The 21st European Carbohydrate Symposium in Paris

Scientific program & ABSTRACT BOOK

... WAITING FOR 2023

Paris, July the 18th 2021,

To the Glycoscience community,

By submitting numerous abstracts and inscribing, despite the sanitary uncertainties prevailing in the last months, you gave an incredible support to the Organizing Committee of Eurocarb 21 in Paris in its tentative to organize an on-site meeting in 2021. It is thus a pleasure to make this "waiting for 2023" Scientific Program & Abstract Book freely available, not only as an acknowledgement of your work and strong attachment to Eurocarb, but most importantly to highlight the dedication of our community towards the advancement of science, even in the difficult conditions we are facing because of the COVID-19 pandemic.

The Organizing Committee would also like to thank:

-All the speakers of plenary, keynote and interdisciplinary duo lectures that accepted to share their recent cutting-edge results.

-The regular generous Eurocarb sponsors that expressed their interest to support an on-site meeting.

-The Maison de la Chimie for all the efforts made to adapt continuously to the evolution of the sanitary regulations to allow an on-site Eurocarb in conditions preventing SARS-CoV-2 transmission.

-The ECO representatives and past Presidents, as well as the National Scientific Committee for their invaluable help in setting up the scientific program.

And, last but not least, I would like to thank Christine LE NARVOR and Vincent FERRIÈRES, for their support and tremendous work as co-chair members in the Organizing Committee.

The Organizing Committee wish you all a profitable and enjoyable reading of this "waiting for 2023" Eurocarb 21 Scientific Program & Abstract Book. May it be a tasty appetizer to the 2023 edition of Eurocarb 21 in Paris, from July 09 to 13, when health and travel conditions will allow us to be **ALL TOGETHER AGAIN**

On behalf of the Organizing Committee

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The National Scientific Committee involves active members of the French Glycoscience Group and proposed a list of Plenary and Keynote Speakers for further approval and amendment by the ECO national representatives and past presidents.

C. Breton (Univ. Grenoble Alpes), K. Bouchemal (Univ. Paris-Saclay), Y. Blériot (Univ. Poitiers), P. Compain (Univ. Strasbourg), S. Colliec-Jouault (IREMER, Nantes), P. Delannoy (Univ. Lille), P-A. Driguez (Sanofi, Paris), R. Daniellou (Univ. Orléans), R. Fauré (INSA Toulouse), T. Fontaine (Institut Pasteur, Paris), C. Grandjean (CNRS, Nantes), S. Gouin (Univ. Nantes), A. Imberty (CERMAV, Grenoble), L. Lay (Univ. Milan), P. Lerouge (Univ. Rouen Normandie), S.Norsikian (ICSN, Gif sur Yvette), F. Pilard (Univ. Picardie Jules Verne), S.Perez (CERMAV, Grenoble), Y. Queneau (ICBMS, Lyon), M. Sollogoub (Sorbonne Université), R. Vivès (IBS, Grenoble), S. Vidal (ICSN, Gif sur Yvette), J. Xie (ENS Paris-Saclay)

European Carbohydrate Organisation

Eurocarb symposia are organized under the auspice of the **European Carbohydrate Organization (ECO)**. The first **Eurocarb** symposium was organized in Austria in 1981. As a biannual meeting, it has evolved from a carbohydrate chemistry forum to a glycoscience meeting which also includes glycobiology and biological chemistry. Nowadays, **Eurocarb** is positioned as a leading symposium at the forefront of Glycosciences at the international level.

In 2008, the **ECO** established The Emil Fischer Carbohydrate Award in in order to honor active carbohydrate scientists distinguished with contributions of excellence.

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Sponsors

They expressed their interest in sponsoring an on-site Eurocarb in Paris in 2021 and we thank them for trusting us:

We thank the Emil Fischer awardee and the nine renowned group leaders that did us the honor of accepting to give a **plenary lecture** in July 2021, despite the uncertainties on the sanitary conditions and travel restrictions.
We wish to see them all in Paris in July 2023 for Eurocarb 21.

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Shanghai Institute of Organic Chemistry,
CHINA

We thank the 14 renowned group leaders, CEO and CSO that did us the honor of accepting to give a **keynote lecture** in July 2021, despite the uncertainties on the sanitary conditions and travel restrictions.
We wish to see them all in Paris in July 2023 for Eurocarb 21.

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PORTUGAL

Manfred WUHRER
Professor
Leiden University Medical Center
NETHERLANDS

We thank the 4 renowned group leaders, that did us the honor of accepting to test the new **"Interdisciplinary Duo Keynote lecture"** format* in July 2021, despite the uncertainties on the sanitary conditions and travel restrictions.
We wish to see them all in Paris in July 2023 for Eurocarb 21.

Interdisciplinary Duo Keynote 1:

Roberto ADAMO
Project Leader
GSK Vaccines,
ITALY

Luigi LAY
Professor
University of Milan,
Department of Chemistry,
ITALY

Interdisciplinary Duo Keynote 2:

Anna BERNARDI
Professor
Università degli Studi di Milano,
ITALY

Franck FIESCHI
Professor
Université Grenoble Alpes,
FRANCE

* Interdisciplinary Duo Keynote Lecture : two speakers with complementary expertise, one communication, double time

Design and Synthesis of Glycan-based Photoaffinity Probes for Exploration of Glycan-Receptor Binding

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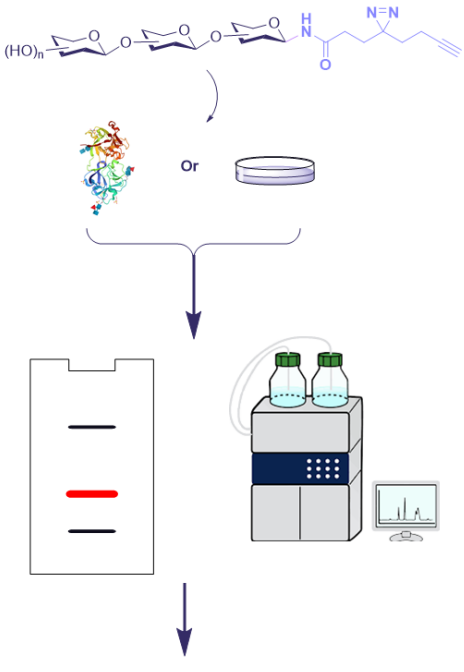
Glycan-protein interactions mediate a range of biologically relevant processes, such as cell-cell signaling, recognition, adhesion, and transport.[1] In the context of human health and disease, abnormal glycosylation of secreted and cell surface-bound glycoproteins results in a myriad of undesirable pathologies.[2] Deciphering the exact molecular mechanisms and receptors of glycan-mediated phenomena is still an ongoing task in glycobiology. To this end, we report a photo-crosslinking strategy to identify glycan-protein interactions and their corresponding receptors in purified proteins and mammalian cells. Photoaffinity labelling (PAL) is powerful non-intrusive technique to capture interactions between probe and their respective targets.[3,4] As part of a program to study such interactions, we have designed and synthesized a library of biologically important glycans equipped with a bifunctional photoactivatable diazirine moiety and a terminal reporter alkyne group. Upon incubation and subsequent photo-crosslinking of our glyco-probes, copper catalyzed click conjugation with a fluorophore allowed for the detection of glycan-labelled proteins via in-gel fluorescence scanning. Here, we demonstrate, as a proof of concept, a selected library of glyco-probes that can be cross-linked with their interacting protein targets in a light-activated manner.



Glycoprobes Workflow

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The catalytic reaction mechanism of the β-galactocerebrosidase enzyme deficient in Krabbe disease

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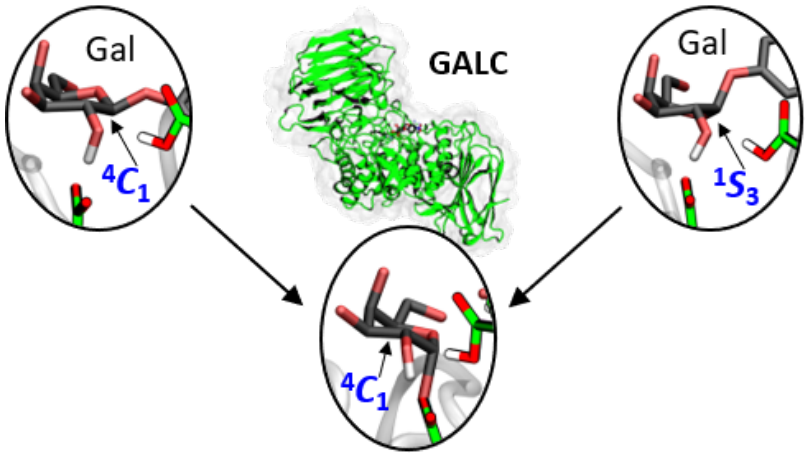
Krabbe disease is a neurodegenerative disorder related to misfunction of β-galactocerebrosidase (GALC), a glycosidase that catalyzes the cleavage of β-galactosidic bonds in glycosphingolipids [1]. Here we uncover the catalytic molecular mechanism of GALC in complex with Gal-β-*p*-nitrophenyl, a substrate analogue, using quantum mechanics/molecular mechanics metadynamics simulations [2]. Our results clarify the unusual chair conformation of the substrate observed in the crystal structure [3] and show that catalysis can take place via two distinct conformational pathways ($^{1}S_{3} \rightarrow [^{4}H_{3}]^{\ddagger} \rightarrow {}^{4}C_{1}$ and $^{4}C_{1} \rightarrow [^{4}H_{3}]^{\ddagger} \rightarrow {}^{4}C_{1}$) with similar free energy barriers because of leaving group flexibility [4]. Preliminary results about the influence of the lipid-transfer saposin A protein to GALC in the catalytic mechanism will be shown. This mechanistic insight will aid in the design of Krabbe diagnosis probes and GALC conformational chaperones.



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Herbaspirillum LPS protects plant host from immune recognition

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Plant microbiota (1,2) is a complex co-association of microorganisms that promotes plant health and growth, primes plant immune response, assures protection from pathogens. Lipopolysaccharides (LPS) (3), outer membrane components of Gram-negative bacteria, are involved in host-microbe interaction events, such as colonization, symbiosis, virulence. How plants immunity discriminates between beneficial and pathogenic microbes and perceives LPS from plant microbiota is still an open question (4). In this frame, we evaluated structure, conformation, membrane properties and immune recognition of the LPS isolated from the plant microbiota member Herbaspirillum sp. Root189. A combined NMR, computational, biophysical and immunological approach showed how LPS is well tolerated by the plant immune system and highlighted the structural features that prevent its immune recognition and how the glycan chain decorations affect LPS physicochemical properties and bioactivity. Herbaspirillum LPS consists of an O-methylated and variously acetylated D-Rhamnose containing polysaccharide chain, whose conformational behavior and flexibility was evaluated using MD simulation and NMR spectroscopy, while structure and properties of reconstituted model membrane, included flexibility, density and water permeability, using Neutron Reflectometry on asymmetric bilayer. We then investigated the LPS immune recognition, demonstrating i) how the O-polysaccharide shielded the LPS to the plant immune system, and ii) how the glycan chain decoration was functional to elude host recognition and favor colonization.

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Parallel Synthesis of Glycans on Membranes via Aerosol Activator Deposition

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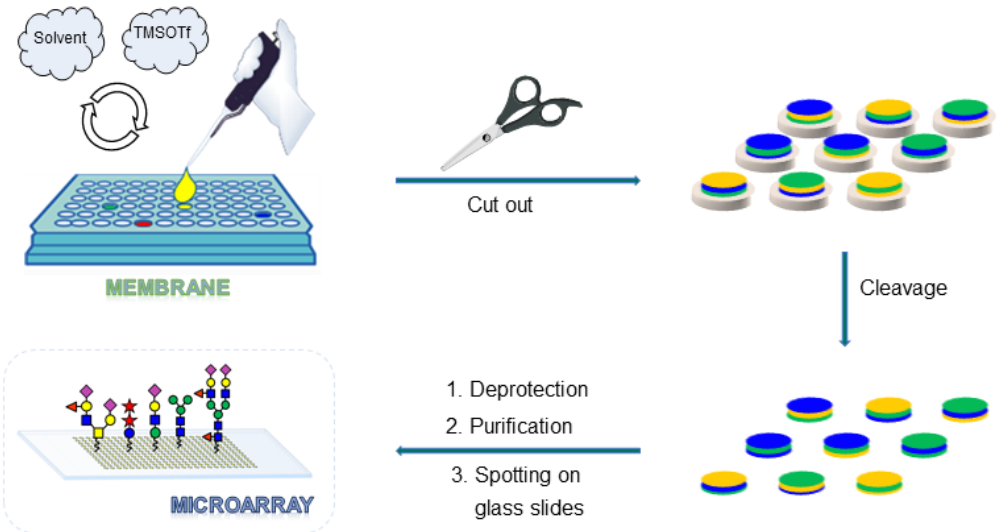
Microarrays are considered to be versatile diagnostic research tools, which allow for high-throughput screening of potential binding partners. Enabled by parallelized automated synthesis, there are a variety of commercial microarray platforms available for peptides and oligonucleotides. However, for glycans,1 there is no parallelized chemical synthesis method available, which limits progress in the field of carbohydrate binding studies. Therefore, we introduce an approach, derived from the peptide SPOT–synthesis,2 to carbohydrate chemistry: spotting various dissolved glycosyl donor building blocks onto a functionalized membrane. Next, using a custom-built chamber, we can perform a parallelized coupling reaction via aerosol activator deposition at low temperatures.3 Afterwards, cleavage, deprotection, and purification of each glycan structure can be achieved. The obtained collections of glycans can be printed in the microarray format to potentially screen hundreds of different glycans. With this approach, we can overcome the limitations of existing methods (automated glycan assembly4, chemoenzymatic synthesis5) to obtain glycan libraries in a fast and parallelized manner, without the need for specific enzymes.



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Prevention of PAINS-induced membrane perturbations by C-glucosylation

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Since Pan-Assay Interference CompoundS (PAINS) were originally described as promiscuous molecules capable of interfering with high-throughput screening results, medicinal chemists have been concerned about investing in natural polyphenol scaffolds as leads for further development.[1] Despite their promising bioactivities in cell-free assays against several targets with therapeutic interest for cancer, diabetes, and Alzheimer's disease, resveratrol and genistein are two good examples of planar compounds, that, lacking catechol or hydroquinone moieties typical of polyphenol PAINS, are believed to display membrane-perturbing effects ultimately leading to subtle alterations in the conformation and function of transmembrane proteins.[2] In this work, we show through fluorescence ratiometric measurements that phloretin, genistein and resveratrol act by decreasing membrane dipole potential, especially in cholesterol-rich domains such as lipid rafts, which play a role in important cellular processes. These results provide a mechanism for their labelling as PAINS through their ability to disrupt cell membrane homeostasis.[3] Furthermore, we present the first synthesis of glucosylresveratrol and demonstrate that polyphenol-promoted membrane dipole potential alterations are fully prevented upon *C*-glucosylation, which opens a new avenue for the exploration of these scaffolds as leads for drug development.[3] The most likely biophysical mechanisms by which the sugar moiety is able to protect the cell membrane are also rationalized and discussed.

Acknowledgements: EU is gratefully acknowledged for having supported the project "Diagnostic and Drug Discovery Initiative for Alzheimer's Disease", FP7-PEOPLE-2013- IAPP, GA 612347 and Fundação para a Ciência e Tecnologia, Portugal, for supporting Centro de Química Estrutural (project UIDB/00100/2020).

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A single bacterial sulfatase is required for metabolism of mucin O-glycans by human gut bacterium

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In the colon, secreted mucus creates a physical barrier that separates the microbiota from the intestinal epithelium [1]. A major component of this colonic mucus is mucin 2 (MUC2), a glycoprotein that contains up to 80% glycans by mass and more than 100 different O-linked glycan structures [2]. Some gut bacteria are able to utilize MUC2 as a nutrient source [3]. However, it remains unclear which enzymes initiate the degradation of the highly complex O-glycans found in mucins. In the colon, these glycans are heavily sulfated [4], but the specific sulfatases that are active on colonic mucins have not been identified. Here, we show that sulfatases are essential to the utilization of colonic mucin O-glycans by the human gut symbiont Bacteroides thetaiotaomicron. We have characterized the activity of 12 different sulfatases encoded by this species, showing that these enzymes are active on all of the known sulfate linkages in colonic O-glycans. Crystal structures of 3 enzymes provide mechanistic insight into the molecular basis of substrate-specificity. Unexpectedly, we found that a single sulfatase is essential for utilization of sulfated O-glycans in vitro and also plays a major role in vivo. Our results provide insight into the mechanisms of mucin degradation by gut bacteria, an important process for both normal microbial gut colonization and diseases such as inflammatory bowel disease (IBD). Sulfatase activity is likely to be a keystone step in bacterial mucin degradation and inhibition of these enzymes may therefore represent a viable therapeutic path for treatment of IBD.

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In-process control analysis of synthetic human milk oligosaccharides by capillary electrophoresis

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Industrial production of synthetic human milk oligosaccharides (HMOs) represents a recently growing interest since they serve as key ingredients in baby formulas and are also utilized as dietary supplements for all age groups. Despite their short oligosaccharide chain lengths, HMO analysis is challenging due to extensive positional and linkage variations. Capillary gel electrophoresis separates analyte molecules based on their hydrodynamic volume to charge ratios, thus, offers excellent resolution for most of such otherwise difficult-to-separate isomers. A conventionally used carbohydrate separation matrix was applied for the in-process control analysis of bacteria enhanced production of 3-fucosyllactose, lacto-N-tetraose and lacto-N-neotetraose. Another example showed the suitability of the method for the in-vivo in-process control of a shake flask and fermentation approach of 2'-fucosyllactose production. In this latter instance, borate complexation was utilized to efficiently separate the 2'- and 3-fucosylated lactose positional isomers. The analysis times for both methods required only a couple of minutes with high resolution and excellent reproducibilities, features representing high importance in carbohydrate type food additive manufacturing in-process control.

Synthesis of Iminosugar Based Glycomimetics as Probes for Activity Based Protein Profiling

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Malfunction of carbohydrate-processing enzymes (CPEs) results in diseases such as, lysosomal storage diseases, immunological diseases, cancer, diabetes or bacterial as well as viral infections. [1] The role and the contribution of the carbohydrate part is still often unknown or unrealised. In this context, activity-based protein profiling (ABPP) has become a versatile tool for evaluating protein activity in living organisms. For instance, Withers and Overkleeft, [2,3] have developed important contributions of such activity-based probes for CPEs. Ligand directed chemistry for protein profiling has been introduced by Hamachi, a method which allows for target selective chemical labelling of proteins under preservation of its activity. [4] In this case, the small molecule probe (Figure 1) is equipped with a recognition part for selective reversible interaction with the active site of the protein of interest and a reporter group (tag) for identification. A chemical reactive group is introduced between the recognition part and the tag enabling covalent binding of the tag to the outside of the active site. [5]

Based on our knowledge about the structure activity relationship of iminosugars with glycosidases, [6] we have designed and synthesised iminosugar based probes for glycoside hydrolases following the ligand directed chemistry concept. Synthetic details as well as preliminary biological studies will be presented.

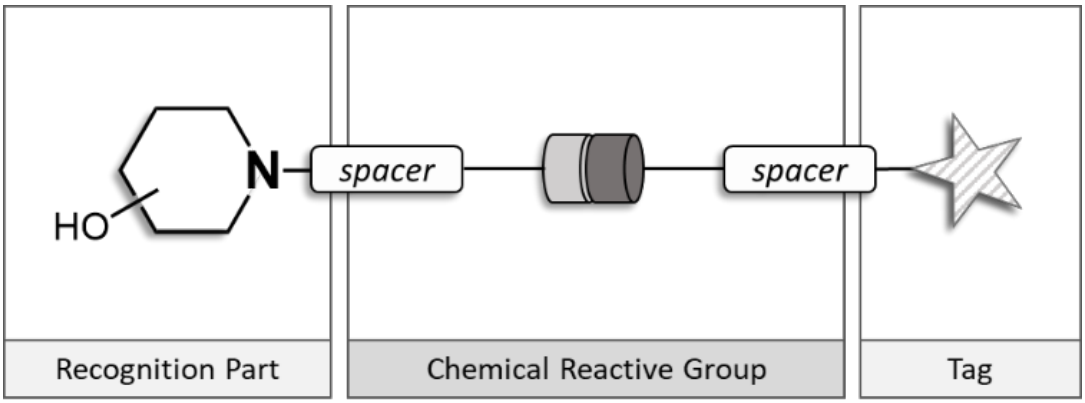

Figure 1: Structural concept of iminosugar based probes.

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Dissecting the Essential Role of Anomeric β-Triflates in Glycosylation Reactions

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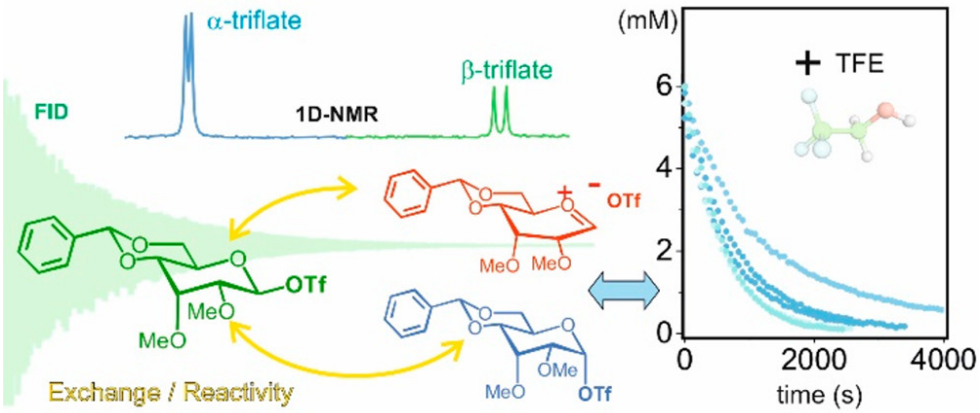
Glycosylations promoted by triflate-generating reagents are widespread synthetic methods for the construction of glycosidic scaffolds and glycoconjugates of biological and chemical interest. These processes are thought to proceed with the participation of a plethora of activated high energy intermediates such as the α- and β-glycosyl triflates, or even increasingly unstable glycosyl oxocarbenium-like species, among which only α-glycosyl triflates have been well characterized under representative reaction conditions. Interestingly, the remaining less accessible intermediates seem to be particularly relevant in α-selective processes, involving weak acceptors. Herein, we report a detailed analysis of several paradigmatic examples of such reactions, employing a combination of chemical, NMR, kinetic and theoretical approaches, culminating in the unprecedented detection and quantification of the true β-glycosyl triflate intermediates within activated donor mixtures. This achievement was further employed as a stepping-stone for the characterization of the triflate anomerization dynamics, which along with the acceptor substitutions, govern the stereochemical outcome of the reaction. The obtained data conclusively show that the formation of the α-glycoside is necessarily preceded by a bimolecular α → β triflate interconversion, which under certain circumstances becomes the rate-limiting step. Overall, our results rule out the prevalence of the Curtin–Hammett fast-exchange assumption, and instead point toward a non-canonical mechanistic scenario evolving along the reaction progress.



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Access to unnatural glycosides by metal-catalyzed functionalisation of glycal substrates

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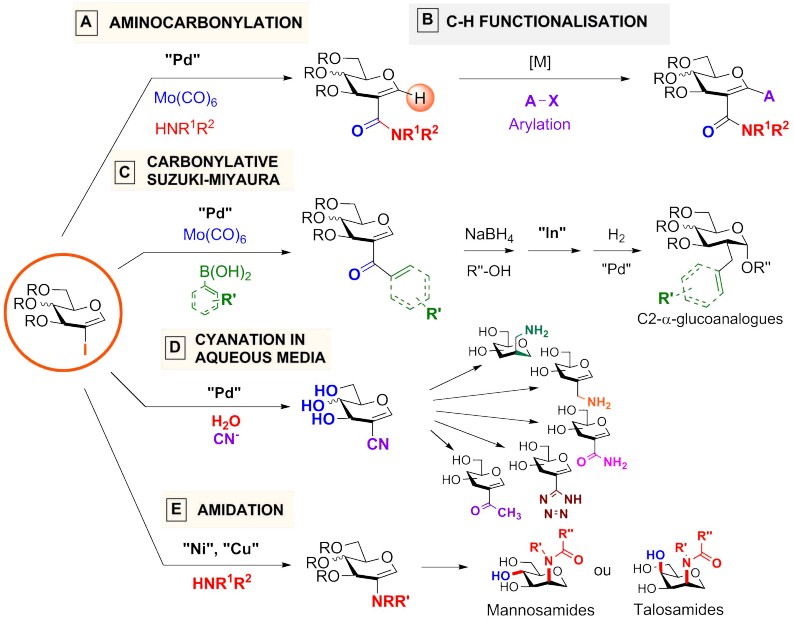
Development of new access to glycoconjugates has become of great interest in synthetic chemistry. In particular, glycoconjugates possessing unnatural bonds are largely studied due to their possible enzymatic and chemical stabilities. To build unnatural bonds on sugars, several strategies were explored, among them metal-catalyzed processes revealed popular powerful tools. By this strategy, we developed novel metal-catalyzed transformations on glycal-type substrates using cross-coupling reactions or C-H functionalisation. Indeed, we used 2-iodoglycal starting substrates to reach C2-amidoglycals via a Pd-catalyzed aminocarbonylation process or 2-ketoglycals via carbonylative Pd-catalyzed Suzuki-Miyaura reaction. Recently, we turned our attention to the development of Pd-catalyzed cross-couplings on unprotected 2-iodoglycals using a Pd-catalyzed cyanation reaction performed in aqueous media leading to unprotected 2-cyanoglycals, which were then derivatized to five glycoanalogue families. Very recently, the creation of C-N bond on 2-iodoglycals was developed thanks to a dual nickel/copper catalysis furnishing mannosamide and talosamide analogues. The C2-amido-glycal scaffolds were then utilized to access to C-aryl/alkenyl-glycosides via a directed Pd-catalyzed C-H functionalization process of the pseudo-anomeric proton. A bidentate amido group was chosen to guide the reactivity on the targeted pseudo-anomeric position and to avoid undesired reactivity. Application of the method to the synthesis of a Dapagliflozin analogue was performed in excellent yield.



Metal-catalyzed reactions on glycal substrates

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The synthesis of hyaluronic acid oligosaccharides and elucidation of their effect on angiogenesis

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Hyaluronic acid (HA) is the glycosaminoglycan built of alternative units of →3)-β-D-GlcNAc-(1→ and →4)-β-D-GlcA-(1→. This biopolymer is known to be essential for regulation of cell-cell interactions, angiogenesis, would healing [1,2] and some others processes. In this communication we report on the synthesis of di-, tri-, tetra- and hexasaccharides 1-4 related to HA (Figure 1). 4,6-p-OMe-Benzylidene-2,3-di-O-benzoyl-glucosyl sulfoxide was used as a donor for the formation of β-D-Glc-(1→3)-β-D-GlcNTCA fragments. Selective removing of p-methoxy-benzylidene protection, oxidation of the primery hydroxyl group by TEMPO-BAIB system followed by methylation led to transformation of Glc unit into GlcA one. Trichloromethyloxazoline donors were used for the formation of β-D-GlcNTCA-(1→4)-β-D-GlcA linkages. Blockwize [1+2], [2+2], and [2+4] strategies were applied for the tri-, tetra-, and hexasaccharides carbohydrate skeleton assembling, respectively. The target compounds were studied as inhibitors of angiogenesis in vitro using endothelial cells and Matrigel as a medium.

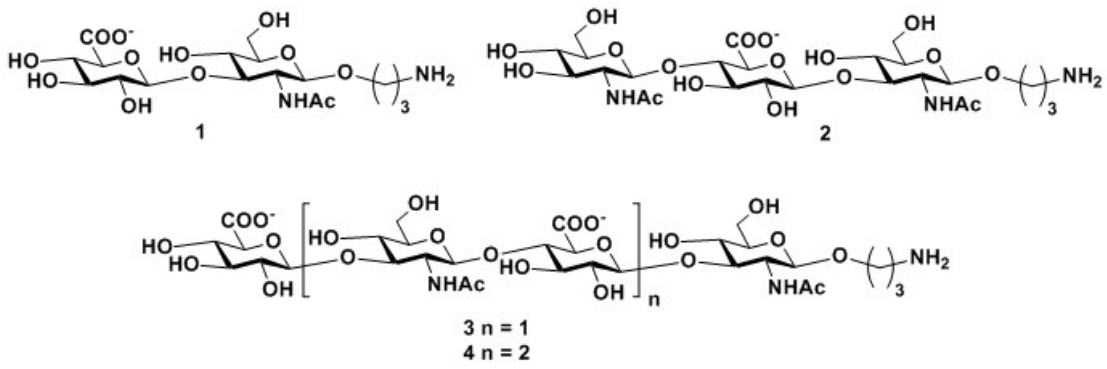

Figure 1. The target oligosaccharides 1-4.

Acknowledgments: This research was funded by RUSSIAN SCIENCE FOUNDATION, grant number 19-73-20240.

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Polarimetry as a method for studying the structure of aqueous carbohydrate solutions

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Using aqueous solutions of D-levoglucosan (1,6-anhydro-D-glucopyranose) as an example, we demonstrated, by comparing results of polarimetry, quantum chemical calculations, HPLC, static and dynamic light scattering, that polarimetry allows one to detect changes in the structure of solutions with changes in concentration (Fig. 1a) [1] and temperature (Fig. 1b) [2], as well as the evolution of the structure of solutions over time [3]. In particular, the phenomenon of the existence of "critical" concentrations and temperatures was discovered at which the specific optical rotation (SR) of the solutions undergo jump-like changes, apparently reflecting rearrangements in the structure of the solution.

We also detected differences in the SR values of ternary levoglucosan–DMSO–water mixtures of different composition (Fig. 1c) which apparently indicate that chiral levoglucosan molecules can be localized in solution domains of different composition and structure (supramers [4]) as evidenced by light scattering data, which leads to differences in the microenvironment of levoglucosan molecules and their conformations. We hypothesize that in the case of aqueous solutions, chiral carbohydrate molecules might act as "probes" that "sense" the slightest changes in their conformation or rearrangement of the environment (in the solvation shell) caused by changes in the structure of water, which at the macroscopic level manifests themselves as changes in SR.

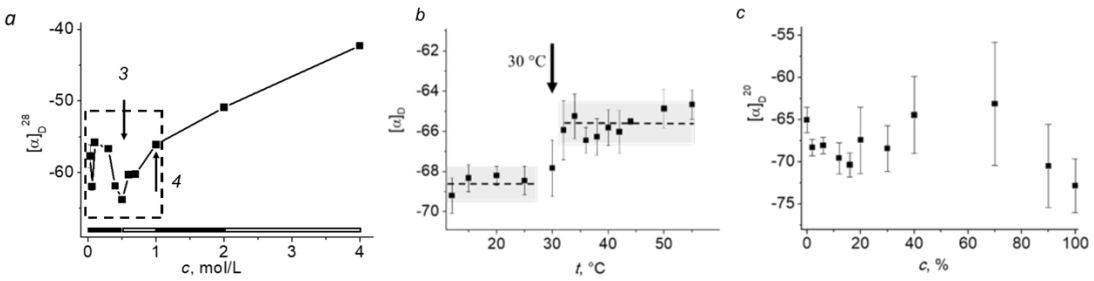

Fig 1. SR of freshly prepared levoglucosan solutions: in water (a, b), in DMSO–water mixtures with various concentrations of DMSO (c).

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Structures of, and vaccine candidates based on, Acinetobacter baumannii capsular polysaccharides

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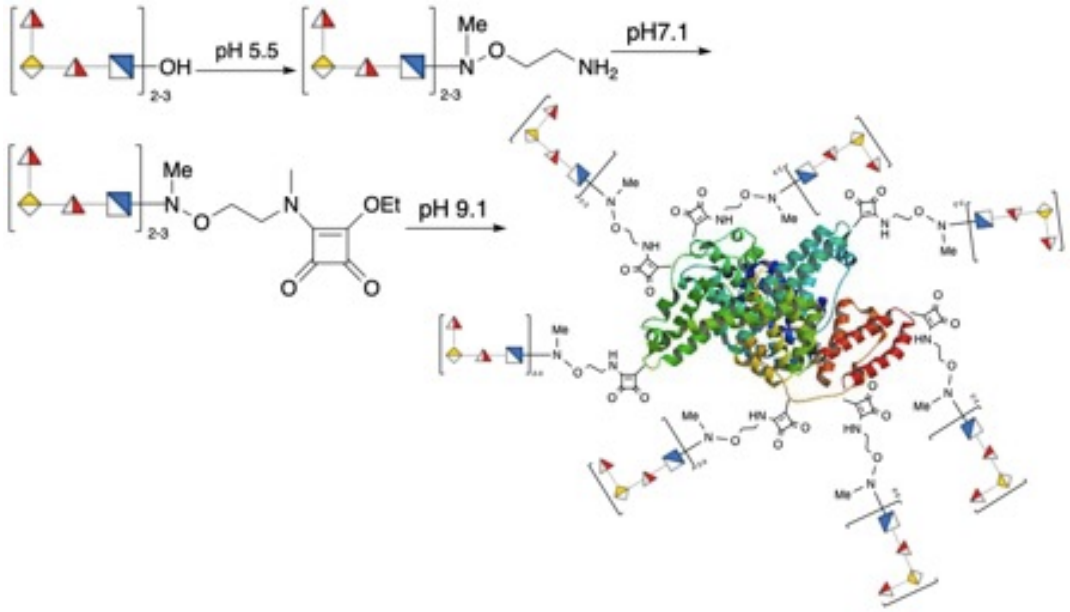
Acinetobacter is a group of germs commonly found in the environment. Acinetobacter baumannii belongs to ESKAPE nosocomial pathogens, that is why WHO includes these bacteria into the Global Priority List of antibiotic-resistant bacteria. The aim of this project is classification of clinical isolates of A. baumannii based on capsular polysaccharides (CPSs) and development on the basis of oligosaccharide fragments of the CPSs of vaccine candidates for prophylaxis of the deceases caused by A. baumannii.

New CPSs were isolated from cells of clinical isolates of A. baumannii of different KL types and purified gel-permeation chromatography. Their structures were either identified by a comparison with published data or de novo established by sugar analysis and selective degradations by chemical methods or recombinant bacteriophage depolymerases along with NMR spectroscopy. The derived oligosaccharides were characterized by high-resolution electrospray ionization mass spectrometry and NMR spectroscopy and conjugated with BSA according to the scheme shown below [1,2]. The immunogenicity of the synthesized glycoconjugates was tested on BALB/c mice using the developed immunization protocol. The experimental animals showed a stable level of immune response during ten months of the observation periods.



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Revealing the human mucinome

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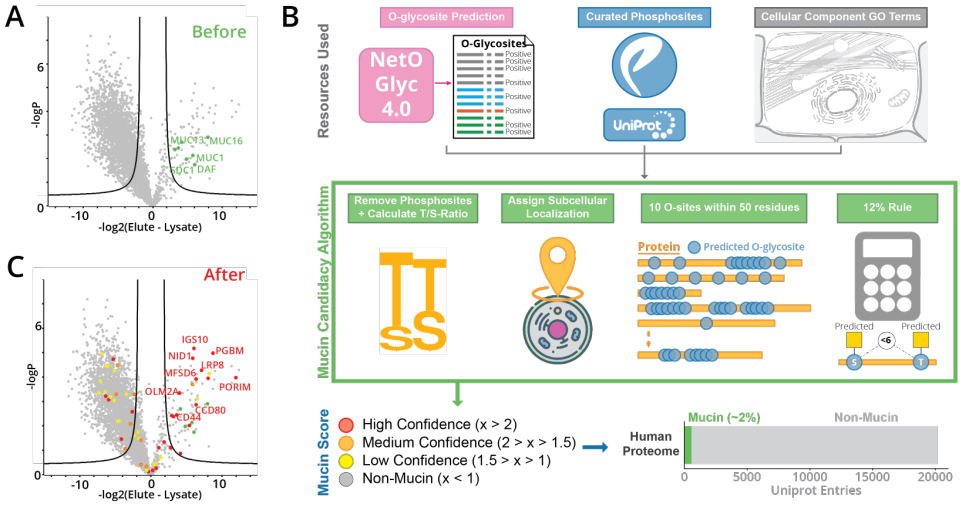
Mucin domains are densely O-glycosylated modular protein domains found in a wide variety of cell surface and secreted proteins. Mucin-domain glycoproteins are key players in a host of human diseases, especially cancer, but the scope of the mucinome remains poorly defined. Recently, we characterized a bacterial mucinase, StcE, and demonstrated that an inactive point mutant retains binding selectivity for mucins. In this work, we leveraged inactive StcE to selectively enrich and identify mucins from complex samples like cell lysate and crude ovarian cancer patient ascites fluid. Our enrichment strategy was further aided by an algorithm to assign confidence to mucin-domain glycoprotein identifications. This mucinomics platform facilitated detection of hundreds of glycopeptides from mucin domains and highly overlapping populations of mucin-domain glycoproteins from ovarian cancer patients. Finally, we used a KRAS dox-inducible system to show which mucins contribute to molecular bulk at the cell surface. Ultimately, we demonstrate our mucinomics approach can reveal key molecular signatures of cancer from in vitro and ex vivo sources.



Mucinomics platform allows for identification of enriched mucins from complex samples

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Glycosynthase-based chemo-enzymatic synthesis of peptidoglycan oligomers

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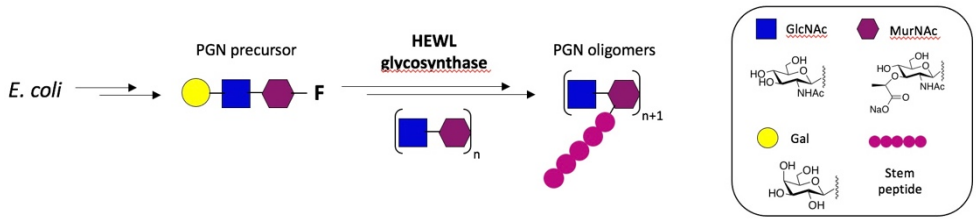
Antibiotic resistance is becoming one of the biggest threats to global health. In 2014, the O'Neill report estimated that deaths due to antimicrobial resistance could rise from approximately 700,000 deaths a year to close to 10 million deaths per year by 2050.[1] Most antibiotics used in human medicine inhibit the peptidoglycan (PGN) biosynthesis. PGN is a giant macromolecule that surrounds the cell of bacteria, and defines their shape. This complex biopolymer is composed of glycan chains made with β-(1,4) linked alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) units that are cross-linked by peptide bridges attached to the lactate moiety of the MurNAc.[2]

To better understand and fight against antibiotic resistance, functional and structural studies of the PGN enzymatic machinery is required. However, the lack of pure and well-defined substrates hampers this process. In the present work, we have designed a straightforward chemo-enzymatic access to well-defined PGN oligomers. Our strategy consists in the controlled and stepwise-glycosylation of a fluorinated donor with various acceptors by a glycosynthase derived from hen egg-white lysozyme (HEWL). Precursors were produced by metabolically engineered Escherichia coli cells followed by few chemical modifications. Using a galactosyl unit as temporary protecting group, an iterative glycosylation process catalyzed by the HEWL-glycosynthase was then implemented to produce a library of size-controlled oligosaccharides. Finally, these oligomers were coupled with relevant PGN stem peptides.



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Engineering loop dynamics to modulate substrate specificity in chitin deacetylases

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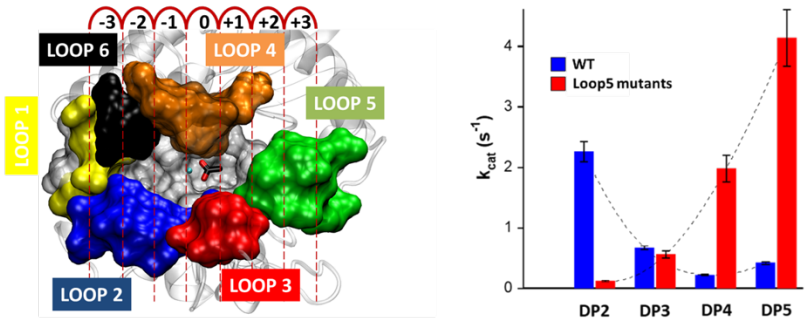
Chitin deacetylases (CDA) are members of family 4 carbohydrate esterases (CE4) which operate by metal-assisted general acid/base catalysis [1]. We are interested in understanding the structural bases of substrate specificity by CE4 enzymes, their mechanism of action and biological functions, as well as the use of engineered variants as biocatalysts.

CDAs exhibit diverse deacetylation patterns, reflecting different specificities and pattern recognition on their substrates, being processive, distributive or deacetylating a single position on chitooligosaccharides (COS) [2]. Because of the influence of different deacetylation patterns in signaling events (*i.e.* pathogenic fungi-host interactions), the availability of a panel of CDAs with defined specificity will provide sequence-defined partially deacetylated COS with broad applications in biomedicine and biotechnology.

Vibrio cholera CDA was the first CE4 enzyme for which E·S complexes were solved by X-ray crystallography, showing that the (α/β)7 fold is decorated by six surface loops that shape the binding site cleft [3]. We proposed the "subsite capping model" to rationalize the differential accessibility of substrates to the binding cleft mediated by these non-conserved loops [3,2]. After identifying Loop5 as the structural element controlling specificity for short substrates [4], here we will report on a rational design to modify specificity towards larger substrates. By biochemical, NMR side-chain (CH_3) relaxation and MD studies, we show that loops dynamics are coupled and their modulation strongly alters specificity.



(Left) Structure of VcCDA showing the 6 loops that shape the binding site cleft. (Right) Change of specificity from WT to a triple mutant at Loop5.

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Hyaluronic acid based non spherical biomimetic devices for drug delivery application

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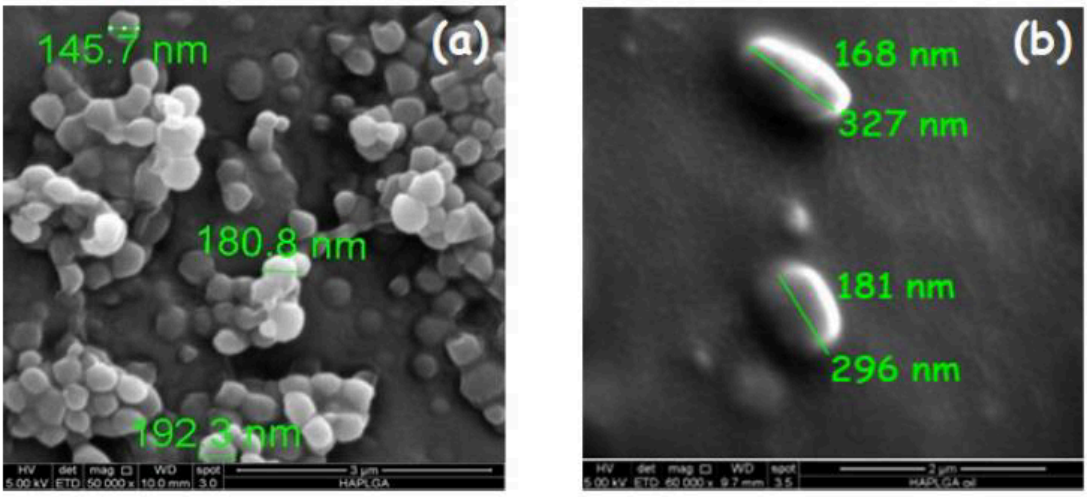
The composition and organization of biological tissues can be the paradigm for the design of smart devices in the biomedical field [1]. Hyaluronic Acid (HA), a main component of extracellular matrix, is a negatively charged naturally occurring polysaccharide, composed of repeating disaccharide units of D-glucuronic acid and N-acetyl glucosamine linked by β(1,4) and β(1,3) glucosidic bonds. HA has been extensively investigated for several biomedical applications due to its unique advantages of non-toxicity, non-immunogenicity, biocompatibility and biodegradability, as well as its susceptibility to chemical modification [2]. In the last decade, the shape of particles has emerged as a new parameter to control the interaction with biologicals systems; particularly, HA-based nanoparticles have a key role in the drug delivery field for cancer therapy. In this frame, HA-based drug delivery systems, consisting of nanoparticles having a spherical and non-spherical shape (Figure 1 a,b), have been realized by conventional and innovative methods such as emulsion and microfluidics, respectively to investigate the effect of shape on the delivery of a new immunostimulant drug. (sulfolipids named Sulfavant [3]) Our results indicate that non-spherical nanoparticles, synthesized by microfluidic method, show both a markedly increase drug/immunostimulant encapsulation efficiency and more efficient internalization in vitro cell culture systems.



