30èmes Journées du Groupe Français des Glycosciences

18 - 22 Mai 2026

— Domaine du Mas de Saboth —
Vers (Lot, Occitanie)

Mot d'accueil

Bienvenue aux 30èmes journées du Groupe Français des Glycosciences.

Dix-huit ans après le congrès organisé à Bonascre, Ax-les-Thermes, sous la Présidence de Pierre Monsan, les journées du GFG reviennent en région Occitanie. Nous sommes très heureux cette fois de vous accueillir à Vers, dans le Lot. Après la montagne, la plaine ! L'esprit demeure toutefois inchangé, un cadre naturel et authentique, particulièrement propice à la réflexion scientifique, tout en favorisant des rencontres informelles et conviviales.

Nous avons souhaité faire de ces journées un moment privilégié de partage et d'échanges, en proposant un programme équilibré associant conférences de chercheuses et chercheurs de renommée internationale, des présentations de jeunes collègues, la remise des prix du GFG ainsi que des sessions de posters, toujours très attendues pour la richesse et la vitalité des discussions qu'elles suscitent.

Nous remercions très chaleureusement nos partenaires privés et institutionnels pour leur confiance et leur soutien, qui ont rendu possible l'organisation de cet événement.
Nous comptons sur vous pour faire vivre l'interdisciplinarité, si chère à notre communauté des glycosciences, et contribuer pleinement au succès de ces journées.

Jérôme Nigou
Pour le Comité d'Organisation

Welcome Address

Welcome to the 30th Meeting of the French Glycosciences Group.

Eighteen years after the conference held in Bonascre, Ax-les-Thermes, under the presidency of Pierre Monsan, the GFG meeting returns to the Occitanie region. We are very pleased to welcome you this time to Vers, in the Lot. After the mountains, the plains! The spirit, however, remains unchanged: a natural and authentic setting, particularly conducive to scientific reflection, while encouraging informal and friendly interactions.

We have designed this meeting as a special opportunity for sharing and exchange, with a balanced program featuring lectures by internationally renowned researchers, presentations by early-career colleagues, the GFG awards ceremony, and poster sessions, always eagerly anticipated for the richness and vitality of the discussions they inspire.

We warmly thank our private and institutional partners for their trust and support, which have made this event possible.

We count on you to foster the interdisciplinarity that is so central to our glycosciences community and to contribute fully to the success of this meeting.

Jérôme Nigou
On behalf of the Organizing Committee

Remerciements

Le comité d'organisation du GFG 2026 remercie vivement les institutions académiques et les partenaires industriels pour leur soutien financier, matériel et pour leur participation.

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Programme scientifique

PROGRAMME DES 30EMES JOURNEES DU GFG 2026 MAS DE SABOTH, LOT – DU 18 AU 22 MAI 2026

Lundi 18 Mai

16h00-18h00	**Accueil – café**
Modérateur : Jérôme Nigou	
18h00-18h15	**Ouverture des 30èmes Journées du GFG**
18h15-18h45	**CI-01. Marcelo Guerin** - *Molecular basis of glycogen metabolism regulation in bacteria*
18h45-19h15	**CI-02. Laurence Mulard** - *Synthetic glycan-based vaccines to combat shigellosis: proof of concept in infants and beyond*
19h30	**Cocktail de bienvenue**
20h00	**Dîner**

Mardi 19 Mai

Modérateur·rice : Laurence Mulard & Romain Vivès	
9h00-9h30	**CI-03. Alexiane Decout** - *Glycan-mediated host-microbiome interactions in women's reproductive health*
9h30-9h45	**CO-01. Amuda Manikandan** - *Synthetic Lipid A disaccharide mimetics for modulating neuroinflammation*
9h45-10h05	**CO-02. Yoann Rombouts** - *Unraveling the role of the C-type lectin DCIR in immunity: From ligand discovery to therapeutic antibody development*
10h05-10h15	**Présentations flash par sponsors** – *Eurobio* *Büchi*
10h15-10h45	**Pause café**
Modérateur·rice : Marie Schuler & Thierry Fontaine	
10h45-11h15	**CI-04. Philippe Compain** - *From seredipitose to superoses: the game of design and chance through glycochemical space*
11h15-11h30	**CO-03. Cyrille Grandjean** - *New generation of glycoconjugate vaccines using pneumococcus as an infection model*

Modérateur : Yoann Rombouts

11h30-12h15	**Présentations flash des posters**
12h30-14h00	**Pause repas**
14h00-16h00	**Session posters**
16h00-16h30	**Pause café**

Modérateur·rice: Yoann Rombouts & Emeline Richard Millot

16h30-17h00	**CI-05. François Foulquier** - *Congenital disorders of glycosylation (CDG): A genetic window into fundamental glycobiology*
17h00-17h15	**CO-04. Marie Morel** - *A new pathogenic variant in SLC35C1 impairs GDP-Fucose transport: Diagnostic and therapeutic implications*
17h15-17h30	**CO-05. Thierry Fontaine** - *β-1,6-glucan, an unexplored and critical cell wall polymer of Candida albicans*
17h30-17h45	**CO-06. Devon Kavanaugh** - *Biochemical characterization of the Escherichia coli surfaceome: a focus on type I fimbriae and flagella*
17h45-18h00	**CO-07. Leïla Bechtella** - *Structural analysis of glycopeptides using cyclic ion mobility spectrometry and tandem MS techniques*
20h00	**Dîner**

Mercredi 20 Mai

Modérateur·rice : Yves Blériot & Christelle Breton

9h00-9h30	**CI-06. Stéphane Vincent** - *Glycomimetics as probes for bacterial and viral infections*
9h30-9h45	**CO-08. Clara de la Tramblais** - *Synthesis and study of multivalent glycoclusters by dynamic combinatorial chemistry*
9h45-10h00	**CO-09. Corinne Lepetit** - *Multivalent galabiosyl-based oligosaccharide glycoclusters as antiadhesive inhibitors of Pseudomonas aeruginosa*
10h00-10h15	**CO-10. David Goyard** - *Screening of multivalent ligands for carbohydrate-binding proteins by microarray*
10h15-10h30	**CO-11. Jonathan Berry** - *Orthogonal photoswitching in a bis-azobenzene glycomacrocycle*
10h30-11h00	**Pause café**

Modérateur·rice : François Foulquier & Sandrine Gulberti

11h00-11h30	**CI-07. Zoeisha Chinoy** - *Inherent selectivities of ST6GALNAC1 for sydnone-Linkered Oglycans*
11h30-11h45	**CO-12. Elodie Mathieu-Rivet** - *Engineering the N-glycosylation pathway in Chlamydomonas reinhardtii to produce humanized glycoproteins*
11h45-12h00	**CO-13. Daphné Gonnet** - *Modulating selectivity in glycoside hydrolases*

12h00-12h15	**CO-14. Clarisse Gosset-Erard** - *Deciphering structure-affinity relationships of cathepsin K–glycosaminoglycans interactions in the context of osteoporosis*
12h15-12h30	**CO-15. Yannick Malbert** - *Innovative Fermentation Platform for Active Oligosaccharide Manufacturing*
12h45-14h15	**Pause repas**
	Après-midi libre ou visite de Saint-Cirq-Lapopie (14h30-18h30)
20h00	**Dîner**

Jeudi 21 Mai

Modérateur·rice : David Bonnaffé & Caroline Nugier-Chauvin

9h00-9h30	**CI-08. Vincent Ferrières** - *A pint of furanosides*
9h30-9h45	**CO-16. Mohammad Krayani** - *Development of a nanomolar inhibitor of NagZ, an enzyme involved in the antibioresistance of P. aeruginosa*
9h45-10h00	**CO-17. Marie Schuler** - *Synthesis of exo-iminoglycals by gold-catalyzed hydroamination*
10h00-10h15	**CO-18. Jérôme Désiré** - *Azalevoglucosan, a usefull scaffold to reach iminosugars with high structural diversity*
10h15-10h45	**Pause café**

Modérateur·rice : Isabelle André & Patrice Lerouge

10h45-11h15	**CI-09. Aria Gheeraert** - *Protein-carbohydrate interactions: from a large-scale structural analysis to deep learning-based predictions*
11h15-11h30	**CO-19. Gianluca Cioci** - *Structural investigations of CAZymes for the synthesis of biopolymers*
11h30-11h45	**CO-20. Arsène Kossov** - *Effect of a single water molecule on the structure of a mannose photosensitizer model and mannose-glutamic acid molecular complex in the gas phase*
11h45-12h00	**CO-21. Cédric Montanier** - *How can we probe the dynamic mechanisms involved in the multienzymatic deconstruction of plant biomass?*
12h15-13h45	**Pause repas**
14h00-16h00	**Session posters**
16h00-16h30	**Pause café**

Modératrice : Stéphanie Norsikian

16h30-17h00	***Prix Bernard Fournet – André Verbert***
	Mathieu Decloquement - *Writers and readers of sialylation: From sialyltransferases enzymatic specificities to Siglecs and their ligands in cancer*
	Ahmed El Rhaz - *Synthesis of thioglycoside glycomimetics as Pseudomonas aeruginosa lectin ligands*

17h00-17h30	***Prix du GFG*** **CI-10. Rebekka Wild** - *Unraveling the step-by-step biosynthesis of glycosaminoglycans at atomic resolution*
17h30-18h30	**Assemblée générale du GFG**
19h00	**Apéritif**
20h00	**Remise des prix des communications orales et des posters Dîner de gala**

Vendredi 22 Mai

Modérateur·rice: Régis Fauré & Aria Gheeraert

9h00-9h30	**CI-11. Bastien Bissaro** - *On the road with carbohydrate oxidases: mechanistic pit stops and functional destinations*
9h30-9h45	**CO-22. Sandrine Gulberti** - *The glycosyltransferase β4GalT7 as a potential target for substrate reduction therapy in mucopolysaccharidoses*
9h45-10h00	**CO-23. Aurore Labourel** - *Exploring the substrate specificity of tandem repeat SusCD transporters (and beyond?)*
10h00-10h15	**CO-24. Emeline Fabre** - *Mycobacterium tuberculosis MtPMT mechanistic insights reveal a WW-like domain and anti-virulence inhibitors*
10h15-10h45	**Pause café**

Modérateur·rice: Jérôme Nigou & Stéphanie Norsikian

10h45-11h15	**CI-12. Samir Messaoudi** - *New catalytic methods for carbohydrate functionalization*
11h15-11h30	**Clôture du GFG30 et quelques mots sur le GFG31**
11h30-12h30	**Repas et départ**

Conférences invitées

Molecular basis of glycogen metabolism regulation in bacteria

Marcelo E. Guerin[1]

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Glycogen metabolism has emerged to allow organisms to save available environmental surplus of carbon and energy, using dedicated glucose polymers as a storage compartment that can be mobilized at future demand. The origins of such adaptive advantage rely on the acquisition of an enzymatic system for the biosynthesis and degradation of glycogen, along with mechanisms to balance the assembly and disassembly rate of this polysaccharide, in order to store and recover glucose according to cell energy needs. The first step in the classical bacterial glycogen biosynthetic pathway is carried out by ADP-glucose pyrophosphorylase. This allosteric enzyme synthesizes ADP-glucose and acts as a point of regulation. The second step is carried out by glycogen synthase, an enzyme that generates linear α-(1→4)-linked glucose chains, whereas the third step is catalyzed by the branching enzyme producing α-(1→6)-linked glucan branches in the polymer. Two enzymes facilitate glycogen degradation: glycogen phosphorylase, which functions as an α-(1g4)-depolymerizing enzyme, and the debranching enzyme that catalyzes the removal of α-(1→6)-linked ramifications. We describe and discuss the structural basis of glycogen metabolism in bacteria to the light of our current knowledge.[1,2]

[1] Cifuente, J.O.; Colleoni, C.; Kalscheuer, R. & Guerin, M.E. *Chem. Rev.* **2024**, 124, 4863-4934. Review.

[2] Cifuente, J.O.; Comino, N.; Trastoy, B.; D'Angelo, C. & Guerin, M.E. *Biochem. J.* **2019**, 476, 2059-2092. Review.

Synthetic glycan-based vaccines to combat shigellosis: proof of concept in infants and beyond

Laurence A. Mulard

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This presentation illustrates the concept and promise of synthetic glycan-based conjugates as an alternative to bacterial polysaccharide-protein conjugate vaccines.

The strategy is exemplified in the context of shigellosis (bacillary dysentery), a major diarrheal disease in low- and middle-income countries. Disease burden calls for a vaccine that would induce broad protection especially in children under the age of five, the population most at risk. *Shigella*, a genus of Gram-negative bacteria, is the causative agent of shigellosis. Protective immunity is believed to be achieved to a large extent by antibodies directed at the *Shigella* O-antigen (O-Ag), making it a prime target for vaccine development.

SF2a-TT15, a semi-synthetic glycoconjugate, was designed to help protect against *Shigella flexneri* serotype 2a (SF2a).[1] Following promising data obtained in a first-in-human study,[2] SF2a-TT15 was shown to be safe, well tolerated, and highly immunogenic in the 9-month-old infant target population living in an endemic setting. Data from this age-descending study will be presented. They support further development enabling serotype broadening to answer the need in the field.[3] Ongoing developments will be highlighted.

Aiming at the next generation synthetic glycan-based bacterial vaccines, recent interest is on *S. flexneri* for which at least 15 serotypes are known. Most serotypes exhibit closely related O-Ags.[4] This presentation discloses an innovative concept whereby key O-Ag repeating unit building blocks featuring type-specific substitutions are built from a single orthogonally protected tetrasaccharide scaffold by means of controlled chemical 1,2-*cis* glucosylation and/or *O*-acetylation of suitable acceptors. Chain elongation of the glucosylated bricks and deprotection delivered linker-equipped oligosaccharides. Conjugation of the latter onto a protein carrier provided sets of potential immunogens representative of multiple *S. flexneri* serotypes. Preclinical data will illustrate the proof of concept for a totally novel broad serotype coverage synthetic glycan-based *S. flexneri* conjugate vaccine prototype.

[1] R. van der Put *et al*, ACS Cent. Sci. **2022**, *8*, 449.
[2] D. Cohen *et al*, *Lancet Infect. Dis.* **2021**, *21*, 546.
[3] A. Phalipon & L. A. Mulard, *NPJ Vaccines*, **2022**, *10*, 403.
[4] A. V. Perepelov *et al*, *FEMS Immunol. Med. Microbiol.* **2012**, *66*, 201.

Glycan-mediated host-microbiome interactions in women's reproductive health

Alexiane Decout[1]

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The vaginal microbiome is a key mediator of women's health and pregnancy outcomes. Dominance of the vaginal microbiota by lactobacilli species, particularly *Lactobacillus crispatus*, prevents colonization by pathogenic bacteria, reduces risk of sexually transmitted infections and in pregnancy, protects against preterm birth and reduces maternal inflammation. However, data are mainly limited to associative findings and there remains a critical need to improve mechanistic understanding of how vaginal microbiota interact with, and at time evade, the host immune system, and identify the drivers of bacterial vaginal colonisation. Using clinical isolates of vaginal pathogens and commensals, we are delineating the glycan-mediated mechanisms promoting *L. crispatus* colonisation and protecting against averse reproductive health outcomes. These data provide new insight into glycobiological mechanisms of microbial associated preterm birth and may offer new targets for novel preventive strategies.

From Serendipitose to Superoses: The game of Design and Chance through Glycochemical Space

Philippe Compain

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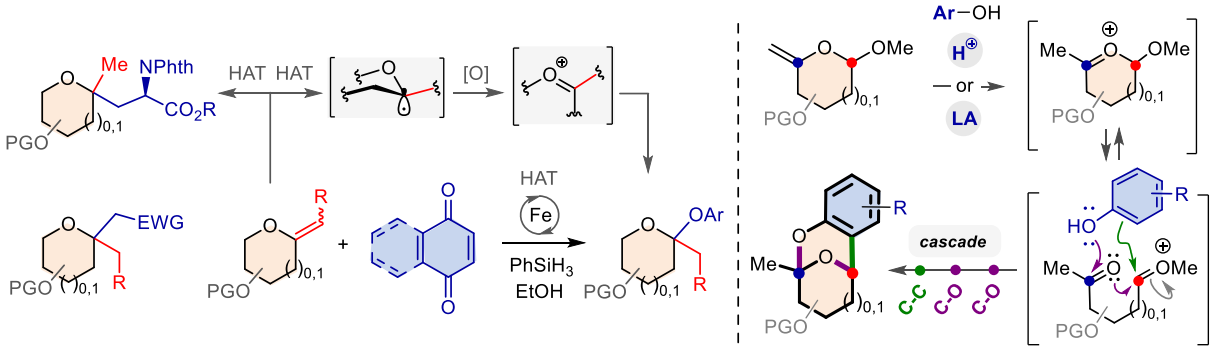
Despite significant progress, the therapeutic exploitation of carbohydrates remains constrained by intrinsic pharmacological limitations. Glycomimetics, which emulate carbohydrate structure, offer a powerful strategy to overcome these barriers.[1] Combined with advanced synthetic methods, glycomimetics are poised to significantly advance glycobiology and meet pressing medical needs. We recently combined innovative synthetic strategies with non-classical carbohydrate architectures to explore new regions of glycochemical space.[2] Efficient iron-hydride hydrogen atom transfer (MHAT) and gold-catalyzed methods were designed to access quaternary (pseudo)anomeric centers,[3] enabling the generation of novel glycomimetic scaffolds.[4]



Additionally, we are investigating the synthetic potential of bifunctional glycosides – an underexplored class of glycosyl donors bearing two (pseudo) anomeric centers – whose reactivity has in some cases emerged from serendipitous observations.[5] These cascade-ready "super sugars" exhibit unique reactivity profiles, and have the potential to significantly expand the glycoscience toolbox.

