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|  **GlyCORE Website QR code**  **Program and Abstracts QR Code** [**GlyCORE Website**](https://pharmacy.olemiss.edu/glycore/)[**Program and Abstracts**](https://pharmacy.olemiss.edu/glycore/summer-mid-south-glycoscience-meeting/2022-mid-south-glycoscience-meeting-program-and-abstracts/)**Program and Abstracts****2022 Mid-South Glycoscience Meeting****July 16, 2022****Zoom QR code** [**Virtual Link**](https://zoom.us/j/99291485458?pwd=WkI1Sis0TE55RDNkUUJscmdRenkrQT09)**ORAL PRESENTATIONS** |
| **Time:**8:15 a.m. | **Presentation:****Welcome and Presentation of Awards: Joshua Sharp** ***Session 1 Chair: Paul Boudreau******Time Keeper: Baharul Islam*** |
| 8:25 a.m. | **Role of ST6GAL1-Mediated Sialylation in Pancreatic Cancer Initiation** *Susan Bellis, University of Alabama at Birmingham, Birmingham, Alabama*  |
| 9:10 a.m. | **Characterization of Novel Glycosphingolipids from Bacteria Using an LCMS-based Approach***Tahir Ali, Paul Boudreau, University of Mississippi, Oxford, Mississippi*  |
| 9:30 a.m. | **Analysis of Different Epimers of Glycosphingolipid LcGg4 by Ion Mobility Mass Spectrometry***Sixue Chen, Tianqi Gao, Aneirin A. Lott, Fanran Huang, Rajendr Rohokale, Qingjiang Li, Hernando J. Olivos, Zhongwu Guo, University of Florida, Gainesville, Florida*  |
| 9:50 a.m. | **Fast Photochemical Oxidation of Carbohydrates (FPOC) for the Characterization of Carbohydrate-Protein Interactions***Joshua S. Sharp, Sandeep K Misra, Hao Liu, Lianyan Xu, Steven D. Townsend, University of Mississippi, Oxford, Mississippi* |
|  |  |
| 10:10 a.m. | Break***Session 2 Chair: Amber Jennings******Time Keeper: Tahir Ali***  |
| 10:25 a.m. | **Biological Evaluation of Dual Responsive Glycopolymeric Nanoparticles for Intracellular Co-Delivery of Vaccine Antigen and Adjuvant***Mehjabeen Hossain, Judith U. De Mel, Oluwaseyi Shofolawe-Bakare, Karan Arora, John T. Wilson, Adam E. Smith, Thomas A. Werfel, University of Mississippi, Oxford, Mississippi*  |
| 10:45 a.m. | **Poster Highlight: Progress Towards the Synthesis of an Acinetobacter Baumanii Lipooligosaccharide***James M. Armstrong II, Brandon J. Conrad, and Justin R. Ragains, Louisiana State University, Baton Rouge, Louisiana*  |
|  |  |
| 10:55 a.m.11:05 a.m. | **Poster Highlight: Engineering High-Affinity Lectenz® Reagents for the Detection and Analysis of Carbohydrates***Christian Gerner-Smidt, Shaza Abnouf, Lu Meng, Sheng-Cheng Wu, Robert J. Woods, and Loretta Yang, Lectenz Bio, Athens, Georgia* **Poster Highlight Investigating the Role of Glycosylation in Myocyte-Environment Interactions During Development and Disease***Joshua Bloomekatz, Tess McCann, Prashanna Koirala, Harini Saravanan, Rabina Shrestha, University of Mississppi, Oxford, Mississippi*  |
| 11:15 a.m.12:00 p.m. 12:30 p.m. | **Lunch and Posters****Poster Session 1** **Lunch Break****Poster Session 2** |
|  | ***Session 3 Chair: Thomas Werfel*** ***Time Keeper: Rohini Dwivedi***  |
| 1:15 p.m. | **Glycosyltransferase Structures as Modular Templates for Diverse Glycan Synthesis***Kelley Moremen,* *Jeong-Yeh Yang, Bhargavi Boruah, Digantkumar Chapla, Renuka Kadirvelraj, Aarya Venkat, Rahil Taujale, Annapoorani Ramiah, Natarajan Kannan, and Zachary A. Wood, University of Georgia, Athens, Georgia*  |
| 2:00 p.m. | **Chemical Probes for Mycobacterial Glycans: from Tuberculosis Tools to Therapeutic Opportunities***Ben Swarts, Central Michigan University, Mount Pleasant, Michigan*  |
| 2:20 p.m. | **Palladium-Catalyzed Stereoselective N- and C- Glycosylation Using Glycals***Qiang Zhang, University at Albany, SUNY, Albany, New York* |
| 2:40 p.m. | **Programmable One-pot Synthesis of Heparin Sulfate and Heparin-derived Commercial Anticoagualnts (Virtual)***Supriya Dey, Hong-Jay Lo, Chi-Huey Wong, Scripps Research Institute, Harvard University, Boston Massachusetts*  |
| 3:00 p.m. | **Break*****Session 4 Chair: Murrell Godfrey******Time Keeper: Hoda Ahmed***  |
| 3:15 p.m. | **Tuning Antibody-Antigen Binding through Glycan Masking***Martina CE, Schoeder CT, Gilchuk I, Crowe JE Jr, Meiler J, Vanderbilt University, Nashville, Tennessee*  |
| 3:35 p.m. | **Multiple Domains of Staphylococcal Superantigen-like Protein 11 (SSL11) Contribute to Neutrophil Inhibition***Chen Yang, Joseph T. Barbieri, Nancy M. Dahms, Chen Chen, Louisiana State University, Baton Rouge, Louisiana*  |
| 3:55 p.m. | **Glycosaminoglycan Based Broad Spectrum Inhibitors of Respiratory Infections** *Poonam Sharma, Stephen Stray, Lance Keller, Courtney Thompson, Ritesh Tandon, University of Mississippi Medical Center, Jackson, Mississippi* |
| 4:15 p.m. | **The Biological Importance of O-GaINAc Glycans** *Richard Cummings, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts*  |

**Poster Presentations**

**Poster 1:**

**Synthesis of Difluorinated Benzopyrans from Simplified Organic Molecule**

*Amena Begum, David A. Colby, University of Mississippi*

**Poster 2:**

**Progress Towards the Synthesis of an Acinetobacter Baumanii Lipooligosaccharide**

*James M. Armstrong II, Brandon j. Conrad, Justin R. Ragains, Louisiana State University*

**Poster 3:**

**Cytocompatibility of Chitosan-Silver Coated Titanium Coupons**

*Emily Coleman, Matthew Atwill, Joel D. Bumgardner, PhD, J. Amber Jennings, PhD, University of Memphis*

**Poster 4:**

**Investigating the role of glycosylation in myocyte-environment interactions during development and disease**

*Joshua Bloomekatz, Tess McCann, Prashanna Koirala, Harini Saravanan, Rabina Shrestha, University of Mississippi*

**Poster 5:**

**LC-MS Identification of Serum Proteins Adsorbed onto Ionic Liquid-Coated Nanoparticles**

*Anh Hoang, Eden tanner, University of Mississippi*

**Poster 6:**

**Engineering High-Affinity Lectenz® Reagents for the Detection and Analysis of Carbohydrates**

*Shaza Abnouf, Christian Gerner-Smidt, Lu Meng, Sheng-Cheng Wu, Robert J. Woods, and Loretta Yang, Lectenz Bio*

**Poster 7**:

**Native** **high molecular weight structure of Pentacta pygmaea fucosylated**

*Rohini Dwivedi, Poonam Sharma, Ritesh Tandon, Vitor H. Pomin, University of Mississippi*

**Poster 8:**

**Fractionation of sulfated galactan from the red alga Botryocladia occidentalis separates its anticoagulant and anti-SARS-CoV-2 properties**

*Seon Beom Kim, Mary Zoepfl, Priyanka Samanta, Fuming Zhang, Ke Xia, Reena Thara, Robert J Linhardt, Robert J Doerksen, Michael A McVoy, Vitor H Pomin, University of Mississippi*

**Poster 9:**

**Isolation and Characterization of Myxobacteria**

*Kayleigh Phillips, Andrew Ahearne, Cole Stevens, University of Mississippi*

**Poster 10:**

**Description and Profiling the Biosynthetic Potential of Archangium Primigenium ATCC 29037**

*Hanan Albataineh, Cole Stevens, University of Mississippi*

**Poster 11:**

**ROS-responsive, glycopolymeric nanoparticles for enhanced drug delivery to macrophages**

*Oluwaseyi Shofolawe-Bakare, Judith U. De Mel, Mehjabeen Hossain, Sushil Mishra, Robert Doerksen, Adam E. Smith, Thomas A. Werfel*, *University of Mississippi*

**Poster 12:**

**Loss of Adventitia Results in an Alteration of Vascular Phenotype Markers Promoting Calcification in an Ex Vivo Model of Type 2 Diabetic Aortic Calcification**

*Christopher X. Adams, Amber M. Kennon, James A. Stewart, University of Mississippi*

**Poster 13:**

**Dextrans as Macromolecular Crowding Agents**

*Randy M. Wadkins, Katherine Morgan, University of Mississippi*

**Poster 14:**

**Prediction of binding free energy in protein-carbohydrate complexes and their mutants using sequence and structural features (Virtual)**

*Siva Shanmugam, K. Veluraja, M. Michael Gromiha, IIT Madras, Chennai, India*

**Poster 15:**

**Receptor for Advanced Glycation Endproducts Signaling Impacts on Healthspan: Early-life Observations**

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

**Poster 16:**

**Synthesis of Polymer Scaffold by Modification of Azlacton Ring with Glycopolymers**

*Sk Arif Mohammad, Alex Fortenberry, Adam E. Smith, Thomas A. Werfel, University of Mississippi*

**Poster 17:**

**Selective 2-desulfation of sulfated fucans under mild acid hydrolysis**

*Marwa Farrag, Seon Beom Kim, Sushil Mishra, Sandeep Misra, Joshua S. Sharp, Robert J. Doerksen and Vitor H. Pomin, University of Mississippi*

**Poster 18:**

**Structural characterization of an α-glucan from the mollusk Marcia hiantina (Lamarck, 1818)**

*Hoda Al. Ahmed, Bernadeth F. Ticar, Ian Black, Anter A. Shami, Sandeep Misra, Christian Heiss, Joshua S. Sharp, Parastoo Azadi, Vitor H. Pomin, University of Mississippi*

**Poster 19:**

**Predicting protein–glycosaminoglycan (GAG) complexes through docking:**

**Methods and challenges**

*Sushil Mishra, Robert J. Doerksen, University of Mississippi*

**Poster 20:**

**Study of Marine Natural Products as Anti-SARS-CoV-2 Agents Using Molecular Modeling** *Priyanka Samanta, Sushil K. Mishra, Rohini Dwivedi, Seon Beom Kim, AyoOluwa O. Aderibigbe, Poonam Sharma, Fuming Zhang, Pavel Kucheryavy, Mary Zoepfl, Ke Xia, Reena Thara, Robert J. Linhardt, Michael A. McVoy, Ritesh Tandon, Vitor H. Pomin, Robert J. Doerksen, University of Mississippi*

**Poster 21:**

**Structural Analysis of Protein-Carbohydrate Interactions by a Novel Inline Liquid Chromatography−Flash Oxidation Approach**

*Suman Choudhary, Sushil K. Mishra, Robert J. Doerksen, Joshua S. Sharp, University of Mississippi*

**Poster 22:**

**Synthesis of 2,2-Difluoroethanols via Hydroxymethylations of Difluoroenolates and Difluorobenzyl Carbanions by Reacting with in situ Generated Formaldehyde**

*Baharul Islam, Hari R. Khatri, Reem A. Alkhodier, Amna T. Adam, and David A. Colby, University of Mississippi*

**Poster 23:**

**Influenza Virus- a Glycan-targetted Treatment for Cancer?**

*Stephen Stray, Christopher Bruni, Kilando Q Chambers, K Ford Gordon, Maggie E Jefferis, Anne Margaret Miller, Evan P Morissey, Lucas E Morrisey, and Pier Paulo Claudio, University of Mississippi Medical Center*

**Poster 24:**

**Analytical and Biophysical Chemistry Research Core**

*Anter A. Shami*, *Sandeep Misra, Joshua Sharp*

**Poster 25:**

**Glycoscience Student Advocates**

*Baharul Islam, Tahir Ali, Kayleigh Phillips, Destinee Manning, Eslam Elhanafy, University of Mississippi*

