PROGRAM AND ABSTRACTS FOR 2020 ANNUAL MEETING OF THE SOCIETY FOR GLYCOBIOLOGY

November 9–12, 2020
Phoenix, AZ, USA

2020 SfG Virtual Meeting Preliminary Schedule

Mon. Nov 9 (Day 1)

TOKYO TIME	ROME TIME	PACIFIC TIME	EASTERN START TIME	EASTERN END TIME	SESSION
23:30	15:30	6:30	9:30	9:50	**Welcome and Introduction - Michael Tiemeyer, *CCRC UGA***
23:30	15:30	6:30	9:50 – 12:36		**Session 1: Glycobiology of Normal and Disordered Development | Chair: Kelly Ten-Hagen, *NIH/NIDCR***
23:50	15:50	6:50	9:50	10:10	KEYNOTE: "POGLUT1 mutations cause myopathy with reduced Notch signaling and α-dystroglycan hypoglycosylation" - Carmen Paradas Lopez, *Biomedical Institute Sevilla*
0:12	16:12	7:12	10:12	10:24	Poster Talk: "Regulation of Notch signaling by O-glycans in the intestine" – Mohd Nauman, *Albert Einstein*
0:26	16:26	7:26	10:26	10:38	Poster Talk: "Generation of an unbiased interactome for the tetratricopeptide repeat domain of the O-GlcNAc transferase indicates a role for the enzyme in intellectual disability" – Hannah Stephen, *University of Georgia*
0:40	16:30	7:30	10:40	10:50	Q&A
10:52	11:12	7:52	10:52	11:12	KEYNOTE: "Aberrations in N-cadherin Processing Drive PMM2-CDG Pathogenesis" - Heather Flanagan-Steet, *Greenwood Genetics Center*
1:14	11:26	8:14	11:14	11:26	Poster Talk: "Functional analyses of TMTC-type protein O-mannosyltransferases in Drosophila model system" – Melissa Koff, *Texas A&M*
1:28	11:40	8:28	11:28	11:40	Poster Talk: "Pituitary gland hyper-O-GlcNAcylation drives growth hormone deficiency" – Eugenia Wulff, *Medical College of Wisconsin*
1:42	17:42	8:42	11:42	12:02	KEYNOTE: "Exploring the mechanisms underlying the NGLY1 loss-of-function phenotypes in Drosophila" - Ashutosh Pandey, *Baylor College of Medicine*
2:04	18:04	9:04	12:04	12:16	Poster Talk: "How N-glycans shape somatosensory dendrites" – Maisha Rahman, *Albert Einstein*
2:26	18:26	9:26	12:26	12:36	Q&A
2:36	18:36	9:36	12:36 – 13:00		***BREAK***
3:00	19:00	10:00	13:00	13:45	**KARL MEYER AWARD LECTURE: Kelley Moremen, *CCRC UGA* | Chair: Michael Tiemeyer, *CCRC UGA***
3:45	19:45	10:45	13:45	15:30	**EXHIBITS & POSTER SESSION 1**
3:45	19:45	10:45	13:45	15:30	**Board of Directors Meeting (Invitation Only)**
3:45	19:45	10:45	13:45	15:30	**Information Session with Q&A: The Common Fund Glycoscience Program: New Tools & Chemical Methods for Doing Glycoscience! Learn how you can beta-test these new methods & technologies. Informational Session with Q & A.**
			15:30 – 16:30		***BREAK***

TOKYO TIME	ROME TIME	PACIFIC TIME	EASTERN START TIME	EASTERN END TIME	SESSION
6:30	22:30	13:30	16:30 – 19:15		**Session 2: Glycoinformatics | Chair: Kiyoko Aoki-Kinoshita, *Soka University***
6:30	22:30	13:30	16:30	17:00	MCP Award Lecture: Cathy Costello, *Boston University*
7:05	23:05	14:05	17:05	17:25	KEYNOTE: "Implications of biosynthetic limits placed on the glycosylation capacities of cells" – Hiren Joshi, *University of Copenhagen*
7:27	23:27	14:27	17:27	17:39	PosterTalk: "A computational model linking the organization of the mammalian glycosylation machinery with glycan processing" – Daniel Ungar, *University of York*
7:41	23:41	14:41	17:41	17:53	PosterTalk: "O-Pair search with MetaMorpheus for O-glycopeptide characterization" – Nicholas Riley, *Stanford University*
8:07	0:07	15:07	17:55	18:05	Q&A
8:29	0:29	15:29	18:07	18:27	KEYNOTE: "A few glycoinformatics pieces fitting in the glyco-puzzle" – Frédérique Lisacek, *Swiss Institute of Bioinformatics*
8:43	0:43	15:43	18:29	18:41	PosterTalk: "Chemical and informatics tools for glycosciences: Glyco-SPOT and GlycoGlyph" – Akul Mehta, *Harvard University*
9:05	1:05	16:05	18:43	19:03	KEYNOTE: "Bridging the Gap: Connecting Glycobiolgy and Glycoinformatics using Semantic Names, Subsumption and Structural Motifs" – Nathan Edwards, *Georgetown University*
8:07	0:07	15:07	19:05	19:15	Q&A
			Tue. Nov 10 (Day 2)		
23:30	15:30	6:30	9:30 – 12:02		**Session 3: Neuroglycobiology | Chair: Ronald Schnaar, *Johns Hopkins University***
23:30	15:30	6:30	9:30	9:50	KEYNOTE: "Gene therapy targeting CD33 reduces amyloid beta pathology and neuroinflammation in Alzheimer's disease" – Ana Griciuc, *Harvard University*
23:52	15:52	6:52	9:52	10:04	PosterTalk: "PTPzeta (phosphacan) carries Siglec-8 ligands in human brain" – Anabel Gonzalez-Gil, *Johns Hopkins University*
0:06	16:06	7:06	10:06	10:26	KEYNOTE: "New Insights into the Role of CD33 in Alzheimer's Disease Susceptibility" – Matt Macauley, *University of Alberta*
0:28	16:28	7:28	10:28	10:40	PosterTalk: "On-tissue microscale glycoproteomics and N-glycan imaging reveal global dysregulation of canine glioma glycoproteomic landscape" – Stacy Malaker, *Stanford University*
0:42	16:42	7:42	10:42	10:52	Q&A
0:54	16:54	7:54	10:54	11:14	KEYNOTE: "Structure and function of perineuronal nets: Unique extracellular matrix structures in the central nervous system" – Rick Matthews, *Upstate Medical University, Syracuse*

TOKYO TIME	ROME TIME	PACIFIC TIME	EASTERN START TIME	EASTERN END TIME	SESSION
1:16	17:16	8:16	11:16	11:28	Poster Talk: "Integrative glycomics and proteomics analysis of myelinated & non-myelinated regions from human control, bipolar disorder & Schizophrenia brain tissues" – Manveen Sethi, *Boston University*
1:30	17:30	8:30	11:30	11:50	KEYNOTE: "Regulation of neural functions by protein glycosylation: Insights from Drosophila model" – Vlad Panin, *Texas A&M*
1:52	17:52	8:52	11:52	12:02	Q&A
2:02	18:02	9:02	12:02 – 13:00		*BREAK*
3:00	19:00	10:00	13:00 – 13:40		**INNOVATOR AWARD LECTURE: Carolyn Bertozzi, *Stanford University* | Chair: Michael Tiemeyer, CCRC UGA**
3:45	19:45	10:45	13:45 – 15:30		***EXHIBITS AND POSTER SESSION 2***
3:45	19:45	10:45	13:45 – 14:35		**GlyGen Demonstration Session: "Computational Resources for Glycoscience"**
3:45	19:45	10:45	14:00 – 15:00		**Glycobiology Editorial Board Meeting (Invitation Only)**
4:40	20:40	11:40	14:40 - 15:30		**Glycomics@ExPasy Demonstration Session: "Comparative tools for glycoprotein and lectin structural profiles"**
5:30	21:30	12:30	15:30 – 16:30		*BREAK*
6:30	22:30	13:30	16:30 – 19:00		**Session 4: Pathways of synthesis, processing, and signaling | Chair: Rebekah Gundry, *University of Nebraska***
6:30	22:30	13:30	16:30	17:05	**KORNFELD AWARD LECTURE: Ajit Varki, UCSD**
7:05	23:05	14:05	17:05	17:25	KEYNOTE: "Structure and mechanism of ER-glycosyltransferases involved in protein N-glycosylation" – Kaspar Locher, *ETH Zurich*
7:27	23:27	14:27	17:27	17:37	Poster Talk: "Novel insights into the fucose metabolism- challenging the old dogma" – Paulina Sosicka, *Sanford-Burnham-Prebys Medical Discovery Institute*
7:39	23:39	14:39	17:39	17:49	Poster Talk: "Investigating the functions of endogenous neuraminidases Neu1 and Neu3 in blood cell and protein homeostasis" – Julia Westman, *Sanford-Burnham-Prebys Medical Discovery Institute*
7:51	23:51	14:51	17:51	18:01	Q&A
8:03	0:03	15:03	18:03	18:23	KEYNOTE: "Target protein O-GlcNAcylation" – Christina Woo, *Harvard University*
8:25	0:25	15:25	18:25	18:35	Poster Talk: "Glycometabolic regulation of the biogenesis of small extracellular vesicles" – Yoichiro Harada, *Osaka International Cancer Institute*
8:37	0:37	15:37	18:37	18:57	KEYNOTE: "Cross-talk of glycosylphosphatidylinositol biosynthesis with glycosphingolipid biosynthesis" – Taroh Kinoshita, *Osaka University*
8:59	0:59	15:59	18:59	19:09	Poster Talk: "Heterozygous De Novo mutations in NUS1 cause a movement disorder with combined lysosomal and glycosylation defects" – Richard Steet, *Greenwood Genetic Center*
9:11	1:11	16:11	19:11	19:21	Q&A

Wed. Nov 11 (Day 3)

TOKYO TIME	ROME TIME	PACIFIC TIME	EASTERN START TIME	EASTERN END TIME	SESSION
23:30	15:30	6:30	9:30 – 11:55		***Session 5: Viral Glycoscience*** **|** ***Chair: Galit Alter, Ragon Institute***
23:30	15:30	6:30	9:30	9:50	KEYNOTE: "Functional role of the glycan shield in the activation of SARS-CoV2 S glycoprotein" – Elisa Fadda, *Maynooth University*
23:52	15:52	6:52	9:52	10:04	Poster Talk: "Analysis of the SARS-CoV-2 spike protein glycan shield reveals implications for immune recognition" – Oliver Grant, *University of Georgia*
0:06	16:06	7:06	10:06	10:18	Poster Talk: "SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2" Thomas Clausen – *University of California San Diego*
0:20	16:20	7:20	10:20	10:40	KEYNOTE: "Structure and dynamics of the SARS-CoV-2 spike protein and its glycan coat" – Gerhard Hummer, *Max Planck*
0:42	16:42	7:42	10:42	10:52	Q&A
0:54	16:54	7:54	10:54	11:06	Poster Talk: "Compromised SARS-CoV-2 placental antibody transfer" – Caroline Atyeo, *Ragon Institute*
1:08	17:08	8:08	11:08	11:20	Poster Talk: "Dendritic cell targeted heparan sulfate alteration reduces inflammation and facilitates pathogen clearance during PR8 influenza A virus infection in vivo" – So Young Kim, *University of California San Diego*
1:22	17:22	8:22	11:22	11:42	KEYNOTE: "Breaking the Glyco-Code of HIV Persistence and Immunopathogenesis" – Mohamed Abdel-Mohsen, *Wistar Institute*
1:47	17:47	8:47	11:47	11:55	Poster Talk: "Determination of the function of the A064R protein produced by Paramecium bursaria chlorella virus" – Immacolata Speciale, *University of Naples Federico II*
1:57	17:57	8:57	11:57	12:07	Q&A
2:07	18:07	9:07	12:07 – 13:00		BREAK
3:00	19:00	10:00	13:00	13:40	**ACHIEVEMENT AWARD LECTURE: Salome Pinho,** ***University of Porto*** **| Chair: Karen Colley,** ***University of Illinois, Chicago***
3:45	19:45	10:45	13:45 – 15:30		***EXHIBITS AND POSTER SESSION 3***
3:45	19:45	10:45	14:00 – 15:00		**WORKSHOP: NIH/NIGMS Information Session for post-docs and early stage investigators, Q&A with Michelle Bond,** ***Program Officer, NIH/NIGMS***
5:30	21:30	12:30	15:30 – 16:30		*BREAK*
6:30	22:30	13:30	16:30 – 19:02		**Session 6: Chemical Glycobiology | Chair: Jennifer Kohler,** ***UTSW***
6:30	22:30	13:30	16:30	16:50	KEYNOTE: "Dissecting O-GalNAc glycosylation by glycosyltransferase bump-and-hole engineering" –Ben Schumann, *Crick Institute*

TOKYO TIME	ROME TIME	PACIFIC TIME	EASTERN START TIME	EASTERN END TIME	SESSION
6:52	22:52	13:52	16:52	17:04	Poster Talk: "Towards the selective tagging of the sialome of living cells using sydnone-modified neuraminic acids" – Frederic Friscourt, *Université de Bordeaux*
7:06	23:06	14:06	17:06	17:26	KEYNOTE: "Chemical editing of proteoglycan architecture" – Mia Huang, *Scripps Florida*
7:28	23:28	14:28	17:28	17:40	Poster Talk: "Polar functional group-containing glycolipid CD1d ligands modulate cytokine-biasing responses" –Yukari Fujimoto, *Keio University*
7:42	23:42	14:42	17:42	17:52	Q&A
7:54	23:54	14:54	17:54	18:14	KEYNOTE: "Automated chemo-enzymatic synthesis of complex glycans offers opportunities for flu surveillance" – Geert-Jan Boons, *CCRC, UGA*
8:16	0:16	15:16	18:16	18:28	Poster Talk: "Occurrence and interactions of zwitterionic modifications of glycans in eukaryotes" – Iain Wilson, *Universität für Bodenkultur*
8:30	0:30	15:30	18:30	18:50	KEYNOTE: "Structural and biochemical insights into the molecular mechanism of polysaccharide O-acetylation" – Breeana Urbanowicz, *CCRC, UGA*
8:52	0:52	15:52	18:52	19:02	Q&A

Thur. Nov 12 (Day 4)

23:30	15:30	6:30	9:30 – 12:10		**Session 7: NCFG Session | Chair: Michael Pierce, University of Georgia and Richard Cummings, *Harvard University***
23:30	15:30	6:30	9:30	9:40	Introduction – Michael Pierce, University of Georgia and Richard Cummings, *Harvard University*
23:40	15:40	6:40	9:40	10:05	"Glycan recognition by glycan-binding proteins: Insights from microarray analyses" – Richard Cummings, *Harvard University*
0:10	16:10	7:10	10:10	10:30	"Glycan receptors for mumps virus and other paramyxoviruses" –Takao Hashiguchi, *Kyushu University*
0:35	16:35	7:35	10:35	10:55	"Making weak antigens strong: exploiting bacterial outer membrane vesicles for discovering glycan-specific antibodies" – Matthew DeLisa, *Cornell University*
1:00	17:00	8:00	11:00	11:20	"The potential role of glycans as therapeutics targets during intestinal inflammation" – Jennifer Brazil, *University of Michigan*
1:25	17:25	8:25	11:25	11:45	"A Caenorhabditis elegans N-glycan shotgun array: Isolation, analysis, creation, and informatics support" –John Cipollo, *FDA-CBER*
1:50	17:50	8:50	11:50	12:10	"New enzymes for cell surface modification: towards universal blood" – Stephen Withers, *University of British Columbia*
2:10	18:10	9:10	12:10	13:00	*BREAK*

TOKYO TIME	ROME TIME	PACIFIC TIME	EASTERN START TIME	EASTERN END TIME	SESSION
3:00	19:00	10:00	13:00	14:00	**Society Business Meeting - Michael Tiemeyer, *CCRC UGA***
3:45	19:45	10:45	13:45 – 15:30		***EXHIBITS AND POSTER SESSION 4***
5:30	21:30	12:30	15:30	16:30	*BREAK*
6:30	22:30	13:30	16:30 – 19:07		**Session 8: Biomes, Inflammation, and Immunity | Chair: Catherine Grimes, *University of Delaware***
6:30	22:30	13:30	16:30	16:50	KEYNOTE: "Mechanisms of sialoglycan degradation and depletion in vaginal dysbiosis" – Amanda Lewis, *UCSD*
6:52	22:52	13:52	16:52	17:04	Poster Talk: "Galectin-8 regulates group B streptococcus uterine outgrowth by engaging microbial sialylated mimics of host glycans" – Nourine Kamili, *Emory University*
7:06	23:06	14:06	17:06	17:26	KEYNOTE: "The microbiota coordinates diurnal rhythms in intestinal innate immunity with the host circadian clock" – John Brooks, *UTSW*
7:28	23:28	14:28	17:28	17:40	Poster Talk: "Harnessing the diversity of streptocooccal sialic-acid-binding adhesins for creating a toolbox of glycan-binding probes to map the host sialome" – Benjamin Cross, *University of Buffalo*
7:42	23:42	14:42	17:42	17:52	Q&A
7:54	23:54	14:54	17:54	18:14	KEYNOTE: "The multifaceted roles of glycans in thrombopoiesis" – Karin Hoffmeister, *MedVersiti Blood Research Institute*
8:16	0:16	15:16	18:16	18:28	Poster Talk: "Glycan-mediated immune evasion in breast cancer" -Amy Paschall, *University of Georgia*
8:30	0:30	15:30	18:30	18:50	KEYNOTE: "Sialic acids in regulation of immune responses" – James Paulson, *Scripps, La Jolla*
8:52	0:52	15:52	18:52	19:02	Q&A
9:02	3:15	16:15	19:02	19:07	**Meeting Dismissal**

(1) Breaking the Glyco-Code of HIV Persistence and Immunopathogenesis

Mohamed Abdel-Mohsen
The Wistar Institute

At the intersection of immunology and glycobiology is "glycoimmunology", an emerging field focused on understanding how immune responses are mediated by glycans and glycan-lectin interactions. How glycans influence immunological functions is increasingly well understood. In a parallel way, in the HIV field, it is increasingly understood how the host immune system controls HIV infectivity, persistence, and immunopathogenesis. However, how the host glycosylation machinery may modulate the persistence and immunopathogenesis of HIV has been mostly overlooked. In this talk, we will discuss four areas in which the links between glycan-lectin interactions and immunology, and between immunology and HIV are well described. These areas are: 1) how glycans on circulating glycoproteins and glycolipids contribute to HIV-associated inflammaging?; 2) how antibody glycosylation impacts HIV persistence?; 3) what is the role of the gut glycome in regulating the homeostatic relationship between the host and its gut microbiota, during HIV infection?; and 4) how T and NK cell-surface glycosylation and glycan-lectin interactions allow HIV-infected cells to evade immunosurveillance? For each area, we will briefly describe these links and then delineate the opportunities for the HIV field, in particular, and the virology field, in general, in investigating potential interactions between glycoimmunology and viral persistence/immunopathogenesis.

(2) The GlyCosmos portal as a partner in the GlySpace Alliance to provide access to integrated glycan-related data resources

Issaku Yamada[2], Masaaki Shiota[1], Daisuke Shimachi[1], Tamiko Ono[1], Shinichiro Tsuchiya[2], Masaaki Matsubara[2], Masae Hosoda[1], Akihiro Fujita[1], Nobuyuki P. Aoki[1], Yu Watanabe[3], Noriaki Fujita[4], Kiyohiko Angata[4], Hiroyuki Kaji[4], Hisashi Narimatsu[4], Shujiro Okuda[3], Kiyoko F. Aoki-Kinoshita[1]
[1]*Soka University;*
[2]*The Noguchi Institute;*
[3]*Niigata University Graduate School of Medical and Dental Sciencesss;*
[4]*National Institute of Advanced Industrial Science and Technology (AIST)*

The GlyCosmos portal allows users to search and browse all kinds of information centered on glycans, such as glycan-related genes, glycoproteins, and information on diseases and pathogens related to them. It has two major sections for Repositories and Data Resources. Under the Repositories section, three repositories are available: GlyTouCan, the international glycan structure repository, GlycoPOST, a registry for raw data from glycan-related mass spectrometry experiments, and UniCarb-DR for registering annotated glycan structures as results of mass spectrometry analysis. Under the Data Resources section, data are classified by data categories, such as Genes/Proteins/Lipids, Glycans/Glycoconjugates, Glycomes, and Pathway/Diseases/Organisms. These categories organize the databases and datasets to types of data. Each individual database is listed under one or more category, while the integrated datasets are listed as GlyCosmos Datasets. In addition to the previously developed JCGGDB, ACGG-DB and GlycoNAVI, semantic web technology integrates the data stored in national and international databases for glycan-related fields. As a result, GlyCosmos can display glycan-related data in an integrated manner, making it easier to find more diverse information. Additionally, GlyCosmos, together with GlyGen in the United States and Glycomics@ExPASy in Europe, is a member of the GlySpace Alliance, an international framework for glycoscience databases. The members of the GlySpace Alliance are committed to freely sharing information in the glycobiology domain. This work has been supported by the Database Integration Coordination Program (DICP) of the Japan Science and Technology Agency (JST) and the National Bioscience Database Center (NBDC) [grant number 17934031].

(4) Automated Chemo-Enzymatic Synthesis of Complex Glycans Offering Opportunities for Flu Surveillance

Frederik Broszeit[2], Rosanne J. van Beek[2], Tiehai Li[1], Lin Liu[1], Kelley W. Moremen[1], Ron A.M. Fouchier[3], Robert P. de Vries[2], Geert-Jan Boons[1]
[1]*University of Georgia, Athens, GA;*
[2]*Utrecht University;*
[3]*Erasmus MC*

We have developed a fully automated process for enzyme-mediated oligosaccharide synthesis that can give easy access to different classes of complex glycans including poly-N-acetyllactosamine derivatives, human milk oligosaccharides, gangliosides and N-glycans. The automated platform uses a catch and release approach in which glycosyltransferase-catalysed reactions are performed in solution and product purification is accomplished by solid phase extraction. Our synthetic methodologies have provided a large collection of synthetic glycans, which were have been printed as a glycan array, and examined for binding of a large number of viral adhesion proteins, including influenza A/H3N2 viruses that have lost the ability to agglutinate erythrocytes critical for antigenic characterization. It revealed that recent A/H3N2 viruses have compensated for decreased binding of the prototypic human receptor by recognizing 2,6-sialosides on extended LacNAc moieties. Erythrocyte glycomics showed

an absence of such glycans, providing a rationale for lack of agglutination by recent A/H3N2 viruses. A glycan remodeling approach installed functional receptors on erythrocytes, allowing antigenic characterization of recent A/H3N2 viruses and confirming the cocirculation of several antigenically different viruses in humans.

(5) The microbiota coordinates diurnal rhythms in intestinal innate immunity with the host circadian clock

John F. Brooks[1], Lora V. Hooper[1,2]
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[2]*Howard Hughes Medical Institute*

Environmental light cycles entrain circadian feeding behaviors in animals that produce rhythms in exposure to foodborne bacteria. It remains unclear whether there are corresponding immunological rhythms that anticipate this microbial exposure. Here, we show that the intestinal microbiota generates diurnal rhythms in innate immunity that synchronize with host feeding rhythms. Rhythmic expression of select antimicrobial proteins was driven by daily rhythms in epithelial attachment by segmented filamentous bacteria (SFB), a member of the mouse intestinal microbiota. Rhythmic SFB attachment was driven by the circadian clock through control of host feeding rhythms. Mechanistically, rhythmic SFB attachment activated an immunological circuit involving type 3 innate lymphoid cells (ILC3). This circuit triggered oscillations in epithelial STAT3 expression that produced rhythmic antimicrobial protein expression and caused resistance to intestinal bacterial infection to vary across the day-night cycle. Thus, host feeding rhythms are synchronized with rhythms in intestinal innate immunity that anticipate exogenous microbial exposure.

(6) Bridging the Gap: Connecting Glycobiolgy and Glycoinformatics using Semantic Names, Subsumption, and Structural Motifs

Nathan Edwards[1], Wenjin Zhang[1]
[1]*Georgetown University, Washington, DC*

Despite many years of effort to create glycan portals, data-resources, and knowledgebases, there remains a fundamental challenge in bridging the gap between the language and terms used by glycobiologists to refer to and describe glycans of interest, and the accessions, identifiers, and other nomenclature used by glycoinformaticians to track, store, and reference glycans. As a central registry of glycan sequences, GlyTouCan has solved the essential issue of stable glycan identifiers, but the resulting accessions, more than 120,000 to-date, cannot easily be explored to find specific glycans. As part of the GlyGen glycan project (http://glygen.org) we have developed tools to help glycobiologists find glycans using familiar terminology and visual, interactive browsing strategies.

The Glycan Naming and Subsumption Ontology (GNOme, http://gnome.glyomics.org) organizes GlyTouCan accessions for interactive browsing, text-based searching, and automated reasoning. A formal OBO Foundry ontology, GNOme also provides a visual, interactive glycan structure browser for quickly finding appropriate glycan accessions using monosaccharide composition, visual selection of appropriate topology, and progressive refinement of glycan linkage details. The GNOme composition browser provides a similar user-interface for finding accessions of glycan compositions. Semantic names, such as Byonic-format composition strings, are associated with specific accessions, but can also be used to find related topologies and structures. For informaticians and tool builders, the interactive browsers permit deep-linking of compositions or accessions, while the formal ontology provides the necessary substrate for automated reasoning about glycans' subsumption relationships.

The GlycoMotif project (http://glycomotif.glyomics.org)aggregates and organizes more than 600 named glycan structural motifs from ten collections, identifying common motifs, motif topology clusters, and motif topologies that differ due to the addition or removal of a single monosaccharide. The GlyGen motif set, based on Cummings (2009), is annotated with each of the citations from this reference, and has been carefully curated to fix common errors in motif naming. We have also defined semantically meaningful motif alignment strategies, including Substructure, Core, Nonreducing-End, and Whole-Glycan, to capture the ways in which these structural motifs are expected to align to glycan structures, and provide precomputed motif alignments for GlyTouCan glycans via a public triple-store resource. Importantly, many of the glycan determinants from Cummings (2009) represent specific antigens with well-understood and accepted names—once aligned, these motifs can be readily associated with GlyTouCan accessions and make their way into GNOme and GlyGen as semantic names and search terms. Furthermore, Core alignment motifs from the GlyGen motif set help assign commonly used glycan classification terminology, such as O-Fucose, to specific glycan structures.

Together, the GNOme and GlycoMotif projects help bridge the gap between glycobiologists' glycans and the accessions representing these glycans in GlyGen and other glycoinformatics resources.

(10) Chemical Editing of Proteoglycan Architecture

Timothy O'Leary[1], Megan Critcher[1], Tesia Stephenson[1], Mia L. Huang[1]
[1]*Scripps Research*

Proteoglycans are multi-facted cell surface macromolecular glycoconjugates that can coordinate many important cellular processes, including mammalian differentiation and development. While much attention has focused on the glycosamino-

glycan chains that decorate proteoglycans, other architectural parameters also play important roles in orchestrating their functional roles. In addition to the distinct binding partners imparted by different poly-sulfated glycosaminoglycans, the protein cores can also serve as unique functional receptors. Furthermore, proteoglycans can be anchored into distinct membrane microdomains that can regulate signal transduction events. We aim to catalogue the corresponding influences in growth factor signaling and differentiation that result from these changes, yet strategies to analyze proteoglycans that consider all these parameters simultaneously are limited. Here, we present a comprehensive approach to study proteoglycan structure and biology by generating semi-synthetic modular proteoglycans that can be displayed on cell surfaces. This approach to create engineered proteoglycans integrates techniques in protein and cell surface engineering, small molecule chemistry, and bioconjugation.

(11) How far can we go with genetic approaches to glycomics?

Hiren J. Joshi[1], Yoshiki Narimatsu[1], Christian Büll[1,2], Henrik Clausen[1]
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[2]*Hubrecht Institute*

Genetic approaches to the glycome take advantage of our knowledge of biosynthetic pathways to bridge the gap between our analytical technologies and the need to describe the full human glycome. However, deriving information on how the glycome is controlled by over 200 genes is difficult to achieve without extra tools. To achieve this, we have made use of data visualisation and in silico tools to make apparent the patterns in the data[1,2]. The "rainbow" glycosylation pathway figure not only functions as a visual map of pathways, but also works as a fully-fledged in-silico framework that can be used to exploit our knowledge of the glycogenome. The visualisation and framework can reveal information about regulatory patterns for gene expression, diseases of glycosylation, and integrate knowledge from new data sources. Integration of this framework into software tools enhances the utility of the software, and enables the interpretation of data from sources such as cell-based glycan arrays[3] (https://glycoradar.glycomics.ku.dk). Finally, we can forecast what limitations on information about the glycome we can reveal using our framework, and how they can be overcome.

References

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(12) Cross-talk of glycosylphosphatidylinositol biosynthesis with glycosphingolipid biosynthesis

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Glycosylphosphatidylinositol (GPI)-anchored proteins and glycosphingolipids interact with each other in the mammalian plasma membranes, forming dynamic microdomains. How their interaction starts in the cells has been unclear. Based on a genome-wide CRISPR-Cas9 genetic screen for genes required for GPI side-chain modification by galactose in the Golgi apparatus, we report that β1,3-galactosyltransferase 4 (B3GALT4), the previously characterized GM1 ganglioside synthase, additionally functions in transferring galactose to the N-acetylgalactosamine side-chain of GPI. Furthermore, B3GALT4 requires lactosylceramide for the efficient GPI side-chain galactosylation. Thus, our work demonstrates previously unexpected functional relationships between GPI-anchored proteins and glycosphingolipids in the Golgi.

(13) Structural and mechanistic studies of OST and ER-based glycosyltransferases involved in protein N-glycosylation

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In eukaryotic protein N-glycosylation, a series of glycosyltransferases catalyze the biosynthesis of a dolichylpyrophosphate-linked oligosaccharide prior to its transfer onto acceptor proteins, which is catalyzed by oligosaccharyltransferase (OST). We present structural and mechanistic studies of yeast ALG6, which adds the first of three glucose moieties onto the DolPPGlcNAc$_2$Man$_9$acceptor. We determined two cryo-EM structures: An apo structure at 3.0 Å resolution and a 3.9 Å structure of ALG6 bound to a synthetic DolPGlc analog. A comparison with reported GT-C structures suggests that GT-C enzymes contain a modular architecture with a conserved and a variable module, each with distinct functional roles. We also determined structures of human OST complexes OST-A and OST-B, which reveal differences that rationalize their distinct cellular functions. The presentation will also cover newer results in our mechanistic investigations.

(14) Structure and function of perineuronal nets: Unique extracellular matrix structures in the central nervous system.

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Perineuronal nets (PNNs), are unique extracellular matrix structures that form exclusively in the central nervous system (CNS). These are complex carbohydrate-enriched macromolecular structures that form in a honeycomb-like pattern on the surface of a limited but specific and unique population of neurons. Despite their limited distribution, they profoundly impact developmental plasticity and multiple different types of learning and memory. Their prominent role in these processes has led to their implication in multiple neuropsychiatric disorders, neurodegenerative disease and recovery after neural injury. However, despite clear evidence for critical roles for PNNs in regulating neural function, their exact molecular composition and structure are not well understood, which, in turn, has made it difficult to determine precise functions of PNNs. PNNs are composed of a hyaluronan backbone saturated with an array of hyaluronan-binding chondroitin sulfate proteoglycans (CSPGs), link proteins, and glycoproteins. Our most recent work has identified a novel and critical role for receptor-type protein tyrosine phosphatase zeta (RPTPζ) in PNN structure and function. RPTPζ and a secreted splice variant (phosphacan), are derived from the Ptprz1 gene and have been implicated in a variety of developmental processes but a role for these proteins in PNNs is completely unknown. Using *Ptprz1* KO mice we investigated PNN formation and structure via immunohistochemistry and biochemical techniques. We found that in the cortex and hippocampus, PNNs were severely disrupted in the absence of RPTPζ/phosphacan. Further analysis using cell culture and biochemical techniques revealed that RPTPζ/phosphacan mediates key interactions between known components of the PNN, in particular tenascin-R, and the cell surface of net-bearing neurons. Therefore, our work demonstrates that RPTPζ/phosphacan is a central player in the formation of PNNs. Overall these studies provide a new and novel model for PNN structure and have important implications for analysis of PNN function.

(17) Exploring the mechanisms underlying the NGLY1 loss-of-function phenotypes in Drosophila

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NGLY1-deficiency, the first known congenital disorder of deglycosylation (CDDG), is caused by mutations in human N-glycanase 1 (NGLY1). It is a multisystem disorder with global developmental delay and other phenotypes including neuropathy, movement disorder, and chronic constipation. However, the pathophysiology of this disease is not well understood, and no mechanism-based therapies have been established for NGLY1 deficiency. *Drosophila Pngl* encodes a homolog of human NGLY1. Previously, we reported that Pngl mutant larvae exhibit developmental defects in the intestine due to mesoderm-specific loss of BMP signaling. Loss of *Pngl* causes lethality (less than 1% survival), as mutant animals die at late larval or pupal stage. However, BMP signaling defects can only explain 20–30% of the lethality in these animals. *Pngl* mutant larvae also exhibit a severe defect in gut clearance, which cannot be explained by impaired BMP signaling. Our recent unpublished observations indicate that impaired gut clearance in Pngl mutants is associated with reduced *AMPK*α level, leading to energy homeostasis defects and impaired gut peristalsis. We have also discovered defects in gut-barrier integrity and a hyper-activated immune response in the intestines of *Pngl* mutant larvae. I will discuss these data and describe how impairment in multiple pathways contribute to the phenotypes and the lethality of *Pngl* mutant *Drosophila*.

(18) Regulation of neural functions by protein glycosylation: Insights from Drosophila model

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Protein glycosylation can affect protein functions by several mechanisms, including regulation of protein folding and stability, effects on trafficking, molecular interactions, and signaling. Several neurological abnormalities associated with congenital defects in glycosylation were identified in recent studies, however molecular and cellular mechanisms underlying these defects remain obscure, which highlights the limitations of functional approaches due to the overwhelming complexity of the nervous system and complicated glycosylation in mammals. A simplified model organism, such as

Drosophila, can help uncover these mechanisms and shed light on new players and associated pathways. One of the pathways with prominent roles in the nervous system is protein O-mannosylation (POM), which is best known for its role in the regulation of Dystroglycan and association with congenital muscular dystrophies. POM defects can also result in pronounced phenotypes in the nervous system, such as abnormal brain development. However, the pathological mechanisms causing these defects are poorly understood. In our studies, we focused on deciphering neurological functions of Protein O-mannosyltransferases 1 and 2 (POMT1–2) that are highly conserved in animals, from *Drosophila* to humans. *Drosophila* Dystroglycan is also conserved in Drosophila as a substrate of POMT1–2. However, our experiments revealed that other functionally important substrates are involved in neural development. Using larval sensory neurons as a model system, we applied a candidate-based screen strategy to elucidate these new functional targets of POM. Our experiments indicated that Receptor Protein Tyrosine Phosphatases (RPTPs) represent a novel group of POM substrates that are involved in the development of axon connectivity and assembly of neural circuits. We analyzed interactions between POMTs and different *RPTP* genes using mutant alleles and RNAi-mediated knockdown genotypes. Interestingly, our results suggested that multiple RPTPs are functionally affected by POMTs. We generated a transgenic RPTP construct for *in vivo* expression and purification experiments. Our experiments indicated that RPTP is a direct target of POMTs that mediates the effect of POM on the development of sensory axons. Our data shed new light on the function of POM in the nervous system. These mechanisms described in *Drosophila* are expected to be conserved in mammals and help understand the pathogenic processes underlying neurological abnormalities caused by POMT defects. This project was supported by grants from NIH (NS099409) to VP, CONACYT-TAMU (2012-037S,19–20-052) to VP and AGH.

(19) POGLUT1 biallelic mutations cause myopathy with reduced Notch signaling and α-dystroglycan hypoglycosylation

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Notch signaling pathway plays critical roles in the highly coordinated muscle regenerative process, maintaining an appropriate population of satellite cells and preventing premature differentiation. Our previous works in showed that addition of O-linked glucose to Notch receptors by protein O-glucosyltransferase 1 (POGLUT1) is required for Notch signaling. We describe the first cohort of 15 patients from nine unrelated families with limb-girdle muscular dystrophy, in which recessive missense mutations in *POGLUT1* gene dramatically reduces the enzymatic activity on Notch. As a result, these patients show a defect in Notch signaling, with a significant depletion of satellite cells resulting in defective muscle regeneration, ultimately leading to muscle dystrophy. We show that the enzymatic activity of POGLUT1 is also essential for the formation of adult muscles by myoblasts in *Drosophila*. Moreover, cross-species overexpression studies in *Drosophila* indicate that mutations impair the ability of human POGLUT1 in rescuing the muscle defects in flies lacking endogenous POGLUT1 activity.

An additional striking result in our patients was the reduced α-dystroglycan glycosylation and functional defect as a receptor, in muscle but not in fibroblasts from skin. This result suggests that the reduction in α-dystroglycan glycosylation is related to the altered timing and dynamic of the muscle regenerative process.

Our cohort showed a broad age at onset, from adult to congenital and infantile forms, and displayed a unique muscle imaging pattern as a defining feature of this muscular dystrophy. These findings broaden the field of pathomechanisms in muscular dystrophy, settle the muscular clinical phenotype linked to POGLUT1 mutations and identify a new class of O-linked glycosylation as an important player.

(20) Protein glycosylation at the interface of inflammation and cancer: mechanisms and clinical applications

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The immune system is guided by a series of co-stimulatory and co-inhibitory pathways. The disruption of the control of these molecular checks and balances can lead to unpredictable autoimmune or cancer states.

The mechanisms underlying the genesis of the loss of immunological tolerance in autoimmunity or the creation of immunosuppressive networks in cancer are still elusive.

We have been deciphering how glycosylation integrates the regulatory networks that govern the innate and adaptive immune responses accounting for autoimmunity and cancer.

Our results in chronic inflammatory disorders such as Inflammatory Bowel Disease revealed that alterations in the branched N-glycosylation pathway regulate the T cell-mediated immune response and was associated with disease severity [Dias AM et al, Human Molecular Genetics 2014]. We further demonstrated that metabolic supplementation of ex vivo mucosal T cells from active Ulcerative Colitis patients with N-acetylglucosamine resulted in enhancement of branched N-glycosylation in the T cell receptor leading to suppression of T cell growth, inhibition of Th1/Th17 immune response and a controlled T cell activity. We also showed that mouse models displaying a deficiency in the branched N-glycosylation pathway (MGAT5−/−; MGAT5+/−) exhibited increased susceptibility to severe forms of colitis and early onset disease. Importantly, the treatment of these mice with GlcNAc reduced disease severity and suppressed disease

progression due to a controlled T cell-mediated immune response at the intestinal mucosa [Dias AM, et al. PNAS 2018; Verhelst X,...,Pinho SS. Gastroenterology 2020].

In a cancer context, our results further support the regulatory properties of branched N-glycans in both in innate and adaptive immune responses. We demonstrated that branched N-glycans are used by colorectal cancer cells to escape immune recognition, instructing the creation of immunosuppressive networks through inhibition of IFNγ. The removal of this "glycan-mask" was found to expose immunogenic glycans that potentiated immune recognition by DC-SIGN-expressing immune cells resulting in an effective anti-tumor immune response (Silva M & Fernandes A, et al. Cancer Immunology Research 2020).

In conclusion, our results demonstrate how glycans regulate both innate and adaptive immune responses [Pereira M & Alves I, et al. Frontiers in Immunology 2018] contributing to inflammatory diseases and cancer, pinpointing glycans as immunomodulatory agents with promising clinical applications in autoimmune diseases and cancer.

(21) 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 cross-talk of different isoenzymes with each other. And since O-GalNAc glycosylation happens early in the secretory pathway, elaboration by downstream GTs introduces heterogeneity that renders unbiased enrichment and (glyco-)proteomics strategies challenging to develop.

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 sites of individual GalNAc-T isoenzymes are enlarged by mutation, creating a "hole" that renders the enzyme compatible with a chemical functionality ("bump") in a synthetic UDP-GalNAc derivative. A traceable chemical handle in the bump allows for the specific detection of glycoproteins by bioorthogonal ligation in the living cell. Chemical MS glycoproteomics enables the characterization of GalNAc-T isoenzyme-specific glycosylation sites and glycan structure. 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. We use these first bioorthogonal O-GalNAc precision tools as reporter probes in superresolution microscopy, chemical glycoproteomics and a genome-wide CRISPR screen, thereby considerably expanding the glycobiology toolbox.

(22) Target protein O-GlcNAcylation

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Over 15% of the cellular proteome is modified by O-linked N-acetyl glucosamine (O-GlcNAc), a post-translational modification (PTM) that consists of a single N-acetyl glucosamine monosaccharide attached to serine or threonine residues of nuclear, cytosolic and mitochondrial proteins. Due to the ubiquitous nature of the modification, O-GlcNAc has been implicated in numerous biological processes, including immune response, cancer progression, neurodegenerative disease, and diabetes. In order to explore the role of O-GlcNAc on glycoproteins in human disease, we have now developed a cellular approach for target protein O-GlcNAcylation. The method uses a nanobody fused to an O-GlcNAc writer or eraser to control O-GlcNAc levels on the desired target protein in cells. Evaluation of the effect of O-GlcNAc on the targeted transcription factors revealed functions in protein stabilization and regulation of protein–protein interactions. Target protein O-GlcNAcylation will accelerate new insights to O-GlcNAc functions and reveal approaches to engineer these biological signals.

(23) 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. In addition to fibronectin, we propose that Drgal9-L1 is binding to other cell surface 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: (a) selective CRD inactivation via mutation, and (b) 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 grants R01GM070589–06 from NIH and IOS-1656720 from NSF to GRV].

(24) Glycosyltransferases within the psrP locus facilitate pneumococcal virulence

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The Pneumococcal Serine-Rich Repeat Protein (PsrP) is a high-molecular weight, glycosylated adhesin that promotes the attachment of *Streptococcus pneumoniae* (Spn) to host cells. PsrP, its associated glycosyltransferases, and dedicated secretion machinery, are encoded in a 37 kb genomic island that is present in ~50% of clinical isolates of Spn. PsrP has been implicated in establishment of lung infection in murine models, although specific roles of the PsrP glycans in disease progression or bacterial physiology have not been elucidated. Moreover, enzymatic specificities of associated glycosyltransferases are yet to be fully characterized. We hypothesized that the glycosyltransferases that modify PsrP are critical for the adhesion properties and infectivity of Spn. Here, we characterize the putative Spn *psrP*-locus glycosyltransferases responsible for *PsrP* glycosylation. We also begin to elucidate their roles in Spn virulence. We show that four glycosyltransferases within the psrP locus are indispensable for Spn biofilm formation, lung epithelial cell adherence, and establishment of lung infection in a mouse model of pneumococcal pneumonia.

(25) Defining the structural basis of N-glycan microheterogeneity through rate monitoring of sugar transfer

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N-glycans are commonly added contranslationally en bloc to eukaryotic glycoproteins via an N-glycosidic linkage to asparagines within a conserved motif (N-X-S/T(C)). These N-glycans are then further processed into a diversity of structures in the endoplasmic reticulum and Golgi apparatus through the addition and removal of additional sugars. These glycans are one of the most common post-translational modifications (PTMs) in eukaryotes, and presentation of differential isoforms are linked to changes in protein solubility, cell–cell/protein–protein interactions, half-life, and immune function. Glycoproteins often harbor multiple N-glycans with divergent isoforms, leading to a site-specific diversity termed 'microheterogeneity'. This microheterogenity can be quite different at different sites of the same polypeptide. Despite advances in glycoproteomics to define microheterogeneity, it remains unclear how protein structure impacts the ability of glycosidases and glycosyltransferases to process the glycans into more mature structures, and how this processing impacts and is responsible for observed microheterogeneity. To address this, our group has developed a workflow in which high yields of high-mannose reporter glycoproteins are produced in *MGAT1*$^-$ HEK293F cells and then further processed *in vitro* using highly purified, appropriate glycosyltransferases and glycosidases. Time-course samples of

these reactions are taken and the resulting glycoproteins are digested to yield glycopeptides that can then be analyzed by tandem mass spectrometry and quantified in MS1. By monitoring the time-dependent addition or removal of individual sugars to individual sites on glycoproteins over time, we can begin to identify local structural features that affect the rate of sugar addition or removal. We have already identified substantial differences in the rate of GlcNAc addition by MGAT1 on different sites of the same protein. Identification of key structural features will aid in the development of glycoprotein therapeutics with defined N-glycan populations through glycoengineering as well as providing a molecular basis for understanding microheterogeneity of N-glycoproteins.

(26) A Strategy Based on Photo-affinity Probe and Proteomics to Identify the Interactome of Non-Saccharide Glycosaminoglycan Mimetics

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Non-Saccharide Glycosaminoglycan Mimetics (NSGMs) are fully synthetic, highly sulfated, and homogeneous mimetics of natural glycosaminoglycans (GAGs). Just as GAG sequences modulate a host of biological responses, cellular growth, inflammation, and coagulation, so also well-designed NSGMs modulate these responses. As with GAGs, the identification of protein binding partners of NSGMs is challenging. We recently showed that G2.2, a fully synthetic and homogeneous NSGM, selectively inhibits colorectal cancer stem cells through structural and functional mimicry a heparan sulfate hexasaccharide (HS06). To identify the human plasma interactome of G2.2, we designed and synthesized a photoactivatable analog of G2.2 based on the diazirine functional group for irreversible conjugation with interacting proteins and an alkyne functional group for fluorescence or affinity tagging using click chemistry. The designed photoaffinity probe of G2.2, labeled as G2.2P, conjugated with only three interacting proteins in a positive control sample containing eight coagulation factors. G2.2P displayed a characteristic concentration-dependence of covalent conjugation suggestive of selective recognition phenomenon. Following this, we explored the use of the probe to elucidate the human plasma interactome of G2.2. Employing an affinity pull-down technique, we obtained G2.2P's bound and unbound fractions, which were subjected to proteomic analysis. We identified 21 human plasma proteins binding to G2.2P probe, which represents a small interactome in comparison to the plausible interactome of several hundred proteins. Interestingly, some 14 proteins in the G2.2P interactome have not yet been known to bind GAGs, which suggests that the known GAG interactome of several hundred proteins is likely to be an underestimation. Overall, the photoaffinity probe combined with proteomics strategy appears to be a powerful technology for identifying protein targets of NSGMs, and possibly for GAGs too, in an unbiased fashion.

(27) Factors modulating the interaction of prions with heparan sulfate and their impact in prion pathogenesis

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Prion diseases are fatal neurodegenerative disorders caused by misfolded prion proteins (PrP^{Sc}) that accumulate in the brain as diffuse deposits (subfibrillar prion strains) or amyloid plaques (fibrillar prion strains), and have selective cell tropism. Fibrillar prions are primarily formed by extracellularly shed, ADAM10-cleaved PrP^{Sc}, while subfibrillar prions are tethered to the plasma membrane by a glycosylphosphatidylinositol anchor. The endogenous glycosaminoglycan, heparan sulfate (HS), selectively binds ADAM10-cleaved PrP in the brain extracellular matrix and vascular basement membrane, facilitating their deposition as parenchymal and vascular plaques. Here, we identify the factors modulating the interaction of fibrillar and subfibrillar prions with HS and define their effect in prion replication and disease progression.

Using liquid chromatography/mass spectrometry, we found that fibrillar prions bound exceedingly higher levels of HS as compared to subfibrillar prions, and that the HS bound to fibrillar prions was generally less sulfated. Heparin promoted the replication of all subfibrillar and fibrillar prions by cell-lysate protein misfolding cyclic amplification (PMCA) in a concentration-dependent fashion. However, heparin desulfated at either the N-, 6-O or 2-O positions failed to promote the in vitro formation of prions, again with no differences between prion strains. We also found that subfibrillar prions require longer heparin chain lengths for efficient prion replication. Finally, we established that ADAM10-cleavage of PrP increases binding affinity for heparin. Together, our in vitro studies point to three major modulators of the PrP:HS interaction: the HS sulfation and length, and the ADAM10-cleavage of prions.

Currently, we are addressing how modulating HS composition in different cell types affect the replication of prions *in vivo*. *N-deacetylase/N-sulfotransferase 1 (Ndst1$^{f/f}$)* mice were bred to mice that express Cre-recombinase under the *tyrosine kinase (Tie2), synapsin1 (syn1) or glial fibrillary acidic protein (GFAP)* promoters in order to decrease or alter the HS sulfation in endothelial cells, neurons and astrocytes, respectively. Mice were intracerebrally infected with subfibrillar and fibrillar prion strains and the prion disease phenotype is being characterized. Our ongoing studies show that desulfating HS is primarily impacting the replication of fibrillar, ADAM10-cleaved prions, resulting in profoundly delayed prion disease progression and altered assembly and spread of prions in brain. Strikingly, these results are also cell-dependent, which renders valuable data on the role of HS in the selective cell tropism of prion strains. Together, our *in vitro* and *in vivo* studies provide a new target for the rational design of neuroprotective therapies based on manipulating HS sulfation to disrupt the interaction of HS with misfolded prion protein.

(28) CarbArrayART—An update on the software tool for carbohydrate microarray data, storage, processing, presentation and reporting

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Glycan arrays have been evolving since 2002 [1]. The technologies provide essential tools to unravel glycan recognition systems in health and in infectious and non-infectious diseases [2–4].

We have developed Carbohydrate micro-Array Analysis and Reporting Tool (CarbArrayART) using Java to address the need for a distributable software tool for carbohydrate microarray data management. The tool capitalizes on processing functions of GRITS Toolbox [5].

The main features of CarbArrayART are:

1. Storage of data from different array formats with raw scan data and related array-specific metadata compliant with guidelines of MIRAGE (Minimum Information Required for A Glycomics Experiment) [6]. These include information on glycan-binding samples, glycan probe lists, array geometry and experimental protocols.
2. Presentation of data as charts, tables and heatmaps based on average glycan-binding intensities automatically generated from scan data and array geometry. There are filtering and sorting functions for glycans.
3. Data reporting in Word, PDF and Excel formats.

To increase versatility, the system enables multiple formats for glycan sequence representations: 2D TEXT [7], GlycoWorkbench Sequence (GWS) [8, 9], Web3 unique representation of carbohydrate structures (WURCS) [10], GlyTouCan ID [11] and GlycoCT {condensed} [12], the latest addition to the list, CFG-IUPAC.

There has been alpha testing for improving the ease of use of CarbArrayART for data entry from arrays that have different layouts. Multiple data sets can be readily compared with side-by-side heatmaps. The web portal for CarbArrayART version 1.0 which will include a download page with user's manuals and related documents is currently being finalized.

New features are being developed in CarbArrayART for uploading data to and downloading from the glycan microarray repository under construction within GlyGen project [13].

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(29) N-glycans on EGF domain-specific *O*-GlcNAc transferase (EOGT) facilitate EOGT maturation and peripheral endoplasmic reticulum localization

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Epidermal growth factor (EGF) domain-specific *O*-GlcNAc transferase (EOGT) is an endoplasmic reticulum (ER)-resident protein that modifies EGF repeats of Notch receptors and thereby regulates Delta-like ligand-mediated Notch signaling. Several EOGT mutations that may affect putative N-glycosylation consensus sites are recorded in the cancer database, but the presence and function of N-glycans in EOGT have not yet been characterized. Here, we identified N-glycosylation sites in mouse EOGT and elucidated their molecular functions. Three predicted N-glycosylation consensus sequences on EOGT are highly conserved among mammalian species. Within these sites, we found that Asn-263 and Asn-354, but not Asn-493, are modified with N-glycans. Lectin blotting, endoglycosidase H digestion, and MS analyses revealed that both residues are modified with oligomannose N-glycans. Loss of an individual N-glycan on EOGT did not affect its ER localization, enzyme activity, and ability to *O*-GlcNAcylate Notch1 in HEK293T cells. However, simultaneous substitution of both N-glycosylation sites affected both EOGT maturation and expression levels without an apparent change in enzymatic activity, suggesting that N-glycosylation at a single site is sufficient for EOGT maturation and expression. Accordingly, a decrease in *O*-GlcNAc stoichiometry was observed in Notch1 co-expressed with an N263Q/N354Q variant compared with wildtype EOGT. Moreover, the N263Q/N354Q variant exhibited altered subcellular distribution within the ER in HEK293T cells, indicating that N-glycosylation of EOGT is required for its ER localization at the cell periphery. These results suggest critical roles of N-glycans in sustaining *O*-GlcNAc transferase function both by maintaining EOGT levels and by ensuring its proper subcellular localization in the ER.

(30) Hypothalamic chondroitin sulfate glycosaminoglycan matrix assembly is required for sustained diabetes remission induced by fibroblast growth factor 1 in rats

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The central nervous system (CNS) plays a critical role in preserving homeostatic control of blood glucose levels, and defective glucoregulatory neurocircuit activity within the hypothalamic arcuate nucleus median eminence (ARC-ME) of the mediobasal hypothalamus (MBH) is implicated in the pathogenesis of type 2 diabetes (T2D). In the ARC-ME of T2D-Zucker Diabetic Fatty (ZDF) rats and high-fat diet, low-dose streptozotocin (HFD/STZ) treated diabetic Wistar rats, we have identified a significant loss in extracellular chondroitin sulfate (CS) glycan containing perineuronal net (PNN) matrices. These CS glycan-rich structures enmesh neuropeptide Y (NPY) glucoregulatory circuits within the ARC-ME and imaging of ARC-ME PNNs show that diabetic ZDF and HFD/STZ rats exhibit a 33% and 16% decrease in *Wisteria floribunda* agglutinin lectin labeling of PNN CS glycans in the medial ARC region, respectively, compared to normoglycemic controls. Distinctive sulfation of the attached CS isomers (0S-, 4S-, 6S-, 4S6S-, 2S6S-CS, dermatan) differentially influences the function of PNNs. Using LC–MS/MS, we provide the first description of hypothalamic CS glycan sulfation patterns and show that T2D-ZDF rats, but not HFD/STZ diabetic Wistar rats, exhibit elevated levels of the Δ4S- isomer, whereas Δ6S- and Δ2S6S-CS isomers are decreased, differences predicted to disrupt protein-GAG interactions involved in neurite outgrowth and favor tissue stiffening in this leptin-deficient rat model of T2D. To determine if ARC-ME PNNs are targets for the blood glucose-lowering effect of fibroblast growth factor 1 (FGF1), we performed intracerebroventricular (icv) injection of FGF1 targeting the lateral ventricle (LV) in T2D-ZDF rats and report that both the hypothalamic PNN CS glycan sulfation patterns and abundance are normalized in association with diabetes remission. Western blot protein analysis shows that this effect corresponds to a 1.7-fold increase in aggrecan protein abundance. To assess the functional significance of ARC-ME PNN restoration by icv FGF1, ZDF rats received an icv FGF1 injection targeting the LV followed by bilateral microinjection of either ChABC, a PNN CS glycan digesting enzyme, or its vehicle control into the ARC-ME. Our finding that ARC-ME PNN digestion blunted the duration of sustained FGF1-induced blood glucose-lowering ($p < 0.05$) identifies hypothalamic PNNs as novel participants in the

central homeostatic control of glucose homeostasis and diabetes remission by FGF1.

(31) An unusual glycan-neoepitope with diagnostic and prognostic value in Systemic Lupus Erythematosus

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Changes in protein glycosylation are a hallmark of many immune-mediated diseases. Glycans are master regulators of the inflammatory response being important danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) crucial for the discrimination between "self" and "non-self". Here we explored whether changes in the cellular glycosylation signature is a phenotype of Lupus Nephritis (LN) and whether an abnormal exposure of specific glycans has diagnostic value for LN further further exploring its prognostic application in Systemic Lupus Erythematosus (SLE).

A comprehensive tissue glycomics characterization was performed in a cohort of human biopsy-proven LN clinical samples from SLE patients and healthy controls through the combination of advanced mass spectrometry imaging (MSI); in situ glyco-characterization and ex vivo glycophenotyping aiming to structurally map the N-glycans repertoire in LN samples, exploring its diagnostic and prognostic applications.

Our results demonstrated that LN samples exhibit a unique glycan signature characterized by an increased abundance and spatial distribution of unusual N-glycans when compared with normal kidney. This exposure of a specific group of glycans in LN occurs at the cell surface of kidney epithelial cells promoting an increased recognition by specific C-type lectin receptors expressed by immune cells. Importantly, we found that this specific glycosignature detected in kidney biopsies at time of diagnosis is able to predict a poor prognosis of SLE, particularly the development of chronic kidney disease (CKD) with 93% of specificity.