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[1]For recent reviews, see: (a) B. Hevey, *Pharmaceuticals* **2019**, *12*, 55. (b) S. Leusmann, P. Ménová, E. Shanin, A. Titz, C. Rademacher, *Chem. Soc. Rev.* **2023**, *52*, 3663.

[2]For an account article, see : P. Compain, *Synlett* **2023**, *34*, 1866.

[3]For a recent review on *gem*-*C*,*C*-glycosides see: D. Hazelard, M. Pascaretti, D. Tardieu, N. Kern, P. Compain, *Org. Biomol. Chem.* **2025**, *23*, 8364.

[4](a) H. Liu, A. G. Laporte, D. Tardieu, D. Hazelard, P. Compain, *J. Org. Chem.* **2022**, *87*, 13178. (b) H. Liu, M. Lang, D. Hazelard, P. Compain, *J. Org. Chem.* **2023**, *88*, 13847. (c) M. Lang, D. Tardieu, B. Pousse, P. Compain, N. Kern, *Chem. Commun.* **2024**, *60*, 3154. (d) M. Lang, S. Walter, D. Hatey, A. Blanc, P. Compain, N. Kern, *Org. Lett.* **2024**, *26*, 38, 8017.

[5](a) H. J. Liu, A. G. Laporte, D. Gónzalez Pinardo, I. Fernández, D. Hazelard, P. Compain, *J. Org. Chem.* **2024**, *89*, 5634. (b) M. Pascaretti, I. Fernández, D. Hazelard, P. Compain *et al. Org. Lett.* **2025**, *27*, 12600.

Congenital Disorders of Glycosylation (CDG): A genetic window into Fundamental Glycobiology

François Foulquier[1]

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Congenital Disorders of Glycosylation (CDG) represent a rapidly expanding group of metabolic diseases caused by defects in the synthesis and attachment of glycans to proteins and lipids. Beyond their severe clinical manifestations, CDGs serve as a powerful genetic lens into fundamental glycobiology, frequently revealing entirely novel regulatory mechanisms and cellular concepts. Central to these disorders are the regulatory processes within the Endoplasmic Reticulum (ER) and the Golgi apparatus—areas where our group has made significant contributions.

This talk will focus on recent advances within the ER, where newly discovered CDGs highlight the stringent quality control mechanisms governing N-glycan precursor assembly and the transfer of oligosaccharides to nascent polypeptides. Specifically, mutations affecting dolichol biosynthesis have recently revealed a completely novel metabolic precursor pathway linked to ER N-glycosyaltion.

Transitioning to the Golgi apparatus, CDGs provide unique insights into the spatial and temporal regulation of glycan maturation. Over the last five years, our group has successfully identified and characterized novel regulatory mechanisms by studying CDG patients. While initial research focused on the direct molecular actors of glycosylation—such as glycosyltransferases, glycosidases, and sugar-nucleotide transporters—our current work identified defects affecting ER-Golgi architecture, multi-enzymatic complex organization and vesicular trafficking. Furthermore, we have recently uncovered the critical role of ER-Golgi metal homeostasis which has recently been evidenced by the characterization of specific metal ion transporters, such as TMEM165 and SLC10A7.

In conclusion, the study of these genetic defects uncovers the fundamental rules of "glycan synthesis" and organelle compartmentalization. This presentation will summarize the paradigm shifts in the field of glycosciences that have been made possible through the study of CDG patients.

Glycomimetics as probes for bacterial and viral infections

Stéphane P. VINCENT[1]

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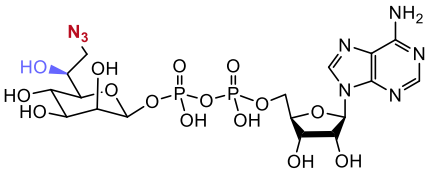
Our research group is specialized in bio-organic chemistry, with a special emphasis in glycosciences. In particular, we are synthesizing fluorinated carbohydrates for the mechanistic and inhibition studies of essential enzymes involved in the bacterial cell wall biosynthesis of important human pathogens.



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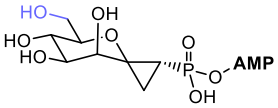

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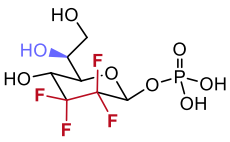

Fluorinated

We have been particularly interested in the biosynthesis of bacterial heptosides present in LPS. We synthesized analogues of ADP-heptose and heptose-1-phosphate as mechanistic probes, labeling reagents and metabolic inhibitors.[1-3]

[1] M. Lacritick, A. Reboul, R. Yahia Boudhar, E. Carlier, J. Fairman, T. Scaillet, S. Subramanian, J. Wouters, B. Staker, X. de Bolle, S.P. Vincent *ACS Chem. Biol*. **2025**, *20*, 1382

[2] J. Cao, S.P. Vincent *Org. Lett.* **2022**, *4*, 4165

[3] T. Li, A. Tikad, H. Fu, J. Milicaj, C. Castro, M. Lacritick, W. Pan, E. Taylor, S.P. Vincent, *Org. Lett.* **2021**, *23*, 1638

Inherent Selectivities of ST6GALNAC1 for Sydnone-Linkered O-Glycans

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[2] *Institut des Sciences Moléculaires, CNRS UMR5255, Talence, France*

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The most common *O*-linked *N*-acetylgalactosamine (*O*-GalNAc) glycans are not only found as dense clusters on mucins, but are also found on most secreted and membrane-bound proteins. *O*-GalNAc glycans are thus involved in almost every aspect of biology, including cell–cell interactions, signal transduction, immune surveillance, host–pathogen interactions, and their aberrant glycosylation have been found to be biomarkers for various cancers.[1,2,3,4] Due to the large structural diversity of biosynthesized *O*-GalNAc glycans, it is very difficult to assign specific functions to individual structures. For instance, it is still unknown how the presentation of the terminal *N*-acetylneuraminic acid (Neu5Ac) and the composition of the underlying sugars can impact its recognition by lectins, namely, Siglecs (a family of immunoglobulin-like lectins, essential regulators of immune cell function in inflammation).[5] One major limitation is the challenging preparation and isolation of well-defined complex O-glycans.

Here we show how the incorporation of the bioorthogonal chemical sydnone[6] linker favorably impacts ST6GALNAC1 for the synthesis of core 1 and core 3 Neu5Ac-α2,6-GalNAc glycans. The small library of core 1 and 3 glycans were attached to microtiter plates, owing to the sydnone tag, and probed for their binding specificities with various Siglecs.

[1] Bagdonaite, I.; Pallesen, E. M. H., et al. *Adv. Exp. Med. Biol.* **2021**, *1325*, 25.

[2] Brockhausen, I., In *Comprehensive Natural Products II*, Liu, H.-W.; Mander, L., Eds. Elsevier: Oxford, **2010**; pp 315.

[3] Jensen, P. H.; Kolarich, D.; Packer, N. H. *Febs. j.* **2010**, *277*, 81.

[4] Reily, C.; Stewart, T. J., et al. *Nat. Rev. Nephrol.* **2019**, *15*, 346.

[5] Varki, A.; Angata, T. *Glycobiology* **2006**, *16*, 1r.

[6] Chinoy, Z. S., Moremen, K. W., and Friscourt, F., *Eur. J. Org. Chem*. **2022**, e202200271.

A Pint of Furanosides

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No one knows carbohydrates like you do! And the further we go, the more this kingdom of surprises and uncertainties expands. Previous synthetic strategies have paved the way for significant technological advancements, complemented by organometallic, photocatalytic, and minimal protection approaches. A deeper understanding of carbohydrate interactions with specific receptors is now enabling us to decipher biological processes, driving the development of bioactive compounds. Structural elucidation also plays a key role in these ongoing advancements.

The world of (hexo)furanosides is fascinating (at least for some of us!). Some impactful molecular-level insights will be shared with you: (1) how the aglycon can influence the progression of a furanosylation reaction [1] (2) how IRMPD and ion mobility in mass spectrometry can rapidly distinguish furanose *vs*. pyranose isomers.[2]

These results were obtained under the support of ANR during the ALGAIMS project involving the teams of D. Ropartz (BIBS, Nantes), J. Boustie and F. Le Dévéhat (ISCR, Rennes), and I. Compagnon (ILM, Lyon).[3]

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Protein-carbohydrate interactions: from a large-scale structural analysis to deep learning-based predictions

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Despite the rapidly growing number of machine learning-based tools in bioinformatics, prediction of protein-carbohydrate interactions remains a particularly challenging task. The difficulty arises from the limited availability of experimental data, as well as from missing or erroneous structural and functional annotations, especially for proteins with highly divergent sequences.

To address this challenge, our group has developed a comprehensive database compiling available information on protein-carbohydrate complexes deposited in the PDB, named DIONYSUS[1]: www.dsimb.inserm.fr/DIONYSUS/. To date, the database contains more than 60,000 experimental structures of protein-carbohydrate complexes. We analyzed their diversity at several levels, including functional classes of interactions, protein sequence identity, and local geometric similarity between binding sites.

These analyses highlight several cases of evolutionary convergence in the local geometry of carbohydrate binding sites and allow us to suggest functional annotations for proteins lacking such information in the existing specialized databases.[2] Moreover, meticulous structure-based analysis allowed us to highlight evolutionary convergence of local geometry of the sugar binding sites as well as to suggest annotations for proteins with missing functional information in the existing specialized databases. Finally, the developed database was used to evaluate both general and carbohydrate-specific deep learning models and to explore new strategies for carbohydrate-binding prediction.

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Prix du GFG 2026

Unraveling the step-by-step biosynthesis of glycosaminoglycans at atomic resolution

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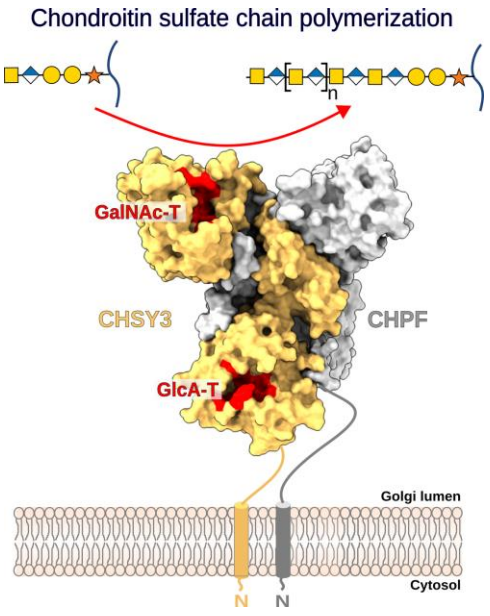
Glycosaminoglycans are complex polysaccharides that are covalently attached to a core protein. My presentation will focus on the biosynthesis of the two glycosaminoglycans: heparan sulfate (HS) and chondroitin sulfate (CS). After the assembly of a common tetrasaccharide linker, the addition of the fifth sugar residue triggers the subsequent chain elongation reaction, during which the long glycan backbone is generated. Extensive modifications of the backbone complete the biosynthesis.

Using cryo-electron microscopy, we determined the structure of the heparan sulfate polymerase complex EXT1-EXT2, revealing the spatial organization of the catalytic domains and their active sites[1]. Our results suggest that HS chain elongation follows a non-processive mechanism.



To learn more about CS chain polymerization, we studied heterodimeric complex formation between the four human CS synthase proteins. We identified four distinct CS polymerase complexes. Functional and structural assays revealed that CHSY1 and CHSY3 harbor catalytic activity, while CHPF and CHPF2 appear to serve only a scaffolding function[2]. As found for HS polymerization, CS chain elongation follows a distributive mechanism.

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On the Road with Carbohydrate Oxidases: Mechanistic Pit Stops and Functional Destinations

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Carbohydrate oxidases (CarbOx) are emerging as powerful and versatile catalysts at the crossroads of carbohydrate chemistry, enzymology, and glycobiology. However, their mechanistic diversity and biological roles remain only partially understood. In this presentation, I will provide an overview of current knowledge on enzymatic carbohydrate oxidation, illustrated by several key recent mechanistic findings.[1,2] I will then describe how my team explores the *in vitro* functional landscape of fungal CarbOx, showing notably how subtle structural variations unlock distinct reactivities toward substrates derived from chitin, pectin, and β-glucans.[3-5] By integrating enzymology, structural biology, and infection biology, we aim at discovering the *in vivo* functional destination of these enzymes. We propose that they do more than catalyze oxidation: they actively shape pathogenic strategies. In particular, I will present experimental evidences indicating that CarbOx play key roles during infection, contributing to fungal cell wall remodelling and modulation of plant immune signals, ultimately enhancing microbial fitness. Overall, this ongoing research redefines CarbOx and related enzymes, hitherto considered as "auxiliary" biocatalysts, as key modulators of biological functions. While highlighting the broad functional potential of CarbOx, this presentation will also underscore the many opportunities ahead for discovering new oxidative biocatalysts for carbohydrate chemistry and for deepening our understanding of their roles in microbial physiology and host–pathogen interactions.

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New Catalytic Methods for Carbohydrate Functionalizations

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The selective functionalization of carbohydrates stands as a pivotal challenge at the intersection of chemistry and biology. Catalysis offers unique opportunities to unlock unprecedented levels of reactivity and selectivity, enabling the precise modification of complex sugar architectures under mild and sustainable conditions. This presentation will highlight our recent advances in catalytic strategies including transition metal catalysis, photocatalysis, and electrocatalysis, that open innovative pathways for carbohydrate functionalization. Particular emphasis will be placed on approaches that achieve site-selective transformations, late-stage diversification, and the generation of novel bioactive molecules.

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2. Pd-catalyzed S-glycosylation of cysteine-containing peptides at room temperature, L. Shen, F. Le Bideau, G. Chen and S. Messaoudi, *Org. Chem. Front*., **2024**, 11, 466-471
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6. Synthesis of Aryl-Thioglycopeptides Through Chemoselective Pd-Mediated Conjugation, Montoir, D.; Amoura, M.; Ababsa, Z.E.-A.; Vishwanath, T. M.; Yen-Pon, E. ; Robert, V.; Beltramo, M. ; Piller, V. ; Alami, M. ; Aucagne, A. and Messaoudi, S. *Chem.Sci*. **2018**, 9, 8753-8759

Prix Bernard Fournet – André Verbert

Writers and readers of sialylation: From Sialyltransferases enzymatic specificities to Siglecs and their ligands in cancer

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Sialylation is an essential glycosylation process that generates a wide diversity of glycoconjugates involved in development, cell communication, and immune regulation. This diversity is primarily dictated by the enzymatic specificities of sialyltransferases ("writers")[1], which control the nature and distribution of sialylated glycans. Understanding how these enzymes shape the sialome is essential to deciphering their biological functions.

During my PhD, I investigated the substrate specificities of the fish polysialyltransferase *Cma* ST8Sia IV, revealing its unique ability to synthesize heterogeneous polysialic acid chains composed of Neu5Ac, Neu5Gc, and Kdn, unlike the strictly homopolymeric structures found in humans[2]. By combining glycoengineering and chemo-enzymatic synthesis of natural and bioorthogonal CMP-sialic acid donors, I developed tools to characterize enzyme activity and demonstrate the formation of diverse polysialic acid structures both *in vitro* and on cell surfaces[2]. Notably, *Cma* ST8Sia IV exhibits higher activity and broader donor substrate specificity than its human counterpart, efficiently polysialylating both *N*- and *O*-glycans to generate long polymers (DP >40) consisting of Neu5Ac and Neu5Gc residues[3]. Given the importance of polySias in multiple health and disease states, *Cma* ST8Sia IV represents a useful biocatalyst for glycoengineering and therapeutic applications.

Since July 2024, my postdoctoral research has investigated how aberrant sialylation is exploited in cancer. Tumor-associated hypersialylation can modulate immune recognition through Siglecs ("readers")[1], a family of fifteen sialic acid-binding lectin receptors expressed on immune cells. Using recombinant Siglecs and quantitative flow cytometry, I profiled ligand expression across more than 70 cancer cell lines, revealing distinct fingerprints of Siglec ligands across cancer types. I analyzed transcriptomic datasets and established correlations between Siglec ligand signatures and glyco-gene expression profiles. These predictions are being tested using overexpression and CRISPR-Cas9 knockout experiments. The objective is to predict Siglec ligands in cancer through an algorithm that can be validated on clinical samples, ultimately enabling the identification of patient-specific sialylation profiles and improving personalized immunotherapy.