**Poster 26:**

**The GlyCORE Imaging Research Core: Bridge the Gap between Advanced Imaging Techniques and Glycoscience**

*Ruofan Cao and Gregg Roman*

**Poster 27:**

**Glycoscience Center of Research Excellence**

*Erin Bradley, Karin Ballering, Joshua S. Sharp, Samir Ross*

**Poster 28:**

**Computational Chemistry and Bioinformatics Research Core**

*Sushil K. Mishra, Priyanka Samanta, and Robert J. Doerksen*

***ABSTRACTS***

***Oral Presentations***

**Role of ST6GAL1-mediated sialylation in pancreatic cancer initiation**

**Susan Bellis**

*University of Alabama at Birmingham, Birmingham, Alabama*

Increased cell surface sialylation is a prevalent feature of tumor cells. The addition of sialic acid to select surface receptors modulates the structure and function of such receptors, leading to changes in intracellular signaling and gene expression. Increased tumor cell sialylation occurs, in part, through the upregulation of sialyltransferases such as ST6GAL1, an enzyme that adds an α2,6 linked sialic acid to N-glycosylated proteins. ST6GAL1 is overexpressed in numerous malignancies, including pancreatic ductal adenocarcinoma (PDAC), and high expression correlates with a poor prognosis. Our group has shown that ST6GAL1 plays a causal role in promoting PDAC initiation and progression in tumor xenograft and genetically-engineered mouse (GEM) models. More specifically, we developed a GEM model with conditional ST6GAL1 expression in the pancreas and crossed this line to the “KC” PDAC model, which expresses oncogenic Kras (Kras G12D ). Mice with dual expression of ST6GAL1 and Kras G12D (”KSC” mice) exhibit greatly accelerated PDAC initiation, progression, and mortality when compared with KC mice. In light of ST6GAL1&#39;s known role in conferring stem/progenitor properties, we hypothesized that ST6GAL1 activity contributes to PDAC initiation by fostering acinar to ductal metaplasia (ADM). During ADM, pancreatic acinar cells de-differentiate into ductal-like, progenitor cells and acquire greater proliferative potential as well as apoptosis resistance. Cells undergoing ADM are particularly vulnerable to neoplastic transformation. Using a variety of approaches and model systems, we uncovered a novel function for ST6GAL1 in

promoting ADM. Our current studies are focused on determining how the sialylation of specific receptors such as EGFR promotes the reprogramming of acinar cells into a progenitor phenotype, and correspondingly, primes acinar cells for oncogenic transformation.

**Characterization of Novel Glycosphingolipids from Bacteria Using an LCMS-based Approach**

**Tahir Ali, Paul Boudreau**

*University of Mississippi, Oxford, Mississippi*

**Introduction:** As exemplified through the COVID-19 pandemic, vaccines are a powerful tool to reduce the prevalence of disease and the risk of death associated with the infectious diseases. A typical vaccine is composed of an antigen that trains the immune system and an adjuvant, adjuvants are necessary to increase the immune response and overall effectiveness of a vaccine. Natural products (NPs) are an important source for novel bioactive molecules, including immune modulating compounds such as α-galactosylceramide, which hold the promise of new adjuvants. Sphingolipids (SLs) are a class of lipids, found in the cell membrane where they play a significant role in the regulation of the various cell functions e.g., signal transmission, immunity, and cell recognition. Glycosphingolipids (GSLs), like α-galactosylceramide, are a group of SLs which are composed of the hydrophobic alkyl tail and the hydrophilic sugar-head. Due to the unique structure, GSLs have ability to engage both dendritic cells and iNKT cells to induce the secretion of Th1- and Th2-type cytokines. It has been reported that GSL with different tails length and sugar head groups can be used to switch between Th1- and Th2-based immune response. In this project, we are isolating and characterizing bacterial sphingolipids, having different lipid architecture and sugar-head-groups in the hopes of finding new bioactive GSLs.

**Methods:** We have cultured various soil bacteria strains to investigate their sphingolipid profiles by using LC-MS/MS-based experiments. The Global Natural Products Social (GNPS) platform was used to compare lipid profiles across these strains by searching the MS/MS data for sphingolipids using matches to library standards and common fragmentation patterns.

**Results:** Among the various strains, NRRL B-54 (Sphingomonas paucimobilis) was found to produce several interesting SLs. A GSL containing glucuronic acid as the sugar moiety is one of the key metabolites, associated with the genus Sphingomonas. GNPS analysis illustrated a cluster showing around 50 precursor ions which were tentatively identified as SLs. With the aid of MS/MS fragmentation data, we have annotated the complete structure of three SLs. The [M+H]+precursor ion of 512 m/z was assigned as ceramide, a sphingolipid without sugar moiety, whereas 688 m/z and 849 m/z were characterized as GSLs with one and two sugar head-groups, respectively. While interestingly, the 849 m/z (parent molecule) was found to be a novel GSL with two sugar-head groups, consisting of glucuronic acid and glucosamine. Work is currently underway to isolate these GSLs to confirm their structure by NMR and evaluate their bioactivity.

**Analysis of Different Epimers of Glycosphingolipid LcGg4 by Ion Mobility Mass Spectrometry**

**Sixue Chen, Tianqi Gao, Aneirin A. Lott, Fanran Huang, Rajendr Rohokale, Qingjiang Li, Hernando J. Olivos, Zhongwu Guo**

*University of Florida, Gainesville, Florida*

Glycosphingolipids (GSLs) are a class of glycolipids with a glycan linked to a lipophilic ceramide. They are major glycolipids on the cell surface with important roles. Aberrant GSL metabolism has been associated with cancer, Alzheimer’s and lysosomal storage diseases. GSLs are biomarkers of undifferentiated malignant myeloid cells useful for the development of new diagnostics and therapies for cancer. LcGg4 is a neutral GSL and cancer antigen. Its epimers GalNAc-LcGg4 and GlcNAc LcGg4, and three lipid forms of GalNAc-LcGg4 were studied by mass spectrometry (MS). Although different forms of GalNAc-LcGg4 carrying homologous (d16:1/18:0) and (d18:1/18:0) lipids were easily separated using liquid chromatography (LC)-MS, LcGg4 and its epimers GalNAc-LcGg4 and GlcNAc LcGg4, which are different only in the C4-configuration of their non-reducing end sugar residues, gave the same product ions in similar intensities, as well as the same LC retention time. It is challenging to differentiate the epimeric GSLs.

Here we applied ion mobility MS analysis using a Bruker trap ion mobility (IM) spectrometry (tims)- Time-of-Flight (TOF) MS and a Waters cyclic IM MS with Travelling Wave (TW) mobility separation. LcGg4, GalNAc-LcGg4, and GlcNAc-LcGg4 were individually dissolved in methanol and introduced into the MS systems through electrospray ionization at a 20 µL/min infusion rate. On the timsTOF, the

capillary voltage, source temperature, and source gas flow rate were 3 kV, 200 °C, and 4 L/min, respectively. On the cyclic IM, the capillary voltage, cone voltage, and source temperature were 2.5 kV, 100 V, and 120 °C, respectively.

LcGg4, GalNAc-LcGg4, GlcNAc-LcGg4 and their mixture were firstly analyzed on the timsTOF system. The [M+H]+ parent ions (m/z 1296.83) were used for IM and all the MS/MS studies. After optimizing the ramp time and accumulation time, we were able to resolve the three isomers separately. When they were mixed, GalNAc-LcGg4 was baseline separated from the other two, but LcGg4 and GlcNAc LcGg4 did not reach a baseline separation. Then, we tried the cyclic IM-MS, which allows the ions to perform multiple passes around the IM cell to increase resolution. An optimal separation of the isomers was achieved after 23 passes. The extracted ion-arrival times for GalNAc-LcGg4, LcGg4, and GlcNAc LcGg4 were 340.76, 345.35, and 338.89 ms, respectively, giving full separation of GlcNAc-LcGg4 and distinguishable partial separation of LcGg4 and GalNAc-LcGg4. In summary, IM-MS was able to efficiently separate and distinguish the GSL epimers. This study has demonstrated the promise of IM MS for isomeric GSL separation and structural characterization.

**Fast Photochemical Oxidation of Carbohydrates (FPOC) for the Characterization of Carbohydrate-Protein Interactions**

**Joshua S. Sharp, Sandeep K Misra, Hao Liu, Lianyan Xu, Steven D. Townsend,**

*University of Mississippi, Oxford, Mississippi*

**Introduction:** Many biological processes are mediated via carbohydrate-protein interactions. However, the study of these interactions is very challenging. This is mainly due to the structural complexity of carbohydrates and the typically low binding affinity of the interactions. There are many approaches to study glycan-protein interactions; however, there are few methods for quickly determining carbohydrate structures that mediate protein-carbohydrate interactions. We are developing a new approach using a mass spectrometry-based fast photochemical oxidation of carbohydrates (FPOC) to monitor changes in carbohydrate solvent accessibility upon binding to a protein target.

**Methods:** The glycan mixture consisted of 25 µM of five trisaccharides: NAG3, isomaltotriose, 1-kestose, raffinose, and melezitose. Four model proteins used in this study were ubiquitin, lysozyme, Griffonia simplicifolia (Gs) lectin, and myoglobin. The samples were irradiated with a KrF excimer laser after the addition of 150 mM hydrogen peroxide. After quenching in a solution containing catalase and methionine amide, the samples were analyzed on a Thermo Orbitrap Fusion Tribrid using a BEH amide HILIC column to separate carbohydrates.

**Results:** Carbohydrates were exposed to hydroxyl radicals generated by flash photolysis of hydrogen peroxide. We then used LC-MS/MS to analyze the oxidation products. We calculated the oxidation of each trisaccharide upon FPOC, in the presence and absence of either binding or non binding proteins. As expected, there was no significant change in the total oxidation of NAG3 when mixed with two non-binding proteins, ubiquitin and myoglobin. Similarly, there was no significant change in the oxidation of the other four non-binding trisaccharides used in this study when mixed with any of our test proteins. The oxidation of NAG3 was significantly decreased (p ≤ 0.05) in the presence of both NAG3-binding proteins, lysozyme and Gs lectin. MS/MS and

chemical reduction data indicate that the primary target of oxidation for NAG3 was the free reducing end. NAG3 bound at the reducing end by lysozyme was much more heavily protected from oxidation compared to that bound at the non-reducing end by Gs lectin. These results are the first examples of hydroxyl radical carbohydrate footprinting and offer a new method for probing protein-carbohydrate interactions. Experiments examining the binding of a synthetic mixture of human milk oligosaccharides are underway, as are methods investigating new photochemical labels for probing other regions of the carbohydrate

**Biological Evaluation of Dual Responsive Glycopolymeric Nanoparticles for Intracellular Co-Delivery of Vaccine Antigen and Adjuvant**

**Mehjabeen Hossain, Judith U. De Mel, Oluwaseyi Shofolawe-Bakare, Karan Arora, John T. Wilson, Adam E. Smith, Thomas A. Werfel**

*University of Mississippi, Oxford, Mississippi*

**Introduction**: Neoantigen peptide vaccines (NPVs) used for boosting immunogenicity in various cancers are prone to aggregation at the site of injection and lack inherent immunogenicity. To address this, immune agonists (i.e. adjuvants) capable of provoking a robust immune response to peptide antigens can be co-delivered. Also, the poor cellular uptake and endosomal entrapment of peptides in cancer cells impacting Major Histocompatibility Complex-I (MHC-I) loading and Cytotoxic T Lymphocyte (CTL) activation can be overcome by pH and reduction-responsive release mechanisms facilitating endosomal escape and cytosolic release of both peptide and adjuvant cargo. Here, we address these barriers to NPV delivery by designing glycopolymeric nanoparticles (NPs) based on stimuli-responsive block copolymers.

**Methods**: The block copolymers, composed of poly[2-(diisopropylamino)ethyl methacrylate]-b poly[(pyridyl disulfide ethyl methacrylate)-co-(methacrylamidoglucopyranose)] (PDPA-b P(PDSMA-co-MAG)), were characterized for composition and molecular weight, and then used to form polymeric micelles. After physicochemical characterization (e.g., NP size, morphology, surface charge, etc.), the NPs were used to co-deliver a model antigen, ovalbumin (OVA), and TLR 7/8 (R848) agonist to dendritic cells (DC 2.4 cell line) in vitro.

