Plenary lecture

Decoding and Modulating Glycosaminoglycan-Protein Interactions

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The remarkable structural diversity of glycosaminoglycans (GAGs) enables selective molecular recognition events that regulate critical biological processes, ranging from development and immune responses to viral invasion and neuroplasticity. We have developed a platform for the automated chemical synthesis and high-throughput biological interrogation of large heparan sulfate (HS) and chondroitin sulfate (CS) oligosaccharide libraries encompassing diverse sulfation patterns. These libraries enable the systematic identification of sulfation motifs that selectively modulate the activities of growth factors, chemokines, and synaptic organizing proteins, revealing new insights into the elusive sulfation code of GAGs and the molecular basis of GAG protein recognition. By deciphering and engineering these interactions, we are developing new strategies to control cell signaling, modulate immune responses, and enhance neuroplasticity.

Oral #1

New Strategies to Discover and Evolve Monoclonal Anti-Glycan Antibodies

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Monoclonal antibodies that target glycans are critical for a variety of basic research and clinical applications, but the field suffers from a deficiency of high-affinity, selective reagents. Traditional hybridoma technology is slow, expensive, and ineffective for many glycans. In vitro approaches such as phage display and yeast display have yielded only limited success for glycan targets. Our group has developed two approaches to discover and evolve anti-glycan antibodies based on the hypothesis that formation of a multivalent complex is critical for success. First, we have developed a multiplexed approach for isolating anti-glycan antibodies from human B cells using panels of DNA-barcoded neoglycoproteins. Second, we have created a directed evolution platform wherein we display full-length, divalent IgG antibodies on the surface of mammalian cells and then capture cells of interest with multivalent glycoconjugate bait. Using this multivalent platform, we engineered improved variants of dinituximab (ch14.18), a therapeutic anti-GD2 antibody used to treat neuroblastoma.

Oral #2

Exploring the Structure and Dynamics in Fungal and Plant Cell Walls Using Solid-State NMR Spectroscopy

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Complex carbohydrate-rich biomaterials, including fungal and plant cell walls, play central roles in microbial survival, antifungal resistance, bioenergy production, and global carbon cycling. However, their insoluble and heterogeneous nature makes native structural characterization exceptionally challenging. Here we combine 13C/15N correlation spectroscopy, fast-MAS 1H detection, Dynamic Nuclear Polarization (DNP), and diverse 13C/2H labeling strategies to directly interrogate intact cell wall architectures across fungal and plant systems. These methods allow us to resolve carbohydrate network remodeling associated with antifungal resistance in fungal pathogens, which cause life-threatening invasive infections in more than two million patients annually worldwide. We found that glucans adopt diverse structural organizations to perform distinct biological functions: α- and β-glucan matrix reorganization enhances resistance to echinocandin drugs in Aspergillus, whereas α-glucans stabilize melanin and capsule incorporation in Cryptococcus. In addition, 1H-detected experiments on Candida albicans and multidrug-resistant Candida auris reveal distinct remodeling responses to caspofungin treatment despite their similar cell wall architectures. These results establish a molecular framework linking polysaccharide organization to fungal stress adaptation and drug resistance. Extending this approach to plant biomass and environmental carbon cycling reveals developmentally regulated lignin-polysaccharide interactions and, through isotope-resolved labeling and DNP, uncovers distinct incorporation pathways of phenylalanine and tyrosine precursors into lignin substructures. DNP-enabled single-hour screening of unlabeled wetland soils further identifies preserved aromatic-carbohydrate cores associated with carbon stabilization over ten thousand years. These studies demonstrate how sensitivity-enhanced solid-state NMR can bridge molecular structure, dynamics, and function to understand biomaterial assembly, stress adaptation, and carbon persistence in complex native materials.

Oral #3

1° Non-Anomeric Glycoboranes: Palladium Catalyzed Synthesis and Functionalization

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Despite the prevalence of carbohydrates in approved drugs, typical carbohydrates are not inherently drug-like. Their preponderance of polar functional groups often limits oral bioavailability, and their glycoside bonds are labile to hydrolysis. Major efforts to address these deficiencies have focused on exchanging labile *O*-glycoside bonds for more stable *C*-glycoside linkages. However, despite recent work illustrating the value of substituting non-anomeric C–O bonds for C–C bonds, highlighted by the recent FDA approval of the reversed glycoside sotagliflozin, methods for modifying non-anomeric positions are comparatively limited. Inspired by boron's ability to take part in a variety of mechanistically distinct C–C bond forming manifolds, we set out to develop robust and scalable methods for synthesizing carbohydrate intermediates borylated at non-anomeric positions (i.e., non-anomeric glycoboranes). To this end, we have developed a palladium-catalyzed deiodinative borylation that proceeds at 25 °C to afford primary pinacol boronic ester glycoboranes in good to excellent yields on millimole scale. Variously protected glucose, galactose, and mannose scaffolds as well as partially unprotected sugars and di- and trisaccharide substrates readily underwent borylation under these conditions. To demonstrate their utility as functionalizable intermediates, we developed a C(sp^2)–C(sp^3) Suzuki coupling of these borylated intermediates with aryl and heteroaryl bromides. Exploration of the electrophile scope showed generality across a variety of aryl and heteroaryl bromides, including challenging pyridyl and unprotected indole electrophiles as well as four tripeptides containing a (hetero)aryl bromide-functionalized amino acid residue. Taken together, this work illustrates the viability of non-anomeric glycoboranes as intermediates in the synthesis of functionalized glycosides and unnatural glycopeptides.

Oral #4

Carbon Nanotube-Amplified Optical Macro-Glycoligands for Probing Specific Carbohydrate-Protein Interactions

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The recognition interactions between cell surface carbohydrates and carbohydrate-binding proteins play a vital role in a multitude of cellular activities, such as immune responses and infections. Our laboratory develops near-infrared fluorescent probes of carbon nanotube-amplified optical macro-glycoligands for detecting specific carbohydrate-protein interactions and understanding their underlying mechanisms that are crucial for discovering therapeutic and diagnostic mechanisms. Carbon nanotubes have intrinsic photoluminescence in the biological transparent window of near-infrared region, enabling the development of label-free assay technology to precisely detect biomolecular interactions. For example, Siglecs are a family of cell surface immunoregulatory receptors that recognize sialoglycan ligands and Siglec recognition is largely implicated as an immunosuppressive pathway of many cancers. Our probes were shown to be effective for differentiating Siglecs, including Siglec-7/9 of highly similar expression patterns, with high sensitivity and selectivity. Additionally, our probes can be a novel tool for probing specific carbohydrate-protein interactions and enable discoveries, including molecular mechanisms of biological processes, potential therapeutic targets, and diagnostic mechanisms.

Oral #5

Anti-inflammatory heparan sulfate octadecasaccharide in influenza infection and secondary bacterial pneumonia

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Influenza A viruses represent a global health threat and cause millions of infection cases and thousands of deaths every year. Furthermore, viral and bacterial co-infection or secondary bacterial infection frequently occurs and is related to worse clinical outcomes including increased mortality. Importantly, a major contributor to influenza associated mortality is secondary bacterial pneumonia, particularly with Staphylococcus aureus, including methicillin resistant S. aureus (MRSA). Viral disruption of epithelial barriers and HMGB1 driven inflammation create a permissive environment for bacterial invasion and uncontrolled bacterial growth.

Current treatment options are seasonal vaccines and antivirals each have limitations. While vaccines are essential to contain viral epidemics, seasonal influenza A vaccines range in effectiveness from 20-60%. Antivirals exert a selective pressure on the virus, which given the high mutation rate of influenza virus, leads to emergence of antiviral resistant strains. Additionally, antiviral mechanism of action targets the virus replication phase and therefore must be given within 48 hours of infection to have clinical benefit. An unmet medical need exists for influenza treatment strategies that target indirect pathogenesis.

Therapeutics directed toward the host response after infection do not face the same selective pressure or narrow therapeutic window as antivirals. This study investigates synthetic heparan sulfate as an indirect anti influenza treatment by targeting HMGB1 mediated inflammation. The compound used is an octadecasaccharide (18 mer) composed of alternating N sulfo glucosamine and 2 O sulfo iduronic acid residues generated by chemoenzymatic synthesis. This same 18 mer has previously demonstrated efficacy in HMGB1 driven inflammatory disease models including acetaminophen induced acute liver injury and CLP induced sepsis.

We show that 18 mer is highly effective at reducing mortality after influenza infection. Compared with a standard antiviral therapeutic, oseltamivir phosphate, 18 mer exhibited a substantially longer therapeutic window and remained.

Oral #6

Applications of catalysis and data science to challenges in carbohydrate chemistry

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My group has a long-standing interest in the development of catalytic methods for site-selective and stereoselective transformations of carbohydrate derivatives. Recent results along this line will be discussed, including N-glycosylations of heterocyclic compounds and C-H functionalizations via photocatalytic hydrogen atom transfer.

In parallel, we have begun to explore applications of computation and data science to challenges in carbohydrate chemistry. I will outline our initial steps in this direction, including Bayesian optimization of enzyme-catalyzed reactions of sugars, and the development of a predictive statistical model for glycosyl donor reactivity.

Oral #7

Organic Reactions Lecture

Merging Enzymatic Catalysis with Iron Catalysis for Highly Stereoselective Heparan Sulfate Oligosaccharide Assembly

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Heparan sulfate (HS) regulates numerous biological processes, but it occurs naturally as a heterogeneous mixture of sulfated complex glycans. Therefore, pursuing a broadly effective approach for homogeneous HS synthesis to advance biological studies has been an outstanding challenge in glycoscience. In this lecture, we will discuss a highly stereoselective and generally applicable HS assembly strategy by merging the catalytic power of sulfotransferases (SULTs) with two distinct iron-catalyzed glycosylation reactions that are recently developed in our lab. Every glycosidic linkage in HS was assembled by one of these iron-catalyzed, entirely stereoselective glycosylation reactions. An array of sulfate groups that are essential to HS's function were installed at their desired locations via the SULT-controlled sulfation of HS precursors. This general approach is showcased in the synthesis of an anticoagulant HS hexasaccharide and other full-length HS octasaccharides and hexadecasaccharides.

Oral #8

Targeting Heparan Sulfate-Viral Protein Interactions for Drug Discovery using SPR biosensor

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Many viruses use the heparan sulfate (HS) on the surface of host cells as co-receptors or facilitator for attachment and initiating cell entry. Therefore, virion-HS interactions have been used as a target for developing broad-spectrum antiviral therapeutics. Here we report the potential anti-SARS-CoV-2, MERS-CoV, monkeypox virus (MPXV) activities of sulfated glycans including Pentosan Polysulfate (PPS) and Mucopolysaccharide Polysulfate (MPS), and marine sulfated glycans extracted from the seaweed, sea cucumbers and sea urchin. The inhibition of these sulfated glycans on interactions between the receptor-binding domain (RBD) of S-protein of SARS-CoV-2 (both WT and different variants), MERS-CoV and heparin, interactions between MPXV A29 and A35 protein and heparin were evaluated using surface plasmon resonance (SPR). The SPR results demonstrate sulfated glycans show strong inhibition on these interactions. The study of molecular interactions between viral proteins and host cell HS is important in developing therapeutics for the prevention and treatment of different viral infections.

Short talk #1

Profiling and Modulating Sialidase Expression and Activity in Macrophages upon LPS Stimulation

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The cell surface is coated with glycans often terminated with sialic acids (Sias), known as sialylation, which plays important roles in a variety of biological processes. Removal of Sias (desialylation) by sialidase, on the other hand, is often involved in a number of pathological pathways. Lipopolysaccharide (LPS) induces endogenous sialidase expression in macrophages, which could result in desialylation of TLR4, thereby initiating the LPS/TLR4 signaling pathway that is responsible for chronic and acute inflammatory disorders that often cause dangerous diseases like sepsis. In this study, we investigated the desialylation profile in THP-1 macrophages upon LPS stimulation. Western blot and ELISA confirmed higher expression of sialidases in THP-1 macrophages upon LPS stimulation. Lectin blot analysis and flow cytometry showed desialylation of cell surface and total proteins of LPS-stimulated THP-1 macrophages. In addition, we investigated the sialidase inhibition effect on the LPS/TLR4 signaling pathway. Flow cytometry confirmed that sialidase inhibitor DANA blocks endogenous sialidase and prevents desialylation of macrophages upon LPS exposure. Additionally, western blot and ELISA assays revealed that DANA treatment decreases NFκB activation and cytokine release. Ultimately, this work shows that sialidase inhibition dampens the LPS-induced inflammatory response.

