The 21st European Carbohydrate Symposium

Paris, 9-13, July 2023

Abstract BOOK

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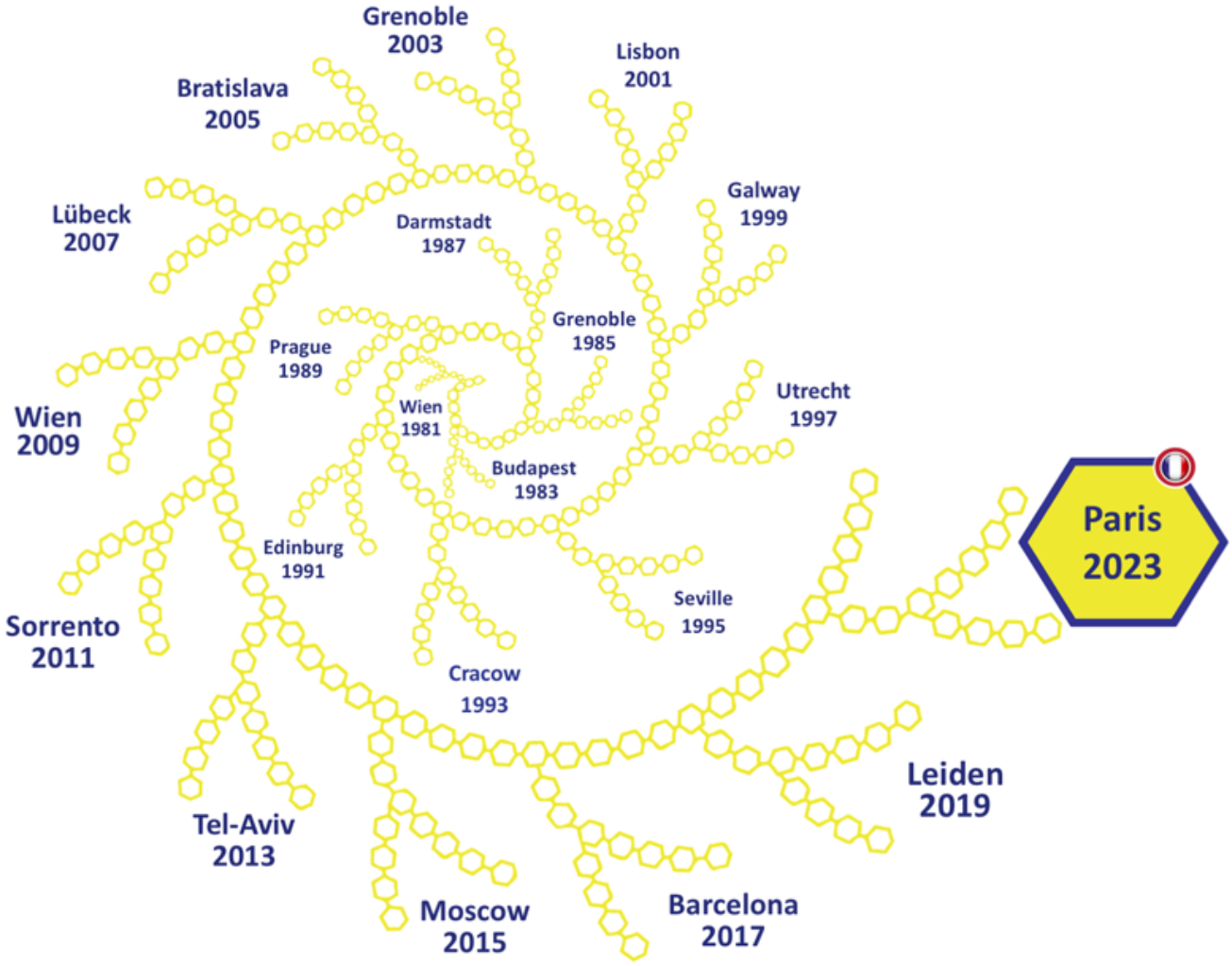
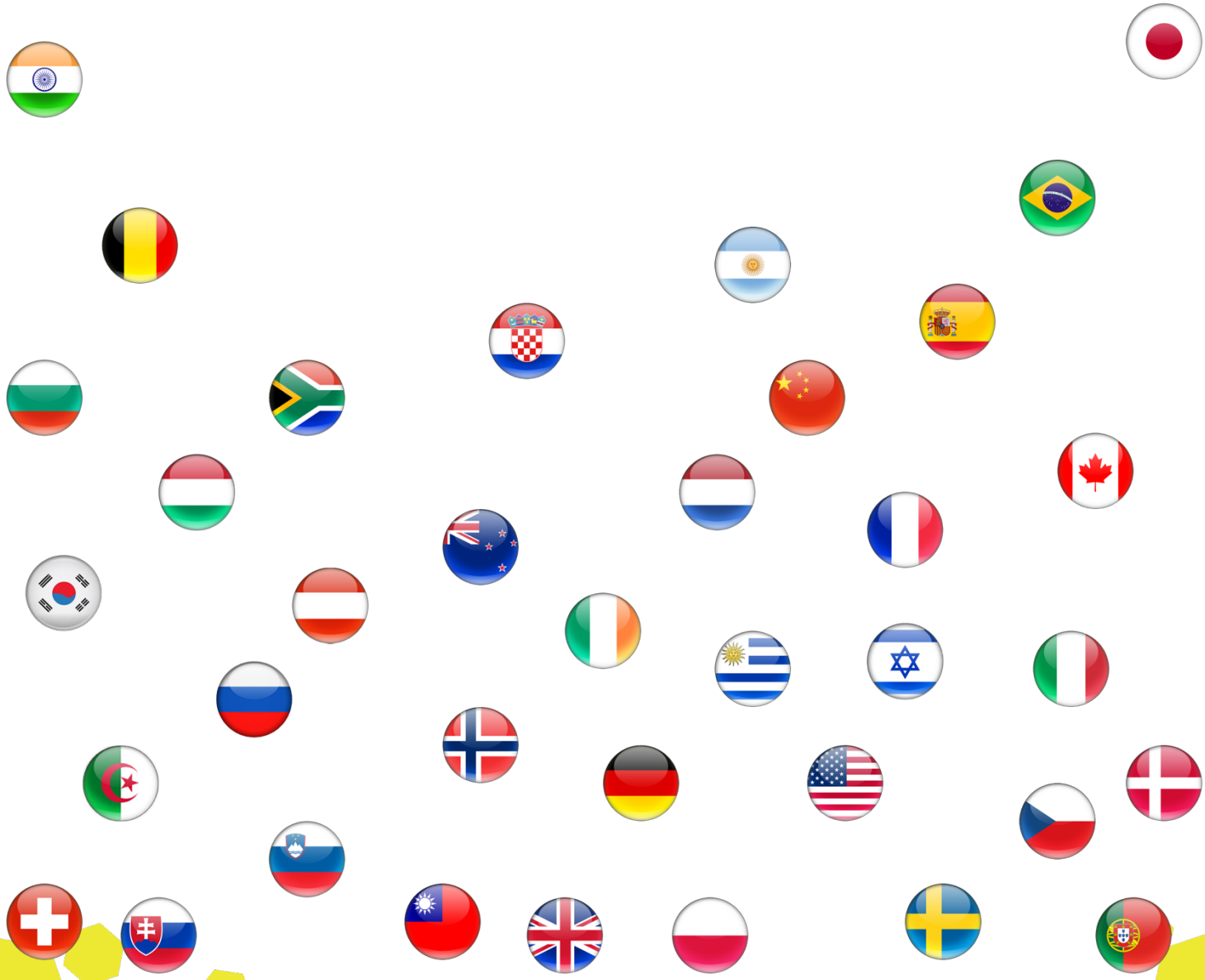


42 years of Eurocarb make this chain grow!