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Revealing the human mucinome

Aurélien BOYANCE [1], Nicholas M. Riley [2], D. Judy Shon [2], Kayvon Pedram [2], Venkatesh Krishnan [3], Oliver Dorigo [3], and Carolyn R. Bertozzi [2,4]

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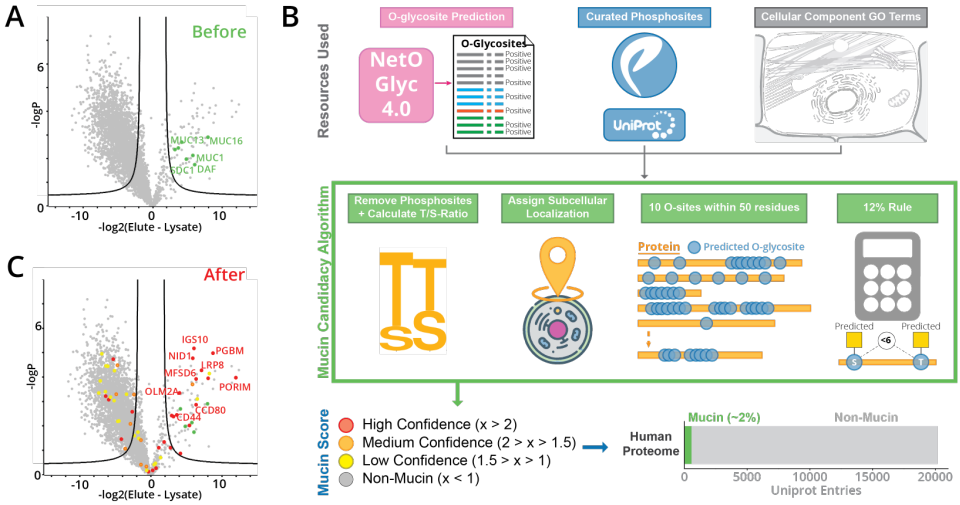
Mucin domains are densely O-glycosylated modular protein domains found in a wide variety of cell surface and secreted proteins. Mucin-domain glycoproteins are key players in a host of human diseases, especially cancer, but the scope of the mucinome remains poorly defined. Recently, we characterized a bacterial mucinase, StcE, and demonstrated that an inactive point mutant retains binding selectivity for mucins. In this work, we leveraged inactive StcE to selectively enrich and identify mucins from complex samples like cell lysate and crude ovarian cancer patient ascites fluid. Our enrichment strategy was further aided by an algorithm to assign confidence to mucin-domain glycoprotein identifications. This mucinomics platform facilitated detection of hundreds of glycopeptides from mucin domains and highly overlapping populations of mucin-domain glycoproteins from ovarian cancer patients. Finally, we used a KRAS dox-inducible system to show which mucins contribute to molecular bulk at the cell surface. Ultimately, we demonstrate our mucinomics approach can reveal key molecular signatures of cancer from in vitro and ex vivo sources.



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Glycomimetics in the discovery of Fucosyltransferase-Specific Inhibition

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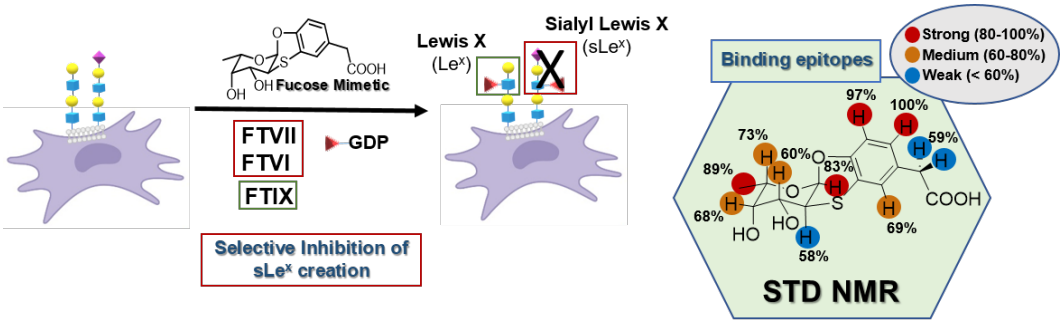
Today, glycomimetics represent a major share of the drug development market, as they mimic the structural information of native carbohydrates and hold vast therapeutic potential.[1] By proper modulation of the mimetic's structure, researchers can overcome the inherent limits of native carbohydrates allowing for the production of drugs with enhanced selectivity, potency, improved pharmacokinetic properties and locked conformation. With this in mind, the possibility to custom modify cell surface glycosylation using non-toxic glycomimetics holds great promise for treatment in a great variety of diseases (*i.e.* autoimmune disorders, inflammation, cancer). Critically, such inhibitors should be selective and target only the desired glycosyltransferase, while leaving other glycosyltransferases untouched, thereby yielding the construction of only requisite glycan products. This communication repurposed a glycomimetic [2], previously used to block bacteria lectin binding [3], to inhibit α-1,3-fucosyltransferase (FT)[4] activity. Of note, we observed[2] that this fucose mimetic selectively and markedly interfered with the creation of sialyl Lewis X (sLe^X) by FTVI and FTVII, but had no effect on the catalytic activity of FTIX, an α-1,3-FT that solely mediates Le^X synthesis,[4] and thereby identified a new generation of selective FT inhibitors. Surprisingly, our findings also indicate that our mimetic and the natural donor substrate (GDP-fucose) do not compete for the same enzymatic binding site, highlighting the need for a deeper understanding of the inhibition mediated by this mimetic.



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In search of DCIR's sweet spot

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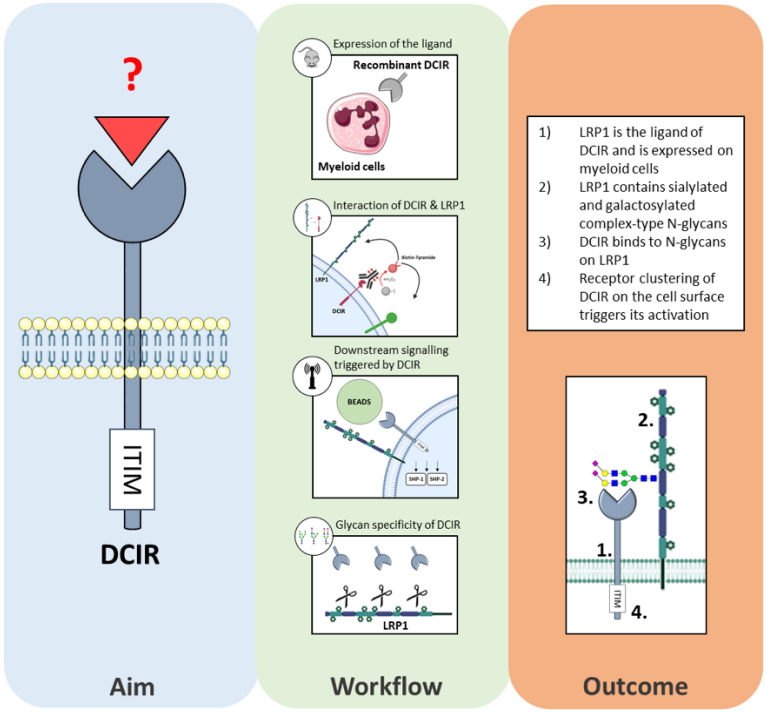
C-type lectin receptors (CLRs) are integral components of the innate immune system through the recognition of self and non-self antigens from pathogens, allergens, cancer cells and damaged tissues. As such, CLRs trigger downstream signalling events that either activate pro-inflammatory responses or inhibit them to avoid excessive inflammation, tissue damage and autoimmunity. The dendritic cell immunoreceptor (DCIR) is one such inhibitory CLR that is implicated in numerous infectious and autoimmune diseases, and whose ligand and signalling remains unknown. To address this, we used pulldown assays and label-free quantitative mass spectrometry to identify the first protein ligand for DCIR: the low-density lipoprotein receptor-related protein 1 (LRP1). Using flow cytometry, microscopy and proximity ligation assay, we found that both DCIR and LRP1 are expressed on the same cells, suggesting these proteins interact in cis. While we were unable to detect activation of DCIR under steady state conditions, stimulation of cells with either BSA- or LRP1-coated microspheres both lead to its activation; reinforcing the concept of a cis interaction. Ligand-blotting studies, demonstrated that DCIR directly binds to LRP1 N-glycans. CE-ESI-MS analysis of LRP1 revealed the presence of sialylated and galactosylated complex-type N-glycans, allowing us to now investigate the specific glycan epitope recognized by DCIR. Collectively, our data provides fundamental insight towards the understanding of the role of DCIR in regulating immune homeostasis.



Chemical Precision Tools to Dissect O-GalNAc glycosylation

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O-GalNAc glycosylation is a major constituent of the cell surface glyco-code. Glycosylation is primed by 20 GalNAc transferase (GalNAc-T) isoenzymes that introduce the first, Ser/Thr-linked GalNAc residue using UDP-GalNAc as a sugar donor. Despite partial redundancy, GalNAc-Ts have been differentially associated with disease, suggesting a pivotal role of isoenzyme-specific protein substrates. However, studying these substrates by mass spectrometry (MS) glycoproteomics approaches is complicated by the cross-talk of different isoenzymes with each other.

Here, we present the development of chemical "precision tools" to dissect the O-GalNAc glycoproteome by methods of modern, quantitative biology.[1] In a structure-based design process, we generate chemically modified monosaccharides to selectively enter particular stages of O-GalNAc glycan biosynthesis.[2,3] Key to our strategy is the use of protein engineering to re-wire cellular nucleotide-sugar metabolism.[2-5] In a tactic called "bump-and-hole engineering", we further generate orthogonal glycosyltransferase mutants (containing "holes") to accommodate bulky chemical tags ("bumps") and profile the substrates of individual GalNAc-T isoenzymes in the living cell.[4,5] Chemical precision tools let us enter methods of quantitative biology, such as CRISPR-KO screens and chemical glycoproteomics, to further understand the determinants of glycosylation and eventually correlate GalNAc-T isoenzyme-specific glycosylation site and ensuing glycan structure in a single experiment.

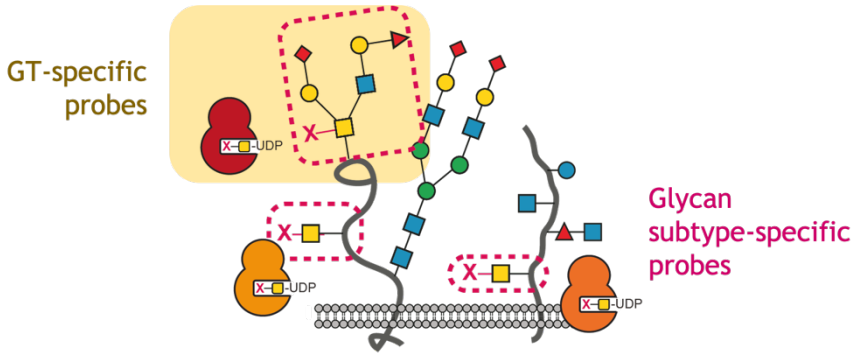

Fig.: Chemical precision tools to dissect O-GalNAc glycosylation.

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Dilock polysaccharides

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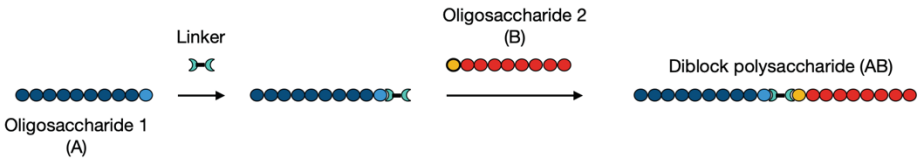
The conjugation at chain termini of two different polysaccharides provides diblock polysaccharides, a new class of precisely engineered polysaccharides. The new architecture provides on one hand new solution and stimuli-responsive self-assembly properties, while retaining key properties such as biodegradability on the other.

The first part of the presentation will focus on the activation of oligosaccharides with bifunctional O,O-1,3-propanediyl-bishydroxylamine (PDHA) or adipic acid dihydrazide (ADH) and the properties of the correspondic conjugates, including their reduction by picoline borane as a standard method. Strategies for attachment of a second oligosaccharide and purification of diblocks are further discussed.

Examples will include chitosan-b-dextran, chitin-b-dextran and alginate-b-dextran diblocks: preparation, purification, structural characterisation, solution properties and self-assembly properties.



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The Oligomannose N-glycans 3D Architecture and its Response to the FcγRIIIa Structural Landscape

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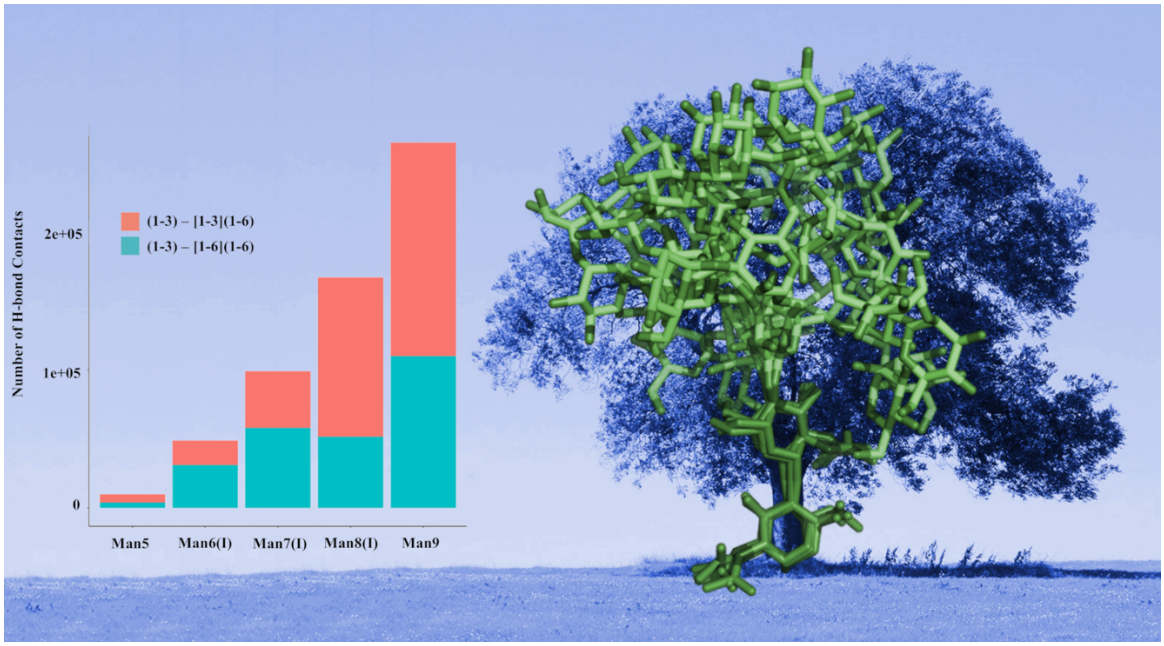
Oligomannoses are evolutionarily the oldest class of N-glycans, In mammalian species oligomannose N-glycans can have up to 9 Man, meanwhile structures can grow to over 200 units in yeast mannan. The highly dynamic nature, branching complexity and 3D structure of oligomannoses have been recently highlighted for their roles in immune escape and infectivity of enveloped viruses, such as SARS-CoV2 and HIV-1. In this work we will discuss the results of over 54 µs of cumulative sampling by molecular dynamics (MD) simulations of unlinked oligomannose N-glycans common in vertebrates. We then discuss the effects of a complex protein surface on their structural equilibria based on over 4 µs cumulative MD sampling of the fully glycosylated CD16a Fc gamma receptor (FcγRIIIa), where the type of glycosylation is known to modulate its binding affinity for IgG1s, regulating the antibody-dependent cellular cytotoxicity (ADCC). Our results show that the protein's structural constraints shift the oligomannoses conformational ensemble to promote conformers that satisfy the steric requirements and hydrogen bonding networks demanded by the protein's surface landscape. More importantly, we find that the protein does not actively distort the N-glycans into structures not populated in the unlinked forms in solution. Ultimately, the highly populated conformations of the Man5 linked glycans support experimental evidence of high levels of hybrid complex forms at N45 and show a specific presentation of the arms at N162, which may be involved in mediating binding affinity to the IgG1 Fc.[1]



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Uncovering novel mechanisms in glycoprocessing enzymes by QM/MM simulations

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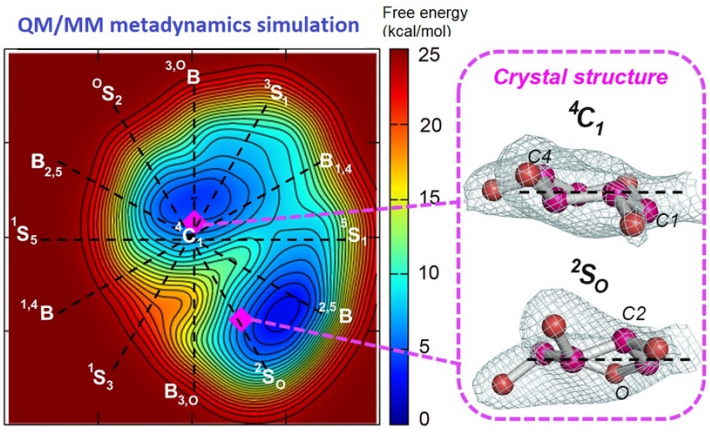
Glycosidases are amongst the most proficient of enzymes, showing typical rate enhancements of up to 10^{17}-fold. Large efforts have been devoted since many decades to uncover their catalytic mechanisms. Most GHs follow the Koshland classical mechanisms for retaining and inverting glycosidases [1] in which two essential carboxylic acid residues catalyze the cleavage of the glycosidic bond. It is also recognized that GHs favor certain distorted sugar shapes or conformations upon binding to the enzyme [2,4]. However, there are notorious exceptions such as GHs following neighboring group participation and GHs with uncommon (e.g. Tyr, Cys) or yet uncharacterized nucleophiles, for which the molecular mechanisms remains elusive. Recently, we have contributed to uncover molecular mechanisms of GHs that either follow non-Koshland mechanisms or challenge the common view of the enzyme recognizing high energy substrate conformations. Here we will report the detailed molecular mechanism of two GHs with uncommon active site nucleophile residues, GH99 endo-α-1,2-mannosidase [5] and GH127 α-L-arabinofuranosidase [6], as well as two exo-acting GHs that, unlike their endo-counterparts, do not require substrate distortion for catalysis, GH59 β-galactocerebrosidase [7] and GH43 exo-oligoxylanase [8]. Our simulations are performed by means of state-of-art simulation techniques such as *ab initio* quantum mechanics/molecular mechanics (QM/MM) metadynamics and are informed by novel structural determinations, providing a detailed atomistic view of enzyme action.



Conformations adopted by the xylopyranosyl ring at the −1 subsite of GH43 exo-oligoxylanase [8].

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Cellulose nanofibers as stabilization agent of a curcuma longa suspension

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The rhizome of curcuma longa L. is used for the extraction of curcumin, a compound that only represents around 3% w/w of the dried rhizome. In order to use the entire rhizome as a food colorant, the development of a curcuma longa suspension was done. Different concentrations of cellulose nanofibers (CNFs) were evaluated (0.1, 0.3, 0.5, 0.7 and 0.9 % w/w). The results established that an increase in the L color coordinate was observed, caused by light scattering, associated with the size of the CNFs. Additionally, we found that the addition of 0.1% w/w CNFs had no effect on the stabilization of the curcuma longa suspension. Additionally, at this concentration, the stabilization index was similar to the suspension without CNFs. This behavior allows us to say that the formation of the three-dimensional network of the nanocellulose at this concentration does not allow the stabilization of the non-solible solids of the suspension. However, as the CNFs concentration increases, the stabilization index grows, and the addition of a 0.9% w/w CNF allowed to obtain a 100 % stable suspension up to 30 days. Finally, the suspensions with CNFs up from 0.3% were characterized by an increment in consistency and higher values of G' than G'', indicating that those suspensions are soft gels.

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Chemical Synthesis and Immunological Evaluation of Novel TACA-αGalCer Cancer Vaccines

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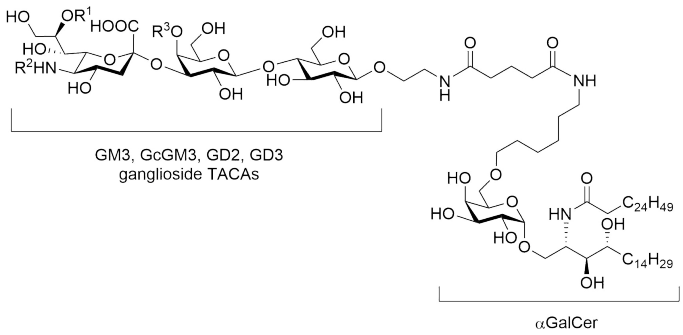
Immunotherapy is revolutionizing cancer therapy by harnessing the power of the innate and adaptive immune system against cancer cells and thus providing a more tumor-selective approach in assistance to traditional treatments.[1] The identification of tumor-associated carbohydrate antigens (TACAs), aberrant glycans decorating the surface of tumor cells, has paved the way for the development of TACA-based cancer vaccines.[2] However, while significant progress has been made, TACA–based cancer vaccines have not yet reached the clinic and addressing some of the limitations that characterize classical approaches in carbohydrate cancer vaccine development can provide access to more effective vaccine candidates. In this context, iNKT cells have emerged as central players in cancer vaccine therapies. Indeed, recent reports have shown that iNKT cell-activating glycolipids, such as α-galactosylceramide (αGalCer), can enhance the immune response against co-delivered cancer antigens, by stimulating iNKT cells to serve as universal T helpers.[3–5] As this strategy appears to be well-suited to break the natural tolerance against TACAs, here we present our synthetic efforts towards the preparation of ganglioside TACAs-αGalCer conjugates, their formulation in liposomes, and their *in vitro* and *in vivo* immunological evaluation. The synthesis relies on the preparation of a suitably functionalized αGalCer moiety, the expeditious synthesis of ganglioside TACAs *via* improved reactions in sialic acid chemistry, and the conjugation of the two components to obtain novel cancer vaccine candidates.



Synthetized TACA-αGC conjugates

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Structural attributes of protein-heparan sulfate interactions: chemo-enzymatic reactions and NMR

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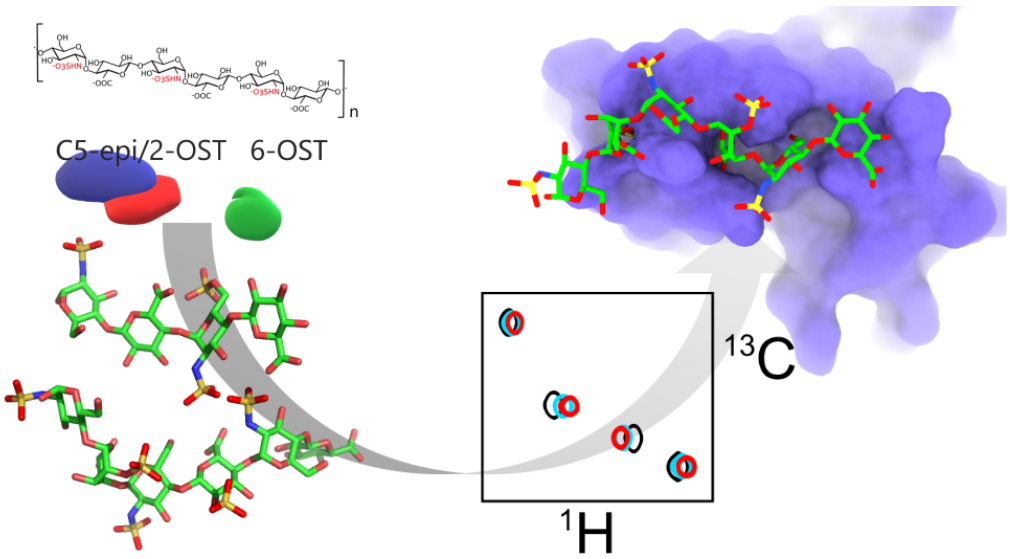
Heparan sulfates (HS) is a polysaccharide found at the cell surface, where it mediates interactions with hundreds of proteins and regulates major pathophysiological processes. HS is highly heterogeneous and structurally complex and examples that define their structure–activity relationships remain limited. Here, to characterize a protein-HS interface and define the corresponding saccharide binding domain, we present a chemoenzymatic approach that generate 13C labeled HS-based oligosaccharide structures. NMR spectroscopy that efficiently discriminates between important or redundant chemical groups in the oligosaccharides, is employed to characterize these molecules alone and in interaction with proteins. Using chemokines as model system, docking based on NMR data on both proteins and oligosaccharides enable the identification of the structural determinant involved in the complex. This study shows that both the position of the sulfo-groups along the chain and their mode of presentation, rather than their overall number, are key determinant and further points out the usefulness of these 13C labeled oligosaccharides in obtaining detailed structural information on HS-protein complexes.



Chemo-enzymatic synthesis of HS oligosaccharides and analysis of their interaction with proteins by NMR

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Investigating the spatial geometry organization within glycoside hydrolases over catalysis

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To deal with the chemical and structural complexity of the plant cell walls (PCWs), biomass-degrading microorganisms produce a vast array of glycoside hydrolases (GHs), some of which are even organized in a large and multi-enzyme membrane-bound complexes, such as cellulosomes. Among the determinants governing the mode of action of GHs on polysaccharides within PCWs, the role played by spatial organization is remaining as poorly understood. We investigate this question by using the Molecular Welding Tool composed of the two small protein Jo and In [1], which spontaneously create an intramolecular isopeptide bond and, incidentally, provide an original means of orientating GH (Fig.1). Firstly, we modulate the distance between GHs immobilized on solid surface and secondly we create chimeric multi-modular GHs, including non-catalytic Carbohydrate-Binding Module (CBM) [2-4]. A large array of complementary technics including Small Angle X-rays Scattering allows us to correlate distance and/or spatial orientation of GHs to i) a tendency of immobilized GH to release preferentially shorter oligosaccharides as compared to free GH, ii) modulate the percentages of xylooligosaccharides produced by different chimeric GHs compared to the catalysts free in solution or, iii) specific spatial arrangements in GH-CBM chimeric enzymes favour activity on soluble polymeric substrates which does not predict the behavior on insoluble PCWs. Our results question the relationship between spatial proximity and synergistic effect as encountered in natural enzymatic systems.

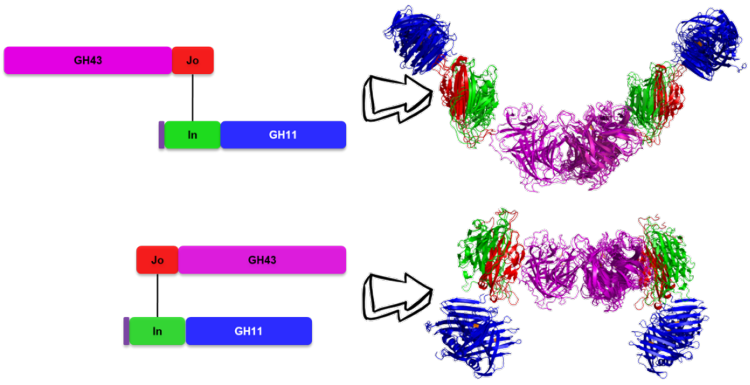

Figure 1: SAXS models of corresponding chimeric GH.

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Enzymes depolymerizing the GAG-mimetic exopolysaccharide GY785 are produced during its biosynthesis

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Some bacterial exopolysaccharides (EPS) are alternative molecules to glycosaminoglycans (GAGs) which are essential in the regulation of animal cell properties and function. Low molecular weight (LMW) and over-sulfated derivatives of the GY785 EPS produced by *Alteromonas infernus* exhibit biological activities similar to GAGs and are valuable in different therapeutic applications [1-4]. These derivatives are currently obtained by chemical methods that can modify the original molecular structure, resulting in a loss of the structural integrity of the derivatives. The use of enzymes for the depolymerization step is an attractive alternative strategy to preserve the original structural features. However, commercial enzymes were unable to depolymerize the GY785 EPS, which presents a very atypical structure. Recently, enzymes that break down the GY785 EPS glycosidic chains have been identified in *A. infernus* cell extracts [5,6]. Various chemical bonds are cleaved during the depolymerization demonstrating the presence of sulfatase, glycoside hydrolase and polysaccharide-lyase activities in the extracts. Results showed that LMW derivatives can be obtained at the laboratory level using crude protein extracts, prepared only from bacterial cells grown on a glucose-supplemented medium. These growth conditions were also required for EPS production suggesting that the biosynthesis of the enzymes is linked to the EPS production. GY785 EPS features and current knowledge of the extracted enzymes will be presented. The overall aim is to develop an optimized depolymerization process

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Nano- and biotechnological approaches to potentiate the immunogenicity of glycoconjugate vaccines

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Bacterial capsular glycoconjugate vaccines have proven efficient, well tolerated and safe in humans including young children and immune-compromised population.1 Current licensed glycoconjugate vaccines are made of poly/oligosaccharides conjugated to a carrier protein which triggers a T-cell dependant immune response to the glycan owing to the activation of the T-helper cells.

The nature of the sugar antigen, its length, its ratio per carrier protein, the conjugation chemistry and the sites at which the carbohydrate antigen is attached impact on both structure and the immune response. In addition, it has long been assumed that formulation, noticeably the particulate nature of a vaccine, can also have an impact.

Considering pneumococcal infection as a model disease [2], we show that combination of site selective mutagenesis or unnatural amino acid incorporation and click chemistries can lead to homogeneous glycoconjugates useful to study structure/immunogenicity relationships [3,4]. We also demonstrate that nanotechnological approaches such as using virus-likeparticles as carrier [5] or glycoconjugate encapsulation into chitosan nanoparticles can improve humoral response by several order of magnitudes compare to glycoconjugate administered alone.

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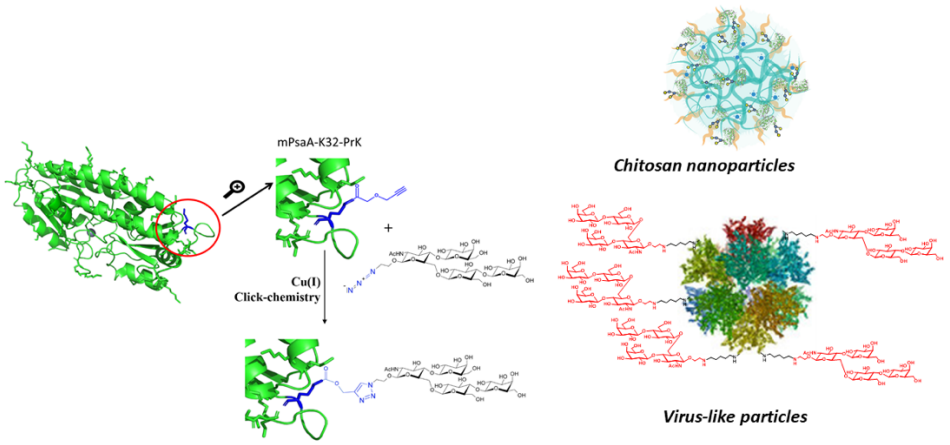

Homogeneous pneumococcal glycoconjugate using combined unnatural amino acid incorporation and click chemistry (left); nanoparticle glycoconjugate vacc

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The Discovery of Lectins from Fungal Pathogens as a Strategy to Identify New Pharmacological Targets

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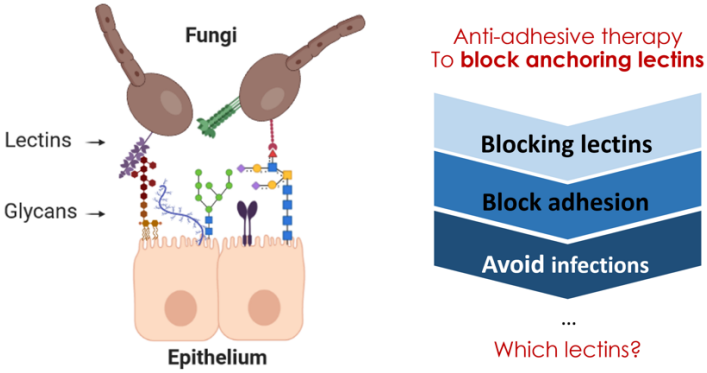
Since lectins are involved early stages of infections, a new therapeutic approach has suggested their inhibition by carbohydrate mimics. Nevertheless, fungal lectins have been poorly studied and their identification reports are scarce compared to those from other organisms. This represents a problem for human life-threatening species such as Aspergillus fumigatus and Scedosporium aspiospermum. Here, we report the identification and characterization of lectins from those pathogens through 2 different strategies;

1. i) In silico prediction of hypothetical lectins by data mining
2. ii) Identification of lectins from fungal extracts

Two new lectins were identified from S. apiospermum by data mining, SapL1: a fucose- binding lectin putatively involved in lung epithelium adhesion and SapL6: a Cyanovirin- like lectin. Those proteins were produced recombinantly in bacteria. Their specificity and affinity were evaluated by glycan array and isothermal titration calorimetry (ITC), and their structure was solved by X-ray crystallography. Affinity chromatography purifications allowed us to recover potential lectins from crude extracts of A. fumigatus and S. aspiospermum. Those were examined by mass spectrometry and the analysis is still in process, but, to date we have identified 4 carbohydrate-binding proteins with potential applications. We expect this study will contribute to a better understanding of glycosylated surface recognition by those pathogens and inspire design targeted therapies.



Of Language Models and Graphs – How Machine Learning can Advance Glycobiology

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As the most diverse (and only non-linear) biological sequence, glycans offer a plenitude of challenges to conventional analysis workflows. Yet, at the same time, this very complexity also implies that glycans carry substantially more information – and associated mechanistic insights – than other biopolymers. Flexible analysis modalities stemming from machine learning, the automatic extraction and utilization of patterns and information from complex data, are perfectly suited to leverage this sophisticated information to link functions to glycan sequences and motifs.

Here, we describe the development and application of state-of-the-art deep learning techniques to glycobiology. Using language models and graph neural networks, we present generalizable and highly accurate sequence-to-function models that can – only using a glycan sequence – predict the taxonomic group a glycan stemmed from, the immunogenicity of glycans, their contribution to bacterial pathogenicity, and their binding to viral proteins. We further demonstrate that our models have learned highly informative representations of glycans that can be used to visualize glycan clusters and aid in the identification of overall trends. Additionally, simple linear models can be applied to these learned representations to achieve competitive results in the prediction of glycan properties.

We envision that our extremely data-efficient deep learning models will contribute toward elucidating sequence-to-function relationships in glycans and accelerate as well as advance the analysis of emerging glycomics efforts.

Glycan-Gold Nanoparticles as Multifunctional Probes and Viral Inhibitors for DCSIGN/R-Glycan Binding

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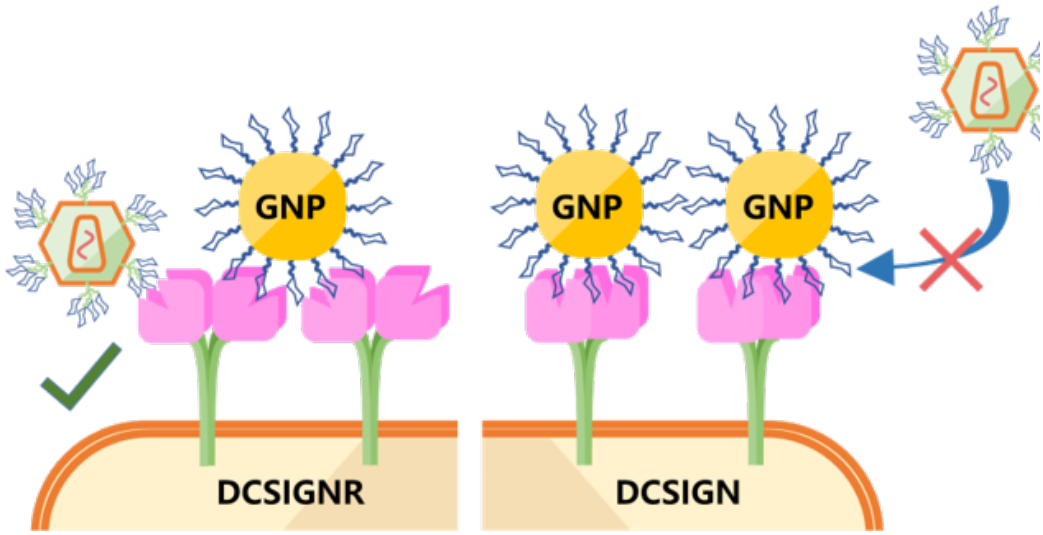
Multivalent lectin-carbohydrate interactions are widespread and critically important in biology. Viruses can display arrays of specific glycans and bind multivalently to multiple multimeric lectins on cell surfaces to enhance binding affinity, leading to cell internalisation. Understanding the underlying structural mechanisms is thus critical to develop specific, multivalent glycan inhibitors that can block such interactions and prevent infection.[1] To illustrate this we chose two closely-related tetrameric lectins, DC-SIGN [2] and DC-SIGNR,[3] which play a key role in facilitating the HIV and Ebola Virus infections. Herein, we report the development of densely glycosylated gold nanoparticles (glycan-GNPs) as multifunctional structural and mechanistic probes for DC-SIGN/R-glycan interactions. We have developed a new fluorescence quenching method for DC-SIGN/R-glycan-GNP binding affinity quantification, revealing that glycan-GNP binding with DC-SIGN can be >1 million-fold stronger than the corresponding monovalent binding. We also show that binding to DC-SIGN gives isolated GNPs but binding to DC-SIGNR produces aggregated GNPs, revealing they have different binding modes.[4] Furthermore, we find that increasing GNP surface glycan density (via dendritic glycan coating) gives significantly enhanced affinity with DC-SIGNR in contrast to DC-SIGN. Finally, we report the glycan-GNPs potently inhibit pseudo-Ebola virus infection of DC-SIGN positive cells (IC50 ~95 pM), placing it as one of the most potent glycoconjugate inhibitors against DC-SIGN-driven Ebola virus infections.[5]



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Synthetic studies on rare amino sugars and zwitterionic Shigella sonnei oligosaccharides

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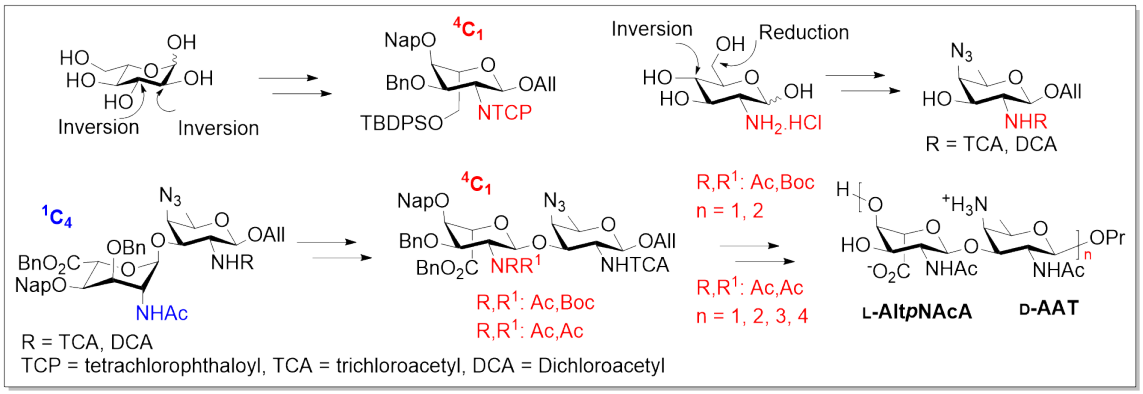
Shigellosis is a major public health burden worldwide and one of the top four causative agent of diarrheal disease in children under 5 years. Fighting against shigellosis, especially S. sonnei, by means of vaccines is envisioned as one of the most cost-effective strategies to reduce burden. A Phase III clinical study with a detoxified lipopolysaccharide-conjugate vaccine was found safe and immunogenic in adults and children older than 3 years.[1] A synthetic carbohydrate-based conjugate vaccine candidate (SF2a-TT15), developed in our laboratory, was shown to be immunogenic against S. flexneri type 2a in adult volunteers.[2] The strategy has been extended to S. sonnei as a possible alternative to the use of the detoxified lipopolysaccharides.

The S. sonnei surface polysaccharide is composed of a zwitterionic disaccharide repeating unit, made of two rare aminodeoxy sugars, a 2-acetamido-2-deoxy-L-altruronic acid (L-AltpNAcA) and a 2-acetamido- 4-amino-2,4,6-trideoxy-D-galactopyranose (AAT).[3] The communication will discuss (i) a novel synthetic strategies to rare aminosugars, including AAT, with emphasis on the versatile scaffold serving as key building block, and (ii) the influence of the N-protection pattern on both the conformation and the reactivity of the flexible L-AltpNAc and L-AltpNAcA moieties within mono- and disaccharide building blocks and (iii) the overall protecting group selection and the successful convergent synthesis of tetra-, hexa- and octasaccharides as portions of the S. sonnei O-specific polysaccharide.[4]



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A Library of Synthetic Doxorubicin Derivatives for Improved Anticancer Treatment

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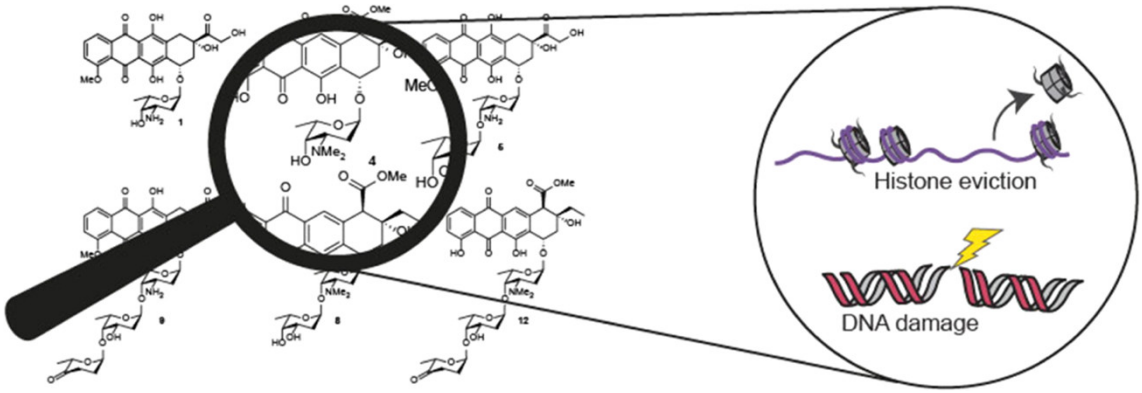
The anthracycline anticancer drug Doxorubicin has been used in the clinic for several decades to treat various cancers and is prescribed to about a million patients every year. However, cumulative cardiotoxicity, is a treatment-limiting side effect of this compound and greatly affects life prospects especially for young, weak and old patients.

For a better understanding of the structure–function relationship of this drug, we synthesised a library of derivatives, in search of more potent and less cardiotoxic variants. These includes structural hybrids between doxorubicin and structurally related trisaccharide anthracycline aclarubicin [1], stereoisomers of (N,N)-dimethyldoxorubicin [2] and a wide range of various other modifications. These saccharides were prepared using IDCP-mediated glycosylation for the sugar chains and Yu's gold-catalyzed alkynylbenzoate glycosylation for attachment to the different aglycones.

We evaluated the capacity of these compounds to induce their two main biological activities: DNA breaks and histone eviction, as well as their cytotoxicity against various human tumor cell lines. Our findings underscore that histone eviction alone, rather than DNA breaks, contributes strongly to the overall cytotoxicity of anthracyclines, and structures containing N,N-dimethylamines are more cytotoxic than their non-methylated counterparts. Finally, N,N-dimethyldoxorubicin showed to be effective against acute myeloid leukemia in living mice without inducing any cardiotoxicity; a very promising discovery for anticancer treatment. [3]



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Synthesis of a trivalent glycocluster containing thiodisaccharides related to heparan sulfate

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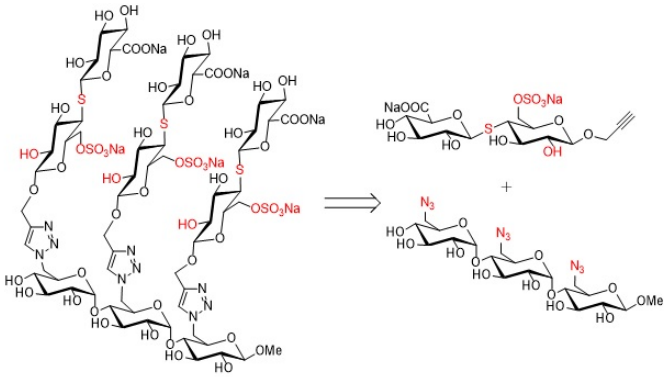
Glycosaminoglycans (GAGs) are involved in the regulation of a large number of biological processes such as inflammation, cell signalling, angiogenesis, viral infection and coagulation [1]. Heparin and heparan sulfate (HS) share the same basic structure, IdoA/GlcA-(1-4)-GlcNAc/GlcNS. Sulfate groups can be located at O-2 of uronic acid residues, at O-6 and/or O-3 of glucosamine units. Heparin is more sulfated than HS and its main uronic acid is IdoA, whereas it is GlcA for HS. Unlike molecules isolated from tissues, pure molecules, derived from organic synthesis, can prevent side effects [2,3] and are very useful tools for understanding the structure-activity relationships of many biological and pharmacological activities.
In our research group, we focus particularly on the synthesis of multivalent thioglycoside analogs. Recently, we developed the synthesis of a thiodisaccharide unit, S-analog of heparane sulfate. After modifying the aglycone part to introduce an azide, the thiodisaccharide was coupled to maltotriose scaffolds carrying one, two or three propargyl groups by CuAAC, affording one monovalent, two different divalent and one trivalent derivatives [4]. Against all expectations, these compounds have shown interesting anti-inflammatory activities.
We report herein the synthesis of a new glycocluster with a sulfated thiodisaccharide unit, S-analog of heparane sulfate. First, the synthesis of a new sulfated thiodisaccharide analog of the HS sequence was performed. For this, a new synthesis strategy is used. All the secondaries hydroxyls are protected with benzoyl groups



Scheme 1: Retrosynthetic approach for the glycocluster preparation.