(32) Seek and destroy, human intelectin-1 surveillance for unique bacterial exocyclic 1,2-diols

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Human intelectin-1 (hIntL-1) is a glycoprotein that is secreted into the intestinal mucosa by goblet cells and is also detected in the blood. Additionally, hIntL-1 expression has been reported in the urinary tract, mesothelium, adrenal cortex, omental tissue and bronchial epithelial cells. Previous work screening the Consortium for Functional Glycomics mammalian and bacterial glycan arrays showed hIntL-1 does not recognize mammalian glycans, including sialic acid, but binds microbe-specific surface glycans containing exocyclic 1,2-diols[1]. Several human pathogens are recognized by the lectin, including *Streptococcus pneumoniae*, *Vibrio cholerae* and *Helicobacter pylori*. Subsequent to binding, hIntL-1 is capable of agglutinating *H. pylori* and increasing phagocytosis of the pathogen[2]. These findings led to the proposed role for hIntL-1 as an innate immune lectin.

Still, our understanding of the effects of hIntL-1 on bacterial biology and its mechanism of pathogen surveillance remain incomplete. To investigate this further, *S. pneumoniae*, the leading cause of bacterial pneumonia worldwide and the first organism shown to bind to hInt-1[1], served as the model for our studies. hIntL-1 binds to *S. pneumoniae* serotype 43 through recognition of the capsular polysaccharide (CPS) glycerol-1-phosphate modification and is capable of agglutinating this strain. In contrast, *S. pneumoniae* serotype 8 lacks any exocyclic 1,2-diol structures within its CPS and is not recognized or agglutinated by the lectin. Through growth curves and spotting assays, our results indicate that hIntL-1 alone is neither bacteriostatic nor bactericidal when incubated with *S. pneumoniae* serotype 43. Additionally, the intelectin showed no adverse effects on bacterial biofilm formation on surfaces or enhancement of complement-mediated

killing. In contrast, hInt-1 substantially enhanced killing of *S. pneumoniae* serotype 43 by professional human phagocytes through opsonophagocytosis and increased bacterial binding to host cells in the A549 lung cancer cell model.

Collectively, these data suggest that the main mechanism of *S. pneumoniae* clearance by hIntL-1 is through agglutination and opsonization of the pathogen leading to increased uptake by innate immune cells. Based on the lectin's broad distribution and recognition of a unique, yet common, microbial modification, we predict the role of hInt-1 will be in host surveillance for any microbe (pathogen or commensal) that is present during an inflammatory event.

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(33) Regulation of inflammatory signaling by the ST6Gal-I sialyltransferase

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The glycosyltransferase, ST6Gal-I, which adds α2,6 sialic acids on to *N*-glycosylated proteins, has been reported to play a vital role in a variety of immunological processes such as granulopoiesis, thymopoiesis, B cell proliferation and antibody production. However, the contribution of ST6Gal-I-mediated sialylation in modulating specific inflammatory signaling pathways is not fully understood. In this study, we identified a novel role for ST6Gal-I in regulating signaling through two main pathways: TNF-α/TNFR1 and LPS/TLR4, both of which are important for monocytes during the innate immune response to pathogens. Utilizing the U937 monocytic cell line, we found that knockdown of ST6Gal-I impaired long-term (2-6 hr) but not short-term (15-30 min) NFκB activation mediated by TNF-α. These data are consistent our prior results showing that α2-6 sialylation of TNFR1 prolongs NFκB signaling in epithelial cells due to a block in TNFR1 internalization. Similar to TNF-α, we found that knockdown of ST6Gal-I also abrogated long-term but not short-term LPS-mediated NFκB activation. Additionally, knockdown of ST6Gal-I impaired activation of other TLR4 effectors such as IRF3 and STAT3 at long-term timepoints. Because knockdown of ST6Gal-I impaired LPS-mediated signaling, we then assessed whether TLR4, the receptor for LPS, is sialylated by ST6Gal-I. By conducting a pull-down of TLR4 using SNA (a lectin that binds to α2,6 sialic acids), we confirmed that TLR4 is a target of ST6Gal-I. To determine whether ST6Gal-I also modulates other inflammatory pathways, we then assessed signaling mediated by the cytokines IFNγ, IL-6, and GM-CSF and found that ST6Gal-I had a limited effect on STAT activation by these cytokines. To confirm our findings in primary cells, we utilized bone marrow derived macrophages (BMDMs) from mice with myeloid-specific deletion of ST6Gal-I (LysMCre/ST6Gal-I$^{fl/fl}$). In corroboration with our data in U937 cells, BMDMs with ST6Gal-I knockout exhibited diminished long-term but not short-term activation of NFκB mediated by TNF and LPS and a decrease in long-term LPS-mediated STAT3 activation. Signaling mediated by IFNγ, IL-6, and GM-CSF was comparable between wild-type and ST6Gal-I knockout BMDMs, in agreement with the data obtained in the U937 model. Taken together, these findings indicate that the sialyltransferase, ST6Gal-I, modulates inflammatory signaling in monocytes/macrophages through specific receptors such as TNFR1 and TLR4 and does not lead to global changes in inflammatory signaling. Ultimately, this contributes to the breadth of literature that highlight ST6Gal-I as a modulator of immune cell signaling and function.

(34) Exploring the role of Reg3g-mediated Heparan Sulfate Polymerization as a target of islet dysfunction

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Offspring of obese mothers are more likely to develop obesity as well as metabolic dysfunctions, including abnormal glucose homeostasis, reduced insulin sensitivity, and impaired beta cell insulin secretion. While the sex-differences have been demonstrated in animal studies, the mechanistic targets underlying these dysfunctions are unknown. We recently found that an increase in islet Regenerating Islet Derived Protein 3-Gamma (Reg3g) in the female offspring of obese murine dams is associated with the maintenance of glucose tolerance as well as islet insulin secretion. As a Type 3 REG, Reg3g functions by binding to the EXTL3 glycotransferase that initiates heparan sulfate GAG polymerization. However, the role of Reg3g and subsequent changes in HSG in modulating offspring islet β-cell health is currently unclear.

We used a diet-induced obesity model to elucidate the role of maternal obesity on offspring islet insulin secretion, Reg3g expression, and pancreatic HS disaccharides compositions. Offspring were evaluated on postnatal day 21 (P21) for insulin/glucose tolerance, glucose-stimulated insulin secretion (GSIS), and islet Reg3g expression. Pancreata were taken for glycosaminoglycan isolation and characterization with SAX-HPLC to determine disaccharides composition as well as

total amount. Offspring were evaluated on postnatal day21 (P21) for glucose/insulin tolerance, glucose-stimulated insulin secretion (GSIS), and islet Reg3g expression. Pancreata were taken for Immunohistochemistry and for glycosaminoglycan isolation and characterization with SAX-HPLC to determine disaccharides composition as well as total amount. Using antibody that targets N-glucosamine of HSG, we found that the increase in islet Reg3g gene correlated with higher islet HSG in females offspring of obese mice. Additionally, the higher islet Reg3g and HSG was associated with maintaince of islet insulin secretion. On the contrary, the male offspring of obese mice did not show significant increase in islet Reg3g and unchanged islet HSG staining. SAX-HPLC analysis revealed that total pancreatic heparan sulfate was lower in the male offspring with glucose intolerance and a 30% decrease in GSIS. The potential for Reg3g and Heparan Sulfate to act as therapeutics were evaluated by injecting the male offspring with saline, recombinant Reg3g, or a heparan sulfate analogue. The pups received recombinant Reg3g or HS showed improvement in both glucose tolerance, supporting the hypothesis of Reg3g being a potential therapeutic target for rescuing offspringislet dysfunction in preclinical model of maternal obesity.

(34-2) Collaboration, Service and Trainings at the Complex Carbohydrate Research Center

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For more than 25 years, the Complex Carbohydrate Research Center (CCRC) has collaborated with universities, federal agencies, and industry from the US and other countries on detailed structural characterization of glycoconjugates. We have expertise in a full range of analytical methodologies needed to conduct comprehensive research in glycobiology. We are equipped with the state-of-the-art instrumentation including a Thermo Orbitrap-Eclipse MS, Orbitrap-Fusion MS, Velos Orbitrap-Elite MS, AB SCIEX TOF/TOF 5800 MALDI-MS, Bruker MALDI-TOF MS, SPR, multiple analytical and preparative separations options, and GC–MS. The CCRC's NMR facility offers outstanding resources for high-field NMR spectroscopy including 900 MHz, 800 MHz, three 600 MHz and a 500 MHz spectrometer. These spectrometers are equipped with variety of probes including a HRMAS probe for solid state analysis and several H-C/H-N cryoprobes and HCN cryoprobes dedicated to biomolecular NMR applications.

Collaborative projects with the CCRC can be very diverse and can include: Glycomics and glycoproteomics, isolation and analysis of Glycosaminoglycans (GAGs) and GAG-derived products, polysaccharides and Lipopolysaccharides (LPS), peptidoglycans, and glycolipids. Staff at the CCRC-AST also excel in the development of novel characterization and isolation methods, depending on the individual client's needs.

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

(35) The predictive power of N-glycan structure profiling can stratify rheumatoid arthritis patient response to methotrexate for informing efficient treatment strategies

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Rheumatoid Arthritis (RA) is an autoimmune disorder, which causes systemic inflammation leading to increased morbidity and mortality. The first-line treatment is methotrexate (MTX), an inhibitor of folate biosynthesis. However, dosing is rarely optimized by providers and, as a monotherapy, this results in an adequate clinical response for only one-third of patients. Treatment escalation is required for those that do not respond sufficiently and commonly includes expensive biopharmaceuticals.

Predictive biomarkers are urgently needed to improve RA treatment efficiency. Specifically, markers that predict response to MTX will allow for earlier intervention with the most appropriate strategy, informing whether MTX dosing strategy requires optimization or whether an alternative therapy is required. Circulating protein glycosylation has been previously reported in RA plasma and specific motifs have been found to correlate with disease severity and progression.

N-glycan structures were quantified by a PGC-LC-ESI-MS method applied to plasma from a cohort of 40 individuals (n = 60 samples): 20 controls and 20 RA patients. For all RA patients, samples were collected pre-treatment, and after 16 weeks of MTX therapy. Of the RA patients, 10 were classified as good responders and 10 were classified as poor responders based on the change in 28-joint Disease Activity Score.

Hierarchical clustering distinguished patients with different MTX responses using the glycan profiles at baseline (pre-treatment). Unblinded analysis revealed 23/45 N-glycan structures significantly differed ($p < 0.01$) between non-RA and RA patients, with many structures consistent with reported analyses of serum-sourced IgG. Uniquely, we observed 5/45 of structures as significantly different between good and poor responders at baseline, supporting the hypothesis that the plasma glycome is a rich source of predictive biomarkers for improving treatment strategies for RA.

These data suggest that specific structures within the plasma N-glycome can be predictive of RA patient response to MTX treatment, allowing discriminative analysis of pre-treatment cohorts.

(36) The glycosylation of human angiotensin converting enzyme 2 (hACE2), the receptor for SARS-CoV-2.

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Understanding the mode of viral attachment, entry, and replication of SARS-CoV-2 is required for the development of new therapeutic strategies to address the COVID-19 pandemic. We are interested to understand the glycobiology of SARS-CoV-2 viral infection and how it can help in development of vaccine and therapeutics. The coronavirus surface spike (S) protein, which facilitates viral attachment, entry and membrane fusion is heavily glycosylated and also plays a critical role in the host immune response. The spike protein is comprised of two protein subunits (S1 and S2) and possess 22 potential N-glycosylation sites and one O-glycosylation site. The S protein of SARS-CoV-2 primarily binds to human angiotensin converting enzyme 2 (hACE2) for the host cell entry. Even though hACE2 has been known for two decades and has been recognized as the entry point of several coronaviruses, limited studies have been available on glycosylation of human and non-human hACE2. Herein, we describe the comparative glycosylation mapping of hACE2 expressed in HEK293 cells and human plasma by both glycoproteomics and glycomics. We have developed methods to easily isolate and purify ACE2 from human plasma which can be used for high throughput screening of ACE2 glycan structures from different individuals. In hACE2 expressed in HEK293 cells, we have quantitatively characterized the N-glycosylation on ACE2 and interestingly also have observed O-glycosylation modification. We have detected glycosylation on all the seven potential N-glycosylation sites, and are the first to report presence of significant O-glycosylation on Thr 730. The presence of charged sialylated O-glycans on Thr 730, which happens to be in the juxtamembrane stalk region of ACE2 could have implications for "ACE2 shedding" and its dimerization. Intriguing questions arise through our data on presence of O-glycans on Thr 730 which could play a role in these processes. To understand the detailed structure of glycan epitopes on hACE2, we have characterized the terminal sialic acid linkages, the presence of bisecting GlcNAc, and the pattern of N-glycan fucosylation. Elucidation of the site-specific glycosylation of hACE2 receptor on human plasma can aid in understanding the role of ACE2 glycosylation and the individual susceptibility to SARS-CoV-2 and possibly in the development of novel therapeutics.

(37) An Enzyme Conjugate Designed to Target Neurodegenerative Gaucher Disease

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Homozygous mutations in the GBA1 gene, which encodes the lysosomal hydrolase glucoslyceramidase (GCase), can result in the lysosomal storage disorder Gaucher disease (GD). Neurodegenerative symptoms occur in two forms of GD, and mutations in a single copy of the gene also contribute to risk of Parkinson's Disease. Enzyme replacement therapy (ERT) using intravenous delivery can alleviate non-neuronal GD symptoms, but the blood–brain barrier presents an obstacle to treating neuronal symptoms in GD as well as Parkinson's Disease. This work aims to address this problem by using recombinant GCase conjugated to Guanidinylated Neomycin (GNeo). GNeo is a small molecule known to enhance cellular uptake and lysosomal delivery through a heparan sulfate-dependent pathway. Recent work has shown that intranasal delivery of GNeo conjugates of another lysosomal enzyme, α-L iduronidase, cleared accumulated substrate throughout the brain of a mouse model much more effectively than unmodified enzyme used in traditional ERT; the approach here for GCase is similar. At this point, recombinant GCase has been expressed and conjugated to GNeo, and the resulting conjugates retain full activity. Uptake is being assessed in human GD fibroblasts, and preliminary results from experiments using enzymatic activity in cell lysate indicate that cell entry does occur. Further experiments will be performed with mixed neuronal cultures from a GD mouse, and we are also preparing a fluorophore-modified version of GNeo to visualize cell entry and localization. Finally, we will assess lysosomal storage and α-synuclein clearance in GD mouse models using the intranasal delivery system described above for iduronidase.

(38) Glycosyltransferase ST6Gal-I promotes epithelial to mesenchymal transition in pancreatic cancer cells

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Tumor-associated glycosyltransferase ST6Gal-I adds α2–6 linked sialic acid to select N-glycosylated cell surface receptors, leading to altered downstream cell signaling. ST6Gal-I is upregulated in multiple tumor types such as pancreatic, colon and ovarian cancers. Previous studies from our lab have shown that ST6Gal-I imparts pro-tumorigenic characteristics such as maintenance of a cancer stem cell phenotype and resistance to apoptotic stimuli including hypoxia and chemotherapy drugs. Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most lethal cancers with a five-year survival rate of ~8%. Our group developed genetically engineered PDAC mouse models with either pancreas-specific expression of oncogenic K-ras (KC) or a combination of oncogenic K-ras and ST6Gal-I overexpression (KSC). KSC mice demonstrate accelerated tumor progression and metastatic disease, leading to a significantly reduced median survival of 4 months for KSC mice as compared to KC mice, with a median survival of 13 months. To investigate the role of ST6Gal-I in PDAC progression, we utilized a human PDAC cell model, Suit2, which has low endogenous ST6Gal-I levels and limited metastatic potential, as well as isogenic, highly metastatic subclones of the Suit2 line, S2–013 and S2-LM7AA. Notably, the metastatic subclones have upregulated ST6Gal-I relative to parental Suit2 cells, suggesting that ST6Gal-I is selected for during the metastatic process. RNA Seq analyses indicated that the two metastatic subclones displayed an enrichment in gene sets corresponding to epithelial to mesenchymal transition (EMT), a process associated with metastatic competence. To interrogate ST6Gal-I's contribution to this process, we conducted RNASeq on Suit2 cells stably transduced with an ST6Gal-I overexpression (OE) vector and found that ST6Gal-I OE was sufficient to induce an EMT phenotype. To solidify a role for ST6Gal-I in EMT, the expression of EMT markers was examined in Suit2-OE cells or empty vector (EV) controls, as well as in S2–013 and S2-LM7AA metastatic cells engineered with either stable ST6Gal-I knockdown (KD) or expression of non-targeting shRNA (shC). As compared with ST6Gal-I deficient cells (Suit2 EV, S2–013 KD, S2-LM7AA KD), cells with high expression of ST6Gal-I (Suit2 OE, S2–013 shC, S2-LM7AA shC) exhibited an upregulation of the mesenchymal markers, snail, slug, N-cadherin and fibronectin, and downregulation of epithelial markers, E-cadherin and occludin. Furthermore, ST6Gal-I-high cells were more invasive than ST6Gal-I-low cells as determined by trans-well invasion assays. One of the major molecular drivers of EMT is the EGFR receptor, which is known to be a ST6Gal-I substrate. We therefore examined the role of α2–6 sialylated EGFR in EMT. Cells with high expression of ST6Gal-I showed elevated levels of basally activated (phosphorylated) EGFR as compared to the ST6Gal-I low lines. Treatment with an EGFR inhibitor, erlotinib, neutralized the differences in EGFR activation, EMT marker expression and cell invasiveness in the Suit2 and S2-LM7AA lines. Lastly, in vivo xenograft studies revealed that ST6Gal-I OE in Suit2 cells promoted greater tumor growth and metastasis relative to EV cells, whereas ST6Gal-I KD in S2–013 and S2-LM7AA lines suppressed tumor growth and metastasis. In conclusion, this study highlights an important pro-tumorigenic role for ST6Gal-I in driving EMT, in part through sialylation of EGFR.

(39) Increased Levels of Galectin Proteins in Sera of Patients with Breast, Lung, and Colon Cancer

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Galectins are a family of carbohydrate-binding proteins, which contain a β-galactoside-binding preference that confers an ability to bind glycans within a variety of biological pathways. Previous studies have shown that levels of different galectins were elevated in a number of cancers. We therefore predicted that galectin levels may be elevated in sera of cancer patients, and the levels of various galectins can be used to phenotype cancer patients. We used cancer patient samples from the Biorepository of the Prisma Health Cancer Institute. Exploratory analysis of mRNA expression of the galectins −1, −3, and − 9 genes (N = 12,820) in breast, lung, and colon cancer tissues using the University of California Santa Cruz Xena database showed the mRNA expression levels are frequently elevated in patients with these malignancies. Given these data, we next aimed to define the protein levels of aforementioned galectins in sera of patients with breast, colon, and lung cancers, using enzyme-linked immunosorbent assay (ELISA). Across ten patient serum samples from each stage (I-IV) of breast, lung, and colon cancer, galectin-1 levels were increased for stages I, III, and IV for breast cancer, stages I, II, and III for lung cancer, and stages I-IV for colon cancer compared to healthy controls. All stages of breast cancer and colon cancer, and stages I, II, and IV of lung cancer had increased levels of galectin-9 compared to healthy

controls. All stages of breast, lung and colon cancer showed significantly elevated levels of galectin-3 compared to healthy controls. These galectin profiles were added to enhance the Prisma Health cancer patient database. Our results highlight differences in the galectin levels in patients with these cancers compared to healthy controls. However, the lack of significant variability between the cancer stages suggests dysregulation of galectins occurs early in oncogenesis. Thus, consistently elevated levels of galectins-1, −3, and − 9 in serum of breast, lung, and colon cancer patients continue to bring attention to galectins as possible markers and therapeutic targets.

(40) Structural and mechanistic insights into LLO-biosynthesis by the glucosyltransferase ALG6

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In eukaryotic protein N-glycosylation, a series of membrane-integral glycosyltransferases catalyze the biosynthesis of a dolichylpyrophosphate-linked oligosaccharide prior to its transfer onto acceptor proteins. The final seven steps are catalyzed in the lumen of the endoplasmic reticulum and require dolichylphosphate-activated sugars as donors. Here we present cryo-EM structures of yeast ALG6 in a substrate-free state and bound to a synthetic substrate analog at 3.0 Å and 3.9 Å resolution. They reveal a new transmembrane fold and suggest a common evolutionary scaffold of GT-C enzymes. The activity of ALG6 was recapitulated in vitro using synthetic analogs of dolichylphosphate- and dolichylpyrophosphate-linked sugars and chemo-enzymatic glycan extension using purified enzymes of the ALG pathway. The dolichylphosphate-glucose-bound ALG6 structure revealed the active site of the enzyme. Functional analysis of ALG6 variants identified an aspartate that is conserved in the GT-C superfamily and probably acts as the catalytic base. Our results define the architecture of ER-luminal GT-C enzymes and provide a structural basis for understanding their catalytic mechanisms.

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(41) An orthogonal approach to examining the Skp1 interactome vastly expands the E3(SCF) ubiquitin ligase repertoire of Dictyostelium and its dependence on the Skp1 glycosylation

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The SCF (Skp1/cullin-1/F-box protein) class of E3 ubiquitin ligases serves a role in regulating the cell cycle and maintaining proteostasis in all eukaryotes. The Skp1 adaptor protein binds to different F-box proteins (FBPs), many of which serve as substrate receptors to meet the degradation needs of the cell. It is not well understood how specific Skp1/FBP combinations are selected for incorporation into the SCF. In Dictyostelium discoideum and other protists, the Skp1 adaptor is cytoplasmically glycosylated with a pentasaccharide on a hydroxyproline in an oxygen dependent manner. NMR and molecular dynamics simulations suggest that this glycan makes the Skp1/FBP interaction more favorable. A previous Skp1 interactome study of wild-type and mutant cells, based on co-immunoprecipitation (co-IP) followed by nLC/MS(2), reported 15 interactors at a late stage of development, of which four were predicted to be FBPs based on sequence features. The degree of interaction of 3 of these 4 appeared to be selectively promoted by Skp1 glycosylation, while another appeared to be decreased, suggesting a role for these proteins in oxygen rich conditions. Here we extend the analysis using an epitope-tagged version of Skp1, which yielded over 103 interactors in growth stage cells. Of these, 29 were predicted to be FBPs, and 14 of these showed 1.5–8-fold increased interaction when Skp1 was glycosylated, suggesting that these FBPs were the primary readers of Skp1 glycosylation. The low degree of overlap between the Skp1 interactomes of growth stage and developed cells indicates a dynamic composition of SCF complexes. Three types of interactors observed in growth stage and developing cells were epitope tagged by genome editing for further analysis. The previously documented FBP FbwD, expected to be a canonical substrate receptor, was found to be reduced in level when Skp1 is glycosylated despite showing increased interaction of the remaining FbwD with Skp1. JcdI, which contains a jumonji C enzyme domain and lacks an obvious substrate receptor domain, was confirmed to be an FBP and was similarly affected by Skp1 glycosylation in reciprocal co-IPs. By additional reciprocal co-IPs, the non-FBP interactor Vwa1 was validated as a FbwD dependent Skp1 interactor and potential SCF(FbwD) substrate. Our new orthogonal approach to analyze the Skp1 interactome has vastly expanded the SCF repertoire and demonstrated its dynamic composition during development. Furthermore, Skp1 glycosylation has been shown selectively enhance steady state interactions with a subset of FBPs, indicating that glycosylation also influences the SCF repertoire, and leads to reduced steady state levels of at least two FBPs.

(42) Synthesis of the Common Polysaccharide Antigen in *Pseudomonas aeruginosa*: D-rhamnosyltransferases WbpX and WbpY work together to polymerize D-Rhamnose.

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The ubiquitous, opportunistic pathogen *Pseudomonas aeruginosa* simultaneously expresses two O-antigenic glycoforms. While the O-specific antigen (OSA) is variable in composition, the Common Polysaccharide Antigen (CPA) is highly conserved and is composed of a homopolymer of D-rhamnose (D-Rha) in trisaccharide repeating units [D-Rha-α-(1–2)-D-Rha-α-(1–3)-D-Rha-α-(1–3)-]$_n$. We have shown that D-Rha-transferase WbpZ transfers the first D-Rha residue from GDP-D-Rha to GlcNAcα-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl. We have now studied the enzymes encoded by wbpX and wbpY genes found in the O antigen biosynthesis gene cluster. We showed that WbpX and WbpY are D-Rha-transferases that differ in their donor and acceptor specificities. They both have the properties of GT-B folded enzymes of the GT4 glycosyltransferase family. Using mass spectrometry, we showed that recombinant His_6-tagged WbpX and WbpY individually transferred a single D-Rha residue to synthetic D-Rhaα1–3GlcNAcα-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl. However, the mixture of WbpX and WbpY was able to catalyze the synthesis of D-Rha polymers. These studies are aimed at elucidating the mechanisms of D-Rha polymer formation in PA, to develop the technology to assemble CPA in vitro, and to inhibit CPA assembly. This work was supported by the Natural Sciences and Engineering Council of Canada.

(43) Probing the Siglec Interactome by Cell-Based Glycan Arrays

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Siglecs are a family of sialic acid-binding receptors expressed throughout the immune system as well as by other cell types. A major function of Siglecs is the modulation of immune cell functions upon binding to sialoglycan ligands. Therefore, Siglecs are important regulators of the immune system, and alterations in Siglec or sialoglycan ligand expression have been associated with autoimmunity, cancer, and neurodegeneration (1, 2). While human Siglecs primarily bind to sialic acid residues on diverse types of glycoproteins and glycolipids that constitute the sialome, the fine binding specificities for elaborated glycan structures of most Siglecs is not fully elucidated. Here, we took advantage of our recently developed cell-based glycan array resource that presents glycans in their natural glycoconjugate context at the cell surface on a large library of glycoengineered isogenic human embryonic kidney 293 (HEK) cells (3, 4). We have generated a sialome sublibrary using combinatorial precise genome editing to express distinct subsets and individual sialyltransferase isoenzyme repertoires. This sublibrary reported sialyltransferase genes required for Siglec binding and provides insights into the biosynthesis of Siglec ligands with predictions of the structural features involved. Moreover, we introduced >40 reporter constructs containing dense O-glycosylation regions of human mucins and other O-glycoproteins into the isogenic cells to probe the influence of clustered O-glycan presentation for Siglec binding. Finally, the sialome sublibrary was further expanded to probe the contribution of glycan-modifications to sialoglycan recognition by the Siglecs. Altogether, this cell-based display of the human sialome enabled dissection of the Siglec interactome in the natural context of the cells and revealed unique genetic and structural features that determine the fine binding specificities of Siglecs. Furthermore we provide evidence for protein context-dependent sialoglycan recognition by Siglecs that can be further explored in biological settings. We will present this cell-based platform for display of the human sialome and insights into the fine binding specificity of the human Siglecs.

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(44) Dissecting molecular determinants of the Galectin-4 glycan interactions and their potential relevance in intestinal inflammation

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Inflammatory Bowel Diseases (IBD), including Crohn's Disease and Ulcerative Colitis, constitute a group of chronic inflammatory disorders that affect the gastrointestinal tract, in which the perpetual state of inflammation constitutes a risk factor for the development of colitisassociated colorectal cancer (CACRC).

In this context, and even though galectin-glycan interactions play a central role as a mediator of pathophysiological processes, their role in IBD and CACRC has been scarcely explored. Galectins, a family of evolutionary-conserved soluble lectins, are involved in immunomodulation, inflammation, pathogen recognition, tumor escape, resistance and metastasis. In particular, Galectin-4 (Gal-4), a tandem-repeat type galectin with two distinct carbohydrate recognition (CRD) domains is expressed in the gastrointestinal tract. This lectin exerts a predominantly proinflammatory role, stimulating T-cells and exacerbating inflammation via recognition of a colitisassociated aberrant glycome.

In this work, we optimized the production and purification of human Gal-4, as well as each of its isolated CRDs. From the different approaches assayed, the expression vector pET-28a-SUMO was finally selected based on the reproducible protocol for tag cleavage using ULP1 protease. Thus, corresponding DNA constructs containing inserts for hGal-4, hGal-N-terminal-CRD or hGal-4-C-terminal-CRD were expressed in *E. coli* BL21 (DE3), and the purification of recombinant proteins was achieved in two chromatography steps, by Ni-NTA affinity and size-exclusion. Then, the activity and glycosidic preferences of recombinant full-length Gal-4 and its CRDs were evaluated by hemagglutination, solid-phase assays and isothermal titration calorimetry. In doing.

so, we identified selective recognition of Gal-4 for particular glycan moieties, which were.

recognized by full length Gal-4 and/or specific carbohydrate recognition domains with high affinity and specificity when compared to other galectins. Furthermore, by molecular docking and molecular dynamics simulations, we characterized the biochemical and biophysical determinants of these interactions in silico, identifying key amino acid residues responsible for glycan recognition. Finally, we evaluated the inhibitory role of these specific glycans in vitro, demonstrating that these oligosaccharides inhibited Gal-4-dependent IL-6 and Granzyme B.

secretion by splenocytes, and in consequence, hampering Gal-4 proinflammatory activity.

In summary, our results provide new insights on the molecular determinants of Gal-4-glycan interactions, and postulate a potential regulation of intestinal inflammation via Gal-4 inhibitors.

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

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Prion proteins cause an infectious and rapidly progressive neurodegenerative disease characterized by prion aggregates, spongiform encephalopathy, dystrophic neurites, and neuronal death. These processes depend on the neuronal expression of prion protein (PrP^C), which exists on the outer leaflet of the cell membrane as a glycosylphosphatidylinositol (GPI)-anchored glycoprotein containing two variably occupied N-linked glycosylation sites on its carboxy terminus. Previous work has shown that glycan modifications may impact PrP aggregation and neuronal toxicity. To investigate the role of glycans in prion-induced neurotoxicity, we engineered a new knockin mouse model that expresses PrP with an additional glycan. This glycan is sensitive to PNGase F digestion, but not to endoglycosidase H digestion, indicating the presence of a complex N-linked glycan. This mouse spontaneously develops neurodegeneration characterized by spongiform encephalopathy in the CA3 region of the hippocampus and dystrophic neurites. In contrast to other murine models of prion disease, this neurodegenerative pathology develops in the absence of PrP aggregates or infectivity, as shown by several in vitro and in vivo methods. Therefore, this model provides the opportunity to investigate the neurotoxic role of PrP^C, uncoupled from its aggregation. We show that although the extra glycan does not affect PrP^C expression, stability, or turnover in cells, primary hippocampal neurons isolated from these mice display signs of excitotoxicity. Additionally, immunoprecipitation-mass spectrometry (IP-MS) studies reveal that introduction of this third glycan significantly alters the PrP interactome as compared to wild-type littermates. These studies hold relevance to both prion diseases and also other neurodegenerative diseases; PrP^C has been shown to act as a cell surface receptor for extracellular aggregated proteins such as amyloid-β in Alzheimer's disease and α-synuclein in Parkinson's disease. Therefore, understanding PrP^C-mediated neurotoxic signaling can aid in understanding not only prion diseases, but also the neurotoxic pathways instigated by other aggregated, misfolded proteins.

(46) Compromised SARS-CoV-2 placental antibody transfer

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SARS-CoV-2 infection in pregnant women is associated with more severe disease compared to age-matched non-pregnant women. Multiple lines of evidence indicate compromised placental antibody transfer during maternal infection. However, whether maternal SARS-CoV-2 infection compromises infant immunity is unclear. Here, we used a systems serology approach to characterize the Fc profile of maternal influenza-, pertussis-, and SARS-CoV-2-specific antibodies transferred to neonates across the placenta. Influenza- and pertussis-specific antibodies were actively transferred but with lower efficiency in SARS-CoV-2 infected women. SARS-CoV-2-specific antibody transfer was not only significantly reduced compared to the transfer of influenza- and pertussis-specific antibodies, but cord titers and functional activity were lower than in maternal plasma. SARS-CoV-2-specific transfer was linked to altered SARS-CoV-2 antibody glycosylation profiles and was partially rescued by infection-induced hypergammaglobulinemia and increased FCGR3A placental expression. These results point to unexpected compensatory immune mechanisms to boost immunity in neonates, providing insights for maternal vaccine design strategies.

(47) GCNT2 suppression in metastatic melanoma is potentiated by hypoxia and correlates with aggressive disease progression

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Metastatic melanoma is a lethal disease with a dismal 5-year survival rate of 25%. Thus, intense efforts are underway to identify biomarkers predictive of early melanoma progression or of a potentially effective therapeutic approach. We recently discovered that loss of cell surface I-antigen (or branched) glycans corresponds with the transition of primary melanoma to metastatic melanoma. I-branched glycans are synthesized by β1,6 N-acetylglucosaminyltransferase 2 (GCNT2) and negatively regulate growth and signaling activity of melanoma cells. Compared with high GCNT2 expression in normal melanocytes, nevi, and early-stage primary melanomas, GCNT2 is conspicuously lost in metastatic melanomas. We hypothesize that GCNT2/I-branch expression weakens the metastatic potential of melanoma cells, compromises binding of pro-melanoma factor, galectin-3 (Gal-3) and is predictive of a non-aggressive melanoma. Using human GCNT2-silenced and -enforced melanoma cell lines, we found that loss of GCNT2/I-branching dramatically increased Gal-3-binding, including binding to pro-metastatic factors, β1 integrin and IGF1R. Growth of human melanoma cells under in vivo low oxygen conditions promoted tumor-initiating cell (TIC) generation, eliminated GCNT2 expression, upregulated stem cell marker KLF4, and increased anti-metastatic genes in spheroid cultures. In a melanoma limiting dilution xenograft assay in immunodeficient mice, we observed a marked reduction in tumor formation in accord with high GCNT2 levels, whereas low GCNT2 levels enabled human tumor formation at significantly less cell inoculum. In addition, we investigated whether loss of GCNT2 on melanoma cells encouraged TIC characteristics and immune suppression. In human PBMC and metastatic melanoma cell co-cultures, we observed increased T regulatory cell generation in GCNT2-low melanoma cell cultures compared to GCNT2-high melanoma cells. To examine whether GCNT2 levels predicted disease progression in melanoma patients, we investigated GCNT2 expression in patient melanomas specimens that had matching clinical outcome data. Using an IHC staining intensity grading system (None, Light, Moderate and Dark) to evaluate GCNT2 expression in primary melanoma tissues, we found that No staining to Light staining in 80% (50/64) of tissues corresponded with Death Due to Melanoma, whereas only 6% of patients (4/64) containing Dark-stained tissues corresponded with Death Due to Melanoma ($p < 0.04$). Altogether, these findings suggest that GCNT2/I-branching can attenuate pro-melanoma activities, including inhibiting TIC and immunosuppressive features, and may be inversely predictive of aggressive melanomas.

(48) Identification of altered membrane sialoglycoproteins in cultured Human Intestinal Epithelial Cells induced by Enteroaggregative *Escherichia coli* infection

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As per CDC reports, diarrhea,has a high incidence rate accounting for 2195 deaths globally, making it the second leading cause of death in children under the age of 5.

Enteroaggregative *Escherichia coli* (EAEC) is an important enteric microbe,associated with widespread cases of severe and continuous diarrheal episodes. Previous studies from our laboratory have reported the involvement of four glycoproteins (Fibronectin, Epidermal growth factor receptor, TSP-1 and GRP-94) in aggregative adherence of EAEC to INT-407 cells. Bacterial infections can modulate host cell glycosylation patterns which can govern susceptibility and resistance to intestinal infections.Thus, predominant alterations in the glycosylation profile of intestinal glycoproteins can be a reliable reflection of physiological and pathological conditions. In the present study, an attempt has been made to identify altered glycoproteins in EAEC infected intestinal epithelial cell lines [INT-407 (Human small intestinal embryonic epithelial cell line) and HCT-15 (Human colon carcinoma cell line)]. Three different EAEC strains were employed, an invasive strain of EAEC (EAEC-T8, clinical isolate), non-invasive prototype strain of EAEC (EAEC-O42) and plasmid cured EAEC-T8 strain (designated as EAEC-pT8). Four different lectins were used to screen differentially expressed membrane glycoproteins in HCT-15 and INT-407 cells infected with different EAEC strains separately from which *Sambucus nigra* agglutinin [SNA, specific for NeuNAcα (2,6) Galβ (1,4) GlcNAc/Glc] was selected as the lectin of choice, as it could detect maximum alteration in the glycosylation pattern of membrane proteins in both the cell lines. Increased reactivity of several SNA interacting membrane glycoprotein spots in EAEC-T8 infected HCT-15 and INT-407 cell lines respectively in comparison to uninfected cells was noted in 2D-PAGE based transblot analysis of the membrane proteins of both the cell lines. Further, identification of these spots was done using MALDI-TOF MS/MS analysis, of which three glycoproteins common to EAEC-T8 infected both the cell lines i.e. Voltage-Dependent Anion-Selective channel (VDAC) Protein 2, Prohibitin-2 and Glucose Regulated protein (GRP) 78, were selected for subsequent studies. The presence of these three proteins was confirmed by western immunoblotting in presence of specific antibody against each protein. Also, the carbohydrate specific binding of SNA with the identified membrane proteins of both the cell lines was confirmed by SNA overlay western blots in presence of 6′sialyl-lactose (SL, a specific trisaccharide inhibitor of SNA), which clearly indicated the presence of α2,6 linked sialic acids in the oligosaccharide portion of identified glycoproteins. Our findings indicate that VDAC-2, Prohibitin-2 and GRP-78 are the altered glycoproteins in the EAEC infected cells. Thus, study on their functional role may be helpful to have a better insight into EAEC pathogenesis.

(49) Staphylococcal Superantigen-like protein 11 inhibitis neutrophil motility

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Staphylococcus aureus (*S. aureus*) is a major opportunistic pathogen of humans and causes an array of diseases, from superficial complications to lethal, invasive infections. An alarming increase in community-acquired (CA) *S. aureus* infections in immune-competent individuals call for better understanding of *S. aureus* virulence mechanisms, as ~30% of the human population are carriers of *S. aureus*. Neutrophils are the most abundant leukocytes in the blood and are the first defense mechanism against *S. aureus* infections. *S. aureus* Superantigen-Like proteins (SSLs) are not mitogenic to T cells and do not bind MHC class II molecule, despite sharing a similar structure with Superantigens (SAgs). Here we show SSL11 from MRSA USA300_FPR3757 induced differentiated human neutrophil-like cells (dHL60) motility arrest by inducing cells adhesion and "locking" cells in the adhesion stage, without inducing oxidative burst. Pre-incubation of SSL11 with the glycan Sialyl Lewis X blocked SSL11 function and de-glycosylation of dHL60 cells by PNGase F abolished SSL11 binding, suggesting that SSL11 functions via interacting with glycans. This is the first description of a bacterial toxin inhibiting neutrophil motility by inducing adhesion and "locking" cells in an adhesion stage. Therefore, this study might provide a new target against *S. aureus* infections.

(50) Endogenous sialidases desialyate T cells, and promote T cell activation and function

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Background: Following immune stimulation, the germinal center (GC) in lymphoid tissue plays a central role in mediating antibody formation, including class-switching and somatic hypermutation leading to increased antibody affinity. These events require the interaction of the surface proteins in the immune synapse between antigen-presenting cells (APCs) and B and T cells. These proteins in the immune synapse are glycosylated, a post-transcriptional modification that covalently attaches diverse carbohydrate chains (oligosaccharides) onto the polypeptide, frequently containing sialic acid (SA) at the non-reducing terminal unit. The highly negatively charged SA moieties can diminish accessibility of surface molecules thus hinder protein-carbohydrate or protein–protein interactions. Their enzymatic removal—desialylation, by microbial neuraminidases (NAs) or endogenous sialidases (NEUs) facilitates these interactions and modulates cellular function. Historically GCs have been identified by their binding of

peanut agglutinin (PNA), a lectin that binds to the subterminal galactose of sialylated proteins.

Hypothesis: Since hypo-sialylation is considered a hallmark of activated T cells, we hypothesized that GC formation requires the activation of endogenous sialidases that remove terminal sialyl residues of T and perhaps B cells and macrophages to promote interactions among these cells. Further, we speculate that T cell-dependent antigens, like glycoconjugate vaccines, were more dependent on desialylated T cells than T-cell-independent antigens, such as polysaccharide vaccines.

Results: We found that LFA-1 on T cells, an important protein in the immune synapse, is sialylated, and antibody blockade of LFA-1/ICAM-1 interaction suppresses the T cell activation. Further, desialylation of T cells in vitro activates LFA-1 and enhances the T cell response to stimulation by peptide-pulsed DCs. During vaccination with a clinically relevant glycoconjugate vaccine, sialidase RNA expression increases along with increased PNA binding to splenocytes and GC B cells, a sign of cell surface desialylation. Administration of the pan-sialidase inhibitor, 2-DN, reduces PNA binding to splenocytes, suppresses GC formation and antibody production against T-dependent antigens (e.g. glycoconjugate vaccine), but not T-independent antigens (e.g. Klebsiella capsular polysaccharides).

Conclusion: We conclude that by removal of sialyl residues of key molecules in the immune synapse, endogenous sialidases play a critical role in GC formation and function. The ability to modulate sialidase activity may find clinical application by increasing cellular interactions in the GC during immunizations (e.g. as an adjuvant) or decreasing sialidase activity during hyperinflammatory states.

(50-2) A diversified lectin repertoire in the softshell clam (*Mya arenaria*): Recognition of sympatric Perkinsus parasite species by galectins and R-type lectins

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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 analysis with currently-known protein sequences of invertebrate galectins, revealed a galectin sequence (MaGal1), with high homology to Manila clam (*Venerupis philippinarum*) galectin. By proteomic (Mass spec) and BLAST analyses we also identified a lactose binding lectin (MaRTL) with high homology to the Mediterranean mussel R-type lectin (MytiLec3). 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].

(51) Selectivity of Heparan Sulfate Recognition of SARS-CoV-2 Spike Glycoprotein

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The COVID-19 pandemic caused by SARS-CoV-2, an enveloped, positive-sense RNA virus, has thwarted early attempts to repurpose current drugs, thereby necessitating a rigorous study of all pathways and mechanisms likely to yield, prevent and treat the disease (Drug Discov. Today (2020) 25:1535–1544). While SARS-CoV-2 entry into host cells is known to involve viral surface spike glycoprotein (SgP) binding to the host cell membrane angiotensin-converting enzyme 2 (ACE2) receptor, heparan sulfate (HS) receptors are also beginning to be appreciated as involved in virus internalization. The SgP is a homo-trimer consisting of S1 and S2 subunits, of which the former contains a receptor-binding domain (RBD) that recognizes ACE2. Application of the dual-filter, genetic algorithm-based computational screening strategy indicated selective recognition of the RBD by HS sequences over other glycosaminoglycans (GAGs). Likewise, exhaustive computational study of the SgP trimer indicates preferential interaction of HS with the RBD over plausible electropositive domains within the trimer. More interestingly, the in silico studies suggested that the RBD favors binding to rare HS residues containing a defined set of sulfate groups in three-dimensional space. Screening of a microarray carrying 24 HS sequences varying with regard to chain length, pattern and number of sulfate groups against S1 and RBD indicated that only 8 HS sequences had reasonable affinity for the RBD. Interestingly, the recognition of these 8 HS sequences for S1 was much weaker possibly suggesting a role for the trimeric form of SgP versus its monomeric form. Whereas the RBD preferred sequences with higher levels of sulfation, a distinct preference was noted for sequences containing a 3-O sulfate group. This work presents a thorough insight into GAG-recognition of SARS-CoV-2 SgP and raises a strong possibility that GAG- or GAG mimetic-based antagonism of SgP-HS receptor interaction may yield a candidate drug.

(52) Synbiotic supplementation with prebiotic *S. commune* derived β-(1,3/1,6)-glucan and probiotic concoction benefits gut microbiota and its associated metabolic activities

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Synbiotics synergistically favors beneficial effects of prebiotics and probiotics towards host metabolic health by modulating gut ecosystem. In this study, we sought to examine the effects of prebiotics (Schizophyllum commune derived ß-(1,3/1,6)-glucan), probiotics (concoction made of eight different bacterial strains) and synbiotics (prebiotics + probiotics) on gut microbiota and its associated metabolic functions through 16S rRNA gene sequences analysis. Results showed that probiotics strains used in this study were detected more in the synbiotic and probiotic treatments, while prebiotic dietary intervention increased the total bacterial abundance and metabolisms related to host immune strengthening. Probiotics and synbiotics dietary interventions enhanced similar metabolisms relating to butanediol and s-adenosyl-l-methionine biosynthesis. Probiotics treatment also showed depleted metabolic activities related to SCFA productions, that were not depleted in prebiotics treatment. With varying differential abundance patterns and metabolic activities across the treatments, our results suggest that synbiotic treatment provide more beneficial effects over probiotics and prebiotics.

(53) Dysfunctional Mucosal Immune Defense in Aspirin Exasperated Respiratory Disease Increases Susceptibility to *Staphylococcus aureus*

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Background: Sinusitis affects 11.6% of adults, chronic rhinosinusitis (CRS) is varied in etiology and clinical presentation. *Staphylococcus aureus* (SA) colonization is increased from 20% to 64% in those with symptoms of CRS compared to those without symptoms. There is up to 87% SA colonization in the upper airway of Aspirin Exacerbated Respiratory Disease (AERD) patients. Airway epithelial cells are the first line of defense and have an important role in differentiating pathogenic bacteria from commensal bacteria. Increased permeability of epithelium, decreased antimicrobial peptide production, and dysregulated mucin secretion are features of CRS. However, the features of mucosal immunity against MRSA in AERD has not been explored completely.

Objective: To determine if innate immune epithelial barrier of AERD airway cells are more susceptible to MRSA.

Methods: To elucidate the mechanism of mucosal dysfunction in AERD to SA, we have developed an air liquid interphase (ALI) culture model of airway epithelial cells. Using this model, we exposed SA to airway epithelial cells from different

clinical patients. Epithelial barrier integrity was evaluated by FITC-Dextran assay in ALI and immunohistochemistry of dapi, occludin, and zo-1 in culture. Antimicrobial peptide, Reg3g production was measured by RT-qPCR and western blot.

Conclusion: Airway epithelium of AERD patients show impaired barrier integrity compared to normal epithelium when exposed to SA with faster transfer of FITC-dextran to basolateral media. In addition, AERD cells were more likely to lose attachment properties in presence of SA shown by immunohistochemistry. Furthermore, growth of SA increased with exposure to AERD airway epithelial cells. Lastly, AERD cells show deficiency of Reg3g induction upon infection with SA, which shows impaired innate mucosal immunity.

Clinical Implication: Airway epithelium of AERD patients are susceptible to SA due to innate immune defects in barrier integrity and impaired production of antimicrobial, Reg3g.

(54) SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2

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The COVID-19 pandemic, caused by the novel respiratory coronavirus 2 (SARS-CoV-2), has swept across the world, resulting in serious clinical morbidities and mortality, as well as widespread disruption to all aspects of society. Understanding the mechanism of SARS-CoV-2 infection could reveal targets to interfere with viral infection and spread. We show that the ectodomain of the SARS-CoV-2 spike (S) protein interacts with both cellular heparan sulfate and angiotensin converting enzyme 2 (ACE2) through its Receptor Binding Domain (RBD). Docking studies suggest a heparin/heparan sulfate-binding site adjacent to the ACE2 binding site. Both ACE2 and heparin can bind independently to spike protein in vitro and a ternary complex can be generated using heparin as a scaffold. Electron micrographs of spike protein suggests that heparin enhances the open conformation of the RBD that binds ACE2. On cells, spike protein binding depends on both heparan sulfate and ACE2. Unfractionated heparin, non-anticoagulant heparin, heparin lyases, and lung heparan sulfate potently block spike protein binding and/or infection by pseudotyped virus and authentic SARS-CoV-2 virus. These findings suggest a model in which viral attachment and infection involves heparan sulfate-dependent enhancement of binding to ACE2 and emphasizes the potential for targeting S protein-HS interactions to attenuate virus infection.

(55) The role of mucin glycosylation in the communication between the gut microbiota and the brain

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A bidirectional communication between the gastrointestinal tract and the brain has long been acknowledged. Several studies showed that the onset of brain diseases such as neurodegenerative disease, stress and anxiety are linked with an altered composition of the gut microbiota. However, the molecular mechanisms underpinning the communication between gut microbes and the brain remain to be elucidated.

Mucin glycosylation plays a critical role in maintaining an homeostatic relationship between the gut microbiota and the host with sialic acid being a major carbohydrate epitope and a target for bacteria. In the brain, sialic acid residues play an important role in neural development, brain plasticity and cell–cell interactions.

Here we used transgenic and gnotobiotic mouse models to explore the importance of intestinal mucin glycosylation in regulating communication between the gut microbiota and the brain focusing on the hypothalamus and the hippocampus.

We showed that mice lacking core 3 mucin glycans (C3GnT $^{-/-}$) display an altered gut barrier, microbiota composition and a decrease in neurons positive for polysylated-neural cell adhesion molecule (PSA-NCAM), a marker of neurogenesis, as well as disorganised dendrites projecting to the upper molecular layer of the hippocampal formation, as compared to C57BL/6 wild-type mice. HPLC analyses of sialic acid derivatives in the caecal content, brain and serum of these mice showed higher level of Neu5Ac and Neu5Gc in the serum and the caecum of C3GnT$^{-/-}$ mice as compared to C57BL/6 wild-type mice. No statistically significant differences were detected in the brain for free sialic

acid but our preliminary results showed that polysialylated NCAM isoform differ in the brain of C3GnT $^{-/-}$ and C3GnT $^{+/+}$ littermates.

To further assess the role of the gut microbiota in this process, we monocolonised germ-free mice with the gut commensal *Ruminococcus gnavus*, a mucin-glycan foraging strain. We showed a significant increase of Neu5Ac and derived metabolites in the caecal content of mice colonised with R. *gnavus* as compared to the germ-free mice, matched per age and gender. No significant differences of free sialic acids derivatives were detected in frontal cortex and middle brain, whereas ialic acid-bound to glycoproteins were increased in the middle brain portion of the gnotobiotic mice. Furthermore, mass spectometry analyses of Muc2 glycosylation revealed a significant decrease in the percentage of sialylated residues in intestinal mucins in the gnotobiotic mice as compared to the germ-free mice, in line with the ability of R. gnavus to release and metabolise sialic acid residues from mucins.

Together these preliminary data provide a support to further explore the role of mucin glycosylation and mucin-derived sialic acid derivatives in the communication between the gut microbiota and the brain.

(56) Mass Spectrometry Imaging of N-Glycans Reveals Racial Discrepancies in Low Grade Prostate Tumors

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Prostate cancer is the most commonly diagnosed cancer in men worldwide. A critical knowledge gap in prostate cancer biology is the molecular events underlining higher incidence and mortality rate in Black men. Identifying molecular features that separate racial disparities is a critical step in prostate cancer research that could lead to predictive biomarkers and personalized therapy. N-linked glycosylation is a required co-translational event during protein folding that modulates many biological processes, such as cell adhesion, immune modulation, cell-matrix interactions, and cell proliferation. Recently, aberrant N-linked glycosylation has been reported in prostate cancers. However, the full clinical implications of dysregulated glycosylation in prostate cancer has yet to be explored. Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is a new and innovative technique that combines mass spectrometry with imaging, enabling the detection of glycans with spatial distribution. Herein, we performed MALDI-MSI analysis to characterize the N-glycan profile from tissue microarrays of over 100 patient tumors banked at the University of Kentucky with over 10 years of follow-up data. We successfully identified 46 unique glycans from readily available formalin-fixed paraffin-embedded prostate tissue, and found significant N-glycan dysregulation between benign and prostate tumor tissue across all patient groups. High mannose as well as tri- and tetra-antennary N-glycans were predominantly found in tumor tissue and correlate with increased tumor grade. Surprisingly, several species of N-glycans were profoundly different between early grade prostate tumors resected from White and Black patients. Further, these glycans predict opposing overall survival between White and Black patients with prostate cancer. These data suggest differential N-linked glycosylation underline the racial disparity of prostate cancer prognosis. Our study highlights the potential clinical applications of MALDI-MSI for digital pathology and biomarker applications, and reveals molecular features that contribute to the racial disparity in diagnosis and survival of prostate cancer patients.

(57) Harnessing the diversity of streptococcal sialic-acid-binding adhesins for creating a toolbox of glycan-binding probes to map the host sialome

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Glycan interactions play an important role in bacterial colonization and biofilm formation in the mouth. Oral streptococci that are pioneer colonizers of tooth surfaces use long fimbrial serine-rich-repeat (SRR) protein adhesins to attach to sialoglycans on salivary mucins adsorbed as part of a protein layer covering tooth enamel. These normally harmless commensal oral streptococci can become pathogenic, causing infective endocarditis, when they enter the blood circulation. Thereupon sialylated mucin-like glycoproteins on the surface of platelets may serve as selective targets for binding and contribute to streptococcal colonization of the injured heart valve endothelium. In this study, we investigated the binding specificities of streptococcal SRR proteins to identify novel adhesins that can be forged into tools for preferential detection of different sialic acid subtypes. To find SRR adhesins that bind to the nonhuman sialic acid N-glycolylneuraminic acid (Neu5Gc), we isolated streptococci from tooth surfaces of chimpanzees and gorillas because these nonhuman primates express Neu5Gc, whereas humans lost the ability to synthesize that sialic acid. Screening of streptococcal isolates was achieved by red blood cell (RBC) agglutination assays with chimpanzee RBCs (Neu5Gc and Neu5Ac) versus human RBCs (Neu5Ac only). Bacteria binding specificities

were confirmed by sialoglycan array binding. The genomes of 45 streptococcal isolates exhibiting distinct phenotypes of sialic acid binding were sequenced. We found that all sialic-acid-binding isolates harbored at least one SRR gene containing a Siglec-like domain. Phylogenetic alignment of the Siglec-like domains revealed a clade for Neu5Gc-specific adhesins distinct from other sialic acid binding phenotypes. Choosing SRRs that were diverse based on hemagglutination phenotype and amino acid sequence, we recombinantly expressed eight SRR binding regions (SRR-BRs) for use as sialic-acid-binding probes. We tested the finer specificity of these SRR-BRs on the sialoglycan microarray, and found them to closely match the binding of their streptococcal strains of origin. Testing our SRR-BRs for binding to natural sialoglycoproteins suggested that our initial toolkit can be used to differentiate sialoglycans from different host species based on the sialic acid subtypes present, and likely their recognition of unique clustered saccharide patches in the sialome. Currently, our tools are being tested in different applications to consolidate sialoglycan specificity and to demonstrate the feasibility of probing diverse biological and pathological samples. In a bioinformatic search of streptococcal genomes using TBLASTN and hidden Markov models, we identified over 200 SRR-encoding genes containing Siglec-like domains. Considering the sequence diversity of Siglec-like domains across the Streptococcus genus, SRR protein adhesins represent a colossal resource for identifying novel glycan-binding tools of bacterial origin.