37 countries, various statuses, different ages... one shared passion

Eurocarb 21



Welcome

To the Glycoscience Community,

FOUR years after Eurocrab20, in Leiden, TWO years after a reprogrammed "lost in COVID19 pandemic" edition, it is our great pleasure to welcome you, AT LAST and ON SITE, to the 21th EUROCARB edition, held under the auspices of the European Carbohydrate Organization. Eurocarb21, hosted at *La Maison de la Chimie*, in Paris, July 9-13 2023, is the first occasion for the international Glycoscience community to meet LIVE after three years of virtual or hybrid meetings. Eurocarb21 is thus a very special edition and THE occasion to renew and strengthen the existing links between established Glycoscientists and integrate the youngest ones in our Glycofamillial network. WELCOME to all of you, let's rejoice and celebrate being together again for the progress of Science and friendship driven by Glycosciences!!

As for the previous editions, Eurocarb21 will bring together scientists from Europe and other parts of the world to share their interests in the field of Glycosciences. Thank you for your strong participation: **600 participants**, including **221 PhD** students (37% the highest score ever!!), coming from **36 different countries**.
The scientific program of Eurocarb21 will cover all diverse aspects of glycosciences and includes 11 renowned Plenary Speakers, 17 Keynote Lecturers, 144 Oral Presenters, 72 Flash Communication (+ Posters) and 224 Poster Presentations.

Eurocarb21 brings its lot of innovations, by introducing the **Interdisciplinary-Duo format**, declined on Duo-Keynote Lectures (4) and Duo-Oral Communications (7). A way to promote and celebrate interdisciplinary research, often at the heart of glyco-based projects, by highlighting excellent and recent collaborative results.

The FOUR recipients of the prestigious "Emil Fisher Award" and "Carbohydrate Research Award" 2021 **and** 2023 will receive their awards and present their remarkable results in Plenary Lectures.

Please note that two workshops will be held on Wednesday the 12th: the GlycoTwinning Workshop and the 2cd-Young Investigator Workshop. The later, following the first one in Leiden, is dedicated to employment of young investigators to develop and strengthen Glycosciences and the burgeoning Glycoeconomy.

The financial support by many organizations, institutions and companies is greatly acknowledged and has been pivotal in keeping students' fees at a minimum level considering the strong worldwide inflation.

Although the Organizing Committee devised a dense scientific program, do not miss the opportunity to visit Paris and learn better its history.

This the third time that a Eurocarb symposium is held in France, after Eurocarb3 (1985) and Eurocarb12 (2003), both in Grenoble. A periodicity of 9 starts to emerge for Eurocarb symposia in France. Will there be a Eurocarb30 organized by French colleagues in 2041? The future will tell us, but let's stay in "the here and now", thus to Eurocarb21 that the Organizing Committee wish you successful, enjoyable and... positively unforgettable.

David BONNAFFÉ, chairman of Eurocarb21
On behalf of the Organizing Committee

General Information

GENERAL INFORMATIONS

REGISTRATION DESK OPENING TIMES

Please go to the registration desk upon arrival to the venue to collect your congress documents.

Registration desk opening times and technical secretariat schedule are outlined below:

- Sunday, July 9th from 14:00 to 19:00
- Monday, July 10th from 8:00 to 19:00
- Tuesday, July 11th from 8:30 to 19:00
- Wednesday, July 12th from 8:30 to 15:00
- Thursday, July 13th from 8:30 to 18:30

If your registration is **NOT confirmed**, please send a proof of your payment to registration-eurocarb2023@cborg.fr and/or let us know if you will pay on-site.

Certificate of attendance will be available on your personal account at the end of the symposium.

PREVIEW ROOM (SALLE 103)

A Preview Room will be available onsite to check your uploaded files, if needed, before it is handed to the technicians of the projection rooms. Please visit it **at least half a day** prior to your presentation. The conference organizers decline all responsibility for presentations that will not be checked in due course.

Opening times

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- Wednesday, July 12th: 8:00 - 9:00, 10:30 - 11:00, 12:30 - 14:00
- Thursday, July 13th: 8:00 - 9:00, 10:30 - 11:00

Instructions to authors

INSTRUCTIONS FOR SPONSOR PRESENTATIONS

Sponsor presentations time slots:

- Please, check the program overview to identify the day of your presentation.
  https://www.eurocarb2023.com/eurocarb-program-abstracts/program-overview/
- You are requested to report to the chair.wo.man in the lecture room 5-10 minutes prior to the start of your session.
- Speakers are requested not to exceed the allocated time: 5 min, no question, for Sponsor Presentations.
  Nb.: the time schedule will be strictly followed and the chair.wo.men will be missioned for that.

Presentations preparation and upload:

- Prepare your slides in landscape orientation. Standard video projection format ratio in 16:9 to maximize the image surface on the screens.
- Only pptx or pdf files will be accepted. If the presentation includes audio (sound or voice), animation or short movie file(s), speakers are advised to upload all files in a single zipped folder and check the links in the preview room (see below).
- Use of your own computer is not possible.
- Speakers are requested to **upload their presentation files at least 24h before the start of their presentation's session** using the appropriate webform:
  https://www.eurocarb2023.com/sponsor-presentation-delivery/

AV equipment at Maison de la Chimie

- PC computers are equipped with pack office 365 (Microsoft Word, Excel, PowerPoint), Acrobat reader and VLC.

Preview room (salle 103)

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We look forward to meeting you in Paris! Please do not hesitate to contact the Symposium contact (contact@eurocarb2021.com), if you have any questions.

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INSTRUCTIONS FOR PLENARY LECTURES

Oral presentations time slots:

- Please, check the program overview to identify the day of your presentation.
https://www.eurocarb2023.com/eurocarb-program-abstracts/program-overview/
- You are requested to report to the chair.wo.man in the lecture room 5-10 minutes prior to the start of your session. Please prepare a brief printed CV at their intention, to be used for your introduction before your lecture.
- Speakers are requested not to exceed the allocated time:
  + Plenary Lectures: 40 min, no questions.

Nb.: the time schedule will be strictly followed and the chair.wo.men will be missioned for that.

Presentations preparation and upload:

- Prepare your slides in landscape orientation. Standard video projection format ratio in 16:9 to maximize the image surface on the screens.

You can upload your presentation file(s), using the appropriate webform:

https://www.eurocarb2023.com/plenary-presentation-delivery/

- Only pptx or pdf files will be accepted. If the presentation includes audio (sound or voice), animation or short movie file(s), speakers are advised to upload all files in a single zipped folder and check the links in the preview room (see below).
- Speakers are requested to **upload their presentation files at least 24h before the start of their presentation's session.**

Alternatively, you may choose to use your own laptop *(see below for technical specifications).*
In this case, please inform us, by filling the same webform:

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AV equipment at *Maison de la Chimie*

- PC computers are equipped with pack office 365 (Microsoft Word, Excel, PowerPoint), Acrobat reader and VLC
- At the desk, there will be:
  + HDMI connection. PLEASE BRING THE SUITABLE ADAPTER (ex: VGA to HDMI, Mini-display to HDMI, USB C to HDMI, Thunderbolt to HDMI...), if your laptop does not support the HDMI connection natively. The conference organizers decline all responsibility in the video connection of non-HDMI compatible laptops.
  + Mini-jack 3.5 for sound (if needed)

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INSTRUCTIONS FOR KEYNOTE and INTERDISCIPLINARY DUO-KEYNOTE LECTURES

Oral presentations time slots:

- Please, check the program overview to identify the day of your presentation.
https://www.eurocarb2023.com/eurocarb-program-abstracts/program-overview/
- You are requested to report to the chair.wo.man in the lecture room 5-10 minutes prior to the start of your session. Please prepare a brief printed CV at their intention, to be used for your introduction before your lecture.
- Speakers are requested not to exceed the allocated time:
  + Interdisciplinary Duo-Keynote Lectures: 40 min (35 + 5 min questions)
  + Keynote Lectures: 25 min (20 + 5 min questions)
  Nb.: the time schedule will be strictly followed due to the nature of the symposium program, which includes 4 parallel sessions running at the same time.