Short talk #2

Stress Responsive Remodeling of Yeast Cell Walls Probed by ^{1}H and ^{13}C solid-state NMR

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Understanding the structural principles that govern yeast cell wall architecture and remodeling is vital for confronting invasive fungal infections, where the cell wall shapes host interactions and limits the effectiveness of current antifungal therapies. Using 13C and 1H-detected solid-state NMR across two evolutionarily distinct yeast models, including the pathogenic species Candida albicans and the fission yeast Schizosaccharomyces pombe, we investigate how fungal cell walls preserve integrity when challenged by metabolic, oxidative-stress, or genetic perturbations.

Phosphate metabolism is an underexplored regulator of C. albicans cell wall structure, particularly in its interplay with oxidative stress, which could identify new opportunities for antifungal intervention. Using 2D 13C-13C CORD spectra of intact C. albicans wildtype cells and mutant devoid of phosphate transporters, revealed extensive reorganization of rigid wall core: Î²-1,3-glucan dominated scaffold characteristic of wildtype cells is replaced in mutant by composite enriched in chitin, Î²-1,6-glucan, and mannose side chains Complementary 2D hcCH TOCSY spectra showed pronounced changes, with reduced structural polymorphism in mutant. Extension to 3D hCCH TOCSY with DIPSI-3 mixing enabled site-specific assignments of individual polysaccharide allomorphs. Upon peroxide exposure, Î±-1,2-mannose units and Î²-1,6-glucan are shifted into rigid phase, with line-broadening of Î±-1,2-mannose by paramagnetic relaxation enhancement (PRE) indicating direct oxidative interactions and the subsequent generation of radicals. These results establish phosphate acquisition as part of oxidative defense and link nutrient sensing and availability to the resilience of fungal cell wall.

In another study, we revealed the function of another carbohydrate polymer, Î±-glucan, in yeast cell wall function. The S. pombe cells lacking Î±-amylase homologs required for Î±-glucan matrix assembly were examined. Solid-state NMR analysis resolved two distinct subtypes of rigid Î±-1,3-glucan (Aa and Ab) within the rigid core. Deletion of Î±-amylase homologs eliminated the Ab subtype and induced a new polymorphic form of Î²-1,3-glucan, revealing compensatory remodeling of the glucan matrix. The mutants also lacked Î±-1,4-linked domains that comprise ~10% of the Î±-glucan polymer and act as bridges between major Î±-1,3-linked segments, thereby losing the ability to extend Î±-glucan chains to generate fibers of appropriate size needed for cell-shape maintenance and cell separation. Together, these results demonstrate the unique power of solid-state NMR to resolve dynamic, stress- and genetics-driven cell wall remodeling in situ, and to define the distinct functions of individual carbohydrate polymers.

Short talk #3

Uncovering Heteroxylan Biosynthesis in Rice through Network-Based Gene Discovery and Protein Interaction Analysis

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Plant cell walls (PCWs) give mechanical strength, flexibility and protection to plant tissues. Heteroxylan (HX) is a major wall polysaccharide in grasses including rice that is necessary for tissue strength and development, but the genes and protein complexes regulating its synthesis remain unknown. Here, we report the identification and characterization of HX-related gene complexes in rice by an integrated genomics and functional validation approach. We created PlantNetX, a rice-centric gene co-expression platform, leveraging bulk RNA-seq data from 70 rice studies to support our effort. PlantNetX combined with gene association network (GAN) analysis revealed putative HX biosynthetic complexes including GT43 and GT47 glycosyltransferase genes, which have been shown to contribute to xylan biosynthesis. To support these predictions, we integratedÂ 9 single-cell RNA-seq datasets into PlantNetX to assess candidate genes in various rice cell types and to investigate if predicted interacting genes are co-expressed in cell types involved in cell wall production. The NAPPA/i-GT-ray system is a high-throughput platform to screen for protein-protein interactions and is used to test for predicted protein interactions. Two predicted GT43/GT47 complexes were initially tested and showed positive interaction signals, proving the effectiveness of this technique for HX-related protein complex validation. The CRISPR/Cas9 gene editing system was also used to investigate specific GT43 genes in Kitaake rice. Mutant lines for GT43-D, GT43-H and GT43-E have been developed and are in characterization and their characteristics demonstrate altered growth, stem morphology, seed development and probable cell wall alterations comparable to previously reported xylan biosynthesis mutants. The PlantNetX-based co-expression analysis, GAN analysis, single-cell transcriptomics, protein interaction tests and CRISPR mutant characterization provide a framework for the identification of HX biosynthesis complexes and better understanding of plant cell wall construction.

Short talk #4

Structure-Based Design and Hydrophobic Encoding of Heparan Sulfate Trisaccharides for Selective Heparanase Modulation

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Heparan sulfate (HS) - protein interactions regulate a wide range of physiological and pathological processes, including extracellular matrix (ECM) remodeling, cell signaling, angiogenesis, and tumor progression. Heparanase (HPSE), a key HS-degrading enzyme, plays a central role in remodeling the HS landscape and has emerged as an important therapeutic target. However, the development of selective HPSE modulators remains challenging due to the structural heterogeneity of HS and the complex interplay between sulfation patterns and protein recognition. Here, we report a structure-based strategy combining hydrophobic encoding with defined HS trisaccharide scaffolds to investigate and selectively modulate heparanase activity. A library of structurally defined HS trisaccharide mimetics was designed based on the molecular features of the HPSE binding site and synthesized with systematically varied glycosidic linkages, sulfation patterns, and strategically positioned hydrophobic substituents. The resulting compounds were evaluated for their ability to modulate HPSE interactions using time-resolved fluorescence resonance energy transfer (TR-FRET) and biolayer interferometry (BLI), complemented by competitive binding and kinetic studies to assess potency and selectivity. Our results demonstrate that hydrophobic encoding can significantly enhance the interaction of HS trisaccharides with HPSE. In particular, the strategic incorporation and positioning of lipophilic groups promote favorable interactions within hydrophobic regions of the HPSE binding site, resulting in improved binding affinity and functional modulation. Structure-activity relationship (SAR) analysis further reveals that the precise spatial arrangement of hydrophobic substituents, in combination with defined sulfation patterns, is critical for optimizing HPSE recognition and selectivity.

Overall, this work establishes a structure-based hydrophobic encoding strategy for the rational design of HS trisaccharides as selective heparanase modulators. These findings provide new insights into the molecular determinants govern HS-HPSE recognition and offer a framework for developing structurally defined HS-based therapeutics.

Short talk #5

Decoding Heparanase-Driven Multiple Myeloma: From Molecular Targeting to Therapeutic Intervention

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Multiple myeloma (MM) is an incurable plasma cell malignancy characterized by progressive bone marrow remodeling, therapeutic resistance, and disease relapse. Heparanase (HPSE), the primary mammalian endoglycosidase responsible for cleaving heparan sulfate, plays a central role in MM progression by promoting extracellular matrix remodeling, syndecan-1 shedding, growth factor signaling, invasion, and angiogenesis. Despite its therapeutic potential, the clinical development of HPSE inhibitors has been limited by poor selectivity and interactions with platelet factor 4 (PF4), highlighting the need for potent and selective HPSE-targeted therapeutics.

To address these limitations, we developed a library of structurally modified, sulfated aminoglycoside-based HPSE inhibitors. Structure-guided incorporation of hydrophobic and negatively charged groups yielded compounds with nanomolar HPSE inhibitory activity and minimal PF4 interactions. Lead compound L17 was subsequently evaluated in aggressive MM models to characterize its therapeutic and mechanistic effects. L17 significantly suppressed MM cell growth, with enhanced activity in three-dimensional tumor models, and reduced invasion, angiogenesis, and tumor cell survival. Transcriptomic and molecular analyses further demonstrated suppression of pathways associated with the HPSEâ€“syndecan-1 axis, extracellular matrix remodeling, and growth factor signaling, providing mechanistic insight into the broader antitumor effects of HPSE inhibition. Preliminary pharmacokinetic and tolerability studies demonstrated favorable systemic exposure with minimal observed toxicity, supporting continued in vivo evaluation.

Collectively, these findings reinforce HPSE as a promising therapeutic target in aggressive MM and demonstrate the potential of L17 to disrupt multiple interconnected mechanisms that drive MM progression. This work provides a foundation for the continued preclinical development of selective HPSE-targeted therapeutics for multiple myeloma.

Short talk #6

Regioselective ring opening of benzylidene with trimethylaluminum: Synthesis of 1-phenylethyl ether as an acid labile hydroxyl protecting group

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Treatment of D-glucose or D-mannose-derived molecules bearing 4,6-O-benzylidene with trimethylaluminum affords a corresponding 1-phenylethyl (PE) ether at C4 and a free alcohol at C6, respectively. Remarkably, the regioselectivity can be reversed in the presence of stoichiometric amount of water to produce 1-PE ether at C6 and free alcohol at C4. Interestingly, opposite regioselectivity was observed on D-galactose derivatives under the same conditions. The resulting 1-PE ethers were found to be acid labile and can be cleaved at room temperature using 5% trifluoracetic acid in methylene chloride. The utilization of 1-PE ether as a hydroxyl protecting group has been demonstrated in the efficient synthesis of a trisaccharide.

Short talk #7

Towards the total synthesis of 2C7 epitopes from Neisseria gonorrhoeae for the development of a carbohydrate-based gonococcal vaccine

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Neisseria gonorrhoeae is a Gram-negative diplococcus responsible for gonorrhea, the second most common bacterial sexually transmitted infection in the world after Chlamydia trachomatis. In 2020, the World Health Organization (WHO) estimated that there were 82.4 million new cases of N. gonorrhoeae infections among adults worldwide. The emergence of multidrug-resistant strains, including those resistant to ceftriaxone - the current last-line therapy, highlights the urgent need for alternative therapeutic and preventive strategies. The 2C7 epitope is a carbohydrate structure found on the lipooligosaccharide (LOS) of N. gonorrhoeae, recognized by the monoclonal antibody (mAb) 2C7, which is expressed by a vast majority of clinical N. gonorrhoeae isolates. It plays a critical role in gonococcal colonization and survival. Its high prevalence and functional importance underscore the potential of the 2C7 epitope as a target for vaccine development and diagnostic applications. However, due to the complex and heterogeneous structures of naturally isolated carbohydrates, the synthetic access to long, branched, and complex surface carbohydrates from N. gonorrhoeae remains a challenging task in chemical synthesis. In this project, we focus on the synthesis of Glycan 1, which is the main motif of 2C7. Glycan 1 presents multiple synthetic challenges, including a long and branched backbone, incorporation of rare sugars (heptose, Kdo), formation of several challenging 1,2-cis glycosidic linkages at secondary hydroxyls, and the presence of diverse polar groups such as carboxylic acids and amides. To address these challenges, we propose to synthesize this highly branched and sterically crowded dodecasaccharide via a strategically designed stereoconvergent [(4+7)+1] assembly approach. Glycan 1 will then be conjugated with Qβ, and the immunobiologic activities of the conjugate will be tested for its efficacy to address the growing challenge of antibiotic-resistant gonorrhea. In this poster, we highlight the successful synthesis of the 2C7 tetrasaccharide and its Qβ conjugate (compound 2), which elicited a specific antibody response in mice, as well as the synthesis of a highly branched intermediate toward the construction of the target 2C7 dodecasaccharide. These advances establish a robust synthetic foundation for the development of carbohydrate-based vaccines to combat drug-resistant gonorrhea.

Short talk #8

Efficient Preparation of Homogenous Antibody Conjugates via Glycosite-Specific Transglycosylation Enabled by Readily Available Glycosyl Donors

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Site-specific antibody conjugation through glycoengineering offers a promising route to generate homogeneous glycosite-specific antibody-drug conjugates (gsADCs) with improved therapeutic indices. Dozens of gsADCs are advancing from preclinical studies to clinical trials. However, current methods involve either multiple enzymes or lengthy preparation of substrates. Herein, we report a novel and synthetically streamlined platform utilizing LacNAc-derived 4,6-acetal glycosyl donors for glycosite-specific transglycosylation mediated by a single enzyme. These glycosyl donors can be synthesized in as few as two steps, representing a major advancement in synthetic accessibility compared to previously reported glycosyl donors, which often require more than 15 steps. Computational analysis showed that the acetal ring restricts conformation, directing donor 7 to a π–π -stabilized groove of the enzyme. Donor 7, along with a positive control, was evaluated in the context of gsADCs, consistently demonstrating potent and selective cytotoxicity toward HER2-positive cancer cells, while sparing HER2-negative cells. Furthermore, donor 7 was successfully adapted to generate glycosite-specific degrader antibody conjugates (gsDACs), highlighting its broad utility. Additional studies revealed that donor 7 produces antibodies with markedly enhanced resistance to Endo S2 mediated hydrolysis. Together, these findings establish a practical and broadly applicable platform for glycosite-specific antibody conjugation, paving the way for next-generation antibody-based therapeutics.

