expression of sialylated core 1 structures. The glycans released from glycosphingolipids were rich in sialylation and were elongated with N-acetyllactosamine repeats in a linear fashion rather than branched. Sialic acids with both α2,3- and α2,6-linkages were detected for the above three glycan types. Additionally, we extracted phosphorylated N-glycans from a human lung and characterized their structural features via MS analysis.

Our work provides fundamental knowledge of the natural glycans present in a human lung. The structural information will assist us to better understand the glycan recognition by influenza viruses within the biological context and will shed light on identifying the decisive factors that contribute to species barrier of influenza infection.

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(112) Structure-function analysis of neutralizing antibodies that confer prophylactic and therapeutic protection against Salmonella Typhi typhoid toxin

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Typhoid toxin is secreted from *Salmonella* Typhi-infected cells during human infection and is suggested to contribute to typhoid disease progression and the establishment of persistent infection. Currently, countermeasures against typhoid toxin are unavailable. Here we generated twelve monoclonal antibodies (MAbs) targeting either the receptor-binding subunit PltB or the nuclease subunit CdtB of typhoid toxin. All MAbs provided the mice protection against a lethal dose challenge of typhoid toxin when MAbs and the toxin were administered simultaneously. The primary mechanisms of protection for MAbs are inhibiting the toxin binding to and/or altering the toxin trafficking in host cells. Epitope locations determined by cryo-electron microscopy explain how two anti-PltB MAbs induce different protection mechanisms. MAbs also protected the mice that were pre-administered with a lethal dose of typhoid toxin. Furthermore, MAbs inhibited typhoid toxin binding to human peripheral blood mononuclear cells. These results highlight the prophylactic and therapeutic potentials of the MAbs.

Yi-An Yang, Angelene F. Richards, Ji Hyun Sim and Tri Nguyen contributed equally to this work.

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(113) Distinct antimicrobial properties of the N- and C-terminal domains of the human protein galectin-9

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Escherichia coli (strain O86:B7), *Klebsiella pneumoniae* (strain KP01), and *Providencia alcalifaciens* (strain PAO5) are gram negative bacteria known to alter their membrane lipopolysaccharides (LPS) to resemble mammalian cell membrane proteins and sugars such as blood group antigens. Previously, several human galectins, including tandem repeat galectin-4 and galectin-8, were found to play a key role in the innate immune response to these self-like antigens through binding to the terminal β-galactoside sugar of LPS and subsequent bacteria killing. However, each galectin domain displays unique binding and killing ability. Human galectin-9 (Gal-9), which consists of distinct N- and C-terminal carbohydrate binding domains, targets KPO1 and PAO5 through both the N- and C-terminal domains. However, only the N-terminal domain targets O86:B7, while the C-terminal domain fails to kill this bacterial strain. This contrast in antimicrobial activity indicates that variation in binding affinity may be responsible for the disparate killing activity of each domain. Binding analysis by flow cytometry demonstrated that Gal-9N and C-domains each bind KPO1, PAO5, as well as O86:B7, while failing to bind to the corresponding negative controls that lack self-like LPS, KPO4, PAO19, and the mutant O86:B7 Waal-, respectively. However, despite binding of both domains to O86:B7, dosing experiments demonstrated dose-dependent antimicrobial activity of both Gal-9 domains to KPO1 and PAO5, but of only Gal-9N to O86:B7. Indeed, Gal-9C failed to efficiently kill O86:B7 at any concentration tested. Assessment of differences in relative binding affinity to microbial antigen by binding of each domain of Gal-9 to immobilized bacteria revealed preferred binding to O86:B7 by the Gal-9N domain compared with Gal-9C. Thus, while binding is required for galectin killing of bacterial targets, variations in binding affinity of galectin to target bacteria may add an additional level of complexity to antimicrobial activity of galectin family members towards various bacterial strains.

(114) Increased antibody response to fucosylated oligosaccharides in inflammatory bowel disease

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Chronic inflammatory diseases of the intestinal tract such as inflammatory bowel disease (IBD) are associated with intestinal dysbiosis and with elevated antibody production towards bacterial and fungal carbohydrates. The underlying processes linking the microbiota with inflammation, however, are still unclear. Surface glycans of intestinal bacteria stimulate the production of carbohydrate-specific antibodies (carbo-ab). The question arises as to whether the overall repertoire of carbo-ab is altered in IBD due to dysbiosis and whether changes in antibody profiles can be linked to the expansion of specific taxonomic groups. Given that bacterial glycans often mimic mammalian glycans, microbiota-specific antibodies can also cross-recognize endogenous mucosal glycans. We studied the repertoire of carbo-ab in IBD patients and healthy controls using an array of human milk oligosaccharides, which share structural epitopes with mucosal glycans. We analyzed carbo-ab of the IgM and IgG classes in the blood of healthy control subjects (n=20) and IBD patients, representing patients with ulcerative colitis (UC) (n=17) and Crohn's disease (CD) (n=23). Whereas an inter-individual diversity of carbo-ab was seen in all groups, we found in CD patients a higher reactivity to oligosaccharides, both for IgG and IgM. This increase mainly reflected a significantly higher reactivity to fucosylated oligosaccharides, while the reactivity to specific sialylated oligosaccharides was decreased. The increased reactivity of carbo-ab was less prominent in UC patients. To address the IgG reactivity to the gut microbiota, we determined the reactivity of UC and CD sera towards selected *Bacteroides* species, which are abundant members of the human gut microbiota and are often associated with IBD. We detected increased IgG reactivity towards specific *Bacteroides* species in CD sera compared to healthy controls. Interestingly, these bacteria were also recognized by the fucose-specific *Aleuria aurantia* lectin, as shown by flow cytometry and lectin blotting, suggesting that they may partially account for the increased reactivity of carbo-ab towards fucosylated oligosaccharides. Our study concluded that *Bacteroides* likely contribute to the increased serum antibody response to fucosylated oligosaccharides in CD.

(115) A Structural Approach to Broadening Glycosyltransferase Binding Specificity

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The multitude of glycans on any cell surface can appear homogeneous overall, but a more site-specific perspective suggests some deliberate diversity. In attempting to understand or infer likely glycosylation events from nucleotide or glycosyltransferase abundance, it may be possible to reproduce the homogeneity of the cell surface but not the specificity of a single protein glycoprofile. We examine the possibility that the diversity of individual protein glycoprofiles is a result of the affinity for glycosyltransferases to various regions of that protein. Using a structural systems biology toolbox (SSBio), we aim to expand our understanding of glycosyltransferase (GT) binding specificity beyond glycan structure specificities, to include the influence of glycoprotein structure and dynamics on GT affinity. In this study, we examine a glycoprotein database with localized annotation of glycosylation sites. For each glycoprotein, we determine which unique and excluded glycan epitopes. Potentially occluded GTs include those decorating other glycosylation sites of the

protein; glycans which are otherwise biochemically feasible. We probe each glycosylation site for structural descriptors including: charge, hydrophobicity, solvent accessibility, secondary structure, thermostability, residue depth and several other attributes. By determining occlusion of GTs on structurally annotated sites, we can learn about the structural specificities of these impactful proteins thereby increasing our capacity to understand and predict their behavior.

(116) Why does loss of POFUT1 trap Notch in the ER of some cells but not others?

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Notch is a transmembrane protein receptor that mediates direct cell-cell interactions and is essential for many cell fate specifications. Notch mediated signaling is activated by direct interaction of Notch with its ligands on adjacent cells. Notch has 36 tandem EGF-like repeats in its extracellular domain, many of which have *O*-fucose glycan modifications. *O*-fucose is added to Notch EGF like-repeats containing the consensus sequence, $C^2XXXX(S/T)C^3$, in the endoplasmic reticulum (ER) by protein *O*-fucosyltransferase1 (human and mice POFUT1, *Drosophila O*-Fut1). Notch is then transported to cell surface for the Notch ligand binding and signaling activation. *O*-fucose modifications are essential for Notch trafficking in some but not all contexts. A temperature sensitive Notch trafficking defect is observed in *O-fut1*^{R245A} mutant in wing imaginal discs of *Drosophila* (1, 2), where Notch accumulates in the ER at 30 degree but not at 25 degreein *O-fut1*^{R245A} mutant cells (1). A Notch trafficking defect is also observed in *POFUT1*-null HEK293T cells, but not mouse *Pofut1*-null ES cells (3, 4). A possible explanation for these cell- and condition-specific differences is expression of chaperones that either retain Notch in the ER or help Notch EGF repeats to fold in the absence of POFUT1. To examine why Notch1 is retained in the ER of *POFUT1*-null HEK293T cells, endogenous NOTCH1 was immunoprecipitated from wild-type and *POFUT1*-null HEK293T cells with immobilized anti-NOTCH1 antibody. The IP was reduced, alkylated and digested with trypsin, and the resulting peptides were analyzed by nano-LC-MS/MS. Interestingly, we found that two ER chaperones, BiP (also called HSPA5 and GRP78) and the thioredoxin-containing endoplasmic reticulum resident protein 44 (ERP44), were significantly increased in *POFUT1*-null mutant versus wild-type HEK293T cells. This result suggests that NOTCH1 is retained in the ER of HEK293T cells in the absence of POFUT1 because it is bound to ER chaperones with ER retention signals. We are checking the contribution of BiP and ERP44 in Notch trafficking. This work was supported by GM061126.

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(117) Aberrant serum glycans as survival prognostics for the haematological cancer, multiple myeloma

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Multiple myeloma (MM) is the second most common haematological cancer worldwide. Although MM is considered incurable, patients can achieve good initial responses to available therapies, often with significant prolongation of survival. The ability to accurately predict patient prognosis is limited and the current, readily available prognostic tests such as the International Staging System, fluorescence *in situ* hybridization and serum lactate dehydrogenase concentration are imprecise. Gene expression profiling on bone marrow (BM) provides additional information but is technically challenging and expensive. Further, BM sampling is expensive, unpleasant for the patient, requires specialist facilities and expertise and may not reveal full information. Minimal residual disease (MRD) is also based on immunophenotyping or molecular analysis of BM and can be the basis of monitoring of remission and treatment planning. Hence an alternative method such as a blood test for prognosis and monitoring MRD would be highly desirable. In this work, serum glycosylation was investigated as a possible target for prognosis and MRD monitoring.

Plasma samples were obtained from healthy volunteers, treated patients with relapsed/ refractory MM and newly diagnosed MM patients with either long-term (overall survival (OS) 57.6-144.2 months) or short-term (OS 1.3-12.3 months) survival (25 samples per cohort). Plasma was fractionated into two fractions, immunoglobulin G (IgG) and albumin- and IgG-depleted (depleted) serum. Lectin microarray profiling of all individual samples demonstrated that there were differences in glycosylation in both the IgG and depleted fractions between all cohorts, with most marked differences between the IgG fractions of each cohort. Individual fractions from each cohort were pooled by protein content and their N-linked oligosaccharide structures were analysed by PNGase F release, 2-aminobenzamide labelling and HPLC analysis. Both IgG and depleted fractions displayed oligosaccharide structural differences between cohorts, again with the most marked differences between the IgG fractions. Several IgG structures were identified as unique to each cohort. We propose that the differences in glycosylation between the different cohorts could provide the basis for predicting disease progress in MM patients and determining the efficacy of treatment choice.

(118) Genetic Alteration of Heparan Sulfate Enhances Antigen Presentation on Dendritic Cells

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Cancer is a leading cause of death worldwide, costing millions of human lives and trillions of dollars in medical care expenditures every year. While recent advances in immunotherapy have shown success, they aren't without drawbacks. For example, checkpoint blockade inhibitors work in only a small subset of patients, and chimeric antigen receptor T (CAR T) cell therapy in carcinoma has been limited by tumor/antigen heterogeneity. Our previous work shows that genetic alteration of heparan sulfate on the surface of dendritic cells (DCs) results in a phenotype characterized by DC maturation, increased tumor-associated effector $CD8^+$ T cells, and reduced tumor growth. Initiation of anti-tumor T cell immunity partly depends on the availability and efficacy of interactions between antigen loaded major histocompatibility complex class I (ag-MHCI) and T cell receptors. Effective ag-MHCI complex presentation is one of the critical steps in forming this primary immunological synapse associated in anti-tumor T cell immunity. In our study, we utilized bone marrow derived DCs from mice bearing a conditional mutation in the heparan sulfate biosynthetic enzyme Ndst1 (N-deacetylase and N-sulfotransferase 1) driven in DCs by transgenic CD11c Cre to examine the effect of the mutation (*Ndst1*f/f *CD11cCre*+) on antigen presentation using flow cytometry. While antigen uptake was reduced in mutant DCs, ag-MHCI presentation was greatly enhanced in comparison with that of wildtype (*Ndst1*f/f *CD11cCre*-) marrow-derived DCs. Our study provides a first look at the potential role of heparan sulfate in regulating antigen presentation. This may eventually help to pave the road to novel forms of cancer immunotherapy.

(119) A Fast, Reliable O-Glycan Analysis Workflow

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Zoetis

Oligosaccharide content, like the amino acid counterpart of glycoproteins, should be characterized and monitored to ensure consistent product safety and functions. N-glycosylation characterization is well established and there are several commercial kits for streamlined N-gylcan profiling. The O-glycosylation analysis, however, is lagging. Unlike N-glycans that are easily released from their protein backbone with PNGase F, O-glycan release has been more challenging due to the lack of such enzymes. Conventional O-glycan preparation is a lengthy process and limited to generating an alditol that is released in the presence of a toxic reagent such as sodium cyanoborohydride or using hydrazine, a highly toxic substance, to obtain O-glycans as reducing sugars. With the increase emphasis of rapid data turnaround and speed to market, a fast and reliable O-glycan workflow is in high demand.

For this poster, we evaluated the new SBio EZ-Glyo® O-glycan Prep kit and compared it to traditional hydrazinolysis O-glycan kits. We analyzed the O-glycans from fetuin, and two recombinant glycoproteins in our biopharmaceutical pipeline. The O-glycans were released by "O-glycan releasing reagent" included in the kit and it took around 5 hours instead of 2-4 days by conventional method. The released O-glycan was then labeled with 2AB and analyzed by LC-fluorescence and mass spec. Our data illustrates the use of this new O-glycan sample preparation method which is more robust and reliable as compared to the conventional releasing method.

(120) Unraveling functions of novel protein O-mannosyltransferases using Drosophila as a model organism

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A recently discovered family of O-mannosyltransferases, transmembrane and tetratricopeptide repeat-containing proteins (TMTCs) includes four similar enzymes that are thought to specifically add O-linked mannose to cadherins and protocadherins, while also targeting some other proteins. Cadherins are type-1 transmembrane glycoproteins that are crucial for cell adhesion and cell migration. They play essential roles in many biological processes and were shown to be involved in pathologies, such as cancer. Many O-linked mannose modifications were identified on cadherins and protocadherins, however, the function of these modifications are not yet understood. Mutations in TMTC genes were recently found to be associated with brain malformations and neurological disorders. However, cellular and molecular mechanisms that underlie functions of TMTCs in vivo remain largely unknown. Considering that TMTCs are evolutionarily conserved in metazoans, we decided to use Drosophila as an experimentally amenable model organism to elucidate functions of TMTC genes.

Like mammals, Drosophila also possesses four TMTC genes (TMTC1-4). These genes encode proteins that are highly homologous to their mammalian counterparts, suggesting that functions of TMTCs are conserved between Drosophila and mammals. Using transgenic and mutant alleles, we have investigated the expression of TMTC1 and TMTC2 genes at different developmental stages. Our results indicated that these genes are expressed in a pattern restricted to the ner-

vous system throughout development. TMTC1 and 2 have a similar expression in the central and peripheral nervous systems of the third instar larvae. Mutations in these genes cause lethality, with the majority of mutants dying during larval stages. We are currently investigating phenotypes of these mutants at early developmental stages, which will elucidate the pathogenic mechanisms underlying lethality and suggest the functional pathways that require these genes. To reveal molecular targets of these genes' activities, we apply a glycoproteomics approach to identify and characterize glycoproteins that have altered O-mannosylation in TMTC1 and TMTC2 mutants. We specifically focus on cadherins as putative functionally important substrates. We will present new data on in vivo functions of TMTCs and their protein substrates. These results are expected to shed light on the cellular and molecular mechanisms of TMTC functions in humans.

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(121) An anti-Tn Antibody Microarray Platform for Early Cancer Detection

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Cancer is a leading cause of death worldwide. However, many of these deaths could be avoided by early detection. Current detection strategies utilize markers that are specific to individual tumor-types, limiting pan-cancer detection. In contrast to most protein and genetic markers, targeting post-translational modifications, such as protein-glycosylation, offers a unique opportunity to target alterations that are conserved across multiple tumor types. In this regard, the tumor-associated carbohydrate antigen Tn is unique in that is expressed across the most common human tumors. Tn consists of a GalNAc residue linked to serine or threonine in a glycoprotein. It is a truncated mucin-type O-glycan that is normally extended in healthy tissues to form the full repertoire of core 1 – 4-based O-glycans. Here we utilize a recently developed anti-Tn monoclonal antibody to develop an anti-Tn antibody microarray platform for early cancer detection. We show that our antibody detects hundreds of the most carcinomas but not healthy controls. Further, using genetically engineered mice, cell lines, and defined glycoproteins, we demonstrate subnanogram sensitivity for Tn-positive glycoproteins from diverse biological specimens. Our anti-Tn antibody microarray represents a promising technology for pan-carcinoma detection.

(122) Lectin microarray-based investigation of protein glycosylation in murine and human biological fluids in response to diet and AGEs

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Lectin microarray-based investigation of protein glycosylation in murine and human biological fluids in response to diet and AGEs

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Advanced Glycation End products (AGEs) exert harmful effects on the human body by altering protein structure and function and by activating selective receptors. AGEs are formed endogenously through non-enzymatic reactions between dietary sugars and cellular biomolecules[1] and exogenously though browning reactions (e.g. cooking) and then taken into the body through diet[2]. The impact of diet on metabolic health is known to be substantial, but little is known about how, or if, AGE pressure alters protein glycosylation. In this work, glycosylation changes in response to AGEs in biological samples are investigated.