Presentations preparation and upload:

- Prepare your slides in landscape orientation. Standard video projection format ratio in 16:9 to maximize the image surface on the screens.

You can upload your presentation file(s), using the appropriate webform:

https://www.eurocarb2023.com/keynote-presentation-delivery/

- Only pptx or pdf files will be accepted. If the presentation includes audio (sound or voice), animation or short movie file(s), speakers are advised to upload all files in a single zipped folder and check the links in the preview room (see below).
- Speakers are requested to **upload their presentation files at least 24h before the start of their presentation's session.**

Alternatively, you may choose to use your own laptop *(see below for technical specifications).*
In this case, please inform us, by filling the same webform:

https://www.eurocarb2023.com/keynote-presentation-delivery/

AV equipment at *Maison de la Chimie*

- PC computers are equipped with pack office 365 (Microsoft Word, Excel, PowerPoint), Acrobat reader and VLC
- At the desk, there will be:
  + HDMI connection. PLEASE BRING THE SUITABLE ADAPTER (ex: VGA to HDMI, Mini-display to HDMI, USB C to HDMI, Thunderbolt to HDMI...), if your laptop does not support the HDMI connection natively. The conference organizers decline all responsibility in the video connection of non-HDMI compatible laptops.
  + Mini-jack 3.5 for sound (if needed)

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INSTRUCTIONS FOR ORAL and INTERDISCIPLINARY DUO-ORAL PRESENTATIONS

Oral presentations time slots:

- Please, check the program overview to identify the day of your presentation.
https://www.eurocarb2023.com/eurocarb-program-abstracts/program-overview/
- You are requested to report to the chair.wo.man in the lecture room 5-10 minutes prior to the start of your session.
- Speakers are requested not to exceed the allocated time:
  +Interdisciplinary Duo-Oral communications: 30 min (25 + 5 min questions)
  +Oral communications: 15 min (12 + 3 min questions)
  Nb.: the time schedule will be strictly followed due to the nature of the symposium program, which includes 4 parallel sessions running at the same time.

Presentations preparation and upload:

- Prepare your slides in landscape orientation. Standard video projection format ratio in 16:9 to maximize the image surface on the screens.
- Only pptx or pdf files will be accepted. If the presentation includes audio (sound or voice), animation or short movie file(s), speakers are advised to upload all files in a single zipped folder and check the links in the preview room (see below).
- Use of your own computer is not possible.
- Speakers are requested to **upload their presentation files at least 24h before the start of their presentation's session** using the appropriate webform:
  https://www.eurocarb2023.com/OL-presentation-delivery/

AV equipment at *Maison de la Chimie*

- PC computers are equipped with pack office 365 (Microsoft Word, Excel, PowerPoint), Acrobat reader and VLC

Preview room (salle 103)

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INSTRUCTIONS FOR POSTER PRESENTATIONS

POSTER FORMAT:

- The recommended size of the posters is DIN A0: 841 mm (wide) x 1189 mm (high).
- The presentation number assigned to your poster should not be placed on your poster. The poster boards will be numbered for you.

Presenting authors are expected to be in front of their posters during the poster session time. Please, check the final scientific program to identify the number attributed to your poster and the day of your poster presentation: https://www.eurocarb2023.com/eurocarb-program-abstracts/program-overview/

SETTING-UP AND TAKING DOWN TIMES:

- Poster presenting authors are responsible for setting-up and taking down their poster. Self-adhesive pads for posting will be offered by the organizer.
- All posters can be fixed on the corresponding board from Sunday 9th from 14h30 and at the latest one hour before the start of the Poster Session.
- Posters which haven't been removed in due time will be detached and dumped. The conference organizers decline all responsibility for non-recovered posters.

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The Organizing Committee

Awards

The 2021's Emil Fischer Award winner :

The 2021 Emil Fischer Award is conferred to **Prof. Antonio MOLINARO**, professor of Organic Chemistry at University of Napoli Federico II and Special Appointed Professor at School of Science of Osaka University.

The Award will be presented to **Prof. MOLINARO** in recognition of his outstanding accomplishments toward the structural elucidation of complex carbohydrates from microbial world, in particular, in the study of structure and role of cell wall of microorganisms in the elicitation of innate immune response in plants and mammals.

When **Prof. Antonio MOLINARO** started his independent career, the investigation of the structure and the mechanisms of action of lipopolysaccharides (LPS) and peptidoglycan (PGN) from plant-pathogen bacteria was still at a descriptive stage and little was known about the mechanisms of perception of these molecules by plants and about the associated signal transduction pathways that trigger plant immunity. By isolating glycoconjugates from Gram-negative bacteria and determining their 3D-structures using state of art NMR and MS methodologies and molecular mechanics and dynamics approaches, **Prof. MOLINARO** was able to elucidate the conformational behavior of the ligand alone and when interacting with a protein, determine its bioactive conformation and elucidate its epitope mapping.

His work led to seminal contributions to the understanding of the molecular basis of elicitation of eukaryotic defenses and the effects of microbial carbohydrate signatures on the immune response of both mammals and plants. Recently, he has moved his interests toward the understanding of the role of LPS in gut microbiota as well as root microbiota symbionts

EC

European Carbohydrate Organisation

The 2023's Emil Fischer Award winner :

The 2023 Emil Fischer Award is conferred to **Prof. Jeroen CODÉE**, professor in Organic Chemistry at Leiden University, The Netherlands, in recognition of his exceptional accomplishments and dedication to bio-organic chemistry in the field of Glycosciences.

Prof. CODÉE's scientific interests range from fundamental organic synthesis to vaccine/drug development and the design and synthesis of glycobiology tools for the investigation of carbohydrate binding targets.

Since the beginning of his career his interest has been focused on the chemical synthesis of complex carbohydrate structures and the development of glycosylation methods, therefore. He contributed significantly in applying techniques of automated synthesis for glycosylation reactions where he achieved the development of a protocol for the automated solid-phase synthesis of oligosaccharides with 1,2-cis-glycosidic linkages, which is one of the most challenging in the context of stereoselectivity and preparatively useful yields. With his excellent knowledge of glycosylation reaction on the molecular basis he established a structure-reactivity-stereoselectivity relationship which allows for rational design of glycosylation reactions. Moreover, using high level computational chemistry, **Prof. CODÉE** achieved in mapping the energy landscape of the complete conformational space of oxocarbenium ions and shedding further light on the insights of the glycosylation reaction.

His fundamental studies to unravel the mechanism of glycosylation reactions led to innovative synthetic routes to assemble complex bacterial glycans. These complex carbohydrate structures and glycoconjugates are useful tools to study their interaction with the host immune system, as antigens and ligands for innate immune system cell receptors as well as to develop glycomimetic conjugates as vaccines against bacterial infections.

EC

European Carbohydrate Organisation

The 2021's Carbohydrate Research Award winner :

Dr Daisuke TAKAHASHI receives this award in recognition of his work on glycosylation chemistry, having developed novel and expedient boron-mediated processes for the stereoselective synthesis of glycosides.

Dr TAKAHASHI is an Associate Professor in the Department of Applied Chemistry at Keio University in Yokohama, Japan. His work focuses on synthetic carbohydrate chemistry and he has developed boron-mediated processes for the stereoselective synthesis of glycosides.