Short talk #9

Chemical Synthesis of Enterobacterial Common Antigen (ECA) toward a Vaccine Against Enterobacteriaceae

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Enterobacteriaceae is a large family of Gram-negative bacteria, including Klebsiella pneumoniae and Escherichia coli. These bacteria are associated with both intestinal and extraintestinal diseases, including urinary tract infections, bloodstream infections, pneumonia, and, in some cases, diarrhea. Alarmingly, several strains of Enterobacteriaceae have developed resistance to antibiotics. Of particular concern is Carbapenem-resistant Enterobacteriaceae (CRE), which is resistant to carbapenems- often considered the drug of last resort. The rapid rise of CRE highlights the urgent need for new preventive strategies. Enterobacteriaceae produce a polysaccharide known as the Enterobacterial Common Antigen (ECA), composed of repeating trisaccharide units [→3)-α-D-Fuc*p*4NAc-(1→4)-β-D-Man*p*NAcA-(1→4)-α-D-Glc*p*NAc-(1→]. It is a conserved surface polysaccharide that plays a critical role in maintaining outer membrane integrity and protecting bacteria against environmental stress, thereby functioning as a virulence factor that supports survival and infection. These unique characteristics make ECA a promising broad-spectrum target for vaccine development against Enterobacteriaceae. In this study, oligosaccharides corresponding to the repeating units of ECA were synthesized through convergent stereoselective glycosylation and subsequently conjugated to the highly immunogenic bacteriophage Qβ carrier to enhance the immunogenicity of the carbohydrate antigen. Immunization with the resulting Qβ-ECA glycoconjugate elicited robust anti-glycan IgG antibody responses, demonstrating the immunogenic potential of synthetic ECA oligosaccharides as vaccine antigens.

Poster #1

Hydrophobic Engineering of Aminoglycoside-Derived Heparan Sulfate Mimetics Enables Potent and Selective Heparanase Inhibition

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Heparanase (HPSE), an extracellular-matrix-remodeling enzyme implicated in tumor progression and metastasis, is an attractive therapeutic target, but development of glycan-based inhibitors has been limited by synthetic complexity and off-target anticoagulant effects. We developed a structure-guided strategy that repurposes the aminoglycoside tobramycin as a synthetically accessible heparan sulfate mimetic. A 35-member library was prepared in 8-9 steps while systematically varying sulfation, hydrophobicity, electronics, and conformational flexibility. Naphthyl capping consistently enhanced HPSE inhibition, and hydrophobic aromatic substitution at the C6 position produced lead compounds with IC_{50} values of 80-140 nM. Selected analogues exhibited >300-400-fold selectivity for HPSE over platelet factor 4 and minimal antithrombin-associated activity. Molecular docking and 200-ns molecular dynamics simulations showed that aromatic groups promote deeper pocket engagement, sulfate shielding, and persistent nonpolar contacts. Subsequent energetic analysis identified van der Waals interactions as a major determinant of potency and HPSE-over-PF4 selectivity. Lead compounds also reduced proliferation in selected HPSE-overexpressing cancer cells. These findings establish hydrophobic engineering as a strategy for developing potent, selective sulfated oligosaccharide-based HPSE inhibitors.

Poster #2

van der Waals Interactions Determine Heparanase-over-Platelet Factor 4 Selectivity in Tobramycin-Based Heparan Sulfate Mimetics

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Heparanase (HPSE) represents a promising anticancer target, yet clinical development of inhibitors has been plagued by off-target binding to plasma proteins, most notably platelet factor 4 (PF4), causing embolisms and thrombosis. Here we present a computational analysis of 33 experimentally validated tobramycin-based heparan sulfate mimetics that reveals how hydrophobic modifications enhance selectivity for HPSE over PF4. Through triplicate 200 ns molecular dynamics simulations and MM-PBSA/GBSA free energies for 20 compounds, five of which were also simulated against PF4, we demonstrate that the van der Waals term, rather than electrostatic complementarity, correlates with binding affinity and with selectivity (ΔΔVDW versus selectivity, r = +0.990, p = 0.001). Capping the scaffold hydroxyls with 2-naphthylmethyl groups improves potency in all twelve matched pairs, from 4.7- to 176-fold, and buries an additional 405 ± 168 Å² of nonpolar surface in the HPSE cleft, which converts that burial into substantially more van der Waals energy than the flat PF4 surface does. Lead compounds 8, 9, and 11 achieve experimental IC_{50} values of 0.10, 0.08, and 0.14 µM, respectively, with >350-fold selectivity, compound 11 reaching 461-fold. MM-PBSA ranks the congeneric Group B compounds accurately (r = −0.956, p = 0.011), and across the 20-compound set the van der Waals component alone is a stronger predictor than either end-state free energy (r = −0.585, p = 0.007). Critically, while MM-PBSA ranks these ligands correctly for HPSE, it fails to rank the same ligands for PF4 binding, consistent with nonspecific binding. These findings demonstrate that incorporating hydrophobic aromatic groups into sulfated oligosaccharide scaffolds can enhance both potency and selectivity, providing a design strategy for developing HPSE inhibitors with reduced off-target effects.

Poster #3

α-Glucans as Structural Scaffolds in Cryptococcus Cell Walls: Insights from ^{13}C and ^{1}H Solid-State NMR

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Cryptococcus neoformans and Cryptococcus gattii are major fungal pathogens that cause life-threatening infections, including cryptococcal meningitis. Their virulence depends on a polysaccharide-rich cell wall, capsule, and melanin's structures absent in humans and attractive targets for antifungal therapy. We used ^{1}H- and ^{13}C-detected multidimensional solid-state NMR, combined with dynamic nuclear polarization (DNP), to resolve the molecular architecture of cryptococcal cell walls in intact cells. We identify five distinct α-1,3-glucan forms with varying dynamics in C. neoformans and define their interactions with capsule polysaccharides and melanin, establishing solid-state NMR as a powerful platform for probing fungal cell wall organization.

To elucidate the role of chitosan, a cell wall polysaccharide, we analyzed wild-type strains and avirulent chitosan-deficient mutants of both species. Chitosan loss disrupts cell morphology and drives species-specific remodeling of the polysaccharide network. Chitosan regulates hydration, flexibility, and polysaccharide composition, altering glucan balance and capsule organization. These findings reveal how α-glucan polymorphism and chitosan-dependent remodeling govern cryptococcal cell wall architecture and provide a structural framework for antifungal target discovery.

Poster #4

Precursor-Resolved Solid-State NMR Reveals Distinct Carbon Routing into Grass Cell Wall Aromatics

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The grass cell wall is a complex carbohydrate-rich composite in which lignin and hydroxycinnamates play critical roles in regulating structure, mechanics, and biomass recalcitrance. However, the metabolic origins of these aromatic components and their incorporation into the intact cell wall remain incompletely understood. Here, we combine ^{13}C-isotope labeling with dynamic nuclear polarization (DNP)-enhanced solid-state NMR to directly trace carbon flow from phenylalanine and tyrosine into the native cell wall of Brachypodium distachyon. Precursor-specific ^{13}C labeling reveals distinct metabolic routing, with phenylalanine preferentially contributing to guaiacyl and syringyl lignin, whereas tyrosine predominantly labels hydroxyphenyl lignin and grass-specific hydroxycinnamates, including ferulates that form key cross-links between lignin and polysaccharides. Two-dimensional ^{13}C-^{13}C correlation spectra resolve these precursor-specific aromatic networks within intact cell walls. Analysis of a p-coumarate 3-hydroxylase (C3H) mutant further demonstrates that disruption of the canonical phenylalanine pathway selectively reduces guaiacyl lignification, while tyrosine-derived aromatic incorporation is largely maintained, highlighting metabolic plasticity in grass cell wall assembly. These findings provide molecular-level insight into precursor-dependent aromatic polymer formation and establish isotope-resolved DNP-enhanced solid-state NMR as a powerful approach for investigating the architecture and biosynthesis of complex carbohydrate-rich plant cell walls.

Poster #5

α-1,3-Glucan-Driven Remodeling of the Conidial Cell Wall in an Aspergillus fumigatus Vaccine Strain Alters Innate Immune Recognition

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Aspergillus fumigatus is a leading cause of invasive aspergillosis in immunocompromised individuals, and current antifungal treatments are constrained by toxicity, emerging resistance, and limited long-term efficacy, with no approved vaccines available. A mutant lacking the sterylglucosidase gene (sglA) has shown potential as a vaccine candidate due to its ability to elicit protective immune responses; however, the underlying structural basis remains poorly understood. In this work, we employ cellular solid-state NMR spectroscopy to investigate the conidial cell wall architecture of the ΔsglA mutant in comparison to the wild-type strain. Our results reveal significant cell wall remodeling in ΔsglA, characterized by elevated α-1,3-glucan content, increased structural heterogeneity, enhanced interactions with β-glucans, reduced hydration, and restricted molecular dynamics. These changes collectively produce a more rigid cell wall framework with decreased β-glucan accessibility. Importantly, these structural alterations correlate with modified neutrophil responses and shifts in innate immune signaling. This study establishes a connection between cell wall organization and immune recognition, providing insights relevant to the development of antifungal immunotherapeutic strategies.

Poster #6

Evaluation of an Aminoglycoside-Based Heparanase Inhibitor in Combination with Cisplatin for Metastatic Prostate Cancer

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Prostate cancer is the second most commonly diagnosed cancer in men worldwide and remains a leading cause of cancer-related mortality, particularly once the disease progresses to a metastatic stage. Despite advances in treatment, current therapies often fail to control disease progression once metastasis occurs, highlighting the need for novel therapeutic strategies for advanced disease.

Heparanase is an enzyme responsible for cleaving heparan sulfate proteoglycan chains found in the extracellular matrix and on cell surfaces, thereby releasing heparan-bound molecules, including growth factors, angiogenic factors, and chemokines. In prostate cancer, this activity promotes tumor cell invasion, angiogenesis, and metastatic dissemination. While heparanase inhibition can also limit tumor growth and survival through blockade of growth-factor release and heparanase-driven survival pathways, its effects on cell death are generally more limited than those of direct cytotoxic agents. Combining heparanase inhibition with cisplatin, a DNA-damaging chemotherapeutic agent, may therefore enhance cytotoxic efficacy while also targeting metastatic mechanisms.

To investigate this, we evaluated L17, an aminoglycoside-based heparanase inhibitor, in combination with cisplatin in prostate cancer cell models. This combination is designed to pair heparanase-mediated inhibition of metastatic mechanisms with cisplatin's direct cytotoxic effect on tumor cells, targeting both processes simultaneously.

The effects of L17 and cisplatin, individually and in combination, are being evaluated using cell viability and combination index analyses. Additional studies will examine cellular processes associated with prostate cancer progression and metastasis. The overall goal is to determine whether combining heparanase inhibition with cisplatin-mediated cytotoxicity represents a promising therapeutic strategy for advanced prostate cancer.