The effect of diet-induced metabolic disorders, such as diabetes, on protein glycosylation was examined in mice with effect of diets high in fructose, high in fat and the influence of pyridoxamine intervention to block the formation of endogenous AGEs investigated. All animals were fed a normal pellet diet for 1 week prior and then allocated to four different dietary regimens. At the end of 12 weeks of dietary manipulation, urine and blood were collected. Glycoproteins from urine and plasma of mice were examined using a multiplexed lectin microarray profiling approach and the resulting relative intensity patterns for each analyzed by multivariate methods[3]. Urinary glycoproteins and plasma glycoproteins produced differential lectin binding patterns that appear to correlate to the animal dietary and metabolic conditions in each biological fluid and unique lectin binding profiles produced.

Saliva, urine and plasma samples were collected from human cohorts, healthy and diabetic, and changes in glycosylation investigated to correlate results with the animal study. Preliminary results confirm that certain glycosylation changes are associated with diet-related groups. This report provides the first evidence of AGEs affecting endogenous glycosylation. Further investigation of which glycoproteins are affected and their direct metabolic or biological impact are needed.

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(123) Metabolomics analysis of the effects of a GalNAc/Man-specific lectin CSL on yeast cells using UPLC–Q-TOF-MS

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Samples of *S. cerevisiaes* cultured with a GalNAc/Man-specific lectin CSL (T group) and without (C group) were analysed using Q-TOF mass spectrometer coupled to UHPLC on a HILIC column in the full survey scan. The MS/MS data were generated using Information Dependent Acquisition (IDA). The ion chromatogram peaks were picked and the metabolites were identified using XCMS software. The results showed that the response intensity and retention time of each chromatogram peak overlapped basically in T group and C group, which means better parallel in the two groups. Principle component analysis (PCA) was performed with T group and C group. In positive and negative ions modes, the data of PC1 and PC2 have obvious separation trend. The results of orthogonal partial least squares discrimination analysis (OPLS-DA) models showed that the models could well distinguish between T group and C group metabolites. The figures of volcano plot of the data in positive and negative ions modes showed that the screened metabolites from T group had significant differences compared from C group (FC>2.0 and $p<0.05$). There were 98 significantly different metabolites in T group compared to C group ($p<0.05$) in positive ions modes, in which 55 metabolites were increased and 43 were decreased. And 79 significantly different metabolites in negative ions modes ($p<0.05$), in which 64 were increased and 15 were decreased. The results of increased α-D-glucose and D-glucose-6-phosphate in T group compared to C group showed that CSL promoted glycolysis in *S. cerevisiaes*. Nicotinamide adenine dinucleotide (NAD^+), L-malic acid and L-aspartate were also significantly increased, which could accelerate the change between NAD^+ and NADH inside and outside the mitochondrial membrane, resulting in less rate of TCA than that of EMP, and thus producing more ethanol. Gutathione (GSH) was a concomitant product during ethanol fermentation, and the content of GSH increased with the ethanol producing. In conclusion, CSL could affect EMP and TCA in *S. cerevisiaes* to produce more ethanol. The work was supported by the National Natural Science Foundation of China (31571916).

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Manila clam lectins, MCL is one of the important humoral factors in the immune system of *Ruditapes philippinarum*, which inhibits the growth of many microorganisms. When MCL is infected by external microorganisms, the amount of MCL will increase dramatically in the body of clams. Determined the temporal pattern of MCL appeared in Manila clam by measuring hemagglutination activity of haemolymph from Manila clam in different months. Different treatment methods of *Shewanella putrefaciens* suspension (SPS) injected, stab wounds and SPS soaking were used to explore the optimal inducing source for stimulating MCL production. The results show that Manila clam contained less MCL in the hemolymph under normal conditions. The U-shaped 96-well plate method could not detect the hemagglutination activity in the hemolymph. Injection of SPS into the Manila clam could stimulate the secretion lectins. The stimulatory effect of SPS injected is better than stab wound. When SPS reaches an optical density of 0.5 at a wavelength of 600 nm, it is the best concentration for stimulating the secretion of MCL from the Manila clam. The work was supported by the National Natural Science Foundation of China (31571916).

(125) Human Airway Siglec-8 Ligands

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Sialoglycan binding proteins of the Siglec family are expressed on overlapping sets of human immune cells and often carry inhibitory domains that downregulate ongoing inflammation when activated by binding to endogenous glycan ligands. Allergic inflammatory airway diseases such as asthma and chronic rhinosinusitis are due in part to infiltration of eosinophils and mast cells, both of which express inhibitory Siglec-8. Previously we purified Siglec-8 sialoglycan ligands from postmortem human airways and demonstrated their anti-inflammatory activity by inducing human eosinophil apoptosis (Gonzalez-Gil et al, Glycobiology 28:786, 2018). We found that Siglec-8 ligands from postmortem human trachea are sialylated galatose-6-sulfated keratan sulfates primarily on the proteoglycan carrier aggrecan. Histochemical Siglec-8-Fc overlay of postmortem human trachea revealed that sialylated keratan sulfates are the sole human Siglec-8 ligands both in human airway cartilage, where aggrecan is expressed, and submucosal glands, where aggrecan is not detected. We hypothesized that Siglec-8 ligands in the submucosal glands and ducts are carried by a different protein that is then secreted onto the human airway mucus layer. We therefore probed for Siglec-8 ligands in nasal lavage flushed from the upper airway mucus layer of inflamed and non-inflamed patients undergoing sinus surgery. A single glycoprotein of ~1,000 kDa was the common Siglec-8 ligand in all human nasal lavage donors. Binding of Siglec-8 to the human nasal lavage ligand was reversed by sialidase or keratanase treatments. Purification and mass spectrometry proteomics identified the protein carrier as DMBT1 (GP340), a heavily O-glycosylated secreted airway glycoprotein. Immunohistochemistry revealed that DMBT1 and Siglec-8 ligands co-express in the submucosal glands but not cartilage of postmortem human trachea and upper airway. Submucosal glands are a major source of airway secretions and presumably the major source of immune regulatory glycans on the human airway mucus layer that dampen allergic inflammation. The quantity of Siglec-8 ligands varied among nasal lavage samples from human donors, indicating potential regulation of the key glycan determinant for Siglec-8 binding. We conclude that all human airway Siglec-8 ligands are sialylated keratan sulfate chains carried either on aggrecan in cartilage or DMBT1 in submucosal glands and on the airway mucus layer. Supp. by NIH grants AI136443, HL107151 & HL141952 (to AGG).

(126) Controlled cortical impact alters the chondroitin sulfate glycosaminoglycan composition in the mouse thalamus

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The brain is limited in its capacity to repair and reorganize neural networks following traumatic brain injury (TBI). Neural networks are maintained, in part, by specialized extracellular matrix (ECM) structures called perineuronal nets (PNN), which are mostly comprised of chondroitin sulfate (CS) glycosaminoglycan (GAG) chains. The CS-GAG chain sulfation pattern can dictate PNN stability, and thereby influence the function of neural networks. Reports indicate that CS-GAG chain sulfation patterns can change after TBI, however, little is known about the anatomical and functional specificity of these changes. In this study, adult male C57BL/6 mice were subjected to a TBI via a controlled cortical impact (CCI) to the somatosensory cortex (SSC). *Wisteria floribunda agglutinin* (WFA) immunostaining was employed to label PNN-associated CS-GAG chains, and a significant reduction in WFA^+ PNNs was observed within the perilesional SSC area. An established thalamocoritcal connection exists between the SSC and the lateral posterior thalamus (LPT). Further investigation of the LPT revealed the appearance of new WFA^+ PNNs at 7 and 28 days after CCI within the ipsilateral LPT, that were not detected in the contralateral LPT. Both ipsilateral and contralateral SSC and LPT were dissected from fixed brain tissue slices and the CS disaccharides were released using chondroitinase ABC (ChABC). Purified CS disaccharides were analyzed with a recently optimized LC-MS/MS+MRM method to differentiate the relative abundance of five specific CS isomers (Δ4S-, Δ6S-, Δ4S6S-, Δ2S6S-, and Δ0S-CS). The ipsilateral SSC and LPT exhibited higher percentages of Δ6S-CS disaccharides compared to the contralateral brain regions, as well as the sham control brain regions. As the thalamocortical axis is critical for sensorimotor function, CCI mice were subjected to the rotarod task to measure sensorimotor performance. Mice exposed to CCI exhibited a decrease in their latency to fall from the rotarod, which inversely correlated to the percentage of Δ6S-CS. Collectively, these data suggest that CCI alters the CS-GAG sulfation pattern within the thalamocortical circuitry, and these changes may contribute to persistent sensorimotor dysfunction. Future studies will investigate novel therapeutic interventions targeting CS-GAG chains to potentially improve outcomes from TBI.

(127) Exploring N-linked glycosylation and protein secretion: kinetic analysis of site-specific N-glycan processing in vivo

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N-glycan processing pathways are localized in the different cellular compartments of the eukaryotic secretory machinery. During secretion, highly specific and differentially localized glycosyltransferases and hydrolases modify the originally transferred $Glc_3Man_9GlcNAc_2$ oligosaccharides of N-glycoproteins in a species-, cell-type-, protein- and site-specific way to yield a precise and site-specific but heterogeneous ensemble of glycostructures. Based on our kinetic analysis of intracellular IgG glycoprocessing (Affolter et al., in press) we have studied intracellular processing of a model protein with 5 N-linked glycans in CHO cells. Experimentally, we combined SILAC and PRM-based glycoproteomics approaches to determine the intracellular turnover-rate of 43 different glycan structures at each of the 5 individual glycosylation sites. These data were then used tomathematically model N-glycan processing along the secretory pathway.

Our results confirmed site-specific N-glycan processing yielding a highly reproducible composition of N-glycans. Importantly, this composition changed upon prolonged cultivation of the expressing cells or upon altering the structure of the model protein by point mutations. We used the experimental data to fit a mathematical model of the N-glycan processing pathway that reflected the specificity as well as the localization of the processing enzymes. In our experimental system, different processing compartments are characterized by different glycan-specific turnover rates. Besides the canonical processing pathways in the ER and the Golgi, our data allowed for the description of: the quality control pathway of protein folding in the ER (ERAD), a direct export and subsequent degradation of (aggregated) glycoproteins to the lysosome, as well as, a potential secretory pathway (most likely Man-6-P dependent) that branches from the trans-Golgi network and involves endosomal processing compartments.

In a final set of experiments, we incorporated a MS-based relative quantification of glycoprocessing enzymes to assess the effect of altered expression levels on site-specific N-glycan profiles of the model protein.

(128) Report from the bench: UC San Diego GlycoBootcamp 2019, a guide for integration of research objectives into hands-on training in laboratory glycomics

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With the increasing call to re-introduce glycans into mainstream biomedical research, a handful of institutions with a critical mass of glycoscience research groups and technical resources and expertise have begun to develop hands-on training courses in laboratory techniques in glycan isolation, analysis and manipulation. Building on existing training activities developed at the Johns Hopkins University and the Complex Carbohydrate Research Center at the University of Georgia, the UC San Diego Glycobiology Research and Training Center recently piloted the GlycoBootcamp, an intensive two-week summer program, focused on developing skills for the isolation, characterization and manipulation of key glycan classes. A unique aspect of this program was the inclusion of a research objective to provide a "real-life" research experience to help the trainees become familiar with workflows in glycan analysis and to aid them with the development of experimental strategies as they begin to include glycans in their own research. The inaugural 2019 GlycoBootcamp was attended by 9 trainees organized into 4 teams. The research objective was to analyze the heart and brain glycomes in a mouse model of Sanfilippo syndrome A, a lysosomal storage disorder, and compare them to those of healthy animals. The poster will report on key aspects of the GlycoBootcamp development and organization as well as the educational and research outcomes of the course.

(129) GlyGen - Computational and Informatics Resources for Glycoscience

Rupali Mahadik
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GlyGen - Computational and Informatics Resources for Glycoscience

Rupali Mahadik.
The University of Georgia Complex Carbohydrate Research Center

Glycan's and glycosylation of proteins play very important role in biological function, development, and disease. However, the study of glycan's and their interaction with proteins has been limited due to the complexity and diversity of the data and distribution of this data in different resources.

To address this issue, The GlyGen project, a new resource supported by the NIH Common Fund, was founded to address this issue and to democratize glycoscience. To this end, we created a comprehensive data repository that integrates distinct types of data into a single resource and allows asking questions across multiple domains (e.g. genomics,

proteomics, glycomics). GlyGen retrieves information from multiple international data sources (including but not limited to EBI, NCBI, GlyTouCan and UniCarbKB) integrate and harmonizes this information in one consistent data collection. This data collection is focusing on human, mouse and rat data and includes information about glycan and protein structures, their annotation with function, enzymes involved in the biosynthesis of glycans, glycosylation of proteins, three-dimensional structures of glycans, proteins, and glycoproteins, and expression of (glyco)proteins in different tissues and disease. Each piece of information is annotated with the original source.

The GlyGen web portal (https://www.glygen.org) provides researchers an easy way to access the freely available integrated information. It allows exploring the data collection and performing unique searches across multiple domains that cannot be executed in any of the integrated databases alone. For each glycan and protein in the dataset, a details page with all integrated information about the protein or glycan is available. The individual pages are interlinked with each other allowing easy data exploration across multiple domains. For example users and browser from the webpage of a glycosylated protein to a glycan structure attached to this protein and from there to other proteins carrying the same glycan. All information on the webpage is linked back to their original source allow to browse to the information pages of a protein/glycan in the other resource which may contain additional data types and information that has not been integrated in GlyGen.

In addition to the browser-based web portal, we also developed machine-readable interfaces allowing programmatic access to the data for bioinformatics data analysis. GlyGen provides a RESTful web service based API (https://api.glygen.org) based on JSON data exchange as well as a SPARQL endpoint providing the integrated information in RDF (https://sparql.glygen.org).

(130) Hyperglycemia enhances cancer immune evasion by inducing alternative macrophage polarization through increased O-GlcNAcylation

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Diabetes mellitus (DM) significantly increases the risk for cancer initiation and cancer progression. Hyperglycemia is the most important characteristic of DM and tightly correlates with a poor prognosis in cancer patients. While enhanced availability of glucose can directly fuel tumor cell proliferation, the effect on the tumor immune microenvironment is less well known. We examined therefore the impact of hyperglycemia on anti-cancer immune responses in a well-defined mouse model. We found that an increase in glucose flux through the Hexosamine Biosynthetic Pathway (HBP) drives cancer progression and immune evasion by altering O-GlcNAcylation in tumor-associated macrophages (TAMs). TAMs were polarized to a tumor-promoting M2 phenotype inhibiting also adaptive anti-cancer immunity. Therapeutic reduction of TAMs abrogated the tumor-promoting effect of hyperglycemia. Inhibition of the HBP or O-GlcNAcylation led to a reversal of M2 polarization by hyperglycemia and inhibited cancer progression in hyperglycemic animals. Analysis of macrophage polarization in patients with DM and colorectal cancer showed an increased number of M2 polarized TAMs compared to colorectal cancer patients without DM. Our results provide a new and targetable mechanism of cancer immune evasion in hyperglycemic cancer patients, advocating for strict control of hyperglycemia in in this patient population.

(131) Galectin 3 is a molecular integrator and tunable transducer in nutrient sensing

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Disease conditions like cancer and diabetes are characterized by dramatically altered nutrient uptake and metabolism. Endomembrane glycosylation and cytoplasmic O-GlcNAcylation play essential roles in nutrient sensing, and in fact, characteristic changes in glycan patterns have been described in disease states. These changes in glycosylation likely have important functional roles and can drive disease progression. However, little is known about the molecular mechanisms underlying glycosylation's role as a nutrient sensor and how these signals are integrated and transduced into biological functional effects.

Galectins are cytoplasmic proteins that bind glycans and are predicted to be O-GlcNAcylated. Hence, their stability, secretion and functions may depend on O-GlcNAcylation. These proteins are secreted by a non-classical secretory mechanism that is not understood. Once outside the cell, galectins bind to N-linked and O-linked glycans via

their terminal galactose residues and modulate numerous extracellular functions like clathrin independent endocytosis (CIE) and cell attachment. As a result, galectins occupy a unique niche in which they can sense changes in glycan patterns and can potentially be targeted by changes in O-GlcNAcylation to alter cellular behavior. As an example, CIE is essential for the survival of nucleated cells, however, there is little known about how this process is regulated. Our preliminary data suggests that galectin 3 plays an important role in modulating CIE. Characterizing glycosylation's unique role in regulating CIE and determining whether galectin 3 secretion can be impacted by O-GlcNAcylation would offer insight into how nutrient sensing via glycosylation can be functionally transduced into cellular effects. *Our hypothesis is that galectin 3 acts to detect and integrate information from all three forms of glycosylation and convert this information into functional effects.*

Our findings indicate that galectin 3 is O-GlcNAcylated, and that changes in O-GlcNAc cycling alter its secretion. Increasing O-GlcNAcylation by knocking down O-GlcNAcase (OGA) leads to decrease in galectin 3 secretion and decreasing O-GlcNAcylation using chemical inhibitors like OSM1 leads to an increase on galectin 3 secretion. Moreover, we noted a significant difference in O-GlcNAcylation status between cytoplasmic and secreted pools of galectin 3. Mouse embryonic fibroblasts isolated from wild type and O-GlcNAcase knock out mice also demonstrated similar galectin 3 secretory characteristics. Finally, O-GlcNAcylation induced changes in galectin 3 secretion lead to changes in CIE, providing proof-of-concept that galectin 3 can act as a transducer of nutrient sensing stimuli coded via O-GlcNAc cycling. These results indicate that O-GlcNAcylation of galectin 3 plays a role in modulating its secretion, thus providing a feedforward/feedback mechanism for transducing nutrient sensing information encoded by glycosylation into biological effects.

(132) New extensions for GRITS Toolbox: MS data annotation for glycosphingolipids and editing of glycan structures and databases

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In recent years glycomics analysis has become more and more important with the awareness of glycans involvement in biological processes, disease and their interaction with other molecules. Mass spectrometry (MS) is one of the major techniques used to study glycan structures. The increase in accuracy and throughput of mass spectrometry has allowed producing larger dataset even faster. To handle this increasing amount of data, software solutions are required to assist experimentalist in the processing and interpretation of these datasets.

Previously, we have developed GRITS Toolbox an extendable software system for processing, interpreting and archiving of glycomics MS data. This system allows glycomics MS datasets (MS/MS, LC-MS/MS, TIM or MS profile data) to be loaded, processed and stored together with its metadata. The core of our MS data analysis system is GELATO, a software module that annotates MS data with glycans and glycan fragments derived from a glycan database. An extensive set of graphical user interfaces can then be used to visualize, review, modify and export the annotated data or to compare the annotations of different samples side by side.

