Overlay of snapshots from MD simulation of the S glycoprotein with site-specific glycosylation. Glycans are shown in ball-and-stick representation: M9 (green), M5 (dark yellow), hybrid (orange), complex (pink). The protein surface is colored according to antibody accessibility from black to red (least to most accessible).

Co-Chairs
Dr. Lara Abramowitz, NIDDK, NIH
&
Dr. Tongzhong Ju, OBP, CDER, FDA

Sponsored by
The NIH Glycobiology Scientific Interest Group, Office of Intramural Research
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The National Institute of Standards and Technology
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Cover art courtesy of Dr. Robert Woods
2020 NIH & FDA Glycoscience Research Day Agenda

May 15, 2020

Co-Chairs
Dr. Lara Abramowitz, NIDDK, NIH & Dr. Tongzhong Ju, OBP, CDER, FDA

Presented virtually via WebEx

Morning Welcome
8:45 a.m., Welcome and Introductions, Dr. Lara Abramowitz, NIDDK & Dr. Tongzhong Ju, FDA Co-Chairs

Session I, Glyco Analytics & Chemistry (1 hr 30 min)
Chair, Dr. Mark Lowenthal, Chemist, NIST

9:00 a.m., Rapid and reproducible imaging mass spectrometry workflows for detecting glycans in tissues, cells and biofluids
  Dr. Richard Drake, Professor, Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina

9:30 a.m., Genetically encoded fluorescent biosensors for nucleotide sugars
  Dr. Hui-wang Ai, Associate Professor, Department of Molecular Physiology and Biological Physics, School of Medicine, University of Virginia

10:00 a.m., Applications of glycan microarray technology to antibody research
  Dr. Jeffrey Gildersleeve, Senior Investigator, NCI, NIH

10:30 a.m., Break

Session II, Glycoproteomics (1hr 30 min)
Chair, Dr. Aleksandra Nita-Lazar, Senior Investigator & Chief, Functional Cellular Networks Section, NIAID

10:45 a.m., CellSurfer: A Platform for Semi-Automated, Quantitative Discovery of Cell Surface N-Glycoproteins from Human Primary Cells
  Dr. Rebekah Gundry, Professor and Vice Chair, Department of Cellular and Integrative Physiology, University of Nebraska Medical Center

11:15 a.m., Machine learning methods for clinical glycoproteomics and beyond
  Dr. Heather Desaire, Professor, Department of Chemistry, University of Kansas

11:45 a.m., Glycopeptide quantification and structural resolution
  Dr. Radoslav Goldman, Professor, Department of Oncology & Department of Biochemistry and Molecular & Cellular Biology, Georgetown University
Lunch (12:15 p.m.) and GlyGen Demo (1 p.m.)

12:15 p.m., Lunch

1:00 p.m., Demonstration of new Glycoscience informatics tools by the NIH Common Fund GlyGen Program

Session III, Glycobiology of Disease and Therapeutics (1hr 20min)
Co-Chairs Dr. Lara Abramowitz, NIDDK, NIH & Dr. Tongzhong Ju, OBP, CDER, FDA

1:30 p.m., Manufacturing biotherapeutics with defined glycosylation to control their quality and safety
Dr Tongzhong Ju, Office of Biotechnology Products, Center for Drug Evaluation and Research, FDA

1:50 p.m., Small molecule inhibition of the oligosaccharyl transferase
Dr. Joseph Contessa, Professor of Therapeutic Radiology and of Pharmacology; Co-Leader, Radiobiology and Radiotherapy, Yale Cancer Center

2:20 p.m., Siglec mediated regulation of immune responses
Dr. James Paulson, Professor and Co-Chair Department of Molecular Medicine, The Scripps Research Institute

Session IV, Human Milk Oligosaccharides (1hr, 30min)
Chair, Dr. Michelle Bond, Program Director, NIGMS, NIH

3:00 p.m., The role of human milk oligosaccharides in the pathogenesis and treatment of necrotizing enterocolitis
Dr. David J. Hackam, Surgeon-in-Chief, Professor of Surgery, Johns Hopkins Children’s Center

3:30 p.m., Application of the membrane permeabilizing ability of HMOs
Dr. Steven Townsend, Assistant Professor of Chemistry, Vanderbilt University

4:00 p.m., Investigating HMO – Enteric Virus Interactions: From Bench to Bedside and Beyond
Dr. Sasirekha Ramani, Assistant Professor, Baylor College of Medicine

4:30 p.m., Closing remarks Dr. Lara Abramowitz, NIDDK & Dr. Tongzhong Ju, FDA

4:45 p.m., Meeting Chairs: Announcement of Abstract Awards

5:00 p.m., Adjourn

This meeting is being sponsored by the NIH Glycobiology Scientific Interest Group and its participating NIH Institutes; the FDA, NIST, and the FAES. The NIH Glycobiology SIG gratefully acknowledges and thanks our judges for donating their time and effort to evaluate abstracts.

This meeting is made possible through generous support from the Food and Drug Administration, the Office of the Director, NIH & FAES.
2020 NIH & FDA Glycoscience Research Day

PRESENTERS
Dr. Lara Abramowitz, PhD
Dr. Lara Abramowitz is a Staff Scientist with the Laboratory of Cell and Molecular Biology at the NIDDK/NIH. She received her B.S. magna cum laude in Biological Science: Molecular Genetics with a distinction in research from the University of Rochester, and her Ph.D. in Cell and Molecular Biology: Genetics and Gene Regulation from the University of Pennsylvania Perelman School of Medicine. After completing her Ph.D. focusing on epigenetics and genomic imprinting in the laboratory of Dr. Marisa Bartolomei, she joined Dr. John Hanover’s laboratory at the NIDDK/NIH to pursue her postdoctoral research. Dr. Abramowitz transitioned to Staff Scientist in 2018 and continues to leverage her expertise in transcriptional regulation to understand how nutrient responsive O-GlcNAc impacts development, stem cell biology and gene expression.

Dr. Hui-wang Ai, PhD
Dr. Ai received his B.S. degree from Tsinghua University in 2003 and Ph.D. degree from the University of Alberta in 2008 (advisor: Robert E. Campbell). He received his postdoctoral training from 2008 to 2011 in the lab of Peter G. Schultz at The Scripps Research Institute. He then became an assistant professor at the University of California, Riverside. In 2017, he moved to the University of Virginia (UVA) Department of Molecular Physiology and Biological Physics as a tenured associate professor. He is a resident faculty member of the Center for Membrane and Cell Physiology. He is also affiliated with the Departments of Chemistry and Biomedical Engineering, and the UVA Cancer Center. His lab uses interdisciplinary approaches in chemistry, engineering, biophysics, and physiology to study and manipulate complex biological systems, with a focus on the development of novel molecular biosensors to peer into cells and organisms to understand their communications. Dr. Ai received the Hellman Fellows Award in 2013, the National Science Foundation CAREER award in 2014, and the American Chemical Society Toxicology Young Investigators Award in 2017.

Dr. Joseph Contessa, PhD
Joseph Contessa is a physician-scientist at Yale University with a clinical practice in radiation oncology. He is Vice Chair for basic science research and the Director of the central nervous system (CNS) radiation therapy division in the Department of Therapeutic Radiology. He has an active practice treating patients with brain tumors and also sees patients with head and neck cancers. He is a member of the Yale Cancer Center’s executive committee and leads the Radiobiology and Radiotherapy Research Program. His laboratory studies mechanisms of tumor cell adaptive responses to cancer therapy, and his laboratory is engaged in high throughput target discovery and chemical biology. A major focus of his laboratory is to study the contributions of N-linked glycosylation to receptor tyrosine kinase (RTK) synthesis, maturation, and function. His laboratory has identified new approaches for quantifying the efficiency of N-glycosylation in intact cells, and this methodology provides a new platform for developing experimental formats that range from microplates to xenografts. Applications of this technology have enabled small molecule and genetic screening, and the discovery of a novel class of small molecule inhibitors that disrupt N-linked glycosylation through inhibition of the oligosaccharyltransferase. Because these inhibitors have very little cellular toxicity, due to incomplete inhibition of the complementary STT3A and STT3B paralogs, they are being actively investigated in tumor model systems.

Dr. Heather Desaire, PhD
I discovered my strong affinity for chemistry at Grinnell College in Grinnell, IA, where I received a Bachelor of Arts degree in 1997. I attended graduate school at the University of California, Berkeley, and developed an admiration for the utility and versatility of mass spectrometry. I completed my Ph.D.
in 2001, spent a few months in an industrial internship, and began my faculty appointment at the University of Kansas in 2002. I became an Associate Professor in 2008, a Professor in 2011, and in 2013, I was named the Dean’s Professor of Chemistry.

My research focuses on the intersection of glycobiology and mass spectrometry, and my driving passion is dreaming up improved ways to use and analyze the data from these experiments. A portion of my group develops new machine learning tools to identify clinically relevant biomarkers from ‘omics data. Others characterize the glycosylation on HIV-1 Env and other high-value glycoproteins. I collaborate extensively, and I enjoy working on problems that require expertise from numerous individuals with divergent backgrounds. On any given day, I am equally likely to be discussing algorithm design, strategies for eliciting broadly neutralizing antibodies, or efficient data acquisition strategies for LC-MS experiments.

Dr. Richard Drake, PhD  
Richard Drake is a Professor in the Department of Cell and Molecular Pharmacology at the Medical University of South Carolina and SmartState Endowed Chair in Proteomics. He received his Ph.D. in biochemistry from the University of Kentucky in 1990, and did post-doctoral training with Alan Elbein in N-linked glycan biosynthesis and glycosyltransferase research. He is an experienced protein biochemist and glycobiologist, with particular expertise in using glycan-targeted mass spectrometry imaging of clinical tissues and biofluids for biomarker discovery and development of new diagnostics. Current efforts are in the large scale application of N-glycan MS imaging methods developed in his laboratory to map the distribution of N-glycans and other glycan classes in prostate, pancreas, liver and breast tumors. The goal is to identify glycan biomarker panels associated with tumor, stroma and immune regions of tumors, in two and three dimensions. Further, these glycan maps are being used to identify the localized glycoprotein carriers, and develop new approaches to effectively isolate and characterize tissue. Efforts are ongoing to adapt the tissue analysis workflows to develop rapid and accessible glycan profiling tools for application to cultured cells, biofluids and immunoarray captured cells and glycoproteins.

Dr. Jeffrey Gildersleeve, PhD  
Jeff Gildersleeve obtained his bachelor’s degree in biology from the University of California at San Diego. He obtained his Ph.D. degree in organic chemistry at Princeton University under the guidance of Professor Dan Kahne, and completed postdoctoral training with Professor Peter Schultz at The Scripps Research Institute. He began his independent career at the National Cancer Institute in 2003 and is currently a Senior Investigator in the Chemical Biology Laboratory. The Gildersleeve group uses chemical approaches and glycan microarray technology to study the development and use of carbohydrate-binding antibodies for diagnosis and treatment of cancer. Dr. Gildersleeve is a recipient of the 2006 NCI Director’s Innovation Award and the 2011 David Y. Gin New Investigator Award from the Division of Carbohydrate Chemistry of the American Chemical Society. He also serves on the Editorial Advisory Board of ACS Central Science, the Editorial Board of the Journal of Biological Chemistry, the Editorial Board of Cell Chemical Biology and the Scientific Advisory Board of the Canadian Glycomics Network.

Dr. Radoslav Goldman, PhD  
Dr. Goldman is Professor of Oncology, Lombardi Comprehensive Cancer Center and directs the Clinical and Translational Glycoscience Research Center (CTGRC) at Georgetown University. His research covers the interface of protein biochemistry, analytical chemistry of glycoproteins, and applications of the
studies to cancer research. Quantification of glycopeptides and their structural resolution is one of the
topics currently studied by researchers in his laboratory and at the CTGRC.

Dr. Rebekah Gundry, PhD, FAHA
Dr. Gundry completed her PhD at the NSF Middle Atlantic Mass Spectrometry laboratory at Johns
Hopkins University School of Medicine and her postdoctoral work at the NHLBI Proteomics Center in the
Division of Cardiology at JHU. As a post-doctoral fellow, she was awarded an NHLBI K99/R00 and
established her independent laboratory at the Medical College of Wisconsin in 2010. In August 2019, she
was recruited to the University of Nebraska Medical Center in Omaha, NE. In addition to her role as
Department Vice Chair, she serves as Assistant Chief of Basic and Translational Research for the Division
of Cardiology and is the inaugural Director of the CardiOmetrics Program which is a collaborative group
that applies mass spectrometry technologies for advancing basic and translational cardiac research and
clinical care. The Gundry lab develops and applies innovative mass spectrometry approaches and
bioinformatic tools to study cell surface glycoproteins and glycans to answer outstanding questions in
cardiac biology, cardiovascular disease and associated comorbidities. Research in the Gundry lab is
funded by the American Heart Association, Juvenile Diabetes Research Foundation, and NHLBI. Rebekah
received the inaugural Robert Cotter Young Investigator Award from US Human Proteome Organization
in 2013, was recently appointed Associate Editor of the Journal of Molecular and Cellular Cardiology,
and is currently Co-Chair of Human Proteome Organization Cardiovascular Initiative.

Dr. David J. Hackam, MD, PhD
David J. Hackam, M.D., Ph.D., is the Garrett Family Professor of Pediatric Surgery at the Johns Hopkins
University, and Pediatric Surgeon-in-Chief and co-Director of the Johns Hopkins Children’s Center. Dr.
Hackam’s clinical practice focuses on complex neonatal surgery. His laboratory is focused on unraveling
the molecular mechanisms that underlie necrotizing enterocolitis, the leading cause of death from
gastrointestinal disease in premature infants. Dr. Hackam’s work has led to the filing of several
international patents, and is been funded by the National Institutes of Health as well as several industry
collaborations.

Dr Tongzhong Ju, PhD
Dr. Tongzhong Ju joined OBP/CDER/FDA as a PI and Senior Staff Fellow in Jan. 2017. Before joining FDA,
he was an Associate Professor in Biochemistry Department at Emory University. His primary research at
Emory focused on the regulation of O-glycosylation and its implication in human diseases, which was a
continuation of over a decade of his fundamental work on purification and cloning Core 1 β3GalT,
discovery of Cosmc, identification of somatic mutations in Cosmc in the blood cells from patients with Tn
syndrome and in human tumor cells. His studies in glycan biosynthesis and biological functions which
were supported by NIH and other funding lead to over 50 peer-reviewed research articles, 5 review
articles published in Nature, Nature Methods, Nature Medicine, PNAS, JCB, Cancer Research, JBC,
Angew Chem Int Ed Engl, and Annu Rev Pathol, among others, as well as 7 book chapters.

Tongzhong received his M.D. from Qingdao University Medical School, China in 1986, and his Ph.D. in
Biochemistry from Fudan University Shanghai Medical School, China in 1994, and his post-doctoral
training with Drs. Bill Canfield and Richard Cummings at the University of Oklahoma Health Sciences
Center from 1997 to 2000.

At FDA, his lab has established the OBP glycobiology research program focusing on: 1) Developing novel
technologies for identification/characterization of glycosylation on biotechnology products; 2) Studying
roles of N- and O-glycosylation (the structural and functional consequences of glycosylation) in protein
drug quality and safety; and 3) Investigating the role of glycosylation in immune regulation and tumor biology.

**Dr. James Paulson, PhD**

James C. Paulson obtained his PhD (Biochemistry) in 1974 from the University of Illinois at Champaign-Urbana, and did post-doctoral work at Duke University Medical Centre, in Durham, North Carolina from 1974-78. From 1978 – 1990 he rose from Assistant Professor to full Professor and vice-chair in the Department of Biological Chemistry at the UCLA School of Medicine where he developed an interest in analysis of receptor specificity of influenza viruses from different host species. From 1990-1999 he served as Vice President and Member Board of Directors of Cytel Corporation, La Jolla, CA. From 1999-present he has been Professor, in the Departments of Cell and Molecular Biology, Chemical Physiology, and Immunology and Microbial Sciences at The Scripps Research Institute, in La Jolla, California. He served as acting President & CEO from 2014-2015 and is currently Cecil and Ida Green Professor and Chair of Molecular Medicine. His current research interests include the roles of glycan binding proteins in the modulation of immune cell signaling, and the receptor specificity of mammalian and animal influenza viruses.

For listing of publications: [https://www.scripps.edu/paulson/publications.html](https://www.scripps.edu/paulson/publications.html)

**Dr. Sasirekha Ramani, PhD**

Dr. Sashi Ramani is an Assistant Professor in the Department of Molecular Virology and Microbiology at Baylor College of Medicine, Houston. Her specific areas of interest are maternal and child health with a focus on gastrointestinal infections and vaccines. The goals of her research program are to understand factors that contribute to disease susceptibility and identify mechanisms to improve immune responses to infectious agents. She is particularly interested in identifying correlates of protection from infection and vaccination and what mediates vaccine failure. Dr. Ramani’s work involves a combination of laboratory and field-based assays, and thus take a complete bench-to-bedside approach to infectious diseases research. Her work on molecular characterization of host glycan-microbial interactions including HMOs focus on (i) understanding the role of host glycans as mediators for susceptibility to infections, (ii) how changes in glycan expression affect infectivity by a pathogen, and (iii) the mechanisms used by the host to block specific interactions between the pathogen and host glycans. Key to this research is the use of human intestinal organoid cultures. In the long term, the goal of her lab is to develop and stimulate new research on the role of glycans and the microbiome in infectious diseases, and identify interventions with translational potential to improve human health.

**Dr. Steven Townsend, PhD**

Steve Townsend completed his PhD at Vanderbilt University under the direction of Gary Sulikowski working on the synthesis of terpenes. Next, Steve completed a postdoctoral research tour with Samuel Danishefsky at Memorial Sloan Kettering Cancer Center and Columbia University. He returned to Vanderbilt in 2014 to begin his independent career, where his group has published 26 articles in the area of chemistry focused glycoscience. At Vanderbilt, he has been awarded the Chancellors Award for Research and the Jeffrey Nordhaus Award for Excellence in Undergraduate teaching. Externally, he was awarded the Ruth Lawrence Award for Excellence in Human Milk Science, a Camille Dreyfus Teacher Scholar Award, and is a member of the C&E News class of 2019.
The NIH Glycobiology Scientific Interest Group
The NIH Glycobiology Scientific Interest Group (SIG) brings together researchers from laboratories across the intramural NIH, FDA, NIST, and local universities who share interests in the glycosciences including those pursuing studies of: glycan structure; synthesis; metabolism; function; and lectin biology. The activities of the Glycobiology SIG are organized by a Steering Committee of volunteers from multiple institutes and federal agencies and highlight the ongoing research and training efforts in the glycosciences taking place across the NIH campus. The SIG maintains a list serve, and website which can be found here <https://oir.nih.gov/sigs/glycobiology-scientific-interest-group>. Looking for a research experience at the NIH? Members of the SIG form a group of training laboratories in the glycosciences. The Glycobiology SIG cross posts activities with the NIH glyco-immunology SIG, the proteomics SIG, the Baltimore-Washington Area Glycobiology Interest Group, and the Chemistry SIG.