**Results**: and Discussion NPs formed from PDPA-b-P(PDSMA-co-MAG) (PDPA: Mn 20 kDa, Đ 1.07 and PDSMA-co-MAG: Mn 10 kDa Đ 1.09) in neutral pH (7.4) were 33.5 ± 0.4 nm in diameter with a surface charge of 3.59 ± 0.29 mV. The NPs exhibited pH-dependent release of Nile Red (a surrogate for R848) from the NP core as well as reduction-sensitive release of OVA antigen. Moreover, the NPs exhibited pH-dependent membrane lysis behavior suggestive of their ability to escape the endolysosomal pathway and deliver antigen into the cytosol of dendritic cells. Lastly, R848/OVA co-delivery via the dual responsive glycopolymeric NPs significantly increased DC cell activation and antigen presentation of OVA epitopes. These results demonstrate the ability of our gylcopolymeric NPs to efficiently deliver vaccine components to DCs, and future work will focus on the immunological outcomes downstream of DC activation.

**Progress Towards the Synthesis of an Acinetobacter Baumanii Lipooligosaccharide**

**James M. Armstrong II, Brandon J. Conrad, and Justin R. Ragains**

*Louisiana State University, Baton Rouge, Louisiana*

**Introduction**: Acinetobacter baumannii is a Gram-negative, multi-drug-resistant bacteria that was placed on the World Health Organization’s watchlist of drug-resistant bacteria. Glycoconjugate vaccines such as those used against Haemophilus influenzae B are used globally and are highly effective. To date, there are no FDA-approved vaccines against A. baumannii, and a glycoconjugate vaccine could represent an effective approach for prevention. Capsular polysaccharides (CPS), lipopolysaccharides (LPS), and lipooligosaccharides (LOS) are glycans found on the surface of bacteria and are important immunological targets for the development of vaccines. Synthesis and immunological evaluation of an A. baumannii LOS can be used to evaluate potential vaccine candidates.

**Methods and Results:** We have produced a trisaccharide representing a truncated A. baumannii lipooligosaccharide through a convergent and stereoselective synthesis. Synthetic challenges addressed in this work include an alpha 1,4-glycosidic linkage between a glucosamine and a Kdo portion as the result of a regioselective glycosylation of a 1,2-cis diol Kdo acceptor.

**Conclusion:** Synthetic strategies presented herein were effective in the production of a trisaccharide representing truncated LOS from A. baumannii. Future immunological experiments will determine the potential for glycoconjugate vaccine development..

**Engineering High-Affinity Lectenz® Reagents for the Detection and Analysis of Carbohydrates**

**Christian Gerner-Smidt, Shaza Abnouf, Lu Meng, Sheng-Cheng Wu, Robert J. Woods, and Loretta Yang**

*Lectenz Bio, Athens, Georgia*

**Introduction:** Glycans containing sialic acids have long been implicated as biomarkers for a variety of diseases, including endocrinal, oral, and colon cancers. As the investigation of the dynamic changes in the levels of glycoforms becomes more commonplace, specific and specific glycan binding proteins are essential to overcome current limitations in the discovery and exploitation of disease-related glycans.

**Methods:** Using computationally-guided design and directed evolution, we have been converting carbohydrate-processing enzymes into catalytically inactive affinity reagents with tunable specificities. These novel LECTin-like, ENZyme-derived reagents have been shown to offer numerous advantages over other carbohydrate-binding reagents: they are high affinity, yet retain the exquisite substrate specificity of the endogenous enzyme, they can be cost-effectively produced, and they can be used as capture reagents for affinity purification.

**Results:** Currently, we have developed and commercialized two sialic acid-specific Lectenz® engineered from a neuraminidase, as well as an α2,6-specific SiaFindTMreagent engineered from a Polyporus squamosus lectin. They have demonstrated in a number of applications such as Western blotting, flow cytometry, immunohistochemistry and affinity chromatography. These applications validate the simplicity, robustness, and accuracy of SiaFindTMreagents in glycoscience research. Supported by NIH grants R44GM113351, R44OD024964, R43GM135984 and R43GM136013.

**Investigating the Role of Glycosylation in Myocyte-Environment Interactions During Development and Disease**

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

*University of Mississippi, Oxford, Mississippi*

Protein glycosylation is important for cardiac and skeletal muscle development and is integral to the pathogenesis of several muscular diseases. For example, cardiac defects are common in congenital disorders of glycosylation (CDG), such as in dystroglycanopathies. And glycan dynamics have been implicated in the progression of diabetic cardiomyopathies triggered by hyperglycemia. We have been studying the role of glycosylation in cardiac and skeletal muscle development in zebrafish at both the individual protein level and the collective glycome level. At the individual-protein level we are interrogating the role of an O-linked glycan present in the oncofetal isoform of Fibronectin; a critical extra-cellular matrix glycoprotein essential for both cardiac and skeletal muscle development and for cardiac fibrosis. Without fibronectin, cardiomyocytes which are specified in bilateral regions of the embryo fail to move to the midline and merge to form a single heart tube. 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 transgenic and CRISPR/Cas9 techniques we are creating a point mutation that specifically inhibits the O-linked glycosylation unique to the oncofetal isoform of fibronectin. We will then assess the role of this mutation by examining fibronectin fibril formation and cardiac and skeletal muscle development. At the collective-level we are taking a holistic approach toward assessing how glycosylation changes during cardiac development and in response to disease. Using functionalized metabolic precursors attached by bio-orthogonal reactions to fluorogenic probes, we are investigating how glycan composition and dynamics change during the development of cardiac and somitic/trunk muscles in zebrafish. Specifically, we are analyzing mesenchymal-to epithelial transitions (MET) and myocyte differentiation in both cardiac and somitic/trunk muscles. We have observed that sialyation is particularly prevalent at the intersomitic trunk muscle boundaries. Furthermore, we are analyzing changes in glycosylation in two disease models – hyperglycemia and cardiac remodeling caused by dilated cardiomyopathy. By uncovering mechanisms by which glycosylation specifically regulates fibronectin’s function and more generally regulates cardiac and skeletal development and disease these studies will not only contribute to elucidating fundamental glycobiology principles they are also likely to contribute to preventive and therapeutic remedies in the future.

**Glycosyltransferase Structures as Modular Templates for Diverse Glycan Synthesis**

**Kelley Moremen, Jeong-Yeh Yang, Bhargavi Boruah, Digantkumar Chapla, Renuka Kadirvelraj, Aarya Venkat, Rahil Taujale, Annapoorani Ramiah, Natarajan Kannan, and Zachary A. Wood**

*University of Georgia, Athens, Georgia*

Asn-linked oligosaccharides are extensively modified during transit through the secretory pathway, first by trimming of the nascent glycan chains and subsequently by initiating and extending multiple oligosaccharide branches from the tri-mannosyl glycan core. Glycan extension is highly ordered and hierarchal based on the precise substrate specificities of the individual biosynthetic enzymes. Few of these enzymes have been well characterized for details of substrate interactions due to challenges associated with their recombinant expression. We developed a modular expression strategy for production of human glycoenzymes as secreted catalytic domain fusion proteins and have recently advanced several of these enzymes to structural studies in complex with substrate analogs. The goals of these studies are to examine the structural basis for substrate recognition and regiospecific glycan modification. Structural studies on several key N-glycan biosynthetic enzymes will be presented, including N-glycan branching (MGAT2), extending (B3GNT2), core modification (FUT8), and capping (FUT9 and ST3GAL6) enzymes as substrate- bound complexes. In each case extensive substrate interactions have been observed that explain their precise specificities for glycan modification. These data demonstrate that mammalian glycosylation enzymes employ omplementary active site modules for glycan donor and acceptor recognition and modification that provide templates for glycan biosynthesis in mammalian cells.

**Chemical Probes for Mycobacterial Glycans: from Tuberculosis Tools to Therapeutic Opportunities**

**Ben Swarts**

*Central Michigan University, Mount Pleasant, Michigan*

Mycobacteria, which cause diseases ranging from tuberculosis to leprosy, have distinctive cell envelopes composed of numerous unique glycans that contribute to bacterial growth and virulence. Several groups, including ours, have developed chemical probes that metabolically label mycobacteria-specific cell surface glycans. These tools have been used to image mycobacterial glycans, probe glycan biosynthesis and remodeling, profile glycan-binding proteins, and rapidly detect mycobacteria. They have also inspired new glycan-targeting therapeutic strategies that may help to address challenging-to-treat mycobacterial infections. This presentation explores how research on chemical probes has led to insights for the development of novel mycobacteria-specific therapeutic strategies, such as metabolic inhibitors and immune-targeting strategies.

**Palladium-Catalyzed Stereoselective N- and C- Glycosylation Using Glycals**

**Qiang Zhang**

*University at Albany, SUNY, Albany, New York*

The critical roles of oligosaccharide incorporation on the biomolecules have been well recognized and received increased attention in recent years. Glycoproteins and glycosylated small molecules have demonstrated the promising antitumor, antiviral, and other biological activities. Stereoselective construction of glycosides have drawn significant attention because of their ability to furnish these structural defined scaffolds in an efficient manner. Recently, palladium-catalyzed stereoselective glycosylation has been developed and applied in the synthesis of complex oligosaccharides and glycoconjugates. In particular, palladium catalyzed Tsuji-Trost reaction has enabled expansion of the classic Ferrier rearrangement in N- and O- glycosylation. However, methodologies for the efficient and stereoselective preparation of uncommon arginine and C- glycosylation remain limited. we present a mild and highly efficient Pd-catalyzed glycosylation strategy for the β-stereoselective synthesis of N- and C- glycosides from readily available 3,4-O-carbonate glycals and vinylogous and guanidium acceptors.



**Programmable One-pot Synthesis of Heparin Sulfate and Heparin-derived Commercial Anticoagualnts (Virtual)**

**Supriya Dey, Hong-Jay Lo, Chi-Huey Wong**

*Scripps Research Institute, Harvard University, Boston Massachusetts*

**Introduction:** The development of a rapid and synthesis of majorly heparin sulfate and heparin derived commercial anticoagulants are important from the standpoint of understanding different biological process, understanding its potential for drug discovery and to cope with the current market demand and cost of anticoagulants. The synthesis of heparin sulfate and anticoagulants (Fondaparinux & Idraparinux) is very challenging due to the difficulty in the regio- and stereoselective glycosylation among the glucosamine, glucuronic acid, and iduronic acid building blocks and the strategic installation of OSO3 and NHSO3 groups.

**Methods:** We have developed a computer program, namely, AUTO-CHO, based on the concept of quantitative determination of the relative reactivity values (RRV) of a thioglycoside donor for the synthesis of heparin sulfate and commercial anticoagulants. We have found that with the aid of AUTO-CHO the synthesis of the complex heparin oligosaccharides was improved both in terms of synthetic steps and yield.

**Results:** With AUTO-CHO software,

(i) The chemical synthesis of heparin pentasaccharides with regiodefined O-sulfation was reported for the first time

(ii) The total synthesis was accomplished in the 22 with 4.2% overall yield which is a very significant improvement compared to previously reported synthetic methodologies and is also scalable.

(iii) Idraparinux was synthesized in shortest possible route (22 steps with 6.8% yield) which has improved the large-scale production of these drugs, will cut down the cost of the production and cope with high market demand. The previously known reported synthesis of Fondaparinux and Idraparinux was 0.1% yield in ~60 synthetic steps and 0.01% yield in -51 synthetic steps, respectively.

**Tuning Antibody-Antigen Binding through Glycan Masking**

**Martina CE, Schoeder CT, Gilchuk I, Crowe JE Jr, Meiler J**

*Vanderbilt University, Nashville, Tennessee*

**Introduction:**

Viruses are able to escape recognition by the immune system by masking their glycoproteins with a glycan shield that blocks the binding of antibodies to amino acid residues. This technique, referred as glycan masking, was recently adopted in the field vaccinology to accomplish a similar result: hiding epitopes of low interest from the immune system. In this work, the ability and efficiency of the glycan masking technique is tested to selectively tune the binding of different antibodies to the same antigen.

**Methods:** The head domain of the hemagglutinin protein (HA) of influenza A H7 was chosen as antigen. This molecule presents four distinct major antigenic sites, targeted by four monoclonal antibodies whose structures in complex with HA are publicly available on RCSB-Protein Data Bank: FluA-20 (pdb ID: 6OCB), H7.5 (6MLM), H7.167 (5V2A) and H7.200 (6UIG).