(58) ATP citrate lyase supports UDP-GlcNAc biosynthesis in A549 cells engaged in epithelial to mesenchymal transition

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Deregulated cellular metabolism is a hallmark of tumors. Cancer cells increase glucose and glutamine flux to provide energy needs and macromolecular synthesis demands. Several studies have been focused on the importance of glycolysis and pentose phosphate pathway. However, a neglected but very important branch of glucose metabolism is the hexosamine biosynthesis pathway (HBP). The HBP is a branch of the glucose metabolic pathway that consumes ~2–5% of the total glucose, generating UDP-GlcNAc as the end product. UDP-GlcNAc is the donor substrate used in multiple glycosylation reactions. Thus, HBP links the altered metabolism with aberrant glycosylation providing a mechanism for cancer cells to sense and respond to microenvironment changes. The mais purpose of the present study is to study the alterations in glucose metabolism during the epithelial to mesenchymal transition (EMT). EMT is the process by which carcinoma cells lose the juxtaposed structure of epithelial tissue, decrease epithelial markers such as E-cadherin, gain mesenchymal markers such as N-cadherin increase motility, invasiveness and resistance to chemotherapy. We have previously published that human alveolar adenocarcinoma cells A549 over activated HBP during EMT and UDP-GlcNAc levels. HBP overactivation requires increased nutrient fluxes to sustain UDP-GlcNAc biosynthesis. ATP citrate lyase (ACL) is the main enzyme that generates Acetyl-CoA in cytoplasm wich is well known to be used for lipid biosynthesis and well described as playing a role in EMT. The acetylation step of UDP-GlcNAc biosynthesis requires Acetyl-CoA but the main source of this molecule during EMT and cancer models remain elusive. In this study we considered to study ACL during EMT because we hypothesize that its activity could support UDP-GlcNAc biosynthesis by providing Acetil-CoA for acetylation step instead of classic notion of Acetil-CoA from lipid metabolism source. We found that ACL increases in EMT and investigated the changes in lipid biosynthesis since ACL generates acetyl-CoA used in the biosynthesis of this macromolecule. We observed the decrease in the following lipids: 2.5 times in fatty acid levels, 5 times in esterified cholesterol levels, 1.5 times in cholesterol levels and 4 times in triacylglycerol (TAG) levels. Due to the requirement of acetyl-CoA for the UDP-GlcNAc biosynthesis, we demonstrated that in ACL inhibition decrease UDP-GlcNAc levels as well as glycoconjugates on the cell surface containing α2,6-linked sialic acid. This decrease of UDP-GlcNAc levels possibly occurs due to the lower availability of acetyl-CoA that is formed as a product of the enzymatic reaction catalyzed by ACL. We also found elevated levels of citrate althought citrate synthase activity is not significantly changed. Taken together, our results indicate that ACL is necessary to sustain UDP-GlcNAc biosynthesis and glycophenotype changes during EMT.

(59) An activity-based proteomics approach to discovering galactosidases in the mouse gut microbiome for important biomedical and industrial applications

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The importance of the gut microbiota is only recently being understood as an important facet to human health. It not only plays an important role in countless diseases, from diabetes to depression, but can also be an excellent source of novel enzymes for use in biomedical research and applications. One limitation, however, is the sheer number of species and the difficulty of growing species individually in a laboratory setting can make it impractical to isolate and study gut microbe enzymes. To overcome this obstacle, we have developed a novel and effective approach to screen the entire gut microbiota for enzymes that have a chosen activity, without needing to grow and isolate bacterial strains individually. We use activity-based probes to specifically target enzymes with a desired function directly in the gut microbiota. The target enzymes, which bind to the probes, are then identified using mass spectrometry, synthesized, and cloned, allowing for immediate transgenic expression in the *E. coli*. We have chosen to isolate alpha and beta galactosidases because of their important biological roles and potential applications. For example, alpha-galactosidases can be used to generate O type from B type blood by enzymatically cleaving off the galactose from the B blood group antigen, converting it to O type blood, the universal donor blood type. Another application of alpha galactosidases is to remove raffinose-like oligosaccharides from food products such as soybeans, molasses and grains to improve nutrition and remove undesirable side-effects, like flatulence and bloating. Removing raffinose-like oligosaccharides from animal feed can help limit methane release into the environment, thereby mitigating one of the causes of global warming. Beta-galactosidase can be used to generate important prebiotics called galactooligosaccharides, which promote the growth of healthy gut bacteria, helping to prevent or treat irritable bowel syndrome or traveler's diarrhea.

(60) Capture-and-Release (CaRe): a rapid and facile method for lectin and glycoprotein identification and purification

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Glycosylated proteins, namely glycoproteins and proteoglycans (collectively called glycoconjugates), play important roles in numerous biological processes. Functions of many glycoconjugates are regulated by their interactions with another group of proteins known as glycan-binding proteins (GBPs). GBPs are broadly divided into two major groups: lectins and glycosaminoglycan-binding proteins (GAGBPs). In order to understand the biological functions of GBPs and their glycosylated binding partners, one must identify these proteins and then obtain them in pure form. The conventional protein purification methods often require longer time, elaborate infrastructure, costly reagents, and larger sample volume. To minimize some of these problems, we recently developed and validated a new method termed Capture and Release (CaRe). This method is time-saving, precise, inexpensive, and it needs relatively small sample volume. CaRe does not require antibodies, solid affinity matrices, specialized detectors, controlled environments and covalent modifications of reagents. 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 specific 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 filtration. The strength of CaRe is rooted in its simplicity, accuracy, expeditiousness and sample economy. CaRe was validated by purifying recombinant human galectin-3 and other known lectins and glycoproteins. This method has the potential to purify other glycoconjugates including proteoglycans. CaRe could be used as a tool for discovering new GBPs and glycoconjugates.

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(61) Glycosylation of SUSD2 and SUSD2's Immune Modulatory Effects in Breast Cancer

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Sushi domain containing 2 (SUSD2), also referred to as "Sushi" protein, is a type one transmembrane protein found to be over expressed in breast cancer cells. It has been implicated in tumor angiogenesis and immune evasion. In addition, it was recently identified as the chaperon protein for Gal-1, a homodimeric beta galactoside binding protein, that has also been associated with tumor angiogenesis, metastasis, and immune evasion. We set out to understand the mechanism of action that allows SUSD2 to chaperon Gal-1 to the surface of cells, as well as the mechanism of action concerning SUSD2's ability to participate in immune modulation. We characterized the N-glycosylation sites on SUSD2. The analysis revealed terminal LacNAc residues in bi-, tri and tetra antennary branches, as well as the presence of terminal sialic acid residues. Of further interest, in naturally expressing SUSD2 breast cancer cell line, SKBR3, analysis revealed the presence of hypersialyation, including, non-canonical cancer associated sialic acids, Neu5Gc and Neu5,9Ac2. In cell lines

in which SUSD2 and Gal-1 had been removed, hypersialylation was reduced, including, 9-O-acetylated sialic acids. SUSD2 and Gal-1 were also found to regulate Siglec binding in breast cancer cell lines, particularly, reducing Siglec 7 and 9 binding in their absence.

(62) Analysis of the dynamics of influenza A virus-receptor interactions: binding receptors for efficient release

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Interactions of influenza A virus (IAV) with sialic acid (SIA) receptors determine viral fitness and host tropism. Binding to a diverse repertoire of mucus decoy receptors and receptors on epithelial host cells is determined by a receptor-binding hemagglutinin (HA) and a receptor-destroying neuraminidase (NA). We recently developed biolayer interferometric analyses to study the dynamics of multivalent influenza A virus-receptor interactions (DOI: 10.1371/journal.ppat.1007233). In the absence of NA activity, virus particles were virtually irreversibly bound to surfaces coated with synthetic sialosides or engineered sialoglycoproteins. Multiple low-affinity HA-SIA interactions resulted in overall extremely high avidity but also permitted a dynamic binding mode, in which NA activity was driving rolling of virus particles over the receptor-surface. Virus dissociation only took place after receptor density of the complete receptor-surface was sufficiently decreased due to NA activity of rolling IAV particles. This self-elution of virus particles reflects the often mentioned, but poorly characterized, HA-NA-receptor balance. Subsequently, we showed that a second SIA binding site (2SBS) located adjacent to the NA catalytic site contributes to NA activity against multivalent substrates and is an important determinant of the HA-NA balance (DOI: 10.1371/journal.ppat.1007860). Of note, the 2SBS is present in most avian viruses, but always lacking in human viruses. Mutation of the 2SBS in avian H5N1 viruses disturbed the HA-NA balance, reduced virus replication, and upon passaging of these viruses selected for compensatory mutations in NA and/or HA that restored the HA-NA balance. Substitutions in HA that were selected reduced binding to avian-type α2,3-linked SIAs. One of these substitutions in HA also resulted in increased binding of α2,6-linked SIAs that are preferred by human viruses (DOI: 10.1371/journal.ppat.1008816). Also for H7N9 and H9N2 viruses in the field, substitutions in the 2SBS of NA preceded, and may have driven, substitutions in receptor binding site of HA that increase binding to human-type receptors. In conclusion, the HA-NA-receptor balance of influenza A viruses can be studied using kinetic biolayer interferometric analyses. These analyses reveal an important contribution of the 2SBS in NA to this balance. 2SBS mutations in NA can drive acquisition of mutations in HA that not only restore the HA-NA balance, but may also confer increased zoonotic potential by concomitantly resulting in enhanced binding to human-type receptors.

(63) Dynamic Isotopic Detection of Amino Sugars with Glutamine (IDAWG) for Released Glycans and O-GlcNAc Modified Proteins

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The Isotopic Detection of Aminosugars With Glutamine (IDAWG) method was originally developed for the glycomics field as a quantitative tool that takes advantage of the hexosamine biosynthetic pathway, isotopically labeling nitrogen-containing glycans in cell culture systems via the use of 15 N-Gln. Here, we present an adaptation of this method, Dynamic IDAWG, that allows for the calculation of half-lives of released glycans in a given sample following analyses by LC–MS/MS. An additional benefit to using this method is that the cycling rates of the post-translational modification O-linked β-N-acetylglucosamine (O-GlcNAc) can also be determined. O-GlcNAc is found on thousands of nuclear and cytosolic proteins in mammals and is thought to be a regulatory modification playing a role in a variety of cellular processes. The modification cycles on and off serine and threonine residues by means of O-GlcNAc Transferase (OGT) and O-GlcNAc Hydrolase (OGA) respectively. O-GlcNAc is thought to be a dynamic modification; that is, the modification exhibits a shorter half-life than the modified protein. However, dynamics have only been evaluated on a small number of O-GlcNAc modified proteins due to the laborious and insensitive methods that are available. Therefore, there is an urgent need in the field to develop high-throughput, sensitive methods to evaluate the dynamics of O-GlcNAc on a global scale. Here, we illustrate the utility of Dynamic IDAWG in cell culture systems to evaluate the turnover of complex N- and O-linked glycans as well as delineating the half-life of O-GlcNAc on nuclear and cytosolic proteins.

(64) The conformational behavior of sialoglycans in interaction with Siglec-like adhesins

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Streptococcus gordonii and Streptococcus sanguinis, commensal species among the normal oral microbiota, become opportunistic pathogens that can cause infective endocarditis (IE) when they enter the bloodstream.[1] The presence on the microbial surface of "Siglec-like" serine-rich repeat adhesins may increase the propensity of streptococci to cause IE. These adhesins contain Siglec-like binding regions (SLBRs) that recognize α2–3 sialylated glycan structures, including O-linked glycans displayed on salivary MUC7, platelet GPIb and several mucin-like plasma proteins.[2] The SLBRs from different strains recognize different repertoires of structures, with some displaying selectivity for a single structure and others showing broader specificity.[3] GspB and Hsa are the Siglec-like serine-rich repeat adhesins of S. gordoni strains M99 and Challis, respectively, that have the ability to mediate Streptococcus adhesion to platelet membrane glycoproteins. The high-resolution crystal structures of these proteins have been published, but they have not fully explained the determinants of ligand specificity.[4,5]

Thus, unveiling the mechanism of glycan recognition by Siglec-like adhesins at the molecular level could represent a starting point to better understand the different selectivity and flexibility of the streptococcal adhesins towards sialoglycans. We explored the recognition and binding of the SLBR of Hsa, which has broader selectivity, to various sialoglycans, such as 3′-sialyllactosamine (3'SLN). The results revealed a conformer selection of 3'SLN upon binding, and we provide a 3D view of the complex. Moreover, we also dissected the molecular recognition of GM1b by the SLBRs of GspB and Hsa in vitro, showing that the ligand adopts a different conformation depending on the accommodating protein binding pocket.

In conclusion, we illustrated how different α2–3 sialylated glycans are recognized bythe SLBRs of GspB and Hsa. These outcomes were achieved by using several techniques, in particular NMR ligand-based methods, such as Saturation Transfer Difference NMR and transferred NOESY, as well as computational approaches, including docking, Molecular Dynamics and CORCEMA-ST analysis.[6,7,8]

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(65) Lipopolysaccharides from Gut Commensals: The Structure and Immunomodulatory Effects on Human Cellular Models

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The gut microbiota guide the development of the host immune system by setting a systemic threshold for immune activation. Lipopolysaccharides (LPSs) from gut Gram-negative bacteria are able to trigger systemic and local proinflammatory and immunomodulatory responses, and this capability strongly relies on their fine structures.[1] Up to now, only a few LPS structures from gut commensals have been elucidated; therefore, the molecular motifs that may be important for LPS – mammalian cell interactions at the gut level are still obscure. Here, we report on the full structure of the LPS isolated from one of the prominent species of the genus *Bacteroides, Bacteroides vulgatus*. This bacterium is known to exert strong immune-modulating properties leading to prevention of colitis-induction in several mouse models for experimental colitis. Furthermore, we previously demonstrated that its isolated LPS does not induce expression of proinflammatory cytokines but actively induces hyporesponsiveness toward subsequent LPS-stimuli in intestinal *lamina propria* $CD11c^+$ cells in murine *in vitro* systems.[2]

Notably, in the same work, we also proved that the administration of purified *B. vulgatus* LPS re-establishes intestinal

immune homeostasis in a mouse model for experimental colitis and correlated these health promoting effects to the poor immunostimulant properties of this LPS exploited through induction of a special type of LPS tolerance in intestinal lamina propria CD11c^{+} cells, via the MD-2/TLR4 receptor complex axis.[2] Given that the administration of the sole *B. vulgatus* LPS in mice caused the restoring of the homeostasis in intestine after severe inflammation, this phenomenon must of course be attributed to its chemical structure. Therefore, we have moved to the establishment of the full structure of this LPS, that is the characterization of its three structural moieties: the glycolipid anchor to the membrane specifically recognized by the host innate immunity receptor MD-2/TLR4 complex (the lipid A), the core oligosaccharide moiety and the polysaccharide chain portion (the O-antigen).

The LPS turned out to consist of a particular and unprecedented chemical structure based on hypoacylated and monophosphorylated lipid A, and with a galactofuranose-containing core oligosaccharide and an O-antigen built up of mannose and rhamnose. The evaluation of the immunological properties of this LPS on human *in vitro* models revealed a very interesting capability to selectively produce anti-inflammatory cytokines and to induce the synergistic activation of both MD-2/TLR4- and TLR2-mediated signaling pathways.[3]

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(66) The impact of two mutations in GM3 synthase on glycosphingolipid biosynthesis and cell signaling associated with human neural cell differentiation

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GM3 Synthase Deficiency is an autosomal recessive neurological disorder characterized by seizures, severe intellectual disability, choreoathetosis, dysmorphic facial features, and altered skin pigmentation. GM3 Synthase Deficiency results from a mutation in the ST3GAL5 gene that encodes GM3 synthase, a glycosphingolipid-specific sialyltransferase. ST3GAL5 adds sialic acid to lactosylceramide to synthesize GM3 ganglioside, which in turn, is used as a substrate by other glycosyltransferases to generate the majority of complex gangliosides. Both GM3 and ST3GAL5 are highly enriched in neural tissues. Fibroblasts from two patients with different mutations in ST3GAL5 were reverted to pluripotency; one patient line was derived from an African-American cohort (Salt & Pepper Syndrome, SP-ST3) and has a point mutation in the S-motif of the sialyltransferase domain, while the other was derived from an Amish cohort (GM3 Synthase Deficiency, A-ST3) and has a mutation that truncates the polypeptide between the L- and S-motifs of the catalytic domain. The resulting induced pluripotent stem cells (iPS cells) were differentiated to neural crest cells (NC cells) to investigate the impact of loss of GM3 on neural-specific glycosylation and cellular signaling responses during differentiation. Consistent with the nature of the mutations and with our previous analysis of patient fibroblasts, GM3 and GM3-derived gangliosides are undetectable in iPS and NC cells derived from both cohorts. More generally, however, glycosphingolipid (GSL) profiles exhibited subtly different compensatory responses in relation to how LacCer is utilized and in the distribution of GSL ceramide heterogeneity. Since GSLs are major elements of plasma membrane signaling domains, we investigated the phosphorylation and expression levels of receptor tyrosine kinases across differentiation of iPS cells to NC cells. For both GM3 deficiency mutations, we detected alterations in the rise and fall of ErbB3 and EGFR signaling compared to the NC differentiation of non-affected human iPS cells. Again, however, the fine details across time and magnitude were subtly different between the two patients. Thus, altered GSL biosynthesis impacts signaling events that drive differentiation and survival of human neural precursors. Additionally, human GM3 deficiency cells provide a novel platform to investigate structure/function relationships that connect GSL diversity to cell signaling.

(67) Synthetically tuning the emergent function of galectin-3

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Galectin-3 is a carbohydrate-binding protein that functions as a modulator of cell behavior in physiological and pathological processes. At the cell surface, galectin-3 can undergo dynamic self-association to form oligomers that crosslink glycans and glycoprotein receptors. Thus, galectin-3 multivalency is central to signal transduction, with oligomerization providing a critical checkpoint for its emergent function. Establishing galectin-3 structure–function relationships, however, is challenged by a lack of chemical tools to precisely control its multivalency. Here we report synthetic galectin-3 oligomers with programmable valency to probe the emergent properties of the native protein. Synthetic oligomers are

constructed through recombinant fusion of peptide sequences encoding alpha helical coiled-coils with 2 through 6 strands to the N-terminus of galectin-3. The N-terminus of galectin-3 was chosen to avoid obstructing the carbohydrate binding pocket of the carbohydrate-recognition domain (CRD) and because it is thought to be the native multimerization domain of the protein. We further fused a green fluorescent protein domain to these constructs so that oligomer glycan-binding can be both visualized and quantitated. First, we established relationships between galectin-3 valency and extracellular signaling activity by measuring the extent to which synthetic oligomers induced death of Jurkat T cells, which express the glycoproteins CD45 and CD7, as well as lymphocyte cell lines lacking CD45 or CD7 (i.e., J45.01 or HuT 78, respectively). We found that synthetic galectin-3 oligomers preferentially engage CD45 to signal cell death, likely due to its abundance and accessibility on the cell surface, whereas the wild-type protein requires both receptors. As a result, synthetic galectin-3 oligomers transduce intracellular signaling cascades through pathways that alter cell fate and function in a manner unique from wild-type galectin-3. While various chemical tools to probe the specificity and affinity of galectin-3 CRD-glycan interactions have been widely explored, we believe that studying the physical interactions of its multivalent form via synthetic means will shed light on the emergent properties of this oligomeric protein.

(68) The dynamic 3D structure of oligomannose N-glycans free and bound to FcγRIII: Epitopes accessibility and shielding effects

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Glycosylation is the most abundant and diverse protein post-translational modification (PTM).N-glycosylation is a type of PTM where the glycans are N-linked to the Asn (N) sidechains of secreted proteins, within the context of NXT(S) sequons. N-glycans can be of three main types, namely oligomannose,complex and hybrid. All of these share a common core structure, deriving from their intricate biosynthetic pathway that diverges in the specific funtionalization of the two main arms, i.e. the $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ arm. In oligomannose N-glycans the two arms are built with only mannose (Man) mono saccharide units, with specific branching patterns and lengths depending on the cell line and on the spatial accessibility of the glycans location. The highly dynamic nature, branching complexity and 3D structure of low processed oligomannose N-glycans, such as Man9 $\rightarrow$ Man5, has been recently highlighted for their roles in immune escape and infectivity of enveloped viruses, such as SARS-CoV2 and HIV-1. The specific architectural features that allow oligomannose N-glycans to perform these tasks is yet unclear, due to their intrinsically disordered nature,which prevents them from being characterized through experimental structural biology. In this work we will discuss the results of over 22.5 μs of molecular dynamics (MD) cumulative sampling of differently processed oligomannose N-glycans free in solution. The extensive conformational sampling allows us to disentangle the microscopic structural disorder and to resolve it within a'glycoblocks' framework [Fogarty CA, Harbison AM and Fadda E., BeilsteinJOC (2020)]. We also discuss the effects of a complex protein surface on these equilibria based on over 8 μs cumulative MD sampling of glycosylated and non-glycosylated FcγRIII systems, also highlighting the role of glycosylation on the protein structure and on its recognition by IgG1s.

(69) Discovering Hotspots to the Optimization of Bioactive Indolocarbazoles

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INTRODUCTION.

Indolocarbazoles (ICZs) are glycosylated natural products presenting wide chemical diversity and broad spectrum of biological activity, notably antitumor, antimicrobial, and antiviral. This class of small ligands has been complemented with a variety of chemical modifications to improve its bioavailability and specificity. While ICZs complexes with target proteins are known, so supporting structure-based optimization of ligands bioactive properties, the conformational properties of the flexible substituents on ICZs, such as carbohydrates, are still partially inaccessible to state of the art structural biology methods. As a complement to these methods, molecular mechanics based calculations may offer a unique set of tools to access flexible molecules conformational ensemble and improve required therapeutics features through conformational-activity relationships.

OBJECTIVES.

Shed light to structural and conformational features of ICZs, offering new insights to refine structure/conformational activity relationships in biological solutions.

MATERIALS AND METHODS.

In order to assess the conformational and solvent-interaction features of ICZs in a pre-complexation state, molecular dynamics was performed through GROMOS force field. Quantum calculations at the MP2 level were implemented by Gaussian and supplied the dihedral potentials and the partial atomic charges, whose parameters were fitted to better reproduce on classical mechanics the rotational bonds profile and the chromophore vector dipole moment of molecules, respectively. Each compound was placed in a box with SPC water molecules and simulations were carried out using GROMACS package for 1 μs.

RESULTS AND DISCUSSION.

The new parameters allowed us to perform simulations of 34 ICZ derivatives presenting different substituents

and glycan moieties. Some structural modification patterns showed to be involved on ICZ-water H-bond, with diverse lifetimes and dihedral freedom degrees. The presence of halogen groups on rebeccamycin analogues increases the carbohydrate-water H-bond lifetimes and affects the bioactive conformation required to poison Top1. Biased molecular dynamics showed multiple carbohydrate puckering on staurosporine analogues with different carbohydrates, including bicycle and tricycle structures, expanding the perception of possible saccharide conformers when compared to NMR data.

CONCLUSION.

Changes regarding H-bonds lifetimes with the solvent could potentially affects the complexation free energy due to the dessolvation energetic costs. The broad perception of ICZs conformation represents a potential factor to be exploited aiming the specificity of these compounds. Together, the dynamic behavior observed for ICZs elucidate chemical and conformational features involved in ICZs complexation process and might help to expand the therapeutic potential of these compounds.

(70) Epitope mapping of sialoglycans in the interaction with Siglecs

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Siglecs (Sialic acid-binding immunoglobulin type lectins) represent a family of cell surface transmembrane receptors belonging to I-type lectins, predominantly expressed by immune system cells [1] They are involved in crucial biological events like cell adhesion and signaling, and are increasingly recognized for their role in helping immune cells to distinguish between "self" and "non-self", dampening autoimmune responses and controlling inflammation [2].

Aberrant sialic acid-Siglec interactions have been thus associated with autoimmune, neurodegenerative and cancer diseases [3] Siglecs functions are modulated by their ability to specifically recognize sialic acids epitopes, mostly found on the terminal moiety of cell surface glycoconjugates. Siglecs are nowadays considered glyco-immune checkpoints for the targeting of sialylated structures over-expressed by tumors.[4] Given their therapeutic potential, an emergent approach for the treatment of autoimmune diseases involves the glycan-based treatments, including the design of specific ligands with high-affinity for the Siglecs binding sites [5].

CD22 (Siglec-2) and Siglec-10, are inhibitory co-receptors of the B-cell receptor and represent attractive candidates for their ability to modulate tolerance to antigens [6] We have investigated the molecular mechanisms underlying sialoglycans recognition by Siglecs using a combination of biophysical, spectroscopic and computational approaches, with the aim to carry out a dynamic characterization of their interaction in solution [7] In this context, we analyzed the binding properties of Siglecs receptors toward with representative sialylated glycans, including 3′/6′-sialyl-N-acetyllactosamine, as well as naturally occurring complex-type N-glycans.

The binding affinity of the Siglecs toward sialyloglycans was evaluated using fluorescence spectroscopy, ELISA and alpha screen assays. NMR spectroscopy, as ligand-based NMR techniques, including STD-NMR and tr-NOESY was used to evaluate the interacting epitope and the bioactive conformation of sialoglycans in solution. Homology modeling, docking and MD studies, together with CORCEMA-ST protocol, were implemented to obtain and validate 3-D ligands/receptor complexes, highlighting the crucial interactions between the binding partners. Comprehensively, our outcomes have improved our knowledge of the molecular interaction occurring between Siglec and sialyloglycans, providing a structural point of view for the design and development of high-affinity ligands able to control the receptor functionality.

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(71) Towards the Selective Tagging of the Sialome of Living Cells using Sydnone-Modified Neuraminic Acids

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The bioorthogonal chemical reporter strategy, which elegantly combines the use of metabolically labeled azido-sugars and 1,3-dipolar cycloadditions with strained alkynes, is emerging as a versatile technology for the labeling and visualization of glycans. Advantages of cyclooctyne-based probes encompass their high reactivity, non-toxicity (metal-free conditions) and synthetic modularity. However, the azido-reporter is not completely biologically inert as it can

react, to varying degrees, with biological functionalities such as thiols. This inherent instability makes the azide functionality a precursor for the potential accumulation of secondary metabolites with unknown biological effects.

In order to address this limitation, while keeping the advantages of the cyclooctyne framework as the reactive probe, we decided to investigate the utilization of other stable 1,3-dipoles as novel reporters. In this context, we present herein the utilization of 3,4-disubstituted sydnones, a singular class of aromatic mesoionic dipoles, as novel chemical reporters for the metabolic oligosaccharide engineering of sialoconjugates in living cells.

The positioning of the reporter on the sialic acid was found to significantly alter its recognition by multiple glycan-processing enzymes, leading to the favored incorporation of the reporter into specific class of sialoproteins. Due to its high biological stability and more selective glycan incorporation, this novel chemical reporter will significantly expand our chemical biology toolbox for investigating the roles of specific sialosides.

(72) Polar functional group-containing glycolipid CD1d ligands modulate cytokine-biasing responses

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The MHC class I-like molecule CD1d is a nonpolymorphic antigen-presenting glycoprotein, and its ligands include glycolipids, such as α-GalCer (eg. KRN7000). The CD1d in complex with the ligands (i.e. lipid antigens) activate natural killer T cells by TCR recognition, leading to the secretion of various cytokines (IFN-γ, IL-4, IL-17A, etc.), which modulate helper T cell differentiation such as Th1, Th2, and Th17. In this study, we report the structure–activity relationship studies of α-GalCer derivatives containing various functional groups in their lipid acyl chains. It was identified that several derivatives are potent CD1d ligands displaying higher cytokine induction levels and/or unique cytokine polarization [1–3]. We have firstly identified a series of novel Th2-biasing CD1d glycolipid ligands based on modification of their lipid part of α-GalCer structure [1–2]. These have shown high binding affinities and efficient Th2 cytokine production, and even truncated acyl chain-containing variants still retain their binding affinities and agonistic activities, which can be associated with an "anchoring effect." i.e. formation of a buried hydrogen bond between a polar group on the acyl chain and the CD1d lipid-binding pocket. The studies also indicated that flexibility of the lipid moiety can affect the binding affinity, the total cytokine production level and/or cytokine biasing. Our analysis with the derivatives also showed that the appearance rates of ligand-CD1d complexes on the cell surface were involved in Th2-biasing responses [2]. We demonstrated that the ligands, having the "anchor" in the shorter lipid part, would be one of the most potent Th2-biasing ligands with keeping the total cytokine induction levels, among the known ligands [2]. In terms of cytokine polarization, we also found that most of the ligands containing polar functional groups displayed Th17-biasing immune responses, as well as Th2-biassing [3]. Our approaches might also be applicable to SAR studies to effectively identify chemical probes for elucidating the function of glycolipid binding proteins, or drug candidates.

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(73) The lipopolysaccharide isolated from Fusobacterium nucleatum ATCC 51191

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Fusobacterium nucleatum is a common member of the oral microbiota [1]. However, this symbiont has been found to cause opportunistic infections such as periodontal diseases and has been implicated in adverse pregnancy outcomes such as preeclampsia, gastrointestinal disorders such as colorectal cancer or appendicitis, as well as cardiovascular diseases, rheumatoid arthritis, respiratory tract infections, Lemierre's syndrome and Alzheimer's disease [2]. F. *nucleatum* has virulence mechanisms that lead to infections outside the mouth [3].

As a Gram-negative bacterium, F. *nucleatum* has an outer membrane layer protecting the bacterium in different environments and its external face is composed mainly by lipopolysaccharides (LPS) [4]. LPS are microbe associated molecular pattern (MAMP) molecules that play a crucial role in the interaction of the host with pathogens but also commensal bacteria as they can be recognized by the innate

immunity response activating the pattern recognition receptors (PRR) [5]. The dual commensal-pathogen behaviour of F. *nucleatum* makes the determination of the LPS structure of this bacterium especially interesting.

The structure of O-antigen and lipid A from *F. nucleatum ssp. animalis* (ATCC 51191) was characterized using a combination of Gas Chromatography—Mass Spectrum (GC–MS) derivatization, Matrix-Assisted Laser Desorption/Ionization (MALDI) and Nuclear Magnetic Resonance (NMR) analytical techniques. The LPS structure of *F. nucleatum* ATCC 51191 displayed great complexity due to the high level of amino functions, acetylation and amino acid presence. In addition, there were various lipid A species with diverse levels of acylation and phosphorylation.

More research is necessary to understand the role that this structure plays in the interaction of this bacterium with host in the context of health and disease.

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(74) Silalylation plays an important role in Glioblastoma maintenance

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Glioblastoma, a grade IV astrocytoma, is a deadly disease with low median survival rate of just 15 months and high recurrence despite aggressive standard of care including maximal surgical resection, radiation and administration of temozolomide, a chemotherapeutic agent. This dismal prognosis and treatment failure is partly linked to inter and intratumoral heterogeneity caused by a subset of cells in GBM called brain tumor initiating cells (BTICs). BTICs have the capacity to self-renew, proliferate, differentiate into multiple lineages, and give rise to tumor in vivo recapitulating the primary tumor. Moreover, BTICs infiltrate the surrounding brain tissue and are left behind during surgical resection. These BTICs are implicated in tumor recurrence, therefore, it is highly crucial to understand the cellular mechanisms of BTICs in order to treat GBM. A mechanism through which BTICs are known to survive in hypoxic and nutrition deprived conditions is by altering posttranslational modifications like altered glycosylation, which is one of the earliest known hallmark of cancer. However, this aspect of cancer cell biology has not been explored in GBM. Among the various glycosyltransferases present in human cells, golgi sialyltransferase ST6Gal-I (beta-galactoside alpha-2,6-sialyltransferase 1) adds sialic acid residues in α2–6 linkage to membrane bound and secreted N-glycans. It has been shown that ST6Gal-I is an important driver of tumorigenic processes such as epithelial to mesenchymal transformation, maintenance of cancer stem cells (CSCs), tumor cell resistance to apoptotic stimuli, chemoresistance, radiation resistance and increased survival of cells exposed to stressors such as hypoxia and serum starvation in various cancers such as pancreatic and ovarian cancer. Role of this potent TIC regulator has not been explored in GBM BTICs.

We hypothesize that ST6Gal-I mediated silalylation of surface receptors in GBM promotes stemness. Data with GBM patient derived xenografts (PDX) depicts increased stemness with high α2,6 sialylation, while, ST6Gal-I knockdown (KD) in GBM PDX lines show decreased in vitro and in vivo GBM growth. These findings strongly implicate ST6Gal-I mediated in BTIC maintenance and GBM tumorigenesis. Determining the mechanistic basis of sialylation-dependent maintenance of BTICs will highlight a novel insight in GBM stemness.

(75) Development of α2,6-Specific Affinity Reagents for the Detection and Enrichment of Sialoglycoconjugates

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Glycans containing sialic acids have long been implicated as biomarkers for a variety of diseases, including endocrinal, oral, and colon cancers. As the investigation of the dynamic changes in the levels of glycoforms becomes more commonplace, specific and sensitive affinity reagents are essential to overcome current limitations in the discovery and exploitation of disease-related glycans. To meet the need for a toolbox of highly specific reliable detection reagents, here we report the development of a series of recombinant SiaFind™ reagents derived from a *Polyporus squamosus* lectin for the detection of α2,6 sialic acid linkages. As recombinant proteins, these reagents can be produced in a highly consistent

manner and offer sensitive and application-tailored alternatives to the *Sambucus nigra* lectin SNA. We have characterized the new SiaFind™ reagents using BioLayer Interferometry analysis, and demonstrate their performance in a variety of detection assays, including ELISA, Western blotting, and immunohistochemistry staining of normal and cancerous tissues. Comparison with existing reagents illustrates the convenience, robustness, and accuracy of the SiaFind™ reagents in glycoscience research.

(76) Analysis of the glycosylation pattern of a clade C HIV-1 envelope gp120 generating autologous serum neutralizing antibodies in immunized rhesus macaques

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The development of an effective vaccine against the human immunodeficiency virus (HIV) remains a critical need with 38 million infected people worldwide in 2019 according to the WHO.

The HIV-1 envelope (Env) glycoprotein (gp) on the viral surface engages with receptors on $CD4^+$ T cells, leading to membrane fusion and allowing the entry of the viral particle into the host cell. Therefore, the Env protein is a key target for neutralizing antibodies. Env is a trimer of gp120-gp41 heterodimers with extensive glycosylation on the gp120 subunit, constituting 50% of its molecular mass. The resulting 'glycan shield' has a strong influence on Env biology and antigenicity. Various studies have highlighted a link between the glycosylation of the transmitted/founder (T/F) or early Env variants and the development of heterologous plasma neutralizing antibodies (nAb) breadth years later. However, much of this work has been based on predicted N-linked glycosylation sites in the amino acid sequence without characterization of the carbohydrate moieties that occupy the Env. Furthermore, there could be clade-specific differences, making it important to characterize diverse Envs from globally predominant clades.

Within this study, we characterized a novel HIV-1 T/F Env derived from an HIV-1 clade C infected individual, Z1800M, who developed a broad plasma neutralizing antibody response. The Z1800M T/F Env gp120 protein was also used in an innovative immunization study and preliminary data revealed that rhesus macaques that were immunized with the gp120 protein following DNA and MVA vaccinations developed autologous neutralizing antibodies. This observation is interesting, as boosting with monomeric HIV-1 gp120 immunogens generally does not elicit this response. Ongoing research includes mapping the neutralizing antibodies to specific regions of gp120 and recovery of monoclonal antibodies.

We applied high-resolution mass spectrometry to different enzymatic digests for identification of the N-linked glycosylation pattern of glycopeptides derived from the Z1800M T/F Env gp120 protein. We were able to confirm site occupancy and site-specific glycosylation for 22 out of 24 N-linked glycosylation sites present on this gp120 via HCD and CID fragmentation methods. We determined the relative abundance of each glycoform on each glycosylation site and identified the glycosylation pattern: All sites were significantly glycosylated, with 20 sites exhibiting mainly complex and hybrid type glycans. However, two sites were occupied by high-mannose type glycans only and suspected to be involved with the formation of the high-mannose patch, which is known to play a role in the trimerization process of Env proteins. Three-dimensional modeling of the elucidated glycosylation pattern onto gp120 to spatially depict the structure for a better understanding of its antigenicity is ongoing.

Overall, this study provides unique insights that are highly relevant to HIV vaccine immunogen design.

(77) The Landscape of N-linked Glycan Synthesis in the Progression of Liver Disease to Hepatocellular Carcinoma: A Computational Approach to Target Identification.

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Approximately one fourth of all protein-coding genes are modified with N-linked glycans in transit to their final destination in the cell. This post-translational modification adds diversity to protein structures with increasingly subtle species-, tissue-, and pathology-specific differences in the observed N-glycoforms of proteins. As such, the changes in glycosylation observed as a consequence of malignant transformation can change the character of the glycoconjugates in a manner that can be exploited for early cancer detection and therapeutic targeting. The liver is a major secretory organ and glycosylated proteins present an interesting target for the serologic detection of liver pathologies, including hepatocellular carcinoma (HCC), and for therapeutic interventions. However, a considerable challenge to discovery efforts is the identification and functional annotation of N-glycoproteins that undergo alterations in the premalignant diseases and HCC.

In this work, we use previously published transcriptomic liver, tumor adjacent, and HCC data from the GTEX (N = 103), CPTAC (N = 159; paired), and TCGA (N = 348) projects to identify the impact of enzymes involved in N-

linked glycan synthesis on the progression of HCC and underlying liver disease. Pearson correlations of enzyme mRNA expression against the expression of phenotypic markers were supplemented by proteomic data of the CPTAC project to assess possible targets impacted by their respective enzymes. At the mRNA level, FUT8 and MGAT5 were among the most consistently significant enzymes associated with the phenotypes observed and were detected at the protein level in previous proteomic work. Both enzymes are positively and significantly correlated with markers associated with the metastatic phenotype (CPTAC/TCGA HCC RNA: FUT8 Avg. Corr = 0.379/0.391, $P < 0.001/0.003$; MGAT5 Avg. Corr = 0.296/0.288, $P < 0.001/0.047$), but significantly and negatively with markers of epithelial and hepatocyte phenotype in HCC datasets (CPTAC/TCGA HCC RNA: FUT8 Avg. Corr = −0.244/−0.183, $P < 0.001/0.001$; MGAT5 Avg. Corr = −0.156/−0.119, $P < 0.001/0.024$). Further investigation shows that a set of N-glycoproteins with the strongest positive correlations with each enzyme at the mRNA and protein levels are enriched for several hallmarks of cancer, most prominently the activation of metastasis and invasion. Finally, we find that N-glycoproteins positively correlated with FUT8 protein expression are core fucosylated in the HCC patient samples of recently published glycoproteomic data. Taken together, our computational approach identifies target glycoproteins associated with FUT8 (n = 142) and MGAT5 (n = 102) mRNA and protein expression which can be used in functional studies of glycosylation in liver cancer, identification of novel targets of interventions, and for further biomarker discovery.

(78) Role of N-glycosylation on structure–function relations of Trypanosoma congolense trans-sialidase

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Trans-sialidases (TS) are an unusual class of sialidases and known to be a major virulence factor playing a key role in the pathogenesis of trypanosomiasis, a devastating disease better known as sleeping sickness in human and nagana in animals. African trypanosomiasis affects humans and livestock in Sub-Saharan Africa and causes serious to life-threatening acute and chronic symptoms when untreated as well as huge economical losses annually. Molecular mechanisms and biological functions of TS are under study since many years. However, little attention has been drawn to the influence of post-translational modifications of TS such as glycosylation. Although it has been reported that several TS carry high-mannose type N-glycans, their biological relevance and function still remains elusive. To study the role of N-glycosylation regarding structure–function relations, we expressed recombinant TS1 of the African animal parasite *Trypanosoma congolense* in CHO Lec1 fibroblasts, known to produce similar high-mannose type N-glycans as described for trypanosomes.

We were able to identify and assign various N-glycosylation sites of recombinant *Trypanosoma congolense* TS1 via MALDI-TOF mass spectrometry. In addition, employing the power of circular dichroism to determine protein secondary structure, we studied the influence of N-glycosylation on protein structure stability. However, the most surprising effect observed, represents the influence of N-glycosylation on TS1 enzymatic activity, which is the transfer of sialic acid from a donor to a receptor glycan. Enzymatically deglycosylated recombinant TS1 showed a two-fold decrease in substrate affinity while maintaining a constant V_{max} relative to the untreated enzyme using fetuin and lactose as sialic acid donor and acceptor substrate, respectively. These observations clearly indicated that N-glycosylation of TS affects the enzyme activity.

(79) A conserved role for AMP-activated protein kinase in NGLY1 deficiency

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Mutations in human N-glycanase 1 (NGLY1) cause a congenital disorder of deglycosylation (CDDG) with global developmental delay and a host of other phenotypes, including neuropathy, movement disorder, and chronic constipation. NGLY1 is known to regulate proteasomal and mitophagy gene expression through activation of a transcription factor called NFE2L1. However, the molecular basis for the impaired energy metabolism and other phenotypes observed in NGLY1 deficiency patients is not well understood, and no mechanism-based therapy exists for this disease. Previously, we have shown that the *Drosophila* homolog of NGLY1 (Pngl) regulates BMP signaling in a tissue-specific manner. In addition, we also reported a severe failure in gut clearance in Pngl mutant larvae, which cannot be explained by impaired BMP signaling. Here, we report that the failure in gut emptying in Pngl mutant is associated with a significant decrease in gut peristalsis. It has previously been reported that mutations in *Drosophila AMP-activated protein kinase α* (AMPKα) result in a similar functional abnormality in larval midguts. Accordingly, we

examined whether decreased AMPK signaling can explain the food accumulation and lethality in *Pngl* mutants. The expression of AMPKα is decreased in *Pngl* mutant midguts, and genetic restoration of AMPK levels in mesoderm significantly rescues the gut clearance and peristalsis and also the lethality of *Pngl* mutants. Importantly, Ngly1 null mouse embryonic fibroblasts (MEFs) also showed decreased AMPKα expression and activation. Both *Pngl* mutant fly guts and *Ngly1* mutant MEFs show impaired energy metabolism, which can be rescued by restoring AMPK level. Experiments in *Drosophila* and MEFs indicate that the AMPK defects caused by *Ngly1* deficiency are independent of NGLY1's role in regulating NFE2L1 activation and proteasomal/mitophagy gene expression. Importantly, fibroblasts from *NGLY1* deficiency patients show reduced *AMPKα* level and decreased mitochondrial respiration compared to control fibroblasts, which can be rescued upon treatment with an AMPK agonist. Collectively, our data establish an evolutionarily-conserved, NFE2L1-independent role for AMPK signaling downstream of NGLY1. The activity of AMPK can be regulated pharmacologically. Therefore, our data suggest that enhancement of AMPK signaling can serve as a potential therapeutic approach in NGLY1 deficiency patients.

(80) Glycometabolic regulation of the biogenesis of small extracellular vesicles

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Small extracellular vesicles (sEVs) play an important role in communication between cells by mediating the unconventional secretion of nucleotides and proteins, which contributes to a wide range of biological and pathological phenomena such as cell differentiation, immune response, neurodegenerative diseases, and tumor progression. The biogenesis of sEVs is regulated by multiple molecular machineries, generating considerably heterogeneous vesicle populations, i.e., exosomes and non-exosomal vesicles, with distinct cargo molecules. However, the role of carbohydrate metabolism in generating such vesicle heterogeneity remains largely elusive. Here we discovered that 2-deoxyglucose (2-DG), a well-known glycolysis inhibitor, suppressed the secretion of non-exosomal vesicles by impairing asparagine-linked glycosylation (N-glycosylation) in mouse melanoma cells. Mechanistically, 2-DG was metabolically incorporated into N-glycan precursors, causing the precursor degradation and partial hypoglycosylation. N-glycosylation blockade by Stt3a silencing was sufficient to inhibit non-exosomal vesicle secretion. In contrast, N-glycosylation blockade barely influenced exosomal secretion of tetraspanin proteins. Functionally, N-glycosylation at the specific sites of the hepatocyte growth factor receptor, a cargo protein of non-exosomal vesicles, facilitated its sorting into the vesicles. These results uncover a link between N-glycosylation and unconventional vesicle secretion, and suggest that N-glycosylation regulates the biogenesis of certain types of sEVs through cargo protein sorting.

(81) Exploring the conformational space of N-glycan's 3D structure through molecular dynamics simulations

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N-glycans share a common core structure, but can differ dramatically in the architecture of their arms. These modifications determine the N-glycans classification into three categories: oligomannose, complex and hybrid. While these different types depend on the level of processing an N-glycan undergoes, species-specific differences in the biosynthetic pathway dictate the exact sequence and branching pattern, designed to meet specific biological needs. The missing link between a specific sequence to its function is the N-glycan 3D structure. Indeed, this information allows us to understand the N-glycan dynamics, its interactions and recognition. Sequence-to-structure relationships of complex carbohydrates are difficult to characterize experimentally, due to their intrinsic structural disorder at the observable timescales. Within this framework, Molecular dynamics (MD) simulations provide a powerful and complementary platform to understand at the atomistic level of detail the glycan conformational propensity and dynamics in function of sequence and branching. Here, I will discuss our work on the characterization of the 3D structure of complex biantennary N-glycans by conventional and enhanced sampling MD simulations[1–3]. In the context of mammalian complex N-glycans, we determined clear sequence-to structure relationships, such as the addition of at terminal b(1–4) Gal to the a(1–6) arm that tightly regulates its accessibility and recognition for further functionalization[1]. Within the context of IgG1 effector function modulation, we were also able to determine through enhanced sampling MD how core fucosylation of the Fc is responsible for

quenching ADCC[2]. Furthermore, we explored how the seemingly subtle modifications found in invertebrates and plants N-glycans do in fact affect dramatically their structure and dynamics relative to their eukaryotic counterparts[3], possibly explaining their immunogenicity. With this insight, I will also discuss how we were able to rationalize and reduce the complexity of the N-glycans dynamic architecture in terms of the local spatial orientation of specific monosaccharides groups as self-contained 3D units, or "glycoblocks" and propose a framework for the control of the N-glycans structure through sequence.

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(82) Human Milk Oligosaccharide (HMO) Conjugates Improve Immune and Metabolic Parameters and Alter the Microbiome in Diet-Induced Obese (DIO) Mice

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Human milk oligosaccharides (HMOs) modulate infant immune responses and feed the developing microbiome. Lacto-N-fucopentoase III (LNFPIII) and Lacto-N-neotetraose (LNnT) are neutral HMOs that differ via the presence of an α1,3-linked fucose on LNFPIII. We developed conjugates of LNFPIII and LNnT, where 10–12 molecules of LNFPIII or LNnT are attached to a 40 kDa dextran carrier (P3DEX/NTDEX). Previously, we showed that LNFPIII conjugates ameliorate inflammation, act on multiple cell types, and are therapeutic in a wide range of murine disease models. Here, based on structural similarity, we asked if NTDEX will improve metabolic parameters similar to P3DEX in diet-induced obese (DIO) mice. Male *C57BL/6* mice were placed on a high-fat diet (HFD) for six weeks, then injected twice per week for eight weeks with 25 µg of 40 kDa dextran (DEX; vehicle control), P3DEX, or NTDEX. Body weights were measured weekly. Fasting blood glucose levels were measured prior to HFD, prior to beginning treatment, and prior to sacrifice. Glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed after four weeks of treatment with two weeks of rest between each test. At sacrifice, we harvested and weighed all organs. P3DEX treatment, but not NTDEX, led to reductions in body weights, organ weights, and fasting blood glucose levels. P3DEX also induced improvements in glucose and insulin tolerance. Upon histological analysis, mice treated with P3DEX exhibited improvements in white adipose tissue (WAT) and accumulated less fat in the liver. P3DEX or NTDEX treatment also induced different profiles of circulating cytokines and diabetic markers. Of note, both P3DEX and NTDEX reduced markers involved in hematopoiesis and chemotaxis, suggesting dampening of an overactive immune response. P3DEX decreased circulating levels of leptin and resistin. In contrast, NTDEX acted on the incretin hormone, gastric inhibitory peptide (GIP), as well as glucagon. P3DEX and NTDEX also exerted differential effects on the gut microbiome. DIO mice treated with NTDEX had reduced absolute abundance in bacteria, whereas mice treated with P3DEX had increased species richness. P3DEX also decreased the ratio of *Firmicutes/Bacterioidetes*. P3DEX treatment increased two phyla of beneficial bacteria, *Bacteroidetes* and *Verrucomicrobia*. LEfSe analysis revealed a myriad of significant biomarkers for DEX, P3DEX, and NTDEX groups. For P3DEX, the most significant biomarkers were of the *Bacteroidetes* phylum. These results suggest that the small difference in structure between P3DEX and NTDEX has drastic effects on the conjugates' modulation of the biome and inflammation. Future work will focus on identifying the receptors for these conjugates and delineating the individual mechanisms by which P3DEX and NTDEX exert their effects.

(83) Cellular substrates of the endoplasmic reticulum protein quality control sensors UGGT1 and UGGT2

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The protein quality control sensors UDP-glucose: glycoprotein glucosyltransferase (UGGT) 1 and 2 are proposed to act as gatekeepers of the early secretory pathway. They initiate rebinding to the carbohydrate-dependent chaperones calnexin and calreticulin that associate with proteins possessing monoglucosylated glycans. The UGGTs control glycoprotein exit from the endoplasmic reticulin (ER) for trafficking to the Golgi or ER retention to provide additional folding opportunities. A quantitative glycoproteomics strategy was used to identify cellular glycoproteins modified by the UGGTs (UGGTomes) at endogenous levels and delineate the specificities of UGGT1 and UGGT2. The UGGTome was comprised of seventy-one mainly large multidomain and heavily glycosylated proteins when compared to the general N-glycome. UGGT1 was the dominant glucosyltransferase with a preference towards large plasma membrane proteins

whereas UGGT2 favored the modification of smaller, soluble lysosomal proteins. This study provides insight into the cellular secretory load that utilizes multiple rounds of carbohydrate-dependent chaperone intervention for proper maturation and helps to define the roles for UGGT1 and UGGT2.

(84) ST6Gal1 expression in precursor B-lineage acute lymphoblastic leukemia

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Normal early human B-cells development from lymphoid progenitors in the bone marrow is dependent upon instructions from elements in the bone marrow microenvironment which include stromal cells as well as extracellular matrix and factors secreted by these cells. Glycosylation is thought to play a key role in such interactions. The sialyltransferase ST6Gal1 is the only enzyme that catalyzes the terminal addition of sialic acids in an α2,6-linkage to galactose on N-glycans. Expression of ST6Gal1 increases as mouse B cells undergo normal B-lineage differentiation. B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is a collective name for leukemias that have differentiation arrest at various stages of early B-cell development and up to 20 different subcategories have been distinguished based on underlying genetic abnormalities. BCP-ALLs were found to have varying levels of ST6Gal1 expression, with some relapse samples showing increased ST6Gal1 expression compared to diagnosis samples of the same patient. To analyze the consequences of increased ST6Gal1 levels, we expressed ST6Gal1 in a diagnosis sample using lentiviral transduction. NSG mice transplanted with these BCP-ALL cells were monitored for survival. Compared to mice transplanted with leukemia cells expressing original ST6Gal1 levels, increased ST6Gal1 expression was associated with significantly reduced survival. A cohort of mice was also treated for 7 weeks with 0.5 mg/kg vincristine chemotherapy to induce remission, then allowed to relapse. Vincristine treatment induced a more rapid remission in mice transplanted with BCP-ALL cells transduced with ST6Gal1, but upon vincristine discontinuation, relapse was detected 14 days later in both groups. Mice with higher ST6Gal1 levels had an increased leukemia burden and lower survival than controls. Long-term vincristine treatment in an ex vivo tissue co-culture model with OP9 bone marrow stromal cells supported increased survival at higher ST6Gal1 expression in these BCP-ALL cells. However, N-linked glycan analysis of the leukemia cells with increased ST6Gal1 expression did not reveal qualitative differences with controls, although some increased sialylation was seen. Surprisingly, RNAseq showed that around 450 genes were differentially expressed (>2 fold, $p > 0.05$, rpkm cutoff =1) in BCP-ALL cells with increased ST6Gal1 levels, with around 60% showing upregulation. These included genes encoding cell surface glycoproteins reported to be substrates of ST6Gal1 sialylation such as Jag2, SLC3A2 and CD109, as well as glycosyltransferases.

(85) Ligand interactions of the endocytic receptor LRP1 can be modulated by GalNAc-T11 mediated O-glycans

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Low-density lipoprotein receptor–related protein-1 (LRP1) is an endocytic receptor for numerous biomolecules that are structurally and functionally diverse. LRP1 is a member of the LDLR-related protein family. These receptors contain clusters of LDLR class A (LA) repeats that provide ligand binding interfaces. We previously demonstrated that short linker regions between the LA repeats contain conserved O-glycan sites selectively controlled by the GalNAc-transferase isoform, GalNAc-T11. To date, linker O-glycans on the receptors LDLR, VLDLR and LRP2 have been shown to modulate ligand interactions. Here we use engineered HEK cells to explore how GalNAc-T11 can modulate the ability of LRP1 to bind and endocytose ligands.

(86) Understanding the Effects of 3-O-Sulfation on Local Microdomains of Heparan Sulfate Using Molecular Dynamics Simulations

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Purpose: 3-*O*-sulfated glucosamine (GlcN3S), present in natural biopolymer heparan sulfate (HS), has been implicated as a critical unit in a number of biologic responses. We hypothesize that its biologic roles arise from novel conformations and dynamics adopted by local microdomains around specific 3-*O*-sulfated sequences.

Methods: A library of 20 unique 3-*O*-sulfated HShexasaccharides and corresponding non-3-*O*-sulfated HS hexasaccharides were parameterized using AMBER GLYCAM-06 and solvated using a TIP3P water box with a 12 Å cutoff. Each sequence was subject to a two-step energy minimization and three-step equilibration, followed by rigorous 1 μs production MD. Torsional angle restraints were implemented to ensure that iduronic acid (IdoA) residues remained either $^1C_4$ or ^2S_O. Trajectory analysis was carried out with various in-house scripts to determine hydrogen bonding (CPPTRAJ), end-to-end distance (EED, CPPTRAJ), minimum

volume enclosing ellipsoid (MVEE, MATLAB), and Na^+ binding interactions (CPPTRAJ).

Results: Depending on the structure of HS sequences, conformational profiles derived from simulations were observed to display multiple minima on a rugged landscape. The analysis of individual trajectories yielded considerable differences in EED, MVEE, and hydrogen bonding parameters suggesting microscopic differences in mobility, dynamic electrostatics, structure of bound water, and torsional angles as a function of sequence structure. Most interestingly, distinct HS hexasaccharides display a novel "C-shaped" geometry induced by tightly bound Na^+ ion(s), wherein two uronic acid (UA) residues in only certain UA-GlcN-UA sequences present the novel epitope. Moreover, this conformation exhibits remarkable stability, in some cases for nearly the entire simulation.

Conclusion: MD of HS offers a unique tool to understand the dynamical behavior of HS sequences. Our studies support the hypothesis that 3-O-sulfation induces novel conformation and dynamics, especially when present in distinct local microdomains. While configurational structure has been exclusively used to interpret biologic functions of HS, the dynamical behavior is likely to be equally important. Our results provide impetus to study a larger library of HS sequences to correlate the structure and dynamics of 3-*O*-sulfated HS with biological responses. This work was supported by grant U01 CA241951.

(87) Toward automated identification of glycan mixtures using multistage mass spectrometry

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Glycans play an essential role in various biological processes. As a unique feature of glycan structures, glycan branching patterns have a great influence on their activities. Comparing to proteins, glycans can be branched multiply rather than simple linear chains, making a tremendous difficulty for their identification. Moreover, mostly glycans in nature exist as mixtures of various branching patterns, which makes it more complicated to cognize branching structures of glycans.

A quantity of bioanalysis techniques has been utilized to determine glycan structures, including high performance liquid chromatography (HPLC), capillary electrophoresis (CE), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR) [2]. Among these techniques, MS is commonly used due to its high sensitivity in the absence of glycan standards. However, tandem mass spectrometry (MS^2) is not always sufficient for identifying fine structures of glycans. Multi-stage mass spectrometry (MS^n, $n > 2$) provides more detailed information about the branching structures by further scanning fragment-ions with unclear sequence and greatly improves the precision and efficiency of glycan identification.

MS^n has been successfully applied to determine glycan branching structures, but most of existing methods can only be used to identify pure glycans rather than mixed glycans. We previously proposed a high-throughput method for glycan structural identification (GIPS) based on multi-stage mass spectrometry [3]. Just like most identification methods, GIPS is only effective for pure glycans. Therefore, determination of components in the glycan mixtures, most of which are isomeric structures is an unsolved and extremely difficult challenge in glycomics.

In this work, we proposed a strategy to determine the contents in glycan mixtures using multi-stage mass spectra. We considered the experimental mass spectra (MS_e) of glycan mixtures as the complete set of peaks and each theoretical spectrum of candidate glycan branching pattern as a subset of peaks. The problem is turned to a set cover problem to find a smallest sub-collection of candidates which is the best explanation of the MS_e. In this strategy, firstly, a denoising module was developed to improve the quality of spectra and reduce the effects of noise. Secondly, a novel score function was designed that carefully considered peak intensity and isotope distributions. Thirdly, an efficient optimized algorithm was designed to find the optimal solution.

We tested our method on ten artificial mixtures of glycan, and contents of eight mixtures were correctly determined with the highest score. For the other two mixtures, an unrelated glycan was incorrectly identified as component of the mixture due to disturbance of noise peaks in the mass spectra, but the scores of true contents of these two mixtures are also within the top five. The test results indicate that our strategy has the potential in identification of glycan mixtures.