As part of an NIH common fund grant we developed an MS data annotation module for glycolipidomics, called DANGO. This module was implemented as an extension (plugin) of GRITS Toolbox allowing reusing all data and metadata processing and visualization component. DANGO annotates MS data with intact glycolipid structures (native or per-methylated) and their fragments. Glycan and lipid portion of the glycolipids are derived from corresponding databases. To visualize, process and compare the annotated MS data a set of graphical displays has been implemented.

Recently, we added another extension (plugin) to GRITS Toolbox, called GlycanBuilder. GlycanBuilder is a graphical user interfaces, which allows editing and saving glycan structures intuitively. Similar software systems are already present as part of other software projects, such as GlycoWorkbench, or web sites, such as GlyTouCan. However, our system does not only allow to draw glycan structures in cartoon representation and save them as images, but also supports the newest SNFG notation, importing from and exporting to different sequence formats. The system also allows loading and editing of GELATO and DANGO glycan databases. This allows users to customize the preinstalled glycan databases with own structures or removed unwanted structures from the existing databases.

The current version of the software system is freely available from our project website: GRITS Tool box: http://www.grits-toolbox.org; and DANGO: http://www.ms-dango.org.

(133) Development of Smart Anti-Glycan Reagents (SAGRs) specific for sialic acid using immunized lampreys

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Studies on expression and structures of cellular glycans are limited by a lack of sensitive tools that can discriminate

specific structural features. In our laboratory, we have developed a robust platform using immunized lampreys (*Petromyzon marinus*) for generating libraries of anti-glycan antibodies. Using a variety of different immunogens, including whole fixed cells, tissue homogenates, and human milk, we have identified three monoclonal lamprey antibodies (VLRBs) which are specific for 2-6 or 2-8 sialic acid linkages. These monoclonal VLRB antibodies were discovered from a yeast surface display (YSD) library that was enriched for glycan specific targets by incubation directly onto the CFG glycan microarray, where antigen specific clones could easily be identified and sequenced. We then generated monoclonal VLRB-Ig chimeras, termed smart anti-glycan reagents (SAGRs), and verified the specificity of each antibody on the NCFG sialic acid and CFG glycan microarrays. Interestingly, the three monoclonal VLRBs have distinct, and non-overlapping binding profiles by glycan microarray screening with the commonly used plant lectin *Sambucus nigra* (SNA). This result was further validated by immunohistochemistry of healthy human tissues, where it is clear that the staining patterns are distinct from that of SNA. Thus, these reagents are a powerful tool for the study of many sialylated glycans that may have been missed by traditional lectin staining. The development of SAGRs and the availability of YSD libraries will enhance future studies on glycan expression by providing sequenced, defined antibodies for a variety of research applications.

(134) A schizophrenia-associated variant in SLC39A8 alters protein N-glycosylation in the mouse brain

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Schizophrenia is a common, heterogenous, and debilitating neuropsychiatric condition characterized by cognitive dysfunction, social withdrawal, and psychosis. Genome wide association studies (GWAS) have identified over 100 loci associated with the disorder, but translating these results into a mechanistic understanding of the disease remains a considerable challenge. The most significantly-associated coding variant identified to date is a missense mutation (A391T) in the manganese (Mn) transporter SLC39A8, present in 8% of the European population. Homozygous loss-of-function mutations in SLC39A8 result in undetectable serum Mn levels and a congenital disorder of glycosylation (CDG) presenting with neurologic symptoms including seizures, intellectual disability, and brain structural abnormalities. Our recent study of the schizophrenia risk variant demonstrates that A391T decreases serum manganese levels and alters plasma N-glycosylation in human carriers. To understandhow the SLC39A8 A391T mutation impacts glycosylation in the brain, we analyzed N- and O-glycans from the frontal cortex, striatum, hippocampus, and cerebellum of C57BL/6 mice homozygous for the A391T missense mutation using MALDI-TOF-MS. Comparison between the four brain regions in wild-type mice revealed significant differences in the relative abundance of several glycan species, emphasizing the importance of analyzing each region independently. We observed an increase in the complexity and branching of N-glycans in the cortex of A391T mice relative to controls, whereas the cerebellum of mutant mice showed decreased complexity of N-glycans. No difference was observed in protein O-glycans between genotypes. These results provide a regional map of the mouse brain protein glycome, and demonstrate that a schizophrenia-associated mutation in *SLC39A8* alters brain glycosylation in a region-specific manner.

(135) Prevalence of rhamnose biosynthesis pathways in completely sequenced genomes and metagenomes

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Rhamnose is a constituent of extracellular polysaccharides, glycoproteins and secondary metabolites. Rhamnose is biosynthesized starting from D-glucose as (d)TDP-L-rhamnose, UDP-L-rhamnose or GDP-D-rhamnose (rml, udp or gdp pathway, respectively). Experimentally characterized enzymes of these three pathways were used to generate hidden Markov model profiles for each enzyme family. These profiles were used to identify rhamnose biosynthesis pathways in 22,061 completely sequenced genomes and 128,195 human-associated / environmental metagenome samples. The rml pathway is found in bacteria, archaea and phages but not in eukaryotes and other viruses. The udp pathway is found only in plants, fungi, algae, nematode and *Acanthamoeba polyphaga* mimivirus. The gdp pathway is found only in *Aneurinibacillus* and *Pseudomonas*. The prevalence of these pathways within different phyla (at least 50 genomes sequenced) vary significantly. The rml pathway is found in >40% genomes of Actinobacteria, Bacteroidetes, Crenarchaeota, Cyanobacteria, Fusobacteria and Proteobacteria but in <20% genomes of Chlamydiae, Euryarchaeota and Tenericutes. The udp pathway is found in all genomes of Streptophyta, <=25% genomes of Ascomycota and Chordata, and none of the genomes of Arthropoda,

Basidiomycota, Chlamydiae and Negarnaviricota. Overall, it is found that the rml pathway, the most prevalent of the three pathways, is found only in 42% and 21% of completely sequenced bacterial and archaeal genomes, respectively. Among the 532 *Pseudomonas* genomes, 161 have both gdp and rml pathways and 295 genomes have only rml pathway. D-rhamnose, synthesized via the gdp pathway, has been reported to be found in the O-antigen lipopolysaccharide of *P. aeruginosa*. Some of the genera in which none of the three pathways are found are *Chlamydia, Helicobacter, Listeria, Mycoplasma, Pasteurella, Rickettsia* and *Staphylococcus*. Analysis of the human-associated metagenomes showed that only 2,385 out of 73,167 samples (3.2%) have the rml pathway. These samples are from faecal, large intestine, oral, saliva and skin biomes. The biomes where the rhamnose pathways are notably absent are digestive, nasopharyngeal, pulmonary, respiratory and vaginal. The presence of the rml pathway is not correlated with the host sex taking into consideration 18,840 samples for which host sex data is available. For the environmental biomes where >=100 samples are available, rhamnose pathways are absent in a majority of biomes. The rml pathway is found in samples from marine, oceanic, soil, sediment, coastal and lake metagenomes, while the udp pathway is found in samples from coastal and marine metagenomes.

(136) GlyGen Data Integration: Creating A Collaborative Environment For Data Generators, Bioinformatics Resources, And Users

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GlyGen is an NIH funded interdisciplinary glycoinformatics project developed to implement a comprehensive data repository with an aim to answer crucial community-wide use cases. GlyGen utilizes novel data integration methodologies to collect and harmonize different types of data to ultimately provide a consolidated view of protein, gene, and glycan in one resource. The data integration infrastructure and protocols allow a systematic and streamlined approach for individual researchers or large molecular biology database developers to submit and integrate their data into GlyGen for further dissemination. Each submission involves collaborative discussions with the provider on data format, quality and standards used followed by the co-development of quality control and integration pipeline to generate a stable, versioned dataset assigned with a unique identifier. Currently, GlyGen has 100 such datasets. These datasets are grouped into different categories (source, annotations, etc.) and are accompanied with detailed documentation (in text and JSON format) to describe the source, usability, and the integration process. All datasets feed into the GlyGen frontend interface (https://glygen.org) and are also available for download through the data interface (https://data.glygen.org/) under CC BY 4.0 license (free to copy, distribute, display and make commercial use with citation. https://glygen.org/license.html).

In addition to providing direct access to the integrated datasets, users can also access GlyGen data programmatically through RESTful web service-based APIs (https://api.glygen.org) and (in-future) SPARQL (https://sparql.glygen.org) endpoint. GlyGen team has developed a comprehensible data model by integrating namespaces from existing protein and glycan ontologies which can be easily extended to accommodate diverse data types and accompanying annotations. The major collaborative efforts integrate glycan-centric (GlyTouCan, ChEBI, PubChem), protein-centric (UniProtKB, RefSeq, PDB) and glycoprotein-centric (UniCarbKB and Protein Ontology) databases and ontologies. Currently, GlyGen includes data associated with Human, Mouse and Rat species (future plans include the addition of HCV and synthesized glycan data). Collection of such diverse datasets allows GlyGen users to not just browse and download data but also perform complex queries across different domains (such as: Which protein(s) bear glycan X?, Which enzyme(s) are involved in synthesizing glycan X?. For details about GlyGen frontend please visit poster presented by Will York and Rupali Mahadik). Researchers interested in integrating their data into GlyGen can reach out to us using our contact page (https://www.glygen.org/contact.html).

(137) Insights into the functions of the Ost3 and Ost6 proteins in the yeast oligosaccharyltransferase

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Oligosaccharyltransferase (OST) is localized in yeast in the Endoplasmic Reticulum (ER), where it catalyzes the posttranslational transfer of an oligosaccharide onto the asparagine in the glycosylation sequon N-X-S/T (X $\neq$P) as soon as the nascent polypeptide chain reaches into the ER lumen. In *Saccharomyces cerevisiae,* OST is an eight subunit protein complex consisting of the subunits Ost1p, Ost2p, Ost3p, Ost4p, Ost5p, Ost6p, Swp1p, Wbp1p and the catalytic Stt3p. Yeast OST exists in two isoforms harboring either Ost3p or Ost6p, which are shown to have different protein substrate specificities.[1,2] Ost3/6p are functional homologs that have oxidoreductase activity that is relevant for site-specific glycosylation.[1,3,4] The structure of the luminal domain of the yeast Ost6p and a human homologue TUSC3 were solved, bringing insights into the oxidoreductase function.[1,4] Interestingly, this thioredoxin domain and the first transmembrane span of Ost3p were not resolved in the cryo-EM structure of the yeast OST complex.[5,6] *In vitro* analysis of the OST activity using lipid-linked oligosaccharide and short peptide substrates revealed a strongly reduced activity of the Ost6p- vs. the Ost3p-containing complex, pointing towards a role of the OST3/6 transmembrane domains in OST function.[7] For a detailed

analysis, we generated yeast strains expressing defined Ost3/6p chimeras. The mutants strains were analyzed with respect to their glycosylation efficiency and OST complex stability using MS-based parallel-reaction monitoring (PRM) combined with SILAC.[8] The results of this analysis as well as a biochemical characterization of purified OST complexes containing hybrid Ost3/6p will be presented.

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(138) The role of 9-O-acetylated glycan receptor moieties in the typhoid toxin binding and intoxication outcomes

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Typhoid toxin is an A_2B_5 toxin secreted from *Salmonella* Typhi-infected cells during human infection and is suggested to contribute to typhoid disease progression and the establishment of chronic infection. To deliver the enzymatic 'A' subunits of the toxin to the site of action in host cells, the receptor-binding 'B' subunit PltB binds to the trisaccharide glycan receptor moieties terminated in N-acetylneuraminic acid (Neu5Ac) that is α2-3 or α2-6 linked to the underlying disaccharide, galactose (Gal) and N-acetylglucosamine (GlcNAc). Neu5Ac is present in both unmodified and modified forms, with 9-O-acetylated Neu5Ac being the most common modification in humans. Here we present that host cells associated with typhoid toxin-mediated clinical signs express both unmodified and 9-O-acetylated glycan receptor moieties. We found that PltB binds to 9-O-acetylated α2-3 glycan receptor moieties with a markedly increased affinity, while the binding affinity to 9-O-acetylated α2-6 glycans is only slightly higher, as compared to the affinities of PltB to the unmodified counterparts respectively. We also present X-ray co-crystal structures of PltB bound to related glycan moieties, which explain the different effects of 9-O-acetylated α2-3 and α2-6 glycan receptor moieties on the toxin binding. Lastly, we demonstrate that the cells exclusively expressing unmodified glycan receptor moieties are less susceptible to typhoid toxin than the cells expressing 9-O-acetylated counterparts, although typhoid toxin intoxicates both cells. These results reveal a fine-tuning mechanism of a bacterial toxin that exploits specific chemical modifications of its glycan receptor moieties for virulence, which provides useful insights into the development of therapeutics against typhoid fever.

(139) Linking maternal sugar consumption to progenies' developmental defect: a focus on OTX2's O-GlcNAcylation.

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Currently, the average American eats around 22 teaspoons of added sugar every day (~30 sugar cubes/day hidden in foods). This modern glucose-rich diet correlates with an increase of obesity, type 2 or gestational diabetes and is measurable throughout generations. Therefore, determining the interplay between diet and metabolic development is of utmost importance for public health. The *O*-GlcNAc post-translational modification is a unique glucose rheostat for cell signaling. The addition of a single residue of N-acetylglucosamine on serine and threonine constitutes a simple but highly effective way to sense glucose concentration and regulate protein function. Indeed, the level of extracellular glucose are reflected by the level of UDP-GlcNAc primary substrate for *O*-GlcNAcylation. To date, thousands of *O*-GlcNAcylated proteins have been identified. Numerous physiological and pathological processes are *O*-GlcNAc-regulated such as cell cycle, transcriptional regulation, diabetes, cardiovascular diseases, neurodegeneration and cancers. While O-GlcNAcylation is known to regulate many developmental factors such as homeobox proteins, we are only starting to unravel the impact of *O*-GlcNAc deregulation on developmental defects.

In this study, we have demonstrated that a progeny's *O*-GlcNAcylation levels are disproportionately affected in response to mother's high sugar diet. Using hyper-*O*-GlcNAcylated cellular and mouse models, the homeobox protein OTX2 was identified as a major *O*-GlcNAcylated protein in the developing brain. We have also shown that both transcription and stability of OTX2 were affected by *O*-GlcNAc variation, resulting in an increased half-life of this protein. Interestingly, we have discovered that while endogenous OTX2 was processed by the proteasome, overexpression in Hela cells resulted in autophagy-mediated degradation. Nevertheless, both degradation processes were inhibited by increased *O*-GlcNAcylation. Using specific domain constructs, we have discovered that the Retention Domain of OTX2 is highly *O*-GlcNAcylated, potentially affecting localization and critical phosphorylation sites. Like many homeobox proteins, OTX2 level needs to be tightly regulated for proper patterning and development. Therefore, deregulation of OTX2 amongst other proteins in brain hyper-*O*-GlcNAcylated mouse results in major developmental defects. Usually supported by a local transient expression of OTX2, pituitary's ontogeny is particularly affected by increase in *O*-GlcNAc level as reflected by post-natal lethality in pituitary hyper-*O*-GlcNAcylated mice.

To summarize, this study highlights the *O*-GlcNAc modification as a nutrient-dependent sensor that regulates the homeobox protein OTX2 during pituitary and brain development.

(140) Succinylation of mycobacterial heteropolysaccharides and its impact on biophysical properties of the cell envelope

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Prokaryotes produce variety of cell wall polysaccharides which play important roles in the cell wall architecture and pathogenesis. Covalent modification of these polysaccharides by distinctive moieties is a strategy that bacteria developed to modify physiological properties of their cell walls in a response to environmental stress factors. These precisely placed modifications can be made by different sugars, amino acids, phosphates or acyl chains and affect either the biosynthesis of polysaccharides, their trafficking within the cell envelope, permeability of the cell wall or immunomodulatory properties of the cell (1).

The cell envelope of human pathogen *Mycobacterium tuberculosis*, the causative agent of tuberculosis, consists of two major heteropolysaccharides, namely arabinogalactan (AG) and lipoarabinomannan (LAM). While AG can be altered by positively charged galactosamine, the nonreducing end of LAM may contain an unusual sugar- methylthioxylose. Whereas enzymes responsible for addition of these moieties have been identified, the biological significance of these groups remains still hypothetical. Extensive analytical studies of AG and LAM reported also the presence of negatively charged succinate groups (2,3). We identified, for the very first time, the succinyltransferase activity responsible for addition of succinyl groups to arabinan domains of AG and LAM. Disruption of the succinyltransferase encoding gene *msmeg_3187* in *M. smegmatis*, a non-pathogenic model of mycobacteria, abolished AG and LAM succinylation without significantly affecting AG and LAM biosynthesis. However, the loss of succinates altered the hydrophobicity and rigidity of the cell envelope of the bacilli as it was similarly reported in transposon mutants of closely related species *M. marinum* and *M. avium* carrying insertions in the orthologous gene whose ability to aggregate and form a biofilm was altered (4,5). Hence, our findings indicate an important role of succinylation of AG and LAM in modulating the cell surface properties of mycobacteria.

Acknowledgements:

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(141) The impact of Thiamet G on cardiac O-GlcNAcylation and heart failure

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Background

The failing heart is characterized by progressive deterioration in pump function, aberrant energy metabolism and disrupted protein expression. *O*-linked N-Acetylglucosamine (*O*-GlcNAc), is a dynamically regulated modification on Ser/Thr residues that has been implicated in cellular stress responses and metabolic disease. Global changes in *O*-GlcNAcylation are observed in various contexts of cardiac pathophysiology including failing, hypertrophic, ischemic, or diabetic cardiomyopathy. Protein *O*-GlcNAcylation is regulated by conserved enzymes OGT (*O*-GlcNAc Transferase) and OGA (*O*-GlcNAcase). Thiamet G (TMG), elevates the *O*-GlcNAc modification in cells and animals by inhibiting OGA. Here, we examine the effects of TMG infusion in heart failure under the working hypothesis that TMG-elevated cardiac O-GlcNAcylation curtails heart failure progression.