The 2019-20 NIH Glycobiology SIG Steering Committee:

Dr. Barchi, NCI, NIH
Dr. Bash, CBER, NIH
Dr. Bewley, NIDDK, NIH
Dr. Blithe, NICHD, NIH
Dr. Bond, NIDDK, NIH
Dr. Cipollo, CBER, FDA
Dr. Geller, NHLBI, NIH
Dr. Gildersleeve, NCI, NIH
Dr. Freedberg, FDA, NIH
Dr. Hanover, NIDDK, NIH
Dr. Ju, CDER, FDA
Dr. Krasnewich, NIGMS, NIH
Dr. Krueger, NCI, NIH
Dr. Kubler-Kielb, NICHD, NIH
Dr. Love, NIAID, NIH
Dr. Lowenthal, NIST
Dr. Marino, NIGMS, NIH, chair

Dr. Melillo, NIGMS, NIH
Dr. Nita-lazar, NIAID, NIH
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Dr. Rao, CDER, FDA
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Dr. Sathyamoorthy, NCI, NIH
Dr. Sheeley, NIDCR, NIH
Dr. Srinivas, NHLBI, NIH
Dr. Robert Stack, DOE
Dr. Roberts, NCI, NIH
Dr. Taylor, NIAID, NIH
Dr. Ten Hagen, NIDCR, NIH
Dr. Vann, CBER, FDA
Dr. Zangmeister, NIST

Join US! Membership in this SIG and on its steering committee is open to all who have interest in the field and time to devote to SIG activities! If interested email: pamela.marino@nih.gov
History of the Glycobiology Interest Group & Glycoscience at NIH
By Diana Blithe and John Hanover

What’s wrong with this picture? DNA → RNA → Protein → → → CELL?
The sequence ignores at least two important classes of macromolecules: Glycans and Lipids. These molecules suffer from a phenomenon known as the “Rodney Dangerfield Syndrome” (they get no respect).

In the early days, investigators studying glycosylated proteins were considered “poor biochemists” because they seemed to have difficulty purifying the sugars away from the proteins. As befitted their biochemical skills, the sessions on glycosylated proteins were scheduled last at major meetings (usually Saturday afternoon).

Analytical technology was a problem and methods for quantifying glycans lagged behind those for proteins and nucleic acids. The inherent structural diversity of complex carbohydrates, the difficulty in determining their sequence, and the absence of a chromophore contributed to the lack of sensitive analytical methods. Furthermore, biosynthesis could not be directly predicted from a template. The development of new alkaline pH HPLC technology for improved analysis of sugar composition expanded the analytical capability of investigators.

In 1987, the NIH Carbohydrate Interest Group was established through the efforts of Drs. Gilbert Ashwell and Vincent Hascall. Activities of the group were further catalyzed by Dr. Mike Bedford. Mike had sold one of the first alkaline pH HPLC units to Drs. John Hanover and Gilbert Ashwell, and he suggested gathering other researchers for a group meeting to share experiences with the technology. Gil and Vince took the lead in organizing the first meeting of what became the NIH Carbohydrate Interest group. The name of the group reflected that of the national association, the Society for Complex Carbohydrates (often confused with advocates for cereal and donuts). Monthly Meetings were held at 4 PM at the FAES Building – adjacent to the NIH campus. The location was chosen because the building allowed wine to be served. Enthusiasm over new analytical alkaline pH HPLC technology brought people together. In addition to the wine, cheese, fruit and crackers were served. Collaborations abounded!

Our colleagues Drs. Y.C. Lee and Saul Roseman had formed a similar Interest Group at Johns Hopkins University, in Baltimore. Upon his return to Hopkins, Dr. Gerald Hart spearheaded the efforts in the Hopkins Medical Campus. In addition to monthly meetings, the two groups (NIH and Johns Hopkins) began to hold annual joint meetings. The first annual meeting entitled “Glycoday” was held May 30th, 1995 in Annapolis, MD. Over 100 attendees were present at the meeting, which was reported on in the NIH Catalyst http://www.nih.gov/catalyst/back/95.07/toc.july95.html

From Carbohydrates to Glycobiology
“The term “Glycobiology” was first coined in 1988 by Rademacher, Parekh, and Dwek to recognize the coming together of the traditional disciplines of carbohydrate chemistry and biochemistry with modern understanding of the cellular and molecular biology of glycans” from Essentials in Glycobiology. Wikipedia, the free encyclopedia, says: “Defined in the broadest sense, glycobiology is the study of the structure, biosynthesis, and biology of saccharides (sugar chains or glycans) that are widely distributed in nature. Sugars or saccharides are essential components of all living things and aspects of the various different roles they play in biology are researched in various different medical, biochemical and biotechnological fields.”
The journal, *Glycobiology* began in September, 1990. The analytical methods bottleneck that had held back the field was eased as more sensitive carbohydrate analysis continued to be developed, and at least two companies (Dionex, and Oxford Glycosystems) sought to improve technologies for carbohydrate analysis. In 1993, the Society for Complex Carbohydrates changed its name to the Society for Glycobiology. Following their lead, the NIH Carbohydrate Interest Group (CIG) changed its name to the Trans NIH Glycobiology Interest Group (GIG).

On September 13-15, 1993, the group organized a major meeting entitled: “Glycobiology: New Perspectives on Human Disease,” held at the National Institutes of Health in Bethesda, Maryland. The conference organizers were: Drs. G. Ashwell, D.L. Blithe, J.A. Hanover, G.W. Hart, V.C. Hascall, G.D. Holt, D.M. Krasnewich and Y.C. Lee. Principal topics included Glycobiology as it relates to:

Infectious diseases — pathogen adhesion, evasion of immune system recognition, suppression of immune system activation.

Human development — tissue organization and outgrowth, differentiation markers, fertilization, glycoprotein hormones.

Cell adhesion, inflammation, and metastasis — metastatic cell adhesion, leukocyte homing, selectins and the inflammatory response, angiogenesis.

Therapeutics — carbohydrates as drugs, chemotherapeutic potential of processing inhibitors, engineering oligosaccharide structures, drug targeting.

Diagnosis — tumorigenesis, markers of metastatic potential, rheumatoid arthritis, metabolic disorders and storage diseases.

GLYCODAY, the annual Washington-Baltimore Glyco-Symposia continued until 1997 (revived by the Baltimore group in 2001). With the advent of numerous other NIH interest groups (7 major and 122 “minor” IGs), activities of the NIH GIG became less frequent and less regular, however, a core of NIH scientists remained active. Seminars, meetings, and announcements for the GIG continued on an ad hoc basis under the leadership of Dr. Diana Blithe. The Baltimore group has remained active as well. In the fall of 2006, Drs. Blithe, Hanover, and Manzoni made a concerted effort to re-establish a regular seminar series for the GIG. The inaugural lecture for the newly established Series was given by Dr. Pamela Stanley. This led to a spirited revival and reorganization of the NIH GIG, with the establishment of a Steering Committee composed of both intra and extramural scientists (Drs. Manzoni, Barchi, Ten Hagen, Hanover, Blithe, Qasba, Srinivas, Sheeley, Sarkar, Huizing, Marino) who proceeded to chart a course for the GIG that included: a seminar series highlighting the second edition of *Essentials of Glycobiology*; a new version of Glycoday, entitled *Glycoscience Research Day*; and a website to accompany an expanded email list. The revamped Glycobiology Scientific Interest Group Steering Committee has continued to expand. A core group of laboratories working in the glycosciences serve as a nucleus for an intramural glycosciences training program and a course in glycobiology given upon request.
The NIH Glycobiology Scientific Interest Group

Training Opportunities in the Glycosciences

The cross-cutting research interests of the intramural NIH make it an ideal environment for the study in the glycosciences. Carbohydrates are one of the three major bio-macromolecules responsible for bulk information transfer in biological systems and glycans are involved in a multitude of cellular functions including: cell recognition; motility/homing to specific tissues; signaling processes; cell differentiation; cell adhesion; microbial pathogenesis; and immunological recognition. Understanding these cellular functions is of great interest to many intramural research programs. If you wish to do postdoctoral training in the glycosciences, you may wish to contact one or more of the following investigators:

**NCGC**

Craig J. Thomas, Ph.D
Chemical Genomics Center
*Expanding the role of small molecules in the glycosciences: small molecule tools directed at elucidating glycosylation pathways*
http://www.ncgc.nih.gov/about/craigt.html

**NCI**

Joseph J. Barchi Jr., Ph.D.
Laboratory of Medicinal Chemistry
Multivalent presentation of tumor-associated carbohydrate antigens (TACA) and TACA-peptide conjugates as modulators of tumor cell adhesion and novel immunogens.
http://ccr.cancer.gov/staff/staff.asp?profileid=6281

Jeffrey C. Gildersleeve, Ph.D.
Laboratory of Medicinal Chemistry
Carbohydrate chemistry and glycobiology: carbohydrate microarray development; carbohydrate-based cancer vaccines; diagnostic agents; therapeutic agents; and identification of cancer biomarkers.
http://ccr.cancer.gov/staff/staff.asp?profileid=7853

Barry O'Keefe, Ph.D.
Molecular Targets Development Program
*Studies of broad spectrum antiviral lectins*
http://ccr.cancer.gov/staff/staff.asp?profileid=7138

Dennis Klinman, M.D., Ph.D.
Laboratory of Experimental Immunology
*CpG Oligonucleotides as Vaccine Adjuvants*
http://ccr.cancer.gov/staff/staff.asp?profileid=12379

David D. Roberts, Ph.D.
Laboratory of Pathology
Heparin sulfate proteoglycans, angiogenesis, immunology, extracellular matrix
http://ccr.cancer.gov/Staff/staff.asp?profileid=5850
Alexander Wlodawer, Ph.D.
Protein Structure Section
Investigating lectins with antiviral activity; a variety of other proteases, ribonucleases, and kinases; and a number of cytokines and cytokine-receptor complexes
http://mcl1.ncifcrf.gov/wlodawer.html

NHGRI
Marjan Huizing, Ph.D.
Cell Biology of Metabolic Disorders Unit
Sialic acid metabolism and human disorders of glycosylation
http://www.genome.gov/11007099

NHLBI
Herbert M. Geller, Ph.D.
Developmental Neurobiology Section
Proteoglycans in the nervous system
http://dir.nhlbi.nih.gov/labs/ldn/hmg.asp

NIAID
James Arthos, Ph.D.
Immunopathogenesis Section
HIV-1 Envelope Interactions with Dendritic Cell Receptors
http://www3.niaid.nih.gov/labs/aboutlabs/lir/immunopathogenesisSection/fauci.htm

David N. Garboczi, Ph.D.
Structural Biology Section
T cell receptor recognition of antigens and their activation in an immune response.
http://www3.niaid.nih.gov/labs/aboutlabs/lig/structuralBiologySection/

Peter Sun, Ph.D.
Structural Immunology Section
Recognition Of The Dendritic Cell Surface Receptor Dc-sign
http://sis.niaid.nih.gov

Jeffery K. Taubenberger, M.D., Ph.D.
Laboratory of Infectious Disease
Studies of Viral Hemagglutinin Binding

NIDCR
John Cisar, Ph.D.
Microbial Receptors Section
Streptococcal Receptors and Adhesins
Kelly Ten Hagen, Ph.D.
Developmental Glycobiology Unit
Role of O-glycosylation during eukaryotic development; mucin-type O-linked glycosylation; O-glycans in Drosophila development; cell adhesion, signaling, and migration; and identification of in vivo substrates destined to be glycosylated

Matthew Hoffman, B.D.S., Ph.D.
Matrix and Morphogenesis Unit
Heparan sulfate and its role in salivary gland development

John Thompson, Ph.D.
Microbial Biochemistry and Genetics Unit
Regulation of sugar transport and metabolism

Nadine L. Samara, Ph.D.
Structural Biochemistry Unit, NIDCR, NIH
Structural and functional studies of glycosylation at the host-microbe interface
https://www.nidcr.nih.gov/research/research-conducted-at-nidcr/investigators/nadine-samara-phd

NIDDK
Carole Bewley, Ph.D.
Laboratory of Bioorganic Chemistry
Specificity and recognition of novel carbohydrate binding proteins.
https://ugsp.nih.gov/scholars_mentors/mentors_d.asp?m=07&id=1427

John A. Hanover, Ph.D.
Laboratory of Cell Biochemistry & Biology
Molecular features of a novel, glycan-dependent, signal transduction cascade.
http://www2.niddk.nih.gov/NIDDKLabs/IntramuralFaculty/HanoverJohn.htm

Paul Kovac, Ph.D.
Carbohydrates Section
Development of conjugate vaccines from synthetic carbohydrate antigens
http://www2.niddk.nih.gov/NIDDKLabs/IntramuralFaculty/KovacPaul.htm

Lawrence A. Tabak D.D.S./Ph.D.
Office of the Director
Structure, biosynthesis & function of complex glycoconjugates; novel glycosyltransferases
http://www2.niddk.nih.gov/NIDDKLabs/IntramuralFaculty/TabakLawrence.htm
FDA

Mustafa Akkoyunlu, M.D./Ph.D.
Laboratory of Bacterial Polysaccharides
Interactions of bacterial polysaccharides with immune system and mechanisms of antibody development against polysaccharide vaccines
https://www.fda.gov/BiologicsBloodVaccines/ScienceResearch/BiologicsResearchAreas/ucm127268.htm

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Laboratory of Bacterial Polysaccharides
Mass spectrometry of carbohydrates, as a tool for characterization of bacterial vaccines and pathogens.
https://www.fda.gov/BiologicsBloodVaccines/ScienceResearch/BiologicsResearchAreas/ucm127264.htm

Daron I. Freedberg, PhD
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New NMR spectroscopy and light scattering technologies to characterize vaccines and blood products.
https://www.fda.gov/BiologicsBloodVaccines/ScienceResearch/BiologicsResearchAreas/ucm127270.htm

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Understanding the synthesis and interactions of bacterial carbohydrates and glycosyltransferases; evaluation and characterization of vaccines.
https://www.fda.gov/BiologicsBloodVaccines/ScienceResearch/BiologicsResearchAreas/ucm127260.htm

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Thank you to Tessa Older for assistance in program production.
Prion diseases are fatal neurodegenerative disorders caused by misfolded prion proteins that accumulate in the brain as diffuse deposits (subfibrillar prions) or amyloid plaques (fibrillar prions). The endogenous glycosaminoglycan, heparan sulfate (HS), enhances parenchymal plaque deposition and accelerates prion disease progression. The interaction of HS to prions is modulated by the HS chain length and sulfation in vitro. Here we determined how manipulating the HS sulfation levels impacts the replication of prions in vivo. Ndst1-SynCre+ mice, which produce poorly sulfated neuronal HS due to a mutation in the NDST1 enzyme, developed a 15% prolonged survival times (1 month) with no changes in the brain lesions, after inoculation with subfibrillar prions. Additionally, Ndst1-SynCre+ mice infected with the fibrillar, plaque-forming strain, showed more than a 3-month prolongation in survival (50% delay) with a redistribution of the prion amyloid plaques from the brain parenchyma to vessels. Importantly, decreasing the sulfation levels on astrocytic HS (Ndst1-GFAPCre+ mice) did not alter the prion disease caused by any subfibrillar prion, which suggests that some prion strains may selectively target neurons through their HS. Finally, we found striking differences in the composition of HS bound to subfibrillar and fibrillar prions which suggests that prion strains selectively bind HS with specific sulfation patterns.
Investigating the Role of 3-O-sulfation in Kidney Using Hs3st3a1; Hs3st3b1 Double Knockout Mice

James Ball; Vaishali Patel; Sophie H. Choi; Matthew P. Hoffman
Matrix and Morphogenesis Section, National Institute for Dental and Craniofacial Research, NIH, DHHS, Bethesda, MD, USA.

Heparan sulfate (HS) proteoglycan is an important component of the glomerular anionic filtration barrier in the kidney glomeruli that includes the glomerular basement membrane (GBM), the fenestrated glomerular endothelial cells and the podocytes. Loss of GBM HS chains is associated with proteinuria in several glomerular diseases and may contribute to the underlying pathology. The sulfated HS chains are modified by sulfotransferases in a highly ordered series of biosynthetic steps resulting in immense structural diversity of sulfate modifications. 3-O-sulfation of HS is the final event and a rare modification carried out by seven 3-O-sulfotransferase isoforms. Isoforms Hs3st3a1 and Hs3st3b1 are abundantly expressed in the adult mouse kidney. We have generated Hs3st3a1 and Hs3st3b1 double-knockout (DKO) mouse to evaluate the importance of 3-O-sulfation in kidney. These mice are viable, fertile, smaller in size with smaller kidneys than the wildtype mice. The DKO mice showed a reduction in the size of the glomeruli. Using the ligand-and-carbohydrate engagement (LACE) assay, which detects the binding of receptor-ligand complexes to endogenous HS, a reduction in FGF10:FGFR2b-Fc complex binding to the glomeruli HS was detected. This suggests that HS is modified in the DKO mice and may affect the signaling complex formation, but preliminary data show that MAPK signaling is not affected in total kidney lysates. Imaging and analysis of GBM by transmission electron microscopy showed alterations in GBM thickness and podocyte morphology compared to that of the wildtype. In addition, preliminary data suggests that kidney function may be affected in the DKO mice as blood urea nitrogen levels are increased and western blot of the urine shows increase in alpha-1 microglobulin, an indicator of proteinuria. Together, our data suggests that 3-O-sulfated epitopes may have a biological function in the kidney glomeruli where they help by maintaining the glomerular filtration barrier.
Glycosylation is involved in maturation of brain-derived neurotrophic factor (BDNF)

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Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057

Brain-derived neurotrophic factor (BDNF) is generated by proteolytic cleavage of a prodomain from the proBDNF precursor either intracellularly by furin-like proteases or extracellularly by plasmin or matrix metalloproteinases. ProBDNF carries a single N-glycosylation sequon (Asn-127) that is located in a highly conserved region proximal to the proteolytic site. To study the proBDNF structure and function, we expressed the protein in HEK293F cells. Using an optimized LC–MS/MS workflow, we demonstrate that secreted proBDNF is fully glycosylated and carries rare N-glycans terminated by GalNAcβ1–4GlcNAcβ1-R (LacdiNAc) extensively modified by terminal sulfation. There is a consensus that this type of glycosylation, carried out by specific GalNAc transferases and sulfotransferases, is protein-specific and restricted to limited number of glycoproteins. LacdiNAc remained dominant structure also on expressed truncated prodomain indicating that this intrinsically disordered region is sufficient determinant of LacdiNAc formation. N-glycosylation site mutation (N127Q) prevented secretion of proBDNF or BDNF, and protein was retained intracellularly as non-cleaved proBDNF. This effect was reproduced in wild-type proBDNF by tunicamycin-induced inhibition of N-glycosylation. Absence of the N-glycan did not affect the kinetics of proBDNF cleavage by furin in vitro, indicating that effects other than a direct furin–proBDNF interaction may regulate proBDNF maturation. On the other hand, surface plasmon resonance analysis revealed that proBDNF deglycosylation reduced the kinetics and affinity to sortilin indicating the role of N-glycans in this interaction. Sortilin is the major intracellular sorting protein in BDNF maturation pathway that is also involved in mediating cytotoxic effects of proBDNF through cell surface p75NTR receptor. The findings of our study reveal that proBDNF carries an unusual type of N-glycans important for its processing and secretion. Our results open new opportunities for functional studies of these protein glycoforms in different cells and tissues and imply novel interacting partners recognizing this glycan epitope.
Establishment of O-glyco-engineered CHO cell line platform for modulating O-glycans on therapeutic proteins

Thomas Biel; Talia Faison; Alicia Matthews; Guozhang Zou; Melissa Pegues; Nicole Azer; Fabiola Gomez; Cyrus Agarabi; Ashutosh V. Rao; Tongzhong Ju
Division of Biotechnology Review and Research 2 and 3, Office of Biotechnology Products, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD

Over 70% of manufactured recombinant therapeutic glycoproteins are produced using Chinese Hamster Ovary (CHO) cells that primarily synthesize mono- and di-sialylated core 1 O-glycan structures on their glycoproteins. While the O-glycans on cellular glycoproteins play critical roles in many biological processes, the role of O-glycans on therapeutic glycoprotein safety and efficacy remains unknown. To investigate whether O-glycans on therapeutic proteins can impact drug safety and efficacy, we generated five clonally-derived, genetically-modified CHO cell lines with the ability to produce different types of O-glycan structures or combinations of O-glycan structures on glycoproteins. Using this O-glycoengineered CHO-K1 cell line platform, we produced and purified six different O-glycovariants of etanercept, an O-glycosylated TNFR2-Fc fusion protein. To confirm that etanercept contained the expected O-glycans, each O-glycovariant of etanercept was subjected to MALDI-TOF-MS, which confirmed that our CHO K1 platform can be utilized to modulate the O-glycosylation of therapeutic proteins. These O-glycovariants of etanercept contained Sialyl Core 1 O-glycans with or without Core 3 structures or Sialyl Core 1 and SialylTn antigen, Tn antigens with or without SialylTn antigens, or Core 3 based O-glycans alone. Collectively, we have established a CHO cell line platform that has potential applications for the development and manufacturing of O-glycoengineered therapeutic proteins, which may improve the potency, stability, safety and reduce the potential immunogenicity profile of protein drug products.
Prion proteins cause an infectious and rapidly progressive neurodegenerative disease characterized by prion aggregates as well as spongiform encephalopathy, dystrophic neurites, and neuronal death. These processes depend on the neuronal expression of prion protein (PrPC), which exists on the outer leaflet of the cell membrane as a glycosylphosphatidylinositol (GPI)-anchored glycoprotein with 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 investigate the role of glycans in prion-induced neurotoxicity, we engineered a new knockin mouse model that expresses PrP with an additional glycan on PrP; this glycan is sensitive to PNGase F digestion, but not to Endoglycosidase H digestion. This mouse spontaneously develops neurodegeneration characterized by spongiform encephalopathy. In contrast to other murine models of prion disease, the brain lesions develop in the absence of PrP aggregates or infectivity, as shown by a fibrillization assay known as RT-QuIC, ThT fluorescence, and inoculation of wild-type mice with brain homogenates. Therefore, this model provides the opportunity to investigate the neurotoxic role of PrPC, uncoupled from its aggregation. We show that although the extra glycan does not affect PrPC expression or stability, primary hippocampal neurons isolated from these mice display symptoms of excitotoxicity such as dendritic retraction and beading. These studies hold relevance not only to diseases of prion aggregation, but also to other neurodegenerative diseases characterized by protein aggregation.
Molly Congdon

Uncovering the expression, localization and biosynthesis of the GalNAc-Tyrosine post-translational modification

Molly D. Congdon1; Li, Xia1, Tiffany Bellomo1, Ruslan Gibadullin1, Thorkell Andresson2, Jeffrey C. Gildersleeve1

1 Chemical Biology Laboratory, NCI, NIH, Frederick, MD 21702, 2 Protein Characterization Laboratory Mass Spectrometry Center, NCI, NIH, Frederick, MD, 21701

Glycosylation is a vital post-translational modification (PTM) found on over 50 percent of all proteins and implicated in a range of biological processes including protein folding and localization, signaling pathways, and cell-cell interactions. Altered glycosylation profiles have been associated with disease progression and are a trademark of cancer. In 2011, a new type of O-linked glycosylation incorporating an N-acetylhexosamine (HexNAc) residue on the side chain oxygen of tyrosine was discovered. One study identified the core HexNAc-Tyr structure of this new PTM, as well as extended oligosaccharides, on Amyloid beta 1-X peptides. Additionally, these peptides were 2.5 times more abundant in cerebrospinal fluid of Alzheimer’s disease patients. In a second study, HexNAC-Tyr peptides were isolated from an engineered Simple Cell line using GalNAc-binding lectins. Furthermore, the GalNAc-Tyr modification was found on over 30 human proteins. Not only do these studies identify a novel O-glycosylation site, they suggest that the GalNAc-Tyr modification may be prevalent, and experience altered expression in some disease states. While these studies provide foundational insight into GalNAc-Tyr expression, little is known about GalNAc-Tyr biological synthesis, function, or expression in normal and disease states. We hypothesize that GalNAc-Tyr is a biologically significant but overlooked PTM due to the lack of GalNAc-Tyr selective tools and the small sampling of human tissues that have been examined. To investigate our hypothesis, we generated a GalNAc-Tyr selective monoclonal antibody, G10C. Based on studies with our glycan microarray, G10C has an apparent KD of 0.1 nM and is completely selective for GalNAc-Tyr versus other glycans possessing terminal GalNAc residues, such as Tn, Forssman, core 5 and Blood group A. Tissue arrays of normal and diseased states, as well as 9 human cancer cell lines were examined with G10C. Surprisingly, our data shows that GalNAc-Tyr is widely expressed intracellularly and in normal human tissue, suggesting it may have a fundamental role in cellular biology. Through immunoprecipitation of cell lysates with G10C, western blots and proteomic analysis we are currently identifying new proteins that possess the GalNAc-Tyr PTM. Finally, by overexpressing and knockout various GalNAc transferases, we are shedding light on the biosynthesis of the GalNAc-Tyr PTM.
Yuanwei Dai

Design and Synthesis of Selectively Fluorinated L-Fucose Analogs That Inhibit Proliferation of Cancer Cells and Angiogenic Cells

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Fucosylation is one of the most important cellular glycosylations involved in many diseases. Small molecule fucosylation inhibitors have shown promise as therapeutic agents in sickle cell disease, arthritis and cancer. We describe in this paper the design and synthesis of a panel of fluorinated fucose analogs bearing fluorine atoms at C2 and/or C6 positions of fucose as metabolic fucosylation inhibitors as well as a preliminary study of their effects on cell proliferation and migration in cancer cells. We found that 6,6-difluorofucose (3) and 6,6,6-trifluorofucose (6) showed significant inhibitory activity against proliferation of human colon cancer cells and human umbilical vein endothelial cells (HUVEC). In contrast, 2-fluorofucose has no effect on all the cell lines we tested. The cancer inhibitory activities of metabolic fucosylation inhibitors such as 3 and 6 demonstrate the possibility of a new approach for cancer therapy. To our knowledge, this is the first systematic study of fluorinated fucose derivatives for the inhibition of the proliferation of human cancer cells and angiogenic cells.
Joshua Dawson

Illuminating the Role of O-GlcNAcylation on PPAR Activation

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Nutrient-sensing O-Linked β-N-acetylglucosamine (O-GlcNAc) is a ubiquitous post-translational modification associated with diabetes, neurodegenerative disease, and cancer. Addition and removal of this sugar is mediated by O-GlcNAc Transferase (OGT) and O-GlcNAcase (OGA) respectively. Oga deletion in mice results in widespread transcriptional changes in neonates especially in growth- and metabolism-related genes. Interestingly, 90% of the Peroxisome Proliferator-Activated Receptor (PPAR) activated genes are deregulated in the neonatal liver of Oga deletion mice. PPAR(α/β/γ) transcription factors have essential roles in regulating the PPAR axis, however the mechanism by which O-GlcNAcylation affects activity of these trans elements and expression of their targets remains elusive. In order to investigate the impacts of changes in O-GlcNAc on transcription of PPAR target genes, two cell-culture based methods were devised to measure PPAR activation. Using this system, we demonstrate that OGA inhibition further promotes ligand activation of PPARα in HepG2 cells. Further, Plin2 expression, analyzed by qRT-PCR, was defined as a reliable endogenous output of PPARα activation. PPAR’s downstream targets provide extensive protection against metabolic diseases in part by promoting insulin sensitivity and enhancing the regulation of energy storage. Implementing this system to study O-GlcNAc’s role in PPAR activation lays promising groundwork to better understand how nutrient status may contribute to the etiology of chronic illnesses like diabetes.
Andrew DelaCourt

MALDI Imaging Mass Spectrometry of N-Glycan Profiles of Molecular Subclasses of Human Hepatocellular Carcinoma

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Introduction

Hepatocellular Carcinoma (HCC) is the second leading cause of cancer deaths globally, and the incidence rate in the US is predicted to exceed 50,000 patients by 2021. Recent work has identified significant changes in N-linked glycosylation directly in HCC tissue by MALDI glycan imaging. However, there was significant heterogeneity between HCC tissues, suggesting a potential correlation between the glycan changes and specific molecular subtypes of HCC. Therefore, this work will analyze HCC tissues, categorized by subtype, in an effort to fit glycosylation patterns within different classes of HCC. Methods

MALDI-IMS imaging was utilized, which uniquely allows for the analysis of spatially mapped N-glycans to paraffin embedded tissues. The sample set of HCC tissues consisted of Hoshida HCC subtypes 1, 2, 3.1 and 3.2 tissues. Tissues were prepared through a previously published protocol that involved antigen retrieval, spraying of PNGase F PrimeTM with a TM-Sprayer, then spraying of a MALDI matrix. This allows for N-glycans to be cleaved while retaining their spatial location on the tissue. Each tissue is then imaged on a Bruker MALDI solariX FT-ICR, and data is analyzed using flexImaging and SCiLS software.

Results

The primary goal of this work was to determine if differences within N-glycan profiles of HCC tissues are related to the differences between Hoshida genetic subtypes. Preliminary data showed tumor-specific glycan changes, yet there was heterogeneity within analyzed HCC tissues regarding the glycan structures. With categorized HCC tissues, glycan MALDI-IMS data showed trends that could approximately separate the subtypes. Subtype 1, which is less differentiated and associated with poor survival, showed more extreme tumor-associated glycan expression compared to surrounding normal and cirrhotic tissue. Using the co-localization feature from SCiLS, subtype 1 tissues showed a higher number of unique glycan structures that associated with the tumor tissue over surrounding normal and cirrhotic tissue. Subtype 1 showed an average of 18.00 ± 8.48 tumor-associated glycans, subtype 2 with 4.25 ± 2.21, and subtype 3 with 2.25 ± 1.71. While the identity of these glycan structures varied, trends emerged, particularly regarding how branched and fucosylated glycans associate to tumor tissue. Overall, this data shows that some of the heterogeneity in glycan expression of HCC tumors may be attributable to differences between genetic subtypes of HCC.
Regulated secretion is a vital process by which proteins, stored in membranous secretory vesicles, are released into the extracellular environment in response to either a hormonal or physiological stimulus. Defects in this process, as well as in the synthesis and maturation of the secreted proteins, can lead to numerous diseases, as this type of secretion occurs throughout various organs and is performed by many types of secretory cells. Exocrine cells, in particular, are often responsible for the synthesis and secretion of large, highly-glycosylated proteins, such as mucins. In the Drosophila salivary glands, these proteins are produced, packaged, and released during the third-instar larval stage, in response to developmentally-regulated pulses of the hormone, 20-hydroxyecdysone. This leads to a distinct spatiotemporal pattern of secretion, in which production and packaging of the mucin proteins begins in the posterior-most tip of the gland and gradually moves toward the anterior-most part of the gland. Therefore, in this study, we are using single-cell RNA sequencing of the Drosophila salivary gland to characterize the gene expression of individual cells at various stages of this secretory process. This will not only allow us to gain a better understanding of how highly-glycosylated proteins such as mucins are synthesized and secreted, but will also provide insight into the process of regulated secretion as a whole.
Mary Hackbarth

Collaborative Development of Therapeutics for Free Sialic Acid Storage Disease

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Lysosomal free sialic acid (SA) storage disease (FSASD) is an autosomal recessive, neurodegenerative, multisystemic disorder caused by defects in the lysosomal SA membrane carrier SLC17A5 (Sialin). SLC17A5 defects cause free SA and secondary metabolites to accumulate in lysosomes. The clinical spectrum ranges from severe infantile onset in infantile sialic acid storage disease (ISSD; elevated urine SA; ~40 reported cases), to a mild, adult form, called Salla disease (moderate elevation of urine SA; ~120 cases). Although sialic acid metabolism, membrane transport, and lysosomal biology have been extensively studied, the pathobiology of FSASD remains poorly understood. Moreover, FSASD is likely underdiagnosed; known patients have experienced a diagnostic delay due to the rarity of the disorder, non-specific clinical symptoms and absence of routine urine SA testing. There is no approved therapy for FSASD. As is typical for orphan diseases, the small population of patients makes it difficult to motivate industries to invest in performing the pre-clinical and clinical studies necessary to develop therapies. On the other hand, multidisciplinary collaborative efforts involving the NIH, academic clinical scientists, and patient advocacy groups have successfully overcome the scientific, clinical and financial challenges facing the development of new drug treatments for rare diseases. Encouraged by these successes, we have initiated a collaborative effort for FSASD. This has allowed us to start creating cell and mouse models, perform basic/translational research, initiate a natural history study to aid in the identification of biomarkers and treatment endpoints, raise awareness for FSASD, and investigate leads on drug candidates. We aim to collect data that incentivize industry to further develop, obtain approval, and commercialize FSASD treatments.
Duc Huynh

Site-specific O-GlcNAc Modification is a Regulatory Bridge between Kelch-like Proteins and Intermediate Filaments

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The Kelch-like (KLHL) protein family, a conserved group of 42 human members, binds to E3 ubiquitin ligase complexes to target protein substrates for proteasomal degradation. Although mutations in KLHL genes cause various human diseases—from cancer to neurodegeneration—their functional regulation remains understudied. Post-translational modifications influence protein fates, yet how they govern KLHL proteins is not fully explored. Our laboratory has examined the modification of KLHL proteins by O-linked-β-N-acetylglucosamine (O-GlcNAc), a form of intracellular glycosylation, governed by O-GlcNAc transferase and O-GlcNAcase, that decorates serine (S) or threonine (T) residues in response to cellular stimuli. Our previous work identified and characterized specific O-GlcNAcylated sites on the KLHL protein gigaxonin that are required for the efficient degradation of its substrates, intermediate filament (IF) proteins that include vimentin and neurofilament light (NF-L). Gigaxonin facilitates the ubiquitination and turnover of IF proteins, as loss-of-function gigaxonin is shown to cause IF accumulation in neurons, resulting in axonal swelling and degeneration in the disease giant axonal neuropathy. Notably, both vimentin and NF-L are modified by O-GlcNAc themselves. By mass spectrometry, photocrosslinking assay and live-cell imaging analysis, we discovered vimentin O-GlcNAcylation site S49 to be required for numerous functions: its self-association, normal IF morphology and cell migration. Our recent data additionally show that, compared to wild type (WT) vimentin, the glycosylation-deficient S49A mutant is a poorer substrate for WT or unglycosylatable gigaxonin alike. This finding demonstrates a potential regulatory bridge between KLHL and IF proteins by O-GlcNAc signaling. In addition, our preliminary result indicate that elevating global O-GlcNAc levels by OGT overexpression may redistribute NF-L into lower-order assembly states and enhance its O-GlcNAcylation significantly, suggesting a control of equilibrium among NF-L assembly states by O-GlcNAc. Taken together, our research proposes O-GlcNAcylation as a protein code for IF assembly, degradation and functions. Future work will characterize functionally important NF-L O-GlcNAcylation sites in homeostasis, changing nutrient availability and disease models. Our long-term goals are to explore site-specific O-GlcNAcylation as a functional link between KLHL and IF proteins in health and pathology.
Seokwon Jo

Pancreatic β-Cell O-GlcNAcase Ablation Perturbs Glucose Homeostasis in High-Fat Diet and Streptozotocin-Induced Diabetes

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O-GlcNAcylation is a dynamic post-translational modification, poised to integrate extracellular signal and nutrient status to exert intracellular response to regulate metabolism and glucose homeostasis. This process is maintained by O-GlcNAc Transferase (OGT) to add and O-GlcNAcase (OGA) to remove the O-GlcNAc moiety from target proteins. Our previous work showed that hypo-O-GlcNAcylation induced by pancreatic β-cell specific deletion of OGT leads to β-cell dysfunction and severe diabetes in mice. We also observed a failure to elicit hyper-insulinemic response to high-fat diet (HFD) feeding, in these OGT loss mice. Therefore, we hypothesized that the hyper-O-GlcNAcylation may confer protection from β-cell failure in conditions driven by HFD-induced obesity and streptozotocin (STZ)-induced diabetes. To test this hypothesis, we generated a mouse model of constitutive β-cell OGA knockout (RIPCre; OGA flox/flox, βOGAKO), where we specifically increased O-GlcNAcylation only in these cells. Under normal chow diet, βOGAKO mice exhibited normal glucose and insulin tolerance without apparent changes in serum insulin or glucose levels under fed and fasting conditions. However, when subjected to HFD metabolic stress, both male and female βOGAKO mice displayed normal body weight gain and insulin sensitivity but developed glucose intolerance that worsened with longer exposure to HFD. βOGAKO mice showed deficit in insulin secretion in vivo, suggesting HFD-precipitated β-cell failure in the OGA loss model. Under the STZ-induced β-cell depletion model, only the female mice developed hyperglycemia, suggesting sex specific role of OGA in the protection against β-cell death. Altogether, we demonstrated that the loss of OGA in β-cells increase its susceptibility to diabetogenic stressors via HFD and STZ, affecting whole body glucose homeostasis. These data reinforce the rheostat model of intracellular glycosyl-modification where too much (OGA loss) or too little (OGT loss) O-GlcNAcylation are both detrimental to a given system.
Su-Ryun Kim

Study on the Role of Core 3 O-glycans in Colorectal Cancer using Cell Models

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O-GalNAc glycosylation, also known as mucin-type O-glycosylation (O-glycosylation) which is characterized by α-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. Furthermore, existing cell lines do not express b3GnT6, and how the b3GnT6 is suppressed in colorectal cancer remains elusive. Herein, we firstly established characterize three colorectal tumor cell lines with the expression of b3GnT6. 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). Furthermore, expression of b3GnT6 in Cosmc-deficient colorectal cancer cell lines caused inhibition of cell growth and migration. Moreover, exogenously expression of CDX1 and CDX2, potential transcription factors for b3GnT6, resulted in the elevated expression of b3GnT6 thereby decreased expression of Tn antigens in the Cosmc-deficient cells. Our ongoing studies will further 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.
O-GlcNAc is a regulatory post-translational modification that is implicated in nervous system disorders including Alzheimer’s disease, and X-linked intellectual disability. This modification regulates diverse cellular processes in response to nutrient levels through its addition by O-GlcNAc transferase (OGT) and removal by the O-GlcNAcase. Genetic studies to examine the role of O-GlcNAc in disease have been complicated by the fact that OGT is an essential enzyme for early development in most animal models. Unlike other species, C. elegans survive to adulthood with loss of O-GlcNAc transferase (ogt-1) function, making them uniquely suited to study this modification. We have generated a CRISPR deletion of ogt-1 and determined that males have a four-fold reduction in mean offspring, with nearly two thirds failing to produce any offspring at all. The ability of a male to reproduce requires their proper development, production of viable sperm, and a complex neurobehavioral mating process. A series of experiments have demonstrated that ogt-1 males develop properly and produce a normal number of functional sperm. However, ogt-1 males transfer fewer sperm to mates than control males. Further, ogt-1 males are slower to initiate mating. These data suggest a defect in mating behavior is the most likely explanation for the phenotype. In support of this hypothesis, we find that neuron-specific expression of ogt-1 in the mutant males was sufficient to restore mating behavior in these males. These studies reveal a crucial role for OGT in complex neurobehavioral processes in C. elegans males and highlight the worm as an integral model organism that can provide insights into the neurological diseases associated with O-GlcNAc deregulation. Further genetic and molecular studies will define the specific neuronal mechanisms impacted by O-GlcNAc in the worm that can be translated into mammalian systems.
Characterization of mutations in GALNT3 that cause hyperphosphatemic familial tumoral calcinosis