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(88) α2–6, but not α2–3, linked sialic acid on TNFR1 death receptor promotes cell survival

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Tumor necrosis factor receptor 1 (TNFR1) death receptor is one of the major receptors that can induce signaling pathways for cell death or survival. The ligand for TNFR1, Tumor necrosis factor-α (TNF-α), is a pro-inflammatory

cytokine produced by immune, and other, cell types. The binding of TNF-α to TNFR1 triggers TNFR1 oligomerization and internalization, which eventually activates apoptosis cascades. Conversely, TNF-α binding to TNFR1 can also induce anti-apoptotic pathways including NFκB and MAPK-stimulating PI3K/Akt networks, which are initiated by surface-bound TNFR1. Prior studies from our group discovered that TNFR1 signaling can be altered by the addition of sialic acid by Golgi sialyltransferases. Specifically, we showed that α2,6 sialylation of TNFR1 by the ST6Gal1 sialyltransferase diverted signaling toward survival by hindering TNFR1 internalization, thereby blocking apoptosis. However, one key question is whether the effects of sialylation are specific to the α2,6 linkage. To determine which linkage of sialic acid on N-glycans affects TNFR1 activity to skew signals to survive, we used HEK293 cell models with CRISPR-mediated deletion of select sialyltransferases. Cell lines were generated to lack ST6Gal-1 and 2 (ΔST6) or ST3Gal-3, 4, and 6 (ΔST3), respectively. ST6Gal-1 and ST6Gal-2 add α2,6 sialic acids on N-glycans while ST3Gal-3, 4, and 6 add α2,3 sialic acids on N-glycans. Data from these cell models show that TNF-α treatment leads to mass apoptosis of ΔST6 cells compared to wild type (WT) and ΔST3 cells (which retain α2,6-linkages), as evidenced by the activation of caspases 8 and 3. Furthermore, the TNF-α induced expression of anti-apoptotic molecules such as XIAP and A20 was diminished in ΔST6 cells relative to WT and ΔST3 cells. In view of prior studies suggesting impaired TNFR1 internalization as the mechanism by which ST6Gal1 blocks apoptosis, we monitored TNFR1 cleavage, a process dependent on receptor internalization. We find that TNFR1 is more rapidly cleaved in ΔST6 cells, consistent with more efficient TNFR1 internalization. Based on these data, we postulate that α2–6, but not α2–3, sialylation on TNFR1 plays a crucial role in regulating the TNF-α dependent switch between cell survival and cell death. Notably, stem cells, and many cancer cells, have enriched levels of α2,6 sialylation, and these populations are also inherently resistant to apoptosis. The anti-apoptotic effects of α2,6 sialylation may be particularly important for protecting cells from TNF-α rich inflammatory milieus, as well as anti-tumor immunity. Collectively, our novel finding of different biological effects of α2,6 and α2,3 linkages could provide insight into new therapeutic directions for treatment of cancer and other diseases.

(89) GlycoSim: a Glycan Pathway Prediction and Glycan Biosynthesis Simulation Tool

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During the last decade, many types of mathematical models for glycosylation have been developed for describing how glycosylation enzymes regulate the diversity of glycan profiles. In our laboratory, we released a web tool called GlycoSim [1], where users can perform glycan pathway prediction (GPP) based on LinearCode®□ format [2], which represents glycans as letters, and execute simulations of glycosylation based on various enzymatic parameters (https://rings.glycoinfo.org/glycosims/index).

However, there were several issues with the web tool. First, it was difficult to verify the hundreds or thousands of predicted glycans manually. Furthermore, the complexity of the mathematical model greatly increased depending on the input. Second, the GPP method was based on our own reaction rules. However, recently, an agreement based and readable extension of LinearCode®□ for reaction rules (LiCoRR) was proposed to integrate many types of reaction rules which had already been published [3]. Therefore, we needed to be able to handle this new format for GPP.

To solve these problems, we firstly added a network pruning method for extraction of the critical glycosylation pathway by comparing the glycans predicted by our GPP program against a given glycan profile, which may be obtained from glycomics experiments such mass spectrometry. In addition, we adopted the LiCoRR representation for integration of reaction rules. These implementations have now made it possible for users to construct pathways using the LiCoRR format and to perform simulations more easily by removing unnecessary pathways (the test version is available here: https://test-glycosim.herokuapp.com/).

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(90) Organic synthesis of exogenous glycans as a quantitative analytic standard and qualitative investigation for non-human type oligosaccharides of biopharmaceuticals

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Glycosylation is well-known biosynthesis as a post-translational modification, and its heterogeneous structures are covalently attached on proteins which are homogeneously created from genetic code in order to provide structural varieties and functional diversities. Expression level and steric size of glycosylation are distinctive in accordance with cell

type and its vital environment, and glycan structure especially shows remarkable difference depend on biological species. At this stage, we receive benefits from biopharmaceuticals produced by culture cells and exogenous organs from animals in the medical field, but it has been known that exogenic antigens derived from animal glycosylation cause neutralization of circulated biopharmaceuticals and induction of immune responses. Despite this situation, even now, safety guideline based on sufficient consideration of the threshold value regarding contamination of exogenous (non-human type) oligosaccharides has not been established because it is difficult to obtain commercially available reagents of structure-defined glycan standards and reliable antibodies.

The αGal and Neu5Gc are familiar epitopes attached on a terminal end of several non-human type oligosaccharides. Immune reaction between these epitopes caused by animal cells and autoantibodies found from host circulation often occurs because human lacks the enzyme activities during the process of evolution with acquired gene mutations. Since there are concerns about risks of anaphylactic shock and attenuation of medicinal effect, the development of reliable glycan standard and appropriate analytical method for the exogenous glyco-antigen has been required. To contribute to the long-standing challenge, we synthesized biantennary core-fucosyl/non-fucosyl N-glycans containing α1–3Gal and α2–6Neu5Gc epitopes at terminals of non-reducing end based on the systematic organic synthesis with applicable glyco-building blocks. As expected to be used for a standard of quantitative analysis, these glycans were fluorescent-labeled with 2-aminobenzamide (2-AB), and their qualities was guaranteed by NMR, MS, and HPLC data. In particular, Neu5Gc-containing glycan is difficult to be purified from general Neu5Ac-containing glycans in biological resources because a difference of one oxygen atom is just only observed, and therefore, the organic synthesis of structure-defined exogeneous glycans seems to have an advantage for the effective large-scale production. In addition, we developed glycan-binding proteins (e.g., anti-glyco antibody) and attempted to apply them to several types of applications. Resulting anti-αGal and anti-Neu5Gc antibodies were evaluated with stereoisomeric oligosaccharides and individual counterparts. Furthermore, developed glycan-binding proteins were shown to be applicable to various detection such as ELISA, WB and immunochromatography.

(91) Mapping glycan-mediated galectin-3 interactions by live cell proximity labeling

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Galectin-3 is a glycan-binding protein (GBP) that binds β-galactoside glycan structures to orchestrate a variety of important biological events, including the activation of hepatic stellate cells and regulating immunological responses. While the requisite glycan epitopes needed to bind galectin-3 have long been elucidated, the cellular glycoproteins that bear these glycan signatures remain unknown. Given the importance of the three-dimensional arrangement of glycans in dictating GBP interactions, strategies that allow the identification of GBP receptors in live cells, where the native glycan presentation and glycoprotein expression are preserved, possess significant advantages over static and artificial systems. Here, we describe the integration of a proximity labeling method and quantitative mass spectrometry to map the glycan and glycoprotein interactors for galectin-3 in live human hepatic stellate cells and peripheral blood mononuclear cells. Understanding the identity of the glycoproteins and defining the structures of the glycans will empower efforts to design and develop selective therapeutics to mitigate galectin-3-mediated biological events.

(92) Galectin-8 regulates group B streptococcus uterine outgrowth by engaging microbial sialylated mimics of host glycans

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Group B streptococcus (GBS), the leading cause of neonatal sepsis and death, possesses a sialylated capsule that serves as a critical and powerful virulence factor. GBS capsular sialic acid mimicing host glycans can engage siglecs to inhibit immune activation. Additionally, limitations to adaptive immunity against this self-like structure may further reduce immune reactivity against sialylated glycans due to tolerance mechanisms that limit self-reactivity. Together, these mechanisms likely facilitate the ability of GBS to persist in the reproductive tracts of 1 in 4 pregnant women, which can lead to devasting consequences in vertically-infected newborns. However, as not all women contract GBS, it remains possible that other immune factors evolved to protect against microbes that utilize sialylated forms of molecular mimicry. Recent studies suggest that galectins may fill gaps in adaptive immunity by serving as innate immune factors toward microbes that use blood group antigen forms of molecular mimicry. However, most galectins recognize non-sialylated

structures, suggesting that these factors may not protect against sialylated forms molecular mimicry. In contrast, our results demonstrate that one galectin family member, galectin-8 (Gal-8), actually possesses strong affinity for sialylated glycans. Using a murine model of GBS colonization along the female reproductive tract, we found that Gal-8 knockout (KO) mice carried a significantly higher GBS burden than WT mice, suggesting a direct involvement of Gal-8 in regulating GBS viability in vivo. Previous studies of Gal-8 antimicrobial activity identified the C terminal domain of Gal-8 (Gal-8C) as possessing killing activity. However, examination of custom microarrays populated with GBS isolated glycans indicated that the N terminal domain of Gal-8 (Gal-8 N) uniquely recognizes microbial sialylated glycans. To better understand the role of each domain in the recognition and targeting of sialylated microbes, we tested each domain individually with GBS. While Gal-8 N intrinsically bound and killed sialylated GBS, Gal-8C failed to bind or impact GBS viability. In contrast, Gal-8C exclusively bound and killed non-sialylated stains of *E. coli*, while failing to impact the viability of GBS. Together these findings suggest that while most immune factors recognize sialic acid as a "self" antigen and are, therefore, ineffective against sialylated microbes, Gal-8 possesses a unique ability to provide innate immunity against sialylated variants of molecular mimicry. Furthermore, the unique and complementary binding preferences of each Gal-8 domain suggest that Gal-8 uniquely evolved to combat sialylated microbes regardless of their surface sialylation level, providing innate immunity against microbes that attempt to switch between sialic acid-based and non-sialic acid forms of molecular mimicry.

(93) Prothrombotic phenotypes in a rat model of Fabry disease

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Fabry disease is the most frequently occurring lysosomal storage disease (LSD) and is caused by mutations in the X-linked gene GLA, which encodes the acid hydrolase α-galactosidase A (α-Gal A). Deficiency of α-Gal A results in the accumulation of terminal α-galactosyl-containing glycosphingolipids (GSLs), primarily globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). As a result of Gb3 and lyso-Gb3 substrate accumulation Fabry patients experience pain crises early in life and develop gastro-intestinal disorders and kidney dysfunction during adolescence, all of which decrease quality-of-life. As adults, Fabry patients often suffer from serious cardio- and cerebrovascular events, which are responsible for a significant decrease in lifespan. Currently, the mechanisms by which GSL accumulation increases the risk of stroke and cardiovascular disease (CVD) remains unclear. Recently, our lab has developed a rat model of Fabry disease that lacks α-Gal A and closely recapitulates disease phenotypes experienced by Fabry patients, including GSL accumulation. Utilizing this model, we have begun to define systemic changes correlating GSL accumulation with an increased risk of stroke and CVD. To quantify the accumulation of GSL species in α-Gal A-deficient (KO) rats, nanospray ionization-mass spectrometry (NSI-MS) was performed. NSI-MS analysis showed that as compared to wild-type (WT) rats, several GSLs accumulated preferentially in the bone marrow of KO rats, including in megakaryocytes, the precursor of platelets. Accumulating GSLs in the bone marrow included the terminal α-galactose containing species Gb3, lyso-Gb3, and blood group B, the ganglioside GM3, and the globoside Gb4. In WT bone marrow samples, Gb3, and lyso-Gb3 were below the limit of quantification, but were estimated to be 122,000-, 5500-fold, higher in KO rats. Blood group B, GM3, and Gb4 were found to be present in WT bone marrow and were elevated 6.3-, 2.1-, and 1.8-fold in KO rats. Consistent with the passage of α-galactose-containing glycoconjugates from megakaryocytes in the bone marrow to platelet progeny in the circulation, NSI-MS analysis of platelet GSL composition showed that KO platelets accumulated the terminal α-galactose containing GSLs Gb3, lyso-Gb3, and blood group B, as well as ganglioside GM3, with fold increases of approximately 7600-, 1100-, 647-, and 2-fold, respectively. Importantly, we found that GSL accumulation in KO rats corresponded with prothrombotic phenotypes that included, 1) an increased abundance of monocytes, macrophages, and neutrophils in the bone marrow and circulation, 2) systemically elevated pro-inflammatory cytokine levels, and 3) increased platelet activation, adhesion, and aggregation in response to the platelet agonist ADP.

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(94) The dynamics of protein paucimannosylation during colorectal cancer progression

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The asparagine-linked paucimannosidic glycans (PMGs, $Man_{1-3}GlcNAc_2Fuc_{0-1}$) have received renewed attention in human glycobiology given our recent discovery of PMGs as significant signatures of human immune cells including neutrophils [1] and monocytes/macrophages [2] and across human cancer types [3] including colorectal cancer (CRC) [4]. While associations reportedly exist between high PMG levels

in the tumor microenvironment and poor CRC prognosis [5], the protein, cell- and stage-specific dynamics of paucimannosylation in CRC remain unknown precluding a detailed understanding of the roles of PMGs in CRC. To this end, we have employed mass spectrometry-based glycomics and glycoproteomics to profile PMGs and PMG-containing proteins in various donor-paired biospecimens including plasma, peripheral blood mononuclear cell (PBMC) and fresh snap-frozen CRC tissue samples in a cohort spanning patients suffering from four different stages of CRC (n = 7/stage) and relevant clinical controls (n = 8). Relatively high PMG levels, in particular driven by elevations of the core-fucosylated PMGs ($Man_{1–3}GlcNAc_2Fuc_1$), were associated with the PBMC and tissue samples from the CRC patients. The non-depleted CRC plasma exhibited elevated signatures of biantennary α2,6-sialoglycans with and without α1,6-fucosylation and tri- and tetra-antennary core-fucosylated sialoglycans which were previously associated with inflammation [6]. Tissue mapping of the glycoproteins identified in the PBMC samples suggested that myeloperoxidase and other monocyte-derived glycoproteins contribute to the CRC-associated elevation of PMGs and pointed to an increased expression of monocytic proteins in advanced stages of CRC. The CRC tissues comprised an over-representation of monocytic and granulocytic proteins across all CRC stages compared to the adjacent non-tumorigenic tissues, suggesting that various immune cell types contribute to the dynamics of the PMG-rich tumour microenvironment in CRC. In conclusion, we have used integrated omics strategies to show that PMG-containing proteins of innate immune cell origin are strong signatures in the CRC tumour microenvironment and may be associated with inflammation and tumour progression. These findings improve our knowledge of the molecular and cellular makeup of the CRC tumour microenvironment and suggest that protein paucimannosylation may play yet to be elucidated roles in CRC progression and dissemination.

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(95) Glycan signatures as mechanistic markers of beta cell injury in diabetes

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Diabetes is denoted by a relative absence or deficiency in the production and release of insulin by pancreatic β-cells. Type 1 diabetes (T1D) results from an autoimmune-mediated death of β-cells that leads to reduced β-cell mass and diminished function. Type-2 diabetes (T2D) progresses from inadequate mediation of blood glucose due to insufficient insulin action. Currently, our understanding of the mechanisms responsible for the loss, dedifferentiation, or inability of β-cells to compensate for increased demand for insulin is incomplete. In T2D, questions remain unanswered regarding why β-cells fail to release insulin in response to glucose in some obese patients, while other obese patients retain metabolically healthy β-cells. Thus, there is an urgent need to investigate the mechanisms of β-cell loss and dysfunction, to identify predictive biomarkers that can be detected during the asymptomatic phase of disease to enable earlier intervention, and to identify novel targets for preventing autoimmune destruction of β-cells and enhancing β-cell function in response to glucose.

Given the important role that protein glycosylation plays in regulating glucose transport and β- cell proliferation, defining protein glycosylation in human serum and islets from T2D, obese, and normal weight donors may reveal potential biomarkers and contribute to our understanding of the mechanisms involved in disease progression. In this study, application of a mass spectrometry-based approach for glycan isomer quantification identified >200 N-glycan structures from human islets. These data revealed four unique N-linked glycan structures that were more abundant in T2D donor islet samples. Glycoproteomic analyses and single cell transcriptional profiling are underway to further define the proteoforms and glycosylation enzymes that are dysregulated in disease. Finally, application of our recently developed bioinformatic tool, GlyCoaster, facilitates the integration of glycomics, glycoproteomics, and transcriptomics data to reveal novel insights linking dysregulation within the glycosylation pathway to disease.

(96) Development of MicroGlycoDB, a database of glycan-related information in microorganisms using RDF

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The Semantic Web allows the Web to be used as a data space where machines can read and process structured web data. In order to realize this Semantic Web for glycan-related data content, it is important to establish rules regarding data structure and organization of the data using standard technologies. In our approach, we use Resource Description Framework (RDF)[1] to describe structured data and express resource relationships using triples of subject, predicate, and object. Moreover, we use ontologies[2] to define the meanings and attributes of the basic vocabulary to describe the resource relationships. RDF makes it easier to interpret and integrate data, allowing datasets created by different people and organizations to be managed in a global web environment. In this study, we aimed to develop a new database of glycan-related information in microorganisms using RDF, called MicroGlycoDB, in order to elucidate various biological processes that occur in vivo in these organisms.

First of all, I newly created an ontology to encapsulate the data at hand utilizing existing ontologies. I created RDF files using data on various microbial transferases and glycosylhydrolases obtained from collaborators. In addition, I depicted the glycan structures using the CSDB/SNFG structure editor[3]. Next, using Docker, I built a mechanism to start a Web server so that the top page can be displayed in a Web browser. Clicking on one species in the list, the biological image of the species will be displayed, and clicking on one part of the body, the glycan-related data related to selected part is displayed.

In the future, in order to respond to the increase of more microbial species and the number of data, we are planning to create a more accurate ontology and improve user friendliness by providing search tools, etc.

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(97) Dendritic cell targeted heparan sulfate alteration reduces inflammation and facilitates pathogen clearance during PR8 influenza A virus infection in vivo.

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Survival outcome of influenza A virus (IAV) infection largely depends on an intricate balance between pathogen-clearance and immunomodulation in the lung. In this study, we demonstrated that dendritic cell targeted genetic alteration of heparan sulfate (HS) (*Ndst1$^{f/f}$ CD11c Cre^{+}:* mutant) inhibits lung inflammation by A/Puerto Rico/8/1934(H1N1) influenza, and resulted in a phenotype characterized by augmented virus-clearance in the setting of lesser lung infiltration by CD4+ regulatory T cells in the early viral lung injury recovery phase. Induction of under-sulfated HS in a model dendritic cell line (Ndst1 silenced DC2.4 cells) resulted in upregulated expression of the antiviral cytokine interferon β relative to control. A major initiation pathway to interferon β expression during IAV infection, CpG dinucleotide dependent TLR9 activation, resulted in greater NF-κB phosphorylation as a result of Ndst1 silencing in DC2.4 cells. Improved pathogen clearance in *Ndst1$^{f/f}$ CD11cCre^{+}* mutant mice was suggested by reduced IAV-AA5H nucleoprotein deposits in lung histopathology sections. These findings establish novel regulatory roles for HS on the dendritic cell surface in innate and adaptive immunity in viral infection. Furthermore, they propose a therapeutic potential for generating DC targeted HS engineering platforms in IAV and other respiratory viruses, including severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2).

(98) The expression of sulfated sialic acids (SiaS) is dynamically regulated in mammalian cells

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Sialic acids (Sias) often modify glycan chains of glycoproteins and glycolipids to play crucial roles in cellular recognition and signaling. One of unique features of Sias that other monosaccharide components of the glycan chains do not show is the structural diversity. Sias consist of N-acetylneuraminic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc), deaminoneuraminic acid (Kdn), and their modified forms with O-acetylation, O-sulfation, O-methylation, O-lactylation, lactonization, and so on. However, very little is known about biological significance of these modified Sia residues, although the natural occurrence of O-acetylated Sias are more frequently studied than other modified Sias (Kitajima et al. Top Curr Chem 367:75–103, 2015; Baumann et al. Nat Commun 6:7673, 2015). As for O-sulfated Sia (SiaS) structures, 8-O-sufation of Neu5Ac and Neu5Gc are known in sea urchin gametes. We previously showed that the SiaS residues were involved in sperm-egg interaction (Maehashi et al. J Biol Chem 278:42050–57, 2003) as well as in regulation of sperm motility (Miyata et al. Glycobiology 14:827–40, 2004; Miyata et al. Glycobiology 16:1229–41, 2006) in sea urchin. In contrast, nothing is known about the occurrence of SiaS in vertebrates, except for bovine gastric

gangliosides [Slomianyet al. Eur J Biochem 119:647–50, 1981]. Thus, our final goal is a comprehensive understanding of the occurrence, biosynthesis and functions of SiaS in vertebrates. In this study, we sought to identify the presence of SiaS in various mammalian cultured cell lines using the antibody 3G9 specifically recognizing Neu5Ac8S. We further examined if the expression of SiaS on mammalian cultured cells was dynamically changed depending on the cultural conditions. The following results were obtained: First, SiaS was expressed in a cell line-dependent and a cell density-dependent manner. Second, in CHO cells, the expression of SiaS was reversibly induced by treatment with the antibiotic G418. Taken together, it is concluded that the expression of SiaS is changed by intrinsic and extrinsic factors in mammalian cells. This is the first demonstration of regulated expression of SiaS.

(99) SH3 domain of FUT8 is required for its enzymatic activity, cell surface localization and association with ribophorin I

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FUT8, a fucosyltransferase localized to the Golgi apparatus, biosynthesizes core fucose in N-glycans. Deficiency of FUT8 results in severe phenotypes, including lung abnormality, and core fucose is profoundly involved in cancer malignancy, indicating that FUT8 critically regulates the functions of glycoproteins in various physiological and pathological processes. However, it remains largely unclear how FUT8 activity is regulated in cells.

FUT8 unusually has an Src homology 3 domain (SH3 domain) in its luminal region, which is usually found in cytosolic signal transduction molecules, and the SH3 domain has not been identified in other glycosyltransferases. In this study, we investigated how this SH3 domain regulates FUT8 functions. We first found that the deletion of SH3 domain abolished FUT8 activity both in cells and in vitro and identified His-535 in the SH3 domain as the critical residue for enzymatic activity of FUT8. Moreover, we unexpectedly observed that although FUT8 is mainly localized to the Golgi, it also partially localizes to the cell surface in an SH3-dependent manner, indicating that the SH3 domain is also implicated in FUT8 localization. Finally, we identified ribophorin I (RPN1), a subunit of the ER-resident oligosaccharyltransferase (OST) complex, as an SH3-dependent binding protein of FUT8. RPN1 knockdown decreased both endogenous FUT8 activity and core fucose levels, indicating that RPN1 positively regulates FUT8 activity. Our results indicate that the SH3 domain critically controls FUT8 catalytic activity and localization and is required for binding by RPN1, which promotes FUT8 activity and core fucosylation (Tomida et al., J. Biol. Chem., 2020, 295, 7992).

(100) Automated MotifFinder Algorithm for Glycan Array Analysis Enables Identification of Complex Binding Determinants on Any Glycan Array Platform

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Glycan array analysis is a challenging bioinformatics problem due to the complexity of glycan determinants that govern protein binding. A number of methods for glycan array analysis have been developed, yet none have been able to capture the complexity and variety of binding determinants found in common lectins; fewer still are able to integrate information from multiple sources of data, with datasets varying protein concentration, glycan content, and glycan presentation. To this end, we developed an automated algorithm built on the MotifFinder program which utilizes a more flexible motif syntax capable of describing nearly any complexity in glycan-binding determinants. Furthermore, the MotifFinder program is capable of analyzing nearly all glycan array structures and formats, which, combined with a novel approach to the combined analysis of datasets, allows for unprecedented use of the available data. Here we demonstrate that this new algorithm is capable of describing a variety of different binding determinants, even those poorly described by other modern methods. This analysis is extended to the analysis of a variety of arrays differing in glycan content and experimental approach, including structure-based glycan grafting methods. We found MotifFinder results consistently showed good agreement both with array results and with historical binding determinants for the proteins. Finally, we give evidence that more detailed summaries of protein binding can be obtained through the combined analysis of data collected at different lectin concentrations and with different arrays.

(101) Dynamic changes in the N-glycome of the rat neocortex throughout ontogeny

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Asparagine-linked glycosylation (N-glycosylation) plays a key role in many neurodevelopmental processes, including neural cell adhesion, neurite outgrowth, and axon targeting. However, little is known about the dynamics of

N-glycosylation during brain development and, in particular, how the N-glycome of the developing neocortex differs from that of the adult. The aim of this study, therefore, was to perform a thorough characterization of N-glycosylation in both the adult and neonatal rat neocortex in order to gain insights into the types of changes occurring in the N-glycome during neurodevelopment. To this end, we used hydrophilic interaction ultra-performance liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry to compare the adult neocortical N-glycome with that of 24 h and 48 h neonates. While the basic features of all the neocortical N-glycoprofiles were generally similar, we discovered numerous quantitative and qualitative differences in the composition of the N-glycomes. We report that the abundance of complex N-glycans is significantly lower in adults compared to neonates. Furthermore, the proportion of charged complex N-glycans is also greatly reduced. This decrease in the abundance of complex N-glycans is offset by a corresponding increase in the proportion of truncated and, to a lesser extent, hybrid N-glycans. Lastly, we report that although the proportion of total oligomannose N-glycans remains constant at around 24%, the distribution of high-mannose subtypes shifts from predominantly large subtypes in neonates to smaller subtypes in the adult. In summary, our findings indicate that N-glycan synthesis in the rat neocortex is fundamentally different in neonates compared to adults with a general shift occurring from large, sialylated N-glycans towards smaller, neutral structures as neonates develop into adults, coupled with a parallel shift towards smaller oligomannose structures. More broadly, our data suggest that the glycosylation requirements of the neocortex vary throughout brain development and that the neuroglycome is dynamic and adapts accordingly.

(102) Characterization of a recombinant IgG autoantibody specific for galactose-deficient IgA1 in IgA nephropathy

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IgG autoantibodies play an important role in IgA nephropathy (IgAN), the most common primary glomerulonephritis worldwide. These autoantibodies recognize aberrantly O-glycosylated IgA1, galactose-deficient in some of the clustered hinge-region O-glycans (Gd-IgA1), the main autoantigen in IgAN, and form high-molecular-weight immune complexes in the circulation. Some of these complexes may deposit in the kidneys and induce glomerular injury. However, the molecular interactions of IgG and Gd-IgA1 in these complexes have not been fully elucidated. Here we characterize structural and functional features of a recombinant IgG (rIgG) autoantibody derived from circulating antibody-secreting cells of a patient with IgAN.

The rIgG autoantibody from an IgAN patient and engineered variants with amino-acid modifications generated by site-directed mutagenesis were produced in the Expi293F system and purified by affinity chromatography. The kinetics of the rIgG binding to Gd-IgA1 were analyzed by surface plasmon resonance (SPR). To solve the 3-D Fab structures of the wild-type and variants of the autoantibody, crystallographic methods were employed. The binding of the panel of rIgGs to Gd-IgA1 was tested by ELISA. To identify the O-glycoforms of Gd-IgA1 recognized by the rIgG, liquid chromatography coupled with mass spectrometry (LC–MS) was used. Biological activity of in vitro-formed rIgG-Gd-IgA1 complexes was tested in vitro using cultured human primary mesangial cells and in vivo using mice injected with preformed complexes.

The SPR indicated an intermediate binding affinity (KD = 3.16 E-07 M) of rIgG for Gd-IgA1. The determined Fab 3-D structures revealed three important elements: an F-containing loop, surface pocket, and complementarity-determining region 3 (CDR3) loop of the heavy chain. Amino-acid mutation revealed two heavy-chain regions critical for Gd-IgA1 binding: a segment at the junction of framework 1 and CDR1 and several residues in CDR3. LC–MS analyses showed that rIgG autoantibody bound to a subset of IgA1 molecules, resulting in enrichment of hinge-region glycoforms with 4 O-glycans, including the galactose-deficient glycoform GalNAc4Gal3. The rIgG autoantibody formed complexes with Gd-IgA1 that stimulated proliferation of primary human mesangial cells (2.88 ± 0.69-fold increase over control). This finding was consistent with the observed increased glomerular cellularity in mice injected with rIgG-Gd-IgA1 complexes (48.4 ± 13.3 nuclei per glomerulus vs. 40.4 ± 10.5 in controls; $P < 0.0001$; $n = 4$ mice in each group).

Our data characterize the structural features of rIgG autoantibody critical for its binding to Gd-IgA1 to form biologically active complexes. These findings and future studies of complexes by cryo-electron microscopy will clarify the fundamental molecular interactions that contribute to formation of these pathogenic immune complexes in patients with IgAN.

(103) Functional analyses of TMTC-type protein O-mannosyltransferases in Drosophila model system

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The family of transmembrane tetratricopeptide repeat-containing proteins (TMTCs) includes four related enzymes that function as glycosyltransferases that add O-linked

mannose to cadherins/protocadherins and some other proteins. Notably absent from this list of targets is α-Dystroglycan, a well-known substrate of O-mannosylation mediated by POMT1 & 2, a different family of protein O-mannosyltransferases, which indicates that TMTCs and POMTs participate in separate functional pathways. Cadherins, the largest group of TMTC substrates, are type-1 transmembrane glycoproteins that are crucial for cell adhesion and cell migration. They play essential roles in many biological processes including neural development and epithelial maintenance. Mutations in cadherins cause various pathologies, from neurological abnormalities to cancer. Although O-linked mannose has been found on cadherins within important functional domains, the role of this modification remains poorly understood. Notably, *TMTC* mutations were recently found to be associated with brain malformations and neurological disorders, which suggests that TMTCs play particularly important roles in the nervous system, possibly via affecting the function of neural cadherins. However, the mechanisms mediated by TMTCs in vivo remain largely unknown. *TMTCs* are evolutionarily conserved in metazoan organisms, including Drosophila, which provides the rationale to use fruit flies as a model system to elucidate the functions of TMTCs in the nervous system.

Drosophila has four TMTC genes that encode homologs of mammalian counterparts. We investigated their functions using mutant alleles and transgenic insertions while focusing on *TMTC1* and *2*. Our initial analyses revealed that these genes are expressed in an overlapping pattern restricted to the nervous system throughout development. Their expression is upregulated in sensory neurons during larval stages. Mutations in these genes are semilethal, causing lethality during larval and pupal stages. Our initial analyses revealed that TMTC1 and 2 mutants have defects in neural connectivity, affecting the wiring of axons. To shed light on the molecular targets of these glycosyltransferase enzymes, we used a glycoproteomics approach that uncovered many glycoprotein substrates showing altered O-mannosylation in *TMTC1/2* mutants, suggesting that they are specific targets of these enzymes. These data also suggested that *TMTC1* and *2* genes likely have pleiotropic functions. Analysis of O-mannosylation sites identified by mass spectrometry approaches supported the hypothesis that cadherins represent a major group of functionally important substrates that are conserved between Drosophila and humans. These conclusions are consistent with the phenotypes of *TMTC1* and *TMTC2*. Taken together, our results shed new light on in vivo functions of TMTCs in *Drosophila*, while suggesting that similar mechanisms may underlie TMTC functions in mammalian organisms, including humans.

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(104) Carcinoembryonic antigen glycosylation: revealing novel features of human glycosylation and cancer origin specificity

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Carcinoembryonic Antigen (CEA) is a biomarker strongly associated with tumour progression and metastasis. Even though N-glycans make up »50% of the entire CEA molecule, current knowledge on CEA specific glycosylation in health and disease is scarce. We show for the first time in an in-depth glycomics and glycoproteomics study that the over 270 different N-glycans identified exhibited antenna fucosylation and sialylation features that allowed a clear CEA body origin assignment. Colon-derived CEA carried a hitherto not described, hexosylated bisected GlcNAc glycoepitope. All analysed CEAs contained a 29th site of N-glycosylation on Asn^{71}, located within a non-canonical ^{71}N-R-Q^{73} sequence motif critical for CD8a binding. Correlation analyses of CEA and glycosyltransferase genes across the TGCA dataset revealed that CEACAM5 and B4GALNT3 expression levels were indicative for survival prediction. Our results open novel opportunities to understand CEA function, its role as a cancer marker but also reveal hitherto unknown aspects of glycobiology.

(105) Siglec-7 contributes to immune modulation induced by F. nucleatum ssp.

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Fusobacterium nucleatum ssp. are gram-negative bacteria prevalent in colon cancer tissues which also found in metastatic colon cancer cells. *F. nucleatum* accelerates tumour progression through the recruitment of tumour infiltrating immune cells. However, the molecular mechanisms underpinning the interaction of *F. nucleatum* ssp. with immune cells remain unclear. Immune cells express a range of lectins, with Siglecs being implicated in tumour progression through their interactions with host-glycans. Here we hypothesised that host immune response is modulated by interactions of *F. nucleatum* ssp. cell surface glycoconjugates with Siglec 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 Siglec-Fcs using flow cytometry. The results revealed that all three *F. nucleatum* strains specifically bound to Siglec-7 in vitro. The binding was significantly reduced in the presence of GD3, suggesting that binding of Siglec-7 with the bacteria is mainly through the V-set domain. Binding to Siglec-7-Fc was also observed using *F. nucleatum* outer-membrane vesicles (OMVs) or purified lipopolysaccharides (LPS) by ELISA-based binding assay or Biolayer Interferometry. In addition, we showed that *F. nucleatum* strains bound to a U937-WT cell population and that binding was reduced against the U937-Siglec-7−/− cells.

Next, we assessed the role of *F. nucleatum* ssp. on the host innate 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 TNFα and IL-8 and cell surface marker induction of CD80, CD86 and PD-L1. Stimulation of human monocyte-derived macrophages (moMφs) showed an M2 macrophage polarization characterised by the induction of IL-10, IL-6 and IL-8 cytokines and upregulation of PD-L1 cell surface marker expression. Further, Siglec-7 RNA-silencing in moDCs showed that F. nucleatum ssp. modulate cytokine levels in a Siglec-7 dependent manner.

Together, these data suggest that Siglec-7 may contribute to the recruitment of tumour infiltrating immune cells and colon tumour progression, and therefore suggest a new therapeutic target in the treatment of CRC.

(106) ST6GAL1 deficiency is protective against gastrointestinal and pulmonary bacterial infections

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Tight control of the immune response is critical to balancing the need for host protection with the risks of collateral tissue damage. Whereas α2,3-linked sialic acids are necessary for early immune functions such as thrombosis and inflammatory cell migration, α2,6-linked sialic acid promotes the resolution of inflammation by suppressing innate immune cell production and priming humoral immunity for antigen presentation. We have previously demonstrated that mice lacking the α2,6-sialyltransferase ST6GAL1 have excess production of neutrophils and pro-inflammatory cytokines, along with exacerbated tissue damage in models of sterile airway and peritoneal inflammation. However, it remains unclear whether expression of ST6GAL1 affects the ability of the host to defend against true infection at mucosal surfaces. Here, we present data from two independent murine models of bacterial infection in which ST6GAL1 deficiency confers a benefit to host defense. In the first, mice were infected with *Citrobacter rodentium*, an attaching and effacing gastrointestinal pathogen that causes subacute colitis. We observed that both global and P1 promoter-restricted loss of ST6GAL1 reduced disease burden and delayed onset of disease. Strikingly, mice with a conditional ST6GAL1 deletion in LysM-expressing myeloid cells were protected completely from infection, with no Citrobacter detectable in the stool. On histological examination, *St6gal1*-KO mice did not differ significantly in colonic hyperplasia or goblet cell loss, but had more evidence of inflammation, with increased leukocyte infiltration, formation of crypt abscesses, and ulceration. Immunofluorescence microscopy also demonstrated increased luminal neutrophil extracellular traps (NETs) in *St6gal1*-KO mice. In line with this, ST6GAL1-deficient neutrophils had increased iNOS expression and spontaneously produced NETs *in vitro*, suggesting a neutrophil-specific phenotype that may explain the *in vivo* findings. In the second model, B cell-deficient mice developed a spontaneous neonatal pneumonia caused by *Pasteurella pneumotropica*, leading to lung abscesses, splenomegaly, blood and tissue neutrophilia, sepsis and death in 45% of mice. Cross-fostering and embryo cross-implantation experiments revealed that passive immunity from maternal immunoglobulins in utero was completely protective against infection, recapitulating key features of human neonatal sepsis. In this setting, ST6GAL1-deficient mice were also significantly protected from death, with loss of only 10–20% of rodents. Collectively, our findings demonstrate that ST6GAL1 attenuates the host response in bacterial infections, with potential implications for the

management of foodborne infections and neonatal sepsis in humans.

(107) Galectin-9 helps govern human B cell adhesion to and migration through human vascular endothelium.

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Humoral immunity is critically driven by the coordinated differentiation of naïve B cells into antibody-secreting plasma cells. For naive B cells to access peripheral lymph node (LN) and reside awaiting activation by pathogenic antigen, there is a requirement for circulating naïve B cells to adhere and be retained in LNs. Though lymphocyte (L)-selectin is considered the LN-homing receptor for all lymphocytes, the singular role of L-selectin in B cell homing to peripheral LN is less clear. There is compelling experimental data suggesting that humoral immune responses and number/size of B cell follicles/germinal centers in L-selectin KO mice are robust. Prior data from our laboratory indicate that circulating naïve B cells express an abundance of i-linear glycans that avidly bind galectin (Gal)-9 and that Gal-9 is expressed at a high level in peripheral LNs. We hypothesize that Gal-9 may play a role in circulating B cell homing to peripheral LNs. In this study, we first analyzed human circulating B cells for their expression of homing molecules, L-selectin and endothelial (E)-selectin-binding glycans, $sLe^{X/A}$, and found that L-selectin was expressed at low level and $sLe^{X/A}$ antigens were absent. We then examined the spatial localization of Gal-9 in human tonsil tissue and peripheral LNs and observed conspicuous expression in/on post-capillary venules and high endothelial venules. Based on this expression pattern and deficits in homing receptor expression, we investigated the ability of Gal-9 and its dual carbohydrate-binding domains to mediate adhesion between human naïve B cells and human vascular endothelial cells. Using a human umbilical vein endothelial cell (HUVEC) model that expresses high levels of Gal-9, we investigated the ability of Gal-9 to mediate adhesion of MACS-sorted CD19+ B cells from PBMC to HUVEC under static and physiologic shear flow assay conditions. We observed Gal-9-dependent adhesion of B cells to HUVEC that was inhibited in the absence of Gal-9 or presence of competitive inhibitor lactose. Furthermore, we investigated whether this pro-adhesive Gal-9 activity promoted chemokine-directed trans-endothelial migration (TEM) through HUVEC monolayers. We, in fact, observed a Gal-9-dependent inhibition of TEM compared with controls, suggesting that Gal-9 was decelerating TEM. Taken together, these data illustrate Gal-9's potential key role in circulating B cell homing to peripheral LN and/or in retention of naïve B cells in LNs. Our studies implicate, for the first time, Gal-9 in the adhesion of human B cells to vascular endothelium and provide a putative mechanism for Gal-9 controlling the efficiency of humoral immune responses.

(108) How do galectins "read" N-glycans in solution, on glycoproteins, at the cell surface and inside cells?

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Galectins contain one or two carbohydrate recognition domains (CRDs) that bind β-galactose containing core disaccharides, like LacNAc, usually with affinities in the 100 μM range. However, galectin affinities for intact glycoproteins in solution, or estimated for the cell surface, are often at least 100 fold higher than for the disaccharide. Multivalency or glycosidic cluster effects cannot explain this difference because they are usually absent or weaker with galectins, when measured with relevant controls. Fine specificity of each galectins for modifications on or near the core disaccharide may enhance (or decrease) binding, but not 100 fold or more. Hence, additional more distant interactions, e.g. within N-glycans or peptide parts of the glycoprotein may contribute. In some cases tri-or tetra-antennary N-glycans are required for galectin to a glycoprotein in solution.

N-glycans are required for galectin binding to CHO cell surfaces as measured at equilibrium at 4 °C. On the other hand, surprisingly, mutation of the enzymes catalyzing the higher branching or e.g. sialylation of N-glycans has relatively little effect on galectin cell surface binding.

In contrast, at 37 °C, fine details of the galectin carbohydrate-binding specificity comes into play. At this temperature, galectins are usually rapidly endocytosed (within minutes), and then may follow different paths inside the cell, such as leading to lysosomes or leading to recycling to the cell surface, and that appears to depend on the fine details of their carbohydrate recognition specificity. A functional example is the different sorting of transferrin glycoforms based on selective binding of galectin-3 to tri-tetranatennary N-glycans. The endocytic pathway, including recycling, is a one way energy requiring process, and, hence galectin binding there may be transient and not at.

equilibrium in contrast to the cell surface-binding measured at 4 °C described above.

In cell biological studies of membrane remodeling and organization during endocytosis most focus has been on the cytosolic face of membranes, so much remains to be analysed and understood regarding the other side facing extracellularly or the lumen of vesicles, where galectins contribute as exemplified above. However, it was also early found that galectins are made as cytosolic proteins, on the "wrong side" of the membranes where there are few galactoside containing glycoconjugates. Galectins have other activities in the cytosol and nucleus (e.g. interaction with autophagy components or ESCRT III complexes). To reach their carbohydrate ligands, galectins reach glycoconjugate ligands by regulated non-classical secretory pathways, and/or accumulate around disrupted vesicles, possibly as part of a secretory mechanism, but also possibly a repair mechanism or as a coupling to autophagy.

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(109) Mass spectrometry-based fast photochemical oxidation of carbohydrates (FPOC) for carbohydrate-protein interaction characterization.

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Introduction.

Carbohydrates exist in various forms in the living organisms, such as glycoproteins, glycolipids, glycosaminoglycans, and other conjugates, which play important roles in biological processes. Most of the processes are mediated and/or modulated via carbohydrate-protein interaction. However, the study of this interaction is very challenging. This is mainly due to the structural complexity of carbohydrates, the dynamic nature of many carbohydrate conformations, and often low binding affinity of the interactions. In order to study and characterize the carbohydrate-protein interaction, we are developing a new approach using mass spectrometry-based fast photochemical oxidation of carbohydrates (FPOC). This method characterizes changes in solvent accessibility of different regions of an oligosaccharide by measuring changes in the apparent rate of formation of carbohydrate-hydroxyl radical oxidation products.

Method:

We used five trisaccharides in this study, including N, N′, N″-triacetylchitotriose (NAG3), isomaltotriose, 1-kestose, raffinose and melezitose along with four model proteins, ubiquitin, lysozyme, lectin (Griffonia simplicifolia-II, GSL-II), and myoglobin. FPOC mixture contained 20 mM of the protein and 40 mM of trisaccharides in 10 mM sodium phosphate buffer, 1 mM adenine, and 17 mM glutamine. The sample was incubated at room temperature for 1 hr and irradiated with KrF excimer laser after the addition of 150 mM hydrogen peroxide. For the control sample, everything was prepared as with the sample, except the laser was not turned on. Samples were quenched immediately after laser irradiation in a solution containing 0.5 mg/ml catalase and 0.5 mg/ml methionine amide. Samples were heated at 95 °C in the presence of 5 mM DTT and digested overnight with protease mixture at an enzyme/protein ratio of 1:100. The digested samples were lyophilized and resuspended in 50% acetonitrile for LC–MS analysis. Samples were analyzed by LC–MS on a Thermo Orbitrap Fusion Tribrid (Thermo Fisher Scientific) coupled with a Dionex Ultimate 3000 liquid chromatography (Thermo Fisher Scientific). Samples were eluted using a BEH Amide HILIC column (50 mm × 1 mm, 1.7 µm, ACQUITY UPLC® BEH Amide, Waters). The spray voltage was 2800, the ion transfer tube temperature was 200 °C and the ions were detected in positive ion mode.

Preliminary data:

To determine the radical shielding effect after binding of the carbohydrates to the protein, we used a model system of lysozyme and NAG3, already known to bind to the lysozyme. As controls, we used myoglobin and ubiquitin proteins that are known not to bind any trisaccharide used in this study. We calculated the oxidation of trisaccharide upon FPOC, in the presence and absence of lysozyme as well as other proteins. As expected, there was no significant change in the total oxidation of NAG3 in the presence of two non-binding proteins, ubiquitin, and myoglobin. Similarly, there was no significant change in the oxidation of the other four trisaccharides, used in this study, with or without proteins. Interestingly, the oxidation of NAG3 was significantly decreased ($p < 0.05$) in the two NAG3-binding proteins, lysozyme and lectin (Griffonia simplicifolia-II, GSL-II), when compared to non-binding proteins myoglobin and ubiquitin. These results clearly indicated that the binding of the glycan to its interacting protein significantly decreases the reactivity of glycan to hydroxyl radicals.

To determine the reactive site of carbohydrate to hydroxyl radicals, we compared the tandem mass fragments of reduced NAG3 against that of reduced (NAG3 + 14 Da). As expected, there were both b and y ions were detected in the non-oxidized precursor ion after reduction. But there were only a series of unoxidized b ions detected in the oxidized precursor ion after reduction of the reducing end. Our data suggested that the oxidation occurred on the reducing-end monosaccharide of NAG3, which is heavily protected by lysozyme binding.

To probe the sensitivity of FPOC to the site of protein binding, we compared the protection to NAG3 from lysozyme and GSL-II. Lysozyme is known to bind GlcNAc via the reducing-end while GSL-II is specific for non-reducing terminal GlcNAc. We calculated the oxidation of NAG3 upon FPOC, in

the presence of lysozyme or GSL-II. While both lysozyme and GSL-II protected NAG3 from oxidation by hydroxyl radicals compared to non-binding proteins, the oxidation amount of NAG3 in lysozyme was significantly lower ($p < 0.05$) than that in GSL-II. This data suggested that our method was sensitive to the binding region of a carbohydrate-binding protein. Studies with other more complex oligosaccharides are ongoing.

Novel aspect:

MS-FPOC footprinting provides a powerful approach to characterize carbohydrate-protein interactions.

(110) An expanding glycosaminoglycan oligosaccharide microarray resource for recognition studies of human pathogenic viruses

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Glycosaminoglycans (GAGs) are highly complex and negatively charged carbohydrate chains abundantly expressed on cell surfaces and in extracellular matrices. They are involved in diverse recognition events in health and disease. They are cell attachment sites for a variety of microorganisms, notably viruses associated with global health threats, such as arboviruses Dengue, Zika and Chikungunya, and also SARS-CoV2 the cause of the current pandemic.

High quality GAG oligosaccharide microarrays are much sought-after for detailed GAG recognition studies. A number of microarray platforms with synthetic heparan sulfate (HS) oligosaccharides have emerged as valuable tools in the field. The GAG oligosaccharide microarray based on the neoglycolipid (NGL) technology [1] is highly complementary to these, in that it covers oligosaccharides derived from different types of natural GAG polysaccharides, including hyaluronic acid, chondroitin sulfates A, B and C, heparin, HS, and keratan sulfate (KS). The clustered and flexible presentation of GAG oligosaccharides in a liposomal formulation renders the microarray system uniquely sensitive. Moreover, the system coupled with the "Beam Search Array" approach can lead to discoveries of hitherto unknown GAG oligosaccharide ligands starting from the ligand positive GAG polysaccharides, an example being the assignment of a KS hexasaccharide antigen as glycan marker for human induced pluripotent stem cells [2].

The GAG NGL probe library at the Imperial College Carbohydrate Microarray Facility currently covers over 120 probes (fractions dp 2 to 20 of the above-mentioned GAG types) and is being further expanded. In this communication, I will give recent research highlights applying our GAG oligosaccharide arrays in recognition studies of human adenovirus 37 [3] and Chikungunya virus [4] in collaborative studies with the groups of Niklas Arnberg (Umeå, Sweden) and Terence Dermody (Pittsburgh). These results contribute to our understanding of different roles of GAGs in the cell attachment processes of the viruses and could facilitate the development of novel antiviral therapeutics.

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(111) Metabolic uptake of dietary N-glycolylneuraminic acid promote colon cancer growth by immune and non-immune mechanisms.

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While the majority of mammals synthesize both *N*-acetylneuraminic sialic acid (Neu5Ac) and N-glycolyl neuraminic sialic acid (Neu5Gc), humans do not synthesize Neu5Gc due to a mutation in the *CMAH* gene, which encodes the only enzyme capable of hydroxylating CMP-Neu5Ac to generate CMP-Neu5Gc. Despite this, Neu5Gc

can be found in healthy human tissues and a higher levels in carcinomas, including colorectal cancers (CRC). Ingestion of red meat is the only known source of Neu5Gc for humans and there is epidemiological evidence that this food group has been associated with CRC risk. We sought to investigate if the metabolic incorporation of Neu5Gc by human colon carcinoma cells could affect signaling pathways relevant to CRC progression. HCT-116 human colon carcinoma cells fed with different concentrations of free Neu5Gc incorporated it into endogenous glycans, and had increased response to Wnt3a stimulation with higher expression of Axin2 and SP5 genes andincreased activation of the Wnt signaling pathway (detected using a plasmid containing the ß-catenin target TCF/LEF promoter region along with the luciferase reporter gene). Further FACS analysis revealed that Neu5Gc incorporation enhanced the binding of Wnt3a to the cell surface suggesting that Neu5Gc incorporation can influence Wnt/Frizzled interaction. The increased response of Neu5Gc-fed cells to Wnt3a resulted in higher cell proliferation rates when compared do Neu5Ac fed. Corroborating these data, we found that human-like *Cmah*$^{-/-}$ mice that spontaneously develop CRC (APC-CPC *Cmah*$^{-/-}$ mice) had a higher number of polyps, that were also bigger in size, if fed with a Neu5Gc-rich diet. These results demonstrate that metabolic uptake after dietary ingestion of Neu5Gc can promote colon cancer growth both *in vitro* and *in vivo*. Moreover, humans present variable levels of serum IgM, IgG and IgA anti-Neu5Gc antibodies. We tested if these antibodies could target Neu5Gc-containing glycans in HCT-116 cells, as an attempt to mimic what would occur in CRC patients eating red meat. We show here that anti-Neu5Gc antibodies from human serum samples deposit at the cell surface of Neu5Gc-fed cells but not Neu5Ac-fed cells, which is followed by sublytic levels of antibody and complement deposition and increased cell proliferation. Taken together, our data suggests that dietary ingestion of Neu5Gc can promote CRC growth by immune and non-immune mechanisms likely contributing to the human specific risk of CRC associated to red meat consumption.

(112) Glycosylated outer membrane vesicles as a tool for glycan-specific antibody discovery

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Many aberrant glycan epitopes are differentially expressed on malignant cells, thereby providing diagnostic and therapeutic targets which motivate the development of affinity reagents that recognize these distinct features. However, whereas a rich and diverse collection of antibodies has been developed for protein antigens, reliable binders that specifically recognize carbohydrates are much less common. While there are ~100 entries for antibodies against N- and O-linked carbohydrates in the Database for Anti-Glycan Reagents (DAGR), collectively these target an extremely small set of unique epitopes. In addition to the known lack of glycan immunogenicity, traditional methods to induce a strong humoral immune response have mostly been unsuccessful. We have previously shown that outer membrane vesicles (OMVs) engineered to surface display O-antigen polysaccharide (O-PS) antigens from Francisella tularensis elicited class-switched IgG antibodies specific to the O-PS that were protective against subsequent challenge with the pathogen.In this study, we leveraged glycosylated OMVs (glycOMVs) displaying bacterial and human glycans for identifying monoclonal IgGs with specificity for each of the different glycan structures. Overall, our results provide evidence that glycOMVs are a useful new tool for anti-glycan antibody discovery efforts.

(113) Site-specific N-glycosylation: kinetic analysis in vivo and influence of glycoprocessing enzymes expression

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N-glycan processing is performed by highly specific glycosyltransferases and hydrolases localized in multiple cellular compartments. It yields a precise and site-specific glycoprofile at each glycosylation sites. To study this process in CHO cells, we combined different omics approaches and determined the intracellular glycan turnover-rate in order tomathematically model N-glycan processing, and to assess the effect of altered expression levels of the glycoenzymes on the site-specific output of the N-glycosylation pathway in the secretory pathway.

We combined SILAC and PRM-based glycoproteomics of a model protein containing five sites of glycosylation. The quantitative data lead to a mathematical model that describes the ER and Golgi processing events as well as ERAD, ERLAD and mannose-6-phosphate dependent pathways. In a complementary set of experiments, we monitored CHO cells over a 10 weeks incubation period and performed MS-based relative quantifications of glycoprocessing enzymes, site-specific glycosylation of our model protein as well as the N-glycome composition. Our results revealed that alterations of processing enzyme expression affected site-specific processing

differently when compared to changes of the overall N-glycome.

(114) Glycomic Studies of the Notch 1 Receptor in Ovarian Cancer

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Glycosylation changes associated with cellular transformation can facilitate the growth and progression of tumors. We have previously discovered ovarian cancers with higher MGAT3 gene expression, which encodes the GnT-III glycosyltransferase responsible for the addition of the bisecting N-linked glycan and unusual bisecting glycan structures are regulating the expansion of the side population or cancer stem cell population through the activation of the Notch signaling pathway. The Notch receptor located on one cell interacts with canonical ligands present on a neighboring cell to initiate the pathway. Glycosylation of the Notch Extracellular Domain (ECD) can either inhibit or augment the activation of Notch by ligands. The Notch ECD is composed of 36 tandemly repeated EGF repeats, many of which are decorated by N-linked glycans, O-fucose, O-glucose, and O-GlcNAc glycans. Recent studies showed that Notch activity is controlled by site specific modification of O-fucose by Fringe. So, to identify all N- and O-linked glycan structures present on Notch in ovarian cancer and define the contribution of these glycans and the enzymes that add these glycans to the progression and growth of ovarian cancer, we created CRISPR/Cas9 knockout of Radical Fringe in the human ovarian cancer cell line OVCAR3-control and OVCAR3-GnTIII shRNA. Our results indicate that removal of Radical Fringe in these cells results in a significant reduction of cell migration and spheroid growth in vitro. We immunopurified NOTCH1 from these cells (WT, GNTIII K.D., and RFNG K.O.), digested them with chymotrypsin and analyzed glycoforms of the peptide from EGF12 using nano-LC–MS/MS and found a small amount of Fringe elongation of O-fucose on EGF 12 in both WT and GNTIII K.D. cell lines, but in RFNG K.O. cell line, elongation of O-fucose on EGF12 is significantly reduced which results in some un-fucosylated peptide.

(115) Evaluation of inhibitors for human Glutamine: Fructose-6-phosphate amidotransferase (hGFAT) selected by virtual screening as a new chemotherapeutic target.

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The hexosamine biosynthetic pathway (HBP) is one of the branches of glucose metabolism that provides the activated monosaccharide uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), an intracellular metabolic sensor used in glycosylation, with the rate limiting enzyme Glutamine: fructose-6-phosphate-amidotransferase (GFAT), composed of two domains (glutaminase and isomerase). Humans express three isoforms (GFAT1, GFAT1Alt and GFAT2) in a specific tissue manner. Recently, studies have shown that GFAT activity/expression plays a role in metabolic disorders, including cancer. Increased expression of GFAT1 has been observed in breast and prostate cancer as well as GFAT2 is positively regulated in pancreatic adenocarcinoma, lung and colon cancer. Our group demonstrated GFAT increasing in epithelial to mesenchymal transition and colon cancer metastasis. Very recently, we published that GFAT is pivotal to M1 to M2 macrophage polarization. These observations strongly suggest GFAT as a potential chemotherapeutic target. However, the most widely used commercial inhibitor, DON (6-diazo-5-oxo-L-norleucine), is not viable for the clinic due to its cytotoxicity and non-selectivity. The main purpose of this work is to test potential inhibitors for GFAT and to evaluate its selectivity for both enzyme isoforms. GFAT inhibitors were be screened by a Structure-based virtual screening method that combined an energy-based docking scoring function with a molecular interaction fingerprint from a smart chemical library of bioactive compounds from the LaSSBio Laboratories at Federal University of Rio de Janeiro (http://www.lassbio.icb.ufrj.br/) based on the structural similarity of isoquinolines. To refine the analysis, we performed the enthalpy calculation of the protein-ligand complexes with the best scores in the docking, using the semi-empirical method PM6. The compounds that obtained the greatest difference in enthalpy variation comparing GFAT isoforms were selected for the *in vitro* inhibition assay. Subsequently, the inhibitory activity of the selected compounds *in silico* was evaluated at concentrations of 10 and 200 μM in human recombinant GFAT2. At a concentration of 200 μM, LASSBio 1799, 1801 and 1809, inhibited GFAT2 activity by 60, 50 and 40%, respectively, corroborating the docking data. Tests performed using 10 μM concentration demonstrated that LASSBio 1801 exhibited a slight inhibitory activity of 30%. Taking together the data highlight LASSBio 1801 as a promising GFAT inhibitor among tested substances. Next steps comprise *in vitro* tests to identify inhibitory effects of 1801 compound besides its specific isoform targeting of GFAT1 or GFAT2. Moreover,

we performed preliminary studies in A549 cells that suggest GFAT inhibition in cell culture.