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New Lipidyl-Cyclodextrins Obtained by Ring Opening of Methyl Oleate Epoxide using Ball Milling

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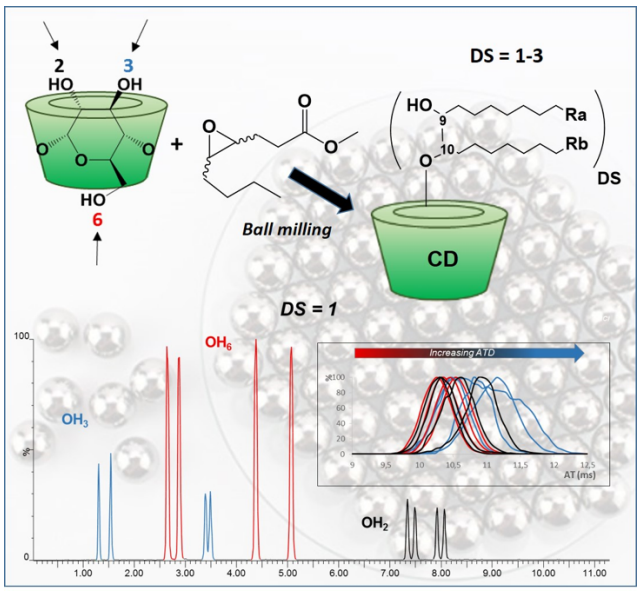
New Lipidyl-CDs derivatives were synthesized using a fatty ester epoxide by means of alternative methods (solvent-free techniques, use of enzymes). The ring opening of methyl oleate epoxide using ball milling leads to easy preparation of bicatenary amphiphilic CDs of low DS. This reaction seems to be highly specific of cyclodextrins in solventless conditions. Furthermore, enzymatic hydrolysis of the terminal ester gives the corresponding carboxylic acid analogs with very good yields and allowing a modulation of the hydrophilicity of the compounds. In our conditions, the coupled reactions can be carried out on free and modified CDs with good efficiency. Despite the low and controlled DS, the final products are obtained in the form of complex mixtures requiring an extensive structural analysis using mainly mass spectrometry and NMR spectroscopy. The powerful IMS-MS technique has been demonstrated in this work to be an effective tool for a better understanding of complex isomer mixtures.

In addition, as part of their use as vectors of active ingredients, these amphiphilic compounds were submitted to a integrity study on an in vitro model of the Blood-Brain-Barrier (i.en the BLEC model) and the intestinal epithelium (Caco-2 model). No toxicity has been demonstrated, suggesting that applications for the vectorization of active ingredients or drugs can be expected. Finally, the properties of these amphiphilic compounds in aqueous biphasic catalysis were also evaluated.



New derivatives of cyclodextrins synthesized by ball-milling conditions and totally deciphered by an structural analysis

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E. Oliva, D. Mathiron, S. Rigaud, E. Monflier, E. Sevin, H. Bricout, S. Tilloy, F. Gosselet, L. Fenart, V. Bonnet, S. Pilard, F. Djedaini-Pilard, (2020), Biomolecules,10(2), 339-357.

Expansion of synthetic glycobiology tools: Altering the donor specificity of a glycosytransferase

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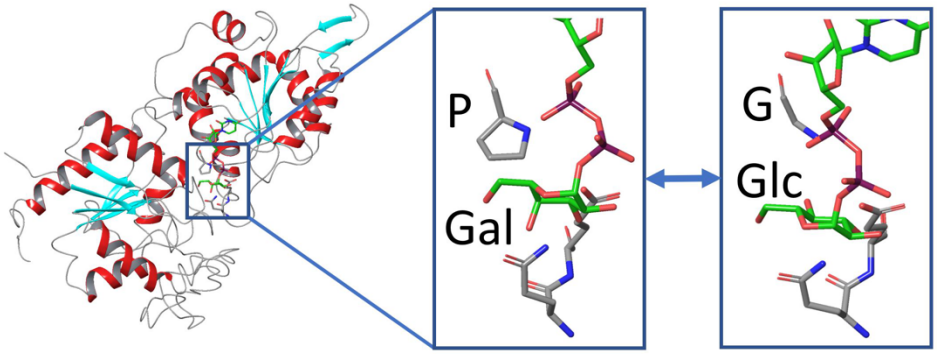
In the context of synthetic glycobiology, Glycosyltransferases (GTs) can be used as tools to glycosylate membrane models such as giant unilamellar vesicles in order to study the glycocalyx. However, due to the challenges in characterization, there is a limited number of available GTs. By changing the sugar donor specificity of existing GTs, we can further expand our library of tools.

MGD1 is a galactosytransferase and its activity to directly glycosylate diacylglycerol (DAG) in a membrane makes it an ideal model for changing the donor specificity from UDP-Galactose to UDP-Glucose. The crystal structure of MGD1 was previously solved and the key residues for donor specificity are identified to be P433, Q455 and E456, aptly named the PQE motif.

By utilising rational protein design based on the crystal structure and comparison of the equivalent 'PQE' motif in closely related glucosyltransferases, a number of mutants were engineered. Activity was detected using a bioluminescence detection assay. A double mutation, P433G and G434A, was able to confer glucosyltransferase activity. Presently the aim is to increase this glucosyltransferase activity to a level equivalent to its galactosyltransferase activity. With the methodology used in this study, similar approaches can be made for other GTs.



MGD1 – Double mutation P433G and G434A confers glucosyltransferase activity

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Non-Carbohydrate Glycomimetics as Inhibitors of Calcium(II)-binding Lectins

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Because of the antimicrobial resistance crisis, lectins are considered novel drug targets. Pseudomonas aeruginosa utilizes LecA and LecB in the infection process. Inhibition of both lectins with carbohydrate derived molecules can reduce biofilm formation to restore antimicrobial susceptibility. Here, we focused on non-carbohydrate inhibitors for LecA to explore new avenues for lectin inhibition. From a screening cascade we obtained one experimentally confirmed hit, a catechol, belonging to the well-known PAINS compounds.

Rigorous analyses validated electron-deficient catechols as millimolar LecA inhibitors. The first co-crystal structure of a non-carbohydrate inhibitor in complex with a bacterial lectin clearly demonstrates the catechol mimicking the binding of natural glycosides with LecA. Importantly, catechol 3 is the first non-carbohydrate lectin ligand that binds bacterial and mammalian calcium(II)-binding lectins, giving rise to this fundamentally new class of glycomimetics.

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Towards the elucidation of the Golgi xylosylation steps in the microalga Chlamydomonas reinhardtii

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Although glycosylation pathways are well described in vertebrates, plants or insects, the knowledge concerning microalgae remains scarce. In the last years, we initiated a pioneer work to decipher the N-glycosylation pathway in the green microalga C. reinhardtii. Mass spectrometry analyses carried out on secreted and membrane-bound proteins showed that mature N-glycans are partially O-methylated Man3GlcNAc2 to Man5GlcNAc2 substituted by one or two xylose (Xyl), and for a minor part by one fucose (Mathieu-Rivet et al., 2013). One Xyl was demonstrated to be transferred by the xylosyltransferase A (XTA) on the core β(1,2)-mannose. Furthermore, another xylosyltransferase candidate named XTB was suggested to be involved in the xylosylation process. Recently, we focused especially on the structural characterization of the xylosylated N-glycans taking advantage of insertional mutants of XTA, XTB, and of the XTA/XTB double-mutant. The combination of mass spectrometry approaches allowed us to identify the major N-glycan structures bearing one or two Xyl. They confirm that XTA is responsible for the addition of the core β(1,2)-Xyl, whereas XTB is involved in the addition of the Xyl onto the linear branch of the N-glycan as well as in the partial addition of the core β(1,2)-Xyl suggesting that this transferase exhibits a low substrate specificity. Finally, the presence of residual xylosylated structures in the double-mutant suggests that an additional XT is involved in the xylosylation process (Lucas et al., 2020).

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Stereoselective β-Mannosylations and β-Rhamnosylations from Glycosyl Hemiacetals mediated by LiI

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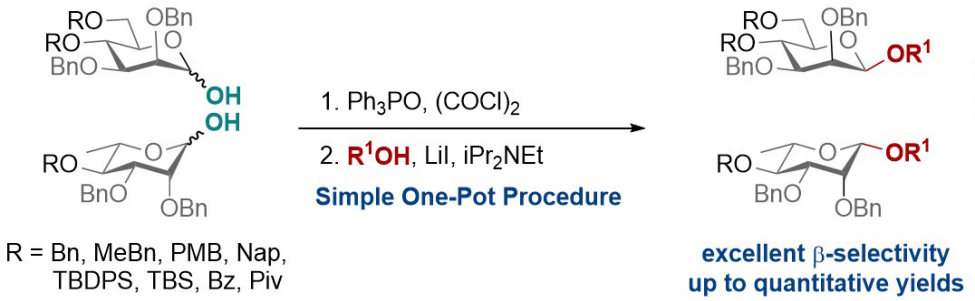
Stereoselective β-mannosylation is one of the most challenging problems in the synthesis of oligosaccharides. Herein, a highly selective synthesis of β-mannosides and β-rhamnosides from glycosyl hemi-acetals is reported, following a one-pot chlorination, iodination, glycosylation sequence employing cheap oxalyl chloride, phosphine oxide and LiI. The present protocol works excellently with a wide range of glycosyl acceptors and with armed glycosyl donors. The method doesn't require conformationally restricted donors or directing groups; it is proposed that the high β-selectivities observed are achieved via an SN2-type reaction of β-glycosyl iodide promoted by lithium iodide.



- Directing groups or restrained donor conformations not required
- Broad donor and acceptor scope
- 29 examples
- Cryogenic conditions not required

Microwave-assisted Acceleration of Automated Glycan Assembly

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Microwave heating has tremendously accelerated solid phase peptide synthesis. Strict control of the low temperature condition provides for a consistent and successful synthesis, albeit with a significant time and power consumption drawbacks, yet auxiliary steps of the synthetic cycle require elevated temperatures. Incorporating a synergistic relationship of microwave heating and constant cooling into an AGA instrument allowed for rapid temperature from -30 °C to +100 °C, such that the synthesis is accelerated by a factor of two. The addition also permitted chemical transformations previously impossible or not practical for AGA. These new possibilities include: 1) rapid sulfation, 2) expanded temporary group portfolio, 3) preparation of oligosaccharides with up to four branches, and 4) obtention of unprotected glycan from solid support.

A rapid synthesis of low-nanomolar divalent LecA inhibitors in four linear steps from D-galactose

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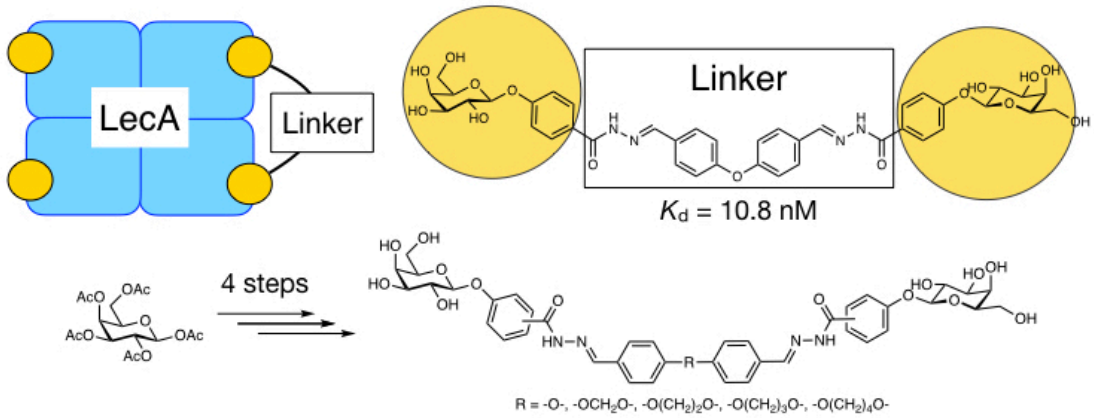
Chronic infections with P*seudomonas aeruginosa* are associated with the formation of bacterial biofilms. The tetrameric *P*. aeruginosa lectin LecA is a virulence factor and an anti-biofilm drug target. Increasing the overall binding affinity by multivalent presentation of binding epitopes can enhance the weak carbohydrate–ligand interactions. Low-nanomolar divalent LecA ligands/inhibitors with up to 260-fold valency-normalized potency boost and excellent selectivity over human galectin-1 were synthesized from D-galactose pentaacetate and benzaldehyde-based linkers in four linear steps.



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Synthetic plant glycan arrays: new tools for elucidating cell wall biosynthesis

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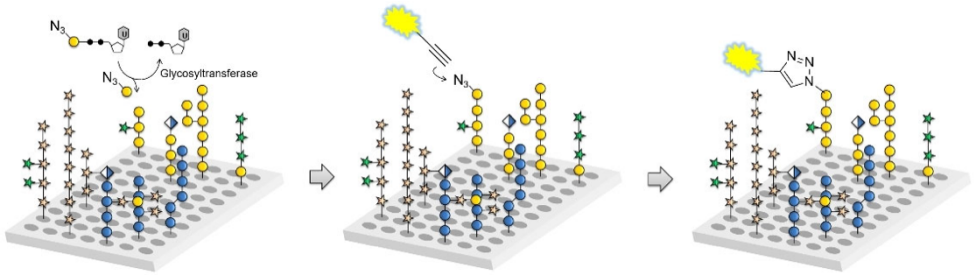
Plant cells are surrounded by a polysaccharide-rich matrix that constitutes the cell wall of all higher plants and provides the richest available resource of fermentable carbohydrates and bio-based materials. Optimal exploitation of this resource requires investigations into the molecular structures, interactions, and biosynthesis of cell wall glycans, which are aided by well-defined and pure glycan samples obtained through chemical synthesis. We have prepared oligosaccharides derived from different classes of cell wall glycans and printed them as microarrays to characterize glycosyltransferases that are involved in plant cell wall biosynthesis. The glycan arrays were incubated with azido-functionalized sugar nucleotide donors and putative glycosyltransferases to enable product detection on chip via click reaction with an alkynyl-modified dye. New glycosyltransferases were identified and reported glycosyltransferase activities corrected or defined more precisely.



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Development of a biphasic glycosyltransferase high-throughput assay

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Many small molecule natural products are decorated with sugar moieties that are essential for their biological activity. A significant number of natural product glycosides and their derivatives are clinically important therapeutics. These natural product glycosides are biosynthesized by the action of glycosyltransferases (GTs). Many natural product GTs have relaxed substrate specificity that can make them valuable biocatalytic tools for altering glycosylation or glycodiversification. The success of glycodiversification greatly depends on the screening method. Towards this aim we have developed a screening tool for assaying GTs in a high-throughput fashion enabled by rapid isolation and detection of chromophoric or fluorescent glycosylated natural products.

Using our novel high-throughput assay, we screened a library of natural product GTs against a panel of precursors to therapeutically important molecules. Two GTs known to prefer flavonoid acceptors showed substrate promiscuity towards anthraquinones. Interestingly, these two enzymes catalyzed the synthesis of novel ε-rhodomycinone glycosides. Considering the pharmaceutical value of the clinically used anthraquinone glycosides that are biosynthesized from an ε-rhodomycinone precursor, and the significance that the sugar moiety has on the biological activity of these drugs, our results are of particular importance towards the glycodiversification of therapeutics in this class. The GTs identified through our strategy and the novel compounds that they produce show promise as biocatalytic tools and new therapeutics.

Lipopolysaccharides from Gut Commensal Bacteria: Structure and Immunomodulatory activity

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Lipopolysaccharides (LPS) from gut commensal bacteria trigger immunomodulatory responses on the basis of their structures. However, only a few gut commensal LPS have been structurally elucidated so far. Therefore, the molecular motifs crucial for LPS–host interactions at the gut level remain obscure. In this communication, I will focus on the LPS of two commensals of the human intestine: Bacteroides vulgatus and Alcaligenes faecalis. I will show that B. vulgatus LPS does not induce proinflammatory cytokines release and that its administration is enough to reestablish intestinal immune homeostasis in a mouse model for experimental colitis. The LPS structural characterization revealed an unprecedented structure based on a hypo-acylated and mono-phosphorylated lipid A, a galactofuranose-containing core OS, and an O-antigen built up of mannose and rhamnose.1,2 In addition, to this particular structure corresponds an intriguing ability, in human in vitro models, to produce antiinflammatory cytokines and to induce the synergistic activation of TLR4- and TLR2-mediated signaling pathways. As for A. faecalis, this is the sole Gram-negative inhabiting gut lymphoid tissues, Peyer's patches (PPs), which are the largest sites for the initiation and regulation of intestinal IgA responses. We have previsouly shown that Alcaligenes LPS is able to maintain a homeostatic environment in PPs, without triggering any harmful response.3 Here I will show that also A. faecalis LPS has an unreported structure whose lipid A has been synthesized and its immunological properties also investigated.4

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Automatization and Self-Maintenance of the O-GlcNAcome catalogue: A Smart Scientific Database

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O-GlcNAcylation is a post-translational modification widely found in proteins across phyla. It consists of the addition of β-N-acetylglucosamine to the hydroxy group of serine or threonine residues. O-GlcNAcylation modulates a myriad of biological processes with more than 5000 O-GlcNAcylated proteins reported in human to date. We recently compiled and published the O-GlcNAc Database (www.oglcnac.mcw.edu) [1], which provides the scientific community with a place to browse all the O-GlcNAcylation data published. This O-GlcNAcome catalogue contains more than 12000 proteins and 9000 O-GlcNAcylation sites from 36 organisms. We provide protein-digest tools, an advanced search mode to match large experimental datasets and we make all content downlabable in multiple formats. We match each O-GlcNAc site against UniProtKB protein sequences (including isoforms) prior to to validate the data we provide. The review and integration of new literature items is accelerated using Machine Learning and Natural Language Processing methods. From a list of frequent expression patterns we described each item with binary values and trained a Neural Network classifier to predict whether one item contains O-GlcNAcylated protein-identification information. Detailed reports are generated and include prediction results, keywords and relevant sentences. Overall, we developed a user-friendly interface and admin-friendly system mostly administrated through automated pipelines. We hope the O-GlcNAc Database will be both a useful resource for the field, and an inspiring framework for scientific developers.

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Synthesis of dimeric Lewis X fragments and their binding to anti-dimLex monoclonal antibodies.

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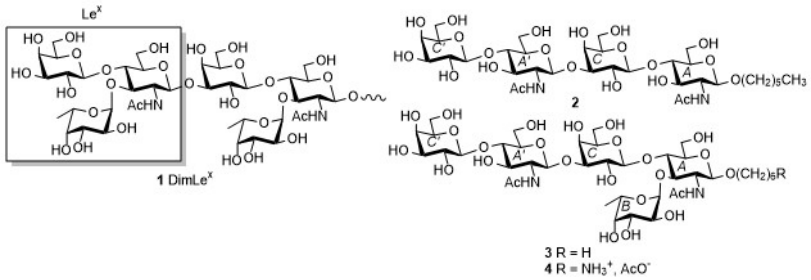
Aberrant glycosylation of tumor cell surface is a universal hallmark of cancer pathogenesis. Several clinical and preclinical studies have shown that antibodies raised in response to tumor- associated carbohydrate antigens (TACAs) can eliminate tumor cells. TACA, dimeric Lewis X (dimLe^x, 1), has been reported to accumulate in colonic and liver adenocarcinomas.[1] Although dimLe^x is tumor specific, it has been shown that the Le^x antigenic determinant expressed at the non-reducing end of dimLe^x, was also displayed at the surface of non-cancerous cells.[1] However, mAbs (SH2, 1G5F6) raised against polyfucosylated type 2 chain oligosaccharides were found to have higher affinity for polymeric Le^x structures than monomeric Le^x.[1-2] Such findings suggest that dimLe^x displays internal epitopes that do not involve the Le^x trisaccharide and could be used for the development of dimLe^x-based cancer immunotherapeutics. In this context, we will describe the preparation of tetra- and pentasaccharide fragments 2-4, which lack the non-reducing end Le^x trisaccharide. Pentasaccharide 4 was conjugated to BSA and used in ELISA titrations to probe the binding specificity of mAbs 1G5F6 and SH2.[3] We will also present our results mapping the epitopes of mAbs 1G5F6 and SH2 using various fragments and analogues of Le^x and dimLe^x.[4]



Dimeric Lewis X and fragments synthesized

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Synthetic, sugar-based Toll-like Receptor 4 (TLR4) modulators: new drug leads targeting infectious and inflammatory diseases

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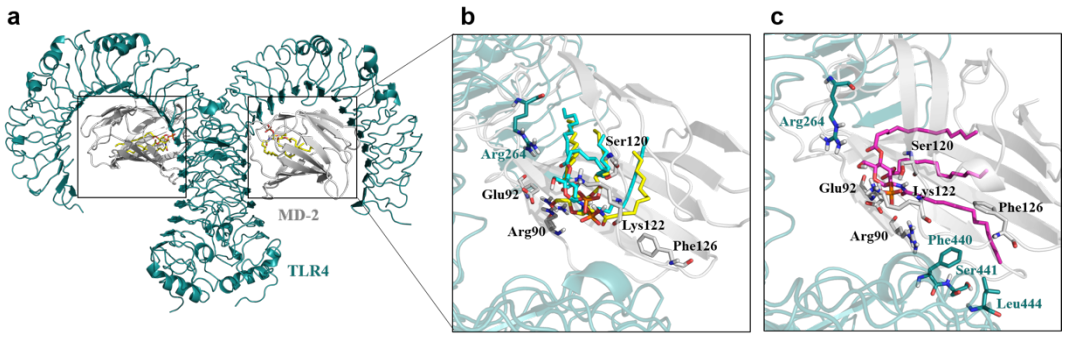
Toll-Like Receptor 4 (TLR4) is one of the receptors of innate immunity, it is activated by Pathogen- and Damage-Associated Molecular Patterns (PAMPs and DAMPs). Mild TLR4 stimulation by non-toxic molecules resembling its natural agonist (lipid A), provided MPLA, a clinically approved vaccine adjuvant. TLR4 excessive activation by Gram-negative bacteria lipopolysaccharide (LPS) leads to sepsis, while TLR4 stimulation by DAMPs is a common mechanism in several inflammatory and autoimmune diseases. TLR4 inhibition by small molecules and antibodies could therefore provide access to innovative therapeutics targeting sepsis, acute and chronic inflammations.[1] TLR4 antagonists can also block the violent inflammation and cytokine storm caused by several viral diseases, including COVID-19. The most recent achievement of our group in the development of synthetic, glycolipid-based TLR4 agonists and antagonists and their preclinical development will be presented. New synthetic, monosaccharide- based TLR4 agonists that potently stimulate innate immunity and are in preclinical development as adjuvants in antiviral and antibacterial vaccines and antagonist[2] that efficiently block inflammation in cell models and in animal models of sepsis, influenza virus lethality,[3] vascular inflammations,[4] neuroinflammations,[5] and inflammatory bowel diseases (IBDs).[6]



Computer-assisted docking of synthetic monosaccharides active as TLR4 modulators to the MD-2/TLR4 dimer

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Selective exoenzymatic labeling of sialic acid on the lipooligosaccharide of Neisseria gonorrhoeae

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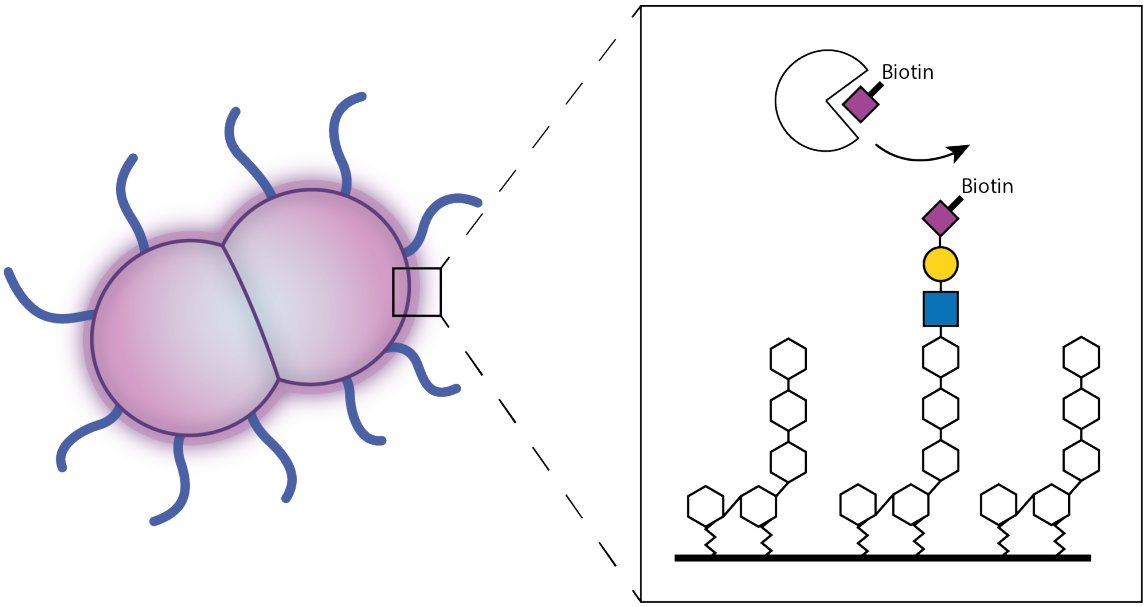
Selective exoenzymatic labeling (SEEL) is a glycoengineering technique that allows for the labeling of specific glycans on the cell surface with chemical reporters. SEEL uses an exogenous recombinant glycosyltransferase to install either a natural monosaccharide or a monosaccharide modified with a chemical reporter, such as a biotin. In our group SEEL has been successfully applied on mammalian cells to identify glycoproteins (a). Here, we report, to our knowledge for the first time, the application of SEEL on bacteria. We have introduced a sialic acid with either an azide or biotin on the lipooligosaccharide of Neisseria gonorrhoeae, which is a relevant human pathogen that is known to decorate itself with sialic acid to evade the host's immune system (b). Through SEEL we were able to introduce a chemical reporter on the lipooligosaccharide of the bacterial cell surface in both un unnatural alpha-2,6-linked sialic acid as the native alpha-2,3-linked sialic acid. We are excited to report our findings on the application of SEEL on Neisseria gonorrhoeae and discuss the future possibilities of this new glycoengineering technique to study bacterial glycobiology.



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N-glycosylation blocks and simultaneously fosters different receptor-ligand binding sites: The cham

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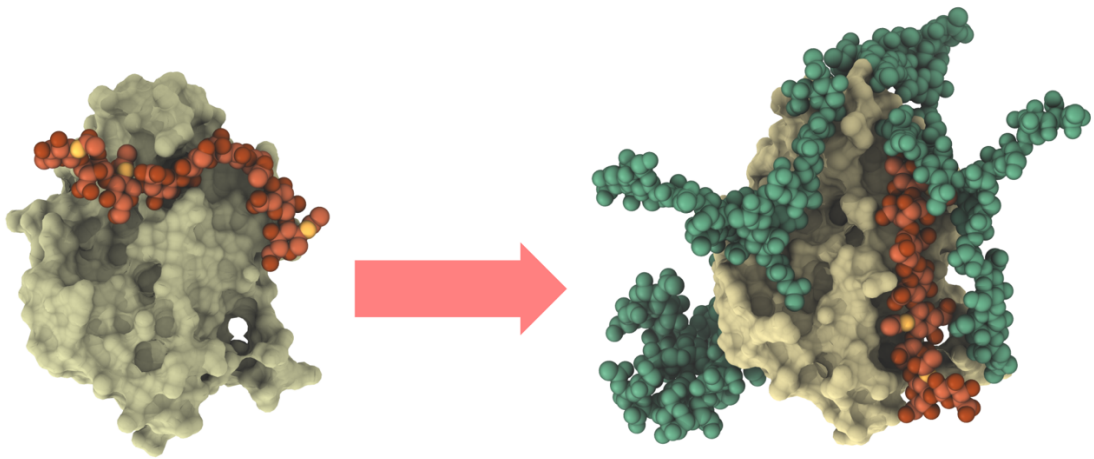
While DNA largely encodes protein structure and function, proteins are also modulated by a complementary and often invisible layer of information encoded by glycans attached to the protein surface. The CD44-hyaluronan complex involved in inflammatory responses and cell migration is a prime example of glycans' significance. CD44 cell surface receptor's ability to bind its ligand, hyaluronan, is modulated by N-glycosylation. Intriguingly, how glycans can regulate the binding process, and the activation of CD44 has remained unclear. In this work, based on massive atomistic simulations and NMR, we provide evidence that CD44 has multiple distinct binding sites for hyaluronan [1] and that N-glycosylation modulates their mutual roles[2]. While non-glycosylated CD44 is found to favor the canonical sub-micromolar binding site, glycosylated CD44 binds hyaluronan with an entirely different micromolar binding site. Our findings show for the first time how glycosylation can alter receptor affinity by shielding specific regions of the host protein, thereby promoting weaker binding modes. The mechanism revealed in this work emphasizes the importance of glycosylation in protein function and constitutes a challenge for protein structure determination where glycosylation is typically not observed.



Change biding site of hyaluronan to CD44 upon glycosylation.

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Determining the reactivity of the acceptor in the glycosylation reaction

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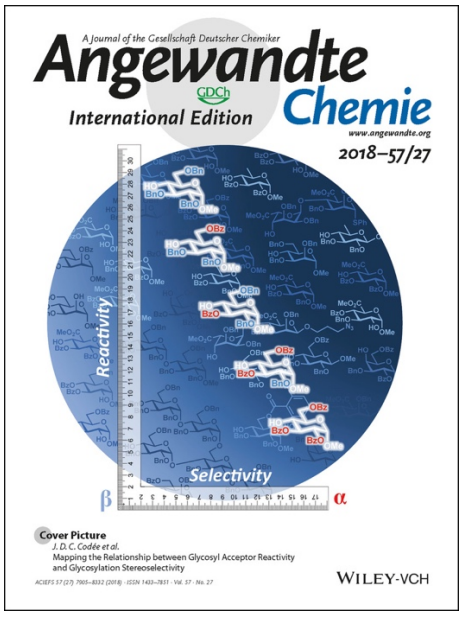
The union of a glycosyl donor and acceptor is arguably one of the most important reactions in the synthesis of larger carbohydrates. However, despite multiple decades of research, stereoselective and high yielding glycosylations remain challenging, time consuming and often require careful tuning of reaction conditions. A key factor in determining the outcome of the glycosylation reaction is the reactivity of both the donor and the acceptor. The reactivity of glycosyl donors is well documented, countless relative reactivity values (RRV) have been determined and anomeric triflates and other reactive species have been characterised. The reactivity of the acceptor on the other hand, is less well understood and systematic studies in this subject are quite scarce. Recently, we have found that the reactivity of acceptors has a profound effect on the stereochemical outcome of the glycosylation reaction. We have then developed a system to measure the reactivity of acceptors based on the stereoselectivity of two conformationally restrained donors, which is heavily dependent on the reactivity of the acceptor. By reacting a large amount of acceptors, which all contain systematic variations in configuration and protecting group pattern, this work shows that the reactivity of the acceptor is dependent on both the configuration and the protecting group pattern in a predictable way. Finally, DFT calculations and competition experiments are used to rationalise the established structure reactivity relationship.



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Development of a 15-minute test for Uropathogenic Escherichia coli using Glycan coated microspheres

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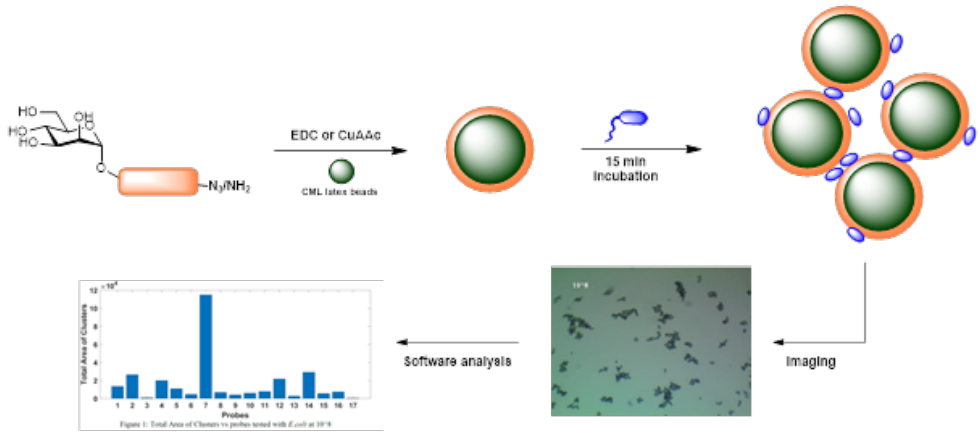
Incidences of Antimicrobial resistance (AMR) are rising and strongly correlated with their inappropriate use, studies have shown that 20-50% of antibiotics are either incorrect or unnecessarily prescribed [1]. As the development of a new antibiotic agent can take 10-15 years and cost over $1 Billion, it is critical to find alternative ways to preserving the effectiveness of today's antibiotics [2-3].

Urinary Tract Infections (UTI) affect an estimated 150 million people every year and Uropathogenic *Escherichia coli* (UPEC) species contribute to >70% of all Urinary Tract Infections. As the second highest rate of prescriptions in primary care, rapid point-of-care diagnostics have an essential role to reduce empirical antibiotic use in UTI [4]

FluoretiQ have developed a rapid 15-minute assay- NANOPLEX- that uses glycan-coated latex microspheres, brightfield microscopy and image processing techniques to selectively identify UPEC species at clinically relevant concentrations (10^5-10^8 CFU/ml). To validate the assay, mannosides were attached with different linker length to latex microspheres. We demonstrated comparable linear agglutination across three *E. coli* clinical isolates at a clinically significant 10^5 CFU/ml and selectivity for *E. coli* against the most prevalent uropathogens in UTI.

NANOPLEX technology is undergoing development and will be translated into a bespoke test kit for clinical use, to enable rapid testing of UTI at the point of care.



NANOPLEX assay

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Synthesis, conformational analysis and complexation study of IminoSugar-Aza-Crowns

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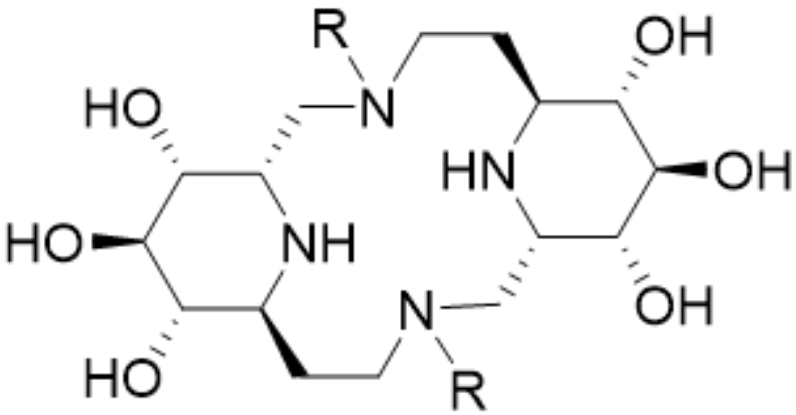
Iminosugars, sugars in which the ring oxygen has been replaced by nitrogen, constitute undoubtedly a major class of sugar analogs. So far, their application has been limited to the medicinal field as these compounds have shown promising therapeutic properties as glycosidases and/or glycosyltransferases inhibitors.[1]

Their sugar-like structure and the presence of an endocyclic nitrogen could be exploited to deliver innovative azacrowns. Aza-crown ethers and cyclams are important molecular receptors for molecular or ionic recognition studies that find applications notably in analytical chemistry as well as in nuclear medicine. The inherent chirality of carbohydrates is very suitable for the design of chiral receptors. Chiral aza-crown ethers incorporating various carbohydrate motifs have received much attention in recent years.[2] We designed a new family of chiral tetraazamacrocycles, coined ISAC for IminoSugar Aza-Crown, incorporating two iminosugars units.

The synthesis of these unprecedented IminoSugar-Aza-Crowns, their conformational analysis and the preliminary study of their chelation properties [3,4] will be presented.



IminoSugar-Aza-Crown Structure

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N-Acetylgalactosamine mimetics: Synthesis and Binding Affinity Toward Amyloidogenesis Prevention

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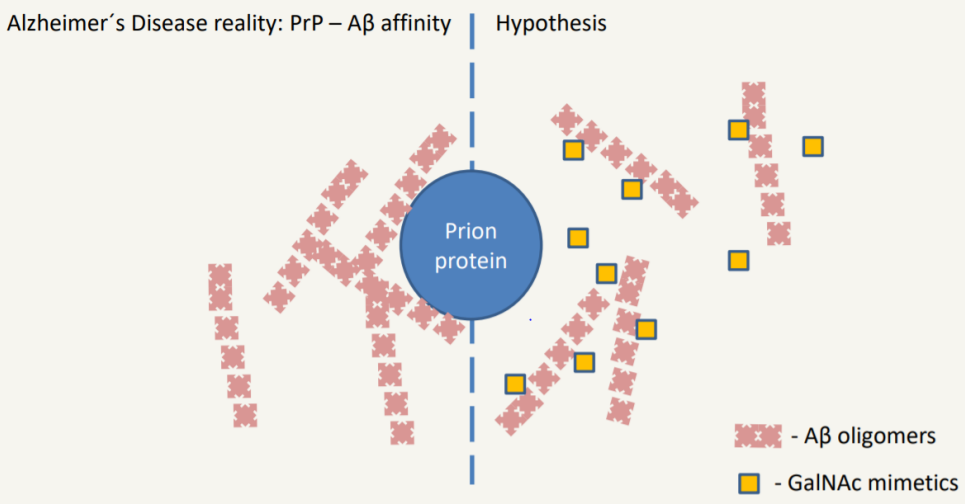
N-acetylgalactosamine (GalNAc) belongs to the group of 2-amino-2-deoxysugars which are found in a wide range of biological structures, playing a role in in cell-cell interaction and receptor induced cell signaling, among other biological processes in health and disease. It was found that GalNAc and other monosaccharides, namely GlcNAc, when attached to Ser/Thr side chain of a prion protein (PrP) via an α-glycosidic linkage, promote the inhibition of amyloidogenesis in Alzheimer's disease (AD), while Gal is ineffective [1]. This uncurable disease is threatening, particularly, the elderly. It is estimated to affect 152 million, by 2050 [2], and research to prevent/treat disease is urgently needed.

In this communication we present synthetic approaches for GalNAc mimetics, including phenyl seleno-, thio- galactosides and phenyl galactosides bearing amide moieties and related groups at position 2. These changes ate the core structure could improve the interactions with the PrP and/or with Aβ1-42. The study of the intermolecular interactions of the new mimetics and Aβ1-42 oligomers was investigated by NMR methods (STD-NMR; F-NMR). In parallel, competition and metabolic experiments were carried out. The potential of the synthesized molecules to act as protein-protein interaction inhibitors will also be discussed, particularly focusing on their ability to directly bind to Aβ, widely accepted as the most neurotoxic type of Aβ aggregates, with potential to disrupt/ prevent Aβ-PrP aggregation, which has been increasingly described as a central element of AD's pathophysiology [3].



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Carbohydrate-functionalised metal complexes: lectin-targeting glycoclusters for therapy & detection

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Carbohydrates can confer metal complexes with many properties, including targeted interactions with biomolecules, such as carbohydrate-binding proteins, as well as well-defined stereochemistry and water-solubility. Carbohydrate–protein interactions are often key to the pathology of bacterial infections.[1] Functionalisation of inorganic complexes with carbohydrate-derived molecules allows some of these properties to be exploited in different ways. We have synthesised various new metal complexes, which aim to harness carbohydrates for potential therapeutic and diagnostic applications.

We have synthesised a series of Ru(II)-centred glycoclusters, presenting four carbohydrate units in a three-dimensional way in order to target bacterial lectins.[2] The ability of these complexes to inhibit biofilm formation by Pseudomonas aeruginosa was assessed and found to be dependent on the carbohydrate motif and spacer units. Building on this work, we have also designed a new class of multivalent luminescent lanthanide-centred glycoclusters (di-, tetra- and hexavalent), for the purpose of detecting lectins, particularly those associated with pathogenic bacteria. This strategy of luminescent detection could give rise to novel diagnostic approaches.

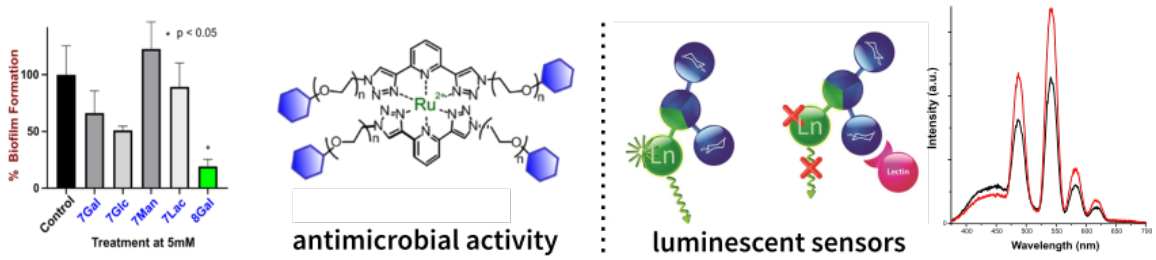

Figure 1. Metal-centred glycoclusters for antimicrobial and diagnostic applications

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Chemoenzymatic Synthesis of Mucin O-Glycans

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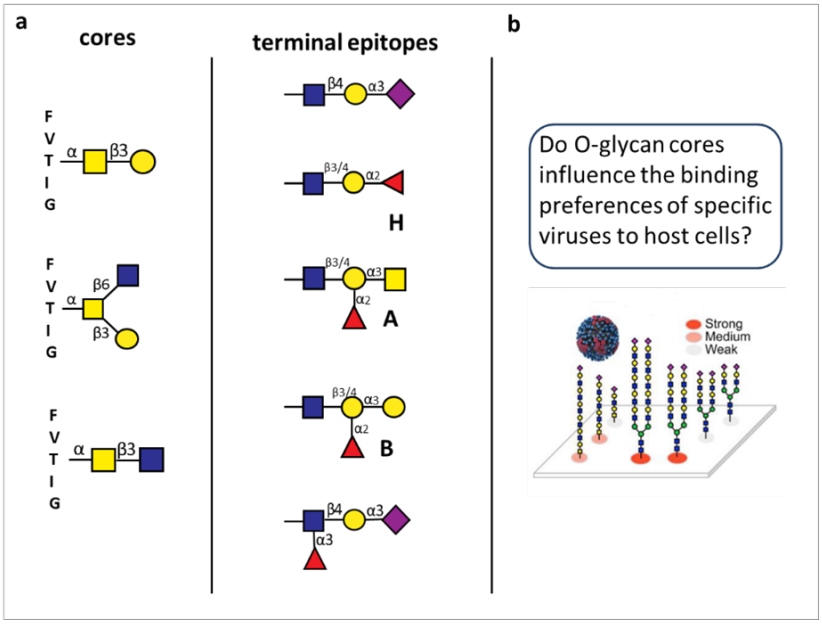
Mucins are involved in many biological processes such as barrier formation, host-pathogen interactions and modulation of immune responses. However, their biological functions at a molecular level are still poorly understood, which in part is due to a lack of methods to synthesize and structurally characterize mucins and their heterogenous O-glycans, respectively.[1] In comparison to N-glycans, Mucin O-glycans are underrepresented in current microarray platforms. To facilitate O-glycan interactomics studies, there is a need for a collection of structurally defined complex O-glycans.[2] To address this deficiency, we have developed a method for the facile preparation of complex O-glycans. The main O-glycan core structures 1 to 3 that have been found in humans were synthesized starting from Tn-antigen. Employing a range of human and bacterial glycosyltransferases, it was possible to extend these core structures to complex O-glycans found in the human gut and in the respiratory tract. We could attach terminal epitopes such as A-, B-, H- and Lewis-antigens as well as α2,3-sialic acids that are known to be recognized by various viruses. This library of O-glycans has been printed as a microarray to investigate how the different O-glycan cores influence binding preferences of various host and microbial glycan binding proteins.