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Hyperphosphatemic familial tumoral calcinosis (HFTC) is a rare autosomal recessive disorder caused by a deficiency of physiologically active fibroct growth factor 23 (FGF23), a hormone responsible for phosphate and vitamin D homeostasis. HFTC manifests across a broad clinical spectrum including, but not limited to, painful subcutaneous masses, calcification around joint spaces, hyperostosis of long bones, systemic inflammation, ocular involvement, and dental pathology. FGF23 undergoes post-translational O-glycosylation by the enzyme UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3) at sites including Thr178, which is thought to stabilize the hormone and protect it from degradation by furin. Previous studies have reported biallelic mutations in GALNT3 that cause HFTC. However, little is known about how these mutations affect the activity, substrate specificity, and stability of GALNT3. To study this, we examined patient mutations in GALNT3 through in silico modeling, protein expression, and biochemical characterization. In silico modeling revealed several mutations that failed to express in cell culture, likely disrupting bonding and resulting in misfolded proteins. Other mutations resulted in stably expressed proteins, but with dramatically lower activity levels. A mutation that cleaves the lectin domain of GALNT3 resulted in a stably expressed protein with reduced but detectable activity. Evidence from this work suggests a potential target for small molecule enhancers of enzymatic activity as a therapeutic treatment of HFTC.
Amber Lockridge

**Lipid Sensitive B-Cell O-GlcNAcylation Patterns in Prediabetic Obesity, Compensatory Hyperinsulinemia and SERCA2-Mediated Fatty Acid Stimulation of Insulin Secretion**

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University of Minnesota

Diet-induced obesity is the primary risk factor for type 2 diabetes, a condition defined by dysfunctional glucose metabolism. An early adaptation to overnutrition and insulin resistance in obesity is the hyperfunction of insulin-secreting β-cells, which is critical for maintaining euglycemia. There are many nutrient-sensing molecules in the β-cell that have been linked to underlying mechanisms of hyperinsulinemia but these disparate pathways lack a unifying contextual timer that can explain the temporal dynamics of insulin hypersecretion, which rises and falls within the early obesity environment of persistent hypernutrition and progressive insulin resistance. We hypothesized such a role for reversible protein O-GlcNAcylation, driven by lipid- and glucose-sensitive substrate production and catalyzed by OGT, which is highly expressed in β-cell containing islet clusters. Subsequently, we used western blotting with the pan-O-GlcNAc RL2 antibody to characterize increased islet O-GlcNAcylation in male and female mice after 6 weeks of high fat diet (HFD), concomitant with the onset of hyperinsulinemia. In islets from 18-week HFD mice, insulin hypersecretion was absent and both O-GlcNAcylation and OGT protein were below the level of standard chow fed controls. We then used constitutive and tamoxifen-induced β-cell OGT-loss mouse models to show that protein O-GlcNAcylation is required for in vivo hyperinsulinemia during the first 3 months of HFD and for in vitro fatty acid stimulated insulin secretion (FASIS). We tested whether FASIS could be rescued by overexpression of Pdx1, a major β-cell transcription factor and known OGT target, but this was not effective. We then examined SERCA2, the Ca2+-ATPase responsible for ER Ca2+ load, which contributes to pro-secretory signaling downstream of the GPR40 lipid receptor and prevents secretion-inhibiting ER stress, which we previously found in βOGT KO islets. We used immunoprecipitation to show that SERCA2 is O-GlcNAcylated in mouse clonal β-cells and human islets and the SERCA2 allosteric activator CDN1163 to rescue FASIS in βOGT KO islets. Our data suggests a hyperlipidemia-responsive O-GlcNAc timer on a subset of β-cell proteins that promotes insulin hypersecretion as a short-term adaptation to maintain glucose homeostasis during early obesity. As obesity progresses, decreased OGT levels may force a period of β-cell rest, a strategy for long-term β-cell survival that is increasingly implicated in diabetic resistance.
Mohit Mathew

O-GlcNAcylation of Galectin-3 and Nutrient Sensing

Mohit Mathew; John Hanover
NIDDK

Disease conditions like cancer and diabetes can be 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 can be 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, be regulated themselves by alterations in O-GlcNAcylation and effect changes in cellular behavior. CIE is essential for the survival of nucleated cells, however, there is little known about how this process is regulated. My preliminary data supports the notion that galectin 3 plays an important role in modulating CIE. Characterizing glycosylation’s unique role in regulating CIE in combination with 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. My hypothesis is that galectin 3 acts to detect and integrate information from all three forms of glycosylation and convert this information into functional effects. My preliminary data indicates that galectin 3 is O-GlcNAcylated, and that changes in O-GlcNAc cycling alters its secretion. Moreover, it was observed that there is a significant difference in O-GlcNAcylation status between cytoplasmic and secreted pools of galectin 3. These results indicate that O-GlcNAcylation of galectin 3 plays a role in modulating its secretion and thus can feedforward/feedback into its function of transducing nutrient sensing information coded into cell surface glycosylation into biological effects.
Carolyn May

Differential Splicing of The Lectin Domain of An O-Glycosyltransferase Modulates Peptide Preferences

Zulfeqhar Syed; Nadine Samara; Kelly Ten Hagen
NIH- NIDCR

Mucin-type O-glycosylation is an essential post-translational modification (PTM) that is required for secretion, extracellular matrix composition and organ growth. O-glycosylation is initiated by a large family of enzymes (GalNAcTs in mammals and PGANTs in Drosophila) that catalyze the addition of the sugar N-acetylgalactosamine (GalNAc), onto the hydroxyl groups of serines or threonines in protein substrates. These enzymes have distinct regions, including the catalytic domain and the C-terminal ricin-like lectin domain. The catalytic region is responsible for binding donor and acceptor substrates and catalyzing the transfer of GalNAc, while the lectin domain is thought to recognize extant GalNAc on previously glycosylated substrates to position the catalytic domain for further glycosylation of unmodified serines and threonines. Previous work from our group has demonstrated that one member of this family in Drosophila undergoes differential splicing within the lectin domain, leading to variants that contain either a positively or negatively charged α subunit. Interestingly, while this splicing event affects only the α subdomain of the lectin region, we find that it influences both peptide and glycopeptide specificity. We demonstrate that each charged enzyme variant has a preference for oppositely charged peptide substrates, and that this preference can be predictably altered by altering peptide charge. We further show that the expression of each splice variant is associated with the expression of appropriately charged mucins in secretory tissues of Drosophila, suggesting that differential splicing may be employed in vivo to ensure O-glycosylation of highly charged substrates. Finally, we demonstrate that each splice variant has unique glycopeptide preferences as well. This study provides the first demonstration that changes within a subregion of the non-catalytic lectin domain can alter the recognition of both peptide and glycopeptide substrates. Moreover, our results elucidate a novel mechanism for modulating substrate preferences of O-glycosyltransferases based on the needs of the cell through alternative splicing within specific subregions of functional domains.
Olurotimi Mesubi

**Excessive O-GlcNAc Promotes Atrial Fibrillation Susceptibility in Diabetic Mice**

Olurotimi Mesubi; Jonathan Granger; Rexford Ahima; Gerald Hart; Mark Anderson

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**Background:** Diabetes mellitus (DM) and atrial fibrillation (AF) are major public health problems that often co-exist and lack adequate therapies. Excessive protein O-GlcNAcylation (OGN) contributes to diabetic cardiomyopathy and arrhythmias by a hyperglycemia dependent pathway where excess glucose activates the hexosamine biosynthetic pathway to increase UDP-N-acetylglucosamine (UDP-GlcNAc). OGN post-translational modification is an evolutionarily conserved nutrient sensing/stress response pathway, catalyzed by the enzyme O-GlcNAc transferase (OGT) and reversed by the enzyme O-GlcNAcase (OGA). The pathophysiologic link between DM and AF is not well understood and the potential role of OGN in AF is untested.

**Methods:** AF susceptibility was assessed by a rapid atrial burst pacing protocol in type 1 (high dose streptozocin) DM (T1D) and type 2 (high fat diet + low dose streptozocin) DM (T2D) mouse models. Results: Atria from both T1D and T2D C57BL/6J male mice demonstrated elevated OGN levels. Both T1D and T2D mice showed similar increased susceptibility to AF compared to non-diabetic controls. For T1D mice [68% (17/25) vs. 25% (6/24), p < 0.01] and for T2D mice [71% (17/24) vs. 38% (8/21), p = 0.04]. Pharmacological inhibition of O-GlcNAc signaling by intraperitoneal injection of diazo-5-oxonorleucine (DON) prevented increased AF susceptibility in T1D mice [30% (6/20), p = 0.02], however DON-treated T2D mice were not protected from DM-primed AF susceptibility [60% (12/20), p = 0.5]. We generated transgenic mice with targeted myocardial overexpression of OGA (Tg-OGA mice). Genetic inhibition of OGN prevented increased AF susceptibility in both T1D mice [30% (7/23), p < 0.02] and T2D mice [36% (8/22), p = 0.04]. To determine potential downstream targets of OGN in AF, we tested the antiarrhythmic potential of DON in non-diabetic S2814D mice with a phosphomimetic CaMKII site genetically engineered into RyR2. These mice showed AF susceptibility at baseline [66% (6/9)] that was rescued by DON treatment [0% (0/6), p = 0.03].

**Conclusion:** These data support a role for OGN in the mechanism of AF in diabetes. Our findings support a view that proarrhythmic consequences of T1D and T2D in AF do not require a highly validated CaMKII target (RyR2 S2814). Further studies to understand the differences in signaling pathways in T1D and T2D that promote AF and to determine specific targets of OGN in proarrhythmic signaling in AF are necessary.
Hyunjin NA

Nutrient driven O-GlcNAc cycling controls DNA damage repair signaling and stem/progenitor cell homeostasis

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Stem/progenitor cells exhibit high proliferation rates, elevated nutrient uptake, altered metabolic flux, and stress-induced genome instability. O-GlcNAcylation is an essential post-translational modification mediated by OGT and OGA which act in a nutrient- and stress-responsive manner. The precise role of O-GlcNAc in adult stem cells and the relationship between O-GlcNAc and the DNA damage response (DDR) is poorly understood. Here, we show that blocked O-GlcNAc cycling leads to elevated insulin signaling, hyperproliferation and DDR activation that mimic the glucose- and oxidative stress-induced response. We discovered a novel feedback mechanism involving key downstream effectors of DDR, ATM, ATR, and CHK1/2, that regulate OGT stability to promote O-GlcNAcylation and elevate DDR. This O-GlcNAc-dependent regulatory pathway is critical for maintaining gut homeostasis in Drosophila and the DDR in mouse ES cells and MEFs. Our findings reveal a conserved mechanistic link between O-GlcNAc cycling, stem cell self-renewal, and DDR with profound implications for stem cell-derived diseases including cancer.
Vaishali Patel

Hs3st3a1; Hs3st3b1 Double Knockout Mice Have Disrupted Ductal Differentiation and Salivary Gland Hypofunction

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The fine control of growth factor function by heparan sulfate (HS) is influenced by structural heterogeneity of sulfated modifications. The most highly sulfated HS epitopes contain 3-O-sulfation. The HS-3-O-sulfotransferase family generates two types of 3-O-sulfated HS epitopes; Hs3st1-like, and Hs3st3-like that bind antithrombin and HSVgD1, respectively. The submandibular gland (SMG) express four of the seven Hs3st isoforms (Hs3st1, Hs3st3a1, Hs3st3b1, Hs3st6). We have previously shown that 3-O-sulfated HS controls epithelial progenitor cell proliferation and expansion during SMG development. We generated compound knockout (DKO) mice of Hs3st3a1 and Hs3st3b1 isoforms to investigate the role of 3-O-sulfated HS in vivo. These mice were viable, fertile and smaller in size with relatively larger SMGs than the wildtype mice. RNA-seq analysis of the SMG showed a reduction in Kit and Krt14, progenitor cell markers, and a decrease in ductal and myoepithelial markers in the DKO compared to wildtype confirming both the histological and immunofluorescent staining data. Pathway enrichment analysis of the differentially expressed genes of the DKO SMGs showed MAPK signaling and calcium reabsorption as two of the dysregulated pathways. We find that indeed calcium-dependent volume change was decreased, and apical-basal polarity was disrupted in the acinar clusters of DKO SMGs. Surprisingly, an increase in binding of FGF10:FGFR2b-Fc complex to endogenous HS in DKO glands was detected compared to wildtype SMGs using a ligand-and-carbohydrate engagement (LACE) assay. This suggests that the HS may be altered in the DKO SMG with loss of Hs3st3a1 and Hs3st3b1 enzymes leading to increased signaling complex. Finally, the DKO mice had reduced saliva flow and increased water intake indicating loss of saliva function. Understanding the 3-O-sulfated code of HS will be useful to manipulate the cellular specificity of HS-binding growth factors and to fine-tune biological responses to enhance progenitor expansion for tissue regeneration.
Mauro Pavao

Non-Anticoagulant Heparan Sulfate from The Ascidian Phallusia nigra Prevents Colon Carcinoma Metastasis in Mice by Disrupting Platelet-Tumor Cell Interaction

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Although metastasis is the primary cause of death on patients with malignant solid tumors, efficient antimetastatic therapies are not clinically available thus far. Sulfated glycosaminoglycans from marine sources have shown promising pharmacological effects, acting in different steps of the metastatic process. Oversulfated dermatan sulfate from ascidians is effective in preventing metastasis by inhibition of P-selectin, a platelet surface protein involved in the platelet-tumor cell emboli formation. We report in this work that the heparan sulfate isolated from the viscera of the ascidian Phallusia nigra drastically attenuates metastasis of colon carcinoma cells in mice. Our in vitro and in vivo assessments demonstrate that the P. nigra glycan has very low anticoagulant and antithrombotic activities and a reduced hypotension potential, although efficiently preventing metastasis. Therefore, it may be a promising candidate for the development of a novel anti-metastatic drug.
Caroline Rothermel

Microct Analysis of Trabecular and Cortical Bone in Mice Deficient for the O-Glycosyltransferase Galnt11

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Vitamin D plays an essential role in bone mineralization and its deficiency is associated with bone diseases such as rickets, osteomalacia, and osteoporosis. In order for vitamin D to carry out its important biological function, it must be converted to its active form within the epithelial cells of the kidney. Circulating 25-(OH) vitamin D3, complexed with vitamin D binding protein, is resorbed into the renal epithelial cells by megalin, an endocytic receptor in the kidney proximal tubules. It has recently been shown that the ability of megalin to bind to vitamin D binding protein depends on O-glycosylation by Galnt11, a member of the family of UDP-GalNAC:polypeptide N-acetylgalactosaminyltransferases that catalyze the addition of GalNAc onto serine and threonine residues. Galnt11 directly glycosylates the ligand-binding site of megalin, and mice deficient for Galnt11 exhibit reduced ligand-binding and age-related decline of megalin. Since the importance of vitamin D for bone health is already well characterized, this recent insight into a mechanism by which Galnt11 modulates vitamin D activity prompted us to investigate the role of Galnt11 on bone morphology. We used micro-computed tomography to compare the bone mineral density and various bone morphometric indices of femurs from Galnt11-deficient and wildtype mice. We observed the general trend that mice deficient for Galnt11 tend to have more trabecular bone and less cortical bone. In particular, Galnt11-deficient mice showed an increase in trabecular thickness, trabecular bone mineral density, and trabecular bone volume, along with a decrease in cortical area and cortical thickness, relative to wildtype mice. These differences were most striking in younger mice. Our findings suggest that the megalin-mediated effect of Galnt11 on vitamin D resorption has downstream effects on bone volume. This preliminary data suggests a role for Galnt11 in establishing and maintaining the integrity of the skeletal system. Further investigation into the mechanisms underlying our observations could have implications for the diagnosis and treatment of bone disease.
The clinical and biochemical phenotyping of MOGS-CDG, congenital disorders of glycosylation with glucosidase I deficiency

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Congenital disorders of glycosylation (CDG) are a group of inborn metabolic errors characterized by the altered protein and lipid glycosylation. Over 135 types of CDG have been reported, but they are often difficult to give diagnosis due to clinically heterogeneity. CDG with N-glycosylation defects can be subdivided into defects of N-linked glycan assembly or transfer (CDG I), and processing (CDG II). N-glycans processing during protein biosynthesis is site-specific, and is important for protein folding, stability, trafficking, localization and oligomerization. MOGS is the gene encoding mannosyl-oligosaccharide glucosidase, the first enzyme in the N-glycosylation that cleaves the distal alpha 1, 2-linked glucose residue from the Glc3Man9GlcNAc2 Oligosaccharide precursor. Mutations in MOGS cause CDGIib, also known as MOGS-CDG. Initial clinical screening of CDG is usually based on the investigation of glycoproteins transferrin, and/or apolipoprotein CIII. These biomarkers do not always detect complex or subtle defects, therefore there is a need to investigate additional glycoproteins. Recently, whole-exome sequencing has led to the identification of unknown etiology of CDGs. Through a multi-center collaboration, we expand the phenotyping of eight patients of MOGS-CDG. Detailed clinical investigations clarified new findings that include developed short stature, craniofacial features (high arched plate or cleft palate with retrognathia), bone (osteopenia, recurrent fractures and defect of osteoclasts phenotype), dental (Delayed eruption, hyperdontia and microdontia). All probands have severe neurodevelopmental phenotype (cortical visual impairment, sensorineural hearing impairment, brainstem dysfunction, microcephaly, early onset epileptic encephalopathy). Brain imaging showed brain atrophy (cortical, white matter, brainstem) in MRI and decreased NAA especially at pons in MRS. Since serum transferrin analysis to detect carbohydrate-deficient transferrin by either isoelectric focusing (IEF) or MALDI-TOF can appear normal, other biochemical methods for supporting diagnosis were employed. In our cohort, urine oligosaccharides analysis by TLC or MALDI-TOF MS showed abnormal pattern in 6/7 patients; these results might suggest that urine oligosaccharide analysis may be the most helpful biochemical test for MOGS-CDG diagnosis. Our work emphasizes the important of detailed clinical, biochemical and genetic investigations for establishing the diagnosis of MOGS-CDG.
Alicia Wong

Genetic ablation of the nutrient-sensor OGT in endocrine progenitors is dispensable for β-cell development but essential for β-cell identity maintenance