Keywords: GFAT, Potential inhibitors, chemotherapeutic target and cancer.

(116) O-GlcNAcAtlas: A Database of Experimentally Identified O-GlcNAc Sites and Proteins

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O-linked β-N-acetylglucosamine (O-GlcNAc) is a post-translational modification (i.e., O-GlcNAcylation) on serine/threonine residues of proteins. Distinct from the traditional glycosylation (i.e., N-glycosylation, O-glycosylation, and GPI-anchored glycosylation), O-GlcNAcylation is a unique intracellular monosaccharide modification without being further elongated into complex sugar structures. O-GlcNAcylation has been documented in all metazoans (including animals, insects and plants), some bacteria, fungi and virus. O-GlcNAcylation is catalyzed by a discrete set of enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). OGT transfers the GlcNAc moiety from UDP-GlcNAc to specific serine/threonine residues of target proteins while OGA removes it. Protein O-GlcNAcylation plays important roles in almost all biochemical processes examined (including transcription, translation, cell cycle, metabolism and signaling). Aberrant O-GlcNAcylation is underlying the etiologies of a number of chronic diseases (including cancer, diabetes, and neurodegenerative disease).

To pinpoint site-specific functions of O-GlcNAcylation, tremendous efforts have been made to map O-GlcNAc sites for either individual proteins of interest or entire proteomes. To appreciate this great endeavor in the last several decades, we have created O-GlcNAcAtlas—a curated database of O-GlcNAcylated proteins and sites. Specifically, we searched articles in PubMed between 1984 and Dec. 2019 with key terms (including 'O-linked β-N-acetylglucosamine', 'O-GlcNAc', 'O-GlcNAcylation'), yielding about 2300 articles. Each journal article was assessed by at least one of the curators. O-GlcNAc sites experimentally identified, together with their protein information and related sample information, were included into the O-GlcNAcAtlas. The current database includes over 12000 unambiguous O-GlcNAc site entries corresponding to >3900 unique proteins. The number of O-GlcNAcylated proteins goes up to 4500, by combining proteins with ambiguous O-GlcNAc sites. We have been working to construct an online searchable database O-GlcNAcAtlas. To our knowledge, O-GlcNAcAtlas is the first curated and comprehensive database encapsulating experimentally identified O-GlcNAc sites and proteins discovered in the past 35 years. We hope it will facilitate both basic and translational research to better understand protein O-GlcNAcylation at the molecular level.

(117) Unraveling the O-glycan diversity in colon cancer tissues and cell lines

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Unraveling the O-glycome in cancer is important for the development of immunotherapies for treating solid tumors and the discovery of cancer-specific glycan structures is critical for improving how these cancers are targeted. The majority of studies deciphering the role of glycosylation in the development and progression of cancer rely on using cell lines as models. However, little attention was given to their O-glycosylation due to its complexity, the presence of isomeric structures as well as lack of enzymatic release methods, making the analysis challenging. Recently we have performed an in-depth O-glycosylation analysis of 26 different colorectal cancer cell lines and found associations of O-glycan phenotypes with cell differentiation. Colon-like cell lines showed high prevalence of I-branched and sialyl Lewis x/a epitope carrying glycans, while most undifferentiated cell lines showed absence of Lewis epitope expression and dominance of truncated sialyl-T and disialyl-T antigens [1]. Moreover, the expression of glycan signatures associated with the expression of glycosyltransferases and transcription factors known to play a role in colon (de-)differentiation, providing a deeper insight into the regulation of glycan biosynthesis in colorectal cancer cell lines [1].

Although it has been proven that cell lines recapitulate the tumours on the genetic and transcriptomic levels [2], their potential to represent glycosylation characteristics of the tumors has not yet been extensively studied. Here, we aimed to map and compare the O-glycosylation signatures of primary cell lines and primary and metastatic colorectal cancers they have been derived from. Furthermore, glycosylation signatures of paired cancer and healthy mucosa were investigated. To ensure a fair comparison, laser capture microdissection was used to enrich for cancer and epithelial cells. The released O-glycans were analysed on a sensitive porous graphitized carbon nano-liquid chromatography coupled to a tandem mass spectrometer using negative electrospray ionization enabling powerful separation of isomeric species, as well as in-depth structural characterization of the epitopes. Our preliminary results show dominance of sialyl-T and disialyl-T antigens both in the cell line and the tumor it has been

derived from, compared to more diverse glycosylation in the healthy colon mucosa including core 3 structures absent from the tumor. This study is currently being expanded to a total of 12 paired CRC tissues and cell lines derived from the same tumors in order to evaluate their potential as glycobiological model systems. Furthermore, the glycosylation differences between paired cancer and healthy colon mucosa from the same patient will be assessed for discovery of cancer-specific glycan antigens.

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(118) Investigating the pathway of CD8+ T-cell apoptosis involving the ST3Gal-I sialyltransferase and Core 1 O-glycan sialylation

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Over 90% of $CD8^+$ T-cells produced during an infection undergo apoptosis during the contraction phase and resolution of a successful immune response. The precise pathway involved in this process is still not known but its modulation is essential to immune system function and homeostasis. Previous studies from this laboratory have shown that the ST3Gal-I sialyltransferase, which catalyzes sialic acid addition to Core 1 O-glycans is linked to post-activation CD8+ T cell apoptosis and is necessary to achieve normal cytotoxic responses. Loss of sialic acids on Core 1 O-glycans is a normal marker of $CD8^+$ T cell activation and results in apoptosis when T cell receptor signaling is subsequently diminished. ST3Gal-I is essential for Core 1 O-glycan sialylation and its deficiency results in widespread apoptosis of peripheral naïve $CD8^+$ T-cells in the absence of immune stimulation and in the absence of signals promoting the formation of $CD8^+$ memory T-cells. Mice lacking ST3Gal-I thus have a deficiency of $CD8^+$ T cells and a deficient cytotoxic immune response. We are investigating the molecular pathway(s) of peripheral $CD8^+$ T-cell apoptosis and homeostasis as controlled by ST3Gal-I. We are identifying the O-glycoprotein substrates of ST3Gal-I among $CD8^+$ T cells and the endogenous receptors and possible lectins involved in this apoptotic and homeostatic mechanism. Current findings will be presented. The understanding of this pathway could be important in the therapeutic regulation of cytotoxic T-cell activity during tissue transplantation and in attenuating pathogenesis in autoimmunedisease.

(119) On-tissue microscale glycoproteomics and N-glycan imaging reveal global dysregulation of canine glioma glycoproteomic landscape

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Here we present an approach to identify N-linked glycoproteins and deduce their spatial localization using a combination of MALDI mass spectrometry N-glycan imaging and microscale glycoproteomic strategies. We subjected formalin-fixed, paraffin-embedded glioma biopsies to on-tissue PNGaseF digestion and MALDI imaging, and found that the glycan HexNAc4-Hex5-NeuAc2 was found to be predominantly expressed in necrotic regions of high-grade canine gliomas, whereas high mannose HexNAc2-Hex5 was predominantly found in benign regions. To determine the underlying sialo-glycoprotein, various regions in adjacent tissue sections were subjected to microdigestion and the extracts were analyzed by LC–MS/MS without further glycopeptide enrichment. Results identified haptoglobin, which is involved in iron scavenging that presents aberrant fucosylation/sialylation in various cancers, as the protein associated with HexNAc4-Hex5-NeuAc2. Additionally, we identified several high-mannose (Hex2-HexNAc5) glycopeptides enriched in benign regions. To the best of our knowledge, this is the first report that directly links glycan imaging with intact glycopeptide identification. In total, our microscale glycoproteomics technique identified over 400 N-glycosylated, O-GalNAcylated, O-mannosylated, and S- and O-GlcNAcylated glycopeptides from over 30 proteins, demonstrating the diverse array of glycosylation present on the tissue slides and the sensitivity of our technique. While N-glycosylation and O-mannosylation were similar between benign and tumor/necrotic sections, S- and O-GlcNAc glycopeptides were significantly deceased in tumor/necrotic sections, whereas sialylated O-GalNAc glycopeptides were significantly upregulated. Ultimately, this proof-of-principle work demonstrates the capability of microscale glycoproteomics to complement MALDI-imaging technologies in understanding dysregulated glycosylation in cancer.

(120) ST6Gal-1 promotes acinar to ductal metaplasia and enhances early tumor progression

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Aberrant glycosylation is one of the earliest known hallmarks of cancer, being first described over 70 years ago. Amongst the most predominate changes in tumor cell

glycosylation is an increase in α2–6 sialyation on N-glycans, mediated primarily by the ST6Gal-1 sialytransferase. ST6Gal-1 expression dramatically alters cellular function via the addition of sialic acid to various surface receptors such as EGFR and TNFR1, resulting in altered signal transduction capabilities. Increased ST6Gal-1 expression has been observed in a variety of cancers, including pancreatic ductal adenocarcinoma (PDAC), and high ST6Gal-1 expression correlates with poor prognosis. Here, utilizing human PDAC tissue arrays, we found a dramatic increase in ST6Gal-1 protein expression in early stages of PDAC compared to normal pancreatic tissue. To investigate the role of ST6Gal-1 in PDAC we generated a novel mouse model with pancreas-specific ST6Gal-I overexpression and crossed them with "KC" mice, which harbor oncogenic $Kras^{G12D}$ expression in the pancreas. Mice with dual expression of ST6Gal-1 and $Kras^{G12D}$ demonstrated markedly accelerated PDAC progression and mortality (compared to KC mice). We hypothesized that ST6Gal-1 expression contributes to an early stage in PDAC; acinar to ductal metaplasia (ADM), in which acinar cells transdifferentiate into progenitor-like, ductal cells more susceptible to oncogenic insult. Organoids derived from ST6Gal-1 knock-in mice demonstrated increased expression of the stem/ductal gene Sox9, which is a critical mediator of ADM, and decreased expression of Ptf1a, a marker of acinar differentiation. Conversely, ST6Gal-1 knock-down in organoids derived from "KC" mice inhibited organoid formation and showed decreased expression of Sox9 and increased expression of Ptf1a. Similarly, ST6Gal-1 overexpression in the acinar 266–6 cell line (a canonical ADM model) increased Sox9 protein expression and decreased Ptf1a expression. *In vivo*,"KC" mice had increased ST6Gal-1 expression in early pancreatic intraepithelial neoplasias (PanINs) and ADM-like lesions while ST6Gal-1 knock-in mice demonstrated co-expression of Sox9 in morphologically normal acini, suggesting that ST6Gal-1 primes acinar cells for ADM. Additionally, we used cerulein to chemically induce ADM in wild-type and ST6Gal-1 knock-in mice. We observed co-expression of ST6Gal-1 and Sox9 in cerulein-induced ADM-lesions but not in untreated mice. Furthermore, cerulein-treated ST6Gal-1 knock-in mice demonstrated a greater number of ADM-like cells compared to cerulein-treated wild-type mice. Finally, RNAseq analysis of mice with dual expression ST6Gal-1 and $Kras^{G12D}$ revealed enhanced expression of ductal genes including Sox9, Krt19, and Cftr, and an increase in stem markers, Hes1 and CD133 when compared to KC mice. Collectively, these findings suggest that ST6Gal-1 expression may act as a molecular switch to drive ADM, thus enhancing PDAC tumor progression.

(121) O-GlcNAc Signaling in Pituitary Corticotrope-Derived Adenoma Function

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The lifetime prevalence of pituitary adenomas has been estimated at a staggering 16.7% of the population. While many of these are subclinical and identified only incidentally on radiology or autopsy, these tumors can be devastating in many patients. Patients with such tumors may experience profound endocrine (related to hyper- or hypopituitarism) and neurologic (related to mass effect) dysfunction. To date, researchers have struggled to identify a common molecular etiology for these typically sporadic tumors. Indeed, no clear genomic or epigenomic markers correlates with their onset or severity. Therefore, we wonder whether the source of dysregulation may be in a cellular signaling pathway common to other tumor types, such as the *O*-GlcNAc post-translational modification. Indeed, this reversible nutrient-sensing modification of serine and threonine residues ubiquitously modifies over 5000 human proteins, including oncogenes and tumor-suppressors. Furthermore, *O*-GlcNAcylation is also upregulated in all cancers studied to date, emphasizing its importance in tumors. Interestingly, *O*-GlcNAcylation also links glucose metabolism to endocrine functions of corticotropes, a subpopulation of pituitary cells generating adenomas. Therefore, we hypothesize that dysregulated *O*-GlcNAc signaling in corticotrope-derived adenomas could mediate tumorigenesis and hypersecretion of ACTH.

Herein, we found that *O*-GlcNAcylation is significantly elevated in corticotropic tumor cells compared to other secreting adenomas. Using corticotropic tumor cells (AtT-20), we then measured the expression of *O*-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively responsible for the addition and removal of *O*-GlcNAc moieties to proteins. In corticotropic normal or tumor cells, corticotropin-releasing hormone (CRH) treatment (100 nM) induces the expression of POMC mRNA leading to production and release of adrenocorticotropic hormone (ACTH). Concomitant to CRH stimulation, we demonstrated and upregulation of OGT ($\sim$1.13 fold increase, $p < 0.01$) and OGA ($\sim$1.22 fold increase, $p < 0.1$), suggesting that *O*-GlcNAc signaling is upregulated in response to CRH treatment. This stresses a significant role for *O*-GlcNAcylation in propagating intracellular signals in corticotrope-derived adenomas. In the context of what is known of *O*-GlcNAc signaling in other tumor types, we are convinced that further investigation of *O*-GlcNAc signaling in AtT-20 cells will provide important insights into tumorigenesis and hypersecretion of such tumors.

(122) Predicting lectin-glycan interactions: A systematic computational characterization of lectin binding sites

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Lectins play central roles in recognizing and interpreting the complex glycan code that directs many biological processes. A detailed understanding of the highly nuanced interactions between lectins and glycans could yield new insights into these processes and potentially how to control

them. While individual lectin-glycan binding interactions are well studied, uncovering overarching principles that differentiate lectin specificity could aid in identifying new roles for existing lectins and generating lectins with novel specificities. We hypothesize that a data-centric approach to studying lectin-glycan interactions will elucidate these binding site features responsible for the specificity of lectin-glycan interactions.

In order to characterize and model lectin binding sites, we used the UniLectin3D database to collect 1,364 lectin structures, representing 412 unique lectins co-complexed with glycan ligands. We cataloged over 4,000 individual lectin-glycan interactions and generated their interaction profiles. Using a custom approach to interrogate the three-dimensional space around the glycan ligands, we combined structural and sequence-based features for each of these interactions. As a result, we have a detailed and comprehensive characterization of each lectin binding site.

For ligands of interest, we then highlighted the enrichment of specific binding site features over the background distribution, thereby inferring which features were most highly associated with lectin specificity towards the selected ligands. To further confirm the biophysical relevance of our lectin binding site characterizations, we related our binding site features to glycan microarray data and found features associated with particular binding patterns. Specifically, these features are predictive of the linker between the array and glycan interfering with the lectin binding. With demonstrated utility for predicting specificity of novel lectin binding sites as well as characterizing nuanced interaction patterns on glycan microarrays, our lectin binding site features capture meaningful information that can be readily used in various applications.

In ongoing work, we are connecting these binding site characterizations to more detailed specificity information from existing glycan microarray data and plan to build a more powerful predictive model of lectin specificity. By using higher throughput specificity profiling than possible from solving complexed lectin-glycan structures, we will be able to identify binding site features responsible for more subtle specificity differences between lectins. Improved knowledge of the determinants of lectin specificity will enable fine-tuned control while engineering novel lectin binding sites that could power increased information from lectin microarrays, more broadly active antiviral or antifungal biotherapeutics, and cancer biomarker diagnostics with increased specificity.

(123) Characterization of O-GlcNAc Transferase Catalytic Domain Variants from XLID Patients

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The O-GlcNAc modification occurs on thousands of nuclear and cytosolic proteins and is added exclusively by the enzyme OGT that is encoded by a single gene on the X-chromosome in humans. Our laboratory, in collaboration with clinicians, initially characterized variants of OGT that were causual for X-linked intellectual disability (XLID). All of our initial XLID-patients encoded variants in the Tetratricopeptide repeat (TPR) domain of OGT. Initial characterization of the TPR variants including thermodynamic stability and kinetic activity showed modest if any significant differences, but transcriptional analyses showed that certain pathways, like those involved in cell fate specification and neurogenesis, were altered. The pervading hypotheses in our group is that these TPR variants may be disrupting key, perhaps cell type-specific, protein–protein interactions that lead to the phenotype. Others have described variants in the catalytic domain of OGT that appear to be causal for XLID. Most recently, we have identified 4 new variants in the catalytic domain of OGT in XLID patients. Based on molecular replacement modeling, we hypothesize that at least 3 of these variants will impact the km for UDP-GlcNAc of OGT. We are currently evaluating the kinetics of these variants, along with determining any changes in O-GlcNAcylation in cellulo, in thermal stability, in post-translational modification of the enzyme and in the protein interactome of OGT. Overall, this project hopes to provide insight into how these mutations lead to the particular phenotype seen in X-linked intellectual disability as well as utilizing natural variants of the OGT protein to better understand its structure and function.

(124) The restricted nature of protein glycosylation in the brain and its implications for neuropsychiatric disorders

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Glycosylation is essential to brain development and function, and genetics implicate glycosylation enzymes in neuropsychiatric disorders ranging from rare Mendelian disorders of glycosylation to common, complex phenotypes. We recently described the genetic association of variants in several glycosylation genes with schizophrenia, highlighting the known biological activity and function of the encoded enzymes as a primer for studies on their contribution to disease. As common variants have small effect size, we set out to generate a detailed understanding of protein glycosylation involving Asn-linked (N-glycans) and Ser/Thr/Tyr-linked (O-glycans) in the wild-type mouse brain as a template for the study of disease-associated variants in murine models. Analysis of brain regions from multiple mice using validated techniques in both sexes revealed an unexpected degree of glycan simplicity. RNAseq analysis in both mice and humans

suggests this simplicity is the result of tight regulation in the expression of glycogene biosynthetic enzymes. These findings begin to reveal the intricacies of protein glycosylation in the brain and provide a roadmap for future studies of common variants in glycosylation genes associated with neuropsychiatric disorders.

(125) Chemical and Informatics Tools for Glycosciences: Glyco-SPOT and GlycoGlyph

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Accessible chemical and informatics tools for glycoscience are greatly desired in order to accelerate and democratize glycoscience. We present the development of Glyco-SPOT synthesis chemical tool for the development of glycopeptide libraries, and GlycoGlyph informatics tool to visualize glycan structures vis-à-vis glycan nomenclature.

Glyco-SPOT (DOI: 10.1016/j.chembiol.2020.06.007): Synthesis of libraries of glycopeptides has been a challenge due to the expense of fmoc-protected glycoamino acids (FPGAAs), their fragility, and the need for small-scale productions with wide diversity. Micromole scale synthesis of glycopeptide libraries is typically expensive, while nanomole scale synthesis using resin based solid phase peptide synthesis is typically inefficient. To overcome these limitations, we have developed a new Glyco-SPOT synthesis method to produce glycopeptide libraries at nanomole (microgram) quantities. In this technique peptides are built on cellulose filter paper as spots (0.5–2 cm in diameter) using iterative cycles of fmoc-chemistry. Special care for is taken during coupling (e.g. inert atmosphere for longer reaction times required for FPGAAs), mild deprotection and release conditions, e.g. to limit side reactions including beta-elimination. We also developed a biopsy method to allow sampling of the reaction products at any time to test for completeness, thus ensuring efficiency. We generated two libraries of glycopeptides: the GP1 library consisting of GlcNAcβ1-modified glycopeptides of 20-mer length; and the GP2 library consisting of glycopeptides which are shorter (10mer) with a larger variety of glycans (1-mer to 7-mer either O-, S- and N-linked) and non-natural modifications, e.g. fluorescein N-terminus. In microarray experiments with these libraries we explored glycopeptide recognition by antibodies, and enzymes. Fluorescently labelled glycopeptides were also used to obtain binding affinities using fluorescence polarization. We are presently using this technique to generate more diverse libraries for ongoing studies.

GlycoGlyph (DOI: 10.1093/bioinformatics/btaa190): Drawing glycan structures and writing glycan names has been a tedious task, often resulting in errors in presentation and reporting. To address this limitation we created GlycoGlyph, which allows drawing glycan structures using a graphical user interface or via nomenclature (CFG linear nomenclature following modified IUPAC condensed format), while producing the other instantly. GlycoGlyph permist saving depicted structures in SVG (vector) format for import into graphic editing softwares easily, and follows the Symbol Nomenclature For Glycans (SNFG) format so as to be useful directly in publications. GlycoGlyph is also able to produce glycan GlycoCT and search various databases such as GlyTouCan, GlyGen and PubChem and ChEBI via GlyGen. The ability to view changes between name and structure in an interactive manner also aids the learning process in glycosciences, and the tool accelerates the teaching of glycoscience to beginners in the field.

(126) A pseudodiastrophic dysplasia is caused by pathogenic variants in calcium-activated nucleotidase-1 and glucuronyltransferase-I

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Glycosaminoglycans (GAGs), including chondroitin sulfate, dermatan sulfate, and heparan sulfate, are covalently attached to the core proteins that form proteoglycans through the common linker region tetrasaccharide, glucuronic acid-galactose-galactose-xylose. The linker region tetrasaccharide is constructed by xylosyltransferase, beta4-galactosyltransferase-7, beta3-galactosyltransferase-6, and beta3-glucuronyltransferase-3 (B3GAT3) using respective donor substrates, uridine diphosphate (UDP)-sugars, which are converted into UDP after a reaction. Calcium-activated UDP nucleotidase 1 (CANT1) hydrolyzes UDP to uridine 5′-monophosphate (UMP) and an inorganic phosphate [1]. Biallelic *CANT1* and *B3GAT3* mutations are known to cause Desbuquois dysplasia and multiple joint dislocations, short stature, and craniofacial dysmorphism with or without congenital heart defects, respectively [2, 3]. Pseudodiastrophic

dysplasia is a severe skeletal dysplasia associated with prenatal manifestation and early lethality [4]. Although pseudodiastrophic dysplasia is classified as a dysplasia with multiple joint dislocations, the molecular pathology of the disorder remains unknown. Here, we identified the biallelic variants in the B3GAT3 (p.Arg169Trp/Arg225X) and CANT1(Glu215Lys), which encode glucuronyltransferase-I (GlcAT-I) and UDP diphosphatase, respectively, by whole exome sequence [5].

The GlcAT-I activities of recombinant p.Arg169Trp- and Arg225X-GlcAT-I proteins were significantly decreased compared to that of wild type-GlcAT-I. Furthermore, cell lysates from the patient fibroblasts showed significantly reduced GlcAT-I activity compared with those from control fibroblasts. The amount of chondroitin sulfate and dermatan sulfate in the fibroblasts from a patient was markedly lower than that in control fibroblasts. Additionally, the nucleotidase activity of recombinant Glu215Lys-CANT1 toward UDP was significantly reduced compared with that of wild type enzyme.

These results suggest that the variants, p.Arg169Trp/Arg225X and p.Glu215Lys, in GlcAT-I and CANT1, respectively, are severe loss-of-function mutations, and cause the disturbance of biosynthesis of GAGs, which results in a pseudodiastrophic dysplasia with the significant phenotypic overlap of conditions within the biosynthetic pathway for proteoglycans.

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(127) Novel Roles for the Golgi Membrane Protein TMEM165 in Control of Migration and Invasion for Breast Carcinoma

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The TMEM165 gene encodes for a multiple pass membrane protein, localized in the Golgi that has been linked to congenital disorders of glycosylation. We have identified TMEM165 as a potential biomarker for breast carcinoma in a previous glycoproteomic study. The TMEM165 protein is not expressed in non-malignant breast tissue, increases slightly in early-stage ductal carcinoma in situ (DCIS) cases, and is highly expressed in invasive ductal breast cancer. Our hypothesis is that TMEM165 confers a growth advantage to breast cancer. In this preliminary study, we created a CRISPR/Cas9 knockout of TMEM165 in the human invasive breast cancer cell line MDAMB231. Our results indicate that the removal of TMEM165 in these cells results in a significant reduction of cell migration and tumor growth in vivo. We also found that TMEM165 alters N-linked glycosylation in breast cancer cells. These studies illustrate new potential functions for this Golgi membrane protein in the control of tumor cell growth and invasion.

(128) Understanding Glycosaminoglycan Binding to SARS-CoV-2 Spike Glycoprotein using Computational Virtual Library Screening and Molecular Dynamics Strategy

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The 2019 novel coronavirus pandemic disease (2019-nCoV) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is proving to be one of the most dangerous viruses in the recent history and there is an urgent need for therapeutics that prevent and stop the infection. The spike glycoprotein (SgP) of SARS-CoV-2 has been shown to bind to heparan sulfate (HS) and heparin (HP). SgP is predicted to possess three potential sites for binding to glycosaminoglycan (GAG); yet, the structural detail of HS/HP sequences interacting with SgP remains unclear. To overcome this, we utilized a computational strategy called as Combinatorial Virtual Library Screening (CVLS) dual filter algorithm, a technology that helps identify specific and non-specific GAG sequences binding to chosen targets. We also added a molecular dynamics (MD) arm to further ascertain the deductions of the CVLS technology. Application of CVLS-MD approach to SgP—HS/HP system led to unique under-

standing on the nature of recognition and origin of specificity for therapeutic targeting. First, we studied an in silico library of 93,312 HS/HP hexasaccharides (HS06s) against all three potential sites of GAG binding to SgP. For all 3 sites, the top 0.01% of sequences was subjected to the additional rigorous runs in triplicate. The predicted poses of HS/HP binding were analyzed using two parameters established to date in our prior works (see Curr. Opin. Struct. Biol. (2018), 50: 91–100), which correspond to in silico affinity (GOLD score) and consistency of binding (i.e., root mean square deviation (RMSD)). Of the three sites, the RBD was found to be the most favored site of interaction with several HS06 sequences having high-affinity and consistency of binding. Interestingly, detailed analysis of these sequences indicated the presence of a 'pharmacophore' consisting of at least three sulfate groups distributed at preferred sites along the HS06 scaffold. MD simulations were performed in the presence of explicit water for >0.5 ms. The results indicate HS/HP—SgP system is a rather stable system as indicated by direct and indirect hydrogen bonding and binding free energy. Overall, the results indicate that there is a strong element of selective recognition of SgP by HS/HP sequences, which may contribute to pathogenicity of the virus. Further, the identification of essential elements of a 'pharmacophore' may help design a HS/HP-based oligosaccharide or a mimetic thereof that antagonizes SARS-CoV-2 infection and spread.

(129) Regulation of Notch Signaling by O-Glycans in the Intestine

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Notch signaling regulates cellular proliferation and differentiation, and determines cell fates. Notch receptors are transmembrane glycoproteins whose extracellular domain contains many EGF repeats. A variety of O-glycans are added by specific glycosyltransferases to consensus sequences in the EGF repeats. Glycosylation regulates the binding of Notch receptors to Notch ligands and thereby controls, or optimizes, Notch signaling. The combined, conditional deletion of *Notch1* and *Notch2* [1] or Notch ligands *Dll1* and *Dll4* [2] disrupt differentiation in the intestinal epithelium. We have shown that Villin-Cre-mediated, conditional deletion of protein O-fucosyltransferase 1 (Pofut1), which transfers fucose to specific EGF repeats in Notch receptors, leads to reduced Notch signaling and an increase in cells of the secretory lineage [3], a phenotype similar to deletion of other Notch pathway members. However, mice with intestinal deletion of *Pofut1* are viable, whereas conditional deletion of RBP-Jk, a downstream transcriptional regulator of Notch signaling is lethal [4]. We are investigating the hypothesis that, in the absence of O-fucose glycans, other O-glycans may support residual Notch signaling. For example, EGF-repeat O-GlcNAc-transferase (EOGT) transfers GlcNAc to Notch receptors, and is required for optimal Notch signaling [5]. We generated CHO cells lacking *Pofut1* or *Pofut1* and *Eogt* using the CRISPR/Cas9 system, and found that binding of Notch ligands was more reduced in the double compared to the single mutant, indicating contributions of both O-fucose and O-GlcNAc glycans to Notch ligand binding, and potentially Notch signaling. We therefore generated mouse mutants lacking *Eogt* alone, *Pofut1* alone, or *Eogt* and *Pofut1* together, in the intestine. *Eogt* null mice did not show significant changes in intestinal epithelium, based on histopathological analysis or expression of genes involved in Notch signaling. Mice conditionally lacking *Pofut1* in intestine gave the Notch-defective phenotype we previously described (3). By contrast, mice lacking both *Eogt* and *Pofut1* in intestine exhibited 50% decreased body weight compared to mice lacking Pofut1 alone at 4 weeks, and did not survive lomg after ∼4 weeks. Furthermore, double knockout of *Pofut1* and *Eogt* showed further downregulation of Notch signaling gene transcripts, markedly-increased numbers of goblet and Paneth cells, and decreased villi length, compared to *Pofut1* deletion alone. Therefore, EOGT and O-GlcNAc glycans are required for optimal Notch signaling in intestine, and are, at least in part, responsible for the Notch signaling that supports the viability of mice lacking *Pofut1* in intestine.

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(130) GlyGen collaborations with NCBI to enhance glycoscientist interactions with PubChem and RefSeq

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Growing recognition of the importance of altered protein glycosylation for the pathophysiology of human

diseases is driving the development of accessible bioinformatics resources in glycosciences. As a result, PubChem and RefSeq are collaborating with glycoinformatics resources such as GlyGen to enrich NCBI's data collection and link existing genes and proteins with glycan structures and functions. While PubChem has more than 500,000 glycan-containing chemical substance records, of which more than 70,000 are annotated as 'biologics', its search modalities and data annotations make it challenging for glycoscientists to find or browse these records in a straightforward manner. Collaborations between PubChem and GlyGen are helping to improve interfaces through a better understanding of glycoscientist use cases, workflows, and desired interfaces. In addition, annotations important to glycoscientists are being contributed to the PubChem project with the help of GlyGen, thereby helping to highlight existing glycan entries and improving glycan data coverage. For example, GlyGen has provided glycan classifications (e.g., N-glycan, O-glycan, hybrid, complex, core 1, etc.) and glycan motifs (e.g., Lewis X, etc) to PubChem compound records. Moreover, GlyGen is also working towards submitting glycoprotein annotations to PubChem protein pages. By submitting such annotations as well as cross-linking both resources at the compound level, it is expected that returned results will be improved and the barrier for both glycobiology experts and non-experts in using these resources will be significantly lowered. This poster outlines on-going and future plans co-developed by the GlyGen consortium and NCBI resources designed to highlight and identify glycan records within PubChem, facilitating access by the biomedical research community, and improving linkage to proteins within RefSeq.

(131) High Resolution Solution State NMR Analysis of Soluble and Insoluble Plant Cell Wall Polysaccharides via Permethylation

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Nuclear Magnetic Resonance (NMR) Spectroscopy plays a critical role in plant call wall polysaccharide structure analysis, providing information at the atomic level that is useful for configuration analysis, molecular weight estimation and accurate anomeric ratio determination. However, certain features of plant cell wall polysaccharides that are essential for cell wall functioning can be detrimental towards a straightforward structure analysis of all cell wall polysaccharide components: the strong microfibril structures of its insoluble cellulosic components and the high molecular weight, extensive branching and tight polysaccharide molecular aggregation due to strong hydrogen bonding. Indeed, cellulose cannot be easily solubilized in common solvents and soluble cell wall polysaccharide components sometimes provide viscous solutions that exhibit severe spectral line broadening that leads to spectral resolution and sensitivity losses. We have discovered that permethylation affords the complete dissolution of both soluble and insoluble polysaccharide fractions of plant cell walls in less viscous organic solvents such as chloroform or acetonitrile. The permethylated polysaccharides can then be analyzed in solution by several analytical techniques including mass spectrometry (MS) and NMR. Permethylation removes intra- and inter-molecular hydrogen bonding by replacing all polysaccharide residue hydroxyl groups with methoxy groups, thus generating non-polar molecules that are soluble in common NMR organic solvents. These solvents with lower viscosity enable fairly rapid isotropic motions of the permethylated material engendering high resolution spectra with the concomitant increase in signal sensitivity. In this presentation, we will show that the high resolution achievable with permethylated cell wall materials, including switchgrass and poplar, afforded the identification of both major and minor cell wall polysaccharide components. The minor components were mostly determined by 2D NMR spectral profiling utilizing a chemical shift database generated with ten permethylated polysaccharide standards. In addition, the high resolution achieved, which is comparable to NMR spectra of soluble des-methylated oligosaccharides acquired in water, allowed facile relative quantitative analysis of both the switchgrass and poplar cell wall components utilizing only a few milligrams of material. The narrow peakwidths also afforded the measurement of $^{1}H—^{1}H$ three bond scalar coupling constants that carry vital structural information.

(132) Inhibition of SARS-CoV-2 viral entry upon blocking N- and O-glycan elaboration

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The Spike protein of SARS-CoV-2, its receptor binding domain (RBD), and its primary receptor ACE2 are extensively glycosylated. The impact of this post-translational modification on viral entry is yet unestablished. We expressed different glycoforms of the Spike-protein and ACE2 in CRISPR-Cas9 glycoengineered cells, and also developed corresponding SARS-CoV-2 pseudovirus. We observed that glycans play a major role in regulating Spike-protein proteolysis. Relative to this, carbohydrate contribution to Spike-ACE2 binding was modest. Blocking N-glycan biosynthesis at the oligomannose stage using both genetic approaches and the small molecule kifunensine dramatically reduced viral entry into ACE2 expressing HEK293T cells. Blocking O-glycan elaboration also partially blocked viral entry. Western blot data suggest that blocking glycan growth may enhance cleavage at the Spike S1-S2 interface. This may lower RBD presentation on virus, reduce binding to host ACE2 receptor and decrease

viral entry. Additional potential glycan dependent mechanisms that regulate viral entry will also be discussed during the presentation. Overall, chemical inhibitors of glycosylation may be evaluated to ameliorate COVID-19 symptoms. [Supported by grants from the NIH].

(133) Hydrocephalus in mouse B3glct mutant model likely caused by defects in multiple B3GLCT targets in ependymal cells and subcommissural organ

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Classical Peters plus syndrome (MIM #261540 PTRPLS) phenotypes include defects in eye development, prominent forehead, hypertelorism, short stature and brachydactyly, with isolated cases of fetal hydrocephalus. PTRPLS is caused by recessive mutations in the β3-glucosyltransferase (B3GLCT) gene. B3GLCT adds glucose to O-linked fucose on properly folded Thrombospondin Type 1 Repeats (TSRs) forming a glucose-fucose disaccharide. The disaccharide is proposed to be important for stabilization of the TSR fold and for efficient trafficking of target proteins. In mice, loss of B3GLCT causes white-spotting and hydrocephalus at high frequency in addition to craniofacial and skeletal abnormalities seen in PTRPLS patients. Reduced function of the secreted metalloprotease ADAMTS20, with 12 predicted B3GLCT-modified TSRs, was proposed to contribute significantly to white-spotting and hydrocephalus in mouse B3glct mutants. In this study, we provided evidence that the flow of cerebrospinal fluid was reduced in B3glct mutant mice, which likely resulted from a combination of altered translational polarity of ependymal cells and abnormalities in the subcommissural organ situated at the entrance to the central aqueduct. Localization of Adamts9 and Adamts20 mRNA provided evidence for impaired function of these related secreted metalloproteases (with 12 of 15 TSRs modified by B3GLCT) in polarization of ependymal cells. Likewise, expression of several B3GLCT targets including ADAMTS members (ADAMTS3, ADAMTS9, ADAMTS20) as well as sco-spondin in the subcommissural organ suggest that impaired function of multiple targets contributes to subcommissural organ abnormalities. Reduced secretion of a sco-spondin fragment (TSR6–9) in cell-based secretion assay using B3GLCT-knocked out HEK-293 T cells suggested that B3GLCT is essential for efficient secretion of sco-spondin. Surprisingly, we observed that sco-spondin, with 16 out of 25 TSRs predicted to be modified by B3GLCT, was secreted together with BiP from the subcommissural organ in B3glct mutants suggesting potential misfolding. Whether secreted sco-spondin in B3glct mutants correctly assembles into Reissner's fiber is yet to be addressed. Compared to ADAMT20, whose secretion in mutant cell lines is severely impacted by loss of B3GLCT, sco-spondin has additional C-mannosylation sites which may help to stabilize the TSR folds and promote trafficking. Our current studies focus on evaluating whether tissues such as the ependymal layer or subcommissural organ, where multiple targets can be simultaneously impacted by loss of B3GLCT, show stress related to unresolved accumulation of unfolded proteins. Combined, the results from these studies underscore the hypothesis that some targets are more susceptible to the effects of mutations in B3GLCT on protein secretion, and provide evidence that defects in CSF flow likely stem from the loss of glucose on more than one target protein in the ependymal layer and subcommissural organ.

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(134) A Dominant Variant in SLC37A4 causes a Novel Congenital Disorder of Glycosylation

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SLC37A4 encodes an endoplasmic reticulum-localized multitransmembrane protein required for transporting glucose-6-phosphate (G6P) into the ER. Together with tissue specific phosphatases, G6P is hydrolyzed to glucose and inorganic phosphate during times of glucose depletion. Recessive pathogenic variants in *SLC37A4* cause an established disorder known as glycogen storage disorder 1b characterized by liver and kidney dysfunction with neutropenia. Here we report seven individuals from four unrelated families presenting with a multifactorial coagulation deficiency with liver dysfunction, who all carried the same heterozygous variant c.1268C > T [p.Arg423Ter] in SLC37A4, but do not show the traditional GSD-Ib phenotype. Serum samples from affected individuals showed profound accumulation of both

high mannose and hybrid type N-glycans; their fibroblasts have normal N-glycans. Due to the tissue specific nature of this disorder, we generated a CRISPR base edited liver cell line harboring the c.1268C > T [p.Arg423Ter] variant. These clonal lines recapitulate the N-glycan abnormalities in secreted glycoproteins, have altered Golgi morphology and intracellular distribution of SLC37A4. Preliminary experiments suggest that Golgi pH is reduced. Here we highlight a dominant mutation in *SLC37A4* that causes a congenital disorder of glycosylation characterized by coagulopathy and liver dysfunction with abnormal serum N-glycans.

(135) DC-SIGN recognizes the Outer Core of *Escherichia coli* Lipooligosaccharide

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It is well known that lectins play an important role in the innate immune system, being involved in the recognition of carbohydrates epitopes exposed on cell surface s [1]. Due to their ability to recognise carbohydrate structures, lectins emerged as potential receptors for bacterial lipopolysaccharides (LPS). LPSs are heat stable amphiphilic molecules known for being the major component of the external leaflet of the Gram-negative bacteria outer membrane. They are one of the main virulence factors of bacteria and they are involved in host–microbe interaction processes [2]. LPSs are constitued by three portions: the lipid A, the core and the so called O-antigen when constituting the smooth form. However, if the O-antigen moiety is missing, they are characterized by a rough form and are known as lipooligosaccharides (LOS). These compositional differences are the consequence of having each motif encoded by different gen clusters [3].

Despite growing interest in investigating the association between host receptor lectins and exogenous glycan ligands, the molecular mechanisms underlying bacterial recognition by human lectins are still not fully understood [4]. Therefore, here is tackled the important question of potential envelope microbial components recognition by lectins, focusing our attention on dendritic cell-specific intracellular adhesion molecules (ICAM)-3 grabbing non-integrin (DC-SIGN).

In detail, a novel molecular interaction between the DC-SIGN and the lipooligosaccharide of *Escherichia coli* strain R1 has been unveiled. Saturation transfer difference NMR spectroscopy analysis, supported by computational studies, demonstrated that DC-SIGN bound to the purified deacylated LOSR1 mainly through recognition of its outer core. These results assess the ability of DC-SIGN to recognise glycan moieties exposed on Gram-negative bacterial surfaces.

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(136) Drug delivery into brain tumors by carbohydrate mimetic peptide IF7

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The blood brain barrier (BBB) is known to tightly control the exchange of substances. Although the blood-tumor barrier (BTB) in the brain tumors is leakier than BBB, it still serves as a serious obstacle for anti-cancer biotherapeutics to reach the tumor site. In the previous studies, we have demonstrated that a carbohydrate-mimetic peptide, designated IF7, targeted to various solid tumors by interaction with the N-terminal domain of annexin A1 expressed on a tumor endothelial cell surface [1]. Since IF7 crossed the endothelial cells by transcytosis, we hypothesized that IF7 may overcome BTB. In this study, we synthesized a monoclonal antibody, designated anti-MC16 antibody, against the N-terminal region of Anxa1. We found the anti-MC16 antibody clearly stained tumor vasculature [2]. Proteomics analysis of the immunoprecipitates by the anti-MC16 antibody of in vivo biotinylation of brain tumor bearing mouse demonstrated that the N-terminal region of Anxa1 is expressed on the luminal side of endothelial cell in the BTB. In a dual tumor mouse model harboring subcutaneous and brain tumors, IF7 conjugated with SN38, an active metabolite of anti-cancer drug CPT-11, significantly suppressed growth of both tumors at a low dose. Furthermore, we found CD8+ cytotoxic T cells infiltrated in the brain tumors in IF7-SN38 treated mouse. These results suggest that IF7 could be a distinct drug delivery system to treat malignant brain tumors.

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(137) The diversity of the Mimiviruses glycosylation is governed by complex gene clusters

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Recently, the traditional concept of viral glycosylation has been subverted by the evidence that giant viruses can independently glycosylate their capsid. Mimivirus is the most prominent example, as it has been established that the fibrils around the viral capsid are covered by complex glycans, synthesized by proteins encoded by the virus itself [1]. Mimivirus paved the way for the study of the fibrils' glycosylation of the *Mimiviridae* family. Here, I will present the complexity of the fibrils' glycosylation by combining two different approaches: on one hand the carbohydrate chemistry to elucidate the constituent sugars and on the other hand the bioinformatic search of the genes encoding the enzymes for their production and modification. Mimivirus, Moumouvirus australiensis and Megavirus chilensis were used as prototypes of the A, B and C clade respectively. The glycosylation genes are organized in cluster for Mimivirus (nine genes) and *M. chilensis* (six genes) [1], while the clade B remained unexplored [2]. As result, we have proved, first, the presence of sugars also for *M. australiensis* (B clade), and then the occurrence of a complex cluster of twelve genes explaining the in vivo data. In addition, we have extended the gene cluster of Mimivirus from nine to twelve genes, thus proving that Mimivurs possesses all the enzymes for the sugars production and their modification.

Finally, a comparative proteomic analysis has revealed that the fibrils glycosylation is clade specific, except for the B clade in which a heterogeneous glycosylation occurs. Regarding the proposed genus *Tupanvirus* of the *Mimiviridae* [3], a bioinformatics research has identified the existence of a genomic region of 49 Kbp, in which at least 10 genes are involved in glycosylation, suggesting that even in this case the fibrils may be glycosylated. Definitively, the understanding of the glycosylation of the *Mimiviridae* family could be considered as a pilot study, which can be extended to other giant DNA viruses, such as Pandoravirus, Pithovirus, Mollivirus, a list in constant growth.

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(138) Comprehensive O-GlcNAc glycoproteomics on NOTCH1 EGF repeats refined the sequons for O-GlcNAcylation and uncovered unique Lewis X epitopes in mammals

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O-GlcNAc modification of Notch receptors regulates Notch ligand interaction in the manner distinct from other forms of O-glycans on epidermal growth factor (EGF)-like repeats of Notch receptors. Although a number of proteins besides Notch receptors are expected to O-GlcNAcylated, only a few proteins were reported to be modified and elongated O-GlcNAc glycans. To extend a view of the specificity and variety of O-GlcNAc glycans, we have conducted a comprehensive analysis of O-GlcNAc glycans on NOTCH1 in mammals. Mass spectrometric analysis of NOTCH1 fragments expressed in HEK293T cells revealed that the several EGF domains with putative O-GlcNAcylation sites are poorly modified with O-GlcNAc. Although amino acid residues immediately before the modification site are preferentially occupied with aromatic residues, Phe and Thr, but not Trp, are permissible for the apparent modification with O-GlcNAc. Furthermore, semi-quantitative analysis of O-GlcNAc glycan isoforms revealed the minor forms of O-GlcNAc glycans with fucose, including a Lewis X structure, in NOTCH1 expressed in several cell lines. The fucosylation of O-GlcNAc glycans was enhanced by expressing FUT1, FUT2, or FUT9 gene. The Lewis X antigen generated by FUT9 on NOTCH1 was independently confirmed by immunoblotting

using an anti-Lewis X antibody. As expected from the similarity in the glycan structures, the Lewis X antigen was detected on O-fucose glycans in addition to O-GlcNAc glycans. Taken together, our results refined the putative consensus sequence for O-GlcNAcylation in mammals and extended the view of structural diversity of functional Notch O-glycans.

(139) The addition of dermatan sulfate promotes neuronal differentiation from mouse and human stem cells.

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Dermatan sulfate (DS) is a one of glycosaminoglycans (GAGs) which are present in the extracellular matrix and on the cell surface. GAGs have been demonstrated to be important in stem cells. Previously, we showed that heparan sulfate plays a key role for the maintenance of the undifferentiated state and regulation of differentiation in mouse embryonic stem cells (mESCs). Chondroitin sulfate has also been to be important for pluripotency and differentiation of mESCs. Keratan sulfate is a marker of human pluripotent stem cells. However, the function of DS in mESCs has not been clarified yet. Dermatan 4 sulfotransferase 1 (D4ST1), which transfers sulfate to the C-4 hydroxyl group of N-acetylgalactosamine of DS, contributes to neuronal differentiation of mouse neural progenitor cells. Therefore, we expected that DS induces neuronal differentiation.

During the neuronal differentiation from mESCs, the expressions of dermatan sulfate epimerase like (Dsel) and D4ST1 were increased. The amounts of disaccharides derived from DS moiety were increased at day12 after neuronal differentiation from mESCs. Then we added purified DS to the culture medium during neuronal differentiation. Expressions of neural stem cell markers (*Nestin* and *Musashi1*), a neural progenitor marker (Pax6), immature neuron markers (*Mash1* and *NeuroD1*), and mature neuron markers (*β-III-tubulin* and *Map2*) were increased at day12 after neuronal differentiated by addition of DS. These results demonstrated that DS promotes neuronal differentiation. Phosphorylation of Erk1/2 and Stat3 was increased and that of Smad5 was decreased at day12 after neuronal differentiation by addition of DS. It suggests that DS promotes activation of Erk1/2 and Stat3 and at the same time inhibited that of Smad5 in neuronal differentiation. Furthermore, the addition of DS caused an increase of neurite length and neurite numbers, indicating that DS promotes neurite outgrowth in neuronal differentiation from mESCs.

Next, we investigated whether DS promotes neuronal differentiation from human stem cells using hNSCs. Expressions of DSE, DSEL, and D4ST1 were increased at day7 after neuronal differentiation from hNSCs. Immunostaining by neuron markers, doublecortin and β-III-tubulin showed that the ratio of each neuron marker positive cells was increased by addition of DS in culture. The length of neurite was not changed differently from mouse neuronal differentiation. In hNSCs migration assay, the number of migrating cells was increased. These results demonstrated that DS promotes neuronal differentiation and neuronal migration, but not neurite outgrowth in hNSCs.

For neurite outgrowth and neuronal migration, we obtained different results in mouse and human. These differences may be depending on species or difference of developmental stage. In conclusion, DS promotes neuronal differentiation in both mouse and human stem cells, suggesting that the addition of DS will give a novel method for efficient neuronal differentiation.

(140) Anti-inflammatory effect of keratan sulfate disaccharide L4 and derivatives through langerin in COPD mouse model

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Chronic obstructive pulmonary disease (COPD) has been the third most common cause of death in the world. The development of chronic bronchitis in the lung caused by long-term tobacco smoke exposure leads to bronchial obstruction and respiratory failure that are not fully reversible. Our research group previously reported that intratracheal administration of keratan sulfate disaccharide L4 and derivatives thereof mitigated the symptoms of elastase-induced COPD model mice. In this study, we performed functional analysis of a C-type lectin receptor, langerin, as a binding molecule with L4 and derivatives to clarify the protective mechanism of L4 against COPD.

We firstly isolated and established mouse bone marrow-derived dendritic cells (BMDCs) as langerin-expressing cells. Using this, we observed downregulation of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα), while upregulation of interleukin-10 (IL-10) after incubation with L4 and derivatives. We also identified macrophage-capping

protein (CapG) as a candidate of langerin binding molecule by immunoprecipitation combined with mass spectrometry analysis. A part of CapG localized in nuclear and bound to the promoter region of IL-6 and TNFa gene in BMDCs, suggesting that CapG suppresses gene expression of IL-6 and TNFa as a transcriptional factor. Additionally, we generated langerin-knockout mice by CRISPR/Cas9. In COPD model using langerin-knockout mice, L4 administration showed protective effects in wild-type but not in langerin-knockout mice, indicating that langerin is a target of L4 in in vivo. These data suggested that anti-inflammatory effect of L4 and derivatives through langerin-CapG axis would be a therapeutic target for COPD.

(141) 3D polymer networks with tunable carbohydrate content to probe extracellular matrix-lectin interactions

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The diverse family of carbohydrate-binding proteins known as lectins can act as signaling molecules to modulate various aspects of cell phenotype and function, including adhesion, migration, differentiation, apoptosis, and proliferation. Within tissues, lectins can bind to glycans on both the extracellular matrix (ECM) and the cell surface. However, little is presently known about the role of lectin-ECM interactions in the context of cell signaling because existing tools to probe them depend on naturally-derived reagents, such as Matrigel or extracted mammalian glycoproteins, which have ill-defined carbohydrate content. Here we will present a synthetic ECM with highly reproducible and user-defined glycan content that can be used to study lectin-ECM interactions. Specifically, we created two-component hydrogels fabricated from mixtures of poly(ethylene glycol) diacrylate and carbohydrate-modified peptide nanofibers. Peptide nanofibers modified with N-acetylglucosamine (GlcNAc) or N-acetyllactosamine (LacNAc) provide hydrogels that selectively capture lectin binding proteins, wheat germ agglutinin (WGA) or galectin-1, respectively. Tuning the GlcNAc or LacNAc content dictates the extent of lectin binding, as well as the duration of lectin retention within the hydrogel. Carbohydrate content can be readily varied by changing either the total concentration of nanofibers or the ratio of glycosylated to non-glycosylated peptides that are co-assembled into nanofibers. In addition, protein absorption can also be controlled by changing PEG molecular weight, with increasing polymer chain length leading to higher lectin binding, likely due to increases in matrix pore size. Collectively, these data demonstrate that glycosylated peptide nanofibers embedded within PEG hydrogels endow specific lectin binding properties for a variety of carbohydrate-binding proteins. We envision this strategy will enable development of synthetic matrices to study the role of lectin-ECM interactions in cell signaling.

(142) Multi-omics analyses of Mixed-lineage Acute Leukemia (MLL) focusing on the importance of glycosylation—a pilot study

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Mixed-lineage Acute Leukemia (MLL) is one of the most high-risk forms of pediatric cancer. Although the long-term survival rates of pediatric acute lymphoblastic leukemia have increased over the past 40 years, the current chemotherapeutic treatment schemes often fail in the treatment of MLL. The biological mechanisms resulting in drug resistance, however, are still not fully understood and there is an urgent need to identify novel diagnostic and therapeutical targets. We have established the first integrated multi-omics investigation of primary patient MLL samples and control precursor B bone-marrow cells from healthy donors, mapping their proteome, transcriptome and glycome.

4–6 million cells from 3 normal bone marrow (BM) and 2 MLL samples were used for analysis on our multi-omics platform. Porous-Graphitised Carbon (PGC) nanoLC-ESI-MS/MS was used for Glycomics analyses after the enzymatic and chemical release of N- and *O*-glycans, respectively. The proteome was explored using RP-LC-ESI-MS/MS analyses, performed after offline high-pH fractionation, in addition to RNA-seq analyses.

Overall, 4225 proteins were identified across the patient MLL and control BM cells, of which 216 were overexpressed in MLL ($p < 0.01$, log2dif > 2). Preliminary analyses of the RNA-seq data matched well with the proteomics findings, revealing significant alterations in expression of glycoprotein signalling receptors and extracellular matrix proteins as well as various transcription factors. High-pH fractionation also

allowed us to identify numerous important glycosyltransferases and expression changes thereof on protein and RNA–seq level. N- and *O*-glycomics revealed overall lower levels of α2–6 sialylated *N*- and *O*-glycans in MLL cells (correlating with ST6Gal1 transcript and *ST6Gal1* protein levels) as well as an increase in Core 2 type O-glycans (correlating with GCNT1 transcript level).

Patient MLL cells exhibit distinct *N*- and *O*-glycan fingerprints, along with specific alterations of receptor tyrosine kinase signalling networks. In addition to well-known MLL glycoprotein markers, our integrated multi-omics workflow identified a number of diagnostic/therapeutic protein candidates that have not previously been described.

(143) Non-nutritive sweeteners disturb P-glycoprotein function and liver detoxification

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Over the course of 9 months, nearly all pregnant women are exposed to either of the following toxins: environmental chemicals, prescription drugs, over-the-counter medicine, social substances, illicit drugs, etcs. In this critical window of development, slight fetal exposures to toxic molecules can be teratogenic, leading to severe neurological and growth defects. In our previously published mouse study, we emphasized that pregnancy consumption of Non-Nutritive Sweeteners (NNSs) increased fetal toxicity, including higher liver's detoxification enzymes and metabolites.

About 35% of adults in the United States acknowledge consuming NNS, seeking to limit sugars consumption and their adverse health effects. While increasingly prevalent in food products, three in four adults cannot identify NNS and are therefore involuntary exposed. Although recognized safe for the general population, NNS's effects on a specific population or period of human development are incompletely understood. According to various studies, NNSs alter gut hormonal secretion, glucose absorption, appetite, kidney function, in vitro insulin secretion, and adipogenesis. Most NNSs circulate in the body and are found in blood, urine, amniotic fluid, and breast milk and bind sweet receptors in target organs. Despite an apparent maternofetal transmission, little is known about NNS consequences during pregnancy on fetal toxicity. To protect the fetus, the placenta and both mother's and fetus' livers are essential cleansing organs. Mainly, transporters such as the P-Glycoprotein (Pgp) are critical to flush foreign metabolites out of the cells and back to mother's blood circulation. In rats, Abou-Donia et al. previously demonstrated that the intestinal Pgp and various Cytochromes P450 expression varies according to sucralose consumption. Therefore we hypothesize that NNSs directly impact Pgp expression and activity in the liver, increasing fetal toxicity.

Using human hepatic cells, we demonstrated that in vitro incubation of a combined sucralose and Acesulfame-K mixture significantly altered detoxification actors' expression, including Aldehyde dehydrogenases and dehydrogenases, the aspartate aminotransferase (AST) or efflux transporters such as the Pgp. Individually, both sucralose and acesulfame-K slightly decrease Pgp expression at low concentration. On the contrary, elevated concentration of both NNS increased Pgp expression in hepatic cells, suggesting an elevated detoxification response and often associated with multi-drug resistant cells. Finally, in a cell-free assay, NNSs inhibited Pgp-mediated drug transport. Altogether, those preliminary data suggested that NNSs disturb drug metabolism by changing Pgp expression and function in vitro, and potentially participate in pregnancy complication and fetal development defects.

