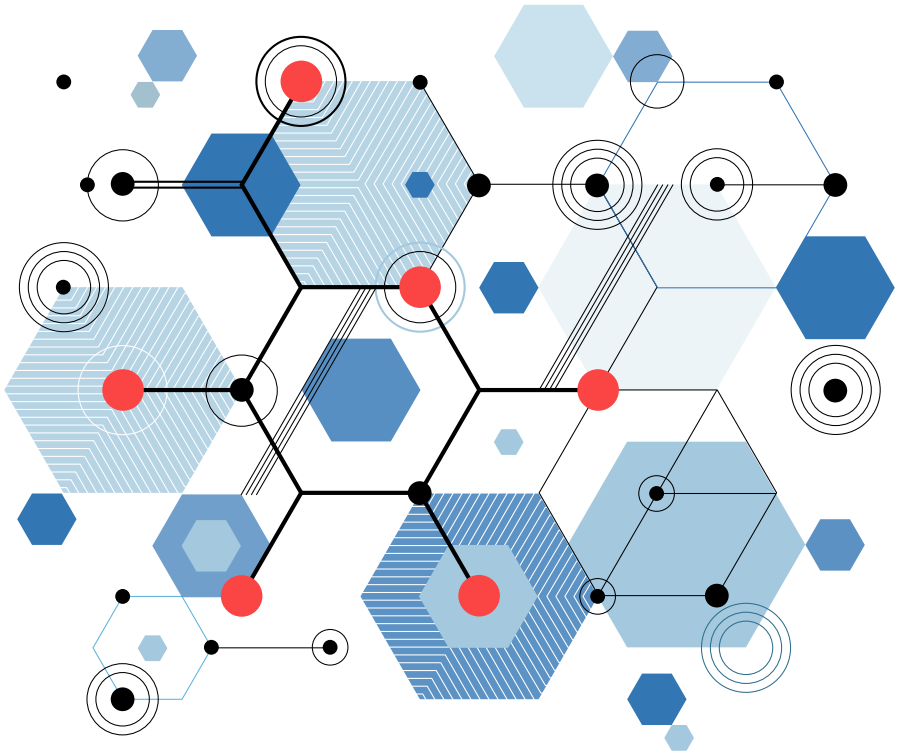

EUROCARB XX

JUNE30 - JULY04

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BOOK OF ABSTRACTS

We gratefully acknowledge our conference sponsors:

PL1

How Carbohydrate-Active Enzymes Work. Insight from Computer Simulation

Carme Rovira[1]

[1]*University of Barcelona, Barcelona, Spain*

Carbohydrate-active enzymes, such as glycoside hydrolases (GHs) and glycosyltransferases (GTs), constitute the main machinery for the degradation and synthesis of glycosidic bonds in nature. They have a myriad of industrial and biotechnological applications, ranging from biofuel production to drug design. In recent years, we have investigated. The catalytic mechanisms in these enzymes using state-of-art simulation techniques such as ab initio quantum mechanics/molecular mechanics (QM/MM) and metadynamics [1], providing a microscopic view of enzyme actionsimulations reveal the nature of the reaction transition states and the conformational itineraries of substrates in GHs [2-4], often encoded in the conformational energy landscape of isolated sugars [2], and complex GT catalytic pathways [5]. In this talk I will highlight some of the work that we have done in last years, in close collaboration with research groups of chemical and structural biology. Sugar conformational dynamics and the formation of short-lived species on the pathway to glycosidic bond formation will be discussed.

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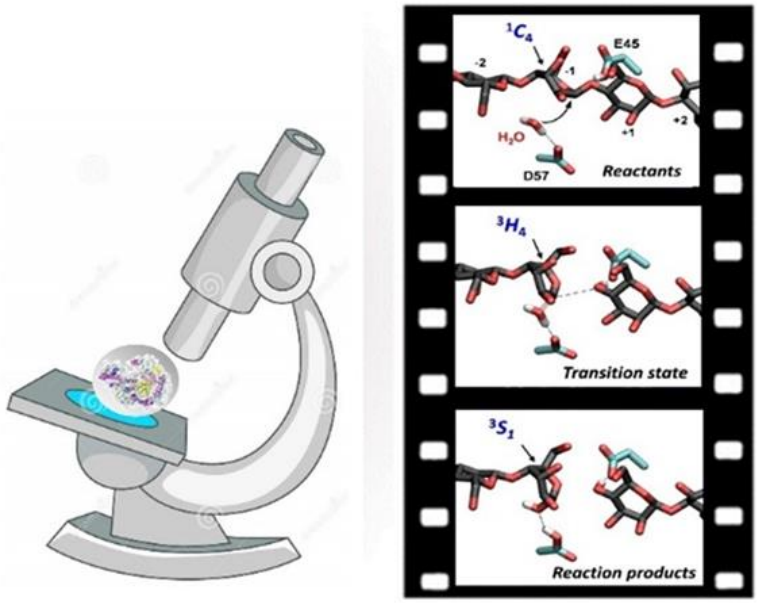

Catalytic itinerary of GH134 β-mannanase [4]. Picture adapted from J. Chem. Inf. Model. 2015, 55, 2218-2226.

PL2

Microbial Glycoconjugates: Structure and Function

Alba Silipo[1]

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The evaluation of the chemical structure of glycoconjugates from microbial cell envelope is key to two interconnected pillars: i) to provide structural insights into the mechanisms governing microbial glycans' recognition and binding by host cognate receptors and ii) to evaluate their contribute to the microbial cell survival as well as their interaction with host receptor(s).
The combined use of biophysical and chemical approaches including NMR spectroscopy, computational techniques and other physical-chemical methodologies is essential to unravel the structure, conformation and molecular recognition features of microbial glycoconjugates as well as their interaction with eukaryotic host.

In Gram negative bacteria, the external leaflet of the outer membrane (OM) is essentially covered by an important glycoconjugate, the lipopolysaccharide (LPS), which represents a bacterial signature and play an important role in any interaction of microbes with the external world. The chemical structures of LPS are highly strain-specific and play a central role in the recognition of both pathogenic and commensal bacteria by diverse eukaryotic hosts, including the recognition of rhizobia by legumes.
The detailed (bio)-molecular characterization of microbial glycoconjugates and a detailed molecular insights into the mechanisms that govern their interaction with host receptors is of primary importance to "tune" the bacterial cell surface initiation or suppression of inflammatory response. Various examples will be here described, with detailed description of the advantages and drawbacks of the application of the different methods and techniques.

PL3

A Chemical Biology Approach Toward Understanding the Roles of Carbohydrates in Neuroplasticity

Linda Hsieh-Wilson[1]

[1]California Institute of Technology, Pasadena, United States

The field of chemical neurobiology is rapidly evolving and providing insights into the molecules and interactions involved in neuronal development, sensory perception, and memory storage. We will describe the development of new synthetic methods and chemical tools to understand how glycosaminoglycans contribute to neuroplasticity, the ability of the brain to adapt and form new neural connections. By combining synthetic organic chemistry, computational chemistry, cell biology, and in vivo biology, we have shown that specific sulfation motifs found within glycosaminoglycan polysaccharides regulate signaling events that underlie processes such as axon regeneration, synaptic plasticity, and the formation of neural circuits.

PL4

A Mass Spectrometry View into Diverse Aspects of Glycobiology

Albert Heck[1]

[1]*Utrecht University, Utrecht, The Netherlands*

Around for more than a century the analytical technique of mass spectrometry is blooming more than ever, and applied in nearly all aspects of the natural and life sciences. In the last two decades mass spectrometry has become routine for the high-throughput analysis of peptides and glycans, and to a lesser extent glycopeptides. However, also intact proteins and even complete protein complexes can nowadays be analyzed. In this lecture, I will describe the emerging role of mass spectrometry with its different technical facets in molecular and structural biology, focusing especially on the analysis of intact glycoproteins. Moreover, I will describe how we use native mass spectrometry to study dynamic protein assemblies, for instnace those involved in complement activation.

Recent developments in mass spectrometry technology have allowed us to analyze intact native glycoproteins and protein complexes using Q-ToF and Orbitrap mass analyzers with very high sensitivity and mass resolving power, enabling us to profile the quality and biosimilarity of protein biotherapeutics, in their native state without requiring much sample preparation. In detail, I will demonstrate how native mass spectrometry can be combined with middle-down proteomics to profile complex structures of various glycoproteins, focusing on mAbs, Erythropoietin and plasma proteins. Thereby I will address the question of how unique each person is, as viewed from each individual's glycoproteome.

PL5

Glycan-Driven Pathogen-Host Interactions and Opportunities for Targeted Interference

Thilo Stehle[1]

[1]University of Tuebingen, Tübingen, Germany

Glycans are or critical importance for many biological processes. They often contribute to physical and structural integrity, extracellular matrix formation, signal transduction, protein folding, information exchange between cells, and pathogen uptake. However, we know little about many of these functions because glycans are notoriously difficult to work with. My presentation will focus on the roles of glycans in viral attachment and bacterial cell wall structure. Many viruses use glycans that are linked to either a protein or a lipid as their primary or secondary receptor. Glycans terminating in sialic acid and its derivatives serve as particularly important receptors for a large number of viruses, including several human pathogens. Glycans are also key modifiers of bacterial cell wall molecules, with relevance for immune evasion.

In the first part of my presentation, I will present recent structure-function analyses of interactions of human pathogenic viruses with sialylated glycan-based cell attachment receptors. In combination with glycan array analyses, structural studies of complexes of viruses with sialylated oligosaccharides provide insights into the parameters that underlie each interaction. These analyses have helped to define common parameters of recognition, and they also serve as a platform for understanding the determinants of specificity. This information is highly useful for the prediction of the location of sialic acid binding sites in viruses for which structural information is still lacking. An improved understanding of the principles that govern the recognition of sialic acid and sialylated oligosaccharides can also advance efforts to develop efficient antiviral agents. Based on our structural data, we have begun to design ligands that can engage viral capsid proteins in adenoviruses and polyomaviruses with in some cases high affinity and increased specificity.

In the second part of my presentation, I will discuss the role of cell wall glycosylation of pathogenic bacteria for immune recognition. We have found that some multidrug-resistant strains of Staphylococcus aureus encode an enzyme called TarP that catalyzes the addition of GlcNAc to D-ribitol phosphate at a particular carbon atom (known as C3) in the ribitol chain of the wall teichoic acid. Normally, GlcNAc is added at a different position, the C4 carbon, by the action of a related enzyme called TarS. Surprisingly, the TarP enzyme is of viral origin and the result of the infection by a bacteriophage. TarP is dominant over its bacterial counterpart TarS. S. aureus is normally held in check because the immune system has the ability to detect it. However, we have found that the form of WTA made by TarP action is less likely to trigger an immune response in mice than is the form of WTA generated by TarS. indicating that TarP is crucial for the capacity of S. aureus to evade host defenses. The high-resolution structural analysis of TarP explains the mechanism of altered glycosylation and forms a template for targeted inhibition of TarP. We expect that our results will help with the identification of invariant S. aureus vaccine antigens and may enable the development of TarP inhibitors as a new strategy for rendering MRSA susceptible to human host defenses.

PL6

LPMOs - From Chitin-Binding Proteins and Sloppy Endoglucanases to Versatile (Per)Oxygenases for Biomass Processing

Vincent G.H. Eijsink[1]

[1]NMBU - Norwegian University Of Life Sciences, Ås, Norway

In 2010, it was shown that proteins previously known as chitin-binding proteins or carbohydrate-binding modules of family 33 (CBM33) and, by analogy, proteins previously classified as glycoside hydrolases of family 61 (GH61), catalyse oxidative cleavage of glycosidic bonds. The discovery of these enzymes, today called lytic polysaccharide monooxygenases (LPMOs), has revolutionized our views on enzymatic processing of polysaccharides, in Nature and industry alike. LPMOs are mono-copper enzymes with intriguing and unprecedented catalytic properties, including a unique ability to break glycoside bonds in crystalline substrates. LPMOs require reducing equivalents and an oxygen-containing co-substrate, which was originally thought to be O_2. Recent studies indicate that LPMOs may in fact be peroxygenases, as H_2O_2-driven LPMO reactions are orders of magnitude faster than O_2-driven reactions [1]. It has also become clear that LPMOs suffer from auto-catalytic inactivation if there is a misbalance between available carbohydrate substrate, reducing power and the oxygen-containing co-substrate. These recent findings shed new light on industrial biomass processing [2] and on the interplay between LPMOs and other oxidoreductases in biomass degrading eco-systems [3]. In this presentation, I will summarize the most recent findings regarding LPMO functionality and discuss knowns and unknowns in the LPMO field [4-6]. Of note, current data indicate that these ubiquitous enzymes may have additional roles, e.g. in microbial pathogenesis, and that their true industrial potential has not yet been harnessed.

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PL7

Saccharides, Pseudosaccharides and Their Mimetics

David Crich[1]

[1]Wayne State University, Detroit, United States

This lecture will cover recent work from the Crich laboratory on the design, synthesis, and evaluation of next generation aminoglycoside antibiotics for the treatment of multidrug resistant infections, including work in the paromomycin and apramycin series.

PL8

Travels Through the Chemistry and Biochemistry of Mannosides

Gideon Davies[1]

[1]*Department of Chemistry, University Of York, York, United Kingdom*

There has much discussion on social media about which sugar is the most biologically and chemically exciting. Most people opt for the sialic acids, whilst a select group favour fucose. Mannose, which plays myriad biological roles and whose chemistry is dominated by its axial O2 group is a strong candidate. Mannans play key structural and recognition roles in biology and cellular function. β-linked mannans are exemplified by robust plant structural polysaccharides, α-linked mannans form the cell wall of yeast and the core of eukaryotic N-glycans (and of the viruses that infect them).

In this lecture I shall review some conformational aspects of mannoside enzymology with examples from α- and β-mannosidases before going on to discuss new, currently unpublished, work in three areas of mannoside chemistry: the enzymology (both mannoside phosphorylases / GDP-Man dependent mannosyltransferases) of the synthesis of β-1,2 linked mannogen in the Leishmanial parasite, the development of new activity-based probes for α-mannosidase inhibitor discovery and the unusual catalytic mechanism of N-glycan processing endomannosidases where evidence for a 1,2 anhydro “epoxide” intermediate will be discussed.

PL9

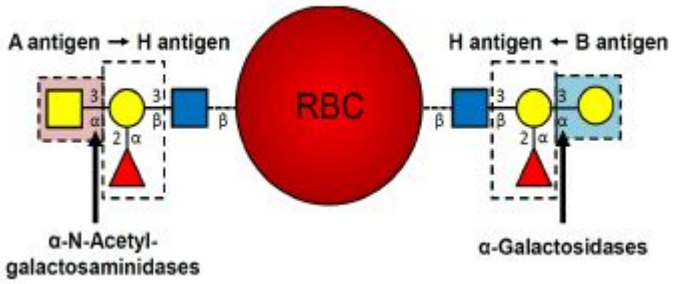
New Enzymes for Cell Surface Modification: Towards Universal Blood

Steve Withers[1], Peter Rahfeld[1], Lyann Sim[1], Haisle Moon[1], Jayachandran Kizhakkedathu[1]

[1]*University Of British Columbia, Vancouver, Canada*

Mammalian cell surfaces are coated in specific sugar structures, many of which function as antigens and are involved in cellular recognition. Important examples are the oligosaccharide A, B, and H antigens present on red blood cells that differentiate the A, B and O blood types. Enzymatic cleavage of the GalNAc and Gal residues from the cell surface would allow conversion of A and B red blood cells, respectively, to O type. Since Type O blood can be universally donated to patients with the same Rh factor, access to efficient enzymes would greatly broaden and simplify blood supply. We have sought such enzymes in metagenomic libraries derived from the human gut microbiome.

Total DNA was extracted from feces samples, fragmented into chunks containing ~30-40 genes (40-50 kB) and transformed into E. coli. After picking colonies into 384 well plates we screened them for enzymes that can be used to remove the Gal or GalNAc residues that function as the antigenic determinants from A and B type red blood cells, thereby generating “universal” O type blood. A set of efficient enzymes of a new class has been identified and characterised and used to convert whole units of A blood to O. These enzymes work approximately 30 times faster than any previously characterized and with high specificity. Further, they function well in whole blood thus can be hopefully integrated into the current blood processing process.



A and B antigens on red blood cells

PL10

The Chemical Diversity of Microbial Glycans

Antonio Molinaro[1]

[1]*University of Napoli Federico II, Napoli, Italy*

Microbial cell surface molecules, such as bacterial lipopolysaccharides and exopolysaccharides and viral glycoproteins, are very important cell wall glycoconjugates and act as microbe associated molecular patterns in eukaryote/prokaryote recognition. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Microbes, differently from eukaryotes, synthesize by their machineries an enormous array of monosaccharide structures/derivatives with which they built up they external cell surface molecules and drive their recognition by any eukaryotic host. Therefore, the chemical study of such glycoconjugates involved as virulence or beneficial microbial factors in animal or plant interactions is a pivotal pre-requisite for the comprehension at molecular level of microbe-eukaryote interaction. [1-4] In this communication, I will show some examples of puzzling mono-, oligo- polysaccharide structures and their 3D arrangements from microbial origins.

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PL 11

Metrology of Carbohydrates - International Initiatives

Sabine Flitsch[1]

[1]University of Manchester , Manchester, United Kingdom

Carbohydrates constitute the largest source of biomass on Earth and their exploitation will be crucial if we are to reduce our dependence on fossil fuels and create a Circular Economy. However, to understand and ultimately exploit carbohydrates, a carbohydrate metrology and measurements 'toolbox' is essential for new research and innovation practices in glycoscience.
CarboMet (https://carbomet.eu) is a CSA programme funded by the European Commission to promote the metrology of carbohydrates for enabling european industries. The objectives include
(i) to establish a european glycoscience user group ; (ii) identify common scientific and technical challenges; (iii) establish a common vision, Roadmap for 2030 and an associated implementation plan; (iv) through knowledge exchange activities facilitate the formation of a cross- sectorial glycoscience metrology platform; (v) establish parallel initiatives to address key glycoscience challenges.

CarboMet will facilitate engagement between key players and stakeholders of the glycoscience community across Europe to identify the current state of the art and in particular future innovation and technological challenges in carbohydrate metrology.

Carbomet has identified three enabling technologies: measurements and analytical, synthesis of standards and bioinformatics and databases. In addition, four bioindustries sectors have been identified as of immediate interest to carbomet where the exploitation of carbohydrates will have huge impact: biopharmaceuticals, precision medicine, healthy lifestyles and sustainable materials.

Any researchers interested in these activities are encouraged to visit the website and engage through social media.

KL1.1

Using Chemistry to Understand Sulfoglycolysis: A Major Pathway in the Biogeochemical Sulfur Cycle

Spencer Williams[1]

[1]*University of Melbourne, Parkville, Australia*

The biogeochemical sulfur cycle describes how sulfur transits through the mineral, atmospheric, hydrologic and biological domains. Within the biological domain an estimated 10 billion tonnes of the sulfur sugar sulfoquinovose (SQ) is formed annually, an amount commensurate with the amino acids cysteine and methionine [1]. SQ forms the head group of the sulfolipid sulfoquinovosyl diacylglyceride (SQDG), which is produced by essentially all photosynthetic organisms, and plays a role in membrane function of photosynthetic organelles and their associated photoproteins. The biosynthesis of SQ is well-established, yet only recently was the first sulfoglycolytic pathway discovered (in E. coli) capable of degrading this important sugar [2]. In this talk I will provide an overview of our efforts to identify new enzymes as part of this pathway and to use chemical methods to develop a detailed molecular understanding of sulfoglycolysis catalysis [3,4,5,6].

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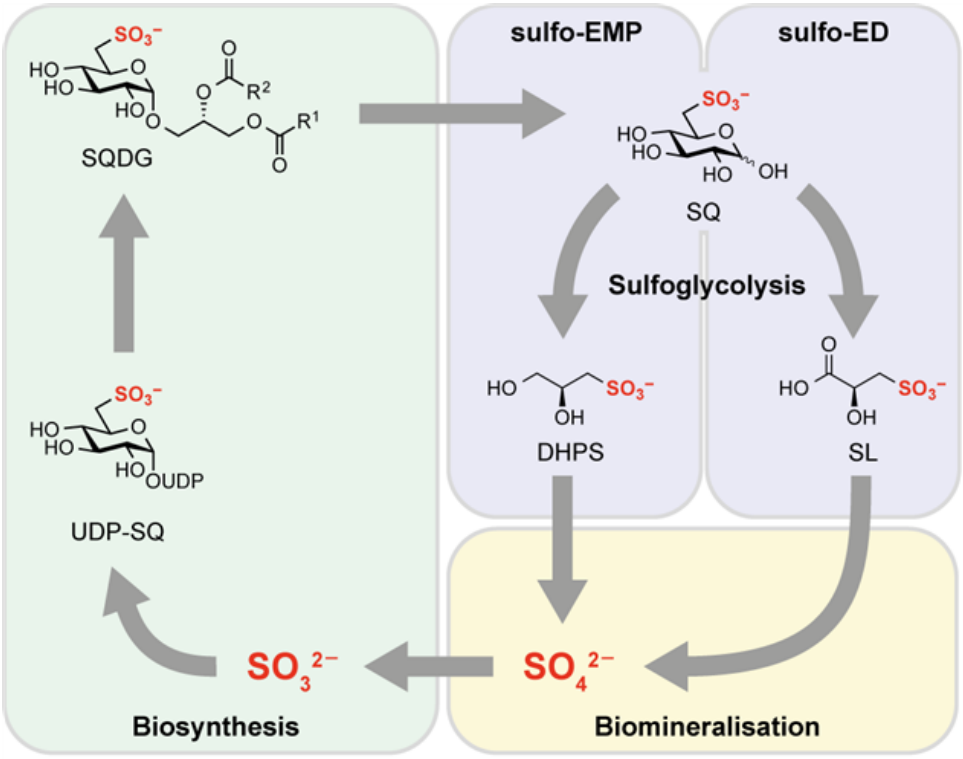

Overview of sulfoquinovose metabolism.

KL1.2

Expeditious Synthesis of Bacterial Glycoconjugates

Suvarn S. Kulkarni[1]

[1]*Indian Institute of Technology Bombay, Mumbai, India*

Bacterial glycoconjugates are comprised of rare D and L deoxy amino sugars, which are not present on the human cell surface.[1] This peculiar structural difference allows discrimination between the pathogen and the host cell and offers avenues for target-specific drug discovery and carbohydrate-based vaccine development.[2] However, they cannot be isolated with sufficient purity in acceptable amounts, and therefore chemical synthesis is a crucial step toward the development of these products. We recently established short and convenient methodologies for the synthesis of orthogonally protected bacterial D and L-deoxy amino hexopyranoside and glycosamine building blocks starting from cheaply available D-mannose and L-rhamnose.[3-5] The one-pot protocols rely on highly regioselective nucleophilic displacements of triflates. These procedures have been applied to the synthesis of various bacterial glycoconjugates.[3-10] (Figure 1) as well as metabolic oligosaccharide engineering for selective detection of bacteria.[8]

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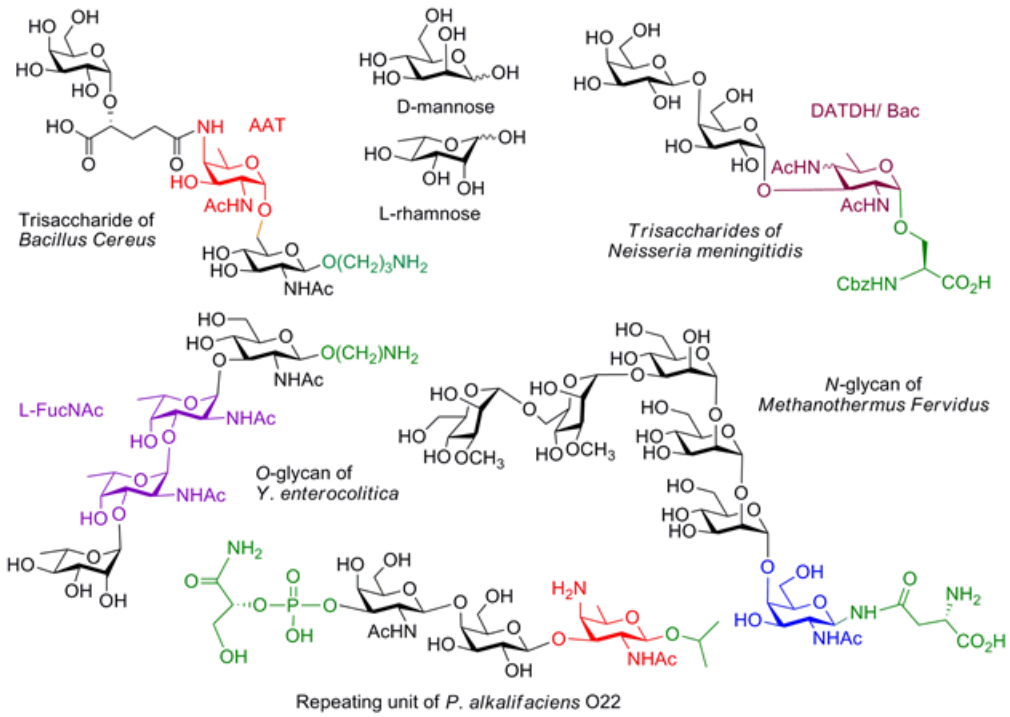

Figure 1. Representative examples of bacterial and archaeal glycans synthesized using the methodology

KL1.3

Glycosphingolipid Analogues as Immunostimulants of NKT Cells

Amadeu Llebaria[1]

[1]MCS, Institute of Advanced Chemistry of Catalonia CSIC, Barcelona, Spain

The stimulation of Natural Killer T cells (NKT cells) with alpha-galactosylceramide (aGC) or analogues has been largely studied over the past 20 years, including some clinical trials on disease treatment for humans, singularly in cancer. Despite the evidences of NKT cell implication in immunological responses in a wide range of diseases, and the strong effect of aGC stimulation, the lack of clear improvement of the treatments effects put down the expectations of the use of aGC or derivatives as adjuvants. The research on new analogues with better pharmacological properties has been objective of different groups in the last years looking for different compounds able to reach a more sustainable immune response and a more polarized profile towards Th1 or Th2 type.
Our group is focused on the design and synthesis of aminocyclitol-ceramide derivatives as non-glycosidic aGC analogues, based on our previously described compounds HS44 and HS161.
Different chemical strategies were followed to obtained several sub-families based on our precedent compounds and their biological activity was studied as NKT cells activators. Good immunological profiles were obtained, with interesting Structure-Activity-Relationship (SAR). Some of them showed promising affinity and their crystal structures in complex with mouse CD1d-TCR were obtained. In light of these results, the new aminocyclitol-ceramide derivatives highlight the relevance that small chemical modifications on the antigen polar part have on their biological response.

KL1.4

Immune Recognition of Bacterial Surface Glycans: Implications for Infection and Therapy

Nina M. Van Sorge[1]

[1]UMCU, Utrecht, The Netherlands

Staphylococcus aureus is a commensal bacterium that colonizes about 30% of the population without any harmful effects. However, S. aureus also represents a major public health concern, due to its ability to cause a wide range of clinical infections combined with the alarming development of antibiotic resistance, which limits treatment options. One of the prime targets for the development of new therapeutic interventions against S. aureus the wall teichoic acids (WTAs), which are abundant glycopolymers that are critical for bacterial physiology, antibiotic resistance and colonization. Commonly, S. aureus expresses WTA composed of a polyribitol-phosphate backbone modified by D-alanine. Structural variation is limited and occurs through glycosylation by the activity of three distinct enzymes: TarM, TarS and TarP [1-2]. Through a multidisciplinary approach, including genetics, microbiology, immunology, and glycobiology, we aim to unravel the molecular interplay between WTAs and host immunity to advance the development of new antimicrobial therapies targeting.

This presentation will highlight some of our recent findings in this area. First, we have identified human Langerin, a receptor unique to skin epidermal Langerhans cells (LCs), as a receptor for β-linked GlcNAc on S. aureus WTA. Functionally, LCs respond with increased cytokine production to S. aureus that express β-GlcNAc-modified WTA. Finally, in a murine epicutaneous infection model, S. aureus strongly upregulated transcript specific cytokine transcripts, which required the presence of both human langerin and WTA β-GlcNAc [3]. Together, these findings provide molecular insight into the unique pro-inflammatory capacities of S. aureus in relation to skin inflammation. The second project demonstrates how the use of chemically synthesized polyribitol-phosphate molecules helps to dissect interactions between WTA and human immune components, specifically human antibodies. In the future, these synthetic WTA molecules will be applied to provide structural insights but could also serve as a platform for identification and optimization of therapeutic antibodies directed against S. aureus WTA.

KL2.1

Synthesis of Oligosaccharides Representative of Plant Cell Wall Glycans

Mads Clausen[1]

[1]*Center for Nanomedicine and Theranostics, DTU Chemistry, Kgs. Lyngby, Denmark*

Plant cell walls are structurally complex and contain a large number of diverse carbohydrate polymers. These plant fibers are a highly valuable bio-resource and the focus of food, energy and health research. We are interested in studying the interplay of plant cell wall carbohydrates with proteins such as enzymes, cell surface lectins, and antibodies. However, detailed molecular level investigations of such interactions are hampered by the heterogeneity and diversity of the polymers of interest. To circumvent this, we target well-defined oligosaccharides with representative structures that can be used for characterizing protein-carbohydrate binding. The presentation will highlight chemical syntheses of plant cell wall oligosaccharides from the group and provide examples of their interactions with proteins.

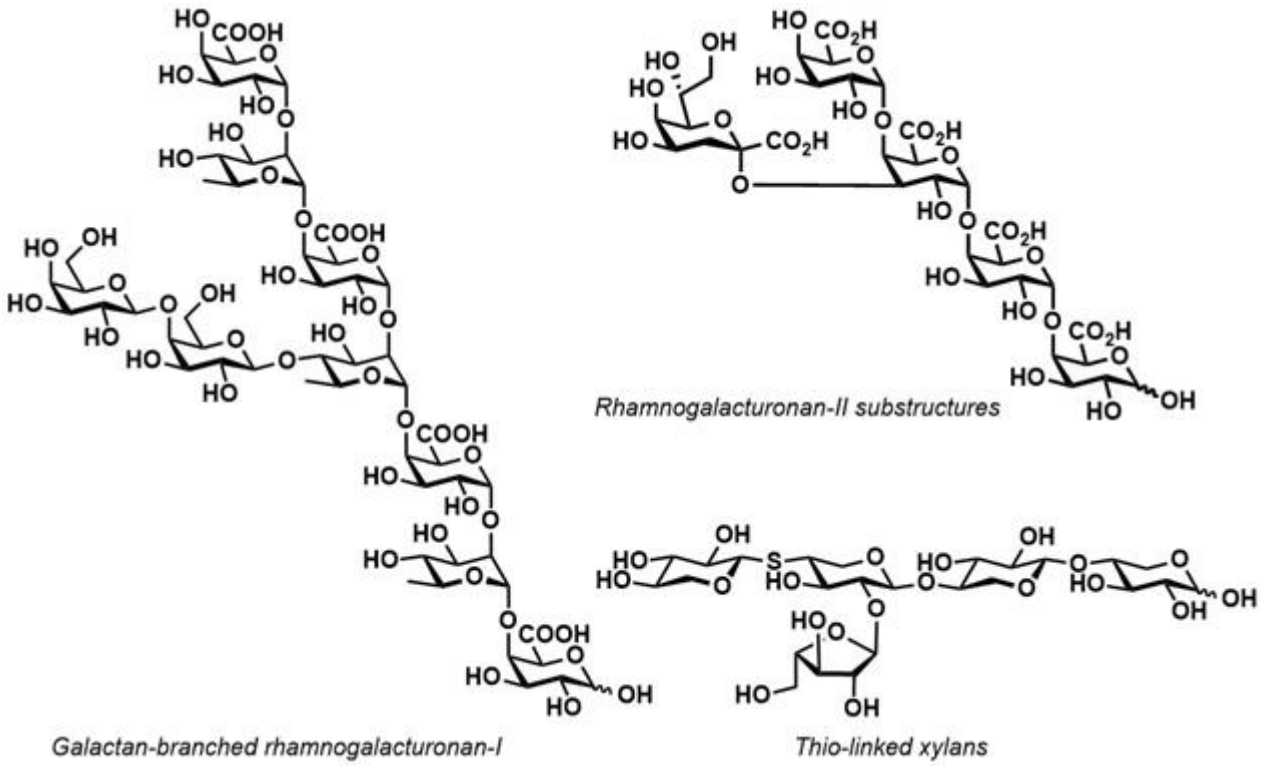

Figure 1. Examples of current synthetic targets.

KL2.2

Structural Studies of Eukaryotic Oligosaccharyltransferase Catalyzing a Key Step in Protein N-Glycosylation

Kaspar Locher[1]

[1]ETH Zurich, Institute of Molecular Biology and Biophysics, Zurich, Switzerland

Eukaryotic oligosaccharyltransferase (OST) is a multi-subunit protein complex embedded in the membrane of the endoplasmic reticulum (ER). It catalyzes the en bloc transfer of a high-mannose oligosaccharide from a dolichol-pyrophosphate carrier onto asparagines located in glycosylation sequons (sequence N-X-S/T) of secretory proteins. Using single-particle cryo-electron microscopy, we determined the structure of a fungal octa-subunit OST complex reconstituted in lipidic nanodiscs at 3.31Å resolution. It revealed the arrangement of the eight subunits and suggested that eukaryotic OST complexes have a conserved architecture. The activity of reconstituted Saccharomyces cerevisiae OST was assessed using an in vitro glycosylation assay with synthetic LLO and peptide substrates. Using the crystal structure of a bacterial homolog of the catalytic STT3 subunit (the PglB protein of Campylobacter lari) as a reference, features critical for the catalytic activity and substrate recognition on STT3 could be identified. The structure not only revealed ordered lipid molecules, but also a large N-glycan attached to a conserved Asn residue within STT3. This glycan lines a cavity that may serve as the glycan-binding pocket for the donor LLO substrate. By docking the structure of yeast OST into previously determined tomography maps, insight into distinct features of OST complexes either involved in co-translocational N-glycosylation (OST complexes associated with the translocon) or in post-translocational N-glycosylation (standalone OST complexes containing a subunit with redox chaperone) could be gained. Our results not only reveal the architecture of the OST complex and suggests roles for the non-catalytic subunits, they also suggest mechanisms by which eukaryotic OST complexes stimulate N-glycosylation over folding of large numbers of secretory proteins.

KL2.3

Modifications Matter: Probing the Effects of Glycosylation on Peptide and Protein Activity

Richard Payne[1]

[1]The University of Sydney, Sydney, Australia

Glycosylation is the most common co- and post-translational modification of polypeptides, with over 50% of human proteins predicted to display covalently bound glycans. Glycoproteins are known to mediate an array of biological recognition events and a number of recently approved biopharmaceuticals contain carbohydrate chains (or carbohydrate mimics) that are critical for activity and/or stability.[1] In addition, aberrant glycosylation is associated with a number of disease states including autoimmune diseases and cancer.

The non-templated enzymatic glycosylation process leads to heterogeneous mixtures of isoforms when glycoproteins are produced in eukaryotic expression systems that hinders the ability to study glycoprotein structure and function in a meaningful way. This has led to significant demand for new tools and technologies to facilitate access to homogeneous glycopeptides and glycoproteins to interrogate the role of individual carbohydrate modifications on structure and function. This talk will outline the use of synthetic technologies developed in our laboratory to access homogeneously glycosylated peptides and proteins for structure-function studies.[2] The synthesis and evaluation of glycopeptide hormones,[3] bacterial glycoproteins,[4] cytokines and thrombin-inhibiting glycoproteins from medicinal leeches[5] will be highlighted.

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KL2.4

Surprising Insights in the Synthesis of Nucleotide Sugars - Lessons from Human Disease

Dirk Lefeber[1]

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Abnormal protein N-glycosylation has been associated with a wide variety of human diseases, such as cancer, diabetes and neurodegenerative disease. The underlying biological mechanisms that control the precise glycan structural abnormalities, in a protein- and tissue-specific manner, are still very poorly understood. Important novel insights in the biochemical mechanisms of protein N-glycosylation have originated from a group of monogenic defects, the Congenital Disorders of Glycosylation (CDG). After initial discovery of many defects in glycosyltransferases, a growing group of additional genetic causes is being identified. These factors cluster in two main groups: defects of Golgi homeostasis1-3 and defects in cellular sugar metabolism4,5.
In our group, we focus on the understanding of protein-specific glycosylation abnormalities via development of N-glycoproteomics, as well as on the tissue-specific mechanisms in sugar metabolism by targeted mass spectrometry. All known sugar metabolites can be sensitively detected in cells, tissues and organisms, for example confirming the presence of CMP-sialic acid in Drosophila. In addition, this resulted in the identification of CDP-ribitol as 10th human nucleotide sugar known to date6. Thus far, ribitol-phosphate had only been shown to be present in bacterial polysaccharides.
We are currently applying this technology to unravel surprising and novel mechanisms in the synthesis and use of human CMP-sialic acid. For example, SLC35A1, the Golgi transporter of CMP-sialic acid, was shown to be also required for O-mannosylation of alpha-Dystroglycan, in a process that is independent from sialic acid7,8. Clinical phenotypes of genetic defects in the sialic acid pathway are contradicting, with an adult myopathy due to GNE mutations, a neurological syndrome due to NANS mutations9, the next enzyme in this pathway, and a neuromuscular disease due to NPL mutations, required for catabolism of sialic acid10. Metabolic tracing with 13C-labeled sugars and chemically modified sugar derivatives, ManNPoc and SiaNPoc is carried out in knock-out cells of the sialic acid pathway, generated via CRISPR/Cas9 genome editing. These studies are generating the first novel insights of tissue-specific mechanisms in the sialic acid pathway, that might explain the contrasting tissue-specific clinical outcome in patients.

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KL4.1

Sugary Coats: Diverse Mechanisms of Polysaccharide Secretion

Jochen Zimmer[1]

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All living systems produce complex carbohydrates as an energy source, protective coat, or adhesive for cell attachment. Polysaccharides displayed on the cell surface are secreted by diverse mechanisms that either directly couple polymer synthesis to export or involve dedicated transporters capable of moving high molecular weight polymers across the plasma membrane. Microbial cellulose biosynthesis and the ABC transporter-mediated secretion of O-antigens exemplify two fundamentally different translocation mechanisms. Both pathways contribute to the formation of protective coats frequently produced by microbial pathogens to reduce the efficacy of their hosts' innate immune responses. Using structural and functional analyses, we obtained detailed insights into diverse polysaccharide secretion mechanisms.

KL4.2

Introducing Sequence-Control into Glycomimetic Polymers and Materials

Laura Hartmann[1]

[1]*Heinrich-Heine-University, Duesseldorf, Germany*

From a chemical point of view, carbohydrates are a highly diverse class of biomacromolecules as they not only use a great number of different building blocks, the monosaccharides, but are assembled into linear and branched structures, oligomers, polymers and glycoconjugate structures. Intentionally reducing this complexity is achieved for so-called multivalent glycomimetics via the attachment of smaller glycan fragments onto synthetic scaffolds. We have introduced the class of precision glycomacromolecules as multivalent glycomimetics that can be easily varied in terms of their scaffold structure, scaffold composition as well as number and kind of glycan fragment attached, parameters known to affect ligand properties such as affinity. This is possible by using a solid phase approach based on tailor-made building blocks and standard Fmoc peptide chemistry giving access to a variety of monodisperse, sequence-controlled glycomacromolecules. In order to mimic the structural diversity of carbohydrates, these glycomacromolecules can then be used again as building blocks to create more complex glycomimetic structures and materials, e.g. through conjugation onto nanoparticles or microgels. The lecture will present the bottom-up synthesis of such glycomimetic materials, starting from small building blocks, solid phase assembly of oligomers going to higher molecular weight structures and will discuss their potential biomedical applications.

KL4.3

Synthetic Plant Glycans as Tools for Cell Wall Biology

Fabian Pfrengle[1,2]

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Plant cells are surrounded by a polysaccharide-rich matrix that constitutes the cell wall of all higher plants. These structurally complex polysaccharides provide an important resource for food, renewable materials, and the generation of bioenergy. Investigations into the structure, function, and biosynthesis of cell wall glycans are aided by well-defined and pure oligosaccharides which are available through chemical synthesis [1].
We have prepared oligosaccharides derived from different cell wall polysaccharides using automated glycan assembly [2-6]. For each class of polysaccharide a collection of oligosaccharide samples were synthesized from a limited set of monosaccharide building blocks. The assembled glycans were subsequently printed as microarray to characterize antibodies that recognize plant cell wall glycans [7] and glycosyltransferases that are involved in plant cell wall biosynthesis.

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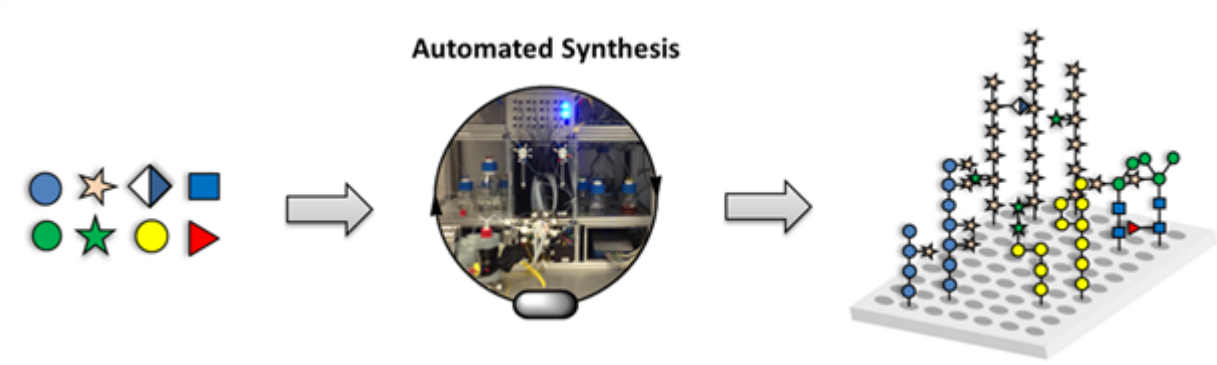

Automated Glycan Assembly (AGA) of plant cell wall oligosaccharides

KL4.4

Computational Investigations of Interfacial Biocatalysis for Plant Cell Wall Polysaccharide Deconstruction

Gregg Beckham

For the last several years, we have been investigating the mechanisms of cellulose degradation by cellulase enzymes using computer simulations, and this talk will cover mechanistic investigations of enzymes that are able to bind to, depolymerize, and hydrolyze the cellulose polymer to soluble sugars as well as mechanistic investigations of esterase enzymes able to process hemicellulose.

KL5.1

Glycomimetics: Useful Tools and Potential Therapeutics

Tanja Maria Wrodnigg[1]

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Glycomimetics are carbohydrate analogs with altered physical, physiological and/or biological properties based on structural changes compared to the respective parent compounds.[1] Iminosugars are glycomimetics with the ring oxygen being replaced by a basic trivalent nitrogen, which are very well known for their manifold applications as tools and therapeutics based on their interactions with glycoside processing enzymes. Representatives of this compound class find application in the elucidation of enzyme mechanisms and disease pathways.[2] For therapeutic purposes they are employed, for example, for the treatment of diabetes Type 2 (Miglitol®) and Gaucher disease Type 1 (Miglustat®). Other representatives have immunoregulating properties and anti-bacterial properties. Furthermore, some are considered to have potential for therapeutic disease management of Alzheimer's as well as Parkinson disease. Recently, glycomimetics have been introduced as probes for activity based protein profiling (ABPP) of glycoprocessing enzymes.[3] Employing these small molecules, ABPP allows for evaluation of protein function on a global cellular level by rapid, sensitive, and selective detection and verification of protein activity rather than abundance.[4]

Our approach towards the design and syntheses of iminosugar based glycomimetics as potential therapeutics as well as probes for activity based protein profiling of glycoprocessing enzymes will be presented.

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KL5.2

Structural Mechanisms of Protein Glycosylation at the ER Membrane

Lin Bai[1], **Huilin Li[1]**

[1]Van Andel Research Institute, Grand Rapids, United States

Most secreted and membrane proteins are synthesized on the ER membrane by ribosomes docked on the translocons. As they pass through the translocons, nascent peptide chains are scanned by two ER-embedded and translocon-associated molecular machines, the oligosaccharyltransferase (OST) complex and the protein mannosyltransferease complex (PMT), for protein N-glycosylation and protein O-mannosylation, respectively. OST is an eight-subunit membrane complex that transfers the oligosaccharide (OS) from the carrier Dol-PP-OS to the Asn-Xaa-Ser/Thr sequon of a nascent polypeptide. And PMT such as the Pmt1-Pmt2 heterodimer or the Pmt4-Pmt4 homodimer transfers a mannose (Man) from the carrier Dol-P-Man to Ser/Thr of a nascent polypeptide. OST is a member of the glycosyltransferase family (GT) 66 and PMT of the GT39 family, both enzyme complexes are inverting transferases with the GT-C structural fold. In my presentation, I will describe the structures and the catalytic mechanisms of the two membrane complexes and provide structural evidences supporting the hypothesis that PMT and OST are evolutionarily related.

KL5.3

Carbohydrate Synthesis: From Oligosaccharide to Polysaccharide

Xin-Shan Ye[1]

[1]*State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China*

Carbohydrates play very important roles in a variety of physiological and pathological processes. Well-defined carbohydrates obtained by chemical synthesis are essential to the understanding of their functions. However, synthesis of carbohydrates is greatly hampered by its insufficient efficiency. We have developed the donor preactivation-based one-pot oligosaccharide synthesis strategy, and this strategy has been established as one of the efficient protocols for oligosaccharide assembly. Using this strategy, quite a lot of complex oligosaccharides with biological activities have been constructed successfully. On the other hand, assembly of longer carbohydrate chains remains one of the most challenging tasks for synthetic chemists. On the basis of oligosaccharide synthesis, we have realized a highly efficient assembly of a 92-mer polysaccharide, which is an essential structural constituent of mycobacterial cell wall, by the preactivation-based one-pot glycosylation protocol. Our work may open an avenue to the synthesis of complex polysaccharides with biological importance that are either difficult or impossible to access through isolation or semisynthesis.

KL5.4

Glycan Scavenging at the Bacterial Cell Surface

Nicole Koropatkin[1]

[1]*University of Michigan Medical School, Ann Arbor, United States*

The mammalian gut Bacteroidetes display a greatly expanded capacity for glycan degradation, with many having the ability to flexibly forage on at least a dozen complex polysaccharides. This glycolytic potential is packaged into discrete polysaccharide utilization loci (PUL) that encode the necessary machinery for the degradation and import of a distinct glycan structure. PUL-encoded protein complexes are referred to as starch utilization (Sus)-like systems after the first such system for starch import described in Bacteroides thetaiotaomicron. Sus-like protein complexes are present in nearly every gut Bacteroidetes yet restricted to this phylum, and their glycan specificity dictates the bacterial metabolic niche. The Sus of B. thetaiotaomicron is perhaps the simplest and most well characterized of these glycan uptake systems, though more recently many other systems that target host mucins, hemicellulose, and even peptides have been elucidated. All are comprised of a putative TonB-dependent transporter and two classes of carbohydrate-binding proteins: the SusD-like proteins and the surface glycan-binding lipoproteins (SGBPs). Within individual Sus-like systems there is some redundant polysaccharide binding between the SusD-like proteins and the SGBPs, yet each protein plays a distinct role in carbohydrate import. Much of our work with the complexes for the acquisition of starch and xyloglucan has revealed that the presence of these proteins, and likely their interactions with the TonB-dependent transporter, are more important than their ability to bind glycan. Through a detailed understanding of how human gut bacteria acquire carbohydrate nutrition in the highly competitive gut ecosystem, we can develop prebiotic and probiotic strategies to manipulate the composition of this community towards improved human health.

KL7.1

Carbohydrate-Derived Nitrones as Synthetic Tools for the Discovery of Novel Classes of Iminosugars

Anaïs Vieira Da Cruz[1,2], Liang Wu[3], Salia Tangara[1,2], Alice Kanazawa[1,2], Jean-Bernard Behr[4], Gideon J. Davies[3], Sandrine Py[1,2]

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Due to their stability in vivo and their activity as glycosidase inhibitors or activators, iminosugars are among the most promising drug candidates for the treatment of diseases such as diabetes, viral infections and lysosomal storage disorders.[1] Recently, our group has identified a series of iminosugars (i.e. compounds 1 and 2), exhibiting a quaternary center in α-position of their nitrogen atom, which proved to be excellent inhibitors (nanomolar Ki) of α-glucosidases with exquisite selectivity.[2]

In this communication, the synthesis of novel iminosugars from carbohydrate-derived nitrones will be presented. The variety of synthetic methods that can be applied to nitrones to prepare bioactive molecules will be emphasized by successful syntheses of original iminosugar scaffolds.[3] Investigation of the biological activities of these new molecules will also be presented, and discussed in light of crystallographic analyses of iminosugar-glycosidase complexes.[4]

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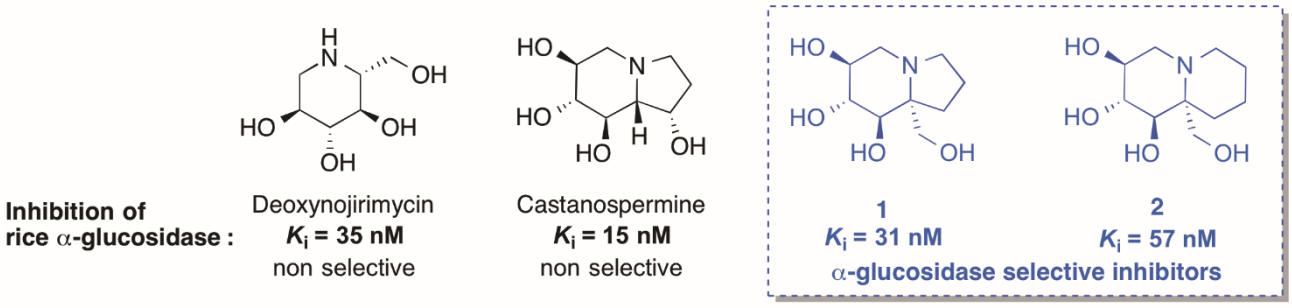

α-glucosidase inhibiting iminosugars

KL7.2

Chitin and Peptidoglycan Deacetylases: Structures, Specificities and Engineering for Biotech Applications

Antoni Planas

Pathogenic bacteria and fungi deacetylate their own cell wall polysaccharides as a strategy to evade the host immune responses at initial stages of infection. Pathogenic bacteria utilize acetylation (6-O-acetylation of MurNAc) and deacetylation (2-N-deacetylation of GlcNAc and/or MurNAc residues) of their cell wall peptidoglycan (PGN) to evade detection by the innate immune system. Likewise, plant pathogenic fungi partially deacetylate their cell wall chitin to be resistant to degradation by plant chitinases or deacetylate the released chitooligosaccahides (COS) to escape recognition by chitin receptors and evade the plant immune responses.
Peptidogycan and chitin deacetylases are members of family 4 carbohydrate esterases (CE4 enzymes) which operate by a metal-assisted general acid/base catalytic mechanism [1]. We are interested in understanding the structural bases of substrate specificity by CE4 enzymes, their mechanism of action and biological functions, as well as the use of engineered variants as biocatalysts.

In this lecture, recent advances towards understanding and engineering substrate specificity of chitin and peptidoglycan deacetylases (CDAs and PGN DAs) will be presented:
- Structural determinants of substrate specificity in CDAs: the subsite capping model to engineer specificity by rational and combinatorial approaches [1-4].
- Peptidoglycan GlcNAc and MurNAc deacetylation specificities. Structure and properties of a novel MurNAc DA with dual specificity [5-6].
- CE4 enzymes active of chitooligosaccharides as biocatalysts for the production of paCOS with defined and novel deacetylation patterns, current targets for a number of biotech applications. [3,7]

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KL7.3

Site-Selective Catalysis in Unprotected Carbohydrates

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In recent years, the application of homogeneous catalysis methods for the functionalisation of complex molecules has seen a steep increase. The modification of complex molecules, most often drugs or natural products, can be very versatile, in particular when the parent compound is available, because it avoids the execution of an entire synthesis route. Nevertheless it poses high demands on the catalysis involved. In carbohydrate chemistry, this approach receives considerable attention as well,[1] also because many (oligo)saccharides are available from nature, though not always in substantial amounts. The challenge here is to discriminate between the (very) similar hydroxyl groups. In our group we focus on the site-selective catalytic oxidation[2] and photo-redox catalysis[3] of unprotected carbohydrates. Oxidation, because a carbonyl function is a versatile starting point for further functionalisation. And photocatalysis, because radical chemistry is mostly compatible with protic solvents and the present hydroxyl groups. Studies have focussed until now mainly on monosaccharides but also results on oligosaccharides[4] will be discussed as it is here where chemical biology can profit the most from this approach.

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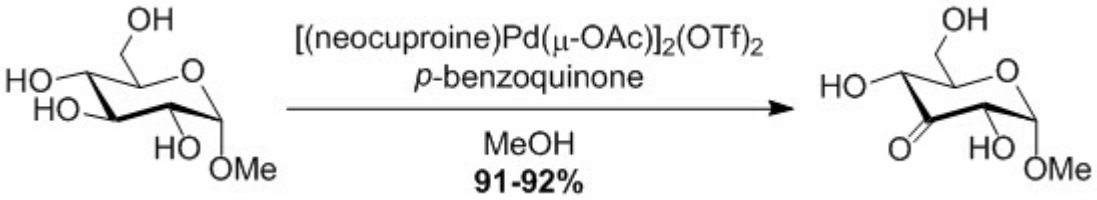

selective palladium-catalyzed oxidation of methyl glucose

KL7.4

Photocrosslinking Sugars for Glycoconjugate Interaction Discovery

Jennifer Kohler[1], Amberlyn Wands[1], Daniela Carroll[1], Nageswari Yarravarapu[1], Atossa Ghorashi[1], Han Wu[1]

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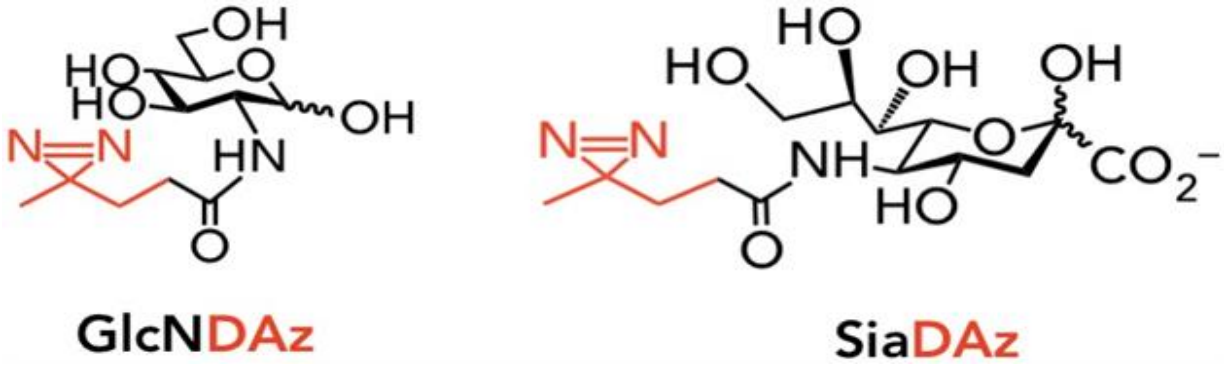
Carbohydrate structures, also known as glycans, are ubiquitous in biology. Cells from all living organisms are coated with glycosylated molecules that dictate extracellular recognition events, and glycosylated molecules play important intracellular roles as well. Despite many critical functions in processes as diverse as development, immunology, cell signaling, neurobiology, and infectious disease, methods to study glycan function lag behind approaches applied to other biomolecules such as proteins and nucleic acids. To facilitate identification of glycan binding partners, we have prepared photocrosslinking sugar analogs of two monosaccharides – sialic acid and N-acetylglucosamine (GlcNAc) – and devised strategies to incorporate these unnatural sugars into cellular glycoconjugates in place of their normal counterparts. Subsequent ultraviolet irradiation of intact cells results in covalent crosslinking of the glycan and its binding partner(s). The covalent complexes can be analyzed by a variety of approaches including immunoblot and mass spectrometry-based proteomics to define glycan-based recognition events that occur in a cellular setting. I will describe the methods that we developed for photocrosslinking sugar incorporation as well as two applications of this technology – (1) defining host cell receptors for cholera toxin and (2) understanding the mechanistic basis of signaling by the intracellular O-GlcNAc modification.



Photocrosslinking sugar analogs

KL8.1

Chemoenzymatic Glycan Editing for Boosting the Efficacy of Cell-Based Cancer Immunotherapy

Peng Wu[1]

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Cancers, such as breast and colon cancer, are chronical diseases that affect millions of people worldwide. Adoptive cell transfer-based therapies have received lots of attention in recent years for treating various types of cancers. Kymriah, a chimeric antigen receptor T-cell (CAR-T) therapy that was approved recently as the first cell-based gene therapy in the United States, is a preeminent example. However, in the tumor microenvironment has evolved numerous strategies to downregulate the cytotoxicity of the transferred T cells. In this talk, I will describe a strategy that is based on chemoenzymatic editing of cell-surface glycans to boost the efficacy of cell-based immunotherapy.

KL8.2

New Orthologs of the Pseudomonas Aeruginosa Lectin Leca in Pathogenic Bacteria

Alexander Titz[1], Ghamdan Beshr[1], Asfandyar Sikandar[1], Julia Gläser[1], Stefanie Wagner[1], Jesko Köhnke[1]

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Lectins play important roles in infections by pathogenic bacteria, for example, in host colonization, persistence and biofilm formation. Among Gram-negative bacteria, numerous homologs of the fuco-/mannophilic Pseudomonas aeruginosa lectin LecB have been reported. In contrast, reports on orthologs from its lectin LecA are rare despite the protein's role in biofilm formation and virulence of the WHO priority 1 pathogen P. aeruginosa.
The entomopathogenic bacterium Photorhabdus luminescens symbiotically lives in insect-infecting Heterorhabditis nematodes and kills the insect host upon invasion by the nematode. The P. luminescens genome harbors the gene plu2096 coding for a novel lectin that we named PllA.[1] This protein has a strict specificity of PllA for α-galactoside-terminating glycoconjugates which could be explained by its crystal structure.
Further, we report a novel ortholog of LecA from the ESKAPE pathogen Enterobacter spp.. This protein has been cloned, expressed and purified. Biochemical characterization and analysis of its glycan binding revealed an unexpected specificity for distinct human blood group antigens and, surprisingly, a lack of galactose-binding which is conserved among the previously characterized relatives LecA and PllA.

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KL8.3

Molecular Basis of Broad Spectrum N-Glycan Specificity and Processing of Igg Antibodies by Endoglycosidase S2

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Therapeutic immunoglobulin G (IgG) antibodies are a prominent and expanding class of drugs used for the treatment of several human disorders including cancer, autoimmunity, and infectious diseases. IgG antibodies are glycoproteins containing a conserved N-linked glycosylation site at residue Asn297 on each of the constant heavy chain 2 (CH2) domains of the fragment crystallizable (Fc) region. The presence of this N-linked glycan is critical for IgG function contributing both to Fc γ receptor binding and activation of the complement pathway. The precise chemical structure of the N-linked glycan modulates the effector functions mediated by the Fc domain. IgG antibodies including those produced for clinical use typically exist as mixtures of more than 20 glycoforms, which significantly impacts their efficacies, stabilities and the effector functions. To better control their therapeutic properties, the chemoenzymatic synthesis of homogeneously N-glycosylated antibodies has been developed.

Streptococcus pyogenes secretes two multidomain antibody-specific endoglycosidases, EndoS and EndoS2, which are central to these pathways. EndoS2 has a broader substrate specificity compared to EndoS, hydrolyzing not only biantennary complex type N-glycans, but also high-mannose, hybrid, and bisecting complex type N-glycans on IgG. Glycosynthase mutants of EndoS2 have also been developed to engineer antibodies with a more diverse set of N-glycans than similar EndoS mutants are capable of creating. In this work, we carried out a detailed study of the structure and function of EndoS and EndoS2, and elucidated the molecular mechanism by which EndoS2 recognizes an expanded repertoire of N-glycans compared to EndoS. Strikingly, this mechanism involves the action not only of the glycoside hydrolase domain, but also that of a carbohydrate binding module, which proved to be essential for IgG recognition and catalysis. These works certainly set the foundation for engineering enzymes to carry out customizable antibody glycosylation reactions for the diagnosis and treatment of human diseases.