Poster #7

Solid-State NMR Reveals Boron-Deficient Remodeling of the Rosa Cell Wall

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Plant cell walls are complex, dynamic structures that provide mechanical support and serve as a renewable source of biomass for the production of fuels, chemicals, and value-added biomaterials. The pectic polysaccharide network within the primary cell wall is a critical determinant of cell wall architecture, hydration, mechanical properties, and interactions with other wall polymers. Boron is an essential micronutrient that contributes directly to cell wall structure through the formation of borate diester cross-links between rhamnogalacturonan-II (RG-II) domains. These 1:2 borate-mediated cross-links connect two RG-II molecules and are essential for maintaining normal cell wall organization and plant growth. Although boron deficiency is known to cause severe developmental abnormalities, including reduced elongation and increased thickening of roots and stems, the molecular mechanisms underlying the associated alterations in primary cell wall structure remain poorly understood. Here, we investigated the effects of boron deficiency on the chemical composition, molecular organization, and dynamics of primary cell walls using magic-angle spinning (MAS) solid-state nuclear magnetic resonance (ssNMR) spectroscopy. Uniformly ^{13}C-labeled Rosa rubiginosa (rose) plants were grown under boron-sufficient and boron-deficient conditions, and their cell wall architecture was characterized directly in the native state using quantitative and multidimensional ssNMR approaches. Analysis of the carbonyl spectral region revealed substantial changes in the esterification and acetylation patterns of pectic polysaccharides in response to boron deficiency. In contrast, the relative abundance and organization of the major rigid wall components, particularly cellulose and hemicellulose, remained largely unchanged, indicating that the primary effects of boron deficiency were localized within the more mobile pectic matrix. Boron-deficient cell walls exhibited pronounced changes in mobile polysaccharide populations, including rhamnose, galacturonic acid, arabinan, and xylan-containing components. The disruption of borate-mediated RG-II cross-linking was associated with weakened pectin-cellulose interactions and reduced motional rigidity of the pectic network, resulting in increased molecular mobility and dynamic behavior. Furthermore, enhanced association of the cell wall matrix with water was observed under boron-deficient conditions, suggesting increased hydration and plasticity of the pectin network. Boron deficiency also resulted in reduced methyl esterification and O-acetylation of galacturonic acid residues, particularly within oligogalacturonide- and rhamnogalacturonan-I-rich regions, demonstrating extensive chemical remodeling of the pectic matrix. Collectively, these findings establish that borate-mediated RG-II cross-linking plays a central role in regulating the chemical composition, intermolecular interactions, hydration, and molecular dynamics of the primary cell wall. Loss of boron-dependent cross-linking substantially alters the organization and physical behavior of pectic polysaccharides while exerting comparatively limited effects on the rigid cellulose-hemicellulose framework. This study provides molecular-level insight into how boron availability regulates plant cell wall architecture and mechanical properties and highlights the importance of pectin remodeling in plant responses to micronutrient deficiency. The findings have broader implications for understanding plant growth and stress responses and may inform strategies for improving agricultural productivity and developing renewable lignocellulosic biomaterials and biofuels.

Poster #8

A Liquid-Phase Iterative Strategy for the Stereospecific, Chromatography-Free Synthesis of Complex Oligosaccharides

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Precise control of anomeric stereochemistry and cumbersome chromatographic purifications remain significant bottlenecks in solution-phase oligosaccharide synthesis. To address these challenges, we report a liquid-phase iterative strategy for the stereospecific synthesis of complex oligosaccharides, combining a novel hydrophobic phytanyl-gallate linker with Qing's directing-group-on-leaving-group (DGLG) glycosylation methodology. The linker exploits the pronounced differential solubility of its polyisoprenoid phytanyl tail between cyclohexane and acetonitrile, enabling simple biphasic CH3CN/cyclohexane extraction to replace column chromatography at every purification step. Employing electrophilic bromine activation with sym-collidine bromonium triflimide at −60 °C, glycosyl donors bearing a chromenone-based leaving group undergo complete SN2-type anomeric inversion in excellent yields. The methodology was applied to the iterative, chromatography-free assembly of (1→6)- and sterically demanding (1→4)-linked glucose tetrasaccharides and extended to the synthesis of a structurally complex L-fucosamine-containing tetrasaccharide analogue of bacterial cell wall polysaccharide repeating units found in Shewanella japonica KMM 3299T and Escherichia coli O172. Validation of the novel L-fucosamine donor across four distinct glycosyl acceptors confirmed exclusive α-selectivity with yields exceeding 90%. The high purity of all intermediates and final products was confirmed by ^{1}H NMR and HRMS throughout. This work demonstrates that the union of a hydrophobic-tag biphasic purification strategy with a broadly applicable stereospecific glycosylation protocol provides a powerful, practical platform for the rapid, chromatography-free assembly of structurally diverse oligosaccharides.

Poster #9

Synthesis of Selective Sialidase Inhibitors

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Sialidases (also known as neuraminidases) are enzymes that catalyze the cleavage of cell-surface glycoconjugates, specifically the sialic acid residues that are located at the terminal positions of those glycoconjugates. The removal of those sialic acid residues allows the sialidases to regulate many different cellular activities. Four types of mammalian sialidases have been discovered: NEU1, NEU2, NEU3, and NEU4. These hNEU enzymes vary in location and presence in different organelles of cells, are encoded by different genes, and have differing functions. While these enzymes have been studied for over forty years, their functionality is still not fully understood. The development of selective sialidase inhibitors would enable more in-depth investigation of the individual functions of specific hNEU enzymes. As these sialidase enzymes are implicated in many diseases, they are very attractive targets for inhibitor development therapeutic application. The goal of this project is to synthesize selective inhibitor for each hNEU (NEU1-NEU4) enzyme. Great success has been found in synthesizing selective hNEU inhibitors by altering the C9 and C5 positions of the 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) compound. This is a key intermediate in this project, which allows us to synthesize various derivatives to create more selective inhibitors. In this presentation, we will discuss the details of the synthesis and characterization of the selective sialidase inhibitors.

Poster #10

Enzymatic Synthesis and Characterization of Chondroitin Sulfate Isoforms and their Effects on APC Generation via Thrombomodulin

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Thrombomodulin (TM) is a proteoglycan that exists on the membrane surface of endothelial cells and takes part in the protein C (PC) anticoagulation pathway, in which it serves to modulate the activity of thrombin, a serine protease, from procoagulant to anticoagulant. Although TM has five domains, only its EGF-like domain (TMD2) is responsible for TM's anticoagulant activity. TMD2 is further divided into six subdomains known as EGF-like domains 1-6, of which only EGF4-6 (TM456) contribute to its anticoagulant activity. However, it is known that chondroitin sulfate (CS), a glycosaminoglycan (GAG) present at TM's serine/threonine-rich domain, also participates in TM-thrombin interactions by binding to and anchoring thrombin to TM, thereby enhancing TM-mediated protein C activation. CS exists as several isoforms (e.g., CS-A, -B, and -C) that vary in their sulfation patterns. In our study, we enzymatically degraded CS-A, -B, and -C into several low molecular weight (LMW) oligosaccharides. We hypothesize that the interaction between thrombin and TM can be altered by varying the molecular weight and sulfation pattern of TM's chondroitin sulfate moiety. Therefore, we utilized an amidolytic PC activation assay to investigate the effects of varying CS sulfation pattern and chain-length on activated PC (APC) generation. Herein, we discuss the synthesis and characterization of various LMW CS isoforms and their activities for thrombin binding and TM-based protein C activation.

Poster #11

Synthesis of Cellular Location-specific Sialidase Inhibitors

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Sialidases (also called neuraminidases) are glycosidases responsible for the removal of sialic acid (Sia) (desialylation) from glycans of either glycoproteins or glycolipids. By desialylation, sialidases are able to modulate the functionality of the Sia-containing molecules and are involved in both physiological and pathological pathways. There are four kinds of mammalian sialidase (Neu1, Neu2, Neu3 or Neu4) based on their cellular locations and substrate specificities. Sialidase inhibitors are useful tools for studying sialidase function and related physiological and pathological pathways. More importantly, effective sialidase inhibitors can be used as drugs to regulate the pathological pathways. Several selective mammalian sialidase inhibitors have been reported. However, they are not cellular localization-specific and thus are less effective in vivo. In this study, we aim to develop of cellular location-specific sialidase inhibitors by modifying the pan sialidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (DANA). Briefly, we modified the C5 position of DANA by introducing a morpholino group, whose nitrogen atom becomes protonated in the acidic lysosomal environment, promoting lysosomal accumulation and thereby enhancing the targeting of DANA toward lysosomal Neu1.

Poster #12

Evaluation of Sialidase Inhibition Effect on TLR4-Mediated NF-κB Signaling in Macrophages

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Toll-like receptor 4 (TLR4) is a pattern recognition receptor on the cell surface of macrophages. By recognizing lipopolysaccharide (LPS) it activates NF-κB signaling pathway, leading to production of pro-inflammatory cytokines such as IL-6 and TNF-α. Activity of neuraminidase 1 (Neu1), a mammalian sialidase, has been recognized as a regulatory step in the activation of this signaling pathway. By removing the terminal sialic acids on TLR-4 receptors, Neu1 facilitates dimerization of TLR4 monomers, leading to activation of the downstream signaling pathway. This study evaluated the effects of sialidase inhibitor, Neu5Ac2en-OAcOMe (a synthetic derivative of the parent compound Neu5Ac2en, also known as DANA), on TLR4-mediated NF-κB activation in Raw Blue macrophage reporter cells upon LPS induction. NF-κB activation was quantified using a secreted embryonic alkaline phosphatase (SEAP) reporter assay in Raw-Blue macrophages. Neu5Ac2en does not show a significant decrease in SEAP activity, however, its derivative demonstrated significant reduction in activity of SEAP. Western blot analysis demonstrated that while the total protein levels TLR4 and NF-κB p65 remained constant across all experimental groups, LPS treatment significantly increased the phosphorylation of the p65 (p-p65) subunit. Both Neu5Ac2en and Neu5Ac2en-OAcOMe reduced the levels of p-p65, with the derivative demonstrating a more significant inhibitory effect. These findings are supported by SEAP reporter assay, which showed a significant reduction in SEAP activity in cells treated with the sialidase inhibitor.

Poster #13

Targeting Heparanase for the Dual Treatment of Pancreatic Cancer and Diabetes

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Pancreatic ductal adenocarcinoma (PDAC) and diabetes are both associated with dysregulated heparan sulfate (HS) homeostasis and heparanase (HPSE) activity, suggesting HPSE as a shared therapeutic target. Analysis of PDAC patient datasets showed elevated HPSE expression in tumors and an association with poor survival. We therefore evaluated L17, a potent sulfated hydrophobic aminoglycan HPSE inhibitor, in pancreatic cancer and diabetes-related models. In PDAC, L17 inhibited tumor cell growth across multiple cell lines with different genetic backgrounds and reduced colony formation and migration, while promoting apoptosis and showing activity in ex vivo models. In parallel, HPSE inhibition suppressed pro-inflammatory macrophage activation and inflammatory cytokine expression. In pancreatic β-cells, L17 reduced mitochondrial reactive oxygen species, preserved F-actin organization, and attenuated apoptosis under HPSE-driven inflammatory conditions. Importantly, in *ex vivo* human pancreatic islets, HPSE inhibition preserved intra-islet HS, reduced oxidative stress, and maintained glucose-stimulated insulin secretion. Together, these findings support HPSE as a therapeutically relevant target in both pancreatic cancer and β-cell dysfunction and highlight L17 as a promising HPSE-targeting strategy for pancreatic cancer and diabetes.

Poster #14

Withdrawn

Poster #15

Convergent Synthesis of the Reducing-End Pentasaccharide Fragment of the Bordetella pertussis Lipooligosaccharide

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Pertussis, caused by Bordetella pertussis, remains a significant public health concern despite widespread vaccination. The lipooligosaccharide (LOS) displayed on the bacterial surface represents an attractive target for the development of next-generation carbohydrate-based pertussis vaccines, as antibodies against LOS can exhibit bactericidal activities.[1,2] To facilitate the development of structurally defined glycan antigens, our group is pursuing the total synthesis of the B. pertussis LOS dodecasaccharide and its structurally defined fragments.[1,2]

Herein, we describe the synthesis of the reducing-end pentasaccharide fragment of the B. pertussis LOS, corresponding to the red-highlighted region of the target molecule. This structurally complex fragment contains a KDO residue, two mannoheptose units, a highly congested branching motif, and an aminopropyl linker for subsequent bioconjugation. A convergent synthetic strategy was developed through sequential glycosylation and selective functional group transformations of appropriately protected monosaccharide building blocks. Particular attention was paid to the sequence of glycosylation and protecting-group manipulation to accommodate the sterically demanding branching structure and the sensitive KDO moiety. The KDO residue was introduced at a late stage, followed by linker installation and global deprotection to furnish the desired reducing-end pentasaccharide.

The successful synthesis of this fragment provides a structurally well-defined building block for the subsequent assembly of the full-length LOS dodecasaccharide and enables future investigation of its potential as a synthetic pertussis glycan antigen.

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Poster #16

Stage-Dependent Cell Wall Remodeling in Aspergillus fumigatus Revealed by Solid-State NMR Using a Protoplast Regeneration Platform

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The fungal cell wall is a sophisticated extracellular structure that underpins cellular integrity, morphogenesis, and host-pathogen interactions, while also serving as a major antifungal target. Although its biochemical composition has been extensively characterized, how individual polysaccharides are assembled into a mature wall during de novo biogenesis remains incompletely understood. Here, we establish a protoplast regeneration platform combined with time-resolved solid-state Nuclear Magnetic Resonance (NMR) spectroscopy to directly monitor cell wall assembly in Aspergillus fumigatus from an initially naked plasma membrane. This synchronized system uncovers an ordered, multistage remodeling process. In the initial phase, α-1,3-glucan accumulates rapidly and forms a nascent peripheral layer. This is followed by a scaffold-building phase in which β-1,3-glucan and chitin increase markedly and coincide with visible wall thickening, consistent with formation of the principal load-bearing framework. During maturation, α-1,3-glucan rises again together with chitosan and mobile components, including galactomannan- and galactosaminogalactan. Genetic and pharmacological perturbations further reveal a highly adaptable compensatory network that preserves wall formation under stress.