(144) Xanthan Gum Digestion by Human Gut Microbiota

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The modern human diet is continuously supplemented with various food additives to improve texture, taste, shelf-life, and processing qualities of consumables. While often assumed to be inert, the impacts of food additives on the metabolism and evolution of symbiotic human gut microbes are only now being deeply investigated. Xanthan gum is a branched polysaccharide with complex sugar linkage structure that is produced by *Xanthamonas campestris*; its unique rheological properties make it useful as a food additive in a wide variety of processed foods at typical concentrations of 0.05–0.5% (w/w). Xanthan gum can also be used as a substitute for gluten in a gluten-free diet, which is a vital component for limiting intestinal inflammation in patients with celiac disease, a lifelong condition estimated to affect 0.7–1.4% of the population and increasing in prevalence. In gluten-free baked goods, xanthan gum can be consumed at up to gram quantities per serving. Here, we show that the ability to utilize xanthan gum is surprisingly common in the individual human gut microbiomes of healthy adults and appears to be contingent on the activity of a single uncultured bacterium that is a member of the *Ruminococcaceae* family. Using a metatranscriptomic approach followed by recombinant enzyme studies, we show that this keystone xanthan gum degrader depolymerizes xanthan gum with a novel glycoside hydrolase family 5 (GH5) enzyme that produces oligosaccharides that it can utilize via a series of additional enzymes. In some cases, oligosaccharide products of this pathway also cross-feed a *Bacteroides* strain equipped

with its own distinct enzymatic pathway. Thus, consumption of a common, generally regarded as safe food additive, which has been used increasingly in the past 50 years, has apparently reinforced the existence of a food web between members of two different phyla of gut bacteria. Our findings have implications for the adaptability of the human gut microbiome to the introduction of new food additives on comparatively short timescales (decades) as well as utilizing the results of these events to engineer new orthogonal niches in members of the human gut microbiota for a future generation of probiotics.

(145) Surveying the unexplored chemical diversity and distribution of nonulosonic acids in prokaryotes

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Nonulosonic acids, commonly referred to as sialic acids, share biosynthetic and structural features, but display an otherwise remarkably large molecular diversity only poorly captured to date [1]. In humans, sialic acids are the terminal sugars involved in many highly important processes such as cellular recognition and immunity. Furthermore, bacterial nonulosonic acids are historically linked to virulence and pathogenicity [2].

Nevertheless, the enormous chemical diversity of prokaryotic nonulosonic acids presents a challenge for existing approaches, and a molecular-level survey has not been achieved to date. Therefore, there are many open questions surrounding the evolution and utilisation of nonulosonic acids in prokaryotes.

Hereby, we demonstrate a novel large-scale discovery approach, which tackles the unexplored chemical diversity of prokaryotic nonulosonic acids. The approach utilises selective chemical labelling, followed by a newly established mass spectrometric scanning approach combined with a dedicated bioinformatics pipeline [3].

Thereby, we provide a first molecular-level comparative study on the frequency and diversity of nonulosonic acids found across different phyla (microbes). The study not only illustrates their surprisingly widespread occurrence in purely environmental microbes, but it also reveals novel ulosonic acid structures and gives insight into species-specific post-synthesis processes [3].

The importance of sialic acids for pathogens, such as C. jejuni, has been well established [4]. However, an understanding of their chemical micro-heterogeneity, as well as their distribution and utilisation in environmental species, is currently only established.

Furthermore, synthetic methods for producing bacterial nonulosonic acids (e.g. for medical studies) are hugely demanding and often of low product yields. The large-scale exploration of these highly important sugars in microbes paves the way for new biosynthetic routes and provides a better understanding of their general utilisation.

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(146) Inhibition of the O-GlcNAc transferase (OGT) activates a p38-stress response pathway in cardiac myocytes

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Cardiomyocyte hypertrophy is a tightly regulated form of cellular growth that allows the heart to cope with increased hemodynamic demands such as those occurring during development and endurance training (physiologic hypertrophy) but also in hypertension, or after a myocardial infarction (pathologic hypertrophy). Key features of cardiomyocyte hypertrophy include enhanced protein synthesis, rearrangement of the sarcomeres and increased myocyte diameter. Multiple signaling pathways coordinate cardiomyocyte hypertrophy, including the phosphorylation signaling cascade of mitogen activated protein kinases (MAPK). Ample evidence implicates MAPKs in both physiologic and pathologic cardiomyocyte hypertrophy via the effector kinases Erk1/2 and p38 respectively.

β-O-linked N-Acetylglucosamine (O-GlcNAc) on S or T residues, is a glyco-PTM that is catalyzed by the O-GlcNAc transferase (OGT) and removed by the O-GlcNAcase (OGA). Apart from targeting S and T via dedicated enzymes, O-GlcNAcylation exhibits several other parallels with phosphorylation, including a highly dynamic nature, inducible responsiveness to environmental cues and widespread intracellular occurrence. Mounting evidence implicates protein O-GlcNAcylation in cardiovascular physiology and pathology, including cardiomyocyte hypertrophy. Here we investigated

the intersections between protein O-GlcNAcylation and activity of Erk1/2 and p38 kinases in neonatal rat ventricular myocytes (NRVM). We utilized the OGT and OGA inhibitors OSMI1 and TMG respectively while NRVM hypertrophy was induced with the Gαq agonist phenylephrine (PE).

Treating NRVMs with 25 μM OSMI1 for 6 hrs increased by nearly 3.5-fold the baseline phosphorylation of p38 (T180/Y182). On the other hand, treatment for 6 hrs with 200 nM TMG did not have a significant impact on p38 phosphorylation. Exposure of NRVMs to 5 μM PE for 30 minutes had a mild effect in inducing p38 phosphorylation by almost 2.0-fold and a stronger effect in inducing Erk1/2 phosphorylation (4-fold). Moreover, pre-treatment of NRVMs with 25 μM OSMI1 for 6 hrs proportionally increased the PE-induced phosphorylation of the two kinases (p38 and Erk1/2) following a 30-minute stimulation. Further investigation in OSMI1-treated NRVMs revealed increased phosphorylation of the p38-downstream target Hsp27 and the transcription factor Creb. Lastly, we found that treating NRVMs with OSMI1 restricts basal and PE-induced protein synthesis and also appears to disrupt the growth response of these cells to PE, as assessed by phalloidin staining of filamentous actin.

Our data indicate that treatment with OSMI1 and by extent short-term inhibition of OGT activate a p38-mediated stress-response pathway in NRVMs. Ongoing efforts seek to identify potential signaling mediators that exhibit reduced O-GlcNAcylation in this system and could serve as direct upstream regulators of p38 in NRVMs.

(147) Glycan-Mediated Immune Evasion in Breast Cancer

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Treatments for advanced stage breast cancer remain largely ineffective due to the ability of the tumor to grow unimpeded and eventually metastasize. One of the reasons breast cancers can develop so quickly is due to downregulation of effector immune responses. Immune regulation can occur through cytokine secretion (such as IL-10), signaling mechanisms, and/or checkpoint (such as through PD-1). Certain suppressor immune cells have been associated with all of these regulatory pathways and are thus implicated in tumor immune escape. Furthermore, the tumor microenvironment has been shown to promote these suppressive mechanisms. This can occur through biochemical groups expressed on the surface of the breast cancer cell, which may act as signaling ligands to receptors on effector immune cells or putative immune suppressor cells. Tn is a tumor-associated carbohydrate antigen previously known to be associated with immune suppression in helminth-infected patients. Tn has also been associated with breast cancer growth and metastasis. One ligand for Tn is CD301b (also known as MGL2), which is expressed on certain dendritic cell and macrophage populations of immune cells. As both Tn and its ligand CD301b have been linked with immune suppression, breast cancer may use this signaling pathway as a mechanism to induce immune suppression. We have investigated the association of Tn and CD301b immune cells with immune suppression and concomitant breast tumor growth. We hypothesized that inhibiting Tn and/or CD301b signaling and interactions can stop breast cancer growth.

We have identified a previously uncharacterized $CD19^{+}CD301b^{+}$ immune cells which accumulate within the breast tumor in mouse models. These cells express extracellular markers associated with regulatory B cells, and express high levels of IL-10. These cells thus represent a suppressive immune cell population specifically within the breast tumor microenvironment. These cells are enriched in cell-line derived tumors expressing higher levels of Tn antigen. We have also observed accumulation of these B cells in a spontaneous tumor model in mice expressing human Muc1 and high Tn antigen. However, ablation of these cells in a DTR mouse model significantly reduces Tn-high tumor growth. Additionally, ablating these cells also reduces accumulation of CD11b + Gr + cells, commonly considered myeloid derived suppressor cells. Our objective is thus to inhibit this Tn-CD301b interaction, overcome immune suppression, and stimulate the anti-tumor immune response.

Our research strategy has elucidated the relationship between Tn expressing tumor tissues, $CD301b^{+}$ B cells in the tumor microenvironment, and the breast tumor growth through inhibition of effector immune cell function. The role of TACAs in cancer is understudied. Extending current knowledge of lectin-expressing regulatory cells and how these immune cells modulate breast cancer in relation to glycoantigens could therefore lead to a breakthrough discovery in how to promote the patient's own immune system to target breast cancer cells at both the primary tumor site and any metastatic lesions.

(148) T antigen regulates pluripotency in mouse embryonic cells by directly modulating galectin-3 mediated frizzled-5 endocytosis

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Mouse embryonic stem cells (ESCs) can differentiate into multiple cell types during development and this pluripotency is regulated by various extrinsic and intrinsic factors. To date, several signaling pathways that control ESC pluripotency have been identified. Among them, Wingless-type (Wnt) signaling maintains the undifferentiated state while at the same time priming ESCs for differentiation via interaction of Wnt ligands with Frizzled receptors. Nonetheless, Wnt activation requires a complex signaling translation, consequently, Wnt signaling regulation is still poorly understood. In addition, Wnt signaling plays a crucial role in development and tumor initiation, thus emphasizing the importance of understanding the mechanisms that modulate Wnt signaling outcome.

Mucin-type O-glycosylation is characterized by the addition of N-acetylgalactosamine (GalNAc) to serine or threonine residues of membrane and secreted proteins. Previously, our and other groups reported that mucin-type O-glycosylation is crucial during development across different species. Moreover, aberrant mucin-type O-glycosylation profiles are a hallmark of tumorigenesis. However, the function of mucin-type O-glycosylation and its relationship with signaling in ESC remains undefined.

In the present study, we identified the elongation pathway via C1GalT1, that synthesizes T antigen (Galβ1–3GalNAc), as the most prominent among mucin-type O-glycosylation modifications in ESCs. Manipulation in the expression of C1GalT1 resulted in the loss of ESC pluripotency. Mechanistically, we observed that galectin-3 binds to T antigen on Wnt receptor Frizzled-5, regulating its endocytosis finely modulating Wnt signaling outcome. Our results provide the first demonstration that mucin-type O-glycosylation controls ESC pluripotency by directly modulating Wnt receptor Frizzled-5 endocytosis, unveiling a direct connection between mucin-type O-glycosylation and Wnt signaling. In conclusion, our findings provide a significant contribution to research in developmental biology and to the development of future therapeutic applications.

(149) Glycosaminoglycan domain mapping of proteoglycans from rat insulinoma cells

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We recently reported on an approach for glycosaminoglycan domain mapping, GAGDoMa, of complex mixtures of chondroitin/dermatan sulfate (CS/DS) primed on xylosides, artificial inducers of GAG biosynthesis. Here, we extended our approach to cover also glycosaminoglycans (GAGs) derived from proteoglycans (PGs). To study PG-derived GAGs, CS/DS from the rat insulinoma cell line, INS-1 832/13, known to produce primarily the PG chromogranin-A, were isolated by anion-exchange chromatography after pronase digestion. After benzonase and hyaluronidase digestion, included in the sample preparation due to the apparent interference by oligonucleotides and hyaluronic acid in the analysis, the GAGs were orthogonally depolymerized and analyzed using nano-flow reversed-phase LC tandem mass spectrometry (nLC–MS/MS) in negative mode. To facilitate the data interpretation, we applied an automated LC–MS peak detection and intensity measurement via the Proteome Discoverer software. This approach effectively provided a detailed structural description of the terminal, internal, and linkage region domains of the CS/DS on chromogranin-A, the major PG produced by the INS-1 832/13 cells. The copolymeric CS/DS GAGs constituted primarily consecutive GlcA-containing disaccharide units, or CS motifs, of which the N-GalNAc residues were 4S-sulfated, interspersed by single IdoA-containing disaccharide units. Furthermore, our data suggest a certain heterogeneity of the secreted GAGs by these cells due to the identification of, not only CS/DS GAGs, but also GAGs entirely of CS character. The extended applicability of the GAGDoMa approach, which allows for the detailed characterizations of PG-derived GAGs, or GAGomes, may greatly increase the knowledge about GAG structures in general, and eventually lead to better understanding of how specific GAG structures are related to biological functions.

(150) Optimization of sample preparation method for transferring N-glycans analysis

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Glycosylation is one of the most common and complex co- and post-translational protein modifications. The glycosylation status is crucial for protein functions, and it changes significantly during inflammation, sepsis and cancers. Human serum transferrin (Tf) is a glycoprotein synthesized mainly by hepatocytes and is involved in iron transport between sites of absorption and delivery. Changes in Tf glycosylation are seen in hepatocellular carcinoma, alcoholism and are a common marker for congenital disorders of glycosylation. Despite that, earlier studies have mostly been focused on presence or absence of sialic acid and Tf glycosylation hasn't been studied in too much depth.

In general, glycoproteins must be isolated from complex samples for their detailed analysis. Even though many

technologies have been developed recently, SDS-PAGE remains one of the most efficient ways to conduct parallel comparison and separate complex samples. It is specifically useful for samples where glycoproteins are available at lower quantities, such as Tf. Moreover, this kind of approach, when used as a second step purification, ensures even higher purity of starting glycoprotein material (in comparison to e.g. in solution deglycosylation after only one step of Tf immunoaffinity purification) since potential glycoprotein contaminants are additionally separated in gel from a glycoprotein band of interest (Tf). After Tf separation by SDS-PAGE its N-glycans can be released directly from the gel band by performing in gel deglycosylation. Released Tf N-glycans are then fluorescently labelled and purified from excess reagents. Labelled Tf N- glycans are analysed using ultra-high-performance liquid chromatography. Here we describe sample preparation method for highly specific analysis of Tf N-glycosylation by employing immunoaffinity purification and in gel deglycosylation.

(151) Mouse Fc and Fab IgG N-glycosylation analysis by capillary gel electrophoresis

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Background: The importance of N-glycosylation for the IgG effector functions is well established. Studies describing associations of total and Fc IgG N-glycosylation with various physiological and pathological conditions in human populations are plentiful, while the knowledge on the importance of Fab glycans is lagging behind. Mouse is the main model animal in biomedical research, and is often used for in vivo studies on IgG glycosylation. Its total IgG N-glycosylation is routinely analyzed by ultraperformance liquid chromatography or capillary gel electrophoresis of released glycans and its Fc IgG N-glycosylation by liquid chromatography mass spectrometry of Fc glycopeptides. Its Fab glycosylation, however, remains underexplored. With the importance of Fab IgG glycans in the pathogenesis of autoimmune and other conditions entering the research spotlight in recent years, there is a growing need for a method enabling in vivo studies on Fab IgG glycosylation in mice.

Aim: The aim of this study was to optimize the analytical method for the analysis of mouse Fab and Fc IgG N-glycosylation based on the method used for human IgG.

Materials and methods: IgG was isolated from the serum of CBAT6T6 mice by affinity chromatography on Protein G monolithic plate. After binding to the IgG-Fc affinity matrix, IgG was digested with SpeB protease into Fab and Fc fractions. The efficiency of protease digestion was confirmed by SDS PAGE. After the Fab and Fc separation, N-glycans were released from each fraction by peptide N-glycosidase F, the efficiency of deglycosylation was determined by SDS PAGE, and the released glycans were labeled with a fluorescent dye, 8-aminopyrene-1,3,6-trisulfonic acid. Labeled glycans were analyzed by capillary gel electrophoresis laser-induced fluorescence on an ABI3130 DNA sequencer.

Results: IgG was successfully cleaved into Fab and Fc fragments. The Fc fragment was only partially deglycosylated, while the level of deglycosylation for the Fab fragment could not be confirmed. N-glycans from both fractions were successfully analyzed. The N-glycosylation pattern of mouse Fab vs Fc roughly corresponds to the glycopattern of human Fab vs Fc fraction, with the Fab fraction bearing the most of highly processed, sialylated N-glycans, and the Fc fraction bearing a bigger proportion of neutral: a-, mono- and digalactosylated N-glycans.

Conclusion: While additional optimization is required to completely deglycosylate both fractions, the initially implemented method for the analysis of mouse Fab and Fc IgG N-glycans is functional. The first analysis shows a distribution of mouse Fab and Fc N-glycans similar to the one observed in human IgG.

(152) Free oligosaccharides of human plasma: their HPLC-profiles in norm and pathology

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Cell protein homeostasis requires a precise balance between anabolic and catabolic processes through thorough regulation of biosynthetic and degradative machinaries. Degradation of misglycosylated and/or misfolded newly synthesized glycoproteins by endoplasmic reticulum- associated degradation and cleavage of mature glycoproteins by endosomal-lysosomal system give rise to free oligosaccharides (FOS)—unbound structural analogues of glycans of glycoconjugates, their biosynthetic precursors and products of degradation. There are mechanisms of complete FOS degradation inside the cell, but some of them flux in blood plasma.

The **main objective** of this work was an investigation of free oligosaccharides of human plasma under normal and pathalogical processes to reveal their structures and ability to reflect intracellular changes in different conditions.

Methods: After plasma deproteinization, FOS were purified and labeled with anthranilic acid (2-AA). The labeled glycans were divided into neutral and acidic species by ion-exchange chromatography on QAE Sephadex (Q25–120) and analyzed by high performance liquid chromatography (HPLC). Chromatographic profiles were characterized in glucose units by comparison with a 2-AA-labeled glucose oligomer ladder derived from a partial hydrolysate of dextran as an external standard.

Results: Free oligosaccharides were found in human plasma both in normal and pathological conditions and were represented by uncharged and negatively charged species. HPLC- profiles of plasma FOS in normal conditions were highly reproducible with stable concentration prevalence of uncharged high-mannose species and the main complex type biantennary N-glycan with two residues of sialic acid among negatively charged glycans. Investigations of plasma FOS in pathological conditions revealed patterns of profile changes in chronic and acute leukemia and cardiovascular diseases. HPLC-profiles of plasma FOS showed individual differences without changes of general pattern of uncharged glycans in chronic leukemia and substantial reduction of this group of glycans in acute stages. A complex type biantennary N -glycan with one residue of sialic acid was a notable glycan in the group of charged oligosaccharides in oncotransformation. In cardiovascular disorders, HPLC-profiles of plasma FOS revealed changes in the patterns depending on the severity of the disease and the treatment used.

Conclusions: This study demonstrated that free oligosaccharides of blood plasma can indicate the intracellular changes of metabolic processes in endoplasmic reticulum and endosomal-lysosomal system under normal and stress conditions. Further investigations of free plasma oligosaccharides in combination with additional approaches in studying of protein quality control could help to understand better their potential for medical diagnostics and therapy monitoring.

(153) Characterization of the Lipopolysaccharide from a chronic strain of the cystic fibrosis pathogen Pandoraea pulmonicola

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Many bacterial species have been isolated and identified as causing pulmonary exacerbations within the lungs of cystic fibrosis patients, with well-known Gram-negative opportunistic species, such as *Pseudomonas aeruginosa* and bacteria of the *Burkholderia cepacia* complex, being well characterized. However, the Pandoraea species are new emerging pathogens causing severe and persistent inflammation, leading to a dramatic damage of the lungs [1]. The molecular mechanisms underlying the high pathogenicity of *Pandoraea* species are still unknown.

As a Gram-negative bacterium, *Pandoraea* sp. express lipopolysaccharides (LPS) on the outer membrane, and its recognition by the host immune system is responsible for the activation of a potent inflammatory response. Importantly, the degree of the inflammatory response strongly relies on the chemical structure of an LPS [2]. The LPS is composed of three distinct domains: the glycolipid moiety (lipid A), which anchors the molecule to the outer membrane, which is covalently linked to an oligosaccharide (core OS)in turn connected to the outermost polysaccharide portion (the O-antigen).

Reported here is the structural characterization of the highly complex lipid A isolated from the LPS of a chronic strain of *P. pulmonicola* (RL 8228), one of the most virulent identified so far among the *Pandoraea* species [3]. Interestingly, the combination of the structural features observed in the lipid A are likely connected to the capability of this strain to persist in the cystic fibrosis lungs avoiding detection by the host immune system as well as its disruption by the most commonly used antibiotics. In addition, the saccharide portion of the LPS from this clinical isolate is being investigated which will enable a comparison to the structural study already carried out on a different strain (LMG 18108); the latter strain was found to produce an LPS with particular O-chain moiety decorated by a five-membered ring aglycon on its trisaccharide repeating unit [4]. Characterization of the fine structures of these important virulence factors is a key initial step in combating these deadly pathogens which target the lungs of cystic fibrosis patients.

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(154) Development of Quantitative Heparan Sulfate BSA Conjugate Arrays through Fluorogenic Azido-coumarin Linker

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Heparan sulfate (HS) glycosaminoglycans engage >500 proteins identified so far and thus regulate numerous physiological processes. HS contains protein-binding domains arising from O- and N-sulfation and uronic acid epimerization of regions of the polysaccharide chain. The fine structure and organization of these domains confers protein binding specificity. HS microarrays have been instrumental in defining protein-HS structure function relationships and recent studies have included synthetic HS oligosaccharides into these glycan microarrays. Since the chemical space of possible HS oligosaccharide binding motifs is enormous, synthetic efforts to generate biologically active structures can be focused through examination of protein binding to biosynthetically-defined full-length HS chains. However, integration of such polyanionic structures into traditional glycan array platforms has been a challenge. Here we report an efficient strategy for site-specific conjugation of HS biosynthetic mutants with enriched sulfation motifs produced in mammalian cells to protein scaffolds to generate neo-proteoglycan mimetics for controlled display in arrays. We conjugated these recombinant HS (rHS) structures to cyclooctyne-BSA protein through azide-alkyne cycloaddition using a fluorogenic small molecule azido-coumarin linker, which allows for quantification of conjugation efficiency and the determination of HS chain-BSA stoichiometry in the resulting glycoconjugates. Immobilization of rHS-BSA onto glass slides or 96-well microtiter plates for microarray and ELISA binding assays, respectively, has validated the specificity of protein interactions with the presented rHS chains according to their sulfation patterns. The ability to characterize and tune rHS-BSA composition and to control the density and presentation of rHS chains on surfaces after immobilization, enhances current HS array platforms. With expanding repertoire of biosynthetic HS mutants becoming available, the presented technique offers a convenient tool for profiling of selectivity in HS-protein interactions and defining biologically relevant HS oligosaccharide structural motifs for synthesis. Supported by NHLBI K12 UCSD Career Development in Glycosciences program K12HL141956.

(155) Using synthetic substrates to probe substrate recognition by the biofilm degrading glycosidase Dispersin B

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Hospital-acquired infections by biofilm forming bacteria continues to be a major health challenge costing an estimated additional $11 billion in health care costs in the USA annually. Bacterial biofilms are sessile surface attached bacteria encased in an extracellular polymeric substance (EPS) consisting of exopolysaccharides, proteins and extracellular DNA that serves to protect the cells and facilitates both cell–cell and cell–surface attachment. Enzymatic breakdown of EPS components has generated interest as a promising strategy to disrupt biofilms. Exopolysaccharides consisting of partially de-N-acetylated β-(1 $\rightarrow$ 6)-poly-N-acetylglucosamine (dPNAG) are a common component of the biofilm EPS 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. In this study, 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.

(156) Multistage specificity ensures that LG domain-binding glycans are built exclusively on alpha-dystroglycan

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Two sites (Thr-317 and Thr-379) on one protein, alpha-dystroglycan, are the only locations in mammalian proteomes on which fully elaborated O-mannose core M3 structures, responsible for extracellular matrix protein and viral spike protein binding, are built. Defects in the enzymes that synthesize these matriglycan capped core M3 structures are causal for a sub-class of congenital muscular dystrophies referred to as secondary or tertiary dystroglycanopathies. To further understand the exceptional degree of specificity constraining biosynthesis of these glycan structures to two sites on one protein, we have expressed, purified, and characterized the substrate specificity of all enzymes in the pathway. In addition, we have enzymatically synthesized (in vitro) fully elaborated, functional, core M3 matriglycan structures beginning

from an O-Man synthetic peptide derived from the alpha-dystroglycan sequence. Our analyses, coupled with existing knowledge, demonstrate how core M3 pathway enzymes use the primary amino acid sequence of the glycoprotein substrate (POMGNT2 and LARGE1), unique underlying glycan acceptor specificity (POMK, FKTN, FKRP, RXYLT1, B4GAT1, and LARGE1), unique sugar nucleotide synthesis and utilization (CRPPA, FKTN, and FKRP), and subcellular localization (POMGNT2, B3GALNT2, POMK) to achieve an unparalled degree of specificity in a post-translational modification pathway. Thus, our results illuminate the multiple mechanisms at play ensuring that only two specific O-man sites on alpha-dystroglycan in unperturbed mammalian cells are modified with the functional glycan capable of serving as the receptor for binding by LG domain-containing extracellular matrix proteins and the spike proteins of certain arenaviruses (e.g. Lassa virus).

(157) Adiponectin is a soluble pattern-recognition protein and is integral to antibacterial immunity

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The trillions of microorganisms that colonize the mammalian intestine control host physiology, metabolism, and immune responses. To maintain a balanced host-microbiota relationship, intestinal epithelial cells secrete antibacterial proteins that limit attachment of pathogenic and opportunistic microbes, thereby limiting access to deeper host tissues. Given the ecological diversity of the intestinal microbiota, there is a limited understanding of the arsenal of antibacterial proteins employed by the intestinal epithelium. By analyzing transcript differences between germ-free and conventionally raised mice, I discovered that the metabolic hormone adiponectin is transcriptionally regulated by the presence of the microbiota. Canonically believed to solely regulate host metabolism, I discovered that adiponectin is secreted into the microbial lumen in both mouse and human feces. Since adiponectin is believed to be produced exclusively by white adipocytes, its presence at the host-microbiota interface suggests adiponectin plays a role in host defense. Adiponectin is structurally-related to C1q-like proteins, a subset of the defense collagen protein family, that act as soluble pattern-recognition proteins that restrict microbial colonization at barrier sites and in blood. Using various *in vitro* methods, I demonstrate that adiponectin is a carbohydrate-binding, pattern-recognition protein that recognizes Gram-negative bacterial polysaccharides. In order to demonstrate its function in vivo, I used the intestinal Gram-negative *Citrobacter rodentium* that colonizes the inner mucus layer of immune-deficient mice. In two mouse models, I show that adiponectin-deficient mice are more susceptible to bacterial colonization of the intestinal mucus layer. Since adiponectin is also abundant in serum, I show that adiponectin-deficient mice are more susceptible against blood-borne Gram-negative bacterial infections. In summary, adiponectin plays a central role in mammalian antibacterial immunity and suggests that the ~15 other mammalian C1q-like proteins may function as soluble, carbohydrate-binding, pattern-recognition proteins.

(158) The Sda synthase B4GalNT2 attenuates maligancy independently of sialyl Lewis X inhibition

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B4GALNT2 mediates the last step of Sd^a antigen biosynthesis by linking GalNAc β1,4 to Gal in a α2,3-sialylated type 2 chain. Sd^a biosynthesis is mutually exclusive with that of the cancer-associated sialyl-Lewis^x (sLe^x) antigen which, in colonic tissues, is mainly synthesized by fucosyltransferae 6 (FUT6). B4GALNT2/Sd^a are highly expressed in normal colon but are down-regulated at a variable extent in colorectal cancer (CRC) tissues. Previous studies indicated that the forced expression of B4GALNT2 in CRC cells replaced sLe^x with Sd^a and reduced malignancy. It was assumed that this effect was due to sLe^x inhibition. We show here an association between higher levels of B4GALNT2 mRNA in CRC and longer survival, according to The Cancer Genome Atlas (TCGA) data. To get insight into the role of B4GALNT2/Sd^a in CRC biology, we genetically engineered three cell lines. LS174T, in which sLe^x is constitutively expressed and partially replaced by Sd^a upon B4GALNT2 transfection; the SW480 and SW620 pair (from the primary and metastatic tumor of the same patient) both lacking sLe^x and Sd^a in which FUT6 or B4GALNT2 transfection generated variants expressing sLe^x or Sd^a. In the B4GALNT transfectants of the three cell lines we observed stemness reduction, while other malignant features were attenuated only in SW620. FUT6 transfection increased wound healing and soft agar growth, but only in SW620. In SW480 and SW620 cells, FUT6 and B4GALNT2 induced gene expression changes, which were largely overlapping but partially divergent. Thus, B4GALNT2/Sd^a attenuate malignancy in a cell line-specific manner, independenty of sLe^x inhibition.

(159) How N-glycans can shape somatosensory dendrites

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Dendrites are essential for the transmission and processing of sensory stimuli through the nervous system. Abnormalities in dendrite structure have been observed in patients with neurological disorders. We use the *C. elegans* PVD somatosensory neurons as a model to study the genetic basis of the tightly regulated mechanisms driving dendrite development. Studies from our lab and others have shown that the multifaceted arrangement of a conserved cell-adhesion complex regulates PVD dendrite branching. Deemed the menorin complex, it includes the skin-derived MNR-1/Menorin and SAX-7/L1CAM, the muscle secreted LECT-2/Chondromodulin II, and the transmembrane receptor, DMA-1/LRR-TM, in PVD. Mutations in any of these genes severely disrupt the stereotyped, candelabra-like arborization of PVD dendrites. In order to identify factors that modulate the activity of the menorin complex, we performed a forward genetic screen to isolate modifiers of a *lect-2/ChM-II* hypomorphic allele. We determined that mutations in *aman-2/Golgi alpha-mannosidase II* enhance the severity of the PVD phenotype in hypomorphic alleles of *lect-2/ChM-II* and *mnr-1/Menorin*, but do not display a mutant phenotype in an otherwise wildtype background. Moreover, exposing hypomorphic animals to Swainsonine, a specific inhibitor of alpha-mannosidase II, results in a similar enhancement. *Aman-2/GM-IIencodes* an enzyme required for the formation of complex N-glycans, and also paucimannose N-glycans in invertebrates. We determined that AMAN-2/GM-II acts cell-autonomously in PVD, and that its enzymatic activity is essential, suggesting that N-glycosylation of a menorin complex component in PVD itself, such as DMA-1/LRR-TM, may be significant. Using endoglycosidases and Western blot analysis, we established that DMA-1/LRR-TM is glycosylated in vivo with primarily high-mannose/hybrid type N-glycans, and that the glycan profile of specifically DMA-1/LRR-TM is altered in animals lacking AMAN-2, with larger, likely mutant N-glycan chains. Thus, we endogenously mutated N-glycosylation sites of DMA-1/LRR-TM in various combinations to determine how the presence or absence of selective glycans can modulate PVD development. Our results reveal that not only is N-glycosylation of DMA-1/LRR-TM required for the normal development of PVD dendrites, but that the N-glycosylation of the 4th site in particular may be essential. We will further characterize the role of *aman-2/GM-II* in the binding of the menorin complex in future pull down assays to gain a fuller understanding of this highly specific function of N-glycosylation in dendrite development. Since Congenital Disorders of Glycosylation (CDG) are known to affect the nervous system, and many patients present with cognitive impairment, it is imperative to understand how glycosylation can modulate dendrite morphogenesis.

(160) Polysialic acid as a therapeutic in heart disease

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The glycan Polysialic acid (polySia) is found exclusively as an α2,8-linked homopolymer on a limited number of cell surface glycoproteins where these proteins function as mediators for ligand-receptor and cell–cell interactions like migration, differentiation, as well as immunological and neurological events. Polysialyltransferases (polySTs) are glycosyltransferases that synthesize these polymers of sialic acid. Bacterial polySTs have been shown to have utility in the modification of therapeutic proteins and tissue regeneration by taking advantage of their substrate promiscuity. Treating cells with bacterial polyST from *Neisseria meningitidis* (PST_{Nm}) along with the activated sugar donor CMP-sialic acid in their extracellular environment deviates from the cell-autonomous glycan production where the endogenous expression of the associated enzyme is not required. These living PolySia modified cells could thus provide a source of remodeled cells capable of migration and repair of injured tissue as shown for motorneurons. Here, we focus on the use of polySia in heart cardiomyocyte for cardiac tissue engineering. We used H9c2 myoblast cell line and modified it with PST_{Nm} where we detect the polysialylation by spinning disc confocal microscopy after staining with the polySia specific lectin derived from inactive bacteriophage endosialidase, EndoNF, which has been fused to Green Fluorescent Protein (GFP) as a reporter (EndoN-GFP). After the synthesis of polySia, PST_{Nm} and the substrate are removed but the polySia lasts for over 72 hours and is not toxic to cells. We observed that the presence of polySia increases H9c2 migration as analyzed by real-time incubator-based single-cell population IncuCyte technology. This effect is coupled to an increased abundance of B1-integrin and also by changes in the signaling of key focal adhesion complexes such as Focal Adhesion Kinase. Strikingly, we find that polySia induces arrest of cell proliferation in H9c2 cells but not in APRE-19 cells, pointing to cell-specific effects. Since H9c2 cells are myoblasts, we are now expanding our work to determine if polySia instead induces differentiation of H9c2 myoblast to adult cardiomyocytes. In summary, modification of cell surfaces with polySia has significant biological consequences, and understanding and exploiting this opens up new therapeutic possibilities.

(161) Structural studies on human oligosaccharyltransferase complexes

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N-glycosylation is one of the most common post-translational modifications in secretory proteins. It starts in the endoplasmic reticulum (ER) with the transfer of the pre-assembled oligosaccharide $GlcNac_2$-Man_9-Glc_3, from

a dolichylpyrophosphate carrier to asparagine residues in the N-glycosylation sequon Asn-X-Ser/Thr, where X can be any amino acid except proline. This transfer is catalyzed by oligosaccharyltransferase (OST), an octameric membrane complex located at the ER membrane. In mammalian cells, two distinct OST complexes co-exist, OST-A and OST-B, which share a core of six transmembrane subunits but feature different catalytic subunits (STT3A or STT3B) and catalyze co-translational or post-translational N-glycosylation, respectively. We devised a strategy to purify homogeneous OST-A and OST-B complexes and elucidated their structures using single-particle cryo-EM, at a resolution of 3.5 Å. While featuring similar architecture, structural differences in their catalytic subunits STT3A and STT3B explain how they interact with specific subunits. STT3A binds the adapter protein DC2 that mediates attachment of OST-A to the translocon, whereas STT3B binds the oxidoreductase MAGT1 that allows OST-B to process partially folded acceptor proteins. In OST-A, ribophorin-I forms a cytoplasmic, 4-helix bundle that can attach to a translating ribosome. In contrast, the equivalent ribophorin-I region is disordered in OST-B. Our results provide the molecular basis of the distinct function of human OST-A and human OST-B [1].

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(162) Enzymatic tools and a microbial system to generate sugar peppered glycoactive antimicrobial peptides

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Glycosylation is undeniably the most abundant type of post translational modification of proteins. The glycan attached to the proteins are often crucial for stability and rigidity of the proteins and provides protection against proteolytic degradation or may play an important role in intracellular localization, cellular signaling, adhesions and immune response of the protein. While glycosylation of proteins is known in eukaryotes since 1930, it is figured out late in archaea and in bacteria in 1985. Yet, the repertoire of glycoproteins, glycans and glycosyltransferases discovered in bacteria is very impressive and much more diverse than what is known in eukaryotes.

In this context, while N-linked and O-linked glycosylation is universal and widespread, S- linked glycosylation is discovered first in bacteria in 2011 and in mammals post 2016. Antimicrobial peptides like Glyocin F, Sublancin 168 and Enterocin 96 are some of the examples of ribosomally synthesized O/S-glycosylated bacteriocins (glycocins) produced by bacteria. These glycocins are stable over wide range of pH and temperature and exhibit antimicrobial activities against variety of microbes including pathogenic and food spoiling bacteria. The glycosyltransfease instrumental in these O/S-linked glycosylation of bacteriocins were first characterized in Bacillus subtilis (SunS), followed by *Bacillus thuringiensis* (ThuS). Our group at CSIR-IMTECH had characterized a third S-glycosyltransferase, EntS from *Enterococcus faecalis* TX104 in 2017. EntS is unique in its ability to transfer as well as extend monosaccharide directly and sequentially on the acceptor peptide at two chemovariant glycosites (Ser/Thr and Cys) in the acceptor peptides. In our studies, a direct correlation between number and type of glycan and the bioactivity of the glycosylated antimicrobial peptide enterocin 96 was identified. Further, using EntS and a recombinant fusion tag, now we have developed an *Escherichia coli* based microbial system (named SELECT-GLYCOCIN) suitable for expression and screening of such O- and S-linked libraries of antimicrobial glycopeptides. Using SELECT-GLYCOCIN, a library of random (1.5 X 10^3) and rational (17) mutants of enterocin 96 was generated and screened for bioactivity against *Listeria monocytogenes*, a food born pathegen. Subsequent comprehensive analysis of selected mutants led to the identification of glyco-variant namely, Ent96-K4_K5insYYGNGV (PedioEnt96) and sequence variants, namely G16E-H24Q and C13T having improved antimicrobial spectrum or activity. For potential applications of glycocins in food, agriculture and healthcare products, the glycocin generating glycosyltransferases are valuable tools for custom glycoengineering. Accordingly, the said microbial system and assay methods being generic in nature can further be adapted for use with known or yet to be discovered glycocins and their enzymes.

(163) Altered glycosylation in Cancer—insights for targeting tumor heterogeneity and novel targets.

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Alterations of glycosylation are common on the cell surface during the carcinogenesis process and are associated with cancer progression and poor prognosis of the patients [1, 2]. Tumor cells express a wide variety of glycosylation alterations, which interfere with key oncogenic processes, contributing to tumor progression and metastization [1, 2].

A variety of genetic and epigenetic mechanisms have been reported to underlie glycosylation features that confer

advantages to cancer cells. We report the latest analyses in human gastrointestinal (GI) cancer cells in order to understand the relevance of the increased sialylation in GI tumors. Detailed data on RTK activation and glycan structures analyses in cancer cells upon sialyltransferases genetic manipulation will be reported [2, 3, 4, 5].

Moreover, the potential use of cancer-associated glycosylation changes in extracellular vesicles (EV) produced by cancer cells have been evaluated. We will report on the impact of the EV isolation strategy on the glycosylation profile of the isolated EV populations [6]. Different methodologies, UC, TEI, ODG and SEC were used to isolate EV populations. We demonstrate that the different isolation approaches enriched EV subpopulations carrying distinct protein and glycosylated profiles [6].

These results disclose novel functional aspects of glycosylation modifications occurring in key proteins in GI cancer and highlights the potential of EV isolation protocols to determine the structural and functional complexity of the EV glycoconjugates in tumor biology, with implications for the clinical setting [1, 6].

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

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The vast majority of cell surface or secreted proteins are N-glycosylated. As secreted glycoproteins age in the circulation, their glycan linkages are progressively hydrolyzed from the terminal position inward. This stepwise exo-glycosidic remodeling starts with neuraminidases, followed by a series of glycosidases to expose different potential lectin ligands. This process was detected among over 600 blood proteins surveyed and published previously. In cases analyzed thus far, the rate of glycan remodeling was different among glycoproteins and inversely linked to their half-lives and abundance. Although some blood glycoproteins normally carry terminal mannose linkages, glycan remodeling with increased molecular age in circulation can increase the amount of terminal mannose present. Most N-glycans measured by mass spectrometry from blood glycoproteins are of the complex type, bi-antennary, and sialylated. Among endocytic lectin receptors that detect glycan ligands on aging glycoproteins, mannose receptors are well described among parenchymal, vascular, and immune cell types. We are investigating the identities and functions of mannosylated blood glycoprotein ligands regulated by the endogenous macrophage mannose receptor (Mrc1). Mrc1 is found on macrophages and specialized cells in most organs as well as the vasculature. It has a broad spectrum of both glycan and protein ligands due to its three distinct binding sites. A cysteine-rich and a fibronectin-like domains bind sulfated glycoproteins and collagens, respectively. The lectin domain of Mrc1 binds mannosylated particles ranging from pathogens and allergens to various but poorly defined endogenous mannosylated blood glycoproteins. Our approach using lectin affinity chromatography of non-denatured blood proteins followed by mass spectrometry has succeeded in identifying the endogenous ligand repertoire of mannosylated blood glycoproteins regulated by the Mrc1 lectin receptor. Meta-analysis of the data spanning biological processes and pathways linked to these ligands successfully predicted the presence of previously undetected novel phenotypes in Mrc1-deficient mice. Increased half-lives and abundance of Mrc1 ligands further marked homeostatic abnormalities contributing to a syndrome of inflammation, vascular damage, high blood pressure, and organ dysfunction, and which may portend life-threatening consequences in altered environmental and experimental contexts. Mrc1 is one of many endocytic lectin receptors in mammals, and each receptor may control physiology and disease by virtue of its unique endogenous ligand repertoire which is now amenable to discovery and investigation.

(165) Unravelling Clostridium thermocellum LysM domains: Structural basis for the recognition of chitin and peptidoglycan

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Lysin Motif (LysM) domains are protein modules that display binding to chitin and peptidoglycan and are widespread in prokaryotes and eukaryotes. LysMs have multiple roles often associated with bacterial cell wall degrading enzymes or proteins involved in signalling and symbiosis or in bacterial spore morphogenesis [1]. LysM domains are classified in the CAZy database (www.cazy.org) as family 50 carbohydrate-binding modules (CBM50). We have identified 15 CBM50 sequences in the genome of Clostridium thermocellum (Ct), an anaerobic thermophilic bacterium highly efficient in the degradation of plant cell wall polysaccharides [2]. CtCBM50 are found associated in tandem or as single domains of modular proteins with sequence similarity to family 18 glycoside hydrolases or to proteins involved in bacterial spore formation. Details of the carbohydrate-binding and functional roles of these CBMs are yet to be elucidated.

To identify the carbohydrate ligands, the CtCBM50 sequences were amplified and recombinantly expressed for high-throughput screening using a sequence-defined neoglycolipid-based carbohydrate microarray [3] comprised of plant, fungal and bacterial cell wall oligosaccharides. Microarray analyses revealed a restricted binding specificity towards β1,4-linked GlcNAc oligosaccharides for 7 of the 15 CtCBM50 and a minimum chain-length requirement of a trisaccharide. The structure of the complex of a CtCBM50 ($CtCBM50_{Cthe_0300}$) with the GlcNAc trisaccharide was solved by X-ray crystallography. This revealed an intermolecular interaction of two CBM molecules with the ligand. These results, combined with analysis by isothermal titration calorimetry (of the native proteins and site-directed mutants) and molecular dynamics simulations, enabled identification of the molecular determinants of the interaction. It was inferred that the CBM, acting in a multimodular way, forms a binding site comprised of up to 6 ligand-binding subsites. Key residues were identified that mediate both chitin and peptidoglycan oligosaccharide recognition and chain-length dependency, through CH-π stacking (Trp11 and Tyr38) and hydrogen bonding interactions, particularly between the side-chain of Asn35 and the N-acetyl group of GlcNAc. The $CtCBM50_{Cthe\_0300}$ binding was favoured by an interchain multivalent assembly in which individual CBM molecules bind in a cooperative manner to longer oligosaccharide chains, providing further evidence of cooperative binding of LysM domains. This integrative work promises to lead to an understanding of the mechanisms of recognition of chitin and peptidoglycan by members of family 50 CtCBMs.

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(166) O-Pair Search with MetaMorpheus for O-glycopeptide Characterization

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Mass spectrometry 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, which offer complementary coverage of both peptide backbone and glycan fragmentation. Existing tools also 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 >2000x over the most widely used commercial glycopeptide search tool (Byonic), requiring <1 min with MetaMorpheus compared to the >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).

(167) The Effects of Stress on Mucin 13 Expression in the Small Intestine

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In the past decade, several studies have demonstrated that the gut microbiome has a profound impact on central nervous system (CNS) function during both homeostatic and pathological conditions. Indeed, the microbiome has been shown to actively contribute to many CNS diseases including: neurodevelopmental disorders, autoimmune diseases, and neurodegenerative diseases. This pathology is often attributed to impaired gut-brain axis communication, mediated by a microbiota dysbiosis. In our lab, using unpredictable chronic mild stress—an animal model for depression—we have shown that a decrease in Lactobacillus correlates with depressive-like behaviors. Furthermore, we have shown that therapeutic supplementation of Lactobacillus is sufficient to correct the observed signs of depression. Chronic stress and the associated production of stress hormones is recognized to increase the risk of developing depression in humans and animal models. While the active role the gut microbiome plays in pathological conditions is becoming clear, therapeutic interventions remain elusive—due in part to the complexity and individuality of the microbiome. In order to provide more effective therapeutic options a key gap in knowledge must be addressed: What is the mechanism mediating microbial composition changes in response to stress? Mucus is an essential layer of glycoproteins, known as mucins, which lines the digestive tract. The mucosal layer has two main functions: first, to serve as a physical barrier against pathogens and the harsh digestive environment; second, to act as a nutrient reservoir and anchor point for commensal bacteria. Both functions are essential for host physiology as the absence of a mucus layer allows for spontaneous disease development and induces sweeping microbiome changes. While several mucins are present in the small intestine, mucin 13 has been shown to play a protective role against host inflammatory events in diseases such as colorectal cancer. As chronic stress is known to cause inflammation, mucin 13 may be equipped to respond to stress induced inflammation and change the composition of the mucosal layer. Based on this, we hypothesize that stress induces a change in muc13 expression in the small intestine of mice. Furthermore, we believe this change in muc13 expression will change the microbial niche to induce dysbiosis and depressive-like behaviors. Here we examine mucin expression in the gastrointestinal tract of mice exposed to chronic mild stress. In order to identify the specific molecules acting within the intestine to change mucin expression, we utilize human cell lines known to expression mucins, Caco2 and HT-29 cells, and treat these cells with agents known to be elevated in stress. Lastly, we use an animal model of reduced mucin expression (Agr2−/−) to determine behavioral consequences of a disrupted mucosal layer without modifying external stressors.

(168) Detecting Glycan Ligands of Siglecs in New Modalities with a Versatile Siglec-Fc Scaffold

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Sialic acid-binding immunoglobulin-type lectins (Siglecs) are immunomodulatory receptors that are regulated by their glycan ligands. A growing connection between Siglecs and human disease motivates improved methods to detect Siglec ligands. As knowledge about Siglec ligands predominantly comes from the use of Siglec-Fc chimeras, improving this scaffold is necessary and we have designed a new versatile set of Siglec-Fc proteins for glycan ligand detection where enhanced sensitivity and selectivity are enabled through multimerization and avoiding Fc receptors. Mutations were made in the Fc domain that prevent Fcγ binding and avoiding secondary antibodies leads to exquisite sialic acid-dependent cell binding, allowing us to fully catalog Siglec ligands on human immune cell subsets from the peripheral

blood and spleen. Using these Siglec-Fc proteins, Siglec ligands were systemically profiled on healthy and cancerous cells and tissues, revealing many unique patterns. These re-engineered Siglec-Fc proteins allow for sensitive and selective sialic acid-dependent binding to cells and tissues, while monomeric, homogenous Siglec fragments derived from these constructs enables glycan interactions to be studied and quantified through the development of a quantitative ligand-binding mass spectrometry assay. Using this assay, the ligand specificities of several Siglecs were clarified. For CD33, we demonstrate that it recognizes both α2–3 and α2–6 sialosides in solution and on cells, which has implications for its link to Alzheimer's disease susceptibility. These new soluble Siglec-Fc proteins are useful probes for studying the glycans ligands of Siglecs in a variety cell-based and biochemical assays and reveal the abundance of their glycan ligands on host cells as self-associated molecular patterns.

(169) Fc γ receptor IIIa/CD16a processing correlates with the expression of glycan-related genes in human natural killer cells

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Engineered natural killer (NK) cells are emerging as a powerful cell-based immunotherapy, including cultured cells with enhanced cytotoxicity resulting from optimizing antibody binding through Fc γ receptor IIIa (CD16a). Receptor processing is critical, affecting surface expression and affinity. Furthermore, it is unclear if modifying the expression of select genes that encode processing enzymes is sufficient to affect key carbohydrate features, notably N-glycans. We identified substantial processing differences with two NK cell lines: NK92 and YTS cells. High resolution analysis of CD16a glycopeptides reveladed that NK92 produced CD16a with a high percentage of underprocessed oligomannose-type glycoforms, and YTS processed to a high level but lacked distinct features found on primary NK cells and monocytes, including increased branching and repeated N-acetyllactosamine structures. Gene expression profiling by RNAseq and qRT-PCR revealed expression levels for glycan-modifying genes which correlated with CD16a glycan composition, including decreased mannosidase gene expression and oligomannose glycoforms in NK92 cells, increased branch fucosylation and FUT7 expression in YTS cells, as well as decreased branching and MGAT4A and MGAT5 expression in HEK293F, NK92 and YTS cells. These results identify a high degree of variability between the processing of a single human protein by different human cell types, in which multiple genes are potentially suitable for manipulation of expression levels in cultured effector cells, providing Golgi conditions as similar as possible to NK cells in the human body. It is then obvious that cultured cells must exhibit appropriate processing to delineate the functional role of individual modifications in cell function.

(170) Glycan-Glycan Interactions in the N-terminal CEACAM1 Domain

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There has been much discussion of the potential importance of carbohydrate-carbohydrate interactions in biological roles ranging from cell adhesion and recognition in early metazoans and carcinomas to host-pathogen interactions. Both low and high affinity interactions have been identified. However, structural details of these interactions remain elusive. Recently we observed that two Man5 N-glycans on the surface of the N-terminal domain of CEACAM1 form an intricate and apparently stable complex during an extended molecular dynamics (MD) trajectory. We have undertaken an NMR study of the system, in an attempt to provide experimental support for this structure. Uniform isotopic labeling, commonly desired for NMR studies and used with bacterial expression systems, is challenging in this situation as properly glycosylated proteins require expression in mammalian cell culture. However, proteins can be sparsely isotopically labeled on select amino acids as well as their glycans by simple supplementation of mammalian growth media with both isotopically labeled glucose and a given amino acid. This produces a limited set of crosspeaks in two-dimensional spectra that can report on short range interactions coming from NOEs or long-range interactions coming from pseudo-contact shifts (PCSs) and residual dipolar couplings (RDCs), when a paramagnetic site is engineered into the protein. We will report our progress on collection of this NMR data and the degree to which it supports specific glycan-glycan interactions.

(171) In vitro binding of T. congolense trans-sialidases to erythrocytes

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The African parasite *Trypanosoma (T.) congolense* infects livestock in sub-Saharan Africa leading to the fatal disease animal African trypanosomiasis (AAT).

It is well known that the parasite attaches to erythrocytes in a sialic acid-dependent manner [1]. However, the

surface molecules responsible for the cell–cell interaction still remain unknown. Possible candidates facilitating the interaction between trypanosomes and erythrocytes are trans-sialidases (TS). These surface-exposed enzymes catalyse the transfer of sialic acids from erythrocytes and other host glycoconjugates to terminal galactose residues on the parasite's surface. This coat of sialic acids is essential for the parasite to survive in the insect vector, the tsetse fly [2].

We established a quantitative erythrocyte binding assay to investigate the in vitro binding of human erythrocytes to the four known TS of *T. congolense* (TconTS1–4 [3]).

Significant binding was observed for TconTS1 and TconTS2. This interaction is sialic acid-dependent since enzymatically desialylated erythrocytes did not bind. Furthermore, binding could be inhibited with 3′-sialyllactose, but not with 6′-sialyllactose. Interestingly, also lactose, a well-known acceptor substrate of TconTS, significantly decreased erythrocyte binding, probably due to trans-sialylation from the erythrocytes' surface to lactose resulting in desialylation of the cells and generating 3′-sialyllactose.

These results provide good evidence that the interaction between trypanosomes and erythrocytes could be mediated by TconTS and that this interaction can be affected by sialic acid acceptor substrates.

Next we want to investigate the binding of TconTS to different erythrocyte species since sialic acid composition and linkage differ between mammalian erythrocytes.

Additionally, first experiments were performed to express TconTS1 in the African trypanosome *T. brucei brucei* which naturally do not bind to erythrocytes. These transgenic trypanosomes will allow us to study parasite-erythrocyte binding in a cellular context.

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(172) Expression of inflammo-modulatory glycans by the epithelial cells of the human choroid plexus

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The pathology of Alzheimer's disease (AD) is complex and involves incompletely understood inflammatory responses. The contributions of inflammatory cells, either resident in the brain (microglia) or recruited from peripheral sources (monocytes/macrophages), are an emerging interest with regard to the initiation and progression of AD. The choroid plexus (CP), which comprises an important part of the interface between the peripheral blood and the cerebrospinal fluid, functions as an immune gateway in the brain and has been proposed to regulate trafficking, activation, and differentiation of inflammatory cells. In a mouse model for aggressive familial AD, we observed upregulated expression of ligands for Siglec-F on CP epithelial cells. Siglec-F, like other members of the Siglec family, binds sialylated glycans to modulate innate and adaptive immune responses in many inflammatory contexts. To explore the role of Siglec ligand expression in normal human CP and in human neurodegenerative disease progression, we have undertaken targeted glycomic and glycoproteomic analysis of Siglec-F and Siglec-9 receptors expressed by choroid plexus papilloma cells (HIBCPP) as well as by 3-D choroid plexus tissue derived from patient induced pluripotent stem cells (choroid plexus organoids, which we call chorganoids). Specific endo-glycosidase digestion and orthogonal biochemical analysis indicates that human CP cells present keratan sulfate ligands for Siglec-9, as well as structurally related ligands for Siglec-F, on more than one polypeptide backbone. The identification of these Siglec ligands in a unique tissue setting presents opportunities for investigating the response of inflammatory cells to disease-related glycan expression.

(173) Using Blender as a Site-Specific 3D Glycoprotein Visualization Software to Create Accurate and Visually Appealing Glycoprotein Renderings

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The prevalence of glycomic and glycoproteomic analysis in combination with mass spectrometry has expedited glycan translational modification studies. About half of all proteins in living organisms contain oligosaccharide modifications, which have a substantial effect on the function of intercellular interactions such as binding, cell replication, and maintaining cellular structural integrity. Visualizing these glycan structures on any given protein in 3D has proved to be a challenging endeavor in glycoconjugate research. Stringent 3D molecular modeling can be used to simulate and visualize how a specific glycoprotein is decorated with experimental glycan structures. This approach can provide powerful and useful insight in the molecular requirements for effective binding, but is limited to depicting single glycoproteins and their interactions with a given binding partner. When the goal is to obtain a visually appealing depiction of a glycoprotein in the context of a whole cell, a more qualitative method is required. In our recent work, we have chosen Blender, an open source 3D computer graphics software, in conjunction with PyMOL, a chemical visualization tool, to render PDB

structures with their unique glycan attachments while in the cell. Modern advancements in fast and efficient real time 3D graphic rendering and a broad holistic toolset makes Blender a well-suited aid for these detailed simulations. Our method allows us to generate any glycan structures including high mannose or complex, and then distribute and duplicate them on specific glycan sites from any pre-analyzed cryogenic electron microscopy PDB structure. We are able to show glycan structures through symbol nomenclature, ball and stick formations, space filling models, as well as hundreds of other stylized representations. Glycans can then be color-coded or grouped to also show distinctions between *N*- and *O*-linked glycans or other structural glycan families. Blender allows the simulation of thousands of glycan structures on hundreds of glycoproteins while inside an intercellular organism. Our method to render these glycans allows for full creative freedom for various stylistic or artistic representations. In conjunction with glycoproteins, we are also able to render accurate lipid bilayers, DNA, RNA, and full cells/organelles. Our method allows us to utilize Blender's expansive 3D platform to comprehensively render these detailed glycoproteins to provide a visually appealing alternative to site specific 3D glyco-imaging. This is not an alternative to glyco-modeling, yet serves as a large-scale tool to depict hundreds of glycoproteins inside of a cell. These advanced 3D glyco-images can be used for journal covers, scientific figures, presentations, or websites, and are readily produced on any desktop or laptop computer without the need for access to a modeling computing facility.