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KL8.4

Chemical Gagobiology: Optimized Chemistry for Tailored Probes

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Heparan Sulfate (HS), a member of the Glycosaminoglycan family, is a linear and sulfated polysaccharide displaying a high level of molecular diversity. HS chains interact and modulate the activity of numerous HS binding proteins (HSBP), amongst which validated therapeutic targets [1]. The selective inhibition of a given protein/HS interaction has been attracting increasing interest as an original therapeutic mode of action, especially since the commercialization of Arixtra® [2], the synthetic version of a pentasaccharide sequence specifically recognizing and activating antithrombin-III. To date, it has not been possible to generalize to other HSBP the strategy used to identify this sequence. In Orsay, we aim at overcoming such a bottleneck by conceiving and synthesizing tailored probes to address different aspects of HS biology and devise therapeutic innovation based on the modulation of HS-HSBP interactions [3]. To reach these aims, we developed new methodologies in organic synthesis and optimized each step by determining their critic parameters [4], thus illustrating that glycochemistry represent an ideal playground to revise central concepts in organic chemistry, as well as to initiate original developments and methodologies.

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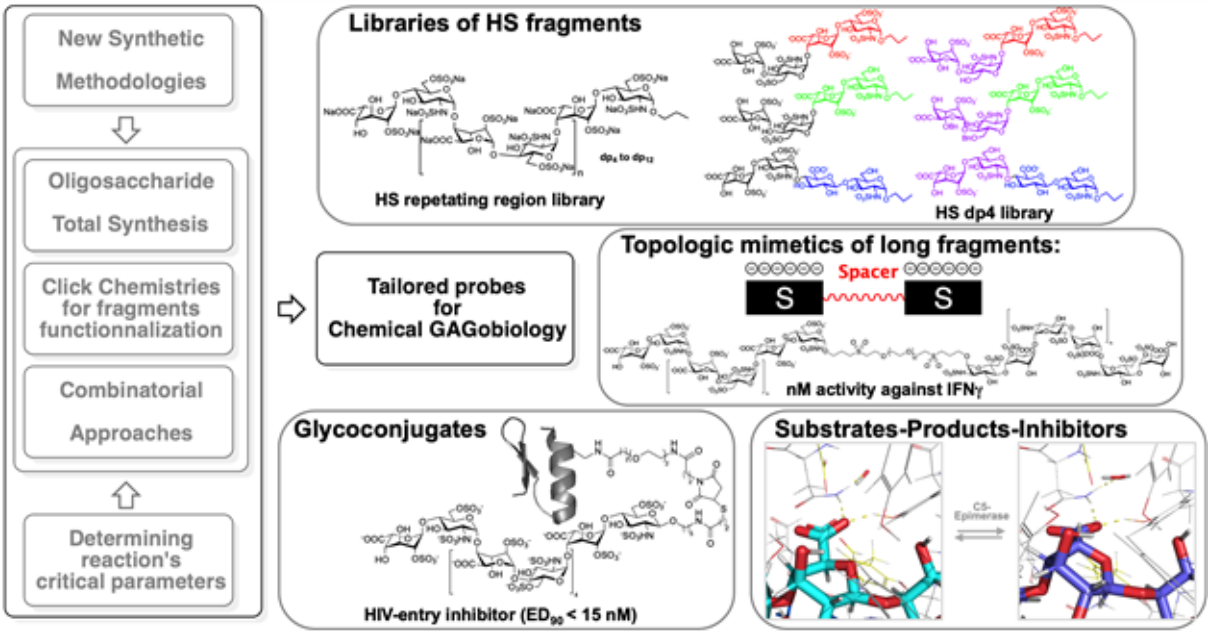

Organic synthesis for tailored probes

KL9.1

The Quest for Pharmacological Chaperones for the Treatment of Pompe Disease

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Pompe disease is an inborn metabolic myopathy caused by deficiency of the lysosomal enzyme acid α-glucosidase (GAA) and characterized by glycogen accumulation in lysosomes causing severe secondary cellular damage resulting in progressive motor handicap and premature death. We have previously determined the high-resolution crystal structure of recombinant human GAA (rhGAA), the standard care of Pompe disease, in its unbound form and in complex with inhibitors [1]. These structures provide the molecular framework for the rationalization of mutations associated with fatal infantile Pompe disease, offering guidance for therapeutic approaches and affording an accurate tool for in silico screening for molecules serving for future therapies. Although since its approval in 2006 enzyme replacement therapy for Pompe disease with rhGAA shows clinical benefits, the treatment is limited by variable patient's response, insufficient targeting and uptake in muscle tissues and immunogenic reactions. Pharmacological chaperone therapy (PCT), based on the concept that small-molecule ligands may block conformational fluctuations of a partially misfolded protein, rescuing its functional state, has been recognized as an appealing alternative therapeutic approach to ERT. Furthermore, pharmacological chaperones can act as enzyme enhancers when co-administered with rhGAA, by favouring enzyme delivery, stability and maturation, making PCT independent from the type of mutation carried by patients. We had already determined the 3-D structure of rhGAA in complex with the pharmacological chaperone N-acetyl-cysteine [2] which provided hints of its stabilizing function at the molecular level. Here we will present our latest results on structural studies of rhGAA in complex with small molecules which bear the potential of serving as selective pharmacological chaperones for the treatment of Pompe patients.

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KL9.2

Dissecting the Molecular Details of O-Galnac Glycosylation by Glycosyltransferase Bump-And-Hole Engineering

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O-GalNAc glycosylation is among the most abundant yet least understood posttranslational modifications. O-glycans contribute to the biophysical properties of the glycocalyx and are crucial mediators of biological processes.[1][2] As part of the glyco-code, O-glycosylation is encoded by a family of 20 polypeptide GalNAc transferase (GalNAcT) isoenzymes that introduce the first, Ser/Thr-linked GalNAc residue.[3] Despite partial redundancy, distinct GalNAcTs have been associated with disease, suggesting a pivotal role of isoenzyme-specific protein substrates. However, studying these substrates by glycoproteome analysis in GalNAcT knockout cell lines is complicated by the cross-talk of different isoenzymes.[4]

Here, a chemical biology method termed “bump-and-hole engineering” is used to dissect the details of GalNAcT isoenzyme specificity in the living cell.[5][6] In a structure-guided process, the active site of a GalNAcT is enlarged by mutation, creating a “hole” that renders the enzyme compatible with a chemical functional group (“bump”) in a synthetic UDP-GalNAc derivative. Extensive structural and functional characterization ensures viability of the orthogonal enzyme-substrate pair to glycosylate native protein substrates. A traceable chemical handle in the bump allows for the specific detection of glycoproteins by bioorthogonal ligation. The GalNAc salvage pathway is programmed to deliver bumped UDP-GalNAc derivatives to the cell, and glycoproteome analysis enables the characterization of GalNAcT isoenzyme-specific glycosylation sites and glycan fine structure in a single experiment.

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KL9.3

Expedient Stereoselective Synthesis of Glycosides. Old Catalysts, New Tricks

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The stereoselective synthesis of glycosides remains one of the biggest challenges in carbohydrate chemistry.(1) The chemical synthesis of complex carbohydrates generally involves the coupling of a fully protected glycosyl donor bearing a leaving group at its anomeric centre, with a suitably protected glycosyl acceptor (R-OH). In many instances, these reactions lead to a mixture of two stereoisomers.

In recent years, our group has endeavoured to develop catalytic and stereoselective methods to address this important synthetic challenge.(2-5) Recent years have seen a steady increase in the application of organocatalysis applied to oligosaccharide synthesis,(3) since the reaction conditions are mild and the careful choice of catalyst can offer significant improvements over traditional methods in terms of atom economy, high yields and control of anomeric selectivity.
Herein, we will report our latest developments on the application of borane catalysis to oligosaccharide synthesis. We will discuss the substrate-controlled direct alpha-stereoselective synthesis of deoxyglycosides from glycal whereby 2,3-Unsaturated alpha-O-glycoside products can be obtained with deactivated glycals at 75 oC in the presence of the catalyst, while 2-deoxyglycosides are formed using activated glycals that bear no leaving group at C-3 at lower temperatures. The reaction proceeds in good to excellent yields via concomitant borane activation of glycal donor and nucleophile acceptor. The method is exemplified with the synthesis of a series of rare and biologically relevant oligosaccharide analogues.

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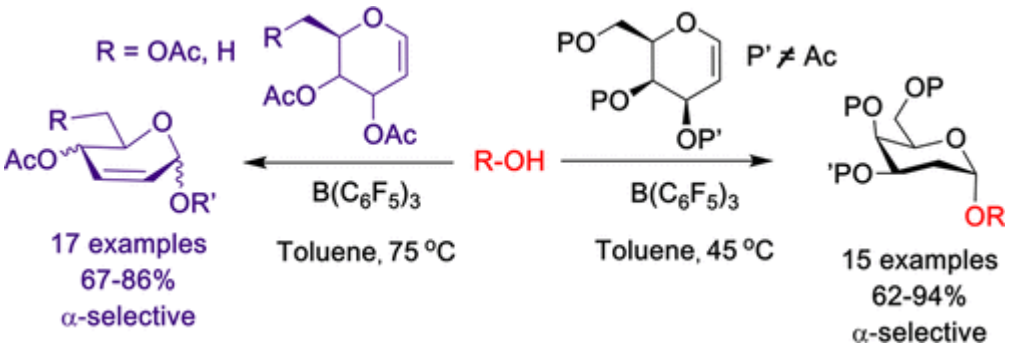

KL9.4

Synthesis and Applications of Heparin-Like Oligosaccharide Fragments

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Heparin/heparan sulfates (H/HS) are linear glycosaminoglycan (GAG) oligosaccharides typically occurring with the backbone modified by a variety of different sulfation patterns. They play diverse regulatory roles in biology. Synthesis of defined HS-like fragments has seen extensive interest from a a range of labs, both for defining new activities, including synthetic anti-coagulants, but also as tools to investigate chemical biology with defined microhetereogeneity. Improved strategies that shorten syntheses are important, as are applications to site-specific diversity, scalability and modifications for conjugation. Here we will describe synthetic entry to long H/HS-like backbones, contributing to expanding the synthetic heparanome, and show how programed synthesis can provide insights into micro-structure specific regulatory effects on HS-dependent biology, and also how synthetic design can afford scalable access to long bioactive oligosaccharides.

This presentation will describe enabling gram-scale syntheses of HS-type dodecasaccharides,1 based on scalable processes for iduronate bulding blocks, and efficient block-style syntheses. Synthetic access to these structurally-varied long HS targets has enabled identification of a structure-specific orthogonal regulation on chemokine-mediated biology1a,3 and evidence for enhanced tumour sensitization in combination therapy,4 and provided tools for PK. Block-synthesis strategy also enables synthetic access to substantially loner H/HS-related oligosaccharides (up to 40-mer backbone).5 This contributes to help establish that that longer HS fragments are viable synthetic targets for biomedical targets and the pursuit in many labs now of designer HS synthesis to identify new structure-specific chemical biology to potentially underpin new glycotherapeutics.

New conformationally-controlled glycosylations using a bicyclic iduronate lactone2 is also described. This facilitates reversing the order of GAG fragment syntheses from the traditional reducing terminal to non-reducing terminal direction. This has also been deployed now to intercept prior H/HS-like fragments, but also as a viable unit for synthesis of Dermatan Sulfate GAG fragments also,6 and so hopefully reagents and strategies here may contribute to providing availability of ranges of synthetic GAG fragments.

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OL1.1.1

Thienyl-Substituted A-Ketoamide: A Less Hydrophobic Photoreactive Group Useful for Analysis of Carbohydrate-Protein Interaction

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Identification of target proteins for bioactive molecules or glycoconjugates is a key issue in chemical (glyco)biology. However, capturing direct interactions between bioactive molecules and their target is still challenging, especially when the target biomolecules are low-abundance proteins and/or low-affinity binding partners. Photo-affinity labeling (PAL) has been widely accepted as one of the most powerful approaches to overcome these challenges, because covalent bond formation via photo-irradiation allows for the detection of reversible interaction, even at cell level. In this study, we report the 2-thienyl α-ketoamide as a less hydrophobic and more compact photo-reactive group, and can be used as an alternative to benzophenone for PAL. Whereas previous reports indicated unfavorable photo-degradation for α-ketoamides, we found that the 2-thienyl group provided low electrophilicity and high photo-stability to α-ketoamides, enabling successful PAL with α-ketoamide. Using an α-ketoamide functionalized mannose and a mannose-binding protein, Concanavalin A, as a model set of interacting partners, we demonstrate the viability of 2-thienyl α-ketoamide as a photoreactive group for the capturing of low-affinity carbohydrate-protein interactions. The most characteristic feature of our new photoaffinity group is its significantly lower non-specific labeling of other proteins, compared to other representative photo-reactive groups.

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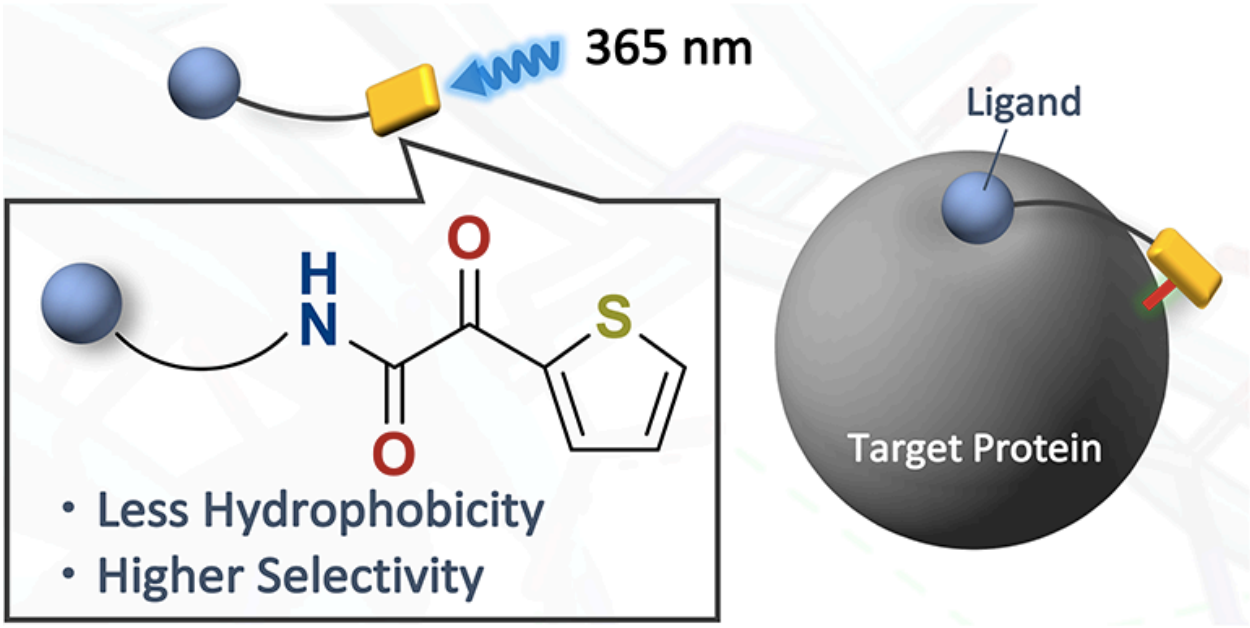

2-Thienyl α-Ketoamide group as a new photo-reactive group

OL1.1.2

Manno-Configured Epi-Cyclophellitol Aziridine Based Fluorescence Polarization Activity-Based Protein Profiling Identifies New Golgi A-Mannosidase Inhibitors

Zachary Armstrong[1], Daniël Lahav[2], Rachel Johnson[1], Chi-Lin Kuo[2], Thomas J. M. Beenakker[2], Casper de Boer[2], Chung-Sing Wong[2], Marjoke F. Debets[2], Mario van der Stelt[2], Jeroen Codée[2], Johannes M. F. G. Aerts[2], Liang Wu[1], Hermen Overkleeft[2], Gideon Davies[1]

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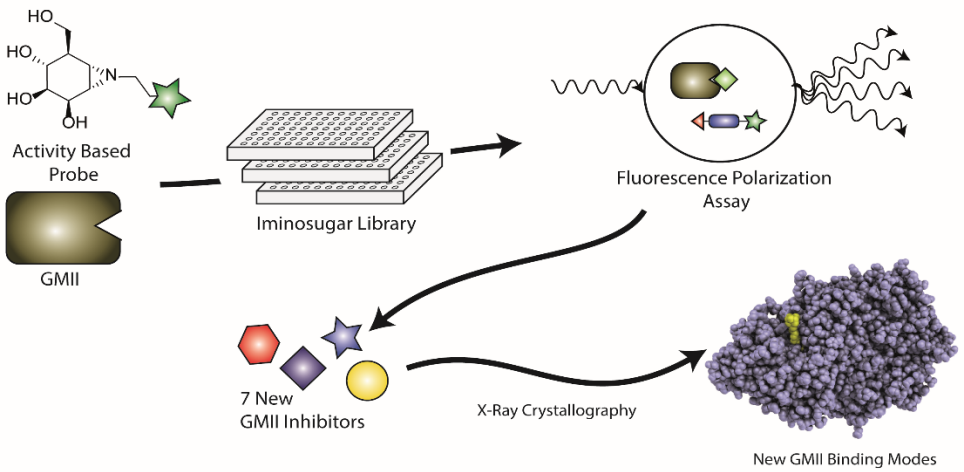
As part of the N-glycan processing pathway, Golgi mannosidase II (GMII) catalyzes the sequential hydrolysis of two mannosyl residues from $GlcNAcMan_5GlcNAc_2$. The product of this hydrolysis ($GlcNAcMan_3GlcNAc_2$) is the precursor for all complex N-glycans, including the branched N-glycans associated with cancer. Inhibitors of GMII reduce the production of complex N-glycans and are therefore potential cancer therapeutics. Despite many studies targeting the inhibition of GMII, potent and selective inhibitors GMII over other alpha-mannosidases remain elusive, and α-mannosidosis-like symptoms due to inhibition of lysosomal alpha-mannosidase are therefore a major concern. It is in this context that we sought to design a fluorescence polarization screen for new GMII inhibitors. We synthesized both manno-epi-cyclophellitol epoxide and aziridine and demonstrated covalent modification and time-dependent inhibition of Drosophila melanogaster GMII (dGMII) with these inhibitors. The manno-epi-cyclophellitol aziridine was then used as a scaffold to design a fluorescent α-mannosidase activity based probe, which was implemented in a fluorescence polarization based screen for dGMII inhibitors. We identified 7 previously unknown inhibitors of dGMII from a library of over 350 iminosugars and investigated their binding modalities through X-ray crystallography. This revealed previously unobserved inhibitor binding modes and promising scaffolds for the generation of selective inhibitors.



OL1.1.3

Sydnone-Modified Neuraminic Acids for a More Selective Metabolic Oligosaccharide Engineering of Living Cells

Frederic Friscourt[1,2]

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The bioorthogonal chemical reporter strategy, which elegantly combines the use of metabolically labeled azido-sugars and 1,3-dipolar cycloadditions with strained alkynes, is emerging as a versatile technology for the labeling and visualization of glycans.[1] Advantages of cyclooctyne-based probes encompass their high reactivity, non-toxicity (metal-free conditions) and synthetic modularity. However, the azido-reporter is not completely biologically inert as it can react, to varying degrees, with biological functionalities such as thiols.[2] This inherent instability makes the azide functionality a precursor for the potential accumulation of secondary metabolites with unknown biological effects.
In order to address this limitation, while keeping the advantages of the cyclooctyne framework as the reactive probe, we decided to investigate the utilization of other stable 1,3-dipoles as novel reporters. In this context, we present herein the utilization of 3,4-disubstituted sydnones, a singular class of aromatic mesoionic dipoles, as novel chemical reporters[3] for the metabolic oligosaccharide engineering (MOE) of sialoconjugates in living cells (Figure).[4]
The positioning of the reporter on the neuraminic acid was found to significantly alter its metabolic fate. Further in vitro enzymatic assays revealed that the 9-modified neuraminic acid is preferentially accepted by the sialyltransferase ST6Gal-I over ST3Gal-IV, leading to the favored incorporation of the reporter into linkage-specific 2,6-N-linked sialoproteins. Due to its high biological stability and more selective glycan incorporation, this novel chemical reporter will significantly expand our chemical biology toolbox for investigating the roles of specific sialosides.

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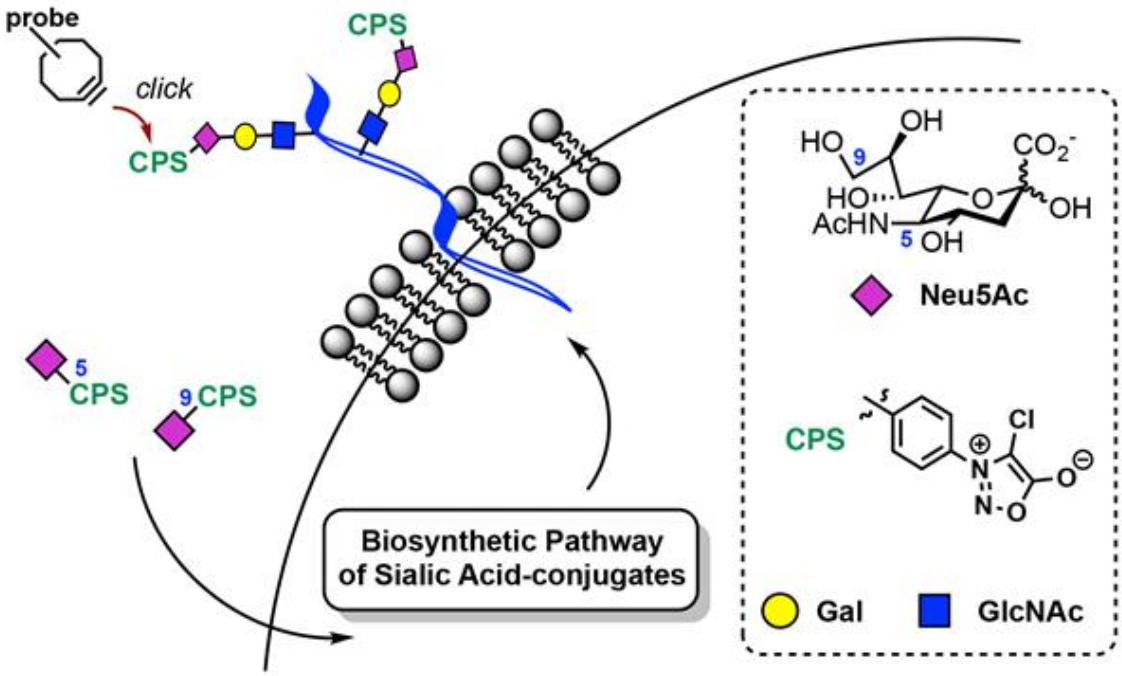

Chemical remodeling of cell-surface sialoconjugates using sydnone-modified sialic acids

OL1.1.4

Developing Carbocyclic Activity-Based Probes for Sialidases

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Sialic acids are a class of monosaccharides that occur on the termini of a wide range of glycans and are vital in intercellular communication. Sialidase enzymes are glycosidases that cleave sialic acids from these glycans and are distributed in various kinds of organisms. Sialidase activity is involved in various diseases[1] that are not yet fully understood and therefore sialidases are an attractive target to study and perturb. Based on the succesfull design of carbocyclic covalent inhibitors and activity-based probes (ABPs) for other glycosdases by Overkleeft et al.[2] we have developed a synthetic route for carbocyclic ABPs for sialidases that we are currently evaluating for its ability to inactivate sialidases.
With the goal of synthesising the carbocyclic epoxide(1, 3) and aziridine(2, 4) mimics of neuraminic acid, we developed a synthetic route starting from a chiral furanone towards a key intermediate allowing for ring closing metathesis[3]. Using a specialised Grubbs catalyst for sterically hindered systems, ring closing metathesis yielded a carbocycle with the desired stereochemistry for neuraminic acid. Further transformations of this carbocycle lead to the covalent sialidase inhibitors 1 and 3 and ABPs 2 and 4.

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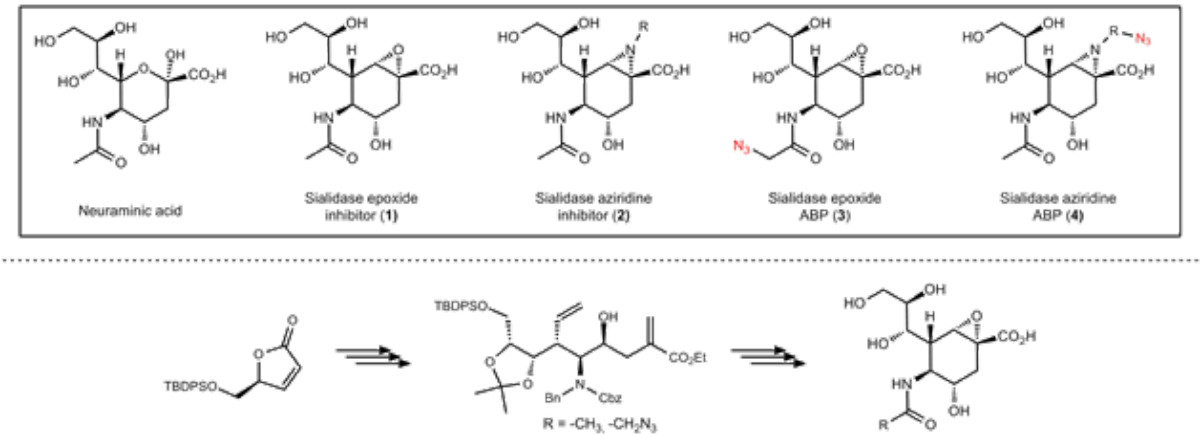

Sialidase inhibitors and ABPs and the synthetic approach towards them

OL1.2.1

Total Synthesis, Structural and Biological Studies of Fragments of T-Cell Dependent Antigen Zwitterionic Polysaccharide Sp1

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Typically, bacterial capsular polysaccharides are considered to be “T-cell-independent antigens” which cannot be used in a vaccine setting, leading to immunological memory, without conjugating to a protein. Zwitterionic polysaccharides (ZPSs) are a rare class of immunomodulatory agents that can provoke a T-cell mediated immune responses through MHC II binding and CD4+ T-cell activation.[1] Zwitterionic polysaccharide Sp1, isolated from the human pathogen Streptococcus pneumoniae, contains repeating units of the trisaccharide below (Figure A).[2] To explore its detailed structure–activity relationship (SAR) and immunomodulatory mechanism, well-defined oligosaccharides of Sp1 are required. The synthesis of Sp1 oligosaccharides is extremely challenging because of the presence of rare monosaccharide constituents (such as trideoxy-diaminogalactose residues), the 1,2-cis-glycosidic bonds, and both positive and negative charges.[3]

Here, we report the synthesis of various oligosaccharide fragments with a varying number of repeating units, with and without O-acetyl groups, using both pre- and post-glycosylation oxidation strategy. We also present its structural studies (molecular dynamics (MD), Molecular Modeling) and the biological evaluation on these ZPS oligosaccharides. Finally, using STD-NMR (Figure B and C), we found that the synthetic Sp1 analogues interact with an anti-Sp1 monoclonal antibody via a groove-type site topology in an extended binding mode.

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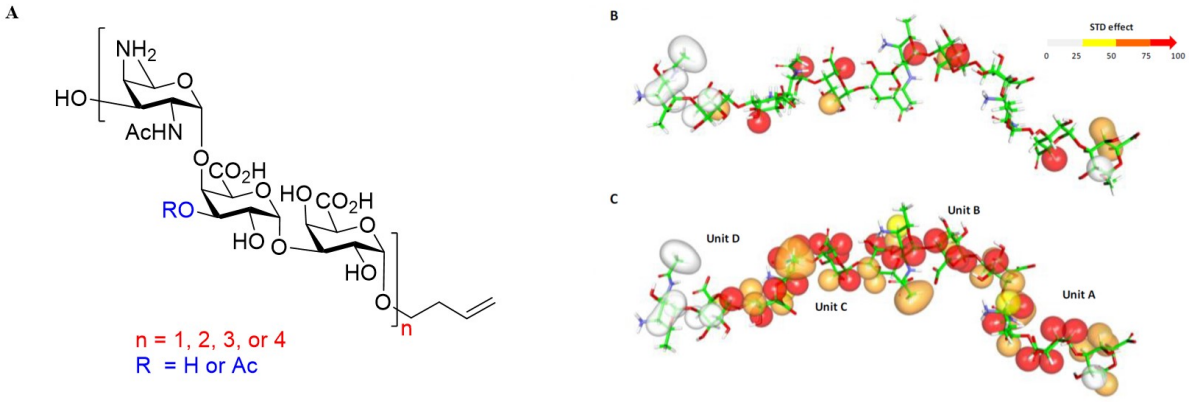

Figure. A) The targets structure of the Sp1. B) Structure of dodecamer. The relative STD effect for non-overlapped protons is indicated by colors. C) Structure of dodecamer, in which the relative STD effect for overlapped protons was equally distributed.

OL1.2.2

Synthesis of an Hiv-1 Related Undecasaccharide Containing an Oligomannosidic Heptasaccharide and A Bacterial Kdo-Lipid A Backbone

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Gp120, present on the surface of the human immunodeficiency virus (HIV), harbors clusters of oligomannose structures that are recognized by several broadly neutralizing antibodies and thus constitutes an important target for HIV-1 vaccine development. Lipooligosaccharide (LOS), isolated from the bacterium Rhizobium radiobacter Rv3, closely resembles these oligomannose structures present on gp120 and immunization of wild-type mice with heat-killed bacteria elicited serum antibodies, which bound to monomeric gp120.[1] In order to boost the immune response against HIV-1 related oligomannosides we have set out to combine a recently identified broadly neutralizing heptamannosidic epitope with a common antigen of the inner core of bacterial lipopolysaccharide.[2] Starting from the Kdo2GlcNAc2 tetrasaccharide 1, a central orthogonally mannose unit was coupled in good yield to OH-5 of the innermost Kdo residue,[3] which was followed by a blockwise coupling with an α-(1→2)-linked mannotriosyl trichloroacetimidate donor to introduce the D1-arm fragment. Deprotection of 3 led to the octasaccharide 5 corresponding to the R. radiobacter Rv3 lipooligosaccharide fragment. Next, glycosylation of O-6 of the inner mannose furnished 4 - harboring the D3-arm extension - followed by global deprotection to give the spacer-equipped undecasaccharide 6 featuring a hybrid structure of viral and bacterial epitopes.

Acknowledgment: Financial support from Austrian Science Fund (FWF, grant P26919-N28) and NIH (R01 AI134299 to RP).

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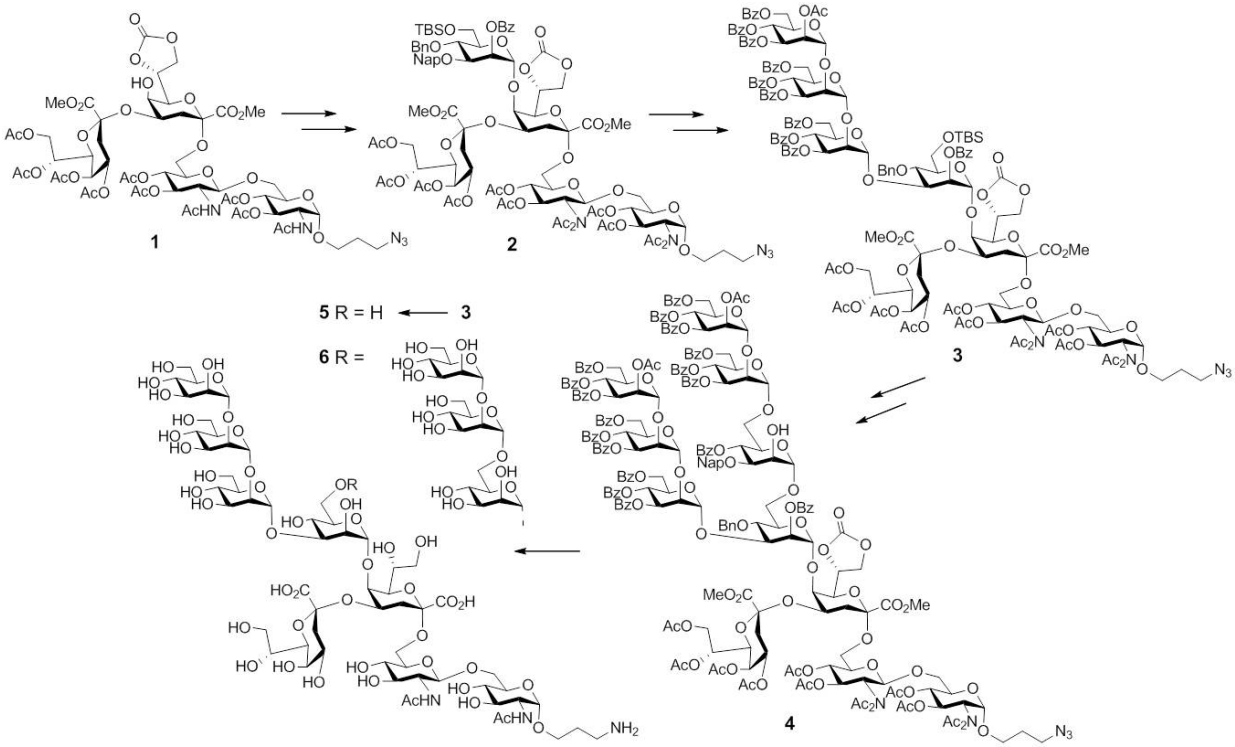

Synthesis of rhizobial octasaccharide 5 and HIV-related undecasaccharide 6

OL1.2.3

Synthesis of Bifidobacterium Adolescentis Eps Fragments Containing Cis-Linked 6-Deoxy-L-Talose

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Exopolysaccharides (EPS) are present on the outside of bacteria, where they can be loosely attached to the cell wall or secreted in the environment. EPS of lactobacilli and bifidobacteria demonstrate several beneficial effects such as antitumor activity, and they serve as prebiotic or as immune modulators.[1] The EPS structure of Bifidobacterium adolescentis, a beneficial strain commonly observed in the gut microbiome, contains 6-deoxy-L-talose (6dTal) residues linked in a 1,2-cis fashion.[2] To understand the biological impact of the cis-linked 6dTal moieties, well-defined structures are needed. Because little is known about the glycosylation properties and preferences of 6dTal, this is the main challenge of this project.

To develop a robust method to attach multiple 6dTal residues through cis-linkages, an efficient protective group strategy, together with a study on the reactivity and selectivity of the resulting 6dTal donors, is essential. To orthogonally protect the C-3 position to allow subsequent elongation, various protective groups were introduced regioselectively. The resulting donors were glycosylated to a set of acceptors using different activation protocols. The best suitable donor was further applied in the oligosaccharide assembly.

This communication will demonstrate the unexpected properties of unusual 6dTal monosaccharides as building blocks and their properties and limitations when used in oligosaccharide synthesis.

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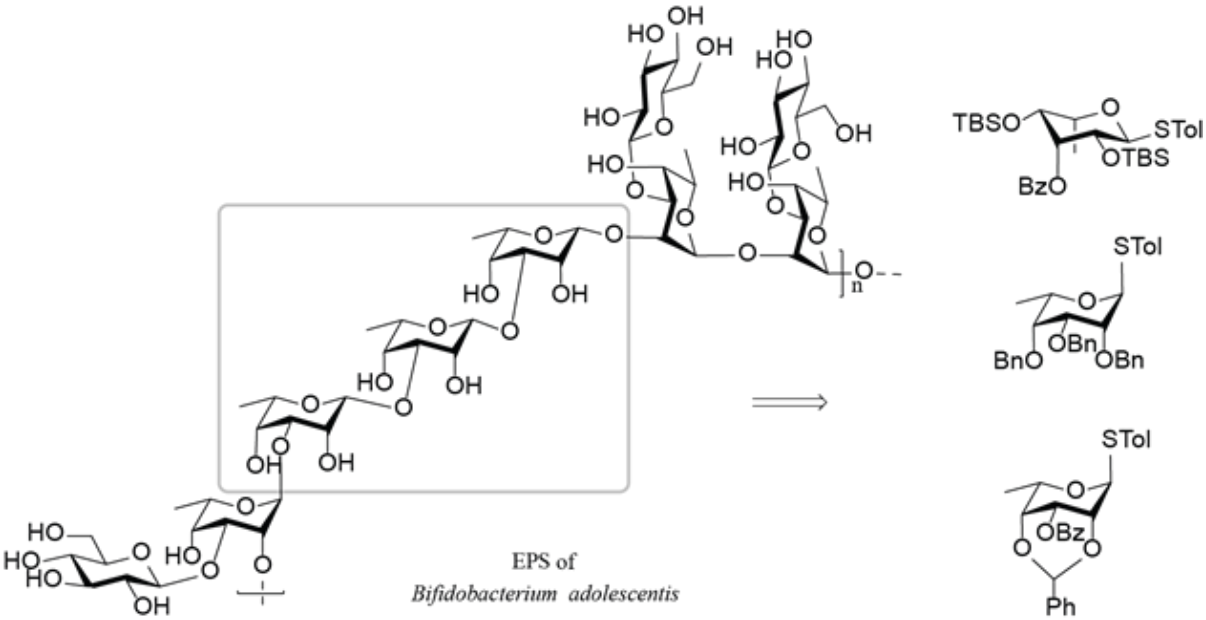

EPS of B. adolescentis and potential donors

OL1.2.4

Synthesis of Group B Streptococcal Oligosaccharide Libraries for Structure-Based Optimization of Vaccine Candidates

Linda Del Bino[1], Davide Oldrini[1], Maria Michelina Raso[2], Filippo Carboni[1], Riccardo De Ricco[1], Rossella Cuffaro[1], Maria Rosaria Romano[1], Immaculada Margarit y Ros[1], Francesco Berti[1], Roberto Adamo[1]

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Despite substantial progresses in the prevention of group B Streptococcus (GBS) diseases with the introduction of intrapartum antibiotic prophylaxis, this pathogen remains a leading cause of neonatal infections. On the basis of variation in the sugar composition of the capsular polysaccharide (CPS), which is a major virulence factor, ten serotypes of GBS have been identified and in the last few years CPS conjugate vaccines representing the most frequent serotypes have been tested in phase I/II clinical studies, showing promise for further development.[1]
The elucidation of polysaccharide epitopes (commonly defined as glycotopes) presented on the bacterial capsule is relevant for understanding the mechanism of action of glycoconjugates and designing synthetic carbohydrate-based vaccines. Recently, an X-ray/NMR based approach was applied to determine a functional epitope of GBS PSIII, which appeared composed of six residues included within two repeating units, paving the way towards the use of synthetic structures for vaccine development.[2]
The repeating units of GBS serotypes Ia, Ib and III share similarities in their sugar composition, such as the NeuNAcβ(1–›3)Gal branch and the GlcNAcβ(1–›3)Gal motif. A flexible and convergent synthetic route could offer access to GBS CPS Ia, Ib and III defined fragments. In particular, we envisaged the regioselective glycosylation of galactose 3-OH to form the disaccharide motif GlcNAcβ(1–›3)Gal as a key step for the synthesis of fragments of the three serotypes. For this reason we designed an alternative synthetic approach taking advantage of the well-recognized major reactivity of Gal 3-OH compared to the 4-OH: a number of glucosamine donors and galactose acceptors with a different pattern of protective groups were screened in order to optimize the regioselective glycosylation and a faster access to the target disaccharide.
The applied innovative synthetic design led to the first synthesis of the pentasaccharide repeating unit of GBS CPS serotype Ib. Furthermore, oligosaccharide libraries from the CPS serotypes Ia and III were prepared. A combined screening approach, including competitive ELISA, competitive SPR and STD-NMR, on the synthetic fragment collections could help to gain new molecular insights into interactions of oligosaccharides with mAbs and to identify optimal or sub-optimal glycotopes for conjugation to carrier proteins and immunological evaluation.

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[2] F. Carboni, R. Adamo, F. Berti et al. PNAS 2017, 114 (19,) 5017-5022

Figure 1. Regioselective approach to disaccharide 3

OL1.3.1

Natural Killer T Cell Activation by CD1d Glycolipid Ligand Based on “Anchoring Effect” of Polar Groups in Lipid Component

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CD1d is a non-polymorphic MHC class I-like molecule present on cells such as dendritic cells, and its ligands include glycolipids such as α-GalCer (KRN7000). Complexes of glycolipid ligands and CD1d are recognized by T cell receptors (TCR) on NKT cells and induce the secretion of various cytokines, including Th1 and Th2 cytokines. Although Th2-biasing CD1d ligands are attractive potential candidates for adjuvants and therapeutic drugs for autoimmune diseases, the number of potent ligands is limited, and their biasing mechanism remain unclear.
In the present research, we have identified a series of novel Th2-biasing CD1d glycolipid ligands based on modification of their lipid part of α-GalCer structure [1,2]. These have shown high binding affinities and efficient Th2 cytokine production, and even truncated acyl chain-containing variants still retain their binding affinities and agonistic activities, which can be associated with an “anchoring effect.” i.e. formation of a buried hydrogen bond between a polar group (eg. amide) on the acyl chain and the CD1d lipid-binding pocket. Our analysis also indicated that the appearance rates of ligand-CD1d complexes on the cell surface were involved in Th2-biasing responses. We demonstrated that the ligands, having the “anchor” in the shorter lipid part, would be one of the most potent Th2-biasing ligands with keeping the total cytokine induction levels, among the known ligands.

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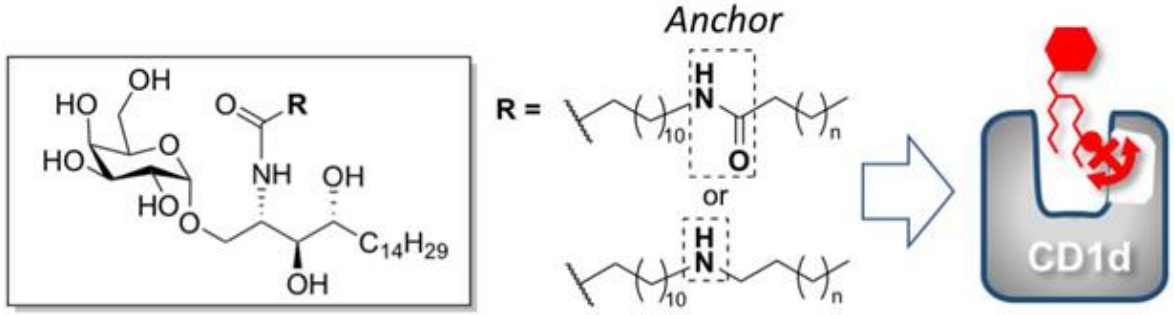

OL1.3.2

Covalent Tlr4-Ligand – Antigen Vaccine-Candidate Conjugates

Niels Reintjens[1], Elena Tondini[2], Nico Meeuwenoord[1], Fabrizio Chiodo[3], Herman Overkleeft[1], Ferry Ossendorp[2], Dmitri Filippov[1], Gijsbert van der Marel[1], Jeroen Codée[1]

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Irrespective of its target, a vaccine usually contains a mix of antigens and an adjuvant, that is required to stimulate the immune system. The exact molecular composition of existing vaccines is largely unknown, complicating the improvement of these vaccines. A promising strategy to generate novel vaccine modalities, having a well-defined and tunable mode of action, entails the conjugation of oligopeptide antigens to innate immunostimulatory agents, such as Toll-like receptor ligands. So far, several TLR agonists have been conjugated to antigenic peptides. Strikingly, the TLR4-ligand Lipid A, one of the most potent immunostimulating agens known to date, has not been explored in peptide-conjugate vaccine modalities [1]. Based on the structure of lipid A, a new class of potent monosaccharide adjuvants has been discovered, the aminoalkyl glucosamine 4-phosphates (AGPs), in which the reducing end glucosamine of lipid A is substituted with a functionalized serine residue [1, 2]. CRX-527 is one of the most potent AGPs and was therefore selected to use as a built-in adjuvant for the generation of novel conjugate vaccine modalities.

Herein, we describe the design, synthesis and immunological evaluation of synthetic long peptide vaccine conjugates 1-4 composed of an antigenic MHC-I peptide (SIINFEKL) and CRX-527 as built-in adjuvant (Figure 1). For its generation we improved the syntheses of the potent TLR4 agonist CRX-527 and (R)-3-alkyloxytetradecanoic acids and developed an efficient conjugation and purification strategy. Immunological evaluation shows that these covalent conjugates effectively initiate dendritic cell maturation and activation through TLR4 leading to enhanced cross presentation. Initial in vivo evaluation of the first AGP-based conjugates has shown the best strategy for this type of conjugates.

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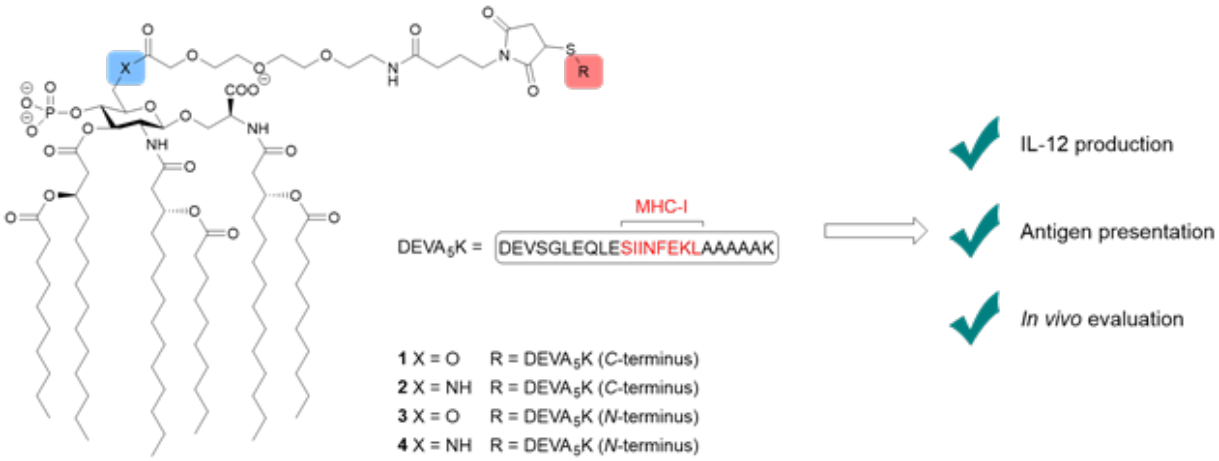

Figure 1. Structure of the synthetic long peptide conjugates 1-4.

OL1.3.3

Development of Alpha-Gal Conjugated Anti-Cancer Antibodies

Koichi Fukase[1], Julinton Sianturi[1], Yoshiyuki Manabe[1], Hao-Sheng Li[1], Li-Ting Chiu[2], Tsung-Che Chang[1], Kento Tokunaga[1], Kazuya Kabayama[1], Masahiro Tanemura[3], Shinji Takamatsu[1], Eiji Miyoshi[1], Shang-Cheng Hung[2]

[1]Osaka University, Toyonaka, Japan, [2]Academia Sinica, Taipei, Taiwan, [3]Osaka Police Hospital, Osaka, Japan

Due to their excellent specificity, antibodies are among the most important molecular targeted drugs for various diseases. In this study, we developed α-gal antibody conjugates that dramatically increased cellular cytotoxicity by recruiting natural antibody through α-gal/anti-Gal antibody interaction [1].
α-Gal epitope (Galα1-3Galβ1-4GlcNAc-R) is expressed abundantly on glycolipids and glycoproteins in non-primate mammals, prosimians, and New World monkeys. Humans do not have α-gal due to inactivation of α-1,3-galactosyltransferase. Instead, they make large quantities of anti-Gal antibodies, which specifically interact with α-gal. Anti-Gal antibodies are the most abundant natural antibodies found in humans (1–2% of total serum IgG and 3–8% of total IgM). Consequently, α-gal antigen causes an acute immune response. α-Gal/anti-Gal antibodies interaction following xenotransplantation from pig to baboon induced hyperacute rejection of the transplanted tissues.
In this study, we applied the ADC (antibody-drug conjugate) concept to develop a novel α-gal-based tumor immunotherapy. Because α-gal antigen causes an extremely strong immune response, anti-tumor antibodies conjugated with α-gal was expected to have high potency against tumors.
After chemical synthesis of α-gal, we achieved concise chemical conjugation of antibodies with α-gal. The synthesized α-gal antibody conjugates efficiently provoked an immune response. The potency of α-gal antibody conjugates depended on the α-gal loading ratio. The larger the amount of α-gal was introduced, the higher the cytotoxicity was observed. Utilization of α-gal dendrimer allowed introduction of large amounts of α-gal epitope to the antibody without loss of affinity for the target cell. The method described here will enable re-development of antibodies to improve their potency.

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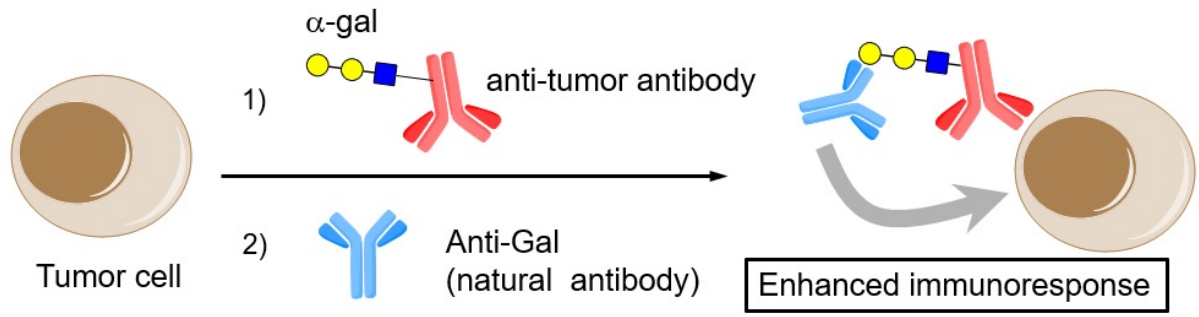

Strategy for tumor immunotherapy using α-gal antibody conjugate.

OL1.3.4

Sulfavants, New Class of Molecular Vaccine Adjuvants: Development in Immunotherapy and Correlation of The Colloidal Self-Assembly with Immune Cell Response

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Adjuvants are components of vaccine that enhance the specific immune response against co-inoculated antigens. Recently, we reported the characterization of a synthetic sulfolipid named Sulfavant A (1) as a promising candidate of a novel class of molecular adjuvants based on the sulfoquinovosyl-diacylglycerol skeleton [1]. Sulfavant A is able to prime human dendritic cells (DC) by a TLR2/TLR4-independent mechanism inducing maturation of DC with expression of high levels of MHC II molecules and upregulation of costimulatory proteins (CD83, CD86) and proinflammatory cytokines (IL-12 and INF-γ) [1]. Moreover 1 triggers an efficient immune response in vivo; in fact mice immunized with OVA associated to Sulfavant A (1:500) produced a titer of anti-OVA Ig comparable to traditional adjuvants. In an experimental model of melanoma, vaccination of C57BL/6 mice by Sulfavant A-adjuvanted hgp10 peptide elicited a protective response with reduction of tumour growth and increase of survival.
Improved synthesis of the sulfolipid scaffold, the preparation of two epimeric analogs named Sulfavant-S (2) and Sulfavant-R (3), as well as a study of self-aggregation of these molecule in water and the effect of this aggregation on biological response will be the object of this communication [2]. In the new synthetic approach, the strategy for Sulfavant A is reduced from 14 to 11 steps with approximately triplication of the overall yield (11%) permitting the preparation of the analogs 2-3 [2]. The new members Sulfavant R and S elicit DC maturation at a concentration of 10 nM, which is 1000 times lower than that of the parent molecule 1. Analysis by Dynamic Light Scattering (DLS) indicates self-assembly of Sulfavants and formation of colloidal particles with a small hydrodynamic radius (Rh~60 nm) for the epimers 2 and 3, and a larger radius (Rh~150 nm) for 1. Further studies on these aggregates were led by Small Angle Neutron Scattering (SANS) and Zeta potential analysis. The formation of colloidal aggregates is responsible for the different immunomodulant behavior of these molecules; it's possible that the particle size can influence the equilibrium with free monomers thus determining the effective concentration of the sulfolipid molecule at the cellular targets and the different immunological efficacy of 1-3. Sulfavants do not show in vitro cytotoxicity at concentrations 105 higher than the dose that triggers maximal immune response, thus predicting a low level of toxicological risk in their formulation in vaccines.

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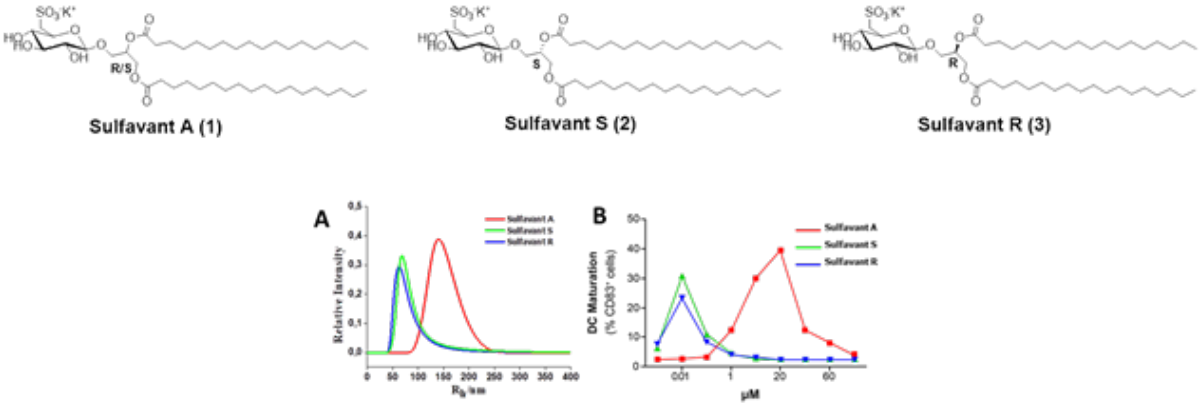

Figure 1. Sulfavants (1-3); (A) hydrodynamic radius of 1-3; (B) biological activity of 1-3

OL1.4.1

Evidences for Antigen-Antibody Molecular Recognition Event in an Antibody-Drug Conjugate System against P. Aureoginosa

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Antimicrobial resistance is a serious and growing problem for the public health, the number of infections from antibiotic-resistant microorganisms has increased significantly in the last few years. The pathogenic gram-negative bacteria, such as Pseudomonas aeruginosa and Acinetobacter baumannii are found resistant to many antibiotics, for this reason the design of new antibiotics, or new strategies to against this class of bacteria are urgently required [1]. Bacterial surface polysaccharide antigens represent one of the most important targets of immunotherapy to prevent bacterial infections, unfortunately the variability of the external part of the polysaccharide antigens, restrict the use of monoclonal antibodies (mAb), while an additional drawback is the lower bactericidal capacity of mAb. Recently a new engineered mAb (VSX) targeting the inner-core glycan of the Lipopolysaccharide (LPS) of P. aeruginosa, was biologically proven to recognise the less-variable part of the glycan antigen, and for this reason potentially targeting a wider class of bacteria. An Antibody-Drug-Coniugate (ADC) system was than designed connecting by covalent link an α-helical peptide able to affect the membrane integrity of P. aeruginosa. Biological tests in-vitro and in-vivo indicate that ADC is active against P. aeruginosa strains and protects mice from P. aeruginosa lung infection. Bacterial cell ELISA test indicates that VSX binds to 23 diverse P. aeruginosa strains, while it does not bind to other common human pathogenic gram-negative bacteria including multiple strains of Escherichia coli, Klebsiella pneumoniae and Salmonella typhimurium. These, and additional molecular biology evidences indicate that VSX recognize the conserved inner-core oligosaccharide, particularly the 2,4-diphospho α-D-Heptose moiety in the LPS present on the outer membrane of P. aeruginosa strains. To understand the molecular reasons by which VSX recognize the inner-core glycan of LPS, and possibly predicting how to tune it, the application of complementary structural biology techniques experimental and theoretical are strongly required. In a first stage the 2,4-diphospho α-D-Heptose monosaccharide is used as a model for the glycan part of LPS. 1H, 1H-STD, and 31P NMR spectra run on high sensitivity spectrometer cryo-probe 600MHz, allow to obtain a nearly atomic description of the interaction by which VSX recognize this glycan, depicting how the two phosphate groups, in particular 4-P, are required for the binding. Molecular Docking, and MD simulation with the “state of the art” GLYCAM06 [2] Force-Field are applied to build a 3D static and dynamic interpretation of the recognition event, which comprehension will be considered preparatory before to include bigger size glycan antigen.

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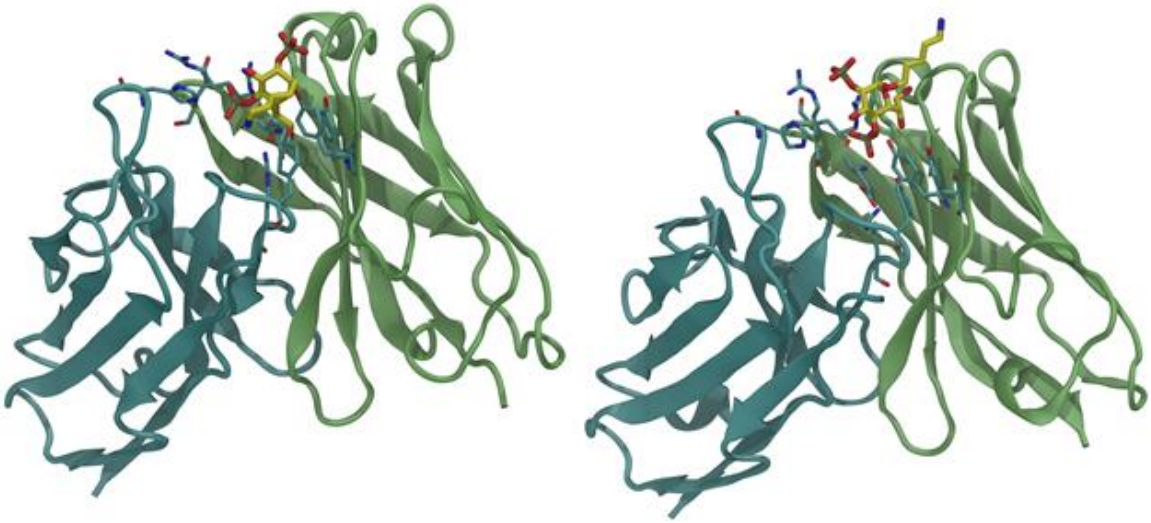

OL1.4.2

Modified Tunicamycins, Streptovirudins and Quinovosamycins: Potent Enhancers of the Penicillin Antibiotics

Neil Price[1], Michael Jackson[1], Todd Naumann[1], John Bannantine[1], Jenny Hering[2], Gisela Branden[3], Margareta Ek[2], Vinayak Singh[5], Valerie Mizrahi[5], Katarina Mikusova[4]

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Tunicamycin is lethal to bacteria and eukaryotes either by blocking cell wall biosynthesis or protein N-glycosylation, respectively. The tunicamycin uracil group mimics the UDP-HexNAc donor substrate, binding to a uridyl binding pocket within PNPT translocase enzymes. This is stabilized by a noncovalent π–π stacking interaction with a conserved Phe within the PNPT active site. We have structurally modified tunicamycins (TunR1 and TunR2) to be less toxic to animal cells, but which retain the antibacterial activity. TunR1 and TunR2 are also potent enhancers of the β-lactam family of antibiotics, resulting in increased activity of penicillins, cephems, and carbapenems. TunR2 in which the uridyl group is converted to a 5,6-dihydrouridyl has low eukaryotic toxicity, and enhanced aminothiazolidyl cephalosporins by >128-fold against B. subtilis and several pathogenic mycobacterial strains.

OL1.4.3

Synthesis and Biological Evaluation of Immunogenic Teichoic Acids Fragments

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Teichoic acids (TAs) are found abundantly as cell wall components of the majority of Gram-positive bacteria.1 Since the arising of antibiotic resistance bacterial strains in nosocomial infections, TAs have been identified as possible antigen candidate in vaccine development. Structurally TAs from S. aureus, E. faecalis and E. faecium share a glycerol-phosphate backbone that can be decorated with different glucosyl substituents. Both native and synthetic glycerol-phosphate TAs have been found to be immunogenic and to raised opsonic antibodies ensuring protection in both active and passive immunization.2 To further understand the binding between teichoic acids and antibodies, a new set of well-defined TAs hexamers was developed. These fragments differ for the position of a glucose moiety along the glycerol-phosphate backbone and for the stereochemistry of the glycerol unit. Using molecular dynamics, different population distributions of low energy conformers were observed among the structural similar diastereoisomers, revealing possible different spatial and chemical properties. Indeed, using a microarray tool,3 the preferential binding of polyclonal antibodies (raised against native TAs from E. faecalis or against a synthetic well-defined glycoconjugate) was affected not only upon the position of the carbohydrate moiety but also for the defined glycerol stereogenic center. Finally, STD-NMR experiments were employed to map the epitope of a glucosyl glycerol-phosphate hexamer and its related monoclonal antibody.