Poster #17

Distinguishing Polymer Turnover from De Novo Biosynthesis Reveals Dynamic Cell Wall Remodeling During Aspergillus fumigatus Conidial Germination

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Dynamic remodeling of extracellular matrices underlies development, environmental adaptation, and host-pathogen interactions, yet distinguishing polymer turnover from de novo biosynthesis in intact cells remains a major challenge. Here, we combined high-resolution solid-state NMR with selective ^{13}C-labeling strategies to distinguish pre-existing cell-wall polymers from newly synthesized polysaccharides during Aspergillus fumigatus conidial germination. Germination was accompanied by substantial remodeling of the rigid cell wall, characterized by decreased β-1,3-glucan and increased chitin and α-1,3-glucan, whereas the mobile wall fraction remained comparatively stable except for the emergence of galactosaminogalactan. Surprisingly, β-1,3-glucan turnover proceeded independently of the major β-1,3-glucanases encoded by the A. fumigatus genome and was dispensable for germination. Instead, isotope-labeling experiments revealed that newly assimilated carbon is preferentially directed toward α-1,3-glucan biosynthesis, whereas deletion of the α-1,3-glucan synthase genes triggered compensatory accumulation of chitin and β-1,3-glucan. These results reveal a compartmentalized cell-wall remodeling program that coordinates selective turnover with de novo polysaccharide synthesis during fungal germination and establish isotope-edited solid-state NMR as a general approach for distinguishing inherited from newly synthesized polymers in complex carbohydrate matrices.

Poster #18

Cyclopropylmethylidene and 1-cyclopropylethylidene as 1,2-cis diol protecting groups in carbohydrate chemistry

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Cyclopropylmethylidene (CPMD) and 1-Cyclopropylethylidene (1-CPED) have been developed as 1,2-cis diol protecting groups in sugar molecules, such as L-rhamnose, D-mannose, D-galactose and L-fucose derivatives. Regioselective ring opening of 5-membered CPMD using trimethylaluminum yields a free hydroxyl group and a 1-cyclopropylethyl (1-CPE) ether. Likewise, ring opening of 5-membered 1-CPED is carried out with DIBAL-H to furnish a free hydroxyl group and a 1-cyclopropylethyl (1-CPE) ether, with an opposite regioselectivity.

Poster #19

Combinatorial Synthesis of a Hyaluronan Based Polysaccharide Library via Ugi Reactions Unlocks Divergent Receptor Selectivity between CD44 and HARE

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Hyaluronan (HA) is a natural extracellular matrix component consisting of repeating disaccharide units of β-1,4-D-glucuronic acid (GlcA)-β-1,3-D-N-acetyl glucosamine (GlcNAc). HA plays important roles in a wide range of biological events, such as inflammation, tumor progression, and immune cell migration through interactions with HA receptors. In addition, HA has been widely used in biomaterials design since it is non-immunogenic, has multiple sites available for versatile chemical modifications, and is biodegradable. As such, studying the biorecognition of HA by its receptors, particularly the impact of chemical modifications on HA-receptor interactions, is of significance to leverage HA in biomedical applications. Here, we developed a new strategy to synthesize a library of HA like polysaccharides using Ugi reaction. The Ugi reaction of HA was optimized to achieve high loading percentages on HA backbone, and a library of HA like polysaccharides was synthesized using 18 amines and 5 isocyanides. Binding affinities to the HA receptors CD44 and HARE were evaluated using competitive ELISAs and biolayer interferometry (BLI). Some lead compounds with strong and selective receptor bindings were identified for further in vitro and in vivo studies. The novel binders hold potential for enhancing targeted drug delivery and modulating HA-mediated biological processes.

Poster #20

Chemoenzymatic Synthesis of 9NHAc-GD2 Antigen for Anticancer Conjugate Vaccine Development

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Harnessing the body's immune system to combat cancer has gained traction due to the overexpression of tumor-associated carbohydrate antigens (TACA) on cancer cell surfaces. One of these antigens is GD2, a ganglioside that appears on the surface of neuroblastoma cells, a type of cancer that starts in certain very early forms of nerve cells. However, the use of anti-GD2 monoclonal antibodies in cancer immunotherapy is hindered by their toxicity, causing pain, cardiovascular issues, fever, and nerve damage in some patients.

To address these toxicity concerns, researchers are exploring alternatives, including a modified GD2 variant called 9-O acetylated GD2 (OAc-GD2). Similar to GD2, OAcGD2 is highly expressed on GD2-positive tumor cells. Importantly, it is not found on the human peripheral nerve system, suggesting that anti-9OAc-GD2 antibodies have the potential to have fewer adverse effects as compared to the corresponding anti-GD2 antibodies. Therefore, OAc-GD2 can be an attractive antigenic target for anti-cancer vaccine design. However, OAc-GD2 synthesis is challenging due to the instability of the 9-OAc group, limiting its use in vaccine development. To date, no such vaccines have been reported.

To overcome this challenge, we developed a derivative of GD2 with a more stable N-acetamide at the C-9 position (9NHAc-GD2) that could serve as a suitable alternative to OAc-GD2 and conducted vaccination studies using NHAc-GD2 conjugated with Qβ. By inducing anti-9NHAc-GD2 antibodies through vaccination, it may be possible to selectively target tumor cells and minimize the adverse effects associated with anti-GD2 antibodies. Our results of 9NHAc-GD2 suggest that the NHAc may be a suitable surrogate of the hydrolytically unstable OAc. Sera from rabbits immunized with Qβ-9NHAc-GD2 showed high IgG titer and strong binding with GD2 expressed tumor cells. *In vitro* and *in vivo* studies showed better anti-tumor therapeutic efficacy of these sera compared with those from rabbits immunized with Qβ-GD2 highlighting the potential of NHAc-GD2 antigen.

Poster #21

Self-Promoted Stereo- and Chemoselective Glycosylation: A Tool for Enhancing Potency of Bioactive Peptides and Small Molecules

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Attaching sugar molecules to peptides[1] and pharmaceuticals,[2] a process known as glycosylation, is essential for improving their pharmacokinetic profile. Towards this, the stereo- and chemoselective glycosylation of peptides remains a critical frontier in synthetic organic chemistry suffering from the challenges of controlling both site selectivity and stereoselectivity when forming sugar-peptide and sugar-small-molecule linkages. Traditional methods often involve extensive manipulation of protecting groups to achieve both stereoselective and chemoselective glycosylation simultaneously. In this context, we report an efficient strategy for glycosylation of peptides that delivers glycopeptides with excellent chemo- and stereoselectivity. By using H-bond forming ability of hydroxyl, thiol and carboxylic acid moiety, optimizing glycosyl donor and reaction conditions, this approach allows precise installation of diverse sugar framework with controlled diastereomeric outcomes. The method tolerates broad functional groups and enables them to access a wide range of glycopeptide architectures which are valuable for probing structure-activity relationships and modulating properties such as solubility and stability. This approach expands the toolkit for peptides modification and offers for an efficient synthetic route to deliver glycopeptides relevant to chemical biology and medicinal chemistry.

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Poster #22

CRISPR Gene Editing for Functional Studies of O-GlcNAcylation on Proteins in Live Cells

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O-GlcNAcylation is a dynamic post-translational modification that modulates a wide range of cellular functions, including transcriptional regulation, signal transduction, and proteostasis in eukaryotes. This process involves the addition and removal of O-linked N-acetylglucosamine (O-GlcNAc) on the serine and threonine residues of nucleocytoplasmic proteins, catalyzed by O-GlcNAc Transferase (OGT) and O-GlcNAcase (OGA), respectively. Perturbations in O-GlcNAc homeostasis through aberrant OGT activity, are implicated in pathological conditions such as cancer, neurodegenerative disorders, and metabolic diseases. However, the small size and widespread distribution of the O-GlcNAc modification across hundreds of cellular proteins is a challenge for specific, functional studies of its biological effects. Our study aims to engineer a high throughput chemical biology platform for the targeted modulation and characterization of O-GlcNAc modifications on specific proteins. By generating stable cell lines expressing mNeonGreen and NanoLuc reporters on O-GlcNAc proteins, this approach will facilitate the investigation of O-GlcNAc's influence on protein stability and localization in a context-specific manner. As a proof-of-concept, we have tagged a small library of O-GlcNAc glycoproteins to assess the effects of high/low/no O-GlcNAc on subcellular localization and stability in live cell, physiological conditions. This platform will help decipher how O-GlcNAc functionally regulates individual proteins and provides a roadmap for unpicking the individual protein effects of this global cellular modification motif. The anticipated outcome is the development of a robust toolkit for systematically interrogating the biological roles of O GlcNAc on key cellular proteins. Our future goal is to advance our understanding of disease mechanisms in which O-GlcNAc plays a role, including cancers and diabetes with hyper-O-GlcNAc and neurodegeneration with hypo-O-GlcNAc.

Poster #23

Development of mQβ-PNAG Conjugate Vaccines and Monoclonal Antibodies against Staphylococcus aureus

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Bacterial infections continue to be a serious global health concern, especially with the rapid increase in antimicrobial resistance. As antibiotics become less effective, it is more important than ever to look for alternative ways to fight infections. Vaccination offers a promising approach to address this challenge. There are still no approved vaccines for many harmful bacteria, including Staphylococcus aureus (S. aureus), which caused about 20,000 deaths due to bloodstream infections in the U.S. in 2017. The increasing prevalence of methicillin-resistant *S. aureus* (MRSA) further emphasizes the need for an effective vaccine. The cell wall of S. aureus, along with many bacterial, fungal, and protozoal pathogens, contains the conserved polysaccharide poly-β-(1-6)-N-acetylglucosamine (PNAG). The PNAG samples isolated from bacteria are heterogeneous in nature with different acetylation patterns. Previously, the Huang group synthesized and evaluated a complete library of 32 structurally defined PNAG pentasaccharides to probe the relationship between acetylation patterns and immunogenicity. Among these, PNAG0, PNAG10, and PNAG26 were evaluated as antigens conjugated to mQβ virus-like particles. In this poster, our rationale for selecting PNAG4 as a vaccine antigen will be presented, which is based on cross-reactivity data showing that antibodies raised against PNAG0, PNAG10, and PNAG26. We will discuss the ELISA-based antibody response of mQβ-PNAG4 conjugates, including durable IgG titers sustained through at least 200 days and reactivity against both synthetic and native PNAG structures, alongside C1q deposition data demonstrating the complement-engaging capacity of the antibodies produced. In addition, the rationale for selecting two candidates of anti-PNAG26 monoclonal antibodies will be discussed, and the ELISA studies on these two antibodies against S. aureus ATCC 29213 will be presented.

Poster #24

Synthesis of a library of heparan sulfate-like oligosaccharides for structure and activity relationship studies

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Heparan sulfate is a linear polysaccharide that consists of a disaccharide repeating unit of glucosamine (GlcN) linked to iduronic (IdoA) or glucuronic acid (GlcA).[1] HS plays pivotal roles in many biological processes like cell proliferation, anticoagulation, inflammation, viral infections, etc.[2] However, the inherent structural complexity and heterogeneity of natural HS oligosaccharides have long posed challenges in elucidating their structure-activity relationships. To address the difficulties of traditional HS oligosaccharide synthesis, HS mimetics have emerged as a promising alternative. Recently, our group has developed a novel synthetic strategy to synthesize a library of HS mimetic pseudo-oligosaccharides for structure-activity relationship studies and to investigate binding to the FGF-2 protein.[3] We developed a highly divergent synthetic route, which will be utilized to synthesize a proposed library of 300 HS-like oligosaccharides that will be available as the largest and most extensive HS mimetics library to date. As an initial demonstration, we successfully synthesized a library of 64 GlcN-GlcA pseudo-hexasaccharides bearing distinct sulfation patterns. These pseudo-hexasaccharides were assembled from a common, orthogonally protected disaccharide epitope using a head-to-tail amide coupling strategy, thereby circumventing the challenges associated with repeated glycosylation reactions during the synthesis of higher-order oligosaccharides.