Methods and Results

In preliminary experiments, heathy adult guinea pigs were implanted with osmotic pumps delivering TMG (5 mg/Kg/-day) or vehicle (10 mM HEPES, pH7.4) for 28 days (time points taken at days 2, 7, 14, and 28). TMG-treated animals had significant increases in cardiac *O*-GlcNAcylation as early as day 2, peaking at 75% increase by day 7 and leveling-off at 60% increase by days 14 and 28. The increases in *O*-GlcNAcylation were mirrored by reciprocal increase in OGA and decrease in OGT, but these changes normalized toward the 28-day time point. The increased *O*-GlcNAcylation did not have any detrimental effect on heart function. In other experiments, adult guinea pigs underwent pressure overload by aortic constriction (AC) and a daily bolus infusion of low-dose isoproterenol (2 mg/Kg/day) for 28 days (ACi model). Western blot analysis in ACi hearts showed an increase by 27% in global protein *O*-GlcNAcylation in ACi vs. sham-i animals. In a third set of experiments sham-i and ACi animals were infused with vehicle or TMG and heart failure progression was followed up for 28 days. Preliminary findings suggest that the mean survival does not differ significantly between vehicle and TMG treated ACi animals. Other endpoints of heart failure, including gravimetric analysis of the heart and lung weights as well as functional indexes (ejection fraction and ventricular wall thickness) are currently being pursued.

Conclusions

The main findings of the present work are: 1) Experimental heart failure in guinea pigs is characterized by increased *O*-GlcNAcylation, 2) Sustained infusion of TMG time-dependently increases *O*-GlcNAcylation in hearts, 3) Increasing *O*-GlcNAcylation by 60-75% has no detrimental impact on cardiac function in healthy animals and 4) TMG infusion has no beneficial or detrimental impact on the survival of ACi animals with heart failure. Future work will examine the profile of the cardiac O-GlcNAcome in ACi animals with or without TMG to identify putative targets that are dynamically regulated by O-GlcNAc in the context of heart failure.

(142) Modified Sialic Acid Expression in Cells and Animals

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Sialic acids (Sia) are found in large amounts both on the cell surface as part of the glycocalyx and in mucus that protects the respiratory and GI tracts. Sia may be chemically modified (including 7,9-*O*-, 9-*O*-, 4-*O*-acetyl (Ac), and/or 5-*N*-glycolyl) and are also attached to glycan chains through different linkages, which vary between hosts and tissues. We have used probes that are specific for modified Sia, as well as HPLC analysis, to survey for the levels of each modified form in cells in culture, and in tissues of different animals. There were wide differences in the levels of each modified form in different tissues of mice (both wild type and cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase (CMAH) knock out), as well as in the blood, erythrocytes and saliva of different animals. We have also developed engineered cell lines that either lack or over-express 7,9-*O*-, 9-*O*-Ac, 4-*O*-Ac Sia or Neu5Gc. MDCK, HEK293, and A549 cells were engineered using CRISPR/Cas9 or expression plasmids. Enzymes included CMAH, CAS1 Domain Containing 1 (CASD1), and sialic acid acetyl esterase (SIAE), and the levels of expression of each modified form in each cell was examined. Some modified forms were located primarily in the golgi compartment of some cells, and on the surface of others. We saw that 7,9-*O*-, 9-*O*-Ac, and 4-*O*-Ac Sia modifications were all highly variable in expression, showing incomplete population homogeneity, and were a small proportion of the total Sia in cultured cells; we are now examining the regulation of their expression and display in different cells.

We have also used those used those cells and other approaches to determine the impact of modified Sia on influenza A virus (IAV) hemagglutinin (HA) binding, neuraminidase (NA) activity, virus growth, and host adaptation. While the importance of Sia α2,3- versus α2,6-linkages in IAV tropism and evolution have been well studied, the roles of modified Sia in IAV host range are poorly understood. The 7,9-*O*-, 9-*O*- or 4-*O*-Ac Sia are expressed in many IAV host tissues, including within the respiratory tissue of humans, particularly in the submucosal glands and mucus. The 7,9-*O*-, and 9-*O*-Ac modifications are also variably expressed in embryonated chicken eggs used to grow vaccine strains, and on MDCK, HEK293, and A549 cells used in IAV research. Neu5Gc and 4-*O*-Ac Sia are highly expressed in some tissues of IAV hosts including pigs, horses, mice and guinea pigs, but not in humans, chickens, or ferrets. The 7,9-*O*-, 9-*O*- and 4-*O*-Ac modifications may all decrease NA cleavage and HA binding, but in a strain specific manner. Over-expression of CMAH resulted in generation of human and canine cells displaying Neu5Gc to between 40 and 90%, similar to those

seen in natural host tissues. We are now assessing the effects of Neu5Gc on HA and NA function, and on virus replication in mice and in cell cultures.

(143) Improved Profiling of Sialylated N-Linked Glycans by Ion Chromatography-Orbitrap Mass Spectrometry

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Introduction:

Good profiling of a recombinant glycoprotein's asparagine-linked (*N*-linked) glycans requires high resolution separation and reliable identification of released glycans. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is a well-established technique for glycan analysis. When coupled to high resolution accurate mass spectrometry (HRAM), it offers simultaneous high resolution separation and reliable identification of glycans. HPAE-PAD is especially effective for separating sialylated glycans, but there is opportunity to increase resolution and improve glycan identification. Here, we evaluated changes to commonly used HPAE-PAD elution conditions to achieve improved resolution. Then effect of glycan structure on resolution was studied leading to new insights on elution conditions to resolve different glycan structures. The results obtained here offer an effective approach to quickly screen the impact of changes in cell culture conditions on sialylation.

Methods:

Enzymatically released glycans were separated on a Thermo Scientific^{TM} Dionex^{TM} CarboPac^{TM} PA200 BioLC^{TM} Analytical Column (3 x 250 mm) attached to a Thermo Scientific Dionex ICS-5000+ HPIC dual IC system. The system was equipped with a Thermo Scientific Dionex ERD 500 electrolytically regenerated desalter. The Thermo Scientific^{TM} Q Exactive^{TM} mass spectrometer was employed in negative mode ESI. Data analysis was performed using SimGlycan software (Premier Biosoft).

Results:

Glycans released from four different glycoproteins, bovine fetuin, bovine thyroglobulin, bovine fibrinogen, and human alpha 1 acid glycoprotein using PNGaseF were used here. We first tested HPAE-PAD separation of released *N*-linked glycans on a CarboPac PA200 column, using a typical sodium hydroxide concentration and temperature, i.e. 100 mM with a gradient of sodium acetate at 30 °C (Standard Condition). We then tested the effect of temperature and sodium hydroxide concentration on resolution. This led to identification of chromatographic conditions which improved resolution. The column effluent containing separated glycans was passed through a salt exchanger to remove sodium ions contained in the effluent prior to mass spectrometry. A Q Exactive Orbitrap mass spectrometer used in negative electrospray mode was coupled to the ion chromatography system. Fragmentation of glycans in the negative mode by HCD provided information-rich MS^2 spectra dominated by glycosidic and cross-ring fragments that frequently revealed linkage information. The possible glycan structures were first identified by SimGlycan high throughput search and score function. The structures were confirmed by annotating the diagnostic fragmentation patterns observed in MS^2 spectra.

Comparison of structures identified under different conditions allowed correlation of glycan structure with observed resolution. For example, fucsolylated glycans were resolved from non fucosylated analogues only under "Standard Condition." And increased hydroxide concentration resulted in change in elution order between a biantennary disialylated glycan and a biantennary monosialylated glycan. In essence, this work presents an effective approach for increasing glycan coverage using HPAE coupled to HRAM mass spectrometry.

(144) Molecular basis for FGF23 site specific glycosylation by GalNAc-T3

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Mucin type O-glycosylation is initiated by a large family (~ 20 isoenzymes in mammals) of polypeptide GalNAc transferases (GalNAc-Ts), that transfer GalNAc onto specific Thr or Ser residues of substrate proteins. Structurally, nearly all GalNAc-Ts contain a catalytic domain and a lectin domain connected by a flexible linker. Our earlier substrate specificity studies have shown that most GalNAc-Ts prefers a C-terminal ~TPGP~ moif and while having unique N-terminal specificities that permit each GalNAc-T to possess an optimal sequence motif. Interestingly, we have further shown that most GalNAc-T activities are enhanced by remote prior glycosylation (>5 residues away from the acceptor) in an N- or C-terminal direction, while the activities of several isoenzymes (GalNAc-T4, T7, T10, T12) are enhanced by neighboring prior glycosylation (<5 residues away). One particular isoenzyme, GalNAc-T3 specifically glycosylates fibroblast growth factor 23 (FGF23) at the Thr178 which neighbors a proprotein convertase (PC) cleavage site (RHT^{178}RêS). This site-specific *O*-glycosylation at Thr178 by GalNAc-T3 inhibits furin PC processing and stabilizes secreted intact FGF23 thus regulating phosphate homeostasis. Here we report, our structural and kinetic studies on GalNAc-T3's glycosylation of this important Thr178 site, which we further show requires prior remote glycosylation at Thr171.

Our kinetic studies unexpectedly revealed that the FGF23 substrate (~FNT*^{171}PIPRRHT^{178}RSAEDD, where T* = GalNAc-Thr, called *T*171-T178*) is actually a very

poor substrate for GalNAc-T3 compared to an optimal T3 substrate (~YAVTPGP~), What makes FGF23 such a poor but selective substrate for GalNAc-T3 is unknown. Our X–ray crystal structure of GalNAc-T3 bound to the FGF23 substrate (*T*171-T178*) revealed specific enzyme-substrate clashes that tend to destabilize the catalytic domain flexible loop required for efficient GalNAc transfer from donor UDP-GalNAc. We also made a series of chimeric FGF23susbtrates containing different optimal GalNAc-T substrate sequences in an attempt to enhance the glycosylation of the T178 by GalNAc-T3. Interestingly, it was found that these substitutions failed to significantly increase activity which we believe is due to the presence of the EDD sequence. Thus Nature has designed a complex system to regulate the function of FGF2315. Key to this control is the finding that FGF23-Thr178 is a relatively poor but selective site, solely glycosylated by GalNAc-T3, and that this glycosylation is primed by prior glycosylation of Thr171. In this manner an otherwise relatively ubiquitous and promiscuous GalNAc-T can glycosylate a site with high specificity by utilizing the unique properties of both its lectin and catalytic domains.

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(145) TRAP Complex Facilitates N-linked Glycosylation Biosynthetic Process During ER-stress

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Introduction

N-linked Glycosylation (NLG) of proteins is necessary for protein folding, localization, stability, and activity. NLG requires multiple enzymatic steps that synthesize and transfer glycan precursors to nascent proteins in the endoplasmic reticulum (ER). We hypothesized that a phenotypic genetic screen to detect aberrant protein glycosylation could be designed and implemented in order to discover novel genetic factors that regulate NLG.

Methods

We established a functional reporter for studying NLG using the bacterial halogenase protein (Halo1N) stably transfected into A459 and HEK293 cells. Fluorescence of this reporter can be detected with Halo ligands under conditions of abnormal NLG. A549 cells with stable expression of Cas9 and Halo1N cells were used to screen 123,411 guide RNAs (gRNAs) targeting 19,050 human genes. Fluorescent cells were sorted with FACS, harvested, and enrichment of corresponding genes was identified through MAGeCK analysis of gRNA deep sequencing in comparison to unsorted controls. Independent clones with target gene knockout were established using similar techniques. Changes in cell surface glycoprotein expression were analyzed by selective exoenzymatic labeling (SEEL) and Mass spectrometry. Functional consequences on select proteins were then evaluated by western blot. RNA sequencing was used to evaluate changes in gene expression and the proximity ligation assay was used to evaluate protein-protein interactions.

Results

The Halo1N reporter displayed increased protein mobility on western blot and robust induction of fluorescence after either tunicamycin or NGI-1 treatment. The reporter was used for a pooled genome CRISPR-Cas9 screen and MAGeCK analysis identified 27 significantly enriched genes; of which 22 are known to be involved in NLG (17 genes required for NLG and 5 genes involved in nucleotide sugar synthesis). In addition, 14 other genes implicated in the NLG pathway had enrichments of single gRNAs but were not significant by the MAGeCK criteria. Members of the TRAP complex (SSR1, SSR2, SSR3, and SSR4) were also significantly enriched in the screen. The role of TRAP in regulating NLG is not well understood therefore we individually knocked-out each TRAP subunit to examine effects on NLG. SEEL identified loss of a subset of glycoproteins at the cell surface of TRAP knockout cells including members of the receptor tyrosine kinases family. Depletion of TRAP subunits reduced glycosylation and ligand induced activation of EGFR but had no effect on IGF-1R, suggesting that TRAP is required for efficient glycosylation of discrete client proteins. Surprisingly, TRAP knockouts lost the glycosylation deficient phenotype over time. Transcriptomic analysis revealed that cellular adaptation with a reduction of ER-stress led to rescue of NLG in TRAP knockout cells, a finding that was confirmed with both pharmacologic and genetic manipulation of ER stress.

Conclusion

We demonstrate that pooled genome CRISPR screening can be used to identify novel components of the NLG machinery. This work provides evidence of a previously unrecognized mechanism for regulating the glycosylation of a subset of glycoproteins, and identifies distinct roles for TRAP subunits in mediating this biosynthetic process under variable conditions in the ER.

(146) Udderly fascinating: relationships between breast milk composition and child development

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Human breast milk is an incredibly rich and complex biofluid composed of many complex carbohydrates, including a diverse repertoire of free human milk oligosaccharides (HMOs). Strikingly, HMOs are not digested by the infant and function as prebiotics for bacterial strains associated with numerous benefits (e.g., protection from enteropathogen infection). Considering the broad variety of beneficial effects of HMOs, and the vast number of factors that affect breast milk composition (e.g., lactation stage, geographical variation), the analysis of HMO diversity and complexity in human milk samples is of utmost relevance.

Using human milk samples from a cohort of Bangladeshi mothers participating in a study on malnutrition and stunting in children, we have characterized breast milk composition in human samples by means of liquid chromatography and high-resolution tandem mass spectrometry analysis (LC-MS/MS). Although the relationship between HMOs and the growth patterns of infants has not been well characterized, some studies have described a negative correlation between the growth phenotype of severely stunted infants and their mother's milk composition. Thus, one of our main goals is to use LC-MS/MS techniques to evaluate modification patterns (fucosylation / sialylation) in HMOs. This approach identified over 100 different glycoforms present in human samples, and these results were used to create glycomic composition profiles of each mother's milk. Our analysis shows a wide diversity of milk composition, with a predominance of fucosylated and sialylated HMOs over neutral non-modified HMOs. Although the five most abundant HMOs, comprising up to 60% of the total HMO content of the samples, are typically low molecular weight (MW) compounds, breast milk samples contain a wide variety of glycoforms of much larger MW. In reality, our results show that human breast milk samples contain on average 80 HMOs, with the highest permethylated masses detected being >5000 mass units. Here we report an easily implemented method, developed for separation, characterization and semi-quantitation of large arrays of HMOs including higher MW sialylated HMOs. Our ultimate goal is to create a simple, high-throughput method, which can be used for full characterization of sialylated and/or fucosylated HMOs. These results demonstrate how current analytical techniques can be used to characterize human milk composition, and shed a new light on the impact of HMOs on the infant's development.

(147) Glycan Engineering reveals that matriglycan alone recapitulates dystroglycan functions ranging from Laminin binding to Lassa Virus infection

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Functional glycosylation of α-dystroglycan is initiated with *O*-linked mannose and terminates with a repeating disaccharide $(\text{-Xyl}\alpha1,3\text{-GlcA}\beta1,3\text{-})_n$ referred to as matriglycan. Using chemoenzymatically defined lengths of matriglycan on a microarray, we demonstrate length dependent matriglycan binding to Laminin and LASV GP1 as well as to the clinically-useful IIH6 antibody. Utilizing a chemoenzymatic approach for the addition of matriglycan to complex N-linked glycans, Fetuin could be converted to a IIH6-positive Laminin-binding glycoprotein. Labeling of N-linked glycoproteins with defined lengths of matriglycan on the surface of intact cells deficient for either α-dystroglycan- or O-Mannose expression allows recovery of infection with a Lassa-pseudovirus. Finally, free matriglycan in a dose and length dependent manner inhibits infection of wildtype cells by the Lassa-pseudovirus. These results indicate that matriglycan alone is necessary and sufficient for IIH6 staining, Laminin and LASV GP1 binding, and Lassa-pseudovirus infection. These studies emphasize the functional importance of glycosylation, independent of the aglycone.

(148) Catalytic deficiency of O-GlcNAc transferase leads to X-linked intellectual disability

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The posttranscriptional modification O-linked β-linked *N*-acetylglucosamine (O-GlcNAcylation), involves the attachment of a single hydroxyl moiety onto serine and threonine of nuclear and cytoplasmic protein. Although this process is regulated by a single pair of enzymes, the O-GlcNAc transferase (OGT) and the hydrolase O-GlcNAcase (OGA), thousands of targets are modified. O-GlcNAcylation is dynamic and reversible. Changes in protein O-GlcNAcylation were shown for example in response to cellular stress, cell cycle and neuron depolarization. O-GlcNAcylation is known to be most abundant in the brain, and it has been linked to both neurodegeneration and neurodevelopment. Recently, mutations in OGT have been found to segregate in patients affected by X-linked intellectual disability (XLID). Affecting 3% of the population, XLID is a developmental disorder, mainly associated with brain development and learning. Although in the last years many mutations have been characterised in the tetratricopeptide (TPR) domain of OGT, little is known about the function of OGT in neurodevelopment and the underpinning mechanism linking OGT mutations to XLID. We have now discovered XLID mutations in the catalytic domain of OGT. Using embryonic stem cells and Drosophila models, we show that these mutations in OGT affect neurite outgrowth in differentiated stem cells.