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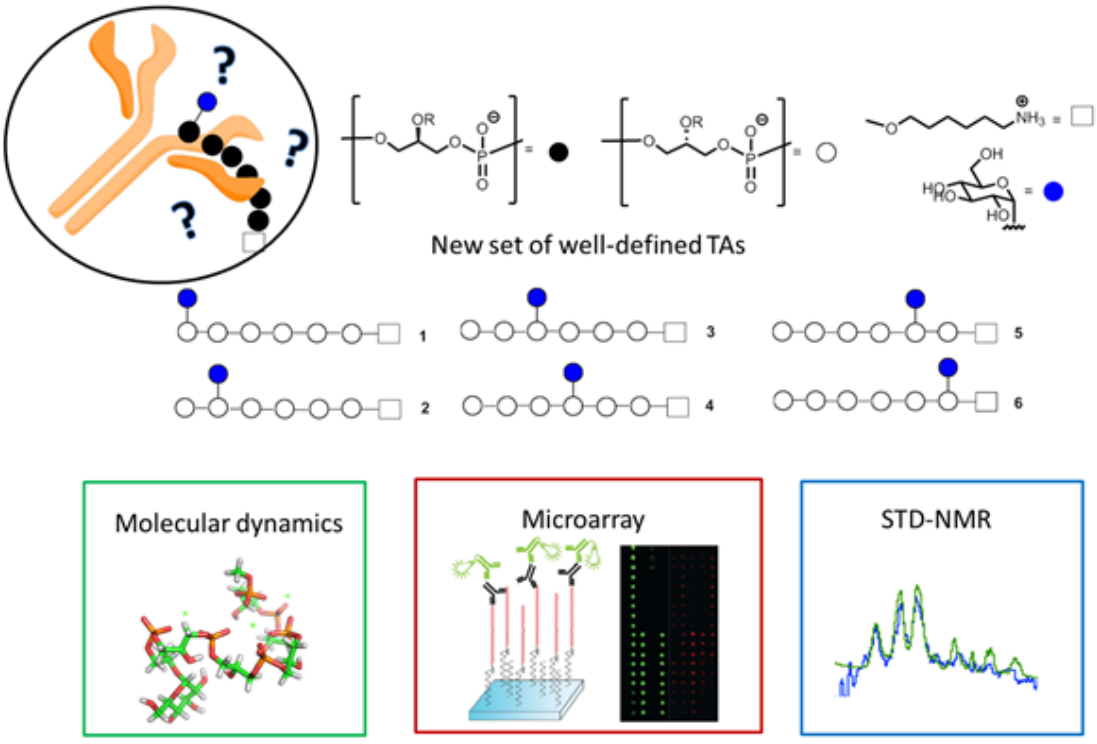

Figure: Synthesis of teichoic acids fragments. Evaluation using molecular dynamics, microarray and STD experiments.

OL1.4.4

Synthesis of Haemophilus Influenzae Type A Oligosaccharides

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The pathogen *Haemophilus influenzae* (Hi) is a major cause of severe diseases, i.e. meningitis, sepsis and otitis, especially affecting young children. Among 6 serotypes, type b (Hib) is the most common and most virulent strain. Additionally, Hib is the first successful example of a vaccine based on synthetic carbohydrate antigens licensed and distributed in Cuba since 2004 with the tradename QuimiHib. [1]

In recent years an increasing rate of infections caused by Hia raised some concern and currently, Hia causes up to 10 % of reported Haemophilus infections. This burden is steadily increasing, and no vaccine targeting Hia is currently available or under development. The capsular polysaccharide (CPS) of Hia is a polymer of 4-β-D-Glc-(1→4)-D-ribitol-5-(PO4→) repeating units and is a potential antigen for a future protein-conjugated polysaccharide vaccine. Furthermore, synthetic oligomers, such as 1 (Scheme 1), are valuable tools to identify a protective epitope within Hia CPS. [2,3]

In this communication we focus on the first described synthesis of oligomers with different chain length such as 1. Crucial and versatile building blocks (2 and 3) were synthesized and further coupled by using phosphoramidite approach. All synthesized oligomers contain a proper N-terminating linker suitable for further protein carrier conjugation. Different approaches and challenges for the oligomerization will be discussed.

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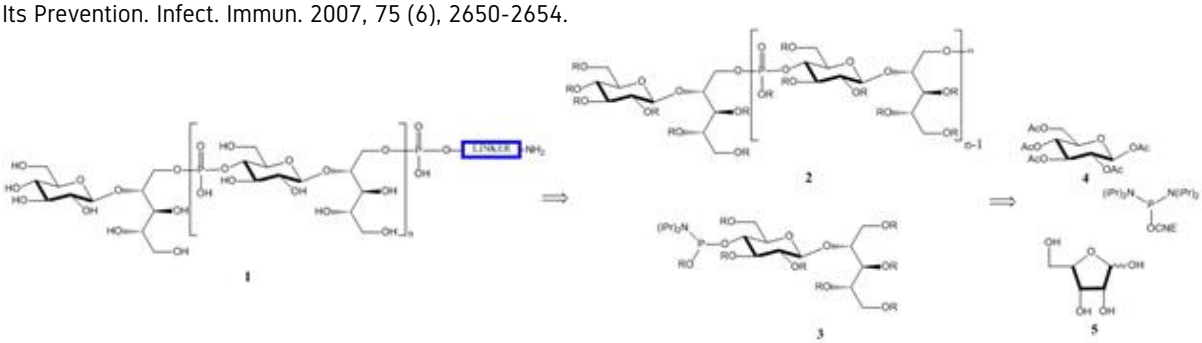

Scheme 1 Retrosynthetic strategy to achieve oligomers of Hia CPS

OL2.1.1

Burkholderia Pseudomallei and Burkholderia Mallei Synthetic Lipopolysaccharide Mimics as Potential Vaccine Candidates

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The Gram-negative bacteria (GNB) Burkholderia pseudomallei (Bp) and Burkholderia mallei (Bm) are the etiologic agents of melioidosis and glanders, respectively, and are endemic in tropical and subtropical regions. Due, among others, to their high mortality rate, their possible inoculation by inhalation, their intrinsic resistance to commonly used antibiotics, and the actual lack of vaccines against these diseases, the Centers for Disease Control and Prevention have classified Bp and Bm as potential bioterrorist weapons [1]. Owing to the dangers that Bp and Bm pose for the society, our project aims to prevent melioidosis and glanders infections by developing synthetic glycoconjugate vaccines against these pathogens.

Diverse polysaccharides are anchored in the outer membrane of GNB cells, including lipopolysaccharides (LPS). The O-antigen (OAg) portion of the LPS constitutes one of the main virulence determinant and protective antigen of Bp and Bm [2]. It consists of a linear heteropolymer whose repeating unit is a disaccharide composed of glucose and diversely acetylated/methylated 6-deoxy-talose moieties. Our team has recently reported the antigenic and immunogenic potential of synthetic oligosaccharides mimicking the minimal intrachain or terminal epitopes of Bp and Bm LPS OAg [3]. On the basis of these previous results, our current hypothesis is that oligosaccharides containing both the major internal and terminal epitopes of Bp and Bm LPS OAg could be suitable candidates for the development of vaccines against melioidosis and glanders. In this context, a synthetic pathway has been developed, which enables the preparation of tetrasaccharides related to Bp and Bm through a [1 + 1 + 1 + 1] glycosylation methodology via the use of orthogonal and participating protecting groups. Regio- and stereoselective reactions were conducted and anomerically pure oligosaccharides were prepared, highlighting the suitability of the developed method for the synthesis of complex oligosaccharides. Antigenicity and immunogenicity assays are soon to be conducted to assess their biological potential. Among other tests, the assembled oligosaccharides will be tested against monoclonal antibodies (mAbs) isolated from melioidosis Thai patients serum. The tetrasaccharides under study could find usefulness in the development of prophylactic measures against melioidosis and glanders, which are all the more relevant in the current global context.

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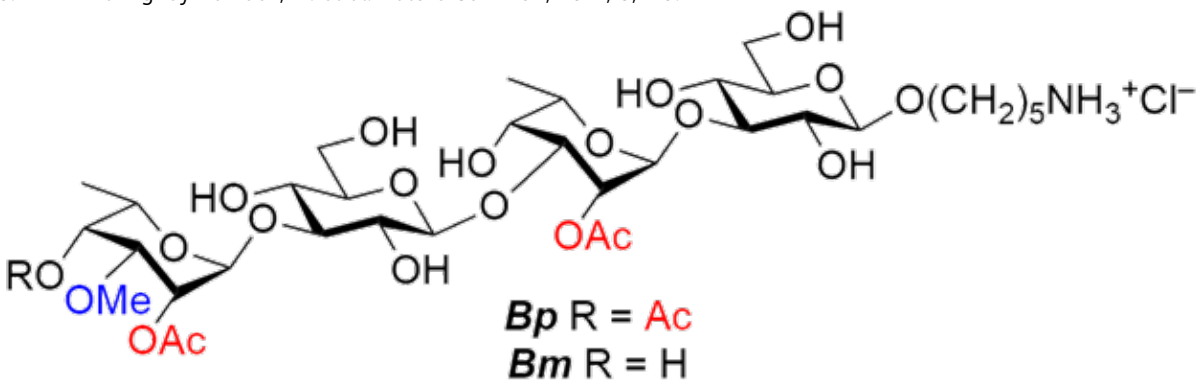

Target tetrasaccharides mimicking Bp and Bm LPS OAg

OL2.1.2

Synthesis of a Pentasaccharidic Repeating Unit of Lipopolysaccharide of Providencia Alcalifaciens O60

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Providencia alcalifaciens along with some of its other species proved to be pathogenic in humans causing variety of serious infections.[1] Pentasaccharide 1 is a repeating unit of lipopolysaccharide (LPS) of Providencia alcalifaciens O60.[2] In order to elucidate the molecular basis and use them as vaccine, synthesis of these kind of glycans is very important.[3] An efficient synthesis of the pentasaccharidic repeating unit 1 of LPS of Providencia alcalifaciens O60 is presented. A convergent [2 + 3] glycosylation strategy was employed to construct a fully protected pentasaccharide 2. The L-serine was installed on glucuronic acid by HATU mediated coupling. Further, a disaccharidic trichloroacetimidate donor and a trisaccharide acceptor were furnished in a linear fashion from respective monosaccharide building blocks in excellent yields. The global deprotection by using Birch condition gave the desired pentasaccharide 1 in good yields. The reactive n-pentenyl group was preserved throught the synthesis to add desired functionalities later on for the bioconjugation, which is a prerequisite for immunological studies.

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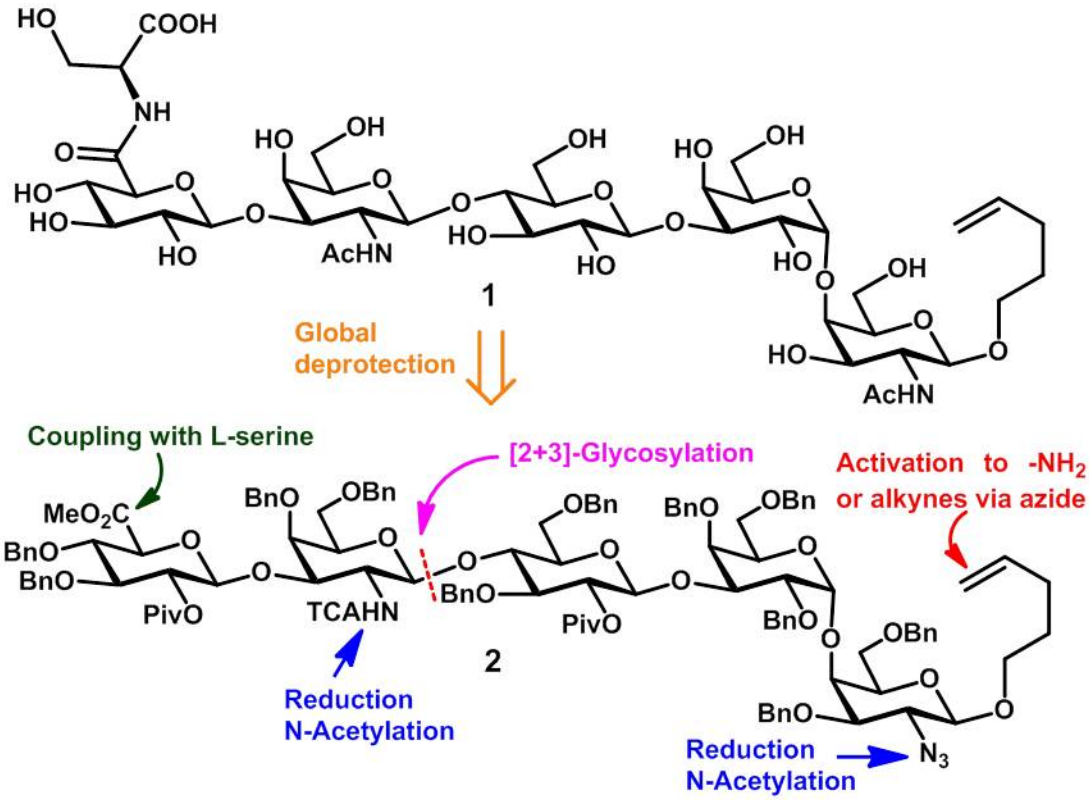

Scheme 1: Structures of the target pentasaccharidce 1 and protected pentasaccharide 2

OL2.2.1

N-Acetylglucosaminyltransferases and Nucleotide Sugar Transporters Form Multi-Enzyme–Multi-Transporter Assemblies in Golgi Membranes In Vivo

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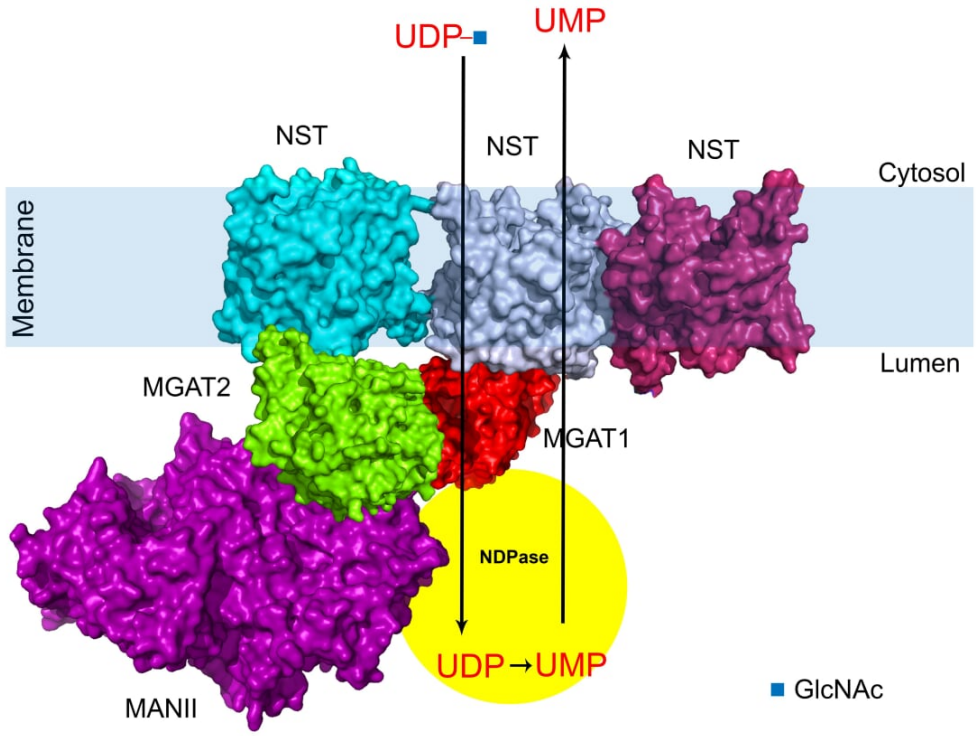
Branching and processing of N-glycans in the medial-Golgi rely both on the transport of the donor UDP-N-acetylglucosamine (UDP-GlcNAc) to the Golgi lumen by the SLC35A3 nucleotide sugar transporter (NST) as well as on the addition of the GlcNAc residue to terminal mannoses in nascent N-glycans by several linkage-specific N-acetyl-glucosaminyltransferases (MGAT1-MGAT5). Previous data indicate that the MGATs and NSTs both form higher order assemblies in the Golgi membranes.

Here, we investigate their specific and mutual interactions using high-throughput FRET- and BiFC-based interaction screens. We show that MGAT1, MGAT2, MGAT3, MGAT4B (but not MGAT5) and Golgi alpha-mannosidase IIX (MAN2A2) form several distinct molecular assemblies with each other and that the MAN2A2 acts as a central hub for the interactions. Similar assemblies were also detected between the NSTs SLC35A2, SLC35A3, and SLC35A4. Using in vivo BiFC-based FRET interaction screens, we also identified novel ternary complexes between the MGATs themselves or between the MGATs and the NSTs. These findings suggest that the MGATs and the NSTs self-assemble into multi-enzyme/multi-transporter complexes in the Golgi membranes in vivo to facilitate efficient synthesis of complex N-glycans.



A schematic model of the multi-enzyme/multi-transporter assemblies in the Golgi membranes of live cells. The complex is shown to import UDP-N-acetylglucosamine (UDP-GlcNAc) into the Golgi lumen, where after MGAT1 is adding the sugar to acceptor (not shown) and freeing UDP. UDP is then transformed to UMP by the nucleotide diphosphatase (shown as part of the same assembly). UMP then drives the import of a new UDP-GlcNAc residue for further catalysis.

OL2.2.2

Predicting N-Glycan Processing Based on Enzyme-Glycan Accessibility

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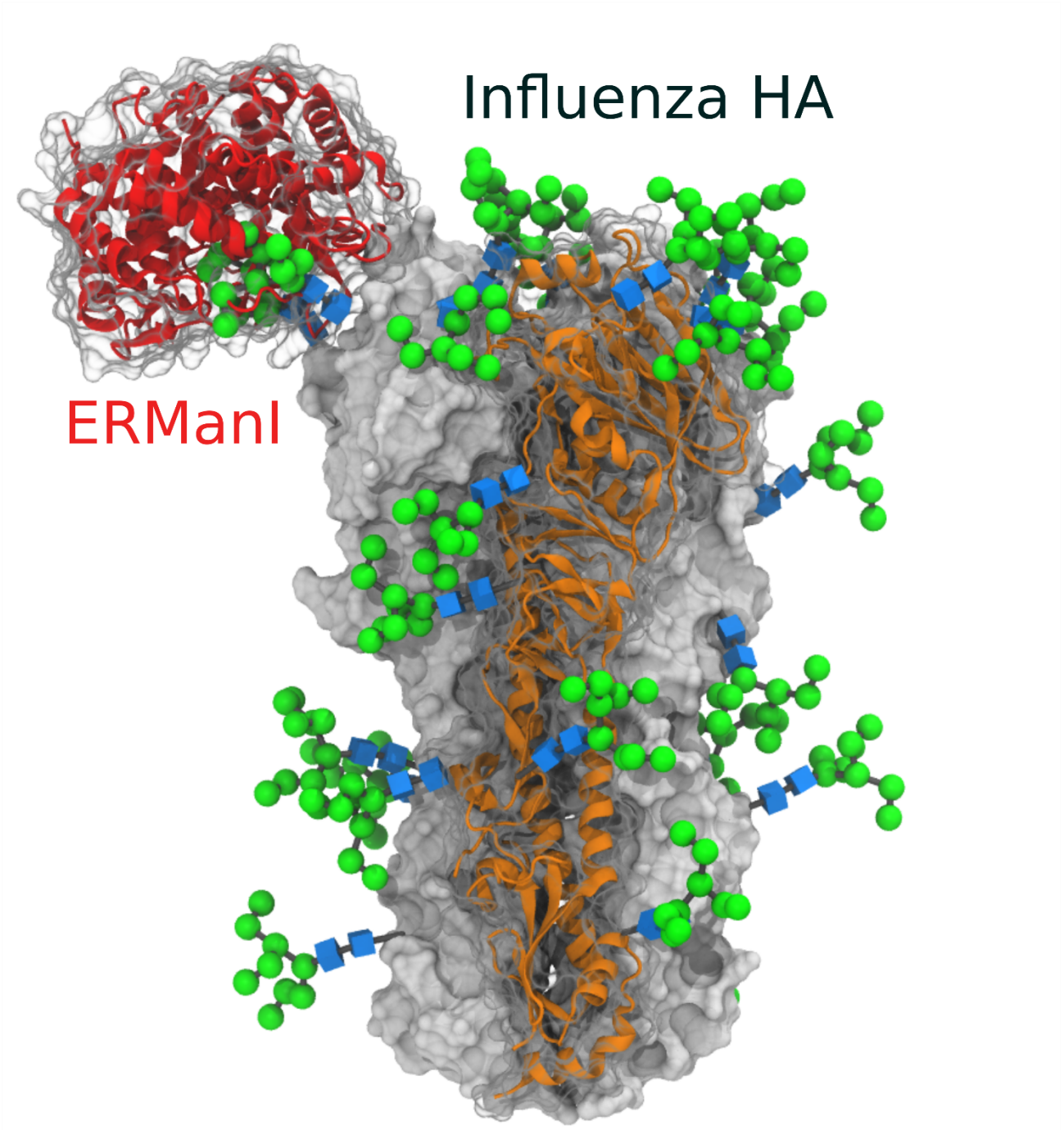
[1]University Of Georgia, Athens, United States

Background:
In this work, computer simulation, glycoproteomics and crystallographic data are combined to show that glycoprotein glycoform distributions depend on the accessibility of N-glycans to the relevant glycosidases in the ER. We illustrate this for three systems: a protein disulfide isomerase precursor named Pdi1p, a hemagglutinin (HA) from influenza A, and the HIV envelope glycoprotein.

Methodology:
We leverage the recently solved 3D structure of ER mannosidase I (ERManI) with molecular dynamics simulations of the glycoproteins, where the enzyme's substrate, Man9GN2, is present at each site. We calculate the percentage of simulation time that ERManI is physically able to bind the glycan, as it samples different shapes throughout the simulation.

Results:
In the case of Pdi1p, the correlation between accessibility to ERManI and the degree of processing is striking. The modeling also predicted that a domain deletion would expose a glycosylation site on a neighboring domain to processing, which was confirmed experimentally. For influenza HA, we were able to rationalize why certain sites remained as Man9GN2. Further, the modeling work was able to propose a 3D model for how the pulmonary collectin SP-D would bind these Man9GN2 glycans and thus neutralize the virus. When applied to the HIV envelope protein, our modeling approach provides insight into formation of the so-called "high-mannose patch".

Conclusion:
The presentation will illustrate the degree to which site-specific glycan processing can be predicted on the basis of 3D structure.



The 3D structure of ER Mannosidase I (Red ribbon with transparent surface) superimposed onto Man9GlcNAc2 (3D-SNFG symbols) on a glycosite of influenza HA (grey surface, with one protomer shown as orange ribbon). The ability of ERManI to bind to a particular Man9GlcNAc2 measured over the course of a molecular dynamics simulation correlates with the experimentally observed degree of processing at that site.

OL2.3.1

Chemical Synthesis of Glypiated and Glycosylated Thy-1 Protein

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Glycosylation is one of the most important post-translational modifications (PTMs) of proteins affecting their physicochemical and biological properties. The biosynthesis of glycoproteins is a complex process that delivers mixture of the same protein sequence with different glycosylation patterns. To overcome the difficulty in the isolation of single glycoproteins from natural sources, synthetic and semi-synthetic strategies are emerging as a powerful alternative for the production of homogeneous glycoproteins. These strategies will help to study the role of carbohydrates in the biological function of glycoproteins. However, there are still limitations to obtain glycoproteins having multiple glycosylations.

Thy-1 (CD90) is a 12 kDa glycoprotein having a glypiation and three N-glycosylations sites. The protein is found on the outer leaflet of the membrane of T-cells and is speculated to participate in cell-cell and cell-matrix interactions, nerve regeneration, apoptosis, metastasis, inflammation, and fibrosis. Furthermore, this glycoprotein can act as a tumor suppressor and be a potential probe for cancer drug targeting. To study the biological function and the effect of glypiation and glycosylations on the folding and activity of Thy-1, we are working on a strategy for the synthesis of homogeneous glypiated and glycosylated Thy-1 variants.

Here, we report a strategy based on a combination of sequential native chemical ligation and chemoenzymatic techniques to obtain Thy-1 having three N-glycosylations. Three peptide fragments were designed, two of them containing N-glycosylated asparagine residues. The fragments were synthesized as peptide hydrazides as thioester precursors using solid phase peptide synthesis (SPPS). The glycosylations were introduced coupling a glycosylated asparagine building block in the specific positions. The challenging Thy-1 fragment 38-103, with two N-glycosylations, was obtained by condensation of the glycopeptide fragments (38-65) and (66-103). In addition to the assembly of the protein, we present the progress for the introduction of differentiated oligosaccharide structures on each glycosylation site using the mutant glycosynthase Endo-M and in the ligation reactions, to obtain a glypiated glycoprotein.

OL2.3.2

GPI-Anchored Proteins Tethered to Lipid Bilayers: Modelling a Complex Interplay of Carbohydrates, Proteins and Lipids

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The glycosylphosphatidylinositol(GPI) anchor is a complex glycolipid abundant on the surface of eukaryotic cells. It serves as an anchor for a variety of proteins, which are connected to a lipid tail through a conserved glycan backbone. GPIs have been thought to contribute to a number of processes involving the cell surface, such as sorting, trafficking or signal transduction, e.g., through the ultimate release of the protein into the extracellular matrix through cleavage by phospholipase C or D. The glycan and also the fatty acid part can be subject to a number of variations, but how to define a structure-function relationship is still an open question. This is mainly because no clear picture exists of how protein and glycan part arrange with respect to the lipid layer[1][2]. Direct experimental evidence is rather scarce, and it appears that atomistic computational modeling through molecular dynamics (MD) simulation would be a convenient method to make progress. However, a GPI anchored protein implies three mutually interacting molecular species in close proximity. Here we show how to construct a modular molecular model of GPIs and GPI anchored proteins that can readily be extended to a broad variety of systems, addressing the micro heterogeneity of GPIs. We do so by creating a hybrid link to which different components with their respective optimized force fields can be attached, such as additional carbohydrates (GLYCAM06), Lipids (Lipid14) and the protein (ffSB14), all sharing the general protocol of the Amber family.
Our results demonstrate that GPI prefers flopping down on the membrane, thereby, strongly interacting with the lipid heads, over standing tall like a lollipop. When attaching green fluorescent protein (GFP) to the GPI, it was seen to lie in close proximity to the bilayer, interacting both with the lipid head and glycan part of the GPI. On extending this model to Toxoplasma gondii GPI, it was seen that the side branch galactosamine was always exposed to the solvent, with barely any interaction with the the bilayer. This finding is in correspondence with experimental studies[3] that show that the galactose residue of the parasite's GPI is the recognition moeity for the binding of Galectin-3 so as to be targeted by macrophages. Our GPI model can be used to study many parasitic and clinically relevant GPIs like trypanosomes and malaria parasites which would pave the way for developing new strategies in vaccine therapy.

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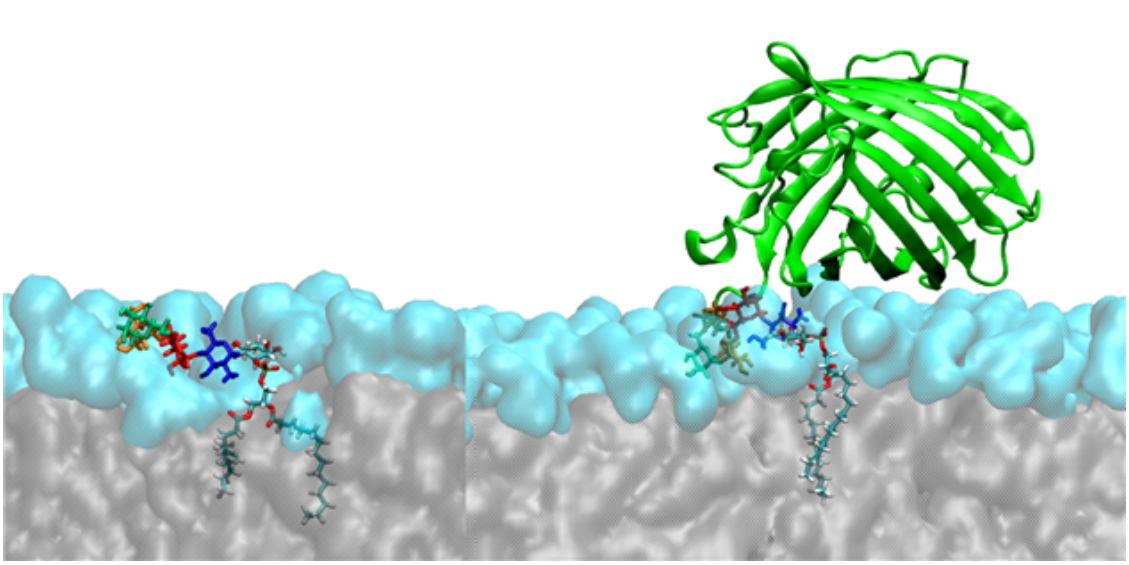

Favored conformations of GPI core (left), and GPI-anchored GFP (right) when inserted in DMPC bilayers.

OL2.4.1

CDP-Paratose 2-Epimerase: Substrate Specificity Beyond Expectation

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Carbohydrate 2-epimerases have evolved to invert the stereochemistry at C-2 on their respective sugar substrates following various catalytic mechanisms. Those mechanisms involve deprotonation/reprotonation, nucleotide elimination/readdition or formation of a transient keto-intermediate, the latter being proposed for CDP-paratose 2-epimerase. An uncharacterized, dimeric homologue of the aforementioned CDP-paratose 2-epimerase (TyvE) originating from Thermodesulfatator atlanticus displaying an expanded substrate scope is sculpting the frame of this research: CDP-glucose was found to function as alternate, readily accessible substrate. The epimerization to CDP-mannose was confirmed by in situ NMR studies as well as the chemo-enzymatic synthesis of CDP-mannose applied in the back-reaction. Catalytic efficiencies ($k_{cat,f}/K_{m,f} \simeq 47.42$ [1/(Ms)], $k_{cat,b}/K_{m,b} \simeq 68.23$ [1/(Ms)]) corresponding to a Haldane relationship of $K_{eq,H} \simeq 0.70$ are in accordance to an equilibrium constant of $K_{eq} \simeq 0.67$ indicating a thermodynamically disfavored forward-reaction. TyvE showing a temperature optimum of 70°C further enabled the observation of kinetic isotope effects at C-2 over a temperature range of 20°C to 80°C resulting in an average KIE of $\simeq 2.5$.

Acknowledgements: This research is part of the EpiSwitch project, jointly funded by the Fund for Scientific Research Flanders (FWO-Vlaanderen, grant n° G0F3417N) and the Austrian Science Fund (FWF; project n° I 3247).

OL2.4.2

Inherited Diseases Caused by Deficiencies In Lysosomal Glycosidases

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A major part of the inherited disorders in metabolism is caused by inherited defects in lysosomal glycosidases. Most prominent are Gaucher disease (lysosomal β-glycosidase deficiency involving the GBA gene and degradation of the lipid glucosylceramide), Pompe disease (lysosomal α-glucosidase deficiency involving the GAA gene and degradation of glycogen) and Fabry disease (lysosomal α-galactosidase A deficiency involving the GLA gene and degradation of globotriaosylceramide).
A frontrunner in developing advanced diagnostics and effective therapies has been, and still is, Gaucher disease. There exists no strict relationship between GBA genotype and phenotypic manifestation of Gaucher disease.

In particular, homozygotes for common milder mutations, like N370S GBA, may express variable disease severity from severe organomegalies and hematological symptoms to a virtual asymptomatic course. This has prompted a search for biomarkers. In Gaucher disease, glucosylceramide laden macrophages (Gaucher cells) are a hallmark of disease. Identified have been as biomarkers specific proteins secreted by these storage cells: chitotriosidase, CCL18 and gp-NMB. These proteins are markedly elevated in plasma of symptomatic patients, assisting confirmation of diagnosis and monitoring of disease. More recently, de-acylated glucosylceramide, named glucosylsphingosine, has been found to be about 200-fold elevated in plasma of Gaucher patients. With sensitive assays, employing 13c-encoded internal standard, glucosylsphingosine can be determined and used to monitor disease status. Several treatments have been developed and are presently registered, including enzyme supplementation and substrate reduction therapy. In development are gene therapy, small compound chaperone and activator therapies. The design and development of activity-based probes specifically labelling glucocerebrosidase is assisting further diagnosis and optimization of therapeutic interventions. Similar approaches are presently copied for other lysosomal glycosidase deficiencies such as Pompe and Fabry disease.

OL3.1.1

Elucidating Structural Carbohydrate Epitopes for Vaccine Design

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Glycoconjugate vaccines are an important and successful countermeasure for control and treatment of infectious diseases. Elucidating the shortest portion of a polysaccharide able to bind to specific functional monoconal antibodies (glycotope) is key for optimal vaccine design. Historically, the determination of glycotopes has been hampered by technical challenges in obtaining well-defined oligosaccharides. New synthetic and depolymerization methods enbales the creation of glycans to study interactions with protective monoclonal antibodies. GBS is a leading cause of invasive infections in pregnant women, newborns, and elderly people, and the capsule is a major virulence factor targeted for vaccine development [1]. GBS PSIII epitope has been historically considered the prototype of a conformational carbohydrate epitope [2]. We have recently applied an integrated approach based on competitive ELISA/Surface Plasmon Resonance/Saturation Transfer NMR/X-ray crystallography to elucidate one epitope from the capsule of Group B Streptococcus type III, whose structure is composed of α-NeuAc-(2→3)-β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-β-D-Glc-(1→4)-β-D-Gal→, with branching at position 6 of GlcNAc. Using a panel of synthetic and semisynthetic structures, we found that the structural epitope consists of 5 disjoined sugar residues, which comprises a single repeating unit, and the GlcNAc moiety of the next consecutive repeat unit, where sialic acid is clearly engaged in antibody binding [3]. Currently we are studying immunogenicity of vaccines based on short GBS PSIII fragments.

In addition, we are using the same approach to map the structural epitope of Neisseria meningitidis serogroup A (MenA), a Gram-negative encapsulated bacterium responsible for epidemic meningitis in the sub-Saharan region of Africa, termed meningitis belt [4]. Despite multivalent and monovalent conjugate vaccines against MenA are now available, the structural minimal epitope of MenA polysaccharide is still unknown [5]. MenA capsular polysaccharide (CPS) consists of (1→6) linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units with O-acetylation predominantly at position 3 [6], therefore the epitope is expected to be linear. Two parameters influence the immunogenicity of MenA conjugates: the oligomer length and the acetylation level [7,8]. Competitive ELISA revealed epitope optimization for oligomers longer than 6 repeating units. The hexamer was also sufficient to deplete the bactericidal activity of serum raised from vaccinated subjects, while inhibition of serum for shorter length oligomers was achieved at higher concentrations. Upon de-O-acetylation of MenA minimal epitope, the kinetics of dissociation increase 1000-fold and no hSBA inhibition was observed, revealing that these moieties play a pivotal role in recognition. STD-NMR effect was stronger for the O-acetyl group (100%) located at C3 followed H3/H4 and acetamide positioned at C2, indicating that these moieties are in close contact with the functional monoclonal antibody. High resolution X-ray crystal structure of a Fab in complex with a phosphosugar linked oligosaccharide revealed a trisaccharide linear epitope, where O-acetyl moieties are engaged in network of hydrogen bond. Efforts in understanding the structural basis of recognition of oligosaccharide epitopes are the foundations for future development of a MenA epitope optimal mimicry.

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OL3.1.2

Identification of the Minimal Glycotope of Streptococcus Pneumoniae 7F Capsular Polysaccharide Using Synthetic Oligosaccharides

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Streptococcus pneumoniae 7F (ST7F), one of the more than 90 described serotypes, was one of the most prevalent serotypes causing infections before the introduction of a 13-valent conjugate vaccine (PCV13/Prevnar13®). Pneumococcal conjugate vaccines based on naturally-derived capsular polysaccharides (CPSs) have been successfully developed [1] and have shown remarkable efficacy. However, novel semi or fully synthetic vaccines represent an alternative offering potential advantages such as higher structural homogeneity and purity of the antigens, simpler conjugation strategies and a highly reproducible and potentially less expensive manufacturing process. Moreover, for many serotypes little is known about the true structures of immunogenic and protective epitopes. In a medicinal chemistry approach aimed at creating new potent semisynthetic glycoconjugate vaccines, the identification of such antigenic determinants is essential. In this regard short synthetic oligosaccharides represent useful tools [2].

In this work a library containing various oligosaccharides, ranging from tri- to hexasaccharides representing fragments of the ST7F CPS repeating unit [3], has been prepared by solution phase techniques. Starting from seven orthogonally protected monosaccharides, a set of disaccharide building blocks has been created. Afterward, a series of [n+2] glycosylations followed by global deprotections furnished all target oligosaccharides. A combination of "state of the art" glycosylation protocols together with recently developed protecting group manipulations allowed the formation of challenging 1,2-cis linkages displayed within the native structure.
Synthesized structures carried a reducing-end linker that enabled covalent immobilization on microarray glass slides [4]. Glycan arrays were probed with a polyvalent human antipneumococcal serum and bindings were visualized and quantified with fluorescence-tagged secondary antibodies. Results of inhibition assays revealed two specific portions of the repeating unit playing a critical role in antibody binding. An additional inhibition assay also showed cross-reactivity of antibodies towards the structurally related CPS from serotype 7A, confirming the presence of shared epitopes between the serotypes.
These results represent a starting point for the rational design of a synthetic antigen which, upon conjugation to an immunogenic carrier protein, could translate into an anti-serotype 7F semisynthetic vaccine candidate.

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OL3.1.3

A Semisynthetic Glycoconjugate Vaccine Against Resistant Klebsiella Pneumoniae St258

Marilda P. Lisboa[1], Jessica Przygodda[1], Bopanna Monnanda[1], Sharavathi G. Parameswarappa[1], Sylvia Oestreich[1], Arun Naini[1], Daniel Knopp[1], Arne Von Bonin[2], Claney L. Pereira[1]

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Klebsiella pneumoniae is a frequent pathogen in antibiotic resistant nosocomial infections and a leading cause of serious bacterial neonatal infections in developing countries. [1],[2] K. pneumoniae is the second most common cause of Gram-negative bloodstream infections, mainly in immunocompromised patients.[3] Vaccines are important tools against antimicrobial resistance and nosocomial infections. Capsular polysaccharides and lipopolysaccharides are key vaccine targets. K. pneumoniae produces a diverse array of capsular polysaccharides or K antigens (78 types) compared to only 9 different lipopolysaccharides (O-antigen). A 24-valent K. pneumoniae capsular polysaccharide vaccine showed safety and immunogenicity in human trials (phase 1), however the protection coverage was not over 70%.[4] Herein, we focus on the development of a semi-synthetic conjugate vaccine against the hypervirulent ST258 strain, which is present in 70% of the infections caused by carbapenem-resistant K. pneumoniae. The predominant LPS O-antigen expressed by the ST258 strain is galactan III (→3)-β-D-Galf-(1→3)-[α-D-Galp-(1→4)]-α-D-Galp-(1→). A series of oligosaccharides containing different lengths of galactan III were synthesized, conjugated to the carrier protein CRM197 and the immunogenicity tested in mice. The vaccine was shown to be immunogenic and cross-reactive against the LPS O-antigen.

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OL3.1.4

Multivalent Glycocalixarenes Bind to Human Anti-19f Antibodies: A Potential Basis for New Immunogenic Systems?

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Streptococcus pneumoniae (SP) bacteria are one of the leading causes of bacterial infections in young children [1]. Their capsular polysaccharides (CPS), whose chemical structure defines the different serotypes, are virulence factors. One of the most virulent SP serotype is the 19F. Its CPS structure is characterized by the trisaccharide repeating unit (→4)-β-D-ManpNAc-(1→4)-α-D-Glcp-(1→2)-α-L-Rhap-(1-OPO_3-→), with phosphodiester bridges connecting a residue of rhamnose at the reducing end of one unit to the N-acetylmannosamine at the non-reducing end of the adjacent one. Among the three monosaccharides, N-acetyl-β-D-mannosamine is supposed to be the immunodominant element. It has been demonstrated that there is an evident effect of the pneumococcal polysaccharide chain length on the immunogenicity [2]. However, to elicit an immune response, a possible alternative to explore respect to the exposition of the long polysaccharide chain could be the multiple presentation of short fragments in a clusterized form, exploiting proper scaffolds. From this point of view, calixarenes have been shown to be versatile platforms for the preparation of multivalent glycoclusters [3]. The possibility of subtly tuning size of the scaffold, valency and geometry of the epitope display, allowed to produce in the past efficient and selective calixarene based ligands, the glycocalixarenes, for carbohydrate recognition proteins [3]. In this work we planned to exploit these macrocycles as scaffolds to design potential immunostimulants against 19F-SP serotype, linking to calixarenes multiple units of N-acetyl-β-D-mannosamine or of the 19F capsular trisaccharide repeating unit as saccharide epitope moieties (see Figure). The synthesis of the ligands with the achievement of structurally well-defined glycocalixarenes and their antigenic properties will be presented evidencing the effect of multivalency in increasing the ability of the single saccharide unit, when attached on the macrocycle, to compete with natural 19F CPS in the binding to specific anti-19F antibody.

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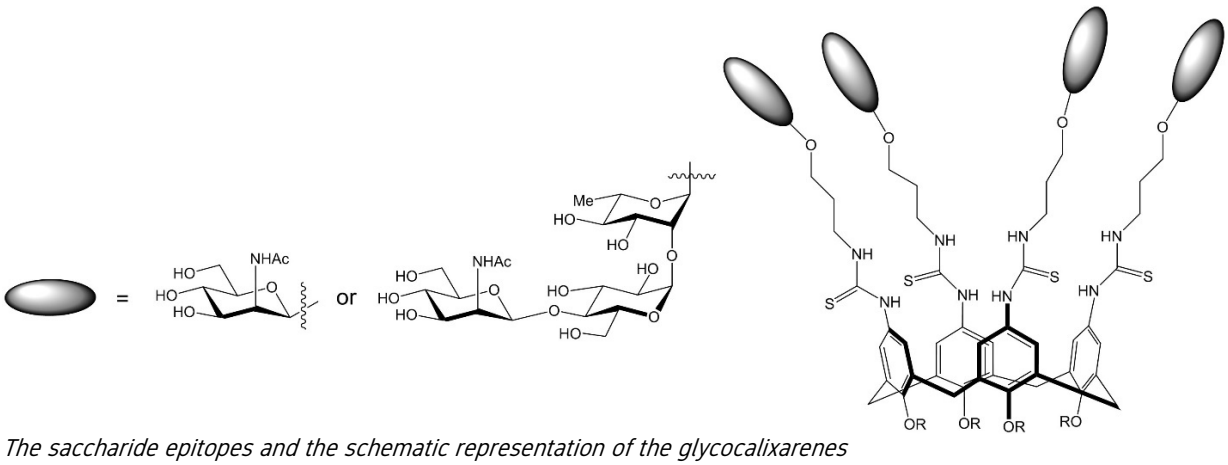

The saccharide epitopes and the schematic representation of the glycocalixarenes

OL3.2.1

NMR Characterization of Allosteric Pathways and a Post Translational Modification Regulating HBGA Recognition Ii GII.4 Human Norovirus

Robert Creutznacher[1], Christoph Müller-Hermes[2], Thilo Stehle[3], Bärbel Blaum[3], Charlotte Uetrecht[4], Thomas Peters[1], **Alvaro Mallagaray[1]**

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Infection with human norovirus (hNoV) is the leading cause of acute gastroenteritis worldwide. Attempts to provide antivirals or vaccines have not been successful so far. Infection of hNoV requires attachment to histo blood group antigens (HBGAs), but how this binding event promotes the infection of host cells is unknown. We employ protein NMR experiments[1] supported by mass spectrometry and crystallography to study HBGA binding to the P-domain of a prevalent virus strain (GII.4).

We have successfully assigned 86% of the backbone NH signals and 100% of the methyl groups in a U-[2H,15N] stereo-selective MILVA methyl group labeled hNoV P-domain (72 KDa). Unexpectedly, our NMR analysis revealed a highly selective transformation of asparagine 373, located in an antigenic loop adjoining the HBGA binding site, into an iso-aspartate residue. This spontaneous post-translational modification (PTM) proceeds with an estimated half-life of a few days at physiological temperatures, independent of the presence of HBGAs but dramatically affecting HBGA recognition. Sequence conservation and the surface-exposed position of this PTM suggest an important role in infection and immune recognition for many norovirus strains.[2]

Binding of HBGAs to hNoV P-domains cause long-range chemical shift perturbations (CSPs) of amino acid residues more than 30 Å away from the binding site, indicating the presence of a subtle allosteric cross-talk. To understand the architecture of the allosteric network we generated a library of 17 naturally occurring soft mutants, which yielded distinct CSP patterns in Methyl-TROSY NMR spectra. A CHESCA-like[3] covariance analysis uncovered functional clusters of coupled residues, as well as a residue-specific dissection of the contribution of each amino acid to allostery. A 2nd order Markov analysis revealed the complete structure of the network, reflecting the flow of the allosteric signalling. Critical junctions in the network can be correlated to biologically significant allosteric sites and pathways.[4] We aim to use this information to identify protein “hot-spots”, which could be targeted as allosteric modulators on hNoV infection.

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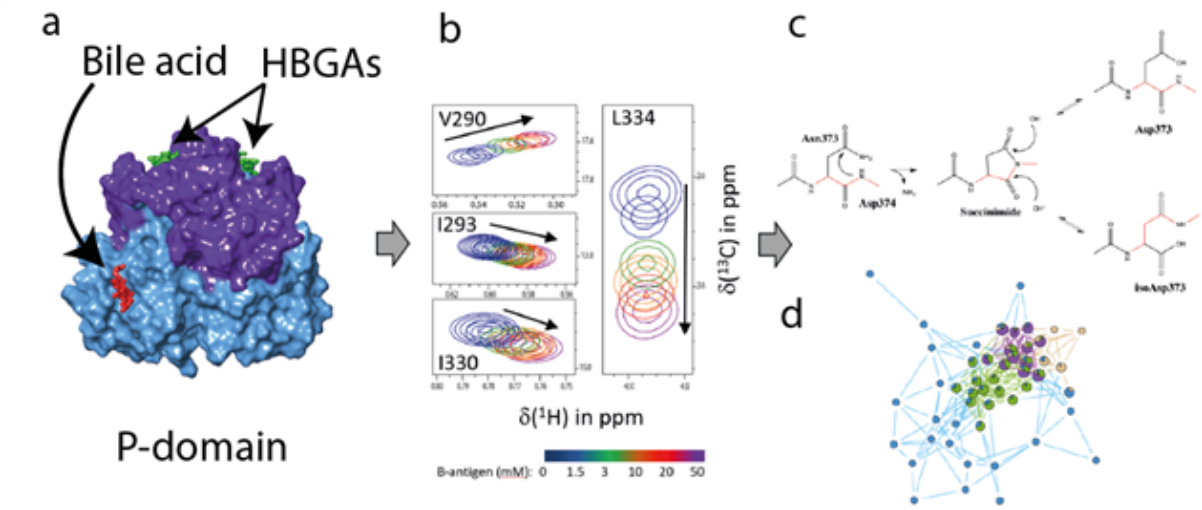

Scheme showing the structure of hNoV P-domains and the work flow. a) The hNoV capsid is decorated with 90 P-domains, each offering four binding sites. Thus, every P-domain can recognize two HBGAs and two bile acid molecules simultaneously (in green and red thick tube representation, respectively). b) Perturbations are introduced by ligand titrations or single-point directed mutagenesis, and NMR CSPs are primarily used as a read-out. The analysis of CSPs combined with MS and crystallography allowed to uncover a hitherto unknown PTM on amino acid 373 that influences HBGA binding (c), and to completely map the allosteric network controlling ligand recognition (d).

OL3.2.2

Unmasking Ligand Conformational Entropy in the Recognition of Blood Group Antigens

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Ligand conformational entropy is generally claimed as an important contribution in carbohydrate recognition events. Indeed, glycans are characterized by an intrinsic flexibility around the glycosidic linkages, and thus, except for isolated cases,[1] the loss of conformational entropy of the sugar upon complex formation strongly impacts the entropy of the binding process. However, this contribution is generally rather difficult to address.
Herein, by employing a multidisciplinary approach combining structural, conformational, binding energy and kinetic information, we have disclosed the role of the conformational entropy in the recognition of the histo blood group antigens A and B by human galectin-3, a lectin of biomedical interest.[2] These rigid natural antigens are pre-organized ligands for hGal-3, being locked in the bioactive conformation. The restriction of the conformational flexibility rendered mostly by the branched Fucose residue,[3] modulates the thermodynamics and kinetics of the binding process, providing the impetus for the high affinity interaction. In fact, the conformational restriction observed for A- and B-BGA reduces the kinetic barrier of the association process and favorably impacts on the binding entropy. Thus, this study highlights the effect of glycan flexibility on the kinetics and thermodynamics of the binding process and provides inspiration for the design of high affinity ligands as antagonists for hGal-3 or other lectins of biomedical relevance.[4]

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OL3.2.3

Vibrational Optical Activity and Saccharides

Jakub Kaminsky[1], Luboš Plamitzer[1], Radek Pohl[1], Vladimír Palivec[1], Hector Martinez-Seara Monne[1]

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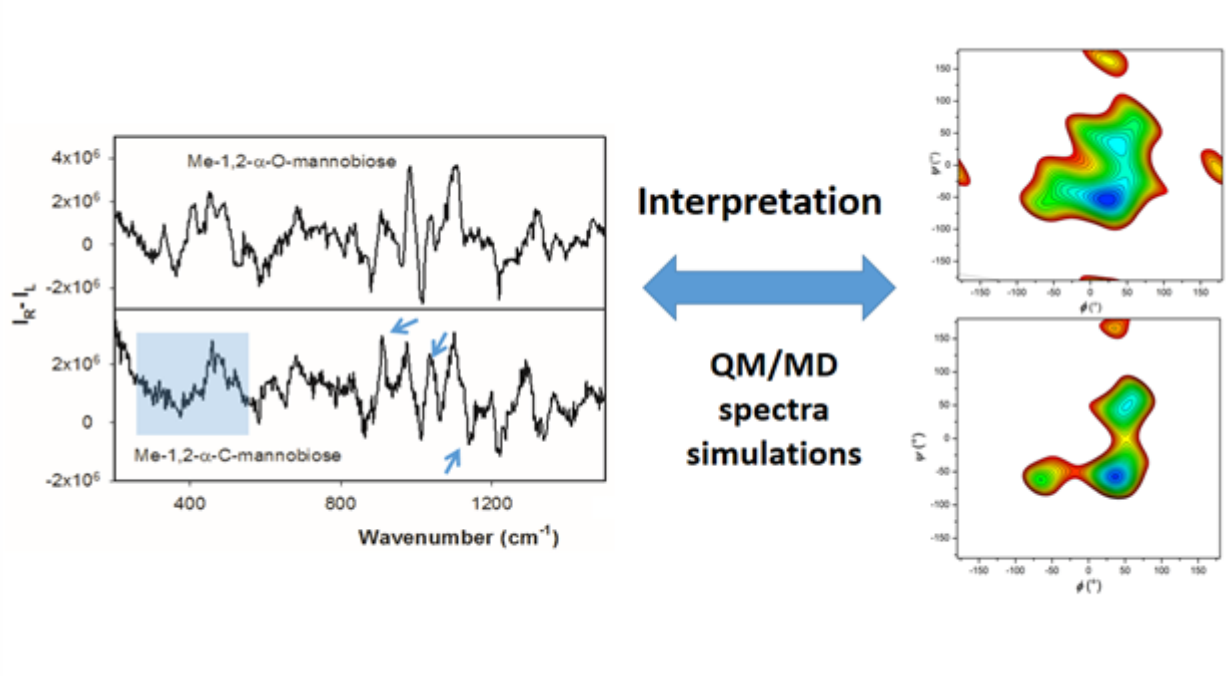
Scientific activities in many research areas are predicted to become increasingly carbohydrates-oriented over the next decade. While the growing impact of carbohydrates on energy, food security and healthcare are already clear, the future development of new carbohydrate-based therapeutics, materials and energy sources will depend heavily on our building a better understanding at the molecular level of the structure-function relationships of carbohydrates. As traditional structural methods (NMR or X-ray) are often difficult to apply to carbohydrates, new approaches are urgently required. The main topic of the talk is to present a new spectroscopic approach for studying carbohydrate structures at the molecular level. We have developed widely applicable yet structurally sensitive approach based on chiral variants of vibrational spectroscopies (especially Raman optical activity and vibrational circular dichroism) for studying carbohydrates that exploit diverse interactions of carbohydrates with circularly polarized light (Figure 1; e.g. Ref. 1). These spectroscopic techniques are able to reveal previously unknown details on all aspects of complex carbohydrate structure and organization, from the hydration of small sugars to inter/intramolecular interactions of oligo- or polysaccharides and glycoproteins that govern their physiological functions.



Structural data extraction from Raman optical activity spectra.

OL3.2.4

Nmr Characterization of Cyanovirin -N/Mannoside Complexes Reveals a Detailed Picture of the Hydrogen Bonding Network

Gustav Nestor[1,2], Taigh Anderson[3], Stefan Oscarson[3], Angela M. Gronenborn[2]

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Carbohydrates used in structural studies by NMR are rarely ^{13}C-labeled, preventing exploitation of the ^{13}C spectral dispersion in 3D or higher order NMR experiments, which would alleviate severe resonance overlap in their ^{1}H NMR spectra. This is in part associated with the lack of easily accessible isotope-labeled sugar molecules. We previously reported a number of attractive advantages of using ^{13}C-labeled carbohydrates to characterize the interactions in protein complexes, comprising ^{15}N-labeled protein. As model system we used the complex of the ^{13}C-labeled Manα(1–2)Manα(1–2)ManαOMe trisaccharide, bound to cyanovirin-N (CV-N). The carbohydrate-protein binding interface was characterized by tailored isotope-filtered NOESY experiments [1] and carbohydrate hydroxyl protons that form hydrogen bonds with the protein were identified and their orientations were determined [2].

In this presentation, we report results about CV-N recognition by Manα(1–2)ManαOMe and Manα(1–2)Manα(1–6)ManαOMe. These two mannosides bind preferentially to the domain B binding site of CV-N, in contrast to Manα(1–2)Manα(1–2)ManαOMe, which binds preferentially to the domain A binding site [1, 2].

Intra- and intermolecular NOEs between carbohydrate ring and hydroxyl protons and protein amide protons were investigated. Five carbohydrate hydroxyl protons were identified (Fig. 1) and for four of them, the dihedral angles were determined. Analysis of the current results in light of previous NMR and crystal structures of the same complexes revealed important differences and similarities. These will be highlighted in the presentation. Overall, our results emphasize the general applicability of our novel approach for characterizing hydrogen bonding in carbohydrate-protein interactions.

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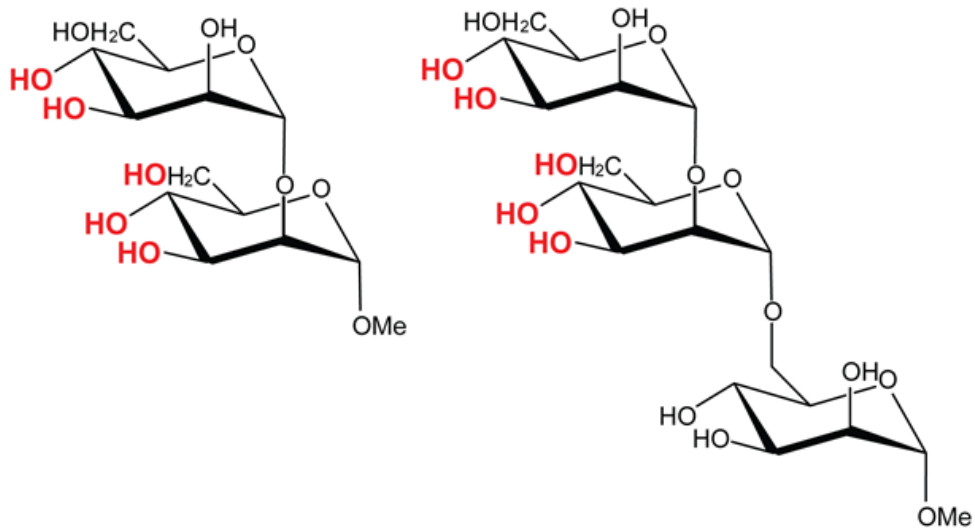

Fig. 1. Formula of Manα(1–2)ManαOMe and Manα(1–2)Manα(1–6)ManαOMe with the experimentally observed hydroxyl protons shown in red bold.

OL3.3.1

Comprehensive N-Glycosylation Characterization of Therapeutic Antibodies by Capillary Electrophoresis and Mass Spectrometry

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Comprehensive characterization of the carbohydrate moiety of protein pharmaceuticals is of high importance, especially when glycosylation changes can impact the biological effect of the drug. Well over half of these new generation drugs are monoclonal antibodies, where the linked carbohydrates not only affect their physicochemical properties and stability, but also their receptor binding activity, circulating half-life and very importantly immunogenicity. State of the art bioanalytical techniques are crucial for N-glycosylation characterization of therapeutic antibodies for the biopharmaceutical industry, especially in clone selection, process development and lot release. N glycosylation analysis, however, represents a very challenging bioanalytical task as glycans are very complex groups of molecules. The lack of chromophore / fluorophore moieties and, in many instances, easily ionizable groups usually require derivatization of carbohydrates before their analysis by high performance bioanalytical techniques. Full structural elucidation of glycans1 (e.g., sequencing) utilizes consecutive enzymatic digestions by sugar and linkage specific exoglycosidases, followed by capillary electrophoresis analysis of the digests.

This presentation will cover the state of the art of liquid phase separation methods for structural elucidation of protein glycosylation, mostly focusing on capillary electrophoresis and its combination with mass spectrometry (CE-MS and CESI-MS). Particular attention will be paid to the comparative interpretation of CE and LC results in case of the analysis of monoclonal antibody therapeutics (both innovative products and biosimilars).2 Comprehensive glycosylation characterization of this recently emerging class of very successful new generation drugs will be discussed in respect to quality by design with the main goal to demonstrate structural and functional equivalence of the products focusing on the analysis of core fucosylation, possible presence of alpha-gal residues and N-glycolylneuraminic acid content. Assisted by the emerging field of glycoinformatics, the identity of glycan structures can be readily assigned relative to their electrophoretic migration time based glucose unit (GU) values. With the aid of such database capillary electrophoresis offers a reliable, precise and traceable glycan structural elucidation method for biotherapeutics and other glycoproteins of biomedical importance.