The orthogonally protected disaccharide epitope 3 was prepared from the GlcN donor 1 and GlcA acceptor 2 using a conventional glycosylation protocol. Controlled modification of this intermediate under different reaction sequences afforded four disaccharide variants with distinct sulfation patterns. These building blocks were subsequently assembled via head-to-tail amide coupling, enabling modular extension to higher oligomers. Using this approach, 64 structurally defined GlcN-GlcA pseudo-hexasaccharides were successfully synthesized. Ongoing efforts are focused on expanding the library by synthesizing an additional 120 HS mimetics incorporating mixed GlcN-GlcA and GlcN-IdoA repeating units. As a key future direction, we aim to integrate automated solid-phase synthesis (ASPS) into the platform to enable the rapid, programmable, and scalable assembly of structurally diverse HS mimetics. This strategy will substantially increase both the size and structural diversity of the library while minimizing manual synthetic effort.

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Poster #25

Synthesis of a Trisaccharide Antigen of Klebsiella pneumoniae K43 CPS via Cs_2CO_3-catalyzed Stereoselective Anomeric O-alkylation

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Stereoselective synthesis of a trisaccharide antigen derived from the capsular polysaccharide (CPS) of Klebsiella pneumoniae serotype K43 has been achieved via cesium carbonate-catalyzed anomeric O-alkylation of D-mannose-derived lactol with secondary electrophile. Notably, a sterically demanding 6-O-tert-butyldiphenylsilyl (TBDPS) ether-protected triflate electrophile was successfully employed, providing the desired disaccharide intermediate in high yield (80%) and exclusive β-selectivity. Subsequent glycosylation and functional group transformations afforded the trisaccharide antigen in good overall yield.

Poster #26

Molecular Architecture of Cell-Wall Carbohydrates and Melanin in Lichtheimia corymbifera, Revealed by Solid-State NMR

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Mucormycoses are life-threatening infections caused by fungi from the order Mucorales. The number of mucormycosis cases is increasing, especially in immunocompromised patients. The molecular composition and structural organization of the cell wall of filamentous fungi underlie the host's ability to identify them as pathogens. Although the organization of the fungal cell wall, composed of 90% polysaccharides, is similar from one fungus to another, small variations condition their ability to trigger pattern recognition receptors.

Despite the clinical importance of Lichtheimia, its cell-wall architecture remains poorly understood at the molecular level. In particular, the exact carbohydrate composition, the organization and interactions of major polysaccharides within the cell wall, and the molecular structure of the associated melanin have not been fully characterized. Understanding these features is important for defining how the cell wall differs between fungal developmental forms and how these structural differences may influence fungal persistence, protection, and host recognition.

In this study, we use multidimensional solid-state nuclear magnetic resonance spectroscopy to investigate the molecular composition and organization of the cell walls of both conidia and mycelia of Lichtheimia corymbifera. Solid-state NMR provides residue-specific structural information while preserving the native, insoluble architecture of the fungal cell wall, allowing characterization and comparison of major polysaccharides and their molecular environments across these two fungal forms.

In addition to cell-wall carbohydrates, we investigate the associated fungal melanin, a structurally heterogeneous pigment that can contribute to protection against environmental stress and host defense mechanisms. By examining carbohydrates and melanin in both conidial and mycelial cell walls, this work aims to develop a more complete molecular-level description of the L. corymbifera cell structural variation during fungal growth and development.

Poster #27

Chemical Synthesis of the α-chain LOS Antigen from *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is a Gram-negative bacterium responsible for gonorrhea, a sexually transmitted infection (STI). In the United States alone, over 600,000 cases were reported in 2023, ranking gonorrhea as the second most frequently reported STI, surpassed only by chlamydia.[1]

A major obstacle in vaccine design is N. gonorrhoeae's extensive phase variation across surface antigens, enabling rapid evasion of antibody recognition. However, the 2C7 epitope presents a promising vaccine target due to its conserved structure and stable expression across most clinical isolates. Targeting this invariant epitope could circumvent the bacterium's evasion strategy and generate protective immunity.[2-4]

To address these challenges, we selected oligosaccharide 1 (derived from the 2C7 epitope) as the synthetic target. We employed flow chemistry and developed adapted methodologies for building block synthesis, expanding flow chemistry applications in oligosaccharide synthesis.[5] A one-pot iterative glycosylation[6] strategy constructed the target tetrasaccharide 4, a key precursor for the synthesis of final dodecasaccharide.

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Poster #28

Assessing the Uptake of Magnetic Glyco-nanoparticles by CD44 Expressing Tumor Cells

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One of the main reasons of death, worldwide, is cancer. Based on current cancer statistics, the future projections of the number of cancer incidences and cancer-related deaths are on the rise. Several factors play a role in the severity of cancer cases, one of which is the stage of detection; the earlier the stage of detection is, the higher the five-year survival rate is. Cancer, traditionally, is detected by a variety of imaging techniques like Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), Single-Photon Emission Computed Tomography (SPECT) and X-ray imaging. However, these techniques have limitations that hinder their ability for early detection of cancer such as low resolution, high background to signal ratios and the use of toxic imaging contrasts and radioactive compounds. In an effort to overcome these limitations, a shift towards the use of nanoparticles has been observed, specifically magnetic iron oxide nanoparticles synthesized via the co-precipitation of iron precursors as they are biocompatible and easily functionalized with specific ligands that allows targeted delivery thus targeted detection. One ligand of note is hyaluronic acid (HA), as it is the endogenous ligand of CD44, a receptor that is overexpressed on the surface of cancer cells; CD44 is generally considered to be a molecular marker of cancer stem cells (CSCs). It is worth mentioning that some HA-derivatives show stronger binding to CD44 compared to HA. Thus, utilizing HA and its derivatives on the surface of the SPIONs allows for the specific targeting of cancer cells via HA-CD44 interactions. One particular application of the functionalized SPIONs is their use as an imaging tracer for Magnetic Particle Imaging (MPI), a newly developed non-invasive technique that detects the signal of superparamagnetic tracers for real time in-vivo imaging with minimal background noise. The synthesized NH2-SPIONs were functionalized with HA and two of its derivatives, namely B1HA and G2HA. The synthesis and the characterization of the SPIONs and the results of competitive ELISA, confocal microscopy and Prussian blue quantification assay proving the binding of the functionalized SPIONs to CD44 as well as cellular uptake will be discussed in the poster.

Poster #29

Design and Synthesis of Soyasaponin-based Vaccine Adjuvant Analogues

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Soyasaponins are complex triterpenoid glycosides found in soybean that have attracted significant interest as naturally derived vaccine adjuvants. Their biological activity is influenced by multiple factors, including the triterpenoid core and the attached carbohydrate moieties. Understanding the contribution of these structural features could enable the development of simpler and more accessible adjuvant candidates.

In this project, four soyasaponins, A1, A2, I, and V, were isolated from soybeans and evaluated for their vaccine adjuvant activity. Comparative evaluation revealed distinct activity profiles, with soyasaponin V exhibiting the highest potency. These findings provided a basis for identifying some of the structural features associated with enhanced activity and guided the design of the first generation of soyasapogenol B-based analogues. To enable analogues synthesis, soyasapogenol B, the triterpenoid core, was obtained from soybean-derived soyasaponins using an established extraction/hydrolysis and purification procedure and was used as a common scaffold for analogues synthesis. A modular synthetic strategy was then employed in which the carbohydrate building blocks were prepared and stereoselectively coupled to the triterpenoid core to generate 12 structurally defined glycosides.

The newly synthesized analogues will be evaluated to establish a systematic structure-activity relationship and to determine how structural changes in the sugar moiety influence their biological activity. By varying the type of sugar, chain length, and glycosidic linkage configuration, these studies will help identify the minimal synthetic carbohydrate motif required to retain strong adjuvant activity, potentially offering a simpler alternative to structurally complex natural saponins such as QS-21.

Poster #30

Synthesis and evaluation of trehalose mycolate analogues as inhibitors of glycolipid metabolism in mycobacteria

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Mycobacterium tuberculosis (Mtb) caused 10 million cases of tuberculosis (TB) disease and 1.6 million fatalities in 2023. The cell envelope of Mtb provides robust protection to Mtb, preventing drugs from diffusing across it. Additionally, Mtb has the inherent ability to form drug-tolerant persister cells. These features make treatment of Mtb very challenging, requiring a six-month course of multiple antibiotics. Novel treatments are needed to target persisters and shorten treatment times. The trehalose catalytic shift (TCS) is an important adaptive response mechanism used by mycobacteria during stress to transition into a drug-tolerant persister state by using trehalose released from cell surface glycolipid breakdown and directing it into the central carbon metabolism to meet energy and redox demands. We previously demonstrated that monosubstituted trehalose analogues, including azido- and amino-trehalose, blocked formation of persister-like Mtb cells through inhibition of TreS, a key enzyme in the TCS pathway that isomerizes trehalose to maltose. Here, we aimed to develop inhibitors of trehalose dimycolate hydrolase (Tdmh), an upstream enzyme in the TCS pathway that hydrolyzes mycolic acid lipids from the glycolipid trehalose dimycolate (TDM), eventually generating trehalose. We designed and synthesized 26 trehalose mycolate analogues with variable linkages (amine, triazole and ester), and different chain lengths C6-C18 and tested their effects on mycobacteria planktonic growth and biofilm formation, the latter being a model of persister formation. Multiple compounds inhibited growth of M. smegmatis and M. tuberculosis in the low-micromolar range, with some compounds exhibiting more potent activity in the biofilm-persister model. Although current studies suggest these analogues do not target Tdmh, future studies will focus on assessing the mechanism and structural basis of antimicrobial action.

Poster #31

Carbon Nanotube-Amplified Optical Macro-Glycoligands: A Versatile Platform for Glycan-Protein Recognition

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Glycan-protein interactions regulate diverse physiological and pathological processes. Understanding these molecular recognition events can provide valuable insight into biological mechanisms and aid in identifying diagnostic and therapeutic targets. However, studying glycan-protein interactions remains challenging due to their weak binding affinities, the lack of well-defined scaffolds to amplify specific interactions, and limitations of assays that rely on labeled components to facilitate detection. Here, we developed multifunctional optical probes by combining the intrinsic near-infrared fluorescence of carbon nanotube scaffolds with the controlled presentation of complexed glycopolymers containing native glycan ligands, including lactose and α2,3- and α2,6-sialyllactose. We investigated the specific interactions of these nanoprobes with various glycan-binding proteins, including viral SARS-CoV-2 spike protein and immune cell receptors Siglec-7 and Siglec-9. We found that nanoprobes amplified glycan recognition with high specificity and apparent affinities, yielding K_D values in the range of 54–688 nM, despite the low glycan densities of the complexed glycopolymers. These findings demonstrate the potential of integrated nanoprobes for label-free detection of pathogen and disease biomarkers, including cancer-associated immune receptors, while offering chemical and optical tunability for diagnostic and therapeutic applications.

Poster #32

Antisense Oligonucleotides (ASOs) to regulate O-GlcNAc Transferase (OGT) expression levels

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Protein O-GlcNAc (O-linked N-acetylglucosamine) modifications serve as dynamic indicators of nutrient flux and cellular stress pathways in human cells and tissues. In humans, the cycling of O-GlcNAc on and off proteins is regulated exclusively by two enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). Elevated O-GlcNAc levels are commonly observed in cancer and diabetes, while decreased O-GlcNAc levels are characteristic of neurodegenerative diseases and X-linked intellectual disability (OGT-XLID). Antisense oligonucleotides (ASOs) are short, synthetic oligonucleotides that are complementary to specific mRNA sequences. They bind to these sequences via Watson-Crick base pairing, thereby modulating gene expression. This study aims to modulate O-GlcNAc sugar levels by controlling OGT splicing patterns using antisense oligonucleotides (ASOs). Transcriptional regulation of OGT may provide therapeutic strategies for OGT-XLID, cancers, and other diseases associated with disrupted O-GlcNAc homeostasis. We hypothesize that ASOs can regulate OGT expression in disease models to maintain O-GlcNAc homeostasis, thereby preventing or treating these conditions. This research involves treating OGT-targeting ASOs to mammalian cell models and quantifying OGT expression at the protein level via immunoblotting, at the RNA level using RT-qPCR, and the transcriptome level using RNA sequencing. We identified three antisense oligonucleotides (ASOs) that upregulate OGT expression in cells and one ASO that downregulates OGT expression. These findings enable subsequent experiments utilizing these ASOs in disease models to achieve transcriptional control of OGT and potentially address related disease conditions resulting from disrupted O-GlcNAc homeostasis.