(149) The function of Golgi alpha-mannosidase II in somatosensory dendrite patterning

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Dendrites are essential for the transmission and processing of sensory stimuli through the nervous system. Abnormalities in dendrite structure have been observed in patients with neurological disorders. We use the *C. elegans* PVD somatosensory neurons as a model to study the genetic basis of the tightly regulated mechanisms driving dendrite development. Studies from our lab and others have shown that the multifaceted arrangement of a conserved cell-adhesion complex regulates PVD dendrite branching. Deemed the menorin complex, it includes the skin-derived MNR-1/Menorin and SAX-7/L1CAM, the muscle secreted LECT-2/Chondromodulin II, and the transmembrane receptor, DMA-1/LRR-TM, in PVD. Mutations in any of these genes severely disrupt the stereotyped, candelabra-like arborization of PVD dendrites. In order to identify factors that modulate the activity of the menorin complex, we performed a forward genetic screen to isolate modifiers of a *lect-2/ChM-II* hypomorphic allele. We determined that mutations in *aman-2/Golgi alpha-mannosidase II* enhance the severity of the PVD phenotype in hypomorphic alleles of *lect-2/ChM-II* and *mnr-1/Menorin*, but do not display a mutant phenotype in an otherwise wildtype background. Moreover, exposing hypomorphic animals to swainsonine, a specific inhibitor of alpha-mannosidase II, results in a similar enhancement. *aman-2/GM-II* encodes an enzyme required for the formation of complex N-glycans, and also paucimannose N-glycans in invertebrates. We determined that AMAN-2 acts cell-autonomously in PVD, and that its enzymatic activity is essential, suggesting that N-glycosylation of a menorin complex component in PVD itself, such as DMA-1/LRR-TM, may be significant. Using endoglycosidases and Western blot analysis, we established that DMA-1/LRR-TM is glycosylated *in vivo* with primarily high-mannose/hybrid type N-glycans, and that the glycan profile of specifically DMA-1 is altered in animals lacking AMAN-2, with larger, likely mutant, N-glycan chains. We are currently using genetic analysis and selectively mutating N-glycosylation sites of DMA-1/LRR-TM to dissect whether the presence of mutant glycan chains, or the absence of wildtype glycan chains, is the potential root cause of an altered protein-protein interaction. We will further characterize the role of *aman-2/GM-II* in the binding of the menorin complex in future pull down assays to gain a fuller understanding of this highly specific function of N-glycosylation in dendrite development. Since Congenital Disorders of Glycosylation (CDG) are known to affect the nervous system, and many patients present with cognitive impairment, it is imperative to understand how glycosylation can modulate dendrite morphogenesis.

(150) REGULATION OF CLATHRIN-MEDIATED ENDOCYTOSIS BY O-LINKED ß-N-ACETYLGLUCOSAMINE MODIFICATIONS

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The function of certain proteins can be regulated through their specific covalent modification with O-linked ß-N-Acetylglucosamine (O-GlcNAc). Modification of proteins with O-GlcNAc is regulated by nutrient availability (e.g. glucose and glutamine) into the hexosamine biosynthetic pathway (HBP). O-GlcNAcylation seems to play an important role in the regulation of various processes in response to metabolic status. How O-GlcNAc modification may regulate endocytosis, and how this regulation may contribute to the maintenance of cellular homeostasis under certain cellular contexts remains poorly understood. Clathrin-mediated endocytosis is an important gatekeeper of cell surface protein composition, thus controlling proliferative signaling and nutrient uptake. We investigated the role of O-GlcNAc in the clathrin-mediated endocytosis of epidermal growth factor receptor and transferrin receptor. Using specific inhibitors to O-GlcNAc transferase and O-GlcNAc

hydrolase, 5-thio-GlcNAc, and Thiamet G respectively, we found that O-GlcNAc distinctly regulates the internalization of different receptors. Through analyzing clathrin-coated pit (CCP) dynamics in cells expressing fluorescently-labeled clathrin, we observed specific control of specific stages of CCP formation and scission by O-GlcNAc modification. Moreover, we can also identify specific CCP proteins that are modified with O-GlcNAc. We find that endocytosis is responsive to the O-GlcNAc modification downstream of the HBP. We are extending our work to study endocytosis under different cellular contexts where we manipulate nutrient availability into the HBP and key enzymes such as glutamine-fructose-6-phosphate transaminase (GFAT). Understanding how clathrin-mediated endocytosis is regulated in the context of cell metabolism and stress by dynamic O-GlcNAc modifications is fundamentally important and could aid in the development of treatments of diseases influenced by altered metabolic statuses like cancer and neurodegenerative diseases.

(151) A First-Generation Sequence Analyses for Carbohydrates

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Protocols are described that provides a precise sequence analyses for carbohydrate samples. The results are linkage and isomer specific and supported with tests on a large assortment of synthetic standards, glycan epitopes, and well characterized natural products. Most recently the focus has been to be fully comprehensive with the inclusion of all isomers. Successful results have been readily explained in the context of organic and physiochemical processes and address the precept of a First-Generation Sequence. In quadrupolar MS instruments low molecular weight bath gases provide a medium to uniformly transfer kinetic energy to trapped ion samples cycling within precise molecular weight trajectories. These increments are transferred uniformly to the total sample which independently adjusts with classical and predictable forms of rearrangement, elimination or simple bond cleavage. These energy adjustments effect the most polar and weakest bonds first proceeding to more stable parts of the molecule with subsequent MS*n* steps. Pathways of disassembly are totally dependent on the ion's inherent structure and the fidelity of disassembly is comparable to those documented in classical organic chemistry reactions. A reversion of such pathways to precursor ions yields a chemically defined sequence. These established details support the basic concept that following low energy bath gas activation, specific and selective reactions disassembly occurs as an independent physiochemical process controlled only by the ions innate structure. Six physiochemical principles complement this approach as an effective carbohydrate sequencing technology: 1.) Sample ions in trapped ion trajectories provide gas phase purification; 2.) Increments in bath gas KE exposes an alternative and unique tool for structural characterization; that of chemical instability; 3.) subsequent steps of disassembly (occurring at higher energies), provides structural detail in core, more stable areas, of a molecule; 4.) Electrospray double and triple charging extends the operating mass range well above most functional epitopes; 5.) These principles and data plots are supported by the quasi-equilibrium theory, (RRKM). Finally, and most interesting; 6.) Such steps of disassembly along with their considered reversion, represents linkage connectivity, a chemical sequence as a searchable mass spectral file. Such shared files will greatly facilitate collaborative research, a feature long missing in glycosylation studies.

(152) Tandem MS Strategies for Intact N- and O-Glycopeptide Characterization

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Intact glycopeptide characterization is an imperative, yet challenging, component to modern glycoproteome analysis. Elucidation of both glycan and peptide modalities often requires multiple tandem mass spectrometry (MS/MS) approaches to identify a single glycopeptide species. Recent efforts have focused on developing fragmentation techniques to allow for rapid elucidation of both moieties to promote high-throughput glycoproteomic investigations. A few studies have highlighted the utility of stepped-collision energy higher energy collisional dissociation (SCE-HCD) and electron transfer dissociation with supplemental HCD activation (EThcD) for large-scale glycoproteomic applications, but these methods still require further investigation to understand their utility in the glycoproteomic toolbox. Both have been used with success in large-scale glycoproteomic experiments, but they each incur some degree of compromise. Here, we systematically explore the advantages and disadvantages of SCE-HCD and EThcD for intact glycopeptide analysis and comment on their suitability for both N- and O-glycoproteomic applications. We compare standard HCD, SCE-HCD, ETD, and EThcD for both N- and O-glycopeptides generated from a panel of glycoprotein standards and glycopeptides enriched from complex HEK 293 lysates. We rely mainly on trypsin to generate glycopeptides due to its ubiquitous use in glycoproteomic workflows, but we also investigate the performance of these MS/MS methods for O-glycopeptides generated using recently described O-glycoproteases. Throughout these comparisons, we evaluate: 1) degree of peptide backbone sequence coverage, 2) degree of glycan sequence coverage, 3) proportion of signal in different fragment ion types (e.g., oxonium ions, Y-type ions, and peptide backbone fragment ions), 4) percentage

of spectra that enable confident glycosite localization, 5) percentage of spectra that contain fragments with glycans (intact or fragments) retained, and 6) proportions of total ion current that can be confidently annotated/explained in identified spectra. With this work we provide data to highlight the strengths and weaknesses of both SCE-HCD and EThcD for intact N- and O-glycopeptide analysis, commenting on which method is best suited for a given application.

(153) A New Generation of Soluble Siglecs for Probing Their Glycan Ligands on Cell Surfaces

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Sialic acid-binding immunoglobulin-type lectins (Siglecs) are a family of cell surface inhibitory receptors found on immune cells whose ability to modulate immune cell function is intimately tied to interactions with sialic acid-containing glycoconjugates via their *N*-terminal domain. Siglecs can modulate immune responses in positive or negative ways depending on sialic acid expression, which is upregulated in diseases such as cancer. This is a growing area of interest, as increased understanding of these mechanisms is needed in order to create new therapeutics. A better understanding of the glycan ligands of Siglecs is needed for elucidating how immune cells are controlled. A common approach is to use Fc-chimeric versions of Siglecs consisting of the extracellular domains of Siglecs fused to the Fc region of an antibody. This platform has advanced our knowledge of glycan ligands of Siglecs, but also has limitations, most notable of which is binding of the Fc to Fc receptors. Here, we present the development of a new versatile set of Siglec-Fc chimeras that includes more versatile features and lacks the drawbacks of finding Fc receptors, which was accomplished by introducing mutations in the Fc domain. We have also incorporated a proteolytic cleavage site between the Siglec and Fc regions to enable the production of monomeric Siglecs and a consensus sequence for enabling site-specific labeling and systematic multimerization. Through various multimerization approaches, we demonstrate that our new Siglec-Fc chimeras leverage avidity effects to enable glycan ligands on the cell surface to be detected in flow cytometry assays that were previously not capable of being detected. Optimized conditions have systematically revealed what Siglec ligands are present on human leukocytes. Through leveraging a large panel of sialyltransferase knock-out cell lines, significant progress has also been made towards identifying the types of glycan ligands identified by individual Siglecs. Moreover, through the generation of soluble Siglec fragments with homogenous glycosylation, a mass spectrometry quantitative approach to studying Siglec-ligand interactions has been validated. With the growing connection between Siglecs in cancer, we expect that these new soluble versions of Siglecs will have many uses in studying their glycans ligands, which are overexpressed on cancer cells.

(154) Hyperglycemia enhances cancer immune evasion by inducing alternative macrophage polarization through increased O-GlcNAcylation

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Diabetes mellitus (DM) significantly increases the risk for cancer initiation and cancer progression. Hyperglycemia is the most important characteristic of DM and tightly correlates with a poor prognosis in cancer patients. While enhanced availability of glucose can directly fuel tumor cell proliferation, the effect on the tumor immune microenvironment is less well known. We examined therefore the impact of hyperglycemia on anti-cancer immune responses in a well-defined mouse model. We found that an increase in glucose flux through the Hexosamine Biosynthetic Pathway (HBP) drives cancer progression and immune evasion by altering O-GlcNAcylation in tumor-associated macrophages (TAMs). TAMs were polarized to a tumor-promoting M2 phenotype inhibiting also adaptive anti-cancer immunity. Therapeutic reduction of TAMs abrogated the tumor-promoting effect of hyperglycemia. Inhibition of the HBP or O-GlcNAcylation led to a reversal of M2 polarization by hyperglycemia and inhibited cancer progression in hyperglycemic animals. Analysis of macrophage polarization in patients with DM and colorectal cancer showed an increased number of M2 polarized TAMs compared to colorectal cancer patients without DM. Our results provide a new and targetable mechanism of cancer immune evasion in hyperglycemic cancer patients, advocating for strict control of hyperglycemia in in this patient population.

(155) Interactions of the Mitogenic Cytokine Pleiotrophin with Structurally-Defined Heparin Oligosaccharides

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The glycosaminoglycan(GAG)- binding cytokine pleiotrophin (PTN) is a neuronal mitogen involved in many facets of growth, development, and repair. Perhaps its most prominent association however, lies with that of angiogenesis and growth of glioblastoma. Architecturally, PTN contains two structured b-sheet domains with basic surfaces that are connected via a short linker, and are flanked by long, flexible termini containing a high density of basic residues, allowing

it to bind GAGs with high affinities. NMR analysis of PTN-GAG binding showed basic clusters within the structured domains are perturbed the most by GAG oligosaccharides. Herein we use heparin oligosaccharides with defined structures to determine a specific binding axis through PTN. Using a labeling scheme that selectively protonates lysines in a deuterated protein, we were able to probe this binding axis through simple solution-state homonuclear NOESY NMR. Initial studies using wild type PTN revealed the flexible termini of PTN also have extensive interactions with GAGs, as only protein-GAG contacts involving those residues are visible in these experiments. Intermolecular NOEs involving the structured domains of PTN were only identified after the truncation of said termini. Current progress has yielded distinct, albeit somewhat ambiguous intermolecular contacts of both uronates and glucosamines of heparin with residues in cluster 2 of the C-terminal domain (CTD) and the linker, both are known to be highly perturbed by GAG oligosaccharides. Also evident, is the potential for GAG-induced homodimerization facilitated by contacts between the N-terminal domain (NTD) and CTD of different PTN molecules. In particular, existing NOE data provides evidence of direct contacts between lysine residues in NTD and CTD that can only be mediated by GAG-binding. Taken together, these data should provide results for the first structure of GAG-bound PTN and clues into how PTN is displayed on the glycocalyx.

(156) Encoded Sialoglycan Microarray Reveals the Differential Sialoglycan Binding Patterns of Phylogenetically-Related Bacterial Exotoxin B Subunits

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Sialic acids (Sias) are nine-carbon-backbone monosaccharides on the vertebrate glycocalyx, involved in a plethora of biological processes and displaying much diversity in modifications, linkage and underlying glycan structure. Slide microarrays allow high-throughput analysis of sialoglycan-protein interactions. As described in the related abstract, a diverse library of more than 250 structurally defined sialyltrisaccharides were chemoenzymatically synthesized with a common vertebrate Sia (Neu5Ac, Neu5Gc and Kdn, and their modified forms) at the non-reducing end. Given the size of our library, microarray data sorting and analysis posed a major challenge for high-throughput studies. We therefore devised a numerical coding system that assigns a unique 9-digit code for individual glycans. The terminal Sias and the underlying two monosaccharides are assigned from the non-reducing to the reducing end, each with three digits to assign a monosaccharide, its modifications, and linkage. We show how this simple code helps in sorting glycans in logical ways, in motif searching of the results, and even in optimizing the initial printing process.

The AB_5 family of toxins are major virulence factors for bacterial pathogens, with cytotoxicity exerted by the A-subunit, and the pentameric B-subunit responsible for binding terminal glycans on host cell surfaces. As described in the related abstract a poor correlation was found between the phylogeny of known B-subunit sequences in available genomes and bacterial species phylogeny, and a stronger correlation was seen with the sialic acid types prominent in susceptible species, when comparing the B-subunits of *Yersinia pestis* (YpeB), *E. coli* subtilase cytotoxin (SubB) and *S. Typhi* (PltB). Further supporting this hypothesis we now show that a related B-subunit (YenB) from *Yersinia enterocolitica* (another pathogen with a very broad host range) recognizes all Neu5Ac and Neu5Gc glycans in our library, including 4-*O*-acetylated Sias not recognized by YpeB. Differential Sia-binding patterns were also observed with other phylogenetically related B-subunits from *S. Typhimurium* (ArtB),*Vibrio cholerae*(CtxB), the cholera family homologue of *E. coli* (EcxB), and *E. coli* heat-labile enterotoxin (EtxB).

While we developed the coding system for the >10,000 possible linear sialyltrisaccharides, it has not escaped our notice that amplifying the calculation to a biantennary branched *N*-glycan with two terminal sialoglycan trisaccharide sequences results in squaring of the number of possibilities, giving $>10^8$ potential combinations. Considering a triantennary branched *N*-glycan with three terminal sialoglycan trisaccharide sequences there could be $>10^{12}$ potential combinations at a single *N*-glycan site on a single protein. While all these possibilities likely do not exist in nature, it is clear that sialoglycans can encode enormous diversity. We suggest that while glycomic approaches are addressing these challenges, naturally occurring probes like these toxin B-subunits can be simpler tools to study the dynamic sialome in biological systems of interest.

(157) Broadening the Landscape of ABO Typing with Multiplexed Lectins

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The discovery of the ABO blood group system occurred during the twentieth century when red blood cells (RBC) antigens were agglutinated with antibodies contained in unrelated serum samples. These antigen-antibody interactions became the foundation to understand the current blood group systems, and the same techniques are still used in the clinical setting today. Further work demonstrated altered expression of the main A, B, O, and AB blood group with group A weakened expression (e.g. A1 and A2) being the most common. Antigen subgroups can unexpectedly develop antibodies that react with blood groups of the same blood group leading to acute hemolytic transfusion reactions during a blood transfusion. Currently, no clinical assays easily predict variations and other glycosylation patterns for glycan blood group antigens. To further characterize glycan red blood cell blood groups and clinically relevant altered expression profiles, we aimed to identify unique glycan signatures attributed to the different blood groups by analyzing surface red blood cell glycans using lectin arrays.

Red blood cell glycoproteins from 80 donors (n=20 for each of the four blood groups) were analyzed using a 7-well glass lectin microarray slide with 45 unique lectins. Donors were blood typed by traditional serological blood banking methods and identified as being either type A, B, O, or AB. Red blood cells were isolated from whole blood samples by centrifugation, purified, and lysed. Membrane-bound proteins were extracted from red blood cell pellets and Cy3- labeled before incubation on the microarray to include traditional ABO blood group typing lectins. Lectin binding affinity was quantified with an evanescent-field fluorescence scanner and data was visualized by principal component analysis using R.

Resulting data showed clustering of predicted lectin ABO glycoprotein assignment compared with traditional serological assays. Interestingly, a subset of the samples did not align with their assigned serological blood type and showed distinct clustering. These outliers were then analyzed by western blotting, which confirmed the weakened or varied expression of the A and B antigens missed by serological blood typing classification. Thus, lectin microarray technology can more finely predict ABO blood groups. This is in contrast to the current simplified understand of ABO blood group expression as present or absent. The lectin microarray broadens the current understanding by delineating gradations in expression that can dictate safer blood transfusions missed by insensitive traditional methods. Future work will evaluate the glycolipid contribution to blood type and resultant changes with disease states for further mechanistic studies overlooked by serological techniques.

(158) Novel insights into the fucose metabolism

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GDP-Fucose used for glycan fucosylation is synthesized *de novo* from either glucose (Glc) or mannose (Man) or utilized directly from salvaged/exogenous fucose (Fuc). Meticulous radioisotope dilution studies performed in the 1970's in HeLa cells using 0.3 μM Fuc showed that the *de novo* pathway provides >90% of GDP-Fuc and salvage <10%.

Employing different Fuc-specific lectins, we showed that at low concentrations (<20 μM), the entry of Fuc into cells relies on transport via GLUT1 (*SLC2A1*), but above this, most Fuc enters via amiloride-sensitive macropinocytosis. Inhibitors of both clathrin- and caveolin-dependent endocytosis had no effect on its uptake. These findings were further confirmed using ^{3}HFuc.