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OL3.3.2

High Throughput Micro-Permethylation of Glycans for Glycomics

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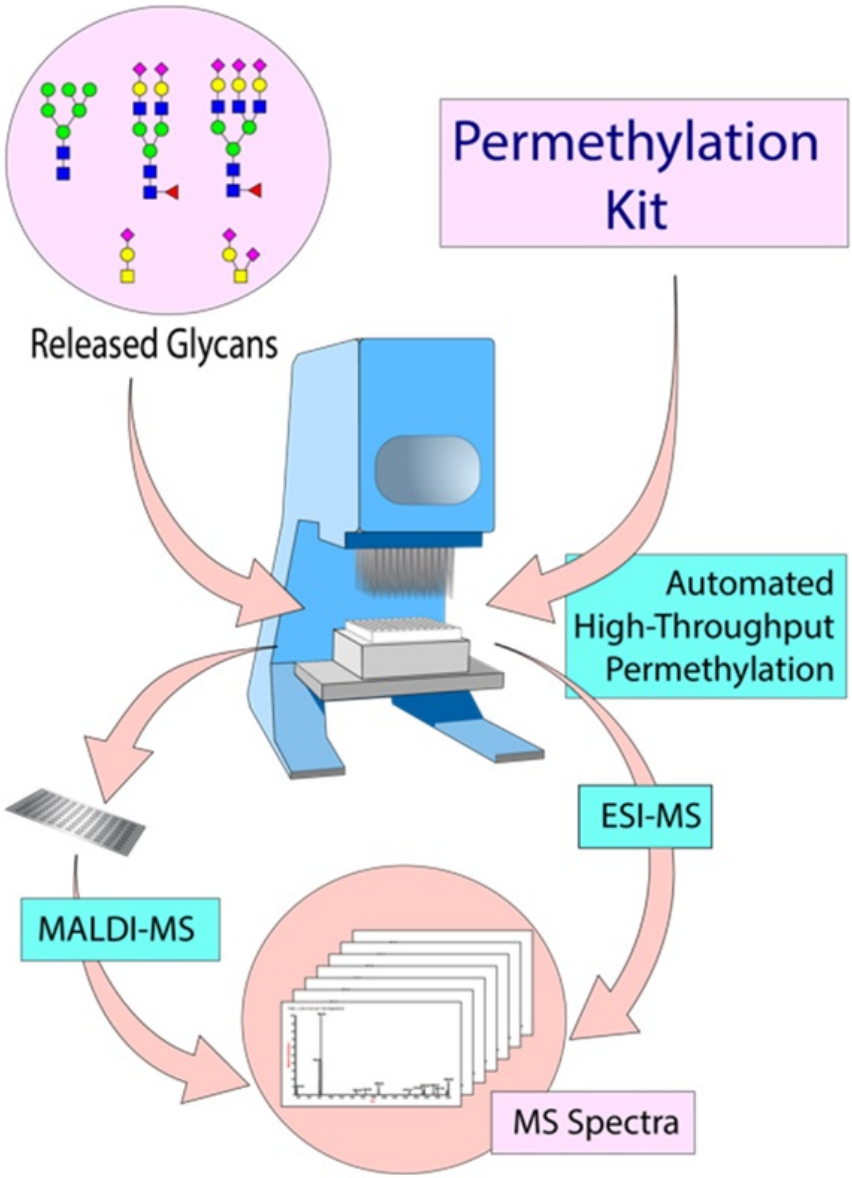
Glycans significantly influence the therapeutic efficacy of glycoprotein by regulating its stability, serum half-life, immunogenicity and biological activity. The demand for the qualitative and quantitative characterization and validation of glycosylation on therapeutic glycoproteins which includes antibodies, vaccines, biomarkers etc. are increasing rapidly. One of the classical yet most informative derivatization methods for glycan characterization is the permethylation of glycans released from glycoproteins and subsequent characterization by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and Electrospray Ionization (ESI-MS). Even though manual permethylation and subsequent processing of glycans is still highly prevalent, all reactions are still performed manually, on one sample at a time. We have developed a cost-effective, simple, sensitive and rapid permethylation kit for the micro-permethylation of glycans released from glycoproteins. Here, we report a protocol for the automation of permethylation reaction in microscale that allows processing of samples in a small amount in a high throughput fashion with minimal handling. The protocol involves releasing of N/O-glycans from glycoprotein samples, the permethylation reaction in microscale, extraction of permethylated glycans directly in a 96 well plate or microcentrifuge tube with significantly shorter processing time and efforts. The permethylated glycans are subsequently analyzed by mass spectrometry. The workflow can be applied to purified glycoproteins or mixtures of glycans or glycopeptides. Automation of permethylation enables high throughput screening of glycosylation for glycomics on a large scale for clinical and biomarker studies which allows for convenient, faster, and reliable characterization of glycoforms.



Schematic for high throughput micro-permethylation of glycans

OL3.3.3

Glycosense: A Rapid And Simple Method for Glycosylation Detection and Monitoring

Loretta Yang[1], Matthew Saunders[1], Sheng-Cheng Wu[2], Lu Meng[2], Christian Gerner-Smidt[2], Robert Woods[3]

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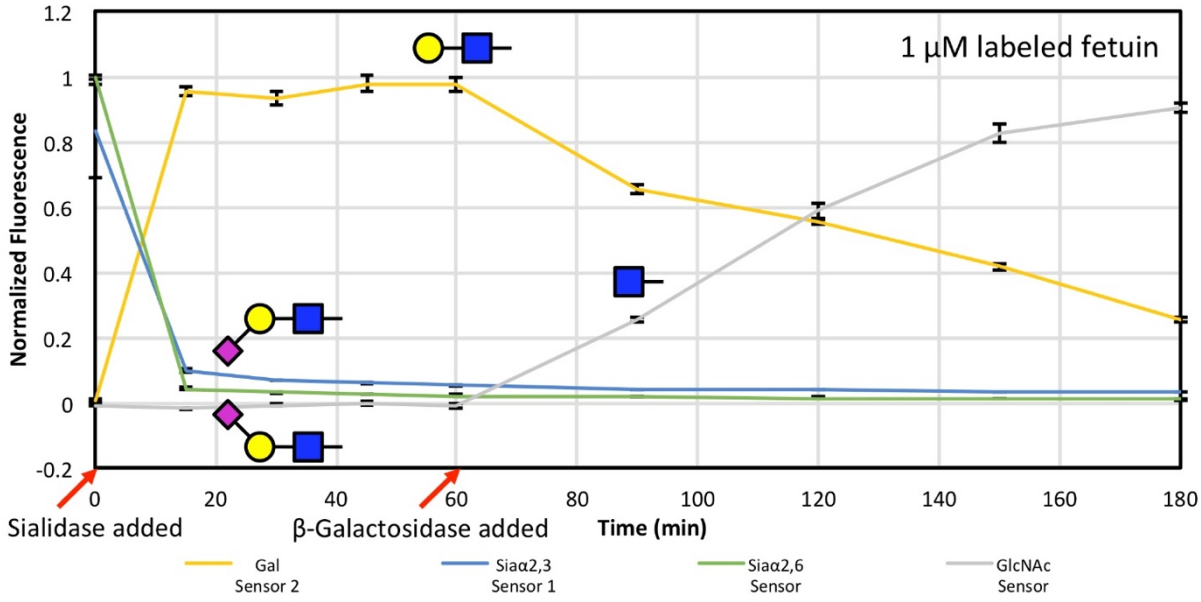
Background:
There is an unmet need for rapid, cost-effective, robust, and easy to use methods for monitoring protein glycosylation during glycoprotein production whether in cell culture or for in vitro glycoengineering. Lectenz Bio has developed an innovative technology called GlycoSense™ to analyze glycoproteins using flow cytometry and multiplex microspheres (beads). This process analytical technology (PAT) does not supplant full glycoprofiling by traditional methods, but provides unique information that can guide process development and advance academic research.

Methods:
The GlycoSense™ method detects binding between glycans and glycan-specific reagents that are conjugated to spectrally-unique beads. By combining the individual bead-based reagents into a multiplex array, a profile of key glycan features can be obtained in a few minutes on a basic cytometer. In addition to established carbohydrate-detection reagents (antibodies and lectins), the GlycoSense™ approach can employ the engineered proteins (known as Lectenz®), developed by Lectenz Bio. Lectenz® are derived from catalytically inactivated glycan-processing enzymes that have been engineered into high affinity glycan binding reagents with well-defined specificities. The conversion of such enzymes into affinity reagents is facilitated by computationally-guided directed evolution. Lectenz® are being developed for a variety of other glycan detection and enrichment applications including affinity chromatography and Western blot.

Results:
We illustrate the performance of the GlycoSense™ method using glycoprotein standards and demonstrate its utility in glycoprotein cell culture, and in in vitro glycoengineering by monitoring changes in glycosylation patterns upon treatment of glycoproteins with glycosidases.

Conclusion:
The GlycoSense™ method offers a unique ability to rapidly detect and monitor changes in glycosylation using only a commonplace benchtop flow cytometer, without the need to purify the glycoprotein analyte or to release the glycans enzymatically.



Fluorescently labeled fetuin treated with sialidase for 1 hour, followed by galactosidase for 2 hours. Error bars are standard deviation values from triplicate time points, background subtracted and normalized to the highest signal for each bead.

OL3.3.4

Linkage-Specific Characterization of Sialylated N-Glycans from Human Plasma by LC-MS

Alan Moran[1,2], Richard Gardner[2], Jennifer Hendel[2], Daniel I R Spencer[2]

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Introduction:
The developing era of precision medicine requires novel disease biomarkers that have molecular characteristics with predictive and/or prognostic value. For example, molecules such as α(2,3)- and α(2,6)- linked sialic acid on N-glycans have been implicated in studies investigating malignant transformation [1]. In these cases, aberrant sialylation can be analysed using several methods including liquid chromatography-mass spectrometry (LC-MS) and MALDI-MS [2]. Various sialic acid chemical modification techniques have been applied in MALDI-MS analyses in order to allow linkage-specific characterization [3]. In comparison, sialic acid linkage differentiation using LC is often based on retention times and/or sequential exoglycosidase digestions [4]. However, few LC-MS methods exist that allow linkage-specific characterization of sialic acids [5].

Methods:
A novel experimental approach for sialic acid linkage differentiation will be presented that involves the selective chemical derivatization of sialylated N-glycans followed by analysis using LC-MS. First, PNGase-F released N-glycans from human plasma were fluorescently labelled using procainamide, followed by removal of excess labelling reagent using a hydrophilic interaction chromatography (HILIC)-based technique (GHP) [6]. Next, ethyl esterification and amidation of sialic acids was carried out with a subsequent GHP membrane clean-up [3]. Finally, modified N-glycans were analysed by HILIC-MS using a (Waters) BEH-glycan column.

Results:
There were prominent differences in the retention times associated with derivatized vs. non-derivatized N-glycans (figure 1). In general, derivatization resulted in overall earlier elution of the sialylated glycans whereby glycans with α(2,6)-linkages were most effected. The final assignment of derivatized sialylated structures was carried out based on three main features: retention times specific to sialic acid linkage, parent ion mass and specific molecular ions. As a result, a more in-depth characterization of human plasma N-glycans could be made. Thus, this study supports the use of chemical modification followed by LC-MS analysis as a platform to characterise α2,3- and α2,6-sialylated glycan isomers. Importantly, this may have implications for precision medicine whereby abnormal changes in these monosaccharides may represent a potential new class of disease biomarker.

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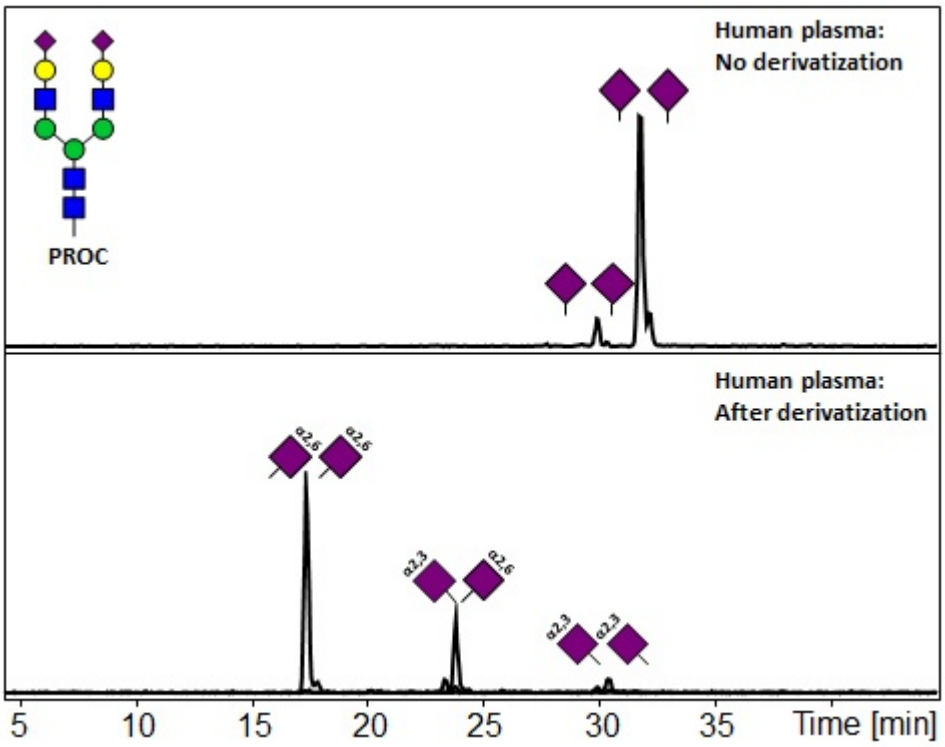

Figure 1: Extracted ion chromatograms of procainamide labelled A2G2S2 N-glycan from human plasma. LC-MS analysis was performed using hydrophilic interaction liquid chromatography mass spectrometry (HILIC-MS) with a (Waters) BEH-glycan column. Top: Underivatized N-glycan is shown. Bottom: Display of derivatized N-glycan(s) for α(2,6) α(2,6) (left), α(2,3) α(2,6) (middle), and α(2,3) α(2,3) (right).

OL3.4.1

N-Heterocyclic Carbene Mediated Activation of Aldoses: Catalyst Controlled Divergence

Markus Draskovits[1], Hubert Kalaus[1], Christian Stanetty[1], Marko D. Mihovilovic[1]

[1]TU Wien, Vienna, Austria

Despite being Nature's biggest chiral pool, there is only a small set of standard carbohydrates, which are readily available (commercially and synthetically). In our research, we focus on the development of new synthetic methodology for the interconversion of abundant sugars to more exotic ones by utilizing the natural reactivity of the aldehyde moiety. Herein, we report our first breakthroughs in the organocatalytic anomeric activation with N-Heterocyclic Carbenes (NHCs, as highly aldehyde selective reagents) yielding defined dehomologation products. Inspired by the reported use of sugars as sacrificial feedstock generating multiple C1-synthons under the mediation of NHCs [1], we have changed perspective and focused on the sugar-based starting materials. Subjecting partially protected aldoses to the activation we delivered a proof of concept for the feasibility of a controlled dehomologation methodology when discovering examples of strong substrate control over the selectivity between the initially targeted pure dehomologation and a competing follow-up reaction - the NHC-triggered redox-lactonization [2] (Figure 1, top). Aiming for a general solution, we targeted catalyst control over this type of divergence as ultimate goal. To facilitate the required evaluation of changes in reaction parameters as well as catalyst structure we developed a high throughput screening based on solid phase extraction, derivatization of sugar compounds and quantification via calibrated GC. This survey, lead to the aspired discovery of two types of catalysts giving either pure dehomologation or alternatively the subsequent redox-lactonization under otherwise identical conditions [3] (Figure 1, bottom). We hope to have set the base for future applications studied by us and others based on NHC-catalysis under the specific and challenging conditions of reducing sugars as reaction partners.

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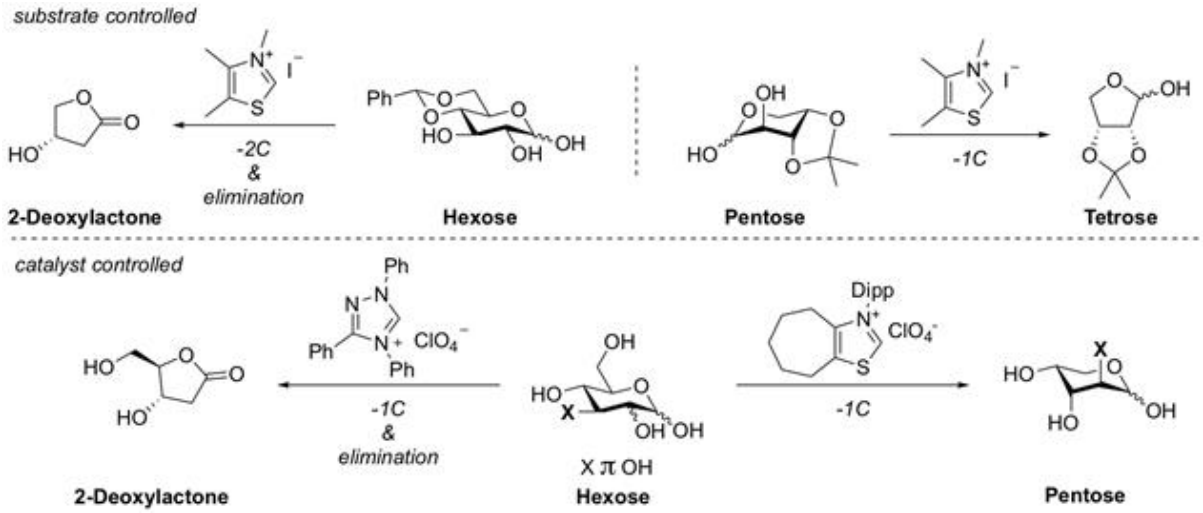

Substrate and catalyst controlled activation of an aldose going either way

OL3.4.2

New Strategies Towards Fine Chemicals from HMF and GMF

Weigang Fan, Charlie Verrier, Lianjie Wang, Eman Dokmak, Sylvie Moebs, Mohammed Ahmar, Florence Popowycz, Yves Queneau[1]

[1]*ICBMS, Univ Lyon, INSA Lyon, UCBL, CNRS, CPE, Villeurbanne, France*

In the frame of our projects dedicated to biobased chemistry, we have studied several reactions using HMF or its glucosylated analog GMF as buiding blocks towards novel fine chemicals [1-4]. This communication gives an overview on our recent in this field.
For the MBH reaction, after a focus on biobased solvents [2], we have turned our attention to solventless conditions. We have also studied the multicomponent version of the aza-MBH reaction using GMF leading to novel α-amino-esters bearing a carbohydrate residue [3].
Considering the high synthetic interest of multicomponent reactions which give straightforward access to complex molecules [5], we have also investigated the Biginelli reaction, which involves a condensation of an aldehyde, a C–H acidic carbonyl compound and an urea leading to dihydropyrimidinones. We show that HMF sensitivity to acidic conditions can be overcome, reporting the first example of HMF in this process, with solvent-free reaction conditions [1].
The exploitation of 5-hydroxymethyl furfural (5-HMF) in aza-Piancatelli and Kabachnik-Fields reactions was also investigated, as well as dipolar cycloaddition of nitrones. Preliminary results on all these directions will be given, with examples of novel bio-based derivatives accessible by them.

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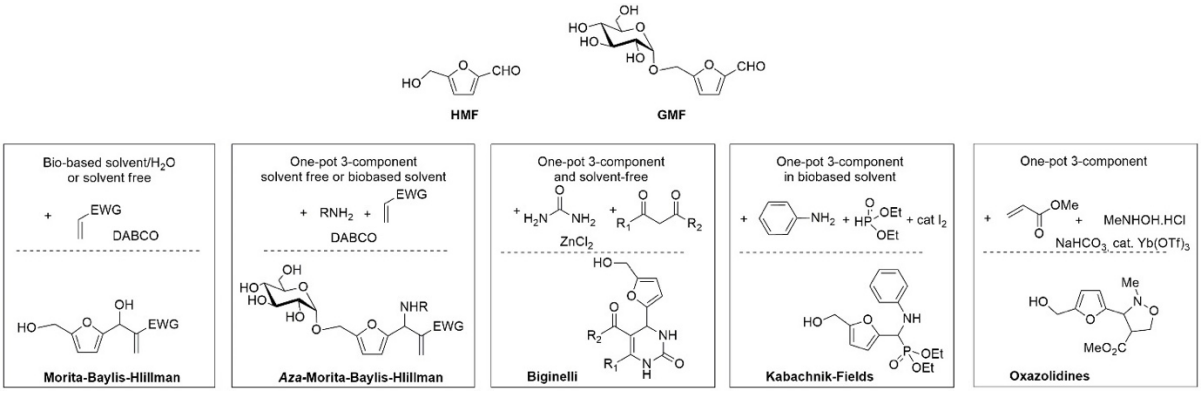

Figure 1 : Various routes from HMF and GMF towards fine chemicals

OL3.4.3

Non-Covalent Catalysis as Powerful Biomimetic Approaches in Strain-Release Glycosylations

Charles C. J. Loh[1]

[1]Max Planck Institute For Molecular Physiology, Department of Chemical Biology, Dortmund, Germany, [2]Technische Universität Dortmund, Fakültät für Chemie und Chemische Biologie, Dortmund, Germany

The research directions in the Loh's research group involve the usage of state-of-the art catalysis concepts to generate challenging carbohydrate based derivatives, and to unravel novel biological activity in pseudo-glycosidic scaffolds. One of the key features is the focus of our research programme on fundamental organic chemistry mechanistic understanding to power development of new glycosidic bond forming concepts, and to address selectivity challenges. Results emerging from our research revealed that non-covalent organocatalysts are extremely versatile in constructing a wide range of O-, N-, C- and S-glycosides exploiting strain-release.

Very recently, we discovered and reported that sub-molar loadings of as low as 0.05 mol% of a charge enhanced thiourea catalyst was highly efficient in accessing a variety of glycosidic derivatives in strain-release glycosylations.[1] A series of understudied strained cyclopropane-fused furanosides and pyranosides were discovered to perform excellently in this methodology, resulting in an array of glycosides generated with high anomeric selectivity. Mechanistic investigations via in-situ NMR approaches revealed deeper intricacies in the pathway, which opened insights into the divergent and the synergistic Brønsted Acid/Hydrogen Bonding nature of thiourea catalysis reminiscent of glycosyl transferases.[2]

Very recently, further exploration into alternative non-covalent catalysis modes such as XB as an enabling tool was fruitful in our endeavors. Lately, application of these pseudo-glycosidic analogues on cell-based phenotypic screens gave unprecedented results. Applying unbiased forward chemical genetics approaches in these strain-release glycosides unraveled new Hedgehog biological activity previously not known in traditional glycobiology.

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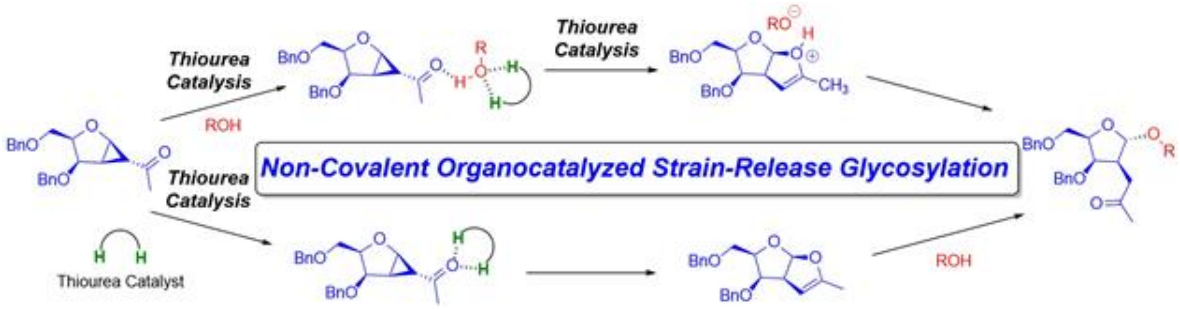

An Ultra-Low Thiourea Catalyzed Strain-Release Glycosylation

OL3.4.4

Assembly of Glycomimetics by Multicomponent Reactions

Ivanka Jeric[1], Kristina Vlahoviček-Kahlina[1], Andreja Jakas[1]

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Carbohydrates are the most abundant class of natural products with a distinctive role in different biological processes, like protein folding, cell–cell communication, and immune response. In addition, synthetic carbohydrate-based compounds found wide application in chemical biology and medicinal chemistry as diagnostics, therapeutics, and vaccines, drug delivery systems, and molecular receptors. Carbohydrates are still relatively untapped pool of new therapeutics, however, advances in functional understanding of carbohydrate-participating biological processes, enabled carbohydrates to take over a prominent role in drug design. Screening of compound libraries is the most available and easy-to-use tool in searching for biologically active molecules. However, a continuous decrease in drug-discovery success suggests deficiencies of currently used libraries. Small-molecule libraries typically represent collections of a large number of structurally similar compounds, that is, the “chemical space” covered is infinitely small. In contract, the ideal chemical library would contain discrete, stereochemically rich, structurally diverse and complex molecules. The concept of expanding the chemical space by developing large collections of structurally diverse molecules is one of the main challenges nowadays.
As a part of our ongoing project, we aim to expand the chemical space of glycomimetics by using multicomponent reactions. Multicomponent reactions (MCRs) offer an attractive one-pot strategy for generating a library of highly functionalized and complex compounds like glycomimetics. Of particular interest is a distinct group of MCRs, isocyanide-based MCRs (e.g., Passerini, Ugi reaction), widely exploited for the generation of drug-like molecules. We developed a smooth multicomponent strategy to access libraries of diverse compounds comprising carbohydrate unit(s) by utilizing, isopropylidene-protected carbohydrate-derived aldehydes and ketones in the Passerini and Ugi reaction.[1] We applied this methodology to access both, simple N-alkylated C-glycosyl amino acid derivatives and densely functionalized glycomimetics bearing up to four carbohydrate. Also, we showed that the strategy can be used for the synthesis of homo- and hetero-multivalent glycomimetics. Access to highly valuable building blocks based on α-hydroxy C-glycosyl acids or more complex systems is elaborated by simple post-condensation methodologies.

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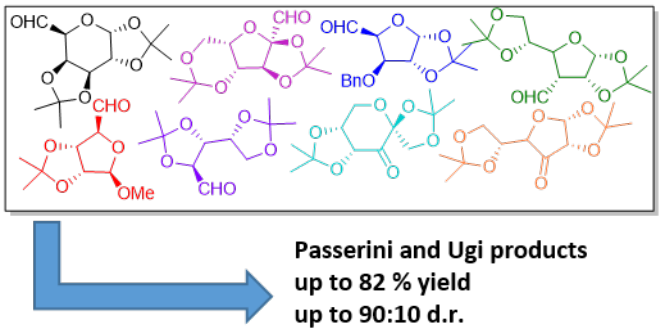

Figure 1. Scope of carbohydrate-derived aldehydes and ketones used in MCRs.

OL4.1.1

Protein Rhamnosylation in Pseudomonas Aeruginosa: A Study on Substrate Recognition Using B-Hairpin Mimetics

Liubov Yakovlieva[1], Thomas Wood[2], Nathaniel Martin[2], Marthe Walvoort[1]

[1]University Of Groningen, Groningen, The Netherlands, [2]Universiteit Leiden, Leiden, The Netherlands

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

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

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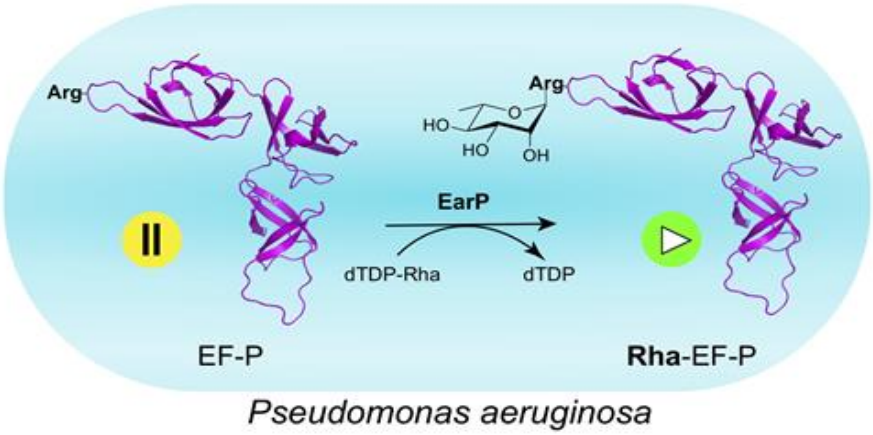

Fig.1 Arginine rhamnosylation as a mechanism to activate elongation factor P in Pseudomonas aeruginosa.

OL4.1.2

Deciphering Galnac O-Glycosylation: From Structure to Function in Human Health & Disease

Helena Coelho[1,2,3], Matilde de las Rivas[4], Ana Diniz[1], Jorge Dias[1], Eurico J. Cabrita[1], Francisco Corzana[5], Jesús Jimenez-Barbero[2,3,6], Ramon Hurtado-Guerrero[4], Filipa Marcelo[1]

[1]UCIBIO Requimte FCT UNL, Caparica, Portugal, [2]CIC bioGUNE , , Spain, [3]Departament of Organic Chemistry II, UPV-EHU, , Spain, [4]BIFI, University of Zaragoza, BIFI-IQFR (CSIC) Joint Unit, , Zaragoza, Spain, [5]Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, Logroño, Spain, [6]Ikerbasque, Basque Foundation for Science, Bilbao, Spain

Glycosylation is the most complex and widespread process of posttranslational modification of proteins and lipids, with an unsurpassed capacity to generate a wide array of structures. [1] The large polypeptide GalNAc-transferase (GalNAc-Ts) family catalyzes the transfer of N-acetylgalactosamine (GalNAc) from a sugar donor UDP-GalNAc to Ser/Thr side chains of cell-surface proteins [2]. Deficiencies and dysregulation of individual GalNAc-Ts have been found to cause diseases and predispositions. Uncontrolled GalNAc-Ts expression alters mucin O-glycosylation influencing a variety of cancer-related functions [2]. Therefore, is vitally needed to understand GalNAc-Ts binding specificities and their mechanism of action in health to further develop inhibitors in diseases such as cancer. In this communication it will be reported the application of NMR methods to follow the mucin O-glycosylation by GalNAc-Ts by using a mucin structure with multiple tandem repeated domains, unveiling new structural, conformational and dynamic insights at atomic level of this biological event. Additionally, it was demonstrated that a GALNT2 mutant (F104S) leading to the inactivation of the enzyme, induces low levels of highdensity lipoprotein cholesterol (HDL-C) in humans.[3] Thus, in this communication it will be also described the molecular basis behind the GalNAc-T2 F104S mutant inactivation, unraveled by the integrative application of X-ray diffraction analysis, molecular modeling and NMR spectroscopic techniques, namely STD-NMR and 19F-NMR [4].

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Acknowledgments: Helena Coelho thanks to the FCT-Portugal for the Projects IF/00780/2015, PTDC/BIA-MIB/31028/2017 and UID/Multi/04378/2019 and to the European commission for the project TOLLerant H2020-MSCETN-642157. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project No 22161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

OL4.1.3

Changing of N-Linked Glycosylation in B1,4-Galt1 Site-Specific Mutant Mouse

Ran Cao[1], Josef Voglmeir[1], Li Liu[1]

[1]*Nanjing Agricultural University, Nanjing, China*

β1,4-GalT1 is type II membrane-bound glycoprotein transferring galactose to acceptor sugars. This enzyme catalyzes the synthesis of lactose or transfers galactose to the terminal GlcNAc of complex-type N-glycans. Previous studies found that β1,4-GalT1 knock-out mouse showed semi-lethality after birth. We obtained a β1,4-GalT1 site-specific mutant mouse modelusing CRISPR/Cas9 in which tyrosine (Y286) were substituted by leucine (L286). This mutation makes β1,4-GalT1 an N-acetylgalactosaminetransferase instead of galactosyltransferase. No lethal deficiency was observed in both heterozygote (+/-) mice and homozygous (-/-) mice. However, homozygous (-/-) mice were unable to give birth and lactation. The further N-glycan profiling showed that homozygous (-/-) mouse serum have no sialylated N-glycans while heterozygote(+/-) mouse showed the similar pattern of N-glycosylation in serum compared with wild-type mouse. The results indicated that the functional changing of galactosyltransferase in homozygous (-/-) mouse leads to a serious interfere of N-glycosylation and might cause the physiological defect of lactation and pregnancy.

OL4.1.4

A High-Throughput Glycosyltransferase Inhibition Assay to Identify Molecules Targeting Fucosylation in Cancer Cell-Surface Modification

David Kwan[1], Xiaohua Zhang[1], Fei Chen[1], Alessandro Petrella[1], Franklin Chacón-Huete[1], Jason Covone[1], Teng-Wei Tsai[2], Ching-Ching Yu[2], Pat Forgione[1]

[1]Concordia University, Montreal, Canada, [2]National Chung Cheng University, Chiayi, Taiwan

In cancers, increased fucosylation (attachment of fucose sugar residues) on cell-surface glycans—resulting from the abnormal upregulation in the expression of specific fucosyltransferase enzymes (FUTs)—is one of the most important types of glycan modifications associated with malignancy. Fucosylated glycans on cell surfaces are involved in a multitude of cellular interactions and signal regulation in normal biological processes. For example, sialyl Lewis-X is a fucosylated cell-surface glycan that is abnormally abundant in some cancers where it has been implicated in facilitating metastasis, allowing circulating tumor cells to bind to the epithelial tissue within blood vessels and invade into secondary sites by taking advantage of glycan-mediated interactions.

To identify inhibitors of FUT enzymes as potential cancer therapeutics, we have developed a novel high-throughput assay that makes use of a fluorogenically labeled oligosaccharide as a probe of fucosylation. This probe, which consists of a 4-methylumbelliferyl glycoside, is recognized and hydrolyzed by specific glycoside hydrolase enzymes to release fluorescent 4-methylumbelliferone, yet when the probe is fucosylated prior to treatment with the glycoside hydrolases, hydrolysis does not occur and no fluorescent signal is produced. We have demonstrated that this assay can be used to measure the inhibition of FUT enzymes by small molecules, since blocking fucosylation will allow glycosidase-catalyzed hydrolysis of the labeled oligosaccharide to produce a fluorescent signal.

Employing this assay, we have screened a focused library of small molecules for inhibitors of a human FUT enzyme involved in the synthesis of sialyl Lewis-X, and demonstrate that our approach can be used to identify potent FUT inhibitors from compound libraries in microtitre plate-format.

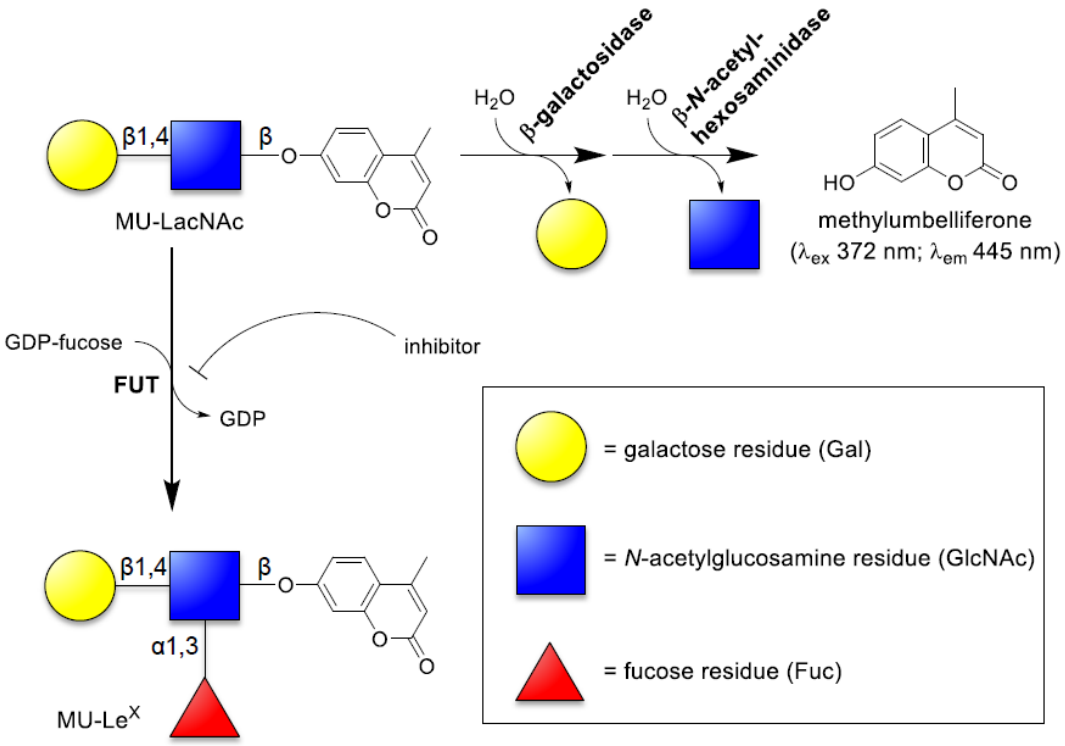

Fig 1: A fluorescence-based inhibition assay of fucosyltransferase activity.

OL4.2.1

Computational Tools to Aid The Design of Glycomimetic Agents

Robert Woods[1]

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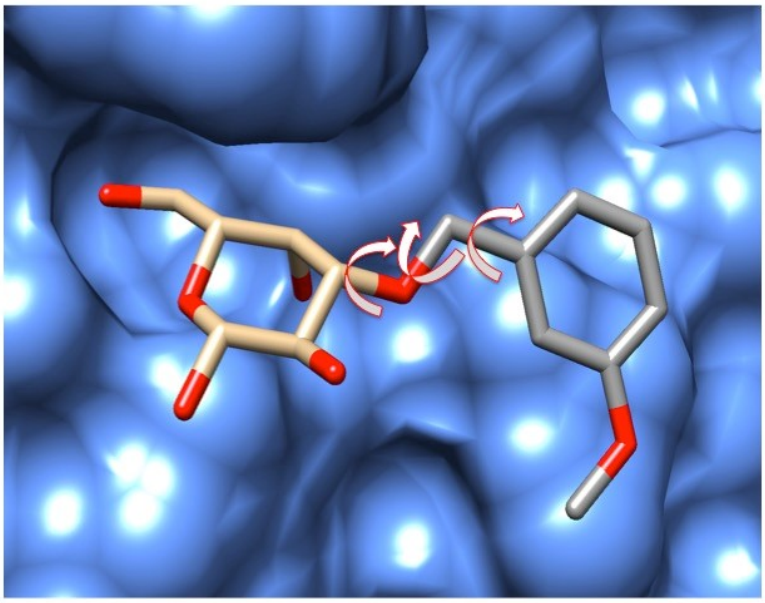
Background:
Carbohydrate-binding proteins (human, bacterial or viral lectins) and carbohydrate-processing enzymes (glycosyltransferases and glycosidases) are important targets for therapeutic intervention, however the creation of drug-like molecules that can competitively inhibit carbohydrate-binding sites is uniquely challenging. Computational approaches that are specifically designed to screen analogs of carbohydrates could be invaluable aids in both increasing the objectivity of the synthetic choices and in prioritizing the synthetic effort required for glycomimetic development. The creation of such a tool is the focus of this presentation.

Methodology:
We present the development of an alternative strategy to ligand docking that leverages the benefits of computational modeling and structural biology. Specifically, we are developing a computational approach that uses carbohydrate-protein co-crystal structures as the basis for lead glycomimetic discovery by modifying the bound oligosaccharide in situ. Interaction energies are computed and compared for several theoretical models (AutoDock v4.2, AutoDock Vina, and AMBER MM-GBSA).

Results:
We compare the performance of both AutoDock scoring functions and the MM-GBSA method applied to inhibitors of Galectin-3 and FimH, and conclude that empirical scoring methods offer important benefits over the more physics-based MM-GBSA approach. While the preliminary results are encouraging, they also indicate that additional energy terms, such as cation-pi, will be necessary for improving performance.

Conclusion:
The presentation will illustrate the degree to which in silico modeling can be applied in the screening and development of glycomimetic compounds.



A glycomimetic created by the addition of a derivative moiety (grey) to a carbohydrate scaffold (beige) shown in the binding site (blue surface) (from PDB ID 5NFA). Once generated, the conformations available to the moiety will be searched (indicated by bond rotation arrows), and the lowest energy conformation identified and scored.

OL4.2.2

New Leads for the Antiadhesive Therapy of Urinary Tract Infections

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Urinary tract infections (UTI) caused by uropathogenic E. coli (UPEC) are among the most common bacterial infections. To date, patients with acute uncomplicated lower UTI are treated with antibiotics to relieve them from infection related symptoms and to prevent exacerbation into life-threatening pyelonephritis or urosepsis. However, the repeated use of antibiotics as a first-line treatment provokes resistance, underlining the need for new strategies to prevent and treat UTI.

The bacterial adhesin FimH, an important virulence factor of UPEC, is located at the distal tip of type 1 pili of uropathogenic E. coli strains (UPECs), which are the major cause of urinary tract infections (UTI). In the initial step of infection, bacteria only weakly interact with the host cell surfaces, still allowing their exploration for optimal nutrition supply. However, when shear forces arise, strong adherence to host cells is necessary to avoid clearance from the bladder. To fulfill these two opposing tasks, FimH relies on a sophisticated allosteric mechanism, fine-tuning its mannose binding affinity according the current requirements through conformational adaption.

Here, we show that the binding affinity, kinetics, and thermodynamics of glycomimetic antagonists to FimH are greatly affected by the conformational dynamics of the protein. Isothermal titration calorimetry (ITC) experiments reveal that strong enthalpy–entropy compensation effects dominate the thermodynamic binding profiles. Kinetic data derived from kinITC analysis gives additional insight into the molecular mechanism of protein–ligand interaction.

Furthermore, we present a series of novel FimH antagonists with high affinity to both disease relevant conformations. Crystallographic and NMR spectroscopic experiments reveal that their unprecedented affinity in the nano- to subnanomolar range is driven by strong π-stacking interactions with the tyrosine gate of FimH. In combination with in vitro pharmacokinetic properties indicative for oral bioavailability, the presented antagonists constitute exhibit promising characteristics for an antiadhesive therapy of UTI.

OL4.2.3

Glycopolymers with Lacnac Branching Discriminate Between Galectins

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LacNAc (Galβ4GlcNAc) is a typical carbohydrate ligand of galectins - human lectins regulating, e.g., intercellular communication, adhesion and signaling [1]. Human galectins participate in a number of pathologies including cancerogenesis, metastatic formation, inflammation or fibrosis. Therefore, they are prospective targets for therapeutical applications where selectivity for one particular galectin is highly desirable. In the present study we synthesized a series of N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers decorated with LacNAc epitope in various concentrations and architectures. The functionalized LacNAc disaccharide was prepared by enzymatic synthesis catalyzed by β-galactosidase from Bacillus circulans [2]. In a structure-affinity relationship study we compare the difference in affinity between LacNAc distributed statistically on the polymer backbone or nested on bi- and trivalent phenyl branches. The affinity of prepared glycopolymers to galectins was determined in ELISA-type assay. We conclude that the manner of the LacNAc presentation on the HPMA copolymer brings a clear discrimination between galectins, reaching affinity in nanomolar range. The prepared selective glycopolymers are attractive for in vivo use due to their good water solubility and lack of toxicity and immunogenicity [3].

Acknowledgement: Support by grant projects LTC17005, LTC18038 and LTC18041 by MSMT CR is gratefully acknowledged.

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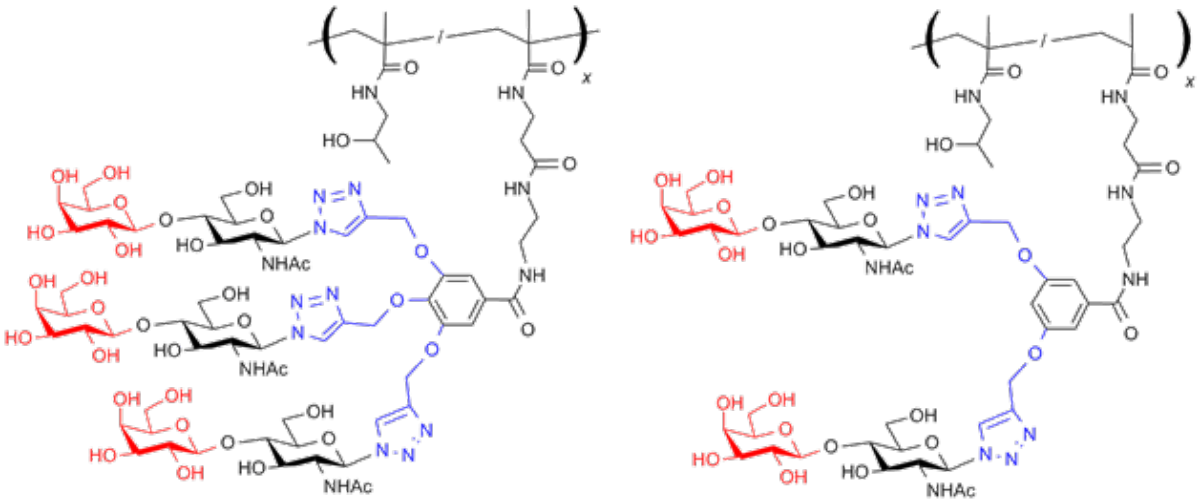

Figure 1. HPMA copolymers decorated with LacNAc epitope on phenyl branches.

OL4.2.4

First X-Ray Crystal Structural Elucidation of the CLEC10A (Hmgl-1) Tn Antigen Binding Mode

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Lectins are the main class of protein which recognize glycans. A member of the C-type lectin family is CLEC10A (h-MGL, CD301), an endocytic receptor located on the surface of immature dendritic cells (DCs) and macrophages. The carbohydrate recognition domain shows exceptional selectivity for α/β GalNAc derivatives, such as Tn antigen and other tumor associated antigens. The binding is calcium dependent and the QPDxW epitope plus the His 284 (full MGL numbering) are indispensable for binding and GalNAc selectivity [1].
In this work we present the first crystal structure of the carbohydrate recognition domain (CRD) of h-CLEC10A with GalNAc derivatives, including the Tn antigen. The X-ray crystal structure reveals that ligands bind in mode typically defined for lectins as binding mode A.
A previous study in solution [2] by NMR and molecular dynamics models led to the proposal of mode B being favoured over mode A (4:1 ratio).
The structure shows that binding mode A has interactions of the GalNAc H-3 and H-4, polarized by coordination to a calcium ion, with the indole ring of Trp 271[3] and also shows hydrogen bonding of the acetamide group to His 286.
The crystal structure packing showed trimerisation of the CRDs. This is independent from the area where the coiled coil neck region would be located, the latter which is believed to induce trimerization. Furthermore, various ligands for the CRD of CLEC10A and the full-length CLEC10A, including glycoculsters, were investigated further by isothermal calorimetry and microscale thermophoresis.
The structural work provides opportunities for structure based ligand or glycocluster design.

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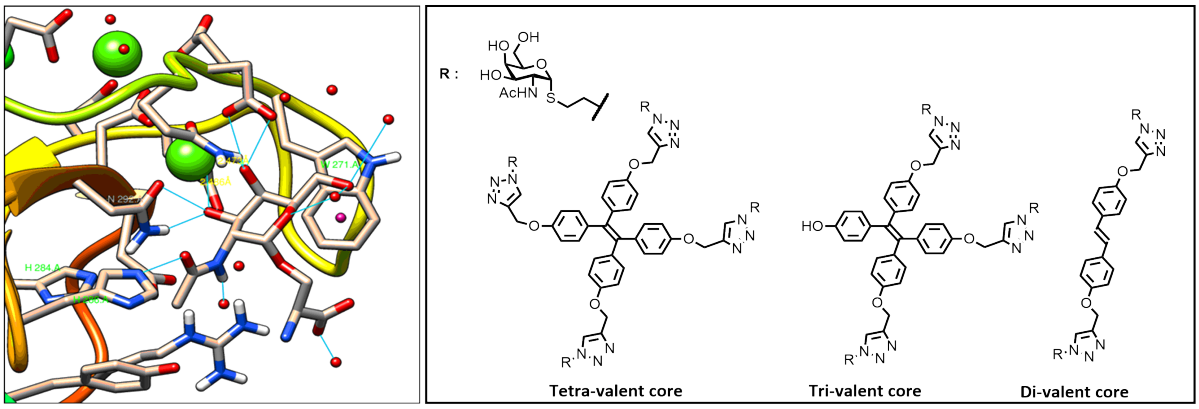

Left: CLEC10A binding with Tn antigen; right: GalNAc multivalent glycoclusters.

OL4.3.1

Synthesis and Applications of a Novel Tandem Ring-Closing Metathesis Cleavable Linker for Solid Phase Synthesizers

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Since the early 1960's, when R. Merrifield first introduced the term "solid phase synthesis", researchers have focused their efforts on establishing new methods and machines to streamline the synthesis of oligomers (peptides, oligonucleotides and oligosaccharides).[1] The linker —used to connect the first synthon to the solid support—plays a key role in solid phase synthesis; the ideal linker system is stable towards all elongation cycle and deprotection conditions and is efficiently cleaved from the solid support at the end of the synthesis under conditions that do not affect the remaining functionalities of the oligomer. To this end, we present in this work a novel tandem Ring-Closing Metathesis (RCM) linker and its applications in biologically relevant oligomer production, such as E. faecalis diheteroglycan oligosaccharides, bacterial β-(1,3)-glucans and bacterial teichoic acid fragments (fig. 1) utilizing automated solid-phase synthesizers. This new RCM-linker is easily synthesized, stable under acidic and basic media, and no additives are necessary during the cleavage from the polymeric support under mild and neutral conditions.[2] It delivers the target compounds -after deprotection- with a suitable amine handle for further conjugation purposes.

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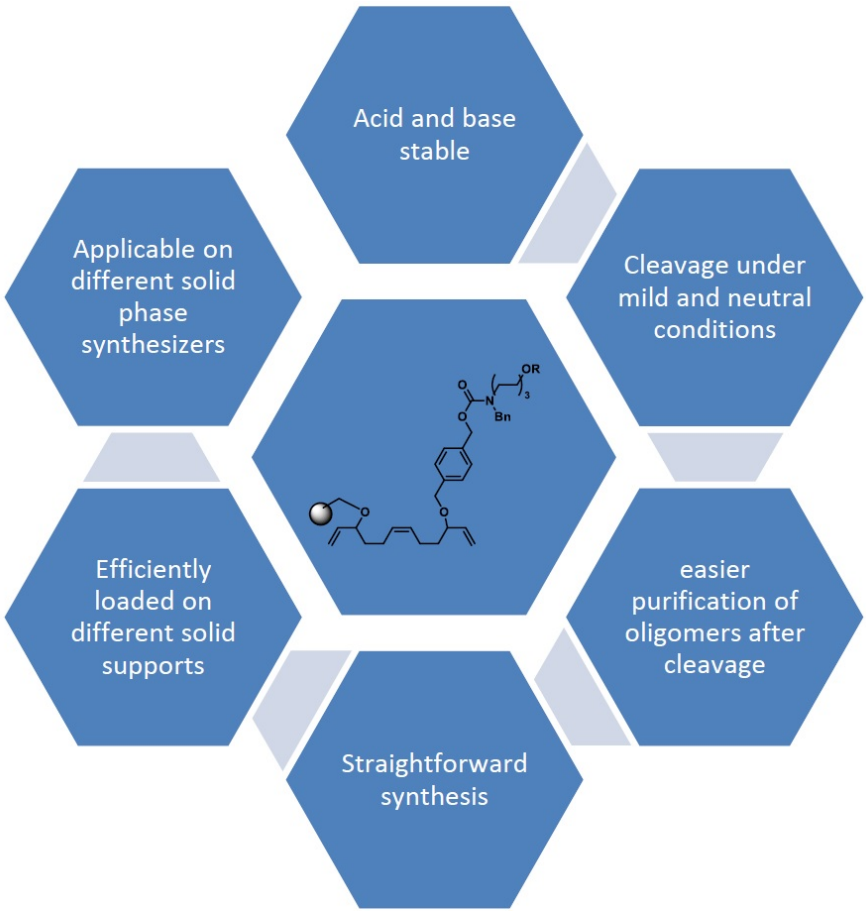

OL4.3.2

Hplc-Based Oligosaccharide Synthesis: Entirely Automated Glycan Assembly

Matteo Panza[1], Keith Stine[1], Alexei Demchenko[1]

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Since the introduction of solid supported synthesis of oligosaccharides in the last century [1], the field has seen a substantial progress with the development of automated devices [2]. The HPLC based system, developed by Demchenko and Stine [3], was upgraded with the implementation of an autosampler allowing for the automated delivery of the promoter for glycosylation reaction [4]. Despite that, the system still lacked the components for full automation.
Presented herein is the introduction of a new computer operated split-valve module and a programmable autosampler in the design of fully automated synthetic sequences (Figure 1a).
Simple programs have been written and combined into more sophisticated sequences to build target glycans (Figure 1b). All the reagents are delivered automatically through the autosampler while the quaternary pump delivers the solvents necessary for the reactions.

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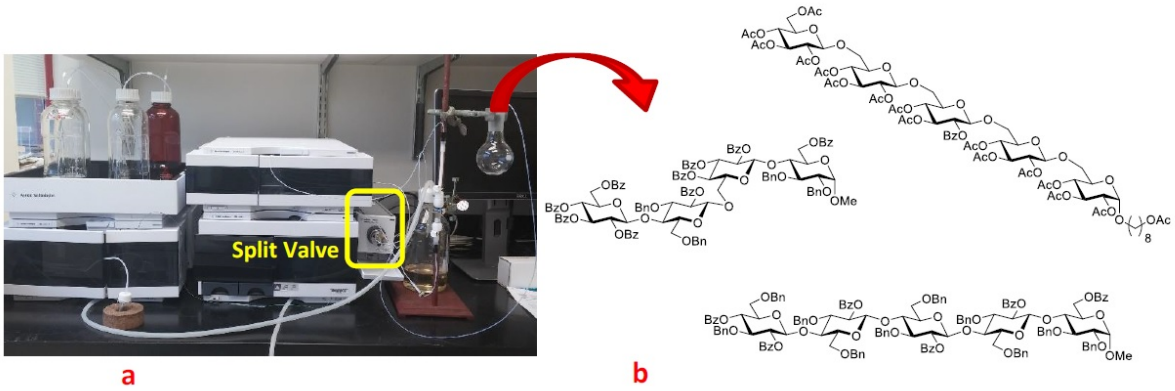

Figure 1. (a) HPLC-based synthesizer equipped with split valve and (b) target glycans

OL4.3.3

Synthesis of Branched Oligosaccharides Related to the Pectic Rhamnogalacturonan I Polysaccharide

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Pectin is one of the major plant cell wall components. It is constituted by highly complex and heterogeneous polysaccharides, which contribute to the modulation of several physiological processes such as cell growth and differentiation, cell-cell adhesion and cell wall support, and defense mechanisms.[1,2] Rhamnogalacturonan I (RG-I) is one of the structural domains of pectic polysaccharides, along with homogalacturonan, rhamnogalacturonan II, xylogalacturonan, and apiogalacturonan. Structurally diverse and complex, RG-I polysaccharides have a backbone consisting of [→2)-α-L-Rhap-(1→4)-α-D-GalA-(1→] disaccharide repeats, with numerous branching side-chains of galactans, arabinans, or arabinogalactans, generally found at the C-4 position of L-rhamnose residues.[3]
Given the structural complexity of RG-I polysaccharides, well-defined synthetic RG-I related oligosaccharides are necessary for investigating enzymes involved in pectin biosynthesis and degradation, as well as for aiding structural analyses of the peptic polysaccharides. While several syntheses of fragments corresponding to the RG-I backbone have been described, the chemical synthesis of large structures with a branched backbone have not been previously reported.[4] Herein, we present our most recent results on the synthesis of a RG-I heptasaccharide backbone by means of a [4+3] glycosylation and late-stage oxidation approach, and the installation of galactan side-chains of different lengths to furnish novel branched RG-I fragments (Figure 1).

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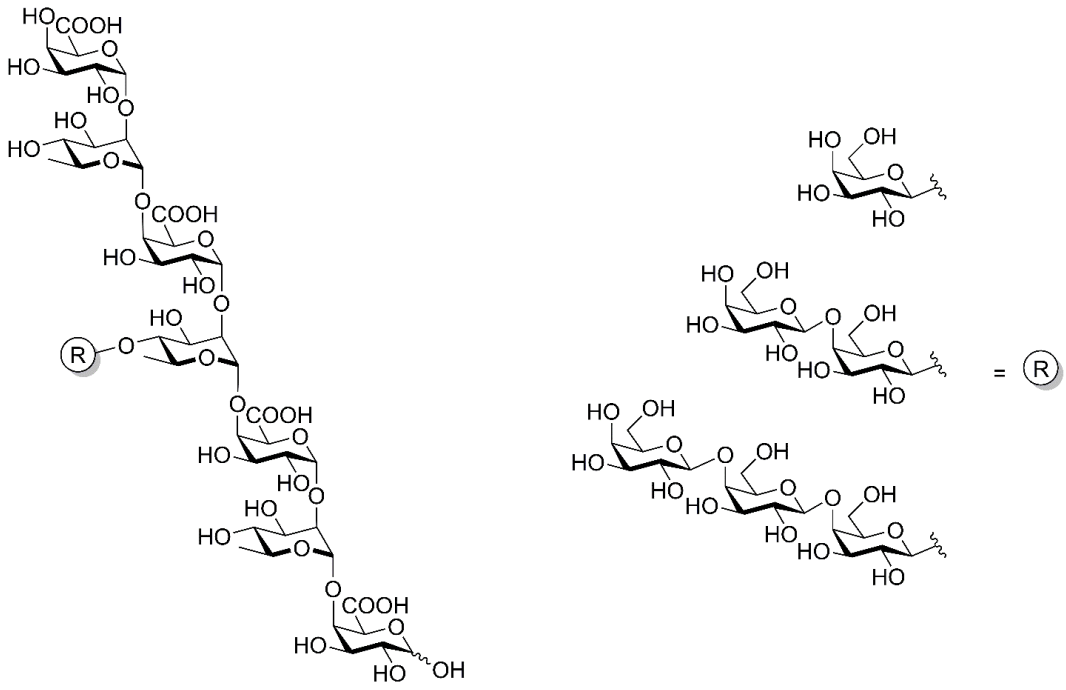

Figure 1: Branched-backbone RG-I fragments

OL4.3.4

Glycosidase-Catalyzed Synthesis of Glycosyl Esters and Phenolic Glycosides

Vladimír Kren[1], Ivan Bassanini[2], Lucie Petraskova[1], Jana Kapesova[1], Sergio Riva[2]

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Glycosides of phenolic acids and of derivatives of hydroxycinnamic acids are ubiquitous in plants, however their isolation from plant material is very tedious. Most of phenolic acid glycosides are glycosylated on the aromatic hydroxyls. However, glycosides attached to the carboxylic moiety can also be rarely found (glycosyl esters; typically β-glucopyrano-sides). Their chemical synthesis is not trivial and involves inherent problems of high lability of glycosyl ester bond, which is incompatible with most acyl protection groups. Glycosides of phenolic acids and of derivatives of hydroxycinnamic acids are ubiquitous in plants, however their isolation from plant material is very tedious. Most of phenolic acid glycosides are glycosylated on the aromatic hydroxyls. However, glycosides attached to the carboxylic moiety can also be rarely found (glycosyl esters; typically β-glucopyrano-sides). Their chemical synthesis is not trivial and involves inherent problems of high lability of glycosyl ester bond, which is incompatible with most acyl protection groups. Enzymatic approach mimicking in vivo biosynthesis employs glucosyltransferases but this method uses expensive UDP-glucose and the yields are low. We have recently described new robust diglycosidase rutinosidase from A. niger, which is able to glycosylate various acceptors including phenols [1] in a good yield using cheap rutin (1) as a glycosyl donor. To our great surprise glycosyl esters were also formed at a reasonable yield. We tested this reaction with a large panel of various phenolic acids and as an example we demonstrate rutinosylation of p-coumaric acid yielding phenolic glycoside (4) and respective glycosyl ester (5). A broader application of this new type of reaction was demonstrated by the synthesis of respective glycosyl esters of p-, m-, o-coumaric acids, ferulic acid and others. Rutinosides can be treated in situ with alpha-L-rhamnosidase (A. terreus) to yield respective β-glucopyranosides. Enzymatic glycosylation of cinnamic acid derivatives is also linked to their (E)-(Z) isomerization in which a quinone intermediate is involved. We describe here probably the first example of glycosylation of a carboxyl group with a glycosidase.

Acknowledgement: Czech Science Foundation project 18-00150S and the joint Czech-Italian AVČR-CNR (V.K. & S.R.) mobility project No. CNR-16-30 are acknowledged.

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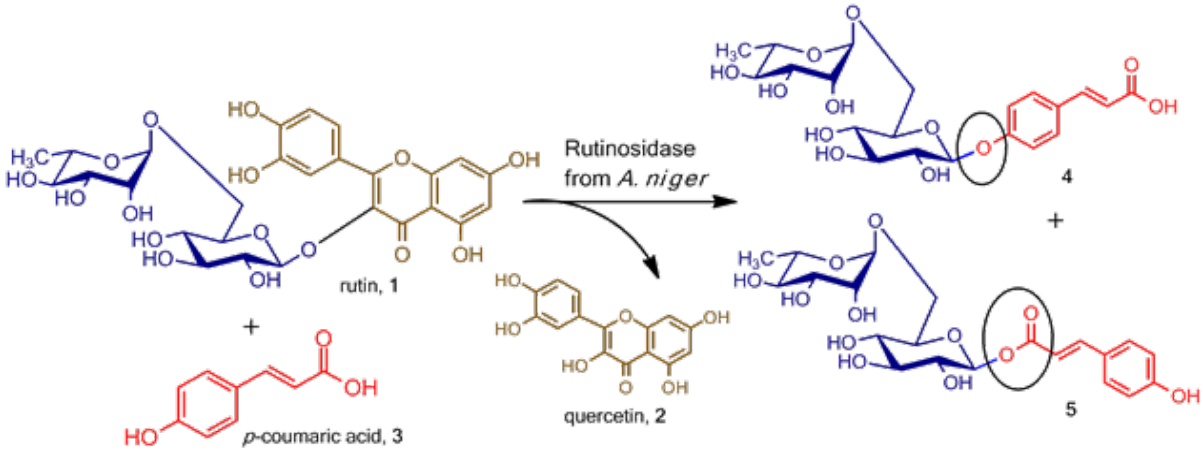

Scheme. Rutinosylation of p-coumaric acid (3) with rutinosidase from A. niger

OL4.4.1

Activity Based Probes for Profiling of (Hemi) Cellulose Active Enzymes

C de Boer[1], S Schröder[1], Nicholas McGregor[2], Jos Reijngoud[1], Jean-Guy Berrin[3], Arthur Ram[1], Jeroen Codée[1], Liang Wu[2], Gideon Davies[2], Herman Overkleeft[1]

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Cyclophellitol, cyclophellitol aziridine and derivatives thereof are selective and potent, covalent mechanism based inhibitors of exo-acting, retaining glycosyl hydrolases. Activity Based Probes (ABPs) based on these inhibitors have contributed tremendously to our understanding of exo-acting enzyme activity in health and disease.[1,2] The construction of cyclophellitol containing carbohydrate oligomers allows the study of endo-acting glycosidases.
In this communication the design and synthesis of dedicated ABPs based on hemicellulose and cellulose motives (the main carbohydrate derived components of plant biomass) is presented. The main challenge is the incorporation of the cyclophellitol warhead in the oligomers and the discovery of reaction conditions that are compatible with the warhead in the late stage of the synthesis. Examples of strategies employed to synthesize different motives will be discussed.
The obtained probes are used to visualize active xylanases, cellulases and xyloglucanases. These probes can aid in the discovery of unknown enzymes acting on recalcitrant biomass leading to higher sugar yields for renewable fuel production.