With the use of Rosetta, a software for protein modeling and design, each of the four binding interfaces was analyzed, ten key residues on the H7-HA head domain were selected and glycan chains were modeled in each position individually. In order to mimic glycan flexibility, each carbohydrate chain was modeled 100 times. Antibodies then were docked to the antigens in the presence of the glycan chain. In all cases, the resulting binding energy was worse compared to the wild-type HA, as expected. Nine out of the ten designs of H7-HA head domain were successfully expressed in human embryonic kidney (HEK) cells. Binding to the antibodies was tested through an enzyme linked imunosorbent assay (ELISA).

**Results:** Out of the 10 glycosylated H7-HA head variants modeled with Rosetta, nine were successfully expressed and purified. Six out of them successfully blocked the binding of the corresponding antibody, in good agreement with the computational data. Each of the four antibodies (H7.200,

FluA-20, H7.5 and H7.167) was blocked by at least one HA variant. This work demonstrates that glycan masking can specifically regulate antigen-antibody binding, and the success of this approach opens new perspectives in the field of vaccine design.

**Multiple Domains of Staphylococcal Superantigen-like Protein 11 (SSL11) Contribute to Neutrophil Inhibition**

**Chen Yang, Joseph T. Barbieri, Nancy M. Dahms, Chen Chen**

*Louisiana State University, Baton Rouge, Louisiana*

**Introduction:** Carbohydrates are the most abundant organic molecules on earth and are critical to a variety of biological processes. The goal of this project is to characterize how human milk, at the chemistry level, protects infants from infectious diseases. At the outset, our initial hypothesis was that human milk oligosaccharides (HMOs) possess antimicrobial and anti-virulence activities. Previously, we discovered that HMOs do indeed modulate bacterial growth and biofilm production. In light of this discovery, three priorities emerged for the program. The first was to characterize the mode of action for antimicrobial activity. The second was to decipher the functional effects of HMO structural diversity. Finally, we set our sights on using HMOs to treat or prevent non-pediatric illness.

**Methods:** A combination of fractionation techniques, chemical synthesis, and industrial partnerships.

**Results**: We determined the identities of several HMOs with potent antimicrobial activity against the important neonate pathogen Group B *Streptococcus* (Group B Strep; GBS). Additionally, we observed that HMOs are effective adjuvants for intracellular-targeting antibiotics against GBS. This included two antibiotics that GBS has evolved resistance to. At their half maximal inhibitory concentration (IC50), heterogeneous HMOs reduced the minimum inhibitory concentration (MIC) of select antibiotics by up to 32-fold. Similarly, we observed that HMOs potentiate the activity of polymyxin B (Gram-negative-selective antibiotic) against GBS (Gram-positive species). Based on these collective discoveries, we hypothesized that HMOs function by increasing bacterial cell permeability, which would be *a novel mode of action for these molecules*. This hypothesis was validated as HMOs were found to increase membrane permeability by around 30% compared to an untreated control. Moreover, HMOs were shown to perturb central metabolism and the biosynthesis of membrane building blocks.

**Glycosaminoglycan Based Broad Spectrum Inhibitors of Respiratory Infections**

**Poonam Sharma, Stephen Stray, Lance Keller, Courtney Thompson, Ritesh Tandon**

*University of Mississippi Medical Center, Jackson, Mississippi*

**Introduction:** Respiratory tract infections are a major public health concern with limited therapeutic options. Currently available drugs are threatened by the development of antiviral or antibiotic resistance. Moreover, respiratory viral and bacterial co-infections and superinfections are linked to higher mortality and morbidity than the viral or bacterial infection itself. The cell surface heparan sulfate proteoglycan (HSPG) represents the first point of contact for many pathogens, including several bacteria and viruses. The purpose of this study is to determine the potential for glycosaminoglycans (GAGs) that are similar in structure to HSPG, to serve as competitive inhibitors for Influenza A virus (IAV) and Streptococcus pneumoniae.

**Methods:** The unfractionated heparin (UFH) was screened for the activity against flu and pneumococci. The antiviral assays were carried out by incubating A/Puerto Rico/8/34 (PR8) and A/Hong Kong/8/68 (HK68) strains of IAV using UFH with a dose range of 0.5 mg/l to 50 mg/l for 1 h at 37°C followed by infection of Madin-Darby Canine Kidney (MDCK) cells. On day 4, the supernatant was harvested for hemagglutination inhibition assays using 1% washed RBCs. For antibacterial assays, Streptococcus pneumoniae non-capsulated R36A strain was incubated with UFH at concentrations of 50 mg/l and 500 mg/l for 1 h at 37°C and the adhesion interference assay was performed by incubating lung cell line A549 with 1 × 107 cfu/ml of bacteria.

**Results:** We found that UFH significantly inhibited IAV even at the lowest concentration tested (0.5 mg/l) while pneumococci were only inhibited significantly at the highest concentration tested (500 mg/l). For IAV, UFH showed at least 10-fold inhibition for H1N1-PR8 strain and 1000-fold inhibition for H3N2-HK68 strain. The UFH at the concentration of 500 mg/l significantly decreased the bacterial attachment of R36A pneumococcal strain. The treated counts were 35 X 103 cfu/ml as compared to mock, 57.67 X 103 cfu/ml. Thus, GAGs may exhibit inhibitory potential for IAV and pneumococci. We will further analyze the effect of degree of polymerization and sulfation of the GAGs on the inhibitory activity of each pathogen. The computational simulations for docking will be performed to determine the influence of GAG sulfation and polymerization as well as any conformational changes in the glycan structures upon binding to the putative receptor/s for each pathogen.

**The Biological Importance of O-GaINAc Glycans**

**Richard Cummings**

 *Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts*

Glycoproteins containing O-linked GalNAc O-glycans are highly abundant and the structures of these O-glycans are critically important to cell adhesion and cell signaling. Deficiencies in such O-glycans typically lead to pathology, depending on whether mutations in these pathways are congenital or arise somatically. Our group has studied the key factors regulating O-glycan production and O-glycan structures in mice and humans, as well as the genetics underlying such regulation. The master regulator of all such O-glycans is the molecular chaperone Cosmc, encoded by *C1GalT1C1*, which has a single client protein the T-synthase, encoded by *C1GalT1*. Cosmc is a transmembrane protein in the endoplasmic reticulum that promotes the proper folding of the T-synthase, allowing it to leave the ER and move to the Golgi, where it causes the elongation of common O-glycans. Defects in Cosmc function, lead to either elimination or disruption of O-glycan elongation. Lack of Cosmc leads to expression of the Tn antigen (GalNAc-alpha-1-Ser/Thr/Tyr), which is a common antigen expressed in human carcinomas, such as in pancreatic cancer and colon carcinomas, and serves as a novel target for immunotherapy. This presentation will focus on the regulatory role of Cosmc, as revealed by human and mouse genetics, and the discovery of a novel *Cosmc*-Congenital Disorder of Glycosylation (*Cosmc*-CDG). In addition, the role of elongated O-glycans in leukocyte trafficking, and autoimmune disorders and the consequences of *Cosmc* mutations will be described. Such studies reveal the critical and essential role of O-GalNAc O-glycans in multiple biological pathways.

***ABSTRACTS***

**Poster Presentations**

**Poster 1:**

**Synthesis of Difluorinated Benzopyrans from Simplified Organic Molecule**

**Amena Begum, David A. Colby**

 *University of Mississippi, Oxford, Mississippi*

**Introduction:** Benzopyrans are a core structure in many bioactive natural products. The Malvidin-3-glucosides are glycosylated antioxidants and play an important role in preventing damage from reactive oxygen species. But the clinical translation causes the de-glycosylation of the nature products. The unstable O-glucoside link is responsible for the hydrolytic reaction. The resulting aglycone cannot pass the blood brain barrier, which eliminates its potential use as an antioxidant and neuroprotective reagent. In organic chemistry, fluorine and carbon forms the strongest bond, which influence conformation, pKa, intrinsic potency, membrane permeability, metabolic pathways and pharmacokinetic properties. The goal of this project is the assembly of difluorinated benzopyrans as simplified derivative of fluorinated glycosylated natural products. The oxygen of the labile glycosyl linkage will be replaced with a difluoromethylene bio-isostere to design more stable derivative. The initial target is to build a molecule that will replace an unstable O-glycoside linkage with a stable CF2-glycoside bond. The simplified organic structure of difluorinated benzopyran can be oxidized to a flavone and into a difluorinated anthocyanin derivative.

**Methodology:** We synthesized the difluoroenolate by the trifluoroacetate release from the pentafluoro-gem-diol precursor. We have some challenges using the methods because of an alpha proton and we ended up with low yield and product with byproduct. Then we proceed to the second-generation method by the direct difluorination for the synthesis of difluoroenolate. Our third-generation method is to develop the difluoroenolate chemistry by trifluoroacetylation and difluorination of methyl ketone.

**Results:** We can successfully synthesize the difluoroenolate using different synthetic strategy. But the limitation of the reaction is low yields and poor stereoselectivities.

Our future direction is to synthesize the difluorinated benzopyrans using the optimized synthetic methodology which will be used to prepare compounds for stability studies.

**Poster 2:**

**Progress Towards the Synthesis of an Acinetobacter Baumanii Lipooligosaccharide**

**James M. Armstrong II, Brandon j. Conrad, Justin R. Ragains**

*Louisiana State University, Baton Rouge Louisiana*

**Introduction:** Acinetobacter baumannii is a Gram-negative, multi-drug-resistant bacteria that was placed on the World Health Organization’s watchlist of drug-resistant bacteria. Glycoconjugate vaccines such as those used against Haemophilus influenzae B are used globally and are highly effective. To date, there are no FDA-approved vaccines against A. baumannii, and a glycoconjugate vaccine could represent an effective approach for prevention. Capsular polysaccharides (CPS), lipopolysaccharides (LPS), and lipooligosaccharides (LOS) are glycans found on the surface of bacteria and are important immunological targets for the development of vaccines. Synthesis and immunological evaluation of an A. baumannii LOS can be used to evaluate potential vaccine candidates.

**Methods and Results:** We have produced a trisaccharide representing a truncated A. baumannii lipooligosaccharide through a convergent and stereoselective synthesis. Synthetic challenges addressed in this work include an alpha 1,4-glycosidic linkage between a glucosamine and a Kdo portion as the result of a regioselective glycosylation of a 1,2-cis diol Kdo acceptor.

**Conclusion:** Synthetic strategies presented herein were effective in the production of a trisaccharide representing truncated LOS from A. baumannii. Future immunological experiments will determine the potential for glycoconjugate vaccine development.

**Poster 3:**

**Cytocompatibility of Chitosan-Silver Coated Titanium Coupons**

**Emily Coleman, Matthew Atwill, Joel D. Bumgardner, PhD, J. Amber Jennings, PhD**

*University of Memphis, Memphis, Tennessee*

**Introduction:** Titanium is commonly used in orthopedics due to its strength, resistance to corrosion, and bone like mechanical properties. Silver ions affect microbials by blocking transport in and out of the cell, inhibiting the production of energy, and interacting with DNA to prevent replication. These characteristics lead to broad spectrum antimicrobial properties against bacteria and fungi and therefore support the advantage of silver ions as an implant coating using chitosan biopolymer as a complexing agent and coating to localize silver.

**Methods:**Treated Coupons: Titanium coupons were polished with 400, 600, 800, and 1200 grit sandpaper before being sonicated in soapy water, acetone, and ethanol to remove oil and residue for 10 minutes each. The coupons were then soaked in 5M NaOH for 24h to allow accumulation of hydroxide reactive groups on the titanium surface and rinsed with deionized (DI) water twice. The coupons were treated with a linking agent and dried for 10 minutes in a 110°C oven. Chitosan-silver solution (Chitozan Health) was added and left to dry overnight. The coated coupons were immersed in phosphate buffer for 1 hour, rinsed with DI water, and dried fully.

Untreated Coupons: Titanium coupons were polished with 400, 600, 800, and 1200 grit sandpaper before being sonicated in soapy water, acetone, and ethanol to remove oil and residue for 10 minutes each. The uncoated coupons were rinsed with DI water and dried fully.

Cytocompatibility: Coupons were UV-sterilized for 20 minutes and washed in cell medium. Soas-2 cells were seeded at 90,000 cells/well in a 12-well plate before exposure to 3 test groups: treated coupons, untreated coupons, and tissue culture plastic (TCP) control. After 24 hours, cell viability was determined using CellTiter-Glo Viability Assay (n=3), and cell morphology was determined using Live/Dead staining (n=1).