We used differentially labelled ^{13}C Glc, Man and Fuc to study contributions of each pathway to Fuc in N-glycans in multiple cell lines. Very low concentrations (0.5-30 μM) of exogenous Fuc progressively inhibit the *de novo* pathway, and completely by ~50 μM. Surprisingly, GDP-Fuc derived from Glc is much more sensitive to this inhibition than that derived from Man, even though both contribute to GDP-Man synthesis. When cultured in 30-50 μM Fuc, cells not only inhibit the *de novo* pathway, but also prefer exogenous Fuc over the salvaged one. They seem to distinguish multiple pools of GDP-Fuc based on their origin.

Using LC-MS/MS, we studied the biosynthesis of >150 fucosylated N-glycans containing 1-4 Fuc residues by comparing the proportion of ^{13}CFuc incorporation at various concentrations for 24h. N-glycans with a single Fuc show a proportional increase in ^{13}C-labeled species with increasing concentration of label. In contrast, glycans with 2, 3, and 4 Fuc each required progressively higher exogenous ^{13}C Fuc to become fully labeled.

Based on LC-MS/MS and lectin staining, 1-6αFuc labels more efficiently with exogenous Fuc than 1-2α, 1-3α and 1-4αFuc, suggesting differential access to exogenous sugar that depends partially on Fuc linkage. K_m differences between fucosyltransferases cannot explain preferential use of GDP-Fuc synthesized from exogenous monosaccharide over *de novo* produced substrate. Rather, it suggests the existence of different, ill-defined pools of GDP-Fuc. All together it raises the question is "internally salvaged" Fuc equivalent to GLUT1-delivered vs. endocytosed Fuc? Their behavior clearly differs from *de novo* synthesized GDP-Fuc.

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(159) The prokaryotic pan-glycome: In silico identification of glycan building blocks in completely sequenced genomes

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The molecular mechanisms by which glycans mediate biological processes is dictated by their structure, which in turn is

regulated by spatio-temporal expression of genes involved in glycan biosynthesis. Identification of enzymes/pathways for the biosynthesis of glycan building blocks is a key step in this direction. A pipeline that utilizes a manually curated database of 1000+ experimentally characterized building block synthesis enzymes was developed. The NCBI prokaryotic RefSeq genome database (12000+ genomes) was mined using a combination of HMM profiles and BLASTp. UDP-GlcNAc, UDP-ManNAc, UDP-Gal*f*, dTDP-L-rhamnose, GDP-L-fucose, CMP-Neu5Ac, UDP-diNAcBac, UDP-GlcA and UDP-GalA are among those that are prevalent across different phyla and genera. Pathway for UDP-L-QuiNAc is found in 180 genomes covering different phyla and genera, and for UDP-FucNAc primarily in S. aureus, implying that these are truly rare sugars. However, no nucleotide sugar is found exclusively in all Terrabacteria. In contrast, CDP-sugars, ADP- and GDP-heptoses, UDP-L-Ara4N, UDP-Fuc4NAc and CMP-diNAcPse and CMP-diNAcLeg are primarily found in Proteobacteria, the other phylum with many sequenced genomes. It has been reported that certain Gram-negative bacteria modify LPS by L-Ara4N addition to gain resistance against cationic antimicrobial peptides. However, UDP-L-Ara4N is absent in Acinetobacter, Vibrio, Shewanella and Campylobacter, among others. This suggests that Shewanella also has evolved alternate approaches for resistance as seen in the case of Acinetobacter, Vibrio and Campylobacter. Some sugars are found only in certain genera/species e.g., CDP-3,6-dideoxy hexoses in S. enterica and Y. pestis, and ADP-heptose in E. coli, S. enterica and K. pneumoniae. CMP-diNAcPse and CMP-diNAcLeg appear to be characteristic of pathogens Campylobacter jejuni and Helicobacter pylori. Building blocks such as GDP-Man, UDP-ManNAc, dTDP-L-rhamnose, CMP-Neu5Ac, UDP-GlcA and GDP-L-fucose are also observed in members of euryarchaeota and TACK group within Archaea. Biosynthesis pathways for none of the 50+ sugars are found in endosymbionts such as Anaplasma, Ehlrichia, Chlamydia, Candidatus and Mollicutes. In contrast, ManNAc and L-QuiNAc are found in Rickettsia, also an endosymbiont. There is no correlation between genome size and presence/absence of glycan building block biosynthesis pathways. Mining genomes of viruses (encoding at least 200 proteins) showed the presence of pathways for the biosynthesis of UDP-L-rhamnose, UDP-viosamine, UDP-ManNAc and UDP-GlcA pathways in six genomes. These results can be used to interpret the correlation between the presence/absence of sugars and phenotype. In addition, these pathways can be utilised to engineer synthetic pathways for glycan synthesis for industrial applications.

(160) Development and Characterization of Sialoglycan Recognizing Probes (SGRPs) with defined specificities towards most predominant mammalian sialoglycans.

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Sialic acids (Sias) are a diverse family of monosaccharides typically found at terminal positions on dense and complex cell surfaces and secreted glycoconjugates in vertebrates. They are involved in numerous biological processes including intercellular interactions, immune responses and regulation, cellular homeostasis, microbial infections, host-pathogen interactions and autoimmunity. Inherent complexities of substitutions, modifications and linkage to underlying glycans, and inadequate techniques for detection have restricted research on Sias. Conventional glycomic methods can destroy or overlook critical Sia substitutions, and efficacy has been limited to a narrow range of sialoglycan structures. Methods for *in situ*detection and analysis of sialoglycans are largely based on plant lectins, sialidases or antibodies, and provide limited options to explore native sialoglycan conformations as they appear in a biological system. A novel set of reagents that recognize different Sia modifications or sialoglycan structures would benefit sialic acid experts and also allow researchers with no glycobiology experience to detect and track sialomes in their systems. Towards this goal we have developed a set of recombinant soluble and stably tagged Sialoglycan Recognizing Probes (SGRPs).

We have defined 9 classes of SGRPs (SGRP1-SGRP9) that each recognize one specific aspect of sialoglycan complexity. These probes can be used to track mammalian sialome changes, using techniques common in most laboratories. We utilized natural proteins such as bacterial adhesins, toxin subunits and viral hemagglutinin-esterases that demonstrate maximum specificity towards mammalian sialoglycans. We selected candidates for SGRPs, experimentally confirmed their specificity and then constructed biotinylated probes, along with corresponding non-binding molecules as internal controls of probe specificity. Binding specificities for probes were confirmed by a sialoglycan microarray displaying more than 200 types of Sia substitutions and linkages, with loss of binding of control molecules for each probe. The final optimized panel of SGRPs is being applied in common detection methods such as ELISA, Western Blotting, FACS analysis and histochemistry. Experimental procedures were optimized to minimize damage to relatively unstable Sia substitutions such as O-acetylation. Our data demonstrate a very high order of specificity of SGRPs towards their target sialoglycans and

results indicate that these probes can discriminate between small structural differences in sialoglycans. Currently, we are working to demonstrate the specificities of this comprehensive set of probes, and to track sialome changes in DSS induced colitis in wild type and CMAH deficient mice. In keeping with the mandate of the NIH Director's Common fund initiative we are also collaborating with BioLegend Inc. to ensure that SGRPs will be commercially available to interested researchers.

(161) Cryo-Electron Microscopy of O-GlcNAc cycling enzymes

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O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) control O-GlcNAcylation, a dynamic post-translational modification (PTM) targeting thousands of nuclear, cytoplasmic and mitochondrial proteins. This PTM targets serine and threonine residues and frequently results in a crosstalk with phosphorylation. The addition of N-acetylglucosamine onto target proteins is performed by OGT, and removal of this residue is catalyzed by OGA. Moreover, protein O-GlcNAcylation is sensitive to UDP-GlcNAc (uridine diphospho-N-acetylglucosamine) concentration, the sugar donor utilized by OGT. This nucleotide sugar is derived from the nutrient dependent hexosamine biosynthetic pathway (HBP) and its concentration is directly influenced by nutrient availability. O-GlcNAcylation modulates cellular homeostasis by changing its target protein activity, interaction with protein partners, localization and/or degradation. As a result, O-GlcNAcylation plays an essential role in many cell signaling pathways and deregulation of this PTM has been linked to insulin resistance, diabetes, cancer, lupus and neurodegenerative diseases. Despite major advances in the field, details of how OGA and OGT interact with and recognize diverse protein substrates is poorly understood. The lack of a detailed protein structure has been a major barrier in investigating OGT and OGA molecular mechanisms. Domains of the O-GlcNAc cycling enzymes have been solved by X-ray crystallography, however, the structure of the intact proteins remains unknown. Here, we optimized a protocol for structural analysis of OGT (110 kDa) and OGA (103kDa) by cryo- Electron Microscopy (cryo-EM). Cryo-EM has emerged as the leading method for determining structure of macromolecules. We have optimized protein purification, particle concentration and freezing parameters. With this analysis we were able to configure a 2D classification of OGA and OGT and we reconstructed a 3D model of OGA which contains compiled catalytic and stalk domain (7Å). The data obtained to date indicates that OGA forms a stable homodimer and our cryo-EM model strongly supports the three partial crystal structures of OGA. Our results suggest that the pseudo-HAT domain of OGA is very flexible, which increases conformational heterogeneity of our samples. Our ongoing experiments focuses on solving the 3D structure of the full length OGA and OGT using chemical crosslinkers, domain replacement, or a substrate peptide to stabilize the flexible region of the proteins.

(162) Therapeutic potential of N-acetylglucosamine as a mitigating treatment for Duchesne Muscular Dystrophy (DMD)

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Duchesne Muscular Dystrophy (DMD) is the most common form of childhood muscular dystrophy and affects about 1 in every 5000 male births with 90% of them forced to use a wheelchair before the age of 15. DMD is a X-linked recessive genetic disease caused by the absence of the dystrophin protein, a key player in the stable yet flexible interaction between muscle fibers and laminin in the cellular matrix. This interaction is critical to protect muscle fibers against damages caused by repeated contractions. In DMD patients, even moderate repeated contractions cause damage, leading to muscle degeneration. The damaged muscles are repaired through myogenesis. Consequently, myogenesis is highly active in DMD patients, and this repeated activation leads to the exhaustion of the myogenic stem cells. A treatment that strengthens the interaction between the muscle fibers and laminin, and that increases the efficiency of myogenesis would reduce this risk of exhaustion.

Galectin-3 (gal-3) is a soluble mammalian lectin that was originally found as a laminin-binding protein. Gal-3 can act as a cell adhesion molecule and modifier of membrane protein dynamics. It is one of the most highly expressed proteins in both myoblasts (differentiating muscle cells) and DMD skeletal muscles while its function(s) in the muscular system have never been investigated.

We first investigated the role of gal-3 in myogenesis, where myoblasts fuse to each other to form myotubes. As low as 0.2μM gal-3 increased the myogenesis of a myoblast cell line (C2C12). Furthermore, myogenesis was impaired in primary myoblasts of gal-3 null mice. Importantly gal-3 supplemen-

tation overcame this deficiency; suggesting the importance of extracellular gal-3 in myogenesis. It has been well established that the biosynthesis of gal-3 ligands is suboptimal due to the low concentration of UDP-N-acetylglucosamine (GlcNAc) in the Golgi apparatus. By increasing extracellular concentration of GlcNAc, it is possible to increase the biosynthesis of gal-3 ligands. Thus, we investigated whether GlcNAc instead of gal-3 can increase myogenesis. Interestingly, as low as 1mM GlcNAc increased the myogenesis of both C2C12 myoblasts and dystrophin-deficient primary myoblasts.

In vivo, we found that lack of gal-3 impaired muscle force production. When *mdx* mice (a DMD mouse model which lack the expression of dystrophin) were treated with GlcNAc for 10 days, the force production of muscles was increased and muscle damage was less severe.

Together, these results indicate an interesting potential of GlcNAc as a therapy to mitigate the progression of DMD. While gene and stem cell therapy approaches for DMD have been slowly advancing, the patients likely have to wait for another decade to reach to those expensive therapies. Thus, it is critical to develop alternative pharmaceutical approaches and we believe that some approaches are glycobiological therapies, such as GlcNAc presented in this study.

(163) Multistage enrichment strategy for sensitive and unambiguous detection of unnatural glycans by both glycoproteomics and glycomics

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Multistage enrichment strategyfor sensitive and unambiguous detection of unnatural glycans by both glycoproteomics and glycomics

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Glycosylation is one of the most important post-translational modification (PTM) of proteins and lipids that occurs in both prokaryotes and eukaryotes and is highly microheterogeneous in nature. Detailed structural characterization is required to understand the role of glycans in various biological processes as well as their potential as disease related biomarkers. Metabolic engineering of glycans is being used by researchers to understand glycome and glycoproteome in the living cells. This process takes advantage of promiscuity of glycosylation machinery of the cells allowing incorporation of unnatural glycans into the cellular biosynthetic machinery. The cell surface labeling and enrichment of glycans can be achieved by treating the cells with unnatural glycan precursors, such as per-*O*-acetylated *N*-azidoacetyl-D-mannosamine (Ac_4ManNAz), per-*O*-acetylated *N*-azidoacetyl-D-glucosamine (Ac_4GlcNAz) per-*O*-acetylated *N*-azidoacetyl-D-galactosamine (Ac_4GalNAz). For detection of these modified unnatural sugars on the glycoproteins, chemical tag-based bioorthogonal techniques such as Cu-assisted as well as Cu-free strain promoted azide–alkyne cycloaddition and Staudinger ligation reactions are the preferred methods. However, the major drawback of these chemical tag based techniques is non-specific background reactions with other highly reactive moieties from the cellular environment leading to ambiguous results. To overcome these limitations, we have developed a tag-free multistage enrichment strategy for both glycoproteomics and glycomics analysis through mass spectrometry (MS) comprised of cellular fractionation, ZIC-HILIC purification and simultaneous conversion of azido functional group to amine functional group. By using this approach, we were able to successfully detect, complex and hybrid types of *N*-linked and core-1 type sialylated *O*-linked with or without unnatural azido sugars as well as natural high mannose type of structures without azido modification on the glycopeptides extracted from PC-3, MCF-7, and Jurkat cells treated with unnatural sugar precursors. This strategy has unraveled new facts about dynamic conversion of modified unnatural sugars in the living cells. In summary, we developed a highly sensitive and efficient method for the detection of metabolically engineered unnatural glycans by MS that can help the discovery of novel disease related biomarkers as well as better understanding of roles of carbohydrates in biological pathways.

(164) Significance of structurally diverse elongation of O-glucose glycans on Notch1 and Notch2

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Biochemical and genetic studies have indicated that *O*-glycosylation such as *O*-glucose, fucose, and GlcNAc is critical for proper Notch signaling; however, it is not fully understood how *O*-glycans regulate Notch receptor functions. To address this question, it is necessary to reveal the actual modifications of *O*-glycans on Notch receptors. Notch receptors are Type-I transmembrane proteins with a large extracellular domain (ECD) that contains 29-36 epidermal growth factor-like (EGF) repeats. Each EGF repeat has a different amino acid sequence, but all have a similar fold with three disulfide bonds via six conserved cysteine residues in a specific pattern. Each *O*-glycan can be attached to distinct serine or threonine residues within their own consensus sequences and be extended to different

structures. Therefore, overall structures of Notch receptors modified with the different types of *O*-glycosylation can be tremendously diverse in structure. Currently, it is not clear to what extent Notch receptors are structurally diverse and how spatiotemporal dynamics of Notch *O*-glycosylation is regulated. Here, we analyzed *O*-glucose glycan structures on Notch1 and Notch2 expressed in HEK293T cells by Orbitrap Fusion mass spectrometer, and successfully revealed the structures and stoichiometries on 15 EGF repeats among 17 EGF repeats with the *O*-glucose consensus sequence of Notch1, and 13 EGF repeats among 17 EGF repeats with the consensus sequence of Notch2. The high levels of *O*-glucose attachment and xylosyl-elongation were detected on the most EGF repeats of Notch1 and Notch2. Unexpectedly, there was previously unreported hexosyl-elongation of *O*-glycans at specific EGF repeats on Notch1 and Notch2. The levels of the hexosyl-elongation on Notch1 and Notch2 increased when both glucoside xylosyltransferases, *GXYLT1* and *GXYLT2*, responsible for xylosyl-elongation were genetically deleted in HEK293T cells, suggesting biosynthetic competition between xylosyl- and hexosyl-elongation on the specific EGF repeats on Notch1 and Notch2. Endogenous expression of Notch1 and Notch2 on the cell surface in HEK293T cells did not change while the cell-surface expression of overexpressed Notch1 and Notch2 decreased in *GXYLT1* and *GXYLT2* double knockout cells compared to that in wild type cells. Consistently, *in vitro* secretion assays showed the secretion defects of both ECDs in *GXYLT1* and *GXYLT2* double knockout cells compared to wild type cells, suggesting a significant role of elongation of *O*-glucose glycans on the Notch ECDs in quality control of Notch receptors. Furthermore, recombinant DLL4 ligand-binding to *GXYLT1* and *GXYLT2* double knockout cells decreased compared to that to wild type cells, suggesting the modulation of Notch receptor-ligand interaction by *O*-glucose elongation. Currently, we are investigating the role of the *O*-glucose elongation pathways in the aspect of Notch receptor activation. Supported in part by JSPS KAKENHI 17H06743 and 19H03176 to H.T.

(165) Antibody-mucinase conjugates for degradation of cancer-related mucins

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Transmembrane mucins are overexpressed in a wide variety of human malignancies and are linked to cell growth, survival, and metastasis. Despite their importance, their size (1-10 MDa) and dense O-glycosylation (around 80% carbohydrate by mass) have limited the study of mucins via traditional methods like enzymatic cleavage, antibodies, and small molecule ligands. Mucin domain-specific proteases such as StcE, a zinc metalloprotease secreted by enterohemorrhagic E. coli, make the study of mucins more tractable and create enticing opportunities for cancer therapy. We have previously shown that StcE degrades mucins – including oncogenic MUC1 and MUC16 – in cell culture and does not degrade globular proteins, such as bovine serum albumin, or non-mucin glycoproteins, such as fetuin. Despite these promising results, it is unlikely that StcE can be systemically administered in an untargeted way, because mucins play integral roles in mucus and the glycocalyx of healthy epithelial cells. Targeting StcE directly to the tumor site will be essential to its development as a potential cancer therapeutic. Antibody-enzyme conjugates have emerged as a strategy to target glycocalyx-modifying enzymes to cells of interest. In order to minimize off-target activity, the antibody-enzyme conjugate specificity must be driven by the antibody-substrate affinity, not by the enzyme-substrate affinity. Here we report the structure-guided site-directed mutagenesis of StcE that, both *in vitro* and *in cellulo*, reduces its activity to the desired levels while maintaining its mucin specificity. We also show progress towards the generation of antibody-StcE conjugates using aldehyde tagging, hydrazino-iso-Pictet Spengler ligation, and Cu-free click chemistry, representing proof-of-concept for the development of antibody-mucinase conjugates.