Poster #33

Chemoenzymatic synthesis of 2,2-difluorotrehalose, a novel trehalose analogue and possible inhibitor of Mycobacterium tuberculosis

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The disaccharide trehalose plays an essential role in the pathogenicity of Mycobacterium tuberculosis, the cause of tuberculosis in humans. Recent studies have found that interfering with trehalose metabolism can inhibit the growth of M. tuberculosis. Our group previously demonstrated that monosubstituted trehalose analogues, such as azido- and amino-trehaloses, have inhibitory activity against various mycobacterial species and can sensitize these organisms to existing antitubercular drugs. Separately, studies have shown that 2,2-difluorosugars may possess inhibitory activity against cognate glycosyltransferase and/or glycoside hydrolase enzymes. Therefore, we aimed to synthesize the novel trehalose analogue 2,2-difluorotrehalose and test the hypothesis that this compound could act as an inhibitor of trehalose-synthesizing or -hydrolyzing enzymes in M. tuberculosis. Here, we report progress toward chemoenzymatic synthesis of 2,2-difluorotrehalose starting from D-mannose, a route which utilizes a trehalose-synthesizing glycosyltransferase enzyme (TreT) in the key glycosidic bond-forming step. A purified product consistent with the expected 2,2-difluorotrehalose structure has been obtained, and ongoing structural characterization is being performed to confirm its identity. These results establish a promising route toward the preparation of 2,2-difluorotrehalose for future evaluation of its activity against mycobacteria.

Poster #34

Semi-synthesis of Homogeneous Glycoproteins and Mimetics by Remodeling Proteoglycans

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Proteoglycans (PGs) are intricate macromolecules decorated with linear polysulfated glycosaminoglycans (GAGs) that play critical roles in orchestrating a wide range of physiological and pathological events such as blood coagulation, growth factor signal transduction, immune modulation, and inflammatory responses. Advances in the utilization of recombinant enzymes and biosynthetic pathways have expedited the production of defined and homogeneous GAGs, helping overcome traditional barriers of chemical synthesis. With significant research focused on the synthesis of GAG chains, the integration with other critical structural elements, most notably the core proteins, has remained comparatively understudied. Consequently, there is a lack of comprehensive structure-function data that addresses the PG architecture as a unified glycoconjugate. Robust synthetic methodologies toward well-defined GAGs and entire PG structures are critical. The present investigation centers on the development of homogeneous proteoglycan mimetics, with the overarching objective of establishing a generalized synthetic strategy. To this end, we will explore several distinct methodologies. The primary methodology relies on the glycoengineering of native proteoglycans to replicate the holistic architecture of these complex biomolecules. Concurrently, a second, divergent strategy evaluates the application of xylosyltransferase-I, the key enzyme initiating glycosaminoglycan biosynthesis, to significantly accelerate the assembly of the tetrasaccharide linkage region. Ultimately, these complementary synthetic strategies aim to achieve the comprehensive assembly of full-length proteoglycan structures.

Poster #35

Elucidating the Role of TaVER2 and Rice Orthologs in Heteroxylan Biosynthesis

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The plant cell wall is a dynamic structure providing strength, shape and protection to the plant cell. Heteroxylan is an important cell-wall polysaccharide in grasses such as rice and wheat where it functions in structural integrity, growth, biomass quality and stress responses. TaVER2, a vernalization-related protein with jacalin and dirigent domains, was a candidate regulator in wheat that may influence xylan production by association with the xylan synthase complex. However, its biological role in living plants, especially in rice, has not been completely demonstrated. In this study, the role of TaVER2 and its rice orthologs Os12g0198700 and Os12g0247700 in heteroxylan biosynthesis and plant growth and development was investigated. Phylogenetic analysis, sequence comparison and 3D structural modelling indicated conservation of the jacalin and dirigent domains between TaVER2 and rice homologs, suggesting possible conservation of function. To test this hypothesis, we generated CRISPR/Cas9 knockout mutants for Os12g0198700 and transgenic rice lines overexpressing TaVER2. Initial observations show visible developmental differences compared to wild-type plants including reduced plant height and altered flowering time. These phenotypes indicate that the regulation of heteroxylan may affect plant growth and architecture in general. We also got the overexpression lines for the orthologs genes and characterizing them now. Molecular, biochemical and microscopic characterization of the mutant and overexpression lines is in progress. The role of these genes in cell wall assembly is being investigated by analyzing heteroxylan content, sugar composition and cell-wall structure. Selected lines will also be grown under biotic stress conditions including Hessian fly infestation to determine if there are any correlations between heteroxylan regulation and plant defense.

Poster #36

Structure-Guided Design, Synthesis, and Evaluation of Hpa2-Derived Peptides as HPSE1 Inhibitors

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Heparanase-1 (HPSE1) is an endo-β-D-glucuronidase that is overexpressed in numerous diseases, including cancer, inflammatory disorders, diabetes, and neurodegenerative diseases. It is the only known mammalian enzyme capable of cleaving heparan sulfate (HS) chains within heparan sulfate proteoglycans (HSPGs). Under normal physiological conditions, HS contributes to extracellular matrix (ECM) integrity and regulates cellular signaling by interacting with growth factors, cytokines, and chemokines. However, dysregulated HPSE1 activity promotes excessive HS degradation and the release of HS-bound signaling molecules, contributing to tumor growth, angiogenesis, invasion, and metastasis. Consequently, HPSE1 has emerged as an attractive therapeutic target for cancer treatment.

Several approaches have been explored to inhibit HPSE1, including small-molecule inhibitors, neutralizing antibodies, and heparin/heparan sulfate mimetics. Although four HS mimetics have advanced to clinical trials, their development has been limited by adverse effects and off-target interactions with other HS-binding proteins, highlighting the need for safer and more selective HPSE1 inhibitors.

Heparanase-2 (Hpa2/HPSE2), a close homolog of HPSE1, provides a potential endogenous strategy for HPSE1 inhibition. Unlike HPSE1, Hpa2 lacks HS-degrading enzymatic activity but binds HS with higher affinity, thereby interfering with HPSE1-mediated HS degradation. Hpa2 has also been associated with the regulation of genes involved in tissue homeostasis and anti-tumor signaling, further supporting its therapeutic potential. Although the complete crystal structure of Hpa2 has not yet been resolved, structural studies have identified several exposed loop regions, designated as Loops 1-7.

To determine whether these regions contribute to HPSE1 inhibition, we evaluated the Hpa2-derived loops using a sulfate-labeled ECM degradation assay. Among the peptides tested, Loops 4 and 5 demonstrated the strongest inhibition of HPSE1-mediated ECM degradation. We subsequently evaluated these peptides using a time-resolved fluorescence resonance energy transfer (TR-FRET) HPSE1 inhibition assay, where Loops 4 and 5 again showed the greatest inhibitory activity, with IC_{50} values of 5.2 and 1.7 µM, respectively.

Together, these results identify Loops 4 and 5 as promising Hpa2-derived scaffolds for the development of novel HPSE1 inhibitors. Ongoing studies will focus on optimizing Loop 4- and Loop 5-based peptides and evaluating their effects on cancer-associated processes, including proliferation, invasion, apoptosis, and angiogenesis.

Poster #37

Designing sugar probes to study time-based effects of metabolic flux in cells.

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N-Acetyl Glucosamine (GlcNAc) is a molecule that is used as a post-translational modification (PTM). This PTM acts dynamically on over 5000 proteins in the human proteome, however the effects of most of these have not been studied. Therefore, tools need to be developed to study the effects of the modification in real time. This research uses protecting groups to keep GlcNAc from being incorporated as a PTM until it is activated. We use light-activated protecting groups for controlled activation. For the light-activated protecting group, we use o-nitrobenzyl groups as the standard group, and we also add a benzofuran ring onto the nitrobenzyl group for increased conjugation. The hypothesis is that these molecules will be able to couple with GlcNAc, be incorporated into the cells, and then be activated in a controlled manner when ready to study O GlcNAcylation events in real-time. So far we have shown that the o-nitrobenzyl group can be readily removed from the 6-position within cells and can then be incorporated into protein O GlcNAcylation. From there, we are working to show that the activation can cause perturbation to O-GlcNAc levels and can be used to study the changes on protein activity.

Poster #38

Substrate-specific inhibition of O-GlcNAcylation Case study: Targeting the OGT-TET1 protein-protein interaction in TNBC

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O-GlcNAcylation is a highly dynamic post-translational modification on serine and threonine residues of nucleocytoplasmic and mitochondrial proteins in response to cellular stress and nutrient flux. O-GlcNAc transferase (OGT) is the sole enzyme responsible for glycosylating proteins with N-acetylglucosamine (GlcNAc). Increased OGT activity leads to hyperglycemia, resulting in metabolic imbalances and cellular responses, including diabetes and cancer. Consequently, inhibiting OGT has become an attractive anti-cancer therapeutic approach. Although OGT inhibition appears promising, the therapeutic relevance of this approach remains to be realized, as global catalytic inhibition of the enzyme would indiscriminately affect thousands of other proteins. To prevent this, we hypothesize that substrate-specific inhibition of O-GlcNAcylation is essential to selectively disrupt OGT's involvement in specific cellular events that drive disease. Our approach involves identifying protein-protein interaction inhibitors (iPPIs) that selectively target the OGT interaction with its partners in specific disease states. In triple-negative breast cancer (TNBC), we previously showed that OGT interacts with the Ten eleven translocase methylcytosine dioxygenase 1 (TET1) enzyme to activate a signaling cascade that drives tumorigenesis in cancer stem cells (CSCs). We also showed that OGT inhibition shuts down the CSC tumorigenic pathway. We further hypothesize that blocking the protein-protein interaction between OGT and TET1 would selectively prevent the O-GlcNAc modification that drives the CSC tumor-inducing pathway. Our strategy involves screening for inhibitors of the OGT/TET1 protein-protein interaction to prevent their association that leads to tumorigenesis. To facilitate this, we have developed a High-Throughput-Ready bioluminescent resonance energy transfer (BRET) assay to screen a library of 10,314 iPPIs from Fr-PPIChem for hits against the OGT/TET1 interaction. Hits from the screen will be validated using orthogonal methods to assess the phenotypic effect of the OGT/TET1 iPPIs. Success in this study would report, for the first time, a substrate-specific inhibitor of the O-GlcNAc modification on proteins and present a new paradigm for OGT inhibition with the potential for reduced side effects.

Poster #39

Synthesis of labeled phosphatidylinositolmannoside (PIM) fragments to probe translocation of PIMs across plasma membrane of mycobacteria cells

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Phosphatidylinositol mannosides (PIMs) are essential glycolipids that serve as precursors for the biosynthesis of the complex lipoglycans lipomannan (LM) and lipoarabinomannan (LAM), major constituents of the mycobacterial cell envelope. These molecules play critical roles in cell wall integrity, bacterial physiology, and host-pathogen interactions. While the early stages of PIM biosynthesis have been extensively characterized, the mechanism by which PIMs are translocated across the plasma membrane for subsequent elaboration into LM and LAM remains largely unknown. In this study, we report on metabolically active PIM-based chemical reporters suitable for click chemistry applications to investigate transport proteins involved in PIM translocation across plasma membrane in Mycobacterium species. Using a modular synthetic approach, we designed and synthesized three phosphatidylinositol mannoside fragments: phosphatidylinositol (PI), PIM_1, and PIM_2, each incorporating an azide- or 7-nitro-2,1,3-benzoxadiazole (NBD)-modified inositol core. The modified inositol scaffold was accessed through an enantiomerically pure Ferrier carbocyclization-mediated approach, which enabled the installation of a flexible alkyl-chain clickable handle for downstream bioorthogonal labeling. These synthetic PIM fragments would be used as metabolic probes to evaluate involvement of candidate proteins in PIM translocation and trafficking in Mycobacterium species by combining metabolic labeling with click chemistry.

Poster #40

Monoclonal Antibody for Broad Neutralization of Fentanyl and Other Opioids of Abuse

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The opioid crisis is an ongoing battle that researchers are aiming to combat, as indicated by the current rise in anti-opioid vaccines. Fentanyl, a highly-potent synthetic opioid, has attributed to a large population of opioid-related deaths. To address the growing threat, we have previously developed an anti-fentanyl vaccine using the highly immunogenic protein carrier, mQÎ² conjugated to a fentanyl hapten to elicit protective anti-fentanyl antibodies. This vaccine generated high antibody titers that bind both fentanyl and derivatives, while avoiding off-target binding with medication-assisted therapy (MAT) medications. Interestingly, protective antibodies demonstrated protection against heroin and etonitazene, including their derivatives, suggesting the potential for broader protection against multiple opioids.

Based on this discovery, we sought to develop a monoclonal antibody derived from this vaccine construct. Monoclonal antibodies provide an alternative to active immunizations by providing a highly specific and effective protection that can be used immediately without requiring the time needed for an immune response to develop. Using hybridoma technology, a multitude of antibodies were generated and one antibody in particular, B7C6, has shown significant protection against fentanyl, with promising activity against other opioids. The activities of this antibody will be presented.