The award was established in 2001 by the Editors and Publisher of Carbohydrate Research and is awarded biannually. **Dr TAKAHASHI** will receive a cheque for $3,000 and an award certificate, which will be presented to him at a future EuroCarb conference.

Congratulations again to **Dr Daisuke TAKAHASHI** on this fantastic achievement.

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The 2023's Carbohydrate Research Award winner :

Prof. Christoph RADEMACHER earned his BSc in Molecular Biotechnology (2004) and MSc in Molecular Life Science (2006) at the University of Lübeck. In 2009, **Prof. RADEMACHER** received his doctorate from the same University, where he performed studies under the supervision of Prof. Thomas Peters in the Department of Chemistry working on virus/carbohydrate interactions using NMR spectroscopy. During these years, he also worked in Prof. David R. Bundle's and Prof. Todd Lowary's laboratories at the Alberta Ingenuity Center for Carbohydrate Science in Edmonton (Canada) and in Dr. Daron Freedberg's group at CBER/FDA in Bethesda (USA).

He then underwent postdoctoral training with Prof. James C. Paulson at The Scripps Research Institute (USA) in the Department of Chemical Physiology, where he entered the field of glycoimmunology.

In December 2011, **Prof. Christoph RADEMACHER** was appointed at the Max Planck Institute of Colloids and Interfaces in the Department of Biomolecular Systems with Prof. Peter H. Seeberger, where he became Emmy-Noether Research Group Leader in June 2012. In 2017, **Prof. RADEMACHER** received an ERC Starting Grant and in 2020 he was appointed full professor at the University of Vienna and the Max F. Perutz Laboratories.

Since 2022 **Prof. RADEMACHER** is part of the NIBR Global Scholar Program. His research is focused on the development and application of novel molecular probes to understand the role of carbohydrates in immune cell regulation with a strong emphasis on molecular drug targeting.

NON SOLUS

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Plenary Lectures

The chemical diversity of microbial glycans

Antonio MOLINARO [1],

[1] Antonio Molinaro Department of Chemical Sciences, University of Napoli Federico II Napoli, ITALY; Department of Chemistry, School of Science, Osaka University, JAPAN

molinaro@unina.it

Microbial cell surface molecules, such as the lipopolysaccharide, are very important cell wall glycoconjugates that act as microbe associated molecular patterns in eukaryotic/microbe recognition. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Microbes differently from Eukaryotes have at their disposal an enormous array of monosaccharide structures/derivative with which they built up they external cell surface molecules and drive their recognition by any eukaryotic host. Therefore, the chemical study of such glycoconjugates involved as virulence or beneficial factors in animal or plant interactions is a pivotal pre-requisite for the comprehension at molecular level of the (innate) immunity mechanisms. [1]

Viral glycoproteins are usually meant to carry on eukaryotic glycans. Indeed, typically, viruses use host-encoded glycosyltransferases and glycosidases to add and remove sugar residues from virus glycoproteins. However, the more recently discovered large and giant viruses broke from this paradigm. Instead, these viruses code for an (almost) autonomous glycosylation pathway. Virus genes include the production of activated sugars, glycosyltransferases and other enzymes able to manipulate sugars at various levels. [2]

In this communication, I will show examples of microbial glycans and their action as immune-elicitors/suppressors of eukaryotic innate immunity as well as new clues about autonomous viral glycans and the machinery involved in their biosynthesis.

By this work, I will also show that structural Glycoscience of microbial world is a fascinating travel through astounding chemical structures with no parallel in any other kingdom.

Bibliographic references:

[1] F. Di Lorenzo, K.A: Duda, R. Lanzetta, A. Silipo, C. De Castro, A. Molinaro (2022), Chem. Rev., (122) 15767-15821.

[2] I. Speciale, A. Notaro, C. Abergel, R. Lanzetta, T.L. Lowary, A. Molinaro, M. Tonetti, J. L. Van Etten, C. De Castro (2022), Chem. Rev. (122) 15717-15766

Dissecting glycosylation reaction rechanisms

Jeroen CODÉE [1],

[1] Leiden Institute of Chemistry, Leiden Univeristy, THE NETHERLANDS

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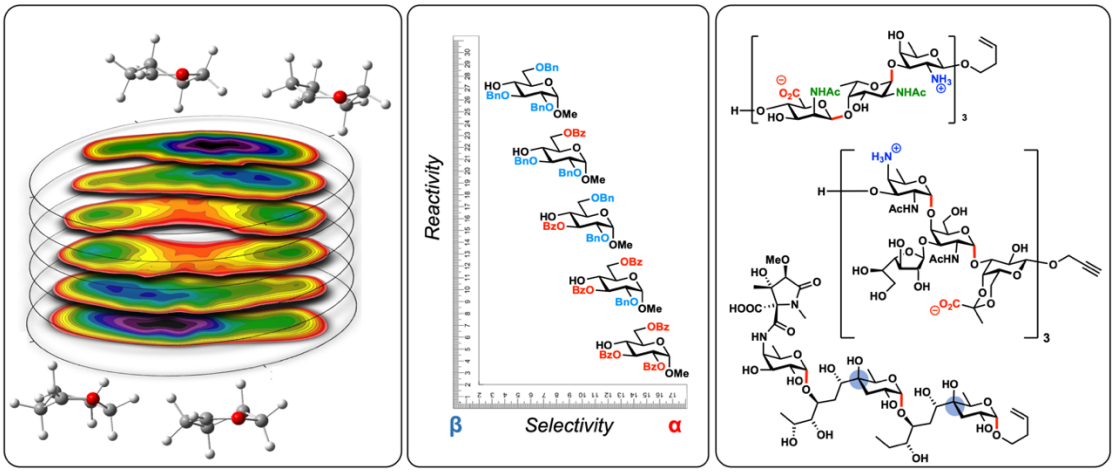
Synthetic oligosaccharides are invaluable tools to unravel their role in biological processes, as components of synthetic vaccines and in the development of carbohydrate-based drugs. Therefore, these fascinating molecules have been the subject of synthetic studies for decades. However, because of the huge structural variety in both the donor and acceptor building blocks there is no general glycosylation method to forge all possible bonds and it often is difficult, if not impossible, to translate glycosylation methodology from one system to another.

To expediate the synthesis of complex oligosaccharides and analogues a better understanding of the glycosylation reaction is imperative. The glycosylation reaction mechanism balances between S_N1 and S_N2 type substitutions and we will here present how subtle structural changes in carbohydrate building blocks impact the reactivity of the building blocks and as a result the stereochemical course of the glycosylation reaction. Using systematic sets of substrates, we have been able to delineate structure-reactivity-stereoselectivity principles and understand the stereoelectronic effects at play in glycosylation reactions.

These have been applied in the development of effective routes of synthesis to assemble complex bacterial glycans of which several illustrative examples will be presented.



Bibliographic references:

1. Total synthesis and structural studies of zwitterionic Bacteroides fragilis polysaccharide A1 fragments, Z. Wang et al. J. Am. Chem. Soc. 2023, doi.org/10.1021/jacs.3c03976.

2. Mapping the effect of configuration and protecting group pattern on glycosyl acceptor reactivity, J. M.A. van Hengst et al. Chem. Sci. 2023, 1532-1542.

3. Reactivity-Stereoselectivity Mapping for the Assembly of Mycobacterium Marinum Lipooligosaccharides, Hansen et al. Angew. Chem. Int. Ed. 2021, 60, 937-945.

