2023 Mid-South Glycoscience Meeting
Thursday, June 29, 2023
University of Mississippi - Thad Cochran Research Center
Program Schedule and Poster Session Information

Time	Speaker	Title
8:15 a.m.- 8:25 a.m.	**Joshua Sharp**	Welcome
	Session 1 - Session Chair: Joshua Bloomekatz	
8:25 a.m.- 9:10 a.m.	**Xuefei Huang**	Stereoselective chemical synthesis of sialyl Lewisa antigen and evaluation of its conjugate with bacteriophage Qβ as a potential anti-cancer vaccine
9:10 a.m.- 9:30 a.m.	**Jing Li**	Impact of Glycosylation on Ion Channel Gating and Assembly: Insights from Molecular Dynamics Simulations
9:30 a.m.- 9:40 a.m.	**Sushil Mishra**	**Poster Highlight:** The Challenges in Predicting Protein-Glycan Binding Energies Through MD Simulations
9:40 a.m.- 10:00 a.m.	**Elex Harris**	Loss of Sialylation Compacts Mucin and Impairs Mucociliary Transport
10:00 a.m.-10:15 a.m.	***Break***	
	Session 2 - Session Chair: Jamie Stewart	
10:15 a.m.- 10:45 a.m.	**Ryan Flynn**	Glycosylated cell surface RNAs
10:45 a.m.- 10:55 a.m.	**Jarrod Barnes**	**Poster Highlight**: O-GlcNAc Regulates Fibrosis Resolution in Idiopathic Pulmonary Fibrosis
10:55 a.m.- 11:05 a.m.	**Erik Alvarez Valenzuela**	**Poster Highlight:** Visible-Light Induced Activation of Selenoglycosides for O-Glycosylation
11:05 a.m.- 11:15 a.m.	**Ally Hernandez**	**Poster Highlight:** Regulation of high mannose N-glycans by redox signaling: Implications for vascular inflammatory diseases
11:15 a.m.-12:00 p.m.	***Poster Session 1***	
12:00 p.m.-12:45 p.m.	***Lunch Break***	
	Session 3 - Session Chair: Jing Li	
12:45 p.m.- 1:15 p.m.	**Fikri Avci**	Immune Interactions of Carbohydrate Antigens in Health and Disease
1:15 p.m.- 1:35 p.m.	**Antim Kumar Maurya**	Structure, anti-SARS-CoV-2, and anticoagulant effects of two sulfated galactans from the red alga Botryocladia occidentalis
1:35 p.m.- 1:55 p.m.	**Joelle Saad**	The role of ST6Gal1 in regulating GBM metabolism including via GLUT3 sialylation
1:55 p.m.- 2:25 p.m.	**Jessica Kramer**	Synthetic mucins: from new chemical routes to engineered cells
2:25 p.m.-3:10 p.m.	***Poster Session 2***	
	Session 4 - Session Chair: Paul Boudreau	
3:10 p.m.- 3:40 p.m.	**Jeffrey Esko** *(Virtual)*	Heparan Sulfate Mosaicism
3:40 p.m.- 4:00 p.m.	**Md. Saqline Mostaq**	Inhibition of GCS Effectively Overcomes Drug Resistance of Colon Cancer Cells Carrying p53 Mutation
4:00 p.m.- 4:20 p.m.	**Amrita Khakurel** *(Virtual)*	GARP complex is Essential for the Maintenance of the Golgi Glycosylation Machinery
4:20 p.m.- 5:05 p.m.	**Umesh Desai**	Designing/Discovering Synthetic, Highly Sulfated Mimetics of Glycosaminoglycans as AntiThrombotic and AntiCancer Agents

Poster Session # 1 - 11:15 a.m. - 12:00 p.m.

Poster #	Presenter	Poster Title
1	Hoda Ahmed	The competitive effect of sulfated glycans on thrombin binding to surface heparin using surface plasmon resonance.
3	Erik Alvarez Valenzuela	Visible-Light Induced Activation of Selenoglycosides for O-Glycosylation
5	Joshua Bloomekatz	The role of glycans in cell state transitions during cardiac development in zebrafish
7	Tiffany Duong	Leveraging Benzyne Chemistry for the Development of 1,2-cis-Selective O-Glycosylation
9	Eslam Elhanafy	Elucidating the Molecular Mechanisms of Gating Regulation of N-Glycosylation on Voltage-Gated Sodium Channels Using Molecular Dynamics Simulations
11	Sushil Mishra	The Challenges in Predicting Protein-Glycan Binding Energies Through MD Simulations
13	Ally Hernandez	Regulation of high mannose N-glycans by redox signaling: Implications for vascular inflammatory diseases
15	Sandeep Misra	Determining the interaction site of a novel Coprinopsis cinerea lectin to Listera innocua peptidoglycan
17	GlyCORE/GSA	Glycoscience Center of Research Excellence & Glycoscience Student Advocates
19	Farhana Taher	Acute COG inactivation unveiled its immediate impact on Golgi and illuminated the nature of intra-Golgi recycling vesicles

Poster Session # 2 - 2:25 p.m. - 3:10 p.m.

Poster #	Presenter	Poster Title
2	Nicole Ashpole	AGE/RAGE Signaling Impacts Cognitive Behaviors Throughout Adulthood
4	Jarrod Barnes	O-GlcNAc Regulates Fibrosis Resolution in Idiopathic Pulmonary Fibrosis
6	Rohini Dwivedi	The sulfated fucan from Thyonella gemmata presents high anti-SARS-CoV-2 but low anticoagulant activity
8	Nidhi Gupta	Acylated Double-Layered Electrospun Chitosan Nanofibrous Membranes reduce burst and extend the duration of elution of hydrophobic therapeutics
10	Marwa Farrag	Structural specificity in anticoagulant and anti-SARS-COV-2 properties of holothurian fucosylated chondroitin sulfate oligosaccharides
12	Joshua Sharp	Probing the interactions of IGF2 with the full-length soluble domains of CI-MPR using hydroxyl radical protein footprinting
14	Joshua Zhu	Ag2O Assisted Gram-Scale Synthesis of Core 2 Containing Glyco-Amino Acid
16	Anter Shami	GlyCORE: Analytical and Biophysical Chemistry Research Core
18	Ruofan Cao	The GLYCORE Imaging CORE: Bridge the gap between advanced imaging techniques and Glycoscience
20	Sushil Mishra	Computational Chemistry and Bioinformatics Research Core

ABSTRACTS
Oral Presentations

Stereoselective chemical synthesis of sialyl Lewis^a antigen and evaluation of its conjugate with bacteriophage Qβ as a potential anti-cancer vaccine

Xuefei Huang,[1,2,3*] Zahra Rashidijahanabad,[1,2] Sherif Ramadan,[1,2,4] Nicholas A. O'Brien,[5] Shuyao Lang,[1,2] Howard Crawford,[6] Jeffrey C. Gildersleeve[5]

[1]*Department of Chemistry,* [2]*Institute for Quantitative Health Science and Engineering, Michigan State University, East Lansing, Michigan 48824, United States*

[3]*Department of Biomedical Engineering, Michigan State University, East Lansing, Michigan 48824, United States*

[4]*Chemistry Department, Faculty of Science, Benha University, Benha, Qaliobiya 13518, Egypt*

[5]*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, Maryland, United States*

[6]*Department of Surgery, Henry Ford Health System, Detroit, Michigan, United States Email: huangxu2@msu.edu*

Introduction: Sialyl Lewis^a (sLe^a), also known as cancer antigen 19-9 (CA19-9), is a tumor associated carbohydrate antigen. The overexpression of sLe^a on the surface of a variety of cancer cells makes it an attractive target for anti-cancer immunotherapy. However, sLe^a based anti-cancer vaccines have been under-explored. In this presentation, the development of a new vaccine targeting sLe^a will be discussed.

Methods: To develop a new vaccine, sLe^a in a conjugable form is needed. Furthermore, to boost the antibody responses against sLe^a, it needs to be conjugated to an immunogenic carrier. The immunogenicity of the conjugate and the protective efficacy are evaluated.

Results: We have developed an efficient stereoselective synthesis of sLe^a with an amine bearing linker by overcoming challenges related to low reactivities of sialic acid donor and stereochemical controls of sialylation. The synthetic sLe^a has been conjugated with a powerful carrier bacteriophage Qβ. Mouse immunization with the Qβ-sLe^a conjugate generated strong and long-lasting anti-sLe^a IgG antibody responses, which were superior to those induced by the corresponding conjugate of sLea with the benchmark carrier keyhole limpet hemocyanin. Antibodies elicited by Qβ-sLe^a were highly selective toward sLe^a structure, could bind strongly with sLe^a expressing cancer cells and human pancreatic cancer tissues, and kill tumor cells via complement mediated cytotoxicity. Furthermore, vaccination with Qβ-sLe^a provided significant tumor protection in a metastatic cancer model in mice demonstrating tumor protection for the first time by a sLe^a based vaccine highlighting the significant potential of sLe^a as a promising cancer antigen.

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Impact of Glycosylation on Ion Channel Gating and Assembly: Insights from Molecular Dynamics Simulations

Eslam Elhanafy, Amin Akbari Ahangar, **Jing Li**

University of Mississippi, Oxford, Mississippi

Abstract:

Alterations in glycosylation patterns play a pivotal role in numerous congenital disorders of glycosylation (CDGs) and have emerged as novel biomarkers for various neurological disorders. Glycosylation exerts significant influence on ion channel function, affecting not only protein folding and trafficking, but also gating properties and protein-protein interactions. However, the underlying structural mechanisms by which glycosylation regulates ion channel assembly and gating continues to be one of the least investigated aspects. To elucidate the modulatory role of glycosylation in ion channel gating and subunit assembly, we employed microsecond-scale molecular dynamics (MD) simulations to investigate the atomic-level structural dynamics of glycans and their interactions

with ion channels. To explore the generality of glycan-protein interactions, we examined two major glycosylated superfamilies, namely voltage-gated and ligand-gated ion channels, along with three types of N-glycans: oligomannose, hybrid, and complex. We modeled and simulated a Nav1.4 channel and a GABAA receptor under specific conditions, and several crucial glycan-protein interactions were observed in both channels. Complex N-glycans attached to voltage-gated sodium channels were found to be terminated by negatively charged sialic acids. Electrostatic potential analysis revealed the presence of negative charge centers above the voltage-sensing domains (VSD), generated by these sialic acids. Notably, strong electrostatic interactions were also observed between sialic acids and positively charged residues at the extracellular loop of VSDs. These interactions likely facilitate the activation of sodium channels. In the case of the GABAA receptor, we observed significant structural impacts of a pore-facing oligomannose N-glycan on the assembly of the pentameric structure. Mutated or hypothetical pentamers with more than two pore-facing glycans exhibited disruption of key native contacts at the subunit interfaces, highlighting the pivotal role of this pore-facing glycan as a molecular determinant in the pentameric assembly of GABAA receptors. Our findings shed light on the structural basis of how glycosylation influences ion channel gating and subunit assembly, providing valuable insights into the functional consequences of glycosylation alterations in ion channels.