(174) Understanding human-specific evolution of CD33/Siglec-3 and its impact on sialoglycan binding and functionality

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CD33 is the founding member of CD33-related-Sialic acid (Sia)-binding Ig-like lectins (Siglecs), which can modulate innate immunity by recognizing endogenous sialoglycans as "self-associated molecular patterns" (SAMPs). CD33-SNPs impact Alzheimer's Disease (AD), wherein the disease-protective derived allele specific to humans increases expression of an alternately spliced isoform (CD33m, with deletion of the Sia-binding V-set domain). While "great ape" genomes do not harbor these SNPs, show fewer changes over >10mya, and express low CD33m, human CD33 harbors ~10 mutations in its V-set domain—suggesting derived hominin-lineage evolution of this gene, which was possibly eventually co-opted to protect cognition of elderly caregivers (The Grandmother Hypothesis). What selection forces originally favored the emergence of this human-specific AD-protective allele? Certain human pathogens have evolved molecular mimicry of SAMPs to evade Siglec-mediated immune responses. Loss of CD33m Sia-binding thus suggests that the V-set domain initially underwent selection to evade such exploitation. Indeed, we found while the ancestral form of CD33 strongly prefers to bind to the non-human sialic acid Neu5Gc, a single amino acid change in hominin CD33 caused a switch to recognition of Neu5Ac, the sialic acid that is enriched in humans due to evolutionary loss of CMAH, the enzyme that converts CMP-Neu5Ac to CMP-Neu5Gc. Human CD33 showed selective binding by the uniquely human pathogen *Neisseria gonorrhoeae* (Ng), which sialylates its surface using host-derived CMP-Neu5Ac to evade immunity. Given negative impacts of gonorrhoea on fertility, we reasoned that evolutionary changes of human CD33 were driven by Ngand/or other human-specific, sialylated pathogens affecting fetuses or neonates, e.g., Group B Streptococcus (GBS). Indeed, GBS strains also bound selectively to human CD33. The high frequency of unique amino acid changes in the human V-set domain suggests continued exploitation of human CD33 by such Neu5Ac sialylated pathogens. An evolutionary scenario consistent with all findings is initial loss of the preferred Neu5Gc CD33 ligand, followed by CD33-upregulation and the switch to Neu5Ac binding. This in turn facilitated emergence of Neu5Ac sialylated pathogens—which then drove hominin-lineage selection towards diverse allelic variations, eventually impacting CD33-signaling pathways, including processes linked with microglial activation in the brain and AD.

(175) Red meat diet affects serum antibodies against the non-human sugar Neu5Gc

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Antibodies against the red meat-derived carbohydrate N-glycolylneuraminic acid (Neu5Gc) exacerbate cancer in 'human-like' mice. Human anti-Neu5Gc IgG and red meat are independently proposed to increase cancer risk, yet how diet affects these antibodies is largely unknown. Based on world global data, we show that colorectal cancer (CRC) incidence and mortality are associated with increased national meat consumption. In a well-defined web-based French large cohort, we used glycomics to measure daily Neu5Gc intake from red meat and dairy, and investigated serum as well as affinity-purified anti-Neu5Gc antibodies. We found that high-Neu5Gc-diet, gender and age affect the specificity, levels and repertoires of anti-Neu5Gc IgG immune responses, but not their affinity. Furthermore, in high-meat diet, anti-Neu5Gc antibodies showed distinct diversity-patterns on glycan microarrays. Altogether, we found a clear link between the levels and repertoire of serum anti-Neu5Gc IgG and Neu5Gc-intake from red meat and dairy. These precise rational methodologies allowed to develop a 'Gcemic index' to simplify assessment of Neu5Gc in foods that could potentially be adapted for dietary recommendations to reduce cancer risk.

(176) Effect of c.303 G > C mutation in functional splice variant of CMP-sialic acid transporter

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The human CMP-sialic acid transporter (hCST) encoded by SLC35A1 is a type III trans-membrane protein antiporter exchanger for CMP-sialic acid/CMP that allows CMP-sialic acid to be transported into the lumen of the Golgi apparatus where it is used for sialyltransferase reactions. Mutations in the hCST cause multi-systemic syndromes characterized by encephalopathy, repeated hemorrhagic incidents, including severe pulmonary hemorrhage and that are classified as Congenital Disorders of Glycosylation (CDG). Currently, three SLC35A1-CDG patients have been confirmed with different heterozygous mutations in SLC35A1. A previously reported mutation c.303 G > C (101Gln > His) in a patient has been shown to reduce CMP-sialic acid transport at 50%. In this work, we evaluated the effect of this mutation in a functional isoform known as del177-hCST. This is isoform lacks 59 amino acids, abrogating the transmembrane domain TMD7 reported to be essential for CMP-Sia transport (V208–G217 stretch and Y214) but it continues being functional. Lec2 cells, a well-known model of deficient CMP-sialic acid transport, were transfected with hCST (Lec2-hCST) or del177-hCST (Lec2-del177hCST), as well as with their mutated counterparts Lec2-hCSTΔ303 or Lec2-del177Δ303. After 24 h of transfection, cells were exposed to the RCA I lectin (10 ng/mL) and cell proliferation was measured through MTT assay. The RCA I lectin is toxic to Lec2 cells because lack of sialic acid residues in the glycans of these cells exposes galactose residues that bind and internalize the lectin. In this assay, resistance to RCA toxicity measured through cell proliferation, is associated to increased sialylation as a result of functional hCST isoforms.

As expected, Lec2-hCST and Lec2-del177hCST showed increased resistance to RCA I toxicity compared to mock Lec2 cells. Interestingly, the Lec2-hCSTΔ303 exhibited an expected reduction in proliferation compared to Lec2-hCST, nonetheless, the Lec2-del177Δ303 showed a similar resistance with respect to Lec2-del177 cells, indicating that the c.303 G > C (101Gln > His) mutation does not seem to affect the functionality of this isoform. Further studies are necessary to understand the effects of mutation in both wt and the del177 isoform as this isoform can be induced through morpholino therapy and could abrogate the deleterious effects of certain mutation in the hCST.

(177) Alternative splicing regulates the substrate preferences and activity of the *Drosophila melanogaster* O-glycosyltransferase PGANT9

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Polypeptide N-acetylgalactosaminyl transferases (GalNAc-Ts) initiate mucin-type O-glycosylation by catalyzing the transfer of N-acetylgalactosamine (GalNAc) from UDP-

GalNAc to Thr/Ser acceptor sites on proteins. These enzymes contain 2 domains that are important for substrate binding and activity: a catalytic domain and a C-terminal lectin domain. Interestingly, GalNAc-Ts do not recognize a consensus sequence or motif and many isoenzymes have unique mechanisms for substrate recognition, binding and active site alignment. One enzyme with a distinct substrate recognition mode is the *Drosophila melanogaster* isoenzyme PGANT9, which influences secretory granule morphology in the salivary glands and has 2 splicing isoforms, PGANT9A and PGANT9B, that differ in the alpha repeat of their lectin domain. PGANT9A-alpha has a net positive charge and PGANT9B-alpha has a net negative charge. Indeed, PGANT9B more efficiently glycosylates a peptide from the mucin Sgs3 than PGANT9A, consistent with the overlapping in vivo localization of PGANT9B and the positively charged mucin Sgs3. The X-ray crystal structures of both enzymes revealed that the charged alpha repeat forms a loop that extends towards the active site in both enzymes, suggesting that the loop dictates substrate specificity by interacting with oppositely charged peptides. To test this hypothesis, we measured the activity of PGANT9A and PGANT9B against a range of Drosophila mucin peptides containing charged residues, as well as artificial peptides containing residues with opposite charges and verified that PGANT9A has high specificity towards peptides with net negative charges. Unexpectedly, PGANT9B was less specific and glycosylated peptides containing both positive and negative net charges. By modeling Sgs3 peptides into the PGANT9A/B active sites, we noticed how the positively charged gating loop could be influencing substrate binding. In PGANT9A, the alpha repeat and the gating loop are both positively charged, explaining its strong preference for negatively charged peptides. In contrast, PGANT9B contains positive charges near the N-terminus of the peptide via the gating loop, and negative charges near the C-terminus via the alpha repeat, explaining why it can glycosylate peptides with a range of net charges. Overall, these studies highlight a novel mechanism of GalNAc-T substrate specificity that is regulated by alternative splicing and dictated by charges in both the catalytic and lectin domains.

(178) Glycoproteomic analysis of N and O glycosylation of the SARS-CoV-2 spike protein

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The outbreak of the Covid-19 pandemic is the reason of the current global health crisis. The development of effective antiviral compounds and vaccines requires detailed descriptive studies of SARS-CoV-2 proteins. The SARS-CoV-2 spike (S) protein mediates virion binding to human cells through its interaction with the cell surface receptor ACE2 and is one of the major immunization targets. The functional virion consists of three S1 and three S2 subunits formed by the furin cleavage of the spike protein at R682, a polybasic cleavage site that differs from the animal version of the protein as well as from the SARS-CoV spike protein 2002. We analyzed the glycoprotein using our newly developed methodology based on low-energy fragmentation and cyclic ion mobility mass spectrometry. Our analysis confirms the O-glycosylation of the spike protein on a threonine (T678) located near the furin cleavage site. This site is occupied by core-1 and core-2 structures. Two other predicted O-glycosites are unoccupied. We identified eight additional O-glycopeptides on the spike glycoprotein and confirmed that the spike protein is heavily N-glycosylated. We were able to identify LacdiNAc, PolyLacNAc, and Sulfated LacdiNAc on several glycosites. In conclusion, our study significantly expands current knowledge of glycosylation of the spike protein and allows the investigation of the effect of O-glycosylation on its proteolytic activation.

(179) Galectin-3 enhances neutrophil motility and extravasation into the airways during Aspergillus fumigatus infection

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Invasive fungal infections are one of the most important causes of infectious mortality worldwide and responsible for up to 1.5 million people each year, which makes one of the most deadly among transmissible diseases. They are caused by opportunistic molds, such as *Aspergillus fumigatus*, which infects patients who are immunocompromised or have chronic lung diseases. Rapid recruitment of neutrophils to the site of infection is critical to clear the fungus, and its appropriate regulation is required to avoid any collateral damage, especially in the case of pulmonary fungal infections while the factors governing the host innate immunity remain poorly defined.

Galectin- 3 is a mammalian soluble β-galactose-binding lectin with both antimicrobial and immunomodulatory activities. We previously reported that galectin-3 facilitates early neutrophil recruitment to the airway infected with *Streptococcal pneumoniaeand* the skin infected with parasite *Leishmania majorin* a spatiotemporal and context-dependent manner [1, 2], while the role of galectin-3 in the defense against molds in the lungs has not been studied. Here we show that galectin-3 expression is markedly up-regulated in mice and humans with pulmonary aspergillosis. Galectin-3 deficient mice displayed increased fungal burden and higher mortality during pulmonary infection. In contrast to previous reports with pathogenic yeast, galectin-3 exhibited no antifungal activity against *A. fumigatus in vitro*. Galectin-3 deficient mice exhibited lower level of neutrophil accumulation in their airways during infection, despite normal numbers of total lung neutrophils. Intravital imaging studies confirmed that galectin-3 was required for normal neutrophil emigration to the airspaces during fungal infection. Adoptive transfer experiments demonstrated that stromal rather than neutrophil-intrinsic galectin-3 was necessary for normal neutrophil entry into the airspaces. Live cell imaging studies revealed that extracellular galectin-3 directly increases neutrophil motility.

Taken together, these data demonstrate that extracellular galectin-3 facilitates recruitment of neutrophils to the site of *A. fumigatus*infection, and reveals a novel role for galectin-3 in host defense against fungal infections.

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(180) GlycoSense™: An inexpensive and easy to use flow cytometry-based simplified glycan profiling platform for monitoring in vitro glycoengineering

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The GlycoSense™ flow cytometry-based platform uses multiplex microspheres and basic two color flow cytometry to give a simplified analysis of terminal glycan residues, or a GlycoPrint of a glycoprotein of interest. By coupling reagents specific for sialic acid, galactose, and N-acetylglucosamine to spectrally distinct microspheres, we can monitor changes to the glycosylation features during enzymatic treatment, or look at differences in terminal glycan composition between samples of similar glycoproteins. Microsphere type (terminal monosaccharide-specific capture reagent) is distinguished by red fluorescence while bound glycoprotein is measured with green fluorescence, either by directly labeling the glycoprotein of interest, or using a labeled secondary detection molecule. We demonstrate that this platform is particularly convenient for monitoring in vitro glycoengineering of several glycoproteins in near real-time as terminal glycan residues such as sialic acid and galactose are enzymatically removed or added in specific linkages. Model glycoproteins show that treatment with sialidase leads to decreases in sialic acid signal, and corresponding increase in galactose signal, as terminal sialic acid is removed and galactose is exposed. Additional treatment with galactosidase shows decrease in galactose signal and a corresponding increase of GlcNAc signal as galactose is removed from the glycan. Treatment of glycoproteins with sialyltransferases or galactosyltransferases also shows increase of linkage specific signals using GlycoSense™. This platform is also useful for comparing differences in the same glycoprotein from different sources or samples.

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(181) PTPζ (phosphacan) carries Siglec-8 ligands in human brain

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Siglecs, sialic acid-binding immunoglobulin-like lectins, are immunoregulatory transmembrane proteins differentially expressed on subsets of immune cells, where most inhibit inflammation via their ITIM motifs upon binding complementary sialoglycan ligands. Microglia, resident macrophages of the brain, express a unique subset of Siglecs that differentiates them from peripheral macrophages and other immune cells. In the brain, microglia clear cellular debris and mediate inflammatory responses through phagocytosis. An interrupted balance between microglia activation and inhibition can contribute to neurological diseases, especially proteinopathies including Alzheimer's disease and Parkinson's disease. Understanding the role of microglial Siglecs and their ligands in regulating microglial cells may provide therapeutic targets for central nervous system disorders. Transcriptomic analysis (Galatro et al, Nature Neurosci 20:1162, 2017) revealed expression of several Siglec genes on adult human microglia, with Siglec-8 and Siglec-10 predominant. Siglec-8 inhibits inflammatory responses by eosinophils and mast cells when crosslinked by antibody or sialoglycan lig-

ands. Likewise, the crosslinking of Siglec-8 on microglia may inhibit microglial activation and regulate ongoing inflammatory events. To identify Siglec-8 ligands, human brain tissue (cerebral cortex) was homogenized in guanidinium hydrochloride solution, the cleared extract dialyzed against urea buffer, treated with or without sialidase, resolved on composite agarose-acrylamide gels and blotted on PVDF membranes. Upon probing with Siglec-8-Fc to reveal Siglec-8 ligands, a single large molecular weight species (~1 MDa) was bound in a sialic acid dependent manner. Brain extract Siglec-8 ligand was purified by size exclusion chromatography followed by affinity capture on Siglec-8-Fc beads. Proteomic mass spectrometry revealed protein tyrosine phosphatase zeta (PTPζ, phosphacan) as the protein carrier for Siglec-8 ligands in the human brain. On lectin overly blots, Siglec-8 ligand comigrated with PTPζ detected by antibodies and both shifted when treated with chondroitinase ABC or PNGase F. Siglec-8 binding to the purified ligand was diminished by treating with sialidase or keratanase I whereas antibody detection of PTPζ was retained, suggesting that a portion of human brain PTPζ carries sialylated keratan sulfate chains that are Siglec-8 ligands. We designate this newly discovered human brain Siglec-8 ligand PTPζ^{S8}. Revealing the functions of Siglec-8 and PTPζ^{S8} in mediating microglia activation in healthy and diseased human brain awaits further studies. Supp. by NIH grant AG062342.

(182) Endo-lysosomal processing of N-glycans on acid alpha-glucosidase is critical to attain the most active enzyme for hydrolyzing glycogen

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Acid alpha-glucosidase (GAA) is a lysosomal glycogen-catabolizing enzyme, a deficiency in which leads to Pompe disease. Pompe disease can be treated with systemic recombinant GAA enzyme replacement therapy (ERT), but data suggests the existing standard of care ERT may be inefficiently delivered to lysosomes in skeletal muscles. Several next-generation GAA ERTs are focused on increasing lysosomal delivery for improved efficacy. Once delivered to lysosomes, GAA undergoes proteolytic cleavage and glycan trimming, yielding a more active enzyme for hydrolyzing glycogen. However, the relative contributions of each of these steps for increasing GAA activity are unclear, though proteolytic processing has been proposed as the primary contributor. To better understand the specific role of glycan processing, we investigated the role of N-glycan trimming in increasing GAA activity towards glycogen. We generated GAA variants with chemically modified terminal sialic acids on N-glycans that were resistant to neuraminidase activity *in vitro*. *In cellulo*, following GAA uptake in Pompe patient fibroblasts, these variants underwent proteolytic cleavage, but not glycan trimming. Lack of glycan trimming resulted in only a partially activated GAA as evidenced by its lower catalytic efficiency compared to fully processed GAA. We also generated enzymatically 'deglycosylated' GAA that showed improved enzyme kinetics towards glycogen without proteolytic processing. These results indicate that both proteolysis and N-glycan processing are required for full GAA activation. Taken together, these data imply that an optimal ERT for treating Pompe disease should have significantly improved lysosomal targeting to all muscles, along with complete proteolytic and glycan processing in lysosomes for optimal glycogen hydrolysis.

(183) Integrative glycomics and proteomics analysis of myelinated & non-myelinated regions from human control, bipolar disorder & Schizophrenia brain tissues

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Chondroitin sulfate proteoglycans (CSPGs) and hyaluronan aggregates compose a significant portion of the brain extracellular matrix (ECM). CSPGs are involved in crucial brain processes such as brain connectivity, axon guidance, and myelination. Furthermore, CSPG abnormalities have been reported in subjects with schizophrenia (SZ), including thalamo-cortical disconnectivity observed in brain imaging studies. We proposed that CSPG expression in the thalamus may play an essential role in the disruption of thalamo-cortical connectivity.

Preliminary immunohistochemistry results on the mediodorsal nucleus (MD), a large thalamic nucleus, of SZ subjects, showed altered organization of myelinated fibre bundles. Thus, we wanted to explore the differences in CS components in myelinated and non-myelinated regions in MD of the thalamus to better understand the role of CSPGs in myelination. In this study, we utilized our recently published on-slide tissue digestion method, coupled with LC–MS/MS analysis. The method allows precise targeting of regions of interest (1 mm diameter or greater) to perform a serial glycomics and proteomics. We worked on specimens from control (n = 15), SZ (n = 15), and bipolar (BP; n = 15) brains that included both caudal and rostral brain regions. Myelinated (MY; n = 3) and

non-myelinated (NM; n = 3) spots were marked on each tissue slide, resulting in 4 samples per subject and a total of 180 samples for LC–MS/MS analysis. The CS disaccharides were released using chondroitinase ABC enzyme by manual on-slide application followed by trypsin digestion to extract peptides. The extracted disaccharides and peptides were desalted and subjected to LC–MS/MS analysis. The relative disaccharide abundance (%) comparisons showed an increase in unsulfated D0a0 for MY compared to NM samples, which was consistent for all three disease groups: CT, SZ, and BP. Interestingly, a striking increase in 6-sulfated D0a6 and a concomitant decrease in 4-sulfated D0a4 disaccharides was observed specifically for SZ-MY samples as compared to other samples in the group. The proteomics results showed some separation for 180 samples on principal component 2 (PC2) analysis plot for both myelin based grouping as well disease based grouping of the samples. The label-free quantitation using Peaks software analysis revealed 25 and 18 differentially expressed for region-specific, and disease-specific groupings, respectively. The heat map showed the distinctive aberration of these proteins between the groups. Interestingly, the extracellular matrix (ECM) components: Collagens: Col3a1, Col1a2 were among the differentially expressed proteins. We plan to further scrutinize the CS and proteomics dataset for myelin-, region-, and disease-specific changes to understand the underlying pathology. To date, no studies have compared myelinated versus non-myelinated regions. The information will contribute to understand neuropathological underpinnings in SZ.

(184) Enhancing the mass spectrometric detection of glycoforms through multi-stage enrichment.

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Glycosylation which is a highly micro-heterogeneous post-translational modification (PTM) on proteins and lipids regulates various biological processes at the cellular level. Detection of glycopeptides and glycans, particularly the low abundant ones, by mass spectrometry (MS) is challenging owing to the microheterogeneity, poor ionization and ion suppressions due to co-eluting peptides. Enrichment for low abundant glycopeptides is often required for the MS-based detection of glycoproteins, particularly from cells and tissues. We developed a multistage enrichment of glycoproteins for the improved detection of glycopeptides by cellular fractionation, subsequent ZIC-HILIC based glycopeptide enrichment, and a spectral enrichment algorithm for the MS data processing. This enabled improved detection of glycopeptides along with very low abundant glycoforms including metabolically incorporated non-natural monosaccharides that are incorporated at low abundance on the N/O-glycopeptides. We demonstrated the applicability of this probe-free strategy by studying the dynamic nature of glycosylation and direct detection of non-natural sugars via glycoproteomics and glycomics. Remarkably, this addressed a significant drawback of bio-orthogonal probe-based method such as non-specific background reactions and ambiguous assignment of non-natural sugars on glycans. Non-natural functional group bearing monosaccharides installed on glycans through promiscuous sialic acid, GalNAc, and GlcNAc biosynthetic pathways on PC-3, Jurkat and MCF-7 cell lines were successfully detected through this approach. Overall, our method allowed the detection of both natural and non-natural sugar bearing N and O- glycopeptides, differentiation of non-natural monosaccharide types on the glycans and also their incorporation efficiency through quantitation. Moreover, we studied interconversion of monosaccharides during their processing through glycan salvage pathway and subsequent incorporation into glycan chains. This method could be conducted in high throughput as few sample processing steps are involved, enabling study of low abundant glycoforms and thereby elucidating their role in cellular functions.

(185) Sialylated O-glycans are critical for mucus integrity and function in the colon

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The colon lumen is occupied by a large and diverse commensal microbiota. Normally, the luminal bacteria are separated from the epithelium and immune cells in the colon by the mucus layer, which consists primarily of mucins that are heavily modified by mucin-type O-linked oligosaccharides (O-glycans). Our published papers have demonstrated that two types of complex O-glycans, i.e. core 1- and core 3-derived O-glycans, are critical for the mucus barrier function (Fu et al. 2011; Bergstrom et al. 2016). Our recent study indicates that the proximal colon-derived O-glycans are essential for the mucus barrier and provide an enclosed ecosystem for microbiota in the whole colon. Sialic acid is an important capping structure on all O-glycans. New unpublished findings in our lab have shown a strong association between O-glycan sialylation status, mucin stability, and mucus barrier function. To further demonstrate the biological function of sialylation, we have generated a novel mouse model with intestinal epithelial-specific deletion of the *Slc35a1* gene that encodes the CMP-sialic acid transporter, which is required for sialylation of glycans in the Golgi. Mice with intestinal epithelial-specificdeficiency of *Slc35a1* had impaired mucus

barrier function and spontaneous colitis. In addition, we found that these mutant mice exhibited delayed tissue regeneration process after dextran sulfate sodium-induce injury in vivo, and altered proliferation of the colonic organoids ex vivo relative to controls. In summary, our data provide new insights into how host sialylated O-glycans regulates mucus barrier homeostasis and function by protecting mucins from bacteria-mediated degradation, and it may suggest the utility of sialylated O-glycans to enhancing mucus barrier function to treat colitis.

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(186) Geometric and energy calculations to predict protein stability and activity changes due to site-specific glycosylation

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Glycosylation affects the properties of a protein from its folding and stability to its intermolecular interactions and function. However, there are a few tools to predict the structural and functional effects of glycosylation at newly introduced glycan acceptor sites. We have calculated five geometric measures and two energy calculations (with Rosetta) to identify predictive factors behind experimentally observed changes in expression, stability, binding and catalytic activity. We compare to shotgun scanning glycomutagenesis experiments on three architecturally diverse proteins: bacterial immunity protein Im7, bovine pancreatic ribonuclease-A, and a human anti-HER2 single-chain Fv antibody. The three systems show different correlations with computed metrics, likely because of their varying rigidities and architectures. The results indicated that interactions between the glycan and the bound protein must occur for the binding activity to be altered (positively or negatively) and that enhanced binding likely arises from multiple low-energy glycan conformations making favorable interactions with the binding partner.

Our work suggests that binding affinity of a protein might be purposefully increased by rational introduction of N-glycans at predetermined locations for glycan-mediated affinity enhancement.

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(187) Assessing Activity of a Neisseria meningitidis Serogroup W Glycosyltransferase with a Homogenous Acceptor by UDP-Glo Bioluminescence Assay

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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. We hypothesize that the capsule polymerase can be used as a new tool for synthesis of sugars in the development of homogenous glycoconjugate vaccines. In this work, we describe our efforts to determine kinetic parameters of the enzyme with an unlabeled homogeneous acceptor using UDP-Glo bioluminescence assay (Promega). These parameters will inform our work to control synthesis by the serogroup W enzyme and produce sugars that can potentially be used in glycoconjugates. Reactions were performed in a 10-minute reaction period to determine Km and Vmax values using sialic acid trimer (DP3) as an acceptor. In our studies with this acceptor, we confirm an increase in product formation with increasing enzyme amounts (0–1250 ng enzyme). Km values for UDP-Galactose and DP3 acceptor were 44.61 μM and 2984.2 μM, respectively. The Vmax values were similar in magnitude for both ~0.01 μM/min. To determine the kinetic parameters of sialyltransferase activity, DP3-Galactose was made enzymatically by reaction of W-enzyme (89.25 μg/mL), with UDP-Galactose and DP3 (each at 4 mM) for 16 h. Product formation was confirmed by the visualization of new spots after TLC using the p-Anisaldehyde sugar staining method. Our data provides initial kinetics data for the DP3 acceptor which was previously unknown and suggests that we can use DP3-derived acceptors as tools to obtain size-controlled sugars. In future work, we will perform kinetic studies with a DP3-Galactose (and longer chain length acceptors) to determine Km and Vmax for acceptor and nucleotide donor sugar substrates. (NIH SC2GM125517).

(188) O-GlcNAcylation induced hypomethylation and changes in chromatin accessibility of transposable elements

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Excess glucose intake in the western diet leads to metabolic disease such as obesity. At the cellular level, increase in glucose intake can also result in overproduction of O-GlcNAc and increase in protein O-GlcNAcylation. Like other protein modifications, O-GlcNAcylation can impact the function of proteins. Recently, O-GlcNAcylation of chromatin regulators has been shown to be required to silence retrotransposon promoters. These observations suggest that alterations of O-GlcNAc levels influence function of transcriptional regulators resulting in changes in gene expression. Here, we explore the additional causes and consequences of increased O-GlcNAc production in obesogenic conditions, mainly increase in inflammation. We treated cells with TNFα to mimic the inflammatory state of obesity and observed a general increase in the levels of O-GlcNAcylatedproteins in TNFα treated cells. To examine the potential impact of increased O-GlcNAcylation on chromatin and transcriptional regulation, we performed ATAC-seq on hepatocytes (HepG2) treated with TNFα and Thiamet-G (TMG; O-GlcNAcase inhibitor), to increase O-GlcNAcylation. Comparative analysis of chromatin accessibility defining differences and similarities revealed O-GlcNAcylation impacts on chromatin and transcriptional regulation. We have furthermore found that TNFα and Thiamet-G leads to specific demethylation and chromatin accessibility changes of transposable element regions. These results broaden our understanding of the role of O-GlcNAcylation in transcriptional and chromatin regulation.

(189) Absence of Polysialic Acid Enhances Survival of Mice after Infection with *Streptococcus pneumoniae*

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Polysialic acid (polySia) is a unique posttranslational modification of a select group of cell surface proteins with a role of guiding cell to cell interactions and sequestering soluble ligands. Its importance in the development of the central nervous system in the form of polysialylated neural cell adhesion molecule (NCAM) is well established. We and others have demonstrated regulated expression of polySia in human and murine leukocytes during cell activation and differentiation and suggested several in vitro roles for this glycan. In this study, we utilized polysialyltransferase IV (ST8Sia IV$^{-/-}$) knock-out mice, which are deficient in polySia in leukocytes, to evaluate the overall impact of polySia during bacterial pneumonia. Wild type (WT) and ST8Sia IV$^{-/-}$ C57/BL mice were infected intratracheally with a lethal dose of *Streptococcus pneumonia (Spn)*. Whereas WT mice became severely ill and died within 4–5 days after infection, ST8Sia IV$^{-/-}$ mice were less susceptible to *Spn* infection, either surviving the infection or becoming ill after a significantly longer period. Consistent with this finding, bronchoalveolar lavage (BAL) of ST8Sia IV$^{-/-}$ mice revealed significantly fewer colony forming units of *Spn*, 24 hr after infection as compared to WT mice. Leukocyte infiltration to the lungs of ST8Sia IV$^{-/-}$ mice was diminished in comparison to WT mice as evident by flow cytometry and immunohistochemistry. This difference in cell migration was corroborated by adoptive transfer of monocytes and neutrophils from WT and ST8Sia IV$^{-/-}$ mice into *Spn*-infected WT mice. Furthermore, BAL fluid from WT and ST8Sia IV$^{-/-}$ mice revealed unique cytokine profiles. One possible explanation for the greater survival of ST8Sia IV$^{-/-}$mice was the significantly greater phagocytosis of Spn that we detected *in vitro* using activated peritoneal macrophages from ST8Sia IV$^{-/-}$ as compared to WT mice. These data demonstrate that polySia likely guides numerous processes on leukocytes during different stages of an immune response, and that its absence on leukocytes has an overall beneficial effect during systemic infection.

(190) An enzymatic toolkit for selective proteolysis, detection, and visualization of mucin-domain glycoproteins

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Mucin domains, characterized by a high density of O-glycosylated serine and threonine residues, are utilized by nearly every cell of the human body to alter the properties of cell surface and secreted proteins. Proteins bearing these modular domains are critical to biological processes involved in embryogenesis, host-pathogen interactions, and disease progression. For example, the mucin MUC1 is overexpressed in >90% of breast tumors and MUC16 is a key clinical biomarker for treatment efficacy and surveillance in ovarian cancer. These correlations have been challenging to uncover and establish, in part due to difficulties associated with the unique biochemical properties of mucins. Mucins are often thousands of amino acids long and > 50% glycosylation by mass, rendering them difficult to purify and proteolyze. In addition, existing staining reagents that recognize specific glycoforms are not selective for glycans in mucin domains while mucin-specific antibodies do not provide a picture of global mucin composition. As a result of these shortcomings,

a comprehensive list of mucin-domain glycoproteins does not exist, and the mechanisms that contribute to the biological and pathological roles of mucins remain underexplored.

To overcome these challenges, we turned to microorganisms that colonize mucosal host environments to find enzymes capable of digesting mucins. We purified candidate mucin-degrading proteases ("mucinases") from pathogens and commensals with selectivity for mucin-type O-glycosylated proteins. Manual validation of peptide sequences from MS/MS spectra revealed the distinct peptide- and glycan-specificities of each bacterial mucinase. These mucinases were then engineered and chemically labeled to generate staining reagents for flow cytometry, Western blot, and immunohistochemistry, enabling the study of dynamic changes in mucin expression in a glycosylation-dependent manner. Successful application of our enzyme-based mucin staining strategy to primary tissue from patients highlights the potential for this approach to deepen our understanding of basic mucin biology and bulk changes that result in disease states.

(191) High expression of ST6Gal-I promotes a stem-like phenotype in pancreatic epithelial cells

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ST6Gal1, a glycosyltransferase that adds α2–6-linked sialic acids to N-glycosylated proteins, is highly expressed in cancer cells, and some stem cell populations, however many differentiated epithelia, such as pancreatic acinar cells, have very low ST6Gal1 expression. Previous studies in our lab have shown that ST6Gal-I acts as a pro-survival factor for pancreatic cancer cells by inhibiting apoptosis induced by growth factor withdrawal, hypoxia, and TNFR1 death receptor signaling. However, little attention has been given to the role of ST6Gal-I in the normal pancreas. In this study, we monitored changes in ST6Gal-I expression as stem cells undergo differentiation, and also interrogated a putative pro-survival function for ST6Gal1 in nonmalignant epithelial cells. To this end, 3D organoid models were established from the pancreatic tissues of wild-type mice or ST6Gal1-overexpressing genetically modified mice (referred to as "SC" mice). Organoid lines are derived from tissue-resident stem cells and maintain a stem cell phenotype during organoid culture. We find that a greater number of organoids was initially formed from SC-derived cells, and these organoids grew more rapidly than organoids from WT cells, implicating ST6Gal1 activity in promoting stemness and cell viability. SC organoids also expressed higher levels of stemness genes such as Sox9 and Hes1. To induce epithelial differentiation, cells from organoids were placed into adherent monolayer culture in media lacking stem cell factors. When WT organoid cells were forced to differentiate, ST6Gal1, in tandem with other stemness genes (e.g., Axin2), was significantly downregulated, supporting ST6Gal-I as a novel biomarker of stemness. Forced cell differentiation is known to induce exit from the cell cycle and ultimately, cell death. Notably, SC, as compared with WT, cells, maintained higher expression of Sox9 and Axin2 stemness genes in monolayer culture, and were protected against differentiation-induced cell death. Preliminary data suggest that one prominent mechanism responsible for suppressing ST6Gal1 expression is methylation of the P3 promoter, which drives expression of the ubiquitously-expressed "YZ" ST6Gal1 isoform. These collective results provide new insight into mechanisms that control both ST6Gal1 expression, and its functional role in promoting a stemness phenotype.

(192) Glycans as immune checkpoints: removal of branched N-glycans enhances immune recognition preventing cancer progression

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Colorectal cancer (CRC) is one of the major causes of morbidity and mortality worldwide, being an important target of study. Alterations of the glycosylation signature of tumor cells have been shown to be instrumental for the regulation of key mechanisms underlying cancer progression (Pinho & Reis, Nature Rev Cancer 2015). We have been characterizing extensively the tumor glycome, demonstrating that malignant cells overexpress the tumor-associated β1,6-GlcNAc branched N-glycans that have been associated with invasive and metastatic phenotypes and with poor prognosis in gastric

cancer patients (Pinho & Reis, Nature Rev Cancer 2015; Carvalho et al, Oncogene 2016; Verhelst et al. Gastroenterology 2019). Although transformed cells can be recognized by the immune system through immunosurveillance, tumor cells are also able to evade to anti-tumor immune response, through the so-called cancer immune escape process (Dunn el al., Nature Immunology 2002). However, it remains elusive the role of this pro-tumoral N-glycan in immune evasion and whether its removal contributes to enhance immune recognition and to unleash an anti-tumor immune response.

Our results showed that along colorectal carcinogenesis cascade there is an overexpression of β1,6GlcNAc branched N-glycans by cancer cells that follow the same tendency as FoxP3-expressing cells. In addition, we established a positive correlation between MGAT5 and FOXP3 mRNA expression in CRC stage. Ex vivo and in vivo experiments demonstrated that branched N-glycans are used by CRC cells to escape immune recognition, instructing the creation of immunosuppressive network. Cancer cell line overexpressing complex branched glycans co-cultures with selected immune cells (PBMC; DC and T cells), demonstrate a suppression of pro-inflammatory responses, by decreasing Th1 differentiation and reducing IFN-γ and IL-8 cytokine production. In accordance, the removal of this "glycan-mask" was shown to expose immunogenic glycan epitopes that potentiates immune recognition by specific C-type lectins receptors expressed in immune cells resulting in an effective anti-tumor immune response. Mouse models that spontaneously develop CRC cancer harboring the absence of Mgat5-mediated branched N-glycans ($Apc^{Min/+}Mgat5^{-/-}$ and $VCMsh2^{LoxP/LoxP}Mgat5^{-/-}$ mice) develop a lower number of tumor lesions with lower size when compared to respective $Mgat5^{+/+}$ controls, by the improvement of anti-cancer immune response (Silva & Fernandes et al; Cancer Immunology Research 2020).

In conclusion, we have revealed a novel glycoimmune-checkpoint in CRC, highlighting the therapeutic efficacy of its deglycosylation to potentiate immune recognition improving cancer immunotherapy.

(193) Novel insights into the fucose metabolism – challenging the old dogma

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

Using differentially labelled ^{13}C-Glc, Man and Fuc we studied contributions of each pathway to Fuc in N-glycans. In absence of exogenous Fuc, *de novo* and salvage equally contribute to fucosylation. Low concentrations of exogenous Fuc progressively inhibit the de novo pathway, and completely by ~50 μM. Surprisingly, GDP-Fuc derived from Glc is more sensitive to this inhibition than that derived from Man, although both contribute to GDP-Man synthesis. Exogenous Fuc is preferred over Fuc located in a separate salvage pathway. Cells seem to distinguish multiple pools of GDP-Fuc based on their origin.

Next, we verified whether observed preference of exogenous Fuc is simply because it increases the total GDP-Fuc pool. No, it did not, even at 50 μM, but we think it selectivity increases a separate GDP-Fuc pool over that of recycled Fuc and *de novo* produced one.

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

Using LC–MS/MS and eight different cell lines, we studied the ongoing biosynthesis of >150 fucosylated N-glycans containing 1–4 Fuc residues by comparing the proportion of ^{13}CFuc incorporation at various concentrations for 24 h. Over the labeling period, cells had to choose between GDP-Fuc synthesized *de novo* or from exogenous fucose. N-glycans with a single Fuc show a proportional increase in ^{13}C-labeled species with increasing concentration of label. To our surprise, glycans with 2, 3, and 4 Fuc each required progressively higher exogenous ^{13}C Fuc to become fully labeled.

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

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(194) Mycobacterial lipoglycan biosynthesis helps maintain cell envelope integrity specifically under biofilm growth conditions

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Mycobacteria, which include the causative agents of severe and widespread human diseases, comprise a clinically important clade of Actinobacteria with distinct cell envelope structure. The success of mycobacterial pathogens and the difficulty in treating them can largely be attributed to a battery of unusual virulence factors and structural elements that make up the cell envelope, including a variety of glycosylated lipids. Lipomannan (LM) and lipoarabinomannan (LAM) are two immune-modulating glycolipids important for virulence in pathogenic mycobacteria such as *Mycobacterium tuberculosis*, and are thought to be core components of the mycobacterial cell envelope. While the biosynthetic pathway of LM and LAM is well characterized and mutants lacking LM and LAM are reported to have altered cell surface characteristics, the structural/physiological role of LM and LAM during growth remains poorly understood. We previously created a conditional gene knockdown strain of *Mycobacterium smegmatis* for the gene *mptA*, which encodes a mannosyltransferase that elongates the mannan backbone of LM, and is required for the biosynthesis of both LM and LAM. While characterizing this mutant, we discovered that knocking down *mptA* expression in Mycobacterium smegmatis biofilm culture, but not in planktonic culture, results in abnormal levels of cell lysis and blebbed cell morphology as the biofilm matures. These defects are rescued in osmo-protective growth medium, demonstrating that the observed lysis is due to a failure of cells to withstand internal turgor pressure. These data show for the first time that mycobacterial lipoglycans are important for maintaining cell envelope integrity specifically under pellicle-biofilm growth conditions. We are currently creating a transposon mutant library of *mptA* knockout mutant to identify mutations that suppress the biofilm defects.

(195) Determination of the function of the A064R protein produced by *Paramecium bursaria* chlorella virus

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Paramecium bursaria chlorella virus 1 (PBCV-1) is a member of the Giant Viruses group: a group of viruses that differ from other viruses in shape, genome size and number of encoded proteins [1]. PBCV-1 is a large dsDNA virus that infects the microalgae *Chlorella variabilis* NC64A [2]. Analysis of its genome revealed that it encodes, at least, six putative glycosyltransferases (GTs), probably involved in glycosylation of its major capsid protein (MCP), named Vp54 (gene *a430l*) [3]. Interestingly, the structure of these glycans differ from all the other N-glycans known so far in the three domains of the life [4].

Comparing the glycan structures of the wild-type PBCV-1 with those from a set of PBCV-1 spontaneous mutants (or antigenic variants) [5], it was possible to establish the role of one of the six GTs: the *a064r* gene. This gene encodes a protein (A064R) of 638 amino acids organized into three domains of approximately 200 aa each, and each with a particular function [5].

Structural analyses, in combination with enzymatic reactions, disclosed that each domain has a transferase activity, demonstrating that the virus PBCV-1 does not rely (at least not entirely) on the host machinery to glycosylate its MCP [6].

These findings provide the first experimental evidence that PBCV-1 encodes a functional GT harbouring three different activities. Thus, the virus PBCV-1 does not rely (at least not entirely) on the host machinery to glycosylate its MCP [7].

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(196) Heterozygous De Novo Mutations in NUS1 Cause a Movement Disorder with Combined Lysosomal and Glycosylation Defects

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NUS1 encodes the Nogo B receptor (NgBR), a multifunctional protein with roles in the formation of N-glycosylation precursors, intracellular cholesterol transport, and in cell adhesion and chemotaxis through its ability to bind the ligand Nogo-B. Variants in *NUS1* have been associated with a congenital disorder of glycosylation, developmental and epileptic encephalopathies, and also suggested to contribute to the pathogenesis of Parkinson's disease. How its diverse functions relate to these different phenotypes is largely unknown. Here we present three patients with *de novo* heterozygous variants in *NUS1* that cause a complex movement disorder characterized by ataxia, seizures, tremor and other neurological features. Using skin fibroblasts from two patients, we show that these variants result in reduced NgBR and NPC2 protein levels, impaired dolichol/polyprenol biosynthesis and N-linked glycosylation, and accumulation of intracellular cholesterol. Overexpression of *NUS1* cDNA bearing one of these variants in HeLa cells (Gly245Val) does not cause any cellular phenotypes, supporting haploinsufficiency as a mechanism for the loss of function variants. Suppression of nus1 in zebrafish embryos results in abnormal swimming behavior, cholesterol accumulation in the nervous system and impaired turnover and/or proteolysis of lysosomal membrane proteins. Reduction of cholesterol buildup following cyclodextrin treatment significantly alleviated lysosomal proteolysis and motility defects. These findings demonstrate that heterozygous *de novo NUS1* variants are associated with a broad phenotypic spectrum in humans. We further demonstrate that movement deficits associated with nus1 haploinsufficiency in zebrafish arise in part from defective efflux of cholesterol from lysosomes, suggesting a possible therapeutic approach.

(197) Generation of an Unbiased Interactome for the Tetratricopeptide Repeat Domain of the O-GlcNAc Transferase Indicates a Role for the Enzyme in Intellectual Disability

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The O-GlcNAc transferase (OGT) is localized to the nucleus and cytoplasm where it regulates nucleocytoplasmic proteins by modifying serine and threonine residues with a non-extended monosaccharide, β-N-Acetyl-Glucosamine (O-GlcNAc). With thousands of known O-GlcNAc modified proteins but only one OGT encoded in the mammalian genome, a prevailing question is how OGT selects its substrates. Prior work has indicated that the N-terminal tetratricopeptide repeat (TPR) domain of OGT, rather than its C-terminal catalytic domain, is responsible for subcellular targeting and substrate selection. Additionally, missense mutations in OGT associated with X-linked intellectual disability (XLID) are primarily localized to the TPR domain without substantial impact on activity or stability of the enzyme, suggesting these mutations might affect TPR protein associations. Therefore, we adapted the BioID proximity proteomic method to identify interactors of a TPR-BirA* fusion protein in HeLa cells. We identified 115 high confidence interactors representing both known and novel O-GlcNAc modified proteins and OGT interactors. The TPR interactors are highly enriched in processes in which OGT has a known role (e.g. chromatin remodeling, cellular survival of heat stress, circadian rhythm), as well as processes in which OGT has yet to be implicated (e.g. pre-mRNA processing). Importantly, the identified TPR interactors are involved in several disease states but most notably are highly enriched in pathologies featuring intellectual disability. These proteins represent candidate interactors that may underlie the mechanism by which mutations in OGT lead to XLID. Furthermore, the identified interactors provide additional evidence of the importance of the TPR domain for OGT targeting and/or substrate selection. Thus, this defined interactome for the TPR domain of OGT serves as a jumping off point for future research exploring the role of OGT, the TPR domain, and its protein interactors in multiple cellular processes and disease mechanisms, including intellectual disability.

(198) Correlation Between Increased Serum Levels of Galectins − 1, −3 and − 9 with the p.Gln472His Mutation in VEGFR-2 in Breast Cancer Patients

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Galectins are members of the B-galactoside-binding protein family, which are capable of binding carbohydrates across a range of biochemical pathways. Fifteen human galectins have been discovered, with galectins −1, −3, and − 9 implicated in cancer progression, metastasis, and angiogenesis, and also in modulating innate and adaptive immune responses. Forty breast cancer patient samples of different stages (I through IV) were obtained from the Biorepository of the Prisma Health Cancer Institute, to measure serum levels of the above-mentioned galectins by ELISA. Average levels of all three galectins were significantly elevated in the breast cancer patients (except for galectin −1 in stage II) compared to healthy controls. Additionally, analysis of DNA sequences of 50 different oncogenes from the same patients was conducted. The galectin profiles and the genetic mutations data were added to enhance the

Prisma Health cancer patient database. We then looked for possible correlations between the levels of these galectins with mutations in these genes. Of the 50 genes analyzed, three of them, encoding VEGFR-2, PI3KCA, and TP53, had the highest number of mutations in these 40 patients. Interestingly, the only type of mutation found in the VEGFR-2 gene, was p.Gln472His. This mutation was present in 11 of 40 patients of all stages (27.5%), and correlated strongly with increased galectin expression as all but two of the 11 patients also had increased serum levels of galectins-1, −3 and − 9. In comparing the averages of serum galectin levels between patients with the aforementioned point mutation against those without the point mutation for each breast cancer stage, all serum level averages were higher for each of the three galectins in patients with the point mutation, except in stage III, where the level averages for all three galectins were lower for patients with the mutation. The most significant elevation was observed for galectin-3 level average in breast cancer stage IV in patients with the Gln472His mutation compared to the patients of the same cancer stage without the mutation. Galectin-1 is thought to promote phosphorylation and cell surface clustering of the VEGFR-2 receptor and is elevated in breast cancers. Galectin-3 prevents internalization of the receptor, and increases angiogenesis in tumor cells, while galectin-9 prevents G2 cell-cycle arrest promoted by both the VEGFR-2 and TP53 pathways. The VEGFR-2 point mutation p.Gln472His has previously been implicated in 95% of VEGFR-2-related mutated colon cancers (Jauhri et. al 2017) and is present in 35% of melanomas (Silva et. al 2016). Our finding further expands these concepts and provides opportunity for further investigation into the possible roles of galectin family members in cancer progression.

(199) Brain glycogen serves as a critical glucosamine cache required for protein glycosylation

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Glycosylation defects are a hallmark of many nervous system diseases. However, the molecular and metabolic basis for this pathology are not fully understood. In this study, we found that N-linked protein glycosylation in the brain is coupled to glucosamine metabolism through glycogenolysis. We discovered that glucosamine is an abundant constituent of brain glycogen, which functions as a glucosamine reservoir for glycosylation precursors. We defined the incorporation of glucosamine into glycogen by glycogen synthase and release by glycogen phosphorylase in vitro by biochemical and structural methodologies, *in situ* in primary astrocytes, and *in vivo* by isotopic tracing and mass spectrometry. Using mouse models of two glycogen storage diseases, we showed that disruption of brain glycogen metabolism causes global decreases in free UDP-N-acetyl-glucosamine and N-linked protein glycosylation. These findings revealed key fundamental biological role for brain glycogen in protein glycosylation with direct relevance to multiple human diseases of the central nervous system.

(200) SWATH investigation of yeast glycogenin-1 and glycogenin-2 functions

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Glycogen is a hyperbranched glucose polymer existing widely in animals, fungi and bacteria. It functions as a sugar buffer to maintain glucose balance. Its biosynthesis starts from glycogenin, a self-glucosylating protein, to form a short chain oligosaccharides which is further elongated by glycogen synthase via $(1 \rightarrow 4)$-α linkages and branched by glycogen branching enzyme via $(1 \rightarrow 6)$-α linkages. There are two isoforms of glycogenin in yeast, Glg1p and Glg2p, and existence of either of them is reported to be sufficient for glycogen priming. However, no functional differences have been found between them. Here, we employed SWATH-MS combined with bioinformatic analysis to investigate their biological roles in yeast. In general, yeast lacking both genes is impaired with pathways including glycolysis, trehalose biosynthesis, and protein folding. Yeast lacking GLG2 is nearly depleted of glycogen and trehalose while the GLG1 KO strain is only partially impaired. In conclusion, our data supports similar roles for GLG1 and GLG2 in regulating metabolism, with glycogen and trehalose biosynthesis as the major functional difference.

(201) Tools for the investigations of sialic acid-protein interactions based on metabolic modulation of sialoglycans

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Sialic acids, a family of nonulosonic acids containing more than 50 distinct chemical structures, are known to participate and regulate many biological and immunological

processes. By virtue of their location, at the termini of glycans on proteins and lipids and the negative charge, sialic acids provide 'the first point-of-contact' for cell–cell, cell-matrix, and cell-pathogen interactions. The roles of sialoglycans are well established in physiological processes such as leukocyte migration, extravasation, and dynamic modulation of cell adhesion. These processes are mediated by specific interaction of sialic acids with a variety of proteins, including selectins and sialic acid-binding immunoglobulin-like lectins (siglecs). Particularly, the interaction of sialyl-Lewis-X (sLeX) glycans present on leukocytes with E-selectin (CD62E) expressed by the endothelial cells is the first step in the extravasation processes. However, the processes governing the extravasation of a selected subset of activated leukocytes are poorly understood.

In addition to the modern genetic, biochemical, and enzymatic tools, the application of synthetic small molecule precursor based metabolic glycan engineering (MGE) in combination with bioorthogonal ligations provides a vital and complementary approach. MGE, first reported by Reutter and pioneered by Bertozzi and others, has become an indispensable tool for chemical glycobiology during the past two decades. Several N-acetyl-D-mannosamine (ManNAc) analogues carrying chemical functional groups including N-acyl, N-azidoacetyl, N-glycolyl, N-thioglycolyl, N-alkynyl, N-alkyl-diazirinyl, and others have been shown to be efficiently metabolized to the respective sialic acids in mammalian cells and animals. However, the chemical space of ManNAc analogues remains under-explored. Herein, we present design, synthesis, and characterization of a panel of ManNAc analogues with modified N-acyl chains and their metabolic processing in a variety of mammalian cells. The metabolic efficiency was measured indirectly using competitive processing of peracetyl N-azidoacetyl-D-mannosamine (Ac_4ManNAz) and strain-promoted azide-alkyne cycloaddition (SPAAC). Expression of N-azidoacetyl-neuraminic acid (NeuAz)-carrying glycans on the Jurkat (human T-lymphoma) cell surface, as measured by flow cytometry after SPAAC, was found to be reduced by ~40–50% upon treatment with the ManNAc analogues. The modified sialic acids were characterized using 1,2-diamino-4,5-methylenedioxybenzene (DMB) labeling and HPLC with fluorescent detection. The changes to cell surface sialoglycans on HL-60 (human acute myeloid leukemia) cells were investigated using anti-CSLEX (CD15s), HECA452 (anti-human cutaneous lymphocyte antigen), and E-selectin by flow cytometry. Results of the effect of metabolic modulators on sialoglycan expression, their affinity to sialic acid binding proteins, and effects on cell adhesion will be discussed in this presentation. Expanded tool box of small molecule ManNAc analogues have the potential to modulate and enable gleaning of fundamental roles of sialoglycans in immunological processes.

(202) The GTXplorer portal to access, navigate and mine evolutionary relationships of fold A glycosyltransferases

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Summary:

Our recent studies (Taujale et al. 2020) on the classification of fold A glycosyltransferase (GT-A) sequences have resulted in a wealth of information on the sequence, structure, function and evolution of these proteins. Here we present GTXplorer, a user-friendly application interface to visualize the evolutionary information for the fold A glycosyltransferases. Access to sequence, classification, alignment and phylogenetic information is maintained through an interactive phylogenetic tree where users can select their clade, family or sub-family of interest. GTXplorer presents a unified package for in-silico analyses of GT-A fold families and provides a means to generate testable hypotheses to conduct targeted biochemical experiments towards the advancement of glycobiology research.

Feature Highlights:

- The landing page of GTXplorer features an interactive visualization of the GT-A phylogenetic tree that is used to access pertinent information about clades, families or subfamilies.
- Download links provide direct access to raw data along with sequences and their alignment.
- The alignment viewer in GTXplorer allows users to visualize stacked weblogos from multiple clades, families or subfamilies overlaid with motif annotations, family constraints and other information to perform comparative sequence analyses and draw associations across families.
- GTXplorer also has a portal for submitting query sequences to perform the following:

1. Identify GT-A fold enzymes and classify them into respective families and subfamilies.
2. Align the query sequences with the GT-A profiles to map the conserved motif positions and family specific constraints.
3. Run query sequences through the machine learning model to predict their donor substrate and identify residue posi-

tions that putatively contribute to donor substrate specificity.

Availability

A demo version of GTXplorer for collecting feedback and review is available at (with limited features): https://uga-gta.netlify.app/

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(203) SIGLEC16 and polysialic acid are linked to increased survival of glioblastoma patients

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Polysialic acid (polySia) is a major regulator of brain development, but also an oncodevelopmental antigen that has been linked to tumor malignancy. In glioblastoma (GB), one of the most aggressive primary brain tumors, polySia has been repeatedly detected, but its frequency of occurrence and prognostic value are controversial. Recently, polySia has been implicated in modulating the activity of microglia and macrophages. In humans, polySia is sensed by microglia and macrophages through the human-specific paired immune receptors Siglec-11 and Siglec-16, coupled to inhibitory (ITIM) and activating (ITAM) signaling, respectively. However, less than 40% of the human population has a *SIGLEC16* allele coding for functional protein expression. Therefore, and because the interactions of tumor cells with tumor-associated microglia and macrophages (TAM) are critical for glioblastoma progression, the current study explores the possible impact of *SIGLEC16* status and polySia on GB outcome. SIGLEC16 status and tumor-associated polySia was analyzed and related to overall survival after primary tumor resection in two independent cohorts of 70 and 100 GB patients, respectively. By immunofluorescence, polySia was detected in about 80% of primary tumors and localized to the tumor cells. About 45% of the patients were heterozygous for a functional *SIGLEC16* allele ($SIGLEC16^{+/P}$) and the corresponding presence or absence of Siglec-16 protein on TAM in GB could be demonstrated using a newly developed Siglec-16-specific antibody. No subjects homozygous for functional *SIGLEC16* alleles were detected in any of the GB cases and compared to the 4.4% of $SIGLEC16^{+/+}$ individuals in the overall population, this genotype was significantly underrepresented in GB. For both cohorts, we found increased overall survival of $SIGLEC16^{+/P}$ patients with polySia-positive tumor cells. Consistent with the activating downstream signaling of Siglec-16, higher expression of TNF was detected in polySia-positive tumors of specifically the $SIGLEC16^{+/P}$ patients. This indicates that proinflammatory activation of TAM causes the better outcome in these patients. We therefore propose prognostic and therapeutic relevance of the polysialic acid-Siglec-16 axis, which may also apply to other polySia-positive tumor entities.

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(204) Biological evaluation and molecular modeling of peptidomimetic compounds as inhibitors for O-GlcNAc transferase (OGT)

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The vital enzyme O-linked β-N-acetylglucosamine transferase (OGT) catalyzes the O-GlcNAcylation of intracellular proteins coupling the metabolic status to cellular signaling and transcription pathways. Aberrant levels of O-GlcNAc and OGT have been linked to metabolic diseases as cancer and diabetes. Therefore, the present work focuses on the study of peptidomimetic compounds derived from isomannide and isosorbide as a novel class of competitive inhibitors of

OGT. Here, we highlight the compound LQMed 330, which presented an IC_{50} of 11.7 μM, compared to the most potent inhibitors found in the literature. The molecular docking studies showed that the most active compound (LQMed 330) realized hydrogen bond interactions with N-Catalytic (Asn557 and His562) and C-Catalytic domain (Gln839, Lys898, Thr922, and Asp925), which form the catalytic binding site.

Molecular dynamics simulation of LQMed 330 into the OGT binding site provided insight into the behavior by which this compound interacts with the enzyme. RMSD values of the inhibitor atoms indicates that LQMed 330 (3.37 ± 0.47 Å) remains stable in the active site with movements of one of the N-carboxybenzyl groups. The analyses of the hydrogen bonds in the OGT_{330} complex over to 80 ns of MDS shows that the most important interactions occurred with residues Gln839 and Lys898 with a lifetime greater than 70%, while interactions with residues Asn557 and Thr922 occurred with a lifetime less than 10% but dispersed throughout MDS (SD3). Therefore, it is possible to suggest that the maintenance of interactions by hydrogen bonding with residues at the N-cat domain could be one of the explanations for the high inhibition activity of LQMed 330.