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O-GlcNAc Transferase (OGT) is a nutrient sensor enzyme that has previously been implicated in pancreatic β-cell function and whole-pancreas development, but little has been done to elucidate its role in the development and maintenance of other endocrine cells in the islet. To explore this, we genetically ablated OGT in endocrine (Ngn3-cre; OGTKOEndo) progenitors in mice. At birth, OGTKOEndo mice exhibited comparable α-cell and β-cell mass and normal islet architecture compared to littermate control, however, by postnatal (p) day 30, developed persistent hyperglycemia, with downward trends in both α-cell and β-cell mass. By p60, the OGTKOEndo mice displayed overt diabetes, accompanied by near-complete reductions in both α-cell and β-cell mass. To elucidate whether bihormonal cells contributed to the initial rise of blood glucose in OGTKOEndo mice, we assessed pancreatic islet architecture and morphology of normoglycemic OGTKOEndo and littermate control mice at p23. While there were no changes in α-cell and β-cell mass between OGTKOEndo and control, α-cells were seen to invade the islet core in OGTKOEndo, and a significant number of cells were observed to simultaneously express both insulin and glucagon. Mechanistically, we conjecture that the ablation of OGT in endocrine cells perturbed protein Pdx1 activity, previously shown to be post-translationally modified by OGT and implicated to play a role in the maintenance of β-cell fate. Indeed, aberrations in Pdx1 protein levels, expression, and localization were observed in the OGTKOEndo mice, with β-cells showing cytoplasmic Pdx1, β-cells lacking Pdx1, and α-cells expressing nuclear Pdx1. Current studies are aiming to rescue β-cell fate phenotype through the overexpression of Pdx1.
Eugenia Wulff

Pituitary Gland Hyper-O-GlcNAcylation drives Growth Hormone Deficiency

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The O-GlcNAc post-translational modification is dynamic and nutrient-sensing, reversibly added onto nucleocytoplasmic and mitochondrial proteins. O-GlcNAc is a unique glucose rheostat for cell signaling since the availability of nucleotide-sugar donor, UDP-GlcNAc, directly reflects extracellular glucose. To date, thousands of O-GlcNAcylated proteins have been identified. Numerous physiological processes are O-GlcNAc-regulated such as development, cell cycle, transcriptional/translational regulation, protein localization and degradation. Moreover, O-GlcNAc deregulation has been linked to pathologies like diabetes, cardiovascular diseases, neurodegeneration and cancers. Therefore, O-GlcNAcylation is a molecular bridge linking dietary glucose levels and proper signaling regulation. Using cellular and mouse models, we have previously delved into the consequences of hyper-O-GlcNAcylation in the brain. Among phenotypes such as early onset obesity and growth defects, the anterior pituitary gland was generally hypotrophic and mice presented signs of Growth Hormone (GH) deficiency. Here, we investigated the importance of O-GlcNAcylation in the anterior pituitary gland by locally knocking out the enzyme that removes the O-GlcNAc modification, O-GlcNAcase (Oga) (OgaΔPit). Despite an initial viability of the Oga KO mice, ~25% of the OgaΔPit failed to be metabolically self-sufficient and died shortly after weaning. These OgaΔPit mice had incomplete penetrance phenotype including impaired eye development, hydrocephalus and growth defects. Finally, GH deficiency was observed in surviving adult OgaΔPit mice compared to wild-type characterized by a decrease of circulating GH and Insulin-like growth factor 1 (IGF-1) levels. Taken together, these results suggest that controlled O-GlcNAcylation level is essential for the anterior pituitary gland development and endocrine function. More importantly, growth hormone-secreting cells (somatotrophs) seemed to be highly affected by O-GlcNAc cycling perturbation. Ongoing characterization of somatotroph-specific Oga KO, and equivalent Ogt KO mice, will further define the importance of the nutrient-dependent O-GlcNAc cycling in pituitary gland’s development and its various hormonal secretion.
Leila Aminova

Enzymes from thermophiles for production of sugar phosphates and activated sugars.

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Recently there has been rapid development in our understanding of the role played by a variety of sugars in biological processes. They are the vital components of all organisms and play a crucial role in structure-and-function of all living beings. Uncovering their role opens new avenues for research and medical applications, not the least of which is the study of viral biology, pathology, and development of new antiviral drugs. Viral glycoproteins and their host receptor glycans are involved in binding and infection and viruses have the ability to hijack the host glycosylation system to hide from our immune system. The ability to modify molecules with sugars may be key in developing new antiviral drugs and carbohydrates that mimic cell surface glycans to bind virus. As a result, there is an urgent need for sugar building blocks to help fuel the study of glycoscience. This includes basic sugars, rare sugars and deoxy-sugars and their activated forms. To meet this need we have successfully synthesized a variety of rare sugars that are important for research and applications in glycobiology. In particular, with we were able to synthesize l-Ribose, l-Galactose, l-Fucose, N-acetyl-d-glucosamine and over a dozen other sugars using engineered enzyme systems. We have also developed an engineered thermostable galactokinase that produces a broad variety of rare sugar-1-phosphates. This enzyme has been modified so that it not only produces its native product, Galactose-1-phosphate, at large scale with yields greater than 150 g/L but can utilize many other sugar substrates including l-glucose, l-arabinose, l-Xylose, 6-azido-6-deoxy-d-Galactose, l-fucose, 2-deoxy-d-Galactose, 6-amino-6-deoxy-d-Glucose and N-acetyl-d-Galactosamine. Finally, the production of activated sugars are important for synthesis of oligosaccharides and glycosylation of small molecules and proteins. d-Galactose-1-phosphate, l-Glucose-1-phosphate and 2-deoxy-d-galactose-1-phosphate were purified and used in a nucleotidytransferase reaction to prepare their respective activated sugar. For example, we were able to achieve conversion rates nearing 100% to produce 150g of UDP-α-D-Galactose. This enzyme also works well with GDP and TTP and we could produce UDP, GDP- and TDP-1-Glucose and 2-deoxy-1-Galactose. Using chemoenzymatic methods we were then able to demonstrate the synthesis of oligosaccharides and glycosylated small molecules using these activated sugars as starting materials.
Dynamic Interplay Between Tn-Glycosylated MUC1 and Human Macrophage Galactose Lectin Unraveled by ITC

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O-Glycosylation of mucins is assumed to serve as a versatile signal via recognition by tissue lectins. Human macrophage galactose-type lectin (hMGL, CD301 or CLEC10A), a C-type lectin expressed by dendritic cells and macrophages, is a receptor of Ser/Thr-linked α-GalNAc (T antigen nouvelle: Tn, CD175) and its α2,6-sialylated derivative (sTn). The relative contributions of the glycan and the peptide backbone to affinity are not well understood. In this study, the chemical synthesis of the tandem-repeat sequence of mucin MUC1, i.e. HGVTSAPDTRPAPGSTAPPA, and site-specific Thr-based glycopeptides facilitated calorimetric study of lectin binding. First, CD spectroscopy revealed stabilization of the polyproline II (PPII) structure with increasing density of the glycan epitope. Binding is an enthalpy-driven process, affinity enhancement occurred for glycopeptides relative to free GalNAc. Size of enthalpic gains were associated to valency, the monoglycosylated peptides had ΔH of about -10 kcal mol⁻¹ and the triply glycosylated ligands had ΔH -30.3 kcal mol⁻¹. Increases in affinity (Kd) by one order of magnitude were determined for mono- (6.9 μM) to triglycosylated (600 nM) MUC1 peptides. Analysis of the kinetic profiles of binding of the MUC1 glycopeptides to hMGL appears to support involvement of a “bind and jump” mechanism. The lifetimes (lower koff values) increase as the density of GalNAc increases along the MUC1 20-mer sequence. In order to assess a role of solvent reorganization to binding to a C-type lectin, experiments were performed in parallel in D2O. Unlike GalNAc binding, H/D exchange resulted in more favorable enthalpic contributions, for the monoglycosylated analogs accompanied by compensating higher entropic penalty. Peptide backbone presence makes a difference, as glycan density was revealed to do. In summary, our results suggest that contact building of MUC1 glycopeptides with hMGL critically depends on structural characteristics in the vicinity of the glycan and its density.

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It is becoming increasingly clear that changes in the glycan composition of glycoproteins in different biofluids are a dynamic metabolic indicator of health and immune status. In recent years, especially for blood-derived fluids, large cohorts of patient samples have been analyzed for total N-glycan content or for N-glycan compositions on immunoglobulin G. Most current analysis methods require significant sample processing, derivatization and purification steps prior to detection. Combined with the amount of time required for these steps, clinical diagnostic applications of this approach for glycans are limited. Using workflows adapted from MALDI imaging mass spectrometry of N-glycans in tissues, we have developed and optimized a novel slide-based approach for analyzing the total N-glycan composition of clinical biofluids like serum and plasma. Serum and plasma samples were de-identified and pooled from healthy donors. Small aliquots of unprocessed samples (1 µL) were mixed with sodium bicarbonate and spotted directly on amine-reactive hydrogel-coated slides. Well modules were attached to the slide, and the samples were washed with Carnoy’s solution and water. A molecular coating of recombinant PNGase F was sprayed onto the slides using an HTX TM Sprayer. After a two-hour incubation, CHCA matrix was applied and native N-glycan profiles were acquired for each spot by MALDI FTICR MS in positive ion mode. Data were visualized and analyzed using SCiLS Lab software. Depending on the sample and preparation conditions, 75 or more N-glycan species in the mass range m/z = 1100-3000 can be detected from a single serum spot (a 1 ul equivalent). Processing times as low as 6 hours are possible, and further optimization can likely decrease this time. Sensitive and reproducible N-glycan profiling is possible with both serum and plasma. The method was adapted for the application of Endo F3 mixed with PNGase F for an alternative form of analysis. The potential application of this method to determine N-glycan differences between clinically relevant sets of biofluids was demonstrated by analyzing pooled serum from non-obese or obese patients with benign breast lesions or breast cancer. The method allows large numbers of samples to be reproducibly analyzed with minimal processing time and sample manipulation requirements, with the long-range goal to develop the approach for routine application to clinical laboratory assays.
Capture-and-Release (CaRe) is a fast and facile method for lectin and glycoprotein purification

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Glycan-binding proteins (GBP) including lectins and glycosaminoglycan-binding proteins (GAGBPs) mediate many biological functions. To study their biological activities and structure-function relationships, researchers must use these proteins in their purest form. Conventional purification techniques have been instrumental in isolating numerous GBPs and glycoproteins. These approaches, however, are time-consuming, consist of multiple steps and often require extensive trial-and-error experimentation. Therefore, methods that are relatively rapid and simple are needed. Here we describe such a method termed “Capture and Release” (CaRe). The strength of CaRe is rooted in its simplicity, accuracy, expeditiousness and sample economy. CaRe purifies target lectins and glycoproteins by utilizing their inherent ability to form spontaneous non-covalently cross-linked complexes with specific multivalent binding partners. The targets (lectins or glycoproteins) are captured in the solution phase by respective multivalent capturing agents, separated from other impurities, and then the captured targets are released from the capturing agents by competitive monovalent ligands. The released targets are separated from the capturing agents by membrane filtration or size exclusion chromatography. CaRe does not require antibodies, solid affinity matrices, specialized detectors, a customized apparatus, controlled environments or functionalization or covalent modification of reagents. CaRe is a time-saving procedure that can purify target proteins even from a few milliliters of crude extracts. We validated CaRe by purifying recombinant human galectin-3 and five other known lectins and also tested CaRe’s ability to purify glycoproteins. Besides purifying lectins and glycoproteins, CaRe has the potential to purify other glycoconjugates including proteoglycans. This technique could also be employed for the detection and purification of non-lectin or non-glycosylated proteins that bind multivalent ligands. Given the ubiquity of GBPs, glycoproteins and proteoglycans in nature, we anticipate that the application of CaRe will be broad. This method combined with proteomic analysis could be a powerful approach for discovering new GBPs and glycoconjugates. Funding: National Science Foundation
Arun Datta

GLYCOMICS WORKBENCH, a grid technology-based workbench for Glycome Analysis.

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A significant number of software tools and databases have been developed over the years for glycome analysis (1). However, full utilization of some of those useful tools is restricted due to the fact that those are not accessible via Web. This also restricts the semantic analysis of a vast amount of experimental data that were generated under the Consortium of Functional Glycomics (CFG), which is now hosted at the National Center for Functional Glycomics (2). This author proposes the development of a grid technology-based infrastructure, termed ‘Glycomics Workbench’, to integrate such useful computational tools and resources that can better serve the Glyco-community. Grid technology offers multiple advantages including high scalability and Web accessibility. Grid infrastructure includes grid services, grid computing, and data grid. Grid computing provides accessibility to High-Performance Computing, such as, XSEDE (3). Grid services built on Open Grid Collaborating Environments (OGCE) are based on several Web service technologies. Our earlier work on neoGrid (4,5) development was built on OGCE. Data grid is a commodity grid that can host exabytes of data that has become essential for glycome analysis. Our work on C-Grid (6,7) development was to fulfill that need. This proposed Glycomics Workbench development, however, needs active participation of researchers for creating ‘molecule page’ of their interest, an effort that can be achieved through a Consortium.

2. National Center for Functional Glycomics (NCFG; https://ncfg.hms.harvard.edu)
3. XSEDE (https://www.xsede.org), an NSF funded program.
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Ashley DeYong

Automated flow syntheses of fucosylated amino acids

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The O-linked fucose post-translational modification was first discovered almost half a century ago when it was isolated from human urine. From there, the importance of this modification has been described through a plethora of biological and analytical studies from isolated material, and it has been shown that this modification is known to play an important role in the Notch signaling pathway, and several diseases are implicated by mutations in Notch including T-cell lymphoblastic leukemia, multiple sclerosis, several heart defects, and breast cancer. Despite knowing the importance of this modification, there still remains information to be gained through chemical synthesis in that it would allow for higher quantities of pure material. This work specifically focuses on the chemical syntheses of fucosylated amino acid building blocks, which can then be incorporated into larger biomolecular structures and probe the structure and function of these systems to gain further insight regarding the importance of these modifications. These building blocks can additionally be used directly in SPPS. Chemical syntheses of these targets can be challenging because of the acid-labile linkage of fucose, and a fast and effective way to synthesize these targets is yet to be determined. An automated flow approach has the potential to improve the syntheses of these building blocks. Flow chemistry can address the long reaction times of the previous syntheses of these targets through efficient heat transfer and better surface area to volume ratio than batch reactions. It would additionally allow for better scalability in synthesizing these building blocks. Scalability and efficiency in these syntheses can address the high commercial cost of these building blocks and make them more accessible. Automating this reaction would allow for more straightforward optimization and reproducibility. Herein we present the progress towards developing a one-step method from commercially available reagents to synthesize O-linked fucosylated amino acid building blocks, including fucose coupled with serine, threonine, hydroxyproline, and tyrosine, using automated flow.
Nathan Edwards

GPTwiki: Glycopeptide Transitions for Targeted LC-MS Quantitation of Site-Specific Protein Glycoforms

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Quantitation of site-specific protein glycoforms by targeted mass-spectrometry techniques requires specialized methods, instrument settings, and prior knowledge of readily observed glycopeptide fragment ions for each glycoform of interest. The Clinical and Translational Glycoscience Research Center aims to address these barriers to adoption by developing methods for glycopeptide MRM, PRM and DIA/SWATH analyses for mass spectrometers widely used in proteomics core facilities; assembling comprehensive libraries of glycopeptide fragmentation spectra; developing informatics tools for extraction and display of glycopeptide transitions with their mass-spectra evidence; and constructing a cloud-based analysis pipeline for glycopeptide DIA/SWATH data. Central to these goals is GPTwiki, a database of glycopeptide transitions with normalized retention-times and spectral evidence. GPTwiki has been built from DDA glycopeptide analyses of human serum and HEK293 cells and by importing the NIST IgG mAb standard glycopeptide library, providing chromatograms and paired EThcD and CID spectral evidence for 1060 glycopeptides from 80 proteins with 85 (composition-based) N-glycans on 180 N-glycopeptides. Glycopeptide transitions extracted from GPTwiki are applied to Thermo Lumos Fusion and Sciex 6600 DIA analyses using the open-source OpenSWATH package to achieve high-throughput quantitation of site-specific protein glycoforms.
Biomolecular structure and conformation play essential roles in biological function. Conceptualization and analysis of biomolecular structure are often approached through the sense of sight. However, visualization of biomolecules represents a barrier to education and research for students who are blind. Recently, we released the TactViz plugin for the widely used Visual Molecular Dynamics (VMD) software to support tactile visualization of protein structures. Now, we have extended TactViz to support tactile visualization of carbohydrate and glycan structures. TactViz applies a representation scheme consistent with the conventions of tactile graphics, and images rendered with TactViz convey concepts of 3D biomolecular structure in variable-height 2D images when printed with widely available Swell Form or Picture in a Flash tactile graphics machines. Further, TactViz supports interactive visualization of protein and carbohydrate structures on electronic refreshable tactile display devices, such as the Graphiti. The development of TactViz has enabled our undergraduate student who is blind to conduct computational biophysical chemistry research with reduced sighted assistance, setting an important precedent and providing essential tools to promote inclusion of individuals who are blind in biology-related STEM fields.
Mallory Kern

Automated Solution-Phase Synthesis of S-Glycosides with Highly Reactive Glycosyl Donors

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Thioglycosides are S-linked glycoside analogs more resistant to chemical and enzymatic hydrolysis than their O-linked counterparts that thereby make attractive targets for carbohydrate-based therapeutic development. Herein, we report the first development of methods for the site-selective incorporation of S-linkages into automated solution-phase oligosaccharide protocols. For the automated synthesis of the oligosaccharides, armed and super-armed glycosyl donors were utilized with minimal side product formation. The protocols were also shown to be compatible with the subsequent formation of S- or O-glycosides in the context of the first synthesis of mannopyranoside trimers that incorporate both S- and O-linkages to allow selective incorporation of an S-glycoside at various stages in an automated oligosaccharide synthesis program.
Teodora Kljaic

Highly Precise and Direct Competitive Kinetic Isotope Effect Measurement Using Whole Molecule MALDI-TOF Mass Spectrometry

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Measurement of kinetic isotope effects (KIEs) is a well-established and powerful tool to study enzyme reaction mechanisms and elucidating transition states. The accurate measurement of KIEs for heavy atoms surrounding the reaction center requires sensitive, precise and quantitative measurements. The widespread use of KIEs to investigate enzyme mechanisms is limited by current methods due to the lack of availability of suitable radioisotope labeled substrates or the requirement for large quantities of multiple isotope labelled substrates. Here, we report a method for the direct measurement of competitive KIEs using a whole molecule MALDI-TOF mass spectrometry. The approach uses isotope labeled internal standards introduced during reaction quenching to improve quantitative measurement of substrate concentrations and isotope ratios. This approach reduces the amount of isotope labeled material required for analysis without the need for purification prior to analysis, making it applicable to a wide variety of enzymatic reactions. We have applied this approach to measure 1-13C KIEs for the glycosyltransferase BshA required for bacillithiol biosynthesis in Gram-positive firmicutes that plays a role in Fosfomycin antibiotic resistance.
Stacy Malaker

Defining the "mucinome": Enzyme toolkit for selective enrichment and analysis of mucin-domain glycoproteins

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Mucin domains are densely O-glycosylated 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. We first expressed and characterized a bacterial mucinase, StcE, and demonstrated that it selectively cleaves mucins in a glycan- and peptide-specific manner. We went on to use 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, and it was found 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.
Control of population dynamics and metabolome in mixed cultures by engineered metabolism of human milk oligosaccharides