The Challenges in Predicting Protein-Glycan Binding Energies Through MD Simulations

Poster Highlight

Sushil K. Mishra[1] and Robert J. Doerksen[1,2]

[1]*Department of BioMolecular Sciences, University of Mississippi, MS-38677, USA;* [2]*Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS-38677, USA*

Protein–glycan interactions play a pivotal role in various biological functions such as adhesion, recognition, differentiation, metastasis, pathogenesis, and immunological responses[1] Understanding these interactions holds great importance in unraveling the underlying mechanisms behind these biological processes. Therefore, approaches for understanding the atomic basis of protein–glycan interactions are of interest not only for theoretical reasons, but also for the design of glycan-based therapeutics and glycan-recognizing reagents in affinity chromatography, analytical approaches, or diagnostics tools. We have assessed the performance of several free-energy calculation approaches[2–4] on protein–glycan complexes and have continued to investigate this by applying Potential of Mean Force (PMF)-based rigorous free-energy calculation approaches.[5] In this presentation, we will demonstrate how binding free-energy calculations can be performed via the geometric route, in which structural restraints are introduced to guide the binding and unbinding of the ligand along the reaction coordinate. We will show how different force fields and simulation lengths affect the performance of such PMF-based method on protein–glycan complexes.

References:

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Loss of Sialylation Compacts Mucin and Impairs Mucociliary Transport

Elex Harris[1], Marina Mazur[1], Stefanie Krick[1], Steven M. Rowe[1*], Jarrod W. Barnes[1*]

[1]*Gregory Fleming James Cystic Fibrosis Research Center, Univ. of Alabama at Birmingham, Birmingham, AL, USA*

Introduction: Mucus stasis is a pathologic hallmark of muco-obstructive diseases, including Cystic Fibrosis (CF). Mucins, the primary constituents of mucus, are extensively O-linked glycosylated and contribute to the gel-forming properties of mucus. Sialylation confers a negative charge on mucins, interacts with the ionic microenvironment and is important for proper mucin maturation. Therefore, changes in sialylation would be expected to alter the biophysical characteristics of mucins. Previous reports suggest that sialylation may be altered in CF; however, the consequences of altered sialylation on mucus transport and physiology in CF have not been determined. In this study, we investigated the consequences of reduced sialylation on mucociliary transport (MCT) and the biophysical characteristics of mucins in the context of CF.

Methods: Mucin charge state and conformation were analyzed by native agarose-PAGE Western blotting and analytical density centrifugation following neuraminidase treatment. Well-differentiated HBECs on ALI were treated with vehicle or 200µM pan sialyltransferase inhibitor (STI, 3Fax-Peracetyl Neu5Ac), 120µM glycolithocholic acid (GA) to inhibit □-2,3 sialylation, or the CFTR modulators elexacaftor/tezacaftor/ivacaftor (ETI) to the basolateral compartment to restore CFTR function. MCT was assessed after 24 or 72 hours by micro-Optical Coherence Tomography (µOCT). Vehicle, 500µM STI, or 300□M GA was instilled intratracheally into wild type (WT) rats daily for 7 days, and then MCT on tracheas was assessed by µOCT. Sialyltransferase expression was analyzed by immunoblotting of proteins isolated from non-CF and CF HBECs with/without ETI.

Results: Removal of sialic acid from mucin by neuraminidase treatment produced a low charge glycoform and increased mucin compaction (defined by its density in the gradient). In control HBECs, treatment with STI or GA significantly reduced MCT after 24 hours. Similar findings were observed on *ex vivo* tracheas in terms of MCT following intratracheal instillation of STI or GA in WT rats for 7 days, indicating importance of mucin sialyation to maintaining mucus clearance. Using CF HBECs, we observed significantly reduced expression of ST3Gal1, but not ST6GalNAc1, compared to non-CF. Interestingly, ST3Gal1 was significantly increased following correction of the CFTR function with ETI.

Conclusion: Loss of sialylation was shown to decrease mucin charge state, increase mucin compaction, and ultimately impairs mucociliary transport. Reduced ST3Gal1 expression was observed in CF HBECs, which was partially restored after ETI treatment suggesting that loss of □-2,3 mucin sialylation may contribute to CF mucus stasis. Reduced mucin sialyation warrants further investigation as a mechanism of mucus stasis in CF and other muco-obstructive diseases.

Glycosylated cell surface RNAs

Ryan Flynn

Boston Children's Hospital and Harvard University, Boston Massachusetts

Glycans modify lipids and proteins to mediate inter- and intramolecular interactions across all domains of life. RNA is not thought to be a major target of glycosylation. Here, we challenge this view with evidence that mammals use RNA as a third scaffold for glycosylation. Using chemical and biochemical approaches, we found that conserved small noncoding RNAs bear sialylated glycans. These "glycoRNAs" were present in multiple cell

types and mammalian species, in cultured cells, and in vivo. GlycoRNA assembly depends on canonical N-glycan biosynthetic machinery and results in structures enriched in sialic acid and fucose. Analysis of living cells revealed that the majority of glycoRNAs were present on the cell surface and can interact with anti-dsRNA antibodies and members of the Siglec receptor family. New chemical tools and insights into the molecular nature of glycoRNAs will be presented, which help to solidify the existence of a direct interface between RNA biology and glycobiology, and an expanded role for RNA in extracellular biology.

O-GlcNAc Regulates Fibrosis Resolution in Idiopathic Pulmonary Fibrosis
Poster Highlight

Shia Vang[1], Elex Harris[1], Molly Easter[1], Seth Bollenbecker[1], Meghan J. Hirsch[1], Patrick H. Howze IV[1], Stefanie Krick[1], and **Jarrod W. Barnes[1]**

[1]Division of Pulmonary, Allergy and Critical Care Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

Rationale- Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease that features scarring and progressive fibrosis, mediated by the extracellular matrix (ECM). This results in impaired lung function ultimately causing respiratory failure and death. Altered cellular metabolism is known to contribute to the IPF pathogenesis and dysregulated nutrient sensing, via altered glucose utilization, has been documented to drive metabolic reprogramming in myofibroblasts, one of the key effector cells in IPF. Interestingly, the O-linked N-Acetylglucosamine (O-GlcNAc) modification of proteins is involved in many cellular processes that may contribute to fibroblast-to-myofibroblast transition (FMT) including metabolism, intracellular/extracellular communication, and growth factor signaling. However, the potential role of O-GlcNAc on these processes during cell transdifferentiation and lung injury has not been studied.

Methods- Lung tissue from IPF and non-IPF control patients was subjected to immunohistochemistry (IHC) to assess O-GlcNAc levels. In addition, O-GlcNAc regulation of FMT markers [alpha smooth muscle actin (α-SMA) and type 1 and type 3 collagen (COL1α1, COL3α1)] were assessed in primary human lung fibroblasts undergoing FMT induction with transforming growth factor-beta 1 (TGF-β1) and O-GlcNAc transferase (OGT) or O-GlcNAc hydrolase (OGA) inhibitors. Lastly, pre-clinical murine models of non-resolving lung fibrosis were examined following OGT knockdown and assessed for fibrosis resolution and matrix metalloprotease (MMP) expression.

Results- O-GlcNAc staining was increased in IPF lung tissue compared to control lungs. In primary human lung fibroblasts, TGF-β1 administration resulted in increased FMT markers (α-SMA, COL1α1, and COL3α1), which were reduced or increased by OGT or OGA inhibition, respectively. Additionally, OGT knockdown in bleomycin treated aged mice resulted in reduced collagen levels and concurrent resolution as assessed by hydroxyproline analysis, expression levels of the aforementioned fibrogenic markers and TGF-β1.

Conclusion- These data suggest that O-GlcNAc regulates TGF-β1 induced FMT including collagen (i.e., COL1α1 and COL3α1) expression and/or turnover. O-GlcNAc may be directly involved in the pathogenesis of IPF and regulation of cellular transdifferentiation. Determining the mechanistic role of the O-GlcNAc modification on these processes is needed, and may identify novel therapies for the treatment of IPF or other fibrotic diseases.

Visible-Light-Induced Activation of Selenoglycosides for *O*-Glycosylation
Poster Highlight

Erik Alvarez Valenzuela, Tiffany Duong, Justin Ragains
Department of Chemistry, Louisiana State University, Baton Rouge, LA, USA

Introduction: In glycoscience, the synthesis of oligo- and polysaccharides has been a longstanding challenge. The most important reaction during multistep synthesis of oligosaccharides is undoubtedly chemical *O*-glycosylation. This reaction has been subjected to extensive research by the synthetic community in efforts to create more efficient and versatile methods. Much of the attention in this area resides in developing novel leaving groups at the anomeric carbon and/or developing novel activation methods. Thioglycosides and selenoglycosides are prized for their stability and amenability toward multistep synthesis in carbohydrate chemistry. However, this very stability makes their activation nontrivial, and strong electrophiles are often required for their activation. As an alternative to strong electrophiles, activation energy can be supplied through

irradiation with visible light as has been shown extensively in the organic chemistry community during the past 15 years. Here, we demonstrate that selenoglycosides can be activated toward *O*-glycosylation with visible-light irradiation in the presence of a stable and commonly used electrophile which is far less reactive than those typically used for ground-state chalcogenoglycoside activation.

Methods: Standard reaction conditions: 0.15 mmol scale, a small vial containing donor, acceptor, reagent A (undisclosed here), and reagent B (undisclosed here) in solvent is irradiated with two blue Kessil lamps as light source. We have synthesized 16 disaccharides thus far. 1HNMR titration and other experiments were conducted for mechanistic insight.

Results: Disaccharides were obtained in yields ranging from 78-99%. A variety of sugar acceptors and linkers were compatible, demonstrating this method's compatibility with functional groups and protecting groups. Limitations have been identified. Key experiments validated the role of light in this reaction.

Conclusion: In conclusion, we developed a novel method for *O*-glycosylation where light is the key source for donor activation. Additives used are commonly used reagents. This method tolerates a range of functional groups and protecting groups which demonstrates its broad applicability for multistep oligosaccharide synthesis.