(a) Components of the O-glycan library (b) Glycan micrroarray studies are used to investigate the role of the O-glycan cores in viral infections [3]

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β-Glucan Grafted Microcapsules, a Tool for Studying the Immunomodulatory Effect of Microbial Cell Wal

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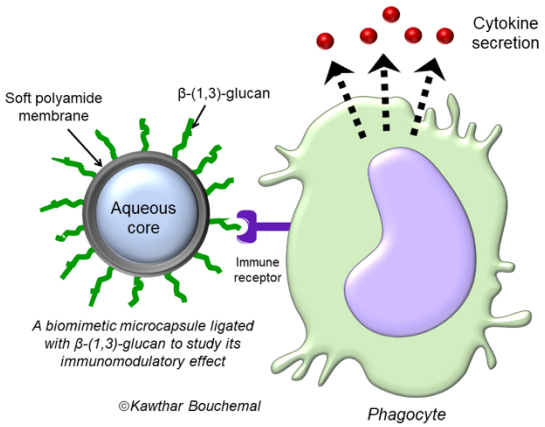
β-(1,3)-Glucan is one of the antigenic components of the bacterial as well as fungal cell wall. We designed microcapsules (MCs) ligated with β-(1,3)-glucan, to study its immunomodulatory effect. The MCs were obtained by interfacial polycondensation between diacyl chloride (sebacoyl chloride and terephtaloyl chloride) and diethylenetriamine in organic and aqueous phases, respectively. Planar films were first designed to optimize monomer compositions and to examine the kinetics of film formation. MCs with aqueous fluorescent core were then obtained upon controlled emulsification–polycondensation reactions using optimized monomer compositions and adding fluorescein into the aqueous phase. The selected MC-formulation was grafted with Curdlan, a linear β-(1,3)-glucan from Agrobacterium species or branched β-(1,3)-glucan isolated from the cell wall of *Aspergillus fumigatus*. These β-(1,3)-glucan grafted MCs were phagocytosed by human monocyte-derived macrophages, and stimulated cytokine secretion. Moreover, the blocking of dectin-1, a β-(1,3)-glucan recognizing receptor, did not completely inhibit the phagocytosis of these β-(1,3)-glucan grafted MCs, suggesting the involvement of other receptors in the recognition and uptake of β-(1,3)-glucan. Overall, grafted MCs are a useful tool for the study of the mechanism of phagocytosis and immunomodulatory effect of the microbial polysaccharides.



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Total synthesis of an agminoside-like glycolipid

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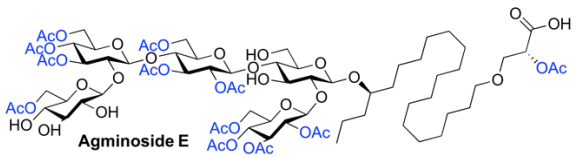
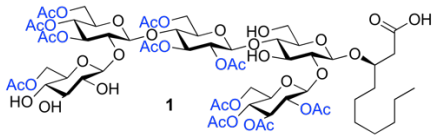
Agminosides are glucolipids produced by the marine sponge Raspailia agminata, which is endemic to New Zealand.[1] Structurally, agminosides possess a lipid chain linked to a branched penta- or hexasaccharide, which is non-stoichiometrically acetylated and exclusively composed of D-glucose residues connected with both β-(1→4) and β-(1→2) linkages. Motivated by the atypical structure of agminosides and by the proven biological properties of structurally related marine glycolipids, we have accomplished the total synthesis of an agminoside-related glucolipid. En route toward the total synthesis of agminosides, our target chimeric compound bears the pentasaccharidic part of agminoside E along with a 3-hydroxydecanoyl chain found within bacterial rhamnolipids.

Our synthesis entails a [3 + 2] glycosylation strategy between a N-phenyl-2,2,2-trifluoroacetimidate trisaccharide donor and a glucolipid acceptor. Both para-methoxybenzyl (PMB) and para-methoxybenzylidene groups were efficiently used to mask the hydroxyl groups prior the acetylation step at a late stage of the synthetic sequence. Furthermore, the judicious use of (2-azidomethyl)benzoyl (AZMB) groups at the C2 position allowed the formation of 1,2-trans¬-glucosidic bonds while preserving the acetylation pattern of glycolipids under orthogonal hydrogenolysis or Staudinger conditions.

This research project stands as a first step toward the total synthesis of a libraries of non-stoichiometrically acetylated glucolipids, which could lead to the discovery of novel molecular entities with potent biological activity.





Structures of agminoside E and our target agminoside-like glucolipid

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Enzymatic synthesis of 1-*O*-acyl galactofuranoses with anti-leishmanial activities

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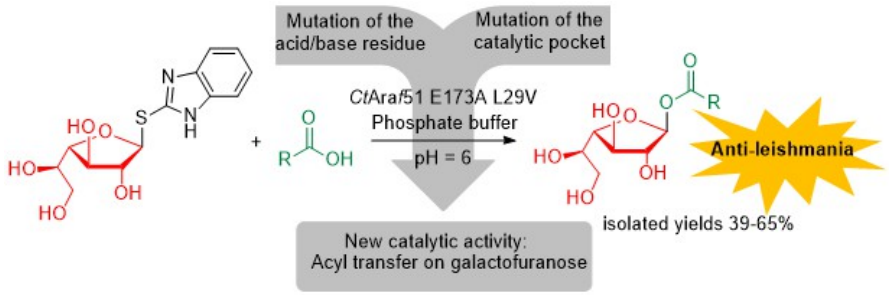
Among identified health threats, leishmaniosis that results from infection with *Leishmania* sp. was pinned out recently by the World Health Organization. Previous works highlighted the parasitostatic activity of simple alkyl β-D-galactofuranosides on Leishmania.[1] In a continuous effort to increase such biological activity, the synthesis and biological evaluation of a new family, the galactofuranosyl esters are reported.

To avoid isomerization of the hexofuranose into its pyranose counterpart, we decided to introduce the acyl chain thanks to a biocatalyzed acylation of a galactofuranosyl donor using an α-L-arabinofuranosidase as catalyst. Nevertheless, the diversion of glycosyl hydrolases to perform anomeric acylation is hampered by the poor nucleophilicity of carboxylic acid and the competition with water. Here we showed that the mutation of the acid/base residue of an α-L-arabinofuranosidase GH51 from *Clostridium thermocellum* (*Ct*Ara*f*51) allowed the *C*-1 acylation of L-arabinose with yields ranging from 18 to 83 %.[2] In order to extend this new catalytic activity to galactofuranose, the affinity of *Ct*Ara*f*51 for this exogeneous substrate was improved by mutation of some amino acids within the catalytic pocket.[3]

Thanks to the combination of both mutations, *C*-1 acylation of thioimidoyl galactofuranoside with various carboxylic acid gave the corresponding acyl galactofuranoses with good yields (39 to 65%). Their biological properties were evaluated and some scaffolds showed improved activities against *Leishmania torentulae* when compared with the alkyl galactofuranosides.



C1-acylation of galactofuranose by double mutant thioligase CtAraf51 E173A L29V

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Novel glycan-based fluorescent nanoprobes for live cell imaging and theranostic tools

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O-Glycosylation is a ubiquitous post-translational modification that is highly dynamic and responsive to cellular stimuli through the action of the cycling enzymes. Expression of specific O-glycans is linked to changes in gene expression in, for example, inflammatory bowel disease, cystic fibrosis and several types of cancer.1 Glycan coated-nanoparticles constitute a good bio-mimetic model of carbohydrate presentation at the cell surface and provide a powerful tool to screen for protein carbohydrate interactions and consequently for the identification of carbohydrate receptors or ligands associated with many inter- and intracellular recognition processes associated to disease. In order to develop and use these glyco-tools for biomedical applications, it is of the utmost importance to have access to structurally defined oligosaccharide-based probes. In recent years, our group has developed fluorescent multivalent O-glycan probes for the screening of O-glycosylation-linked interactions in live cells and more recently in bacteria. In this presentation, we will discuss our more recent results on the synthesis of fluorescent nanoparticles and their potential as theranostic tools and plant biology applications.2

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Synthesis of oligosaccharides representative of plant cell wall glycans rhamnogalacturonan I and II

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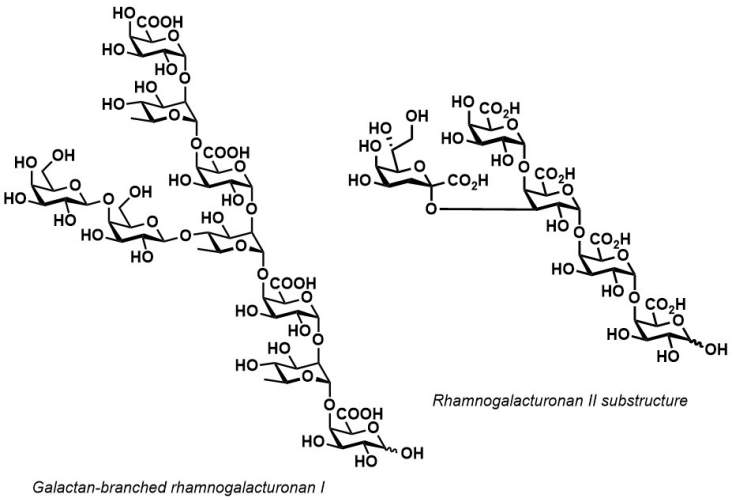
Plant cell walls are structurally complex and contain a large number of diverse carbohydrate polymers. These plant fibers are a highly valuable bio-resource and the focus of food, energy and health research. We are interested in studying the interplay of plant cell wall carbohydrates with proteins such 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 structures found integral to rhamnogalacturonan I and II. The first has a backbone of alternating L-Rha and D-GalA with branching D-Gal and the latter a backbone of D-GalA and a Kdo substitution.



Examples of oligosaccharide structures

Transglycosylation activity of engineered Bifidobacterium lacto-N-biosidase mutants at donor subsite

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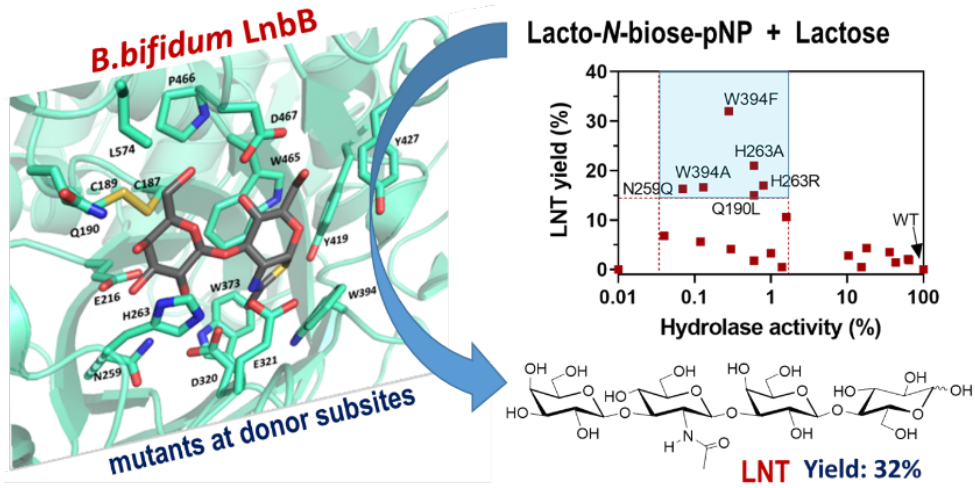
The health benefits of human milk oligosaccharides (HMOs) make them attractive targets as supplements for infant formula milks. However, HMOs synthesis is still challenging, and only two HMOs have been marketed. Engineering glycosidases into transglycosylases may provide biocatalytic routes to the synthesis of complex oligosaccharides. Lacto-N-biosidase from Bifidubacterium bifidum (LnbB) is a GH20 enzyme present in the gut microbiota of breast-fed infants that hydrolyzes lacto-N-tetraose (LNT), the core structure of the most abundant type I HMOs. Based on the crystal structure of LnbB in complexes with LNB and LNB-thiazoline [1] and our previous study on the conservation of active site topologies in family GH20 [2], here we report a mutational study in the donor subsites of the substrate binding cleft with the aim of reducing hydrolytic activity and conferring transglycosylation activity for the synthesis of LNT from p-nitrophenyl β-lacto-N-bioside and lactose. As compared with the wt enzyme with negligible transglycosylation activity, mutants with residual hydrolase activity within 0.05% to 1.6% than the wild-type enzyme result in transglycosylating enzymes with LNT yields in the range of 10-30%. Mutations of a key Trp residue, located in subsite -1 next to the catalytic residues, have a large impact on the transglycosylation/hydrolysis ratio, with a Trp to Phe being the best mutant as biocatalyst producing LNT in 32% yield [3]. It is the first reported transglycosylating LnbB enzyme variant, amenable to further engineering for practical enzymatic synthesis of LNT.



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Bicyclic DNJ-based GlcNAc mimetics as a new family of potent and selective O-GlcNAcase inhibitors

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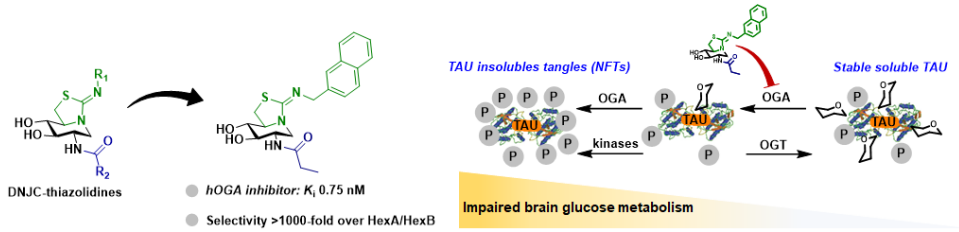
O-GlcNAc is abundant in the brain and more specifically on proteins implicated in the etiology of several diseases including Alzheimer's disease and cancers. Levels of phosphorylation of tau, a key protein implicated in various neurological pathologies known as the tauopathies, are regulated by the levels of O-GlcNAc on tau. Maintaining healthy O-GlcNAc levels by selective inhibition of human O-GlcNAcase (hOGA) could help block pathological hyperphosphorylation of tau, thereby avoiding the formation of tau oligomers and neurofibrillary tangles (NFTs) that are associated with neurodegeneration. Small molecule OGA inhibitors, such as Thiamet-G1 or the recently developed MK-8719,2 have been considered for treatment of AD and related tauopathies. In this work, we expand the scope of iminosugar-type hOGA inhibitors to a new prototype that combines the most advantageous structural features already identified in several known hOGA inhibitor families. We keep a common 2-deoxy-2-acylamino 1-deoxynojirimycin (DNJ) core with the piperidine nitrogen engaged in a five-membered cyclic isothiourea moiety, namely 6S,5N-alkyliminomethylidene-2-carboxamido-1,2-dideoxynojirimycin (DNJC-thiazolidine). We also show that varying the carboxamide N-acyl group enables generating molecular diversity and fine tuning of inhibitory properties. The chemical synthesis, hOGA inhibitory properties in vitro, and ligand-protein structural analysis will be presented.



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Experimental and in silico insights into the molecular mechanisms of mannuronan C-5 epimerase AlgE4

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Mannuronan C-5 epimerases catalyse the epimerization of mannuronate into its C-5 epimer guluronate in the polysaccharide alginate. The enzyme tailor alginate on the polymer level to needed function in alginate-producing organisms. The understanding of the molecular mechanism of the enzymes' action along polymer chains is required for rational enzyme design that could be used for designing alginate for biotechnological applications. In this interdisciplinary study, we rigorously characterize the extracellular processive epimerase AlgE4 from A. vinelandii by combining experimental and molecular modelling approaches. We find that charged residues lining the binding groove interact in an extensive hydrogen bonding network that is essential in the interaction with docked substrates. Furthermore, molecular dynamics simulations of substrate dissociation provide insight into the enzyme's movement along the substrate chain. The results obtained by the in silico methods were applied as rational approach to direct subsequent site directed mutagenesis and experimental analysis. Alanine mutants of charged residues are compared with respect to their initial rates and product profiles obtained from NMR-studies, and characteristics of their substrate interactions obtained with isothermal titration calorimetry and optical tweezers. The modelling and experimental studies complement each other, resulting in a better understanding of the role of individual residues in binding and movement along the alginate chains, which could aid in generation of engineered enzymes for alginate modification.

Access to new C-Glycosides from unprotected sugars via a Green Chemistry approach

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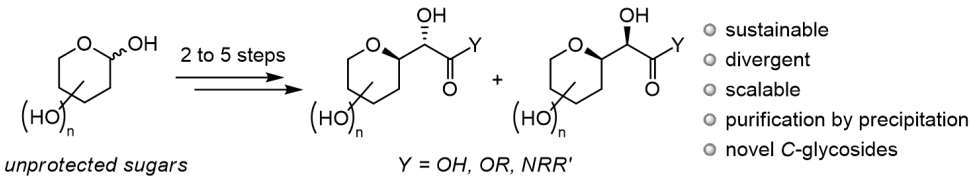
Carbohydrates are valuable and versatile building blocks that have notably been used for a variety of industrial applications. In the cosmetic industry, they are commonly utilized as active ingredients in skin and hair care products. In addition to their biological and physico-chemical functions in living organisms, carbohydrate derivatives are also of interest in terms of sustainable chemistry. Hence, the development of new sugar derivatives is clearly key to many areas, including the cosmetic industry.

To this end, an efficient, divergent and straightforward access to novel C-glycosides has been developed, namely alpha-hydroxy carboxamide and carboxylic acid derivatives, via a green and scalable process from unprotected carbohydrates (1). The method involves condensation of 1,3-dimethylbarbituric acid with unprotected sugars followed by subsequent barbiturate oxidative cleavage in the same pot. Further expanding of the chemistry led to the development of efficient entries to diastereoisomerically pure C-glycosyl-alpha-hydroxy esters or amides through nucleophilic attack on a readily available and versatile key lactone intermediate.

Finally, this methodology fulfills L'Oréal's commitment to Green Chemistry resumed in 3 pillars (2): 1/ it uses renewable or potentially renewable raw materials, 2/ the process can be environmentally friendly, and 3/ the final compounds show favorable in silico environmental impact.

Cosmetic applications of these novel C-glycosides are ongoing.



Mucinomics General process in 2 to 5 steps

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Identification of N-glycan oligomannoside isomers in the diatom Phaeodactylum tricornutum

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Microalgae are emerging systems for the production of recombinant proteins like monoclonal antibodies. In this blue biotech context, the characterization of the host cell N-glycosylation machinery and of the microalgae-made biopharmaceuticals, which are mainly glycoprotein-based products, requires efficient analytical methodologies dedicated to the profiling of the N-glycans. In this work, in order to gain knowledge regarding its N-glycosylation pathway, we profile the protein N-linked oligosaccharides isolated from the diatom Phaeodactylum tricornutum that is currently used successfully to produce functional glycosylated monoclonal antibodies. The combination of ion mobility spectrometry–mass Spectrometry and electrospray ionization-multistage tandem mass spectrometry allows us to decipher the detailed structure of the oligomannoside isomers and to demonstrate that the processing of the oligomannosides N-linked to proteins occurs in this diatom as reported in mammals. Therefore, P. tricornutum synthesizes human-like oligomannosides in contrast to other microalgae species. This represent an advantage as an alternative ecofriendly expression system for the production of biopharmaceuticals intended to be use for human therapy.

Deoxyfluorinated N-acetyllactosamines as Advantageous Carbohydrate-based Probes for Human Galectins

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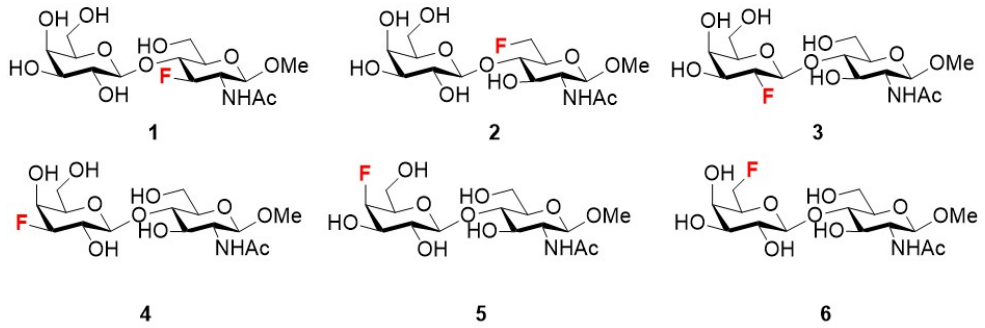
Human galectins (hGals) are carbohydrate-binding proteins playing key roles in a plethora of physiological processes. They are able to modulate immune responses or neoplastic transformation processes via molecular recognition of galactoside-containing glycans.[1] Therefore, the development of their selective inhibitors has become a focus of pharmaceutical research. However, the preparation of inhibitors targeting individual hGals remains challenging as 12 hGals featuring similar substrate specificities have been identified. A deeper understanding of subtle differences between individual hGals could provide guidelines for such development and deoxyfluorinated carbohydrates are established tools, capable of providing such information.[2]

In this work, a complete series of mono-deoxyfluorinated N-acetyllactosamine analogues have been prepared. The synthesis of each analogue required approximately 15 synthetic steps, including deoxyfluorination of monosaccharide precursors and chemical glycosylation. Their binding affinities to hGal-1 and hGal-3 were determined by ELISA assay and 19F NMR T2-filter techniques which enabled the identification of hydroxyl groups essential for the interaction with hGals, and also permitted to compare both tested hGals in terms of the importance of these key hydroxyl groups in the recognition events. Furthermore, the library is also a perfect tool to study the molecular origin of recognition events via epitope-mapping 19F NMR techniques.[2]



The series of prepared N-acetyllactosamine analogues 1–6.

Support from MEYS (Project No. LTC20052 and LTC20072, COST Action CA18103 INNOGLY) is acknowledged.

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Calcium Bridging Carbohydrates and Lectins: Classical Molecular Dynamics with Effective Polarisation

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The developments of additive carbohydrate force fields increased the reliability of molecular dynamics (MD) simulations of protein-carbohydrate complexes [1]. The presence of bridging Ca^{2+} ions can, however, pose problems for structural and energetic description due to quantum effects, such as polarisation and charge transfer [2, 3]. To overcome this limitation, we had developed Ca^{2+} parameters with effective electronic polarisation for use with additive force fields [2] and applied them to pharmacologically important calcium-dependent lectins LecA/LecB of *Pseudomonas* bacterium in complex with carbohydrate and glycomimetics (Fig. 1) [4, 5]. Such a treatment improved the structural description of the binding site of LecB/Lewis x complex in submicrosecond MD (evaluated by the $Ca^{2+}\cdots Ca^{2+}$ distance, Fig. 1B, black/red – standard Ca^{2+} parameters; blue/green – new ones [2]; yellow – crystal distance). For the modelled LecA/glycomimetic complexes, MD gave insights into the interplay of linker geometry and length for an optimal divalent lectin binding. To conclude, including Ca^{2+} polarisation in modelling protein-carbohydrate complexes qualitatively corrects the previous deficiencies and can be leveraged for reliable design of glycomimetics.

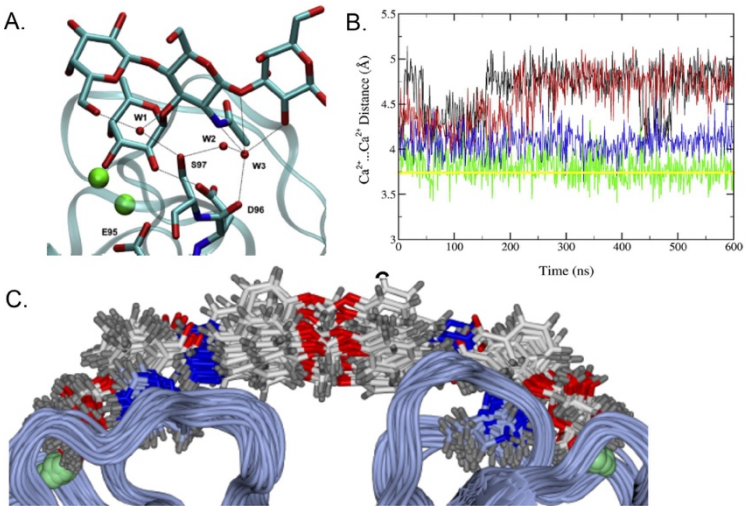

Figure 1. **A.** LecB/Lewis x crystal, Ca^{2+} green spheres (PDB: 5A70) [4], **B.** Performance of Ca^{2+} parameters [2] in MD, **C.** LecA/glycomimetic complex modeled by MD [5]

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A Concise and Efficient Synthetic Strategy for Isoiminosugars

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Isoiminosugars (A) are sugar analogues in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group. Selected structures of this compound class, e.g. C-5a-chain extended derivatives of 4-epi-isofagomie (1), have been proven as highly potential pharmacological chaperones for the treatment of GM1 gangliosidosis [1,2]. As a matter of fact, the indicated structural characteristics remain to challenge the synthesis of isoiminosugars (A), compared to related structures such as iminosugars (B). However, valuable synthetic strategies towards this compound class have been reported [3-6]. In context with our interest in the design and synthesis of such structures, we have found an efficient and concise synthetic approach towards isoiminosugars (A). This method can be applied on different configurations and allows variations in the reaction sequence, opening the avenue to various modifications in the substitution pattern. Herein, synthetic details as well as the scope and limitations of this approach will be presented.

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Computational and experimental analysis of the interactions between glycosaminoglycans and drugs

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Glycosaminoglycans (GAGs), long unbranched periodic anion polysaccharides composed of repeating disaccharide units [1], are a class of chemical compounds involved in several pathological conditions including Alzheimer's and Parkinson diseases [2]. GAGs play a crucial role in cellular communication through interactions with proteins in the extracellular matrix, they participate in multiple cell signaling processes affecting cellular adhesion, proliferation and communication [3]. GAGs also increase the ability of peptides to integrate into membranes and modify drug activity [4]. Based on that results, Tarbell et al. suggested an important role of GAGs in vascular diseases as atherosclerosis, stroke, hypertension, kidney disease, sepsis and cancer [5]. Therefore, GAG structures can be used for design of new drugs to control and enhance regeneration processes in a variety of disorders.

Despite the great potential of GAGs, little is known about the molecular mechanisms of their interactions with small molecules. Experimental methods are not always able to provide sufficient information about the nature of interactions in GAG-small molecule systems, for which only limited structural data are available. Therefore, for these studies, we decided to use computational chemistry approaches such as molecular docking, molecular dynamics as well as specific free energy analysis. We chose several drug molecules and conducted detailed evaluations to expand available knowledge about the molecular aspects of the interactions between GAGs and small molecules.

This work was financed by Sonata Bis project UMO-2018/30/E/ST4/00037
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Theoretical and experimental insights into Glycosaminoglycan - APRIL protein interactions

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APRIL, a proliferation-inducing ligand, is a peculiar member of the tumor necrosis factor superfamily, since it requires the binding to glycosaminoglycans (GAGs), to efficiently signal into target cells. [1] The GAG binding region of APRIL has been located at the N-terminus constituted by a lysine stretch commonly found in GAG ligands. GAGs with their multiple sulfate groups act as a bridge that connects APRIL trimers mediating oligomerization. As heparan sulfate (HS) proteoglycan and chondroitin sulfate (CS) proteoglycan are ones of the APRIL's co-receptors [2] it is important to understand the nature of the interactions in these complexes which are involved in various diseases at atomistic level.

We analyzed and confirmed the binding of native and truncated version of APRIL to GAGs from HS class with the model heparin (HP) molecule and from the CS class with the disulfated CSe and monosulfated CSc molecules and found out that HP is the strongest binder while CSc binding is the weakest. APRIL's C-terminus was revealed to contribute to GAG binding as well. Two novel GAG-specific docking approaches were applied to verify the data obtained by the conventional docking to challenge the possibility of multiple GAG molecule binding to a single APRIL trimer and to reveal binding poses of long GAG molecules of 48-mer which was previously unobtainable with traditional rigid docking.

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Electrochemical strategies for Studying the Effect of Modifications on Glycans Binding Preferences

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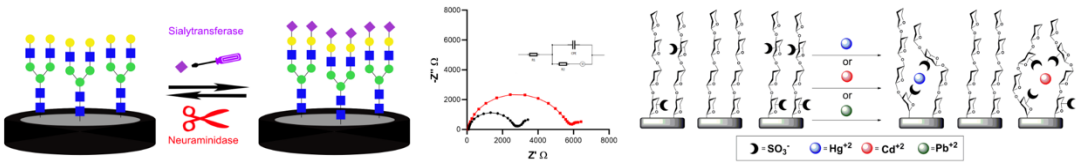
Modifications of complex glycans govern their binding preferences and biological activities. Evaluating the effect of sulfation and sialylation on glycans interaction preferences is not straightforward. First, obtaining modified complex glycans in sufficient quantity is not easy. Second, many interactions are too weak to study using standard bioanalytical tools. Third, interactions studies usually requires glycan labeling. We develop label-free electrochemical tools for studying the effect of glycans modifications on interaction preferences. Assembly of complex glycans on electrodes enables studying glycan interaction with proteins, metal ions and even enzymatic processed by the electrochemical impedance spectroscopy. The use of surface chemistry tools allows utilizing only minute quantities of complex glycans while the electrochemical signal enables label-free sensing of even very weak interactions by following the changes in the glycan layer hence differs significantly from classic analytical methods. We determined the effects of sulfation patterns on glycosaminoglycans-heavy metal ions interactions using electrochemical impedance spectroscopy and other surface chemistry methods. We developed tools to follow time-dependent enzymatic sialylation processes by transforming glycan attachment and hydrolysis into a measurable electrochemical signal. Applying highly sensitive electrochemical tools to elucidate the effect of glycans modification on binding preferences offers a new way of studying glycobiology



Electrochemical impedance spectroscopy was used to study the interaction preferences of sulfated and sialylated glycans

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

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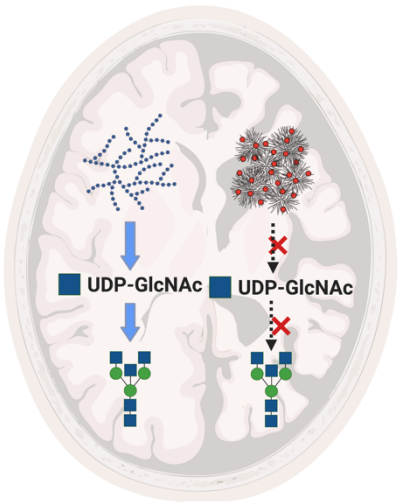
Glycosylation defects are a hallmark of many nervous system diseases. However, the molecular and metabolic basis for this pathology are not fully understood. In this study, we found that N-linked protein glycosylation in the brain is coupled to glucosamine metabolism through glycogenolysis. We discovered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

Brain glycogen is comprised of glucose and glucosamine. Glycogen storage diseases impacting the brain result in PGBs that sequester both.



Mechanistic Studies of the Base-Catalysed Isomerization of Aldoses to Ketoses

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The isomerization of aldoses into ketoses presents an atom-efficient method to produce rare or highly demanded ketoses from often readily available aldoses. Suitable chemo-catalysts may present a feasible alternative to the currently employed enzymes.[1] Here we explore the reaction mechanism and kinetics in the presence of bases to facilitate a rational catalyst and process design.

Our work focuses on the mechanistic and kinetic investigation of the isomerization of D-glucose to D-fructose in the presence of aq. NaOH as a model system. We employ a combination of kinetic studies at various conditions (variation of pH, temperature, concentration of substrate, ionic strength) with operando NMR- and UV/Vis-spectroscopy. Kinetic investigations identified D-mannose and D-allulose as co-products of the isomerization. Increasing the pH value accelerates the reaction and decreases the activation energy. In accordance with previous results,[2] the equilibrium shifts towards D-fructose at higher temperatures, although thermal decomposition can lead to a decrease in selectivity. Moreover, increasing the ionic strength was found to accelerate the isomerization. Our results suggest that it is caused by a decrease of the monosaccharides pKa values in the presence of salts. As previously described,[3] the isomerization occurs via enediol anion intermediates, showing an absorbance at 312 nm. We synthesized partially deuterated D-glucose and D-fructose to perform operando 1H NMR-experiments allowing for spectroscopic study of the mechanism.[4]

Applications of ketoses and influences investigated in this study.

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Revealing the human mucinome

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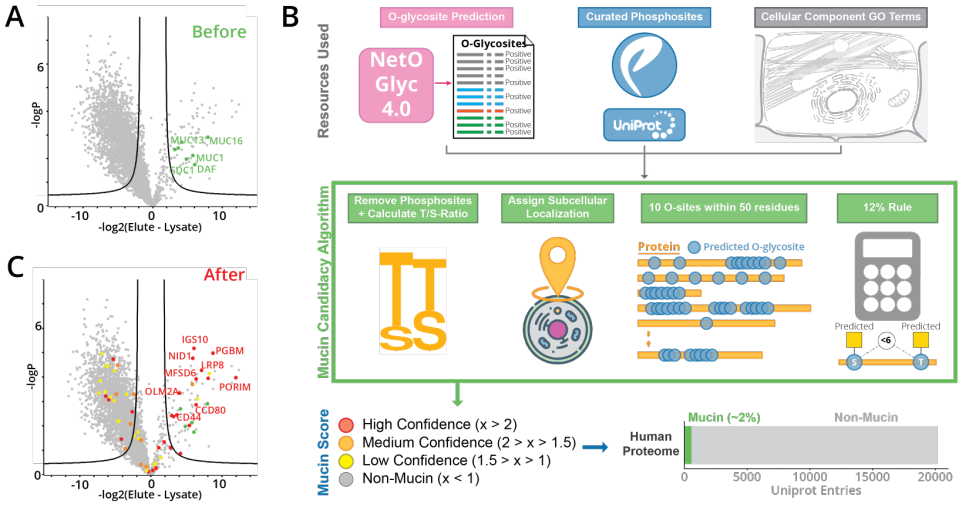
Mucin domains are densely O-glycosylated modular protein domains found in a wide variety of cell surface and secreted proteins. Mucin-domain glycoproteins are key players in a host of human diseases, especially cancer, but the scope of the mucinome remains poorly defined. Recently, we characterized a bacterial mucinase, StcE, and demonstrated that an inactive point mutant retains binding selectivity for mucins. In this work, we leveraged inactive StcE to selectively enrich and identify mucins from complex samples like cell lysate and crude ovarian cancer patient ascites fluid. Our enrichment strategy was further aided by an algorithm to assign confidence to mucin-domain glycoprotein identifications. This mucinomics platform facilitated detection of hundreds of glycopeptides from mucin domains and highly overlapping populations of mucin-domain glycoproteins from ovarian cancer patients. Finally, we used a KRAS dox-inducible system to show which mucins contribute to molecular bulk at the cell surface. Ultimately, we demonstrate our mucinomics approach can reveal key molecular signatures of cancer from in vitro and ex vivo sources.



Mucinomics platform allows for identification of enriched mucins from complex samples

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How does glycan-glycan interaction modify protein behavior?

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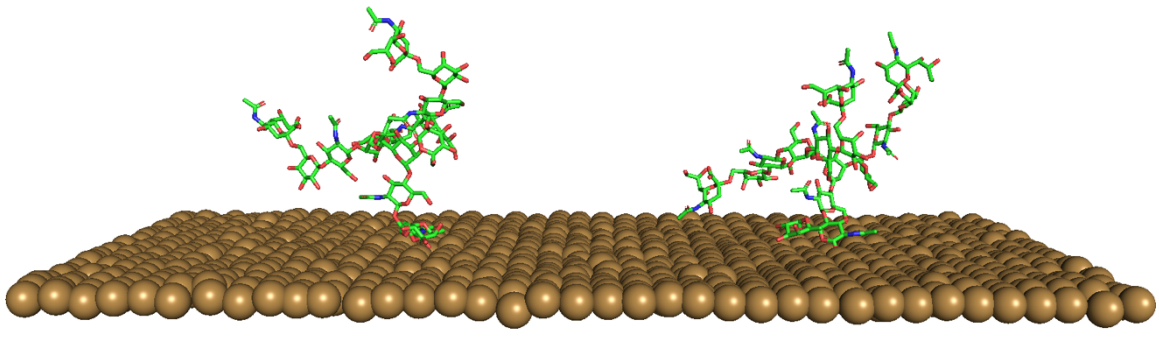
Glycosylation is an essential feature in regulating many biological processes via modification of the protein behavior. It is known that the interplay between the sugars and the protein surface can reshape the structural properties of the latter and thus alter its function. However, it has yet to be discovered if this protein behavior relates to specific sugar-sugar interactions, or even their specific protein surface distributions. For instance, the formation of sugar antennae can hinder some interactions, whereas the repulsion between sugar moieties could free space and ease them. Solving such a problem is not trivial, as it is hard to characterize the glycosylation motifs bound to proteins. Sugars, in general, do not crystallize together with the proteins when using standard protocols and are very challenging to deconvolute via NMR. Furthermore, glycosylations often vary within a protein. For these reasons, computational methods could be an alternative approach suitable to shed light on this question.

We use classical molecular dynamics simulations of several complex sugar antennae bound to a surface that mimics the protein. Then, we change the distance between two nearby sugar antennae. By monitoring the number of contacts between them, we evaluate their tendency to form aggregates or repeal each other. We compare our findings with glycosylation information in the Protein Data Bank (PDB).

Our results show that almost half of the glycosylation sites lie at distances that allow the glycans to interact with others, with a maximum around 5nm. The maximum coincides wi ith the distance at which the sugars interact with each other weakly in our simulations. However, this observation might be biased as the antennae used were all 7 monosaccharides long, and other sizes might be interesting to explore. Also, 92% of the antennae sites are within a distance of 15nm to another site. All of this indicates that the distribution of the glycosylation spots is not random and aims to foster sugar-sugar interactions. Overall, we have found that glycosylation sites try to cluster around others, potentially indicating that sugar-sugar interactions may play a biological role.



Elucidation of the Chemical Structure of Lipopolysaccharides isolated from Gut Bacteria

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The human gut microbiota harbours a complex community of microorganisms which influences human physiology, metabolism, nutrition, and immune function [1]. To remain immunologically tolerant to these commensal bacteria and preserve a symbiotic relationship elaborate biochemical mechanisms are involved. A key mechanism involves the bacterial lipopolysaccharides (LPSs), key components of the Gram-negative bacteria cell wall. LPS is a potent ligand for the host receptor Toll-like receptor 4/myeloid differentiation factor 2 (TLR4/MD2) complex [2]. These interactions are vital for the initiation of immune response to pathogens but how LPS from commensal bacteria interact with TLR4/MD2 complex is still unclear. In this communication I will show results regarding the structural elucidation, through Mass Spectrometry and NMR, of the LPS isolated from two human commensal species. Veillonella parvula and Bacteroides thetaiotaomicron [3-5]. Both have been demonstrated to have significant beneficial roles. Accordingly, our previous work shows Bacteroides vulgatus produces LPS which does not elicit a potent proinflammatory response and is structurally different to pathogen-derived LPS [5]. Since LPS are involved in the interaction between bacteria and the host, an in-depth investigation of the full structure of the LPS is a first but essential step to understand the basis of virulence and symbiotic behaviour. To this aim, LPSs have been extracted from V. parvula and B. thetaiotaomicron, both grown in our laboratories, and then undergone a full structural elucidation

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Detecting and Dissecting Native Microbial Xyloglucan Degrading Systems using Activity-Based Probes

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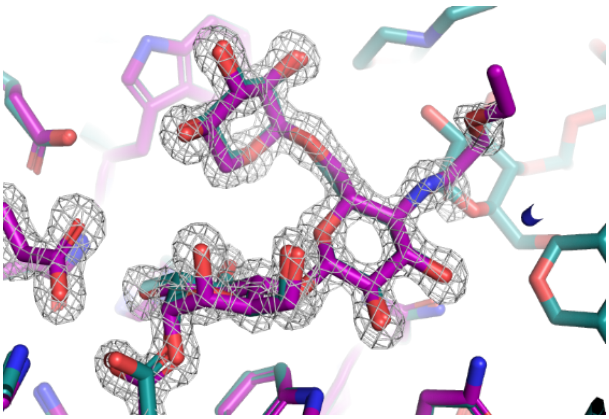
The identification and characterisation of key enzymes produced by carbohydrate-degrading organisms remains a significant challenge. High-throughput omics-based methods such as RNA-Seq and sequence-based inference generate large collections of genes of interest. However, when looking for key enzymes, such data-rich techniques often present a needle-in-a-haystack problem: which enzyme(s) are actually produced and function with the specificity of interest? Activity-based protein profiling (ABPP) enables the detection and identification of active enzymes displaying target specificities within complex biosamples. Building on the recently described cyclophellitol-derived endo-glycosidase activity-based probe architecture [1,2], a new probe for xyloglucanases, vanguard endo-glucanases involved in the utilisation of the ubiquitous plant polysaccharide xyloglucan, is presented. The designed minimal probe scaffold forms a near-perfect mimic of the glycosyl enzyme intermediate upon addition to the enzymatic catalytic nucleophile. It is selectively recognised by xyloglucanases, and resists exo-hydrolase degradation. We present the application of this and other probes to the sensitive detection and identification of xyloglucanases and their accessory exo-hydrolases within whole cell lysates and secretomes. This work demonstrates the dissection of the native architecture of complex xyloglucan utilisation systems, facilitating in vivo investigations of substrate-responsiveness. Furthermore, our results highlight some remarkable structural features of native bacterial endo-glucanases.



Crystal structure of the xyloglucanase probe bound to a xyloglucanase (purple) overlaid with the glycosyl enzyme intermediate structure (cyan)

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2-Acetamido-2-deoxy-D-iminosugar C-glycosides: recent synthetic approaches and perspectives

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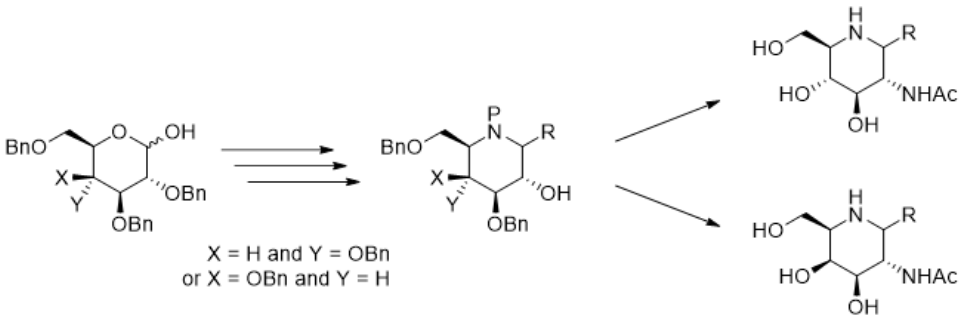
Whereas iminosugars are ranking among the most powerful glycosidase inhibitors [1], their promising C-glycoside derivatives have not yet shown their full potential as glycosidase or glycosyltransferase inhibitors. One of the main drawbacks associated with iminosugar-C-glycosides is their multistep sequence synthesis. This is even more obvious with 2-acetamido-2-deoxy-D-iminosugars, due to the presence of the sensitive NHAc moiety.

Because of the key biological role of GlcNAc and GalNAc and the valuable potential of relevant iminosugar analogues able to interfere with enzymes of high therapeutic interest [2], our group is interested in developing new access to this class of compounds.

Since the first syntheses of GlcNAc and GalNAc homoiminosugar analogues [3], we have shortened the synthetic sequences and enlarged molecular diversity of iminosugar-C-glycosides leading to L-derivatives [4]. These results open new perspectives in the mimicry of GlcNAc and GalNAc-derived glycoconjugates. Our last results in this field will be presented.



General strategy to access 2-acetamido-2-deoxy-D-iminosugar C-glycosides

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One pot synthesis of thio-glycosides via aziridine opening reactions

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Difficult synthesis and metabolic instability are major drawbacks in development of functional carbohydrate mimics. To combat this, new synthetic strategies and development of more stable analogs of natural carbohydrates are on the rise.

Our group has developed a new facile approach for synthesis of thio-glycosides, creating a class of pseudo-glycosidic compounds with improved hydrolytic and enzymatic stability and potential for further functionalization. [1]

In the developed procedure, formation of the thiol and opening of the aziridine are combined in a one pot reaction, providing an efficient and operationally simple alternative to classical glycosylation methods. The aziridine ring selectively opens when attacked by a glycosyl thiol generated in situ from a corresponding glycosyl thio-acetate, affording a single product via trans-diaxial opening.

We explored the reaction and optimized the conditions for various mono- and disaccharides, generating a library of compounds with general structure of N-linked-pseudo-thio-glycosides (Figure 1). Unexpected anomeric isomerization of glycosyl thiols under the reaction conditions was observed and the influence of temperature, base and solvent on the isomerization was investigated.

The synthesized glycomimetics can be potentially used for various applications, the N-linked tether embedded in the structure can be exploited for further functionalization as pseudo-glycopeptides or multivalent constructs. They are currently being investigated as DC-SIGN ligands, in development of novel rhamnose-based immunotherapeutics, etc.