Development and application of boron-mediated aglycon delivery method

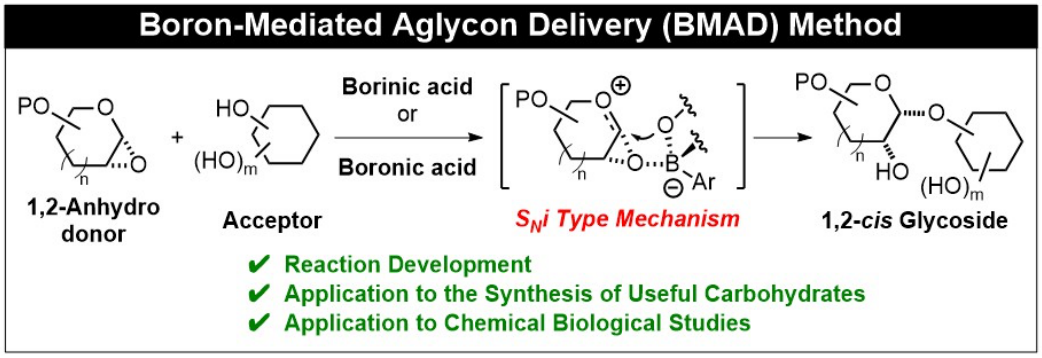
Daisuke TAKAHASHI [1],

[1] Keio University, Yokohama, Japan

dtak@applc.keio.ac.jp

1,2-*cis* Glycosides are found in a variety of biologically active natural products, pharmaceuticals, and highly functional materials. Therefore, there is an urgent need to develop efficient 1,2-*cis*-glycosylation methods to elucidate the precise biological roles and structure-activity relationships of these glycosides, as well as to create new lead compounds for pharmaceutical and/or functional materials by derivatization of these glycosides.

In this context, we have developed a novel 1,2-*cis* stereoselective glycosylation method, named the boron-mediated aglycon delivery (BMAD) method, using organoboron compounds and 1,2-anhydro donors.[1,2] Specifically, we have been working on the development of 1,2-*cis*-stereoselective glycosylations using arylborinic acids and their applications to the synthesis of useful glycosides. In addition, we have been working on the development of boronic acid-catalyzed regio- and 1,2-*cis*-stereoselective glycosylations which simultaneously control not only 1,2-*cis* stereoselectivity but also regioselectivity of the hydroxyl groups in the glycosyl acceptor, and their applications to the synthesis of useful glycosides. Furthermore, recently, we are also working on the application of this method to chemical biological research utilizing this method as a late-stage glycosylation of biologically active natural glycosides.[3] In this talk, I would like to introduce the development and application of the BMAD method, including the latest research.[4]



Bibliographic references:

*[1] Review: *D. Takahashi, M. Tanaka, N. Nishi, *K. Toshima (2017), Carbohydr. Res. (452) 64-77.*
*[2] Review: *D. Takahashi, K. Inaba, *K. Toshima (2022), Carbohydr. Res. (518) 108579.*
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Glycomimetic ligands for mammalian C-type lectins

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Many C-type lectin receptors (CLRs) are essential components of the innate immune system that recognize and bind to pathogen-associated molecular patterns present on the surface of microorganisms. These receptors are also involved in several physiological processes, including cell adhesion, migration, and differentiation. Due to their role in disease and immunity, CLRs have emerged as potential targets for therapeutic intervention. However, the development of CLR-targeting drugs has been hampered by the lack of selective ligands.

In recent years, glycomimetic ligands have emerged as promising candidates for CLR targeting. These molecules are designed to mimic the structure and function of natural glycans and offer advantages over their natural substrates, including increased specificity, stability and bioavailability. Alternatively, allosteric modulation of CLRs has been reported and may offer an additional concept for the development of CLR targeted therapeutics.

I will present our approaches to developing glycomimetic and allosteric ligands for CLRs. Examples from immune cell targeted delivery of therapeutics will be covered, where CLR ligand are conjugated to lipid nanoparticle or other carriers. The ligand binds to the CLR on the target cells, leading to internalization and release of the payload intracellularly. This strategy offers several advantages over conventional drug delivery methods, including increased specificity and reduced toxicity as well as enhanced efficacy.

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Synthetic studies of glycans and glycoconjugates for regulation of immune responses

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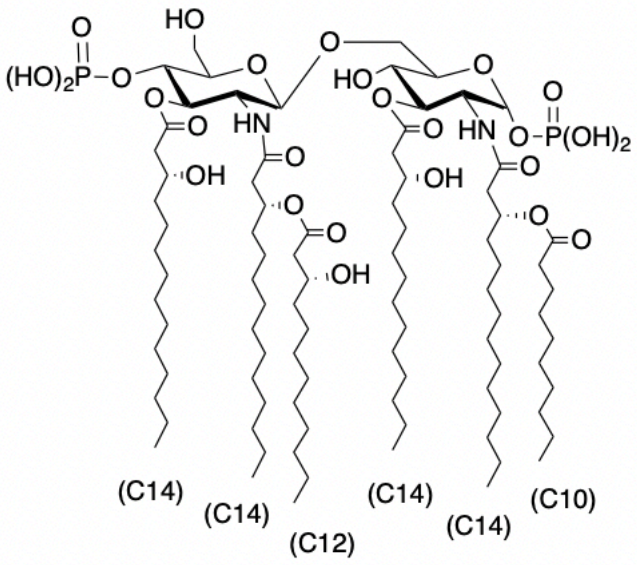
We have examined the synthesis of glycans in microbes and animals to determine the active components responsible for immunostimulatory activities, as well as their application in adjuvants and vaccines.

We have focused on developing new adjuvants using low-toxic LPS, derived from parasitic bacteria as well as the symbiotic Alcaligenes species found in the Peyer's patches, and have conducted synthesis and functional studies on its active constituent, lipid A. Our research has shown that lipid A from the symbiotic A. faecalis possesses mild immunostimulatory effects with low inflammatory properties, making it a promising candidate for a novel adjuvant.[1] Specifically, A. faecalis lipid A was found to activate both mucosal and systemic immunity, and intranasal vaccines containing this adjuvant demonstrated excellent protective effects against pathogens in a mouse model.

Furthermore, we describe the synthesis and biological activity of other low-inflammatory lipid A molecules such as Acetobacter lipid A and Campylobacter jejuni lipid A.

Self-adjuvanted vaccines, in which an antigen and an adjuvant are linked through covalent bonding or physical association, have emerged as a new strategy for vaccine development. We have prepared several self-adjuvanting vaccines that consist of an antigen, adjuvant, and T cell epitope as conjugated, liposomal, and viral capsid mimic vaccines.[2] Their efficacy in producing antibodies in mice with little inflammatory response demonstrates the potency of self-adjuvanted vaccines.



Alcaligenes faecalis lipid A

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Fine-tuning the spike: Rebuilding glycosylation from structure to function by high performance computing

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The extensive glycosylation of fusion proteins, known as glycan shield, is a trademark feature of enveloped viruses, which use it used to hide from the hosts' immune system, facilitating viral pathogenesis. The SARS-CoV-2 spike (S) protein provides a very particular case within this context. S is heavily shielded, yet, because of its mechanism of action, not as effectively as fusion glycoproteins of "evasion strong" enveloped viruses, dangerously exposing its receptor binding domain (RBD) for binding the main receptor ACE2[1].

Understanding the specific functions of the SARS-CoV-2 S unique glycosylation pattern is tricky because the glycans' intrinsic conformational disorder prevents structural characterisation. In this talk I will focus primarily on how we used high-performance computing (HPC) molecular simulations to advance our knowledge on the role of glycosylation in the SARS-CoV-2 infection [2,3]. I will focus in particular on how we identified the unique functional role of the glycan shield in the activation of the S glycoprotein, and on how specific changes in the nature and topology of the glycan shield affect fusion, fine-tuning the S. Furthermore, I will discuss how changes in the glycan shield topology are intertwined with the S evolution [3,4], and have and are increasing SARS-CoV-2 infectivity along the phylogenetic tree by enhancing viral evasion and S fitness or both.