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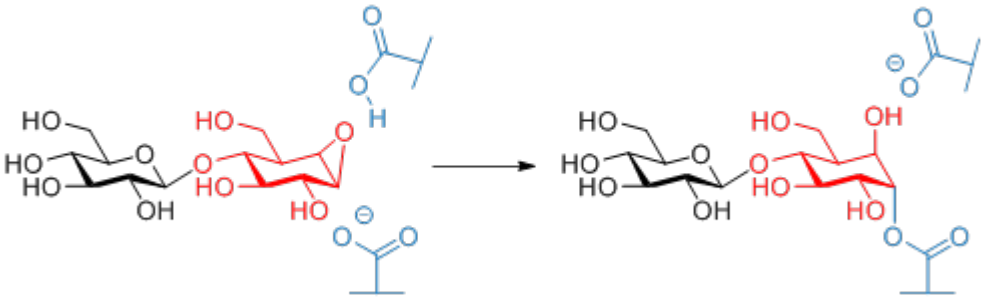

OL4.4.2

Discovery, Biochemical and Structural Characterization of a New LPMO Family with Novel Features Raises Parallels to Cu-Proteins of Unrelated Function

Kristian Frandsen[1,2], Aurore Labourel[1], Tobias Tanddrup[2], Mireille Haon[1], Sacha Grisel[1], Marie-Noëlle Rosso[1], Bernard Henrissat[4,5], Francis Martin[3], Jean-Guy Berrin[1], Leila Lo Leggio[2]

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Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent [1] redox enzymes that oxidatively [2] modify carbohydrate biomass and play a key role in nature e.g. during fungal degradation of plant polysaccharides. The LPMO active site contains a copper atom coordinated by the "Histidine brace" motif (His brace, composed of an N-terminal histidine and a second histidine) highly conserved in members across all families. Currently, six LPMO families exist (AA9-AA11 and AA13-AA15 in the CAZy database [3]) and members are found in fungi, bacteria, viruses and recently in arthropod species (e.g insects) [4,5].

Recently we discovered a new LPMO family found in various lineages of both saprotrophic and ectomycorrhizal fungi. Members of this family invariably harbor a C-terminal glycosylphosphatidylinositol (GPI) anchor. Through transcriptomic analysis, we found that in some ectomycorrhizal fungi the genes encoding these new LPMO family members are upregulated during ectomycorrhizal- and fruiting body formation and immunolabelling experiments revealed that the corresponding LPMO proteins locate to the interface between fungal hyphae and tree rootlet cells, suggesting a role in the symbiosis-related cell wall remodelling.
In this presentation, we focus on the structural characterization of one family member from the saprotroph basidiomycete fungus Laetisaria arvalis, with demonstrated LPMO activity on cellulose. We have determined the X-ray crystal structures of this new LPMO (in three different crystal forms) which show an antiparallel β-sheet topology typical of LPMOs. However, LaLPMO has a diminished active site surface compared to other LPMOs. In addition, all structures show the copper coordinated by a canonical His brace, but also an unusual Asp ligand. Bioinformatic analyses show that the majority of the sequences in the family have a similar Asp ligand, but that a subgroup of sequence appear to have a third His instead of the Asp ligand. These active site arrangements are reminiscent both of an unrelated protein CopC likely involved in copper homeostasis [6], but also of particulate methane monooxygenases (pMMOs) which has a monocopper site with three His ligands [7].
The results from our work expand the known biological and structural diversity of LPMOs and challenges the current view on their functional roles.

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OL4.4.3

Potential Processive Catalysis by an Exo-Hydrolase Identified by Experiment and Computation

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In our group, we apply computational techniques (molecular modelling methods) to study different aspects of carbohydrates: from mechanistic aspects of the reactions catalysed by CAZy enzymes to recognition processes involving carbohydrates or their mimics. After some years studying the mechanism of retaining glycosyltransferases [1], more recently, we have also investigated the transglycosylation and hydrolysis reactions of a family GH1 β-glicosidase [2] and the product exit pathway of a plant exo β-glucosidases of family GH1 (HvExoI). This latter study, done in collaboration with Prof. M. Hrmova (University of Adelaide), has led us to suggest a potential processive mechanism for this polysaccharide hydrolysing enzyme thanks to the identification of a putative transient lateral cavity next to the -1/+1 subsites that would allow the exit of the hydrolyzed glucose product (Fig. 1).
To the best of our kowledge, and contrary to the case of endoglucanases or cellobiohydrolases, this is a mechanism that has not been proposed for any exo-acting hydrolase. The finding could have implication for the biotechnological use of such enzymes (e.g. in biomass degradation).

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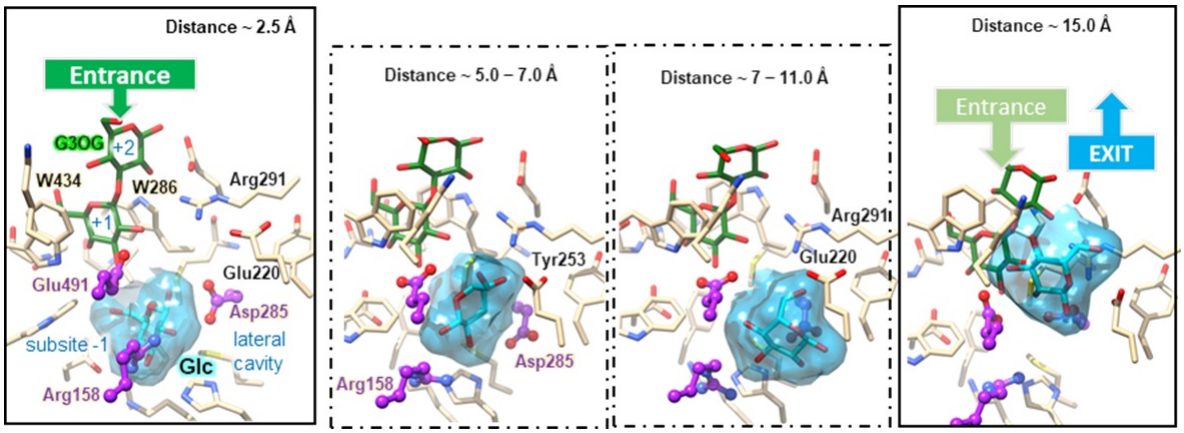

Figure 1. Newly identified exit path for Glc product bound at -1 subsite of HvExoI.

OL4.4.4

Catalysis by a Rigid Enzyme

F. Ben Bdira[1], A. N. Volkov[2], E. AB[3], S. Schröder[1], J. Codee[1], H.S. Overkleeft[1], J. M. F. G. Aerts[1], H. van Ingen[4], M. Ubbink[1]

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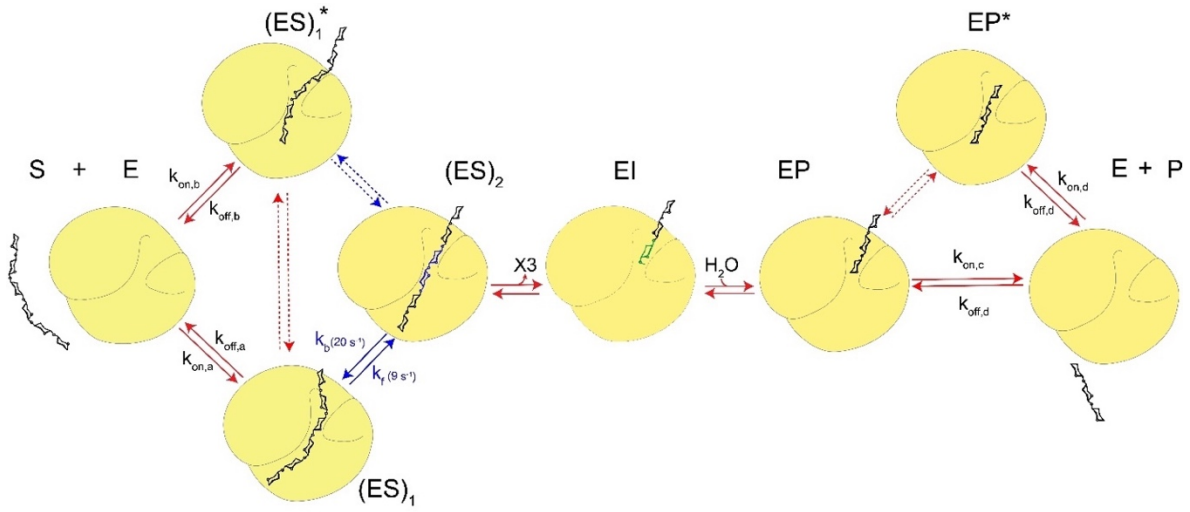
Many enzymes are dynamic entities, sampling conformational states that are relevant for catalytic activity. Nevertheless, crystal structures of catalytic intermediates suggest that other enzymes do not require structural changes for activity. The single domain enzyme xylanase from Bacillus circulans (BCX) is involved in the degradation of hemicellulose. It is demonstrated that BCX in solution is undergoing minimal structural changes during catalysis. A rigid protein matrix provides a frame for fast substrate binding, followed slow distortion induced by the enzyme to enable hydrolysis. Thus, the dynamics of the substrate rather than the enzyme are essential for catalysis.



OL5.1.1

Lipophilicity of Fluorinated Carbohydrates and Derivatives

Bruno Linclau[1]

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Lipophilicity is a much used parameter in drug development, and defined as the octanol-water partition coefficient. It is a measure for membrane permeability, and a proxy for solubility and ADME properties, which determine to a large degree dosing, and thus toxicity effects. Until recently, drug development was strongly focused on bioactivity optimisation, to the extent that lipophilicity of drug candidates was allowed to rise to unsuitable levels (referred to as “molecular obesity”). Indeed, increasing lipophilicity is correlated with stronger binding but it is also correlated with unfavourable solubility and ADME properties. Nowadays bioactivity and lipophilicity are optimised simultaneously in the drug discovery process. The introduction of fluorine in organic compounds is one of the ways to achieve this.
On the contrary, carbohydrates are too hydrophilic, and we all know that the pharmaceutical industry is, in general, not keen to work with carbohydrates partly for that reason. However, the lipophilicity of carbohydrate derivatives is not well-established. In fact, there are, to the best of our knowledge, almost no such data in the literature, and lipophilicity is typically not included in protein-carbohydrate binding considerations. This may be due to the difficult lipophilicity measurement of (the non-UV active) carbohydrates.
Sugar deoxyfluorination will increase lipophilicity, and we became interested in quantifying the extent of these expected changes. This led to the development of a suitable, convenient and accurate 19F NMR based direct logP determination method.[1] This will be explained in the presentation, followed by a discussion of results that include influence of sugar ring stereochemistry, fluorination position, fluorination motif, and anomeric configuration, on the sugar lipophilicity. We will also introduce the concept of “anomeric-specific lipophilicities” of reducing sugars, which will be discussed based on measured values.
We believe that these data provide an extra level of information that may be useful in the interpretation of binding affinity data of modified carbohydrates, and to the optimisation of physical properties of carbohydrate analogues to facilitate the application of carbohydrates in Medicine.

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OL5.1.2

Stereoselective B,B-1,1' Glycosylation for the Synthesis of Lipid A Mimetics

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Toll-like Receptor 4 (TLR4)-mediated pro-inflammatory signaling plays a crucial role in the pathogenesis of numerous inflammatory and autoimmune diseases, which highlights its significance as a target for therapeutics as well as vaccine adjuvants [1;2]. Our preliminary immunobiological tests proved that Lipid A mimetics based on the β,β-1,1'-linked diglucosamine act as powerful TLR4 agonists. Chemical glycosylation towards 1,1'-linked disaccharides is challenging due to possible formation of four diastereomeric products. Existing approaches to 1,1'-linked disaccharides focus on establishing α,α-1,1' and α,β-1,1' linkages using mostly simply protected monosaccharides. In our case an orthogonal protective group pattern was crucial for the synthesis of Lipid A mimetics with complex substitution pattern.

The 1,2-trans selectivity on the side of the glycosyl donor was readily achieved by application of 2-N-carbamate protected imidate donors, whereas the β-stereoselectivity on the side of the glycosyl acceptor required preparation of the anomerically enriched hemiacetals where the β-configuration was trapped via quick glycosylation reaction to afford β,β-1,1' diglucosamines. We established a facile approach towards β-configured GlcN hemiacetals which includes deallylation of protected β-allyl glycosides with retention of configuration via terminal olefin isomerization and ozonolysis followed by methanolysis of the formyl group [3]. The latter were glycosylated with retention of β-configuration by reaction with GlcN-imidate donors. Alternatively, the superior reactivity of the β-configured anomeric OH-group in 2-azido protected GlcN-hemiacetal acceptors which were obtained by NBS-mediated hydrolysis of thioglycosides allowed for stereoselective high-yielding β,β-1,1' glycosylation. Application of conformation-constraining protecting groups (4,6-O-DTBS- or 4,6-O-benzylidene acetal) in both acceptor and donor molecules was decisive for attaining high β,β-1,1' stereoselectivity. Non-symmetric orthogonally protected β,β-1,1'-linked diglucosamines were used as scaffolds for the divergent synthesis of bioactive Lipid A mimetics.

Financial support by Austrian Science Fund (Grant FWF P-28915) is gratefully acknowledged.

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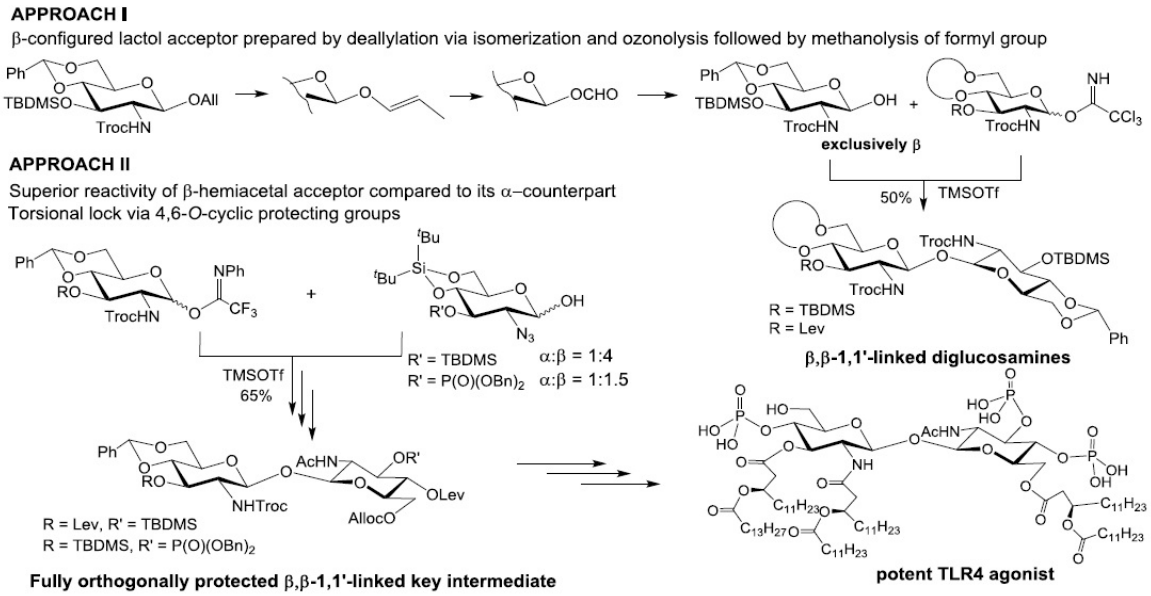

OL5.2.1

Capsular Polysaccharides of the Nosocomial Pathogen Acinetobacter Baumannii: Structure, Genetics of Biosynthesis and Cleavage by Bacteriophage Tailspike Receptor Proteins

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Acinetobacter baumannii is gram-negative conditionally pathogenic bacteria, which cause nosocomial infections, such as pneumonia, wound and catheter-related urinary tract infections, peritonitis, meningitis, endocarditis, and bloodstream infections. Treatment of the infections is complicated by the ability of the bacteria to acquire and accumulate various mechanisms of antibiotic resistance, which poses a serious public health problem. One of the virulence factors of A. baumannii is a capsular polysaccharide (CPS) composed of many oligosaccharide repeats (K units), which forms a thick protective layer around the bacterial cell. Due to polymorphism of the capsule gene locus (K locus, KL) CPS structures are highly diverse (by now more than 120 KL types have been identified). The CPSs are ligands of tailspike receptor proteins of bacteriophages specific to A. baumannii, which bind to and cleave the CPS prior to infection of bacterial cells. The aim of this work was elucidation of CPS structures, characterization of the K loci and mechanisms of the CPS cleavage with bacteriophage tailspike receptor proteins.

The CPS structures of 30 A. baumannii strains of various KL types were established by sugar analysis along with one- and two-dimensional NMR spectroscopy and selective cleavage by Smith degradation or solvolysis with trifluoroacetic acid. The CPSs were found to be built up of tri- to octa-saccharide K units containing common sugars (D-Glc, D-Gal, D-Man, D-GlcNAc, D-GalNAc), 6-deoxy hexoses (L-Rha, L-6dTal), uronic (GlcA) and amino uronic (GalNAcA) acids, derivatives of 6-deoxyamino hexoses (L-FucN, D-Fuc3N, D-QuiN4N) and 5,7-diamino-3,5,7,9-teradeoxynon-2-ulosonic acids (Pse and Leg). A number of A. baumannii CPSs are structurally and genetically related to each other. In some of them, the same K units are linked in different modes (by different linkages or between different monosaccharides). Others differ in the position of a glycosidic linkage between monosaccharides within K units or in the nature of an N-acyl group on an amino sugar. Accordingly, the K loci of strains with the related CPSs show the same organization and contain the same genes but those for polymerases (Wzy), the corresponding glycosyltransferases or acyltransferases are polymorphous. In addition, the CPSs may differ in the presence or absence of a side-chain glycosyl group or an O-acetyl group. The former is added to the K unit by an additional glycosyltransferase encoded in the K locus, whereas O-acetylation is ensured by an acyltransferase encoded by a gene located outside the K locus.

The CPSs were cleaved with recombinant bacteriophage tailspike receptor proteins that possess a polysaccharide-depolymerasing activity, and structures of derived oligosaccharides were established by NMR spectroscopy and high-resolution electrospray ionization mass spectrometry. All depolymerases studied were found to cleave specifically the CPSs of A. baumannii by the hydrolytic mechanism to give monomers or/and oligomers of the K units. The proteins studied may be considered as potential therapeutic agents, and the reducing oligosaccharides obtained by the enzymatic cleavage of the CPSs as components of glycoconjugate vaccines against infections caused by A. baumannii.

OL5.2.2

Challenges with Pseudaminic Acid: From Its Synthesis to Biological Studies

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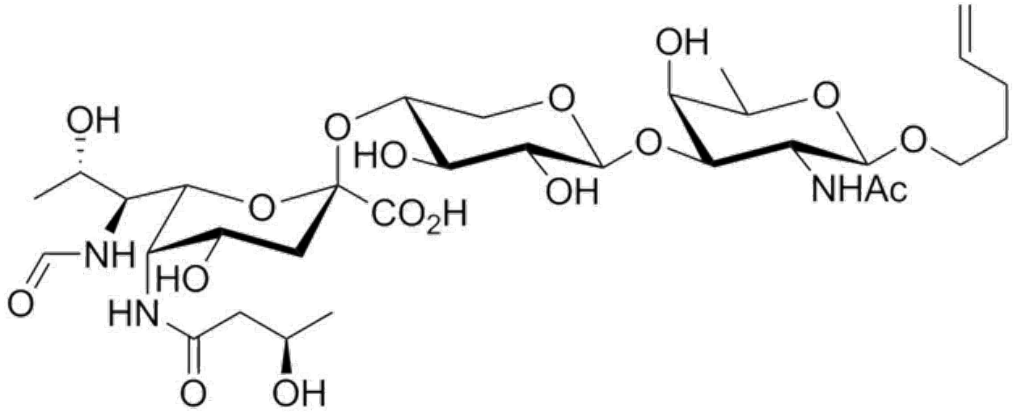
Whereas mammalian oligosaccharides consist of only ten different monosaccharide units (e.g. Glc, Gal, Man, GlcNAc etc.)[1] bacteria present a plethora of different monosaccharides.[2] Many of them are highly desoxygenated, bear amino groups, quaternary carbon centers or longer carbon chains. This feature allows to detect bacteria by carbohydrate-specific antibodies since many of these sugars are not found in mammals. One of these bacterial monosaccharides found in the flagellum of Helicobacter pylori and other bacteria is pseudaminic acid (Pse).[3] Pse is crucial for the assembly of functional flagella, and is required for bacterial motility and invasion of the host intestinal tract. The talk will tackle our synthetic endeavors towards the synthesis of a building block for pseudaminic acid, its incorporation into a trisaccharide found in pilins (Figure 1) and biological studies with azido derivatives.



Trisaccharidic pilin structure including Pse found in Pseudomonas aeruginosa.

OL5.3.1

A-Selective Glycosylation with B-Glycosyl Sulfonium Ions Prepared via Intramolecular Alkylation

Sam Moons[1], Rens Mensink[1], Jeroen Bruekers[1], Maurits Vercammen[1], Laura Jansen[1], Thomas Boltje[1]

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The major challenge in the chemical synthesis of oligosaccharides is the stereoselective synthesis of the glycosidic bond. 1,2-trans glycosides can be synthesized with high selectivity by the use of neighboring group participation of a C-2 acyl group and is applicable to gluco- and manno-type sugars. In contrast, the stereoselective synthesis of 1,2-cis glycosides remains more challenging. By utilizing neighboring group participation using C-2 chiral auxiliaries, the stereoselective synthesis of 1,2-cis gluco[1]- and manno[2]-type sugars could be achieved. These reactions proceed via an intermediate sulfonium ion, which is depicted in scheme 1. This intermediate can be prepared by making three disconnections, of which two have already been explored[1,3].
Using a thioglycoside precursor, β-sulfonium ion intermediates could by prepared utilizing an unexplored disconnection. The synthetic route to this glycoside donors allows for a late stage introduction of the C-2 auxiliary, giving more control over the outcome of the stereoselectivity using a single glycosyl donor. Using low-temperature NMR studies, the activation of the glycosyl donor, as well as the formation of the β-sulfonium ion were investigated. Subsequently, the influence of the thioaryl moiety, as well as the benzyl protecting groups on the stereoselectivity was explored.

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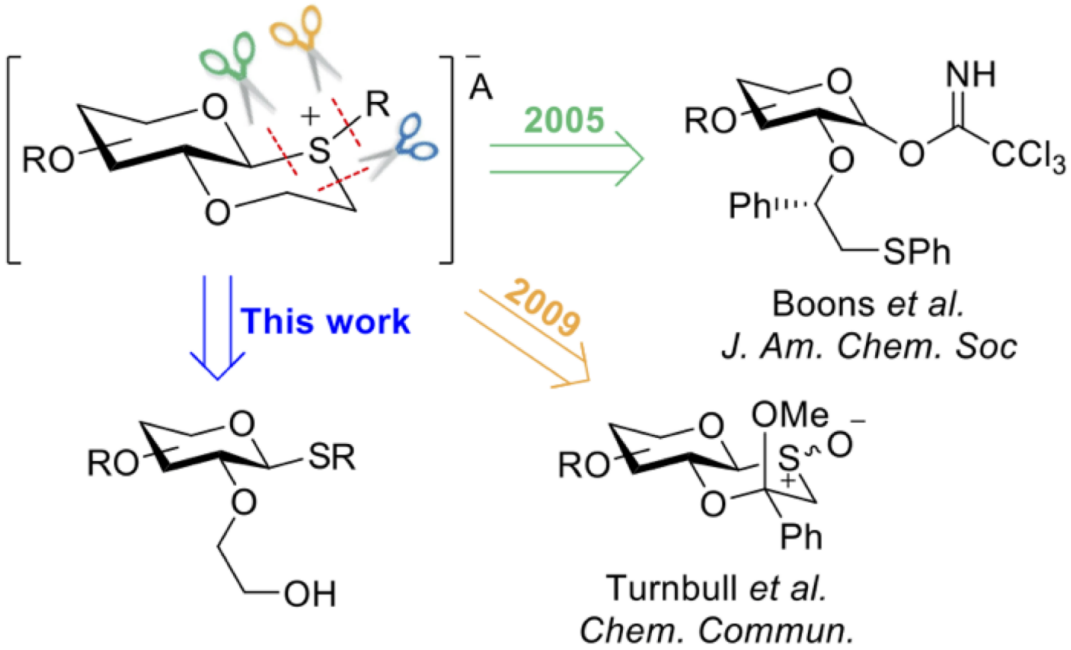

OL5.3.2

Reagent Controlled Stereoselective Synthesis of 1,2-Cis-Glucoside

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The development of a general glycosylation method that allows for the stereoselective construction of glycosidic linkages is a tremendous challenge. Because of the differences in steric and electronic properties of the building blocks used the outcome of a glycosylation reaction can greatly vary when switching form one glycosyl donor-acceptor pair to another. We developed a strategy to install cis-glucosidic linkages in a fully stereoselective fashion that is under direct control of the reagents used to activate a singly type of donor building block. The activating reagents are tuned to the intrinsic reactivity of the acceptor alcohol to match the reactivity of the glycosylating agent with the reactivity of the incoming nucleophile. A protecting group strategy is introduced that is based on the sole use of benzyl-ether type protecting groups to circumvent changes in reactivity as a result of the protecting groups. For the stereoselective construction of the α-glucosyl linkages to a secondary alcohol, a per-benzylated glucosyl imidate donor is activated with a combination of trimethylsilyltriflate and formamide (DMF or N-methyl-N-phenylformamide), while activation of the same imidate donor with trimethylsilyl iodide in the presence of triphenylphospine oxide allows for the stereoselective cis-glucosylation of primary alcohols. The effectiveness of the strategy is illustrated in the modular synthesis of a linear 1,6- 1,4-, 1,3- and 1,2-linked oligoglucosides as well as α-linked glucosamines, present Mycobacterium tuberculosis, Aspergillus fumigatus and Enterococcus faecalis derived antigenic carbohydrates.

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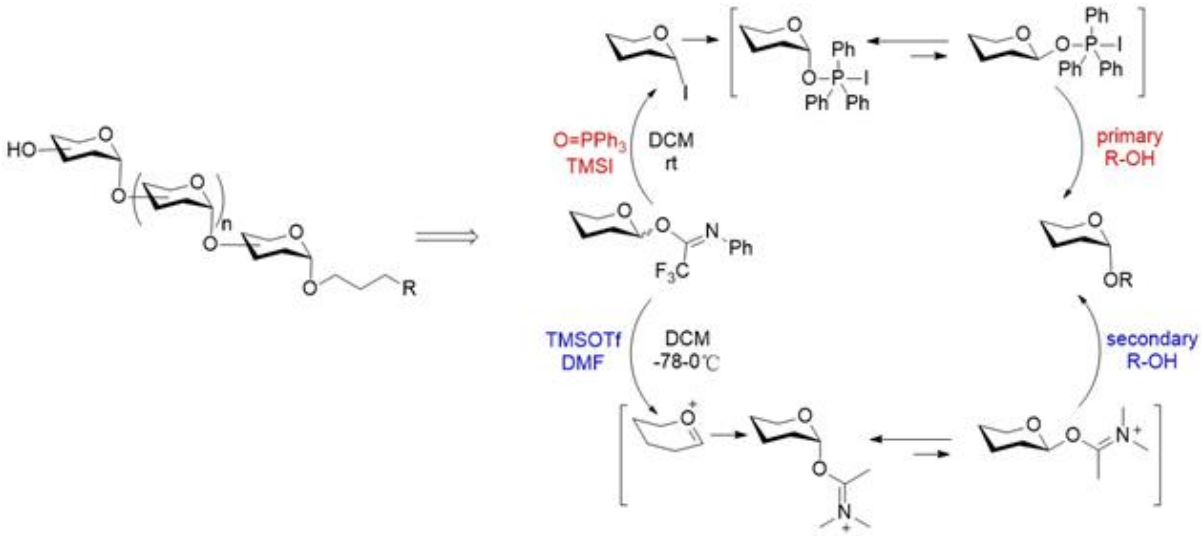

Figure 1. 1,2-cis-oligosaccharides and proposed glycosylation mechanism

OL5.4.1

Differential Bacterial Capture and Transport Preferences Facilitate Co-Growth on Dietary Xylan in the Human Gut

Maher Abou Hachem[1], Maria Louise Leth[1], Morten Ejby[1], Christopher Workman[1], David Adrian Ewald[1], Signe Schultz Pedersen[1], Claus Sternberg[1], Martin Iain Bahl[2], Tine Rask Licht[2], Finn Lillelund Aachmann[3], Bjørge Westereng[4]

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Maria L. Leth1, Morten Ejby1, Christopher Workman1, David A. Ewald1, Signe, S. Pedersen1, Claus Sternberg1, Martin I., Bahl2, Tine Rask Licht2, Finn L. Aachmann3, B., Westereng, Maher Abou Hachem1

Metabolism of dietary glycans is pivotal in shaping the human gut microbiota. However, the mechanisms that promote competition for glycans among gut commensals remain unclear. Roseburia intestinalis, an abundant butyrate-producing Firmicute, is a key degrader of the major dietary fibre xylan. Despite the association of this taxon to a healthy microbiota, insight is lacking into its glycan utilization machinery. This study examined the apparatus that confers the growth of R. intestinalis on arabino- and glucuronoxylans.
R. intestinalis displays a large cell-attached modular xylanase that promotes multivalent and dynamic association to xylan via four xylan-binding modules, one of which represents a previously unknown family of xylan binding modules with lower affinity than canonical xylan binding modules from known families.
This xylanase of glycoside hydrolase 10, which is conserved in the R. intestinalis species, operates in concert with an ATP-binding cassette (ABC) transporter to mediate breakdown and selective internalization of xylan oligomers. The solute binding protein of this ABC transporter was characterized to reveal the binding preference for oligomers of 4–5 xylosyl units, which is different from the corresponding transport protein from a model xylan-degrading Bacteroides commensal that targets larger ligands. Although R. intestinalis and the Bacteroides competitor co-grew in a mixed culture on xylan, R. intestinalis dominated on the preferred transport substrate xylotetraose. These findings highlight the differentiation of capture and transport preferences as a possible strategy to facilitate co-growth on abundant dietary fibres and may offer a unique route to manipulate the microbiota based on glycan transport preferences in therapeutic interventions to boost distinct taxa.

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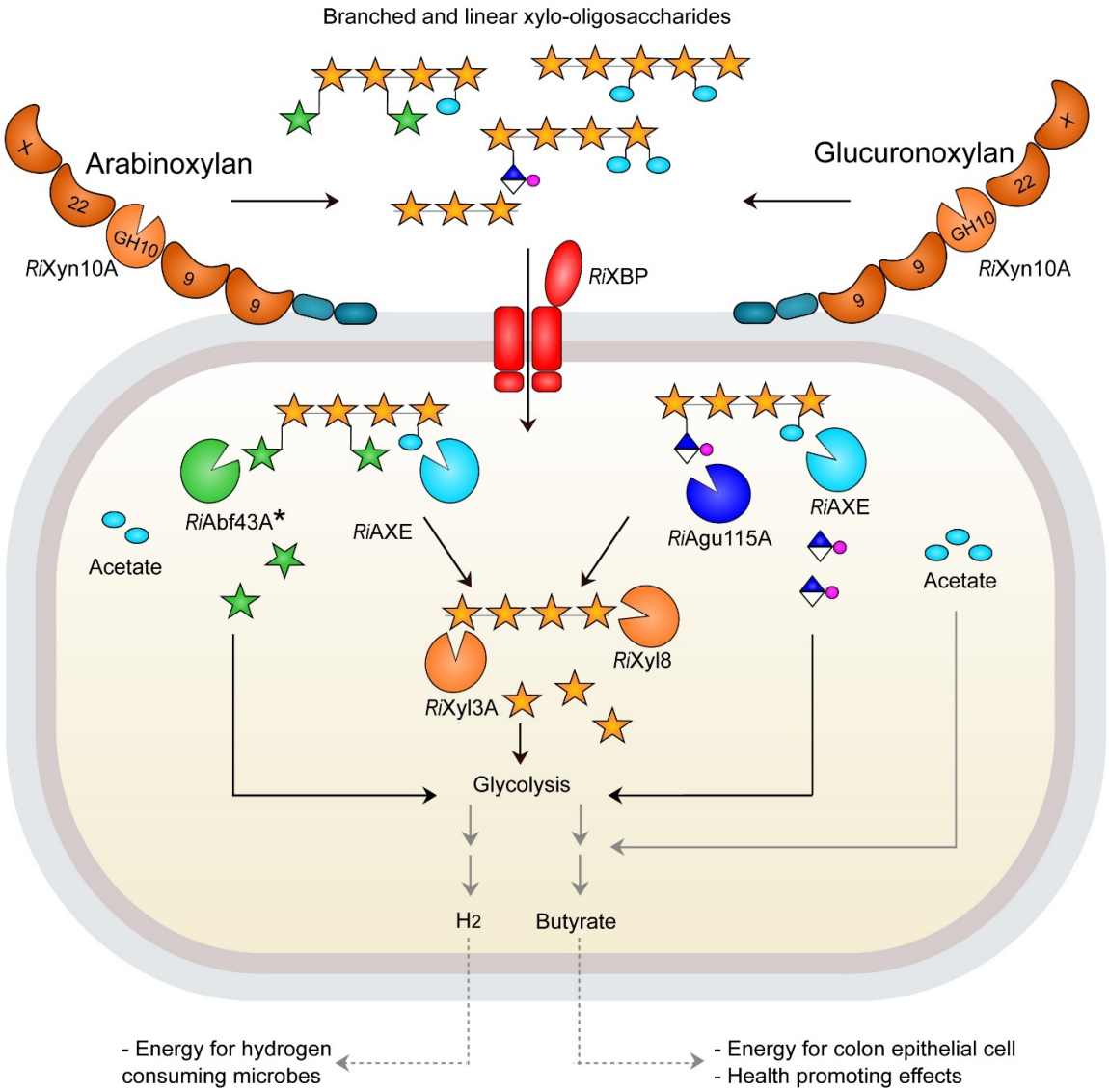

A model of xylan utilisation model in the human gut commensal R. intestinalis.

OL5.4.2

Surface Exposed Mannoside Hydrolases of Human Gut Bacteria

Henrik Stalbrand[1], Viktoria Bagenholm[1], Mathias Wiemann[1], Abhishek Bhattacharya[1], Derek Logan[1]

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Galactomannans are hemicellulosic dietary fibers which can be fermented in the gut (Fåk et al. 2015). Here we report on the structure-function of enzyme systems for galactomannan digestion among common human gut bacteria, results which contribute to the design of prebiotics.

Gut bacteria may have different synergistic strategies for mannoside and galactomannan digestion. The studied Bifidobacteria express single surface exposed beta-mannanases from either of two glycoside hydrolase (GH) families: GH5 or GH26 (Kulcinskaja et al. 2013, Morrill et al. 2015). Bacteroides ovatus, on the other hand, expresses several GHs from a polysaccharide utilisation locus (PUL), a gene-cluster which is essential for galactomannan utilization (Reddy et al. 2016, Bågenholm et al. 2017). The GHs, two beta-mannanases (BoMan26A, BoMan26B) and an alpha-galactosidase (BoGal36A), act in a sequential manner. The two GH26 beta-mannanases were characterised, including solving the TIM-barrel crystal structures, contributing to a model of the combined function of the enzymes and binding proteins of this galactomannan PUL. BoMan26B is exposed on the cell surface (analysed by immuno-fluorescence) and makes the initial endo-attack on highly branched guar gum galactomannan, explained by the open and extended active site cleft visible in the recent crystal structure. Oligosaccharide products generated by BoMan26B bind (Kd 4 mM by micro-thermophoresis) to a surface-exposed glycan-binding SusD-homolog, predicted to guide saccharide import to the periplasm, where BoMan26A acts in synergy with the periplasmic BoGal36A (Bagenholm et al 2017) and efficiently releases mainly mannobiose from the oligo-saccharides via endo-/exo-hydrolysis, as suggested by time-course product analyses using anion-exchange chromatography (HPAEC) and MALDI mass spectrometry combined with solvent isotope labelling using 18O-water in hydrolysis reactions.

The narrow active site cleft of BoMan26A (Bågenholm et al 2017) and active site loop flexibility, recently studied with 1H, 13C, and 15N NMR, contribute to explain the different mode of attack compared to BoMan26B and selected GH5 beta-mannanases.

Phylogenetic analysis place BoMan26A and BoMan26B in different clades of family GH26, from which we can extrapolate a potentially similar set up with two distinct GH26 beta-mannanases in PUL-encoded systems among several other Bacteroidetes. The genetic data guided the selection of gut bacteria which then were fed on (galacto)-mannosides in co-cultures to address potential synergistic cross-feeding between species. For this, glycan conversion and up-take were analysed with HPAEC.

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OL6.1.1

Exploring Synthetic Application of A1,3/4-Fucosyltransferase to Construct a Library of Fucosylated Glycans

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This work focuses on the synthetic application of L-fucose-containing glycans by Helicobacter pylori α1,3/4-fucosyltransferase (FucTIII). By combining of the sequential one-pot enzymatic system of human milk oligosaccharide (HMOs) production,[1] FucTIII was utilized to synthesize various fucosylated HMOs such as lacto-N-fucopentose V (LNFP V), lacto-N-neofucopentose V (LNnFP V), lacto-N-difucohexaose II (LNDFH II), lacto-N-neodifucohexaose II (LNnDFH II) and LNnDFH III; the other important application of L-fucose-containing glycan synthesis – Lewis antigens such as Lewis X, Lewis Y, Lewis a, Lewis b, sialyl Lewis X, sialyl Lewis a and their derivatives were also achieved. Enzyme kinetics data showed that the catalytic efficiency (kcat/Km) of FucTIII on type II N-acetyl lactosamine (LacNAc) was 50 times higher over the type I LacNAc. Noticeably, the enzyme kinetics revealed that the additional GlcNAc on the non-reducing end of the acceptors would enhance the catalytic efficiency of FucTIII on the glycan acceptors. The availability of structurally defined fucosylated glycans would offer a practical approach for investigating future biological applications.

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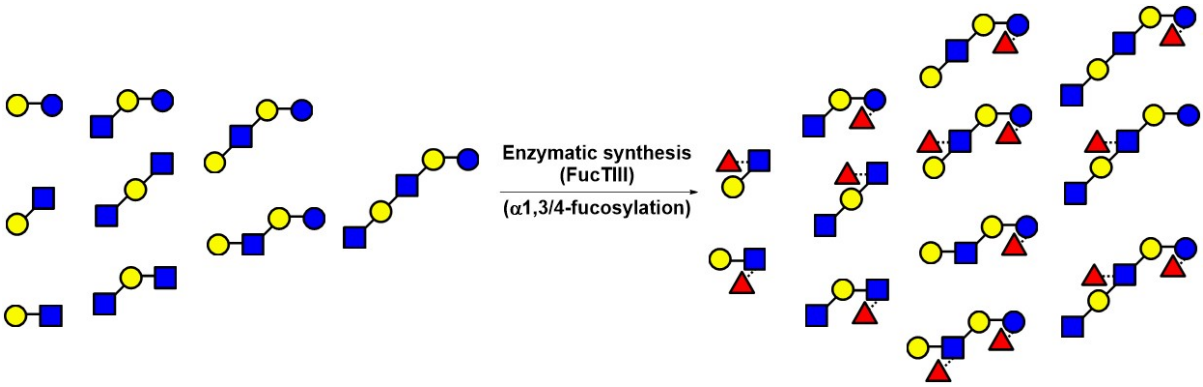

FucTIII-catalyzed enzymatic synthesis of fucosylated glycans

OL6.1.2

Alternative Application of Aldolase or Synthase in Optimized Multi-Enzyme Cascade Production of 3'-Sialyllactose

Sabine Schelch[1], Stefanie Gross Belduma[1,2], Manuel Eibinger[2], Jürgen Kuballa[3], Barbara Petschacher[1,2], Bernd Nidetzky[1,2]

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Multi-enzyme cascades starting from a cost-effective substrate are regarded as valuable biotechnological production systems for complex oligosaccharides. Enzyme cascades have been described in literature for decades, however, there has been little attempt to optimize flux through the cascade based on systematic evaluation of process efficiency. Ideally kinetic modelling can guide cascade optimization and minimize experimental effort.
Here we focused on systematic optimization of 3'-sialylactose production by multi-enzyme cascades in a one pot approach. 3'-sialyllactose is a major human milk oligosaccharide and the simplest target for biotechnological sialoside synthesis [1]. Sialoside syntheses with sialyltransferases depend on high cost nucleotide donor substrates and therefore are ideally integrated in multi-enzyme cascades enabling donor synthesis from a cost-efficient substrate. In the here used cascade 3'-sialyllactose production started with sialic acid production from N-acetyl mannosamine. Sialic acid was then converted to the activated sialyl donor CMP-N-acetylneuraminic acid and in a final step N-acetylneuraminic acid was transferred to the acceptor substrate by the sialyltransferase. Previously, a sialic acid aldolase was used in the initial step for sialic acid production [2]. Sialic acid aldolases use cost efficient pyruvate as a co-substrate, however, an unfavorable thermodynamic equilibrium on the substrate side decreases the reactant flux through the cascade.
In an alternative approach we replaced the sialic acid aldolase by a sialic acid synthase for sialic acid formation from N-acetyl mannosamine. Sialic acid synthases rely on the more cost intensive phosphoenolpyruvate as co-substrate, however the thermodynamic equilibrium of the catalyzed reaction is clearly on the product side. A comparison of alternative application of synthase or aldolase in a one-pot approach for 3'-sialyllactose synthesis showed that the production rate with the synthase significantly increased compared to the aldolase with high 3'-sialyllactose production yields for both enzymes.
In the here optimized multi enzyme 3'-sialyllactose synthesis a strong focus was put on the quantitative analysis of reactants. Analysis results supported development of a Michaelis-Menten kinetics based model. The kinetic model will guide fine-tuning of enzyme-ratio and minimizing substrate usage in order to optimize productivity of the cascade.

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OL6.1.3

Enzymatic N-Acylation and N-Transacylation of D-Glucosamine

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We previously reported the isolation and characterization of a bacterial deacetylase (CmCBDA), which catalyzes selectively the hydrolysis of GlcNAc to glucosamine under mild reaction conditions[1]. This CmCBDA deacetylase was further studied and demonstrated to catalyze the N-acylation of unprotected glucosamine and to N-transacylate unprotected N-acetylglucosamine at ambient temperatures. A wide range of N-acylglucosamine derivatives bearing aliphatic chains or different functional groups suitable for further incorporation reactions were obtained in high conversion rates. Furthermore, CmCBDA catalyzed the N-acylation of glucosamine and was used in an enzymatic cascade for the synthesis of sialosides.

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R = -CH_3 (2), -CH_2CH_3 (3), -$CH_2CH_2CH_3$ (4), -$CH_2(CH_2)_3CH_3$ (5), -CH_2N_3 (6), -CH_2OH (7), -CH_2SH (8)

CmCBDA-catalyzed acylation of glucosamine 1 to provide N-acylglucosamines 2-8.

OL6.2.1

Synthesis of a New Class of Thio-Glycomimetics Through One-Pot Aziridine Opening Reactions

Nives Hribernik[1], Alice Tamburrini[1], Ermelinda Falletta[1], Francesca Vasile[1], Anna Bernardi[1]

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As the most abundant natural products, sugars encode a large amount of biochemical information. Development of glycomimetic structures allows manipulating their structural complexity, thus producing tools for investigation of biological processes and new drug development. The need for molecules with improved drug-like characteristics and metabolic stability has put thio-glycomimetics in the spotlight. [1,2]
The synthetic potential of thioglycosides presents further benefits; as they are stable under a wide range of conditions and yet interconvertible in a number of useful ways. Our group has recently developed a novel one-pot aziridine opening approach to the synthesis of N-linked-pseudo-thio-glycosides, providing an alternative to standard glycosylation procedures. [3] We successfully synthetized a first example of this new class of glycomimetics, N-linked-pseudo-thio-dimannoside 1 (Figure 1), addressed against DC-SIGN (Dendritic Cell-Specific ICAM3 Grabbing Non integrin). C-type lectin DC-SIGN is involved in recognition of viruses and pathogens on mucosal level and plays a role in HIV-1 transmission. [2] We showed that compound 1 binds to DC-SIGN with the same affinity of natural substrate 1,2-mannobioside and its previous mimic pseudo-1,2-dimannoside. [3]
Synthesis of these compounds requires a peracetylated thioglycoside and a conformationally locked aziridine 3 (Figure 1). In a facile one-pot reaction the aziridine ring selectively opens when attacked by a glycosyl thiol generated in situ, affording a single product via trans-diaxial opening.
We explored the scope of the reaction and optimized the conditions for various mono- and disaccharides, generating a library of compounds with the general structure of 4. These novel compounds can be potentially recognized by a wide range of lectins while providing resistance to glycosidases. Our current objective is to further exploit potentials of the structure for synthesis of pseudo-glycopeptides.
All the synthetic and mechanistic aspects will be discussed as well as possibilities to additionally functionalize these compounds.

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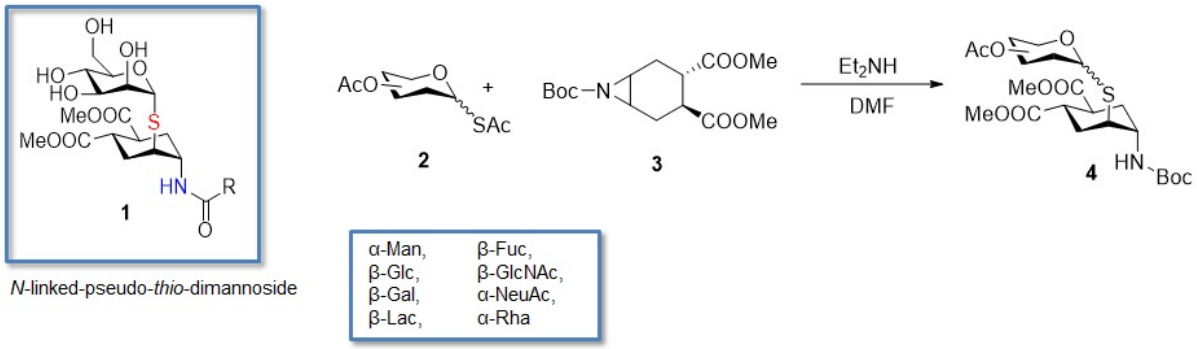

Figure 1: Structure of N-linked-pseudo-thio-dimannoside 1 and one-pot aziridine opening reaction.

OL6.2.2

Structural Study Of Bc2l-C N-Terminal Leads the Way Towards the Design of Antagonists

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Multi-drug resistant (MDR) pathogens have become a high-profile threat to public health. Rapidly spreading worldwide, they are responsible for increased mortality in hospital-acquired infections. The “anti-adhesion” therapy is a new tactic to fight against bacterial infections. It aims to block the infectious process at its initial stage by preventing bacteria from adhering to host tissues via lectin-glycoconjugate interactions.

Burkholderia cenocepacia is a MDR, Gram-negative, opportunistic bacterium usually responsible for “Cepacia Syndrome”; a condition affecting cystic fibrosis patients in which rapid decline of respiratory function can lead to respiratory failure and even death. B. cenocepacia features a carbohydrate-binding protein with dual specificity: BC2L-C. The two distinct carbohydrate recognition domains (CRDs) presented by this superlectin play a role in cell-adhesion: while the LecB-like C-terminal domain specifically binds to bacterial mannosides, the N-terminal domain binds to human fucosides.[1,2] BC2L-C’s N-terminal domain is particularly interesting because of its affinity for human histo-blood group epitopes. Additionally, it presents a novel fucose binding site and a fold previously unseen in lectins.[1]

In order to perform rational design of antagonists for BC2L-C-Nter, extensive structural study of its binding site is necessary. In turn, this required the production and purification of a new recombinant form of BC2L-C-Nter. Isothermal titration calorimetry (ITC) confirmed micromolar affinity for fucosylated oligosaccharides as well as millimolar affinity for monosaccharides, corroborating previous studies and thus, validating the new construct.

Additionally, it allowed co-crystallization with oligosaccharides for the first time. Crystal structures of the complexes with H-type 1 and H-type 3 blood-group antigens were solved by X-ray crystallography at 1.6 and 1.9 Å resolutions, respectively (see Figure 1). Analysis of the contributions of the additional glycan moieties offered insights at the atomic level on how the gain of affinity can be rationalized, leading the way towards high-affinity ligand design. Furthermore, the vicinity of the CRD was mapped and two potential binding pockets were identified. Thanks to this information, fragment screening provided a small library of ligand candidates for the two CRD-adjacent sites.[3] With experimental validation of the fragments under way, synthetic approaches are being tested to connect the monosaccharide core to fragments. Our latest results will be described in the communication.

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(3) Work performed by K. Lal as part of the collaboration between the present project and his PhD thesis: “Design of antagonists for bacterial lectin BC2L-C by molecular modeling”.
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765581.

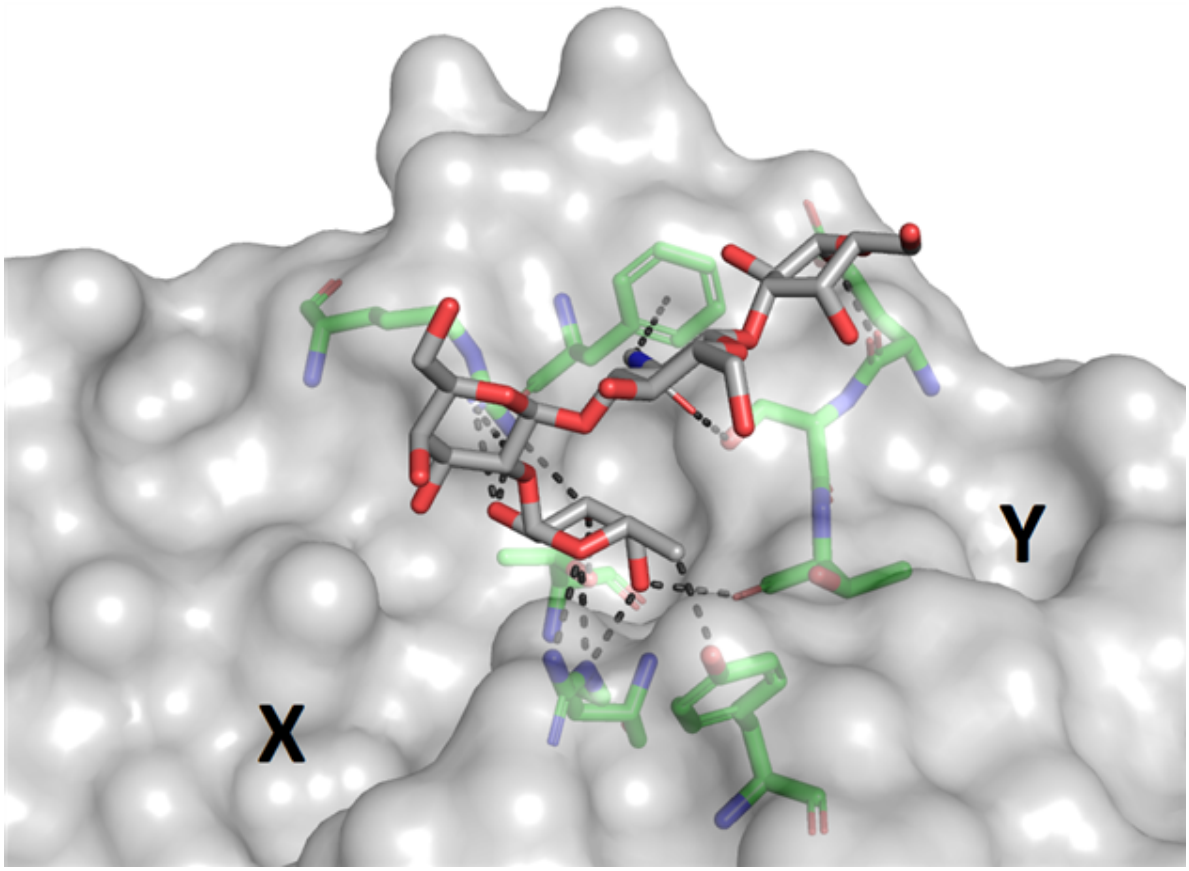

Figure 1. Binding of H-type 1 tetrasaccharide to BC2L-C-Nter. CRD-adjacent sites X and Y are potential fragment binding sites.

OL6.2.3

Allosteric Modulation of Mammalian C-Type Lectins

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Many members of the C-type lectin family are expressed on cells of the innate immune system. These cell surface receptors promote pathogen recognition and, in some cases, also endocytosis. This recognition process is based on the interaction of the lectins with glycans found on the invading pathogen, often sufficient avidity is gained by homooligomerization of the receptor. On a molecular level, a central Ca2+ located in the carbohydrate binding site allows for the coordination of the glycan. Once endocytosed, this Ca2+ coordination can be perturbed by endosomal acidification and reduction of local Ca2+ concentration. While Ca2+ sequestering directly impairs carbohydrate recognition, pH-dependent loss of the receptor multimerization state results indirectly in cargo release in the endo-/lysosomal pathway. Taken together, C-type lectins harbor several molecular determinants in their overall architecture to promote pathogen binding, but also to enhance cargo release, rendering these receptors rather flexible on various time scales.

Here, we will summarize our efforts to make use of the structural plasticity of C-type lectins for the development of small molecule modulators of their receptor function. Previously, we have highlighted the discrepancy between the static picture of these proteins as inferred from X-ray crystallography and the experimental description of susceptibility of these proteins for drug-like molecule binding [1]. In particular, we found that besides the shallow and featureless carbohydrate recognition site, several secondary sites exist that are partially druggable and offer possibilities for inhibitor design against C-type lectins [2]. These insights are complemented by our studies into the receptor flexibility using protein NMR in combination with molecular dynamics simulations revealing an allosteric network of communicating amino acid sidechains. This network regulates the Ca2+ affinity and partially its pH sensitivity in human Langerin [3]. In summary, based on these insights on existence of druggable secondary sites and the presence of allostery, we were able to develop a series of allosteric inhibitors of murine Langerin in the low micromolar affinity regime [4].

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OL6.2.4

Strategies for the Development of N-Glycomimetics

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[1]CIC BiomaGUNE, San Sebastian, Spain, [2]Université Grenoble Alpes, CEA, CNRS, IBS, , Grenoble, France

A growing number of studies including glycan array binding assays illustrate how larger oligosaccharides are bound by lectins with far higher affinity than smaller fragments or monosaccharides. Oligosaccharide ligands presented with a defined surface density can give rise to very selective binding events on cell surfaces. Based on the hypothesis that complex N-glycans can engage in multiple interactions with carbohydrate binding proteins that exceed binding to the primary lectin binding site, we have developed strategies for the synthesis of N-glycomimetics as high affinity lectin targeting probes and inhibitors. Here I present the results of our on-chip and solution phase synthesis of a library of N-glycan mimetics and initial binding assays with plant and human C-type lectins.

OL6.3.1

Defining the SN1 Side of Glycosylation Reactions: Stereoselectivity of Glycopyranosyl Cations

Thomas Hansen[1], Ludivine Lebedel[2], Wouter A. Remmerswaal[1], Stefan van der Vorm[1], Dennis P. A. Wander[1], Mark Somers[1], Herman S. Overkleeft[1], Dmitri V. Filippov[1], Jerome Désiré[2], Agnès Martin-Mingot[2], Yves Blériot[2], Gijs A. van der Marel[1], Sébastien Thibaudeau[2], Jeroen D. C. Codée[1]

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Glycosyl cations – also known as glycosyl oxocarbenium ions – are key reactive intermediates in the glycosylation reaction but given their high reactivity and fleeting nature these species remain poorly understood and their role in shaping the stereochemical outcome of a glycosylation reaction enigmatic. We here present on a computational method that relates the stereochemical outcome of reactions involving these species to the full ensemble of conformations these species can adopt. This computational method maps the complete conformational energy landscape (CEL) and predicts the stereoselectivity of SN1-type glycosylation reactions in a quantitative manner (Figure 1). The vast majority of glycosyl oxocarbenium ions, including those derived from L-fucose, L-rhamnose, D-glucose, D-mannose and D-galactose, are stereoselectively attacked to provide 1,2-cis glycoside products. Experimental evidence for the computed privileged conformers is obtained by the generation and direct spectroscopic characterization of selected glycosyl oxocarbenium ions under superacid conditions. The fundamental insight offered by the calculations into the structure and reactivity of glycosyl oxocarbenium ions, and the intrinsic cis-selectivity of these reactive species will pave the way for the development of new and improved glycosylation chemistry, enabling the more effective generation of oligosaccharides to fuel glycobiology and glycobiotechnology research.

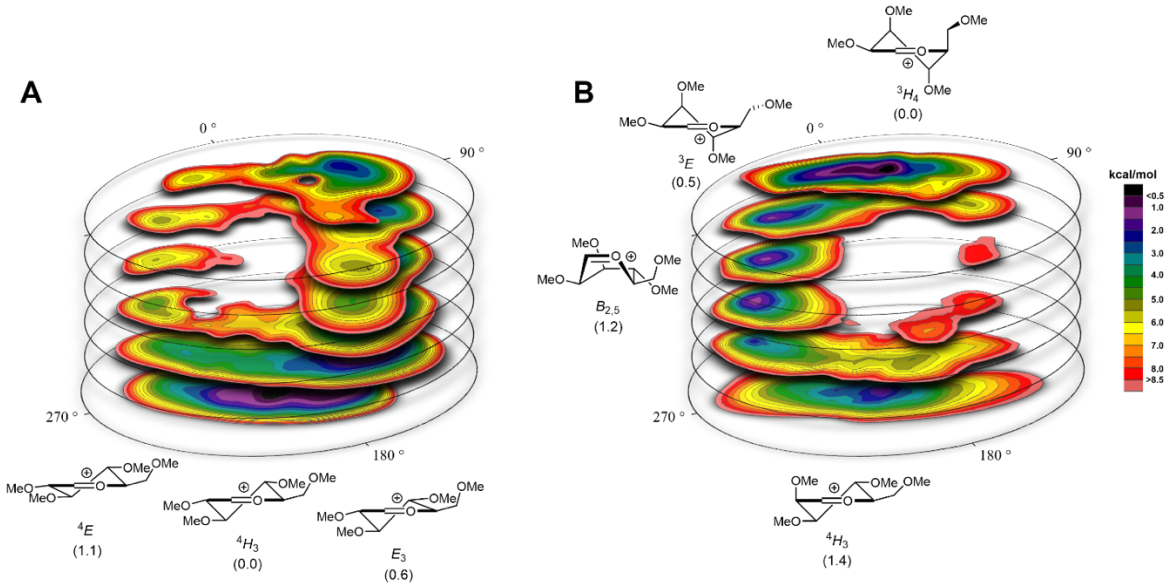

Figure 1. Mapping the conformational preference and reactivity of the glucosyl (A) and mannosyl (B) oxocarbenium ions to understand the role of these reactive intermediates in the assembly of complex oligosaccharides.