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The synbiotic relationship between human milk oligosaccharides (HMOs) and probiotic bifidobacteria exemplifies prebiotic control of microbial community dynamics. Inspired by this example, we have engineered the well-known probiotic, E. coli Nissle, to metabolize HMOs and used this metabolism to control population dynamics and protein expression in mixed cultures. We accomplish this using a unique whole-cell biosensor which provides linkage-specific, quantitative detection of several trisaccharide and tetrasaccharide HMOs. Addition of these complex substrates to synthetic microbial consortia orthogonally controls growth rate or protein expression of particular strains. We can also mimic selfish, altruistic, and social cheating ecological behaviors in these communities. In addition, we performed further metabolic engineering on our target probiotic, enabling production of short-chain fatty acids from HMOs as sole carbon sources, and recapitulating an important function of the infant gut microbiota. Combining the two approaches, we show that by controlling population dynamics, we can control the metabolome of the mixed culture. Finally, we applied our high-throughput screen to enhance the activity and substrate specificity of glycosidases, demonstrating the potential of this method to expand the glycobiology toolbox. This work lays the groundwork for creation of a unique ecological niche for engineered live biotherapeutics in the resource-limited gut environment as well as for the prebiotic manipulation of population dynamics in natural and engineered microbial communities.
Daniel Mattox

Structural binding determinants of lectins: A survey of lectin-glycan interactions in the PDB

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Lectins are central to recognizing and interpreting the complex glycan code that directs so many biological processes. To better study these processes, we need a thorough understanding of the highly nuanced interactions between lectins and glycans. While individual lectin-glycan binding interactions are well studied, we are still striving to uncover overarching principles that can differentiate more global binding patterns. At present, robust data-driven models for examining lectin specificities are lacking, in part due to the complexity of glycan structures, difficulty differentiating between similar glycans, and the relatively recent arrival of glycan microarrays for studying lectin binding. We hypothesize that through a data-centric approach to studying lectin-glycan interactions, we can learn what features drive binding interactions and be able to predict and engineer novel interactions. As the number of solved structures in the Protein Databank (PDB) grows, there are increasing opportunities to glean more information about the biophysical determinants of lectin specificity. In this project, we have identified 1,376 lectin structures co-complexed with a glycan ligand through the UniLectin3D database. We thoroughly characterized these lectin-glycan interactions along with the general binding site. For each glycan, we then looked for the enrichment of specific lectin features or sets of features over the background distribution, implying an association with specificity. In future work, we plan to link the characterization of these binding pockets to more detailed specificity information from glycan microarrays hosted by the Consortium for Functional Glycomics. Having more general knowledge about the determinants of lectins' specificities will enable the prediction of novel protein-glycan interactions and advance lectins for use in diagnostics and antiviral therapeutics.
Mana Mukherjee

Synthesis of the complete O-antigen of Vibrio cholerae O1, serotype Inaba

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The O-SPs of the two strains of V. cholerae O1, Ogawa and Inaba, consist of a chain of 15-20 (1-2)-α-linked repeats of 4-amino-4,6-dideoxy-D-mannopyranose (D-perosamine), {1} where the amino group is acylated with 3-deoxy-L-glycero-tetronic acid. {2} The two serotypes differ in that the terminal, upstream residue is methylated at O-2 in the Ogawa O-SP. Here we report the first synthesis of the 18-mer of the monosaccharide repeat, which is, essentially, the complete O-antigen of serotype Inaba. On the way towards the octadecasaccharide, we used a mono-, tri- and hexasaccharide glycosyl donors, containing 4-azido group as a latent amino group. These were coupled with suitable glycosyl acceptors. The dodecasaccharide was built via coupling of a hexasaccharide donor and a hexasaccharide acceptor. The corresponding octadecasaccharide was built via coupling of a hexasaccharide donor and a dodecasaccharide acceptor. D-Perosamine being a rare sugar, we had to make its immediate precursor on a few hundred gram scale from the commercially available methyl α-D-mannopyranoside. Many reaction protocols involved have been substantially improved, compared to the conversions developed earlier, and gave the desired products in excellent yields and with high stereoselectivity. Most intermediates were obtained in the analytically pure state and were fully characterized for the first time. References 1. a) Manning, P.A.; Stroehrer, U. H.; Morona, R.; Ed. Wachsmuth, I. K.; Blake, P. A.; Olsvik, O. Vibri cholerae and cholera, American society for microbiology, Washington DC, 1984, p. 77.; b) Dick Jr., W. E.; Beuret, M.; Ed. Cruse, J. M.; Lewis, R. E. Conjugate Vaccines, 1989, 10, p. 48. 2. Kenne, L.; Lindberg, B.; Unger, P.; Gustafsson, B.; Holme, T. Carbohydr. Res., 1982, 100, 341.
Carbohydrates play a major role and occur in nature with high complexity. The fingerprinting of carbohydrates to elucidate its structural features is often challenging. Also, databases containing information on sequences of carbohydrate oligosaccharide and glycoconjugate structures are not often publicly available. MS coupled to LC has become the robust method of analysis for carbohydrates for many biological samples. However, this method alone is insufficient especially when known standards are not available. This study employed the LC-MS and hybrid-searched-based ‘bootstrapping’ method for identification and annotation of oligosaccharides and glycoconjugates in complex matrices for the development of MS library building of different carbohydrate structures. The separations were performed on a HILIC-UHPLC system coupled to an Orbitrap MS for oligosaccharides from human and other mammals, released glycans from human serum and gangliosides from bovine and porcine. Data analysis tools like hybrid search ‘bootstrap’ identification method along with in-house programs were used in this study. The key to the analysis of compounds not in the library was the DeltaMass value generated by the hybrid search, which is the difference in mass between the query and library compounds. When scores were >800, and DM values corresponded to multiples of common glycan components, spectra were manually examined and assigned putative structures. For illustration, a library searching of unknown spectra of oligosaccharides was done and resulted in 74 annotated milk oligosaccharides. The newly hybrid-search bootstrapping method was applied. This involved hybrid searching of the previous library, which was itself constructed using the hybrid search of oligosaccharide spectra in the NIST 17 Library. The coverage of oligosaccharides was significantly extended using milks from a variety of mammals. This new method led to the identification of another 145 HMOs, including an additional 80 HMOs from reanalysis of human milk. The newly identified HMO structures contain 9 to 18 monosaccharide units with core structures: lacto-N-dodecaose and lacto-N-tetradodecaose carrying fucose residues. These have not been reported to be present in human milk. The newly identified compounds were added to a freely available MS database of 219 HMOs. Such a ‘bootstrap’ approach appears to provide a general means of expanding libraries for other classes of compounds such as glycolipids and released glycans.
Nicholas Riley

**O-Pair Searching with MetaMorpheus for O-glycopeptide Characterization**

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Mass spectrometry (MS) is the gold standard for interrogating the glycoproteome, enabling the localization of glycans to specific glycosites. Yet, standard approaches for interpreting tandem MS spectra are ill-suited for features inherent to O-glycosylation, including O-glycan heterogeneity and dense stretches of O-glycosylation in serine/threonine rich sequences. Current analysis pipelines are unable to search for multiply glycosylated peptides within reasonable time frames even for simple mixtures of O-glycoproteins, much less for proteome-scale experiments. Moreover, current software tools for O-glycopeptide identification fail to capitalize on modern MS-acquisition methods, e.g., combinations of collision-based and electron-based dissociation within the same analysis, and they lack the ability to confidently localize glycosites within multiply glycosylated O-glycopeptides. Here we describe the O-Pair Search strategy implemented in the MetaMorpheus platform. Using paired collision- and electron-based dissociation spectra collected for the same precursor ion, O-Pair Search identifies O-glycopeptides in four steps: 1) rapid identification of peptide candidates using a fragment ion indexing search strategy, 2) determination of possible O-glycans present on peptide candidates using combined glycan total masses, 3) localization of individual O-glycans to specific O-glycosites using graph theory for spectra from electron-driven dissociation, and 4) calculation of probability-based localization scores for each localized glycosite, a first for glycopeptides. With O-Pair Search, we show that search times for O-glycopeptides from simple mixtures can be reduced by >1000x over the most widely used commercial glycopeptide search tool (Byonic), requiring 12 hours using Byonic. Additionally, O-Pair Search identifies more O-glycopeptide identifications than Byonic and reports localization levels that indicate if all (Level 1), at least one (Level 2), or none (Level 3) of the O-glycosites are confidently localized — a feature previously unavailable on any other platform. We further demonstrate the utility of O-Pair Search by performing searches using larger glycan databases, larger protein databases, and O-glycoproteomic data from complex mixtures (i.e., searches that are not practical in Byonic). Our software is free, reliable, fast, completely open-source, user-friendly, and readily accessible to researchers.
Abstract Number 44

Miloslav Sanda

Low collision energy fragmentation in the structure-specific glycoproteomics analysis

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Mass spectrometry (MS) is one of the most efficient tools used in the current studies of glycoproteins and their structure identification. Collision energy (CE) is a crucial instrument parameter that can be exploited to improve structural resolution because different linkages of glycan units show different stability under CID/HCD fragmentation. Here we report the utility of CE modulation for qualitative and quantitative analysis of site- and structure- specific glycoforms of proteins. Using CE modulation, we were able to break selectively specific glycan linkages on intact glycopeptides and get structure-specific mass spectrometric signals. Structure- and CE- specific oxonium ions provide sufficient information for the resolution of outer arm structure motifs. Glycopeptides obtained from protein standards, plasma, cell lysates and cell secretomes were measured using EThed, HCD and CID with multiple collision energy on the Orbitrap Fusion Lumos (Thermo), 6600 TripleTOF (Sciex) and timsTOF mass spectrometers. Glycopeptides were separated using capillary reverse phase (C18) nano-chromatography prior to mass spectrometry analysis. Data were processed using Bionic software and all structure specific fragments confirmed manually. We document that modulation of CID/HCD fragmentation of N-glycans attached to specific peptides provides structure-specific information which is not retrieved at high CE typical of proteomic or glycoproteomic workflows. Under low CE fragmentation conditions, we retrieve fragments diagnostic of common outer arm structural motifs which can resolve isobaric structures attached to the same peptide. We expect that glycopeptide quantification, utilizing the structure-specific ions, will allow quantification of isobaric glycopeptides using previously published methods based on B- and Y-ion quantification (refs). Our results suggest that inclusion of the relative intensity of oxonium ions at defined CE settings to the scoring algorithm would improve automated identification of structure-specific ions in current software tools.
Benjamin Schumann

Dissecting O-GalNAc glycosylation by glycosyltransferase engineering

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O-GalNAc glycosylation is a major constituent of the cell surface glyco-code. Glycosylation is primed by 20 GalNAc transferase (GalNAc-T) isoenzymes that introduce the first, Ser/Thr-linked GalNAc residue using UDP-GalNAc as a sugar donor. Despite partial redundancy, GalNAc-Ts have been differentially associated with disease, suggesting a pivotal role of isoenzyme-specific protein substrates. However, studying these substrates by mass spectrometry (MS) glycoproteomics approaches is complicated by the crosstalk of different isoenzymes with each other. Here, a chemical biology method termed “bump-and-hole engineering” is used to dissect the details of GalNAc-T isoenzyme specificity in the living cell. In a structure-guided process, the active site of a GalNAc-T is enlarged by mutation, creating a “hole” that renders the enzyme compatible with a chemical functionality (“bump”) in a synthetic UDP-GalNAc derivative. Structural and functional characterization ensures viability of the orthogonal enzyme-substrate pair to glycosylate native protein substrates. A traceable chemical handle in the bump allows for the specific detection of glycoproteins by bioorthogonal ligation. The GalNAc salvage pathway is re-programmed to deliver bumped UDP-GalNAc derivatives to the cell, and MS glycoproteomics enables the characterization of GalNAc-T isoenzyme-specific glycosylation sites and glycan structure in a single experiment. We further show that the chemical handle can be tailored to suppress epimerization to the corresponding UDP-GlcNAc derivatives, thereby considerably reducing the complexity of glycoprotein labeling. This process yields the second generation of bumped GalNAc derivatives as precision tools to investigate the biology of O-GalNAc glycans.
Neisseria meningitidis is a Gram-negative bacterium that causes meningitis. Our overall goal is increased understanding of the N. meningitidis serogroup W capsule polymerase. This enzyme creates the capsular polysaccharide found in this serogroup. During the reaction, the enzyme transfers galactose and sialic acid from two nucleotide donor sugars (UDP-Galactose and CMP-Sialic Acid) to an acceptor. This capsule polymerase can be used as a new tool for glycoconjugate vaccine development. In this work, we describe our efforts to determine kinetic parameters of the enzyme which are currently unknown. We have optimized commercially available bioluminescence-based assay to investigate activity of the enzyme (UDP-Glo by Promega). Reactions were performed to determine Km and Vmax values in a 10-minute reaction. To simplify our studies, we have recently moved to use of a homogeneous acceptor, a sialic acid trimer (DP3). In our studies with this acceptor, we confirm an increase in product formation with increasing enzyme amounts (0-1250 ng enzyme). Km and Vmax values were determined for UDP-Galactose (44.61 µM and 0.009457 µM/min) and DP3 acceptor (2984.2 µM and 0.01099 µM/min). We also made DP3-Galactose as a modified acceptor by chemoenzymatic reaction of W capsule polymerase. In future work, we will perform kinetic studies with this modified acceptor (DP3-Gal) to determine Km and Vmax for this acceptor and CMP-Sialic acid. Research support by: NIH ULIMD009605; NIH 1SC2GM125517-01
Nandini Singh

Exploring the Role of Amyloid-Beta Precursor Protein (APP) O-Glycosylation in Alzheimer’s Disease

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The amyloid-β precursor protein (APP) is a transmembrane protein that can undergo proteolytic cleavage to determine its fate in Alzheimer’s disease (AD) pathogenesis. Only 10% of the protein undergoes proteolysis by β- and γ-secretases to produce amyloid-β peptides, which is a hallmark of AD, whereas the remaining 90% will undergo proteolysis by α-secretase and γ-secretases, resulting in the non-amyloidogenic pathway. Recently, it was found that the four threonine residues Thr633, Thr651, Thr652, and Thr659, in the vicinity of the β-secretase cleavage site (Met671-Asp672) of APP, are modified by complex mucin-type O-glycans. Moreover, an increase of up to 2.5 times in Tyr681 glycosylation, located within the Aβ42 region of APP, was found in AD patients in comparison to the non-AD patients. These findings suggest the possible role of O-glycosylation in APP proteolytic processing. Therefore, we synthesized native and Swedish-double-mutated (Met671Asn and Asp672Leu) APP (glyco)peptides with O-GalNAc and studied conformational changes, secretase activity, and aggregation kinetics using circular dichroism, enzyme kinetics and Thioflavin T assays, respectively. Our results show that the non-glycosylated peptide analogs in water show characteristics of β-sheet conformation, however, when there is a site-specific O-glycosylation on Thr, Ser or Tyr residues of their counterparts, there is a change in conformation that resembles a mixture of α-helix and random coil. All peptides adopt an α-helix in 50/50 (v/v) trifluoroethanol/water mixture (membrane environment) and random coil in phosphate buffer, pH 7.4 (physiological environment). Furthermore, the level of β-secretase activity significantly increases for the glycosylated analogs containing the Swedish mutation compared to their non-glycosylated counterparts. Lastly, the glycosylated analogs impact the protein’s aggregation kinetics by decreasing its lag phase and taking an early onset for aggregation. In conclusion, our results suggest that APP’s site-specific O-glycosylation along with Swedish mutation can induce a conformational change in the protein and increase its proteolytic processing by β-secretase, rendering its fate towards the amyloidogenic pathway. Our long-term goals are to assess the role of certain patterns of multiple glycosylation sites in close proximity of the relevant secretases cleavage sites and develop specific inhibitors of the amyloid or activators of the non-amyloid pathways.
Nitin Supekar

Deducing the N- and O- glycosylation profile of the spike protein of novel coronavirus SARS-CoV-2

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The acute emergence of the novel coronavirus pandemic caused by SARS-CoV-2 requires the development of new therapeutic strategies to prevent rapid progress of mortalities. The most interesting target for this endeavor is the surface protein of the coronavirus, namely spike (S) protein. It facilitates viral attachment, entry and membrane fusion and plays a critical role in the elicitation of the host immune response. The spike protein is comprised of two protein subunits (S1 and S2), which together possess 22 potential N-glycosylation sites. Our study reports the glycosylation mapping on spike protein subunits S1 and S2 expressed in human cells through high resolution mass spectrometry: Not only have we characterized the N-glycosylation profile quantitatively on spike protein, but we observed strong evidence for O-glycosylation at two sites on the receptor binding domain (RBD) of spike protein subunit S1 – the crucial viral attachment location. Our data on the N- and O- glycosylation is strengthened by extensive manual interpretation of each glycopeptide spectra in addition to using bioinformatics tools to confirm the complexity of glycosylation in the spike protein. The understanding of the glycan repertoire on the spike protein, particularly in the RBD domain of the spike protein of SARS-CoV-2, provides insights into the viral binding studies and aids the interpretation of their outcome. The glycosylation mapping of angiotensin I-converting enzyme 2 (ACE2) is currently underway. Furthermore, it propels research towards the development of suitable immunogens for vaccine design and development.
Glycans serve a variety of functional roles in a wide range of biological processes and are known to be important in many diseases. Although both the speed and sophistication of data acquisition have been improved a lot due to ongoing technological advances in recent years, there is a lack of integrated resources that often hinders glycobiologist’s understanding of the complex roles glycosylation plays. GlyGen is a glycoinformatics knowledgebase, supported by the NIH Common Fund, to promote glycoscience research and facilitate knowledge discovery in glycobiology. Its strength lies in its integrated, scalable, sustainable and cross-disciplinary platform that provides tools and integrated data to address specific glycoscience questions perplexing both glycobiologists and non-glycobiologists. The knowledgebase collects data and knowledge from diverse sources to integrate and generate a comprehensive data repository. GlyGen integrates multiple types of data, in glycan, glycoprotein and glycan domain. By establishing international cooperation with database providers from different domains and glycoscience researchers, GlyGen can help solve glycobiology questions that previously could only be answered through extensive literature-based research and manual data collection from different sources. GlyGen utilizes commonly used standards and methodologies to integrate and harmonize data that fulfills FAIRsharing requirements. The collected datasets are filtered with stringent quality control (QC) along with the creation of a dataset BioCompute Object (BCO) to provide detailed documentation of the data processing workflow and streamline data sharing. An intuitive web-based interface (https://glygen.org/) has been developed to visually represent the data and the connections between datasets. In addition, to the browser-based interface the RESTful web services-based APIs and SPARQL endpoints, provide programmatic access to integrated datasets. To benefit the broadest possible user population, a query interface “Quick Searches” has been implemented to suggest likely “use-case” questions. By starting a question like “Which proteins have been shown to bear glycan X and which site is this glycan attached to?”, GlyGen can provide answers by doing a complex yet quick query across multiple datasets and domains without showing the complexity behind the searching, which makes it more user-friendly.
Metabolic Engineering Challenges to extending N-glycan pathways in CHO cells

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In mammalian cells, N-glycans are capped with sialic acids with one to three repeated N-acetyllactosamine units (LacNAc). LacNAc involved in various cellular functions in differentiation, metastasis, immune response and tumorigenesis, such as working as a scaffold for other oligosaccharides to display and a binding motif for galectins. Moreover, LacNAc can also affect therapeutic protein’s enzymatic activity and circulatory half-life. The cellular functions of poly-LacNAc is limited as is the understanding of its biosynthesis regarding its plasticity and/or competition with other Golgi glycosylation machinery for available precursor N-glycans.