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Chemical synthesis of marine saponins

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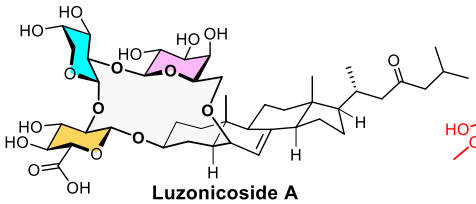
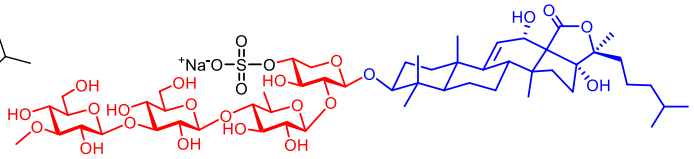
Marine saponins are characteristic metabolites of the slow-moving starfish and sea cucumbers. These complex glycosides of steroids or triterpenes are believed to be the defense chemicals against parasites and predators, therefore are expected to exhibit a wide range of biological and pharmacological properties.

Isolation of sufficient amounts of homogeneous marine saponins from the natural sources is a formidable task, such hampering in depth studies on their activities.

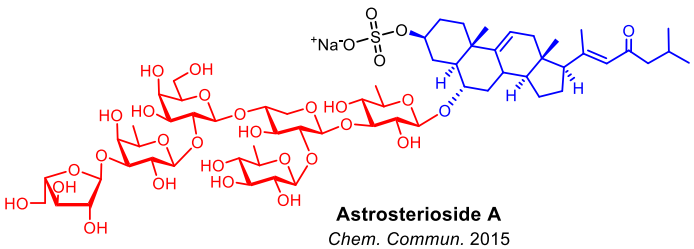
Here I present our long efforts on the chemical synthesis of the major types of these marine natural products, including asterosaponins (e.g., astrosterioside A), polyhydroxysteroid saponins (e.g., linckosides A), cyclic saponins (e.g., luzonicoside A), and sea cucumber saponins (e.g., echinoside A). Highlighted are the stereoselective glycosylation reactions employed in the successful synthesis.



Luzonicoside A
Angew. Chem. 2022



Echinoside A
Angew. Chem. 2017



Astrosterioside A
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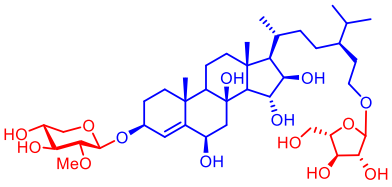

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Application of biocatalysis in carbohydrate chemistry

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Carbohydrate active enzymes provide useful tools for the analysis and synthesis of glycans, and the toolbox of these biocatalysts is expanding in terms of reaction diversity and substrate range. Whilst enzymes have been used widely for native biochemical reactions, it is now increasingly possible to design and engineer biocatalysts that can also be used for non-natural reactions with much wider substrate scope [1,2].

This lecture will discuss a variety of applications of biocatalysts to the synthesis of modified carbohydrates such as protected sugar building blocks [3], deoxy-fluoro sugars [4], imino sugars [5,6] and glycoconjugates such as glycoproteins [7,8] and antibodies [9].

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A genetic entry point to harness the untapped potential in glycobiology

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Breakthroughs in O-glycobiology have gone hand in hand with the development of new technologies, such as advancements in mass spectrometry and the facilitation of genetic engineering in mammalian cell lines. High-throughput glycoproteomics has enabled us to draw a comprehensive map of O-glycosylation and mining this information has supported the discovery of functions related to site-specific O-glycans, such as protection from proteolytic cleavage and modulation of receptor functions. Yet, there is still much to discover.

Among the important next challenges will be to define the contextual functions of glycans in cellular metabolism, host-microbiome interactions, and different stages of cellular differentiation. We have used a genetic entry point and targeted specific glycogenes to generate a library of cell and tissue models that selectively differ in their capacity to produce specific glycan structures.

The engineered libraries can be used to define how glycan-binding proteins recognize glycans in the context of the cellular membrane and how specific glycans impact tissue formation and homeostasis. Furthermore, the engineered cells are instrumental in developing new glycan-based treatments, including potent immunotherapies targeting cancer-associated O-glycans.

Using synthetic oligosaccharides to accelerate the investigation of heparan sulfate biology

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Heparan sulfate is a sulfated polysaccharide that participates in a wide range of physiological and pathophysiological functions. A central issue in the investigation of the biology of heparan sulfate is to determine the contribution of specific sulfate saccharide sequences to the functions. Our laboratory has developed a chemoenzymatic method to synthesize homogeneous oligosaccharides.

The method has been shown for its ability of preparing structurally complex oligosaccharides in gram quantities, providing a new technology for the development of heparan sulfate-based therapeutics. In addition, we use this synthetic approach to prepare ^{13}C-labeled disaccharide and oligosaccharide calibrants to improve the LC-MS/MS-based analysis of heparan sulfate isolated from biological sources. The inclusion of ^{13}C-labeled calibrants permits the quantitation of heparan sulfate. In the presentation, I will discuss how to exploit the anti-inflammatory activity of a synthetic 18-mer (octadeca-saccharide) in mouse models:

1) protecting against acute liver injury caused by drug overdose;

2) preventing organ damage from sepsis.

I will also show an example to connect 3-O-sulfated heparan sulfate, and Alzheimer's disease. The findings offer new lines of evidence for justifying the efforts for developing heparan sulfate-based therapeutics.

Automated Glycan Assembly as enabling technology for the glycosciences

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Automated glycan assembly (AGA)[1] allows for preparation of diverse oligo- and polysaccharides[2] on a solid support employing a synthesizer.[3] Microwave-heating accelerates capping, deprotection and functionalization steps of AGA.[4]

A better understanding of glycosylation reactions is needed in order to optimize coupling steps and thereby shorten overall assembly times. We developed a continuous flow set-up to optimize glycosylations using minimal amounts of material while achieving high reproducibility.[5]

The data obtained using this set-up helped us to quantitate 13 parameters that influence glycosylations and enabled the use of machine learning techniques as a basis for predicting glycosylation outcomes.[6] Currently, reactivity and optimal glycosylation temperatures are correlated in order to further accelerate AGA.[7] Access to ever more complex glycans including cis-linked polysaccharides[8] and complex N-glycans[9]are enabling fundamental investigations into the structure and function of polysaccharide materials,[10] vaccines[11]and diagnostics.

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Interdisciplinary Duo-Keynote and Keynote Lectures

Controlling the C-type lectin receptors in infections & immunity: selective glycomimetic antagonists

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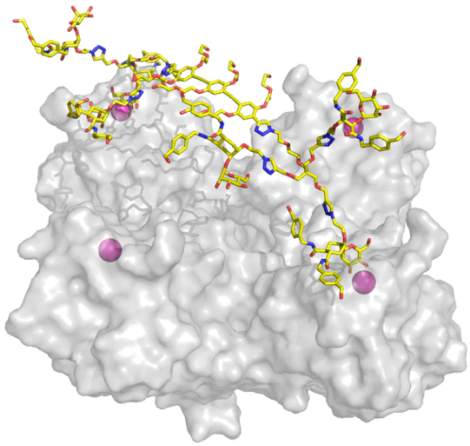
C-type lectin receptors (CLRs) are a large family of Pattern Recognition Receptors, dedicated to the detection of carbohydrate-based motifs, using a Ca^{2+} ion for recognition. Innate immune cells express a variety of CLRs, which shape the immune response, often in cross-talk with TLRs. Some pathogens have found strategies to circumvent CLR's role or even to use them in their infection process. This is the case of several deadly viruses, like HIV and SARS-CoV2. Subversion of several CLRs has been reported, including MGL, L-SIGN and especially DC-SIGN, which is the most widely reported co-receptors facilitating viral infection. During the past 15 years, our groups were engaged to develop glycomimetic ligands able to interfere with DC-SIGN recognition, thus inhibiting its role in viral infections.[1–3]This project took us from the design of glycomimetic able to mimic the monovalent oligosaccharide ligands of DC-SIGN[4], to the refinement of their activity by structural optimization[5] and of their multivalent presentation[6]. Biophysical methods were devised, optimized to produce structure-activity-relationship for the designed mimics and to account for the dynamic and the different topology of the receptors presentation[7,8]. Structures from crystallographic studies were used in the design to increase the affinity and selectivity of ligands for a specific lectin. In the process, we have developed general principles for the selective design of potent glycomimetic ligands for CLRs and tools for their study. This duo presentation will summarize the history of this scientific journey.