Poster #41

The cross-talk between O-GlcNAcylation and skeletal muscle insulin signaling in models of T2D

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Type 2 diabetes (T2D) has been a major health challenge for centuries. Despite advances in science and medicine, T2D remains without a proper cure and accounts for a significant percentage of mortalities and morbidities in the current world. Prolonged insulin resistance in insulin-sensitive tissues is a key precursor to T2D. Skeletal muscle plays a key role in glucose homeostasis, taking up a significant proportion of blood glucose in response to insulin stimulation. Elevated O-GlcNAcylation is implicated in T2D. In this study, our overall goal is to discover sugar-driven biochemical pathways in insulin resistance and investigate novel approaches to reverse insulin resistance in skeletal muscle. We hypothesize that modulation of O-GlcNAcylation will reverse skeletal muscle insulin resistance in T2D. We are using pharmacological O-GlcNAc modulation by inhibition of the two enzymes involved in the O-GlcNAcylation process: O-GlcNAc Transferase (OGT) and O-GlcNAcase (OGA). We employ differentiated C2C12 mouse myoblasts as a model system for skeletal muscle, OSMI-4 and 5SGlcNHex as OGT inhibitors, and Thiamet G (TMG) as the OGA inhibitor in this study. We carried out inhibitor cytotoxicity and dose-response studies for the C2C12 cell line and established safe and effective working concentrations. Moreover, we observed changes in OGT expression in response to OGT and OGA inhibition as a compensatory mechanism to maintain O-GlcNAc homeostasis. By performing metabolite assays, we determined the impacts of O-GlcNAc modulation on lactate and glucose uptake levels. Lactate levels did not vary depending on the inhibitor treatments, whereas OGA inhibition increased, and OGT inhibition decreased glucose uptake without insulin stimulation. Moreover, we are examining fatty acid-induced and high glucose-induced insulin resistance in C2C12 cells as an in-vitro model for insulin resistance studies. This project will provide mechanistic knowledge about the interplay between insulin signaling and O-GlcNAcylation, muscle O-GlcNAcome, and new therapeutic approaches for T2D.

Poster #42

GlycoID2: An Optimized Proximity Labelling Platform for Cell Compartment Proteome Mapping

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Living organisms adapt and regulate cells by modifying proteins with GlcNAc, a monosaccharide that adds sugar to serine and threonine residues. Understanding how this process affects cell well-being is important, as deregulation has been implicated in the onset and progression of degenerative diseases like cancer. Studying the spatiotemporal effects of O-GlcNAc in cells is challenging because it occurs at thousands of O-GlcNAc sites and is regulated by just two enzymes: O-GlcNAc transferase and O-GlcNAcase. The difficulty of investigating these dynamics motivated the Fehl Lab to develop GlycoID, an intracellular proximity-labelling tool. We noted that GlycoID, a construct combining GafD with increased affinity for GlcNAc and mTurbo, an enzyme biotinylating intracellular proteins and their interaction partners within the nucleus and cytosol, produced approximately 100 hits, which is seen as a relatively low count. This led the group to consider human O-GlcNAcase, the enzyme that cleaves GlcNAc from serine or threonine residues, reverting O-GlcNAc to its native state. We mutated its catalytic residue, D174 (aspartic acid), to D174N (asparagine) and hypothesised that this would render the enzyme catalytically inactive while retaining its binding to O-GlcNAc. We also linked it to a MiniTurbo to biotinylate its interacting partners and complexes in cells, and added a localisation sequence to the N-terminus of the constructs to generate constructs that localise to the cytosol and nucleus, termed Cyt-GlycoID2 and Nuc-GlycoID2. Results show that these constructs labelled over 350 hits and exhibit rapid labelling kinetics, confirmed within 15 minutes. In addition, we have confirmed expression, localisation, and O-GlcNAc functionality, and we are currently deploying them for in-proteomic mapping in cells.

Poster #43

Synthetic Partially N-Acetylated Oligoglucosamines to Produce Conjugate Vaccines against Staphylococcus aureus

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Staphylococcus aureus infections and its antibiotic resistant variants are a public health concern around the world with no available vaccines. Poly-β-(1-6)-N-acetylglucosamine (PNAG), is a major component of the biofilm formed by Staphylococcus aureus, and a well-preserved structure in the biofilm of many other bacterial strains. It is well known that antibodies against PNAG can display effective bactericidal activities. In order to develop structurally defined antigen for vaccine design, our group successfully pursued synthesis of a library of 32 PNAG pentasaccharides with defined acetylation patterns before.[1] In that project we utilized binding affinity to monoclonal antibody (mAb) F598 to determine potential effective vaccine candidates. mAb F598 has a binding pocket able to contain 3 glucosamine units only.[2] Based on this, in this poster, the synthesis of 8 trisaccharide PNAG structures with defined acetylation patterns will be discussed.

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Poster #44

Comparison of the Synthesis of D-Galactose Building Block Between Flow and Batch Approaches

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Neisseria gonorrhoeae (N. Gonorrhoeae) is a Gram-negative bacterium responsible for the Sexually Transmitted Infection (STI) known as gonorrhea. N. Gonorrhoeae exhibits an extensive phase variation across surface antigens, enabling rapid evasion of antibody recognition. However, the 2C7 epitope presents a promising vaccine target due to its conserved structure and stable expression across most clinical isolates.

We selected tetrasaccharide 1 from the 2C7 epitope as the synthetic target. This oligosaccharide chain has synthesized from three different monosaccharide building blocks (3, 4 and 5), found in the alpha-chain. As the galactose sugar is present twice in the chain, I first focused on this vital building block. The synthesis of β-D-Galactose was compared using two different synthetic strategies, flow chemistry and batch. The synthetic scheme of the targeted compound 4 was adopted to flow synthesis from the pre-existing batch synthesis strategy. The greatest challenge was to optimize the reaction scheme into the chemical environment needed to run the reaction smoothly, in order to achieve a high yield in a faster reaction time.

In this poster I will present the results of multiple trials of the different conditions used to adopt the flow set-up for the synthesis of the target compound. Our target is an improved reproducibility in the synthesis of glycan 4.

Poster #45

A Novel Bacteriophage Mutant-Based Broad-Spectrum Conjugate Vaccine Against *Salmonella Enterica*

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Salmonellosis, caused by Salmonella enterica, is a global health threat responsible for over 95 million cases and approximately 150,000 deaths annually. It can lead to symptoms such as diarrhea, nausea, fever, gastrointestinal bleeding, and death in severe cases. Compounding the issue, rising antimicrobial resistance among Salmonella strains has emerged as a serious public health concern. The CDC has classified the pathogen as a serious threat, while the World Health Organization (WHO) has designated it as a high-priority pathogen. IASO Therapeutics, Inc., in collaboration with Professor Xuefei Huang's laboratory at Michigan State University, has developed a next-generation conjugate vaccine. This vaccine uses a mutant version of the virus-like particle, mQβ, as a highly immunogenic carrier, paired with a synthetic trisaccharide antigen that mimics the conserved O-antigen structure of the Salmonella lipopolysaccharide (LPS). Iaso's first-generation vaccine has elicited strong immune responses in both mice and rabbits, outperforming conventional protein-carrier-based vaccines. It also demonstrates functional protection against non-typhoidal Salmonella (NTS) strains and safeguards mice against lethal infections. To expand the vaccine's protective scope, a second-generation version was developed. This updated formulation not only targets NTS strains but also includes protection against Salmonella Paratyphi A, a typhoidal strain, thus making it a broad-spectrum vaccine.

Poster #46

Structure-Based Optimization of Lablaboside F Derivatives for Enhanced Solubility and Vaccine Adjuvant Activity

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Vaccine adjuvants are used in conjunction with subunit vaccines to provide a robust immune response, control the type of immune response, and extend the length of immunity. Adjuvant choice plays a vital role in formulating an effective vaccine and controlling the immune response; however, the limited amounts of safe and cost-effective adjuvants have created a need for the development of new vaccine adjuvants. Lablaboside F is a natural saponin vaccine adjuvant that has been synthesized by our lab along with eight structural derivatives. Compounds S1 and S5 have demonstrated high adjuvant activity and poor solubility. Furthermore, while S5 has an excellent safety profile, S1 suffers from some cytotoxicity. Hydrophilic motifs were added to improve S5's solubility (S5-1 to S5-4) and a variety of structural derivatives were prepared to determine the key structural features for adjuvant activity (S5-5 to S5-9). A series of S1 derivatives were also prepared to explore the effect of different sugars on the cytotoxicity and solubility (S1-1 to S1-6). Solubility of S1 and S5 derivatives was determined using in vitro shake flask method, and cytotoxicity will be determined in vitro with differentiated THP-1 cells. These studies will develop potential vaccine adjuvants, establish how each sugar affects solubility and cytotoxicity, and clarify the impact of glycosylation patterns and hydrophilic motifs on adjuvant activity.

Poster #47

Unveiling the Neuroprotective Potential of L-Rhamnose and Its Derivatives in Alzheimer's Disease Pathogenesis

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As the global population ages, Alzheimer's Disease (AD) poses a critical challenge, driven by inflammation, regulated by the Nrf2 signaling pathway, amyloid-β-induced oxidative stress causing atrophy of neurites, and mitochondrial dysfunction. Recent publications report L-Rha derivatives involved in alleviating AD's amyloid-β-induced oxidative stress and mitochondrial dysfunction amongst other factors, serving as evidence of this carbohydrate's significant role in neuroprotection.

The present study aims to elucidate the neuroprotective effects of L-Rha and its derivatives, focusing on their ability to engage Nrf2, an essential regulatory system protecting cells from oxidative stress. Our preliminary findings indicate that L-Rha not only enhances neurite outgrowth but also mitigates neurite atrophy and oxidative stress, indicating its potential to support neuronal health and integrity in the face of neurodegeneration and preventing the progression of the disease.

In pursuit of this, we propose two specific aims. First, a variety of L-Rha-containing molecules will be synthesized to optimize structure-activity relationships (SARs), using a cutting-edge microwave-assisted synthesis method. A group of extracted glycoproteins will be used along with the above-mentioned library to probe the correlation between molecular structure and neuroprotective efficacy. Second, the influence of these derivatives on the activation of Nrf2 and downstream antioxidant responses will be assessed, using Nrf2 knockout mouse models as a control to assess the importance of this pathway in the progression of the disease.

This research holds promise not only for advancing our understanding of L-Rha's potential as a therapeutic agent for AD but also for enhancing the broader landscape of neuroprotective strategies. By targeting the Nrf2 pathway, L-Rha and its derivatives could pave the way toward innovative and effective interventions for combating neurodegenerative diseases, offering avenues for improved patient outcomes.

Poster #48

Entirely Carbohydrate-Based Conjugate Vaccines Targeting Cancer-Associated Antigens

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The surface of cancer cells is covered in abnormal carbohydrate antigens that facilitate tumor growth, immune evasion, and metastasis. Overexpressed and often specific to cancer cells, these tumor-associated carbohydrate antigens (TACAs) offer a valuable handle for targeted immunotherapy.

These TACAs, a set of twenty to thirty common structures, play key roles at all stages of disease progression, as such, they have garnered growing interest as vaccine and monoclonal antibody targets. Sialyl Tn (STn) is a tumor associated carbohydrate antigen that is overexpressed in a variety of carcinomas such as breast, ovarian, and colon cancer. In normal tissue, STn is not detectable, which makes it an ideal target for cancer immunotherapies.

Like most carbohydrates, TACAs such as STn are incapable of being processed by the Major Histocompatibility Complex Class II (MHCII) and are thus T-cell independent, making it challenging for the immune system to mount a competent immune response towards them. This limitation is typically bypassed by conjugating the antigen(s) to a more immunogenic carrier, typically a protein. This approach often backfires, since the protein carrier itself dominates the resulting immune response at the expense of antibody production against the target TACA, leading to poor immune responses towards the desired carbohydrate target.

This issue was overcome by this group by linking the desired antigen (STn) to a carbohydrate-based carrier (PS A1), a Zwitterionic Polysaccharide (ZPS), which are rare bacterial capsular polysaccharides, isolated from Bacteroides fragilis, that display alternating charges on adjacent monosaccharides. Unlike other carbohydrates, PS A1 can be processed by MHCII and trigger CD4+ T-cells, immune memory and antibody isotype switching to high-affinity IgG1, IgG2a, IgG3, making an entirely carbohydrate, semi-synthetic STn-PS A1 conjugate vaccine possible.