Here we employed multiple genetic strategies including glyco-gene knockouts and knockins to achieve multi-dimensional modification of the glycosylation in CHO cells. Especially, previous studies indicated that β-1,3-N-acetylglucosaminyltransferase 2 (B3GNT2) and β-1,4-galactotransferase 1 (B4GALT1) are two of the primary glycosyltransferases involved in generating LacNAc units. In the current study, knocking out sialyltransferase genes slightly enhanced the LacNAc content (≥4 repeats per glycan) on recombinant EPO protein. Next, the role of single and dual-overexpression of B3GNT2 and B4GALT1 was explored in recombinant EPO-expressing CHO cells. While overexpression of B4GALT1 slightly enhanced the levels of large glycans on recombinant EPO, overexpression of B3GNT2 in EPO-expressing CHO cells significantly decreased the recombinant EPO LacNAc content, resulting in N-glycans terminating primarily with GlcNAc, a limited number of Gal termini, and nearly undetectable sialylation, which was also observed in sialyltransferases knock-out cell lines. B3GNT2 overexpression also enhanced intracellular UDP-GlcNAc and CMP-Neu5Ac content while slightly lowering UDP-Gal content. Furthermore, B3GNT2 could not be overexpressed as measured at both the transcriptional or translational levels following initial B4GALT1 expression. Expression of B3GNT2 following initial expression of B4GALT1 was also problematic in that transcriptional and translational analysis indicated the accumulation of truncated B3GNT2 missing a section of the B3GNT2 trans-Golgi lumen domain while transmembrane and cytoplasmic domains were present.

The addition of one or more recombinant glycosyltransferase genes may have an unexpected influence on the expression and activities of glycosyltransferases, which can disrupt the nucleotide sugar levels and lead to unexpected modifications of the resulting N-glycan patterns.
Weiming Yang

EXoO-Tn: Tag-n-Map the Tn Antigen in the Human Proteome and Pancreatic Cancer Sera

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Tn antigen (Tn), a single N-acetylgalactosamine (GalNAc) monosaccharide attached to protein Ser/Thr residues, is found on most solid tumors yet rarely detected in adult tissues as reported in previous studies, featuring it one of the most distinctive signatures of cancer. Although it is important in cancer, Tn-glycosylation sites are not entirely clear owing to the lack of suitable technology. Knowing the Tn-glycosylation sites may empower the future development of prevention, diagnostics, and therapeutics of cancer and other diseases associated with the expression of Tn. Here, we introduce a technology named EXoO-Tn for large-scale mapping of Tn-glycosylation sites. EXoO-Tn utilizes glycosyltransferase C1GalT1 and isotopically-labeled UDP-Gal(13C6) to tag and convert Tn to Gal(13C6)-Tn, which gives rise to a unique glycan mass. This exquisite Gal(13C6)-Tn structure is then recognized by a human-gut-bacterial enzyme, called OpeRATOR, that cleaves N-terminals of the Gal(13C6)-Tn-occupied Ser/Thr residues to yield site-containing glycopeptides. The effectiveness of EXoO-Tn was benchmarked by analyzing Jurkat cells, where 947 Tn-glycosylation sites from 480 glycoproteins were mapped. The EXoO-Tn was further applied for studying pancreatic cancer sera, where Tn-glycoproteins were found to have a difference between cancer and the control sera. Given the significance of Tn in diseases, EXoO-Tn is anticipated to have a broad utility in clinical investigations of diseases.
Antibodies are proteins produced by the immune system that are capable of binding to foreign invaders, typically other proteins or carbohydrates with high affinity and specificity which is central to their function. We use a combination of enhanced-sampling molecular-dynamics (MD) simulation and site-identification by ligand competitive saturation (SILCS) FragMaps to explore and identify conformations of the Fc, its mode of binding to glycan, and its interaction with EndoS and EndoS2, two endo-b-N-acetylglucosaminidase. Both EndoS and EndoS2 are secreted by the human pathogen Streptococcus pyogenes to deglycosylate the conserved N-linked glycan at N297 of IgG Fc to evade the immune system. Studies include the glycan in isolation and the Fc with both one and two glycans, thereby providing information on the impact of glycan to the Fc stability, impact of Fc on glycan conformational preference, and insight on the protection of glycosylation on aggregation of the Fc. The results provide conformations of glycan that could be accessible for both FC binding and to the Endoglycosidases.
Preethi Chandran

Velcro and Slime like interactions in N-glycan shields

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The surface of cells and pathogens is covered with short polymers of sugars known as glycans, which are attached to proteins and lipid in the membrane. Greater than 70% of the glycans belongs to the class of N-glycans, which have a core of three branched mannose sugars followed by distal sequences either rich in mannose (high mannose type) or having repetitions of N-acetylglucosamine and galactose capped by sialic acid or SA (complex type). Long-range slime-like adhesion between SA-SA residues and the short-range Velcro-like adhesions between man-man residues were observed in studies with sugar monolayers with ill-defined presentation. Our goal is to determine if and how these adhesions manifest when the two sugars are present in complex N-glycan architecture accompanied by the other N-glycan sugars. The complex N-glycan shields of two pseudo-typed HIV virus were brought together and pulled apart in force spectroscopy. The change in interaction patterns was monitored as the shields were cleaved to expose the mannose core in one or both viruses, and further cleaved to remove the mannose core. At high rates of approach, slime-like retraction forces were observed between two complex glycan shields, attributable to self-adhesion between the terminal SA sugars. At low rates, however, the retraction forces shifted to Velcro-like man-man adhesion. Bare-tip indentation experiments confirm that complex glycan shield becomes more penetrable at low rates, allowing adhesion patterns of the mannose core to dominate. Slime-like SA-SA adhesions were lost when sugars distal to the mannose core were removed. Velcro-like man-man adhesions were lost when the mannose core was removed. While penetrability of the glycan shield affected adhesion patterns in force spectroscopy, the aggregation between freely diffusing viruses in solution is dominated by terminal sugars. The consistency between ill-defined monolayer and defined N-glycan presentations suggests that these rules are applicable for bioengineered systems with sugar coating.
Invertebrates display effective innate immunity for defense against microbial infection with a diversified repertoire of soluble and cell-associated lectins mediate binding interactions with potential pathogens. Among them, the highly conserved galectins are key to multiple biological functions, including pathogen recognition and regulation of immune responses. We previously showed that the galectins CvGal1 and CvGal2 from the eastern oyster (Crassostrea virginica) preferentially bind ABH blood group oligosaccharides and play a significant role in infections by the parasite Perkinsus marinus. CvGals bind to “self” glycans on the hemocyte surface, and recognize potential microbial pathogens and unicellular algae. They preferentially bind to P. marinus trophozoites, but not to P. chesapeaki, a sympatric species mostly prevalent in the soft-shell clam Mya arenaria. We recently isolated a novel galectin from the softshell clam (MaGal1) that strongly cross-reacts with anti-CvGals antibodies, and preferentially binds to Gal(α1-3/4)Gal(β1-3/4)GlcNAc. Consistently, it preferentially binds to asialofetuin over PSM, the preferred ligand for CvGals. We hypothesize that the differential recognition of Perkinsus species by the oyster and clam lectins facilitate parasite entry and infectivity by carbohydrate-based parasite mimicry in a host-preferential manner, and are responsible for their relative prevalence and pathogenicity in oyster and clam species. To extend our knowledge about the diversity of the lectin repertoire of the bivalves, we performed a transcriptomic analysis on M. arenaria by RNAseq. Annotated with seven databases, 167 unigenes are identified as lectin or lectin-like proteins: 60 C-type lectins, 8 galectins, and 3 R-type lectins. Blast with currently-known protein sequences of invertebrate galectins, revealed the potential MaGal1 sequence, with high homology to Manila clam (Venerupis philippinarum) galectin. We also identified a lactose binding lectin (MaRTL) with high homology to the Mediterranean mussel R-type lectin (MytiLec3) through Mass spec and BLAST analysis. Current studies are aimed at the structural basis for the CvGal1, CvGal2, MaGal1, and MaRTL differential recognition Perkinsus species. [Supported by grants IOS-0822257, IOS-1063729, and IOS-1656720 from NSF, and grant 5R01GM070589-06 from NIH to GRV. We are grateful to Dr. Richard D. Cummings and Dr. Jamie Heimburg-Molinaro, NCFG, for glycan array analysis].
TCR signal strength and nutrient availability interact to regulate complex N-glycosylation in recently activated CD4+ T cells

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TCR signaling strength can regulate CD4+ T cell differentiation by mechanisms that are not well understood. In the time between initial stimulation and the first cell division, TCR stimulation of CD4+ T cells causes changes in several forms of protein glycosylation, including a dramatic increase in complex N-glycosylation. N-glycosylation status can regulate the expression and/or activity of many cell-surface molecules involved in T cell activation and differentiation, including cytokine receptors and the TCR itself. Furthermore the availability of nutrients that feed into glycosylation pathways, particularly glutamine, are known to affect CD4+ T cell differentiation in complex ways. Therefore, we hypothesized that TCR signal strength may direct CD4+ differentiation by restricting the availability of sugar precursors necessary for complex N-glycosylation. To test this hypothesis, we stimulated human PBMCs with anti-CD3 and anti-CD28 for four days, then stained the proliferating cells with the lectin L-PHA, which binds the most highly branched complex N-glycans. We find that the strength of TCR signaling determines the persistence of high levels on complex N-glycosylation as cells divide. Strong TCR stimulus leads to decreased complex N-glycosylation after the first few cell divisions, while a weak TCR stimulus leads to more sustained complex N-glycosylation. This phenomenon occurs in both naive and memory CD4+ T cells. Addition of nutrients can increase overall L-PHA binding, but does not prevent decreased binding with proliferation in the presence of a strong TCR stimulus. Experiments using conditioned media suggest that a strong TCR stimulus leads to the secretion of a soluble factor that suppresses complex N-glycosylation as cells divide. Indeed, treatment with a variety of cytokines can suppress complex N-glycosylation as cells divide, including IL-12 and IL-4. These results suggest that rather than directly regulating complex N-glycosylation, TCR signal strength results in the production of secondary factors that influence metabolism in ways that affect glycosylation. As they divide, strongly stimulated T cells acquire the ability to respond to these factors, while weakly stimulated T cells do not. Ongoing experiments will attempt to identify the factor(s) required for this effect and the signaling pathways they employ, as well as the precise metabolic pathway changes that lead to differences in complex N-glycosylation.
Galectins are a family of β-galactosyl-binding lectins with members (galectin-1 to -15) classified as proto-, chimera- and tandem-repeat types based on the organization of their carbohydrate recognition domain (CRD). They have been implicated in diverse processes including development, immune regulation, and cancer. Recently, galectins have been identified as pattern recognition receptors (PRRs), being upregulated during certain infections, binding to galactose residues exposed on pathogen surfaces, and acting as defense factors. For some pathogens, however, these protective role(s) have been subverted to facilitate their attachment to and entry into host cells. The protective role of the prototype galectin-1 (Gal1) against influenza A virus (IAV) infection has been previously reported. Studies in our lab, however, have shown that upon exposure of airway epithelial cells to IAV, both Gal1 and the chimera type galectin-3 (Gal3) are secreted to the host airway lumen, where they bind to the cell surface galactosyl moieties exposed as a result of desialylation by the viral neuraminidase. Both galectins can directly cross-link the pneumococci to the epithelial cell surface, potentially promoting a secondary bacterial infection. We proposed that this pneumococcal cross-linking was achieved due to galectin dimerization (Gal1) or oligomerization (Gal3), resulting in multivalent proteins. The protective role of the tandem-repeat galectin-9 (Gal9) against IAV infections has also been previously reported, but the mechanism(s) involved are not fully understood. We propose that as a multivalent binding protein, Gal9 may also be able to crosslink pathogens to the desialylated cell surface, and enhance pathogen adhesion and entry. In an in vitro model containing neuraminidase-treated and untreated glycoproteins we found that Gal9 cross-linked IAV strain PR8 to desialylated asialofetuin, compensating for the reduced adhesion as a result of desialylation. We are currently testing the capacity of Gal9 to cross-link PR8 to desialylated A549 airway epithelial cells. Experiments to investigate the Gal9-mediated IAV adhesion will be followed by the assessment of Gal9-mediated viral entry and replication. [Supported by Fulbright Fellowship grant ID: PS00219036 to MI, and grants IOS-0822257, IOS-1063729, and IOS-1656720 from NSF, and grant 5R01GM070589-06 from NIH to GRV].
Colorectal cancer (CRC) is one of the three most common cancer types worldwide and the bacteria Fusobacterium nucleatum is detected in around 45% of the cases\textsuperscript{1,2}. F. nucleatum is involved in CRC development through immunomodulation but the underlying molecular interactions are poorly understood\textsuperscript{3}. In this project we tested the hypothesis that F. nucleatum mediates immune response by interaction of its cell surface glycoconjugates with lectins expressed on innate immune cells. We first analysed the binding of three F. nucleatum strains (ATCC 25586, 10953 and 51191) to a panel of recombinant human lectins (Dectin-2, Galectin-3 and Siglec-7) using flow cytometry. The results showed that the majority of F. nucleatum ssp. population specifically bound to Siglec-7-Fc and to a lesser degree to the other lectins tested. Furthermore, we showed that the extracted outer-membrane vesicles (OMVs) of F. nucleatum strains as well as the F. nucleatum ATCC 51191-derived lipopolysaccharide (LPS) bound to the soluble Siglec-7-Fc using ELISA-based binding assay. The O-antigen characterization of the F. nucleatum ATCC 51191-LPS showed a trisaccharide repeating unit made of two aminuronic monosaccharides and one 6-deoxy-2,4-diamino unit with a heterogenous 4N-acetylation pattern. Next, we assessed the role of F. nucleatum ssp. on the host immune response. Stimulation of human monocyte-derived dendritic cells (moDCs) with F. nucleatum strains/OMVs/LPS showed induction of a pro-inflammatory profile with increased cytokine production of TNF\textalpha{} and IL-8 and cell surface marker induction of CD80, CD86 and PD-L1. Further, the stimulation of human monocyte-derived macrophages (moMfs) showed a tumour associated profile characterized by the cytokine induction of IL-10, IL-6 and IL-8 and the upregulation of PD-L1 cell surface marker expression. We showed that Siglec-7 is expressed on moDCs and moMfs and our preliminary data using Siglec-7 RNA-silencing in moDCs showed that stimulation with F. nucleatum ATCC 10953 caused a modulation in cytokine levels in a Siglec-7 dependent manner. Together these data support a role for Siglec-7 in mediating F. nucleatum immune evasion in the tumour microenvironment.

Andrew Lees

Simplifying Conjugation: Ready to Conjugate CRM197 Carrier Protein

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Conjugate vaccines consist of carbohydrates, peptides or other poorly immunogenic antigens chemically linked to a carrier protein, which provides T cell help. Conjugate vaccines have proven remarkably effective in preventing disease but are costly to develop and manufacture. Fina Biosolutions’ expertise and focus is to facilitate the research and development and reduce the cost of conjugate vaccines. We have developed a novel E. coli expression system with an oxidative intracellular environment that can express disulfide bonded proteins as intracellular, soluble, properly-folded proteins and have used the strain to produce conjugate vaccine carrier proteins, including CRM197 (EcoCRM®) and recombinant tetanus toxin heavy chain (rTTHc). EcoCRM® has been extensively compared to CRM197 obtained from multiple manufacturers, produced in differing cell expression systems (Hickey et al, J Pharm Sci. 107, 1806, 2018) and is being used for several conjugate vaccines in development targeting S. pneumoniae, Group B Strep, malaria and vaccines for drugs of abuse. To further promote conjugate vaccine research, we have developed and formulated maleimide-derivatized CRM197 which can be frozen and ready to use for conjugation. The CRM197 is derivatized with ~15 maleimide groups, allowing conjugates of thiol-haptens (e.g., cysteine peptides and thiol-oligosaccharides) to be readily prepared with minimal effort. Here we show that in the formulation buffer, the maleimide is stable for >6 hrs at 4°C and can be subjected to at least 10 freeze-thaw cycles without loss of the maleimide of reactivity. We also demonstrate the immunogenicity of conjugates that have been made from the frozen CRM-maleimide. Ready-to-conjugate carrier proteins will simplify the preparation of conjugates by non-specialist researchers. We are also preparing to manufacture CRM-maleimide as a GMP reagent that will facilitate its use in clinical trials, especially phase 1 trials. This product will eliminate the need for the development of the labeling process and associated analytical assays.
**Scott Walsh**

*Allosteric-like Binding and Signaling Behavior Among the N-linked Glycosylation Sites of the Interleukin-7 Receptor*

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Protein glycosylation is the most extensive form of post-translational modification that occurs in a eukaryotic cell. It is essential to understand how glycosylation affects protein structure and function. One key glycoprotein in our adaptive immune system, is the interleukin-7 receptor (IL-7R), which is a shared cytokine receptor, that functions in IL-7 and thymic stromal lymphopoietin (TSLP) signaling pathways. In humans, IL-7R is essential for T cell development, survival, and homeostasis. Aberrant IL-7 and TSLP signaling have been implicated in severe combined immunodeficiency and driving disease progression of autoimmune conditions and cancers. In a key breakthrough, we discovered that asparagine (N)-linked glycosylation of human IL-7R enhances its binding affinity to human interleukin-7 (IL-7) by more than 300-fold relative to the unglycosylated receptor. Interestingly, crystal structures of IL-7 bound to glycosylated and unglycosylated forms of IL-7R revealed that the receptor’s N-glycans do not result in large structural differences, and none of the receptor’s N-glycans interacts directly within the binding interfaces. Further results show that N-glycosylation of IL-7R is essential for productive IL-7 signal transduction and binding of TSLP proteins. These results strongly suggest that N-glycosylation modulates the receptor’s binding affinities and signaling potential through an indirect or “allosteric-like” mechanism about which little is known. Six asparagines of human IL-7R can be N-glycosylated and mutagenesis studies indicate that a single N-glycan is the most critical in the receptor’s association with IL-7. Strikingly, we obtained further data that subsets of N-glycans on IL-7R, other than those attached to the key asparagine; cause IL-7R to bind more tightly to IL-7 than predicted from strict binding additivity. This indirect or allosteric-like mechanism of enhanced energetic coupling among different N-glycans of IL-7R represents a potential new binding phenomenon for N-glycoproteins. We are determining the structural and biophysical bases of this mechanism and its effects on IL-7R function in IL-7 and TSLP signaling in deciphering IL-7R’s “N-glycosylation code”. We will test our understanding of N-glycosylation effects on IL-7R function using a collaborative, comprehensive structural, biophysical, and cell biological approach.
Robert Woods

3D Models of glycosylated SARS-CoV-2 spike protein suggest challenges and opportunities for vaccine development

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Here we present 3D structures of several glycoforms of the spike (S) protein SARS-CoV-2, based on reported 3D structures for the S protein and on glycomics data for the protein produced in HEK293 cells. We also report structures for glycoforms that represent those present in the nascent glycoproteins (prior to enzymatic modifications in the Golgi and ER), as well as those that are commonly observed on antigens present in other viruses. We have subjected these models to MD simulations to take into account protein and glycan plasticity and compare the extent to which glycan microheterogeneity impacts epitope exposure. Lastly, we have identified the peptides in the S protein that are likely to be presented in human leukocyte antigen (HLA) complexes, and discuss the role of S protein glycosylation in modulating the adaptive immune response to the SARS-CoV-2 virus or to a related vaccine.
The zebrafish tandem-repeat galectin 9 (Drgal9-L1) promotes in vitro attachment and infection of the infectious hematopoietic necrosis virus (IHNV)