One of the possible chelation binding mode of Polyman26 on DC-SIGN tetramer

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Not all Noroviruses like it sweet

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In this talk, we will show how mass spectrometry and NMR spectroscopy complement each other in the study of carbohydrate-protein interactions. In many viral infections, specific carbohydrate-protein interactions play an important role. As an example, we will discuss glycan recognition by noroviruses. Noroviruses belong to the family of *Caliciviridae*, non-enveloped viruses with single-stranded positive-sense RNA. The viral capsid consists of 180 copies of the major capsid protein VP1, which assemble into icosahedral particles that envelop the viral RNA. The binding site for HBGAs is located in the so-called protruding domain of VP1 and has been the subject of many crystal structure studies. However, the binding affinities reported have been inconsistent. At one extreme, mass spectrometry detects binding for the same system while NMR spectroscopy does not.

We will explain the reasons for the observed discrepancies [1] and present reliable and reproducible binding affinities[2]. We will then show how mass spectrometry provides new insights into the glycan-induced structural dynamics of VP1 that are not readily available from other techniques [3]. Our joint studies show that mass spectrometry and NMR spectroscopy provide highly complementary insights into the process of HBGA-binding by norovirus capsid proteins.

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Lectins and lectomes: from structural glycobiology to glycoinformatics

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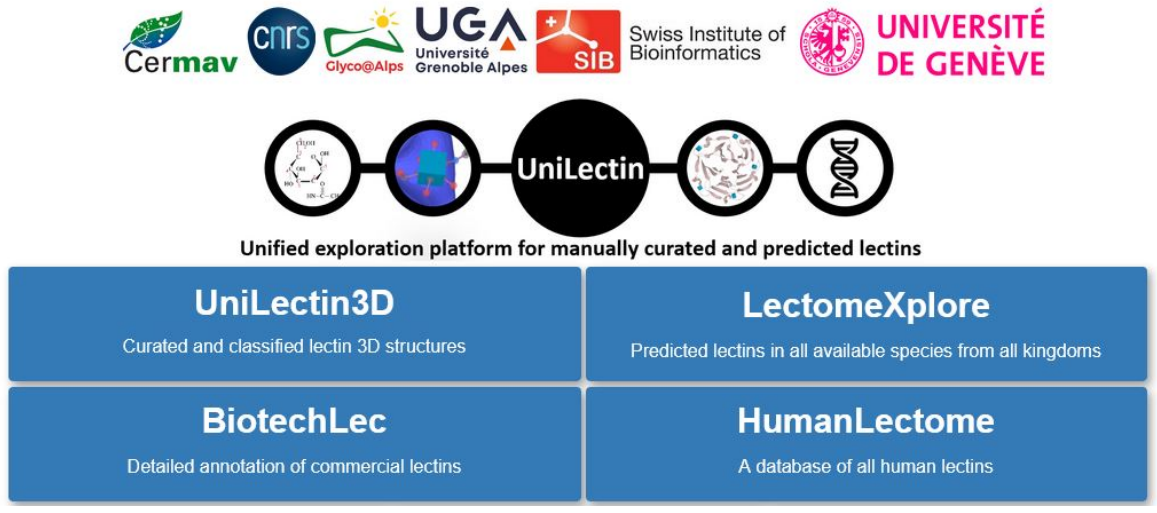
The ability of lectins for deciphering the structural message embedded in complex glycans is a remarkable source of protein fold diversity. Thousands of 3D-structures of lectin-glycan ligand complexes are available from X-ray crystallography and NMR and since 2019, stored in UniLectin3D, a searchable database providing binding information linked to protein sequence and structure as well as glycoscience databases [1]. Despite the interest of identifying new lectins in a larger range of organisms, the poor quality of sequence annotation in databases hinders lectin detection in newly sequenced organisms.

The limited size of functional domains and the low level of sequence similarity challenge usual bioinformatics tools. To meet this type of challenges, our two groups co-develop the UniLectin portal. We first built upon UniLectin3D to define a new structure-based classification, and used the latter to design a sequence-based lectin prediction software.

The result of screening millions of sequences of the NBCI-nr and UniProt databases can be found in LectomeXplore, a database proved invaluable for detecting lectins in fungal or microbial communities (3) or identifying lectins with new functional properties (4). Recently, we launched the HumanLectome database gathering almost 200 curated or putative human lectins, with information of specificity, expression and structure and the BiotechLec guide describing commercial lectins classically used as carbohydrate-recognition tools.



UniLectin portal: logo and modules on homepage (unilectin.unige.ch)

Acknowledgment:

Eurocarb21 thanks the Organic Chemistry Division of the French Chemical Society for its financial support.

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Structural studies to guide glycoconjugate vaccine design

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Glycoconjugate vaccines are an important and successful means for prevention of infectious disease, including pneumoniae, meningitidis and salmonellosis. Understanding at atomic level the binding between microbial carbohydrates and specific functional monoclonal antibodies can direct vaccine design, particularly when synthetic carbohydrates are used. Recently we have applied structural studies to identify the minimal epitope of group B *Streptococcus* type III polysaccharide. (GBS PSIII) is a leading cause of invasive infections in pregnant women, newborns, and elderly people, and the capsule is a major virulence factor targeted for vaccine development [1]. GBS PSIII epitope has been historically considered the prototype of a complex conformational carbohydrate epitope [2]. Through an integrated approach based on competitive ELISA/Surface Plasmon Resonance/Saturation Transfer NMR/X-ray we elucidated a structural epitope consisting of a hexasaccharide constituted of a single repeating unit, and the glucosamine moiety of the next consecutive repeat unit [3]. Based on this data a conjugate vaccine from the short hexasaccharide epitope was prepared and elicited in mice functional antibodies comparably to a polysaccharide conjugate [4]. Likewise, structural studies carried out for serotypes Ia and Ib showed that the polymeric nature of the polysaccharide can strongly impact epitope presentation [5].

A similar approach allowed also to map the structural epitope of *Neisseria meningitidis* serogroup A and X, which are responsible for epidemic meningitis in the sub-Saharan region of Africa, known as meningitis belt [6],[7]. Studies are ongoing to gain this type of information also on structurally similar sialylated W and Y polysaccharides. Structural data can be exploited to guide synthetic carbohydrate vaccine design [8].

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Cell-specific bioorthogonal tagging of glycoproteins

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Alterations in glycoprotein expression and composition are an undisputed corollary of cancer development. Consequently, some of the most faithful tumor biomarkers are heavily glycosylated. Understanding cancer-related changes of the glycoproteome is paramount but hampered by limitations in cellular model systems: Technological advances in mass spectrometry have allowed profiling of glycoproteomes but are often restricted to isolated cells that do not adequately reflect the interaction between tumor and microenvironment. Co-culture systems in vitro or in vivo better reflect the physiological environment but the glycoproteomes of cells from the same host organism cannot be meaningfully discerned to distinguish tumor from associated cells.