**Results**: The Saos-2 cell viability for treated coupons was not statistically different than untreated coupons and was about 70 percent of the TCP control. Live/Dead staining also produced similar results, with mostly living cells in all groups.

**Poster 4:**

**Investigating the role of glycosylation in myocyte-environment interactions during development and disease**

**Joshua Bloomekatz, Tess McCann, Prashanna Koirala, Harini Saravanan, Rabina Shrestha,** *University of Mississippi, Oxford, Mississippi*

Protein glycosylation is important for cardiac and skeletal muscle development and is integral to the pathogenesis of several muscular diseases. For example, cardiac defects are common in congenital disorders of glycosylation (CDG), such as in dystroglycanopathies. And glycan dynamics have been implicated in the progression of diabetic cardiomyopathies triggered by hyperglycemia. We have been studying the role of glycosylation in cardiac and skeletal muscle development in zebrafish at both the individual protein level and the collective glycome level. At the individual-protein level we are interrogating the role of an O-linked glycan present in the oncofetal isoform of Fibronectin; a critical extra-cellular matrix glycoprotein essential for both cardiac and skeletal muscle development and for cardiac fibrosis. Without fibronectin, cardiomyocytes which are specified in bilateral regions of the embryo fail to move to the midline and merge to form a single heart tube. 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 transgenic and CRISPR/Cas9 techniques we are creating a point mutation that specifically inhibits the O-linked glycosylation unique to the oncofetal isoform of fibronectin. We will then assess the role of this mutation by examining fibronectin fibril formation and cardiac and skeletal muscle development. At the collective-level we are taking a holistic approach toward assessing how glycosylation changes during cardiac development and in response to disease. Using functionalized metabolic precursors attached by bio-orthogonal reactions to fluorogenic probes, we are investigating how glycan composition and dynamics change during the development of cardiac and somitic/trunk muscles in zebrafish. Specifically, we are analyzing mesenchymal-to epithelial transitions (MET) and myocyte differentiation in both cardiac and somitic/trunk muscles. We have observed that sialyation is particularly prevalent at the intersomitic trunk muscle boundaries. Furthermore, we are analyzing changes in glycosylation in two disease models – hyperglycemia and cardiac remodeling caused by dilated cardiomyopathy. By uncovering mechanisms by which glycosylation specifically regulates fibronectin’s function and more generally regulates cardiac and skeletal development and disease these studies will not only contribute to elucidating fundamental glycobiology principles they are also likely to contribute to preventive and therapeutic remedies in the future.

**Poster 5:**

**LC-MS Identification of Serum Proteins Adsorbed onto Ionic Liquid-Coated Nanoparticles**

**Anh Hoang, Eden Tanner**

*University of Mississippi, Oxford, Mississippi*

Nanocarriers are promising candidates for drug delivery due to their size and tunable surface characteristics. However, when they are intravenously injected, few particles make it to their designated location. This is because upon entering the bloodstream, the serum in the blood, which is rich with a diversity of proteins, adsorbs onto the particles’ surfaces forming a protein corona. Many of the attached proteins trigger the mobile immune system and are removed by macrophages, and many particles are then filtered out by the liver and kidneys. Ionic Liquids (ILs), which consist of asymmetric, bulky components that are liquid <100°C, are emerging as a promising surface modification technology that can be used to reduce serum protein adsorption. Solvent evaporation of Poly Lactic-co-Glycolic Acid polymer in organic solvent acetonitrile was used to make bare NPs. A single drop of ILs was added into the batch after the polymer aggregated. Due to the negative charge of the terminal carboxylic acid group, the ILs simultaneously coat each of the particles. The IL-capped NPs were then centrifuged to eliminate unbound IL in the NP solution. We tested the protein adsorption using 0.1 % sodium azide whole mouse serum by incubating with NPs at a ratio of 1:1. After each round, the upper non-adsorbed serum was carefully removed and replaced with an equal amount of 1X PBS. Liquid Chromatography-Mass Spectrometry (LC-MS) was used to identify specific serum proteins adsorbed onto the NPs. The results indicated that IL-coated NPs were able to enrich new proteins from the serum, and the identity of the proteins was determined by the chemical identity of the IL. These findings could provide the identity of adsorbed proteins, and enable directed drug delivery.

**Poster 6:**

**Engineering High-Affinity Lectenz® Reagents for the Detection and Analysis of Carbohydrates**

**Shaza Abnouf, Christian Gerner-Smidt, Lu Meng, Sheng-Cheng Wu, Robert J. Woods, and Loretta Yang**

*Lectenz Bio, Athens, Georgia*

**Introduction:** Glycans containing sialic acids have long been implicated as biomarkers for a variety of diseases, including endocrinal, oral, and colon cancers. As the investigation of the dynamic changes in the levels of glycoforms becomes more commonplace, specific and specific glycan binding proteins are essential to overcome current limitations in the discovery and exploitation of disease-related glycans.

**Methods:** Using computationally-guided design and directed evolution, we have been converting carbohydrate-processing enzymes into catalytically inactive affinity reagents with tunable specificities. These novel LECTin-like, ENZyme-derived reagents have been shown to offer numerous advantages over other carbohydrate-binding reagents: they are high affinity, yet retain the exquisite substrate specificity of the endogenous enzyme, they can be cost-effectively produced, and they can be used as capture reagents for affinity purification.

**Results:** Currently, we have developed and commercialized two sialic acid-specific Lectenz® engineered from a neuraminidase, as well as an α2,6-specific SiaFindTMreagent engineered from a Polyporus squamosus lectin. They have demonstrated in a number of applications such as Western blotting, flow cytometry, immunohistochemistry and affinity chromatography. These applications validate the simplicity, robustness, and accuracy of SiaFindTMreagents in glycoscience research. Supported by NIH grants R44GM113351, R44OD024964, R43GM135984 and R43GM136013

**Poster 7**:

**Native** **high molecular weight structure of Pentacta pygmaea fucosylated**

**Rohini Dwivedi, Poonam Sharma, Ritesh Tandon, Vitor H. Pomin**

*University of Mississippi, Oxford, Mississippi*

**Introduction:** Fucosylated chondroitin sulfates (FucCS) are unique marine glycosaminoglycans that exhibit diverse biological functions, including antiviral and anticoagulant activity. Sulfation pattern and molecular weight (MW) are key structural elements that play a role in the biological outcomes of FucCSs. We have reported recently the structure, moderate anticoagulant and potent anti-SARS-CoV-2 (Wuhan strain) activity of a new FucCS isolated from the body wall of the sea cucumber Pentacta Pygmaea (PpFucCS). In this subsequent study, we perform free-radical depolymerization of PpFucCS to generate oligosaccharides to conduct advanced structure-activity relationship studies. This rationale can facilitate assessment of specific structural features of PpFucCS regarding its antiviral and anticlotting effects. In this work, investigations were extended to the more infectious SARS-CoV 2 Delta strain.

**Methods:** PpFucCS was isolated by proteolytic digestion of the sea cucumber body wall and fractionated by anion-exchange liquid chromatography. The native polysaccharide was depolymerized by copper-based Fenton method. The obtained oligosaccharides were fractionated by size-exclusion chromatography using Bio-Gel P-10 column. MW distribution of fractionated oligosaccharides was estimated by polyacrylamide gel electrophoresis, and structural analysis was performed by 1H NMR spectroscopy. Anticoagulant activity of oligosaccharides was measured by activated partial thromboplastin time (APTT) and serpin mediated protease inhibition assays. Anti-SARS-CoV-2 activity was examined in HEK-293T hACE2 cells using a baculovirus pseudotyped with SARS-CoV-2 Delta variant spike protein containing a green fluorescence protein reporter.

**Results:** Free radical depolymerization of PpFucCS by copper-based Fenton method was successfully employed to generate oligosaccharides with shorter chain lengths and conserved structural integrity like native PpFucCS. Depolymerized PpFucCS oligosaccharides upon fractionation were pooled in to four fractions with different MWs (Fr1>Fr2>Fr3>Fr4). 1D 1H NMR spectra confirmed the presence of the two types of monofucose branches (α-Fuc2,4- disulfated and α-Fuc4-sulfated) and one difucose branch (α-Fuc2,4-disulfated-(1→4)-α-Fuc), attached to the C3 position of the glucuronic acid linked to N-acetylgalactosamine unit in the backbone of all fractions, except Fr4. Fraction 4 exhibited an upfield 1H shift of the fucose anomeric peaks suggesting possible desulfation. The large 1H chemical shift displacement and position of the α-Fuc2,4S unit in the difucosyl branch indicated selective 2-O-desulfation solely at this residue. The PpFucCS oligosaccharides fractions exhibited a significant reduction of their anti-SARS-CoV-2 and anticoagulant activities as compared to the native high MW PpFucCS structure. Our results suggest that native high MW of PpFucCS is a key structural element to achieve the maximal antiviral and anticoagulant effects.

**Poster 8:**

**Fractionation of sulfated galactan from the red alga Botryocladia occidentalis separates its anticoagulant and anti-SARS-CoV-2 properties**

**Seon Beom Kim, Mary Zoepfl, Priyanka Samanta, Fuming Zhang, Ke Xia, Reena Thara, Robert J. Linhardt, Robert J. Doerksen, Michael A. McVoy, Vitor H. Pomin**

*University of Mississippi, Oxford, Mississippi*

**Introduction:** Sulfation pattern and molecular weight play a key role in the biological actions of sulfated glycans. Besides anticoagulant effects, certain sulfated glycans can also exhibit anti-SARS-CoV-2 properties. The sulfated galactan isolated from the red alga Botryocladia occidentalis (BoSG) is well known to present potential anticoagulant activity due to the enhanced inhibition of blood factors IIa and Xa by the two serpins, antithrombin and heparin cofactor II. To develop a more selective antiviral carbohydrate, an efficient strategy to separate these two actions is required.

**Method:** After extraction and purification, BoSG was subjected to mild acid hydrolysis to generate oligosaccharides. Fractionation was achieved by size-exclusion chromatography. NMR spectra were acquired at 50℃ in a 500 MHz spectrometer. Activated partial thromboplastin time (aPTT) of the BoSG fragments was performed. The serpin-mediated inhibitory activity of BoSG and fragments were assayed as anti-IIa and anti-Xa. Anti-SARS-CoV-2 activity was measured by incubating BoSG and fragments with green fluorencent protein (GFP)-expressing virus-like particle (VLP) pseudotyped with spike proteins from SARS-CoV-2, then infecting ACE2- expressing HEK293 cells and measuring total GFP fluorescence three days post infection. Solution competition analysis between heparin and soluble BoSG-derived samples was performed using SPR. In brief, different viral S-proteins (wild type, L452R, N501Y, and K417T/E484K/N501Y mutants) were mixed with sulfated glycans and injected over a heparin sensor chip. Results related to binding affinity were collected and interpreted in terms of response units from the sensorgrams.

**Results:** The lowest MW fraction was found to be primarily composed of octasaccharides of monosulfated monosaccharides. Unlike heparin or native BoSG, hydrolyzed BoSG products showed weak anticoagulant activities as seen by aPTT and inhibitory assays using purified cofactors. In contrast, lower MW BoSG-derivatives retained anti-SARS-CoV-2 activity. SPR confirmed that longer chains are necessary for BoSG to interact with coagulation cofactors but is not required for interactions with certain S-protein variants. We observed distinct affinities of BoSG derivatives for the S-proteins of different SARS-CoV-2 strains, including WT, N501Y (Alpha), K417T/E484K/N501Y (Gamma), and L542R (Delta) mutants, and stronger affinity for the N501Y-containing variants. Docking of the four possible monosulfated BoSG disaccharides in interactions with the N501Y mutant S-protein predicted potential binding poses of the BoSG constructs and favorable binding near the 501Y residue. Our results demonstrate that depolymerization and fractionation of BoSG are an effective strategy to segregate its anticoagulant and anti-SARS-CoV-2 actions.