Regulation of high mannose N-glycans by redox signaling: Implications for vascular inflammatory diseases

Poster Highlight

Alexandria L. Hernandez-Nichols[1,2], *Alana Franceski[3], Jarrod Barnes[4], Rakesh P. Patel[1,2]*

[1,2]Department of Pathology and Center for Free Radical Biology, University of Alabama at Birmingham
[3]Division of Hematology and Oncology, Department of Medicine, Heershink School of Medicine, University of Alabama, Birmingham, AL
[4]Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

N-linked glycosylation is a ubiquitous co- and post-translational modification of both cell surface and secreted proteins. Multiple glycoforms are created including proteins with complex, hybrid or high mannose (HM) type N-glycans. Expression of HM type protein N-glycoforms on the cell surface has been demonstrated in various inflammatory and autoimmune diseases, and their roles in disease pathogenesis have been proposed. However, the mechanism(s) regulating formation of HM N-glycans are less understood. Using two different model systems, we tested the hypothesis that redox signaling, specifically hydrogen peroxide (H2O2), regulates the expression of HM type glycoforms on the cell surface. In the first model, endothelial cells (ECs) were stimulated with TNFα, which led to formation of both HM and complex type N-glycoforms on the surface adhesion molecule, ICAM-1. HM ICAM-1 mediates selective adhesion of pro-inflammatory (CD16+) monocytes, which is an important step in atherogenesis. Scavenging or prevention of H2O2 production in ECs attenuated formation of only HM ICAM1 and the subsequent CD16+ monocyte adhesion. No effect of H2O2 on complex ICAM-1 was observed. TNFα led to transient inhibition of class 1α mannosidase activity that coincided with generation of HM ICAM-1. This effect was also prevented by H2O2 scavenging. Moreover, addition of H2O2 (1-100μM) also led to transient inhibition of class 1α mannosidase activity. These data suggest that H2O2 modulates early steps of the N-glycan biosynthesis to change resulting N-glycoforms in ECs.

In the second model, we tested whether H2O2 affected surface HM expression in red blood cells (RBCs). Recent studies have linked surface HM structures on RBCs to anemia in sickle cell disease. Treatment of healthy donor RBCs with H2O2 led to a rapid (5-10min) increase in surface HM structures assessed by FACS. This rapid effect, alongside the fact that RBCs are enucleated, suggest H2O2 stimulates redistribution of the HM structures in RBCs from inside the cell to outside. This effect was prevented by pre-treatment with carbon monoxide, indicating a role for heme redox cycling. Additionally, RBCs from sickle-cell disease (SCD) patients in crisis had higher basal HM expression when compared to stable post-crisis samples. Moreover, RBCs expressing HM structures showed higher adhesion to ECs suggesting a role in vascular plugging/dysfunction. In conclusion, we posit a role for H2O2 in the formation of HM N-glycans and consequential mechanisms that contribute to inflammatory disease.

Immune Interactions of Carbohydrate Antigens in Health and Disease

Fikri Avci
Emory University, Atlanta, Georgia

Avci Lab seeks to address essential research questions at the interface of immunology and glycobiology. We aim to explore the treatment of and protection from infectious diseases and cancer by understanding key molecular and cellular interactions between the immune system and carbohydrate antigens associated with microbes or cancerous cells. Avci Lab also investigates immunoregulatory properties of glycans associated with symbiotic bacteria inhabiting the host gastrointestinal tract to enable the development and healthy functioning of the immune system. Our research program delineates immune mechanisms involved in carbohydrate-mediated effector and regulatory immune responses and designs and tests prophylactic and therapeutic agents targeting model pathogens, symbionts, and cancers.

Structure, anti-SARS-CoV-2, and anticoagulant effects of two sulfated galactans from the red alga *Botryocladia occidentalis*

Antim K. Maurya[a], Poonam Sharma[b], Priyanka Samanta[a], Anter A. Shami[a], Sandeep K. Misra[a], Fuming Zhang[c], Reena Thara[a], Deepak Kumar[b], Deling Shi[c], Robert J. Linhardt[c], Joshua S. Sharp[a,d,e], Robert J. Doerksen[a,d], Ritesh Tandon[a,b,f], Vitor H. Pomin[a,d],*

[a] *Department of BioMolecular Sciences, University of Mississippi, Oxford, Mississippi, USA*
[b] *Center for Immunology and Microbial Research, Department of Cell and Molecular Biology, University of Mississippi Medical Center, Jackson, Mississippi, USA*
[c] *Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York, USA*
[d] *Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, Oxford, MS, USA*
[e] *Department of Chemistry and Biochemistry, University of Mississippi, Oxford, MS, USA*
[f] *Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA*

Introduction: The structure of the sulfated galactan from the red alga *Botryocladia occidentalis* (BoSG) was originally proposed as a simple repeating disaccharide of alternating 4-linked α-galactopyranose (Gal*p*) and 3-linked β-Gal*p* units with variable sulfation pattern. Abundance was estimated only for the α-Gal*p* units: one-third of 2,3-disulfation and one-third of 2-monosulfation. Since this work was published approximately two decades ago, numerous publications, including those reporting other potential biological applications such as antithrombotic, antimalarial, and neuroprotective effects, have appeared in the literature. In this current work, we re-investigate the structure, and the anticoagulant and anti-SARS-CoV-2 properties of the two BoSG fractions in more detail.

Method: Sulfated galactans (BoSG Fr1 and Fr2) were isolated from the *B. occidentalis* by papainolysis and purified by anion-exchange chromatography. To successfully accomplish our goals, a multi-faceted approach composed of various timely techniques was employed. These techniques are 1D and 2D homonuclear and heteronuclear 1H- and 13C-based nuclear magnetic resonance (NMR) spectroscopy, high-performance size-exclusion chromatography/multi-angle light scattering, polyacrylamide gel electrophoresis, *in vitro* anti-SARS-CoV-2 assays using wild-type and delta SARS-CoV-2 S-proteins in pseudotyped lentivirus vectors in HEK-293T-hACE2 cells monitored by green fluorescent protein, *in vitro* anticoagulant activities seen by activated partial thromboplastin time and inhibitory assays using purified blood serpins (antithrombin, and heparin cofactor II) and proteases (thrombin, IIa, and factor Xa), computational docking and competitive surface plasmon resonance (SPR) spectroscopy.

Results: The structure of BoSG Fr1 and Fr2 was revised by NMR. The current data reveals a much more complex structure for both BoSG fractions than the first proposition. A more complex sulfation pattern was noted in BoSG Fr2 along with the occurrence of 4-linked α-3,6-anhydro-Gal*p* (AnGal*p*) units. Despite higher sulfation content, BoSG Fr2 showed slightly reduced *in vitro* anti-SARS-CoV-2 activities against both wild-type and delta variants, and significantly reduced anticoagulant activity as compared to BoSG Fr1. The reduction in both bioactivities is attributed to the presence of the AnGal*p* unit. Docking scores from computational

simulations using BoSG disaccharide constructs on wild-type and delta S-proteins, and binding analysis through competitive SPR assays using blood (co)-factors (antithrombin, heparin cofactor II and thrombin) and four S-proteins (wild-type, delta, gamma, and omicron) strongly support the conclusion about the deleterious impact of the AnGal*p* unit.

The role of ST6Gal1 in regulating GBM metabolism including via GLUT3 sialylation

Joelle Saad, MSc., *Sajina GC, Ph.D., Kaysaw Tuy, Travis Nemkov, Ph.D., Victor Darley-Usmar, Ph.D., Susan Bellis, Ph.D., & Anita Hjelmeland, Ph.D.*
University of Alabama at Birmingham, Birmingham, Alabama

Glioblastoma (GBM) is the most frequent and highly malignant primary brain tumor that predominantly occurs in individuals over 50 years of age. Despite the use of the current standard of care, involving maximal surgical resection followed by radiation and chemotherapy with temozolomide, the median survival rate is only around 15 months. This poor patient prognosis is partly driven by the therapeutically resistant subpopulation of brain tumor initiating cells (BTICs) that have properties of tumor stem cells. Alterations in metabolic profiles represent one mechanism underlying this resistance. Elucidating regulators of BTICs metabolism provides potential targets for therapeutic intervention, and glycosylation is one such regulator. Glycosylation is a post-translational modification in the Golgi, where a saccharide is added to a protein destined for cellular membrane. Beta-galactoside α2,6-sialyltransferase 1 (ST6Gal1) is a glycosyltransferase responsible for adding α2,6 linked sialic acid to the terminal galactose of specific cell-surface and secreted proteins, resulting in protein sialylation. Numerous studies, including publications from our group, have established the critical role of ST6Gal1 in malignancies. Our laboratory has demonstrated that ST6Gal1 is upregulated in GBM, specifically in the BTICs population, and that it regulates a subset of cell-surface proteins associated with BTICs maintenance. However, impacts of sialyltransferase activity on GBM metabolism remains unexplored. To decipher the role of α2,6 sialylation in regulating GBM metabolism, we have acquired metabolomics profile from GBM patient derived xenografts with ST6Gal1 modulation. Our global metabolomic profile data shows increased oxidative stress and decreased fatty acid oxidation upon knocking down ST6Gal1 or inhibiting sialyltransferase activity via the pan sialyltransferase inhibitor, 3Fax-Peracetyl Neu5Ac. This is the first report linking changes in sialyltransferase to global metabolome effect in GBM. It has been established that α2,6 sialylation by ST6Gal1 can alter conformation, clustering, and retention of glycoproteins, and we have made a novel discovery that one of its downstream targets is glucose transporter 3 (GLUT3). Our past studies have confirmed that GLUT3 is upregulated in GBM leading to higher BTICs survival during glucose deprivation. However, the role of GLUT3 sialylation in altering GLUT3 receptor dynamics and modulating BTICs metabolism remains unknown, and the mechanism by which ST6Gal1-mediated α2,6 sialylation promotes BTICs survival under restricted nutrient environments requires further investigation. In addition, we aim to study GLUT3 cell-surface dynamics after ST6Gal1 modulation using flow cytometry and confocal microscopy. Determining the role of sialylation in GBM metabolism and survival will uncover novel GBM reprogramming pathways and potential therapeutic targets.

Synthetic mucins: from new chemical routes to engineered cells

Jessica R. Kramer
Asst. Prof. of Biomedical Engineering, University of Utah, Salt Lake City, Utah

Mucus is essential for life and serves as a barrier to hydrate, lubricate, and protect tissues. Mucin glycoproteins are the major component of mucus. There are 20+ mucin genes with variable expression patterns, splicing, and post-translational glycosylation that result in structures with discrete biochemical functions. Mucins play roles in infection, immunity, inflammation and cancer. Such diversity has challenged study of structure-function relationships. The Kramer lab is developing scalable methods, based on polymerization of amino acid *N*-carboxyanhydrides, to synthesize glycoproteins that capture the chemical and physical properties of native mucins. We are utilizing these synthetic mucins to engineer the glycocalyx of live cells to shed light on the role of glycans in health and disease. Areas of focus for our lab are progression of epithelial cancers, and infection processes in cystic fibrosis and COVID-19.