OL6.3.2

Stereochemical Study of Chemical Glycosylation with C-3 and C-4 Deoxofluorinated 2-Azido-2-Deoxy-Hexopyranosyl Donors

Martin Kurfirt[1], Jindřich Karban[1], Lucie Červenková-Šťastná[1], Martin Dračínský[2]

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Aminosugars are appreciably abundant in nature. They play crucial roles in many essential biological events. Derivatives of α and β linked D-glucosamine and D-galactosamine represent typical carbohydrate structures occurring in glycoconjugates on the cell surface and in the extracellular matrix. These aminosugars participate in processes like tumor metastasis or immune response which are currently intensively studied [1]. Research into these processes necessitates carbohydrate derivatives including appropriate carbohydrate mimics. Fluorinated sugars seem to be suitable for this purpose. A replacement of a hydroxyl group by fluorine (deoxofluorination) represents an established and often very useful way to modulate chemical as well as biological properties of the parent carbohydrates. Deoxofluorinated sugars often successfully mimic natural carbohydrates due to similarities between oxygen and fluorine. This makes them very promising compounds for studying carbohydrate-protein recognition processes by advanced ^{19}F-NMR epitope mapping techniques [2].
The synthesis of oligosaccharides and glycoconjugates containing fluorinated analogs of D-glucosamine and D-galactosamine requires a preparation of deoxofluorinated 2-amino-2-deoxyhexopyranosyl donors end examination of their reactivity. Corresponding donors were prepared from 3-fluoro or 4-fluoro 1,6-anhydro-2-azido-2-deoxy-D-gluco- and galactopyranoses. Preactivation protocol utilizing diphenyl sulfoxide/triflic anhydride (Ph_2SO/Tf_2O) [3] was used and the results obtained by glycosylation of common model carbohydrate acceptors [4] will be presented. A preparation of multivalent deoxofluorinated glycoclusters employing the results obtained in the model glycosylations will be also presented and a discussion of stereoselectivity based on a computation study of the proposed reaction intermediates will be included.

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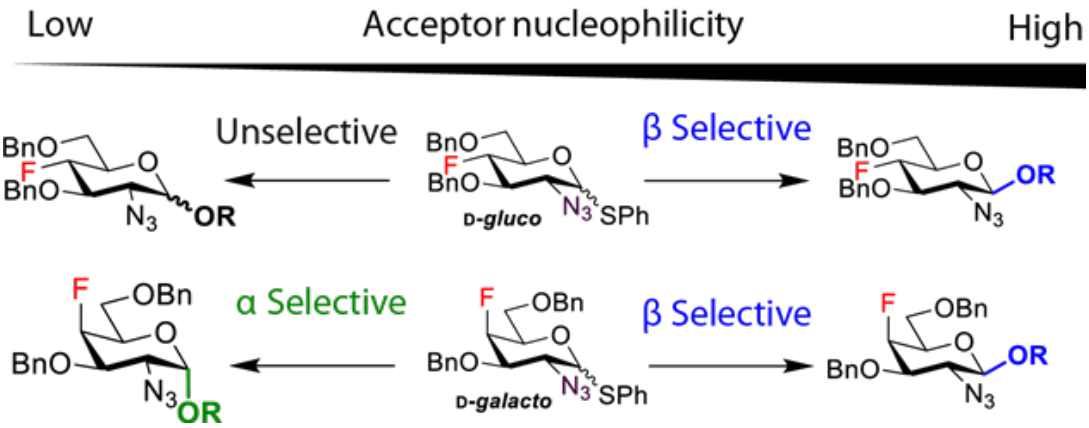

Fig.1 - Important results of the glycosylation stereoselectivity study.

OL6.3.3

Prediction of Glycosylation Reactions via Reactivity and Intermediate

Cheng-Chung Wang[1]

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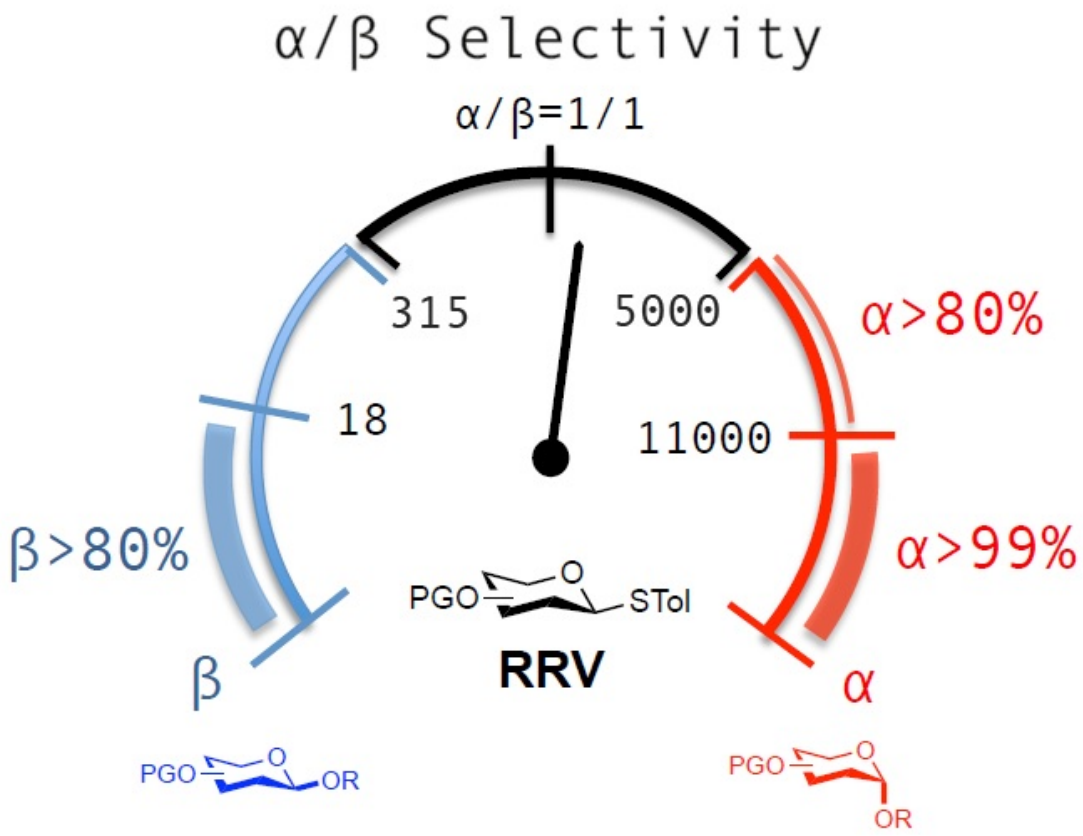
The combination of thioglycoside and NIS/TfOH promoter system is one of the most commonly used glycosylation reactions to construct challenging glycosidic bond in chemical carbohydrate synthesis. However, over the past three decades, the mechanism remains unclear with glycosyl triflate commonly believed as intermediate. Even if glycosyl triflate exhibits high reactivity, stereocontrolled glycosylation is still a long-standing challenge because the behaviors and effects of the byproducts present in the reaction system were unknown. In this paper, for the first time, we confirm that glycosyl iodide is the real intermediate dominating glycosylation with its role in this glycosylation system being clarified. The relative quantities of glycosyl triflate to iodide greatly influence stereoselectivity. Intermediate transformation is governed by numerous factors, including donor reactivity, temperature, activation time, TfOH, and promotors (NXS). Based on our findings, stereoselectivity is now predictable on various thioglycosides, as indicated by their relative reactivity values (RRVs). We also disclosed new guidelines to achieve intermediate-controlled stereoselective glycosylation reactions.



A prediction of stereoselectivity and intermediate distribution by using relative reactivity value of donors (RRV)

OL6.3.4

Glycosylation Intermediates and Mechanisms in Uronic Acid Derivatives

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Uronic acids are important constituents of polysaccharides found on the cell membranes of different organisms. To prepare uronic acid containing oligosaccharides, uronic acid 6,3-lactones can be employed as they display a fixed conformation and a unique reactivity and stereoselectivity. The mechanism of glycosylation of uronic acid 6,3-lactones and derivatives thereof is established using a combination of techniques including IR ion spectroscopy combined with quantum-chemical calculations and variable-temperature nuclear magnetic resonance (VT NMR) spectroscopy. The role of these intermediates in glycosylation is assayed by varying the activation protocol and acceptor nucleophilicity. The observed trends show analogy to the well-studied 4,6-benzylidene glycosides and may be used to guide the development of next-generation stereoselective glycosyl donors.

OL6.4.1

Structural and Functional Roles of the Interaction of Human Papillomavirus Capsids with Heparan Sulfates

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Human papillomaviruses (HPV) are small non-enveloped DNA viruses that can cause a variety of anogenital cancers. During initial infection, HPV16, the best-studied and most prevalent cancer causing type, requires heparan sulfates (HS) for initial binding to host cells. The interaction of HPV16 virions with HS not only enables initial attachment, but also triggers a crucial conformational change in the viral capsid that we termed structural activation. This structural activation facilitates three subsequent alterations of the capsid, which include further conformational but also proteolytic changes in both capsid proteins. These changes are thought to prepare the virus for engaging the elusive internalization receptor, endocytic uptake into host cells and eventual uncoating of the viral genome. Since virus engagement of HS proteoglycans (HSPGs) occurs independently of the protein backbone, we asked, whether the virus would require a specific HS sulfation pattern for the binding and subsequent structural activation of the virus. Employing complimentary approaches using siRNA-mediated knockdown and overexpression of sulfotransferases/sulfatases as well as specifically sulfated/desulfated HS analogues of different length, we found that an HS chain consisting of between 20 and 40 saccharide units was required for stable binding and structural activation. In addition, N- and 6-O-sulfations were needed for HPV infection, whereas only N-sulfation was crucial for the induction of structural activation. Interestingly, 2-O-sulfation and isomerization to iduronic acid appeared to be detrimental for HPV infection. The potential role of 3-O-sulfation is still being investigated. Nevertheless, our work so far already demonstrated that a specific sulfation pattern is required for HPV16 binding and structural activation.

OL6.4.2

Determination of The A064r Gene Function Produced by Paramecium Bursaria Chlorella Virus

Immacolata Speciale[1], Garry Duncan[2], Michela Tonetti[3], Maria Elena Laugieri[3], Todd Lowary[4], Sicheng Lin[4], Antonio Molinaro[1], Cristina De Castro[5], James Van Etten[2]

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The chlorovirus Paramecium bursaria chlorella virus 1 (PBCV-1), is a large dsDNA virus that infects the microalgae Chlorella variabilis NC64A.[1] It is a member of the giant virus group, a class of viruses that differ from the others in shape, genome size and number of encoded proteins.[2] It's main feature is that unlike to other viruses, PBCV-1 encodes most, if not all, of the components required to glycosylate its major capsid protein (MCP), named Vp54 (gene a430l).[3] Noteworthy, Vp54 is decorated with four glycans, that are different from all other N-glycans known so far in the three domains of the life.[4]

Recent analysis about the PBCV-1 gene, has disclosed that it encodes, at least, six putative glycosyltransferases[1], presumably involved in glycosylation of the its major capsid protein Vp54. One of them, the A064R, catched our attention because 18 of 21 antigenic variants of PBCV-1 present mutations in this gene or it was deleted.[5] Comparing the wild-type glycan structures from PBCV-1 with those from a set of PBCV-1 mutants, that have truncated glycan structures, it was possible to address the role of the whole a064r gene. This gene encodes a protein of 638 amino acids organized in three domains [4], each with a particular function.

Combining structural analyses with enzymatic reactions, it is now established that the domain 1 encodes for a β-(1,4)-L-rhamnosyl transferase onto a xylose acceptor,[5] in contrast with previous data, which supposed that such domain encoded for a glucosyl-transferase.[6]
Domain 2 has no resemblance with any known glycosyltransferase, and it encodes for a α-(1,2)-L-rhamnosyl transferase that recognizes rhamnose as acceptor, while domain 3 has a double methyltransferase activity and acts on a terminal rhamnose unit.

This communication will present the strategy used to evaluate the activity of this complex protein.

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OL6.4.4

Pentavalent Sialic Acid Conjugates Inhibit Viruses that Cause Highly Contagious Eye Infections

Emil Johansson[1], Rémi Caraballo[1], Georg Zocher[2], Weixing Qian[1], Nitesh Mistry[3], Niklas Arnberg[3], Thilo Stehle[2], Mikael Elofsson[1]

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Members of the Adenoviridae (Ad8, Ad19, Ad37) and Picornaviridae (enterovirus 70 and Coxsackievirus A24 variant) families are responsible for causing the highly contagious eye infections epidemic keratoconjuctivitis (EKC), and acute hemorrhagic conjunctivitis (AHC), respectively.[1],[2] The infections are generally self-limiting and spontaneously resolve in 1-2 weeks. However, symptoms may be severe and AHC can result in neurological impairments such as acute flaccid paralysis, and EKC-causing adenoviruses are potentially life-threatening in immunocompromised individuals.[2],[3],[4] To date, no antiviral agents or vaccines are available for treating adenovirus and picornavirus infections.[5]
Coxsackievirus A24 variant (CVA24v) has been reported as the main causative agent of AHC. To facilitate attachment, CVA24v binds to glycans terminating in 5-N-acetylneuraminic acid (sialic acid) via recognition sites that cluster in a pentameric geometry around the five-fold symmetry axis of the viral capsid.[2] Thus, we hypothesized novel pentavalent sialic acid conjugates could effectively chelate the sialic acid binding sites of the virion thereby blocking access to epitopes on host cells. The design is heavily inspired by ME0462, a potent trivalent sialic acid attachment inhibitor of Ad37.[6] Biological data indicates that the novel pentavalent sialic acid conjugates inhibit infection of human corneal epithelial cells by CVA24v (IC50 10-160 μM), and x-ray crystallography confirmed binding to the virion. Interestingly, the pentavalent sialic acid conjugates are highly efficient inhibitors of Ad37 (IC50 18-190 nM) supporting development of general inhibitors for two of the major causative agents EKC and AHC.

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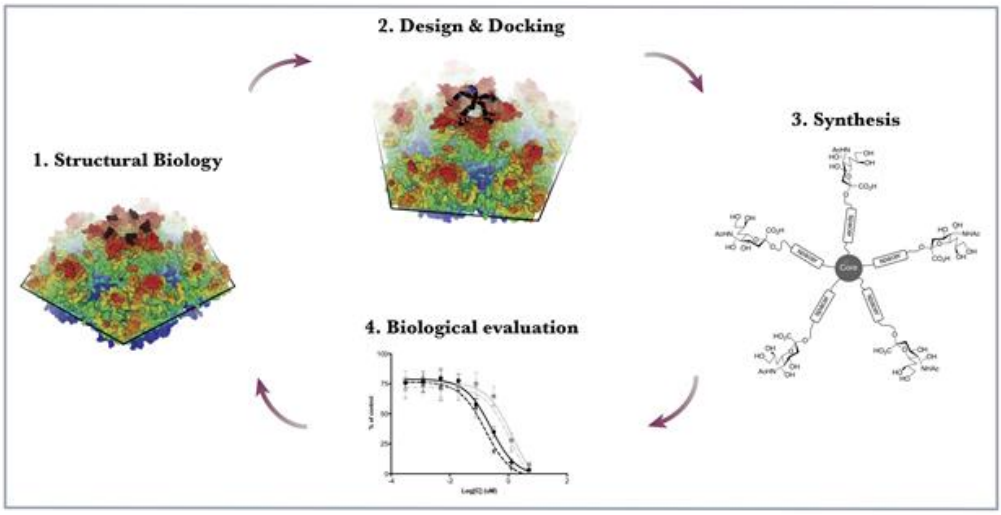

Graphical Abstract

OL7.1.1

New Approaches to Iminoglycoside Mimics by way of Organotin Intermediates

Justyna Jaszczyk[1], Sizhe Li[1], Cyril Nicolas[1], Olivier Martin[1]

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Sugar analogs with nitrogen in the ring, or iminosugars, are now recognized as major carbohydrate mimics and are gaining increasing importance as potential or proven thera-peutic agents for a diversity of diseases such as lysosomal disease, diabetes, viral infections and others. In this context, our group has been actively investigating new iminosugar derivatives designed to exhibit higher efficiency and selectivity towards specific enzymes, and has focused on the synthesis of iminoglycosides mimics. Of particular interest are derivatives in which the 'aglycone' is linked to the anomeric carbon by a C-C-bond (imino-C-glycosides). Such compounds have been shown to demonstrate exquisite selectivity and potent activity as glycosidase inhibitors for example towards GCase, the enzyme involved in Gaucher disease.
In recent work, we have demonstrated that N-sulfinylglycosylamines constituted favorable precursors for the stereocontrolled synthesis of iminosugar-C-glycosides of defined 'pseudoanomeric' configuration [1][2]. In continuation of this work, we have investigated the addition of organotin reagent such as tributyltinlithium to various glycosylamines. These reactions were found to be very efficient and highly stereoselective, with control by the sulfinyl group, leading to chain extended stannylated aminoalditols (Scheme 1).
These intermediates could be exploited directly for organometallic coupling reactions or cyclized to give novel 1-C-stannyl iminosugar derivatives; pyrrolidines and piperidines derivatives were thus prepared, with a well-defined □- or □-configuration. These derivatives constitute remarkable precursors for the diversity-oriented synthesis of glycoside mimics: conditions of Pd-mediated Stille coupling of these organometallic derivatives of iminosugars with various ar(o)yl halides were optimized and applied to the synthesis of a small library of novel imino-C-glycosyl aromatic compounds. Details of these investigations will be illustrated in this presentation.
Acknowledgments: a grant from Labex SYNORG (ANR-11-LABX-0029) and a stipend from the French Ministry of Higher Education and Research are gratefully acknowledged.

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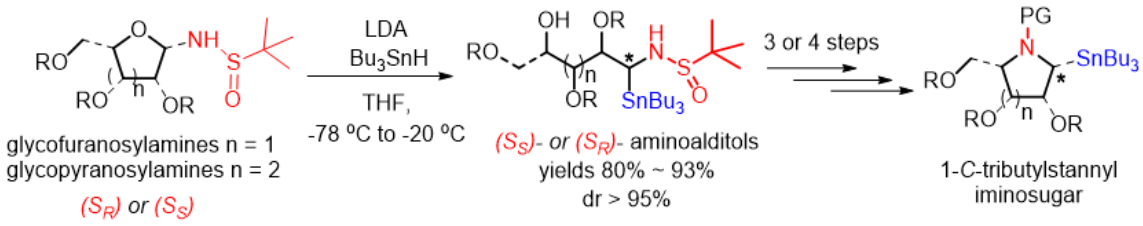

Scheme 1. Synthetic methodology

OL7.1.2

A Novel Class of Powerful and Selective Hexosaminidase and Protein O-Glcnacase Inhibitors: N-Modified Cyclopentanoid Sugar Analogs

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Half of the nearly sixty lysosomal storage diseases described to date are caused by the lack or catalytic incompetence of carbohydrate processing enzymes such as glycosidases, phosphorylases as well as other hydrolases which manipulate sugar moieties. Hexosaminidases as well as O-GlcNAcase are involved in the chemical manipulation or catalytic removal of N-acetyl-D-glucosaminyl or N-acetyl-D-galactosaminyl residues from degradation-bound polysaccharides and glycoconjugates.

Regulation of O-GlcNAcase, which belongs to the family of hexosaminidases, prevents the tau protein from hyperphosphorylation which is a pathological hallmark for diseases such as Alzheimer's. [1] It has been shown that inhibition of O-GlcNAcase enhances the autophagy in the brain and thus shows a benefit in neurodegenerative diseases such as Parkinson's. [2] Selective inhibition of O-GlcNAcase can be achieved by elongation of the C-2 substituent to exploit a deep pocket at the active site of the enzyme. [3] In this presentation the synthesis as well as the biological activities of novel and selective cyclopentanoid O-GlcNAcase inhibitors (1) as well as hexosaminidase inhibitors (2) will be presented.

Figure 1: Synthesized O-GlcNAcase and hexosaminidase inhibitors.

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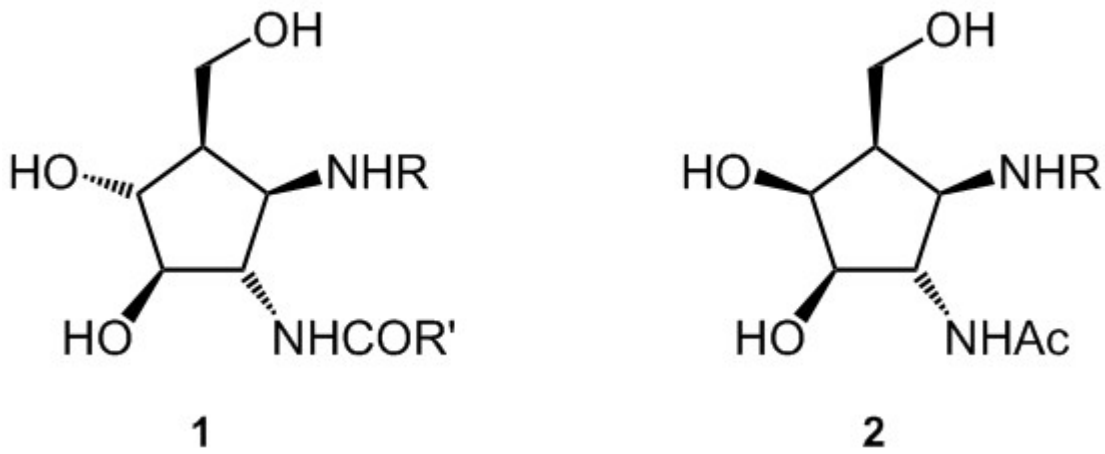

Figure 1: Synthesized O-GlcNAcase and hexosaminidase inhibitors.

OL7.1.3

Rational Design of Glycosidase Pharmacological Chaperones: From Covalent Cyclic Sulfates to Reversible Cyclic Sulfamidates

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"Cyclosulfates", carbohydrate analogues bearing a cyclic sulfate as an electrophilic trap, have recently been described as a conceptually new class of glycosidase inhibitors. α-Glu-cyclosulftate adopts a 4C1 conformation and covalently inhibits α-glucosidases in a potent and selective manner by mimicking the Michaelis conformation.[1] Modifying the cyclophellitol core to galactose configuration has resulted in the generation of α-Gal A irreversible inhibitors (Figure 1A). Exploration of diverse bioisosteres and reducing the electrophilicity of the cyclic sulfate has afforded a novel competitive α-gal A inhibitor that acts as a chaperone for Fabry Disease (Figure 1B). Herein, we present a novel class of reversible glycosidase inhibitors, which we have validated by metadynamics simulations, 3-D crystal structure analysis in complex with recombinant human α-galactosidase (Fabrazyme), activity-based protein profile[2] as well as in vitro and in situ cell experiments. Remarkably, α-gal-cyclosulfamidate behaves as a pharmacological chaperone for Fabry disease: α-Gal A activity is increased when cells are treated with recombinant enzyme supplemented with α-Gal-cyclosulfamidate and toxic metabolite levels (Gb3 and LysoGb3) are corrected in classical mutant R301X Fabry cell lines. Variation of the cyclosulfamidate configuration provides diverse glycosidase pharmacological chaperones.

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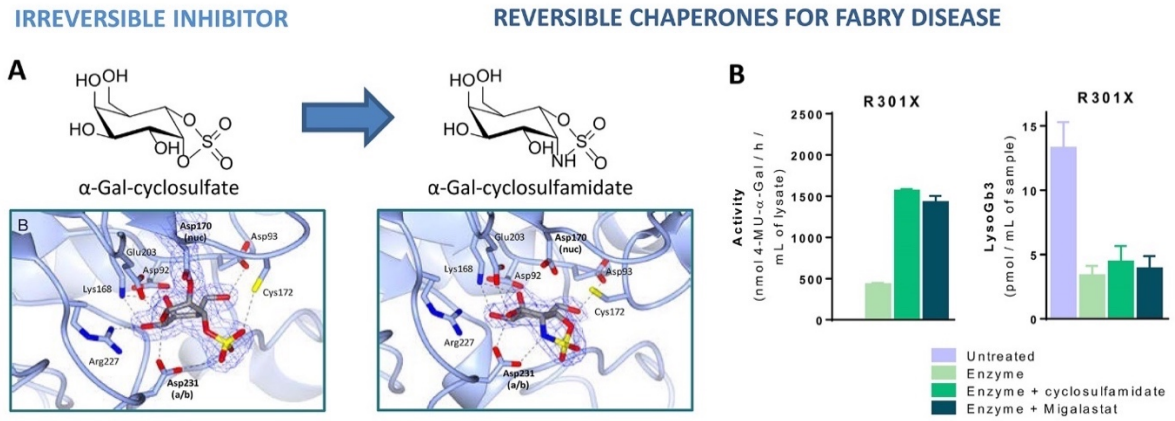

Figure 1. From covalent α-Gal-cyclosulfate to competitive α-Gal-cyclosulfamidate. A. Crystal structures of human α-Gal A bound to α-Gal-cyclosulfate and α-Gal-cyclosulfamidate in a covalent and reversible manner respectively. B. Chaperone effect: α-Gal A activity is increased when cells are treated with ERT supplemented with α-Gal-cyclosulfamidate and toxic LysoGb3 levels are corrected.

OL7.1.4

Design, Synthesis and Biological Evaluation of Heparan Sulfate Fragments Analogs (or Derivatives) as Human Endosulfatatases Inhibitors

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Heparan Sulfate proteoglycans (HSPGs) interact with many proteins, especially growth factors or cytokines via specific sulfation patterns. They can be remodeled in the pericellular space by a novel class of extracellular enzymes, the Endosulfatases (HSulf 1 and 2), which selectively remove the 6-O-sulfate groups from glucosamine residues within the HSPGs chains [1-2]. HSulfs expression/production is deregulated in many human cancers including breast, lung, ovarian and hepatocarcinoma [3-4]. Thus, this family of enzymes represents an interesting therapeutic target.
Most of sulfatases require a post-translational modification of a cysteine residue to a catalytically formylglycine residue to be active [5]. One of the most efficient classes of sulfatases inhibitors are sulfamate compounds. For Endosulfatases, very few molecules have been reported as inhibitors and no selectivity towards Endosulfatases was found with the most potent one [6].
In order to increase the selectivity and to obtain an efficient inhibitor, we designed heparan sulfate fragments library bearing a 6-O-sulfamate at the non-reducing end (see figure 1). The synthesis and biological evaluation of these analogs will be presented.

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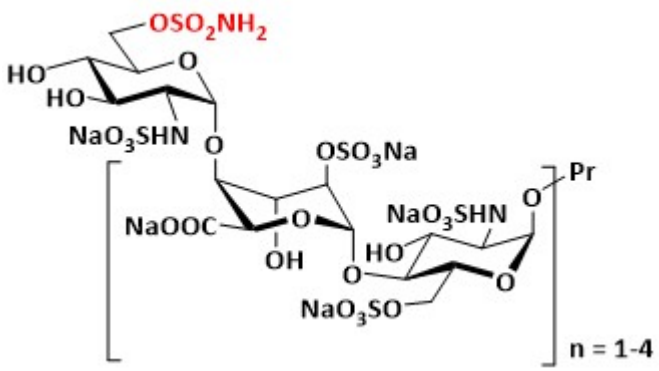

Figure 1 : Heparan sulfate fragments library bearing a 6-O-sulfamate

OL7.2.1

The Molecular Mechanism of Substrate Recognition and Catalysis of the Membrane Acyltransferase Pata from Mycobacteria

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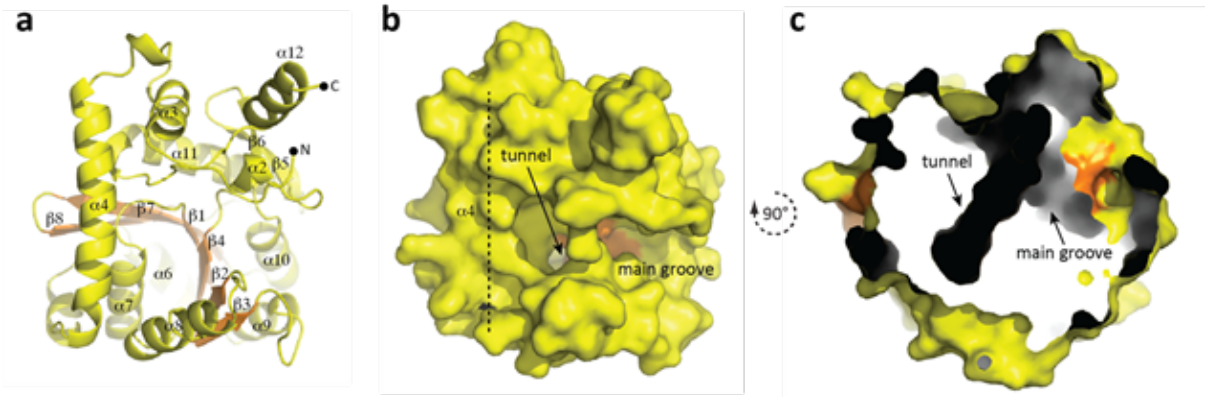
The biosynthesis of phospholipids and glycolipids are critical pathways for virtually all cell membranes. PatA is an essential membrane associated acyltransferase involved in the biosynthesis of mycobacterial phosphatidyl-myo-inositol mannosides (PIMs). The enzyme transfers a palmitoyl moiety from palmitoyl–CoA to the 6-position of the mannose ring linked to 2-position of inositol in PIM1/PIM2.
We report here the crystal structures of PatA from Mycobacterium smegmatis in the presence of a nonhydrolyzable palmitoyl–CoA analog and 6-O-palmitoyl-α-D-mannopyranoside, unraveling the acyl donor and acceptor binding mechanism.
The structures reveal an a/b architecture, with the acyl chain deeply buried into a hydrophobic pocket that runs perpendicular to a long groove where the active site is located. Enzyme catalysis is mediated by an unprecedented charge relay system, which markedly diverges from the canonical HX4D motif.
By the use of combined structural and quantum-mechanics/molecular-mechanics (QM/MM) metadynamics, we unravel the catalytic mechanism of PatA at the atomic-electronic level. Our studies provide a detailed structural rationale for a stepwise reaction, with the generation of a tetrahedral transition state for the rate-determining step. Altogether, our work establishes the mechanistic basis of substrate/membrane recognition and catalysis for an important family of acyltransferases, providing exciting possibilities for inhibitor design.



Overall structure of PatA. a. Cartoon representation showing the general fold and secondary structure organization of PatA. Secondary structure elements are labelled. The central core b-sheet is shown in orange. b. Surface representation of PatA showing the location of the main groove and the active site. The groove entrance is flanked by two important a-helices, a11– a12. The groove ends up into a cavity mainly flanked by a4. c. The main groove runs perpendicular to a hydrophobic tunnel, which is deeply buried into the core of PatA.

OL7.2.2

Novel Nanomolar Inhibitors of the Gram Negative Bacterial Heptose Pathway

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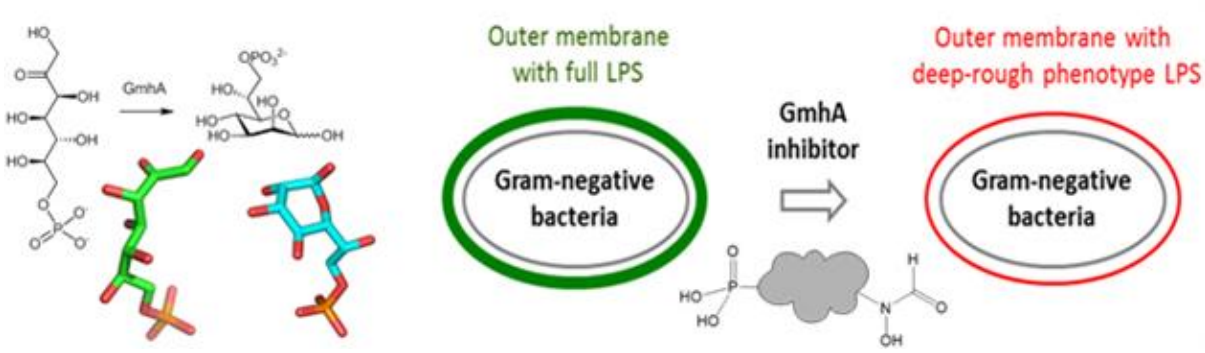
A threat to global health is presently associated with the increase of multidrug-resistant bacteria, for several of which common antibiotics are not effective anymore.[1] Novel approaches, such as the antivirulence concept, are therefore urgently needed to identify bacterial targets and develop appropriate compounds with new modes of action. Various isomers of heptoses play crucial roles in bacteria. Glycero-D-manno-heptoses have been found in capsular polysaccharides, O-antigens and the core region of lipopolysaccharide (LPS).[2] The biosynthesis of heptoses and the nucleotide-activated ADP- and GDP-heptoses has been elucidated in great detail lead.[3] The first enzymatic step common to all pathways is catalyzed by the sedoheptulose-7-phosphate isomerase GmhA, which notably requires a zinc atom for activity.[4]



Antivirulence approach aided by new potential antimicrobial substance

Starting from suitable ribose and hexose precursors, a series of derivatives, based on GmhA's open chain substrate sedoheptulose-7-phosphate and a zinc chelating formylhydroxamate scaffold have been prepared as terminal phosphate and phosphonate analogues, some of which were able to interfere with the heptose biosynthesis in a nanomolar concentration range. Bacteria grown in the presence of one of these inhibitors and glucose-6-phosphate to activate the UhpT transport system expressed a deep rough phenotype and were effectively sensitized to erythromycin.[5] These findings further support the search for innovative ways to fight bacterial infections.[6]

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OL7.2.3

Gut Microbiota Lipopolysaccharides: A New Concept of Endotoxins from the Inside

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The Gut Microbiota (GM) is an essential actor in the modern concept of human health driving many host physiological and pathological processes, including immune system modulation. Accumulating evidences highlighted that studies of the immune system during health or disease cannot ignore our GM.[1] Initial sensing of microbes by the host immunity is mediated by the recognition of microbial-associated molecular patterns, such as lipopolysaccharides (LPS), which are highly conserved among bacteria, thus shared by both commensals and pathogens. The LPS structure strongly influences the biological effects on the host immune system. Defined LPS structures can act as potent agonists on the immune receptors whereas other can operate as antagonists reducing or inhibiting the cytokine production otherwise induced by toxic LPSs.[2,3] Thus, a crucial question to address is how the immune system distinguishes between permanently established commensals LPS and pathogens LPS.
The elucidation of the structure and the immunological activity of LPS isolated from gut microbes will bear new advances in the medicinal chemistry and in the field of search of new molecules able to antagonize pathogens LPS effect, as well as of GM LPS-inspired molecules able to prevent uncontrolled host immune response against our microbiota.[1] This will also shed light on the structure-activity relationship of LPS itself, which is an open question in immunology field. In particular, this will improve the knowledge of the still poorly investigated GM world, giving insights in the host-microbe interaction mechanisms both at intestinal and systemic level furnishing, in parallel, information about the elicitation/modulation of immune response triggered by pathogens and commensals LPS, thus improving the overall knowledge of the immune system.[1]

In this communication, I will show some very recently elucidated GM LPS structures and their immunological properties that revealed to express unique and interesting features.
Insights gained from the structural and molecular analysis of the isolated and characterized GM LPS and, in general, of GM LPSs might help to chemically design novel inflammation-silencing drugs as a potential alternative therapeutic approach for the treatment of inflammatory disorders.

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OL7.2.4

Synthesis of D-Arabinofuranosylated Probes Towards the Functional Analysis of Mycobacterial Arabinan Degrading Enzymes

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Degrading enzymes of arabinan from mycobacterial cell wall had been identified [1] and their function began to attract our attention from not only a biological but also a synthetic point of view. Mycobacterial arabinan as the substrate of the enzymes involves only D-form of arabinose whereas only L-arabinose is one of the components of plant cell wall and has been found as a common constituent of both arabinogalactan (AG) and lipoarabinomannan (LAM), which are attracting particular attention among furanoside-containing glycans [2]. The functions and the mechanisms of the enzymes have not been reported in detail yet due to the limited supply of pure substrate with well defined structure for the precise biological and enzymatic studies of mycobacterial arabinan degrading enzymes. Here, we wish to report the synthesis of D-arabinofuranosylated probes towards the functional analysis of mycobacterial arabinan degrading enzymes.

We have been already studying on the synthesis of mycobacterial [3–5] and plant oligo-D-arabinofuranosides. The construction of substrate probe including some typical fragment structures with long and short as well as linear and branched structures, which possess 1,2-acetonde in the reducing residue as the small tag moiety for MASS analysis, has been achieved in stereoselective manner. As we reported previously the synthesis of docosasaccharide arabinan ($Araf_{22}$) fragment [5], we synthesized PNP D-Araf and various mycobacterial arabinan fragments as the substrate basically using the similar synthetic methodology. Synthesis of liner $Araf_3$ and $Araf_9$ and also branched $Araf_5$ and $Araf_8$ has been completed in stereoselective manner. As one of the expected product during the cleavage of $Araf_8$ and $Araf_5$-acetonide by arabinanase [1], a branched $Araf_4$ derivative has been synthesized, also as methyl glycoside.

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OL7.3.1

Transition Metal-Catalyzed Approaches to Functionalyze Glycosyl Thiols

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Thioglycosides are valuable glycomimetic derivatives that have attracted much attention as mimetics of O-glycosides. Thioglycosides are among the most important glycomimetics reported to date due to their powerful activities. Owing to their importance, building up methods for their construction has drawn considerable attention. However, the classical methods of their synthesis presenting many shortcomings such as the use of harsh acidic media, and are generally limited in substrate scope. Therefore, metal-catalyzed coupling of thiosugars with halogenated electrophiles can be a superior and highly versatile approach to the sunthesis of thioglycosides. The palladium-catalyzed Buchwald–Hartwig–Migita cross-coupling reaction has become an effective tool in industrial and academic research giving acces to natural products and novel materials as well as a wide number of pharmaceuticals currently on the market. Advances in this coupling reactions have been driven by the discovery of a new class of ligands, which are able to promote reactions with a variety of substrates including nitrogen-, sulfur-, and oxygen-containing nucleophiles. In this context, our group explored the ability of the G3-XantPhos palladium precatalyst[1] to promote the coupling of unprotected thiosugars with various halide partners at room temperature and in aquous medium. Some of these results will be present in this orale communication.[2]

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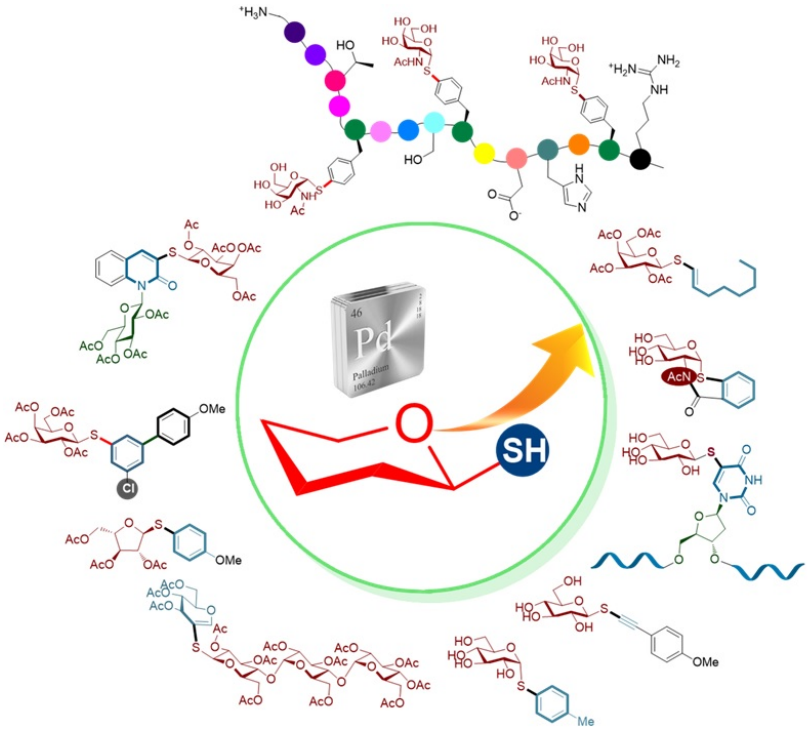

OL7.3.2

Sugars: Sweet Tales From an Aldehyde's Perspective

Christian Stanetty[1], Markus Draskovits[1], Hubert Kalaus[1], Alexander Reichetseder[1], Marko Mihovilovic[1]

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Our research focuses on the development of new synthetic methodology for the interconversion of abundant sugars to more exotic ones utilizing the natural reactivity of the aldehyde moiety. The open chain content generally represents a minute minority in reducing sugars' equilibria (dominated by the hemiacetal forms), which is usually too small to be determined efficiently and/or accurately. To overcome this issue, we have developed an assay based on the adduct formation of a strongly UV-active aminal species which thereby translates the open chain proportion into a readily measurable reaction rate (see Figure 1, middle). [1] We have confirmed the validity of the approach measuring all parent hexoses and pentoses and exhibiting a high degree of consistency with literature data.
Next, we went on to apply the assay to real cases from the field of indium mediated acyloxyallylation [2] (Figure 1, bottom) and our current study of the organocatalytic anomeric activation by N-heterocyclic carbenes (NHCs) which we are developing into a controlled dehomologation methodology (Figure 1, top). [3] In both areas we have encountered unexpected reaction outcomes which could not be explained by the sole chemical vicinity of the aldehyde function but could be correlated to the differences in the ratios of available open chain content within relevant starting materials, intermediates and products.

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[2] Draskovits, M.; Stanetty, C.;* Baxendale, I. R.; Mihovilovic, M. D; J. Org. Chem. 2018, 83 (5), 2647-2659.
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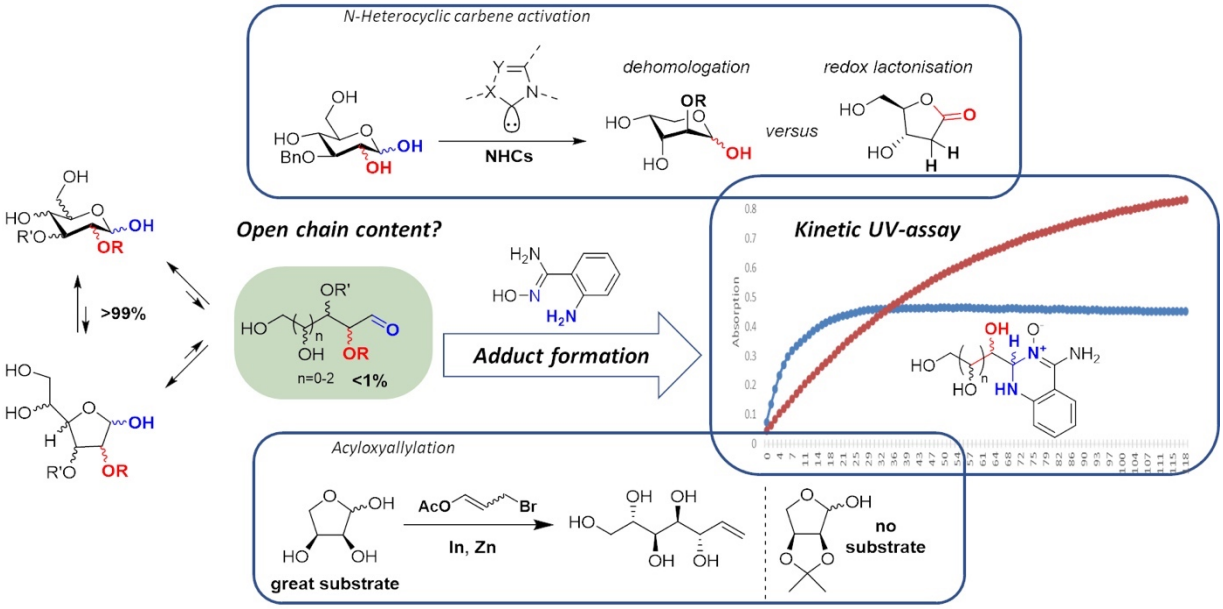

Figure 1: Indirect determination of open chain content and questions addressed by the developed UV-assay

OL7.3.3

Zirconium-Catalyzed Site-Selective Deprotection of Primary Acetates by Dibal-H

Thomas Lecourt[1]

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Carbohydrates, and other polyols, with a single free primary position and a polyacetylated framework are highly relevant synthetic intermediates for the preparation of numerous bioactive compounds, natural products, and biomaterials. However, their preparation suffers from several drawbacks. Indeed, classical approaches based on the manipulation of protecting groups have a high environmental impact and synthetic cost.[1] Moreover, alternative routes involving the regio- and chemoselective deprotection of peracetylated substrates have a relatively narrow scope, and require advanced technologies, like immobilisation of esterases on solid supports, to be amenable on large scales.[2] We report herein that a mixture of Cp_2ZrCl_2 and DIBAL-H in THF at -20°C induce a site-selective deprotection of the primary position of peracetylated compounds. This transformation, relying on cheap, easy to handle, and commercially available reagents, de-O-acetylate pyranosidic and furanosidic scaffolds, as well as non-carbohydrate substrates, like amino-acids and terpene derivatives. Achieved under very mild reaction conditions, this reductive process is also tolerant towards numerous functional groups (acetals, ethers, benzoates and pivalates, NHAc, NHBoc, N_3, nitro, cyano…).[3] Mechanistic studies also reveal that this site-selective de-O-acetylation process is induced by a zirconium/aluminum mixed-metal hydride, and finally lead to the development of reaction conditions catalytic in zirconium.[4]

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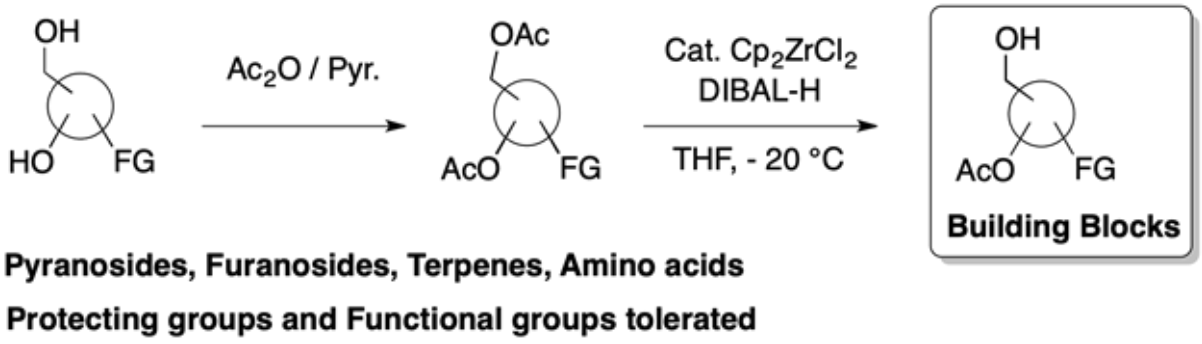

Zirconium-Catalyzed Site-Selective De-O-Acetylation by DIBAL-H

OL7.3.4

Problems Encountered in Global Deprotections of GXM Oligosaccharides

Conor Joseph Crawford[1], Stefan Oscarson[1]

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In a project aimed with synthesising a library of capsular polysaccharide fragments based on glucuronoxylomannan (GXM) polymer of Cryptococcus neoformans, we encountered a series of issues in the hydrogenolysis (global deprotection) of the benzyl ethers. Namely, the preservation of our desired 6-O-acetylation pattern in the target structures and the saturation of benzyl and napthyl rings to their corresponding ether (Figure 1).
This led to our efforts in finding a high quality source palladium on carbon catalyst in the hope of rectifying these issues. In this presentation details regarding the synthesis of GXM oligosaccharides will be presented with applications including FRET probes.[1] Also presented will be a procedure where the palladium catalysts reactivity is tuned through controlled poisoning. This gave rise to an efficient catalyst in terms of increased yield, reaction rate and chemoselectivity under hydrogenolysis conditions.

1. Guazzelli, L., Ulc, R., Rydner, L. & Oscarson, S. A synthetic strategy to xylose-containing thioglycoside tri- and tetrasaccharide building blocks corresponding to Cryptococcus neoformans capsular polysaccharide structures. Org. Biomol. Chem. 13, 6598–6610 (2015).

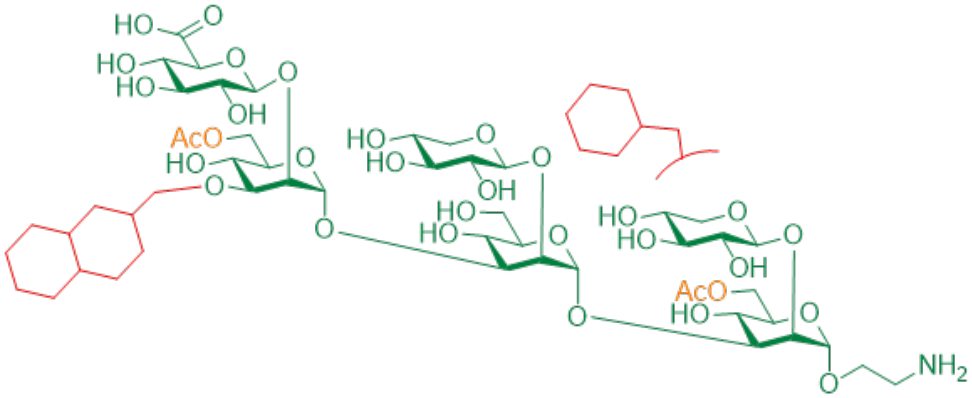

Figure 1. Example of structure obtained upon global hydrogenolysis. In green desired structure and in orange the desired acetylation pattern. In red the undesired saturation of the napthyl and benzyl rings.

OL7.4.1

Synthesis of Microbial Sialic Acids for the Study of their Glycobiology

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Pseudaminic acid (Pse) and Legionaminic acid (Leg) are nine-carbon diamino α-ketone monosaccharides that are found as important terminal components of cell surface glycoconjugates in various human-associated microbes such as C. jejuni and M. smithii. However, the exact biological roles of Pse and Leg are not yet fully understood and research in this area is constrained by the difficulties in obtaining Pse and Leg. To further explore their glycobiological significance, we have developed a robust synthesis route for Pse and its derivatives that allows for modifications to create molecular probe derivatives that will allow investigation of its microbial glycobiology. Moreover, we also modified a previously reported synthesis of Leg[1] to create probes that allow for metabolic labeling of Leg.

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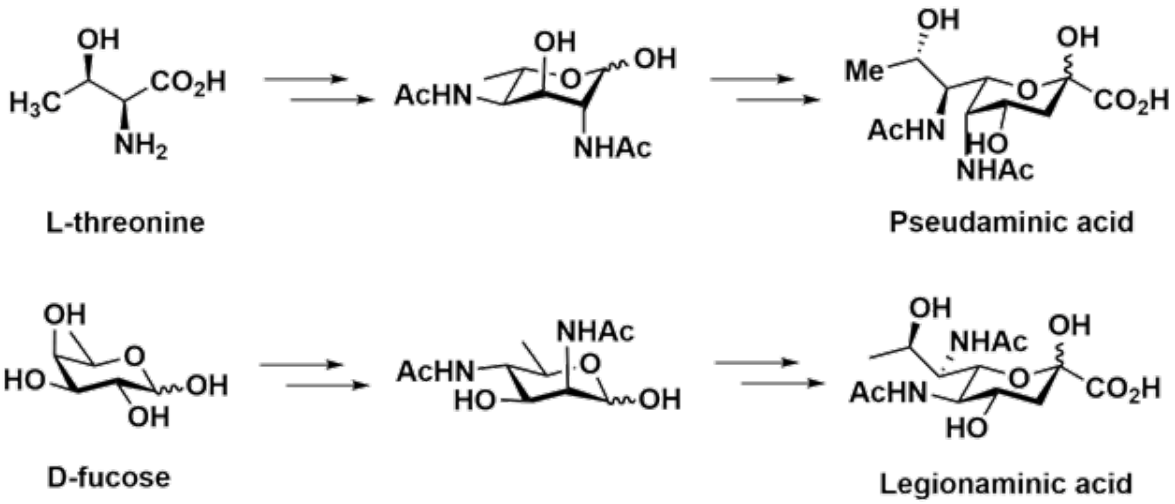

The synthesis overview of Pseudaminic acid and Legionaminic acid.

OL7.4.2

Surveying the Spectrum of Nonulosonic Acids Throughout the Phylogenetic Tree Of Life Using All-Ion-Reaction-Monitoring (Airm) Mass Spectrometry

Hugo Kleikamp[1], Yuemei Lin[1], Mark van Loosdrecht[1], Martin Pabst[1]

[1]TU Delft, Delft, The Netherlands

Over the past decades nonulosonic acids have been increasingly found in (pathogenic) prokaryotes, decorating the contact surface to their external environments. A recent phylogenetic large-scale study, covering nearly 1,000 microbial genomes, discovered a surprisingly wide distribution of an ancient core nonulosonic acid biosynthetic pathway, which was shown to be widely spread amongst both bacteria and archaea. However, predicted ORFs often do not encode for functional entities, and most importantly, genomics alone does not reveal the important post synthesis processing events, which make nonulosonic acids so unique.

Here, we provide a first large-scale comparative study and molecular signature of the broad diversity of nonulosonic acids, by surveying a large number of species throughout the phylogenetic tree of life, using high-resolution mass spectrometry. We established a fully open, but nonulosonic acid specific approach (called all ion reaction monitoring, AIRM), capable of differentiating between different classes of nonulosonic acids, as well as species-specific post synthesis processing events. Furthermore, the high specificity of the assay allows to directly analyse from crude cell lysates.

This study illustrates not only the common utilisation of nonulosonic acids between various species but also gives insight into species specific post-synthesis processes. Though the significance of bacterial sialic acids in host/pathogen interactions has been established over the past decades, our study aims to provide further evidence for their wide-spread utilisation in environmental species.

Key words: nonulosonic acids; pseudaminic acid; neuraminic acid; legionaminic acid; acinetaminic acid; prokaryotes; mass spectrometry; sialo-omics.

Significance
• There are many open questions surrounding the evolution and utilisation of nonulosonic acids in prokaryotes. Unfortunately, the analysis with conventional methods is a laborious process. We developed therefore a fast but highly specific assay for the discovery of novel nonulosonic acid variants.
• We provide a comparative large-scale study on the molecular level demonstrating the diversity and distribution of different classes of nonulosonic acids across species, including purely environmental samples;
• The developed assay serves as a tool to explore complex samples and communities to study the broader ecological context of this prominent, but still poorly analysed class of sugars.

OL7.4.3

New Avenues to Characterize the Conformation and Recognition Features of Multi-Antennary and Extended N-Glycans by Using NMR

Angeles Canales[1], Beatriz Fernández de Toro[2], Wenjie Peng[6], Andrew Thompson[4], Theodoros Karagiannis[5], Javier Cañada[2], Carlo Unverzagt[5], James Paulson[4], Jesús Jiménez-Barbero[3]

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Complex glycosylation patterns containing multiantennary N-glycans are typically found in mature glycoproteins. However, the structural characterization of these glycans is rather challenging. Usually, standard NMR and X-ray diffraction techniques fail to provide specific answers on the structure and molecular recognition features due to intrinsic attributes of the glycan. As a promising approach, carbohydrates conjugated to lanthanide binding tags have revealed high potential toward this aim. This methodology has first been applied to the study of small oligosaccharides (di-, tri- and tetrasaccharides). [1-3] Proceeding from this experimental basis, we have extended this concept to the level of high degree branching and long chain N-glycans. The unprecedented resolution obtained in the spectra has allowed us to perform conformational and interaction analysis of complex carbohydrates such as: tetraantennary N-glycans and a sialylated tetradecasaccharide N-glycan presenting two LacNAc repetitions at each arm. [4-5] The latter is especially relevant since it has been identified as the receptor of the hemagglutinin protein of pathogenic influenza viruses. [5]

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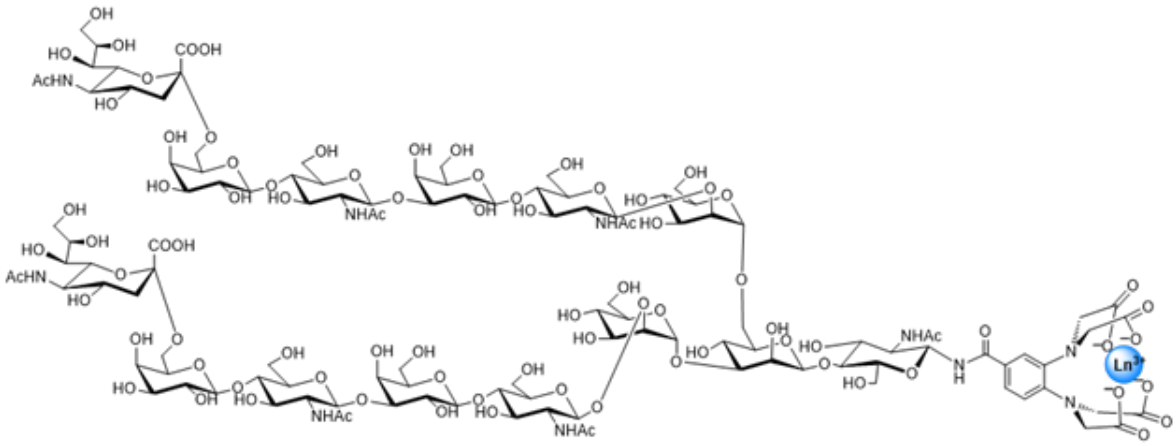
[5] B. Fernández de Toro et al. Angew. Chem. Int. Ed. Engl. 2018, 57(46), 15051-15055.



Sialylated tetradecasaccharide N-glycan derivative

OL7.4.4

Cell-Based Display of the Human Sialome

Christian Büll[1], Julie van Coillie[1], Rebecca Nason[1], Lingbo Sun[1], Henrik Clausen[1], Yoshiki Narimatsu[1]

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Human cells produce a repertoire of sialoglycans with high structural and functional diversity that forms a subclass within the Glycome - the Sialome. The structural diversity of the Sialome derives from the chemically diverse sialic acid types (e.g. Neu5Ac, Neu5Gc, Neu5,9Ac2), linkage types (α2-3/6/8) produced by twenty sialyltransferase isoenzymes, and underlying glycan types that are utilized in a combinatorial manner for sialoglycan synthesis. The vast structural diversity of the Sialome is mirrored by its diverse biological functions on the molecular and cellular level such as regulating the biochemical properties of glycoproteins or cell-cell and cell-extracellular matrix interactions. Moreover, the Sialome is at the center of numerous molecular interactions with endogenous sialic acid-binding lectins such as the Siglecs that are vital for immune cell function. On the contrary, the Sialome is exploited by pathogens that express sialic acid-binding lectins to attach to host cells and aberrations of the Sialome are associated with autoimmunity, neurodegeneration and cancer. Many functions of the Sialome, however, remain elusive and the fine binding specificities of sialic acid-binding lectins, such as the fourteen Siglec family members, to specific sialoglycan types in their natural glycan environment at the cell surface are poorly understood. We have developed a library of isogenic HEK293 cells through precise gene-editing/engineering with combinatorial knock-in/out of glycosyltransferase genes to display distinct features of the Sialome in the natural glycoconjugate context. This cell-based sialoglycan array is used to dissect the fine binding specificities of sialic acid-binding lectins in the natural glycan context at the cell surface. We will present our genetic engineering platform for the cellular display of the human Sialome and its interactions with sialic acid-binding proteins with particular focus on the immunommodulatory Siglec family.

OL8.1.1

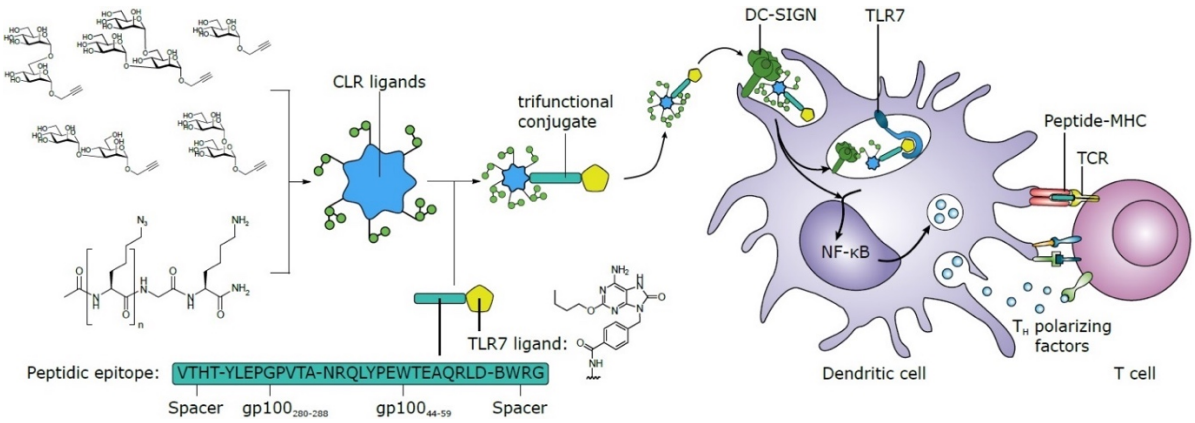
Development of Oligomannose-Peptide Antigen-TLR7-Antigen-Conjugates to Simultaneous Target DC-SIGN and TLR7 For Improved Dendritic Cell Vaccines

Tim P. Hogervorst[1], Eveline R.J. Li[2], Silvia Achilli[3], Corinne Deniaud[3], Sandra J. van Vliet[2], Gijs A. van der Marel[1], Herman S. Overkleeft[1], Dmitri Filippov[1], Yvette van Kooyk[2], Jeroen D.C. Codée[1]

[1]Department of Bio-organic Synthesis, Leiden University, Leiden, The Netherlands, [2]Department of Molecular Cell Biology and Immunology, Cancer Center Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands, [3]Membranes & Immunity Team, Institut de Biologie Structurale, Grenoble, France

With the increasing achievements in the field of immunotherapy, dendritic cells (DC) play an important role as initiators of the adaptive immune response. Induction of the adaptive immune response relies on the recognition of pathogens by DCs via pathogen recognising receptors (PRRs). The conjugation of antigen with immune stimulatory agents is a frequently applied strategy to enhance the immunogenicity of the antigen. Such conjugates have successfully targeted members of the toll-like receptors (TLRs), C-type lectin receptors (CLRs) and NOD-like receptors. Here we describe the synthesis and evaluation of peptide-conjugates that can simultaneous target TLR7 and DC-SIGN and the effect on DC maturation, cytokine profile and antigen (cross-)presentation.