**Poster 9:**

**Isolation and Characterization of Myxobacteria**

**Kayleigh Phillips, Andrew Ahearne, Cole Stevens**

*University of Mississippi, Oxford, Mississippi*

**Introduction:**Myxobacteria play an intricate role in structuring microbial community composition either directly by predation of other soil microorganisms or indirectly through nutrient cycling of complex macromolecules such as cellulose. Their complex lifestyle has resulted in the largest genomes amongst bacteria, replete with biosynthetic gene clusters, offering a reservoir of undiscovered natural products. However, improper characterization relying on fruiting body morphology has resulted in confusion and rediscovery of common myxobacteria species. Going towards a combined approach utilizing morphology, enzymatic activity, and whole comparison genomics can help clarify the classification of taxonomic relationships as well as identify prime targets for natural product discovery

**Methods:**Novel myxobacteria were isolated from various sources throughout the United States. Isolation of predatory myxobacteria was performed by inoculating soil samples on water agar plates streaked with Escherichia coli. After a few days, myxobacteria swarms were seen consuming the E.coli bait, and swarm edges were repeatedly transferred to fresh VY/4 agar for purification. Isolation of cellulose-degrading bacteria was performed by placing filter paper on ST21, mineral salt agar, and incubating until degradation of the filter paper and build-up fruiting bodies were present. Purification was performed by transferring fruiting bodies from original samples and baiting again with filter paper until swarms are pure and transferred to a nutrient-rich media. DNA extraction was performed using the Nucleobond HMW DNA extraction kit from Macherey-Nagel. Long read sequencing was performed on Pacbio sequel, and reads were assembled into contigs using the HGAP 9.0.0 protocol. The taxonomic assessment was performed using the type strain genome server (TYGS) for dDDH prediction and phylogenetic tree construction. Average nucleotide identity was calculated using the Kostas lab's online ANI matrix calculator.

**Results:** Digital DNA-DNA hybridization and average nucleotide identity data suggests these newly isolated myxobacteria belong within the genus of Pyxidicoccus, Corallococcus, Stigmatella, Archangium, Nannocystis, Polyangium and Myxococcus. We are in the process of doing enzymatic activity and growth conditions to characterize them for proper type strain designation.

**Poster 10:**

**Description and Profiling the biosynthetic potential of Archangium primigenium ATCC 29037**

**Hanan Albataineh, Cole Stevens**

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**Introduction:** Whole-genome sequencing (WGS) of bacteria has become a more accessible, affordable tool in modern microbiology. The powerful combination of WGS and bioinformatics-driven analysis of sequence data has delivered unexpected insights into microbial communities. Myxobacteria are a unique group of Proteobacteria that are best known for their large genomes, well-coordinated social behavior, and ability to predate other microbes. Genome sequencing of myxobacteria has demonstrated that they are a “gifted” taxon as their genomes house multiple biosynthetic gene clusters (BGCs) for the production of specialized metabolites with drug-like properties. To fully access novel specialized metabolites from myxobacteria, it is important to understand the relationships between myxobacterial evolution, taxonomy, and genomic variation. Here we report the full genome, description, and metabolic capacities of the myxobacteria Archangium primigenium ATCC 29037.

**Methods:** Genomic DNA was isolated and sequenced and annotated using the NCBI Prokaryotic Genome Annotation Pipeline. Sequencing data have been deposited in NCBI under the accession numbers JADWYI000000000.1. Growth characteristics were assessed at various temperatures, pH values, and salinities at pH 7.5 and 30°C on VY/4 agar. The biochemical properties of strains were characterized using the API 20E and API ZYM kits. The genomes were analyzed by the AntiSMASH platform to assess specialized metabolite gene clusters using the “relaxed” strictness setting. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) scores were calculated. Genome BLAST Distance Phylogeny (GBDP) minimum evolution tree was constructed. These three metrics were applied to analyze and compare the genome of A. primigenium to other myxobacteria. A. primigenium was cultivated in 500 ml CYH media in 2 L shake flasks (10 flasks, total 5 L). After 10 days of incubation at 30 °C and 110 rpm, the culture was complemented with 40 ml of a 50% v/v sterilized XAD-16 resin and shaken for 4 days. Total fermentation period is 14 days. To get the extract, the pellet was extracted with methanol and concentrated under reduced pressure.The crude extract was fractionated using Sephadex LH-20.

**Results:** Comparative genomics results suggest previously classified as A. primigenium to instead be a novel member of the genus Melittangium. The taxonomic polyphasic approach was applied to characterize and describe A. primigenium for the first time. Utilizing mass spectrometry data, we assess the biosynthetic potential of this strain and we describe our plan to isolate pharmacologically active metabolites.

**Poster 11:**

**ROS-responsive, glycopolymeric nanoparticles for enhanced drug delivery to macrophages**

**Oluwaseyi Shofolawe-Bakare, Judith U. De Mel, Mehjabeen Hossain, Sushil Mishra, Robert Doerksen, Adam E. Smith, Thoas A. Werfel**

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**Introduction:** The modulation of macrophage activity has emerged as a promising therapeutic strategy to combat a variety of inflammation-related diseases (asthma, Crohn’s disease, cancer) whose pathogenesis is heavily dependent on these actions of macrophages. Despite the tremendous potential macrophage-modulation therapies, they are plagued by poor pharmacokinetics and off target toxicities that hamper their efficacy. Drug delivery vehicles that can effectively transport these therapeutics specifically to macrophages are therefore essential to improve the efficacy of these drugs. Here, we synthesize nanoparticles (NPs) that target macrophages through the polymeric glucose ligands that form the corona and selectively release drugs in response to oxidative species. This smart drug delivery platforms enables preferential delivery to macrophages thereby improving the therapeutic index of the delivered drug.

**Methods:** The NPs are made from diblock copolymers consisting of poly (propylene sulfide) (PPS), which forms the NP core and enables hydrophobic drug loading, and polymethacrylamidoglucopyranose (PMAG), which imparts stability to the NPs and enables macrophage-specific delivery. P(PS-b-MAG) was synthesized through a combination of anionic polymerization and reversible addition fragmentation transfer (RAFT) polymerization. P(PS-b PEGMA) was used as a control polymer. Physicochemical characterization of the polymers was performed by 1H NMR, and GPC. NPs were formed by the nanoprecipitation/dialysis [P(PS-b MAG)] and solvent evaporation method [P(PS-b-PEGMA)]. NP characterization was performed through DLS and cryoTEM. The cytoxicity of the polymers were assessed using a glow assay. The serum stability was measured by incubating the NPs in different concentrations (0-40%) of FBS and measuring the loss of fluorescence with time using a plate reader. Likewise, the ROS responsiveness of the nanoparticles was measured at vary doses of H2O2 (0-30wt% H2O2). Cellular internalization studies were performed by incubating the NPs in serum with naïve macrophages for 2 h then analyzing uptake using flow cytometry.

**Results:** P(PS-b-MAG) diblock copolymers were successfully synthesized and had a molecular weight of about 18kDa (Mn). The P(PS-b-MAG) copolymer formed micelles of 129nm. CryoTEM images corroborated the sizes obtained from DLS. The cytocompatbility studies showed that the polymer exhibited negligible cytoxicity. The serum stability studies showed that the NPs are stable in 40% serum after 6 h. The NPs showed dose-dependent cargo release in response to H2O2. The P(PS-b MAG) NPs showed a 2-fold higher uptake in macrophages compared to P(PS-b-PEGMA) NPs after 2h.

**Poster 12:**

**Loss of Adventitia Results in an Alteration of Vascular Phenotype Markers Promoting Calcification in an Ex Vivo Model of Type 2 Diabetic Aortic Calcification**

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**Introduction:** As the prevalence of type 2 diabetes (T2DM) rises, greater numbers of individuals are at risk for increased cardiovascular complications, such as vascular calcification. Diabetes-mediated vascular calcification is often studied using medial layer vascular smooth muscles cells (VSMCs) or adventitial layer fibroblasts (AFBs) in in vitro models of the calcification. In these modeling systems, VSMCs and AFBs are studied separately; however, in in vivo setting these cell types are in physical contact and conduct cellular communication. Previous studies by our laboratory have demonstrated calcification of these cell types are differentially regulated by AGE-RAGE signaling mechanisms.

**Methods:** The goal of this study was to elucidate the phenotypic changes occurring when whole aortic rings were cultured in an ex vivo setting with and without the adventitial layer. Mouse thoracic aorta rings were calcified for 7 days with and without the adventitia present. Histological analysis, immunohistochemistry staining, and western blotting experiments were conducted to determine differences in smooth muscle cell phenotype marker, α-SMA, and bone protein, OPN.

**Results:** Data revealed the loss of the adventitial layer with calcification and exogenous AGE treatment caused an increase in calcification as well as an increase in α-SMA in non-diabetic aortic rings. Diabetic aortic rings without adventitia had increased calcification despite a loss in α-SMA expression due to calcification and AGE treatment. Removing adventitia appeared to increase calcification, which was exacerbated by AGE treatment even in RAGE knockout mice.

**Conclusion:** AFBs present in the adventitial layer may act in a protective manner to limit aortic calcification. While further investigation is required to determine the role for AGE- RAGE signaling and the adventitia in the calcification response in diabetes, we can conclude the loss of the adventitial layer promoted ex vivo calcification as a result in altered phenotype markers in T2DM.

**Poster 13:**

**Dextrans as Macromolecular Crowding Agents**

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**Introduction:** DNA is the hereditary material in all organisms. In the textbook Watson and Crick model, DNA exists as two strands coiled into a double helix. However, DNA has been found to exist in four-stranded secondary forms, often known as G-quadruplexes (G4) and/or i-motifs (iM). The iM are formed under acidic conditions when sets of cytosines pair with other, protonated cytosines, and their backbones are coiled around each other to make a four-stranded structure. The pKa value for iM in dilute solutions is approximately 6.5, but iM can exist at higher, more physiological pH values under crowded conditions. Within the interior of a cell, a large percentage of total cellular volume is a dense mixture of macromolecules and solutes, including glycogens or starches. In contrast, a typical setup for an in vitro biochemical experiment is a dilute solution.

**Methods and Results**: To best mimic cellular crowding in biochemical experiments, crowding agents like Ficoll, Polyethylene Glycol (PEG) and Dextran can be used. In this study, Dextran was explored to try and understand whether it could be an effective crowding agent in the study of iM. However, a biological contaminant was found in some commercial sources of Dextran, which became an impediment to our iM studies. Here we discuss the general concept of Dextrans as crowding agents and our cautions on how to use them properly in experiments.

**Poster 14:**

**Prediction of binding free energy in protein-carbohydrate complexes and their mutants using sequence and structural features (Virtual)**

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**Introduction:** Protein-carbohydrate interactions perform several cellular and biological functions and their structure and function are mainly dictated by their binding affinity. Elucidating the factors influencing the binding affinity and predicting their binding free energy provide deep insights for understanding the recognition mechanism.

**Methods:** We have collected data on binding affinity of protein-carbohydrate complexes, ProCaff (<http://web.iitm.ac.in/bioinfo2/procaff/> ), which contains 3713 entries on dissociation constant (Kd), Gibbs free energy change (ΔG), experimental conditions, sequence, structure and literature information. Further, we developed models for predicting the binding affinity of wild-type and mutants by relating structure and sequence-based features, respectively.

**Results:** We used a set of 389 complexes from ProCaff database and related with structure-based features. We found that binding site residues, accessible surface area (ASA), interactions between atoms and energy contributions are important to understand the binding affinity. We developed a multiple regression method, PCA-Pred, which showed an average correlation and mean absolute error (MAE) of 0.73 and 1.15 kcal/mol, respectively, between experimental and predicted ΔG on a jackknife test. Further, we validated our method using blind data set of 40 complexes and we obtained a correlation and MAE of 0.88 and 1.02 kcal/mol, respectively. It is available at <https://web.iitm.ac.in/bioinfo2/pcapred/> Further, we have developed a model for predicting binding free energy change upon mutation (ΔΔG) using the sequence-based features (PCA-MutPred). Our analysis showed that ASA, mutation preference, secondary structure, conservation score, hydrophobicity and contact energies are essential for predicting ΔΔG values. It is available at <https://web.iitm.ac.in/bioinfo2/pcamutpred> PCA-MutPred showed an average correlation of 0.74 and MAE of 0.70 kcal/mol on 10-fold cross-validation. We have validated our method using test data set of 124 mutations, which showed a correlation of 0.79 and MAE of 0.56 kcal/mol. These methods are helpful to understand the affinity of carbohydrates, disease-causing mutations, designing carbohydrate-based therapeutics for immune-related diseases and gene therapy.