Heparan Sulfate Mosaicism

Jeffrey D. Esko[1], Charlotte B. Spliid[1], Sanjay Mehta[2,3], Mark M. Fuster[3,4], Cameron Martino[5,6,7], Lee Euyhyun, Hyeri You, Nharae Lee[1], Ivan Florentino[1], Claire L. Morris[1], Tara Schwartz[5], Sawyer Farmer[5], Gail Ackermann[5], Rob Knight[5,6,8,9], and Tatiana Hurtado De Mendoza[10]

[1] Department of Cellular and Molecular Medicine, [2] Department of Medicine, Division of Infectious Diseases and Global Public Health, [3] VA San Diego Healthcare system and Veterans Medical Research Foundation, [4] Department of Medicine, Division of Pulmonary & Critical Care, [5] Department of Pediatrics, [6] Center for Microbiome Innovation, [7] Bioinformatics and Systems Biology Program, [8] Department of Bioengineering, [9] Department of Computer Science and Engineering, [10]Department of Surgery, University of California San Diego, La Jolla, CA, USA

Introduction. Inbred mice have organotypic heparan sulfate signatures but exhibit little if any variation across individual mice. Similarly, heparan sulfate composition varies across different human organs and in different cells based on compositional studies in various tumor cell lines. Studies of variation in heparan sulfate composition across individuals have been limited.

Methods. To address this question, we have begun to examine variation in heparan sulfate content and composition in different organs across individual human organ donors. To expand the power of these studies, we collected saliva from 98 human study participants stratified by age, gender, and race.

Results. Although our studies of human organs are limited by donor availability, large variation in composition and content has already been noted in spleen, intestine, lung, heart and other organs across individual donors. Studies of saliva samples revealed large variation in the quantity of heparan sulfate, which increased with age. They also showed a wide range in composition across all age groups. In contrast, salivary heparan sulfate quantity and composition did not differ significantly with gender, ethnicity or race. Studies of mutant mice lacking enzymes involved in heparan sulfate formation show variation in composition less than or equal to that observed across human donors.

Implications. These findings demonstrate a surprisingly wide variation in heparan sulfate content and composition in saliva across the sampled segment of the human population and confirm other findings that show variation in content and composition of glycosaminoglycans in blood and urine. The variation in our "heparanomes" suggests that environmental factors, health status, age and likely genetic background impact heparan sulfate biosynthesis and turnover. It also raises the question of whether our heparanomic signatures affect normal physiology and pathophysiology and might account for idiopathic diseases and disorders in humans.

Inhibition of GCS Effectively Overcomes Drug Resistance of Colon Cancer Cells Carrying p53 Mutation

Md. Saqline Mostaq[a], Amanda Raphael[a], Celine Asbury[b], Anish Gupta[b], Lin Kang[b], Yong-Yu Liu[a]

[a]School of Pharmaceutical and Toxicological Sciences, University of Louisiana at Monroe College of Pharmacy, Monroe, LA 71203
[b]Biomedical Research, VCOM Louisiana, Monroe, LA 71203

The protein p53 encoded from tumor suppressor *TP53* is critical in preventing tumorigenesis and tumor progression. Notably, missense mutations of *TP53* detected in over 50% of cancer cases can promote drug resistance and tumor metastasis. Glucosylceramide synthase (GCS), encoded by the *UGCG* gene, is a rate-limiting enzyme in the synthesis of glycosphingolipids (GSLs). Overexpression of GCS responding to chemotherapy is highly correlated to cancer drug resistance and progression. It is hypothesized that knocking out the *UGCG* gene and inhibiting GCS could reverse the drug resistance of cancer with p53 mutations. By utilizing CRISPR/cas9 to knockout *UGCG* and applying new generation inhibitor (Genzyme-667161) to supress GCS activity, we observed that GCS inhibition overcome drug resistance of colon cancer cells (WiDr) carrying a p53 mutation (R273+/+). The WiDr/UGCG-KO (knock out) cells displayed an approximately 2-fold lower IC50 value for oxaliplatin, and an approximately 4-fold reduction in cell migration rate, compared to the WiDr parental cells. Furthermore, UGCG-KO or GCS inhibitor treatments significantly sensitized oxaliplatin effects, reducing tumor volumes to approximately 63% and 69% respectively, compared to WiDr tumors treated solely with oxaliplatin. Single-nucleus RNA-sequencing (snRNA-seq) revealed a reduction to 89% in the abundance of stem-like cells in WiDr/UGCG-KO tumor, compared to WiDr tumor exposed to oxaliplatin. These findings suggest

that Cer-glycosylation by GCS plays a crucial role in promoting cancer drug resistance during chemotherapy. Further studies will identify key molecules involved in the genesis of cancer stem cells and elucidate how Cer-glycosylation modulates mutant protein expression of *TP53*. Such insights could pave the way for developing novel strategies targeting Cer-glycosylation in cancer therapeutics.

GARP complex is Essential for the Maintenance of the Golgi Glycosylation Machinery

Amrita Khakurel[1*], Tetyana Kudlyk[1*], Vladimir V. Lupashin[1**]

[1]*University of Arkansas for Medical Sciences, Department of Physiology and Cell Biology, Little Rock, Arkansas, US*

The Golgi Associated Retrograde Protein (GARP) complex is a multi-subunit vesicle tethering complex localized in the *trans*-Golgi Network (TGN) and is believed to function by tethering the retrograde vesicles arriving from the endosomes to the TGN. However, the role of GARP complex in Golgi glycosylation has not been investigated. We hypothesized that GARP complex is involved in maintaining Golgi glycosylation machinery. In this study, we employed CRISPR/Cas9 strategy to knock out (KO) GARP complex subunits in three different human cell lines. Our findings surprisingly demonstrated severe defects in Golgi modification and secretion of *N*- and *O*-glycosylated proteins in GARP deficient cells. We observed a decrease in the stability of Golgi enzymes and resident glycoproteins in GARP-KO cells. We also observed reduced retention of several key glycosylation enzymes (MGAT1, GALNT2, B4GALT1 and ST6GAL1) in the Golgi of GARP-KOs. A RUSH (Retention Using Selective Hooks) assay showed that, in GARP-KO cells, B4GALT1 is not retained at the Golgi complex but instead is missorted to the endolysosomal system indicating that GARP complex is a critical regulator of Golgi glycosylation machinery. Significantly, expression of the missing GARP subunit in the GARP-KO cell corrected all observed defects. These findings reveal that GARP complex is essential for the maintenance of the Golgi glycosylation machinery.

Designing/Discovering Synthetic, Highly Sulfated Mimetics of Glycosaminoglycans as AntiThrombotic and AntiCancer Agents

Umesh R. Desai, PhD

Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298

Natural, highly sulfated glycosaminoglycans (GAGs), including heparin/heparan sulfate (Hp/HS), are thought of as drugs because of their polymeric and highly anionic structure. In fact, drug developers shy away from such structures. In 1998 we started our GAG-based drug discovery program with a defined goal of developing a highly promising synthetic, non-saccharide mimetic of Hp/HS as an anticoagulant. In the past 25 years or so, we have realized one such molecule that exhibit excellent antithrombotic effects in vivo by selectively targeting coagulation factor XIa (J Thromb Haemost 2019, 17:2110-22; J. Med. Chem. 2014, 57:4805-18). Simultaneously, we initiated a program around 2010 on selectively targeting cancer stem cells (CSCs) relying on libraries of GAG oligosaccharides (Oncotarget 2016, 7:84608-22) or synthetic GAG mimetics (ACS Chem Biol 2014, 15:1826-33). This campaign has now yielded a highly selective inhibitor of colorectal CSCs, which prevents cancer relapse in vivo by targeting three tyrosine kinase receptors (TKRs) in a concerted manner (Mol. Cancer Ther. 2019, 18:51-61; Angew. Chem. Int. Ed. 2022, 61:e202211320). In the process, we have discovered and/or developed several principles/technologies on GAG recognition of proteins (J. Med. Chem. 2023, 66:4503-31), GAG mimetic synthesis (J. Med. Chem. 2019, 60:641-57; J. Med. Chem. 2011; 54:5522-31), computational high-throughput screening of GAGs (Curr Opin Struct Biol 2018, 50:91-100; Curr Opin Struct Biol 2022, 74:102356), and oral delivery of GAG mimetics (J. Med. Chem. 2023, 66:1321-38). This talk will be directed towards a student learner who might benefit from a synopsis of lessons gained in following an off-the-cuff route, especially in drug discovery and development.

ABSTRACTS
Poster Presentations

Poster 1:

The competitive effect of sulfated glycans on thrombin binding to surface heparin using surface plasmon resonance

Hoda Al. Ahmed[1] and Vitor H. Pomin[1,2]

[1]*Department of BioMolecular Sciences, University of Mississippi, Oxford, Mississippi, USA.*
[2]*Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, Oxford, Mississippi, USA.*

Introduction: Marine organisms are a rich source of biologically active compounds, including unique sulfated glycans endowed with potential medical applications. The marine sulfated glycans (MSGs) show unique structures composed of defined oligosaccharide building blocks and specific biological effects such as those seen in clotting inhibition. The anticoagulant effect of MSGs depends ultimately on certain structural features such as molecular weight, monosaccharide composition, glycosidic linkage, sulfation pattern and levels of fucosylation. Sulfated fucan (SF), sulfated galactan (SG), and fucosylated chondroitin sulfate (FucCS), extracted from algae sea urchins and sea cucumbers, are classes of MSGs widely used in research. In this work, we aim to test the competitive inhibitory effects of 4 FucCS, 4 SF and 2 SG on the binding interaction of the main coagulation factor, thrombin (IIa) to surface heparin using surface plasmon resonance (SPR).

Methods: Preparation of streptavidin (SA)-biotinylated heparin SPR sensor chip: SA chip was prepared by injecting SA (0.5 µM) to biotin sensor chip. Biotinylated heparin was prepared by conjugating its reducing end to amine-PEG3-biotin. The purified biotinylated heparin (50 µg/ml) was immobilized to SA sensor chip. Factor IIa was diluted in HBS-EP buffer at varying concentrations and injected at a flow rate of 20 µl/min at 25 oC. At the end of each sample injection, HBS-EP buffer was flowed over the sensor surface to facilitate dissociation and then the sensor surface was regenerated by injecting 2 M NaCl. **SPR solution competition study of sulfated glycans versus surface heparin:** Factor IIa (200 nM) was mixed with different concentrations of unfractionated heparin (UFH), low molecular weight heparin (LMWH) and the MSGs in HBS-EP buffer. The mixtures injected over a heparin sensor chip at a flow rate of 20 µl/min. The dissociation and regeneration were performed after each run. For each set of competition experiments, a control experiment using IIa only was performed to make sure the surface was completely regenerated and that the results obtained between runs were comparable. EC50 values reflecting the concentration of the competing analyte resulting in a 50% decrease in protein binding, measured in response units (RUs), were calculated from the RU plots as a function of sulfated glycan concentration.

Results: Our results showed that the dissociation constant of IIa binding to surface heparin was 102 nM (± 0.00179 nM). The EC50 of UFH, LMWH, PpFucCS and HfFucCS in nanomolar concentration were respectively 86.7 (± 0.0894), 2120 (± 0.744), 1510 (± 1.89) and 1150 (± 1.08). The other MSGs will be tested shortly and from the expected set of data, we will be able to identify structural motifs within the MSGs responsible for high affinity with IIa.