Synthesis of Thioglycoside Glycomimetics as *Pseudomonas aeruginosa* Lectin Ligands

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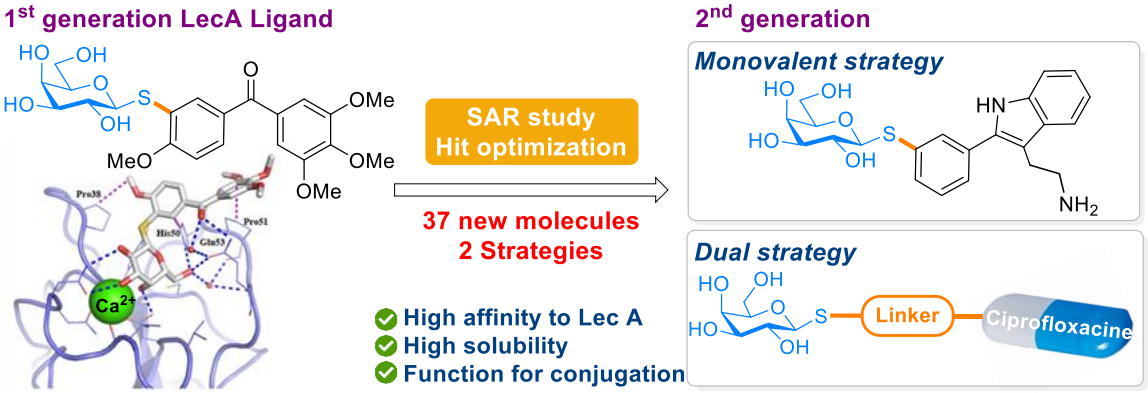
Severe respiratory infections caused by *Pseudomonas aeruginosa* are a major cause for concern, especially in immunodeficient patients.[1] The adherence of this pathogen to epithelial cells, mediated by lectins A (LecA) and B (LecB), promotes biofilm formation, leading to pulmonary clearance disruption and complicating antibiotic treatment.[2] Particularely, glycomimetic inhibitors that block lectin-glycan interactions can prevent adhesion and biofilm formation.

During this project, we pursued a medicinal chemistry program in the group targeting the LecA of *Pseudomonas aeruginosa*. A first generation of aryl thiogalactosides displaying unprecedented micromolar affinity toward LecA was reported in 2023 in our group,[3] although limitations in physicochemical properties remained. We focused on the design and synthesis of a second generation of 37 thiogalactoside derivatives, prepared using a cross-coupling strategy developed in our group (*Scheme 1*). Two complementary approaches were pursued: the optimization of monovalent ligands to improve solubility and binding affinity, and the development of dual thiogalactoside–ciprofloxacin conjugates combining lectin inhibition with antibiotic activity. These compounds were synthesized and evaluated for their binding affinity toward LecA, providing new insights for the development of new therapeutic strategies against *Pseudomonas aeruginosa*.



Scheme 1. *Development of a second generation of ligands targeting the LecA of Pseudomonas aeruginosa*

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Communications orales

Synthetic Lipid A Disaccharide Mimetics for Modulating Neuroinflammation

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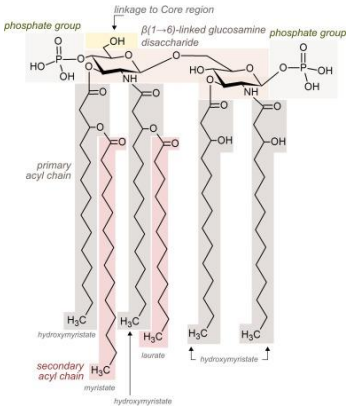
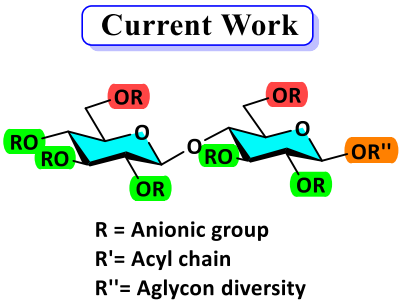
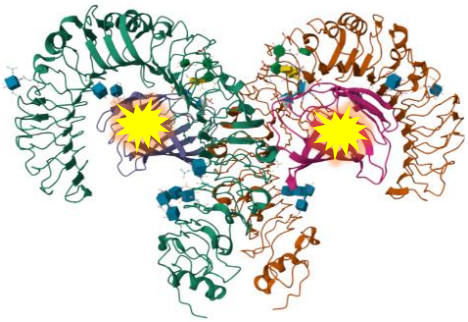
Neuroinflammation is a central pathological feature underlying the progression of major neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease and multiple sclerosis. The pattern-recognition receptor Toll-Like Receptor 4 (TLR4), a key mediator of innate immune signaling, has emerged as a critical regulator of both systemic and neuroinflammatory responses.[1] Activation of TLR4 within the central nervous system drives the amplification of pro-inflammatory signaling cascades, positioning this receptor as a compelling target for therapeutic intervention.

In our current research, we report the design and synthesis of a structurally defined class of anionic disaccharide-based modulators of TLR4 signaling.[2] These compounds are derived from the bacterial lipid A architecture and are built on a D-(+)-cellobiose scaffold, selected for its stereochemical and topological similarity to the native lipid A backbone. Strategic functionalization enables the incorporation of anionic substituents to mimic the phosphate pattern critical for TLR4/MD-2 recognition.[3] Biological assays, complemented by molecular modeling and docking studies, indicate that these mimetics engage the TLR4/MD-2 complex, suggesting a capacity to modulate receptor activation.







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Unraveling the role of the C-type lectin DCIR in immunity: From ligand discovery to therapeutic antibody development

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The dendritic cell immunoreceptor (DCIR/CLEC4A), a C-type lectin receptor expressed by myeloid cells (monocytes, macrophages, dendritic cells, granulocytes), is implicated in diverse diseases including chronic inflammation, autoimmunity, allergy, infection, and cancer. For instance, in 2017, we showed DCIR's role in regulating immune responses and bacterial clearance during tuberculosis (TB), a global leading cause of death.[1] Yet, its physiological ligand remained unknown, obscuring its precise immunoregulatory role and therapeutic potential.

We discovered LRP1, a ubiquitously expressed, highly glycosylated receptor, as a conserved endogenous ligand for human DCIR and murine DCIR1.[2] DCIR binds LRP1 via galactose-terminated biantennary *N*-glycans. Using X-ray crystallography, small-angle scattering and site-directed mutagenesis, we revealed DCIR's dimeric structure and atypical glycan-binding mechanism, identifying critical contact residues.

We also generated 16 synthetic single-domain antibodies (sdAbs) against human and murine DCIR using ribosome and phage display on recombinant lectins and live cells. Most sdAbs block DCIR-glycan interactions, indicating binding to the carbohydrate recognition site. Interestingly, one sdAb behaves as an allosteric modulator and broadens hDCIR's spectrum of carbohydrate recognition.

Currently, we are evaluating the agonist/antagonist properties of lead sdAbs, focusing on their effects on TB-associated lung inflammation *in vivo*. These sdAbs may not only highlight DCIR's role in immunity but also offer novel therapeutic strategies for TB and other diseases.

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New generation of glycoconjugate vaccines using pneumococcus as an infection model

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The pneumococcal conjugate vaccine (PCV) has an excellent safety profile and confers high protection against vaccine serotypes. However, inclusion of protein antigens from *Streptococcus pneumoniae* combined with potent adjuvants and suitable delivery system are expected to both extend protection to non-vaccine serotype strains and stimulate a broader immune response, thus more effective in young children, elderly, and immuno-compromised populations.

We will describe our own contributions based on cysteine mutagenesis and non-canonical amino acid incorporation approaches to provide homogeneous glycoconjugates and control the structure/immunology relationships (Figure A),[1,2] encapsulation in chitosan nanoparticles to improve the humoral response by a factor of 10 to 100 (Figure B),[3] use of flagellin to prepare self-adjuvantingvaccines (Figure C)[4]

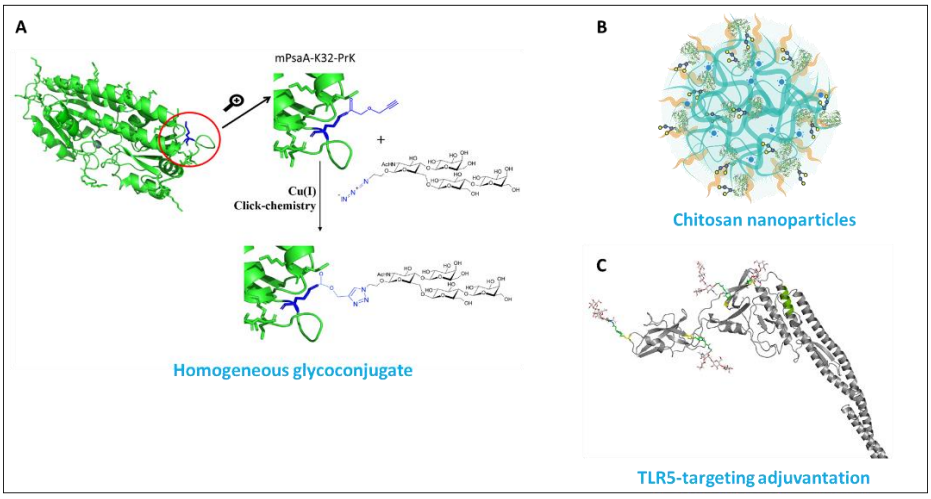

Figure. Different strategies towards next generation of pneumococcal glycoconjugates vaccines

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A new pathogenic variant in SLC35C1 impairs GDP-Fucose transport: Diagnostic and therapeutic implications

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Glycosylation is an essential cellular process in which monosaccharides are attached to proteins or lipids, shaping their structure and function. Congenital disorders of glycosylation (CDG) arise from defects in this complex biosynthetic machinery, resulting in highly heterogeneous clinical manifestations. Among them, fucosylation defects, are particularly rare. The most frequent form, SLC35C1-CDG, also known as Golgi GDP-fucose transporter deficiency (OMIM #266265), is an autosomal recessive condition typically characterized by dysmorphic features, short stature, and variable degrees of developmental and cognitive impairment. Here, we describe a new case of SLC35C1-CDG, confirmed through combined biochemical and genetic analyses along with studies on the patient's fibroblasts. These investigations identified a novel pathogenic mutation impairing GDP-fucose transport into the Golgi apparatus and led to a therapeutic proposal based on L-fucose supplementation, offering promising therapeutic strategy for this patient and for expanding our understanding of this pathway.

β-1,6-glucan, an unexplored and critical cell wall polymer of *Candida albicans*

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The cell wall of human fungal pathogens plays critical roles as an architectural scaffold and as a target and modulator of the host immune response. Although the cell wall of the pathogenic yeast *Candida albicans* is intensively studied, one of the major fibrillar components in its cell wall, β-1,6-glucan, has been largely neglected, its biosynthetic pathway remains totally unknow. Our data show that β-1,6-glucan is essential for bilayered cell wall organization, cell wall integrity and filamentous growth. The set-up of a cell wall analytic approach allowed a comparative investigation of cell wall composition and β-1,6-glucan structure between growth conditions and cell wall mutants. For the first time, we show that β-1,6-glucan production compensates the defect in mannan elongation in the outer layer of the cell wall. In addition, β-1,6-glucan dynamics are also coordinated by host environmental stimuli and stresses with wall remodeling, where the regulation of β-1,6-glucan structure and chain length is a crucial process. As we point out that β-1,6-glucan is exposed at the yeast surface and modulates immune response, β-1,6-glucan must be considered a key factor in host-pathogen interactions[1]

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Biochemical characterization of the *Escherichia coli* surfaceome: a focus on type I fimbriae and flagella

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The *Escherichia coli* surfaceome consists mainly of the large surface organelles expressed by the organism to navigate and interact with the surrounding environment. The current study focuses on type I fimbriae and flagella. These large polymeric surface organelles are composed of hundreds to thousands of subunits, with their large size often preventing them from being studied in their native form. Recent studies are accumulating which demonstrate the glycosylation of surface proteins or virulence factors in pathogens, including *E. coli*.

Using biochemical and glycobiological techniques, including biotin-hydrazide labeling of glycans and chemical and glycosidase treatments, we demonstrate (i) the presence of a well-defined and chemically resistant FimA oligomer in several strains of pathogenic and non-pathogenic *E. coli*, (ii) the major subunit of type I fimbriae, FimA, in pathogenic and laboratory strains is recognized by concanavalin A, (iii) standard methods to remove *N*-glycans (PNGase F) or a broad-specificity mannosidase fail to remove the glycan structure, despite the treatments resulting in altered migration in SDS-PAGE, (iv) PNGase F treatment results in a novel 32 kDa band recognized by anti-FliC antiserum.

While the exact identity of the glycan(s) and their site of attachment currently elude detection by conventional glycomics/glycoproteomics, the current findings highlight a potential additional layer of complexity of the surface (glyco) proteome of the commensal or adhesive and invasive *E. coli* strains studied.

Structural analysis of glycopeptides using cyclic ion mobility spectrometry and tandem MS techniques

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Introduction: The structural deciphering of protein glycosylation at the molecular level is a major challenge for their exploitation in the biomedical field. The characterization of glycoproteins is limited by the inherent complexity of glycans and their high degree of isomerism. Glycopeptide analysis by mass spectrometry (MS) can help identifying the localization of glycosylation sites and the glycan's monosaccharide composition. However, regio- and stereochemical information remains largely inaccessible. Hyphenation with ion mobility spectrometry (IMS) has recently demonstrated its potential for distinguishing glycosylation motifs.[1,2] In addition, recent advances in instrumentation improved its resolving power, for instance with cyclic IMS. Collision-cross sections (CCS), which can be extracted from IMS experiments, provide structural information on the ions and can be calculated theoretically.[3] Here, we aim to study the behavior of glycopeptide fragments using high resolution IMS and molecular modelling, ultimately tracing them back to the intact glycopeptide structures.

Methods: Glycopeptides are separated by hydrophilic interaction liquid chromatography (HILIC), ionized in positive mode and fragmented prior to IMS experiments using a SELECT SERIES Cyclic IMS instrument (Waters). Experimental CCSs are collected. In parallel, modelized glycopeptide fragments are generated through theoretical calculations and their CCSs are computed using optimized protocols.

Results and Impacts: Our experiments using collision-induced or electron-capture dissociations (CID, ECD) yield promising fragmentation data. First results show on simple structures that we are able to produce glycopeptide fragments and separate isomeric motifs. The modelisation of isomeric fragments nicely illustrate their propensity to display a compact or extended conformation, aligning with their separation in IMS experiments. This work will provide the basis for the construction of a CCS database, to provide detailed structural identification of protein glycosylation.

Novelty: High-resolution IMS is combined with CCS computation of diagnostic fragments. Until now, little use has been made of IMS on glycopeptide fragments and theoretical CCSs for analytical purposes.

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Synthesis and study of multivalent glycoclusters by dynamic combinatorial chemistry

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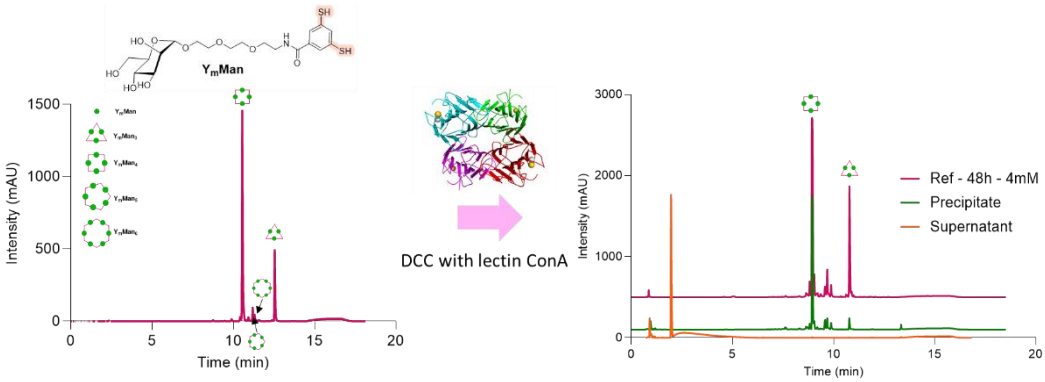
Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium that mainly affects immunocompromised patients and is involved in nosocomial infections such as lung infections critical in cystic fibrosis patients. The World Health Organization listed Pseudomonas aeruginosa as a high-priority bacterial pathogen due to its multiple resistance to antibiotics. In order to counteract this resistance, anti-biofilm and anti-adhesive approaches are being explored. The adhesion of Pseudomonas aeruginosa to host cells is mediated by two soluble lectins (LecA and LecB).[1] Several multivalent glycoclusters have been synthesized and have shown potential therapeutic applications as anti-infective agents.[2] To move away from a classicals methods for lectin ligand design, dynamic combinatorial chemistry (DCC) has been investigated for the generation of a library of potential multivalent glycoclusters through thermodynamically controlled reversible covalent bonds.[3] A first generation of multivalent glycoclusters, composed of a 1,4-dithiophenol aromatic core displaying glycosylated arms, synthesized using DCC, have shown promising results. The study demonstrated the predominant formation of macrocycles X_3 and X_4, linked by disulfide bridges. The glycoclusters were evaluated by ITC and showed an affinity in the nanomolar range.[4] With the aim of obtaining greater diversity, monovalent building blocks were synthesized using the same method.[5] Preliminary studies have shown the formation of larger macrocycles up to hexamers and amplification of tetramers by the lectin ConA.