(166) Bacterial glycoengineering for the synthesis of a potential cancer vaccine glycoepitope

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The recent discovery of a cytoplasmic pathway for N-linked protein glycosylation in *Actinobacillus pleuropneumoniae* combined with its functional transfer into *Escherichia coli* marked the beginning of a new era in the engineering of glycoproteins in prokaryotes (1). The central enzyme, known as N-glycosyltransferase (NGT), modifies distinct asparagine residues with a single β-linked glucose within the consensus sequon Asn-X-Ser/Thr (X $\neq$ Pro) (2). Artificial biosynthetic pathways for site-specific glycosylation of proteins are developed by combining NGT-based glucosylation with additional bacterial glycosyltransferases from various sources.

In this project we aim to synthesize globopentaose (Gb_5), a tumor-associated glycan antigen found on the cell surfaces of at least 15 different cancer types (3). The pentasaccharide has the following structure Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc. The designed biosynthetic pathway starts from N-linked lactose, which is provided by the combined actions of the NGT and a β1,4-galactosyltransferase (LgtB) from *Neisseria meningitidis* (4). The lactose structure can be further elongated with a single α1,4-linked galactose residue using LgtC from *Haemophilus influenza*. To finalize the total enzymatic synthesis of Gb_5, LgtD from

H. influenza is added. LgtD has double specificity, first as a β1,3-N-acetylgalactosaminyltransferase, and second, as a β1,3-galactosyltransferase when Gb_4 serves as the acceptor substrate.

The artificial synthesis pathway for the enzymatic assembly of Gb_5 will be combined with a multivalent protein carrier for the production of a cancer vaccine candidate within the cytoplasm of *E. coli*. This structure will be tested for the production of a long lasting and high-affinity anti-glycan antibody response.

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(167) An Approach to Determine the True Degree of Polymerization of Highly Unstable Polysialic Acids

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It is more than 35 years since the discovery that polysialic acid (polySia, a homopolymer of α2-8-linked sialic acid residues) is selectively added to N-glycans on the Neural Cell Adhesion Molecule (NCAM), and later shown to be downregulated after embryonic development, but retained in neurogenic niches in adult brains. PolySia forms a highly hydrated structure on the cell surface with a steric effect that directly affects cell-cell contacts and interactions of cell surface receptors and matrix components ("repulsive field"), while also sequestering and concentrating soluble ligands like FGF2 and BDNF ("attractive field"), enabling critical modulatory roles in many neural processes. PolySia-N-glycan structures depend on expression and activity of two enzymes ST8Sia-II and ST8Sia-IV, which act either independently or cooperatively. While the degree of polymerization (DP) clearly affects the biological function, how to accurately characterize DP is not clear. The popular reagent DMB selectively labels sialic acids, producing a covalent derivative that can be separated by HPLC with a quantitative fluorescence readout, an approach usually applied to glycosidically-bound monomeric sialic acids after release under acidic conditions. Polysialic acid chains with exposed reducing termini can also be derivatized with DMB, but applications to biological samples of polySia-glycoproteins first requires release of polySia chains from the underlying glycan. To date, use of acid hydrolysis to accomplish this release has complicated by the high instability of the internal α2-8-linkages of polySia, which undergo spontaneous hydrolysis of C2 glycosidic linkages, likely self-catalyzed by the adjacent C1 carboxylate in a temperature and pH dependent manner. PolySia chains of DP>400 in embryonic brain were earlier shown by enzymatic release using endo-β-galactosidase, but this enzyme only releases the rare polySia chains linked via a poly-N-acetyllactosamine motif. In contrast, standard analysis using acid hydrolysis release and DMB derivatization under acidic conditions typically yields polySia fragments of DP ~30-50. We have developed an approach to protect internal α2-8sia linkages of long polySia chains during acid hydrolysis conditions and subsequent DMB derivatization by initially using ice cold-acidic conditions wherein the C9 hydroxyl group of each sialic acid is known to undergoes lactonization with the carboxylate of the adjacent sialic acid to form a stable 6-carbon ring structure. This polylactonized molecule remains stable under acid hydrolysis release and during DMB derivatization under acidic conditions, potentially unlocking a novel method to more accurately analyze DP of polySia structures in biological samples—by enabling acid release of lactonized chains followed by base hydrolysis to break the lactones just before HPLC analysis.

(168) Rapid Evolution of Bacterial Exotoxin B Subunits Independent of A subunits: Sialic Acid Binding Preferences Correlate with Host Range and Intrinsic Toxicity

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Rapid Evolution of Bacterial Exotoxin B Subunits Independent of A subunits: Sialic Acid Binding Preferences Correlate with Host Range and Intrinsic Toxicity

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Several well-known AB bacterial toxins have cytotoxic A subunits that are delivered to the cytosol, following initial B subunit binding to terminal sialic acid-presenting glycans on host cell surfaces. We find that phylogenetic analysis of known and predicted B subunits in available genomes showed a poor correlation not only with associated A subunits, but even with that of the bacterial species phylogeny, suggesting ongoing combinatorial lateral gene transfer in different lineages. In some instances, such as an exported protein of *Yersinia pestis* (YpeB) we also noted that there appears to be no associated A subunit encoded in the genome. *Y. pestis* is the etiologic agent of Plague, responsible for major devastating epidemics in human history and is of global importance to public health and biodefense. Plague can never be eradicated because of the adaptability of the organism to infect more than 200 known host species. We noted that YpeB shares 58% identity/79% similarity with the homopentameric B subunit of Subtilase cytotoxin, an AB_5 toxin of *E. coli*, which also has 48% identity/68% similarity with that of the A_2B_5 *S. Typhi* typhoid toxin. We previously studied binding of such toxin B_5 pentamers to a sialoglycan microarray and showed selective binding to different kinds of sialic acids that happen to be enriched in corresponding host cells, such as *N*-acetylneuraminic acid (Neu5Ac; dominant in humans) or *N*-glycolylneuraminic acid (Neu5Gc; prominent in ruminant mammals and rodents). Consistent with the much broader host range of *Y. pestis,* we have now found that YpeB binds to essentially all types of sialic acids studied including Neu5Ac, Neu5Gc and their modified versions. The only exception appears to be 4-O-acetylated sialic acid-containing glycans, to which YpeB does not bind, and which are relatively enriched in guinea pigs and horses, species that may be relatively resistant to serious infection. Notably, we find that YpeB toxin alone can cause cytotoxicity that is abolished by mutations eliminating sialic acid recognition, suggesting that both uncontrolled cell proliferation and cell death might be mediated simply via lectin-like cross-linking of cell surface glycoprotein receptors. These findings may help explain the wide host range of *Y. pestis* and could be important for understanding pathogenic mechanisms. Taken together, the data suggest evolutionary Red Queen effects that are driving ongoing rapid evolution of both host sialic acids and pathogen toxin binding properties. Further studies of this clade of B_5 toxins are revealing additional correlations of sialic acid binding preferences with host range and intrinsic toxicity, and molecular modeling studies are underway to define amino acid residues that determine binding differences.

(169) Production of sialylated O-glycans on therapeutic proteins in E. coli

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As glycosylation is a crucial property of many therapeutic proteins and can extend their circulation half-life, it is beneficial to add these glycans during the production of these proteins. As an alternative to costly mammalian cell systems that effect correct glycosylation, we are developing a synthetic biology approach to engineer an *E. coli* strain that will produce sialylated *O*-linked sugars on recombinant glycoproteins. This will be accomplished by introducing an *O*-glycosylation operon into *E. coli*, encoding various eukaryotic and bacterial glycosyltransferases, and accessory enzymes involved in donor substrate synthesis.

In previous studies, we have shown the successful addition of T-antigen (Gal-β1,3-GalNAc-α-O-Ser/Thr) on recombinantly expressed Interferon α2b and human growth hormone using a dual plasmid approach; one encoding the target protein and a second encoding the O-glycosylation machinery. We are now exploring the potential of modifying the *O*-glycosylation operon to elaborate sialylated core 1 structures. Towards this aim, we have engineered our host *E. coli* strain to produce the CMP-Neu5Ac donor and constructed O-glycosylation operons incorporating α-2,3-sialyltransferase and α-2,6-sialyltransferase to produce disialyl-core 1 modified proteins.

(170) Dissecting dendritic cell sialic acid-mediated interactions in antitumor immunity

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The immune system is vital for the antitumor response, as it has the capability to recognize and eliminate transformed tumor cells. However, tumor cells can manipulate multiple pathways for their survival, and escape host immunesurveillance. Upregulation of surface molecules to target inhibitory receptors on immune cells such as PD-1/PD-L1 axis can lead to tumor immune escape. Siglec receptors are a family of surface molecules, which bind to sialylated glycans (sialoglycans). They are found widely spread on cells of the immune system in mice and humans. Most of these Siglec receptors can also transmit inhibitory signals to dampen immune cell activation. Previous work in our group showed that human Siglec-9 and its functional paralog mouse Siglec-E can dampen immune responses to tumor cells via inhibition

of neutrophils and T cells [1,2]. Here, we are trying to characterize the expression and function of Siglec receptors on different subsets of dendritic cells(DCs) in cancer patients and mouse models and study their function. Using preclinical mouse tumor models, we observed an increase of DCs expressing Siglec-E during tumor progression. This was most pronounced in type 2 conventional DC type 2(cDC2s), which represents the dominant intratumoral DC subset. Further studies include functional analysis of genetic mouse models including DC-specific overexpression of human Siglec-9 and conditional deletion of Siglec-E in different cDC subsets. In addition, we are studying the function of inhibitory Siglec receptors in murine and human DCs by employing Siglec-blocking antibodies. Our analysis will provide important information on how manipulation of the sialoglycan-Siglec pathway could improve antitumor immunity.

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(171) Characterization of the type 3 Streptococcus pneumoniae capsule degrading glycoside hydrolase

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Paenibacillus sp. 32352 is a soil-dwelling bacterium capable of producing an enzyme, Pn3Pase, that degrades the capsular polysaccharide of type 3 *Streptococcus pneumoniae* (Pn3P). Recent reports on Pn3Pase have demonstrated its initial characterization and potential for protection against highly virulent type 3 pneumococcus infections. Our initial experiments revealed this enzyme functions as an exo-b1,4-glucuronidase cleaving the b(1,4) linkage between glucuronic acid and glucose. However, mechanism of action for this enzyme is still unknown. Here, we set out to determine the catalytic residues and domains of this protein. Pn3Pase shows no significant sequence similarity to known glycoside hydrolase families, the closest family being GH39 with alignments showing 12% identity within the glycoside hydrolase superfamily domain. This suggests that Pn3Pase belongs to a new carbohydrate-active enzyme (CAZy) glycoside hydrolase family. Site directed mutagenesis studies revealed two catalytic residues along with truncation mutants defining essential domains for function. Pn3Pase and mutants were screened for substrate binding specificity and impact of substrate size on binding and kinetics. This study will determine the Pn3Pase activity at the structural and mechanistic level to establish a new CAZy glycoside hydrolase family with Pn3Pase being its first member. This study will also serves towards generating Pn3Pase derivatives with optimal activity and pharmacokinetics aiding in the use of Pn3Pase as an alternative therapeutic approach against type 3 *S. pneumoniae* infections.

(172) Skp1 isoforms are differentially modified by a dual function prolyl 4-hydroxylase/N-acetylglucosaminyltransferase in a plant pathogen

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Skp1 is hydroxylated by an O_2-dependent prolyl hydroxylase (PhyA) that contributes to O_2-sensing in the social amoeba *Dictyostelium* and the mammalian pathogen *Toxoplasma gondii*. Hydroxylated Skp1 (HO-Skp1) is subject to glycosylation and the resulting pentasaccharide affects Skp1 conformation in a way that influences association of Skp1 with F-box proteins, and potentially the assembly of E3(SCF) ubiquitin ligase complexes that mediate the poly-ubiquitination of target proteins that are degraded in the 26S-proteasome. To investigate the generality and specificity of these modifications, we analyzed proteins from the oomycete *Pythium ultimum*, an important crop plant pathogen. Genomic sequences postulated to encode *Pythium*'s putative PhyA and first glycosyltransferase in the predicted five-enzyme pathway, a cytoplasmic αGlcNAc-transferase (Gnt1-), predict a bifunctional enzyme (Phgt) that, when expressed in *Dictyostelium*, rescued knockouts of *phyA* but not *gnt1*. Though recombinant Phgt was also unable to glycosylate *Dictyostelium* HO-Skp1, it could hydrolyze UDP-GlcNAc and modify a synthetic hydroxypeptide from *Dictyostelium* Skp1, suggesting it was a true GlcNAc-transferase as predicted. *Pythium* encodes two highly similar Skp1 isoforms, but only *Pythium* Skp1A was efficiently modified *in vitro*. While kinetic analysis revealed no evidence for processive processing of Skp1, the physical linkage of the two activities implies dedication to Skp1 *in vivo*. These findings i) indicate a widespread occurrence of the Skp1 modification pathway across protist phylogeny, ii) suggest that both Gnt1 and PhyA are specific for Skp1, and iii) indicate that the second Skp1 provides a bypass mechanism for O_2-regulation in *Pythium* and other protists that conserve this gene.

(173) Investigating the functions of endogenous neuraminidases Neu1 and Neu3 in blood cell and protein homeostasis

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Neuraminidases cleave terminal sialic acid linkages on the glycans of glycoproteins and glycolipids. In some cases this exposes underlying galactose linkages that can be recognized by lectins including the endocytic hepatic Ashwell-Morell receptor that thereby modulate protein function through endocytic clearance. Neuraminidases are widely distributed in vertebrates and microorganisms, although the neuraminidases of mammalian origin seems to differ from the microbial variants in genetic sequences and enzymatic properties. Four mammalian neuraminidases, encoded by *NEU1-4*, have been identified and characterized. Mice deficient in the lysosomal resident Neu1 have been reported with molecular features resembling sialidosis, a severe human lysosomal storage disorder, whilst Neu3 deficiency does not result in an obvious physiological phenotype. Neu1 is found in a complex with β-galactosidase and protective protein/cathepsin A in the lysosome. The subcellular localization of Neu3 has been suggested to be the plasma membrane and endosomal compartments. Interestingly, the membrane attachment mechanism and topology is yet to be fully clarified, however Neu3 is commonly reported to be membrane-associated. A variety of mammalian cell types express neuraminidases, including endothelial cells as well as hematopoietic lineages spanning platelets, monocytes, and the erythrocyte surface. Both Neu1 and Neu3 have been found in the bloodstream and in extracellular compartments, by one or more secretion mechanisms that are not fully defined. We have previously noted that circulating neuraminidase activity is measurable in normal mammalian blood at physiological pH, and desialylate plasma proteins with increasing molecular age, and this was further demonstrated to result in lectin-mediated clearance in the determinations of glycoprotein half-lives. To further investigate the roles of each of these mammalian neuraminidases, we are using multiple approaches in Neu1- and Neu3-deficient mice, including measurements with novel lectins that detect different types of sialic acid linkages and their modifications, among the endothelium and the surfaces of hematopoietic cells in parallel with comparative blood proteomics, flow cytometry, and other molecular identification strategies. These studies will be presented with our current findings that may provide further information regarding mammalian Neu activation and function in glycoprotein homeostasis.

(174) Expanding the toolkit for studying polysialic acid reveals polysialylated proteins in unexpected places

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Polysialic acid (polySia) is a long homopolymer consisting of α2,8-linked sialic acid with tightly regulated expression in humans. In healthy adults, it occurs on cell surface glycoproteins in neuronal and immune tissues, where it imparts a migratory phenotype that is important for axon formation, synaptogenesis and immune system function. PolySia is also present in many cancers and its overexpression correlates with significantly increased metastasis and poor prognosis. However, current methods for detection of polySia and identification of polysialylated proteins are hampered by the large, anionic and acid-sensitive nature of polySia. We developed a robust method for purifying polysialylated proteins that uses a metabolic labelling strategy to introduce a bioorthogonal functionality into polySia, which resulted in the identification of seven previously unknown polysialylated proteins in the MCF-7 breast cancer cell line. To validate these proteins, we developed an improved sandwich ELISA that allows for the relative quantification of polySia as well as specific polysialylated proteins in complex mixtures without any pretreatment or harsh conditions. The ELISA was used to validate the presence of polysialylated NCAM in human and fetal bovine serum as well as in a wide variety of cancer cells lines. These methods substantially improve our ability to investigate the biology of polysialic acid.

(175) Insights into A Novel Molecular Based Recognition of 6'-sulfo sLeX

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The binding of sialylated glycans to selectins and siglecs mediates inflammatory responses. While the selectins prefer to bind to sialyl Lewis X (sLe^x), the siglec ligands show a wider diversity in glycan motifs. Many siglecs either do not tolerate the presence of fucose in sLex or prefer to have a 6-O-sulfate moiety in the epitope. In contrast, fucose is essential for selectin binding, while the presence of 6-O-sulfation abrogates binding. Elucidating the molecular mechanisms for their binding specificities is crucial for understanding their biological functions and specificities.

Here we used molecular modeling to design a double mutant of E-selectin (E92A&E107A) that when expressed showed binding preference for 6'-sulfo sLe^x that were indistinguishable from that displayed by siglec-8. The result confirmed the theoretical prediction that the aversion of endogenous E-selectin for 6'-sulfo sLe^x arises from strong electrostatic repulsions between the conserved acidic residues in

the binding site and the sulfate group at the galactose 6-O position.

Structural analyses of the E-selectin/siglec-8 – 6'-sulfo sLe^x complexes indicated that the glycan ligand maintains the same conformation in each, but forms completely unique set of protein-glycan interactions in each system. Therefore, a new binding scheme for 6'-sulfo sLe^x was discovered in E-selectin, which is different from that in siglec-8. The results indicate that identical specificities may be displayed through completely different binding motifs, and indicate a role for computational studies in the design of high-specificity carbohydrate-binding proteins.