Poster 2:

AGE/RAGE Signaling Impacts Cognitive Behaviors Throughout Adulthood

Miguel A. De Leon, Cellas A. Hayes, Brandon G. Ashmore, Nyah Morgan, James A. Stewart, Jr., **Nicole M. Ashpole**
University of Mississippi, Oxford, Mississippi

Introduction: Natural accumulation of advanced glycated endproducts (AGEs) throughout our life is negatively associated cognitive function and overall health. Preclinical studies suggest that blockade of AGE signaling can lessen the burden of several pathologies commonly observed in advanced aging. However, longitudinal studies have not yet clarified whether a reduction in AGE signaling across a healthy lifespan will have a direct impact on aging and behavior. Here, we investigated the effects of AGE signaling across the lifespan to determine if they delay or accelerate cognitive and physical impairments.

Methods: We utilized cohorts of male and female mice deficient in the cognate age receptor- RAGE. RAGE knock-out mice and wild-type controls were enrolled in the study at three months of age and behavioral endpoints (learning and memory, anxiety-like behaviors, circadian rhythm, nociception) were monitored at various stages of adulthood.

Results: Early in adulthood, RAGE KO mice showed significant impairments in spatial learning, with increased errors made in the radial arm water maze as well as decreased success in completing the task. These impairments in spatial learning persisted across adulthood and were observed in both male and female mice. Unlike spatial learning, working memory was not impacted in either female or male RAGE KO mice, as the novel object tasks revealed no significant differences. There were also no differences in motor coordination on the rotarod. When circadian function was assessed at 14 months of age, female RAGE KO mice were more active on free running wheels than wild-type controls. No differences were observed in the males. Interestingly, there was a treatment by phase interaction in the circadian study as female RAGE KO mice ran significantly more in the dark phase than their wild-type controls and significantly less in the light phase than wild-type controls, suggesting a tighter restriction on their daily running period. Further studies are needed to explore light sensitivity and circadian rhythm entrainment in these mice. A similar paradoxical effect was observed in the open field test. While there were no differences in the percent time spend in the center vs periphery, RAGE KO females were far less active in the brightly-lit arena, which may suggest altered sensitivity to visual stimuli. Both male and female RAGE KO mice showed signs of visual impairment in late adulthood. Additionally, male KO mice showed increased mechanical sensitivity. Overall, our study suggests that lifelong reductions in AGE/RAGE signaling has direct effects on multiple behaviors in healthy adult mice.

Poster 3:

Visible-Light-Induced Activation of Selenoglycosides for *O*-Glycosylation

Erik Alvarez Valenzuela, Tiffany Duong, Justin Ragains
Department of Chemistry, Louisiana State University, Baton Rouge, LA, USA

Introduction: In glycoscience, the synthesis of oligo- and polysaccharides has been a longstanding challenge. The most important reaction during multistep synthesis of oligosaccharides is undoubtedly chemical *O*-glycosylation. This reaction has been subjected to extensive research by the synthetic community in efforts to create more efficient and versatile methods. Much of the attention in this area resides in developing novel leaving groups at the anomeric carbon and/or developing novel activation methods. Thioglycosides and selenoglycosides are prized for their stability and amenability toward multistep synthesis in carbohydrate chemistry. However, this very stability makes their activation nontrivial, and strong electrophiles are often required for their activation. As an alternative to strong electrophiles, activation energy can be supplied through irradiation with visible light as has been shown extensively in the organic chemistry community during the past 15 years. Here, we demonstrate that selenoglycosides can be activated toward *O*-glycosylation with visible-light irradiation in the presence of a stable and commonly used electrophile which is far less reactive than those typically used for ground-state chalcogenoglycoside activation.

Methods: Standard reaction conditions: 0.15 mmol scale, a small vial containing donor, acceptor, reagent A (undisclosed here), and reagent B (undisclosed here) in solvent is irradiated with two blue Kessil lamps as light source. We have synthesized 16 disaccharides thus far. 1HNMR titration and other experiments were conducted for mechanistic insight.

Results: Disaccharides were obtained in yields ranging from 78-99%. A variety of sugar acceptors and linkers were compatible, demonstrating this method's compatibility with functional groups and protecting groups. Limitations have been identified. Key experiments validated the role of light in this reaction.

Conclusion: In conclusion, we developed a novel method for *O*-glycosylation where light is the key source for donor activation. Additives used are commonly used reagents. This method tolerates a range of functional groups and protecting groups which demonstrates its broad applicability for multistep oligosaccharide synthesis.

Poster 4:

O-GlcNAc Regulates Fibrosis Resolution in Idiopathic Pulmonary Fibrosis

Shia Vang[1], Elex Harris[1], Molly Easter[1], Seth Bollenbecker[1], Meghan J. Hirsch[1], Patrick H. Howze IV[1], Stefanie Krick[1], and **Jarrod W. Barnes[1]**

[1]*Division of Pulmonary, Allergy and Critical Care Medicine, University of Alabama at Birmingham, Birmingham, AL, USA*

Rationale- Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease that features scarring and progressive fibrosis, mediated by the extracellular matrix (ECM). This results in impaired lung function ultimately causing respiratory failure and death. Altered cellular metabolism is known to contribute to the IPF pathogenesis and dysregulated nutrient sensing, via altered glucose utilization, has been documented to drive metabolic reprogramming in myofibroblasts, one of the key effector cells in IPF. Interestingly, the O-linked N-Acetylglucosamine (O-GlcNAc) modification of proteins is involved in many cellular processes that may contribute to fibroblast-to-myofibroblast transition (FMT) including metabolism, intracellular/extracellular communication, and growth factor signaling. However, the potential role of O-GlcNAc on these processes during cell transdifferentiation and lung injury has not been studied.

Methods- Lung tissue from IPF and non-IPF control patients was subjected to immunohistochemistry (IHC) to assess O-GlcNAc levels. In addition, O-GlcNAc regulation of FMT markers [alpha smooth muscle actin (α-SMA) and type 1 and type 3 collagen (COL1α1, COL3α1)] were assessed in primary human lung fibroblasts undergoing FMT induction with transforming growth factor-beta 1 (TGF-β1) and O-GlcNAc transferase (OGT) or O-GlcNAc hydrolase (OGA) inhibitors. Lastly, pre-clinical murine models of non-resolving lung fibrosis were examined following OGT knockdown and assessed for fibrosis resolution and matrix metalloprotease (MMP) expression.

Results- O-GlcNAc staining was increased in IPF lung tissue compared to control lungs. In primary human lung fibroblasts, TGF-β1 administration resulted in increased FMT markers (α-SMA, COL1α1, and COL3α1), which were reduced or increased by OGT or OGA inhibition, respectively. Additionally, OGT knockdown in bleomycin treated aged mice resulted in reduced collagen levels and concurrent resolution as assessed by hydroxyproline analysis, expression levels of the aforementioned fibrogenic markers and TGF-β1.

Conclusion- These data suggest that O-GlcNAc regulates TGF-β1 induced FMT including collagen (i.e., COL1α1 and COL3α1) expression and/or turnover. O-GlcNAc may be directly involved in the pathogenesis of IPF and regulation of cellular transdifferentiation. Determining the mechanistic role of the O-GlcNAc modification on these processes is needed, and may identify novel therapies for the treatment of IPF or other fibrotic diseases.

Poster 5:

The role of glycans in cell state transitions during cardiac development in zebrafish

Joshua Bloomekatz[1*], Tess McCann[1], Harini Saravanan[1], Prashanna Koirala[1], Rabina Shrestha[1]

[1]*Department of Biology, University of Mississippi, University, MS 38677*
[*]*Corresponding author – josh@olemiss.edu*

Introduction: Cell-state transitions involve the coordinated transformation of cellular identity, physiology, and morphology. These transitions, for example epithelial-to-mesenchymal transitions (EMT) or myocardial differentiation are central to cardiac development and disease pathogenesis, including in the de-differentiation of cardiomyocytes and the fibrosis generated during fetal hyperglycemia. We are examining the role of glycosylation, both at the individual and collective level, in cardiac development and disease progression. Specifically, we are examining: the role of O-linked glycosylation in the V-domain of fibronectin, the dynamics of the cardiomyocyte glycocalyx and the role of O-GlcNAc in the cell-state transitions occurring during cardiomyocyte development and during hyperglycemia-mediated disease pathogenesis.

Methods and results: The oncofetal isoform of fibronectin has been found to be specifically expressed during development and cancer. Unique to this isoform is an O-linked glycan. However, the role of this isoform and the O-linked glycan unique to it has not been elucidated. We are using transgenic and genetic approaches along with mass-spectrometry to determine the function of this oncofetal-isoform and the structure of the attached glycan. Using functionalized metabolic precursors attached by bio-orthogonal reactions to fluorogenic probes,

we are taking a holistic approach to investigate how glycan composition and dynamics change during the development of cardiac and trunk muscles in zebrafish. Specifically, we are analyzing mesenchymal-to-epithelial transitions (MET) and myocyte differentiation in both cardiac and trunk muscles. We have observed that sialyation is particularly prevalent at the intersomitic trunk muscle boundaries and at cell boundaries in the ventricle and outflow tract of the zebrafish heart. However, this enrichment in the heart is lost in our zebrafish model of fetal-hyperglycemia, indicating metabolic-dysfunction due to increased glucose levels causes disruptions in the cardiomyocyte glycocalyx. In the future we plan to combine these studies with proteomic studies analyzing dynamics of the glycocalyx during cardiac differentiation and disease pathogenesis.

Poster 6:

The sulfated fucan from *Thyonella gemmata* presents high anti-SARS-CoV-2 but low anticoagulant activity

Rohini Dwivedi[1], Marwa Farrag[1,2], Poonam Sharma[3], Deling Shi[4], Sandeep Misra[1], Priya Ray[3], Jayanti Shukla[3], Fuming Zhang[4], Robert Linhardt[4], Joshua Sharp[1,5], Ritesh Tandon[1,3], Vitor Pomin[1,5]

1Department of BioMolecular Sciences, University of Mississippi, Oxford, Mississippi, USA
2 Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71515, Egypt
3Department of Microbiology and Immunology, University of Mississippi Medical Center, Jackson, Mississippi, USA
4Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York, USA
5Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, Oxford, MS, USA

Introduction: Marine sulfated glycans (MSG) can exhibit antiviral activities such as those against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectivity, but also anticoagulant properties. Research and discovery of naturally low anticoagulant MSGs still retaining promising antiviral activities is a strategy to develop a more selective antiviral agent. In this study, we isolated a new sulfated fucan (TgSF) and a fucosylated chondroitin sulfate (TgFucCS) from the sea cucumber *Thyonella gemmata*. Structural characterization, anticoagulant and anti-SARS-CoV-2 activities were also performed.