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Multivalent Galabiosyl-Based Oligosaccharide Glycoclusters as Anti-Adhesive Inhibitors of *Pseudomonas aeruginosa*

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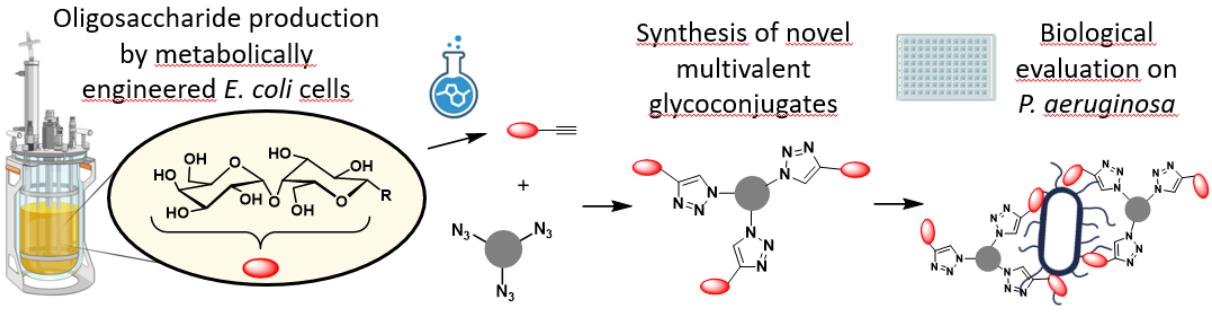
Pseudomonas aeruginosa is an opportunistic pathogen commonly found in soil, water, and plants. While generally harmless in healthy individuals, it can cause severe infections in immunocompromised patients, particularly in hospital settings where it is responsible for major nosocomial infections. Current treatments mainly rely on antibiotics; however, the intrinsic and acquired resistance[1] of *P. aeruginosa* significantly limits their efficacy. Consequently, *P. aeruginosa* is listed as a priority pathogen by the World Health Organization (WHO), highlighting the need for alternative therapeutic strategies. Among these, anti-adhesive strategies aim to prevent bacterial attachment to host cells by targeting bacterial lectins involved in colonization. LecA[2] is a galactose-binding tetrameric lectin involved in adhesion, infection and biofilm formation in *P. aeruginosa.* Most reported glycoconjugates targeting this lectin display terminal galactose monosaccharides, often in multivalent presentations, to mimic host glycans. However, LecA naturally recognizes a more complex oligosaccharide, the globotriose (Gb3)[3], present on human cell surfaces. The development of inhibitors displaying longer saccharide chains remains poorly explored due to their synthetic complexity. This limitation has been overcome by the development of the *E. coli* cell factory approach.[4] Using this strategy, we report the synthesis of galabiosyl-derived oligosaccharides and their chemical assembly into multivalent glycoclusters. These compounds were evaluated for their affinity toward LecA using a competitive binding assay and for their ability to inhibit *P. aeruginosa* biofilm formation.



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Screening of multivalent ligands for carbohydrate-binding proteins by microarray

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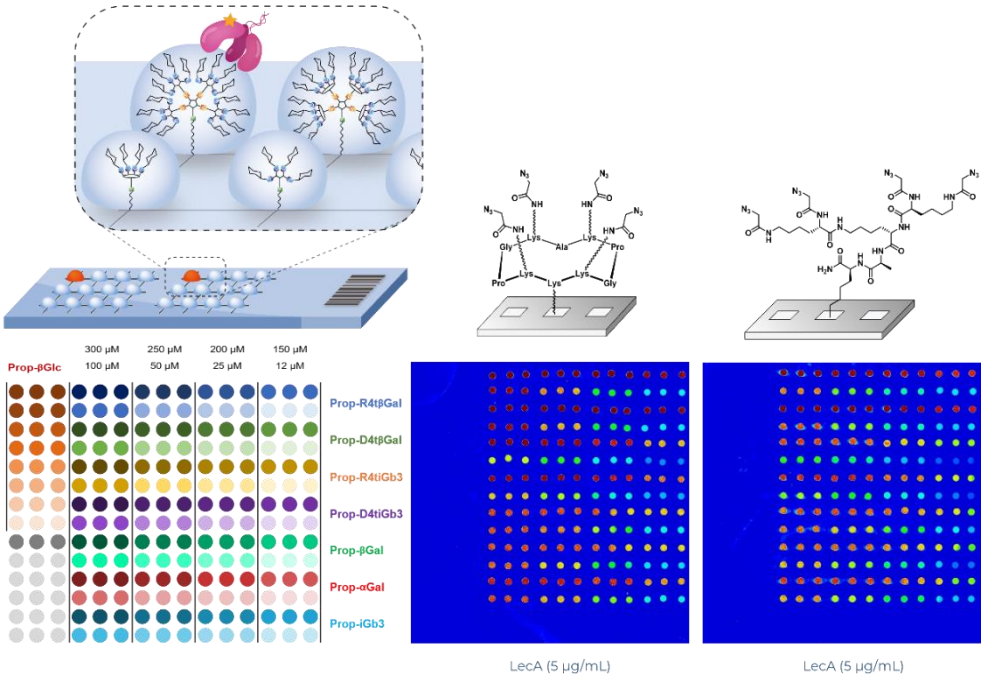
This project aims to develop a new tool for the design and evaluation of carbohydrate-based bioactive compounds. This tool, based on microarray technology, allows for a fast and easy evaluation of a large number of multivalent structures against a number of biologically relevant carbohydrate-binding proteins such as bacterial lectins, and human enzymes and antibodies. The overall objective being the identification of high affinity molecules against bacterial adhesion, carbohydrate-processing pathologies and cancer. The most innovative part of the project is to develop on-surface synthetic methodologies that will allow the preparation of supramolecular structures directly on the glass slide using iterative chemical ligations. This alternative method offers many advantages by avoiding numerous purification steps and decreasing the quantities of material needed as solution syntheses are limited to the preparation of functionalized synthons. The development of oxime ligations and CuAAC reactions on the surface gave access to a wide variety of potentially active molecules. Currently our efforts are focusing on developing a MALDI compatible surface in order to fully characterize the compounds synthesized on the slides and therefore assess the efficiency of this methodology.



Orthogonal photoswitching in a *bis*-azobenzene glycomacrocycle

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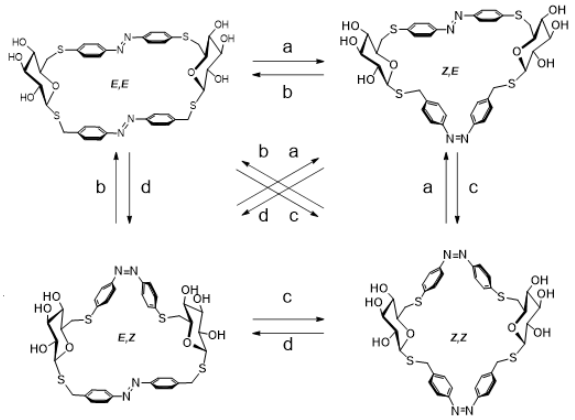
Azobenzene derivatives (AB) are well-known photochromic compounds that rely on reversible *trans* (stretch) / *cis* (bent) photoisomerization to operate geometry changes of large amplitude. When such molecules are embedded in a macrocyclic scaffold, the ring conformation is altered upon isomerization, which results in a unique modulation of the molecular properties (e.g. host-guest interactions, self-assembly, chiroptical propeties…).[1] The recently disclosed glycoazobenzene macrocycles (glycoAB) are of particular interest because they are prepared from biosourced carbohydrate building blocks, are polyfunctionnal and chiral.[1,2] In the last years, chemists have turned their attention to multiphotochromic compounds and orthogonal photoswitching. In general, multi-state switching is achieved with a single stimulus when using photochromic units of different nature,[3a] or on a same photoswitch but with different stimuli.[3b,c]

In this work, we studied a prototypical glycoAB macrocycle incorporating two distinct AB units and thus existing as mixtures of four isomers (EE, ZE, EZ and ZZ) under light exposure (Figure 1). The macrocycle was carefully studied in solution (DMSO) by UV/vis and NMR spectroscopy and we observed selective and orthogonal photoswitching at four distinct wavelengths (340, 365, 415 and 530 nm), with over 50% yield in each major isomer, with light as the only fuel. Circular dichroism measurements also revealed that each photoisomerization step leads to a distinct chiroptical pattern. Finally, we performed a comprehensive photokinetic study by combining spectroscopy experiments, simulation and data fitting. This afforded concentration profiles of the respective isomers over the course of light irradiation and the corresponding quantum yields of each photochemical process.



a = 340 nm; b = 530 nm; c = 365 nm; d = 415 nm

Figure 1 : Four state photoswitching of a glycoAB macrocycle

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Engineering the *N*-glycosylation pathway in *Chlamydomonas reinhardtii* to produce humanized glycoproteins

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Microalgae are considered as attractive expression systems for the production of biologics. Indeed, they are photosynthetic unicellular organisms that do not require costly and complex media for growing, and are able to secrete glycosylated proteins. Some biologics have already been successfully produced in the green microalga *Chlamydomonas reinhardtii* that is a model for studying cellular biological processes[1]. Structural analysis of glycans *N*-linked to *C. reinhardtii* proteins has revealed mainly oligomannoside structures, the main one being a non-canonical $Man_5GlcNAc_2$. In addition, mature *N*-glycans harbouring β(1,2)-xylose and α(1,3)-fucose residues are synthesized through a *N*-acetyl-glucosaminyltransferase (GnT)-independent pathway[2]. Thus, in the context of the bioproduction of recombinant therapeutics, engineering the *N*-glycosylation pathway in *C. reinhardtii* is necessary to obtain glycoproteins harbouring humanized *N*-glycans. We first performed a knock-out strategy by selecting *C. reinhardtii* mutants in key Golgi glycosyltransferases that are responsible for the transfer of β(1,2)-xylose and α(1,3)-fucose residues[3]. Therefore, we initiated knock-in strategies by expression in specific Golgi compartments of heterologous glyco-enzymes to get the chassis $GlcNAc_2Man_3GlcNAc_2$ since it is the glycan substrate for the expression of human glycosyltransferases involved in the transfer of decorations such as galactose. In this last purpose, the first step implies the accumulation of $Man_3GlcNAc_2$ [4] that constitutes a prerequisite, for further expression of heterologous GnT required for the transfer of terminal *N*-acetylglucosamine residues. This presentation will summarize these recent results, as well as the on-going work carried out to complete the humanization of *N*-glycans in this model microalga. The key future issues such as the targeting mechanisms of glycoenzymes into the secretory system will be discussed.

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Modulating selectivity in glycoside hydrolases

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The vast diversity of retaining GHs constitutes a pool of enzyme candidates with the potential to synthesize almost any glycosidic bond. However, most GHs primarily hydrolyze glycosidic bonds, with transglycosylation being a concomitant minority reaction. Fortunately, GHs are amenable to protein engineering strategies aimed at modifying their catalytic potential.[1] To further generalize these strategies, we are investigating the use of amino acid acceptors in a chemoenzymatic synthesis route designed to procure glycosylated amino acids. Our ambition fits into a wider strategic framework involving genetic code expansion and the synthesis of site-specific glycosylated proteins.

To achieve the first step in our strategy, we set out to identify suitable enzyme candidates for protein engineering. A selection pipeline involving an initial literature search was implemented, focusing on GH families 20 and 84. Following selection, eight recombinant GHs were expressed, purified and screened to reveal the ability to transfer Ser-*N*HBOC onto D-GlcNAc. This process yielded two candidates, one from each GH family. Afterwards, a semi-rational engineering approach was designed and implemented. This multi-pronged strategy comprises two complementary computational steps designed to pinpoint potential targets for site-specific mutagenesis. The first was achieved using the *in silico* tool, *BindScan*.[2] The second involved a sequence-based approach to detect conserved residues.[3] The results from both strategies were consolidated, along with available structural data, and a restricted list of targets and substitutions was established. Site-directed mutagenesis was performed and mutant enzymes are currently being screened for altered hydrolytic activity and transglycosylation potential.

This work is supported by a public grant managed by the National Research Agency under the 'Investissements d'Avenir' programme with the reference ANR-18-EURE-0021.

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Deciphering structure-affinity relationships of cathepsin K–glycosaminoglycans interactions in the context of osteoporosis

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Cathepsin K (Cat K) is a key protease involved in type I collagen degradation in bone tissue, and its excessive activity contributes to osteoporosis[1]. The enzymatic activity of Cat K is regulated by interactions with glycosaminoglycans (GAGs), sulfated polysaccharides that bind to the enzyme allosteric site. These interactions modulate enzyme stability and active-site accessibility, leading to either enhanced or inhibited collagenolytic activity[1], thereby making Cat K/GAG complexes attractive therapeutic targets. However, the structural features of chondroitin sulfate (CS) governing Cat K recognition and the underlying mechanism of allosteric regulation remain incompletely understood.

We have developed a label-free C-PAGE gel-based assay to investigate structure–activity relationships[2] in Cat K–GAG interactions. In parallel, mass photometry was used as an orthogonal single-molecule approach to directly probe Cat K/CS complex formation in solution. Together, these complementary techniques enable the screening of linear and multivalent CS architectures with defined sequences, revealing the influence of chain length, sulfation pattern, and multivalency on complex formation and selectivity across GAG libraries. This combined strategy allows rapid prioritization of biologically relevant GAG structures for further quantitative and structural characterization.

Overall, this study establishes a broadly applicable analytical strategy for probing GAG–protein interactions in glycosciences and for guiding the rational selection of novel Cat K modulators. Building on these screening results, future work will extend toward the determination of binding constants and complex stoichiometries using affinity capillary electrophoresis coupled to UV detection (ACE-UV) and mass spectrometry (ACE-MS).

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Innovative Fermentation Platform for Active Oligosaccharide Manufacturing

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Oligosaccharides are structurally diverse carbohydrates ubiquitously found across all forms of life, where they fulfill a wide range of biological functions. Harnessing this diversity offers significant opportunities for innovation but chemical synthesis and extraction for obtaining structurally defined oligosaccharides are often limited by low yields, high energy consumption, and sustainability concerns.

At Sweetech, we have developed a proprietary and innovative platform technology leveraging glycoside phosphorylases (GPs) to enable the production of a broad spectrum of pure oligosaccharides. GPs are reversible enzymes that catalyze glycosidic bond cleavage via phosphorolysis in the presence of inorganic phosphate (Pi), as well as glycosidic bond formation through reverse phosphorolysis using sugar-1-phosphate (S1P) as a glycosyl donor. [1] The industrial application of reverse phosphorolysis is, however, constrained by the high cost and limited availability of S1P, as well as unfavorable reaction equilibria. To overcome these limitations, GPs were implemented within specifically engineered microbial cell factories. *Escherichia coli* strains were designed to endogenously generate S1P from low-cost, renewable feedstocks, while promoting oligosaccharide synthesis through product secretion. [2] Combined with precision fermentation, it enables scalable production from gram to industrial quantities of highly pure oligosaccharides previously inaccessible at scale. Furthermore, our work on GPs provides the first demonstration that they can utilize S1P both as a glycosyl donor and as an acceptor, enabling the synthesis of a novel class of phosphorylated oligosaccharides not previously reported. [3]

Initial biological characterization of selected oligosaccharides produced using this platform reveals promising effects on key skin cell types, supporting their potential for advanced cosmetic formulations. Overall, this work highlights how a proprietary and scalable bioproduction platform can unlock access to unprecedented oligosaccharide structures with strong potential for healthcare applications.

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Development of a nanomolar inhibitor of NagZ, an enzyme involved in the antibioresistance of *P. aeruginosa*

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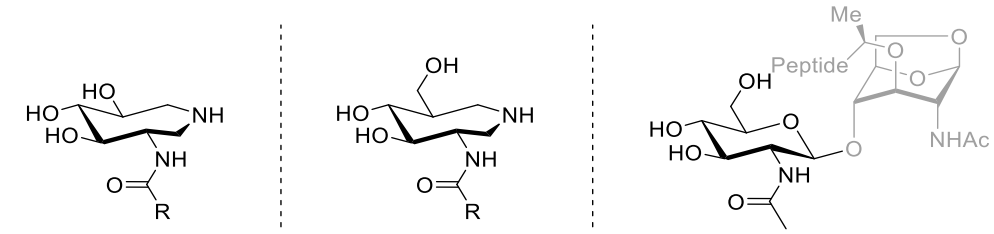
Antibiotic resistance has become a major global public health threat, particularly in Gram-negative bacteria such as *Pseudomonas aeruginosa*, whose survival depends on the continuous remodeling of their cell envelope, a primary target of β-lactam antibiotics.[1] However, the extensive use of these antibiotics promotes the emergence of resistance mechanisms, notably through the overproduction of the β-lactamase AmpC, which is capable of inactivating these drugs. This process is regulated by the glycosyl hydrolase NagZ, which generates a key inducer required for AmpC expression. Therefore, targeting NagZ represents a promising strategy to limit bacterial resistance and restore the efficacy of antibiotic treatments.[2]

We previously synthesized a series of NagZ inhibitors based on a polyhydroxylated azepane scaffold designed to mimic the enzymatic transition state.[3] We recently designed a second generation of NagZ inhibitors.[4] Among the new compounds, one molecule exhibits an IC_{50} value < 10 nM. We will present herein their design, synthesis and biological evaluation.