(176) Detecting substrate glycans of fucosyltransferases on glycoproteins with fluorescent fucose

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Fucosylation and sialylation are common terminal modifications on various glycans and play important biological roles in cellular communication, cell migration, and cancer metastasis. Detecting the substrate glycans of fucosyltransferases is important for understanding how they are regulated in response to different growth conditions and external stimuli. Previously, we described direct fluorescent glycan labeling (DFGL) using enzymatic incorporation of fluorescent sialic acids (*Glycobiology*, cwz058, https://doi.org/10.1093/glycob/cwz058). Here we report the extension of our work on DFGL to fluorescent fucoses using recombinant fucosyltransferases. Specifically, we describe the detection of substrate glycans of FUT8 on therapeutic antibodies. We further describe the detection of high mannose glycans on glycoproteins by enzymatic conversion of high mannose glycans to the substrate glycans of FUT8. By establishing a series of precursor glycans, we demonstrate the substrate specificities of FUT8. Finally, using enzymatic incorporation of both fluorescent sialic acids and fluorescent fucoses, we demonstrate the interplay between fucosylation and sialylation. The two labeling strategies are similar in principle but complementary in applications.

(177) Characterization of Erythropoietic Activity and In Vivo Neuroprotective Effects of Plant-produced Asialo-rhuEPO

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Mammalian cell-produced recombinant human erythropoietin ($rhuEPO^M$) best known for its use in treatment of anemia, has also been shown to protect various organs from diverse injuries. However, the adverse effects such as thrombosis and hypertension associated with its erythropoietic activity have prevented its use for cell/tissue-protective purposes. Asialo-rhuEPO, a desialylated form of rhuEPO, has been proven to protect various organs from diverse injuries. However, its protective effects could not be translated into clinical practice because it is not available in large quantities for research and clinical studies. We took advantage of lack of sialylation capacity in plants to produce asialo-rhuEPO by co-expressing human *EPO* and *GalT* encoding β1,4-galactosyltransferase in tobacco plants. Our previous *in vitro* cytoprotection assays showed that the plant-produced asialo-rhuEPO (asialo-$rhuEPO^P$) displays ~2-fold better cytoprotection to neuronal-like cells (Kittur et al., PLoS One, 2013), pancreatic β-cells (Arthur et al., Front. Pharmacol., 2017) and cardiomyocytes (Kittur et al., Biochem. Biophys. Rep., 2019) than $rhuEPO^M$. In this study, we confirmed that asialo-$rhuEPO^P$ lacked erythropoietic activity by repeated parental administration in BALB/c mice. Using a mouse model of middle cerebral artery occlusion (MCAO) ischemia/reperfusion (I/R) injury, we assessed neuroprotective effects of asialo-$rhuEPO^P$ by determining neurological deficits, brain edema and infraction volume. The asialo-$rhuEPO^P$ (44 μg/kg body weight, i.v.) was injected at 1 h after infarction, and TTC staining was performed 24 hours after reperfusion. The same dose of $rhuEPO^M$ was used as a positive control. Asialo-$rhuEPO^P$ displayed 52.2% neuroprotection by reducing infarction volume (15.9%) compared to saline-treated mice (33.2%), and was similar to $rhuEPO^M$ (15.4% infarction volume, 53.7% neuroprotection). Furthermore, asialo-$rhuEPO^P$ dramatically reduced neurological deficits and edema. Currently, we are working on neuroprotective mechanism(s) of asialo-$rhuEPO^P$. Our results indicate that asialo-$rhuEPO^P$ lacking erythropoietic activity could be a promising neuroprotective agent, which can be expected to be used for a pilot clinical trial in the near future.

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(178) GlycoNAVI: Three-Dimensional Structure and Heterogeneity of Glycans in Glycoprotein

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We will be reporting a database on the three-dimensional structure and abundance ratios of glycan structures of glycoproteins. In terms of 3D-structure, we have developed software tools to analyze and store these data from the Protein Data Bank (PDB). The 3D structure of glycans and modification sites have been analyzed and extracted from input data using this tool. After converting the extracted glycan structures into WURCS, the glycan structures were

assigned accession numbers of the international glycan structure repository GlyTouCan.

Next, the abundance ratio of glycans were examined. Glycoprotein glycans are heterogeneous and glycans of different structure are known to modify proteins at the same modification site. These data on glycoproteins and glycans are stored as extracted from the literature.

We developed a database that associates the 3D structure of glycans with abundance and disease information. These data are available from the GlycoNAVI website (https://glyconavi.org). In addition, GlycoNAVI is a resource of the Glycoscience Portal (GlyCosmos: https://glycosmos.org), developed with the support of JST / NBDC.

(179) GlycoSense^{TM}: A flow cytometry-based technology for rapid and simplified glycan profiling

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Protein glycosylation is a non-templated co-/post-translational modification in which the glycans can differ greatly depending on monosaccharide composition, linkage types, and branching. Analysis of glycans on both pharmaceutical biologics and glycoproteins of research interest is typically outsourced to dedicated core facilities, and is mass spectrometry- or HPLC-based. The backlog at such facilities and the expertise and expense required are serious hindrances to high-throughput glycan analysis.

The GlycoSense^{TM} method uses spectrally unique microspheres coupled with different glycan recognition molecules to provide a fast, simplified analysis of glycosylation using flow cytometry. The glycoprotein of interest, or its secondary detection reagent, is labeled with a 488 nm excitable tag (FITC, Alexa Fluor 488, DyLight 488, GFP) and incubated with the GlycoSense^{TM} multiplex bead set. The beads are resolved using the red fluorescence (633-640 nm excited) channel, and the binding of the glycoprotein analyte to each bead is measured in the green fluorescence channel. This method can be used on many commonly available flow cytometers, does not require glycoprotein pre-purification or enzymatic release of the glycans from the protein, and benefits from minimal or no wash steps.

The GlycoSense^{TM} method is not intended to replace full characterization by traditional techniques, rather its strength is in providing a quick assessment of glycosylation features that can be used to measure changes in glycan composition, such as occur during glycoprotein production or during *in vitro* glycoengineering reactions in which glycans are modified by enzyme treatment. Here we demonstrate the use of the GlycoSense^{TM} method to monitor the glycoengineering of a model glycoprotein, as well as detecting differences between well-characterized glycoproteins.

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(180) Bioluminescent biochemical and cell-based assays for glycosylation studies

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Monitoring cellular signaling events can help better understand cell behavior in health and disease states. Traditional assays to study glycosylation can be tedious, require multiple washing steps, and not easily adaptable to HTS. Here we describe application of bioluminescent approaches to study Glycosyltransferase enzyme activities using biochemical assays and to measure glycosylation using a novel cell-based immunoassay. These bioluminescent assays take less than two hours to complete in a homogeneous "Add and Read" format. This presentation will show how cellular mechanisms driven by PTM enzymes in general and glcnacylation in particular can be addressed by analyzing these enzyme's cellular activity using NanoLuc luciferase based immunoassays. We will also discuss how the bioluminescent nucleotide assays that detect UDP, GDP, UMP and CMP as means to measure glycosyltransferase activities can streamline studies on GT specificity of transfer of different sugars to different acceptors. Development of these bioluminescent detection assays will enable the investigation of key signaling pathways and the study of a large number of PTM enzymes in general and GTs in particular, which may have significant impact on diverse areas of glycobiology research.

(181) Peptidoglycan fragment microarray platform for human immune system investigation

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The interactions between the human immune system and bacteria are essential for numerous diseases, such as Crohn's disease and Lyme arthritis. The human immune system utilizes pieces of the bacterial cell wall, specifically peptidoglycan (PG), to generate an immune response. Serum antibodies that recognize antigens play a fundamental role in immune defense, homeostasis, and autoimmunity; they serve as potential biomarkers for a variety of medical applications. The data shows that bacterial PG is also used in an adaptive immune response to generate specific PG-antibodies, as these fragments have been found in the serum. Consequently, we are synthesizing new chemical probes based on PG to better understand their response against serum antibodies. The objective here is to develop novel techniques for the production of cell wall derived libraries and then use those molecules as tools for the investigation of the adaptive immune response. The power of microarrays lays in their unprecedented capacity to simultaneously interrogate tens to hundreds of thousands of immobilized probes. Here we developed the PG microarray using synthetic PG molecules

with linkers constructed at varying positions and then applied to NHS-functionalized glass slides. This array platform is essential for the rapid screen for biomarkers and antigen responsible for autoimmune diseases. Applications of this array platform are highlighted by providing a high throughput method to interrogate potential biomarkers for autoimmune diseases as well as the analysis of human humoral immunity to the pathogenic PG.

(182) Genome editing of primary neutrophils derived from CD34+ human hematopoietic stem cells

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Studying the biology of primary human peripheral blood neutrophils is challenging since these cells have a short half-life in circulation, cannot be cultured *ex vivo* and are difficult to manipulate at the genetic level. To address this challenge, we optimized differentiation protocols to obtain human neutrophils starting from CD34+ human hematopoietic stem/progenitor cells (hHSCs) isolated from umbilical cord blood. The differentiated cells were characterized using transcript measurements, quantifying cell surface glycan and protein expression, cytospins and selectin binding functional assays. CRISPR-Cas9 ribonucleoprotein (RNP) based methods were then developed to edit these cells during the differentiation process by knocking-out candidate E-selectin ligands that were previously identified using murine models: CD43, CD44 and PSGL-1. These candidate ligands were either knocked-out alone or in tandem using RNPs. Confocal, multi-color, microfluidic cell rolling and adhesion assays performed with genome edited cells demonstrate that CD44, but not CD43 and PSGL-1, is an E-selectin ligand on these primary human neutrophils. In order to validate this observation, cell-free, gain-of-function bead rolling studies were performed over E-selectin substrates by coupling CD44-Fc produced in different HEK293T cell lines onto 5μm polystyrene beads. Here, CD44-Fc produced by HEK cells over-expressing FUT7, but not constructs expressed in wild-type HEK cells lacking FUT7 activity, supported robust bead rolling on E-selectin under flow. The data demonstrate that CD44 is a functional E-selectin ligand on primary human neutrophils. Targeting CD44 may help fine-tune neutrophil function during inflammation and cancer.

(183) The development of chemo-enzymatic method for simultaneously profiling N- and O-glycans on therapeutic glycoproteins

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N-Glycosylation is a critical quality attribute (CQA) of therapeutic proteins such as monoclonal antibodies (mAb)s by impacting their efficacy, half-life, and stability. Many therapeutic proteins are also O-glycosylated in addition to its N-glycosylation, yet, the role of O-glycosylation in the quality and safety of protein drugs remains elusive. Much effort has been deployed to characterize N-glycosylations and to understand their impacts on drug quality and safety, however, characterization of O-glycans is still very challenging due to lack of a universal enzyme to release the O-glycans from glycoproteins. Herein, we developed a chemo-enzymatic approach to simultaneously analyze both N-and O-glycan profiles of protein drugs. Using Fetuin which is a glycoprotein, abatacept and etanercept which are therapeutic Fc-fusion glycoproteins, we were able to analyze both N-glycans and O-glycans quantitively and qualitatively in a single setting by mass spectrometry, MALDI-TOF-MS. In addition to the reported glycan structures, our data also revealed the presence of trace amount of bisecting GlcNAc and non-human glycan structures, e.g. the terminal galactose-α-1,3-galactose (α-Gal) and N-glycolylneuraminic Acid (Neu5Gc), in N- or both N- and O- glycans of abatacept respectively. Our novel method is feasible and reproducible. We are currently testing and validating the method with more therapeutic fusion proteins, and will eventually standardize the experimental procedures for manufacturers to assess glycosylations of their protein drugs. This method will not only facilitate manufacturing and developing high quality protein drugs in industries, but will also enable the reviewers to adequately assess the quality and safety of therapeutic proteins with regard to their glycosylations.

(184) Masked glycosylated proteins related to glycogen in serum of controls, Pompe disease patients and Pompe disease mice

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Following incubation of glycogen with rhGAA until no more carbohydrate is detected by HPAEC-PAD masked glycosylated proteins are detected following boiling in 0.1N HCl or treatment with proteases. The masked material in the medium binds to Dowex 50W-X8 which is evidence of a charge such as a protein but it is not bound by Concanavalin A. Glycogen does bind Concanavalin A. Given its characteristics the presence of this material in serum was suspected. These masked oligosaccharides are present in serum either in an HCl extract or in a trypsin or chymotrypsin digests. The characteristics of the serum material were the same as the material from the *in vitro* incubation medium. One oligosaccharide, which can not be degraded further by rhGAA, from the incubation medium and serum co-elute. The oligosaccharides contain *m*-inositol, *e*-inositol, sorbitol, mannose, galactose and glucose with three of them also containing xylose. The presence of

this glycosylated protein in serum is taken as an indication of a fraction of glycogen being degraded outside the lysosome and outside of the cell. Several other masked glycosylated proteins are exposed in the same manner. The oligosaccharide which appears to be an end product from GAA degradation of glycogen is present in the serum of Pompe disease patients, receiving enzyme replacement therapy (ERT) but it is not present in the serum of Pompe mice which are not receiving ERT. The Pompe mice do have a series of masked glycosylated proteins which elute later on HPAEC-PAD in the region where maltooligosaccharides of DP from about 5 to 9 elute. The Pompe patients on ERT also have these oligosaccharides present but in significantly lower relative quantities than the mice not on ERT. This relative difference is suggestive of improvement of the patients on ERT but yet short of a complete restoration of the normal state. Analysis of these later eluting oligosaccharides reveals that they are not the maltooligosaccharides which might be suspected. When the oligosaccharides were isolated they were found to have monosaccharide compositions similar to the oligosaccharides described earlier with inositol, sorbitol and smaller amounts of hexoses. When the collected oligosaccharides were then incubated with trypsin and re-chromatographed each was eluted in at least three to five carbohydrate peaks which when isolated and hydrolyzed with 4N TFA had differing monosaccharide compositions. All of them had inositol but only three had sorbitol and all had small amounts of hexoses. These results indicate that each of these glycosylated proteins has multiple glycosylation sites with differing compositions. The relationship of these complex glycosylated proteins and their relationship to glycogen in normal and Pompe disease patients and mice is under investigation. Questions are raised about the possible interactions of glycogen or possible degradation of cytoplasmic glycogen.

(185) Oligosaccharides released from corn starch on gelatinization

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During the Christmas holidays in 2016, tamales made from masa prepared by a well known market in Southern California did not hold together and fell apart. This situation received wide publicity since tamales are a major holiday food in the Hispanic community. Investigation revealed that the starchy endosperm of the corn used to make the masa was defective since compared to the starchy endosperm of good corn on cooking it did not release a fraction of oligosaccharides in the degree of polymerization range of 7 to about 26. A search of the starch literature indicated that very little is published about the polysaccharides released from starch on gelatinization. Comments in publications related to oligosaccharide release on gelatinization stated: 1.) *The granules are observed to swell, absorb water, lose crystallinity, and to leach amylose or 2). With all starches, leaching of polysaccharide (amylose and/or amylopectin, depending on the starch) was highly correlated with swelling factor.* For this reason commercial corn starch was investigated. The glucans released by cooking ranged from DP7 up to about DP26. This is the same range of the glucans where the significant difference between the good corn and the bad corn was apparent. The conclusion from these experiments is that there is a distinct population of oligosaccharides that is released from corn starch on gelatinization. Kingsford's cornstarch was boiled for 30 minutes then centrifuged at 16,000 x g for 5 minutes and the separated gel on the top of the starch was removed with a spatula. The gel was centrifuged again and the liquid above the gel was removed. The gel was then extracted with water by stirring and centrifugation. The extract was passed over a Dowex 50W X8 column. After the water wash the column was eluted first with 0.5N NH_4OH and then with 2N NH_4OH. The first fraction, the water wash, was the only fraction that contained the unique population of oligosaccharides from DP7 to DP26 on HPAEC-PAD. Nine following fractions (2.9 ml) were collected and hydrolyzed with 4N TFA for three hours at 120°. Every fraction eluted after the water wash was found to contain protein and significant amounts of glucose. They all contained *myo*-inositol, iditol and sorbitol. One fraction had a small amount of sorbitol but a large amount of iditol and two fractions contained small amounts of both iditol and sorbitol. In other work in this laboratory a fraction with similar composition containing protein, *myo*-inositol, iditol, sorbitol and hexoses has been identified in hydrolyzates of corn starch and glycogen. This is believed to be the first report of protein involvement in starch gelatinization.

(199) A bi-to-mono CRD transition in GAL-9 potentiates mesenchymal invasion of breast cancer epithelia

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Aberrant expression and functions of glycans and their binding proteins (lectins) represent one of the earliest 'hallmarks' of cancer. Galectins are a conserved family of lectins that can bind to β-galactosides. A special class of galectins known as tandem-repeat (GAL-4, -8, -9, and -12 in humans) can bind two distinct β-galactosides simultaneously and play intricate roles in physiological and pathological contexts. In this study, we asked if one or more tandem repeat galectins regulate breast tumor progression based on earlier reports of their differential expression. Upon mimicking a spectrum of progression from homeostatic breast- to invasive cancerous architectures by culturing HMLE (immortalised breast epithelial cell line), MCF7 (non-invasive breast cancer cell line), MDA-MB-231 (metastatic cell line) in laminin-rich ECM- and Type 1 collagen- rich scaffold gels, we observed that expression of the gene encoding GAL-9 tracked invasiveness of probed

cells. Breast cancer patient samples (especially with a 'triple negative' (ER^- /PR^- /$HER2^-$) histotype) showed higher levels of GAL-9 when compared with matched adjacent normal tissues. Perturbing GAL-9 levels in cancer epithelia showed its positive correlation with their adhesion to- and invasion within- laminin-rich matrices. Within a complex bimatrix scaffold that mimics the epithelial-basement membrane-stromal matrix organization, GAL-9 preferentially enhanced the solitary over collective invasion of cancer epithelia. To dissect which carbohydrate recognition domain (CRD) is involved in regulation of cancer invasion, we generated GAL-9 mutants with deletion of individual CRDs or the intervening linker. Only the misexpression of the N-terminal CRD of GAL-9 (and not the C-terminal CRD or linker deletion) is able to increase cancer invasion similar to full length GAL-9 overexpression. We also observed that GAL-9 in human and murine invasive cancer cells was cleaved into individual CRDs, as opposed to expression of primarily uncleaved biCRD forms in untransformed and non-invasive transformed cells. Our results, in the light of a strongly predicted protease-susceptibility of the GAL-9 linker region suggest that the N-CRD of GAL-9 that is free of the C-CRD potentiates the mesenchymal invasion of cancer epithelia through stromal-like milieu.