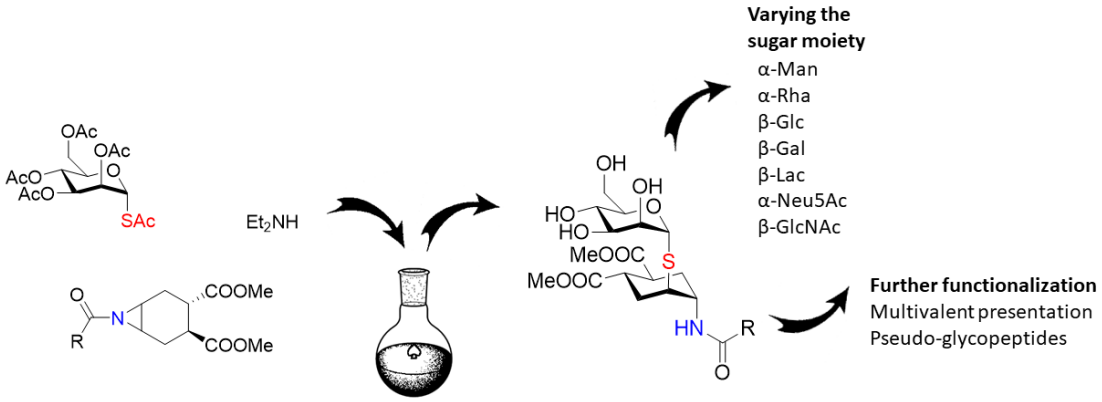

Figure 1: The principle of the one pot aziridine opening reaction.

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Detection of the rare galactofuranose in lichen polysaccharides using MS-IR

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Characterizing all isomerisms present in carbohydrates remains a major analytical today. Of particular interest to biologists, chemists and physicists is the ring-size of carbohydrates. The most abundant form for hexoses is a 6-membered ring, also called pyranose, which requires lesser energy to biosynthesize. Yet the furanose form (5-membered ring) is also found in some species like lichens. The presence of such rare forms, with a higher energy cost for the organism, seems to correlate with remarkable biological resistance properties.

The AlGAIMS project funded by ANR brings together an interdisciplinary consortium of lichen biologist, carbohydrate chemists, analytical chemists and molecular physicists, which aims at developing MS-based analytical strategies for the detection of galactofuranose in lichen polysaccharides. [1] In this context, I will present my PhD project, which consists of applying the MS-IR approach developed in Lyon, [2] for the identification of the ring size of galactose. Diagnostic MS-IR signatures of synthetic saccharides models were obtained. [3,4] Computational studies were carried out to explain these signatures. The application of this approach to the detection of galactofuranose in lichen extracts will be discussed.

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Variable Post-translational modifications of SARS-CoV-2 nucleocapsid protein

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease (COVID-19), started in 2019 in China and quickly spread into a global pandemic. Nucleocapsid protein (N protein) is highly conserved and the most abundant protein in coronaviruses and thus a potential target for both vaccine and point-of-care diagnostics. We have performed comprehensive glycomics, glycoproteomics and proteomics experiments on two different N protein preparations. Both were expressed in HEK293 cells, one was in-house expressed and purified without a signal peptide sequence and the other was commercially produced with a signal peptide channeling it through the secretory pathway.

Our results show completely different PTMs on the two N protein preparations. The commercial product contained extensive N- and O-linked glycosylation, as well as O-phosphorylation on site Thr393. Conversely, our own N Protein had O-phosphorylation at Ser176 and no glycosylation, highlighting the importance of knowing the provenance of any commercial protein to be used for scientific or clinical studies. Recent studies have indicated that N protein can serve as an important diagnostic marker for coronavirus disease and as a major immunogen by priming protective immune responses. Characterization of N protein can provide useful insights for understanding the roles of PTMs on viral pathogenesis, vaccine design and development point-of-care diagnostics.

Engineering sialylated lipid nanoparticles toward a new cure for Influenza

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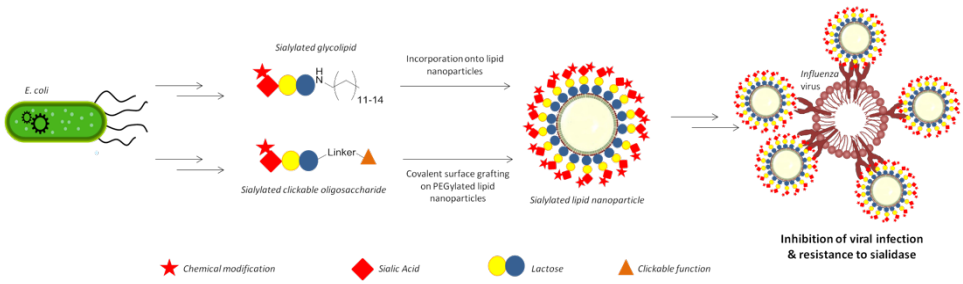
Lipid nanoparticles (LNPs) are now important players in modern medicine as they are easily producible at large scale and show low toxicity potential. Among them, Lipidots can be prepared with specified diameter, long term stability, and used for the vectorization of different bioactive compounds [1-3].

Sialic acid, the terminal carbohydrate of glycans in mammalian cells, is considered as "immunosuppressive", preventing serum protein degradation. Therefore, it can be used to make stealthy particles. Sialylated oligosaccharides are also known to bind with human Influenza virus hemagglutinins [4-5]. Consequently, sialylated LNPs would represent the most efficient multivalent decoy ligand for the development of a preventive treatment based on the saturation of the virus surface.

We describe herein the chemo-biotechnological production of several oligosaccharides incorporating a native or modified neuraminic acids with metabolically engineered E. coli cells. More precisely N-modified sialylated oligosaccharides have been targeted as they are resistant to viral sialidase [6]. Then two different pathways have been explored. On the one hand, the oligosaccharides have been coupled to aliphatic chains of different lengths. These glycolipids have been incorporated onto lipid nanoparticles as replacers of PEG-stearate normally used as surfactant in Lipidots. On the other hand, the oligosaccharides have been coupled to PEGylated Lipidots by surface grafting. In both cases, particles have been characterized in term of physico-chemical properties, stability and biological activity.



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Multivalent glycomimetics for scavenging of tumorigenic galectin-3

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Galectin-3 plays a crucial role in cancerogenesis, and its targeting is prospective for cancer diagnostics and therapy. Multivalent presentation of glycans can strongly increase the affinity to galectin-3, and further strengthening of interaction may be reached through aryl substitutions in the carbohydrate molecule [1]. We established a new, as yet undescribed chemoenzymatic method to produce selective C-3-substituted N,N'-diacetyllactosamine glycomimetics and coupled them to human serum albumin. The β-N-acetylhexosaminidase from Talaromyces flavus had the unique ability to efficiently synthesize the C-3-propargylated disaccharide, which was further conjugated with various aryl residues via click chemistry. Coupling to human serum albumin afforded multivalent neo-glycoproteins with up to 21,000-fold increased inhibitory potency compared to the lactose standard. Surface plasmon resonance brought further information on the kinetics of galectin-3 inhibition. The potential of prepared neo-glycoproteins to target galectin-3 was demonstrated on colorectal adenocarcinoma cells. The neo-glycoproteins efficiently scavenged exogenous galectin-3 in the microenvironment of cancer cells, inhibiting its interaction with the cell surface, and protecting T-lymphocytes against galectin-3-induced apoptosis [2]. Due to their straightforward synthesis, selectivity, non-toxicity, and high efficiency for targeting exogenous galectin-3, the neo-glycoproteins are prospective for application in the immunomodulatory treatment of galectin-3-overexpressing cancers.

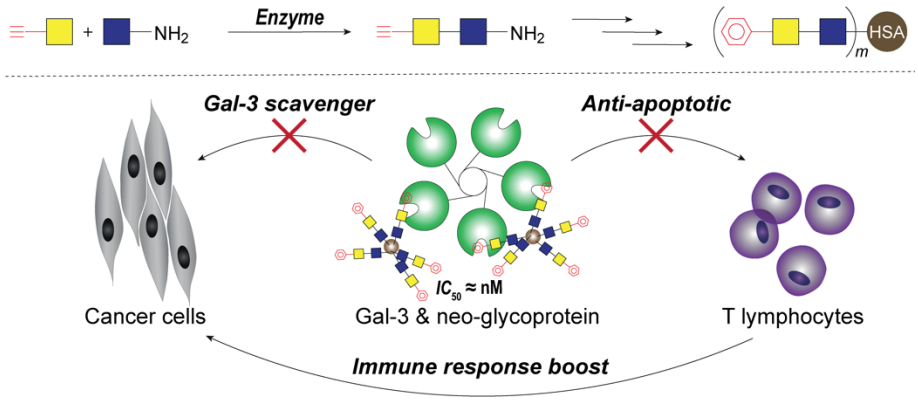

Figure 1: Schematic synthesis and biological impact of glycomimetic-carrying neo-glycoproteins.

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Midkine interaction with CS model synthetic tetrasaccharides and their mimetics.

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Midkine (MK) is a neurotrophic factor that participates in embryonic CNS development and neural stem cell regulation. MK is strongly expressed from mid-gestation until birth or associated with the pathogenesis of malignant diseases in adults. Recently midkine was demonstrated to interact with sulfated glycosaminoglycans (GAGs), mainly with heparin and chondroitin sulfate (CS), this being its natural ligand in the CNS. Among CS, there is some selectivity; CS-E is active while CS-A is not. Using synthetic tetrasaccharides as models of CS-E and CS-T and mimics of them by the inclusion of aromatics moieties in the basic carbohydrate skeleton. We carried out a structural study of this library by NMR and MD methods, concluding that the 3D-structure is a helix with four residues per turn that can be considered linear. We have studied the tetrasaccharide-midkine complexes by ligand observed NMR techniques and concluded that the shape of the ligands does not change upon binding. The ligand orientation into the complex is very variable. It is placed inside the central cavity of midkine formed by the two structured beta-sheets domains linked by an intrinsically disordered region (IDR) that is also involved in the binding. Docking analysis confirmed the participation of aromatics residues from MK together with electrostatic interactions. Finally, we test the biological activity by stimulating the MK expression using CS tetrasaccharides and the capacity of several tetrasaccharides in enhancing the growth stimulation effect of MK in NIH3T3 cells

Which role play hydroxide ions during sugar isomerization in presence of solid catalysts?

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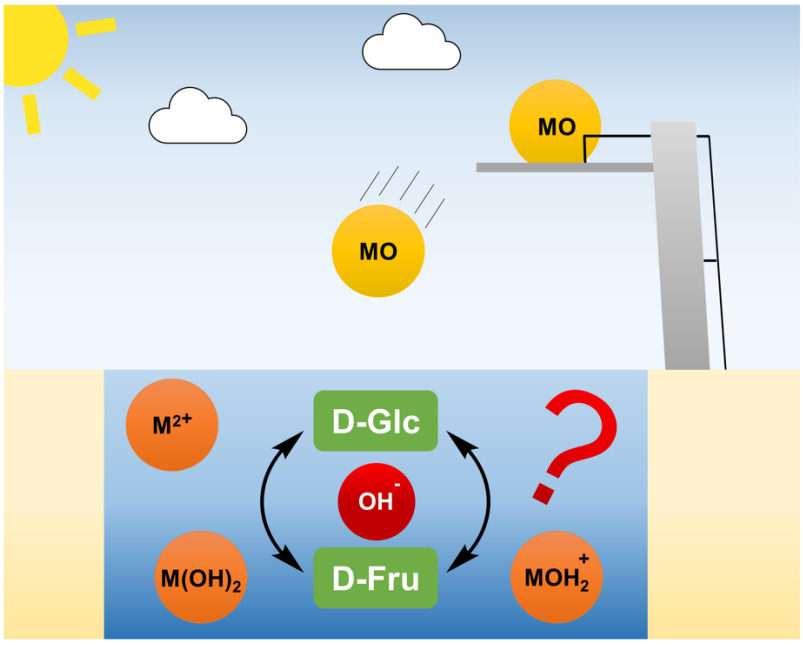
A key step in the concept of cellulose valorization is the conversion of D-glucose into D-fructose.[1-2] Especially solid base materials have gained in attention, as these materials are regarded as highly active and inexpensive catalysts for this reaction. However, the catalysis mechanism is not yet fully understood, which hinders the knowledge-driven development of a base catalyst. In this work, we studied the isomerization of D-glucose into D-fructose and vice versa in the presence of MgO, CaO, SrO and Ba(OH)2 catalysts. Isomerization was carried out under batch conditions at 40 °C with 10wt.% aqueous solution of substrate and systematic variation of the catalyst mass. Besides determination of the reaction rates of the isomerization, the evolution of the pH values and concentration of leached metal was monitored during reaction. Our results indicate homogeneous catalysis by hydroxide ions released by a partial dissolution of the materials. The poorly soluble MgO (pKs 9.8) continuously generates hydroxide ions during the reaction, while the more soluble CaO (pKs 5.3), SrO (pKs 3.2) and Ba(OH)2 (pKs 3.6) generate alkalinity directly upon immersion in the reaction solution. Comparable conversion-selectivity plots were observed for isomerization in presence of the catalysts used. The applicability of filtration tests and contact tests to estimate the contributions of homogeneous and heterogeneous catalysis is discussed. For isomerization catalyzed by bases, deceptive results of these tests may be obtained due to in situ generation of catalytically active species.



Graphical abstract

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Fluorescence-quenched substrates for quantitative monitoring of glycosidases activity in live cells

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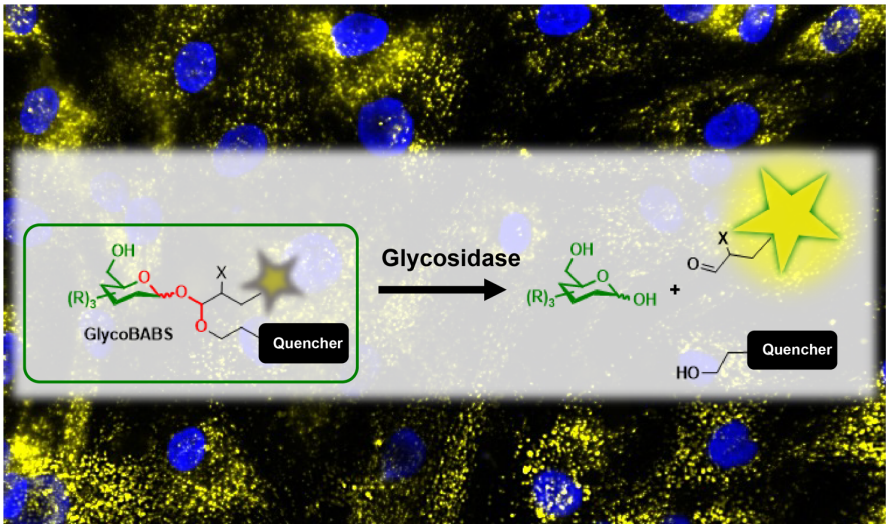
Enzymes act in a complex and crowded environment within cells, where trafficking and an array of binding partners and protein modifications regulate their activities. However, characterization of their activity relies almost entirely on assays carried out *in vitro*, in absence of cellular factors, and predominantly using purified enzymes.(1) Accordingly, a complete understanding of enzyme function and regulation requires quality tools able to evaluate and quantify their activity within living cells.

We describe here a strategy for live-cell quantitative monitoring of the activity of the disease-relevant human α-galactosidase (GalA) and α-*N*-acetylgalactosaminidase (NAGal) using bright and modularly-assembled Glyco-Bis-Acetal-Based Substrates (BABS).(2) We illustrate the benefit of BABS substrates in living cells to *(i)* precisely measure engagement of target enzymes by inhibitors, *(ii)* assess the efficiency of pharmacological chaperones within live patient primary cells, and *(iii)* highlight the importance of *in cellulo* quantitation with the use of chemical perturbogens of the secretory pathway.



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Acid-promoted ring contraction in O-silylated S-galactopyranosides: scope and limitations

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A reliable access to biologically significant oligosaccharides, which include monosaccharide residues in furanose form, depends on the availability of the respective building blocks. A recently discovered [1] reaction of pyranose ring contraction in ethyl 1-thio-beta-D-galactopyranosides containing bulky silyl substituents (TIPS or TBDPS) at both O-2 and O-3 with retention of aglycone under mildly acidic conditions (aqueous TFA in dichlorometane (DCM)) creates new opportunities for the synthesis of partially protected galactofuranose thioglycosides.

In the continuation of this research, here we report that the reaction rates of acid-promoted pyranose ring contraction in 2,3-di-O-silylated S-galactopyranosides to the corresponding furanosides are strongly influenced by anomeric configuration and aglycone structure. Galactopyranose thioglycosides with beta-configuration undergo ring contraction faster than their counterparts with alpha-configuration. Derivatives with electron-donating alkyl substituents in aromatic aglycone can be involved in the ring contraction reaction under milder conditions (aqueous TFA in DCM). Noteworthy, the use of anhydrous TFA in DCM made possible a pyranose ring contraction also in galactopyranose derivatives with phenyl or 4-chlorophenyl aglycones, while galactopyranose thioglycosides bearing strongly electron-withdrawing 4-nitrophenyl aglycone did not undergo ring contraction under identical conditions.

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Probing viral receptors DC-SIGN/R-glycan interactions using polyvalent glycan gold nanoparticles

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Multivalent protein-carbohydrate interactions mediate the first pathogen-host cell contacts, which ultimately lead to infection. Elucidating their underlying mechanisms is thus of great importance, allowing us to design specific glycoconjugates that can potently block such interactions to prevent infection (1). Unfortunately, the structural mechanisms of some key lectins remain poorly understood, due to a lack of effective method to probe such complex and flexible cell membrane proteins. These proteins include DC-SIGN (2) and DC-SIGNR (3), a pair of closely-related tetrameric lectins which play a key role in facilitating the HIV, HCV, Ebola and Zika virus infections. Herein, we report how glycan-nanoparticle size affects their DC-SIGN/R binding. We have prepared a series of glycan-gold nanoparticles (GNPs) by self-assembly of lipoic acid-oligo-(ethylene glycol)-glycans (LA-EGn-glycan, n = 2, 4; glycan = mannose, dimannose) onto different size GNPs (e.g. 5, 13, and 27 nm) to study their binding with DC-SIGN/R (dye-labelled). By exploiting GNP's strong fluorescence quenching as readout, we find that the binding affinity between DC-SIGN/R and glycan-GNPs is increased with the increasing GNP size (e.g. 27 > 13 > 5 nm). Moreover, the 13 nm glycan-GNPs binds even more strongly with DC-SIGNR than with DC-SIGN, which is opposite to those observed with glycan-QDs (4) and 5 nm glycan-GNPs, suggesting size play a critical role in controlling DC-SIGNR binding. Finally, we report that the EGn linker length also plays an important role in controlling DC-SIGNR binding (5).

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Asymmetric Synthesis of Heterocycles using carbohydrate as chiral scaffolds

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The development of new asymmetric methods to construct chiral heterocycles continues to attract carbohydrate chemists due to their crucial in biological and medicinal chemistry sciences. Chiral heterocyclic compounds play an essential role in numerous biological processes, e. g. as enzyme cofactors, bases of nucleotides etc. Moreover, biological efficacy of many pharmaceuticals is based on heterocyclic systems. In the events, chiral heterocyclic systems are widely used as substructures of some pharmaceuticals or as drugs which are in medical use. However, the syntheses of chiral heterocycles suffer from low yields or lack in regio- and stereoselectivity or multistep procedures, needed to achieve the target molecule. Therefore, there is a high demand for developing efficient and diastereoselective strategies for the synthesis of chiral heterocyclic systems in enantiopure form especially for the medicinal industry. Herein, we demonstrate a variety of examples for regio- and stereoselective transformations of 2,3-anhydropentoses delivering a series of chiral heterocyclic systems. Such carbohydrate-derived chiral templates can be further transformed into naturally occurring compounds or to interested biologically active drugs (Fig. 1).

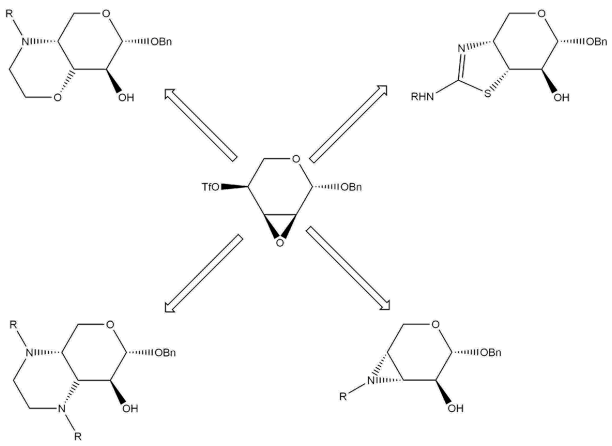

Fig. 1. Representation of new chiral heterocycles, synthesized from 2,3-anhydropentoses, used as a chiral scaffold.

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Structure Guided Development of Novel Glycosidase Active Compounds for Lysosomal Storage Disorders

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Lysosomal glycoside hydrolases are a small class of carbohydrate active enzymes which degrade glycolipids1. Deficiencies in these enzymes lead to a range of lysosomal storage disorders such as Gaucher (GD) and Fabry disease (FD), which affect the major organs resulting in premature death2,3. Although there is no cure for these disorders, progress is being made in diagnostic/therapeutic strategies4. However, this requires an understanding of how the activity of these enzymes influence disease states. In this regard, my PhD focussed on the development of novel activity-based probes (ABPs), inhibitors and chaperones for the study of β-glucocerebrosidase (GBA) and α- Galactosidase A (α-Gal), the enzymes associated with GD and FD. Through protein x-ray crystallography and biophysical assays, a novel class of reversible α-Gal cyclosulfamidate inhibitors were developed as molecular chaperones for potential use in FD chaperone therapy5. Additionally, a number of potent C6-substituted cyclophellitol inhibitors of GBA were developed for the generation of neuropathic GD animal models in zebrafish6. During my PhD, I also sought to address the lack of non-clinical GBA available for GD research by establishing an insect-baculovirus expression vector system7. The resulting GBA formulation exhibits comparable biochemical/biophysical properties to pharmaceutical products and crystallises readily, facilitating the structural analysis of multiple GBA active compounds. This system therefore circumvents the need for therapeutic GBA formulations for future biochemical and structural GBA studies.

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Synthesis of AI-2 Derived Sugar Prodrugs and Chemical Probes

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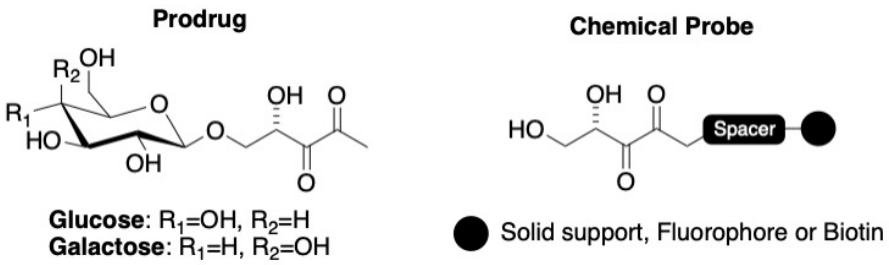
Autoinducer-2 (AI-2),[1,2] is a signalling molecule for bacterial inter-species communication. Understanding the molecular mechanisms that bacteria use to communicate and therefore regulate their group behaviours can lead to the development of new therapies to control bacterial infections. We hypothesise that AI-2 plays an important role in controlling colonisation and homeostasis of the gut microbiota contributing to protective properties against pathogens.[1] We present the synthetic strategy to obtain new beta glycoside analogues of AI-2(Fig.1) that will function as prodrugs to deliver intact AI-2 to the gut, taking advantage of beta-glycosidases produced there. The anomeric selectivity of the glycosylation reaction between AI-2 precursors and different thioglycosides has been studied.

Another aim is to develop chemical probes using different synthetic strategies (Fig.1).[4] These probes will allow the identification of new AI-2 receptors. Despite the conservation of the AI-2 production machinery in a wide variety of bacteria, at the moment only two classes of protein receptors for AI-2 have been identified.[1,3] However, evidence that bacteria that do not have the known receptors respond to AI-2, suggests the existence of other classes of AI-2 receptors.[1,4] We describe the synthesis of new AI-2 derived molecules that will either allow the modulation of gut microbiota composition or increase the knowledge on AI-2 mediated bacterial quorum sensing. Both approaches will be relevant for the development of new and effective strategies to manipulate bacterial behaviours.

Figure 1



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Acetyl group migration across the glycosidic bond

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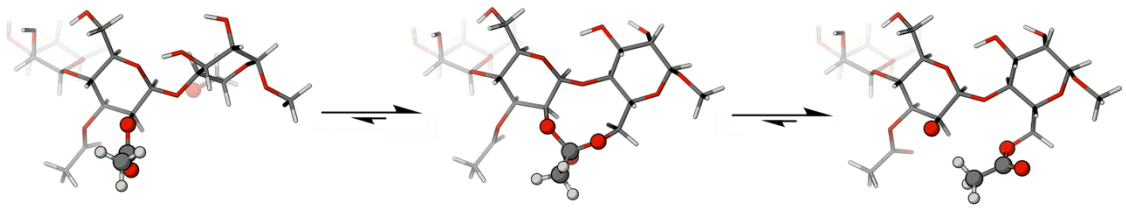
The well-known acyl group migration phenomenon was first demonstrated in carbohydrates by Fischer in 1920 [1]. Since then, most of the studies performed have focused on monosaccharides and in only a few cases on oligo- or polysaccharides [2]. In monosaccharides, the acyl groups have a large preference for the primary position. In the absence of primary hydroxyl groups, a more even equilibrium may take place between the secondary hydroxyl groups. Studies on oligo- and polysaccharides have indicated that the migration mainly takes place within the same monosaccharide units, although detailed studies have been lacking. Recently, we demonstrated for the first time that intramolecular acetyl group migration may also take place between the different monosaccharide units of oligosaccharides, across the glycosidic bond [3].

Many of the natural polysaccharides, including xylans, glucans and mannans, contain acetyl groups in different positions of their backbones [4]. These naturally occurring polysaccharides often display a range of biological roles and activities, which several studies have linked to the presence of acetyl groups [5]. The recently demonstrated acetyl group migration between saccharide units could help to understand the regulation of the biological activity of polysaccharides, both within the plant and also in therapeutical use.

Here, we provide closer insights into the reaction kinetics of the migration process in the oligosaccharide model compounds and discuss the expansion of our studies towards acetyl group migration also in the native polysaccharides.



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Glycomimetics to Inhibit Influenza Infection

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As a potential alternative to current flu antivirals, we are developing glycomimetics that block the interaction between the viral hemagglutinin (HA) and the host glycans that serve as flu-receptors. This interaction is essential for viral adhesion, and the universal receptor for flu A [15] and B [16] viruses is Sia-Gal (sialic acid, Sia, a.k.a. neuraminic acid, linked to galactose, Gal). HAs from either human (H1N1) or avian-infective (N5N1) flu strains bind to di- or trisaccharides terminating in Sia-Gal regardless of whether the Sia-Gal linkage is α2,6 or α2,3. This observation suggested that a small Sia-Gal glycomimetic might be developed that could inhibit a broad range of flu A strains, including flu B, and was critical in the design of our glycomimetic framework (FB127).

Here were report that FB127 shows dose-dependent inhibition of the infection of MDCK cells by a range of flu viruses from group 1 (H1N1, H5N1), group 2 (H3N2), and flu B, and that the influenza neutralizing ability of FB127 is within the same range as reported for glycodendrimers. Because FB127 is a synthetic small molecule, it can be rationally modified, and manufactured with a high degree of quality control and reproducibility. In this regard, FB127 is amenable to a number of formulations, including aerosol, oral, and intravenous routes that provide great flexibility in drug delivery.

Targeting sialoglycan – Siglec interactions to shape host immunity

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Siglecs (Sialic acid-binding immunoglobulin type lectins) are a family of cell surface transmembrane receptors belonging to I-type lectins, predominantly expressed by immune cells. [1-2] Individual family members exhibit preferences for sialosides of various linkages to underlying glycan motifs, but the physiological ligands they interact with are largely unknown.

Many Siglecs, such as Siglec-2, Siglec-7 and Siglec-10, are inhibitory receptors involved in the down-regulation of cell signalling upon the interaction with sialylated glycans that act as determinants of self. Interestingly, clinically relevant pathogens possess the ability to decorate their surface with glycans that mimic self-associated molecular patterns, bind to inhibitory Siglecs, and escape immune surveillances. Thus, Siglecs are nowadays considered glyco-immune checkpoints and exhibit a great therapeutic potential for the treatment of autoimmune, neurodegenerative and cancer diseases.

In this context, we investigated the molecular mechanisms underlying sialoglycans recognition by Siglecs using a combination of biophysical, spectroscopic and computational approaches, with the aim to carry out a dynamic characterization of their interactions in solution. [3-5] Our outcomes provide a structural point of view for the design and development of high-affinity ligands able to control the receptor functionality.

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Probing molecular recognition of sialoglycans by Nuclear Magnetic Resonance and Molecular Modelling

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The interplay between sialoglycans and their receptors lays the foundation for numerous molecular recognition processes like cell–cell interactions, information transfer and pathogenesis.[1] Of particular interest is their role as selective mediators in disease, defense, and symbiosis,[2] making sialoglycans ideal candidates to design effective diagnostic tools or therapeutic drugs. To date, several sialic acid-based drugs have already been designed and currently employed as therapeutics, diagnostics and vaccines and many others are expected to come in the next years.[3]

To this purpose, the atomic level characterization of sialoglycans binding upon their receptors sets the basis for the design and development for novel therapeutics. In this context, we applied nuclear magnetic resonance (NMR), computational and biophysical techniques to provide a deeper comprehension of the biological processes underlying sialoglycans recognition by therapeutically relevant sialic acid binding proteins, such as Siglecs[4][5] (Sialic acid binding immunoglobulin-type lectins) as well as neuraminidases.[7] Our studies allowed for the rigorous definition of the bioactive conformation and the binding epitope of natural occurring sialoglycans and their analogues and afforded the atomistic description of the 3D complexes structures. The obtained outcomes represented a first step toward the development of more effective drugs to prevent life threatening diseases, including cancer and autoimmune disorders.[8]

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Convenient Access to Amphiphilic b-C-glycosylbarbiturates for LCD-biosensors and Hydrogels

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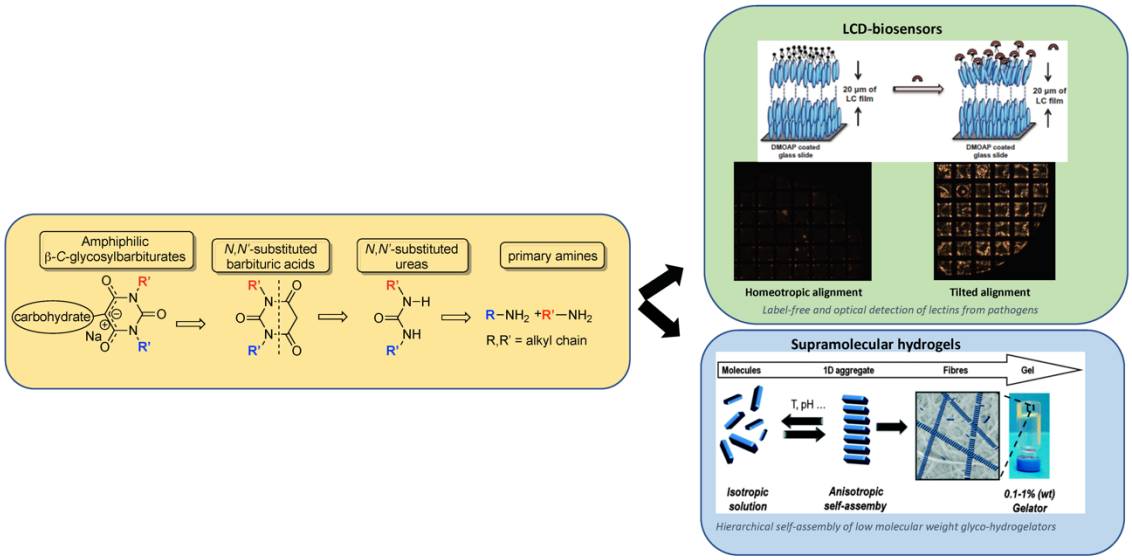
Synthesis of glycoconjugates is certainly one of most performed in carbohydrate chemistry because of their multiple roles in biological processes. In fact, they are mainly exposed at the cell surface which interact with surrounding environment such as lectins, growth factors, enzymes and so on. Thus, reducing-end modification of carbohydrates is highly mandatory and glycochemists are still looking for efficient synthetic methodologies.

In this communication, Knoevenagel condensation onto free protecting-group carbohydrates will be highlighted by using a modular chemical platform consisting in N,N'-substituted barbituric acids.[1],[2] The ease of synthesis of barbituric acid derivatives and related b-C-glycosylbarbiturates allows to consider this "glyco-click" coupling as a valuable chemical toolbox for glycosciences and some examples of applications will be exposed.In our group, since a decade, we developed sugar-based amphiphiles for their ability to self-assemble into glyco-nanostructures. The barbituric acid-mediated Knoevenagel condensation approach allows us to design libraries of glyco-amphiphiles obtained in water.

Preliminary results will show how those glyco-amphiphiles have been used as active layers adsorbed at the hydrophobic thermotropic liquid crystal/aqueous interface to develop specific recognition patterns with targeted lectins leading to a simple, sensitive, label-free, and fast optical detection method. Stimuli-responsive glyco-hydrogelators have been developed too, owing to self-assembly through non-covalent interactions into supramolecular hydrogels.



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Synthesis of glycopeptides through chemoselective Pd-mediated conjugation

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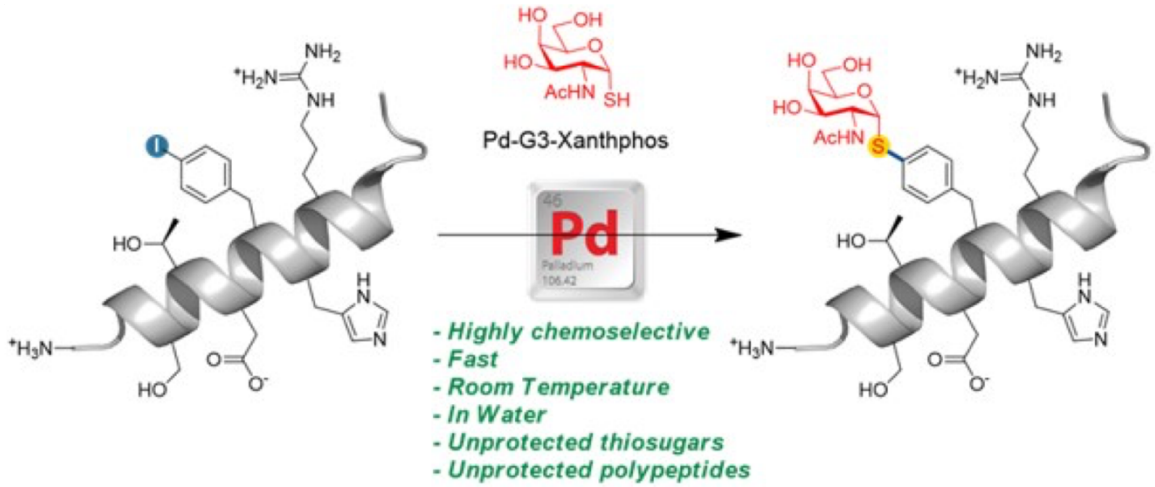
We describe herein the development of a Pd-catalyzed coupling methodology for the glycoconjugation of unprotected peptides under mild reaction conditions. This operationally simple process occurs under semi-aqueous conditions and displays wide substrate scope. The strategy has been successfully applied to both the thioglycosylation of unprotected long peptides and the generation of thioglyco-aminoacid building blocks, including those suitable for solid phase peptide synthesis. To demonstrate the broad potential of this technique for late stage functionalization, we successfully incorporated challenging unprotected β-S-GlcNAc- and α-S-GalNAc- derivatives into very long unprotected peptides. This study opens the way to new applications in chemical biology, considering the well-recognized resistance of S-glycosides towards both enzymatic and chemical degradation as compared to their O-glycoside counterparts.



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Glyco-PAINT: Single molecule imaging of glycan-lectin-interactions on cells

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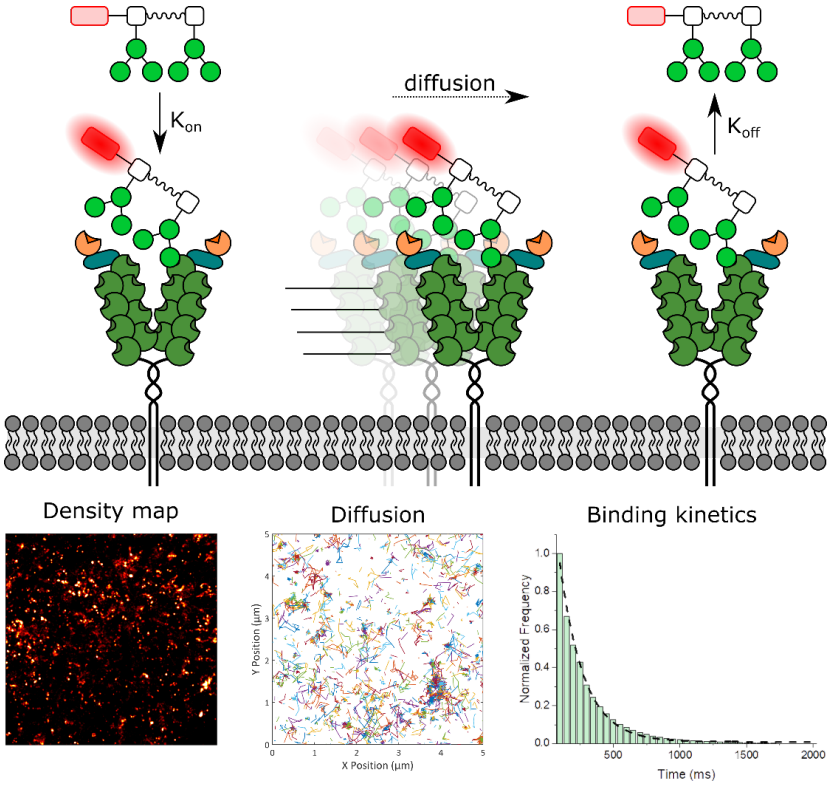
The mannose receptor (CD206) is an endocytic receptor involved in host/pathogen recognition in the immune system and has been targeted for vaccine and drug delivery purposes. It binds to different carbohydrate ligands with relatively low affinity, which has made identification of optimal ligands difficult. Here we introduce a PAINT super-resolution microscopy method to capture the weak glycan-lectin interactions at the single molecule level in living cells (glyco-PAINT). Glyco-PAINT exploits the weak and reversible sugar binding to directly achieve single molecule detection and quantification in cells and has been used to establishment the relative kon and koff rates of a synthesized library of oligomannoside and 4-sulfo-N-acetyl galactosamine probes, as well as the diffusion coefficient of the receptor-sugar complex. The uptake of the ligands is correlated to the binding affinity and residence time of the ligands to establish structure-function relations for the different synthetic glycans. It is revealed how the sugar multivalency as well as the presentation geometry can be optimized to achieve binding and internalization. Overall, Glyco-PAINT represents a powerful approach to study weak glycan-lectin interactions on the surface of living cells that can be potentially extended to a variety of lectin-sugar interactions.



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Microarray-based evaluation of HexNAc-O-tyrosine specific rabbit antibodies

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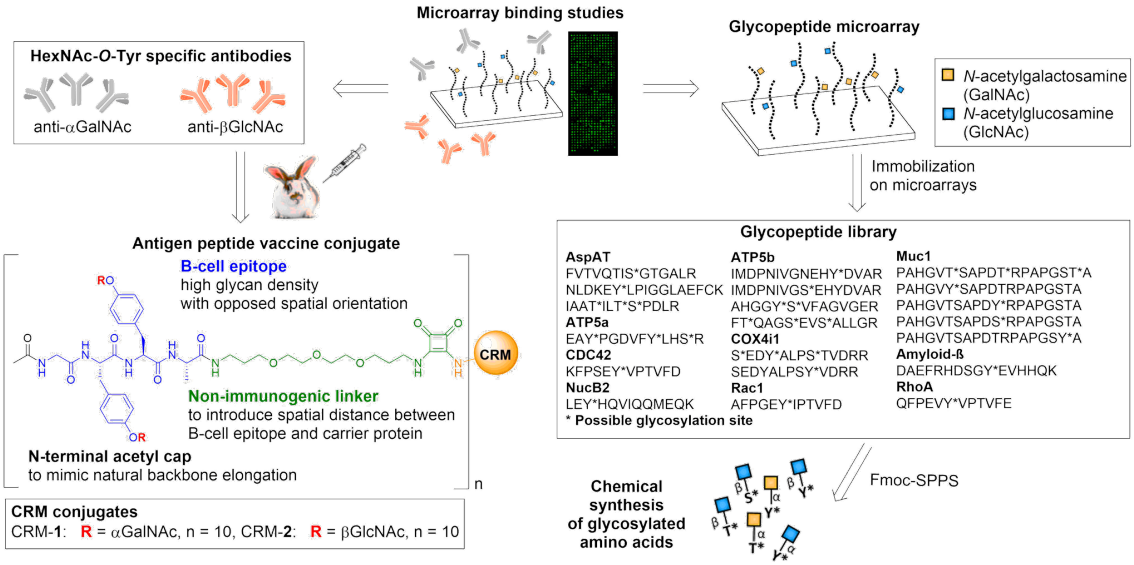
Protein glycosylation is one of the most abundant and diverse forms of post-translational modifications. Usually, *O*-glycans are attached to the protein backbone via serine or threonine. In 2011, a new type of protein *O*-glycosylation has been reported where the carbohydrate *N*-acetylhexosamine was attached to the amino acid tyrosine (HexNAc-*O*-Tyr).[1] Although this modification has been discovered on several glycoproteins, the knowledge about its biological roles, biosynthesis and expression is limited.[2-5] A reason for this is the lack of efficient methods to determine the exact structures and linkages of the attached carbohydrates, and to specifically detect and enrich this modification. In this study, we generated HexNAc-*O*-Tyr specific antibodies to selectively detect and profile peptides and proteins carrying this modification. Therefore, we induced polyclonal HexNAc-*O*-Tyr specific rabbit antibodies using antigen HexNAc-*O*-Tyr glycopeptide conjugates, and confirmed their HexNAc-*O*-Tyr specificity using glycopeptide microarrays. Consequently, we prepared a peptide library including peptide sequences carrying α-GalNAc, α- or β-GlcNAc on serine, threonine or tyrosine, and immobilized it on microarrays. Finally, we showed that the obtained HexNAc-*O*-Tyr specific antibodies can be applied to selectively detect this modification on protein level. Therefore, the antibodies were purified by affinity chromatography, re-evaluated by microarray assays and used to specifically detect αGlcNAc-*O*-Tyr modified RhoA using Western blot.



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Multivalent inhibitors of bacterial sialidases

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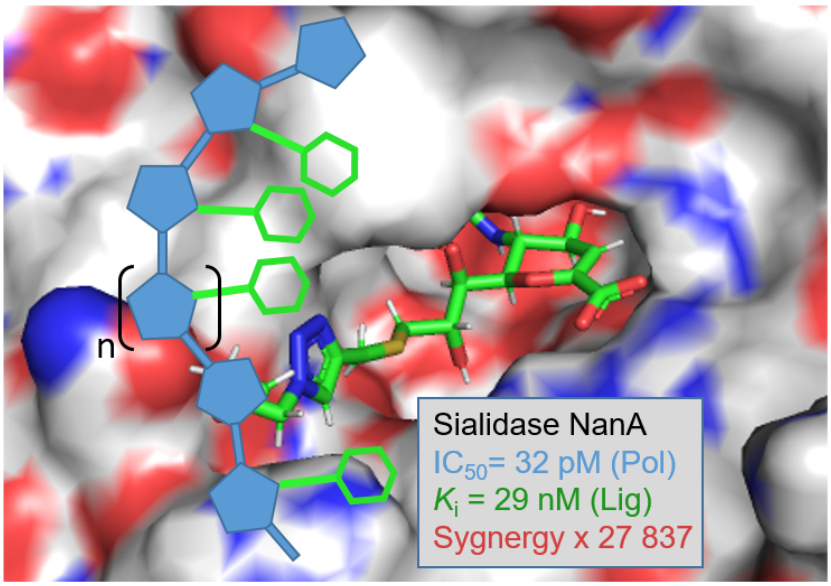
Sialidases (SA) are a family of exoglycosidases hydrolysing sialic acids and are common virulent factors expressed by bacteria, virus and parasites. The catalytic domain (CAT) of bacterial SA from the GH33 family is often flanked with a lectinic domain or carbohydrate-binding modole (CBM). The CBM was shown to significantly improve the SA catalytic efficiency for polysialylated surface.[1]

In this work, we developed non-hydrolysable multi- and polyvalent thiosialosides to bind in the CAT and CBM of bacterial SA. The polymeric thiosialosides led to highly potent inhibitors of Streptococcus pneumoniae SA (NanA) with high synergistic multivalent effects.[2] Surprisingly, the synergistic interaction was shown to operate in the CAT domain exclusively, and not in the flanked CBM. Thus, we developed a second generation of CAT-selective polyvalent compounds based on a potent transition-state analogue of sialyl substrates (2-deoxy-2,3-didehydro-N-acetylneuraminic acid = DANA). [3] Poly-DANA inhibits the activity of NanA and B. thetaiotaomicron (BtSA) at picomolar and low nanomolar levels when expressed in moles of molecules and DANA, respectively. They surpassed the inhibitory potential of the monovalent reference DANA by more than four orders of magnitudes. These results extends the concept of multivalency to this important class of enzymes.