Methods: TgSF and TgFucCS were isolated by proteolytic digestion of the sea cucumber body wall and fractionated by anion-exchange chromatography. Structural characterization was performed by both 1D and 2D nuclear magnetic resonance spectroscopy. Molecular weights were determined by polyacrylamide gel electrophoresis and multi-angle light scattering. Anticoagulant activity was measured by activated partial thromboplastin time and serpin-mediated inhibitory assays of blood proteases. Anti SARS-CoV-2 activity was examined using a pseudotyped SARS-CoV-2 baculoviral vector in HEK293T cells monitored by green fluorescence protein. Anti-SARS-CoV-2 action was mechanistically investigated by surface plasmon resonance using both wild-type and mutant spike proteins.

Results: TgFucCS structure is predominantly (70%) composed of a chondroitin 4-sulfate. Interestingly, 33% of the glucuronic acid residues were substituted at the C3 position with two types of α-fucose (Fuc) branches: Fuc2,4S (35%), and Fuc4S (65%), where S = SO3-. TgSF is structurally comprised of 6 major types of Fuc units: Fuc2,4S (10%), two Fuc4S (35%), one Fuc4S (15%), two Fuc2S (40%) units linked mostly by α(1→3) linkage and minor α(1→2) linkage. TgSF showed negligible anticoagulant action, lower than unfractionated heparin. Like heparin, both TgFucCS and TgSG showed efficacious anti-SARS-CoV-2 activities.

Poster 7:

Leveraging Benzyne Chemistry for the Development of 1,2-*cis* Selective *O*-Glycosylation

Tiffany Duong, Erik Alvarez Valenzuela, Justin R. Ragains
Department of Chemistry, Louisiana State University, Baton Rouge, LA

Introduction: In the realm of chemical *O*-glycosylation, 1,2-*cis*-selectivity is a subject of ongoing investigation. While there have been many ingenious solutions to this problem, an approach that avoids specialized auxiliaries and protecting groups or expensive reagents is especially desirable. Herein, we demonstrate that reaction of benzylchalcogenoglycosides with benzyne results in highly 1,2-*cis*-selective *O*-glycosylation. This is an operatively straightforward and mechanistically novel approach to the formation of 1,2-cis *O*-glycosidic linkages.

Methods: Benzylchalcogenoglycosides were combined with Kobayashi's reagent (a benzyne precursor), a fluoride source, and alcohol nucleophiles in organic solvent. *O*-Glycosides rich in the 1,2-*cis* linkage resulted, and these products were purified with silica gel chromatography. Estimation of stereoselectivity was determined with $_1$H NMR integration of distinct signals from the respective 1,2-*cis* and 1,2-*trans* products in both the crude and purified samples.

Results: We have shown that benzylchalcogenoglycosides (*S*- and *Se*-glycosides) can be activated with *in-situ* generated benzyne to afford *O*-glycosides in a highly 1,2-*cis* selective fashion. This method has been demonstrated across a range of substrates.

Conclusion: This work represents a mechanistically novel and user-friendly approach to *O*-glycosylation in which benzyne serves as a critical intermediate in chalcogenoglycoside activation. This method does not require special auxiliaries, protecting groups, or indirect multistep processes to obtain product in a highly 1,2-*cis* selective manner.

Poster 8:

Acylated Double-Layered Electrospun Chitosan Nanofibrous Membranes reduce burst and extend the duration of elution of hydrophobic therapeutics

Nidhi Gupta, Rabeta Yeasmin, Jessica Amber Jennings
Biomedical Engineering, Herff College of Engineering, The University of Memphis, Memphis, Tennessee, USA.

Wound dressings serve to protect tissue from contamination, alleviate pain and facilitate wound healing. Electrospun chitosan nanofibrous membranes (ESCM) are advantageous for wound healing applications because of their high degree of biocompatibility, biodegradability, ability to mimic extracellular matrix, and increased surface area. However, their use is often limited due to their extreme hydrophilic nature causing them to lose their nanofibrous structure. In this study, chitosan membranes were modified by acylation reactions using fatty acid anhydrides of different chain lengths to increase hydrophobicity of the membranes and protect nanofibrous structure in aqueous environment. Loading directly acylated biopolymer ESCM with antimicrobial and anaesthetic agents may provide protection from contaminants, pain management until and during hospitalization. Adsorption of trans-2-decenoic acid (T2DA), an inhibitor of *Staphylococcus aureus* biofilm, to acylated ESCM is a viable approach to treating musculoskeletal infections by slowly releasing biofilm-inhibiting fatty acid. Further, effect of the single and double-layered ESCM is evaluated against the release of T2DA. We hypothesize that T2DA will be released slowly from double-layered ESCM and more rapidly in acidic environments mimicking wounds, thereby extending the wound dressing functionality.

ESCM of varying thickness were prepared by electrospinning a 5.5% (w/v) solution of 86% chitosan and a 7:3 (v/v) mixture of trifluoracetic acid and dichloromethane. Single and double-layered ESCM were stabilized through acylation using fatty acids anhydrides of different chain lengths: hexanoic, octanoic and decanoic acid anhydrides, followed by loading with T2DA by immersion. The modified membranes were characterized using scanning electron microscopy, Fourier transform infrared spectroscopy, contact angle and elemental analysis to confirm the addition of the modification groups. The degree of substitution of the fatty acid chain were estimated from the elemental analysis by XPS. T2DA release profile is monitored through reverse phase high-pressure liquid chromatography.

The double-layered ESCM adsorbed almost two-fold of T2DA compared to the single-layered membrane but released slower than it. The single-layered ESCM has an initial burst releasing more than 50% of adsorbed T2DA within 3hr. The T2DA release was slower as the length of the fatty acid anhydride chain increased. The long chain fatty acid modified double-layered ESCM exhibited comparatively slow elution of anti-biofilm agent and thereby maintain their therapeutic sustainability in the system beyond wound care protocol time. This can significantly reduce the cost and time of wound care. In future, it could also serve as a promising support to load with hydrophobic drugs for orthopedic wound surgical dressing applications.

Poster 9:

Elucidating the Molecular Mechanisms of Gating Regulation of N-Glycosylation on Voltage-Gated Sodium Channels Using Molecular Dynamics Simulations

Eslam Elhanafy and Jing Li
Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, MS, 38677

Voltage-gated sodium channels (VGSCs) are essential for the initiation and propagation of action potentials in excitable cells. VGSCs are differentially sialylated in muscle and heart cells, a phenomenon thought to contribute to the distinctive electrical signaling properties of these two cell types. In this study, we used molecular dynamics (MD) simulations to investigate how glycans alter ion channels, both structurally and dynamically. We investigated the effects of differential sialylation on the structure and function of VGSCs. Using the cryo-EM structure for human Nav1.4 (PDB ID: 6AGF), we built a model of the glycosylated channel (α subunit) embedded in the lipid bilayer. The glycans were attached to eleven N-glycosylation sites, and their structure and composition were the most abundant form from previous glycomic profiling analyses of Nav channels. MD simulations of the Nav1.4 channel were performed with and without sialylated glycans for 1 microsecond. An electrostatic potential analysis showed that the sialic acids generated some negative charge centers above the voltage-sensing domains (VSDs), which might facilitate their activation. Notably, a strong electrostatic interaction is observed between a sialic acid and arginine R1058, located at the extracellular loop of VSD3. Interestingly, while R1058 has not been associated with any disease-related mutation in Nav1.4, equivalent mutations (R1245Q/V in Nav1.1 and R1232Q/W in Nav1.5) are linked to severe epilepsy (Dravet syndrome) and cardiac arrhythmia (Brugada syndrome), respectively. Our findings suggest that differential sialylation of VGSCs can modulate their structure and function. Further studies are needed to investigate the role of differential sialylation in VGSC-related diseases.

Poster 10:

Structural specificity in anticoagulant and anti-SARS-COV-2 properties of holothurian fucosylated chondroitin sulfate oligosaccharides

Marwa Farrag[1,2], Rohini Dwivedi[1], Poonam Sharma[3], Deepak Kumar[3], Ritesh Tandon[1,3,4], Vitor H. Pomin[1,5]

[1]*Department of BioMolecular Sciences, University of Mississippi, Oxford, Mississippi, USA*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.*
[3]*Department of Microbiology and Immunology, University of Mississippi Medical Center, Jackson, Mississippi, USA*
[4]*Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA*
[5]*Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, Oxford, MS, USA*

Introduction: Fucosylated chondroitin sulfate (FucCS) is a unique glycosaminoglycan found primarily in sea cucumbers. This marine sulfated glycan is composed of a chondroitin sulfate backbone decorated with fucosyl branches attached to the glucuronic acid. FucCS has been shown to be effective against severe acute respiratory syndrome coronavirus (SARS-CoV-2), however, they are also endowed with potential anticoagulant effects which may lead to bleeding risks when explored as antivirals. Previous studies have suggested that molecular weight reduction of these glycans through depolymerization may offer an effective way to gain a selective anti-SARS-CoV-2 activity while minimizing the associated anticoagulant effects. In this work, we aim to generate oligosaccharides of the FucCS from *Holothuria floridana* (HfFucCS) and investigate its potential inhibitory properties against blood clotting and SARS-CoV-2 infection.

Methods: Oligosaccharides were prepared by free-radical depolymerization using copper-based Fenton reaction. 1D ^{1}H NMR spectra were employed in structural analysis. Anticoagulant actions were monitored by activated partial thromboplastin time, as well as protease (factors Xa and IIa) inhibition by serine protease inhibitors (antithrombin, and heparin cofactor II) in the presence of the sulfated carbohydrates. Anti-SARS-CoV-

2 effects were measured by the concentration-response inhibitory curves of HEK-293T-human angiotensin-converting enzyme-2 cells infected with a baculovirus pseudo-typed SARS-CoV-2 wild-type and delta variant spike (S)-proteins. The cytotoxicity of native HfFucCS and its oligosaccharides was also assessed.

Results: In our study, we were able to generate a HfFucCS oligosaccharide fraction devoid of high anticoagulant effect but still retaining considerable anti-SARS-CoV-2 actions against both variants.

Poster 11:

The Challenges in Predicting Protein-Glycan Binding Energies Through MD Simulations

Sushil K. Mishra[1] and Robert J. Doerksen[1,2]

[1]*Department of BioMolecular Sciences, University of Mississippi, MS-38677, USA;* [2]*Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS-38677, USA*

Protein–glycan interactions play a pivotal role in various biological functions such as adhesion, recognition, differentiation, metastasis, pathogenesis, and immunological responses.1 Understanding these interactions holds great importance in unraveling the underlying mechanisms behind these biological processes. Therefore, approaches for understanding the atomic basis of protein–glycan interactions are of interest not only for theoretical reasons, but also for the design of glycan-based therapeutics and glycan-recognizing reagents in affinity chromatography, analytical approaches, or diagnostics tools. We have assessed the performance of several free-energy calculation approaches2–4 on protein–glycan complexes and have continued to investigate this by applying Potential of Mean Force (PMF)-based rigorous free-energy calculation approaches.5 In this presentation, we will demonstrate how binding free-energy calculations can be performed via the geometric route, in which structural restraints are introduced to guide the binding and unbinding of the ligand along the reaction coordinate. We will show how different force fields and simulation lengths affect the performance of such PMF-based method on protein–glycan complexes.