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Synthesis of *exo*-iminoglycals by gold-catalyzed hydroamination

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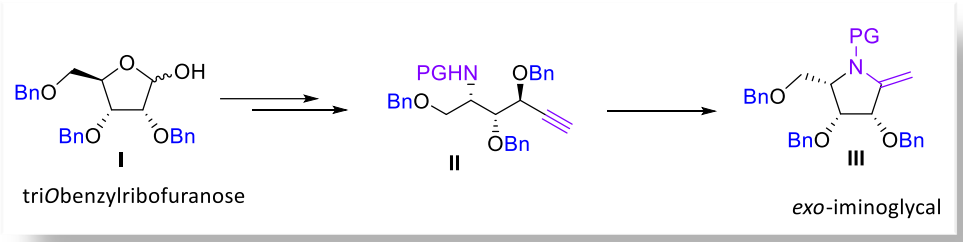
Glycals are unsaturated sugars that possess a carbon-carbon double bond at the anomeric position. The synthesis of both *endo*- and *exo*-glycals has been widely studied due to the high synthetic potential of these structures, notably as precursors of glycomimetics such as *C*-glycosides. [1] By analogy, iminoglycals are compounds in which the endocyclic oxygen atom has been replaced by a nitrogen. Due to the limited number of preparative methods available to date, iminoglycals are less described, and most of the reported structures are *endo*-iminoglycals.[2] In this context, our aim is to prepare innovative *exo*-iminoglycals[3] **III** as potential glycoside hydrolases inhibitors. Indeed, those compounds combine several key structural features for mimicking the enzymatic transition state, such as sp^2-character at the anomeric center and replacement of the endocyclic oxygen with a nitrogen. Our synthetic strategy relies on a novel gold-catalyzed hydroamination[4] from carbohydrate-based precursors **II**.



We will present here our recent results about a) the development of efficient synthetic route towards precursors **II** starting from protected sugars **I** and b) the outcome of the hydroamination reaction towards the synthesis of *exo*-iminoglycals **III**.

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Azalevoglucosan, a usefull scaffold to reach iminosugars with high structural diversity

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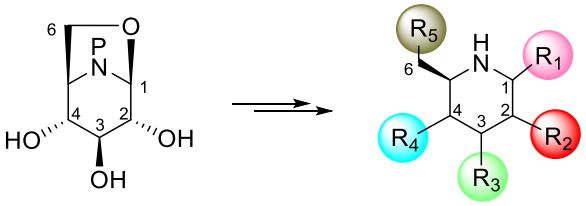
Iminosugars, notably six-membered polyhydroxylated piperidines, are one of the most promising classes of carbohydrate analogs for therapeutic purposes.[1] While structural diversity has been extensively introduced at N, C1 and C6 positions to identify and validate new biological leads,[2,3] C2, C3 and C4 positions have been poorly scrutinized according to their more difficult chemical access.

In this context, we have developed a robust synthesis of an azalevoglucosan scaffold,[4,5] a bicyclic 1,6-anhydro iminosugar in which the trans diaxial arrangement of the secondary hydroxyl groups allows unprecedented introduction of structural diversity in a regio- and stereoselective manner at C2, C3 and C4 positions. Further bicycle ring opening allows additional decoration of the C1 and C6 positions to generate highly substituted piperidine iminosugars.



Azalevoglucosan scaffold — Polyfunctionalized iminosugars

The synthesis of the key azalevoglucosan scaffold, its decoration at C2, C3 and C4 positions and its ring opening to access polyfunctionalized iminosugars will be presented.[6]

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Structural Investigations of CAZymes for the Synthesis of Biopolymers

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Polymers are ubiquitous in modern life, with applications ranging from everyday consumer products to advanced technologies. The urgent need for sustainable and renewable alternatives to petroleum-based polymers is driving research on polysaccharides for applications in the food, health, and environmental sectors. Carbohydrate-active enzymes (CAZymes), capable of polymerization, glycosylation, phosphorylation and related modifications, offer powerful biosynthetic routes to tailored polysaccharides. However, engineering these enzymes requires a detailed understanding of their catalytic mechanisms, substrate interactions and reaction dynamics—features that are often obscured by their complex, multidomain, and highly dynamic architectures.

At TBI's Biocatalysis team, we are developing an integrative approach to characterize these enzymes using a suite of structural biology techniques, including cryo-electron microscopy, X-ray crystallography, small-angle X-ray scattering, and nuclear magnetic resonance, complemented by biochemical assays and molecular modeling. We will present recent insights into key enzyme families such as glucansucrases [1], glycoside phosphorylases [2,3], and glucan kinases, demonstrating how these complementary methods elucidate enzyme conformational and reaction dynamics during catalysis. This integrated approach paves the way for rational enzyme engineering toward the sustainable synthesis of biopolymers and carbohydrate-based compounds.

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Effect of a single water molecule on the structure of a Mannose photosensitizer model and Mannose-Glutamic Acid molecular complex in the gas phase

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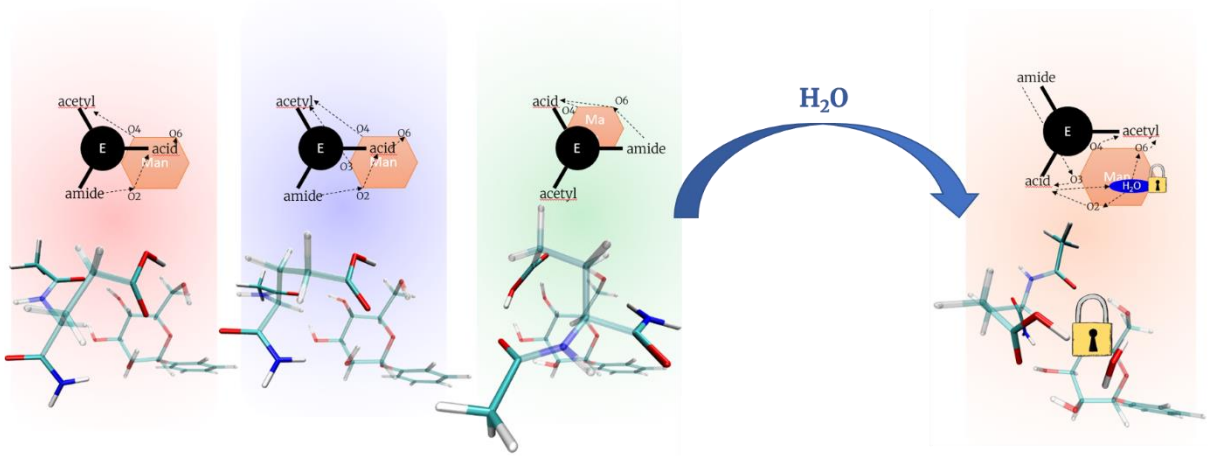
The interactions between sugars and peptides are ubiquitous in biology. The structural preferences of these molecular complexes are often linked to biological responses. A variety of non-covalent interactions is involved in the stabilization of these kind of structures, ranging from weak London dispersion forces, to stronger dipolar interactions, such as $\sigma\pi$ interactions or hydrogen bonds. Vibrational spectroscopy is a powerful tool to study these interactions, as it is very sensitive to the local environment of the molecules.

To avoid the interference of the solvent bulk water molecules and disentangle the contributions of the molecules from their environment, we first isolate our systems in the gas phase and progressively reintroduce water molecules one by one in a bottom-up approach. The systems are cooled down, probed with laser IR-UV action spectroscopy techniques and mass-detected with a Time of Flight (TOF). This gives us structure-specific IR spectra, that can be assigned to the most stable structures at low temperature, with some density functional theory (DFT) calculations.

We studied a photosensitizer model with mannose, designed to fight against retinoblastoma cancer. Then, we looked at the interactions between a phenyl-mannose and a glutamic acid. The acidic part of the glutamic acid in interaction with the sugar has shown interesting spectroscopic signatures, with a large acidic band and Fermi resonances. The influence of water on these structures is a complete reorganization of the structures, and a locking of one new most favorable structure.



How can we probe the dynamic mechanisms involved in the multienzymatic deconstruction of plant biomass?

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To address the intricate complexity of plant cell walls (PCWs), lignocellulolytic microorganisms that utilize polysaccharides as a carbon source produce a wide array of glycoside hydrolases (GHs), often organized either as free multimodular enzymes or as multienzymatic complexes. In both strategies, the spatial arrangement and relative distance between enzymes play a central role in the dynamic deconstruction of complex, insoluble plant polysaccharides.

To decipher the synergistic effects of enzyme spatial proximity, we propose three complementary approaches. First, engineered enzymes are immobilized on functionalized elastomeric surfaces that allow control over their orientation. A custom-built device is used to stretch these surfaces, thereby modulating the distance between immobilized enzymes. Experiments using natural polysaccharides reveal activity variations depending on the degree of stretching, providing insights into the role of spatial proximity in enzyme dynamics[1].

A second approach involves locking both spatial proximity and topology between GHs using a pair of small proteins that spontaneously form intramolecular isopeptide bonds. This strategy offers a unique means to geometrically constrain the spatial organization between multiple GHs. The most promising complexes are further characterized by small-angle X-ray scattering (SAXS), while their product profiles are analyzed by mass spectrometry[2].

The third strategy aims to visualize differences in GH targeting within wheat straw *in situ*. This is achieved through immunolabeling[3] and X-ray microtomography[4], enabling the observation of plant cell wall modifications at different stages of enzymatic deconstruction

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The glycosyltransferase β4GalT7 as a potential target for substrate reduction therapy in mucopolysaccharidoses

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Mucopolysaccharidoses (MPS) are a group of inherited lysosomal storage diseases caused by the deficiency of enzymes involved in the degradation of **glycosaminoglycans (GAG)**. Their abnormal accumulation in tissues leads to cellular damages and progressive multiple organ dysfunction. Enzyme Replacement Therapy, consisting in injection of functional recombinant enzyme, has been developed for several types of MPS, but shows limitations that are mainly due to the low tissue distribution of the enzyme. **Substrate Reduction Therapy (SRT)**, using small molecules able to cross the blood brain barrier and inhibit GAG biosynthesis to reduce their accumulation in tissues, represents a suitable alternative in MPS treatment.

In this context, we propose **a new approach of SRT** by developing small hydrophobic molecules to specifically inhibit the β1,4-galactosyltransferase 7 (β4GalT7), a key enzyme involved in GAG biosynthesis initiation. This would aim to prevent GAG accumulation (limiting their synthesis) and reduce the symptoms of most MPS, including neurological effects. Using an *in vitro* high throughput screening of the Prestwick Chemical Library, we have identified a set of **β4GalT7 inhibitory candidates**. The selected hits have been further tested *in cellulo* on dermal fibroblasts for their ability to inhibit GAG synthesis. Two of these hit candidates showed significant, inhibitory effect on the glycosylation of decorin, a model proteoglycan in our cell assays. These compounds will be the starting point to search for potential inhibitory chemical families of analogues and hit optimization. The study of physicochemical and pharmacokinetic properties of β4GalT7 inhibitors will initiate ADME-Toxicity tests on the best identified molecules to provide non-toxic drug candidates.

We hope that this project will provide original molecules that could help treating both the somatic and neurologic symptoms of MPS, by reducing GAG biosynthesis and limiting GAG accumulation. This will open avenues to new therapeutic strategies for MPS by positioning glycosyltransferases (like β4GalT7) as potential therapeutic targets. These pharmacological compounds could be used alone or in combination with others therapeutics to treat severe forms of MPS and contribute to the improvement of the quality of life of MPS patients.

Project funded by the French Foundation of Rare Diseases

Exploring the substrate specificity of tandem repeat SusCD transporters (and beyond?)

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Members of the *Bacteroidota* phylum have colonized all types of habitats and substrate niches and are considered primary degraders of polysaccharides. A unique feature of their genomes is the presence of **Polysaccharide Utilization Loci** (PUL) which are clusters of co-regulated genes encoding a complement of cell Surface Glycan-Binding Proteins (SGBPs, including the **SusD-like**), TonB-dependent transporters (**SusC-like**), Carbohydrate Active Enzymes (CAZymes) and carbohydrate sensors/ transcriptional regulators.

The marine flavobacterium ***Zobellia galactanivorans* Dsij^T** is a model organism for the bioconversion of algal polysaccharides[1], for which genomic and transcriptomic data are available, as well as genetic tools to delete and complement genes. We have identified a PUL (locus tag *Zg*208-*Zg*215) containing a **tandem repeat SusCD (trSusCD)**, whose characterization will contribute to deepen the understanding of the SusCD transport system. To date, it is commonly accepted that each PUL targets a single substrate. Yet, PUL encoding tandem repeat SusCDs question this definition as they might target different substrates. While the *Zg*208-*Zg*215 PUL contains only one enzyme, a GH5 characterized as an endo-β-glucanase most active on barley mixed-linked glucan (MLG)[2], it is upregulated in presence of laminarin, MLG and xylan[3]. Compared to the wild-type strain, a *trSusCD* deletion mutant features a longer lag phase when grown with MLG or xylan, thus showing that these transporters are involved in the uptake of the corresponding oligosaccharides. Additionally, *Zg*213_SusD was previously shown to bind to xyloglucan although *Z. galactanivorans* is not able to grow on this substrate. We showed for the first time that the bacterium can grow on xyloglucan oligosaccharides (XGO), but forms aggregates. Strikingly, the *trSusCD* deletion mutant was also able to grow on this substrate, but with only minor aggregation. This indicates that these SusCD are not essential for XGO uptake but might have a role in signal transduction not directly linked to carbohydrate metabolism.

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M. tuberculosis MtPMT Mechanistic Insights Reveal a WW-Like Domain and Anti-Virulence Inhibitors

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Protein *O*-mannosylation (POM) is crucial for the virulence of *Mycobacterium tuberculosis* (Mtb)[1] and is mediated by the membrane enzyme MtPMT. It catalyzes the transfer of a single mannose residue from a polyprenol phospho-mannosyl donor to the hydroxyl groups of selected Ser/Thr residues in acceptor proteins during their translocation across the membrane. The molecular basis of its activity and substrate specificity, however, remained unknown.

We developed a robust *in vivo* phenotypic assay using a model mannosylated protein substrate whose glycosylation is quantified by Enzyme-Linked Lectin Assay (ELLA), validated by LC-MS. This approach enabled direct measurement of MtPMT activity in living mycobacteria, providing a powerful tool for functional characterization and inhibitor high-throughput screening[2]. MtPMT belongs to the CAZy GT39 family of GT-C glycosyltransferases, but lacks the eukaryotic MIR domain. Importantly, mechanistic analyses confirmed the catalytic mechanism and led us to identify a functional WW-like domain, unprecedented in prokaryotes, which recognizes proline-rich sequences signing *O*-mannosylation sites. This finding defines a novel mechanism of substrate recognition specificity and suggests a possible evolutionary relationship with eukaryotic WW modules[3].

Based on these insights, we identified pyrrole-based inhibitors of MtPMT activity *in vivo*, without observable impact on bacterial growth or protein secretion[2].

Together, this work combines innovative *in vivo* screening, structural insights, and targeted inhibition, establishing MtPMT as a promising antivirulence target. These findings open new therapeutic avenues against tuberculosis and demonstrate a strategy to exploit bacterial glycosylation for drug discovery.

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Communications par affiche

Synthesis and biological evaluation of iminosugar-based analogues of UDP-GlcNAc targeting OGT

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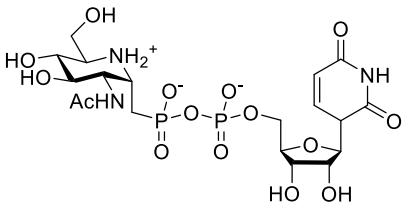
O-GlcNAc transferase (OGT) is an essential mammalian enzyme that regulates numerous cellular processes through the attachment of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) residues to nuclear and cytoplasmic proteins.[1] Inhibitors of OGT are needed as research tools and for evaluating the potential of OGT as a therapeutic target.
Capitalizing on our recent synthesis of 1-*C*-phophonomethyl iminosugars[2] and our expertise in iminosugar *C*-glycosides synthesis, we present herein a series of iminosugar-based analogues of UDP-GlcNAc. Iminosugars are known to mimic the glycosyl oxocarbenium character of putative glycosyltransferase transition state but this property has been scarcely explored for the development of OGT inhibitors to the best of our knowledge. The synthesis of glycomimics and their biological evaluation as OGT inhibitors will be presented.



iminosugar-based analogue of UDP-GlcNAc

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STRUCTURAL AND FUNCTIONAL STUDY OF SULFATASES TARGETING COMPLEX POLYSACCHARIDES

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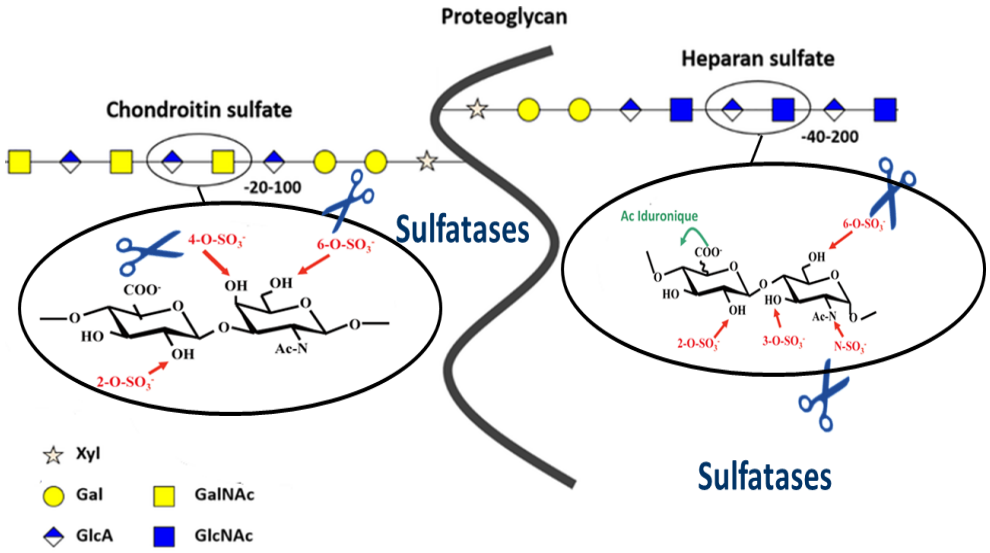
Sulfatases are key enzymes in sulfate metabolism, capable of removing sulfate groups from sulfate esters and sulfamates, and of modifying specific sulfation patterns of glycosaminoglycans (GAGs). GAGs are complex polysaccharides found on the cell surface and within the extracellular matrix, where they play a central role in numerous biological and pathophysiological processes. They interact with a wide range of proteins (such as growth factors and cytokines), thereby modulating their activity and contributing to mechanisms such as cell signaling, regulation of inflammation, neuronal development, and extracellular matrix organization. Protein–GAG interactions rely on precise saccharide motifs and specific sulfation patterns. By selectively removing certain sulfate groups, sulfatases can alter or finely modulate GAG function.