**Poster 15:**

**Receptor for Advanced Glycation Endproducts Signaling Impacts on Healthspan: Early-life Observations**

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**Introduction:** As individuals age, the extent of advanced glycan end product (AGE) accumulation throughout the body is associated with reduced health. It has been suggested that disruption of AGE signaling can avert the onset of cognitive impairment associated with diabetes as well as the bone damaging effects of osteoarthritis and other age-related disease states. Few studies have examined the role of AGEs in ‘normal’ aging processes in the absence of disease. Thus, our lab is examining the effects of AGE signaling on healthy aging. We hypothesize that reductions in AGE signaling will delay the onset of age-related cognitive and physical impairments, as well as the onset of senescence, inflammation, and other molecular hallmarks of aging.

**Methods:** To address this, cohorts of male and female WT and receptor for advanced glycation end products (RAGE) KO mice (C57/Bl6J background) were bred and we are observing phenotypic aging across their lifespan. At 3 months of age, behavioral and physical assessments were made in a young cross-sectional cohort. Behavioral assessments included rotarod motoror coordination testing and cognitive testing in the radial arm water maze and novel object/novel location tasks. Body weights and overall activity are assessed as well, and circulating levels of cytokines were quantified using the FlexMap3D multiplex system.

**Results:** Consistent with previous reports, RAGE-KO mice weighed significantly more than age matched WT controls and the increased weight of female RAGE-KO mice has persisted through at least 9 months of age. Despite this difference in weight, no significant effect on motor coordination was observed on the rotarod. Assessments of cognitive function in the radial arm water maze revealed sex and genotype differences between the RAGE KO and WT mice. Minimal differences were observed in circulating levels of pro- and anti-inflammatory cytokines/chemokines thus far. The baseline data from the young mice will allow us to gain a clearer picture of the role that AGEs has on the onset of age-related pathologies in the months to come. Our future studies include locomotor, cognitive, and frailty assessments in and advanced age. Moreover, we will be comparing the extent of inflammation, senescence, and oxidative stress in key tissues across these three time points in order to better understand the contribution of AGEs to biological aging processes.

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**Poster 16:**

**Synthesis of Polymer Scaffold by Modification of Azlacton Ring with Glycopolymers**

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**Introduction:** Polymer scaffold-based functional materials have a wide range of applications, including biomaterial surfaces and sensors. The use of well-defined polymer "brushes" formed by anchoring polymer chains to a surface by one of their ends being addressed for in situ functionalization. Polymers containing azlactone or oxazolone functionality have become more valuable for designing functional materials quickly and modularly. Azlactone functionalized materials can be used to provide a wide range of chemical functionality to soluble polymers, surfaces/interfaces, and insoluble supports via ring-opening reactions with a variety of different nucleophilic species (e.g., hydroxyl groups, primary amines, and thiol functionality). Specifically, poly(2-vinyl-4,4-dimethylazlactone) (PVDMA) chains, which feature a reactive azlactone ring at each repeat unit, are connected to a surface and functionalized with a variety of small molecules, mainly amines or peptides, to create brushes. In current approach, we have developed amide, ester and thioester containing brushes by modification of PVDMA with N,N-Dimethylethylenediamine (DMED), 2-Dimethylaminoethanol (DMAE) and 2-(Dimethylamino)ethanethiol hydrochloride (DMAT.HCl) respectively. Specially, we explore the impact of temperature and pH on the rate of degradation of PVDMA-based charge shifting polycations to acquire a better understanding of their hydrolytic kinetics. Next step of this work is to developed polymer scaffold based on glycopolymers modified PVDMA.

**Methods:** The polymer was synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization technique. Small molecules were attached by post-polymerization modification of PVDMA block. Polymer and modified polymer both characterized by NMR, FTIR techniques. Molecular weight of the polymers was measured by GPC.

**Results and Discussion:** Successful synthesis of polymer and modified polymer was justified by NMR and FTIR spectra. Besides, GPC characterization justified synthesis of a well-defined and narrow disperse polymer structure. 1H NMR spectra depicts all the functional moieties showing base catalysed hydrolytic degradation. At elevated temperatures and high pH, thioester shows the fastest degradation compared to others.

**Poster 17:**

**Selective 2-desulfation of sulfated fucans under mild acid hydrolysis**

**Marwa Farrag, Seon Beom Kim, Sushil Mishra, Sandeep Misra, Joshua S. Sharp, Robert J. Doerksen and Vitor H. Pomin**

 *University of Mississippi, Oxford, Mississippi*

**Introduction:** Sulfated fucans (SFs) are polysaccharides composed of sulfated fucose units. SFs are found exclusively in marine organisms such as sea cucumbers, sea urchins and brown algae. While algal SFs are highly heterogeneous, regular and repeating structures can be found in invertebrates. Production of oligosaccharides is a key step for advanced structure-activity relationship studies. Controlled chemical hydrolysis such as mild acid hydrolysis is usually employed in depolymerization of SFs since specific fucanases capable to cleave these polymers are not well described. In this work we investigate the production of oligosaccharides from three holothurian 2- and/or 4-sulfated 3-linked alpha SFs isolated from Lytechinus variegatus (LvSF), Isostichopus badionotus (IbSF) and Holothuria floridana (HfSF) by multiple methods including 1D 1H and 2D 1H 13C nuclear magnetic resonance (NMR) spectra, polyacrylamide gel electrophoresis (PAGE), size-exclusion chromatography (SEC), liquid chromatography-mass spectrometry (LC-MS), and molecular dynamics (MD).

**Methods:** The crude polysaccharide extracts were obtained by enzymatic digestions of the body wall of the sea cucumbers and egg jelly of the female sea urchins. Purification was performed by anion-exchange chromatography using a DEAE Sephacel column. A series of reactions were performed at 60oC at different time intervals using 0.05 M H2SO4 (IbSF and HfSF) and 0.01M HCl (LvSF and HfSF), then neutralized with ice-cold NaOH, to optimize conditions for depolymerization. The hydrolyzed products were analyzed by PAGE (22%) at which the approximate molecular weights (MW) were estimated by comparison against various MW standards. 1D 1H and 2D 1H 13C heteronuclear single quantum coherence (HSQC) spectra were acquired for 9h-depolymerized IbSF, and 2h- and 5h-depolymerized HfSF. Scaled-up digestions were prepared for SEC fractionation in Bio-Gel P-10 column. Specific fractions were desalted using a Sephadex G-15 column prior to 2D 1H 13C HSQC NMR and LC-MS analysis. The LvSF-derived octasaccharide was derived from a previous work (J Struct Biol. 2020, 209(1):107407).

**Results:** As determined by PAGE, the best time of mild acid hydrolysis employed to produce oligosaccharides suitable for further fractionation in SEC Bio Gel P-10 column was 9h with 0.05 M H2SO4 for IbSF, and 5h with 0.01M HCl for HfSF. NMR data confirmed that the HfSF products were the same regardless of the acid employed. NMR and LC-MS data have confirmed selective 2-desulfation in just one specific fucose unit of all three SFs. Preliminary MD results obtained by computational simulations have suggested higher accessibility and exposure to acidification for the 2-

sulfate sites more susceptible to desulfation.

**Poster 18:**

**Structural characterization of an α-glucan from the mollusk Marcia hiantina (Lamarck, 1818)**

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**Introduction:** Marcia hiantina (Lamarck, 1818), or hiant venus, is a mollusk bivalve belonging to the family Veneridae (venus clams). Over 500 species of venus clams are known, most of which are edible, and many of which are used as food sources. M. hiantina is widely distributed along the Indo-West Pacific from the Gulf of Aden to Papua New Guinea, north to southern Japan and south to Queensland. Up to this report, no description regarding the isolation and structural characterization of the main polysaccharide of M. hiatina has been published. In this work, we isolated the main carbohydrate from M. hiantina and characterized its structure by multiple nuclear magnetic resonance (NMR) methods together with gas chromatography (GC)-mass spectrometry (MS) and size exclusion chromatography with multi angle light scattering (SEC-MALS). The NMR methods used in this work were the following: one-dimensional (1D) 1H and two-dimensional (2D) 1H- and 13C-related experiments, including 1H 1H correlation spectroscopy (COSY), 1H 1H total correlation spectroscopy (TOCSY), 1H 1H nuclear Overhauser effect spectroscopy (NOESY), 1H 13C heteronuclear single-quantum coherence (HSQC), and 1H 13C HSQC-NOESY.

**Methods:** The polysaccharide from M. hiantina was isolated using hot water extraction method and purified using SEC, Sephadex G-15 column. The purified polysaccharide was structurally characterized by multiple NMR methods (1D 1H, 1H 1H COSY, 1H 1H TOCSY, 1H 1H NOESY, 1H 13C HSQC and 1H 13C HSQC-NOESY. All NMR spectra were recorded at a cryogenic probe at 50oC on a Bruker 500 MHz equipment, with the sample dissolved in 99.8% deuterium oxide. The glycosidic linkage was confirmed by methylation analysis using GC-MS of the partially methylated alditol acetate derivatives of the purified polysaccharide. Molecular weight was determined using HPLC SEC-MALS system.

**Results:** The analysis from NMR, monosaccharide composition, methylation analyses and size-exclusion chromatography on the pure M. hiantina-derived glycan confirmed a branched polysaccharide exclusively composed of glucose (Glc), mostly 4-linked in its backbone, but also with minor amounts of linkages at C3 or C4,6 positions. Weight-averaged molar mass of M. hiantina polysaccharide was calculated to be 573 kDa (± 4.67%). These results have indicated a glycogen-liked glycan with a small portion of the unique 3-linked Glc units as the main carbohydrate in the mollusk M. hiantina.

**Poster 19:**

**Predicting protein–glycosaminoglycan (GAG) complexes through docking:**

**Methods and challenges**

**Sushil Mishra, Robert J. Doerksen**

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**Introduction:** Glycoscience continues to emerge as a high-value information-rich field providing medical insight in the post-genomic era. Among the glycans, glycosaminoglycans (GAGs) represent a large family of highly sulfated, complex, linear, periodic polysaccharides that display a variety of important biological roles via interaction with protein targets. One of the recent examples is that heparan sulfate, itself a GAG, facilitates SARS-CoV-2 spike protein binding to the ACE2 receptor which triggers coronavirus infection (1). Not only this, but certain other kinds of GAGs have also been found to inhibit SARS-CoV-2 activity considerably and have been proposed as potential therapeutics (2). Computational modeling is an effective tool in studying biological systems but the nature of these long periodic linear and negatively charged polysaccharides makes it challenging to model GAG systems alone or their complexes with proteins (3). Docking is an essential tool for understanding protein–GAG interactions, but there has been a lack of validation studies to show the reliability of docking programs in predicting protein–GAG complexes. In this work, we will show some of the challenges and limitations of current software in modeling protein–GAG interactions.

**Methods:** We have performed docking of 80 protein–GAG complexes using different docking software and dockingstrategies. Predicted docked conformations of the GAGs were compared with their conformation in thecrystal structure. The root means square deviation (RMSD) between docked poses and crystal structurepose has been used to quantify the accuracy of the docking programs.

**Results:** The software performed moderately in docking GAGs to proteins showing average RMSD of about ~5 Å from the crystal structure poses. We will present details on the performance of different docking programs and docking strategies on protein–GAG complexes.

**Poster 20:**

**Study of Marine Natural Products as Anti-SARS-CoV-2 Agents Using Molecular Modeling**

**Priyanka Samanta, Sushil K. Mishra, Rohini Dwivedi, Seon Beom Kim, AyoOluwa O. Aderibigbe, Poonam Sharma, Fuming Zhang, Pavel Kucheryavy, Mary Zoepfl, Ke Xia, Reena Thara, Robert J. Linhardt, Michael A. McVoy, Ritesh Tandon, Vitor H. Pomin, Robert J. Doerksen**

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**Introduction:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has created a global pandemic. Viral entry into the host cell is mediated by spike glycoprotein (SGP). Carbohydrate small molecules were found to bind to the receptor binding domain (RBD) of SGP which also interacts with angiotensin-converting enzyme 2 (ACE2), forming a ternary complex. Marine natural products (NPs) isolated from Pentacta pygmaea, Isostichopus badionotus and Botryocladia occidentalis have exhibited anti-SARS-CoV-2 activities, presumably by blocking viral entry mediated through SGP–heparan sulfate interactions. Here we report a collection of computational studies conducted as part of a collaborative effort to investigate the effects of these glycans isolated from sea cucumber and red alga species on the wild type (WT) and N501Y mutant SGP RBD.