We synthesised and evaluated an array of oligomannose containing clusters that vary in number (ranging from 1-6 copies) and type of mannoside (mono-, di- and tri-mannosides). The affinity of the clusters toward DC-SIGN, and rate of uptake were assessed by SPR and FACS. Based on these studies, a selection was made of the DC-SIGN targeting-constructs to be conjugated to a model epitope (the melanoma tumour antigen GP100). The simultaneous incorporation of a TLR7 agonist allowed us to test for synergistic effects. Our results show that simultaneous targeting of TLR7 with DC-SIGN does not result in synergy and the correlation between affinity of the mannose clusters and antigen presentation of the cluster-conjugates seems to be non-linear. The conjugates that showed the highest affinity for DC-SIGN did not lead to maximal antigen (cross-) presentation and even hampered T cell activation, whereas a moderate binder resulted in enhanced presentation. Our results suggest that targeting antigens towards DCs using DC-SIGN is not just a matter of enhancing the affinity of the carbohydrate ligand-conjugates and our results could contribute to the design of future glycan-based immunotherapeutic drugs.



OL8.1.2

Targeting Glycosylated Motifs on Cancer Cells Using Innovative Lectins Fragments for Theranostic Applications

François Bulteau[1,2,3], Michel Thepaut[1], Maxime Henry[2], David Goyard[3], Olivier Renaudet[3], Jean-Luc Coll[2], Franck Fieschi[1]

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We estimate about 3.91 million new cases of cancer in Europe in 2018. And 1.93 million cancer deaths. Half of these are breast, colorectal, prostate and lung cancers.
Currently, several ligands such as RGD peptides, folic acid and recombinant antibodies are used to target cancer cells based on the fact that they recognize receptors overexpressed on the surface of cancer cells. In the present work, our strategy consists in the generation of new tools that target the glycosylation profile of cell surface epitopes.
Many cellular features are altered during the process of carcinogenesis and in particular the glycosylation profile of the membrane of the cancer cells. Notably, it has been observed in many cancers (colon, breast and uterus) appearance of new specific glycosylations patterns (not present on healthy cells)[1][2]. Among them, of particular importance is the Thomsen-nouveau (Tn) antigen, it's a N-acetylgalactosamine link to the serine or a threonine[3]. Tn antigen is an O glycosylation types.
In the other side, they are proteins, named lectins, which bind specifically and reversibly with sugars. The MGL (macrophage galactose type C-type lectin) can bind with N-acetylgalactosamine).
The innovative goal of my project is to use MGL to target and to deliver molecules of interest such as fluorescent contrast agents and/or therapeutic agents. However, the antigen is not considered as a biomarker yet.
In preliminary experiments, recombinant extracellular domain of MGL constructs have been produced (trimeric) and validated for their functionality. They have been then tested by flow cytometry and confocal microscopy for their specificity toward cancer cell lines positive or negative for the Tn antigen.
The first results show a strong and specific labelling of the Tn-positive cell lines by the MGL.
This strategy is very promising for various applications in oncology. Next steps will be to test our new MGL constructs on 3D spheroid cell cultures and in tumor bearing animals. Later, we will clone the carbohydrate recognition domain (CRD) of this lectin, produce it in E. coli, and load 4 or 6 copies of this CRD on artificial chemical platforms (RAFT) in order to generate multivalent recognition motifs and regioselectivity.

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OL8.1.3

Conquering Hypersialylation Induced by Tumor Related Hst3gal1 by Custom-Made Sufex Warheads

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Inadequate sialylation is known to correlate with a wide variety of serious infectious as well as non-infectious diseases.[1]
Considerable effort has been expended to investigate the role of ST3Gal1 in various types of cancer. Among them are breast-, ovarian-, prostatic- and pancreatic cancer to only mention a few of them.[2] ST3Gal1 was found to enhance cell growth and angiogenesis, and contribute to metastasis by concealing the cells from the immune system.[3] Additionally, elevated expression of this sialyltransferase leads to resistances against established chemotherapeutics.[4]
Subsequently, the selective inhibition of this human glycosyltransferase is a highly desirable objective. Regarding covalent inhibition, tyrosine is a reasonable target for this intention because of its low abundance and amphiphilic structure. Human sialyltransferases share CMP-Neu5Ac as donor and consequently its binding site is conserved among these GT29 enzymes. Accordingly, a possible way of discriminating between different ST enzymes to accomplish the desired selectivity is addressing the substrate binding site. In hST3Gal1 (Figure 1a) there are three tyrosine residues involved in the catalysis of the transfer of sialic acid from CMP-Neu5Ac onto the acceptor glycoside R-Galβ1,3-GalNAc. Two of them (Y230, Y266) contribute to substrate and one (Y191) to donor binding. Both residues are inevitable for catalysis. Sequence alignment reveals that this constitution of the active site is unique among siayltransferases and is thus a promising target. Sharpless et al. recently renewed the concept of Sulfur-(VI)-Fluoride-Exchange (SuFEx) reactions as a click like option, which was initially introduced by Steinkopf et al. in 1927[5]. In preliminary experiments, we found, that besides minor side reactions, the three tyrosine residues located in the active site are modified by a SuFEx reagent. By further refinement and targeting not only one tyrosine residue per SuFEx warhead, but introducing two fluorosulfates in a bifunctional warhead for dual reaction with Y230 and Y266 (Figure1b), we intend to highly enhance selectivity and binding affinity. Further selectivity will be aspired to by enhancing the proximity through coupling the SuFEx reagent with a suitable carbohydrate, for instance sialic acid, Galβ1,3-GalNAc and derivatives thereof. These moieties will be introduced by copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC), facilitating quick and adjustable variation of the selectivity-providing structure as well as enabling implementation of different warheads.

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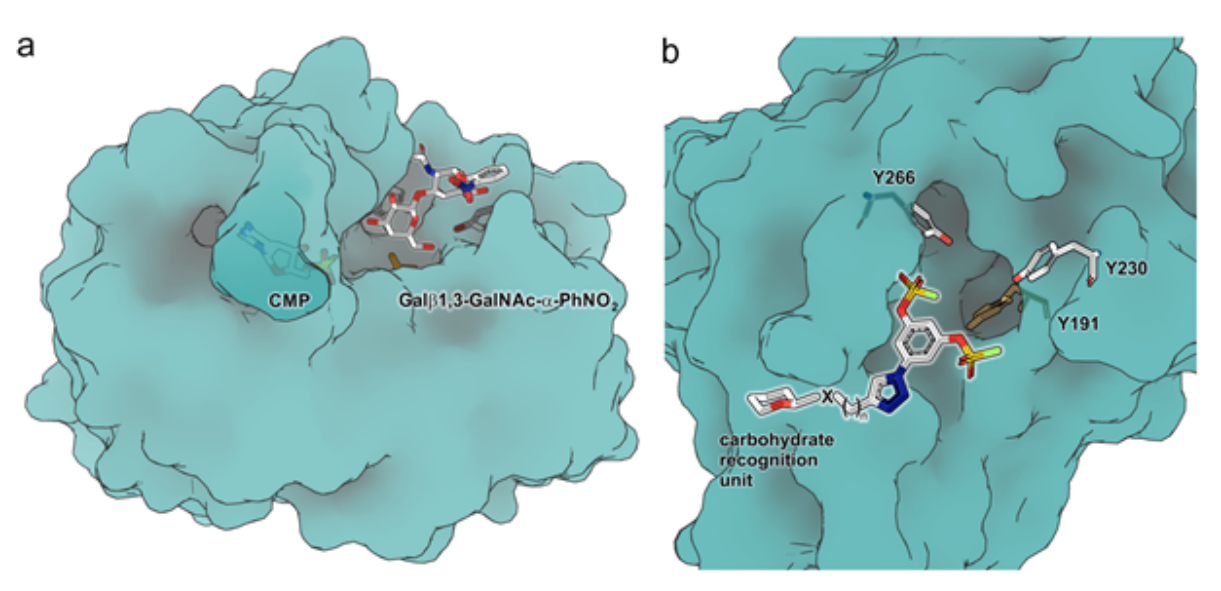

Figure 1: a. Overview of the active site of hST3Gal1, CMP and acceptor Galβ1,3GalNAcα-PhNO2 from pST3Gal1 (PDB 2wnb). b. schematic representation of the inhibition concept; the alkyne functionality will be introduced with a flexible linker at different positions of the saccharide. A homology model of hST3Gal1 was generated with I-TASSER (Iterative Threading ASSEmbly Refinement) [6] using porcine ST3Gal1 as template (2WNB).

OL8.1.4

Doxorubicin and Aclarubicin: Shuffling Antracyclin Glycans for Improved Cytotoxic Agents

Dennis Wander[1], Sabina van der Zanden[2], Gijs van der Marel[1], Jeroen Codee[1], Jacques Neefjes[2], Hermen Overkleeft[1]

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Doxorubicin is one of the Topoisomerase II inhibitors that are used for the treatment of various types of cancer, including leukaemia and non-Hodgkin lymphoma. According to its typical mechanism of action, intercalation occurs into the DNA, their target topoisomerase is trapped, thereby generating DNA double-strand breaks and ultimately cell death. As effective and popular as this drug is, its usage is hugely limited by the cumulative cardiotoxicity it brings along.

It was recently shown that these anthracyclines are able to induce histone eviction from chromatin [1]. Amongst the consequences are a marked delay in DNA repair and diverse epigenetic changes. This additional activity of the anthracyclines may explain the difference in potency and side-effects between these drugs and structurally different Topo II inhibitors.

We have developed a flexible methodology that allows for the preparation of mono-, di- and trisaccharide analogues of doxorubicin and aclarubicin (a related anthracycline trisaccharide). Structural variation in the target compounds has been achieved by swapping and shuffling of the sugar sequence and varying the alkylation pattern on the amine functionality. The saccharides can be assembled by iterative stereoselective couplings using thiophenyl donors, which can then be coupled to the desired aglycon using Yu's ortho-alkynyl benzoate donor methodology [2].

A combined cell-biology and bio-informatic pipeline allows us to gain more insight in the biological and cytotoxic properties of the designer antracyclines, in search of better chemotherapeutic agents with diminished side-effects.

This has so far led to the discovery of a doxorubicin-aclarubicin hybrid structure that lacks the classical DSB mechanism, yet maintains its histone evicting property and cytotoxicity. Moreover, its cardiotoxic property has shown to be abolished in vivo in mice. Efficacy studies on human AML xenografts in mice are currently ongoing with the hope of ultimately providing non-cardiotoxic anthracycline anti-cancer treatment for humans.

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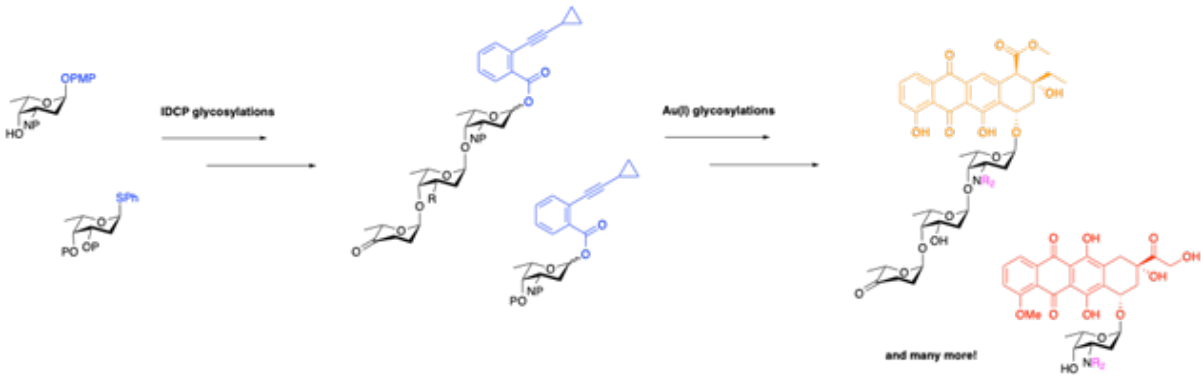


Figure 1: Retrosynthesis towards doxorubicin and aclarubicin analogs/hybrids.

OL8.2.1

Architecture and Evolution of Blade Assembly in B-Propeller Lectins

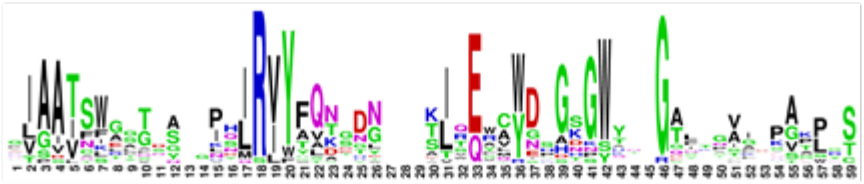
François Bonnardel[1,2], Atul Kumar[3], Michaela Wimmerova[3], Martina Lahmann[4], Serge Pérez[1], Annabelle Varrot[1], Frédérique Lisacek[2], **Anne Imberty[1]**

[1]Cermav-cnrs, Grenoble, France, [2]Swiss Institute of Bioinformatics, Geneva, Switzerland, [3]CEITEC, Masaryk University, Brno, Czech Republic, [4]University of Bangor, Bangor, United Kingdom

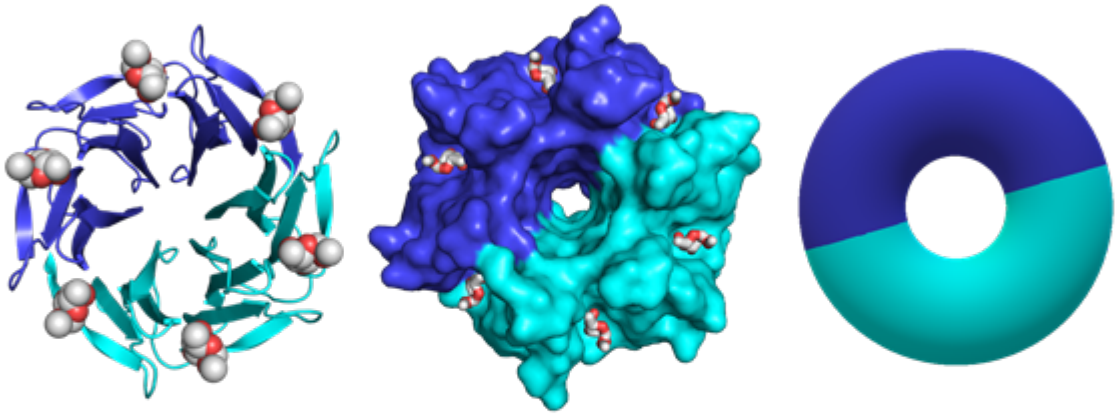
Lectins with a β-propeller fold bind glycans on the cell surface through multivalent binding sites and appropriate directionality. These proteins are formed by repeats of short domains, raising questions about evolutionary duplication. However, these repeats are difficult to detect in translated genomes and seldom correctly annotated in sequence databases. To address these issues, a database has been developped and is now available on internet https://www.unilectin.eu/propeller/.

We defined the blade signature of the five types of β-propellers using 3D-structural data. With these templates, we predicted 3887 β-propeller lectins in 1889 species and organised this new information in a searchable online database. The data reveals a widespread distribution of β-propeller lectins across species. Prediction also emphasises multiple architectures and led to uncover a novel β-propeller assembly scenario. This was confirmed by producing and characterizing a predicted protein coded in the genome of Kordia zhangzhouensis. The crystal structure shows a new intermediate in the evolution of β-propeller assembly and demonstrates the power of our tools

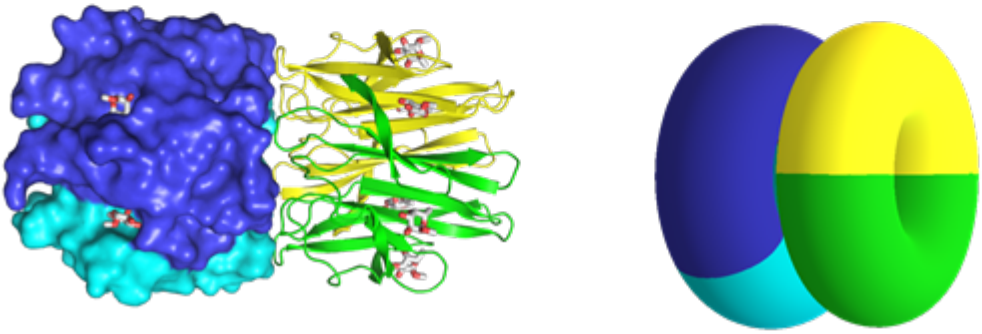
Conserved blade motif for PropLec6A β-propeller familly



Identification of a β-propeller lectin with a unique new assembly



Two propellers assemble together as a tetramer



Discovery of new b-propeller lectin assembly from data base prediction

OL8.2.2

Glycoprofile Analysis of an Intact Glycoprotein as Inferred by NMR Spectroscopy

Luca Unione[1], Ana Ardá[2], MariaPia Lenza[2], Pedro Urquiza[2], Ana Laín[2], Juan Manuel Falcón[2], Oscar Millet[2], Jesús Jiménez-Barbero[2]

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Protein N-glycosylation stands out for its intrinsic and functionally related heterogeneity. Despite its biomedical interest, glycoprofile analysis remains a major scientific challenge, especially when looking for structure/function relationships within the intact glycoprotein. Herein, we present a strategy to delineate the N-glycans composition in intact glycoproteins, under physiological conditions, using solution state nuclear magnetic resonance (NMR) spectroscopy. The employed methodology allowed to dissect the glycan pattern of the IgE high affinity receptor (Fc□RI□) expressed in human HEK 293 cells, identifying the presence and relative abundance of specific glycan epitopes. Furthermore, the presentation and dynamics at the glycoprotein surface demonstrates that the N-glycans are essentially solvent exposed and properly presented as targets for N-glycan receptors molecules, such as lectins. In line, we also report the first NMR-based study of the interactions between an intact glycoprotein and a lectin in solution. This approach provides novel opportunities to directly detect specific glycosylation alterations as function of endo or exogenous factors and opens new avenues for the detection of specific N-glycan prognostic biomarkers and for the studies of glycan-protein interactions, accounting for the role of the glycan heterogeneity and their presentation in native glycoconjugates.

OL8.2.3

Explorations in Multivalency with High Profile Medicinal Target Lectins of P. Aeruginosa and Influenza A Virus

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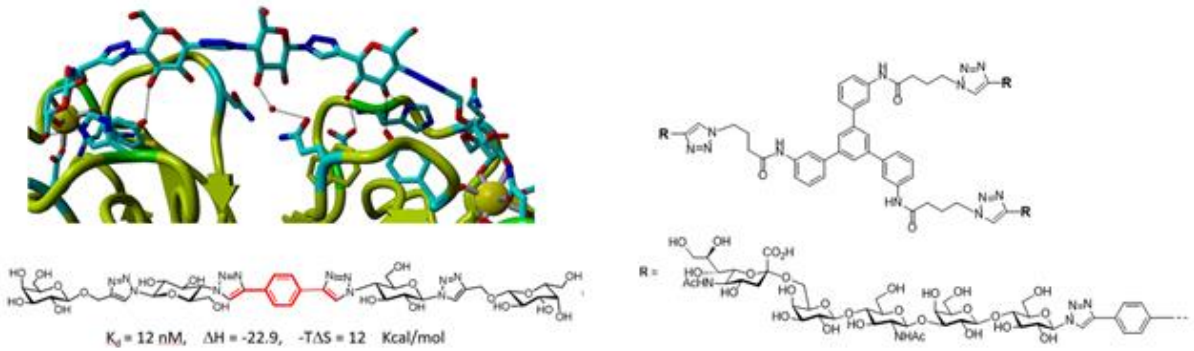
Numerous carbohydrate binding proteins play decisive roles in the pathogenicity of microorganisms, including bacteria, fungi and viruses. With the uncovering of many of the specificities of these proteins, the possibilities for intervention increases dramatically. This alternative antipathogenic intervention is further driven by the increasing resistance of pathogens against antibiotic and antiviral agents aimed at killing the pathogens. In many cases the carbohydrate binding proteins attaches to cell surface carbohydrates, in a multivalent fashion, to induce colonization and invasion. Multivalent inhibitors are becoming increasingly effective as the rules of multivalency are increasingly being understood. We here will discuss the design and thermodynamic binding, and infection inhibition of aspects of multivalent ligands of the virulence factor lectin LecA of the problematic ESKAPE pathogen P. aeruginosa [1]. Major multivalency effects were observed of approaching 3 orders of magnitude, and varying contributions of enthalpy and entropy. Furthermore, calculations were performed that predict the multivalency effect and correlated well with experiment. The hemagglutinin protein (HA) of the influenza A virus is our other medicinally important target. For this trivalent protein target with widely separated binding sites, large sialyl-LacNac based ligands were synthesized and linked to scaffold molecules. Enhanced inhibition of ca. 400 fold was observed, indicating that chelation between binding sites was achieved. Results will be put in perspective to prior efforts.

[1] G. Yu, A.C. Vicini, R.J. Pieters, 'Assembling of divalent ligands and their Effect on Divalent Binding to Pseudomonas aeruginosa Lectin LecA', J. Org. Chem. 2019, acs.joc.8b02727.



Left: X-ray of LecA bound to a divalent ligand and exemplary structure and binding parameters. Right: Example of trivalent HA inhibitor.

OL8.2.4

The Synthesis of C-Analogues of Thiodigalactoside (TDG) as Novel Enzymatically Stable Galectin-1 Inhibitors

Kamil Parkan[1], Marcela Pávová[2], Petr Pachl[2], Radek Pohl[2]

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Over the last two decades, human galectins (particularly galectin-1, 3, 7 and 9) have become attractive targets for anticancer and anti-inflammatory drugs development. They are defined by their affinity for β-D-galactopyranoside moieties and their possession of a conserved carbohydrate-recognition domain (CRD). Since lactose and thiodigalactoside (TDG) are known scaffold in preparation of galectin-1 and galectin-3 inhibitors, our goal was therefore preparation of their carba-analogs as enzymatically stable glycomimetics.[1]
Our approach for the synthesis of C-analogues of TDG 1 employs Pd-catalyzed sp2-sp3 cross-coupling reaction of 9-BBN derivative 2 with corresponding 1-iodoglycals 3. The cross-coupling products 4 with one endocyclic double bond allow access to various C-glycosides 1 using various stereoselective transformations. We have found that CDG (β-D-Galp-C-(1→1)-β-D-Galp) occupies in free unbound state predominantly one conformation, which is very similar to the conformation of TDG, and therefore perfectly arranged to fit into the binding site of galectin-1. However, the determination of binding affinity of CDG (Kd = 416 mM) to galectin-1 by ITC showed repeatedly Kd worse than for lactose (Kd = 327 mM). Moreover, TDG binds to galectin-1 about 6 times stronger than lactose (Kd = 57 mM). This difference in binding affinities of CDG and TDG might be therefore attributed to the special geometric arrangement in the proximity of sulfide bridge. We have tested prepared glycomimetics in our optimized viral entry assay using LuSIV cells infected with HIV-1 and in red blood cells hemagglutination assay. Both assays show that CDG has a comparable effect as TDG. These biochemical and biological findings, together with molecular modeling, will serve as a basis for further synthesizing novel glycomimetics with improved efficiency, stability and bioavailability.

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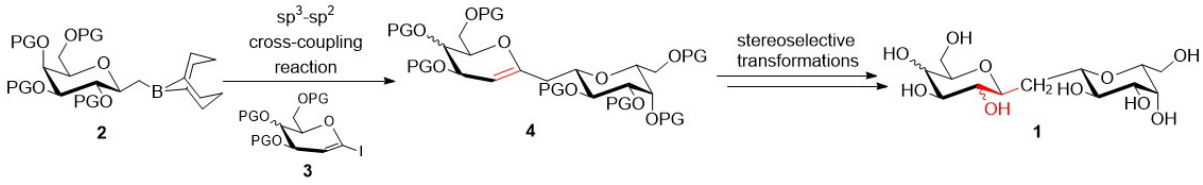


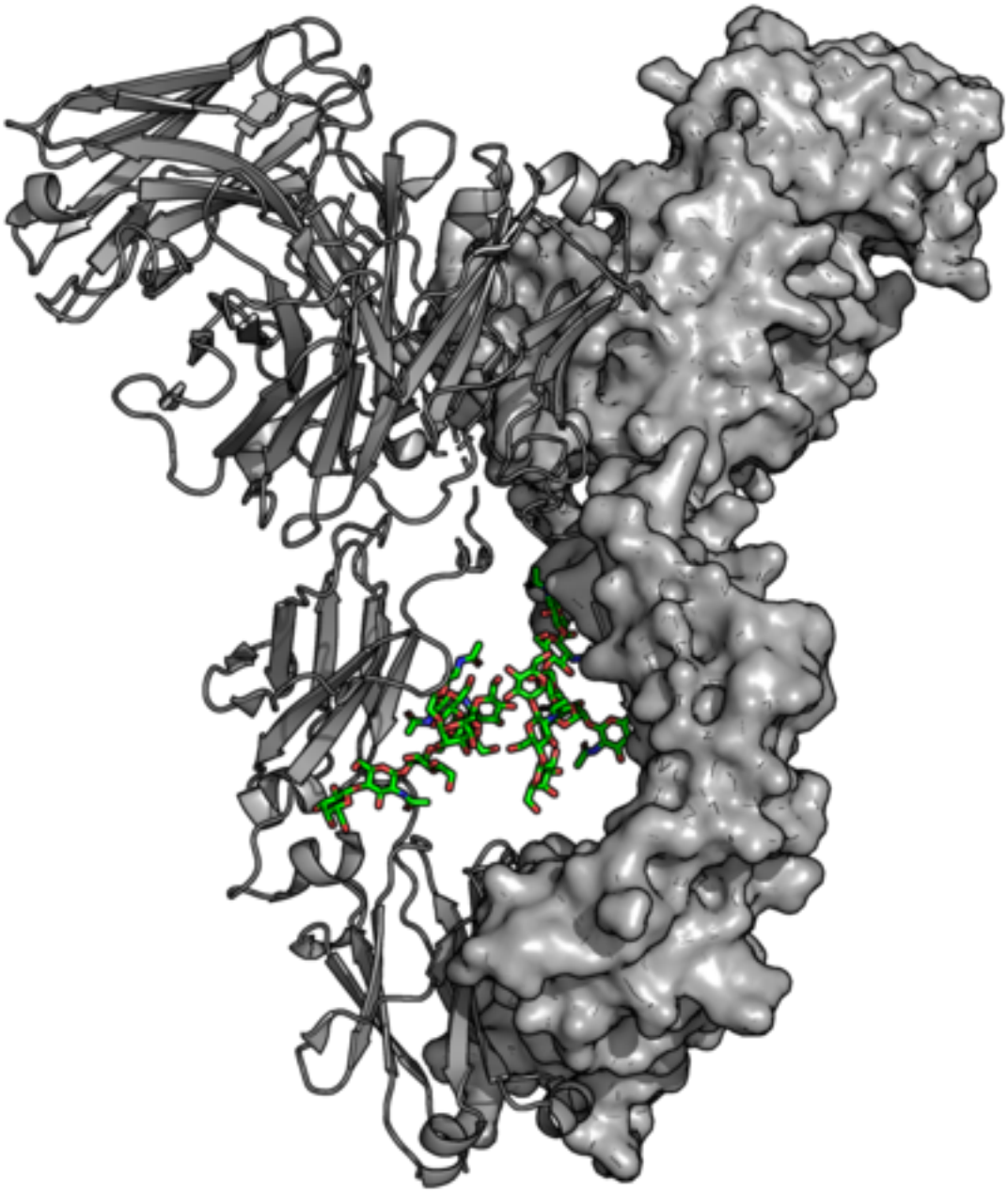
OL8.3.1

Sequence-To-Structure Dependence of Isolated Igg Fc Complex Biantennary N-Glycans: A Molecular Dynamics Study

Aoife Harbison, Lorna Brosnan, Keith Fenlon, **Elisa Fadda**[1]

[1]*Maynooth University, Maynooth, Ireland*

Fc glycosylation of human immunoglobulins G (IgGs) is essential for their structural integrity and activity. Interestingly, the specific nature of the Fc glycoforms is known to modulate the IgG effector function and inflammatory properties. Indeed, while core-fucosylation of IgG Fc-glycans greatly affects the antibody-dependent cell-mediated cytotoxicity (ADCC) function, with obvious repercussions in the design of therapeutic antibodies, sialylation can reverse the antibody inflammatory response, and galactosylation levels have been linked to aging, to the onset of inflammation, and to the predisposition to rheumatoid arthritis. Within the framework of a structure-to-function relationship, we have studied the role of the N-glycan sequence on its intrinsic conformational propensity. Here we report the results of a systematic study, based on extensive molecular dynamics (MD) simulations in excess of 62 μs of cumulative simulation time, on the effect of sequence on the structure and dynamics of increasingly larger, complex biantennary N-glycoforms isolated from the protein, i.e. from chitobiose to the larger N-glycan species commonly found in the Fc region of human IgGs. Our results show that while core fucosylation and sialylation do not affect the intrinsic dynamics of the unlinked N-glycans, galactosylation of the α(1-6) arm shifts dramatically its conformational equilibrium from an outstretched to a folded conformation. These findings are in agreement with and can help rationalize recent experimental evidence showing a differential recognition of positional isomers in glycan array data and also the preference of sialyltransferase for the more reachable, outstretched α(1-3) arm in both isolated, and Fc-bound N-glycans. Furthermore, I will also discuss recent results of large scale simulations, showing the effects of the IgG Fc region's architecture on the glycans' conformational propensity with implications on molecular recognition and ADCC sequence dependence.



Structure of the intact human IgG B12 antibody (PDB 1hzh) with Fc glycans highlighted in an all-atom (sticks) representation.

OL8.3.2

Increased Autoantibody Variable Domain N-Glycosylation in Autoimmune Diseases

Karin van Schie[1], Lise Hafkenscheid[1], Rochelle Vergroesen[1], Linda Slot[1], Albert Bondt[2], Theresa Kissel[1], Theo Rispens[3,4], Hans Ulrich Scherer[1], Manfred Wuhrer[2], René Toes[1]

[1]Department of Rheumatology, Leiden University Medical Center (LUMC), Leiden, The Netherlands, [2]Center for Proteomics and Metabolomics, Leiden University Medical Center (LUMC), Leiden, The Netherlands, [3] Department of Immunopathology, Sanquin Research, Amsterdam, The Netherlands, [4]AMC-Sanquin Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

The most prominent autoantibodies in rheumatoid arthritis, ACPA (anti-citrullinated protein antibodies), were recently found to harbour a remarkably high amount of glycans in the variable domain. For these glycans to be expressed, ACPA B cells need to introduce glycosylation sites in the B cell receptor (BCR) through somatic hypermutation. DNA sequence analysis of the ACPA BCR repertoire showed that over 90% of the sequences contained a glycosylation site in the variable domain, whereas tetanus-specific B cells from RA patients showed no glycosylation sites. This striking observation has been confirmed on protein level using HILIC-UHPLC, showing that 93% of APCA-IgG bears one or more glycans in the variable domain.
Notably, we now demonstrated that increased ACPA variable domain glycosylation is exclusive to RA patients and healthy individuals transitioning to RA, while absent in ACPA+ individuals that remain healthy, suggesting a direct link between glycosylation and disease pathogenesis.
We furthermore showed that 1) introduction of these glycosylation sites was not due to random accumulation, 2) glycosylation sites are rarely located in the antigen binding site and 3) variable domain glycans do not improve affinity for autoantigens. This implies that introduction of ACPA variable domain glycans is driven by other selection mechanisms than classical antigen selection.
On DNA level, others have recently shown that increased variable domain glycosylation-sites are also found in the total BCR repertoire of patients with other autoimmune diseases than rheumatoid arthritis. Using HILIC-UHPLC and nano-LC-MS, we are currently investigating the glycosylation profile of these other autoantibody types. This would reveal whether these glycosylation-sites are (solely) derived from autoantibodies, and whether these sites are actually glycosylated.
Understanding the strong selection on variable domain glycosylated autoantibodies could be the key to understanding the breach of tolerance and one of the origins of autoimmunity.

OL8.3.3

Site-Specific Glycosylation Mapping of Human Fc Gamma Receptor Iiib

David Falck[1], Iwona Wójcik[1], Thomas Sénard[1], Erik L de Graaf[2], George MC Janssen[1], Arnoud H de Ru[1], Gillian Dekkers[2], Yassene Mohammed[1], Peter A van Veelen[1], Gestur Vidarsson[2], Manfred Wuhrer[1]

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Fc gamma receptors (FcγRs) govern key functions of the human immune system. For example, triggering of FcγRIIIb on neutrophils by immunoglobulin G (IgG) induces phagocytosis and degranulation. Allelic polymorphisms of FcγRIIIb have been associated with autoimmune diseases. The influence of antibody glycosylation on FcγR binding is well established and led to therapeutic advances. Glycosylation of the FcγR itself also influences these interactions as it is essential for the 50-fold increased affinity of afucosylated IgG towards FcγRIIIa. Despite these crucial roles, FcγR glycosylation in humans is largely uncharacterized.
We are mapping the glycosylation of human FcγRs as a basis for studying its impact on IgG effector functions and its involvement in autoimmune diseases. Here, we present the glycosylation of FcγRIIIb obtained from the neutrophils of healthy donors. Our study represents one of the first site-specific glycosylation studies of a cellular FcγR and covers more glycosylation sites than recent reports [1].
Neutrophils were extracted from healthy donor plasma by density gradient centrifugation. FcγRIIIb was then purified by immunoprecipitation and subjected to in-gel glycoproteomics analysis. Proteolytic cleavage by chymotrypsin and endoproteinase GluC yielded separate glycopeptides for each theoretical glycosylation site. These were analysed by liquid chromatography – mass spectrometry (LC–MS) using an orbitrap Fusion Lumos MS. MS/MS spectra were obtained by high-energy collision induced dissociation at three energy levels and assigned by combining software tools and manual interpretation. All identified FcγR glycopeptide compositions were quantified on the MS level using LaCyTools.
The FcγRIIIb glycosylation appears to be quite different at the different glycosylation sites. For example, the presence of high mannose type (HM) glycans could be confirmed for FcγRIIIb and was attributed mainly to site N45[1]. In contrast, N162 and N169 showed mainly di- and triantennary complex glycans. In the future, it will be interesting to also look at functional consequences of altered glycosylation at specific sites and its role in specific (patho)physiological conditions.

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OL8.3.4

Carbohydrate-Mediated Lysosomal Protein Trafficking, and Modifications to Improve Therapies

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[1]University Of Canterbury, Christchurch, New Zealand

The majority of lysosomal enzymes are targeted to the lysosome by post-translational tagging with N-glycans terminated in mannose-6-phosphate (M6P) residues and interaction with the mannose-6-phosphate receptors (M6PRs) [1]. Some current enzyme replacement therapies (ERTs) for lysosomal storage disorders are limited in their efficacy by the extent to which the recombinant enzymes bear the M6P-terminated glycans that are required for effective trafficking. Improving the targeting of recombinant enzymes to the lysosome through the M6PRs is therefore of considerable interest.
A variety of avenues of investigation have been pursued, including the chemical conjugation of naturally-derived or synthetic oligosaccharides containing M6P [2]. Our own studies have focused on synthetic applications of the endo-beta-N-acetylglucosaminidase enzymes (ENGases)[3]; an approach which we have pioneered over the past 15 years for the production of defined homogenous glycoproteins and glycopeptides. I will discuss ongoing studies in the area [4][5], which centre on the production of N-glycans that are terminated in M6P residues, and their subsequent attachment to proteins using ENGase enzymes (Fig. 1).

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[4] Priyanka, P.; Parsons, T. B.; Miller, A.; Platt, F. M.; Fairbanks, A. J. Angew. Chem. Int. Ed. 2016, 55 (16), 5058.
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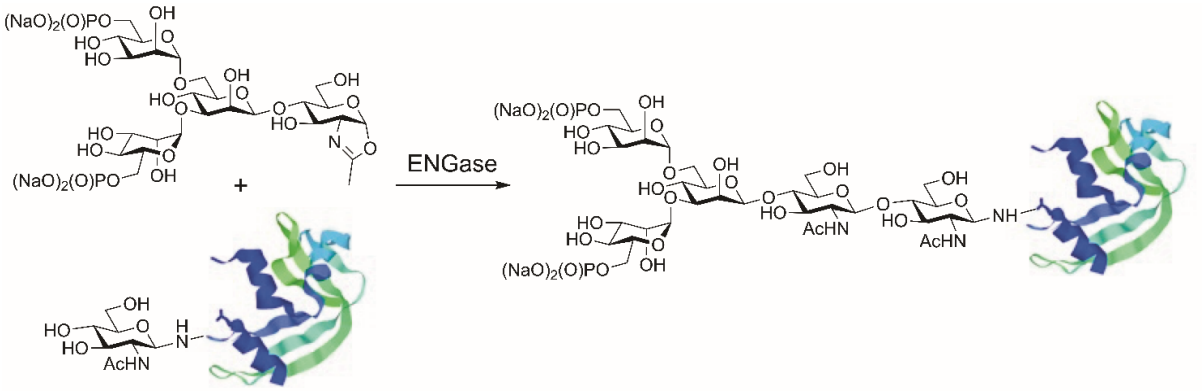

Fig. 1 Synthesis of a phosphorylated glycoprotein using an ENGase

OL8.4.1

Modeling Insights into Molecular Mechanisms Underlying the Role of Glycosaminoglycans in Cell Signaling Processes

Sergey Samsonov[1]

[1]University Of Gdansk, Gdansk, Poland

Glycosaminoglycans (GAGs) represent a particular class of linear anionic periodic polysaccharides made up of repetative disaccharide units which could be sulfated in different positions and therefore determine their structural and functional properties. GAGs are located in the extracellular matrix of the cell, where they participate in many cell signaling processes via interactions with their protein targets such as cytokines and growth factors. Despite the high biological relevance of these interactions, molecular mechanisms underlying them are not well understood due to the challenging nature of these molecules for the experimental analysis. Therefore, modeling is very useful to complement the experiment and to provide new insights in protein-GAG systems. Although molecular modeling of protein-GAG interactions also experiences difficulties due to the scarcity of the computational methods for these systems in comparison to other biomolecular complexes, we established and successfully applied molecular docking and molecular dynamics-based protocols to the complexes of GAGs with the following protein targets: IL-8, SDF-1, VEGF, FGF1, sclerostin, TIMP-3, BMP-2, cathepsin K and others (most representative publications are provided below in References). Such modeling studies exploiting the available corresponding experimental data allowed to complement, explain and rationally guide the further experimental analysis. Here, we present an interdisciplinary work, where we provide modeling insights into the molecular mechanisms underlying the role of GAGs in cell signaling processes, where several protein targets are involved: CXCL-14, VEGF and cathepsin K. Our results contribute to deepening the general understanding of the molecular interactions in protein-GAG systems and, in perspective, could be very useful for the development of novel approaches within the area of regenerative medicine.

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OL8.4.2

NMR Studies Unravel the Binding of Glycosaminoglycans to CXCL14

Anja Penk[1], Sergey A. Samsonov[2], Daniel Huster[1], Lars Baumann[1]

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CXCL14, a soluble 9.4 kDa protein, is a novel, highly conserved chemokine with unprecedented features. Despite exhibiting the typical chemokine fold, its flexible N terminus consists of only two amino acids; a domain that is usually attributed to chemokine receptor activation. CXCL14 is a positive allosteric modulator of the receptor CXCR4 and a growing body of evidence points towards a multitude of immunomodulatory activities of CXCL14 such as homeostatic immune surveillance and elimination of early neoplasmic transformations in skin and mucosae. Its strong anti-angiogenic properties may be a target for cancer therapy.
In the current study, we investigated the interaction of CXCL14 with different glycosaminoglycans (GAGs) in order to understand the molecular basis of GAG-binding. To this end, we cloned, expressed and refolded CXCL14 with final yields of up to 4 mg highly pure protein per liter E. coli batch culture.
We used ^{15}N-isotopically labelled CXCL14 to investigate the interaction of GAGs with this protein via NMR chemical shift perturbation (CSP). The recorded ^{1}H-^{15}N HSQC NMR spectra show well resolved signals with a single peak for each backbone amide of the protein. Upon titration of GAG ligands, peaks near the binding site are expected to shift in the spectrum due to their altered electronical environment or secondary structure changes induced by ligand binding. These CSPs were monitored for chondroitinsulfate A/C and D as well as heparin and dermatan sulfate for varying protein/GAG ratios.
Interestingly, the observable CSPs with the various hexameric GAGs (dp6) reveal that the amino acids of CXCL14 are influenced differently. Especially the pattern for heparin suggests a different primary binding site compared to the other GAGs. Thus, we assume that varying sulfation patterns of the GAGs confer specificity beyond simple electrostatic interactions. This hypothesis is also supported by complementary computational methods (docking and molecular dynamics-based studies).
However, the differences in the amino acid pattern are less pronounced, if longer GAGs (dp10) are investigated. Furthermore, titration experiments of heparin dp10 were not feasible due to an immense loss in NMR signal intensity. Upon heparin dp10 titration the solution remains clear and no line broadening in the spectra was observed. This indicates a tendency of CXCL14 to form stable, fast relaxing, high molecular weight oligomers in the presence of heparin, which were also shown in a preliminary CXCL14 crosslinking experiment.
Hence, CXCL14 shows differences in the binding site for the investigated GAGs and this binding seems to be closely related to the oligomerization behaviour of CXCL14. Therefore, CXCL14 and GAGs provide a promising system to investigate a possible specificity of GAG protein interactions.

OL8.4.3

Exploration of the Biosynthesis of Glucosaminoglycans

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Proteoglycans (PG) are large macromolecules that consist of a core protein decorated by glycosaminoglycans (GAG), i.e. large linear, negatively charged polymers of repeating disaccharide units. The PGs are found on the cell surface as well as in the extracellular matrix with important roles in the regulation of growth factor signaling, and cell-cell interactions.

The biosynthesis of GAG chains is initiated by xylosylation of a serine residue in the PG protein. The xylosylated protein is then further glycosylated to form a linker tetrasaccharide. This linker is then polymerized to form the full-length GAGs. The variable lengths of GAGs together with post-synthetic modifications result in extensive diversity.

Interestingly, the biosynthesis of GAG chains can also be initiated by xylosides that act as acceptors in the first galactosylation step by the enzyme β4GalT7, and thus provide a simplified model system for GAG biosynthesis.

In this lecture we will present our recent findings on the key enzymes β4GalT7 and DS-epi1 (Figure 1), the use of xylosides as tools for understanding the biosynthesis of GAGs, and engineering of specific GAG-chains for use as anticoagulants. [1-4]

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[4] A. Persson, et al. J. Biol. Chem. 2018, 293, 10202-10219

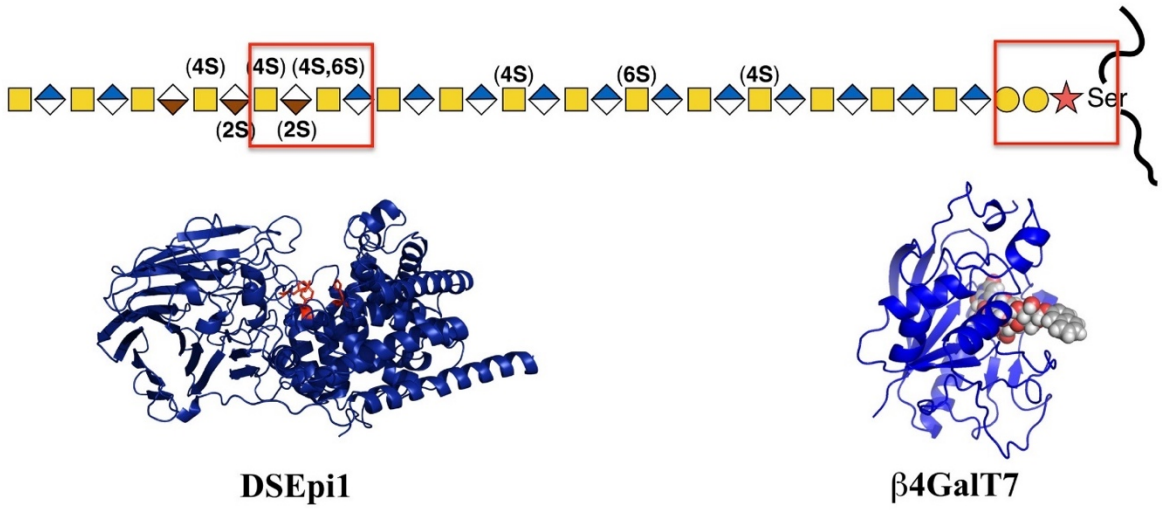

Figure 1: Proteoglycans are composed of glycosaminoglycan chains linked to a core protein via the carbohydrate xylose

OL8.4.4

Exploring the Glycosaminoglycan Conformational Space: A Pipeline to Generate 3D Model and Identify Patterns of Amino-Acids Involved in GAG-Protein Complexes

Serge Perez[1], Olivier Clerc[2], Julien Mariethoz[3], Alain Rivet[1], Frédérique Lisacek[3], Sylvie Ricard-Blum[2]

[1]CERMAV-CNRS, Grenoble, France, [2]University Claude Bernard Lyon 1, Lyon, France, [3]SIB Swiss Institute of Bioinformatics, Geneva, Switzerland

Mammalian glycosaminoglycans (GAGs) are linear complex polysaccharides comprising heparan sulfate, heparin, dermatan sulfate, chondroitin sulfate, keratan sulfate and hyaluronic acid. They bind to numerous proteins and these interactions mediate their biological activities. GAG–protein interaction data reported in the literature are curated mostly in MatrixDB database (http://matrixdb. univ-lyon1.fr/). However, a standard nomenclature and a machine-readable format of GAGs together with bioinformatics tools for mining these interaction data are lacking.

We report here the building of an automated pipeline to (i) standardize the format of GAG sequences interacting with proteins manually curated from the literature, (ii) translate them into the machine-readable GlycoCT format and into SNFG (Symbol Nomenclature For Glycan) images and (iii) convert their sequences into a format processed by a builder generating three-dimensional structures of polysaccharides based on a repertoire of conformations experimentally validated by data extracted from crystallized GAG–protein complexes. We have developed a converter to automatically translate the GlycoCT code of a GAG sequence into the input file required to construct a three-dimensional model [1].

From the analysis of the 3D structures of GAG-protein complexes, we could identify the signatures of the patterns of amino-acids most closely involved in the interactions with the sulfate groups. The significance of such signatures has been corroborated by the calculations of the electrostatic surfaces of the proteins. This agreement opens the road to implementing pattern searches in protein-binding GAG sequences.

While strengthening the implementation of GAGs in the field of glycobioinformatics, these new developments are a step forward towards bridging glycomics with interatomics [2].

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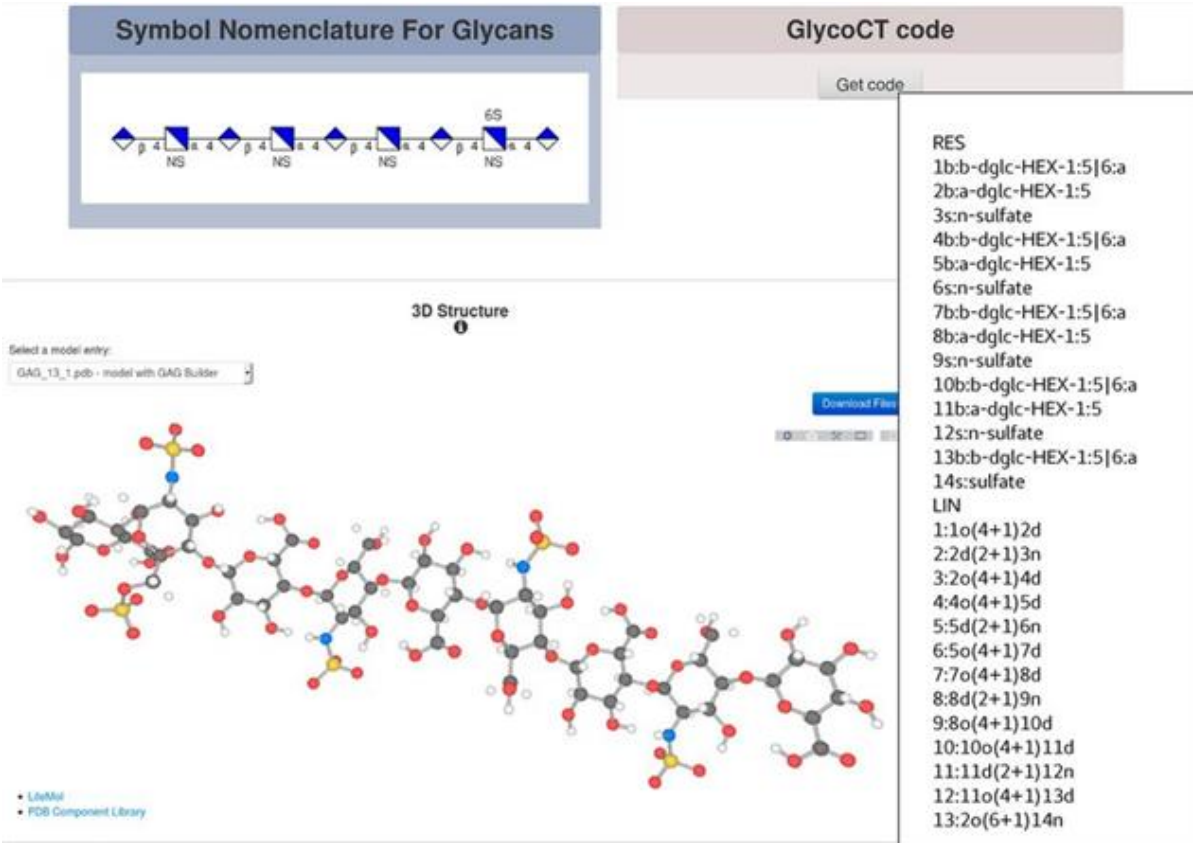

From sequence to 3D structure

OL9.1.1

Extending the Chemical Space of Cazymes: How and Why?

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Standard protein engineering usually involves the substitution of one or more amino acids by other amino acids chosen from the remaining nineteen common alternatives. While this approach has a proven track record, it is nevertheless extremely limited by the chemical space offered by the 20 canonical amino acids.
Over the last 25 years, techniques have been developed to site specifically introduce non-canonical amino acids (ncAA) into proteins, while using the existing genetic code [1,2]. Advantageously, these approaches provide a means to explore chemical space, create new catalytic opportunities and perform bioorthogonal conjugate reactions or assemblies.
Despite the increasing use of ncAA, this approach has rarely been used to engineer carbohydrate-active enzymes (CAZymes). In work aimed at extending the chemical space of CAZymes, we are studying the production of nCAA-bearing glycoside hydrolases and carbohydrate-binding modules [3]. In this presentation, we will describe current progress towards this goal, focussing both on the methodological aspects and on prospects for the future creation of artificial enzymes displaying new functions or architectures.

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OL9.1.2

Ph-Responsive Gcase Inhibitors as Potential Pharmacological Chaperones for Gaucher Disease

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Many genetic diseases are caused by mutations that result in improperly folded proteins, with key examples being the lysosomal storage diseases (LSDs), each one of them due to the deficiency of an enzyme involved in the stepwise degradation of glycolipids or glycosaminoglycans in the lysosome. In these cases, degradation stops and the residual macromolecule remains trapped within the cell [1]. Gaucher disease, the most prevalent of these rare diseases (ca. 1/40000 births), is a consequence of missense mutations in the encoding gene GBA1, ultimately causing insufficient glucocerebrosidase (GCase) activity [2]. This enzyme is a retaining beta-glucosidase responsible for catalyzing the last step in the degradation of glycosphingolipids [3]. The work proposed here represents an alternative to the currently employed enzyme replacement therapy (ERT), and is based on the use of small molecules that specifically bind the functional form of the enzyme, compensating for the destabilizing effects of the mutation, thus stabilizing the folded form. This then allows more enzyme to avoid endoplasmic reticulum-associated degradation and travel to the lysosome, where it can degrade its substrate. This is the basis of the enzyme enhancement therapy (EET), which has the potential for being much less expensive than ERT and for treating neuronal forms of the disease [4].
Recently, the Withers lab reported on a library of iminoxylitol derivatives as potent inhibitors and chaperones of GCase. The synthetic strategy relied on a common iminosugar-alkene precursor (IMX), prepared from D-xylose in seven synthetic steps, from which 16 dideoxyiminoxylitols bearing various different lipophilic substituents were generated in the last step via a divergent thiol-ene coupling. Enzyme kinetic analyses revealed that a number of these products were potent, low-nanomolar inhibitors of human GCase that stabilize the enzyme to thermal denaturation by up to 20 ºC. Moreover, cell-based assays conducted on Gaucher disease patient-derived fibroblasts demonstrated that administration of the compounds can increase lysosomal GCase activity levels by therapeutically relevant amounts [5]. Interestingly, NMR studies revealed that the conformation of these iminoxylitols in solution depends on the pH value, and that a proper 5C2 conformation, mimicking the 4C1 conformation of beta-glucopyranose, is only adopted at high pH values (free amine), while the opposite chair conformation (2C5), displaying most of its substituents in an axial configuration, is prevalent at low pH (protonated amine). This conformational change is related to the amine pKa, and therefore engineering of its basicity allowed the formation of second generation iminoxylitols with pKa values in the range of 7.0-4.5, thus optimizing inhibition towards GCase at the physiological conditions of the ER, but showing lower affinity at the more acidic enviroment of the lysosome. In this communication, I shall present progress in this direction, as well as supporting in vitro and in vivo results.

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OL9.2.1

Imidazolium-Based Tags as Probes to Harness Glycosyltransferase Activity in Human Breast Milk

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Carbohydrates have become important targets for novel therapeutics[1], due to their diversity in nature and their involvement in a myriad of biological processes, such as cell-cell communication, fertilisation, immunity to pathogenic attack.[2] However only limited carbohydrate-based drugs have been developed, mainly due to the lack of efficient methods to obtain structurally defined samples by either chemical synthesis or isolation, for biological screening.

Our group has developed imidazolium-based Tags (ITags) for the chemical and enzymatic synthesis of oligosaccharide whereby the ITag serves a dual purpose as soluble purification support and as mass spectrometry reporter which eliminates the need for expensive radioactive or fluorescent probes, and to facilitate product isolation, as a purification handle.[3,4] (see Figure 1) These ITags have been shown to be compatible with several glycosyltransferases.
Herein, we report the synthesis of a new class of ITag labels, which can be used to harness the natural biosynthetic machinery present in human breast milk (i.e. glycosyltransferases) to access biologically important oligosaccharides.

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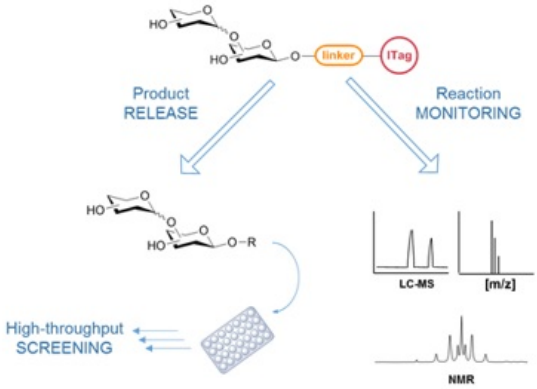

Figure 1. General scheme for ITag-supported synthesis and reaction monitoring[3,4]

OL9.2.2

Hen Egg-White Lysozyme Engineering for the Synthesis of Chitinoligosaccharides

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Chitinoligosaccharides (COs), β-1,4-linked oligomers of N-acetylglucosamine, are an important class of signaling molecules involved in plant-biosphere cell-cell interactions. COs having a degree of polymerisation (DP) from 6 to 8 are potent inducers of immune responses in rice and wheat among others [1]. Despite their biological interest and potential agronomical usefulness, COs with well-defined structure remain poorly accessible. While chemical or enzymatic degradation of chitin allows the production of COs of DP 2 to 6, higher oligomers are hardly accessible by depolymerisation methodologies. Therefore, enzymatic synthesis of COs has been a matter of research by exploiting the transglycosylation activity of retaining glycoside hydrolases (GH).
In the present work, we report the engineering and expression of Hen Egg-White Lysozyme (HEWL, GH-22) in the methylotrophic yeast Pichia pastoris. Site-directed mutagenesis on the essential aspartate 52 [2] was carried out, three mutants devoid of hydrolytic activity were produced and one of them (D52S) displayed an efficient glycosynthase activity. Polycondensation reactions of α-chitintriosyl fluoride led to the formation of COs up to DP 15. Afterwards, we took advantage that a de-N-acetylated oligomer at the non-reducing end cannot behave as an acceptor for HEWL (+1 subsite does not accept a glucosaminyl residue). In a one-pot sequential procedure, the donor was first specifically de-N-acetylated at the non-reducing end by the action of Nod B chitin deacetylase, and then condensed on COs acceptors with mutant HEWL to give single addition products with size varying from hexa- to octamer.

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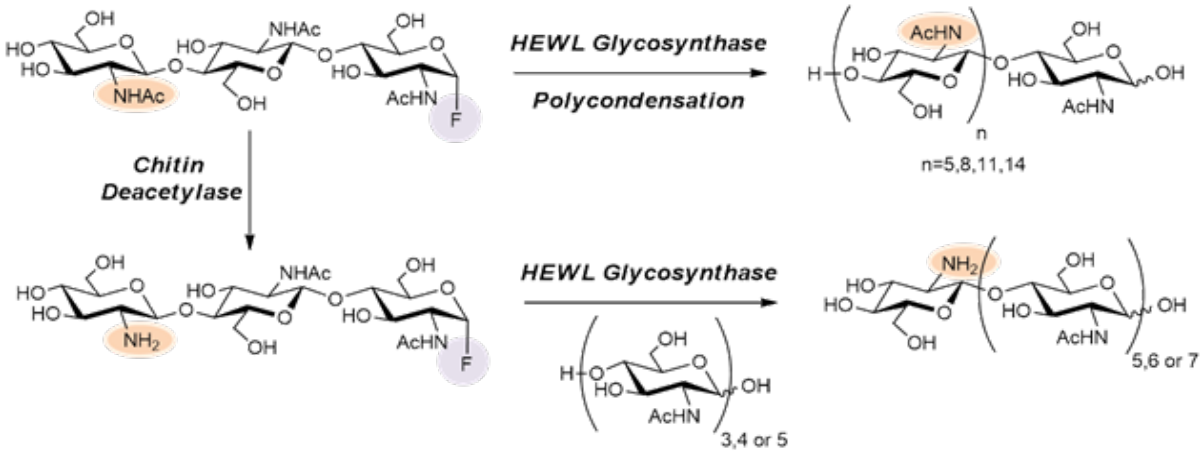

Figure 1. Synthesis of long chitinoligosaccharides by HEWL glycosynthase.