The identification of LQMed 330 allows us to consider it as a new lead compound from the peptidomimetic class for future design and synthesis of OGT inhibitors. Therefore, as further directions, we consider the experimental studies on toxicity, carrying out tests on cells and improving some pharmacokinetic parameters, finding a balance between solubility, permeability and size.

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(205) The L-rhamnose biosynthetic pathway in Trichomonas vaginalis

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Trichomonas vaginalis (Tv) is a flagellated parasitic protozoan; it represents the most common non-viral agent for sexually transmitted diseases in the world. The global prevalence has been estimated at 8,1% for woman and 1,0% for men, with about hat 97% of cases occurring in developing countries with limited access to health care, where Tv has been associated with an increased risk of HIV infections. Moreover, Tv can cause low weight at birth and prematurity during pregnancy and induces an augmented risk of cervical and prostate cancer. Metronidazole and related molecules are currently used for therapy; however, persistent and recurrent infections are presently observed, due to emerging antimicrobial insensitive TV strains [1]. Resistance to nitroimidazole based molecules is of great concern, since few alternatives to standard therapy exist; thus, new targets for drug development are required.

Adhesion of the protozoan to the host epithelial tissues is mediated by specific proteins and by the lipophosphoglycan (LPG). LPG is anchored to the cell surface by an inositol-ceramide structure and it is composed by several different monosaccharides. Tv mutants with modified LPG composition show lower ability to adhere to host epithelial cells and to induce inflammation [2]. The exact structure of LPG is still debated, but it has been proposed to contain a α1,3 poly-L-rhamnose backbone [3]. Thus, interference with L-rhamnose metabolism, by altering LPG structure, could be potentially exploited as a new therapeutic target. This is of particular interest since many studies are now focusing on the identification of new compounds inhibiting the L-rhamnose biosynthetic pathway in bacteria, to overcome the increasing antibiotic resistance [4]. Thus, identification and characterization of the enzymes involved in L-rhamnose production in Tv will allow testing of the lead compounds identified for the bacterial systems.

We have identified and characterized the first enzyme of the L-rhamnose pathway in Tv, the UDP-D-glucose 4,6-dehydratase (UGD), which converts the substrate to the first intermediate UDP-4-keto-6-deoxy-D-glucose. As already reported for other dehydratases, Tv UGD can use also dTDP-D-glucose as substrate, albeit with lower efficiency. Preliminary data on the following enzymes of the pathway suggest that Tv L-rhamnose biosynthesis, at variance with other eukaryotic pathways, occurs following a bacterial-like strategy, which includes three separate activities: the 4,6-dehydratase, a 3,5-epimerase and a NADPH-dependent 4-reductase. Due to high degree of gene duplication, typical of Tv genome, several putative epimerases and reductases are present. Experiments are in progress to characterize also these proteins.

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(206) 3'-Sialyllactose on Notch: NOTCH1 functions as a scafold of O-linked, 3′-sialyllactosylated glycans

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Notch signaling is important for the development and homeostasis of multicellular organisms. Dysregulation of Notch signaling leads to various human diseases. Genetic and biochemical studies have revealed that O-linked glycosylation on Notch receptors is essential for the transduction of Notch signaling. However, it is not fully understood how O-linked glycans regulate the activity of Notch signaling. To pursue this, we need to know the sites and structures of O-linked glycans on Notch receptors. Here, our mass spectrometric analysis of proteolytic digests derived from mouse NOTCH1 and 2 overexpressed in HEK293T cells revealed that many of the epidermal growth factor-like (EGF) repeats of NOTCH1 and 2 are modified with O-glucose (Glc), O-fucose (Fuc), and O-GlcNAc glycans at different stoichiometries. For example, most of the EGF repeats with the O-Glc consensus sequence are modified with a conventional trisaccharide (Xyla1–3Xyla1–3Glc-O), but a subset of EGF repeats are not. Surprisingly, we discovered sialylated, hexosylated O-Glc glycans specifically attached to the O-Glc site of EGF10 within the ligand-binding region of NOTCH1, but not NOTCH2. Further chemical analyses on b-elimination-released glycans indicated that the structure of the novel O-Glc glycans appeared to be Neu5Aca2–3Galb1–4Glc-O. Genetic deletion of both GXYLT1 and GXYLT2 in HEK293T cells increased the ratio of the novel glycans on EGF10 in NOTCH1, suggesting the competition between xylosyl-extension and galactosyl-extension for O-Glc glycans. Currently, we are identifying the enzyme(s) responsible for the biosynthesis of this galactosyl-extension. The novel glycans may confer a previously unknown function specifically on NOTCH1.

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(207) Hyper-Truncated N-Acetyl-β-D-Glucosamine Signatures Augment the Activity and Inhibition Potential of Neutrophil Granule Myeloperoxidase

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Myeloperoxidase (MPO) plays essential roles in neutrophil-mediated immunity via the generation of reactive oxidation products [1]. Complex carbohydrates decorate MPO at discrete sites, but their functional relevance remain elusive [2]. To this end, we have characterised the structure-biosynthesis-activity relationship of neutrophil MPO (nMPO). Mass spectrometry demonstrated that nMPO carries both characteristic under-processed oligomannosidic- and hyper-truncated paucimannosidic- and N-acetyl-β-D-glucosamine (GlcNAc) core-type asparagine-linked glycans. Occlusion of the Asn355/Asn391-glycosylation sites and the Asn323-/Asn483-glycans, located in the MPO dimerisation interface, was found to affect the local glycan processing, thereby providing a molecular basis of the site-specific nMPO glycosylation. Native mass spectrometry, mass photometry, and glycopeptide profiling revealed significant molecular complexity of diprotomeric nMPO arising from heterogeneous glycosylation, oxidation, chlorination and polypeptide truncation variants, and a previously unreported low-abundance monomer. Longitudinal profiling of maturing, mature, granule-separated, and pathogen-stimulated neutrophils demonstrated that nMPO is dynamically expressed during granulopoiesis, unevenly distributed across granules and degranulated upon activation. We also show that proMPO-to-MPO maturation occurs during early/mid-stage granulopoiesis. While similar global MPO glycosylation was observed across conditions, the conserved Asn355-/Asn391-sites displayed elevated hyper-truncated GlcNAc signatures, which correlated with higher enzyme activities of MPO in distinct granule populations. Endoglycosidase H-mediated truncation of the Asn355-/Asn391-glycans generated a glycoform of nMPO that recapitulated the activity gain and showed that nMPO carrying hyper-truncated glycans

at these positions exhibits increased thermal stability, polypeptide accessibility, and ceruloplasmin-mediated inhibition potential relative to native nMPO. Finally, structural modelling revealed that hyper-truncated Asn355-glycans positioned in the MPO-ceruloplasmin interface are critical for uninterrupted inhibition. Here, through an innovative and comprehensive approach, we report novel functional roles of the peculiar MPO glycosylation, providing new insight into neutrophil-mediated immunity.

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(208) A computational model linking the organization of the mammalian glycosylation machinery with glycan processing

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The Golgi apparatus is the central organelle for eukaryotic glycan processing. Correct processing requires non-uniform enzyme distributions in different cisternae of the Golgi. Enzyme organization is often modified during both physiological and pathological adjustments of the cell's condition, leading to altered glycan profiles. Yet our lack of understanding regarding the enzyme sorting mechanisms prevents us from assessing what features of enzyme organization will critically affect glycan processing and the resulting heterogeneous distribution of glycans. We have developed a systems biology approach to probe enzyme organization in the Golgi by computationally modelling the synthesis of glycan structures produced by the organelle [1]. The relative abundance of glycan structures in the cell, measured using MALDI mass spectrometry, are used as the primary data in the modelling.

The model uses a stochastic simulation algorithm to capture the competition between enzymes when modifying the emerging glycans. Success for each enzyme in this competition is weighted by an effective activity (EA) parameter. Importantly, the EA captures the enzyme's intrinsic turnover rate, Leloir-donor levels and the amount of the enzyme as a single value. For each enzyme we define a separate EA for each cisterna, and Approximate Bayesian Computation (ABC) is then used to adjust all EA values to fit the computed to a measured glycan profile. This will provide information on relative enzyme abundances in the different cisternae when turnover rates and culture conditions (and thus Leloir-donor levels) do not change. Use of the ABC is crucial, as it can deal with highly uncertain starting parameters, while providing information on the shift of these parameters during fitting. This allowed us to investigate how the organization of a set of 11 N-glycan biosynthetic enzymes changed in the Golgi apparatus of mutant HEK293 cells in which intra-Golgi enzyme sorting was perturbed. The model predicted a marked decrease in the EAs of three enzymes, and we were able to confirm this experimentally for two of these by demonstrating a reduction in the overall levels of galactosyltransferase (GalT) and N-acetyl-glucosamintransferase 1. As the model treats each cisterna separately, we could also extract localization information, and in turn experimentally confirmed a predicted shift for GalT towards the cis-Golgi in the mutant cells.

In current efforts, we are investigating altered enzyme sorting using mutations found in congenital glycosylation disorder patients as well as biochemically generated mutations that alter enzyme trafficking. We are also using the model to predict what changes in enzyme organisation are needed to rationally engineer the glycan output, in particular for therapeutic glycoproteins.

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(209) Phylogenetic analysis of the del177 splice variant of CST in mammals

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The CMP-sialic acid transporter (CST) is a member of the Solute Carrier family SLC35. It is a highly conserved type III trans-membrane antiporter responsible for translocating CMP-sialic acid/CMP into the Golgi lumen. The membrane topology has been identified for the murine CST (mCST) and recently its crystallography model was reported [1, 2].

In humans, CST (hCST) has 337 aa and exhibits 4 splicing isoforms. The del177 isoform (del177-hCST) presents skipping of exon 6 that results in a loss of 59 amino acids but without a change in the ORF and conserving its C-terminal. Interestingly, the del177 isoform preserves the ability to

transport CMP-sialic acid in contrast to the non-functional del177 isoform expressed in Lec2 cells a well known cell line deficient in CMP-sialic acid transport (*Cricetulus griseus* del177-haCST), and that is 91% homologue with the del177-hCST [3].

The del177-CST isoform has been only reported in humans and hamster cells but it may be expressed by other mammals. Here, we predicted the del177 isoform sequences of *Canis lupus familiaris*, *Macaca mulatta*, *Sus scrofa*, *Mus musculus*, Pongo abelii and *Bos taurus* using orthologs sequences from hCST reported by NCBI. We analyzed conserved and non-conserved regions with respect to the del177-haCST and del177-hCST.

The alignment between the del177-hCST and del177 isoform from *Mus musculus*, *Macaca mulatta*, Cricetulus griseus, *Canis lupus familiaris*, *Sus scrofa*, Pongo abelii and *Bos taurus* revealed similarity of 99%, 94%, 91%, 94%, 99%, 95%, respectively.

The phylogenetic analysis of predictive del177 isoforms in primates showed a narrow range of values between them but distant clades hCST were identified for *Mus musculus* and Cricetulus griseus. Also, the del177-haCST sequence was closer to del177 isoform from *Canis lupus familiaris*.

The conformational analysis between del177-hCST and del177-haCST allowed to determinate that not exist differences in amino acid residues reported as essential in substrate recognition of CST, which suggesting that loss-of-function of del177-haCST and the functionality of del177-hCST is due to difference in structural domains.

This work contributes to propose key domains conserved between del177-hCST and other mammals to establish amino acid residues involved in functional del177 splice variant of hCST.

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(210) O-GlcNAcylation as new target for glioblastoma cell lines

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Glioblastoma (GBM) is the most aggressive primary malignant brain tumor, and the treatments available are ineffective. Temozolamide (TMZ) is the main chemotherapy used in GBM treatment, but has a high resistance rate. Recent evidence suggests that autophagy is strongly related to GBM tumorigenesis and its resistance to TMZ. To note, both tumorigenesis and autophagy have been associated with protein *O*-GlcNAcylation in other tumor types. O-GlcNAcylation is a dynamic process of adding β-N-acetylglucosamine-*O*-linked to serine and threonine residues of intracellular proteins. The O-GlcNAc addition is catalyzed by *O*-GlcNAc transferase (OGT), and *O*-GlcNAcase (OGA) catalyzes its removal. In this work, we used two GBM strains U87MG (commercial lineage) and GBM11 (lineage from a tumor recurrence process), and human astrocytes as non-tumoral control. First, we investigated the levels of *O*-GlcNAcylation, OGT, OGA and GFAT, the rate-limiting enzyme of the Hexosamine Biosynthetic Pathway (HBP). *O*-GlcNAcylation, OGT, OGA and GFAT are increased in two GBM strains when compared to untransformed human astrocytes. The increase of *O*-GlcNAc levels by treatment with Thiamet G (OGA inhibitor) generated a significant increase in the number of GBM cells without altering the profile of the cell cycle and death. Then, we investigated the influence of OGA inhibition in the autophagy activation by analyzing the protein markers p62 and LC3. Our results demonstrate an autophagic activation by treatment with Thiamet G. The correlation between the increase in *O*-GlcNAc and autophagy was confirmed by associating the treatment of Thiamet G with an autophagy inhibitor Chloroquine (CQ). These results motivated us to verify the impact of the reduction of *O*-GlcNAc in the cell viability, proliferation, cell death and resistance to TMZ. Then, GBM cells lines were incubated with Osmi-1, and showed lower viability and proliferation in 2D and 3D culture models. In addition, Osmi-1 induced cell death by apoptosis and sensitized GBM cells to TMZ. The action of Osmi-1 on astrocyte control cells was also evaluated, however, no reduction in viability and proliferation of these cells was observed. The results represent an important step towards understanding the role of *O*-GlcNAc in molecular aspects of GBM tumorigenesis including autophagy, cell viability and response to TMZ chemotherapy with possible therapeutic implications.

(211) New Insights into Immunological Involvement in Congenital Disorders of Glycosylation (CDG) from a People-Centric Approach

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Background: Congenital disorders of glycosylation (CDG) are an expanding family of over 150 rare diseases with variable phenotypes and severity. For most CDG, immunological involvement remains a vastly elusive topic, mainly due to lack of robust data.

Objective: To characterize immune-related manifestations' prevalence, relevance, and quality-of-life (QoL) impact by developing and adopting an innovative people-centric approach which entailed the direct harvesting of the knowledge and experience of CDG patients, caregivers and other lay citizens.

Methods: We developed electronic questionnaires (e-questionnaires) targeting (1) CDG patients and (2) the general "healthy" population. The e-questionnaires development relied on a detailed literature revision and on the continuous input fromtwo well-established advisory committees, made up by i) medical and scientific experts, and ii) CDG family members. The e-questionnaires were divided into several sub-sections, including infections, allergies, autoimmune diseases, vaccination and QoL impact of immunological manifestations.

Results: We included 209 CDG patients/caregivers and 349 healthy participants. The most prevalent CDG was PMM2-CDG (n = 122/209). About half of PMM2-CDG patients (n = 65/122) described relevant infections with a noteworthy occurrence of gastrointestinal tract (GI) infections (63.1%, n = 41/65). Infection burden and QoL impact were shown to be correlated with more severe clinical phenotypes and with a set of relevant non-immune PMM2-CDG signs. Autoimmune diseases had a marginal presence in PMM2-CDG (2.5%, n = 3/122), all being GI-related. Allergy prevalence was also low in PMM2-CDG (33%, n = 41/122) except for food allergies (26.8%, n = 11/41 of PMM2-CDG and 10.8%, n = 17/158 of controls). High vaccination compliance with greater perceived ineffectiveness (28.3%, n = 17/60) and more severe adverse reactions were described in PMM2-CDG.

Conclusion: This people-centric approach went beyond the confirmation of literature findings, but created new insights into immunological involvement in CDG, namely by highlighting the possible link between the immune and GI systems in PMM2-CDG. Our results emphasized the importance of patient/caregiver knowledge and raised several red flags about immunological management.

(212) O-glycan synthesis on therapeutic proteins—in bacteria

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The presence of terminal sialic acid on glycans is a crucial modification that can help serum stability of therapeutic glycoproteins. As an alternative to costly mammalian cell systems that effect correct glycosylation, we are engineering an *E. coli* strain that is capable of adding simple *O*-linked sugars onto recombinant glycoproteins. This will be accomplished by introducing an *O*-glycosylation operon into *E. coli*, encoding various eukaryotic and bacterial glycosyltransferases, and accessory enzymes involved in donor substrate synthesis.

In preliminary studies, we have shown the successful addition of T-antigen (Gal-β1,3-GalNAc-α-O-Ser/Thr) on recombinantly expressed Interferon a2b and human growth hormone using a dual plasmid approach; one encoding the target protein and a second encoding the O-glycosylation machinery. We are now exploring the potential of modifying the *O*-glycosylation operon to further produce sialylated T-antigen structures. Towards this aim, we have engineered our host *E. coli* strain to produce the CMP-Neu5Ac donor and constructed new O-glycosylation operons incorporating various α-2,3- and α-2,6 sialyltransferases to produce disialylated core 1 modified proteins.

(213) The study of glycosphingolipids-glycan signature in different human acute myeloid leukemia cell lines using PGC LC–MS/MS in negative ion mode

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Glycosphingolipids (GSLs) are information-bearing biomolecules that play critical roles in embryonic development, signal transduction and carcinogenesis. The abnormal expression of GSLs is correlated with development of diverse cancers, including leukemia [1].

Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder of haemopoietic progenitor cells and the most common malignant myeloid disorder in adults. Previous studies indicate that the aberrant expression of certain GSLs is associated with the differentiation of AML cells [2]. However, the biosynthetic regulation and expression patterns of GSLs in AML and their biological relevance are still poorly explored due to their complexity, the presence of multiple isomeric structures as well as tedious analytical procedures.

In this study, we performed in-depth GSL glycosylation analysis for 19 AML cell lines to investigate the variation in glycosylation phenotypes and their association with expression levels of glycotransferases and transcription factors. For this, we extracted GSLs from cell lines. Glycan head groups were enzymatically released followed by reduction and purification. Glycans were analyzed by porous graphitized carbon liquid chromatography with negative ion mode electrospray ionization tandem mass spectrometry [3].

Our results show strikingly different GSL-glycan profiles for the 19 AML cell lines. High expression of gangliosides was observed in KG1, NB4, U937, and TF1 cell lines, while neolacto-series was expressed in all AML cell lines. In addition, the cell lines THP1,TF1, and M07e expressed high levels of globosides. The GSL glycan signatures showed some expected associations between globosides and A4GALT and between oligosialylated gangliosides and ST8SIA1. Besides, relatively strong correlation is seen for oligosialylated gangliosides with transcription factors GATA1 and RUNX1 which are both important players in the development of blood cells. The expression of Lewis antigens showed a positive association with the transcription factor MYC-associated factor X (MAX).

In conclusion, a large diversity was observed for the GSL glycan profiles of 19 AML cell lines and the correlation between the expression of GSLs-glycans with related genes provides insights into the regulation of glycosphingolipid expression. Further research is needed to dissect the role of GSLs in hematopoiesis and associated malignancies.

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(214) O-GlcNAc governs myofibroblast differentiation by regulating the expression and function of Serum Response Factor—A potential role in the pathogenesis of Scleroderma

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Scleroderma, also known as systemic sclerosis, is a chronic connective tissue disorder characterized by hyper-activation of fibroblasts and multi-organ fibrosis that has the highest morbidity and mortality rate among all rheumatic diseases. The pathogenesis of scleroderma is poorly understood and the efficacy of current therapeutic regimens is unsatisfactory.Dysregulated glucose metabolism is a metabolic feature for scleroderma. Glucose is utilized by several pathways in the cell, including the hexosamine biosynthetic pathway that produces UDP-GlcNAc, the essential substrate for O-linked β-N-acetylglucosamine (O-GlcNAc) modifications of proteins, which are carried out by O-GlcNAc transferase (OGT), and removed by O-GlcNAcase (OGA). Dysregulated levels of O-GlcNAc have crucial roles in chronic diseases including diabetes, cancer, cardiovascular diseases and neurological degenerative disorders such as Alzheimer's disease. However, whether dysregulated O-GlcNAc is involved in the hyperactivity of fibroblasts and gives rise to the onset of scleroderma remains unknown.

To explore the role of O-GlcNAc in regulating fibroblast function and skin fibrosis, primary mouse skin fibroblasts were isolated from wild-type C57BL/6 mice and grown in vitro. Inhibition of OGT expression by RNA interference (RNAi) significantly reduced the expression of α-SMA, an established marker for myofibroblast differentiation, and disrupted the formation of α-SMA (+) stress fibers in primary skin fibroblasts. Chemical inhibition of OGT with OSMI-1 yielded a consistent effect that protein abundance of α-SMA was decreased in fibroblasts. Inhibition of OGT by either RNAi or OSMI-1 also reduced the expression of Serum Response Factor (SRF), the key transcription factor that governs myofibroblast differentiation. In addition, the typical nuclear accumulation pattern of SRF was disrupted, and the binding between SRF and its cofactor, Myocardin-related Transcription Factor (MRTF) was dissociated in the cells treated with OGT RNAi.

We investigated the effect of manipulation of protein O-GlcNAcylation on skin fibrosis, using the well-accepted mouse model of bleomycin-induced skin fibrosis. The global level of O-GlcNAc increased significantly in mouse skin treated with bleomycin for 7 days, as compared with the control group. Next, we treated the mice with either OSMI-1 or Thiamet-G (another OGA inhibitors), prior to bleomycin treatment, once daily for 21 days. At the end of treatments, the severities of fibrosis in treated skins were assessed by histology and compared between the different treatment groups. Although OSMI treatment did not significantly reduce skin fibrosis, OGA inhibition by

Thiamet-G significantly enhanced the fibrosis induced by bleomycin, which is consistent with our in vitro finding that a higher level of O-GlcNAc promotes myofibroblast differentiation.

In summary, we observed that a normal level of O-GlcNAc is required to maintain SRF expression and function, and is necessary for myofibroblast differentiation in vitro. Our in vivo study showed that the levels of O-GlcNAc in mouse skin are increased upon bleomycin treatment. OGA inhibition by Thiamet-G treatment significantly exacerbates the skin fibrosis induced by bleomycin. These findings provided evidence that a dysregulated level of O-GlcNAc may promote fibroblast function and give rise to the development of skin fibrosis in scleroderma.

(215) A high-throughput small molecule screen identifies novel modulators of glycosaminoglycan expression

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Glycosaminoglycans (GAGs) are long, linear polysaccharides that are ubiquitously expressed on the cell surface and in the extracellular matrix and are a key component of the glycocalyx. These highly sulfated carbohydrates play important roles in many cellular processes, and defects in their biosynthesis and degradation have been implicated in certain disease states, such as sepsis and rare genetic disorders. Small molecule modulators of GAG expression could serve as powerful tools to restore and regulate the architecture and function of the glycocalyx as a novel therapeutic approach. To find novel modulators of GAG biosynthesis, we screened a small molecule library of ~57,000 compounds using Chinese hamster ovary cells and quantified fibroblast growth factor-2 (FGF2) binding to cell surface heparan sulfate using fluorescence-based imaging as a phenotypic readout. High-throughput screening resulted in the discovery of several N-aryl-2-aminothiazoles that enhanced HS expression up to 3-fold on the cell surface and displayed favorable "drug-like" physicochemical properties with minimal cytotoxicity. Structure–activity relationship studies with the most promising hit identified new active analogs and determined key physicochemical properties for their biological activity. Overall, these studies provide a promising new avenue for the development of novel therapies for targeting the glycocalyx and treating disorders involving glycosaminoglycan deficiencies.

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

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Sialic acids are negatively charged monosaccharides covalently attached to glycoproteins and glycolipids. They are involved in many biological and pathological processes including the masking of glycan ligands of multiple endocytic lectin receptors as well as the formation of ligands for other lectin receptors. The enzymatic hydrolysis of sialic acids is mediated by neuraminidases (sialidases) which are present in normal blood plasma and sera. Among circulating glycoproteins, de-sialylation with increased molecular age exposes underlying galactose linkages that can be recognized by various endocytic lectins including the Ashwell-Morell receptor of hepatocytes. This interaction thereby modulates the abundance and half-life of many circulating blood glycoproteins. Neuraminidases are widely distributed in vertebrates and microorganisms. Four mammalian neuraminidases, encoded by NEU1–4, have been identified and are expressed in various intracellular and extracellular compartments with Neu1 and Neu3 previously detected in circulation. Mice deficient in Neu1 have been reported with molecular features resembling sialidosis, a severe human lysosomal storage disorder linked to Neu1 mutations, whilst Neu3 deficiency does not result in an overt disease phenotype. To further investigate the roles of these mammalian neuraminidases in blood protein homeostasis, we are using multiple experimental approaches incorporating studies of Neu1- and Neu3-deficient mice. The plasma proteome is being investigated using ELISA, HPLC and GC–MS to identify and measure the sialic acid linkages among circulating glycoproteins. Changes in blood plasma protein abundance are under further study to identify those glycoproteins bearing increased sialylation with altered half-lives. Our studies include the identification of previously undetected and potential phenotypes caused by changes in blood protein homeostasis. Our current findings will be presented as they provide increased understanding of mechanisms controlling the plasma proteome and its modulation by glycoprotein sialylation and de-sialylation in health and disease.

(217) Immunoglobulin G subclass-specific glycosylation changes in primary epithelial ovarian cancer

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Epithelial ovarian cancer (EOC) remains one of the most lethal cancer types in women. In the absence of biomarkers able to reliably detect it at an early stage, altered glycosylation of proteins attracted attention as a potential source of complementary screening markers. In the present study, we investigated the subclass-specific EOC-related glycosylation changes in IgG, the most abundant glycoprotein in human serum. To this end, IgG was isolated from sera of 87 EOC patients and 74 age-and sex-matched healthy controls. To separate IgG2 and IgG3, whose peptide backbones encompassing the N_{297}-glycosylation are frequently identical, we performed a two-step affinity purification employing Protein A and Protein G Sepharose. After tryptic digestion, generated IgG glycopeptides were enriched using cotton-HILIC columns and measured by MALDI-TOF-MS. EOC-related glycosylation changes were monitored at the level of total agalactosylation, monogalactosylation, digalactosylation, sialylation, bisection and fucosylation, calculated separately for each IgG subclass. Aside from EOC-related increase in agalactosylation/decrease in monogalactosylation and digalactosylation observed in all IgG subclasses, some subclass-specific trends were detected. Glycosylation of IgG1 was found to be most strongly affected in EOC, as it exhibited the highest number of significant differences between healthy controls and EOC patients. For instance, it was the only subclass that showed a significant EOC-related decrease in sialylation and a significant increase in fucosylation. Additionally, IgG1 agalactosylation showed the strongest association with CA125, the routine ovarian cancer maker. Interestingly, IgG2 and IgG3 that in previous studies were often investigated collectively, showed distinct glycosylation patterns. In particular, IgG_3 was characterized by notably higher sialylation, which decreased significantly in EOC patients. In conclusion, our study indicates that IgG subclasses exhibit subtly distinct glycosylation patterns of EOC-related alterations. Additionally, our results show that simultaneous analysis of IgG_2 and IgG_3 might lead to wrong conclusions as these two subclasses exhibit noticeably different glycosylation phenotypes.

(218) A new ELISA assay demonstrates sex differences in the concentration of serum polysialic acid

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Male and female immune systems are strikingly different and yet little is known about sex differences in immune glycans, though glycans play central roles in regulating the immune response. Polysialic acid (polySia) occurs on the majority of leukocytes and is a potent immunomodulatory glycan which enables cell migration and serves as an immune checkpoint. Due to widespread influence of polySia on the immune system, we aimed to characterize its levels in serum, its presence on specific proteins, and differences in the amounts of polySia in male and female serum. However, polySia is difficult to quantify and detect on specific proteins, which makes it challenging to elucidate the molecular details of polySia function. We developed a sandwich ELISA that allows for the quantification of polySia as well as specific polysialylated proteins in complex mixtures without any pretreatment or harsh conditions. The assay is quick, linear, and robust under a wide variety of conditions and gave a limit of detection of approximately 0.2 ng polySia per mL of serum. We then quantified polySia and polysialylated CD56 in human and mouse serum. These studies strongly support our hypothesis of differences in glycosylation between the sexes as significantly less polySia was observed in female samples than in male samples.

(219) Peak Filtering, Peak Annotation, and Wildcard Search for Glycoproteomics

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Introduction

Glycopeptides in peptide or digested protein samples pose unique analytical and bioinformatic challenges beyond those posed by unmodified peptides or peptides with smaller, binary posttranslational modifications. These challenges include finding glycopeptide spectra when they are underrepresented among all tandem MS spectra, assigning spectra with unanticipated glycans that are not in the initial glycan database, and finding, scoring, and labeling diagnostic peaks in tandem mass spectra. Here we discuss recent improvements to Byonic, a glycoproteomics search program, that address these three issues. Byonic now supports filtering spectra by m/z peaks so that the user can focus attention to spectra with diagnostic peaks; for example, the presence of at least two out of three peaks for HexNAc (204.087 Da), NeuAc-H_2O (274.092 Da), and HexNAc-Hex (366.139 Da) within a set mass tolerance (e.g., ± 0.01 Daltons). A new glycan "wildcard" search allows an unspecified mass within a user-set mass range to be applied to N- or O-linked glycans and enables assignment of spectra with unanticipated glycans. And finally, Byonic now supports user-specified peak annotation from user-defined posttranslational modifications.

Methods.

The new search features were added to Byonic's existing code base, which is written in C++. The performance was tested with publicly available data, including proteomic and peptidomic data from rat and human origin. Performance metrics included the time taken to perform the search and the number of total identifications that pass below a 1% false discovery rate using target-decoy methodology.

Preliminary data.

The new features incorporated into the Byonic code base provided improvement in either search speed, sensitivity, or both. Peak filtering, or rather spectrum filtering by diagnostic peaks, improved search speeds up to 20X and, in some cases, led to the identification of novel glycopeptides when spectra were pre-filtered for diagnostic oxonium ions. The speed boost is not seen in all analyses, especially smaller data sets and protein databases, as Byonic is preconfigured to perform spectrum scoring in parallel based on a user-specified allotment of processing cores. The ability to encode custom peak scoring and annotation proved useful for chemoproteomic analyses, where chemically modified precursors produce conserved fragment ions. Using data from HexNAz-modified glycopeptides, as well as a new probe for prenylation, we demonstrate that this new scoring paradigm is particularly useful for the identification of modified precursors that represent a minute fraction of the total precursor population. Lastly, we have found that the glycan wildcard search is able to detect novel glycans that are not contained in the original glycan search space. Furthermore, this feature is also able to increase the sensitivity for glycopeptides that are modified with a database glycan, but are lost in a non-wild card search due to large error in the precursor monoisotopic mass, which is often inferred from incomplete isotope clusters with low signal-to-noise ratio.

Novel aspect.

Pre-search spectrum filtering, Custom peak scoring, Glycan wildcard search.

(220) Occurrence and interactions of zwitterionic modifications of glycans in eukaryotes

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Zwitterionic modifications of glycans, such as phosphorylcholine (PC), phosphoethanolamine (PE) and aminoethylphosphonate (AEP) are known on bacterial polysaccharides, but are actually also quite widespread in eukaryotes. Examples include N- and O-glycans from invertebrates and fungi, N-glycans from some protists, glycosaminoglycan chains from nematodes, glycolipids from a range of invertebrates and fungi as well as GPI anchors even from mammals. Recently, we have reported the occurrence of PC- or PE-containing N-glycans from insects (lepidopterans as well as honeybee royal jelly and venom) and filarial nematodes (Dirofilaria), whereby the zwitterionic modifications also occur on anionic glycans capped with glucuronic acid. Complementary to the glycomics analyses, we have obtained the first array data on natural oligosaccharides from these organisms. We show that human serum amyloid P binds glycans derived from royal jelly (the food for honeybee queens, but also used as a beauty product), while human C-reactive protein (CRP) interacts with the glycans of the canine heartworm (a mosquito-borne parasite). On the other hand, synthetic short phosphorylcholine-modified glycoconjugates are recognized not only by CRP, but by IgG and IgM in the sera of some helminth-infected animals. Not only are such arrays complementary to current resources, but they have high potential to yield new insights into the glycan-mediated interactions of invertebrates with mammals.

(221) Designing multiscale synthetic hydrogels to model bacterial invasion into macrophages in context of inflammatory bowel disorders

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The immune system is a complex network of various cellular components that must differentiate between pathogenic bacteria and the commensal bacteria of our microbiome, where misrecognition is linked to inflammatory disorders. Fragments of bacterial cell wall peptidoglycan bind to pattern recognition receptors within macrophages, leading to immune activation. Typically, these interactions are studied using two-dimensional invasion assays on tissue culture plastic plates; however, the dimensionality and 'stiffness' of these traditional culture systems may not be representative of the cell-microenvironment interactions that exist in vivo. In order to better simulate the native cellular environment, three-dimensional cell culture systems can be used to control key biochemical and mechanical properties of the cell microenvironment. Here, we established an approach for the encapsulation and culture of human macrophages in three dimensions within well-defined, biomimetic hydrogels decorated with integrin binding peptides that mimic the natural in vivo environment and linked with cell-degradable sites for cell-driven migration and remodeling to probe macrophage response to bacterial invasion. These hydrogels were established for monocyte encapsulation and differentiation into macrophages in 3D culture with high viability. Bacteria invaded into macrophages permitted in situ peptidoglycan labeling of peptidoglycan. Macrophages exhibited biologically-relevant cytokine release in response

to bacteria. This multi-dimensional bacteria-macrophage coculture system will prove useful in future studies to observe bacterial fragment production and localization in the cell at the carbohydrate level for insights into how our immune system recognizes and misrecognizes bacteria.

(222) Analysis of the SARS-CoV-2 spike protein glycan shield reveals implications for immune recognition

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We present an analysis of the impact of glycan microheterogeneity on the antigenicity of the spike (S) glycoprotein from SARS-CoV-2 and compare these predictions to reported antibody epitopes [1]. Despite the relatively modest contribution of the glycans to the total molecular weight of the S trimer (17% for the HEK293 glycoform) they shield approximately 40% of the protein surface.

The 3D structures show that the protein surface is extensively shielded from antibody recognition by glycans, with the notable exception of the ACE2 receptor binding domain, and also that the degree of shielding is largely insensitive to the specific glycoform. Lastly, we have identified peptides in the S glycoprotein that are likely to be presented in human leukocyte antigen (HLA) complexes, and discuss the role of S protein glycosylation in potentially modulating the innate and adaptive immune response to the SARS-CoV-2 virus or to a related vaccine.

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(223) Galectin-7 Targets Bacteria Expressing Carbohydrate Self-Antigens

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The ability of adaptive immunity to target a nearly infinite range of antigens is only tempered by tolerance mechanisms that reduce autoimmunity. However, this creates a gap in adaptive immunity towards microbes that engage in molecular mimicry by decorating themselves in self-like antigens. Recent studies suggest that innate immune factors in the intestinal lumen can protect the host against microbes that in particular utilize ABO(H) molecular mimicry. To test whether similar factors exist in the skin, we examined the binding specificity and overall antimicrobial activity of galectin-7 (Gal-7), a member of the galectin family of soluble innate immune lectins that is highly expressed in keratinized epithelium. Our results demonstrate that Gal-7 recognizes human blood group ABO(H) antigens, with particularly high affinity for the H antigen when analyzed on the CFG and ABH glycan microarrays. Gal-7 also recognized microbial glycans containing blood group motifs when isolated and printed in a microbial glycan microarray (MGM) format. However, these interactions were not limited to blood group antigens, as Gal-7 recognized an entire range of microbial glycans with distinct mammalian-like glycan structures on the MGM. Interactions observed on the MGM accurately predicted actual interactions with intact microbes, with Gal-7 exhibiting the ability to bind to unique strains of *Escherichia coli*, *Klebsiella pneumoniae*, Providencia alcalifaciens and *Streptococcus pneumoniae*, all of which express distinct forms of self-like antigens. Gal-7 recognition of microbes appeared to be strain specific, as engagement of related strains that fail to express self-like antigens failed to occur. Similar to binding, Gal-7 induced the rapid microbial death of each strain that expressed self-like antigens, while failing to alter the viability of related strains decorated with unrelated antigens. In contrast, Gal-7 likewise bound red blood cells, yet failed to induce any detectable change in membrane integrity. Taken together, these results demonstrate that Gal-7 possesses the unique ability to intrinsically recognize and kill microbes that utilize molecular mimicry, while failing to similarly alter the membrane integrity of host cells. Gal-7 may therefore provide an example of innate immunity against molecular mimicry along keratinized epithelial surfaces.

(224) Pituitary Gland Hyper-O-GlcNAcylation drives Growth Hormone Deficiency

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To date, at least5000 human proteins are O-GlcNAc-modified. Reversibly added to nucleocytoplasmic and mitochondrial proteins, *O*-GlcNAcylation regulates their function, stability, and interaction according to nutrient input. Indeed, this modification is a unique glucose rheostat for cell signaling relying on the availability of UDP-GlcNAc, itself reflecting extracellular glucose. 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 between dietary glucose level 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 specifically knocking out in this organ the enzyme that removes the O-GlcNAc modification, *O*-GlcNAcase (Oga) ($Oga^{\Delta Pit}$). Female mice appear to be more heavily affected by this perturbation in pituitary gland's *O*-GlcNAcylation during development, since only about 16% of viable females were $Oga^{\Delta Pit}$ versus the expected 25% Mendelian distribution. Interestingly, those viable female $Oga^{\Delta Pit}$ reached adulthood with no obvious detrimental phenotypes. On the other hand, male $Oga^{\Delta Pit}$ were mostly viable. However, post-weaning mortality was about 20% for $Oga^{\Delta Pit}$ male, suggesting failure to become metabolically self-sufficient. In addition, these $Oga^{\Delta Pit}$ male mice demonstrated incomplete penetrance phenotypes, including impaired eye development, hydrocephalus, and growth defects. Furthermore, GH deficiency was observed in living adult $Oga^{\Delta Pit}$mice compared to wild-type characterized by a decrease of circulating GH and Insulin-like growth factor 1 levels, particularly significant for adult female $Oga^{\Delta Pit}$mice. Altogether, these results suggest a sexually dimorphic nature of *O*-GlcNAc-dependent GH regulation. Finally, we have identified a critical factor in pituitary development, OTX2, that is modified by *O*-GlcNAcylation. This modification regulates the stability of this homeobox transcription factor, and its dysregulation acts to imbalance the pituitary gland's proper development.

Taken together, these results suggest that controlled *O*-GlcNAcylation level is essential for the anterior pituitary gland development, proper endocrine function, and metabolic regulation. 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.

(225) A single de novo mutation in COG4 disrupts selective protein trafficking and early development in zebrafish via non-canonical Wnt signaling

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Biallelic mutations in COG4cause a severe congenital disorder of glycosylation (CDG) with early lethality. In stark contrast, Saul-Wilson syndrome (SWS), is a rare skeletal dysplasia with progeroid and a dwarf-like appearance, is caused by the identical, heterozygous dominant de novo variant (p.Gly516Arg) in COG4. COG4 is one of 8 subunits in the Conserved Oligomeric Golgi (COG) complex involved in intracellular vesicular transport. Although overall protein secretion and N-glycosylation appeared normal in SWS-COG4 patient fibroblasts, Golgi architecture, selective protein trafficking, proteoglycan modification and collagen secretion is altered.

To extend these observations to a more relevant cell type, we used CRISPR to knock-in (KI) the SWS-mutation into chondrosarcoma cells. Like SWS fibroblasts, SWS chondrosarcoma cells had abnormal protein trafficking, proteoglycan and collagen 1A1 secretion. Interestingly, both fibroblasts and KI cells showed a two-fold increase in glypicans, based on heparinase III digestion.

We then moved to zebrafish to investigate developmental defects of the SWS mutation. The zebrafish Cog4 is 72% identical to human COG4 and the SWS mutation site is conserved across vertebrate species. Since the SWS mutation is dominant, comparable overexpression of human WT-COG4 and SWS-COG4 provides a practical first step for further analysis. Embryos mis-expressing SWS-COG4 show convergent & extension defects during gastrulation, shortened body length at larvae stages, and abnormal chondrocyte alignment in mature jaw cartilage. In addition, SWS-COG4 mis-expressing embryos showed dose-dependent increases in wnt4 transcripts. This finding points to the non-canonical Wnt signaling pathway in SWS pathogenesis. Overexpression of wnt4 mRNA in zebrafish embryos also caused shortened body length and abnormal chondrocyte alignment, much like SWS-COG4 mis-expression.

With these insights, we returned to patient fibroblasts and SWS-engineered chondrocytes and found they also increased WNT4 transcripts. Interestingly, WNT4 secretion was drastically impaired in both cell models, indicating that SWS mutation in COG4 affects WNT4 trafficking. Additional components of non-canonical Wnt signaling pathway, such as pRAC1 and pp38, are also increased. Data in these three systems all suggest a disturbed non-canonical wnt signaling could contribute to the pathogenesis of SWS. In addition, we are interested in how COG4 modulates glypican expression levels, which are implicated in early embryogenesis and linked with non-canonical Wnt signaling. Our disease-modeling studies combining both human and zebrafish system provide promising molecular and cellular leads to a mechanistic understanding of SWS pathology.

(226) Protein rhamnosylation in *Pseudomonas aeruginosa*: a study on substrate recognition using β-hairpin mimetics.

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Protein glycosylation is a ubiquitous post-translational modification present in all three domains of life. In prokaryotes, glycans are frequently present on extracellular proteins to engage in host–microbe interactions. Glycans are also found inside the cell, where they help modulate bacterial homeostasis by tuning protein function. Notably, bacteria utilize a large variety of carbohydrate moieties and linkages, many of which are not found in eukaryotic systems. One such example is the recently reported arginine-rhamnose motif detected in a protein from *Pseudomonas aeruginosa* [1], a previously unprecedented modification in both bacteria and eukaryotes. Rhamnose is transferred from TDP-l-Rha to a specific arginine by the action of the glycosyltransferase EarP. The rhamnosylated arginine motif is essential for bacterial protein biosynthesis and is predicted to be conserved in a number of bacterial pathogens.

In this talk I will describe our efforts towards understanding the key recognition elements of the bacterial protein glycosyltransferase EarP. By employing enzyme activity assays, substrate mimics, and NMR techniques we aim to expand the knowledge of bacterial glycosyltransferases as a first step to targeted inhibitor design against clinically relevant gram-negative pathogens.

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(227) GlycoTree: Infrastructure Supporting Semantic Annotation of Glycan Structures

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GlycoTree is one part of the semantic technology being developed by the GlyGen initiative. It enables semantic annotation of glycans by specifying their structures as collections of prototypes representing their chemical components. We frequently use semantic prototypes in everyday conversation. For example, we say that a baseball team is composed of exactly nine players, whose positions (pitcher, catcher, etc.) are given titles, each title referring to a semantic prototype. Thus, a baseball team is very generally represented as a set of nine prototypes. The 'catcher' prototype is partially defined by asserting that 'a catcher wears a mask', allowing one to infer that the catcher on an arbitrarily chosen baseball team wears a mask, without explicitly stating this fact. A similar approach allows the structure of a glycan to be represented as a set of canonical glycosyl residue prototypes, each of which defines the chemical structure of a residue (e.g., b-D-Man*p*) and its chemical context (e.g., attachment to another canonical residue at a specific site). This allows each residue in the 'canonical glycan tree' to be unambiguously defined and named. Representing an arbitrarily chosen glycan (e.g., GlyTouCan ID: G78059CC) as a set of such canonical residues and asserting that, e.g., the canonical 'core b-D-Man*p*' residue is transferred to the growing glycan by the enzyme 'ALG1' allows one to infer that this mannosyl residue in the glycan (G78059CC) was transferred by ALG1. This approach has allowed the identities of enzymes involved in the biosynthesis of more than 3850 glycans to be inferred and published on the GlyGen web site. As part of the GlyGen initiative, we continue to extend the GlycoTree approach, for example, by adding new residues to the canonical trees and annotating many of these residues with extensive biosynthetic information. At present, we disseminate this type of information via the GlyGen Sandbox web site: https://raw.githack.com/CCRC-will/glycoTree/master/portal/explore.html?G78059CC.

GlycoTree is based on concepts originally introduced by Takahashi and Kato (2003, https://doi.org/10.4052/tigg.15.235), and extended by implementing a formal description logics approach (York, et al. 2004, https://unit.aist.go.jp/brd/jp/GTRC/HGPI/ws1/pro.html). A rigorous implementation of glycoTree was developed as the GlycO ontology (https://bioportal.bioontology.org/ontologies/GLYCO) and leveraged to enable curation of glycan structure records using the Qrator software (Eavenson, et al., 2015, PMID: 25165068).

Our current work includes exploring emergent properties of each canonical tree to infer additional biosynthetic information (e.g., the order of glycosyl transfer reactions in biosynthetic pathways) and developing user interfaces to facilitate the dissemination and presentation of this more detailed information.

(228) Display of the Human Mucinome With Defined O-Glycans by Gene Engineered Cells

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Mucins are a large family of heavily O-glycosylated proteins that cover all mucosal surfaces and constitute the major macromolecules in body fluids. Mucins serve in lubrication, barrier functions, containment, feeding, and clearance of microorganisms at the epithelial frontline. Mucins are primarily defined by their tandem repeat (TR) domains that are densely decorated with O-glycans and present the essential binding opportunities and informational cues for microorganisms via their adhesins. However, these O-glycodomains is essentially limited to results from studies with simple oligosaccharides without the protein context of mucins and the higher-order features presented by dense O-glycan motifs. We have developed a cell-based platform for the display and production of human TR O-glycodomains (around 150–200 amino acids) with tunable structures and patterns of O-glycans using membrane-bound and secreted GFP-tagged constructs expressed in glycoengineered HEK293 cells. The recombinant mucin TRs revealed surprisingly high fidelity in complete decoration of O-glycans as well as in the designed O-glycan structures, which for the simplest glycoforms enabled intact mass analysis. The availability of defined mucin TR O-glycodomains enables us to decode the structures and functions of mucins in directing microbiomes and lead to new strategies for molecular dissection of specific roles of microbial adhesins, glycoside hydrolases, and glycopeptidases.

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(229) ST6GAL1 Is Involved in Intestinal Stem Cell Protection and Regeneration from Injury

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The epithelium of the small intestine needs to mediate efficient nutrient uptake while maintaining the barrier integrity to prevent entry of pathogens and toxins. These critical functions necessitate the complete renewal of the epithelium from intestinal stem cells (ISCs) every 48–72 hrs, a trait rendering it highly susceptible to damage from insults such as radiation or chemical exposure. Current therapeutic approaches to intestinal damages are mainly supportive. Definitive therapies to protect the intestine or to promote rejuvenation are lacking, and this is due at least in part to incomplete knowledge on how ISCs are governed in gut regeneration. We have recently identified a glycan-modifying enzyme, the sialyltransferase ST6GAL1, which protects mouse from death by gastrointestinal acute radiation syndrome (GI-ARS). Curiously, the ST6GAL1 originates elsewhere because the adult mouse small intestine natively expresses negligible ST6GAL1 if any at all. In ex vivo organoid cultures, extracellular ST6GAL1 promotes ISC quiescence and protects the ISC-originated organoids from irradiation damage. At resting (non-irradiated) state, addition of recombinant ST6GAL1 potentiates ISC self-renewal in these organoids. On the other hand, lack of functional ST6GAL1 results in up-regulated cell cycle signaling pathways and DNA repair signaling pathways, supporting the observed hypersensitivity of the St6gal1-KO intestine to irradiation-damage. The work forms the essential foundational premise for discovery of ST6GAL1 and other glycan-modification enzymes as novel therapeutic drugs to intestinal epithelial damage, such as from GI-ARS, and provides guidance for development of therapeutic delivery methods of such drugs.

(230) Protective effects of sulfated hyaluronic acid on growth factors and its application for tissue culture.

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Glycosaminoglycans (GAGs) play an important role in cell signaling, proliferation, and tissue formation by protecting and enhancing growth factor signals such as fibroblast growth factor (FGF). Although proteoglycans and GAGs in biological systems have significant functions in tissue formation, natural extracellular matrices may not function sufficiently at damaged sites or in the context of *in vitro* organ regeneration. Sulfated hyaluronic acid (HA) is an artificial compound that binds to several growth factors and show physiological effects; its potential application in hydrogels, among others is extensively studied [1–3]. Recently, Miura et al. used sulfated HA to achieve the feeder-free cultivation of human-induced pluripotent stem cells (iPSCs) without the addition of FGF2(bFGF) [4].

We synthesized sulfated HA molecules with different degrees of sulfation to examine their effects *in vitro*. Of note, this synthetic sulfated HA does not contain any animal-derived components, as it is obtained from a microbiological source. The degree of sulfation was controlled via adjustment of the reaction; the number of sulfation sites was adjusted from 1.3 to 2.0 and 2.1 to 3.9 in disaccharide units named hyaluronic acid low sulfated (HA-LS) and hyaluronic acid high sulfated (HA-HS).

The binding properties of the sulfated HA to FGF2 were compared with the binding properties of other GAGs. HA-HS showed higher binding to FGF2 than heparin and heparan sulfate. In contrast, HA-LS showed lower binding to FGF2 than heparin. This indicates that the bond strength of this material can be changed via the adjustment of the degree of sulfation. This material was considered capable of binding to and was expected to have a protective effect on growth factors. In fact, sulfated HA demonstrated protective effect on the stability of growth factors such as FGF2, FGF1, KGF, IL-6, and BMP2. Interestingly, this protective effect was altered depending on the combination of growth factors and HA with different degrees of sulfation. For instance, HA-HS had no protective effect on BMP2, whereas HA-LS had no protective effect on IL-6. A more detailed analysis of the binding properties and application of this material in the context of tissue culture is currently ongoing.

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(231) A study of causal relationships between human IgG N-glycosylation traits and twelve associated diseases

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Immunoglobulin G is a major component of humoral immunity and the most abundant N-glycosylated antibody in the blood plasma of healthy humans. Each IgG molecule has two conservative N-glycosylation sites in the fragment crystalizable region (Fc). Moreover, the variable fragment antigen-binding (Fab) of 15–20% of IgG molecules is N-glycosylated. The structure of attached N-glycans affects the affinity of IgG to its ligands, antigen-binding, immune complex formation and modulates immune response.

IgG N-glycosylation profile is inherited as a complex polygenic trait and recent genome-wide association studies (GWAS) discovered a number of genetic variants associated with the composition of IgG N-glycome. At the same time, N-glycosylation of IgG is altered in various physiological and pathological conditions, like ageing, cancer, inflammatory and autoimmune diseases, etc. It is still unclear, whether the observed differences in the N-glycome composition drive the onset of the pathologies, or if the changes in IgG glycosylation are induced by diseases. In this study we applied the two-sample Mendelian Randomization (MR) approach to investigate the causal relationships between IgG N-glycosylation traits and the risk of 12 inflammatory, autoimmune, cardiovascular and neurodegenerative diseases using summary statistics from publicly available GWAS.

MR analysis of causal effect of IgG glycosylation on the disease risk did not yield any signals passing the nominal statistical significance threshold. The reverse analysis pointed out the causal link of systemic lupus erythematosus (SLE) risk on bisection of IgG N-glycans (IgG_B) in the discovery round of the analysis (N = 8090), with estimated effect of 0.131 standard deviation units of IgG_B per log odds units of SLE risk (p = 2.24e-09). To confirm the detected causal signal we performed two rounds of sensitivity analyses. First, we used a refined set of genetic variants associated with SLE risk to estimate the causal effect, and confirmed the effect with p-value of 1.24e-05. Next we used the same refined set of genetic variants and summery statistics for IgG_B from a replication cohort (N = 3147), where the causal effect of SLE on IgG_B was no longer significant (p = 0.49). In conclusion, the observed genetic support for causal relationships between IgG N-glycosylation and disease risk was limited and further studies are required.

(232) Cosmc Controls B Cell Homing

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The molecular mechanisms regulating lymphocyte homing into lymph nodes are only partly understood. Here, we report that B cell-specific deletion of the X-linked gene, *Cosmc*, and the consequent decrease of protein O-glycosylation, induces developmental blocks of mouse B cells. After transfer into wild-type recipient, *Cosmc*-null B cells fail to home to lymph nodes as well as non-lymphoid organs. Enzymatic desialylation of wild-type B cells blocks their migration into lymph nodes, indicating a requirement of sialylated O-glycans for proper trafficking. Mechanistically, *Cosmc*-deficient B cells have normal rolling and firm arrest on high endothelium venules (HEV), thereby attributing their inefficient trafficking to alterations in the subsequent transendothelial migration step. Finally, *Cosmc*-null B cells have defective chemokine signaling responses. Our results thus demonstrate that *Cosmc* and its effects on O-glycosylation are important for controlling B cell homing.

(233) Peters Plus Syndrome Mutations Affects the Stability and Function of Human Beta-1,3-glucosyltransferase

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Peters plus syndrome (PPS) is a severe autosomal recessive congenital disorder of glycosylation characterized by multiple structural anomalies, including Peters anomaly (an ocular anterior segment malformation), disproportionate short stature, brachydactyly, dysmorphic facial features, developmental delay, and variable other systemic abnormalities. PPS patients have mutations in the β1,3-glucosyltransferase (B3GLCT) gene, which encodes an enzyme that catalyzes the addition of a glucose to an O-linked fucose on thrombospondin type-1 repeats (TSRs), forming an unusual Glucoseβ1–3Fucose (GlcFuc) disaccharide on TSRs. Protein O-fucosyltransferase 2 (POFUT2) is the enzyme that adds the O-fucose to a serine or threonine in the consensus sequence $C^1XX(S/T)C^2$ found in TSRs, where C^1 and C^2 are the first two of six conserved cysteines in TSRs. Currently, 49 human proteins contain the consensus sequence for this GlcFuc disaccharide modification. Half of these proteins belong to ADAMTS/ADAMTS-like family of extracellular matrix proteins that have critical roles in mediating angiogenesis and developmental processes. Previous results demonstrate that secretion of several ADAMTS/L proteins, in particular ADAMTS20 TSR2–8, is dependent on the presence of POFUT2 and B3GLCT. Since both POFUT2 and B3GLCT are localized in endoplasmic reticulum and only modify folded TSRs, this leads to the proposal that both POFUT2 and B3GLCT assist the folding of TSRs, modulating the secretion of TSR-containing proteins. We introduced missense and truncation PPS mutations individually into plasmids encoding human B3GLCT and analyzed their effects using both cell-based secretion assays and in vitro enzyme activity assays. Our results showed that these PPS mutants not only abolished their ability to rescue ADAMTS20 TSR2–8 secretion from $B3GLCT^{-/-}$ cells, but also greatly reduced mutant protein levels compared to wild type (WT) B3GLCT within the cells. Mutant enzymes harboring mutations associated with Peters Plus Spectrum, which is a milder form of PPS with patients who have a subset of PPS phenotypes, all rescued ADAMTS20 TSR2–8 secretion and had the same protein levels within the cells as WT B3GLCT. Results from enzyme activity assays demonstrated that some PPS mutants lost enzymatic activity completely whereas spectrum mutants retained enzymatic activity as the WT enzyme. On-going experiments are comparing the stabilities of mutant enzymes with wild type using in vitro stability assays. Overall, by analyzing the impact of PPS and Peters Plus Spectrum mutations on B3GLCT, we are aiming to understand the molecular mechanism of the disease and advance the development of novel therapeutics to alleviate the symptoms of PPS as well as other congenital disorders of glycosylation. This project is supported by NIH grant HD096030.

(234) Virus-Receptor Interactions of Glycosylated SARS-CoV-2 Spike and Human ACE2 Receptor

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SARS-CoV-2 betacoronavirus uses its highly glycosylated trimeric Spike protein to bind to the cell surface receptor ACE2 glycoprotein and facilitate host cell entry. We utilized glycomics-informed glycoproteomics to characterize site-specific microheterogeneity of glycosylation for a recom-

binant trimer Spike mimetic immunogen and for a soluble version of human ACE2. We combined this information with bioinformatic analyses of natural variants and with existing 3D-structures of both glycoproteins to generate molecular dynamics simulations of each glycoprotein alone and interacting with one another. Our results highlight roles for glycans in sterically masking polypeptide epitopes and directly modulating Spike-ACE2 interactions. Furthermore, our results illustrate the impact of viral evolution and divergence on Spike glycosylation, as well as the influence of natural variants on ACE2 receptor glycosylation. Taken together, these data can facilitate immunogen design to achieve antibody neutralization and inform therapeutic strategies to inhibit viral infection.

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