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Poster 12:

Probing the interactions of IGF2 with the full-length soluble domains of CI-MPR using hydroxyl radical protein footprinting

Sandeep K. Misra[1], Richard Bohnsack[2], Nancy M. Dahms[2] and **Joshua S. Sharp**[1,3,4]

[1]*Glycoscience Center of Research Excellence, University of Mississippi School of Pharmacy, University, MS 38677*
[2]*Department of Chemistry and Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226*
[3]*Department of BioMolecular Sciences, University of Mississippi, University, MS 38677*
[4]*Department of Chemistry and Biochemistry, University of Mississippi, University, MS 38677*

The cation-independent mannose 6-phosphate receptor (CI-MPR) is a transmembrane P-type lectin that is responsible for trafficking proteins with M6P-tagged N-linked glycans to late endosomes. CI-MPR is also responsible for internalizing the non-glycosylated insulin-like growth factor 2 (IGF2), a small protein hormone that promotes cell proliferation in many tissues. A loss of CI-MPR binding to IGF2 can lead to increased bioavailability of IGF2, promoting angiogenesis. Through this mechanism, CI-MPR plays a role as a natural tumor suppressor.

CI-MPR is approximately 300 kDa in size, with a large extracellular region comprised of fifteen repeating domains. In our previous work, we used hydroxyl radical protein footprinting (HRPF) to analyze the binding of M6P-tagged glycoproteins to domains 1-5, as well as examine the conformational changes upon pH decrease that result in release of M6P-tagged cargo. HRPF is a flexible, mass spectrometry-based technique that allows for changes in protein topography (i.e. solvent-exposed surfaces) to be measured between different conformational states by measuring the changes in the rate of reaction with hydroxyl radicals at many different sites of oxidation simultaneously. Here, we move to examining the entire extracellular region of CI-MPR, looking at both binding of IGF2 at neutral pH as well as at the conformational changes of the full extracellular region upon a drop to pH 4.5. Our preliminary HRPF results show that binding of IGF2 results in local reorganization around the binding site, with some regions in domains 6, 8 and 11 showing protection upon binding while another peptide in domain 11 shows exposure. Distal conformational changes are also observed in domains 4, 5 and 12 suggesting either allosteric changes or a compacted unbound structure not reflected in the cryo-EM model. Upon a drop in pH, we observe a large-scale reorganization of CI-MPR, with exposures in domains 1, 4, 5, and 12 and a mix of protection and exposure in the central domains surrounding the IGF2 binding region that mimic topographical changes observed upon IGF2 binding. We are currently following up on these results to improve our statistical power, which we will then use to generate models of CI-MPR structure-function relationships.

Poster 13:

Regulation of high mannose N-glycans by redox signaling: Implications for vascular inflammatory diseases

Alexandria L. Hernandez-Nichols[1,2], *Alana Franceski*[3], *Jarrod Barnes*[4], *Rakesh P. Patel*[1,2]

[1,2]Department of Pathology and Center for Free Radical Biology, University of Alabama at Birmingham
[3]Division of Hematology and Oncology, Department of Medicine, Heershink School of Medicine, University of Alabama, Birmingham, AL
[4]Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

N-linked glycosylation is a ubiquitous co- and post-translational modification of both cell surface and secreted proteins. Multiple glycoforms are created including proteins with complex, hybrid or high mannose (HM) type N-glycans. Expression of HM type protein N-glycoforms on the cell surface has been demonstrated in various inflammatory and autoimmune diseases, and their roles in disease pathogenesis have been proposed. However, the mechanism(s) regulating formation of HM N-glycans are less understood. Using two different model systems, we tested the hypothesis that redox signaling, specifically hydrogen peroxide (H2O2), regulates the expression of HM type glycoforms on the cell surface. In the first model, endothelial cells (ECs) were stimulated with TNFα, which led to formation of both HM and complex type N-glycoforms on the surface adhesion molecule, ICAM-1. HM ICAM-1 mediates selective adhesion of pro-inflammatory (CD16+) monocytes, which is an important step in atherogenesis. Scavenging or prevention of H2O2 production in ECs attenuated formation of only HM ICAM1 and the subsequent CD16+ monocyte adhesion. No effect of H2O2 on complex ICAM-1 was observed. TNFα led to transient inhibition of class 1α mannosidase activity that coincided with generation of HM ICAM-1. This effect was also prevented by H2O2 scavenging. Moreover, addition of H2O2 (1-100μM) also led to transient inhibition of class 1α mannosidase activity. These data suggest that H2O2 modulates early steps of the N-glycan biosynthesis to change resulting N-glycoforms in ECs.

In the second model, we tested whether H2O2 affected surface HM expression in red blood cells (RBCs). Recent studies have linked surface HM structures on RBCs to anemia in sickle cell disease. Treatment of healthy donor RBCs with H2O2 led to a rapid (5-10min) increase in surface HM structures assessed by FACS. This rapid effect, alongside the fact that RBCs are enucleated, suggest H2O2 stimulates redistribution of the HM structures in RBCs from inside the cell to outside. This effect was prevented by pre-treatment with carbon monoxide, indicating a role for heme redox cycling. Additionally, RBCs from sickle-cell disease (SCD) patients in crisis had higher

basal HM expression when compared to stable post-crisis samples. Moreover, RBCs expressing HM structures showed higher adhesion to ECs suggesting a role in vascular plugging/dysfunction. In conclusion, we posit a role for H2O2 in the formation of HM N-glycans and consequential mechanisms that contribute to inflammatory disease.

Poster 14

Ag2O Assisted Gram-Scale Synthesis of Core 2 Containing Glyco-Amino Acid

Joshua Zhu, Lisa Nicholson, Anna Rediger, Brooke Van Engen, and Noah Mulder
Departments of Chemistry, Dordt University, Sioux Center, IA 51250

O-linked glycosylation is a widely existing glycoform on human cell surface. Alpha-linked N-acetylgalactosamine (GalNAc) on Serine/Theorine is one of the major types of O-linked glycosylation such as T/Tn antigens. O-GalNAc glycans can be derived into branched sugars, therefore, 8 core structures of O-GalNAc glycans are commonly found in biological systems. Among the 8 core structures, core 2 structure has been found on human glycoproteins with critical biological functions. For example, core 2 containing glycans are commonly required for certain selectin-mediated interactions between endothelial cells and leukocytes. In order to study the detailed functions of core 2 containing glycans, synthetic core 2 containing glycans are needed. These structure-defined sugars can provide precise information of how the sugars are involved in their biological functions. However, it is a challenging task for synthesizing glycopeptides with core 2 containing glycans. One of the major obstacles is the large-scale synthesis of core 2 containing glyco-amino acid. In our study, we applied Ag2O for constructing the alpha linkage between 2-azido-Gal and Fmoc-Serine which is essential for later core 2 synthesis. With the use of Ag2O, per-acetylated 2-azido-galactosyl chloride is used as the sugar donor instead of using other types of glycoside donors. The advantage of using 2-azido-galactosyl chloride as the donor is that the azido group is introduced by NaN3 instead of Tf-N3 as Tf-N3 has higher potential of explosive than NaN3. Another advantage is that the synthesis of 2-azido-galactosyl chloride can be easily done in more than 15-gram scale reactions and only one column chromatography purification for three-step reactions. After introduction of the α-linked sugar, the following β1-3 Gal and β1-6 GlcNAc can be assembled through traditional glycosylation methods. So far, we have achieved the synthesis of per-acetylated core 2 containing Fmoc-Serine in more than 1.9-gram scale, and the synthesized glyco-amino acid has been successfully used for the synthesis of glycosylated CCR5 N-terminal peptides.

Poster 15

Determining the interaction site of a novel *Coprinopsis cinerea* lectin to *Listera innocua* peptidoglycan

Sandeep K. Misra[1]; Sushil K. Mishra[1]; Nika Janez[2]; Robert J. Doerksen[1]; Jerica Sabotic[2]; Joshua S. Sharp[1]

[1]University of Mississippi, University, MS; [2]Jozef Stefan Institute, Jamova cesta 39, Slovenia

Introduction: Lectins are proteins that reversibly bind to carbohydrates and glycoconjugates, often with relatively low affinity. Understanding how they recognize carbohydrates selectively is important for fully understanding the importance of lectin-carbohydrate interactions, which play many important roles in a wide range of biological processes. A novel protein purified from *Coprinopsis cinerea* showed sequence similarity to known lectins suggesting that it may be a lectin. To verify that the protein is a lectin and to identify the peptidoglycan binding site of this protein, as well as any conformational changes to this protein upon binding, we employed hydroxyl radical protein footprinting (HRPF) and molecular docking.

Methods: The samples, containing 5 µM of the protein, 1 mM adenine in 50 µM phosphate buffer, pH 7.8, with or without 15 µM peptidoglycan, and 100 mM hydrogen peroxide, were irradiated using a Fox Protein Footprinting System (GenNext Technologies). The samples were collected into a quench solution containing 35 mM methionine amide and 0.3 mg/mL catalase. After quench, the samples were heat denatured, divided into two equal parts and digested with trypsin and chymotrypsin separately for 16 hr. 0.1% formic acid was added to the samples and the samples were run on an Orbitrap Exploris mass spectrometer coupled with a Dionex Ultimate 3000 nanoLC system (Thermo Fisher, CA).

Results: The dosimetry of the samples, as measure by adenine absorbance, with or without peptidoglycan was comparable, indicating no need for compensation for radical scavenging. Both trypsin and chymotrypsin were

used, achieving more than 83% sequence coverage of the protein. We detected a total of nine peptides oxidized and one peptide was detected with no oxidation by the hydroxyl radicals. Three peptides (corresponding to protein residues 39-48, 49-58, and 82-104) were significantly protected from oxidation modification ($p \leq 0.05$) in the presence of peptidoglycan, with the other peptides showing no significant changes in oxidation upon peptidoglycan binding. This result validates that the protein is binding to the peptidoglycan, and suggests that these protected regions are either directly involved in binding to peptidoglycan or are affected due to allosteric changes upon binding to peptidoglycan. No regions of exposure upon peptidoglycan binding were detected. Molecular dynamics simulation studies identified three amino acids in the protected regions (F42, W50, W95) that may play a direct role in the binding of this protein to peptidoglycan. Current mutagenesis studies are underway to validate the importance of these residues in protein-peptidoglycan interactions.