OL9.3.1

Programmable Intermediate-Controlled Sialylation Reactions

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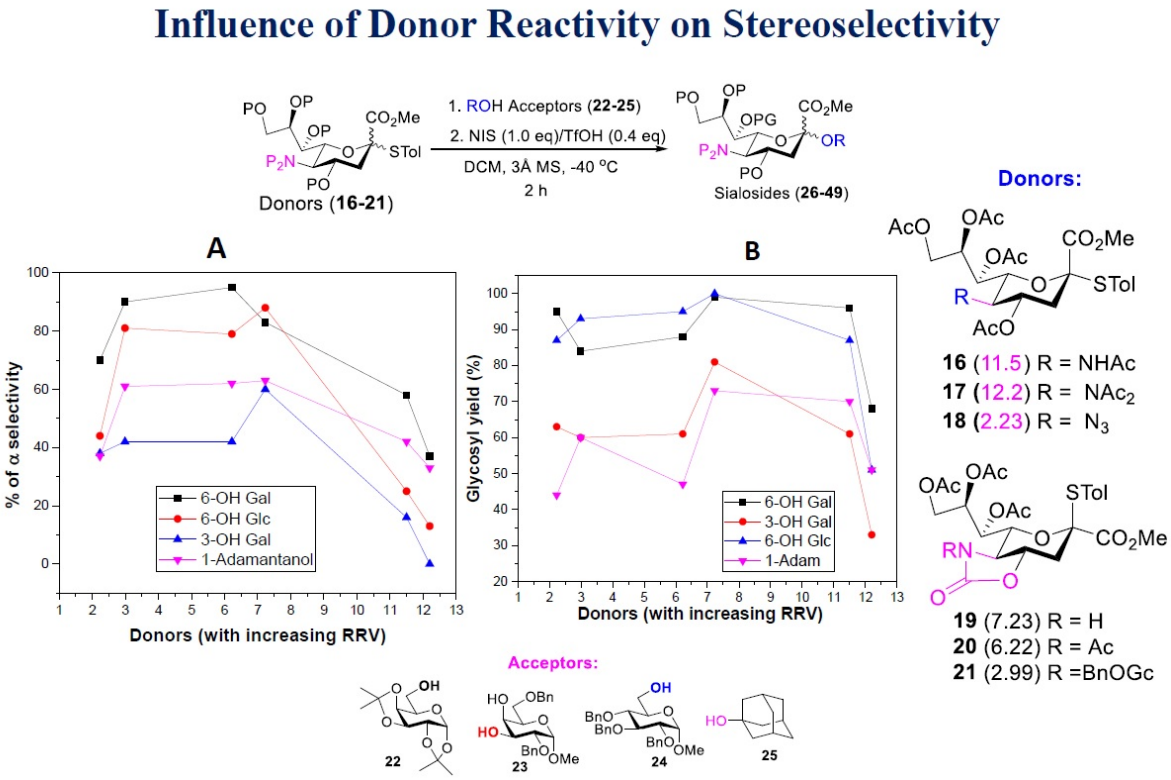
Although tremendous efforts have been made for the efficient preparation of natural sialosides, controlling the stereochemical outcome of sialylation is still the most challenging task. Herein, we developed a new strategy performing high stereoselectivity and yield of O-sialylation on several p-tolyl thiosialosides in NIS/TfOH system by using their corresponding RRV as an indicator. In the reaction mechanism, we further confirmed that unexpected glycosyl halide intermediates were generated with the participation of halogens by using low temperature NMR technique, which greatly enhanced the α-selectivity. Eventually, a precise mechanism was proposed with numerous crucial factors including donor functionalities, acceptors, promoters (NXS, X = Cl, Br, I), temperature, and activation time.



OL9.3.2

Nano- and Mesoscale Structuring in Reaction Solutions: Possibilities to Modulate the Outcome of Glycosylation and Other Reactions with Carbohydrate Derivatives

Leonid Kononov[1], Anna Orlova[1], Elena Stepanova[2], Elena Kononova[3], Daniil Ahiadorme[1], Polina Abronina[1], Ilya Myachin[1], Nikolay Kondakov[1], Oleg Segida[1], Anatoly Filippov[1]

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It is currently well established [1-4] that most aqueous and non-aqueous macroscopically homogeneous solutions of various low-molecular mass substances can be structured at nano- and mesoscale level (the size of inhomogeneities, aka supramers [4], ranges from ca. 1 nm to 10^2–10^3 nm). This novel type of very subtle (if judged by the energies involved, which are comparable with kT or even lower [2]) but spontaneous and powerful structuring of liquids was overlooked for a long time by researchers, and only very recently its importance for chemical reactions has been found and emphasized [4, 5].
We have been developing an approach, which explicitly accounts for the structure of a reaction solution and is based on the hypothesis that in many cases the real reactive species in solution are non-covalently-bonded supramolecular aggregates, supramers [4], rather than isolated molecules of reagents. According to the supramer approach, molecular structure and reaction conditions determine the structure of the resulting supramers, hence their chemical properties. An overview of the published and novel data will be presented, which suggests that the reaction solution structure cannot be ignored when analyzing the results of glycosylation and other reactions with carbohydrate derivatives. For example, a change in the quality of reaction solvent can (1) lead to substantial changes in density of supramers hence to dramatic reduction of reactivity of a glycosyl donor upon dilution [6] or (2) promote an efficient pyranose-to-furanose ring contraction [7]. An abrupt change in the structure of supramers of glycosyl donor, which occurs at the “critical” concentration, can lead to a switch of glycosylation pathway [8] resulting in dramatic changes in reaction time and stereoselectivity [9, 10]. Protective groups pattern is also capable of influencing the structure hence properties of suparmers. N-Acetyl- and N,N-diacetylsialyl chlorides form supramers differing in size and density, which results in dramatic differences in their reactivity [11]. Apparent nucleophilicity of glycosyl acceptors can be influenced by groups remote from the reacting hydroxy group but capable of influencing the structure of reaction solution [12].
This work was supported by the Russian Science Foundation (Project No. 16-13-10244-P).

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OL9.4.1

Towards the Synthesis of Specific Heparan Sulfate Sequences

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Sulfated polysaccharides such as heparan sulfates (HS) have many roles in mammalian cells. One of these roles is as factors in cell signalling cascades, regulating processes including haemostasis and angiogenesis[1]. They are often found as heparan sulfate proteoglycans (HSPG) on the outer surface of a cell, and present in the extracellular matrix[2]. Their ubiquity and specificity make them potential pharmaceutical targets, but there has been relatively limited development in this space. This is primarily a result of synthetic challenges in accessing specific HS sequences, as such a synthesis requires absolute regio- and stereospecific control in reactions.

In this presentation, we will discuss synthetic strategies used to access novel polysaccharides with specific functionality. A major factor in our plan is the use of disaccharide building blocks with a robust, orthogonal protecting group strategy[3]. Following glycosylation of disaccharide building blocks to the required length, these protecting groups allow access to specific sites on the polysaccharide for functionalisation. This includes the recent incorporation of the acetoxymethyl benzoate (AMB) protecting group, allowing access to a new position of interest within our existing strategy.

We will discuss some of the successes and challenges encountered during our synthetic approach, and the recent adaption of AMB to access new positions for sulfation.

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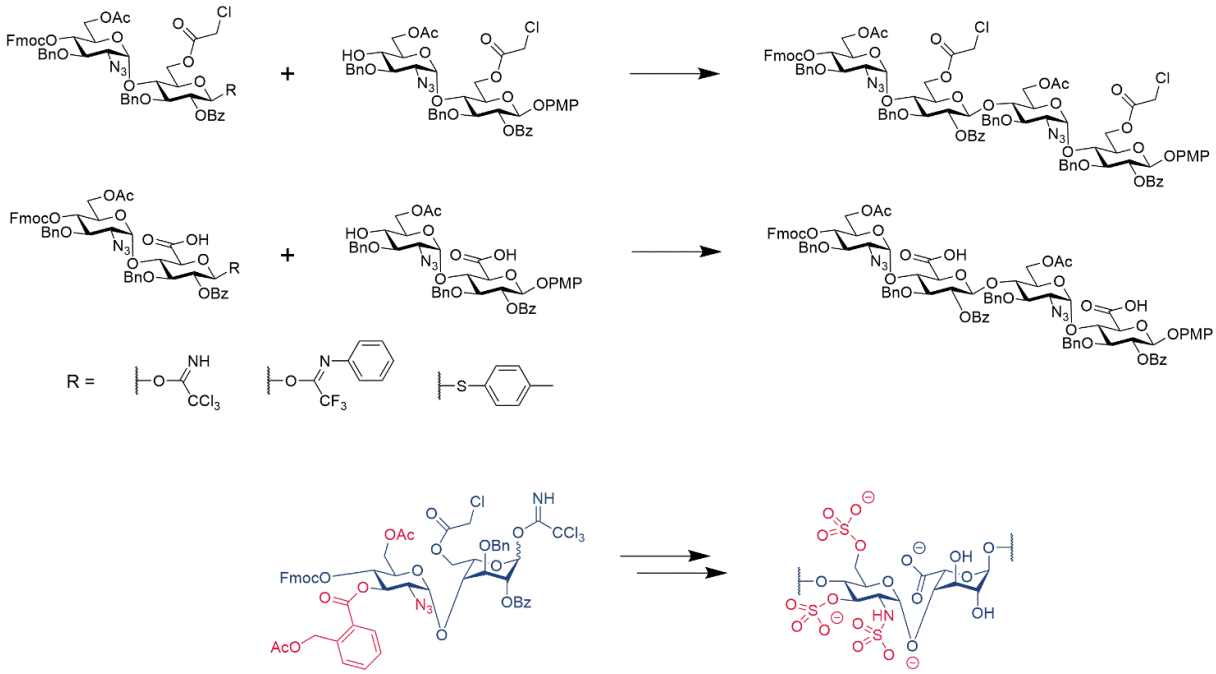

A figure showing the use of an orthogonal protecting group strategy on disaccharide building blocks to generate specific sulfated oligosaccharide sequences.

OL9.4.2

Highly Efficient and Controllable Method for Sulfation of Complex Carbohydrates

Chih-Wei Chang[1], Rajaratnam Premraj[1], Paul D. Madge[1], Robin J. Thomson[1], Mark von Itzstein[1]

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Sulfated glycosaminoglycans (GAGs) and polysaccharides are important biological molecules, a number of which, for example heparin, Fondaparinux Sodium (ARIXTRA®) and Pentosan Polysulfate Sodium (ELMIRON®), have been in clinical use for decades. A variety of sulfated compounds based on new scaffolds have also been recently developed for treatment of various diseases [1,2]. A key step in the preparation of homogeneous sulfated carbohydrates is efficient, reproducible and scalable chemical O- and N-sulfation method. A significant difficulty that arises during attempts to sulfate polyfunctional substrates using conventional approaches is incomplete conversion. Therefore, a reliable and more controllable sulfation protocol is an unmet need for synthetic chemists.
In this presentation we describe a new chemical sulfation method, developed to eliminate this synthetic bottleneck in GAG synthesis. First, we identified that the dipolar solvent, N,N-dimethylformamide (DMF), widely used as a bulk solvent in O-sulfation reactions, while enabling solubilization of the reactants, also uninstalls the newly-formed sulfate groups on reaction substrates. To investigate this problem, we conducted a mechanistic study using real-time NMR spectroscopy to monitor the progress of sulfation reactions distinguishing between conventional and modified approaches. The intriguing findings from the NMR study led us to establish a new sulfation protocol which can efficiently sulfate a wide range of complex carbohydrate substrates with reproducible full conversion. This newly-developed sulfation method both attenuates adverse side reactions and prevents loss of sulfate groups potentially initiated by dipolar solvents throughout the course of reaction, work-up and purification.
The new sulfation method has been successfully applied to O- and N-sulfation of a wide range of substrates [3]. Among these, a library of discrete homogeneous heparan sulfate fragments ranging from mono to octa-saccharides was prepared, which has been exploited to uncover the specific binding interactions of pure GAG fragments with proteins and in metallo-organic complexes [4,5,6]. We also successfully extended this new protocol to sulfate a polyol substrate on a scale of 232 grams in the academic lab and transferred the technology to GMP production (7 kg scale) of a lead drug candidate currently in a human clinical trial.
Fig. Examples of O- and N-sulfation

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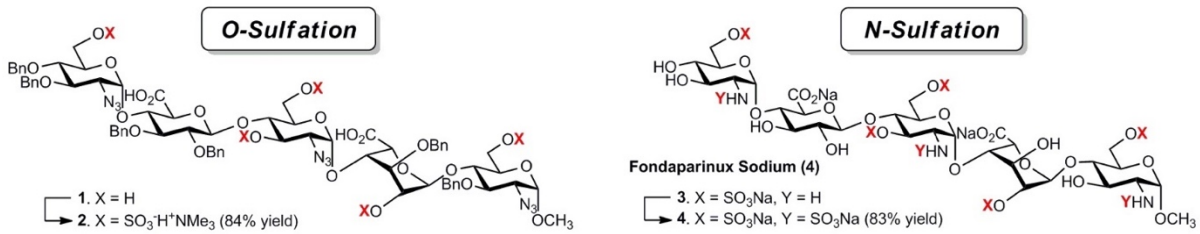

Fig. Examples of O- and N-sulfation

OL10.1.1

Structure of the O-Repeating Unit and the Lipid A Isolated From Fusobacterium Nucleatum Atcc 51191

Pilar Garcia-Vello[1], Dimitra Lamprinaki[2], Flaviana Di Lorenzo[1], Antonio Molinaro[1], Nathalie Juge[2], Cristina De Castro[3]

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INTRODUCTION: Fusobacterium nucleatum is a common member of the oral microbiota [1]. However, this symbiont has been found to cause opportunistic infections such as periodontal diseases and has been implicated in adverse pregnancy outcomes such as preeclampsia, gastrointestinal disorders such as colorectal cancer or appendicitis, as well as cardiovascular diseases, rheumatoid arthritis, respiratory tract infections, Lemierre's syndrome and Alzheimer's disease [2]. F. nucleatum has virulence mechanisms that lead to infections outside the mouth [3].
As a Gram-negative bacterium, F. nucleatum has an outer membrane layer protecting the bacterium in different environments and its external face is composed mainly by lipopolysaccharides (LPS) [4]. LPS are microbe associated molecular pattern (MAMP) molecules that play a crucial role in the interaction of the host with pathogens but also commensal bacteria as they can be recognized by the innate immunity response activating the pattern recognition receptors (PRR) [5]. The dual commensal-pathogen behaviour of F. nucleatum makes the determination of the LPS structure of this bacterium especially interesting.
METHODS: The structure of O-antigen and lipid A from F. nucleatum ssp. animalis (ATCC 51191) was characterized using a combination of Gas Chromatography - Mass Spectrum (GC-MS) derivatization, Matrix-Assisted Laser Desorption/Ionization (MALDI) and Nuclear Magnetic Resonance (NMR) analytical techniques.
RESULTS: Analysis of the NMR disclosed the presence of a repeating unit composed by three monosaccharides, apparently in contrast with the presence in the proton spectrum of about six signals in the anomeric region with no stoichiometric proportions. Analysis of the connectivity by combining COSY, TOCSY and NOESY spectra established that three different sugars were present: β-GlcpNAcA, β-GlcpNAc3NAlaA and α-FucpNAc4NAc. Importantly, the amino function at position 4 of α-FucpNAc4NAc is acetylated in a not stoichiometric fashion: depending on the presence of the acetyl, the chemical shifts of all signals change, giving a spectrum with six anomeric signals. Also, the Lipid A was analysed by MALDI.
CONCLUSIONS: The LPS structure of F. nucleatum ATCC 51191 displays great complexity due to the high level of amino functions, the partial acetylation of one of them and the presence of an amino acid, Ala. More research is necessary to understand the role that this structure plays in the interaction of this bacterium with host in the context of health and disease.

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OL10.1.2

Synthetic 1,1′-Linked Disaccharide – Based Glycolipids for Immunomodulation

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Immunomodulation is a promising strategy for therapy of acute and chronic diseases ranging from antibiotic-resistant infections to autoimmune disorders and cancer. We aim at coordinating the immune response to infection and chronic inflammation by targeting the lipopolysaccharide (LPS)-sensing innate immune receptors Toll-like receptor 4 (TLR4) and caspase-4 (as well as its murine homolog caspase-11). The transmembrane TLR4 complex and the cytosolic cysteine protease caspase-4 are responsible for propagating the immediate immune response to Gram-negative infection which is aimed at bacterial clearance. Dysregulated TLR4 and caspase-4 signaling is involved in the pathogenesis of numerous chronic and acute inflammatory disorders; besides, activation of TLR4 and caspase-4/11 plays the central role in the initiation and pathophysiology of sepsis syndrome.[1-3] Sepsis is increasingly reputed as the final common pathway to death from infection and remains the leading cause of mortality in intensive care units. Recent studies revealed that the deficiency of the TLR4-driven inflammation, rather than inefficient pathogen clearance, is responsible for the severity of Gram-negative sepsis. It has been also recognized that the progression of many chronic and autoimmune diseases is related to non-resolving inflammation, which underscores TLR4 and caspase-4 directed immunotherapy as promising approach for treatment of variety of disorders.

Using crystal-structure based design we developed unique 1,1'-linked disaccharide based glycolipids as TLR4 and caspase-4/11 ligands with pico- to nanomolar affinity for respective innate immune receptors. We synthesized a library of anionic glycolipids derived from β,α-1,1′-, α,α-1,1′ and β,β-1,1′ - linked disaccharide scaffolds which can either potently induce the TLR4-mediated intracellular signaling, or inhibit the TLR4- and/or caspase-4/11 mediated inflammation and fully block the endotoxic action of LPS. We found that the specific 3D-molecular shape of the non-reducing disaccharide scaffold (which is inflicted by the anomeric configuration around the rigid 1,1′-glycosidic linkage: αα, ββ, or βα) is decisive for the expression of agonist or antagonist activities.[4,5]

The chemistry behind the synthesis of 1,1′-linked disaccharide-derived innate immune modulators involves the challenging stereoselective 1,1′-glycosylation to attain fully orthogonally protected non-symmetrical disaccharide scaffolds having β,α-1,1′-, α,α-1,1′ or β,β-1,1′ configuration. Matching the reactivities of glycosyl donor and acceptor pairs by varying the protecting group pattern and employing conformationally locked hemiacetal-acceptors was decisive for achieving high glycosylation stereoselectivity and yields. The disaccharide scaffolds were subsequently decorated by phosphate groups and long-chain β-hydroxy-, β-acyloxyacyl or β-keto- lipid residues of variable length.

Manipulating the 3D-structure and the phosphorylation status of the disaccharide-derived anionic glycolipids allowed for discovery of first caspase-4/11 antagonists as potential anti-sepsis drug candidates and for development of powerful inducers of the TLR4 signaling and caspase-4/11 activation as potential immunotherapeutics and vaccine adjuvants.

Financial support by Austrian Science Fund (Grant FWF P-28915) is gratefully acknowledged.

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OL10.1.3

Investigation of 4-Amino-4-Deoxy-L-Arabinose Transferases from Gram-Negative Bacteria Involved in Antibiotic Resistance

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Modification of bacterial lipopolysaccharide (LPS) by addition of 4-Amino-4-deoxy-L-arabinose (Ara4N) to lipid A and Kdo/Ko inner core units has been implicated as a major cause of antibiotic resistance in Gram-negative bacteria due to the masking of anionic charges.[1-3] 4-Amino-4-deoxy-L-arabinose transferases (ArnT) for which functional and structural information is still scarce, utilize α-Ara4N undecaprenylphosphate as donor substrate. In order to study the enzyme mechanism and explore substrate inhibition, a series of both anomeric forms of Ara4N-phosphodiester linked derivatives have been synthesized. First, TIPDS-protected hemiacetal were converted into a separable mixture of the corresponding α and β H-phosphonates[4], followed by formation of the phosphodiester derivatives in fair yields upon PivCl mediated activation and oxidation. Cleavage of the silyl ether and conversion of the 4-azido into the 4-amino group through a Staudinger reduction gave the target compounds containing linear alkyl and isoprenyl lipid extensions (Fig. 1A). Preliminary activity assays using a crude membrane preparation of ArnT and Kdo2-lipid A (KLA) as acceptor and α-L-Ara4N-P-neryl 4α as donor resulted in a robust in vitro conversion of 40% of the Kdo2-lipid A precursor to mono-substituted Kdo2-lipid A containing a single L-Ara4N unit (Fig. 1B).

Acknowledgment: Financial support from Austrian Science Fund (FWF, grant P28826-N28).

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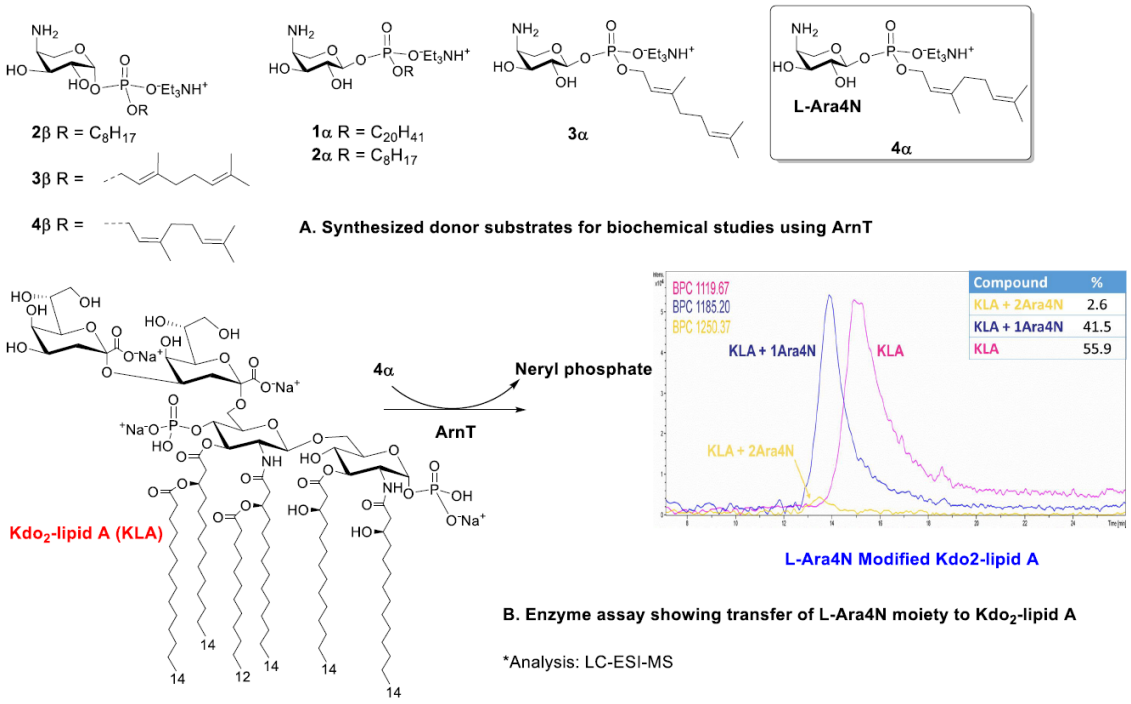

Figure 1. Synthetic L-Ara4N donor substrates (A) and enzymatic transfer of 4α to Kdo2-lipid A (B).

OL10.1.4

Heptose Glycomimetics: Synthesis and Biological Evaluations

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The glycero-D-mannoheptose core structure is exclusively found in bacteria, either with the 6-D configuration as in 1 or with a 6-L configuration as in ADP-heptose 3. Nucleotide-sugar 3 is the substrate of bacterial heptosyltransferases that play a key role in the biosynthesis of Lipopolysaccharide (LPS). From a medical prospective this bacterial biosynthetic pathway is important because: 1) the inhibition of the heptose processing enzymes leads to a dramatic phenotypic change that blocks the virulence of some major pathogenic gram-negative bacteria 2) It has been recently shown that heptose metabolites such as 1-3 can activate the innate immune response through a TIFA-dependent pathway.[1]
Based on the biological relevance of the bacterial heptosides, our laboratory has synthesized different types of heptose glycomimetics illustrated in Figure 1: multivalent species based on a glycofullerene core structure 4,[2] highly fluorinated D-heptoside 5 and heptosyltransferase inhibitor 6.[3] Novel heptose scaffolds are now available in our laboratory and their biological evaluations will be discussed.

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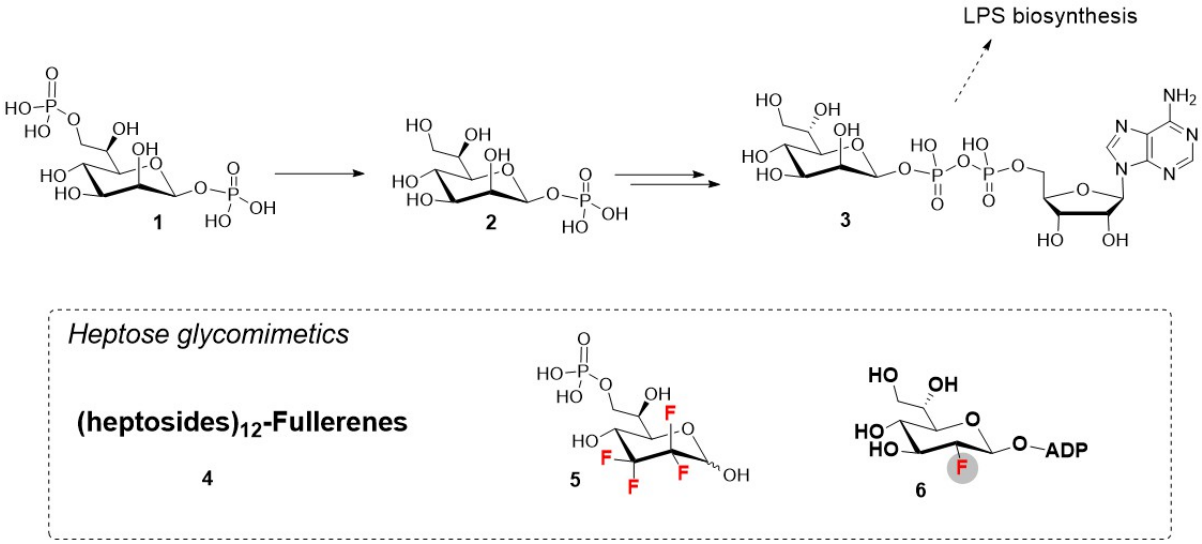

Figure 1. Natural bacterial heposides 1-3 and synthetic heptose analogues 4-6.

OL10.2.1

Structure of A Glycomimetic/DC-SIGN Complex Highlights Potency and Selectivity Improvements Designed by Fragment-Based Virtual Screening

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C-type Lectin Receptors (CLRs) are carbohydrate-binding proteins expressed on the surface of Dendritic Cells (DCs). They recognize pathogens or damaged cells by interacting with glycan features. The encounter between the CLR and its ligand is the necessary step for the activation of the adaptive immune system and, therefore, CLRs are attractive targets to tailor immunity response. Molecules selective to each individual CLR have to be developed and one approach is based on the synthesis of glycomimetics that mimic the natural sugar.
Recently, our group has addressed the question of the overlapping affinity between DC-SIGN and langerin, in the context of HIV recognition. A rational design approach was exploited and used to selectively target DC-SIGN in disfavour to langerin [1].
A deeper affinity towards DC-SIGN was sought. We used fragment-based virtual screening approach to identify interesting moieties that could positively interact with the protein in the vicinity of the primary binding site. The best fragment candidate combines the presence of an ammonium ion and an aromatic ring. On the basis of these fragments, new glycomimetics, incorporating these feature into previously first generation compounds, were produced and tested toward DC-SIGN and Langerin CLRs. Thanks to SPR competition assay and/or ITC measurements, one compound in particular, called Man69, have been identified as really promising. SPR analysis revealed an IC50 of 80 µM, with a selective inhibition of DC-SIGN and ITC experiments confirmed the µM range of the interaction (Kd of 57 µM). Finally, the crystal structure of DC-SIGN co-crystallized with Man069 was obtained, highlighting an unexpected and unprecedented binding mode that will be presented and analysed in detail. Analysis, by Analytical ultracentrifugation, of the interaction between Man69 and DC-SIGN in solution allowed to fully understand the binding properties and to state on the properties observed within the crystal structure.
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OL10.2.2

Synthesis of B-Lewis B to Investigate Binding Preferences of Helicobacter Pylori

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Helicobacter pylori, a Gram-negative bacterium found in the stomach, is a major cause of gastritis, peptic ulcers and stomach cancer.[1] The bacterium uses a membrane-bound lectin, Blood-group Antigen Binding Adhesin (BabA), to bind to Lewis b structures displayed on the surfaces of host cells.[2] By varying the pH of the environment of BabA, it was possible to change its binding preferences towards Lewis b structures.[3] Multivalent presentations of the Lewis b hexasaccharide, along with A- and B-Lewis b, will allow variable pH studies to be performed and determine how the binding preferences of BabA change.

A linear synthesis of B-Lewis b will be presented. Preparation of the heptasaccharide commenced with a 3'-OH lactoside acceptor, obtained via reductive ring-opening of a 3',4'-endo-O-benzylidene acetal. NIS/AgOTf-promoted glycosylations yielded the desired 1,2-trans linkages while Lemieux's halide-assisted glycosylation conditions were used to install the 1,2-cis linkages.[4] Upon global deprotection by Pd-catalysed hydrogenolysis, isothiocyanate methodology was employed to facilitate conjugation of the glycan to Bovine Serum Albumin (BSA).

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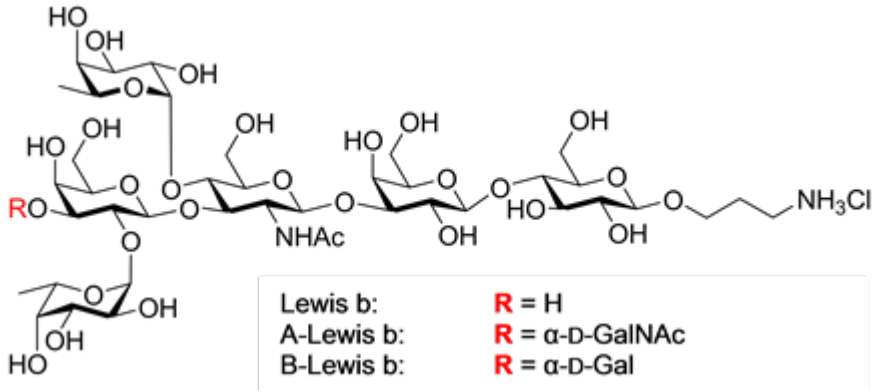

Figure 1 – Lewis b structures attached to an amino-propyl hydrochloride spacer.

OL10.2.3

NMR Studies of the Specific Interaction of Dc-Sign with the Blood Group A/B Antigens

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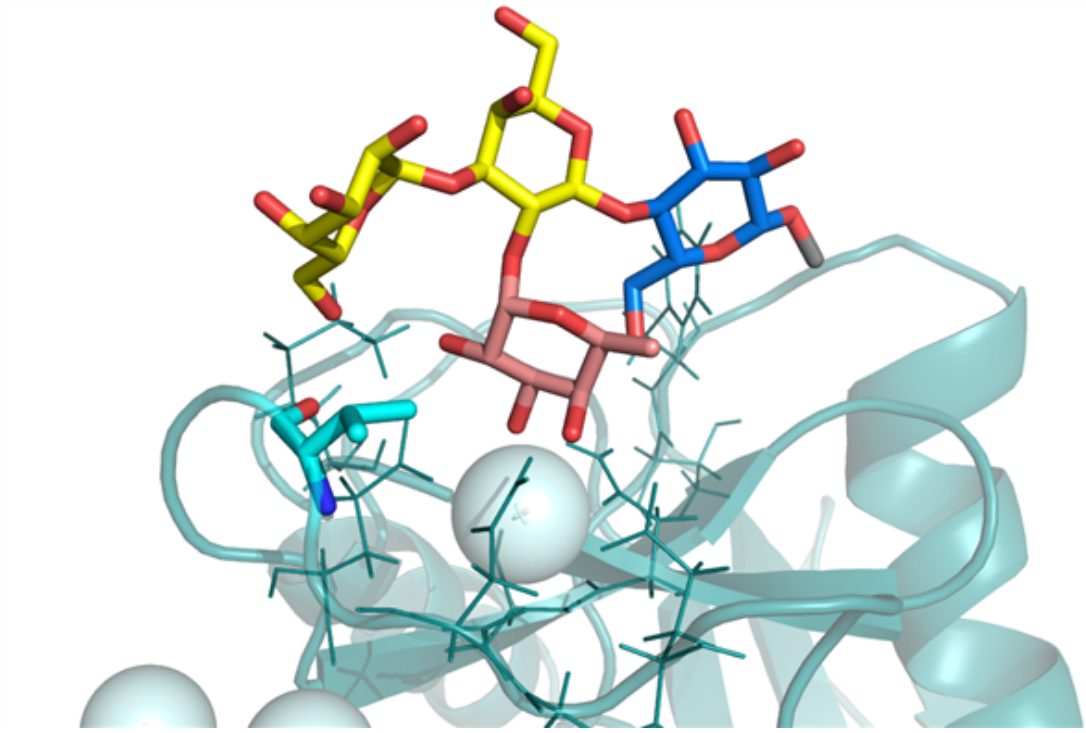
The DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin) protein is a key receptor of the C-type lectin receptor (CLR) family. This human lectin acts as a pathogen recognition receptor (PRR) through the specific interaction with complex oligosaccharides distributed on the pathogen surface, via its calcium-dependent binding site. At the same time, its role in the immune modulation and homeostasis has been also described by means of specific targeting of endogenous glycoproteins like ICAM-3.1
The broad specificity in binding is one particular feature of this lectin. Previous glycan array data2,3 agree with the existence of two main ligands for DCSIGN: L-fucose (Fuc) and D-mannose (Man). Fuc is commonly found at the terminal positions of Lewis-type and ABH-type antigens in mammalian cells, while Man is the major component of the highly branched oligosaccharides attached to membrane glycoproteins in different pathogens, as gp120 in HIV. In addition, these studies also speculated on how glycan presentation and subtle structural differences between similar Fuc- or Man-containing motifs might tune or modify the binding affinities4. In this regard, the interaction details at a molecular level, especially for the endogenous Fuc-containing partners, remain rather unexplored.
We have unraveled the key structural details of the molecular recognition event of the A- and B- histo blood group antigens by DC-SIGN. We have employed a variety of NMR methods assisted by molecular modeling protocols to define the binding features of these antigens to the carbohydrate recognition domain of DC-SIGN. This methodology has allowed us to propose a well-defined binding model for both blood groups. These results provide the initial milestones for forthcoming studies with other ligands, including longer and branched saccharides.



Proposed binding pose for the Blood Group B type VI tetrasaccharide interacting with DC-SIGN.

OL10.2.4

Exploring Bacterial Lectin Recognition Events of Synthetic Mucin Glycopeptide Ligands

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Mucins are densely glycosylated proteins that populate the cell-surface of epithelial tissues.[1] The extracellular tandem repeat peptide regions rich on proline, threonine and serine residues characterize the mucins. By display of O-glycans often organized in a multivalent fashion, the mucins and mucin like glycoproteins are involved in a plethora of cell-surface binding events.[2] Glycans on mucins often act as ligands for invading pathogens, studies of such interactions are useful for characterization of microbes and viruses as well as to develop new anti-adhesive drugs. By chemical synthesis of well-defined glycan and glycopeptide probes we aim to identify and map the functions of mucins and their interacting binding partners involved in infection processes.

In recent years we have developed efficient total synthesis strategies to construct over 300 different mucin O-glycopeptides modified with short tumor-associated glycan structures and more complex elongated mucin core structures.[3-6] Using enzymes, the elongated core structures were further diversified by fucosylation, sialylation and polyLacNAc. The synthetic glycopeptides have been immobilized on biocompatible hydrogel slides that display the glycopeptides in a multivalent mode. Our recent microarray analysis results evaluating bacterial lectin recognition of mucin glycopeptide epitopes will be presented at Eurocarb.

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OL10.3.1

Cello-Oligosaccharide Synthesis with Degree of Polymerization Control Using Reverse Phosphorolysis by Coupled Phosphorylases

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Soluble cellodextrins (linear β-1,4-D-gluco-oligosaccharides) have interesting applications as ingredients for human and animal nutrition. Their bottom-up synthesis from glucose is promising for bulk production, but to ensure a completely water-soluble product via degree of polymerization (DP) control (DP ≤ 6) presents a challenge. Here, we show controlled polymerization of cellodextrins with DP centered at 3 - 5 (~95 wt.% of total product) from glucose and α-glucose 1-phosphate (αGlc1-P) using coupled cellobiose and cellodextrin phosphorylase. For the purpose of efficient synthesis, kinetic and thermodynamic restrictions upon αGlc1-P utilization were overcome (from 53% to ≥ 90% conversion) by in situ removal of the phosphate released via precipitation with Mg2+. The product DP was controlled by molar ratio of glucose/αGlc1-P (~0.25; 50 mM glucose) used in the reaction. As result, the yield of soluble cellodextrins was 3.3 g/g glucose attained at 30.0 g/L total product concentration and 97% theoretical utilization of the substrates used. In addition, completely insoluble cellodextrins (DP ~10) were synthesized by keeping the glucose concentration low (1 - 10 mM; 200 mM αGlc1-P). In summary, this study provides the basis for an efficient and product DP-controlled biocatalytic synthesis of cellodextrins from expedient substrates.

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OL10.3.2

Structural Basis of Glycogen Biosynthesis Regulation in Bacteria

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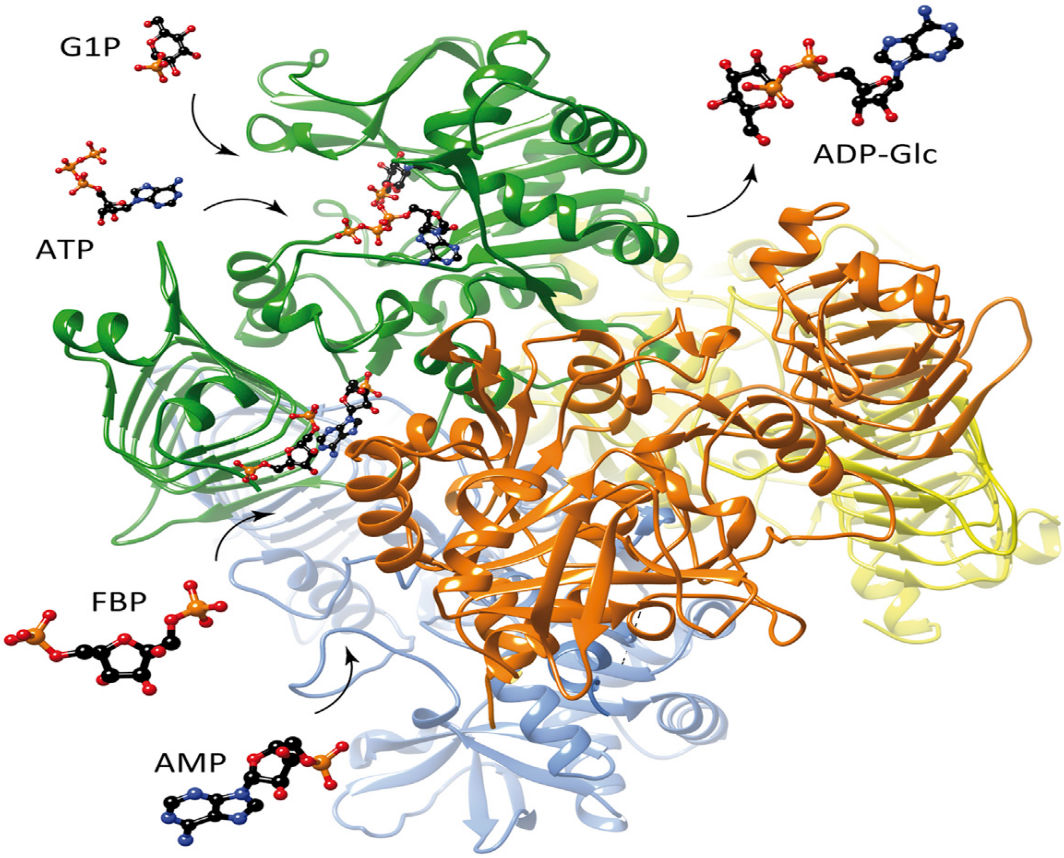
ADP-glucose pyrophosphorylase (AGPase) catalyzes the rate-limiting step of bacterial glycogen and plant starch biosynthesis, the most common carbon storage polysaccharides in nature. A major challenge is to understand how AGPase activity is regulated by metabolites in the energetic flux within the cell.
Here we report the first crystal structures of the paradigmatic homotetrameric AGPase from Escherichia coli in complex with its physiological positive and negative allosteric regulators, fructose-1,6-bisphosphate (FBP) and AMP, and sucrose in the active site.
FBP and AMP bind to partially overlapping sites located in a deep cleft between glycosyltransferase A-like and left-handed beta-helix domains of neighboring protomers, accounting for the fact that sensitivity to inhibition by AMP is modulated by the concentration of the activator FBP. Single point mutations of key residues in the AMP-binding site decrease its inhibitory effect but also clearly abolish the overall AMP-mediated stabilization effect in wild-type EcAGPase. Single point mutations of key residues for FBP binding did not revert the AMP-mediated stabilization. Strikingly, an EcAGPase-R130A mutant displayed a dramatic increase in activity when compared with wild-type EcAGPase, and this increase correlated with a significant increment of glycogen content in vivo.
Altogether, we propose a model in which the energy reporters regulate EcAGPase catalytic activity by intra-protomer interactions and inter-protomer crosstalk, with a sensory motif and two regulatory loops playing a prominent role.



OL10.3.3

Engineering Three GH84 Enzymes into Glycoside Phosphorylases and Understanding the Nature of the Reaction Intermediate

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Carbohydrate active enzymes (CAZymes) include glycoside hydrolases (GH), auxiliary activities, e.g. oxidases (AA), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE) and glycoside phosphorylases. Contrarily to the other enzyme categories, glycoside phosphorylases do not have their own separate classification but are mingling within GT (GT4, 35) and GH (GH3, 13, 65, 94, 112, 130) [1]. However, the conversion of a GH or a GT into a glycoside phosphorylase has yet to be reported. Here, we converted three bacterial GH84s from Streptococcus pyogenes (SpOGA), Oceanicola granulosus (OgOGA) and Thermobaculum terrenum (TtOGA) (sharing 29–33% identity pairwise) into glycoside phosphorylases. While OgOGA and TtOGA variants display both hydrolysis and phosphorolysis, SpOGA became a pure phosphorylase with a reasonable activity having kcat = 2.7 ± 0.2 s–1, which is comparable with the only known natural retaining β-glycoside phosphorylases, that belongs to GH3 [2].

To understand what enabled this conversion, metadynamics QM(DFT)/MM simulations were performed on TtOGA and its variant. Our calculations reproduced the experimental data and showed that the electrostatic potential in the active site is the main driver towards the specificity shift. The computed molecular mechanism of hydrolysis/phosphorylation showed that both mutant and native enzymes feature an oxazolinium ion -and not a neutral oxazoline [3,4]- as an intermediate. Understanding the molecular details of the GH84 mechanism is important for the design of inhibitors against the clinically relevant human GH84. Indeed, GH84 are involved in protein regulation through O-GlcNAc removal on Ser/Thr, a modification that controls protein (in)activation and phosphorylation [5].
Acknowledgements: grants from MINECO and the Novo Nordisk foundation supported this research.

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OL10.3.4

Insight into Substrates Binding in B-1,3 and B-1,4-Glucan Phosphorylases by STD NMR Spectroscopy

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Glycoside phosphorylases (EC 2.4.x.x) are enzymes able to carry out the reversible phosphorolysis of glucan polymers. Importantly, their ability to build up short-to-medium length sugar chains from donor and acceptor substrates makes them powerful biological tools for the synthesis of new sugars with functional groups introduced in a selective way. The synthesis of cellodextrin oligomers from GH94 cellodextrin phosphorylase (CDP, EC 2.4.1.49) involves the elongation of short β-(1→4)-glucans (acceptors) through the addition of glucose units donated from α-D-glucose-1-phosphate (donor).[1] Recently, the first X ray crystal structure of CDP bound to cellotetraose has been published, allowing a better understanding of the enzyme molecular recognition.[2] Previous studies have covered acceptors permissiveness and donor specificity as well, probing the efficiency of CDP to carry out the synthesis of cellulose derivatives. In addition, a new family of β-1,3-phosphorylase was recently identified and named GH149, which is able to synthesise β-(1→3)-glucans oligomers.[3] To be able to exploit the interesting catalytic properties of these enzymes in the synthesis of novel glucan derivatives, a profound understanding of the molecular basis of substrate recognition in solution is still needed for a diverse sets of potential substrates.
In this study, STD NMR experiments for the investigation of the molecular recognition of substrates by β-1,3- and β-1,4-phosphorylases were performed, and binding epitope maps of the different ligands were obtained. In the case of CDP, several donors (α-D-glucose-1-phosphate, α-D-galactose-1-phosphate, α-D-mannose-1-phosphate, etc.) as well as acceptors (glucose, cellobiose, laminaribiose and cellotriose) have been investigated. Moreover, we focused on cellotriose to look into ligand orientation and solvent-accessibility in the binding pocket by average DEEP-STD NMR methodology[4] and LOGSY-titration experiments[5], respectively. Finally, we probed the impact of inorganic phosphate in the ligands binding affinity. The obtained experimental data were compared to molecular docking calculations run both with and without inorganic phosphate, in order to reveal its impact on ligands conformation and orientation. The same study was started for β-1,3- phosphorylase, focusing on the donors and acceptors binding epitope investigation.
The collected data show differences in the protein contacts to the α and β-anomers at the reducing ring, as well as to β-(1→4) and β-(1→3)-glucans and highlights enzyme preferences for binding recognition. Cellobiose and cellotriose β-anomers showed a spread binding epitope all along the ligand, suggesting a more complex binding mode. The ligand orientation, with the non-reducing ring inside the binding pocket, was confirmed through DEEP-STD NMR and the analysis of water exposure. On the other hand, combined analysis with docking calculations unveiled the existence in solution of an unproductive binding mode, where the acceptors enter the binding pocket via the reducing ring. Interestingly, the presence of inorganic phosphate enhances ligand binding affinity and affects the conformation around the inter-glycosidic linkage.
Overall, the combination of STD NMR experiments and molecular modelling calculations has allowed us: first, to obtain insights on enzyme-ligand interactions for small molecules reluctant to co-crystallize, and second, to detect and characterize an additional unproductive binding mode of the enzyme acceptors.

[1] M. Hiraishi et al. / Carbohydrate Research 344 (2009) 2468–2473
[2] O'Neill et al. / Carbohydrate Research 451 (2017) 118-132
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[4] Monaco et al. / Angew. Chem. Int.Ed. (2017), 56, 15289—15293
[5] Geist, L. et al. / J. Med. Chem (2017)., 60, 8708-8715

OL10.4.1

New 3d Printable Glyco-Biomaterials: Synthesis and Characterization of Scaffolds for Advanced 3d Organoids and In Vitro Models

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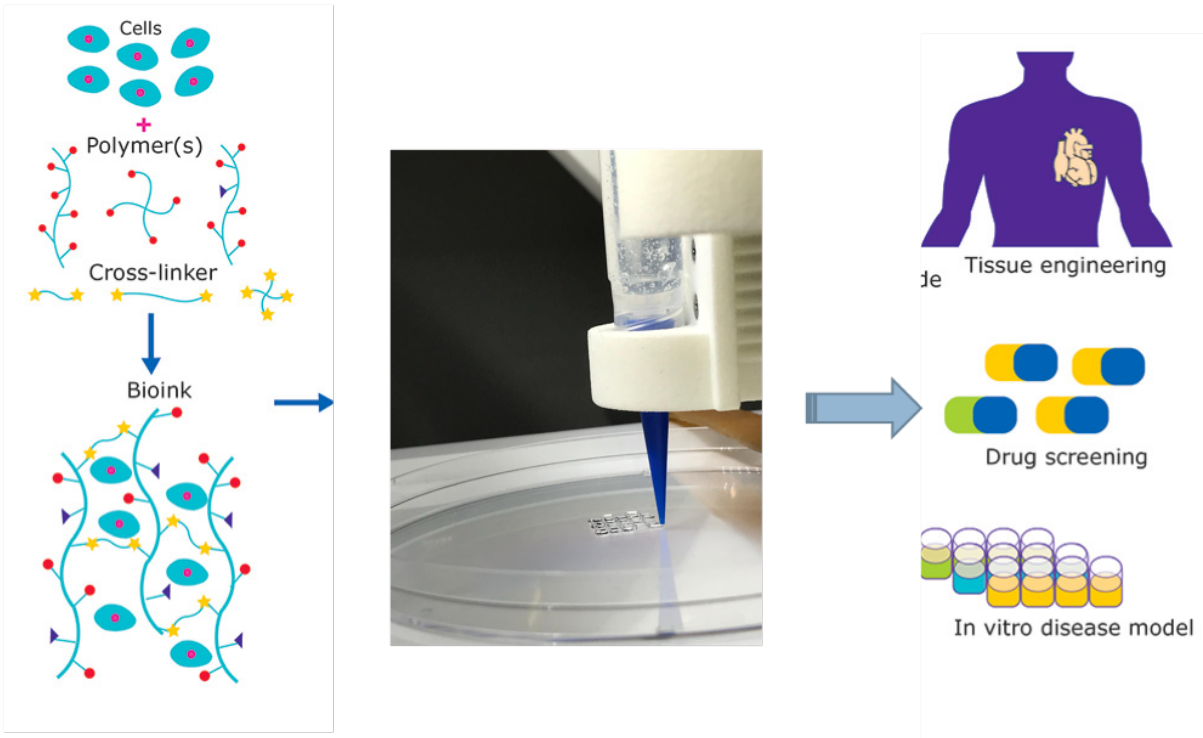
Glycans are modulators of cell-cell and cell-Extracellular matrix (ECM) interactions. The ability to mimic the structural and biochemical role of ECM is one of the way to control specific cell fates, inducing the formation of functional tissues. Both, physical and biochemical motifs of ECM can be employed in order to stimulate organoids formation or tissue constructs. A wide variety of glycans (polysaccharides, glycoproteins and proteoglycans) are involved in cell - microenvironment interactions and finely tune cell fate in physiological and pathological states. Three-dimensional (3D) cell cultures have recently gained attention based on their potential to recapitulate tissue physiology and functions. 3D scaffold supports, cell aggregate systems and hydrogels are good candidates to support the formation of functional tissues and organoids for studying the effects of drugs and bioactive agents on 3D in vitro models. The design of new hybrid glycopolymers that can be formulated as scaffolds or bioprintable materials is of interest in the development of new materials employable in 3D functional in vitro constructs. Herein we present the design, the synthesis and the characterization of new hybrids bioprintable Glycobiomaterials and their evaluation for advanced 3D in vitro cell models.



GlycoBiomaterials for in vitro 3D in vitro models

OL10.4.2

Synthetic Carbohydrate-Based Materials

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Polysaccharides are the most abundant organic materials in nature, yet correlations between their three-dimensional structure and macroscopic properties have not been established. Automated glycan assembly (AGA) enables the preparation of well-defined oligo- and polysaccharides resembling natural as well as unnatural structures [1]. A collection of related compounds, modified at specific positions of the chain, is presented (Fig1). These synthetic glycans are ideal probes for the fundamental study of polysaccharides, shedding light on how the modification patterns affect the polysaccharides properties (i.e. three dimensional shape). Molecular modelling simulations and NMR analysis show that different classes of polysaccharides adopt fundamentally different conformations, drastically altered by single-site substitutions [2].

Moreover, these synthetic oligosaccharides are shown to self-assemble into nanostructures of varying morphologies. Well-defined differences in chain length, monomer modification, and aggregation methods yield glycomaterials with distinct shapes and properties. These novel synthetic materials show unexpected excitation-dependent optical properties in a broad range within the visible spectrum, illustrating their potential for use in optical devices and imaging applications.

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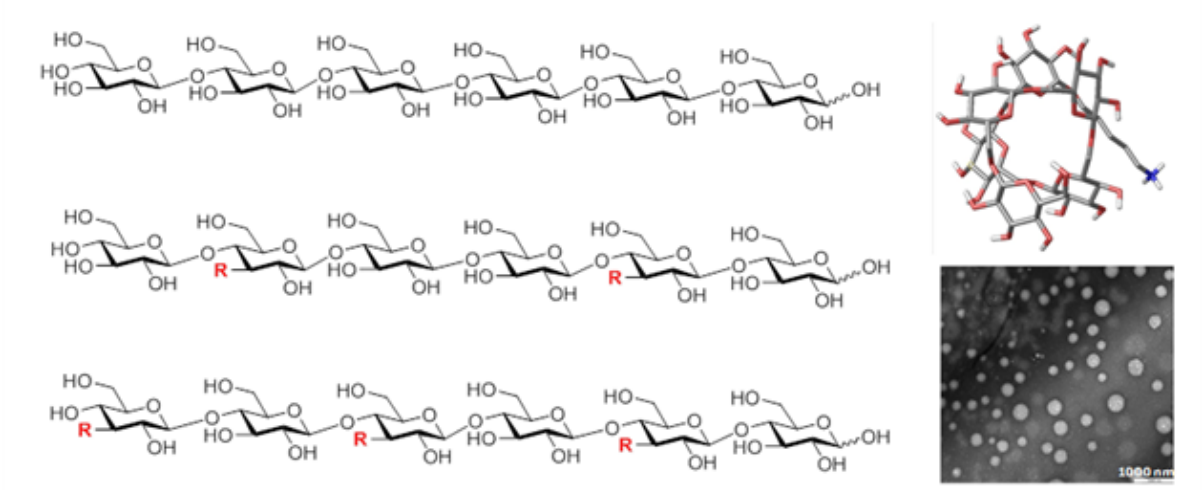

Synthetic cellulose oligomers with different modification patterns. Molecular dynamic simulations and oligosaccharide-based nanoparticles (NPs).

OL10.4.3

Structural Modulations of Laminarin to Improve Biostimulation/Bioprotection Properties

Vincent Ferrieres[1], Laurent Legentil[1], Grégory Lecollinet[2], Sophie Trouvelot[3], Xavier Daire[3], Franck Paris[1]

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It is well known that β-(1→3)-glucans are able to enhance immune defense in mammals, so that these natural polysaccharides find applications for instance in immunotherapy to fight cancers. Rationalization of the observed effects comes from the discovery of receptors such as Dectin-1, CR3 [1]. Interestingly, it was also shown that laminarin and sulfated derivatives thereof can be used in the bioprotection and/or biostimulation if the plant kingdom. Unfortunately, these efficiency is significantly limited both in standardized or real field conditions. One major explanation is probably a limited penetration through the leaf cuticle following spray application. We have demonstrated that penetration through leaves was improved thanks to formulation efforts and by spraying the solutions on abaxial surface [2].
In order to favor penetration bioactive laminarin in plants, we propose to present the synthesis of laminarin-conjugates characterized by (1) increased hydrophobicity (partial acylation with long chains, peracetylation, permethylation) of (2) the presence of negative charges (sulfation, carboxymethylation, sulfoxyethylation). The biological properties related to their elicitation or bioprotection , as well as biphotonic studies with luminescent derivatives, will be also presented.

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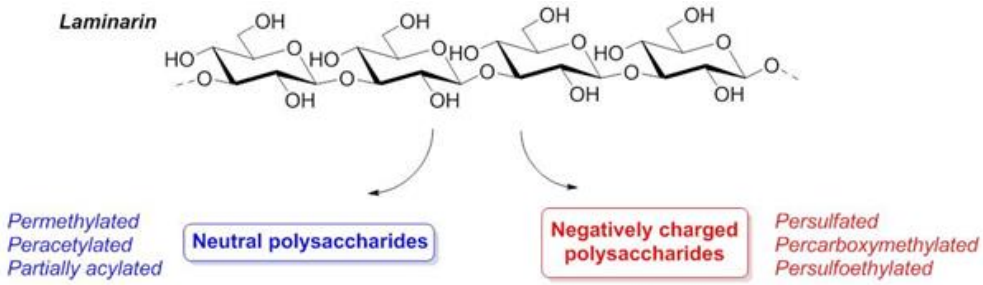
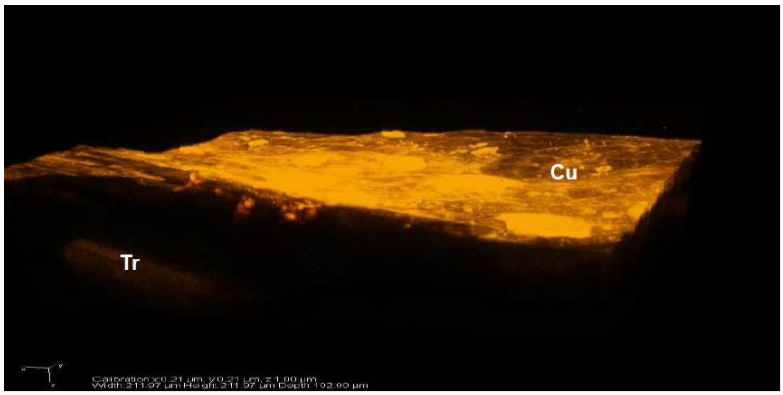
[2] F. Paris, Y. Krzyzaniak, C. Gauvrit, F. Jamois, F. Domergue, J. Joubes, V. Ferrieres, M. Adrian, L. Legentil, X. Daire, S. Trouvelot. An ethoxylated surfactant enhances the penetration of the sulfated laminarin through leaf cuticle and stomata, leading to increased induced resistance against grapevine downy mildew. Physiol. Plant 156 (2016), 338-350.



Visualization of abaxial plant leaf using fluorescent-labeled permethylated laminarin.



Laminarin-conjugates: structures and biphotonic imagery

OL10.4.4

Synthetically Modified Chitosan for Controlling Planktonic Bacteria and Bacterial Biofilms

Priyanka Sahariah[1], Martha Á. Hjálmarsdóttir[2], Már Másson[1], Rikke L. Meyer[3]

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Chitosan is a linear biopolymer with many interesting properties like biocompatibility, non-toxicity, biodegradability, including antimicrobial activity. In recent years, it has been shown that chemical modification of chitosan can lead to significant improvement of its antimicrobial effect. The aim of this study was therefore, to selectively modify the amino group of chitosan by various cationic and lipophilic moieties to obtain derivatives and conjugates having improved antimicrobial activity than chitosan itself.

The synthesis was performed by using 3,6-O-di-tertiarybutyldimethylsilyl protected chitosan as a precursor and the various modifications like trimethylation, guanidinylation, quaternisation[1] and multiple functionalization[2] was carried out at the 2-amino position of chitosan in a controlled manner. The structural modification of the chitosan derivatives was confirmed using FT-IR, 1H-NMR and 2D-NMR spectroscopy, and their average molecular weight was measured by size exclusion chromatography.

Initial assessment of these conjugates towards a panel of clinically important bacterial strains like Staphylococcus aureus (S. aureus, MRSA and MSSA), Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis and clinical A. baumannii isolate showed bactericidal effect at an optimized ratio of the quaternary ammoniumyl group and the lipophilic functionality. We observed that presence of higher degree of substitution and shorter hydrophobic alkyl chains significantly improved the antimicrobial activity, whereas the introduction of spacers between the functional group and the polymer backbone caused a significant reduction in the activity. Simultaneously, we could also control the toxicity of the derivatives by slight alterations in the ratio of the attached moieties. Such polymer conjugates having tunable antimicrobial properties can emerge as promising antibacterial agents. The excellent activity of some of the chitosan derivatives towards planktonic cells led us to further explore their efficacies towards bacterial biofilms. We have recently investigated how the combination of different functional groups influenced chitosan's efficacy against preformed S. aureus biofilms[3]. The antibiofilm effect of the cationic chitosan derivatives was greatly enhanced in presence of hydrophobic groups (alkyl chains), and the extent of their effect was determined by the ratio and length of the alkyl chains. Living and dead cells were visualized by fluorescence staining, and three-dimensional imaging of biofilms confirmed the accessibility and antimicrobial effect of chitosan derivatives with alkyl chains in the full depth of the biofilms.

We combined the above results to develop an overall structure-activity relationship for these polymers towards planktonic bacteria and bacterial biofilms[4]. Further exploration into the control of biofilms of different bacterial strains utilizing such modified biopolymers are in progress.

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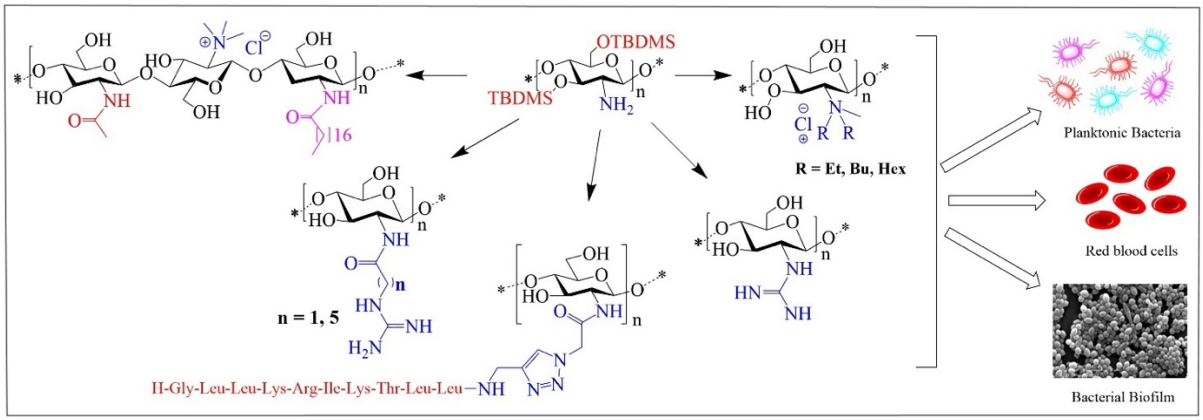

Scheme 1. Schematic representation of the synthesized chitosan derivatives.

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