**Methods:** Starting from an X-ray crystal structure of the RBD–ACE2 complex, models of WT and N501Y mutant SGP RBD were built. To investigate the static and dynamic behavior of the RBD– NP interactions, blind and site-targeted molecular docking using diverse docking programs (Glide, AutoDock Vina or ClusPro) were carried out, followed by extensive all-atom molecular dynamics (MD) simulations with two force fields (CHARMM36 or Glycam06) in explicit solvent and binding free energy calculations.

**Results:** Conformations of the NPs varied considerably when studied in water or in complex with RBD. Five NP binding sites on the RBD were identified from the blind docking studies, using heparin disaccharide as a probe. Each binding site was extensively studied using site-targeted docking followed by MD simulations and binding free energy calculations, which identified important interacting RBD residues. The differences caused by varying the force field for the marine NPs were also investigated. The NPs’ binding specificities towards SARS-CoV-2 variants were explained. The results of the docking and MD studies were in excellent agreement with previously published reports of the heparin binding sites of SGP. Statistical analyses of the stability of various protein–NP complexes helped to differentiate pseudo- vs.real- binding sites. Our results will provide significant insights into the importance of extensive dynamics calculations in order to move beyond the limitations of molecular docking.

**Poster 21:**

**Structural Analysis of Protein-Carbohydrate Interactions by a Novel Inline Liquid Chromatography−Flash Oxidation Approach**

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**Introduction:**Low-affinity or non-stoichiometric protein-ligand interactions are challenging to probe by covalent labeling due to the conformational heterogeneity in solution. These challenges are common in the analysis of protein-carbohydrate interactions. Here, we describe a method coupling a FOX Protein Footprinting System with inline LC to probe the binding of unfractionated heparin (UFH) with antithrombin III (ATIII). Size-exclusion chromatography (SEC) is used to separate ligand-bound and unbound protein fractions with FOX to individually footprint each conformer as it elutes from the column. Using the UFH-ATIII system, we seek to validate that we can achieve accurate footprints of each conformer co-existing in solution.

**Methods:** Human ATIII protein and its UFH ligand were mixed in a 6:1 ratio and incubated at room temperature for half an hour. Separation of bound and unbound conformers was performed using SEC column attached to HPLC system at a flow rate of 14 μL/min. A Peek microTee mixer was installed after the UV detector, with one inlet port leading to SEC column, one inlet port leading to FPOP reagent (660 mM hydrogen peroxide, 10 mM adenine) flowing at a flow rate of 2.5 μL/min, and outlet port coupled to the FOX system (GenNext Technologies) for UV exposure. The samples were collected into vials containing quenching solution (0.5 μg/μL catalase and 35mM methionine amide), and subjected to trypsin digestion for LC-MS analysis.

**Results:** SEC separation of bound and unbound conformers was successfully achieved by an isocratic gradient of 100 mM sodium phosphate (pH 6.8) for 90 min. The mixed sample showed two major peaks at 60 min and 69 min for UFH-ATIII complex and pure ATIII, respectively. Quantification showed an average of approximately 2.5 ATIII proteins bound per heparin molecule, indicating possible ATIII dimerization on the heparin molecule. Eluents from central portion of each peak were subjected to inline Flash OXidation (FOX) labeling by UV exposure using a lamp voltage of 800 V and 2 HZ frequency with 25% exclusion volume. The data analysis revealed a significant decrease in oxidation levels in thirteen peptides of ligand bound ATIII in comparison to unbound ATIII. The known fondaparinux binding site is protected, as are other distal regions of the protein. This indicates that widespread topographical changes are induced in ATIII upon UFH binding and are not restricted to the binding site only. Validation of these results is in progress by MD simulations and SASA value determinations for ligand-bound and unbound ATIII structures.

**Poster 22:**

**Synthesis of 2,2-Difluoroethanols via Hydroxymethylations of Difluoroenolates and Difluorobenzyl Carbanions by Reacting with in situ Generated Formaldehyde**

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**Introduction:** Fluorinated alcohols are valuable reagents in synthetic organic chemistry. A myriad of organic reactions has been shown to be promoted using fluorinated alcohols. Although a number of examples of environment-friendly and catalyst-free reactions using fluorinated alcohols exist in the literature, the utilities of such reagents are primarily limited to trifluoroethanol and hexafluoro-2-propanol. Hence the research avenue of synthesizing substituted fluorinated alcohols remains under-explored. Installing a CH2OH group, also called hydroxymethylation, is a significant synthetic process to functionalize organic compounds. Hydroxymethylation has been widely used in total synthesis of natural products and drug discovery. To perform hydroxymethylation, a common strategy is to use formaldehyde or a reagent that serves as a formaldehyde equivalent. Dimethylsulfoxide (DMSO) or DMSO-like structures have also been found to serve as a source of in situ generation of formaldehyde. However, the conventional methods utilizing DMSO for formaldehyde generation require excess heat and produce many side products which reduce their applicability and scope. Fluorine incorporation on organic molecules has garnered popularity in medicinal chemistry since fluorine improves drug-like qualities. Although monofluorination and trifluoromethylation are ubiquitous, methods for difluoromethylation of organic molecules are less common.

**Methods:** We previously showed that pentafluoro-gem-diols fragment into α,α-difluoroenolates intermediates in a mild condition. These reactive α,α-difluoroenolates react with aldehydes, imines, and electrophilic halogen sources. Herein, we present a reactivity of α,α-difluoroenolates with in situ generated formaldehyde to construct 2,2-difluoroethanols. Difluorobenzyl carbanions are also compatible with the reaction.

**Results:** Using cesium carbonate and bromine in DMSO to promote formaldehyde generation, pentafluoro-gem-diols were hydroxymethylated to assemble corresponding 2,2-difluoroethanols in 25–68% isolated yields. In conclusion, we present a method for the hydroxymethylation of difluoroenolates and difluorobenzyl carbanions by reacting with formaldehyde generated *in situ* to construct 2,2-difluoroethanols.

**Poster 23:**

**Influenza Virus- a Glycan-targetted Treatment for Cancer?**

**Stephen Stray, Christopher Bruni, Kilando Q. Chambers, K. Ford Gordon, Maggie E. Jefferis, Anne Margaret Miller, Evan P. Morissey, Lucas E. Morrisey, Pier Paulo Claudio**

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**Introduction:** Glioblastoma (GB) is the most common malignant primary tumor of the adult brain, with a median survival time of 16-18 months post diagnosis. Three carbohydrate surface antigens, CD133, CD15, and A2B5, have been implicated as markers for GB progenitor or cancer stem cells (CSC’s). The A2B5 epitope is known promote proliferation, migration, and clonogenicity in GB, and is dependent on the expression of �-2,8sialyltransferase-3 (ST8SIA3), and thus is likely to consist at least in part of �-2,8 linked sialic acid (�-2,8SA). Influenza viruses are known to bind cell-surface sialic acids via the viral hemagglutinin (HA) surface glycoprotein.

**Methods:** We tested the ability of a panel of influenza A, B, and C viruses to inhibit the growth of an array of GB cell lines. GB cell lines were treated with serially diluted influenza virus, then gown understandard conditions for 96 hours, whereupon cell monolayers were stained with Crystal Violet todetermine the relative number of cells present. The presence of ST8SIA3 mRNA wasdetermined by harvesting total RNA from cell cultures, synthesizing cDNA using oligo dTprimers, and performing PCR with ST8SIA3-specific oligonucleotide primers.

**Results:** Different viruses inhibited individual cell lines to differing extents. A virus strain previously shownto bind �-2,8SA significantly inhibited three out of five cancer cell lines tested. Preliminary datashowed the presence of ST8SIA3 mRNA in the GB cell lines, suggesting that �-2,8SA maymediate the effect on GB cell lines in vitro. We hypothesize that cell-surface �-2,8SA may allowus to target GB CSC in vivo using oncolytic influenza viruses. The ability to eradicate GB CSC’shas the potential to reduce or eliminate recurrence of GB after surgery, with the likely effect ofprolonging patient survival.

**Poster 24:**

**Analytical and Biophysical Chemistry Research Core**

**Anter A. Shami, Sandeep Misra, Joshua Sharp**

*University of Mississippi*

The University of Mississippi has established the Glycoscience Center of Research Excellence (GlyCORE) by Center of Biomedical Research Excellence (COBRE) award from NIH 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 Orbitrap Exploris 240 system coupled to Dionex Ultimate 3000 nano-UHPLC system for LC-MS/MS analyses. We also recently have installed Nicoya OpenSPR system for the surface plasmon resonance spectroscopy and FlexMap 3D system for the multiplexing of protein/gene measurements. We are collaborating with the researchers of Ole Miss in a wide variety of research projects encompassing proteomics, structural analyses, metabolomics and glycomics utilizing these instruments. A few of the representative projects include the identification of protein oxidation, untargeted metabolomics, molecular weight determination of polysaccharide, and identification of glycosylation patterns on isolated glycoproteins.

We have recently installed a UHPLC system with a Corona Veo RS Charged Aerosol Detector. It can be used for the universal detection of non-volatile analytes with poor optical properties, such as carbohydrates and polymers. Using this detector, concentrations of the oligosaccharides and other analytes of interest can be measured against any known standard because response is near uniform and independent of chemical structure.

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 25:**

**Glycoscience Student Advocates**

**Baharul Islam, Tahir Ali, Kayleigh Phillips, Destinee Manning, Eslam Elhanafy**

*University of Mississippi*

Glycoscience Student Advocates (GSA) is comprised of graduate and undergraduate students interested in or conducting glycoscience-related research at The University of Mississippi. The purpose of this student organization is to provide opportunities to promote glycoscience knowledge by encouraging research, education, and training of interdisciplinary glycoscience trainees.

Glycoscience is an emerging interdisciplinary field of research that focuses on a better understanding of the structures, functions, and biomedical utilities of glycans and glycoconjugates. Glycans—also known as carbohydrates, saccharides, or sugars—play central roles in many biological processes and have properties useful in various applications.

The research area is relatively new, but interest is rapidly growing as scientists have begun to appreciate how glycans play a critical role in nearly all aspects of biology and as glycoscience is evolving to be a cornerstone of modern chemical, biological, and biomedical sciences. Despite being an important part of nearly all biological functions, the study of glycans has been limited until the convergence of integrated efforts of multidisciplinary sciences. One such paradigm of effort is the formation of the Glycoscience Center of Research Excellence (GlyCORE) at the University of Mississippi.

With the facilities of GlyCORE\* at our disposal, we, a group of students interested in glycoscience, are working to promote the field by encouraging research, education, and training of interdisciplinary glycoscience trainees.

**Poster 26:**

**The GlyCORE Imaging Research Core: Bridge the gap between advanced imaging techniques and Glycoscience**

**Ruofan Cao and Gregg Roman**

*University of Mississippi*

The objective of the GlyCORE Imaging Core is to promote and enhance the growth of glycoscience projects at the University of Mississippif, and throughout the mid-south region. The Core brings together 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 imagine 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) and Fluorescence Recovery After Photo Bleaching (FRAP), and image processing for presentation. The Core also contains a Zeiss Axio Imager M1 widefield microscope with epifluorescent, dark-field, phase-contrast and birght-field imaging functions. This M1 Axio imager is well suited for the general morphometric analysis of tissues. The GlyCORE Imaging Research Core also house 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 will also offer many services including but not limited to new imaging technique development, image processing and specimen preparation consultation etc. Amongst 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 microfluidic device, histology sectioning and immunostaining. Extra information and services could be found on the core website <https://gic.olemiss.edu/>

**Poster 27:**

**Glycoscience Center of Research Excellence**

**Erin Bradley, Karin Ballering, Joshua S. Sharp, Samir Ross**

*University of Mississippi*

Recognizing the opportunity to contribute 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 hots three central Research Cores to support these investigators with cutting-edge biomedical research tools. Our goal to lower the barriers for 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, rather develop the infrastructure and dedicated expertise necessary to support glycoscience 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.

**Poster 28:**

**Computational Chemistry and Bioinformatics Research Core**

**Sushil K. Mishra, Priyanka Samanta, Robert J. Doerksen**

*University of Mississippi*

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. In this poster, we will provide an overview of the capabilities of CCBRC in terms of computations that we can support and/or perform and the training we provide. Notably, we show our hardware resources that encompass several standalone workstations, servers, and a list of available 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.