Poster 16

GlyCORE: Analytical and Biophysical Chemistry Research Core

Anter Shami, Sandeep K. Misra, Joshua S. Sharp
The University of Mississippi, Oxford, Mississippi

The University of Mississippi established the Glycoscience Center of Research Excellence (GlyCORE) in 2020. The Analytical & Biophysical Chemistry Research Core is one of the three dedicated research cores created in GlyCORE to support the glycoscience work of researchers at the University of Mississippi and throughout the Mid-South region. We currently operate a high resolution Thermo Orbitrap Exploris 240 system coupled to Dionex Ultimate 3000 nano-UHPLC system for LC-MS/MS analyses. We also operate an analytical Ultimate 3000 UHPLC system with UV/Vis, fluorescence, and charged aerosol detection for the sensitive measurement of analytes with poor optical properties such as glycans. This system also includes a Wyatt RI/MALS detector for SEC-MALS analysis. We offer a Nicoya OpenSPR system for surface plasmon resonance spectroscopy to measure binding kinetics, and a FlexMap 3D system for multiplex analyses. We are collaborating with researchers across the country in a wide variety of research projects encompassing glycomics, proteomics, protein structural analyses, and metabolomics using these instruments. A few examples of the projects our Core has handled for investigators include the identification of protein modification, untargeted metabolomics, molecular weight determination of polysaccharides, and identification of glycosylation patterns on isolated glycoproteins.

We are happy to consult researchers within the University of Mississippi and the Mid-South region to discuss their needs and help in supporting and advancing their research projects through services offered by the Analytical and Biophysical Chemistry Research Core.

Poster 17

Glycoscience Center of Research Excellence (GlyCORE)

Joshua S. Sharp, Samir A. Ross, Karin King Ballering
The University of Mississippi, Oxford, MS

Carbohydrates are one of the four major classes of biomolecules and play a central role in almost all biological systems, from their central role in energy metabolism to roles in cell-cell communication and protein regulation that we are only beginning to understand.

Sustained and impactful innovation in glycoscience requires a concentration of both specific core research capabilities, as well as expertise in the chemistry, biochemistry, and cellular biology of carbohydrates and carbohydrate conjugates. The University of Mississippi hosts accomplished faculty in glycoscience in a wide variety of disciplines, including glycoside natural products isolation and characterization, glycan analysis, protein-carbohydrate interactions, and physiological responses to protein glycation. Additionally, we have reached a critical concentration at the University of Mississippi of faculty members whose interests intersect with glycoscience from a variety of disciplines. This confluence has presented an opportunity to nurture these interests and develop a core of expertise necessary for the long-term development of institutional excellence in glycoscience, leveraging our unique history and resources in natural products.

Recognizing the opportunity to contribute to the future of glycoscience research, The University of Mississippi has established the Glycoscience Center of Research Excellence (GlyCORE), a new University-wide NIH COBRE Phase 1 center to study how carbohydrates and carbohydrate-containing molecules affect human

health. GlyCORE supports investigators in glycoscience through direct funding of selected research projects, establishes mentors for early career investigators, supports the recruitment of new faculty in glycoscience, and develops local and regional meetings for investigators to discuss their work. GlyCORE also hosts three central Research Cores to support these investigators with cutting-edge biomedical research tools. Our goal to lower the barriers to entry to glycoscience for researchers across the spectrum of biomedical research is what makes GlyCORE innovative; we do not solely serve the dedicated glycoscience community, but rather develop the infrastructure and dedicated expertise necessary to support glycoscience research from a diverse community.

In addition to our primary goal of serving The University of Mississippi, GlyCORE seeks to develop, support, and foster glycoscience throughout the Mid-South region (including Mississippi, Alabama, Arkansas, Louisiana, Tennessee, Kentucky, and Missouri). We encourage students, faculty, and scientists in the region to contact us regarding potential collaborations, participation in seminars and lecture series, and any other ways in which we can help support and promote glycoscience research at your institution.

Glycoscience Student Advocates (GSA)

Mario Djugovski, President; Hoda Ahmed, Vice President; Shiva Akhlaghi, Secretary;
Destinee Manning, Treasurer; Marwa Farrag, Event Manager
The University of Mississippi, Oxford, Mississippi

Glycoscience Student Advocates (GSA) is comprised of graduate and undergraduate students interested in or conducting glycoscience-related research at The University of Mississippi. GSA strives to promote the field of glycoscience by encouraging the research, education, and training of interdisciplinary glycoscience trainees, as well as hosting seminars and knowledgeable speakers during monthly meetings.

Poster 18

The GLYCORE Imaging CORE: Bridge the gap between advanced imaging techniques and Glycoscience

Ruofan Cao, Gregg W. Roman
The University of Mississippi, Oxford, Mississippi

The GlyCORE Imaging Core aims to promote and enhance the growth of glycoscience projects at the University of Mississippi and throughout the mid-south region. The Core combines new and existing advanced microscopes into a University-wide central platform, offering a wide range of advanced imaging techniques. The Core currently administrates three microscopes, one imaging cytometer, and a computer workstation dedicated to image analysis. The primary microscope is an inverted **Leica SP8 confocal microscope** with a white light laser, piezo encoded stage, resonant scanner, objective inverter, adaptive focus control (AFC), and ultrasensitive HyD detectors. This confocal can handle most fluorescent imaging applications, including Z-stack, time-lapse, spectrum screening, and tiling/stitching with robust (AFC), sensitive and super-fast imaging (up to 28 fps) capabilities. Software modules within the system will assist many advanced imaging techniques, such as Fluorescent Resonant Energy Transfer (FRET), Fluorescence Recovery After Photo Bleaching (FRAP), and image processing for presentation. We also updated the Leica SP8 with a deconvolution-lightening module which would increase the imaging resolution down to 140 nm. The Core also contains a **Zeiss Axio Imager M1 widefield microscope** with epifluorescent, dark-field, phase-contrast, and bright-field imaging functions. This M1 Axio imager is well suited for the general morphometric analysis of tissues. The GlyCORE Imaging Research Core also houses a **Zeiss Discovery v.12 Stereomicroscope** capable of bright field and fluorescent microscopy, with ZEN2.3 software and extended focus technique for increasing depth of field in thick specimens of larger tissues and structures, and the morphometric analysis of these structures. The **FlowCam imaging cytometer** provides a high-throughput screening/analysis platform for suspended particles and cells. Most commonly used image processing software, including Fiji, Photoshop, Zen Lite, and LAS X, are free to access on our **top-level image workstation**. The GlyCORE Imaging Research Core also offers many services, including but not limited to new imaging technique development, image processing, specimen preparation consultation, etc. The most important will be consulting with the investigators on how the data can be best collected and published. We have been establishing new imaging techniques in the Core, including expansion microscopy to enhance our confocal resolution to 70 nm, imaging coagulation with a

microfluidic device, histology sectioning, and immunostaining. Extra information and services can be found on the core website https://gic.olemiss.edu/

Poster 19

Acute COG inactivation unveiled its immediate impact on Golgi and illuminated the nature of intra-Golgi recycling vesicles

(Virtual Poster)

Farhana Taher Sumya, Irina D. Pokrovskaya, Zinia D'Souza and Vladimir V. Lupashin*

University of Arkansas for Medical Sciences, Little Rock, Arkansas

Conserved Oligomeric Golgi (COG) complex orchestrates intra-Golgi retrograde trafficking and glycosylation of macromolecules, but the detailed mechanism of COG action is unknown. Previous studies employed prolonged protein knock-out and knock-down approaches which may potentially generate indirect/suppressed mutant phenotypes. To achieve a fast depletion of COG4 in human cells, the auxin-inducible degradation system was employed. This approach allows a very fast (30 min) and efficient depletion of COG4, which provide the ability to accumulate COG complex dependent (CCD) vesicles and investigate primary cellular defects associated with the acute depletion of COG complex subunits. We have applied a comprehensive set of biochemical and microscopy (superresolution and electron) approaches to reveal the effect of acute COG4 depletion on other COG subunits, components of trafficking machinery and Golgi resident proteins. The results revealed that upon COG4 depletion, both lobe A and lobe B COG subcomplexes were mislocated from Golgi. As predicted, v-SNAREs (GS15, GS28) were relocalized into CCD vesicles while t-SNAREs (STX5, YKT6) remained attached to the Golgi membrane. Coiled-coil tethers also demonstrated differential response: t-tethers GM130 and P115 were associated with the Golgi, while the v-tethers both giantin and golgin-84 show off-Golgi patterns in COG4 depleted cells. Surprisingly, the majority of COG complex-interacting Golgi Rab-GTPases (Rab2a, Rab6a, Rab30a) did not relocate to vesicular fractions after acute COG4 depletion. Interestingly, another COG-interacting Rab, Rab1b was found to be enriched in vesicular fraction only after 2 h of COG4 depletion which is much later compared to Golgi v-SNAREs, enzymes and other Golgi proteins. These results indicate that COG interacting Rabs are primarily acting from the acceptor Golgi membrane during the intra-Golgi vesicle recycling process. Importantly, all tested Golgi enzymes (B4GalT1, MGAT1, GalNT2, FUT 8) and Golgi resident proteins (GPP130, TMEM165, SDF4, TGN46) were significantly relocalized from Golgi into CCD vesicles. Accumulated CCD vesicles were relatively stable - partial degradation of vesicle content was observed only after prolonged (24-48 hours) COG depletion. The vesicle degradation coincided with detectable protein glycosylation defects, mirroring results obtained with COG KO cells. Superresolution microscopy analysis of CCD vesicles failed to detect any significant co-localization of MGAT1 and B4GALT1, indicating that medial and trans-Golgi enzymes recycle in different populations of CCD vesicles. This prediction was confirmed by using sucrose velocity gradient for vesicle separation. Moreover, Acute COG depletion significantly affected three Golgi-based vesicular coats-COPI, AP1, and GGA, suggesting that COG uniquely orchestrates tethering of multiple types of intra-Golgi CCD vesicles produced by different coat machineries. In summary, the study revealed the first detailed view of primary cellular defects associated with COG complex dysfunction and uncover heterogeneous nature of vesicular intermediates that recycle Golgi enzymes and other resident Golgi proteins in human cells.

Poster 20

Computational Chemistry and Bioinformatics Research Core

Sushil K. Mishra[1], Priyanka Samanta[1], Mohamed Jahan[1] and Robert J. Doerksen[1,2]

[1]Department of BioMolecular Sciences, University of Mississippi, MS-38677, USA; [2]Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS-38677, USA

Molecular modeling approaches to study protein–carbohydrate interactions are essential for understanding their direct role in biological functions like pathogen adhesion, host-recognition, cell-differentiation, metastasis, and immunological recognition.1-2 The Computational Chemistry and Bioinformatics Research Core (CCBRC), one of the three research cores of the Glycoscience Center of Research Excellence, supports performing computations on a wide range of glycoscience topics. This poster provides an overview of CCBRC's computational capabilities, training, and available resources (including workstations, servers, and software).

We also present a brief overview of computational approaches being used by CCBRC on some of the ongoing projects that can foster and encourage glycoscience research in the Mid-South region of the USA.

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