The main objective of this project is to identify and characterize new sulfatases that specifically target GAGs. These enzymes will be used to develop molecular tools to study the structure–function relationship of GAGs and to better understand how their sulfation patterns determine their interactions with proteins. In addition, we are currently investigating the structural differences between endosulfatases and exosulfatases, with the aim of engineering exosulfatases into endosulfatases, thereby expanding the toolbox available for GAG analysis.



Seven-Membered Iminosugars as Tools to Study Conformational Pathways of Glycosidases

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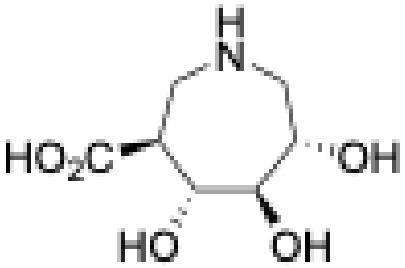
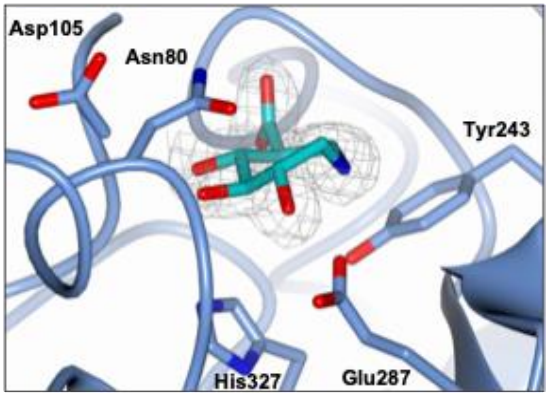
Glycoside hydrolases (GHs) are responsible for the degradation or hydrolysis of glycosidic bonds in carbohydrates.[1] While their catalytic mechanisms are usually similar, operating by means of acid/base catalysis with retention or inversion of the anomeric configuration, there has been increasing evidence that several GHs are adapted to recognize energetically higher conformations of carbohydrate units before hydrolysis.[2] In particular, for pyranoses, the saccharide unit binding at subsite -1 adopts a distorted (boat or skew) conformation instead of the relaxed $^4C_1$ chair conformation. This has been observed in several complexes of retaining and inverting GHs, as well as carbohydrate-bound biological receptors.[3] To study the conformational itinerary of these enzymes further, we have developed conformationally flexible seven-membered iminosugars that not only inhibited the target enzymes but also provided insighful information.[4] Herein, we will present our last results (synthesis, inhibition, X-ray structure) concerning glycuronidases, a subclass of glycosidases with high therapeutic potential.[5]





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Chemical tools to characterize and identify the lysosomal oligosaccharide transporter (LOST)

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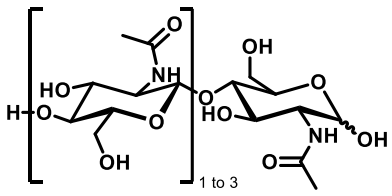
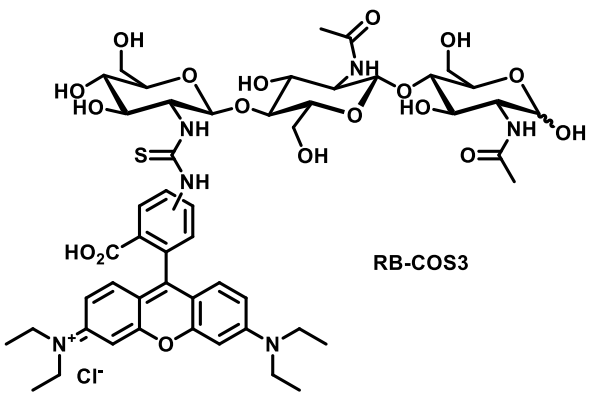
Small polymannose-type oligosaccharides (fOS) that are generated during protein *N*-glycosylation are transported from the cytosol into lysosomes to be degraded[1]. Presently, lysosomal oligosaccharide transport (LOST) remains to be identified at the molecular level. In terms of its activity, *in vitro* studies using rat liver lysosomes revealed that radioactive [^{3}H]Man$_5$-GlcNAc ([^{3}H]M$_5$) import is blocked by GlcNAc but not by mannose. Moreover, [^{3}H]M$_5$ transport is blocked efficiently by chitooligosaccharides containing 2 to 4 residues of β1-4 linked GlcNAc (COS2-4) with intact reducing end[2]. These data indicate that LOST may have a wider tolerance for the substrate than initially imagined and suggest that it could potentially have diverse transport functions. However, characterizing LOST faces important obstacles as the protein, or protein complex, involved has not been identified. To have a better understanding of this process, we are developing fluorescent[3] or photoactivable chemical tools to characterize and identify LOST proteins.



COS(2-4)



RB-COS3

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Towards the development of chondroitin sulfate biosynthesis inhibitors

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Chondroitin sulfate (CS) chains are long and linear polysaccharides, localized at the cell surface and in the extracellular matrix. These chains are involved in a wide range of cellular mechanisms such as cellular signaling, morphogenesis, extracellular matrix remodeling and neuronal plasticity. They are covalently attached to a core protein through a serine residue. CS chain elongation consists of the repetitive addition of glucuronic acid and N-acetylgalactosamine, and is catalyzed by four heterodimeric enzyme complexes. Each of these complexes is composed of one chondroitin sulfate synthase protein (CHSY1 or CHSY3), containing glycosyltransferase activity, and one chondroitin polymerizing factor (CHPF or CHPF2), presumably playing a structural role. A recent structure of the CHSY3-CHPF complex revealed the arrangement of the catalytic sites suggesting a non-processive distributive mechanism for CS polymerization[1]. Importantly, CS polymerase proteins have been shown to be highly expressed in different cancer types, for example CHSY1 in colorectal and gastric cancers[2,3]. My aim is to develop inhibitors of the CS polymerase complex using different strategies: produce AI-predicted protein binders, screen a library of small chemical molecules and generate nanobodies against the complex. The potency of the potential inhibitors will be evaluated using in vitro activity assays. An ultimate goal will be to determine cryo-electron microscopy structures of inhibitor bound CS polymerase complexes. The identified inhibitors may lead to new therapeutic strategies for several cancer types, atherosclerosis and chronic inflammatory diseases of the skin[4].

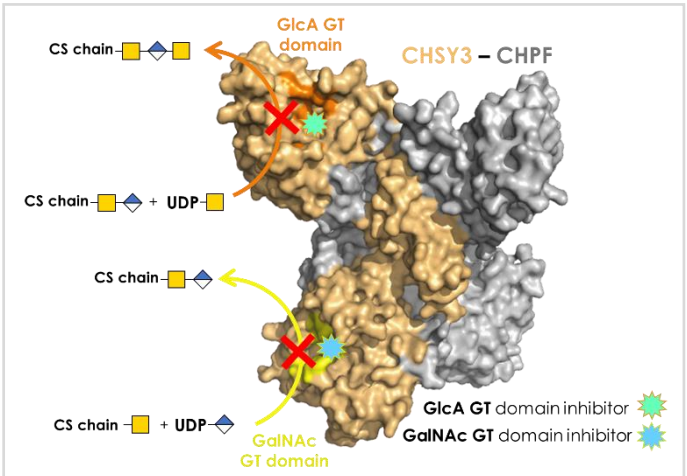

Fig 1 : Schematic representation of potential inhibition sites for CS chain elongation.

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MANNOSE-SPECIFIC BORONOLECTINS: DESIGN AND SYNTHESIS OF BIS-BORONIC ACIDS

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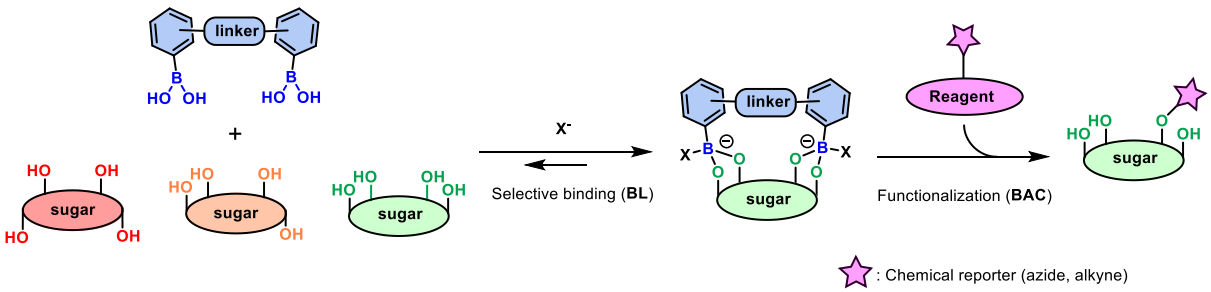
Glycoproteins practically cover all eukaryotic cells and are responsible for numerous cellular communication and recognition events. Glycobiology underwent significant development at the end of the last century but remains hampered by the high structural complexity of glycans.[1] The need for new tools to study the structure and function of glycoproteins is therefore crucial, given that certain diseases result from glycosylation defects or are marked by specific surface oligosaccharides.[2] The recent development of Metabolic Oligosaccharide Engineering (MOE) by Carolyn Bertozzi and coworkers represents a significant breakthrough allowing the study of native glycans in living cells.[33] Our goal is to develop an alternative chemical-based bioconjugation method. To specifically functionalize saccharides of interest, we aim to use boronolectins (BL) for their selectivity coupled with Boronic Acid Catalytic methods (BAC) to ensure reactivity.[4]



In this communication, we will disclose our latest results regarding the design, the synthesis and the reactivity of mannose-specific boronolectins.

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Design of radiolabeled glycoside derivatives for hepatic fibrosis imaging

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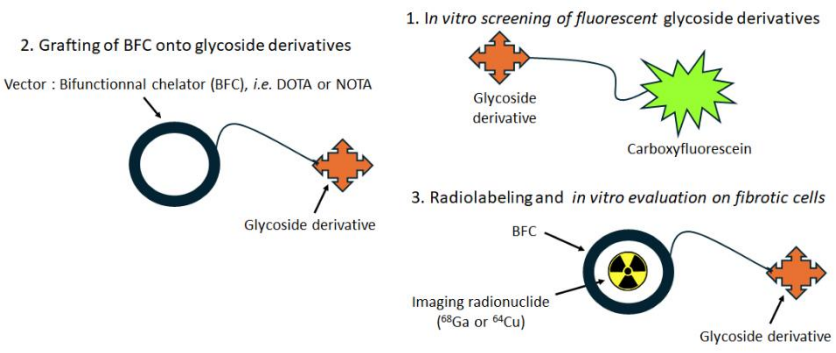
Hepatic fibrosis is a wound-healing response to chronic liver injury that can progress to cirrhosis and contribute to the development of cancer[1]. Chronic liver diseases are often diagnosed late, at the stage of hepatic complications, with poor short-term survival. Early-stage fibrosis, however, is reversible but difficult to diagnose accurately using conventional imaging tools. Liver biopsy is considered the gold standard for assessing hepatic fibrosis, but it has many drawbacks, while serum markers lack precision. In contrast, molecular imaging offers a promising non-invasive approach to visualize the onset and early changes of fibrosis *in vivo*, thereby facilitating (i) early diagnosis, (ii) staging of hepatic fibrosis, and (iii) monitoring of treatment response. Promising biological targets for such a strategy include receptors on hepatic stellate cells, macrophages, and extracellular matrix components such as collagens[2].

The objectives of this work are therefore to develop glycoside derivatives targeting activated stellate cells through such receptors, enabling imaging of fibrosis and the processes leading to it. Fluorescent derivatives from the COrInt team's library will be first synthesized in order to evaluate putative interaction with different hepatic cell lines. The most promising glycoside derivatives will subsequently be modified to be radiolabeled with gallium-68 and copper-64, positron-emitting radionuclides of interest for PET imaging. The radiotracers thus prepared will be evaluated *in vitro* (stability, cellular uptake).



The first results regarding the synthesis and characterization of glycoside derivatives will be presented. In addition, the synthesis of two platforms onto which both the glycoside derivatives and the bifunctional chelators (enabling radiolabeling with gallium-68 and copper-64) will be conjugated will also be described.

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Non-canonical amino acids: a novel source of chemical diversity to create novel protein properties

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Conventional protein engineering involving the substitution of one or more amino acids is powerful, but limited by the genetic code, which provides 20 amino acids. One option to expand chemical diversity is site-specific incorporation of non-canonical amino acids.[1] The use of the existing genetic code makes this strategy attractive and amenable for protein engineering aimed at the creation of novel molecular assemblies and new catalytic capabilities.

In this work we discuss prospects for the creation of proteins bearing designer metal ion-binding sites, or targeted sites for further post-translational glycosylation and labelling.[2]

These works were performed in the framework of the Carnot 3BCAR project NeoZYM, PRC ANR project LABEL (ANR-22-CE43-0012-01), INRAE project GLYCOzYME, and the European Union's Horizon 2020 research and innovation program (PEARL, Programme for EArly-stage Researchers in Lille).

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DIONYSUS: A guide to using the protein–carbohydrate interfaces structural database

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Obtaining structural data related to sugar-binding sites on the surface of proteins is challenging due to the low affinities between these macromolecules. Additionally, the wide variety of sugars complicates the identification of the endogenous substrates of a sugar-interacting protein. One way to address these issues is to develop data-driven approaches to predict these interactions in the absence of data

To address these challenges, we developed DIONYSUS: a structural database of protein–carbohydrate interfaces[1]. In addition to extensively cataloging and annotating all existing interaction sites found in the Protein Data Bank, DIONYSUS also classifies them[2]. This classification is a valuable tool for identifying which sugars can bind to a site and determining that site associated function. We have developed a comparison method allowing users to overlay their active site of interest onto the sites in the database, which helps annotate and understand its function.

In this poster presentation, we will demonstrate how to use DIONYSUS for different scientific applications. We can show you how to use DIONYSUS advanced search features, explore its classification system, suggest binding site annotations for a target of interest, and analyze the glycosylation patterns found in the PDB for a protein with a given Uniprot ID.

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Reevaluation of mycobacterial Phosphatidyl-*myo*-Inositol Mannosides (PIMs) as Toll-Like Receptor 2 (TLR2) agonists

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The innate immune system detects pathogens through pattern recognition receptors (PRR), such as Toll-like receptor 2 (TLR2), which recognizes lipoproteins, lipopeptides, but also lipoglycans from *Mycobacterium tuberculosis* (Mtb), including phosphatidyl-*myo*-inositol mannosides (PIM), lipomannan (LM) and lipoarabinomannan (LAM). Lipopeptides, featuring N-terminal cysteine acylation, activate TLR2 through heterodimers with TLR1 (triacylated forms) or TLR6 (diacylated forms). Structural studies confirm these interactions, linking lipid structure to immune signaling. We previously demonstrated that mycobacterial lipoglycans, taken as a whole, are *bona fide* pathogen-associated molecular pattern (PAMP) contributing to innate immune detection of mycobacteria via TLR2. However, unlike LM, PIM and LAM are weak TLR2 agonists as compared to bacterial lipoproteins (EC_{50} in the µg/ml vs ng/ml range). Therefore, a contamination of purified PIM and LAM samples by trace amounts of highly active lipopeptides is formally difficult to rule out.