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Galectins are β-galactoside-binding lectins characterized by a unique sequence motif in their carbohydrate recognition domains (CRDs). Tandem-repeat galectins display two CRDs joined by a linker peptide that are similar but not identical in binding specificity. Zebrafish (Danio rerio) is an excellent model for studying galectins as it possesses orthologous genes and expresses all three classes. Previous work revealed that Drgal9-L1 interacts directly with the IHNV envelope glycoprotein to promote viral attachment to the fish epithelial cell surface. We hypothesized that Drgal9-L1 crosslinks the virion glycoprotein to a truncated fibronectin on the cell surface, enhancing viral attachment and infectivity. We also propose that Drgal9-L1 is binding to other galectin ligands, that may serve as alternate receptors thereby increasing viral residence time on the surface and thus increasing infectivity. To determine binding specificity of Drgal9-L1’s N- and C-terminal CRDs, two approaches were used: selective CRD inactivation via mutation and enzymatic cleavage at the peptide linker. Glycan array analysis revealed that all proteins had a strong binding preference for terminal and internal Galβ1-4GlcNAc but there were differences between the two CRDs. Plaque assays revealed that two active CRDs are required for crosslinking to occur. Several potential galectin receptors were identified on the EPC cell surface, including fibronectin which Drgal9-L1 bound to in a carbohydrate-dependent manner. Incubation of IHNV with Drgal9-L1 led to increased binding to fibronectin and the EPC cell surface. We are also investigating the protective role of epithelial mucus glycans as a “decoy” for preventing DrGal9-mediated viral attachment to the epithelium. All three galectin classes have been detected in the zebrafish epithelial mucus and exogenous Drgal9 as well as IHNV were found to bind it in a carbohydrate-dependent manner. In a plaque assay mucus coating reduced the number of IHNV plaques on the EPC cells in a concentration and volume dependent manner. This research has wide ranging applications for aquaculture disease management and alternative vaccine development. We are grateful to Dr. Richard D. Cummings, Dr. David Smith and Dr. Jamie Heimburg-Molinaro,Core H-CFG and NCFG, for glycan array analysis [Supported by grant R01GM070589-06 from NIH to GRV]
Mindy Engevik

*Akkermansia muciniphila* promotes *Clostridioides difficile* mucin utilization

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Background: *Clostridioides difficile* is an enteric pathogen causing post-antibiotic diarrhea and colitis. Several pathogens have been demonstrated to adhere and colonize the intestinal mucus. However few studies have addressed this interplay with *C. difficile*. As mucus is among the first lines of epithelial defense, this information may hold the key for *C. difficile* host colonization and be a potential preventative treatment target. Methods & Results: Using CFDA-SE fluorescently tagged bacteria, we demonstrated in vitro that *C. difficile* is capable of adhering to human MUC2. In vivo we identified co-localization of *C. difficile* with colonic MUC2 in infected C57B6J mice. To identify microbes that may be interacting with *C. difficile* in the intestinal mucus layer we generated biome reactor communities with human stool with the addition of mucin-coated inserts. 16S rDNA sequencing identified that *C. difficile* formed mucus-associated biofilms with several mucin-degrading microbes. We reasoned that these microbes may be releasing mucin oligosaccharides that might serve as a chemoattractant. In order to ascertain which mucin-components *C. difficile* was attract to, we used CFDA-Se tagged *C. difficile* in chemotaxis capillary assays. We found that *C. difficile* R20291 chemotaxes towards intestinal MUC2 derived from the mucus-secreting human cell lines HT29-MTX, LS174T and T84 as well as mucin monosaccharides, including: fucose, mannose, glucose, galactose, N-acetyl-galactosamine (GalNAc), and N-acetyl-glucosamine (GlcNAc) and sialic acid (NANA). The highest levels of chemotaxis was found with mannose and GlcNAc. We confirmed our chemotaxis phenotype using live cell imaging of CFDA-SE tagged *C. difficile* in IBIDI chambers. Although *C. difficile* lacked the glycosyl hydrolases required to degrade mucin glycans, co-cultures with the mucin-degrading *Akkermansia muciniphila*, which was identified in our 16S sequencing, allowed *C. difficile* to grow in media containing purified MUC2, but lacking glucose. qPCR analysis of chemotaxis and adherence genes revealed that upregulation of key genes in response to both MUC2 and *A. muciniphila*-mucin metabolites. Conclusions: Collectively, these studies suggest that *C. difficile* is chemoattracted to and adheres to mucins, where it interacts with mucin-degrading microbes which likely promote its colonization. These data point to the potential role of modulating intestinal mucus as a novel therapeutic for *C. difficile* infection.
Kristen Engevik

**Abstract Number 63**

**Kristen Engevik; Mindy Engevik; Lori Banks; Alexandra Chang-Graham; Jacob Perry; Diane Hutchinson; Nadim Adjami; Joseph Petrosino; Joseph Hyser**

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**Background:** Studies have identified changes within the gut microbiome in response to diarrheal-inducing bacterial pathogens. However, examination of the microbiome in response to viral pathogens remains understudied. Compounding this, many studies use fecal samples to assess microbiome composition; which may not accurately mirror changes within the small intestine, the primary site for most enteric virus infections. As a result, the functional significance of small intestinal microbiome shifts during infection is not well defined.

**Methods & Results:** To address these gaps, rotavirus-infected neonatal mice were examined for changes in bacterial community dynamics, host gene expression, and tissue recovery during infection. Profiling bacterial communities using 16S rRNA sequencing suggested significant and distinct changes in ileal communities in response to rotavirus infection. At one-day post-infection, we observed a loss in *Lactobacillus* species from the ileum, but an increase in *Bacteroides* and *Akkermansia*, both of which exhibit mucin-digesting capabilities. Concomitant with the bacterial community shifts, we observed a loss of mucin-filled goblet cells in the small intestine at day 1, with recovery occurring by day 3. To address whether the lack of mucin staining was due to depletion of stored *Muc2* granules or loss of goblet cells, we examined several goblet cell markers by qPCR. Although we observed decreased levels of *Muc2*-positive goblet cells by immunostaining, we found no change in *Muc2* mRNA levels at 1 and 3 dpi. Additionally, no changes were observed in goblet cell secreted factors *Tff3* or *Relm-β* expression in the rotavirus-infected mice compared to control mice, indicating that the goblet cells were still present. These data point to granule expulsion from the goblet cells, rather than a loss of goblet cells by differentiation or apoptosis. Rotavirus infection of mucin-producing cell lines (HT29-MTX, LS174T and T84) and human intestinal enteroid (HIE) monolayers stimulated release of stored mucin granules, similar to in vivo findings. In vitro, incubation of mucins with *Bacteroides* or *Akkermansia* members resulted in significant glycan degradation, which altered the binding capacity of rotavirus in silico and in vitro to MA104 cells. Conclusions: Taken together, these data suggest that the response to and recovery from rotavirus-diarrhea is unique between sub-compartmental of the GI tract and may be influenced by mucin-digesting microbes.
Desialylated Host-Derived MUC1 Ectodomain and Bacterially-Derived Flagellin are Biomarkers for Airway Colonization and Infection with Pseudomonas aeruginosa in Mechanically Ventilated and Cystic Fibrosis Patients

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Background: MUC1 is a highly sialylated cell surface mucin comprised of an NH2-terminal ectodomain (MUC1-ED) coupled to a COOH-terminal MUC1 cytoplasmic domain. We previously established that MUC1 is a signal-transducing airway epithelial cell surface receptor for Pseudomonas aeruginosa (Pa)-expressed flagellin. Engagement of MUC1 by flagellin stimulates rapid desialylation of the MUC1-ED by NEU1, the predominant sialidase in human airway epithelia. NEU1-mediated MUC1-ED desialylation unmasks a Gly-Ser protease recognition site in its juxtamembranous region, promoting MUC1-ED shedding from the cell surface as a soluble decoy receptor that competitively inhibits Pa binding to cell-associated MUC1. Here, we evaluated MUC1-ED and Pa flagellin levels in bronchoalveolar lavage fluid (BALF) as diagnostic biomarkers of Pa colonization and infection in patients with ventilator-associated pneumonia (VAP) and cystic fibrosis (CF). Results: BALF MUC1-ED levels in Pa-colonized/infected VAP/CF patients were 5.6-fold greater compared with patients colonized/infected with other microorganisms, and 7.7-fold greater compared with noncolonized/uninfected patients. BALF was superior to tracheal aspirate for measuring MUC1-ED levels. Pa stimulation of in vitro cultures of human airway epithelia increased MUC1-ED levels in cell culture supernatants up to 13.1-fold, compared with the PBS control. The FlaA flagellin-expressing PAK bacterial strain and FlaB flagellin-expressing PA01 strain stimulated comparable MUC1-ED levels in vitro. A flagellin-deficient PAK mutant provoked dramatically reduced MUC1-ED levels, compared with the flagellin-expressing wild-type strain, and purified FlaA and FlaB flagellins recapitulated the effect of intact Pa bacteria. Concurrent with increased MUC1-ED levels in BALF of Pa-colonized/infected VAP/CF patients, greater levels of Pa FlaA and FlaB flagellins also were detected, providing a basis for estimating Pa lung burden in the absence of quantitative microbial cultures. Finally, an E. coli-expressed, nonglycosylated, human recombinant MUC1-ED dose-dependently inhibited multiple Pa flagellin-dependent processes, including bacterial motility and adhesion to airway epithelia, and IL-8 production, and enhanced neutrophil-mediated Pa phagocytosis, without affecting bacterial growth. Conclusions: These results indicate that the MUC1-ED and Pa-derived flagellin constitute biomarkers of Pa airway colonization and infection in VAP and CF patients.
Lisa Parsons

N-glycan sub-type as a pathogenic factor in Influenza

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Pulmonary surfactant protein D (SP-D) is an innate immune protein that binds to and neutralizes influenza type A viruses (IAV) carrying key hemagglutinin (HA) head region high mannose glycans. Little is known about HA glycosylation of subtypes such as H6 and H11 which have been found in birds and swine but not yet in humans. Through reassortment these subtypes could be the source of the next human pandemic. Here we investigate the SP-D activities of recombinant human mnSP-D and mutant hnSP-D against a range of HA subtypes, including H2N1, H5N1, H6N1, H11N9, an avian H3N8 and a human seasonal H3N2 subtype. N-glycosylation of HAs were characterized by LC/MSE to reveal site specific glycosylation patterns and their relationship to SP-D activity.
Myles Poulin

Using synthetic substrates to probe substrate recognition by the biofilm degrading glycosidase DspB

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Bacterial biofilms consist of sessile, surface attached bacteria encased in an extracellular polymeric substance (EPS) composed of exopolysaccharides, proteins and extracellular DNA. The EPS protects the cells and facilitates both cell–cell and cell–surface attachment. Enzymatic breakdown of EPS components by protease, nuclease and glycosidase enzymes is being explored as a promising strategy to disrupt biofilms. A common component of bacterial biofilm EPS are exopolysaccharides consisting of partially de-N-acetylated β-(1»6)-poly-N-acetylglucosamine (dPNAG), which are present in biofilms of both gram-positive and gram-negative human pathogens, including Staphylococcus epidermidis and Staphylococcus aureus. There are only two enzymes that specifically hydrolyze dPNAG reported to date, and relatively little is known about the specific interactions required for substrate recognition and specificity in these enzymes. Here, we use a combination of chemically defined synthetic dPNAG analogs and targeted site-directed mutagenesis studies to probe the mechanism of substrate recognition by Dispersin B (DspB), a PNAG specific exo-glycosidase. We identified negatively charged patches that play a role in substrate recognition in DspB both in vitro and in biofilm dispersal. These studies provide new insight into the recognition mechanism of deacetylated exopolysaccharides by DspB and their degradation that can aid in the development of more efficient dPNAG hydrolase enzymes.
E Tian

Loss of the disease-associated glycosyltransferase Galnt3 alters Muc10 glycosylation and the composition of the oral microbiome

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The importance of the microbiome in health and its disruption in disease is continuing to be elucidated. However, the multitude of host and environmental factors that influence the microbiome are still largely unknown. Here, we examined UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase 3 (Galnt3)-deficient mice, which serve as a model for the disease hyperphosphatemic familial tumoral calcinosis (HFTC). In HFTC, loss of GALNT3 activity in the bone is thought to lead to altered glycosylation of the phosphate-regulating hormone fibroblast growth factor 23 (FGF23), resulting in hyperphosphatemia and subdermal calcified tumors. However, GALNT3 is expressed in other tissues in addition to bone, suggesting that systemic loss could result in other pathologies. Using semiquantitative real-time PCR, we found that Galnt3 is the major O-glycosyltransferase expressed in the secretory cells of salivary glands. Additionally, 16S rRNA gene sequencing revealed that the loss of Galnt3 resulted in changes in the structure, composition, and stability of the oral microbiome. Moreover, we identified the major secreted salivary mucin, Muc10, as an in vivo substrate of Galnt3. Given that mucins and their O-glycans are known to interact with various microbes, our results suggest that loss of Galnt3 decreases glycosylation of Muc10, which alters the composition and stability of the oral microbiome. Considering that oral findings have been documented in HFTC patients, our study suggests that investigating GALNT3-mediated changes in the oral microbiome may be warranted.
Bacterial biofilms are crucial to pathogenesis and surface colonization by many bacteria. A network of polysaccharides and other biopolymers provides structural support for these biofilms. Poly-N-acetylglucosamine (PNAG) is one of the most important structural exopolysaccharides of E. coli biofilms. PNAG is synthesized by a series of proteins expressed from the pga operon. PgaC and PgaD proteins together form the PNAG synthase, a glycosyltransferase complex that spans the cytosolic membrane. This enzyme catalyzes the addition of GlcNAc residues onto the growing chain from UDP-GlcNAc and facilitates PNAG export into the periplasm. Although the PgaCD function has been predicted based on its homology to other biofilm glycosyltransferases, empirical validation is needed to understand its function. The PgaCD enzyme contains conserved DXD, TED, and QXXRW motifs common to glycosyltransferase family 2 enzymes. Mutants of these conserved motifs created by site-directed mutagenesis exhibit a loss of activity, indicating that the catalytic residues predicted by these sequence motifs are involved in the PNAG biosynthesis. To further examine the structure of PNAG synthase we will use two approaches – topological studies and crystallography. Selective cysteine accessibility method (SCAM) experiments reveal the topological orientation of the two proteins, informing the possible role of PgaD. Crystallography requires preparation of a large quantity of pure protein, which is often challenging for membrane complexes such as PgaCD. Here, we present a workflow for PgaCD detergent solubilization and purification using IMAC chromatography, which is a critical step in preparation for future PgaCD crystallography trials. The approaches developed herein lay the groundwork for further understanding of PgaCD structure and function, which can inform future approaches for combating bacterial biofilms.
Sexually transmitted infections account for a high percentage of the total acute contracted infections globally each year. Of these 357 million STI’s, approximately 78 million of those are gonorrhea infections caused by the gram-negative bacteria Neisseria gonorrhoeae. In recent years, gonorrhea has steadily become resistant to almost all classes of antibiotics. The emergence of multiple drug-resistant gonorrhea has significantly complicated the treatment of gonorrhea making development of novel treatment methods for controlling of N. gonorrhoea infections an urgent global priority. In this current study, we evaluated the characteristics and efficacy of adhesin-specific glycan-conjugated iron oxide nanoparticles for selective-binding and -inactivation of N. gonorrhoeae via magnetically-mediated energy delivery (MagMED).

Iron oxide magnetic nanoparticles (MNPs) coated with polyethylene oxide based polymer and functionalized with bacterial adhesin-specific glycans GalNAcβ1-4Galβ1-4Glc-NAc-propargyl (aGM2-MNPs), which has been shown to bind specifically to adhesins of N. gonorrhoeae, were synthesized and characterized using Dynamic Light Scattering, Zeta Potential, Fourier Transform Infrared Spectroscopy, Ultraviolet-Visible Spectroscopy, Transmission Electron Microscope, and Hydrogen Nuclear Magnetic Resonance. To determine the potential applications of MagMED for treatment of infections caused by N. gonorrhoeae, the binding characteristics of aGM2-MNPs to N. gonorrhoeae FA1090 and selective killing of FA1090 via MagMED were evaluated. Specific aggregation of FA1090 was observed when interacting with aGM2-MNPs. Subsequently, aGM2-MNPs were used along with alternating magnetic field (AMF) for targeted killing of FA1090. Killing of FA1090 was dependent on concentration of aGM2-MNPs and duration of AMF. Clinically relevant reduction of FA1090 was achieved after 30 minutes of AMF exposure in the presence of aGM2-MNPs. Extensive cell membrane damage was observed after AMF exposure in the presence of aGM2-MNPs. These results suggest that bacterial specific glycoconjugate MNPs along with AMF can be efficiently employed as novel non-antibiotic platform for inactivation of N. gonorrhoeae. In addition, we also 3D printed MagMED compatible device and demonstrated the feasibility of utilizing 17β-estradiol treated BALB/C (immunocompetent) and SKH1 (hairless) mice as models for studying N. gonorrhoeae infections.
Haiyang Wu

Fucosidases from the human gut symbiont Ruminococcus gnavus

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The availability and repartition of fucosylated glycans within the gastrointestinal tract contributes to the adaptation of gut bacteria species to ecological niches. To access this source of nutrients, gut bacteria encode α-L-fucosidases (fucosidases) which catalyze the hydrolysis of terminal α-L-fucosidic linkages. We determined the substrate and linkage specificities of fucosidases from the human gut symbiont Ruminococcus gnavus. Sequence similarity network identified strain-specific fucosidases in R. gnavus ATCC 29149 and E1 strains that were further validated enzymatically against a range of defined oligosaccharides and glycoconjugates. Using a combination of glycan microarrays, mass spectrometry, isothermal titration calorimetry, crystallographic and saturation transfer difference NMR approaches, we identified a fucosidase with the capacity to recognize sialic acid-terminated fucosylated glycans (sialyl Lewis x / a epitopes) and hydrolyze α1-3/4 fucosyl linkages in these substrates without the need to remove sialic acid. Molecular dynamics (MD) simulation and docking showed that sLeX could be accommodated within the binding site of the enzyme. This specificity may contribute to the adaptation of R. gnavus strains to the infant and adult gut and has potential applications in diagnostic glycomic assays for diabetes and certain cancers.
Characterization of C. neoformans single motif expressing exopolysaccharides

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The polysaccharide capsule (PS) of the pathogenic fungus Cryptococcus neoformansis a critical virulence factor. Our current understanding of the capsule comes majorly from structural analyses that describe substituted mannose triads thought to make up the repeating units of the dominant polymer glucuronoxylomannan (GXM), and cellular studies showing the effects of the capsule in vivo during infection. However, recent work suggests that the use of cetyl trimethylammonium bromide (CTAB) detergent for exopolysaccharide (EPS) isolation, as first done by Cherniak, et al. and which much of our structural knowledge is based, alter the chemical and physical structure of the EPS (Cherniak et al., 1998; Frases et al., 2008; Wear et al., 2019). Updating the filtration protocol described by Frases et al. to isolate the EPS in a more native state, we characterized the GXM EPS from C. neoformans strains. By characterizing the EPS of strains expressing exclusively one polysaccharide motif (as identified by Cherniak et al.) using nuclear magnetic resonance (NMR) spectroscopy, matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrometry, and dynamic light scattering (DLS), we can compare the classical CTAB extraction to our native isolation. With this comparison we are working to determine if single-motif expressing strains truly contain one type of polysaccharide, and what the three-dimensional structure of these polymers are. Our focus on exclusively single-motif expressing strains significantly simplifies the study of the biochemical composition of the EPS, and has important ramifications for our current understanding of the PS structure as well as identification of potential structures that confer resistance to host protective mechanisms.