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The architecture of starch blocklets follows phyllotaxic rules

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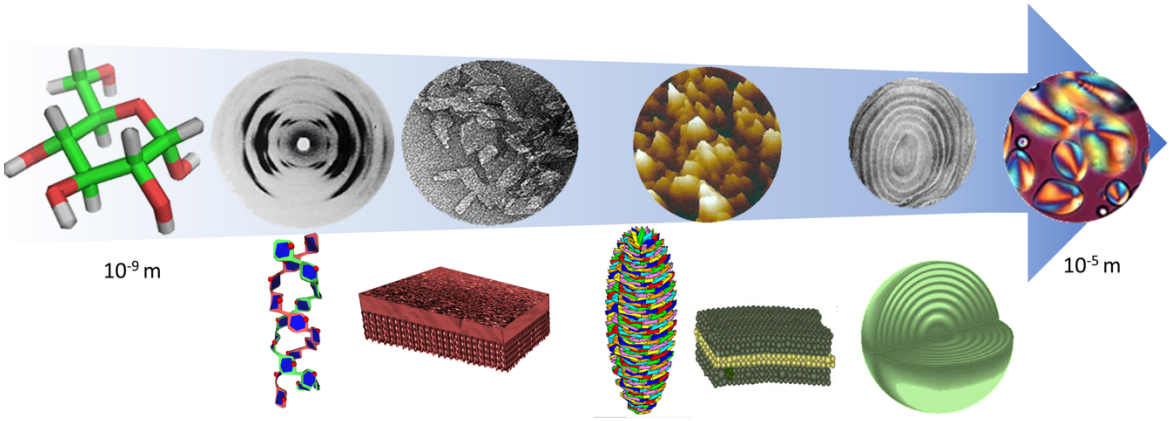
The starch granule is Nature's way to store energy in green plants over long periods. Irrespective of their origins, starches display distinct structural features: the fingerprints of organisation levels over six orders of magnitude. We hypothesised that Nature retains hierarchical material structures at all levels and that some general rules control these structures' morphogenesis. We considered the occurrence of «phyllotaxis» like features that would develop at scales ranging from nano to micrometres and developed a novel geometric model capable of building complex structures from simple components. According to the Fibonacci Golden Angle, we applied it to form several Golden Spirals and derived theoretical models to simulate scattering patterns.

A Golden Spiral Ellipsoids, constructed with elements made up of parallel stranded double-helices, displayed shapes, sizes and high compactness reminiscent of the most intriguing structural element: the 'blocklet'. The convergence between the experimental findings and the theoretical construction suggests that the «phyllotactic» model represents an amylopectin macromolecule, with a high molecular weight in agreement with experimental measurements.

While establishing a viable model of a consistent hierarchical organisation over four orders of magnitude, the present results offer a new 3-dimensional vision to reconsider previously experimentally reported data and extend our understanding of the structures' complexity and the underlying biosynthetic events.



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Advances in molecular docking of glycosaminoglycans

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Glycosaminoglycans (GAGs) represent a class of linear anionic periodic polysaccharides playing a crucial role in many biologically relevant processes carried out in the extracellular matrix of the cell though their interactions with proteins. Due to the lack of the structural data on protein-GAG complexes molecular docking could be a powerful approach in the structural analysis of these intermolecular systems. However, because of GAG high flexibility, long length, charged nature requiring proper description of electrostatic and solvent effects, diverse sulfation patterns, pseudo-symmetry and periodicity, lack of specific tools developed for these systems, docking GAGs remains a challenge. In the recent years, we have contributed to the pool of *in silico* methods aimed to deal with the docking of these systems specifically. First, we analyzed the prediction power of 14 conventional docking software on a dataset of protein-GAG structures. Then, we proposed novel GAG-specific approaches including: targeted molecular dynamics (MD)-based Dynamic Molecular docking approach accounting for the full flexibility of both ligand and receptor as well as for the solvent explicitly; Hamiltonian Replica Exchange MD where van der Waals radii are increased through the replicas allowing for more rigorous sampling of the protein surface leaving GAGs flexible and being independent of the GAG length; fragment-based approach where GAG trimeric sequences are docked with a conventional docking approach and then assembled together into longer GAG chains.

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Bacterial glycosylation: An overview on bacterial sweet side

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Glycosylation is a prevalent protein posttranslational modification found in all domains of life. Advances in genomics and mass spectrometry have revealed several types of glycosylation systems in bacteria. Among prokaryotes, general glycosylation systems have been reported in pathogenic bacteria, including *Acinetobacter baumanii*[1], *Helicobacter pylori*[2], *Campylobacter jejuni*[3] as well as commensal bacteria such as *Bacteroides fragilis*[4], indicating their abundance across the kingdom. However, the reasons why bacterial proteins are posttranslationally modified remains poorly defined. In this talk, I will address our extensive work in understanding bacterial glycosylation systems, using *C. jejuni* as a model organism. Our studies showed that *N*-linked glycosylation plays a role in maintaining proteome stability. Abrogation of *N*-linked glycosylation reduced multidrug efflux pump activity and substantially affected host-microbe interaction[5]. In depth studies revealed *N*-linked glycans role in promoting protein-protein interaction and enhancing protein thermostability[6]. This altogether affirms the pivotal role played by *N*-linked glycosylation in maintaining bacterial physiology in as well as deepens our knowledge of the emergence of bacterial posttransilational modification.

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Synthetic antigens related to Streptococcus suis type 9 glycans for conjugate vaccine development

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Infections caused by Streptococcus suis (S. suis) are responsible for severe economic losses in the swine industry [1]. Moreover, as an emerging zoonotic pathogen, it has also been a global indirect threat to human health [2]. S. suis has been classified into 35 serotypes based on the chemical composition of the capsular polysaccharide (CPS) structures. Serotype 9 is the most invasive serotype in Europe. In the case of S. suis serotype 9 infections, antibiotics are so far the only choice for treatment. However, the abuse of antibiotics will lead to bacterial resistance and various side effects [3]. To overcome these deficiencies, glycoconjugate vaccines provide an attractive preventing option.

S. suis serotype 9 CPS repeating unit consists of a branched tetrasaccharide with a phosphorylated D-glucitol residue. The presence of a labile C-4-keto sugar and the phosphodiester group pose considerable synthetic challenges. However, the corresponding reduced form exhibited improved stability, while maintaining the ability to induce an immune response against the CPS [4]. So we focused on the synthesis of the C4-axial reduced form of the repeating unit its analogues. Here we describe the design and first chemical synthesis of a library containing eight synthetic oligosaccharides resembling the CPS to aid the development of an anti-S. suis serotype 9 semisynthetic conjugate vaccine. Synthesized antigens carried a reducing-end linker that enabled covalent immobilization on microarray glass slides to evaluate the characteristics of antibody binding.

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Janus lectins as a scaffold for double specificity

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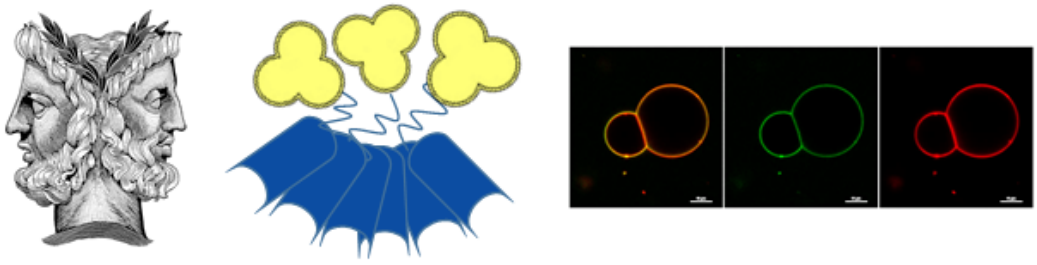
Lectins are sugar-binding proteins occurring in all types of organisms. They present a large panel of biological functions and can be a powerful tool for developing biotechnological and therapeutic applications. Although lectins came across a solution how to improve their affinity by multivalency [1], most of the natural lectins display specificity for one type of saccharide.

Using synthetic biology tools, a new type of protein, called Janus lectin was recently created [2]. The first Janus lectin was engineered as chimeric bispecific lectin which can bind independently fucosylated and sialylated glycoconjugates.

Continuing with this approach, we can now create new artificial lectins with dual specificity, regulate their valency and reach avidity effect. In order to explore new combinations, we are investigating several lectin families based on tandem repeat lectins with β–propeller fold, β-trefoil fold, and others. The suitable candidates for Janus lectins were selected by bioinformatics analysis [3]. The gene sequence was designed and protein was produced in bacteria and purified by a series of chromatographies. The function of Janus lectin was characterized by biophysical analysis.

The new Janus lectin possesses specificities for α-galactose and fucose and can interact with these saccharides simultaneously. Moreover, the crosslink of giant unilamellar vesicles was observed. Due to their dual specificity and multivalency, Janus lectins open new possibilities in the applications like cell targeting, drug delivery, and the construction of biosensors.



Mucinomics Crosslinking of giant unilamellar vesicles by Janus lectin

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Phthalazinone-Derivatives Bind the Galectin-8N CRD with Excellent Selectivity

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Galectins are sugar-binding proteins with specificity for β-galactoside sugars. Galectin-8 is a member that has immunomodulatory properties and is involved in tumour development and metastasis. The interactions that galectin-8 engages in to assert its function are a combination of protein–protein interactions and carbohydrate recognition.(1) To delineate the role of galectin-8 sugar-binding, selective binders of the galectin-8 N-terminal carbohydrate recognition domain (CRD) are important tools.

Here we present the design and synthesis of a series of highly selective galectin-8N ligands with a phthalazinone scaffold. We will explain the selectivity amongst these highly conserved galectin CRDs along with our molecular simulations studies. This unprecedented selectivity for the first time allows delineating the biological function of carbohydrate binding of galectin-8 in disease models. This series will feed the hit-to-lead optimisation in the drug discovery pipeline and may inspire those looking for selectivity within the galectin family.

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Revealing the human mucinome

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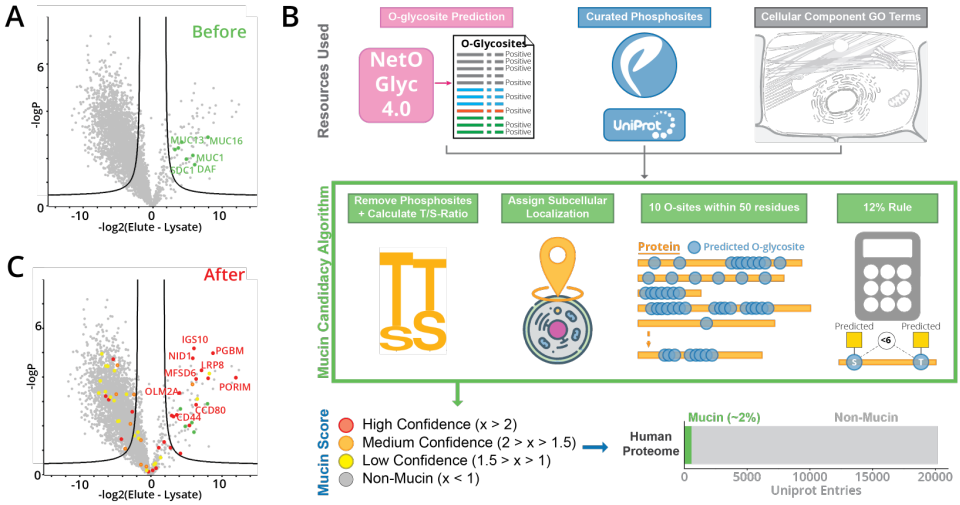
Mucin domains are densely O-glycosylated modular protein domains found in a wide variety of cell surface and secreted proteins. Mucin-domain glycoproteins are key players in a host of human diseases, especially cancer, but the scope of the mucinome remains poorly defined. Recently, we characterized a bacterial mucinase, StcE, and demonstrated that an inactive point mutant retains binding selectivity for mucins. In this work, we leveraged inactive StcE to selectively enrich and identify mucins from complex samples like cell lysate and crude ovarian cancer patient ascites fluid. Our enrichment strategy was further aided by an algorithm to assign confidence to mucin-domain glycoprotein identifications. This mucinomics platform facilitated detection of hundreds of glycopeptides from mucin domains and highly overlapping populations of mucin-domain glycoproteins from ovarian cancer patients. Finally, we used a KRAS dox-inducible system to show which mucins contribute to molecular bulk at the cell surface. Ultimately, we demonstrate our mucinomics approach can reveal key molecular signatures of cancer from in vitro and ex vivo sources.



Mucinomics platform allows for identification of enriched mucins from complex samples

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O-antigen polysaccharides of outer membrane vesicles as mediators of bacteriophage infection

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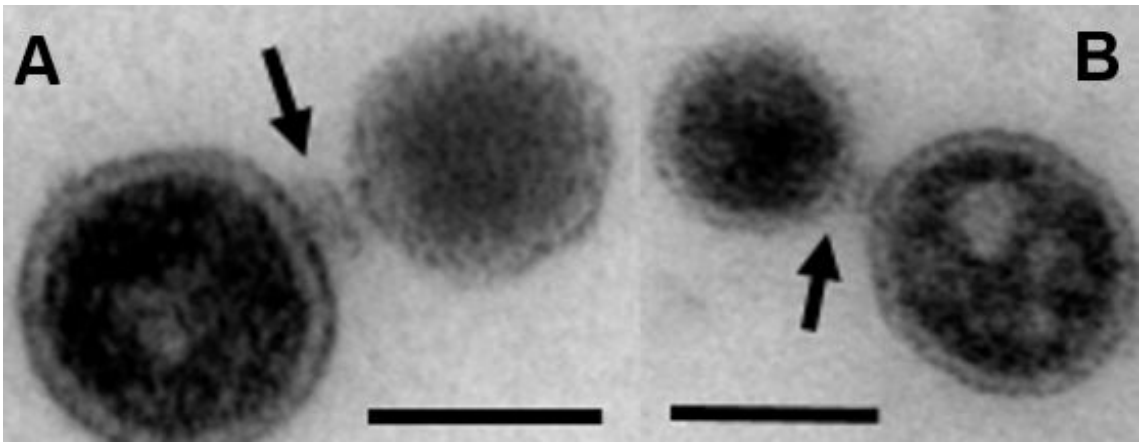
Bacteriophages exploit a large number of different bacterial glycan envelope structures as receptors for surface attachment. Consequently, bacterial surface glycans represent a major control point for the defense against phage attack. One strategy for phage population control is the production of outer membrane vesicles (OMVs) that contain their cell surface glycan receptors. In Gram-negative host bacteria, O-antigen-specific bacteriophages address lipopolysaccharide (LPS) to initiate infection, thus relying on an essential outer membrane glycan building block as receptor that is constantly present also in OMVs. In this work, we have analyzed how Salmonella (S.) bacteriophages interact with OMVs. Bacteria may distribute the phage glycan receptor differently to OMVs, depending on the actual cellular status. In in vitro assays, OMVs effectively reduced the number of infective phage particles. Fluorescence spectroscopy showed that presence of the O-antigen polysaccharide receptor was mandatory for inactivation. However, phage inactivation was only in part due to genome release into the vesicular lumen. In contrast, if phages were challenged with non-vesicular preparations of lipopolysaccharides containing the O-antigen receptor they lost their genomes completely, although more slowly than with OMVs. This stresses that glycan receptor presentation, as part of a lipid membrane, is an important feature for regulation of bacterial virus entry.



Electron microscopy of OMV-bound Salmonella bacteriophage P22 without (A) or after (B) genome ejection. Arrows show phage baseplate. Bar: 50 nm.

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Multichromophoric carbohydrates as fluorescent & reversible photoswitches for optical applications

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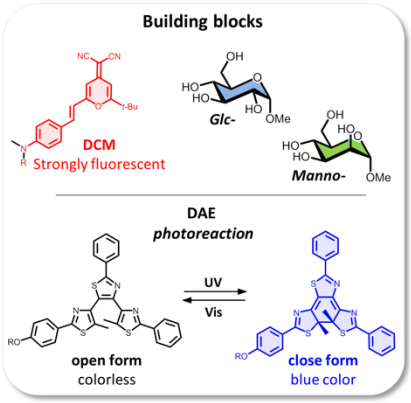
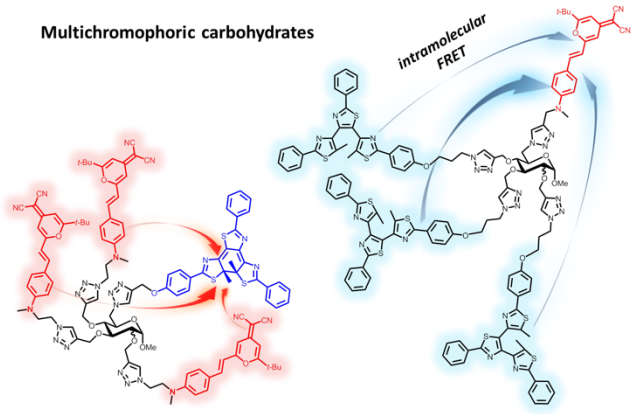
The development of photosensitive molecular systems attracts considerable interest in the growing fields of the photopharmacology and the photoresponsive nanotechnologies (optical memories and super-resolution imaging).[1],[2]

Because of their optical transparency and their structural diversity, carbohydrates appear as very interesting building blocks for the construction of novel photosensitive molecules bearing multichromophoric units. As a continuing program on the development of fluorescent photoswitchable molecules for biological and optical applications, we have designed and synthesized multichromophoric architectures based on carbohydrates derivatives.[3],[4]

To take advantage of the intramolecular energy transfer (FRET) possibilities between fluorophores and molecular photoswitches, we have combined photochromism of diarylethene (DAE) and fluorescence properties of dicyanomethylene (DCM) units on a single sugar unit. Thanks to the capability of DCM to photoisomerize as function of the wavelength of illumination,[5] we have discovered a new property: the fluorescent hysteresis effect. Hysteresis effect is very appealing for its fundamental aspects and can potentially find applications in “intelligent” molecular material. In order to caracterize it, the ratio of the linked chromophores (DAE/DCM) and the monosaccharidic platforms (glc- and manno-) were explored. Synthesis of these photoswitchable multichromophoric carbohydrates as well as their photochemical and photophysical properties by absorption and fluorescence under light illumination will be presented.





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Total Synthesis of Tiacumicin B: Study of the Challenging beta-Selective Glycosylations

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During the last decades, bacterial resistance to antibiotics has re-emerged as a serious biomedical risk impacting our quality of life. Tiacumicin B is an atypical glycosylated macrolide antibiotic that was approved by the U.S. FDA in 2011 for the treatment of nosocomial diarrhea associated with Clostridium difficile. Tiacumicin B inhibits RNA polymerase by interacting with the "switch" region blocking RNA synthesis and then killing bacteria.[1] Despite this promising biological activity, only one synthetic access to Tiacumicin B has been reported in 2020.[2] .This project aims at providing an efficient synthetic access to Tiacumicin B through the development of novel synthetic strategies and methodologies. Our strategy is based on our experience with the synthesis of the tiacumicin B aglycone and on unique 1,2-*cis*-glycosylation steps. It features the conclusive use of sulfoxide anomeric leaving-groups in combination with a remote 3-*O*-picoloyl group on the donors allowing highly beta-selective rhamnosylation and noviosylation that rely on H-bond-mediated Aglycone Delivery (HAD). [3] (Figure 1).

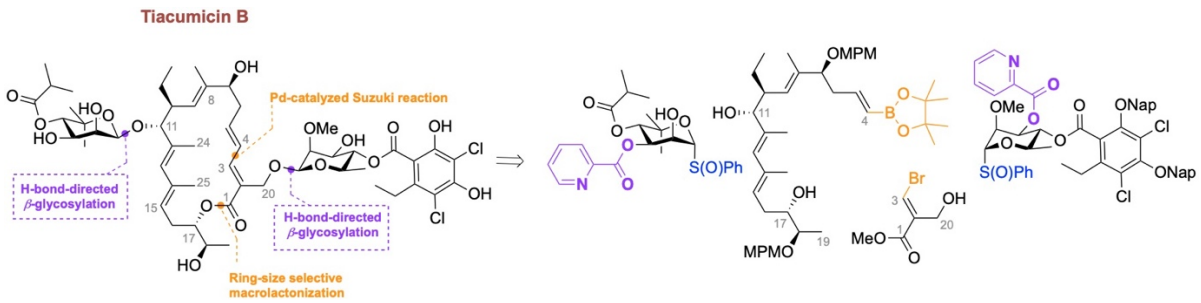

Figure 1

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Towards iminosugar based probes for profiling of glycoprocessing enzymes

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Activity based protein profiling (ABPP) is a powerful and efficient tool for evaluating enzyme activity in living systems.[1] With respect to glycoprocessing enzymes (CPE) valuable ABPP strategies have been developed for use in medicinal diagnosis, in high-throughput inhibitor discovery, in enzyme discovery and mechanistic evaluations.[2] In a broader context, ligand directed chemistry (LDC) for protein labelling has been introduced by Hamachi and co workers.[3] By this method the protein of interest is covalently tagged with a reporter group outside its active site region thus allowing to preserve the enzymes activity after labelling. We aim for applying this method to glycoprocessing proteins. Therefore, we have designed and synthesised iminosugar based probes featuring the respective components of ligand directed chemistry probes and examined their ability on a model enzyme, β-glucosidase from almonds, in a proof of concept study. Results towards iminosugar based ligand directed chemistry probes for activity based profiling of glycoprocessing enzymes will be presented.

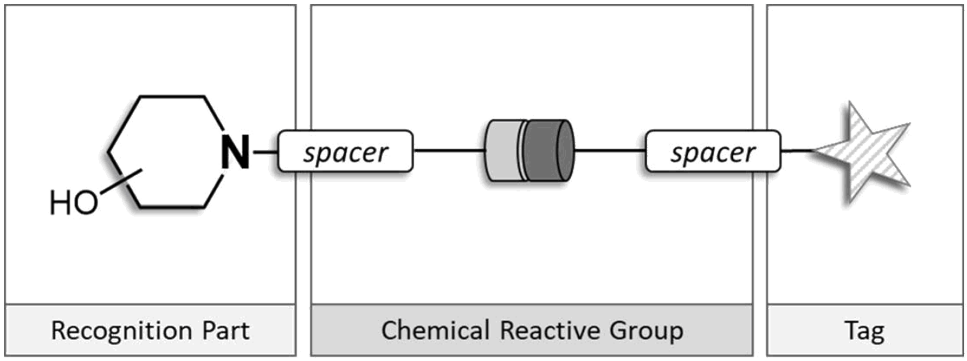

Figure 1: Structural concept of iminosugar based ligand directed chemistry probes.

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Binding Specificities of LecB and TcdA Towards Fucosylated Mucin Glycopeptides

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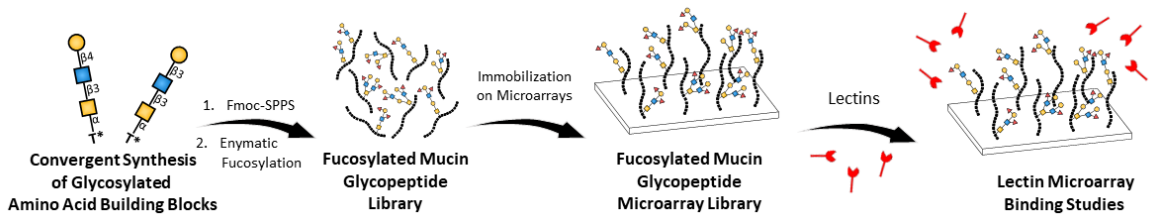
Mucins are densely O-glycosylated membrane-bound or secreted proteins ubiquitously found on the epithelial cell surface.[1] They are part of the innate immune system and play major roles as protective barriers to defend the host against invading pathogens.[2] However, bacteria and viruses have co-evolved with the human host and developed strategies to promote virulence for instance by adhering to carbohydrate ligands on the host cell-surface via pathogenic lectins. In order to improve our understanding of the pathogenic adhesion processes on a molecular level, we study the interactions between pathogenic lectins and carbohydrate ligands presented on mucin peptide backbones. In the presented study binding specificities of two bacterial lectins that recognize and bind to fucose containing glycans were evaluated: LecB from Pseudomonas aeruginosa, an opportunistic bacterium that is a major player in airway diseases[3-5], and toxin A from Clostridium difficile (TcdA), a bacterium that causes gastrointestinal disorders.[6] Selected synthetic mucin core and extended core glycopeptides were enzymatically modified with Lewis a, Lewis x, or H-type motifs as well as bi-fucosylated Lewis b and Lewis y structures. Then the glycopeptides were immobilized on microarray slides and applied to evaluate the LecB and TcdA binding preferences. Clear preferences for unique fucosylated binding epitopes and impact of underlying mucin core structures were found. Our detailed findings will be presented at Eurocarb.



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Inline Lectin Ligands: A New Approach for Precipitation-Free High-Affinity Multivalent Binding

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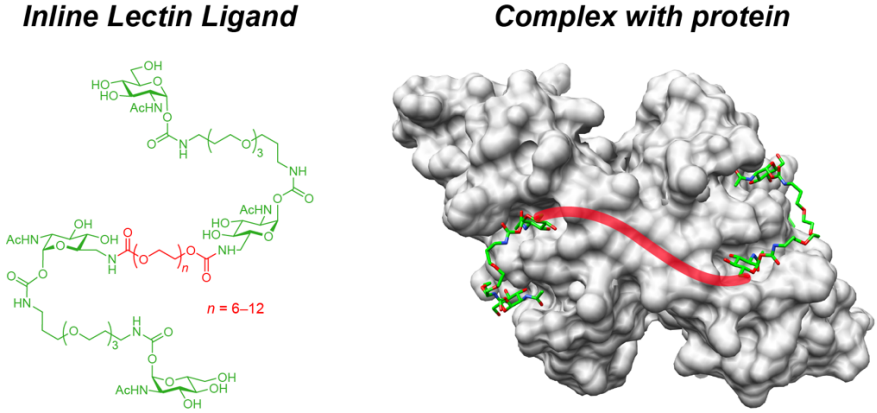
Multivalency is a key concept in biology. Artificial multivalent ligands often have drastically enhanced binding affinities enabling them to interfere with the natural systems [1]. However, they can also cause crosslinking of receptor proteins. This becomes critical when the receptor-ligand complexes precipitate. Plaque formation due to precipitation of proteins is known to result in numerous fatal diseases. Previously, we used wheat germ agglutinin (WGA) as a model lectin to study multivalent carbohydrate-protein interactions. To unravel the molecular details of multivalent binding to WGA, we combined a whole set of analytical techniques [2] including X-ray crystallography [3] and distance measurements in the nanometer range by EPR spectroscopy using spin-labeled carbohydrates [4].

Here, we present a new design of high-potency multivalent ligands featuring an inline arrangement of ligand epitopes with exceptionally high binding affinities in the low nanomolar range [5]. This design is especially efficient in terms of molecular size because the carbohydrates are directly attached to each other with no need for a central scaffold. With a multi-methodological approach, we show that precipitation of the receptor is prevented, and we distinguish distinct binding modes of the ligands. In particular, we elucidate a unique chelating binding mode, where four receptor binding sites are simultaneously bridged by one multivalent ligand molecule. The new design concept has great potential for the development of high-potency multivalent inhibitors as future therapeutics.



Inline lectin ligand and its binding to the protein

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Structural elucidation of cellulose derivatives complexes with metal ions

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Cellulose is the most abundant polymer on earth and a symbolic system in the field of polymers and macromolecules. Chemo-, chemoenzymatic and automated chemical syntheses approaches have been reported for the decoration of cellulose with functional groups. In specific, the introduction of chelating groups (i.e. carboxymethyl groups) into the cellulose backbone enhances cellulose ability to interact and to form complexes with metal ions.[1] Nonetheless, structural details of the cellulose-metal complex formation have yet to be elucidated.

The structural characterisation of cellulose derivatives at different length scales can be achieved by using complementary analytical techniques (TEM, IR AFT, AFM, PXRD and NMR). Among those, NMR spectroscopy is a valuable technique due to its ability to detect different domains, define packing and conformation of cellulose and cellulose derivatives. [2-4]

In this work, we combined electron microscopy (EM), Raman spectroscopy, PXRD and advanced NMR spectroscopy to obtain morphological, long range and short range structural information about the metal complexation to carboxylate groups in carboxymethyl cellulose. In addition, to better understand the mechanism governing the formation of the cellulose-metal complexes, we performed Molecular Dynamics (MD) simulations. The findings reported here elucidate the structure and dynamics of this system in aqueous environment.

Acknowledgments: This research was funded by the University of Padova STARS grant “SensCo”.

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From Microtiter Plates to Biosensors – Glycosylation Caught in the Act

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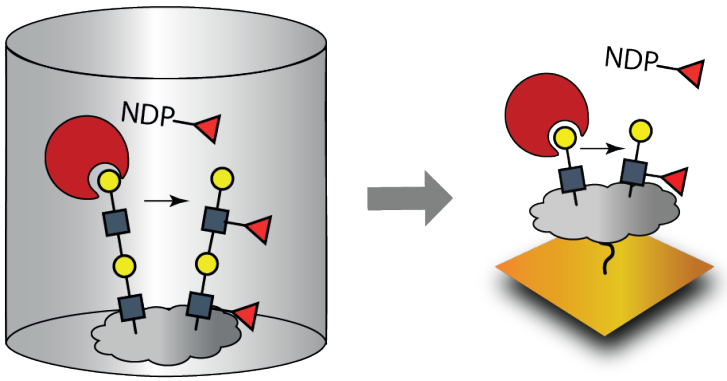
Approaches to diagnosis of human diseases call for fast and feasible screening methods. Some pathogenic biomolecules, for example the *Clostridium difficile* toxin TcdA, bind to glycans on the surfaces of human cells and induce destructive processes. Binding of the toxin occurs via the carbohydrate recognition domain that binds multiple ligands simultaneously. Hence, multivalent presentation of ligands is beneficial to increase the binding strength between the two binding partners.

Since efficient ligands are still unknown for some pathogens, screening of glycan libraries is crucial. We developed a method to assemble a variety of glycan epitopes on-plate, using *N*-acetyllactosamine (Galβ1-3/4GlcNAcβ1-3Galβ1-4GlcNAc) neo-glycoproteins as basis. Glycans were modified by a toolbox of glycosyltransferases; new epitopes were verified with specific lectins and tested with TcdA. We found the Lewis^{y}-Lewis^{x}-neo-glycoprotein (Fucα1-2Galβ1-4[Fucα1-3]GlcNAcβ1-3Galβ1-4[Fucα1-3]GlcNAc) to be a promising ligand. In cell cultures, the neo-glycoprotein was able to protect human cells from TcdA [1].

To further investigate the processes during interaction of different binding partners, we introduced a biosensor for electrochemical impedance spectroscopy. This system facilitates real-time monitoring of binding kinetics and enabled us to analyze binding of the single layer compounds, especially between glycans and their binding partners (enzymes and lectins) [2]. The developed biosensors present potential tools for the analysis and diagnosis of human pathogen-related diseases.



Glycosylation of neo-glycoproteins on-plate and on a biosensor chip

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Lectin array for quality control of recombinant therapeutic proteins

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A biological medicinal product that treats human diseases is known as biotherapeutic, like monoclonal antibodies. Their production occurs in host cellular systems that generate recombinant proteins with variable glycosylation. This could affect the therapeutic efficiency by increasing the in vivo clearance or inducing a dangerous immune response. Regulatory agencies more and more stress the importance of ensuring the batch to batch reliability and compare the glycosylation profiles.

Lectin array assay represents a relevant method for the in-process quality control of glycosylation during the biotechnological production of recombinant human protein. Lectins recognize and bind to specific glycans on intact glycosylated protein. A limitation is the lack of commercial availability of lectins that specifically recognize unique sugar structures.

We aim to produce lectins for the detection of potential immunogenic glycans like Neu5Gc and α-1,3-Gal epitopes. Those lectins are either extracted from natural sources or produced recombinantly in genetically engineered strains of E. coli. They are immobilized on a glass slide or 96-well plate to build a lectin array based on the GLYcoDiag technology platform, the GLYcoPROFILE. It is an inhibition assay, where the biotherapeutics are applied unlabelled to the plate in the presence of a fluorescent competitor, highly specific for the lectin. This method allows a fast and high-throughput screening of the glycan signatures on the recombinant therapeutic proteins, and quality control from the early research phases to the production.

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Synthesis of immunodominant MOG peptides bearing a DC-SIGN binding glycan on asparagine

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In the neurodegenerative autoimmune disease multiple sclerosis (MS) the protein myelin oligodendrocyte glycoprotein (MOG) has been identified as the key autoantigen1. However, the protein's precise role is still not entirely understood. Previous work from our group shows that heterogeneously fucosylated variants of MOG bind to the immune-regulating DC-SIGN receptor, inducing immunotolerance towards MOG2. We have also recently shown that the immunodominant peptide MOG35-55 shows amyloid-like aggregation behavior when site-specifically citrullinated3. Hence, we aimed to investigate the effect of homogeneous glycosylation of MOG35-55 on immune homeostasis and citrullination-driven amyloid-like aggregation.

To this end, we synthesized MOG31-55, the immunodominant peptide N-terminally extended to include the natural N-glycosylation site (Asn-31). We have synthesized Fmoc-Asn building blocks decorated with various protected glycans, including a derivative of the known DC-SIGN ligand Lewis X. The combination of acid labile and ester protecting groups used during oligosaccharide assembly enabled minimal protecting group manipulation to convert the glycosyl azides into SPPS-compatible Fmoc-Asn derivatives. Here, I will present the synthesis of these glycosylated asparagine building blocks, their application in SPPS of homogeneously glycosylated MOG peptides and the impact of N-glycosylation of MOG on amyloid-like aggregation. Furthermore, we show that a monovalent Lewis X ligand on a MOG peptide is sufficient to bind to DC-SIGN in vitro and initiate an anti-inflammatory response4.

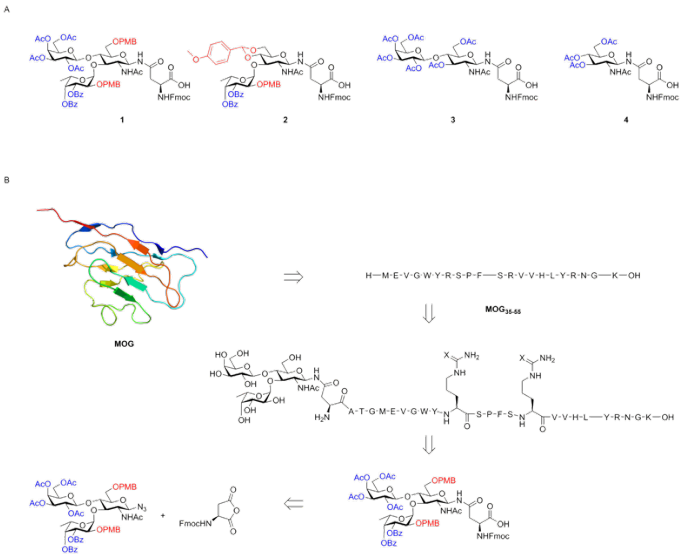

Figure 1: A) Glycosylated Fmoc-Asn derivatives synthesized in this study. Compound 1 is asparagine decorated with a protected LeX. Acid labile protecting groups are highlighted in red and ester protecting groups in blue. B) Retrosynthetic analysis showing the approach taken for the synthesis of MOG derived glycopeptides starting from protected glycosyl azide to N-glycosylated peptide. X is either NH (Arg) or O (Cit). MOG PDB-ID: 1OJ0

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Original biobased surfactant design from carbohydrate-based furanic platform molecules

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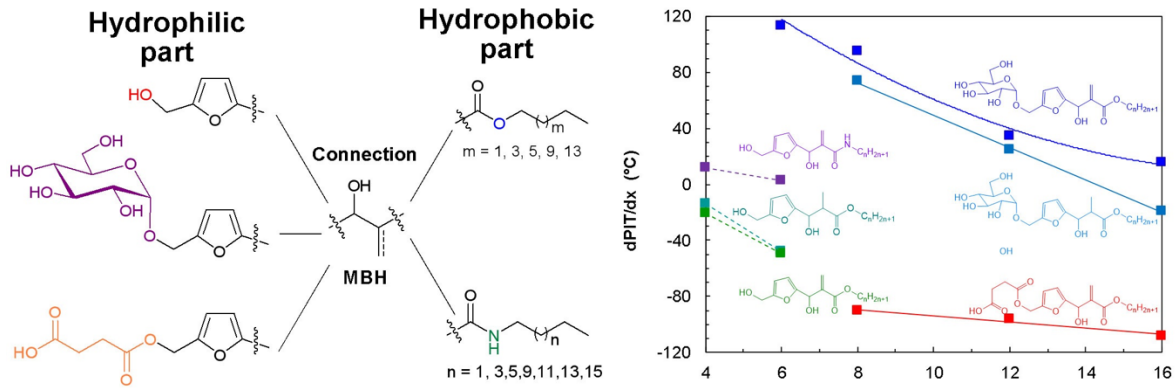
Carbohydrate-based furanic platform molecules offer original scaffolds for the design of novel chemical architectures. In this area, we studied several reactions using HMF or its glucosylated analog GMF as building blocks, such as the Morita-Baylis-Hillman (MBH) reaction,[1] the Biginelli reaction [2] and dipolar cycloadditions of HMF-based aldonitrones.[3]

Using the Morita-Baylis-Hillman (MBH) reaction, a series of new furanic amphiphiles has been prepared from the coupling of HMF, GMF or SMF (succinate derivative of HMF) as biobased furanic aldehydes with hydrophobic activated alkenes. Structural variations include the level of polarity on the furanic substrate (HMF, GMF and SMF), the type of activated alkenes (ester or amide) and the alkyl chain length and level of saturation. The physicochemical properties of these original systems were evaluated. Some of them exhibit interesting abilities to lower the water surface tension. Using the PIT-slope method, the newly synthesized compounds were compared to other types of surfactants, including previous series of carbohydrate-based amphiphiles. [4,5]



Structural variations in novel MBH adducts from HMF, GMF and SMF, and comparison of their amphiphilicity through the PIT slope method

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Studies on Bacterial Pseudaminic Acid

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Pseudaminic acid (Pse) has been identified in a variety of pathogenic bacteria as important surface glycan. Belonging to the nonulosonic acid carbohydrate family, Pse is structurally related to its well-known congener, sialic acid. Anomerically, Pse exists with both α and β configuration in native glycoconjugates with variable substitution patterns at N5 and N7. Such structural diversity makes the structure-function relationship of Pse-containing glycans both intriguing but also challenging. The biological and evolutional significances of bacterial pseudaminic acid and its glycoconjugates remain largely unexplored, due to the lack of synthetic access to pseudaminic acid and the structurally-defined pseudaminylated glycoconjuates. In 2017, we developed a facile and scalable de novo synthesis of pseudaminic acid (16 steps in 11%). Furthermore, we developed a robust methodology for the “perfect” stereocontrolled chemical glycosidation of pseudaminic acid to afford both (only) α- and (only) β- glycosides. This was achieved through the use of a common glycosyl donor (5N-azide)/7NCbz Pse thioglycoside) through the simple modulation of the reaction conditions. The power of the methodology is highlighted through the stereocontrolled preparation of a large number of α- and β-pseudaminylated substrates encompassing a variety of biologically-relevant linkages. In addition, we developed the synthetic route to altrosamine derivatives, Alt-4NAz and Alt-2NAz as chemical reporters, which are chemically synthesized for the first time.

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Synthesis of a library of Pel heptasaccharides from Pseudomonas aeruginosa

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Pseudomonas aeruginosa, an opportunistic Gram-pathogen can cause both acute and chronic infections, which rarely cause disease in healthy persons, but can multiply easily in immunocompromised patients. P. aeruginosa can become resistant to certain antibiotics due to its ability to form biofilm, which complicates the treatment of its infections.[1] In the biofilm formation three exopolysaccharides are synthesized that consists of alginate, Pel and Psl. In the biofilm formation three exopolysaccharides are synthesized that consists of alginate, Pel and Psl. Pel is positively charged and composed of 1,4-linked partially de-acetylated α-N-acetyl-galactosamine (GalNAc) and α-N-acetyl-glucosamine (GlcNAc) moieties, present in a ±6:1 ratio (Figure 1A).[2] To unravel their role in biofilm formation, to elucidate their biosynthesis and possibly as synthetic antigens in the generation of potential P. aeruginosa vaccines, a library of Pel heptasaccharides was assembled featuring α-glucosamine, α-N-acetyl glucosamine, α-galactosamine and α-N-acetyl galactosamine linkages (Figure 1B). Key features of the synthetic strategy include the use of di-tert-butylsilylidene directed α-glycosylation methodology and regioselective benzylation reactions. Varied deprotection strategies provided 4 sets of heptamers, showing the chemistry developed to be applicable to any type of Pel-target.

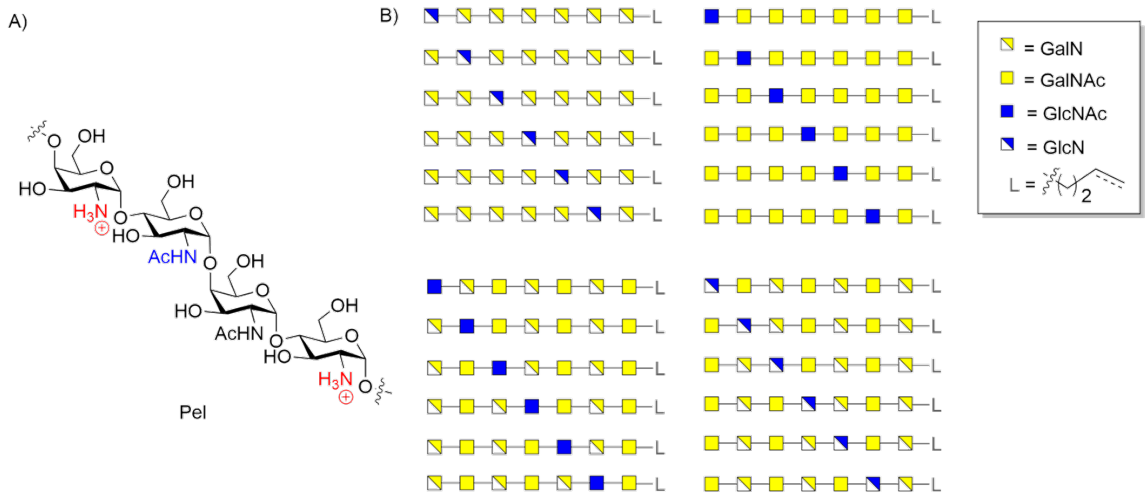

Figure 1. A) Structure of Pel. B) Structures of designed Pel oligomers.

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Expanding the Scope of Glucan Synthesis by Automated Glycan Assembly

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Polysaccharides are Nature´s most abundant biomaterials essential for plant cell wall construction and energy storage. Seemingly minor structural differences result in entirely different functions: cellulose, a β (1-4) linked glucose polymer, forms fibrils that can support large trees, while amylose, an α (1-4) linked glucose polymer forms soft hollow fibers used for energy storage. A detailed understanding of polysaccharide structures requires pure materials that cannot be isolated from natural sources. Automated Glycan Assembly (AGA) is a powerful method for the quick production of well-defined natural and unnatural oligosaccharides. Here we reported the recent progress on the synthesis of ionic trans-linked glycans analogues of cellulose, and the stereoselective installation of multiple cis-glycosidic linkages present in amylose. Using thioglycoside building blocks with optimized conditions, we prepared cellulose analogs with a well-defined charge pattern. We also achieved excellent stereoselectivity during the synthesis of linear and branched α-glucan polymers with up to 20 cis-glycosidic linkages. The molecules prepared in this study will serve as probes to understand the biosynthesis and the structure of glucans, and candidates for further material study.