We wished to reevaluate the TLR2 agonist activity of PIM, and more generally mycobacterial lipoglycans. We placed a particular focus on phosphatidyl-*myo*-inositol di-mannosides (PIM_2) because they harbor the simplest structure of this family of molecules accumulating in the cell envelope and because we previously discovered that an unconventional di-acylated form, not naturally found in the Mtb envelope, is generated during the lysosomal processing of PIM_2 before their presentation by CD1b proteins to T cells. This suggests that the repertoire of PIM_2 encountered by immune cells extends beyond the acyl-forms produced by bacteria, raising the question of whether the PIM acyl-forms generated in the antigen-presenting cells during infection act as TLR2 agonists.

In this study, a large set of molecules were tested for their ability to trigger TLR2 activation in HEK-TLR2 cells, including: i) purified natural PIM_2 acyl-forms from Mtb, ii) unconventional PIM_2 acyl-forms enzymatically-generated from Mtb tetra-acylated PIM_2, iii) a synthetic analog of tetra-acylated PIM_2, and iv) PIM_6, LM and LAM purified from various mycobacterial species. Surprisingly, the synthetic acyl-form was found to be inactive. We thus submitted PIM samples to an H_2O_2 treatment, which abolishes lipopeptide activity while preserving the structural integrity of PIM. Activities of all the PIM_2 acyl-forms were totally abolished. This prompted us to apply the same approach to PIM_6, LM, and LAM. Using compounds purified from various mycobacterial species or strains, we observed that LM activity was barely affected H_2O_2 treatment. In contrast, PIM_6 activity was entirely abolished, whereas LAM retained only residual activity. Altogether, we conclude that lipoglycan-mediated recognition of mycobacteria by TLR2 mostly relies on LM.

Understanding the regulation and quality control of protein *N*-glycosylation in *Chlamydomonas reinhardtii*

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The protein *N*-glycosylation pathway is one of the most important co- and post-translational modification of proteins in eukaryotes[1]. Glycoproteins are first produced in the endoplasmic reticulum (ER) where molecular actors are responsible for the proper folding and 3D conformation of proteins before their transfer into the Golgi apparatus[2]. The growing interest of using *C. reinhardtii* as an emerging cell biofactory for the industrial production of recombinant glycoproteins requires overall an in-depth understanding and analysis of protein *N*-glycosylation in this organism. Despite a relatively well-investigated *N*-glycosylation pathway in *C. reinhardtii*[3], many glyco-enzymes and lectins involved in the *N*-glycosylation pathway in the ER remain uncharacterized. This study aims to elucidate the regulatory mechanisms of *N*-glycosylation in the ER of *C. reinhardtii*, with a particular focus on protein quality control (QC). To do so, we are combining functional genetics of key molecular actors (e.g., enzymes, lectins) and multi-omics approaches (transcriptomics, proteomics, glycomics) to unravel the regulation of protein *N*-glycosylation in microalgae. Specifically, we will: (i) define the functional roles of ER molecular actors, such as ER-lectins calnexin and calreticulin, which are not yet functionally characterized in microalgae, using mutant approach strategy (ii) understand the interplay/regulation between the truncated glycan precursor ($Glc_3Man_5GlcNAc_2$), its monoglucosylated intermediates, and ER-resident lectins, which orchestrate protein folding and QC. This will be achieved through integrated approaches allowing identifying and mapping regulatory cellular pathways including the *N*-glycosylation. These results will provide insights 1- into the physiological role of protein *N*-glycosylation in microalgae and 2- into the molecular processes ensuring accurate protein folding, a critical step toward optimizing microalgae as efficient platforms for biopharmaceutical production.

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Development of a fluorescent labeling strategy for qualitative and quantitative analysis of glycosaminoglycans

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Glycosaminoglycans (GAG) are linear and highly sulfated polysaccharides expressed as proteoglycan in the extracellular matrix (ECM) when attached to core proteins. GAGs are critical for ECM organization and play important roles in cell signaling. Their structural features, including chain length, sulphation patterns and relative abundance are increasingly recognized as potential biomarkers in inflammation, cancer and rare genetic diseases.

Current analytical approaches rely mainly on radioactive S-35 GAG chain labeling or the use of exogenous xyloside analogs, such as 4-methylumbelliferyl-xylose, to prime GAG biosynthesis in cells, which both present limitations.

Here, we aim to establish a fluorescent method to analyze GAG chain length produced by cultured cells without disrupting endogenous glycosyltransferase activity. The strategy involves: (1) natural production of proteoglycans by cells, (2) extraction and purification of secreted proteoglycans, (3) enzymatic digestion of core proteins to generate short peptides that are still linked with their GAG chain and which N-terminal amino group is retained free, (4) covalent labeling using amino-reactive probes. Analysis of length and quantity of GAG is performed with Size Exclusion HPLC coupled to a fluorescence detector.

No comparable approach has been demonstrated yet in the literature, underscoring the exploratory nature of this work. Preliminary experiments based on the literature demonstrated the feasibility of reductive-end labeling, but revealed solubility constraints. The project focuses on optimizing reaction conditions and implementing chromatographic strategies to efficiently separate and quantify fluorescent-labeled GAG.

The expected outcomes of this project include achieving efficient labeling yields (> 50%) and establishing a workflow enabling qualitative and quantitative GAG analysis within 2-3 weeks. Further, this method will support size-profiling of heparan sulfate and chondroitin/dermatan sulfate chains and may contribute to the development of GAG-based diagnostic tools. Ultimately, this project aims to position GAGs as accessible, biologically meaningful biomarkers through a robust, non-destructive fluorescent labeling strategy.

This project is funded by the Lorraine University's call for proposal "EMERGENCE".

Microbial synthesis of a saccharidic platform towards anti-adhesive multivalent glycans

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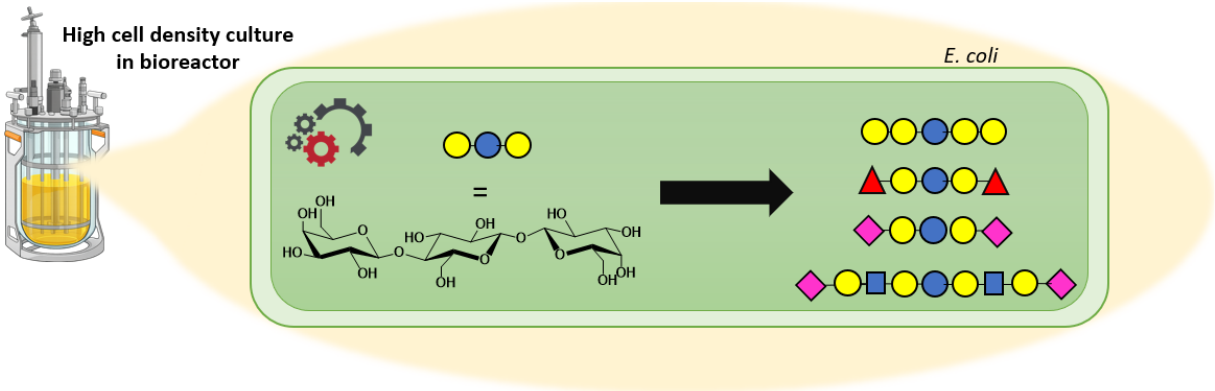
Many pathogens, including *influenza* viruses and *Pseudomonas aeruginosa bacterium,* exploit cell-surface glycans as initial attachment sites to initiate infection. Current anti-infective therapies mainly rely on vaccines and antibiotics, but rising resistance highlights the need for alternative strategies targeting these early adhesion events. Anti-adhesive therapy based on multivalent glycoconjugates is a promising approach to block pathogen infection.[1] Chemical synthesis remains the most widely used method for producing such glycoconjugates, despite involving complex multi-step procedures. As a result, their industrial development remains limited. As an alternative, the CBO team at CERMAV has developed strong expertise in chemo-biotechnological strategies for glycoconjugate synthesis, combining the production of complex oligosaccharides in metabolically engineered *E. coli* [2] with multivalent presentation achieved through chemical conjugation approaches.[3]

In this work, we aim to develop a new strategy for producing multivalent glycans enzymatically in *E. coli*, eliminating the need for any chemical steps. An unexpected side-activity of the β-1,4-galactosyltransferase from *Neisseria meningitidis* (LgtB)[4] on the anomeric hydroxyl of lactose, was used to produce for the first time the key trisaccharide Gal-β-1,4-Glc-β-1,1-β-Gal as core platform. Further modification of this platform with carbohydrate receptors targeting *influenza* viruses and *Pseudomonas aeruginosa* will be presented.



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Synthesis and Evaluation of alkyl *C*-hexofuranosides as potential Inhibitors of Galactofuranose-Containing Pathogens.

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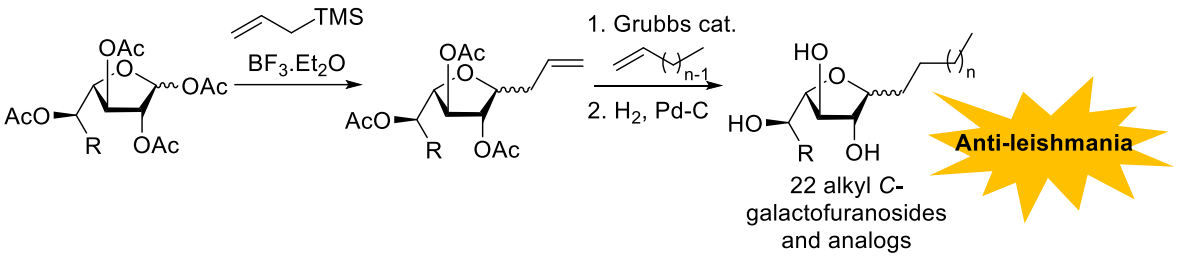
The glycocalyx of pathogenic microorganisms of the genus Leishmania and Trypanosoma exhibited a common feature, the presence of the rare carbohydrate galactofuranose within their glycophosphatidylinositol anchor.[1] In order to target the biochemical machinery responsible for the biosynthesis and incorporation of this rare carbohydrate, a library of 22 stable alkyl *C*-galactofuranosides and analogues was synthesized.[2] The key synthetic steps to access such β-d-furanosyl lipids relied on the *C*-1 allylation of glycosyl acetates, followed by cross-metathesis with α-olefins and hydrogenation. Their biological evaluation against *Leishmania* and *Trypanosoma* revealed IC_{50} values in the range of 50 µM against both parasites. Notably, three compounds showed promising antimicrobial efficacy with IC_{50} values and selectivity index comparable to the conventional drugs, highlighting their promising therapeutic efficacy.



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An approach based on enzymatic digestion highlights the crucial role of pectin in pollen tube adhesion

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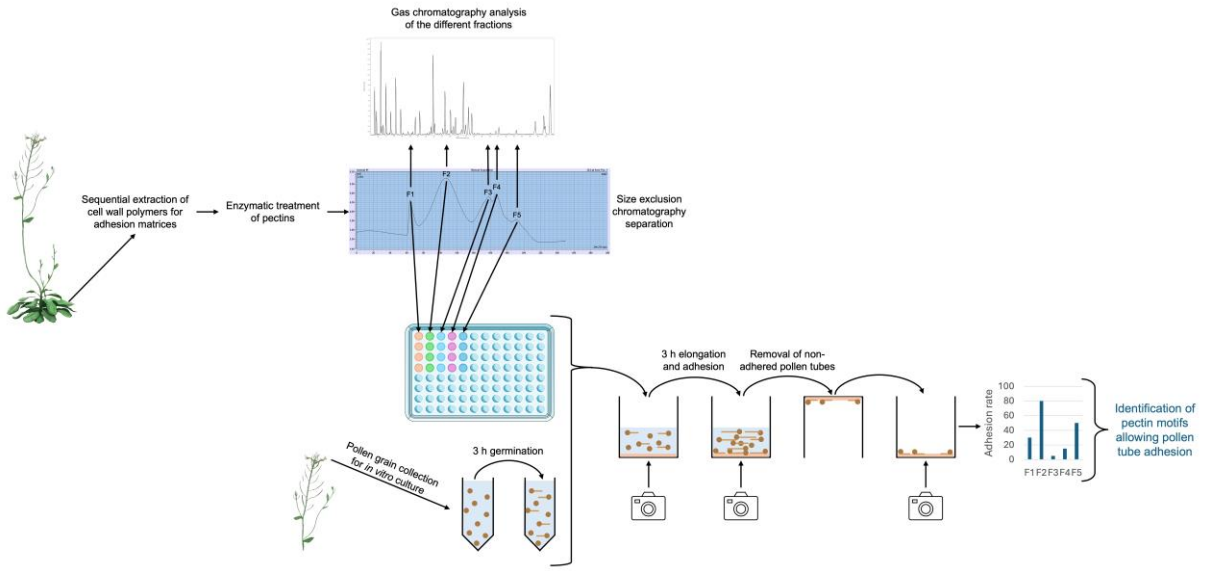
Summary In the context of climate change and global population growth, sustainably feeding the world's population has become an critical challenge. A deeper understanding of sexual plant reproduction is a key factor for improving seed and fruit production, which form the cornerstone of human and animal nutrition. During sexual reproduction, pollen grains land on the stigma, where they adhere, germinate, and generate a pollen tube. The pollen tube transports male gametes through the style and ovary toward the ovules, guided by several mechanisms, including adhesion to the cell walls of female tissues. This adhesion is thought to be mediated by cell wall polysaccharides, particularly pectic polysaccharides. However, the specific pectic structures required for pollen tube adhesion remain poorly characterized.

To address this, we adapted an *in vitro* pollen tube adhesion assay using an artificial matrix enriched in pectin-containing cell wall extracts from wild-type *Arabidopsis thaliana* plants and mutants impaired in pectin biosynthesis or remodelling. The deconstruction of these pectin-enriched extracts with specific glycoside hydrolase enzymatic treatments followed by Size Exclusion Chromatography has allowed the identification of pectin motifs with different compositions and molecular weights specifically involved in pollen tube adhesion.



N-glycan xylosylation in the diatom *Phaeodactylum tricornutum*

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In 2024, the biopharmaceutical market value reached above $469 billion. This dynamic market includes monoclonal antibodies (mAbs) either originators or biosimilars, hormones, nucleic acid and engineered cell-based products. Currently, most of the mAbs are produced in mammalian cells, such as Chinese Hamster Ovary (CHO) cells. However, production in CHO cells is expensive and present potential risk of viral contamination. This explains the growing interest for the development of cost-effective, safer and more sustainable expression systems, such as microalgae. Since they are photosynthetic eukaryotic cells, their culture in photobioreactors is inexpensive. In addition, microalgae perform efficient folding and *N*-glycosylation of proteins. Moreover, microalgae are classified as Generally Recognized as Safe strains and, therefore, microalgae, such as the diatom *Phaeodactylum tricornutum,* have emerged as alternative cell factories for the production of biologics. So far, analyses of glycans *N*-linked to *P. tricornutum* proteins revealed mainly high mannose type *N*-glycans both on endogenous glycoproteins[1] and mAbs[2]. However, bioinformatic approaches allowed the identification of a putative sequence coding for a xylosyltransferase (PtXylT) in the *P. tricornutum* genome[3]. *N*-glycan xylosylation of plant-derived therapeutic proteins has been demonstrated to induce immune responses[4]. Therefore, the present research project aims to investigate the xylosylation processing of *Phaeodactylum tricornutum N*-glycans, that is currently poorly understood. In contrast, plant core β(1,2)-xylosyltransferases (XylT) have been well-studied. In this context, we performed mass spectrometry analyses to identify xylosylated protein *N*-glycans in *P. tricornutum* Pt3 ecotype. Moreover, functional complementation of a tobacco mutant impaired in XylT activity with the gene coding for PtXylT confirmed that this glycosyltransferase is a core β(1,2)-xylosyltransferase.

This work is funded by the European Union under Grant Agreement 101119499 through the Marie Sklodowska-Curie Actions Glyco-N project. The authors gratefully acknowledge Nicole Raven and the Fraunhofer Institute for Molecular Biology and Applied Ecology IME (Aachen, Germany) for providing seeds of *Nicotiana benthamania* XylT/FucT double mutant.

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UDP-L-Iduronic Acid: New Synthesis of a Potential Substrate or Inhibitor of EXT1–EXT2 Polymerase.

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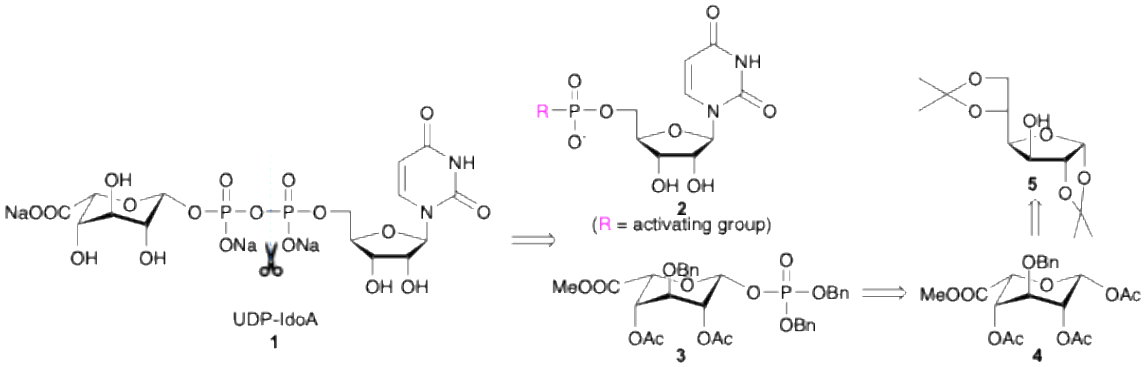
Here we report an efficient synthesis of UDP-IdoA **1**, starting from methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-β-L-Idopuranuronate **4**, easily available on a large scale from commercial 1,2,5,6-di-*O*-isopropylidene-α-D-glucofuranose **5**.[1,2] The key steps involve selective anomeric deacetylation, stereoselective anomeric phosphorylation and smart activation of UMP.