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Superacid as a unique tool to observe and characterize various glycosyl cations in a condensed phase

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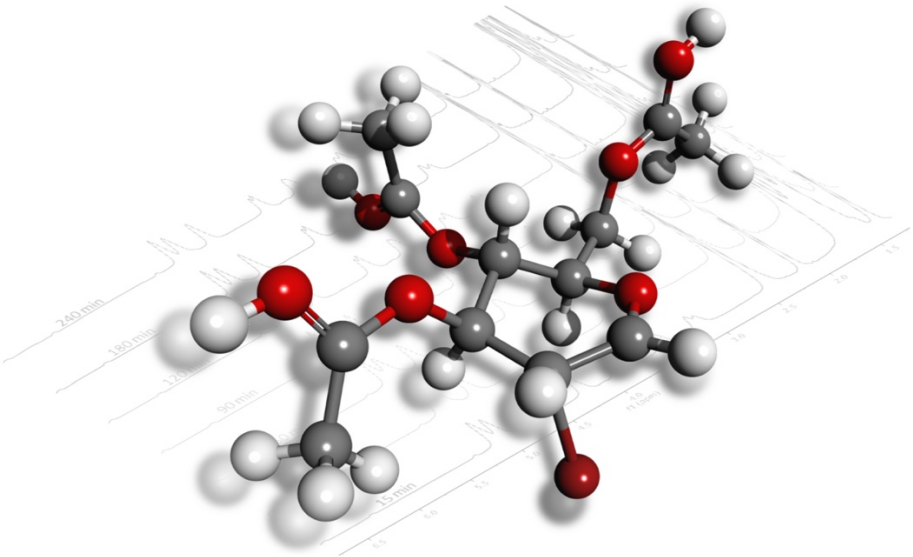
Glycosyl cations, ionic species strongly debated,[1] are transient ionic species formed during the enzymatic and chemical formation/hydrolysis of the glycosidic bond, transformations that are central to glycosciences. The high reactivity and short lifetime of these elusive species in organic solvents make them very challenging to characterize using spectroscopic techniques.[2] Their observation is of significant interest to gain insight into glycosylation intermediates at the atomic level. Combining superacid and carbohydrate chemistries, we have recently been able to observe and characterize for the first time such elusive species in a condensed phase.[3] Our recent results regarding this approach applied to various carbohydrates will be presented.



Structure of a polyprotonated peracetylated 2-bromo-2-deoxyglucosyl cation generated in HF/SbF5 superacid and deduced from NMR and DFT calculations

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Procainamide fluorescent labeling for identification of low abundant non-invasive glycan biomarkers

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Invasive laparoscopic surgery is currently the only method for endometriosis diagnosis. Urine, as the filtration of the blood plasma, could be a novel source of non-invasive biomarker candidates. IgG is a low abundant protein of urine therefore, a new method was required for glycan analysis. InstantPC and AQC (aminoquinoline carbamate) utilize reactive NHS-carbamate chemistry to form a covalent urea linkage between the fluorescent tag and a glycosylamine moiety that transiently resides on the reducing end GlcNAc following N-glycan release by PNGase F. This strategy significantly improves labeling speed and sensitivity, but it is sensitive to pH in solvents which contain -NH2 group. ProA (4-amino-N-[2-(diethylamino)ethyl] benzamide) operates with the same mechanism as 2-AB (2-Aminobenzamide) to bind to the reducing end of a glycan using Schiff base chemistry. ProA shows increased fluorescence and ionization performance, which can be explained by the fact that it contains a basic tertiary amine tail, hence it provides higher sensitivity during mass spectrometry and liquid chromatography separation. Based on preliminary results, we chose ProA to develop a novel method for urinary IgG labeling. IgG comparisons may provide information on whether serum or urine is a more suitable biomarker candidate for endometriosis as there is a high demand to discover novel non-invasive biomarkers for this disease. Glycosylation of control IgG differed between urine and serum in galactosylation and sialylation which may impact IgG function and its diagnostic value.

Exopolysaccharide-based growth factor delivery systems for tissue engineering applications

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Growth factors constitute a key element in tissue engineering strategies since they stimulate essential cellular processes (proliferation, migration, differentiation). With the aim to enhance both their bioactivity and bioavailability, encapsulation of growth factors in delivery systems is explored [1]. In this way, we are currently developing microgels based on a marine bacterial exopolysaccharide (EPS) [2]. The atypical structure of this EPS with the presence of uronic acids and sulfate groups confers unique biological glycosaminoglycan-mimetic and gelling properties. To encapsulate proteins in mild conditions through a one-step process, a microfluidic technique was selected. To further enhance their bioactivity, growth factors were incubated with highly sulfated low-molecular weight EPS derivatives before their incorporation within the microgels. Release kinetics from various microcarriers were obtained and the bioactivity of the released growth factor was assessed. Finally, in the context of a bone repair approach, the efficacy of growth factor-loaded microgels to stimulate proliferation and osteogenic differentiation of human bone-marrow derived mesenchymal stem cells was evaluated.

In parallel, Atomic Force Microscopy was used to understand the relation between the EPS structure and its growth factor binding properties [3]. The EPS-protein interaction strength was estimated from imaging and single-molecule force spectroscopy, while mechanical properties of microgels were measured to get an overview of the structure-properties relations.

Acknowledgment. Financial supports were provided by ANR, the French National Research Agency within th framework of the FunCapsul project (ANR-17-CE08-0001).

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Lectins and lectomes: from structural glycobiology to glycoinformatics

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The ability of lectins for deciphering the structural message embedded in complex glycans is a remarkable source of protein fold diversity. Thousands of 3D-structures of lectin-glycan ligand complexes are available from X-ray crystallography and NMR [1] and we recently demonstrated that neutron crystallography associated with perdeuterated carbohydrate reveals new details of the interaction [2]. This approach is helpful in curating the content of UniLectin3D, a searchable database providing structural information crosslinked to protein sequence and structure as well as glycoscience databases [1]. Furthermore, the identification of new lectins in organisms is of interest due to their applications. However, they are poorly annotated in sequence databases and consequently lacking in newly sequenced organisms. The limited size of functional domains and the low level of sequence similarity challenge usual bioinformatics tools.

Our two groups co-developed the UniLectin portal to build upon UniLectin3D and define a new structure-based classification organized in 109 classes. The portal also includes two specialised modules reflecting the results of lectin prediction from amino acid sequences. The first, named PropLec, contains predicted β-propeller lectins spanning six classes of lectins [3]. The second, named LectomeXplore includes the result of screening millions of sequences of the NBCI-nr and UniProt databases for each of the 109 curated lectin classes [4]. These preliminary results show that lectins are more widespread across kingdoms than presently documented.

UniLectin platform logo

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Understanding and engineering specificities in CAZymes: experiments and simulations

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CAZymes specificities and mechanisms often require a combination of methodologies to be understood and/or engineered. Here we will focus on two specific cases where experimental data (residues conservation, protein structures, mutagenesis & kinetics) were combined with molecular dynamics simulations [1] to uncover and rationalize engineered CAZymes specificities.

First, a hydrolytic GH84 O-GlcNAcase was converted into a phosphorylase by a single point mutation; and calculations allowed to rationalize this novel conversion.[2] The active site electrostatics was modified so that phosphate ions were able to reach the engineered catalytic center and react. By contrast, in the case of the native enzyme phosphates were precluded to visit the active site, and even if that would occur, phosphorylation turned out to be both thermodynamically and kinetically unfavored.

Secondly, we analyzed what distinguishes the O-, N-, and S-glycosylation catalyzed by a glycosyl transferase from family GT1. While each of these specificities was the result of a single nucleophilic substitution in the same active site, the mechanisms between these three activities were different. We engineered mono-specific N- and S-glycosylating variants.[3] The factors that discriminate between the different enzymatic specificities were revealed by molecular modelling.

Overall, we aim to comment and exemplify the synergy between experimental enzymologists and theoreticians to push further the investigation of CAZymes.

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Sulfated Glycomimetics as Antivirals

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Many pathogens such as parasites, bacteria, and viruses have evolved to interact with carbohydrate polymers presented on the surface of cells. These glycosaminoglycans are often used by pathogens to adhere to host cells and to facilitate infection. Glycomimetics, which can be designed to mimic these glycans, have the potential to attenuate pathogen engagement and infection by engaging and blocking pathogen lectins for host glycans. Here, we present the synthesis of a series of short-chain sulfated glycooligomers and long-chain sulfated glycopolymers as glycomimetics of sulfated glycosylaminoglycans, and the evaluation of their role in inhibiting viral infection. Sulfated glycooligomers were synthesized via solid supported polymer synthesis, while sulfated glycopolymers were prepared RAFT polymerization. The resulting sulfated glycomimetics were evaluated for their potential and mechanism to inhibit viral infection. Our results using a human papillomavirus type 16 (HPV-16), the major cause of cervical cancer, as a model demonstrate that both classes of compounds can prevent viral infection with different efficiencies. Interestingly, while the glycopolymers prevented binding to cellular glycans as expected, oligomeric glycomimetics interfered with infection at a post-binding step. Extension of this work to other viruses including herpes simplex virus (HSV), influenza A virus (IAV), and Merkel Cell polyomavirus (MCPyV) revealed a broad range of antiviral effects. Current efforts are focused on the investigation of the broad-spectrum antiviral effects of these compounds.

MGAT5 - Structures, Simulations, Inhibition

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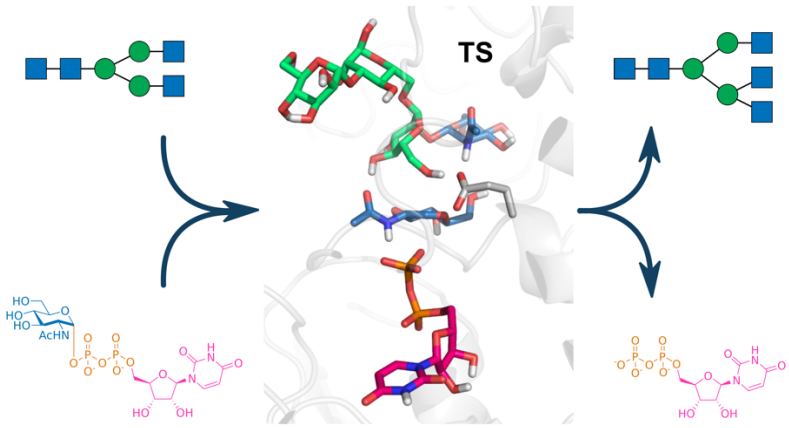
Protein N-glycosylation is one of the most prevalent classes of post-translational modification, capable of exerting profound biological effects at the molecular, cellular and organismal level. a-Mannoside b-1,6-N-acetylglucosaminyltransferase V (MGAT5, also GnT-V) is a key glycosyltransferase involved in complex tetra-antennary N-glycan formation, which strongly promotes cancer malignancy when overexpressed (1). Despite intense interest, the mechanistic details of MGAT5 catalysis have not been well characterized, limiting the scope for therapeutic exploitation. We conducted a comprehensive crystallographic analysis of MGAT5 substrate interactions, solving structures complexed to both donor and acceptor subsite ligands. Our data reveal a previously unforeseen role for donor induced loop movements in controlling MGAT5 acceptor substrate engagement. Molecular dynamics and quantum mechanics/molecular mechanics simulations were also carried out to dissect the molecular details of MGAT5 activity. These simulations highlight key enzyme residues involved in catalysis and allow us to map the considerable conformational distortions imposed upon the sugar substrate during transfer (2).

Finally, we carried out a preliminary inhibitor screening campaign against MGAT5, using a bespoke library of '3-D fragments' (3). We identify novel scaffolds that may enable specific targeting of MGAT5 vs related glycosyltransferases. Our combined structural/computational/chemical approach hints at routes towards the rational development of MGAT5 inhibitors to correct cancer-associated N- glycosylation.



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Organogels formed by association of alkylglycosides and aromatic boronic acids

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Gels can be described as liquid trapped into a 3D network of crystalline fibers. When the 3D network is structured by weak interactions (physical gels) despite of covalent bonding (chemical gels), the gelation phenomena is reversible. In addition, the physical gel will be also sensitive to its environment (pH, temperature, analytes). Thus, physical organogels are known as a promising source of soft materials with applications in medicine, food industry or analytical chemistry.[1] These types of gels can be obtained by adding a small amount of small molecules (less than 5% wt) called "organogelators" into an organic solvent. Sugar-based derivatives are already known for their potential as remarkable organogelators.[2] Here we describe an easy synthesis of a new class of organogelators obtained by esterification of a glycolipid with an aromatic boronic acid. We synthesized a series of sugar-boronate derivatives which permitted to investigate both the impact of the alkyl chain and the impact of the aromatic part on the gelation properties. Thanks to the boronate function, our organogels are water-sensitive and depending on the chemical structure, they showed different behavior against hydrolysis. In addition, some members of this organogelator family bear fluorescent properties. We also fully characterized the gels by rheometry, electron microscopy (SEM) and X-ray diffraction.[3]

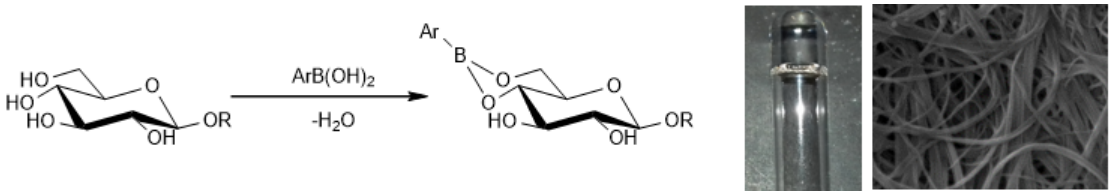

Figure: Synthesis of arylboronate glucosides, gelation in toluene and SEM image of the fibrillar network

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Towards understanding the regulation of the N-glycosylation pathway in regards to the huge diversity

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N-glycosylation is a key post-translational modification of proteins occurring throughout the living kingdom in Eukaryotes, Bacteria and Archea. In Eukaryotes, this process consists in the addition of an oligosaccharide precursor to an asparagine (Asn) belonging to the consensus sequence Asn-X-Ser/Thr/Cys where X corresponds to any amino acid except a proline or an aspartic acid. In eukaryotes, the N-glycosylation starts in the endoplasmic reticulum leading to the transfer via the oligosaccharyltransferase complex of an oligosaccharide precursor to the nascent protein. It continues within the Golgi apparatus where maturation of N-glycans occurs by the addition of various residues and monosaccharide modification depending on the phylum to which the species belongs. This work focuses on the Golgi maturation of the N-glycans and describes recent findings regarding the huge diversity of the N-glycan structures depending on the eukaryotic clade that the organisms belong to. This comparison naturally leads to the questioning of differential regulation of the N-glycosylation process between eukaryotic clades. To tackle this point, we also highlight recent findings on the regulation of the protein N-glycosylation, especially in mammals, through manganese and calcium homeostasis and the specific role endorse by TMEM165. Through this work, we position the importance of Mn homeostasis for the Golgi glycosylation machinery and address the function of TMEM165 in this homeostasis in mammals as well as in other eukaryotes for which orthologues of TMEM165 have been identified.

Chemoenzymatic synthesis of oligomers of GM3 analogues for biological evaluation

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Glycosphingolipids (GSLs) are ubiquitous components on animal cell membranes and exposed on the outer surface of cell membranes. Ganglioside GM3, the first and simplest member in the metabolic series of a GSLs family, contains sialic acid, lactose and ceramide. GM3 has a strong impact on the occurrence and development of human cancer. GM3 is not only overexpressed in several types of cancer but also inhibits tumor cell growth through anti-angiogenesis or motility. In particular, the effect of GM3 on EGFR signaling is essential for cancer[1]. Previously our laboratory prepared a series of GM3 analogues, among them, several compounds display interesting activity against tumour growth. In the present study, we would like to improve the biological activity of this type of the compounds by using the multivalency effect. We screened out mannose-containing analogues with better anti-tumor activity from the previously synthesized GM3 analogues[2], and further synthesized mannose-containing analogues oligomers, in order to study their antitumor activities and search new leading compounds for cancer therapy.
We firstly synthesized novel mannose-containing GM3 analogues by enzymatic hydrolysis and chemical procedures. Next, conjugation of mannose-containing analogues to multivalent skeleton through the click reaction was carried out. At last, dimer, trimer and tetramer containing mannose analogues were synthesized.

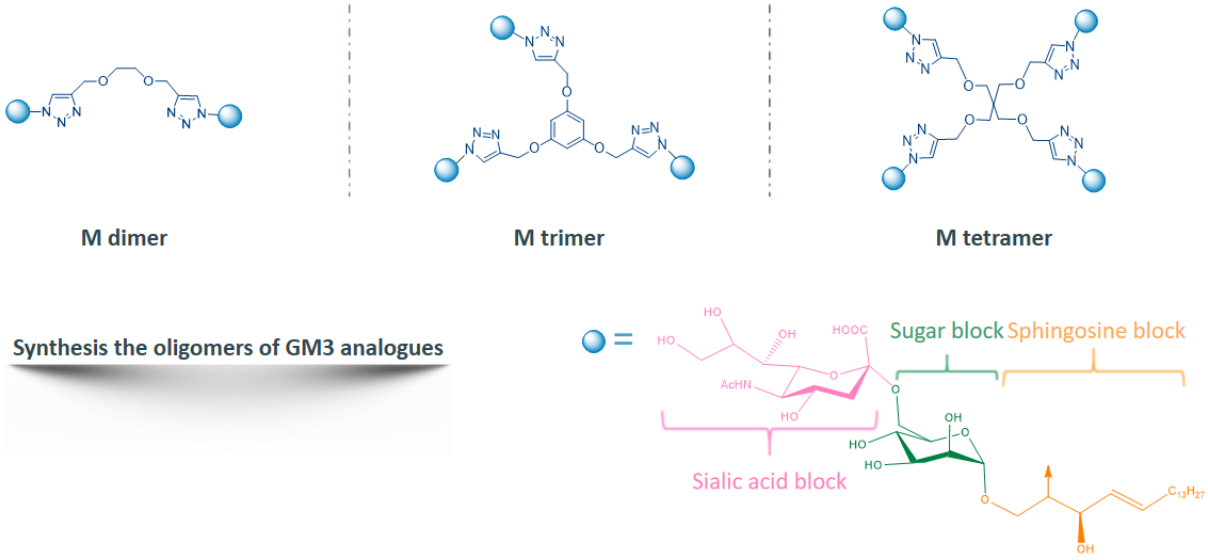

Figure 1 Synthesis the oligomers of GM3 analogues

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Cleavage of capsular polysaccharides of *Acinetobacter baumannii* by phage and prophage depolymerases

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Acinetobacter baumannii is one of the most serious opportunistic pathogens responsible for nosocomial infections in the modern healthcare system by causing diseases such as ventilator-associated pneumonia and meningitis with high mortality. The “last-line” antibiotics including carbapenem and colistin for the management of Gram-negative pathogen prevalence are getting less effective. Hence, a new therapy is urgently required to overcome the failure of antibiotic treatment such as phage therapy. One of the virulence factors of *A. baumannii* is a capsular polysaccharide (CPS), 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. The aim of this work was the establishment of the biochemical basis for phage therapy of the infections caused by *A. baumannii*.

Capsular polysaccharides of twelve KL-types of *A. baumannii* whose structures had been established earlier were cleaved with recombinant bacteriophage or prophage-derived depolymerases. The oligosaccharide products were fractionated by gel-permeation chromatography and studied by high-resolution electrospray ionization mass spectrometry and NMR spectroscopy. It was found that all depolymerases studied possess a glycosidase activity and cleave specifically the CPSs of *A. baumannii* by the hydrolytic mechanism to give a monomer or/and oligomers of the K units.

This work was supported by the Russian Science Foundation (Project No. 19-14-00273).

Unleashing the power of porous graphitic carbon for isomer specific N-glycan analysis

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The importance of protein glycosylation in the biomedical field demands for methods capable of assigning the isomeric structures of N-glycans. The biological relevance of different glycan isomers and the importance of their analysis was shown by several studies[1,2].

Coupled with MS, the high shape selectivity of porous graphitic carbon (PGC), exceeding other stationary phases commonly used in glycan analysis, makes it a very promising stationary phase for the analysis of isomeric N-glycans. Though being one of the most powerful methods for in-depth glycome analysis, PGC-ESI-MS is marked with serious blemish: The poor reproducibility of retention times with fluctuations often in the minute-range while retention time differences of isomers often are in the sub-minute range. To overcome this problem, isotope labelled N-glycans spanning the whole retention time window, were used as “sign posts” to normalize the retention times and project a given run onto a reference chromatogram. This strategy allowed to use chromatographic retention on PGC as the primary criterion for structural assignment and to create a normalized retention time-library.

Because of the biological relevance[3], we focused primarily on the widespread glycan composition of five hexoses, four N-acetylhexosamines and one fucose residue (H5N4F1), which may adopt to over forty different structures in the mammalian N-glycome. This method narrowed the assignment space to only very few, often just one possible isomer, making the identification of isomers an easy task.

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Developing molecular tools to study Kdo hydrolases in Gram-negative bacteria

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Glycosphingolipids Infections caused by antibiotic resistant Gram-negative bacteria are an ever increasing source of morbidity and mortality worldwide. It is therefore crucial to study the factors involved in bacterial pathogenesis and antibiotic resistance in order to identify new therapeutic targets for the development of novel antibacterials.

An essential feature in Gram-negative bacteria is their lipopolysaccharide (LPS) that plays a crucial role in bacterial survival and infection. Within the inner core of the LPS the monosaccharide, 3-deoxy-D-manno-octulosonic acid (Kdo), is found.

Kdo can be present in the LPS as a single unit or as multiple units. Recently it was discovered that the number of Kdo residues in some infectious bacteria is modified by a Kdo hydrolases(1) and that this modification appears to be related to infection and bacterial prevalence.(2) However, little is currently known about how, why and which bacteria perform this modification and how this affects bacterial resistance and infection.

In this study we developed a tool that can help us answer these questions. We synthesized a Kdo-based activity based-probe that was designed based on the strategy developed by Withers et al.(3) with a fluorine atom adjacent to the anomeric center of Kdo (C-3) and a highly reactive leaving group at C-2. We are currently evaluating the probe for its ability to isolate, label and inactivate Kdo hydrolases in Gram-negative bacteria.

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Chemoenzymatic synthesis of ^{13}C-enriched sialosides to follow their metabolic fate in gut microbiota

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N-acetylneuraminic acid (Neu5Ac) is found in the gut mucus layer1 and its removal from the terminal position of *O*-glycans is necessary for gut microbiota to gain access to the underlying residies.[1,2] Neu5Ac is also found in human milk oligosaccharides (HMOs) such as 3'-sialyl lactose (3'-SL) and 6'-sialyl lactose (6'-SL).[3] HMOs represent a class of glycans important for early colonisation of gut microbiota and have direct effect towards the neonates' health.[4]

Studying the metabolic fate of Neu5Ac as part of *O*-glycans and HMOs in gut microbiota enables the mapping of the Neu5Ac flux within our gut microbiota, what Neu5Ac metabolites are produced and their subsequent uptake by other bacteria (cross-feeding) or the human host. In particular, the catabolism of glycans into short chain fatty acids (SCFAs) by gut bacteria is of great interest, as SCFAs have been shown to play a multifaceted role in affecting the health of the human host (i.e. gut-brain axis).[5]

In this study we set out to use isotope-ratio mass spectrometry to study the metabolites produced by gut microbiota fed with either 3'-SL and 6'-SL that contain a ^{13}C-enriched Neu5Ac. This should enable the identification of metabolites produced in the Neu5Ac catabolism pathway by the gut microbiota communities.

To obtain sufficient amount of these ^{13}C labelled HMOs, we have developed a facile chemoenzymatic synthesis route towards 3'-SL and 6'-SL starting from the cheap and commercially available [U-^{13}C]glucose via the key peracetylated [$^{13}C_6$]ManNAc intermediate which could be obtained in gram scale.

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Photoswitchable sp2-iminosugars

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sp2-Iminosugar are structural, chemical and functional mimics of monosaccharides. Throughout the last years, different series of derivatives have been synthesized in order to evaluate the influence of modifications on the binding potency and selectivity towards different targets, including enzymes, lectins and antibodies. sp2-Iminosugar-type glycoconjugates bearing O-, S-, N, or C-anomeric aglycone groups, have shown potential as drug leads against cancer, parasitic diseases, and inflammatory disorders [1]. Alternatively, 1-deoxy-sp2-iminosugars have shown potential as pharmacological chaperones for the treatment of lysosomal storage diseases [2]. Based on this background, we have addressed the design and synthesis of light-responsive sp2-iminosugar glycomimetics suitable for photopharmacological strategies.

Photopharmacology, an emerging strategy for the treatment of different pathologies, relies on the introduction of a photoswitchable unit into the molecular structure of the bioactive compound itself[3]. We planned to insert an azobenzene photoresponsive element that can switch between the E and the Z configuration, to achieve spatiotemporal control of the overall molecular topology and, consequently, of the biological activity by irradiation at the appropriate wavelength [4].

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Schematic representation of the interaction between enzymes and photoswitchable sp2-iminosugars (A) and structures of some sp2-iminosugars synthetized

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Exploration of Glycosphingolipid-Glycans Signatures of Human Acute Myeloid Leukemia Cell Lines

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Glycosphingolipids (GSLs) are information-bearing biomolecules that play critical roles in embryonic development, signal transduction and carcinogenesis. The abnormal expression of GSLs is correlated with development of diverse cancers, including leukemia.1 Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of haemopoietic progenitor cells and the most common malignant myeloid disorder in adults. Previous studies indicate that the aberrant expression of certain GSLs is associated with the differentiation of AML cells.2 However, the biosynthetic regulation and expression patterns of GSLs in AML and their biological relevance are still poorly explored due to their complexity, the presence of multiple isomeric structures as well as tedious analytical procedures.

In this study, we performed in-depth GSL glycosylation analysis for 19 AML cell lines to investigate the variation in glycosylation phenotypes and their association with expression levels of glycotransferases and transcription factors. For this, GSLs were extracted from cell lines, glycan head groups were enzymatically released followed by reduction and purification. Glycans were analyzed by PGC-LC-MS/MS in negative ion mode.3

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

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The role of Glycosaminoglycan orientation on its interactions with protein target

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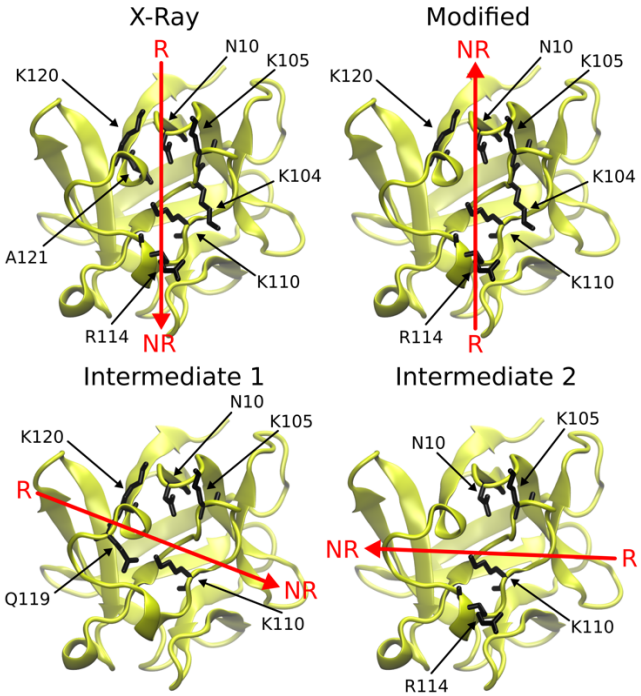
Glycosaminoglycans (GAGs) are long unbranched periodic and negatively charged polysaccharides present in extracellular matrix as well as in lysosomes, in which they are involved in many biologically-relevant processes. They interact with respective protein targets such as cathepsins, growth factors or interleukins via predominantly electrostatic interactions.

GAGs are well described in literature and numerous studies both experimental and computational successfully characterised many aspects of protein-GAG interactions. However, each approach has its own challenges due to characteristic properties of GAGs. While analysing GAGs with theoretical chemistry methods, it is important to know that these polysaccharides are very flexible as well as mobile. Due to their negative charge, they interact with positively charged amino acid residues with long flexible side chains, which increases the computational cost of simulations. Additionally, it might be possible for a GAG to be bound at one binding region but with different poses. Special case of such scenario is when GAG can be bound in two orientations antiparallel to each other.

In this study we performed a series of microsecond timescale molecular dynamics simulations for two protein-heparin (HP) systems of different GAG length, orientation and position on the protein surface. We also characterised the energetic profile of GAG orientation charge as well as GAG dissociation. We proposed that GAG can be bound in orientation opposite to known crystallographic structures that were also studied.



The most stable orientations of HP in complex with FGF-1. Red arrows shows the position of HP chain with its direction on the protein surface.

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Janus lectin mediated liposomal fusion to cancer cells

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Not only is it crucial to develop more efficient and new anticancer drugs, but it is also essential to develop ways to precisely deliver drugs to cancer cells and understand this process's fundamentals. The aberrant overexpression of specific glycosphingolipids tightly associates with the initiation of a tumour and its malignant transformation. Therefore, the carbohydrate-binding proteins known as lectins are currently in the spotlight as targeted drug delivery tools. Naturally, we asked whether the drug-loaded liposomes can be used for targeted cancer therapy via lectin-induced membrane fusion?

This project aims to deliver drugs, loaded in liposomes, to the cytosol of H1299 lung epithelial cells via Janus lectin-induced membrane fusion and subsequently kill the cancer cells. We previously designed and produced a Janus lectin (Fig.1A), a chimeric and trimeric construct containing a fucose-specific RSL domain and a sialic acid-specific CBM40 domain (1). Sialic acid is widely present in Sialyl Lewis x and Sialyl Lewis a epitopes, which are overexpressed in non-small-cell lung cancer cells (2). We incubated the JN with human non-small-cell lung carcinoma cell line (H1299) and FSL-A (Function-Spacer-Lipid comprised of blood group A trisaccharide) functionalized liposomes. We expect the Janus lectin's two specificities to bridge the liposome's fucosylated surface and the sialylated surface of cancer cells. Our studies demonstrate that Janus lectin crosslinks H1299 cells with FSL-A liposomes (Fig.1B). Next, we are aiming to obtain vesicle fusion to cancer cells via Janus lectin.

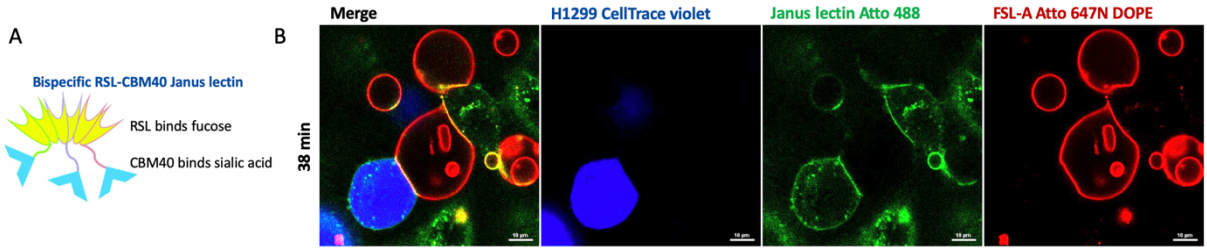

Figure 1. A) Bispecific RSL-CBM40 Janus lectin. B) Janus lectin crosslinks FSL-A liposomes to H1299 cells. Janus lectin is enriched at the interface.

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Identification and characterisation of the first mollusc T-synthase

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Identification and characterisation of the first mollusc glycoprotein-N- acetylgalactosamine β1,3-galactosyltransferase (T-synthase)

Glycoprotein-N-acetylgalactosamine β1,3-galactosyltransferase (T-synthase, EC 2.4.1.122) catalyses the transfer of the monosaccharide galactose from UDP-Gal to GalNAc-Ser/Thr, synthesising the core 1 O-glycan structure (T-antigen) of mucin-type O-glycans. Although such structures have been described in snails, so far, no core 1 β1,3-galactosyltransferase has been determined there. The sequence of the enzyme was identified by a BlastP search of the NCBI Biomphalaria glabrata database using the human T-synthase sequence (NP_064541.1) as a template. The gene codes for a 388 amino acids long transmembrane protein with two putative N-glycosylation sites. The coding sequence was synthesised and expressed in Sf9 cells. The expression product of the putative enzyme displayed core 1 β1,3-galactosyltransferase activity using pNP-α-GalNAc as the substrate. The enzyme showed high sequence homology (49,40% with Homo sapiens, 49,14% with Caenorhabditis elegans, 53,69% with Drosophila melanogaster) and similar biochemical parameters with previously characterised T-synthases from other species.

In this study, we present the identification, cloning, expression and characterisation of the glycoprotein-N-acetylgalactosamine β1,3-galactosyltransferase from the fresh water snail Biomphalaria glabrata, which is the first cloned T-synthase from mollusc origin.

This project is supported by the Austrian Science Fund (FWF): [P33239-B].

Effect of dietary chitosan oligosaccharide supplementation on transcriptomic profiles of the porcine

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Chitosan oligosaccharide (COS), a natural oligosaccharide derived from chitin, has attracted considerable research interest since it can improve the reproductive performance in sows. However, the molecular mechanism still remains unclear. In this study, we explored the effect of dietary COS supplementation on gene expression profiles in porcine feotus by using RNA-Sequencing (RNA-Seq). The analysis acquired 14,926,964 and 15,140,699 clean reads from control foetus and COS foetus libraries, respectively. A total of 2001 differentially expressed genes (DEGs) were identified (FDR ≤ 0.001, |log2Ratio| ≥ 1). There were 815 up-regulated and 1186 down-regulated genes in the COS foetus compared with the control foetus. A large number of these DEGs were involved in cellular process, single-organism process, cell and binding. Furthermore, pathway analysis indicated that these DEGs were significantly enriched in 38 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Collectively, these novel findings not only furthered our understanding of the molecular mechanisms of COS on foetal survival and health, but also provided theoretical foundation for developing functional oligosaccharides in livestock industry.

Design, synthesis and study of glycosylated dynamic macrocycles as antibacterial agents

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Pseudomonas aeruginosa is a well-known pathogen responsible for numerous diseases. It is most notably found in hospitals as a cause for nosocomial infection . While the bacterium is rather inoffensive for healthy people, immune-compromised patients are much more exposed to such infections which can be lethal. Moreover, it is quite difficult to cure an infection involving Pseudomonas aeruginosa due to its high resistance to antibiotics.

Anti-adhesive strategies consist in inhibiting the adhesion of bacteria to host cells. Two soluble multivalent lectins (LecA and LecB) have been identified in this process and several multivalent glycoclusters have been designed to bind these lectins with in vivo applications towards potential therapeutic anti-infectious agents.

While typical medicinal chemistry approaches involve synthesis and inhibition assay of each inhibitor candidates, dynamic combinatorial chemistry provides a rapid access to an equilibrating mixture of potential inhibitors through reversible covalent bonds under thermodynamic control. We have designed dynamic combinatorial libraries of glycoclusters self-assembling through disulfide bonds, which composition can be modified upon an external stimulus such as a multivalent lectin.

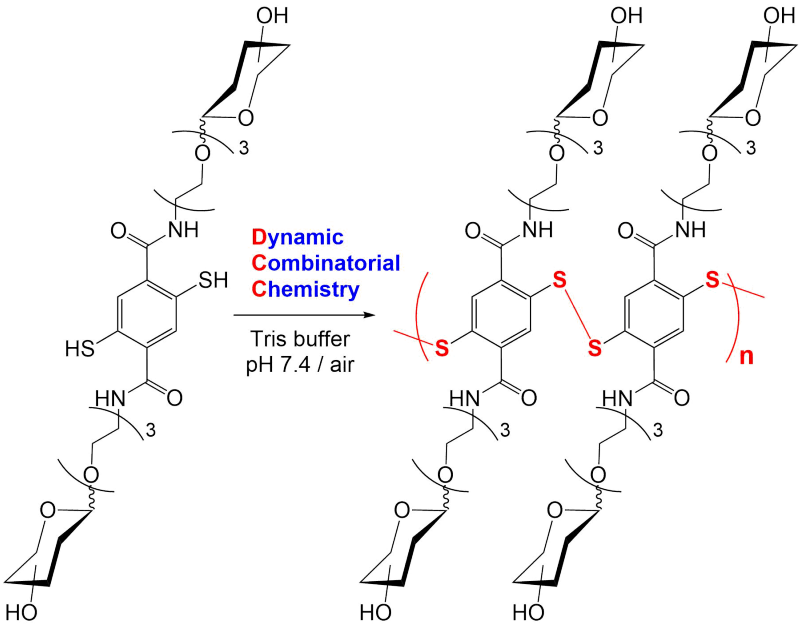

Figure 1 Synthesis the oligomers of GM3 analogues

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Heparanase inhibition by glycol-split and poly-carboxylic synthetic heparin fragments

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Overexpression of heparanase favors malignancy through cleavage of heparan sulfate and release of growth factors, cytokines and other bioactive molecules [1]. Heparanase inhibitors are therefore potential drugs and one of them, roneparstat, has reached clinical development. Roneparstat was obtained from pig mucosal heparin modified to avoid anticoagulant activity. The critical feature for heparanase inhibition by roneparstat is the presence of periodate oxidized/borohydride reduced uronic acid units (glycol-split units, abbreviated gs). We have shown that synthetic trisaccharides GlcN-UA-GlcN, become (weak) heparanase inhibitors after periodate glycol splitting followed by reduction to di-alcohol [2]. To check whether the nature of the uronic acid, iduronic or glucuronic, influenced inhibition we have prepared the epimeric trisaccharides GlcNS6S-IdoA-GlcNS6S and GlcNS6S-GlcA-GlcNS6S and the corresponding gs derivatives. Heparanase inhibition assay indicated that ido configuration favored heparanase inhibition while gluco configurated compounds were inactive. The conformation of these compounds has been studied combining NMR ($^3J_{HH}$ coupling constants and NOEs measurements) and MD simulation. These experimental constrains allowed to select oligosaccharides-heparanase models obtained by molecular docking and MD simulation figuring out the molecular aspect of their different inhibition activities.

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Synthesis of MUC1-derived glycopeptide bearing a novel triazole STn analog

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Mucins are high molecular weight glycoproteins having as a fundamental characteristic the linkage between sugar αGalNAc and amino acid residues of Ser/Thr. In tumors the mucin biosynthesis is deregulated, which modifies the O-glycosylation pattern of their glycoproteins, resulting in abnormal and incomplete glycans. These are called Tumor-Associated Carbohydrates Antigens ("TACAs"), such as Tn, sialyl-Tn and TF antigens (Fig 1A) and are continuously expressed in tumor cells and generally absent in healthy tissues [1]. Among tumor mucins MUC1 is the most investigated, being composed of tandem repeat regions with the HGVTSAPDTRPAPGSTAPPA sequence. The synthesis of MUC1 glycopeptides bearing modified tumor-associated carbohydrate antigens (TACAs) represents an effective strategy to develop potential antitumor vaccines that trigger strong immune response [1,2]. In this context, we present herein the multistep synthesis of the triazole glycosyl amino acid Neu5Ac-α/ß2-triazole-6-ßGalNAc-ThrOH 1 as STn antigen analog, along with its assembly on the corresponding MUC1 peptide (PDTRP) to give NAcProAsp [Neu5Acα/ß2-triazole-6-ßGalNAc]ThrArgProGlyOH 2 (Fig 1B).

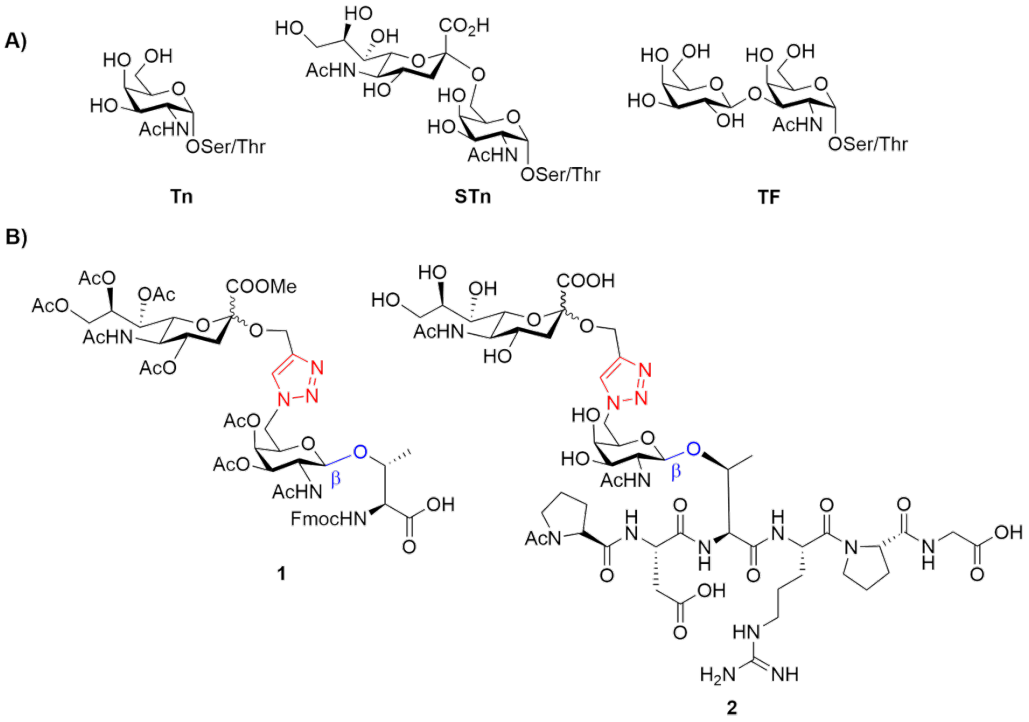

Figure 1. (A) Representation of the main Tumor-Associated Carbohydrate Antigens (TACAs). (B) Chemical structures of the target products 1 and 2.

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Glycosylated pyrimidinones as potential acetylcholinesterase inhibitors and antitumoral molecules

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C-Nucleosides and analogues constitute an important class of natural and non-natural products, many of which exhibit relevant biological activities [1,2]. In this communication, the preparation of oxo/thioxodihydropyrimidines linked to furanoses with D-mano configuration are presented. For this, the reaction of dialdofuranoses with an alpha-keto ester and urea or thiourea allowed the high yield obtention of the target molecules containing the 2-oxo- or 2-thioxo-pyrimidine system. The potential interest of these molecules in Alzheimer´s disease and/or in cancer treatment were then explored. Interestingly, an acetylated glycosyl(thioxo)dihydropyrimidine of this group of compounds led to a relevant acetylcholinesterase inhibition at 100 µg/mL. Therefore, further modifications on this skeleton should be considered in the development of more potent inhibitors of this enzyme. On the other hand, despite being nucleosides, these compounds have generally low to moderate antiproliferative effects on breast (MCF-7 and T47D) and prostate (LNCaP) cancer cells and on normal human dermal fibroblasts. However, within these, a relevant result was observed for a glycosyl(thioxo)dihydropyrimidine, which led to a relevant decrease on the proliferation of MCF-7 and LNCaP cell lines and can be a promising structure for the development of more potent and selective antiproliferative agents.

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Digestive glycosylhydrolases from crab visceral waste

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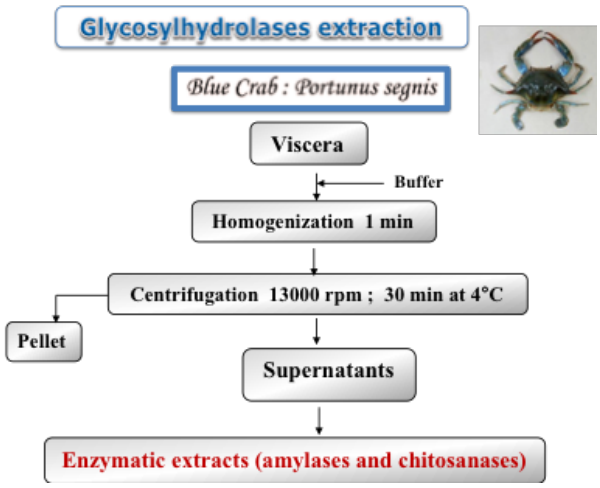
Owing to its vast diversity of organisms and habitats, the marine world is a rich source of valuable carbohydrates-active enzymes, particularly, α-amylases (EC 3.2.1.1) and chitosanases (EC 3.2.1.132), endo-acting hydrolases which randomly cleave α-(1,4) and β-(1,4) glycosidic bonds of starch and chitosan polymers, respectively, resulting in oligosaccharides with varying lengths [1]. These classes of enzymes are of great industrial importance in functional foods, health care medicines and other industries [2].

A novel digestive α-amylase (BCA) from blue crab viscera was purified to homogeneity with a final purification fold of 424.02 and specific activity of 1390.8 U mg−1. BCA, showing a molecular weight of 45 kDa, possesses desirable biotechnological features, such as optimal temperature of 50 °C, interesting thermal stability which is enhanced in the presence of starch, and broad pH range stability. The enzyme displayed K_m and V_{max} values, of 7.5 ± 0.25 mg mL^{-1} and 2000 ± 23 µmol min^{-1} mg^{-1} for potato starch, respectively. It was found to be efficient in the enhancement of the antioxidant potential of oat flour, in terms of polyphenols content and antiradical activity. On the other hand, we reported the extraction and biochemical characterization of crude chitosanase from the blue crab viscera, as well as its application in the production of biologically active chitooligosaccharides from shrimp shells chitosan.

This study provides novel features with other marine-derived enzymes and a better understanding of the biodegradability of carbohydrates in marine environments.



Digestive enzymes extraction

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Process development for high yield fermentation of active recombinant lectins expressed in E. coli

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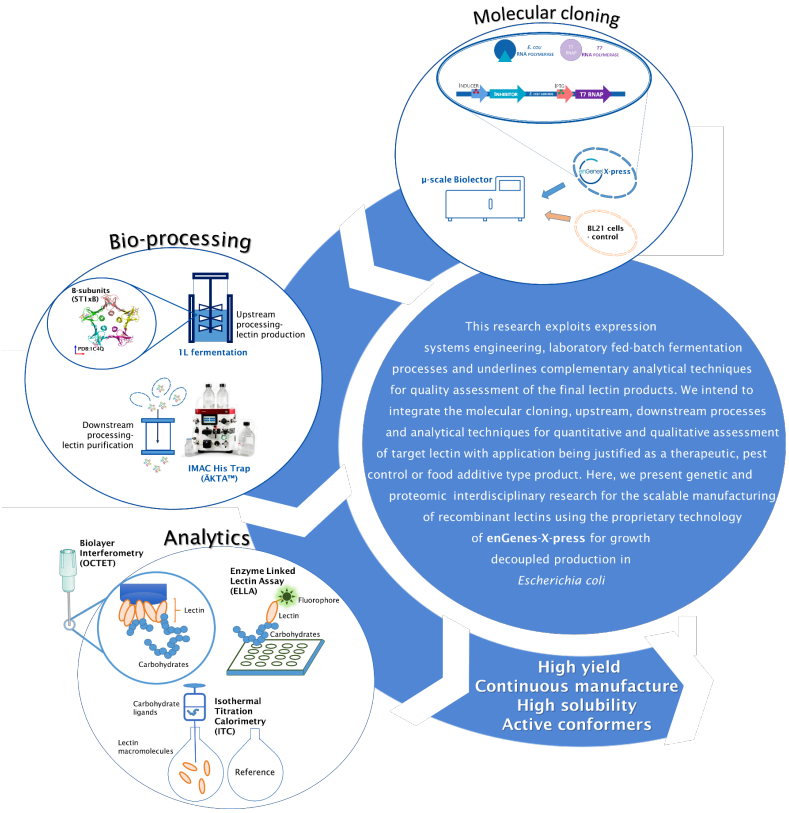
Protein-carbohydrates interactions pave the way for many pathogens and pathogen-like entries inside the eukaryotic systems. Such sugar-binding lectins provide means for finding novel cancer treatment, vaccine strategies, protein-based therapeutics, and pest control applications. Here, we present genetic and proteomic interdisciplinary research for the scalable manufacturing of recombinant lectins using the proprietary technology of enGenes-X-press for growth decoupled production in Escherichia coli. This synthetic biology-based approach for over-expression of recombinant lectins from the BL21 (DE3) cells with inducible inhibition of host RNA polymerase blocks the host messenger RNA production. Overexpression of the lectins is then propagated by the orthogonal T7 RNA polymerase. Shutting down of the host RNA polymerase is achieved by L-arabinose-inducible expression of the T7 phage-derived Gp2 protein from a genome-integrated site. Here, we evaluated the production of a panel of lectins in high-throughput µ-scale fed-batch-like cultivation and 1 L benchtop-bioreactor fed-batch cultivation. In our studies, we will validate the activity and selectiveness of the produced lectins to their sugar-based binding partner with the analytical methods to deduct the kinetics and thermodynamic profiles of those interactomes. We intend to establish manufacturing protocols for the soluble production of active lectins that can be easily up-scaled to pilot-production scale if necessary.



This project receives funding from the European Union's Horizon 2020 research and innovation programe under the Marie Skłodowska-Curie grant agreement No 814029.

Development of 1,2-*cis*-Selective Galactosaminylation with Fluorinated Thiodonors

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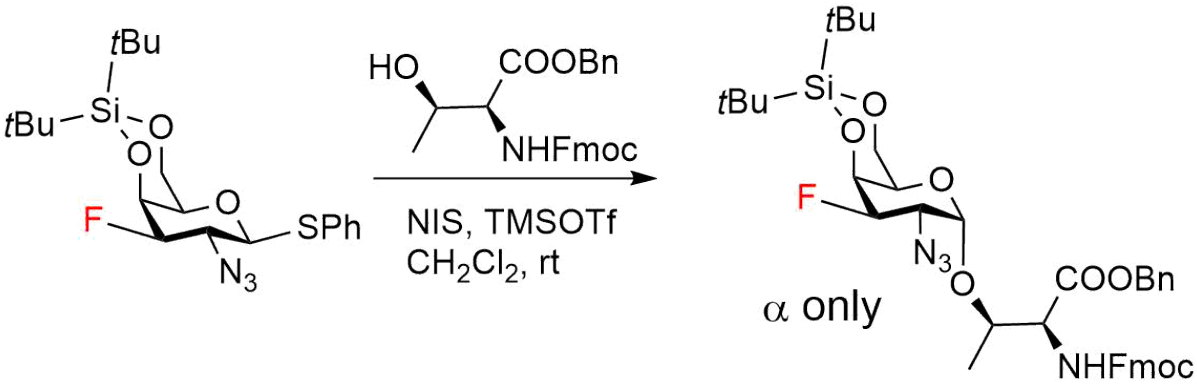
The Tn antigen (GalNAcα1-Thr/Ser) is frequently expressed on the surface of tumor cells but occurs rarely on normal cells rendering it an attractive epitope for antitumor vaccines [1]. The low immunogenicity of the Tn antigen may be enhanced by deoxyfluorination of the GalNAc moiety. Because the stereoselective synthesis of an α-linked fluorinated GalNAc is difficult, we prepared a panel of C3 and C4 deoxyfluorinated galactosazide-based thiodonors and evaluated their stereoselectivity in the glycosylation of carbohydrate acceptors and threonine derivatives. Glycosylation with C4 fluoro thiodonors gave minimal to modest α-stereoselectivity that could not be improved using acyl and silyl protection at O3 and O6. Glycosylation with 4,6-di-O-di-tert-butylsilylene-protected C3 fluoro thiodonors resulted in high α-selectivity and reaffirmed the strong α-directing effect of this protective group in glycosylation with galacto-configured glycosyl donors.



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sp^2-Iminosugar-based mannobioside glycomimetics as functional C-type lectin ligands

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Dendritic cells play a key role in the induction of adaptive immune responses against pathogens. The recognition process is based on the interaction of C-type lectins at DC membrane, such as Langerin and DC-SIGN, and the glycans present on pathogens. For instance, Langerin mediates the capture of HIV-1, leading to its internalization and delivery to Birbeck granules avoiding the virus transmission, whereas DC-SIGN facilitates the HIV transmission to T-cells [1]. These mechanisms are promoted by the interaction of the lectins with the $(Man)_9(GlcNAc)_2$ oligosaccharide at the surface of gp120 VIH-1 glycoprotein, which mainly involves the terminal mannobioside residues (Manα1,2Man). Langerin and DC-SIGN have the classical Ca^{2+}-dependent carbohydrate binding domain of C-type lectins, where the mannobioside can be coordinated to Ca^{2+} through OH-3 and OH-4 of either of both Man residues (dual binding mode) [2]. Taking advantages of the unique properties of sp^2-iminosugar glycomimetics, especially their ability to engage in glycosylation reactions affording metabolically stable α-linked pseudoglycosides [3], we have prepared three different families of pseudomannobiosides, incorporating one or two sp^2-iminosugar motifs, in mono (Figure 1, left) and multivalent (by click conjugation with β-cyclodextrin, Figure 1, right). The conformational properties of these glycomimetics have been analysed by NMR and computational methods, and their interactions with Langerin and DC-SIGN have been investigated using Saturation Transfer Difference (STD) and transferred-NOESY methodologies [4].

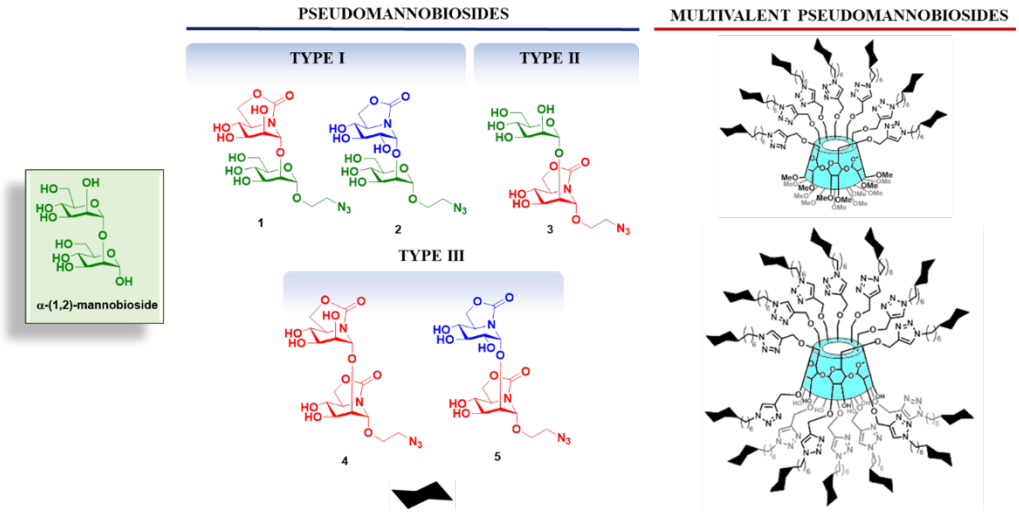

Figure 1. Families of sp^2 iminosugar-based mannobioside glycomimetics prepared in this work.

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The NMR and in silico study of binding specificity in protein-glycosaminoglycan systems

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Glycosaminoglycans (GAGs) represent a class of linear, periodic, anionic polysaccharides, which are essential components of the extracellular matrix. Their function in the body is mediated by interactions with proteins including growth factors and chemokines. However, protein-GAG interactions are still not well characterized at the molecular level and the question to what extent those interactions are specific remains. For some protein-GAG systems, the interaction affinity is mostly electrostatics-driven and thus unspecific. For other systems, it has been shown that a change in GAG type and sulfation pattern affects the ability of GAGs to bind their protein partners.

The goal of this study is to determine to what extent the interaction between heparin, a glycosaminoglycan, and the chemokine interleukin-8 (IL8) is specific. This is investigated using computational approaches in combination with experimental data from NMR methods based on heteronuclear single quantum coherence (^{1}H-^{15}N-HSQC) spectroscopy titration. The computational methods employed include molecular docking, molecular dynamics simulations and estimation of the energy of the bound complexes. Simultaneously, the binding of a series of anionic peptides, which potentially mimic heparin, towards IL8 is analyzed, to help investigate the specificity of IL8-GAG interactions.

Preliminary results show differences in binding strength of the heparin-IL8 complex compared to the anionic peptides, which suggests specificity of the heparin-IL8 binding and the influence of more factors than electrostatics alone.

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Production of cellulose nanofibers from banana rachis and its application for oil in water Pickering

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Cellulose nanofibers were isolated from banana rachis as an alternative to use this agroindustrial residue to obtain a new material. The isolation process includes chemical treatments with KOH, $NaClO_2$, and HCl to remove the non-cellulosic components of the rachis. Subsequently, the obtained suspension was mechanically treated by Ultra Fine Friction Grinding (Supermasscolloider) in order to have a homogeneous suspension of cellulose nanofibers (CNFs). According to the results, CNFs presented a high aspect ratio, and its rheological characterization showed a gel-alike behavior with high potential to stabilize Pickering emulsion. Therefore, the CNFs were evaluated as Pickering agent to develop an oil in water emulsion with lycopene extracted from guava in the oil phase. To obtain the emulsion, three treatments were evaluated (High shear mixing-HSM, Ultra fine friction grinding-UFFG and High Pressure Homogenization-HPH). Additionally, the concentration of CNFs to obtain a stabilized emulsion, depends on the treatment, since they have different mechanisms that are responsible for the size reduction of the drops and the homogenization. From the three mechanisms evaluated to develop Pickering emulsion, the HPH contributes to a more homogeneous emulsion with smaller oil drop sizes according to optical observations, which are more easily adsorbed by the nanofibers allowing the stabilization of the emulsion even after 14 days of storage.

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

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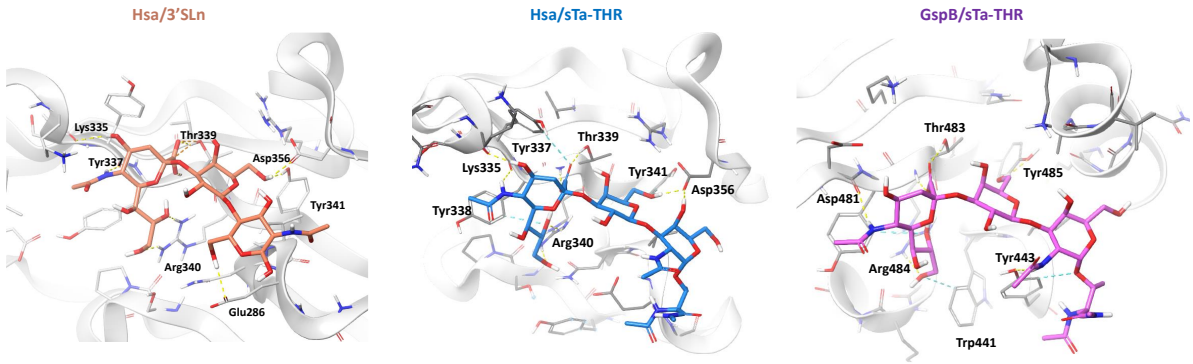
Streptococcus gordonii and Streptococcus sanguinis, commensal species among the normal oral microbiota, become opportunistic pathogens that can cause infective endocarditis (IE) when they enter the bloodstream [1]. The presence on the microbial surface of "Siglec-like" serine-rich repeat adhesins may increase the propensity of streptococci to cause IE. These adhesins contain Siglec-like binding regions (SLBRs) that recognize α2-3 sialylated glycan structures, including O-linked glycans displayed on salivary MUC7, platelet GPIb and several mucin-like plasma proteins [2].

GspB and Hsa are Siglec-like serine-rich repeat adhesins of S. gordonii strains M99 and Challis, respectively, that can mediate Streptococcus adhesion to platelet membrane glycoproteins. Although their high-resolution crystal structures have been published [3,4], they have not fully explained the determinants of ligand specificity. Thus, unveiling the molecular mechanism of host glycans recognition by Siglec-like adhesins represents a prerequisite to deep understand the different selectivity and flexibility of the streptococcal adhesins towards sialoglycans. We indeed explored the recognition and binding process of SLBRs of GspB, highly selective, and Hsa, which instead shows broader sialoglycans specificity. Our outcomes were achieved by a combination of NMR ligand-based methods, such as Saturation Transfer Difference NMR, WaterLogsy and transferred NOESY, as well as computational approaches, including CORCEMA-ST analysis, docking and Molecular Dynamics [5,6].



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The Carbohydrate-Binding Mode of the lectin SapL1: a New Drug Target from Scedosporium apiospermum

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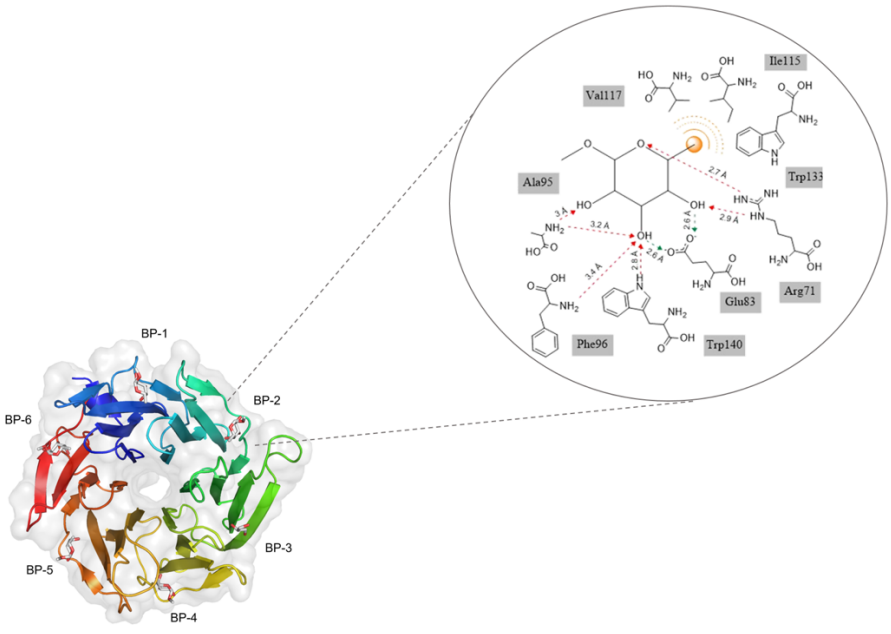
Scedosporium apiospermum is an emerging opportunistic fungal pathogen responsible for human life-threatening infections. It is resistant to several antifungals. A new therapeutically route explored is the development of antiadhesive glycomolecules targeting lectins involved in the recognition and adhesion to the host glycoconjugates. Here, we present the identification and characterization of the first lectin from S. apiospermum (SapL1). SapL1 is homologous to the conidial surface lectin from Aspergillus fumigatus (FleA) known to be involved in the adhesion to human lung epithelium. Both lectins are dimeric and strictly specific for fucosylated carbohydrates and display strong affinity by Lewis and blood groups antigens, which are abundant on pulmonary mucins. SapL1 structure exhibits a 6 bladed β-propeller fold with six non-equivalent binding sites. They all share the necessary features for fucose recognition such as a triad of conserved residues involved in essential hydrogen bonds and hydrophobic interactions. We determined the structural basis responsible for the higher affinity of some binding sites. This information contributes to a better understanding of glycosylated surface recognition by Scedosporium, and will help for the design of glycodrugs targeting SapL1 inhibition.



SapL1 monomer

DC-SIGN recognition of bacterial LPS

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It is well known that lectins play an important role in the innate immune system, being involved in the recognition of carbohydrates epitopes exposed on cell surfaces.[1] Due to their ability to recognise carbohydrate structures, lectins emerged as potential receptors for bacterial lipopolysaccharides (LPS). LPSs are heat stable amphiphilic molecules known for being the major component of the external leaflet of the Gram-negative bacteria outer. Being involved in host-microbe interaction processes,[2] LPSs are constituted by three biosynthetically and functionally distinct portions: the lipid A, the core and O-specific polysaccharide chain (O-antigen). LPS is defined as smooth-type (S LPS) or rough-type LPS (R-LPS or Lipooligosaccharide, LOS) if the O-chain is absent. [3] Despite the growing interest in investigating the association between host receptor lectins and exogenous glycan ligands, the molecular mechanisms underlying bacterial recognition by human lectins are still not fully understood.[4] Therefore, we aim at tackling the important question of recognition of microbial envelope glycoconjugates by lectins, focusing here on the study of molecular recognition of bacterial LPS by DC-SIGN, a C-type lectin expressed by dendritic cells.

We chose to focus on the LPS from various bacterial strains, as Escherichia coli and Bacterioides vulgatus. In detail, the interaction between the DC-SIGN and the lipooligosaccharide of Escherichia coli strain R1 has been unveiled by means of NMR spectroscopy and MD simulation among others.

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Effects of CoCl2 on regioselective tosylations of oligosaccharides

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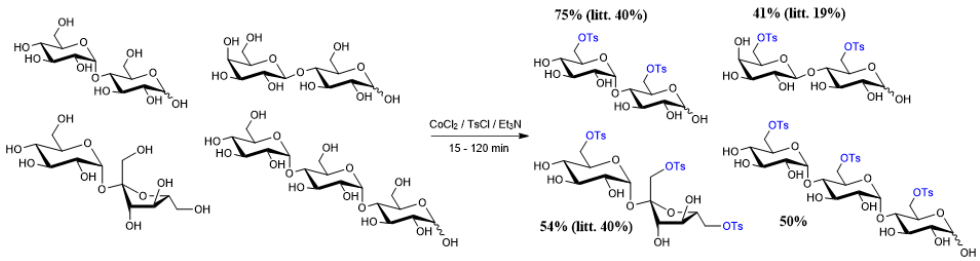
In carbohydrate chemistry, tosylation reactions are popular as they are very useful for the preparation of high-added value precursors. The introduction of a tosyl functional group is usually achieved by the reaction of a carbohydrate-containing a free hydroxyl group with TsCl in pyridine [1]. Depending on the reactivity of the substrate, the reaction conditions can be adapted by selecting the appropriate temperature and stirring time. Catalysts such as DMAP or triethylamine have been used to reduce the reaction times [1,2]. However, when several hydroxyl functions are accessible, this procedure may result in complex mixtures of tosylated products [1,3]. Recently, we developed a regioselective tosylation of primary positions of di-and trisaccharides by using $CoCl_2$ in catalytic amounts. This addition minimizes the formation of polytosylated products and improves the yields of selective tosylated compounds. In this talk, I will present the detailed synthesis of different tosylates obtained, and the Effect of Cobalt II chloride on the regioselectivity tosylation will be discussed.

Financial support: Jamal EL-ABID thanks thetionale, de l'enseignement supérieur et de la recherche and we all thank the university of Picardy Jules Verne for the financial support.



Regioselectivity tosylation of unprotected oligosaccharides

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The hydrotrope-mediated, low-temperature, aqueous dissolution of maize starch

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Complete aqueous dissolution of starch at low temperatures is difficult. After the high-temperature dissolution of starch in an autoclave, aqueous starch solutions after cooling are not stable towards aggregation and crystallization, eventually leading to precipitation. We recently found out that with the addition of sodium salicylate (NaSal), MS spontaneously gelatinizes at room temperature.

Differential scanning calorimetry (DSC) and polarized optical microscopy (POM) of starch dispersions in NaSal solution were used to demonstrate the room temperature gelatinization of MS at different concentrations of MS and NaSAl. The DSC gelatinization peak shifts to lower temperatures and the gelatinization enthalpy decreases with increasing NaSal concentration. POM images confirm the same trend through the disappearance of native starch granule birefringence. The minimal NaSal concentration to induce complete room temperature MS dissolution was found to be around 15-20 wt%. The starch concentration has little influence on the amount of NaSal needed to dissolve it. The effect of the NaSal solution on the MS molecular weight was checked with HPSEC.

It is speculated that, because of its amphiphilic character, NaSal enhances the solubility of MS in water by association with the more hydrophobic MS moieties, much like urea, which has also been used to enhance starch dissolution in basic aqueous media. As such small molecules do not tend to form micelles in water, they are called hydrotropes, rather than surfactants. Further investigations into the dissolution mechanism are necessary.

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Diastereoselective Pd-Catalyzed Anomeric C(sp^3)–H Activation: Synthesis of α-(Hetero)aryl C-Glycosides

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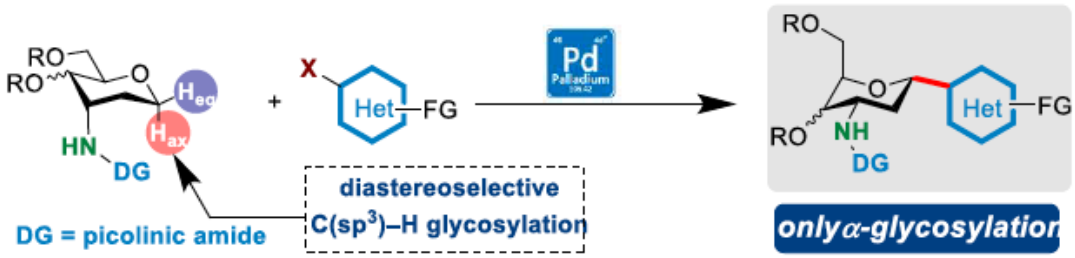
Anomeric C–H bond activation is an unsolved long-standing synthetic challenge. Herein, we report a diastereoselective Pd-catalyzed anomeric C(sp^3)–H activation methodology that allows the synthesis of elusive C-(hetero)aryl glycosides with an exclusive α-selectivity.

C-(Hetero)aryl glycosides belong to a privileged class of carbohydrates in which the biologically labile C–O anomeric acetal bond is substituted by a C–C bond[1]. This strong glycosidic bond confers to such compounds a significantly enhanced in vivo resistance toward basic, acidic, and enzymatic hydrolysis[2]. A plethora of C-aryl glycosides are found in nature[3] and serve as key substrates in various biological mechanisms or are used as antitumor4, antibiotic5 and type II antidiabetic agents6.



- Perfect control of the α-configuration
- Aryl and heterocycles halides
- Easy accessible 3-amidosugars
- Broad tolerance to FG
- DFT calculations
- Easy removable DG
- First applications to α–dapagliflozin

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Structure-Based Design of Anti-Adhesive Glycomimetic Inhibitors of Bacterial Super Lectin (BC2L-C)

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Burkholderia cenocepacia is a gram-negative bacterium responsible for deadly lung infections in immunocompromised or cystic fibrosis patients, which presents extreme resistance to almost all clinically used antibiotics.[1] B. cenocepacia produces a lectin called BC2L-C which acts as a virulence factor involved in adhesion to host cell and subsequent infection process.[2] The N-terminal domain (BC2L-C-nt) of the lectin has been characterized as a novel fucose-binding domain with a TNF-α-like architecture while the C-terminal domain specifically binds to the mannose.[2] Therefore, BC2L-C-nt is an interesting target for designing molecules for anti-adhesive therapy which can prevent lectin mediated bacterial adhesion to the host epithelium. Structure-based virtual screening of a small fragment library identified potential hits predicted to bind in a new region (site X, Fig.1) in the vicinity of the fucose binding site.[3] The interaction of the fragments with the protein domain was confirmed using several biophysical techniques including STD-NMR. The X-ray structure of BC2L-C-nt complexed with one of the identified fragments confirmed binding at the expected location and therefore the druggability of site X. Connecting the fragments to the fucose core was first performed in silico, resulting in the design of several fucose-derivatives. Experimental validation through chemical synthesis, bioassays and structural characterisation is in progress.

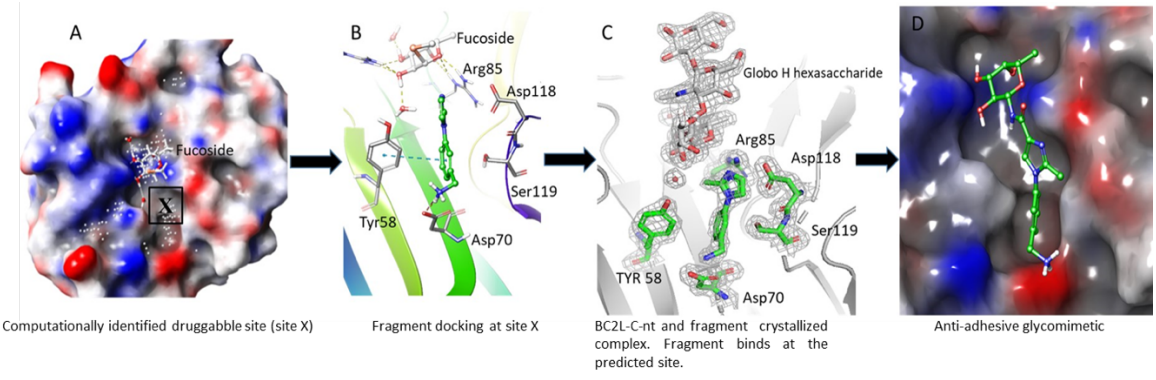

Figure 1. Strategies for the fragment-based design of glycomimetic inhibitor against BC2L-C-nt.

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A trip into the nano-bio interface of cyclodextrin-mediated nonspherical nanomaterials

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β-(1,3)-Glucan is one of the antigenic components of the bacterial as well as fungal cell wall. We designed microcapsules (MCs) ligated with β-(1,3)-glucan, to study its immunomodulatory effect. The MCs were obtained by interfacial polycondensation between diacyl chloride (sebacoyl chloride and terephtaloyl chloride) and diethylenetriamine in organic and aqueous phases, respectively. Planar films were first designed to optimize monomer compositions and to examine the kinetics of film formation. MCs with aqueous fluorescent core were then obtained upon controlled emulsification–polycondensation reactions using optimized monomer compositions and adding fluorescein into the aqueous phase. The selected MC-formulation was grafted with Curdlan, a linear β-(1,3)-glucan from Agrobacterium species or branched β-(1,3)-glucan isolated from the cell wall of Aspergillus fumigatus. These β-(1,3)-glucan grafted MCs were phagocytosed by human monocyte-derived macrophages, and stimulated cytokine secretion. Moreover, the blocking of dectin-1, a β-(1,3)-glucan recognizing receptor, did not completely inhibit the phagocytosis of these β-(1,3)-glucan grafted MCs, suggesting the involvement of other receptors in the recognition and uptake of β-(1,3)-glucan. Overall, grafted MCs are a useful tool for the study of the mechanism of phagocytosis and immunomodulatory effect of the microbial polysaccharides.

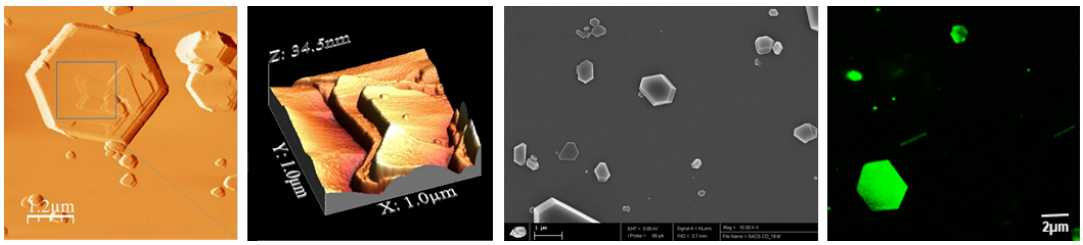

Figure 1: AFM, SEM and CLSM images of chitosan nanomaterials composed of stearyl chitosan/α-cyclodextrin.

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New hypertransglycosylating β-N-acetylhexosaminidase variants for the synthesis of chitooligomers

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The β-*N*-acetylhexosaminidase (EC 3.2.1.52, GH20) from *Aspergillus oryzae* (*Ao*Hex) has previously shown a remarkable synthetic ability. However, in the wild-type enzyme the yields of the transglycosylation reactions are significantly lowered by the concurrent hydrolysis of both the substrate and products. To solve this problem, four putative transglycosidase variants of *Ao*Hex were designed, aiming at suppressing the hydrolytic activity and retaining transglycosylation activity of these enzymes. At first, two types of mutants were generated: Y445F and Y445N mutants of the active-site tyrosine residue stabilizing the oxazoline reaction intermediate, and two mutants were designed at the aglycon-binding site (F453W, V306W) introducing tryptophan residues to improve binding of the acceptor sugar in the transglycosylation reaction. The transglycosylation activity of the recombinant enzymes was tested with *p*NP-GlcNAc employed both as a donor and acceptor. All of the prepared enzymes exhibited decreased hydrolytic activity and catalyzed transglycosylation reactions with higher yields than the parent enzyme. Based on the acquired results, five combined double mutants of *Ao*Hex were prepared, four of them proved to be highly efficient transglycosidases. This is the first report on the hypertransglycosylating mutants of the aglycon-binding site of a GH20 β-*N*- acetylhexosaminidase able to synthesize valuable chitooligomers.

Support from the Czech Science Foundation (grant 20-00477S) and from the Czech Ministry of Education, Youth and Sports (LTC19035) is acknowledged.

Computational insights into the role of calcium ions in protein–glycosaminoglycan complexes

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Glycosaminoglycans (GAGs) are linear, periodic, anionic polysaccharides which are composed of periodic disaccharide units. They are key players in countless biological processes ongoing in the extracellular matrix. With regard to computational approaches, GAGs are known to be very demanding molecules due to their high periodicity, flexibility, predominantly electrostatic-driven nature of interactions with their protein counterparts and potential multipose binding. Moreover, the molecular mechanisms underlying GAG-mediated interactions are not fully known yet, and experimental techniques alone are usually insufficient to gain insights into them. The objective of this study was to characterize protein–ion–GAG complexes for the systems where ions are recognized to be directly involved in GAG binding. Molecular docking, molecular dynamics and free energy calculation approaches were applied to model and rigorously analyse the interactions between annexins (II and V), calcium ions (Ca2+) and heparin (HP). The computational results confirm that the presence of Ca2+ influences the annexin-HP binding site to a great extent. This study presents a general computational pipeline to disclose the complexity of protein–GAG interactions and helps to grasp the role of ions involved at the atomic level. The shortcomings of the applied protocols are characterized and discussed pointing at the challenges recurring in the state-of-the-art in silico tools to study protein–ion–GAG systems.

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Tackling the chemical diversity of microbial sialic acids – a universal large-scale survey approach

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Nonulosonic acids (NulOs), commonly referred to as sialic acids, share many biosynthetic and structural features across all domains of life, but display an otherwise remarkably large molecular diversity only poorly captured date.[1] In humans, sialic acids are involved in processes such as cellular protection, immunity and brain development. On the other hand, sialic acids are commonly associated with bacterial pathogens and viral infections.

However, the remarkably large chemical diversity of microbial nonulosonic acids challenges their large-scale discovery and molecular level methods have been lacking so far.

Here, we demonstrate a newly established sialo-omics approach and provide a first comparative study including non-pathogenic species of prokaryotes.[2] Thereby, we illustrate their surprisingly widespread occurrence and moreover open the window to yet undescribed sialic acid derivatives.[2] The importance of sialic acids for pathogens, such as C. jejuni, has been well established.[3] However, an understanding of their micro-heterogeneity, and distribution and utilisation in purely environmental microbes is currently only established.

Synthetic routes for sialic acids are highly demanding and often of low product yields. Ultimately, our new approach enables a large-scale exploration of alternative microbial sources including new biosynthetic routes.

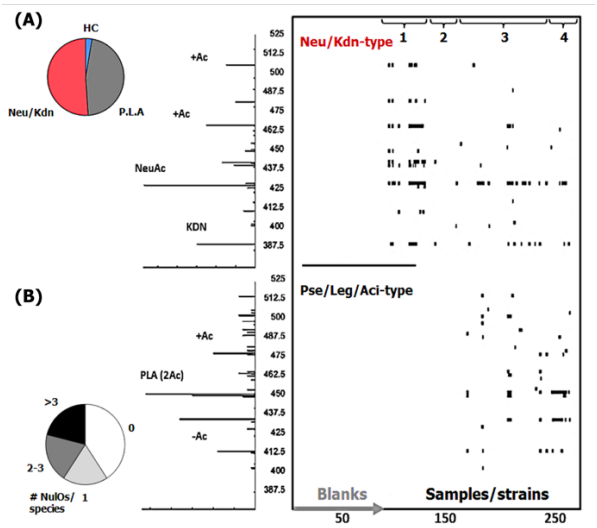

FIGURE: The figure shows the outcome of a large-scale survey (A) The upper part of the hit-map shows Kdn and Neu-like sialic acids and the lower map is filtered for Pse/Leg-type sialic acid identifications. The same data are visualised as binned spectra on the left. The numbers indicate samples which were grouped into taxa (duplicates): (1) references and animal cells; (2) plants, fungi and algae; (3) Archaea, alpha-, beta-, gamma-, delta- and epsilon proteobacteria, actinobacteria, firmicutes, cyanobacteria; (4) enrichment /communities. Circle graphs: Sialic acids were found at high frequency throughout environmental samples. (C) Kdn/Neu-type sugars were observed with comparable frequency to bacterial-type NulOs. While the former were found in eukaryotes and prokaryotes, the bacterial types were exclusive to prokaryotes.[2]

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The role of spike protein glycosylation in modulating SARS-CoV-2 antigenicity and adhesion

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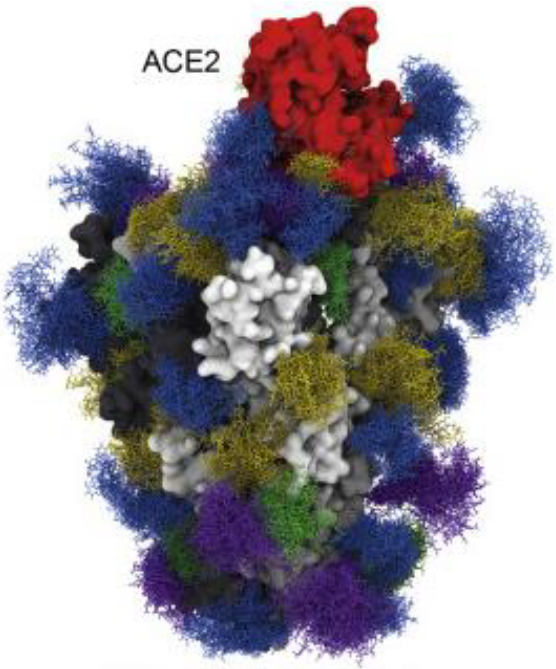
Viral envelop proteins frequently cloak their surfaces with complex sugars (N-linked glycans) in order to evade host immune surveillance, and to suppress the number of viable immunogenic peptide sequences. However, some level of exposure of the surface of the adhesion domain is essential for binding to the host, and thus glycan shielding of these regions can't be exploited without the potential of reducing viral fitness. Remarkably in the case of the SARS-CoV-2 Spike glycoprotein glycosylation also appears to serve a functional role by promoting adhesion to the host receptor protein angiotensin-converting enzyme 2 (ACE2). The mechanisms of the roles of glycosylation in modulating the antigenicity [1] and adhesion [2] of SARS-CoV-2 are examined using molecular dynamics simulations of the glycoproteins, and probed by point mutagenesis and Biolayer Interferometry.



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Synthesis of Difunctionnalized hexa, hepta or octasaccharides by cyclodextrin ring opening.

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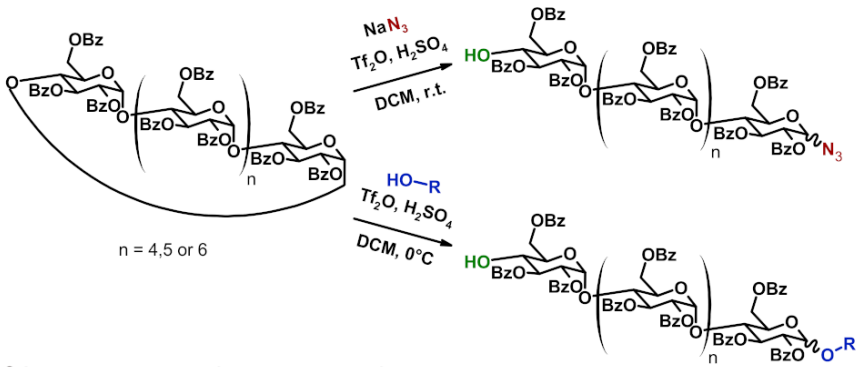
Oligosaccharides have many interests in food and health fields, but also, more recently, in materials science and nanotechnologies for more extensive applications. Obtaining pure oligosaccharides both from natural sources and by multistep oligosaccharide synthesis remains challenging. In one hand, after the hydrolysis of a polysaccharide, the complex mixture of oligosaccharides must be fractionated by exclusion chromatography. On the other hand, total synthesis allowed to obtain pure compounds, but the yield of the sequential glycosylations strongly decreased with the growth of the oligosaccharide chain, limiting the quantity of the final product. Even the automated oligosaccharide synthesis is limited by the availability of building blocks. For many years now, our laboratory has developed chemistry for modification1 and opening of cyclodextrins.2,3 The synthesis of oligosaccharides by cyclodextrin (CD) ring opening is indeed, an efficient alternative to obtain these ones. Starting from α-, β-, or γ-CD, it can afford oligomaltosides containing 6, 7 or 8 glucose units, respectively. We have therefore investigated a new opening method of CD leading to asymmetrically difunctionalized oligomaltosides. Here, we report the optimization of the synthesis of various easily graftable and non-protected oligomaltosides. The synthesis is efficient even at multi-gram scale and versatile with various useful functionalizations.



- One-pot opening and functionalization of the reducing end
- Unprotect non-reducing end with retention of configuration
- Work with the three native cyclodextrins

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Dioxolenium ions in stereoselective glycosylation reactions

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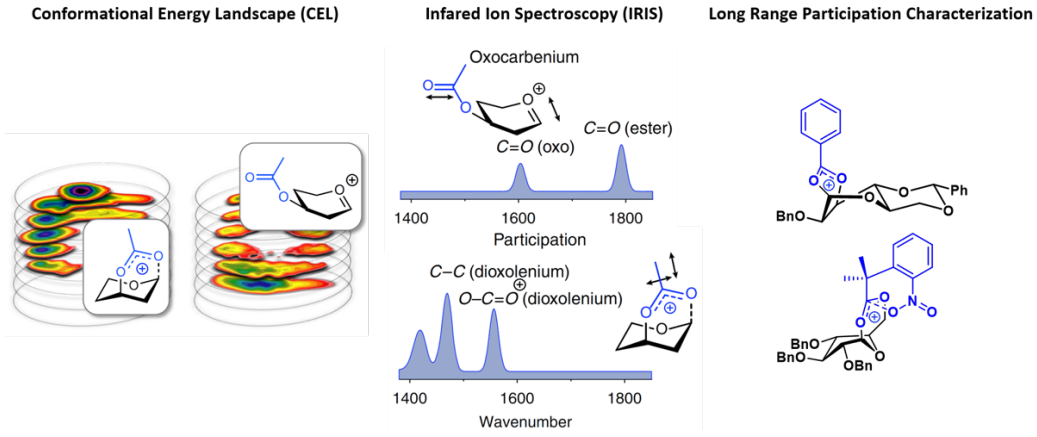
The influence of distal acyl protecting groups (PGs) on the stereoselectivity of glycosylation reactions has been well documented although the origin of the stereodirecting effect is subject to considerable debate.[1,2] Direct evidence for dioxolenium ion formation giving rise to a reaction mechanism driven by long range participation (LRP) is relatively scarce.[3] Recently, we described an approach to study dioxolenium ion formation and LRP using a combination of infrared ion spectroscopy (IRIS), DFT calculations and glycosylation experiments.[4] We used this setup to probe the stereodirecting effect of the O-3 acyl groups of 4,6-O-benzylidene manno- and glucosides. It was found that a benzoate group at the C-3-O has a strong stereodirecting effect on glycosylations of the mannose donor, but virtually no effect on corresponding glucosides. IRIS shows that both donors can form dioxolenium ions, but the mannosyl ions are more stable than their glucose counterparts. The transition state barrier for dioxolenium ion formation is significantly lower for mannosyl ions compared to glucosyl ions. These data show that the mannosyl dioxolenium ions form more readily, providing an explanation for the disparate behavior of the manno- and glucosyl donors. Using the same approach we probed the stereodirecting effect of the recently introduced 2,2-dimethyl-2-(*ortho*-nitrophenyl)acetyl (DMNPA) PG.[5,6] We found that dioxolenium ions formed from the DMNPA group, are stabilized by the nitro group ("double participation") enabling LRP from the glucosyl C-6 position.



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Consumption of non-human sugar Neu5Gc from red-meat affect serum anti-Neu5Gc antibodies in human

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

Cysteine Nucleophiles in Glycosidase Catalysis: Mechanistic Application of a Covalent Inhibitor

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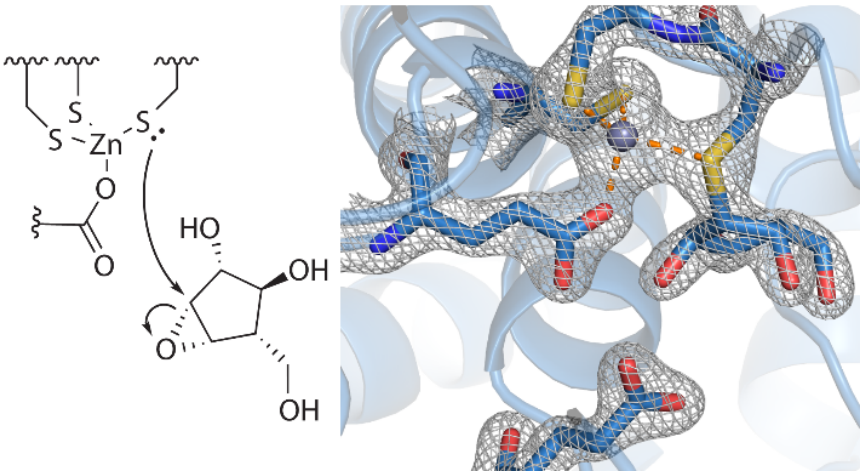
New mechanisms in glycoside hydrolysis are rare. Since the generalised description of the retaining mechanism of glycoside hydrolysis by Daniel Koshland in 1953[1], the only known enzyme-derived electron donors have been the carboxyl groups of aspartate or glutamate or, for certain sialidases, the phenolic oxygen of tyrosine. Recently, the crystal structure of a retaining β-L-arabinofuranosidase from Bifidobacterium longum (HypBA1) reported by Ito et al. in 2014 revealed a Zn(Cys)3Glu coordination complex adjacent to the anomeric carbon of a bound L-arabinose ligand.[2] This suggested the use of a mechanism running through a thioglycosyl enzyme intermediate (tGEI). The proposed mechanism has remained controversial due to the well-known stability of thioglycosidic linkages and the lack of experimental evidence for the existence of the tGEI. To investigate the structure of the tGEI and the mechanism of tGEI breakdown, we synthetised a β-L-arabinofuranoside-configured cyclophellitol derivative. This compound is a potent irreversible inhibitor of HypBA1, enabling the determination of the structure of a tGEI-like complex. Informed by these coordinates, QM/MM simulations show that zinc-assisted cleavage of the thioglycosidic linkage by base-catalysed attack of a water molecule is a viable mechanism, made possible by the fine-tuning of cysteine nucleophilicity via coordination to Zn(II).[3]



(Left) Hypothesized mechanism of covalent inhibition. (Right) X-ray crystal structure of HypBA1 following inhibitor treatment

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