Heparan sulfate (HS) is a sulfated glycosaminoglycan that interacts and regulates the activity of numerous proteins (HSBP, e.g. growth factors and chemokines). HS biosynthesis allows the expression of a huge structural repertoire and is tightly regulated, thus allowing selective interactions with HSBP depending on the cell type and its activation.[3] HS chain elongation is catalyzed by the EXT1–EXT2 complex using UDP-D-glucuronic acid (UDP-GlcA) and UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc).[1,4] L-iduronic acid (IdoA)—a key determinant of HS conformation flexibility and biological activity—is generated after polymerization by the action of glucuronyl-C5-epimerase.[5] UDP-L-iduronic acid (UDP-IdoA) is thus not needed nor naturally occurring. A first synthesis has been reported, but, due to contamination by the GlcA nucleotide, the possibility of the EXT1–EXT2 to use UDP-IdoA as substrate is still a matter of debate.[6] A new synthesis was thus needed to address this question and potentially open up new opportunities for the chemoenzymatic synthesis of HS fragments based on direct incorporation of L-IdoA during polymerization.[7]

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PAGés : One « Glyco-toolbox », many « Glycotools »

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PAGés, established in 2012 within the UGSF, provides broad expertise in the structural analysis required in the field of Glycoscience. Since 2020, it has operated as the platform PAGés-P3M within the Plateformes Lilloises en Biologie et Santé (PLBS[1], UAR2014-US41). Its capabilities have been further strengthened through partnerships with local proteomics platforms, enabling the delivery of multi-level, wide-range, in-depth analyses of glycosylated protein.

Today, PAGés–P3M is the only platform in France recognized by the national IBiSA[2] infrastructure for its expertise in glycoconjugate and polysaccharide analysis, as well as in glycomics and glycoproteomics. Its mission is to optimize[3], develop[4], and design innovative tools to address a wide range of questions related to the composition and fine structure of glycoconjugates from diverse biological sources - including plants, bacteria [5], mammals, algae, yeasts, fungi - and across multiple classes, such as polysaccharides, *O*- or *N*-linked glycans, free or conjugated glycans, glycolipids, hemicelluloses, lipopolysaccharides (LPS), starch, amylopectin…

To achieve these objectives, the platform relies on a team of nine engineers and researchers specialized in the glycoconjugate analysis and benefits from comprehensive analytical and chemical resources, including chromatography, mass spectrometry, and NMR spectroscopy facilities. PAGés-P3M collaborates with both academic research groups and industrial R&D partners through scientific collaborations, service provision, and participation in national funding programs, including those from ANR, Institut Carnot, or other agencies. We invite you to explore our services and expertise to support and advance your research in glycobiology.

PAGés Plateforme Analyses Glycoconjugués UAR2014 US41 PLBS

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Lectin array and WTA-based neoglycoproteins to define *S. aureus* glycan signatures

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Staphyloccocus aureus is a dominant opportunistic human pathogen responsible for a wide range of infections, ranging from secondary skin conditions to critical, life-threatening pathological conditions such as sepsis[1]. This bacterium shows antibiotic resistance, causing several deaths each year; and the ongoing failure of antibody-based therapies and vaccines highlights major gaps, particularly in the understanding of protective immunity and the identification of suitable antigens. *S. aureus* is a Gram-positive bacterium that exposes on its surface major glycopolymers, wall teichoic acids (WTAs), which, due to their limited structural variability, high accessibility and widespread presence across strain, emerge as a pivotal immune targets[2]. For this reason, the identification of *S. aureus* WTA-glycan signatures on the bacterial cell surface and, in the same time, the study of their interactions with carbohydrate-binding proteins expressed by host cells has been considered. To achieve this, glycoprofiling of various *S. aureus* strains was carried out using the lectin platform developed by GLYcoDiag, alongside the synthesis and characterisation of WTA-derived glycoconjugates for binding and avidity studies. In the first part of the project, glycoprofiling of *S. aureus* strains was performed using a well-defined method, namely NHS-ester labelling of the cells and followed by incubation on a microplate lectin array cointaning natural and human recombinant lectins. Using fluorescence readout, it has been possible to identify the cell-specific glycan signature under controlled culture conditions, as the results reported will show. At the same time, the synthesis of WTA-based neoglycoproteins, through strain-promoted azide-alkyne ciclyaddition (SPAAC), has been initiated from the BSA-DBCO-sulfo-NHS ester coupling. In this context, different coupling tests are in progress in order to achieve a coupling degree of around 40 DBCO-sulfo-NHS ester per BSA molecule. The results of this trials will be reported.

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Exploration of Fungal Cell Walls remodelling via characterization pipelines and Carbohydrate Active Enzymes case studies

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Filamentous fungi are major actors in a wide range of biological processes, including the global carbon cycle, life-sustaining (e.g., symbiosis), and life-threatening (e.g. pathogenesis) mechanisms. In all these processes, the fungal cell wall (FCW) — which provides mechanical and physicochemical resistance — serves as the primary interface between the cell and its environment. Its composition and dynamics are therefore critical. FCWs are mainly composed of carbohydrates (e.g., chitin, linear/branched α- and β-glucans) and glycoproteins, whose assembly and remodeling are largely mediated by Carbohydrate-Active enZymes (CAZymes). However, the diversity of FCW architectures and the enzymatic mechanisms governing their remodeling remain poorly understood.

In this presentation, we will summarize our ongoing efforts to establish miniaturized and optimized pipelines for exploring FCW biodiversity, dynamics, and the role of CAZymes in their remodeling[1]. Our approach combines biochemical, immunochemical, and enzymatic tools to characterize FCW structures and a dedicated pipeline for the production of recombinant CAZymes, enabling functional studies of these enzymes.

As a case study, we will present our work on a family of CAZymes from the plant pathogen *Colletotrichum orbiculare*, which targets chitin oligomers in the FCW. This example illustrates how our integrated pipelines can uncover novel enzymatic activities and their implications for FCW plasticity.[2,3]

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A study of dolichol synthesis in yeast: a close link with the ergosterol pathway.

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Glycosylation is a necessary and complex cellular process in which monosaccharides are transferred onto proteins or lipids. This evolutionarily conserved mechanism can lead to congenital defects of glycosylation (CDG) when disrupted. Dolichol, a long-chain lipid, is crucial for the first steps in N-glycosylation, notably the LLO-precursor assembly, essential for the oligosaccharide transfer onto the protein in the endoplasmic reticulum[1]. Genetic deficiencies in genes such as SRD5A3 or the newly discovered DHRSX, involved in dolichol metabolism, have been associated with atypical clinical phenotypes of CDG as well as severe N-glycosylation defects.[2,3] This has helped us to identify that dolichol synthesis requires a three-step detour involving polyprenal and dolichal as metabolites. Identification and characterization of the yeast orthologues for SRD5A3 and DHRSX led us to demonstrate the occurrence of a similar dolichol synthesis pathway in yeast, with marked differences (Wilson et al., 2026, *in press*). Eventually, our unpublished results in yeast highlight an unexpected crosstalk between ergosterol metabolism and N-glycosylation that could help further development of therapies for CDG patients suffering from dolichol biosynthesis defects[4,5].

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Identification of bacterial cell envelope factors that influence tuberculosis disease and treatment outcome

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Mycobacterium tuberculosis (Mtb) factors, including cell envelope lipids and lipoglycans, play a central role in host-pathogen interactions in both cellular and animal models. They are therefore assumed to be key determinants of clinical outcomes, influencing tuberculosis (TB) disease progression and response to treatment in humans. However, this has remained insufficiently explored.

Within the SMA-TB EU-funded project (GA847762), a Phase 2b, double-blind, placebo-controlled clinical trial (NCT04575519) evaluating ibuprofen and acetylsalicylic acid as anti-inflammatory adjunctive therapies to standard-of-care treatment for pulmonary TB, we collected 150 baseline Mtb clinical isolates in Georgia and South Africa. The strains were cultured on solid medium, and lipoglycans and lipids were quantified using a combination of dot-blot assays and lipidomic analyses to investigate the relationship between bacterial component variability and patient clinical data. The inflammatory potential of strains was also measured by their ability to induce NF-kB activation in the human macrophage THP-1 cell line.

Strikingly, we observed that the production of several lipids, including phthiocerol dimycocerosates (DIM), triacylglycerols (TAG), sulfoglycolipids (SGL), and phosphatidylinositol mannosides (PIM), correlated with baseline disease severity scores and with specific blood parameters. Moreover, among patients in the placebo arm, those showing poor TBscore evolution at weeks 8 and 24 of treatment were infected with strains producing higher levels of lipoarabinomannan (LAM). Similarly, patients in the placebo arm who exhibited unfavourable chest X-ray evolution at week 8 were infected with strains displaying a higher inflammatory potential. In both cases, these associations were abolished in the ibuprofen and acetylsalicylic acid arms, highlighting the impact of adjunctive anti-inflammatory therapy.

Altogether, our findings strongly suggest that bacterial factors, along with the intrinsic inflammatory potential of Mtb strains, influence human TB disease progression and treatment outcomes. Such pathogen-derived biomarkers could support the development of urgently needed patient-stratification strategies to improve TB clinical management and therapeutic success.

Synthesis and evaluation of Schweinfurthine-OSW-1 hybrids

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We report the first synthesis of hybrid molecules combining two biologically active compounds, Schweinfurthin E (SW-E) and OSW-1, both known for their strong antiproliferative effects. Schweinfurthins are natural prenylated stilbenes, some containing a hexahydroxanthene (HHX) moiety essential for activity.[1] Their pharmacological profile differs from conventional chemotherapy, targeting a novel pathway involving the OSBP protein,[2] which regulates cholesterol transport between the endoplasmic reticulum and Golgi apparatus. OSW-1 is a steroid bearing a disaccharide moiety critical for its cytotoxicity, likely contributing to hydrophobic clustering and enhanced potency.[3] The hybrids were synthesized via a CuAAC reaction using a polyfunctionalized alkyne derived from SW-E and azido sugars (**Figure 1**). We also developed an efficient method for preparing the OSW-1 disaccharide by sequential functionalization of L-arabinose, using a boronic ester[4] as a switchable protective/activating group. This approach streamlines existing synthetic routes by reducing steps and purifications. Biological evaluation showed that some hybrids exhibit significantly higher cytotoxicity than SW-E in a glioblastoma cell line. Finally, molecular modeling studies were performed to rationalize these results.[5]

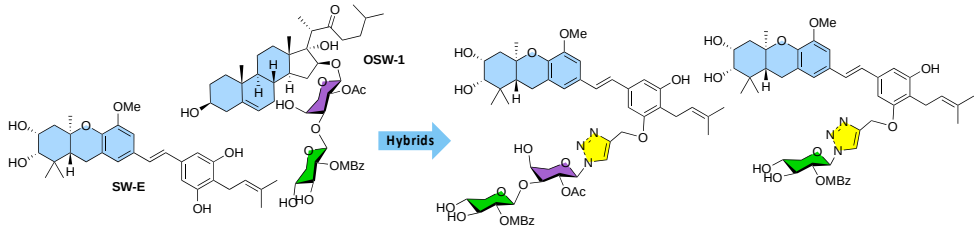

Figure 1

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From furcellarans to succinylated galactans: eco-friendly process for the valorization of algal polysaccharides

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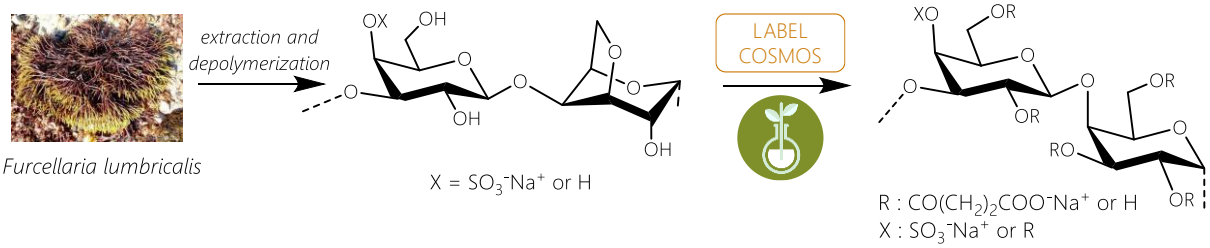
In recent years, the cosmetics industry has faced growing environmental awareness, prompting the development of more sustainable synthesis processes and novel active ingredients. The PhD research presented here was conducted in collaboration with CODIF International, a company specializing in marine-derived cosmetic actives, with the aim of developing environmentally responsible approaches consistent with green chemistry principles and evolving cosmetic regulations.

This work focused on the marine polysaccharide furcellaran, extracted from the red alga *Furcellaria lumbricalis*. Following depolymerization and detailed characterization of the resulting oligosaccharides, structural modifications were performed through grafting of selected carboxylic acids. Depending on the nature of the fatty chain, different synthetic strategies were optimized to meet both green chemistry criteria and cosmetic regulatory requirements.[1] In particular, succinyl moieties were grafted from the tetrabutylammonium salt of this oligofurcellaran. Detailed NMR analysis of the resulting product unexpectedly revealed the disappearance of the anhydro bridges, thus linear succinylated and sulfated galactans were obtained. The biological properties of these original oligosaccharides were finally evaluated to assess their potential as cosmetic active ingredients.



Furcellaria lumbricalis

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Tracing glycosylation events in *Chlamydomonas reinhardtii* using reporter glycoproteins targeted to different compartments of the secretory pathway

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Microalgae are eukaryotic photosynthetic and unicellular organisms that are intended as a cost-effective expression system for producing recombinant glycoproteins, such as glycosylated therapeutics. In this context post-translational modifications such as *N*-glycosylation, which are essential for the function of drugs, need to be well-understood[1]. In this study, we aim at deciphering the step by step *N*-glycosylation events in the green microalga *Chlamydomonas reinhardtii*. To do so, we developed an original approach based on the glycosylation analysis of a reporter protein expressed in specific compartments of the secretory system. Human erythropoietin (hEPO) or phytohemagglutinin (PHA) fused to a fluorescent protein (FP) were used as reporter glycoproteins. Various constructions were developed using the Golden Gate cloning technology allowing the expression of these reporter glycoproteins fused to either a C-terminal HDEL sequence for its retrieval in the endoplasmic reticulum (ER), or a 'cytoplasmic, transmembrane, stem' (CTS) domain for its targeting in specific Golgi cisternae of the microalgae. *C. reinhardtii* was transformed with these constructions and reporter glycoproteins were purified from positive clones. They were therefore submitted to a glycoproteomic analysis by Liquid-Chromatography coupled to Mass Spectrometry (LC-MS/MS) to characterize the micro- and macroheterogeneity of the glycosylation of reporter glycoproteins. We present here the first results obtained for hEPO expressed either in the ER, or in specific Golgi compartments. This original approach allows us to determine the step by step events of the *N*-glycosylation pathway in *C. reinhardtii* leading to the specific glycosylation profile in this microalga.

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Mycobacteria Recognition by Host C-type Lectins: Exploring the Lateral Dynamics of Surface-Exposed Glycan Ligands

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Background: *Mycobacterium tuberculosis* (Mtb) displays surface glycans that interact with host C-type lectin receptors (CTLs), some of which promote protective immune responses and others favour infection. We recently found that the nanoscale clustering of these glycans is crucial for recognition by the CTL DC-SIGN[1]. To get further insights into how CTL ligand organization at the bacterial surface may influence recognition by host CTLs, we now asked how the lateral distribution of surface-exposed glycans changes as a function of time, bacterial elongation and division.

Approaches & Results: To assess glycan dynamics, we labelled surface-exposed oxidizable carbohydrates on live model *Mycobacterium smegmatis* cells with AlexaFluor hydrazide and imaged them using time-lapse confocal microscopy. Carbohydrates remained associated with the original cell envelope, with little signal in newly synthesized, elongating regions. Atomic force microscopy (AFM) revealed stable hydrophilic and hydrophobic nanodomains on single cells, established during new cell envelope synthesis at the poles, with no lateral displacement over 8 hours. Fluorescence Recovery After Photobleaching (FRAP) and time-lapse confocal imaging of fluorescent lectin-labelled bacteria showed extremely slow lateral diffusion of glycans and exclusive association of fluorescence with the old cell wall, even after 16 hours of outgrowth. These findings were replicated in formaldehyde-inactivated Mtb.

Implications: The static lateral organization of mycobacterial surface glycans occurs on a timescale exceeding cell wall elongation and division. This stasis may enable stable nanodomain formation, facilitating functional recognition by CTLs. Further, given the known asymmetry in cell envelope synthesis at mycobacterial poles, which yields phenotypic heterogeneity in cell age and size[2], stasis in glycan lateral dynamics may contribute to this heterogeneity on the bacterial surface, with implications for interactions with CTLs expressed on diverse host cells.

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