Here, we report the development of chemical “Precision Tools” that allow for Bio-Orthogonal Cell-specific Tagging of Glycoproteins (BOCTAG). We equip cells with an artificial metabolic pathway to biosynthesize chemically tagged UDP-GalNAc analogues. Engineered glycosyltransferases accommodate these chemical tags, allowing to selectively study the glycoproteome of transfected cells in the presence of bystander cells. We extensively validate BOCTAG as a strategy for cell-specific imaging in co-culture and to selectively annotate cell-specific glycosylation sites by mass spectrometry. BOCTAG serves to visualize and profile the cancer-specific glycoproteome in co-culture in vivo and in vitro without cell sorting and in secretome, unraveling the importance of glycosylation as a modulator of cellular function.

Recently, we have applied the principles of BOCTAG and other chemical tools to unveil O-GalNAc glycosylation as a modulator of the proteolytic maturation of SARS-CoV-2 spike, majorly influencing the mutational trajectory of variants of concern including Alpha, Delta and Omicron. Our work highlights the outstanding relevance of chemistry for glycobiology.

Acknowledgment:

EMBO Young Investigator Lecture

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Statistical analysis: a new perspective on stereoselective glycosylation reactions

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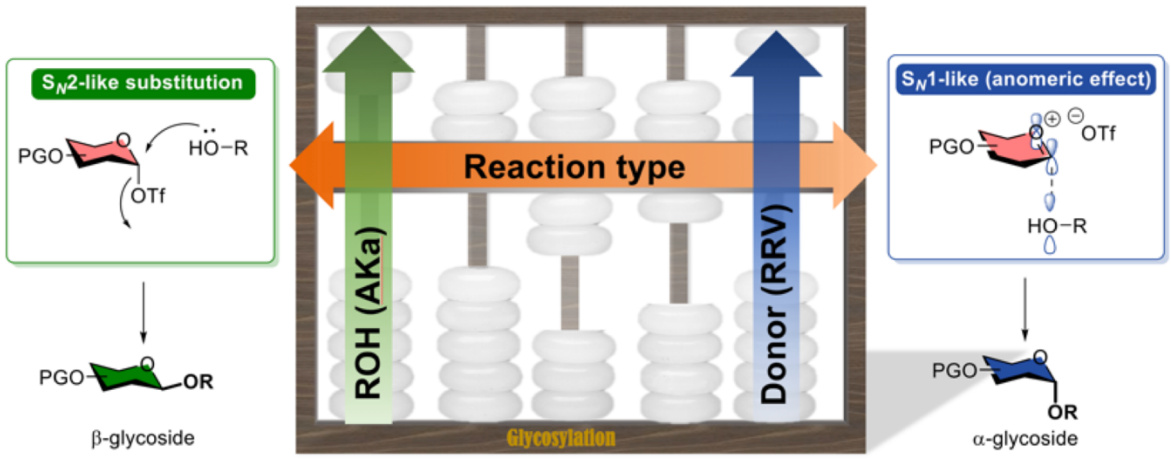
Carbohydrates are ubiquitous biomolecules that mediate numerous biological processes and exhibit important pathogenic effects. However, chemical synthesis of glycoconjugates has been hampered by challenges in glycosylation reaction, of which the stereoselectivity and yield are paramount but unpredictable. We develop a database composed of relative reactivity value (RRV) [1-5] and acceptor nucleophilic constant (AKa)[6] to quantify the reactivity of glycosyl donors and nucleophilicity of hydroxyl groups in glycosylation influenced by the steric, electronic and structural effects, providing a connection between experiments and computer algorithm. A diverse range of glycosylation donors and acceptors with well-defined reactivity and promotors were organized and processed by the designed program "GlycoComputer" for prediction of glycosylation reaction without involving sophisticated computational processing. The applicability of this system was further tested by the synthesis of a Lewis A skeleton to show that the stereoselectivity and yield can be accurately estimated.[6]



A predictive platform for stereoselective glycosylation reactions using RRV and Aka

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Glycans' immunomodulatory role in altered glycosylation: potential therapeutic target and biomarkers

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Our group investigates the role of glycans in immunopathology, focused on diseases such as cancer and congenital disorders of glycosylation (CDG). We identified subgroups of cancer patients with aberrant sialylated glycan profiles associated with poor prognosis and immunosuppression. In triple-negative breast cancer (TNBC), patients expressing sialyl-Tn (STn) have lower overall survival. Moreover, STn+ TNBC cell lines have higher proliferation rates and decreased expression of c-myc, a regulator of proliferation and immune response. Additionally, higher expression of the *ST6GALNAC1* gene in patients positively correlates with infiltration of regulatory T cells and M2 macrophages, which are pro-tumoral and immunosuppressive. Another subgroup of high sialyl Lewis X-expressing TNBC patients also exhibited poor prognosis and higher proliferation rates. Interestingly, an anti-STn mAb that we developed overcomes immunosuppression and leverages the immune response in a preclinical breast cancer model, suggesting glycan blockade's effectiveness as a therapeutic approach in cancer.

Although sialylated glycans associate with poor cancer prognosis and immunosuppression, mechanisms underlying sialic acid deficiency remain unclear. Interestingly, we found increased Th1 profiles in models of sialic acid shortage and in GNE -CDG reinforcing the immunomodulatory role of sialic acid. In PMM2-CDG, where N-glycan defects occur, patients often experience immune-related clinical issues that worsen other symptoms. We identified altered immune-related genes and the MAPK signalling downstream to the TNF-α receptor, which may account for inefficient infections/inflammation control.

Overall, our findings highlight the importance of glycans in immunopathology and identify potential therapeutic targets and biomarkers for developing glycan-targeted therapies.

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Converging technologies in the design and the development of novel aminoglycoside antibiotics

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Evolutionary selection pressures resulted in bacteria able to kill other bacteria by the synthesis of complex carbohydrates. The pharmacological optimization of these natural products has resulted in semisynthetic derivatives that have in some cases been developed into efficacious medicinal products. But no matter how great an antibacterial therapeutic is, evolutionary forces remain in power and bacterial resistance to chemotherapy emerges soon after a new drug is launched, demanding continuous and relentless efforts in designing the next generation of antibacterial carbohydrates. Developing a 21st-century aminoglycoside antibiotic of high clinical utility requires substantial improvements to patient safety and bacterial resistance when compared to existing drugs, without compromising its potent and rapid bactericidal activity, broad-spectrum coverage, or stability. Here, we present a multidimensional approach in aminoglycoside drug design integrating technology platforms that assess or predict activity in a specific host environment, selective affinity for bacterial over mitochondrial ribosomes, drug safety, and evasion of resistance due to aminoglycoside modifying enzymes, RNA methyltransferases, and efflux. The carbohydrate chemistry required to synthesize complex new chemical entities is complemented by chemoenzymatic catalysis and metabolic engineering of biosynthetic pathways to achieve scalability in manufacturing and acceptable cost of goods, warranting availability to patients and communities that are affected the most by drug-resistant bacteria.

From stereocontrolled glycosylation to automated chemical synthesis of glycans

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From the building blocks of nature to disease-battling pharmaceuticals, carbohydrates have had a profound impact on evolution, society, economy, and human health. Numerous applications of these essential biomolecules in many areas of science and technology exist, most of which can be found at the forefront of therapeutic agent and diagnostic platform development. Although carbohydrates are desirable for the pharmaceutical and biomedical communities, these molecules are very challenging targets for chemists because of the need for functionalization, protecting and leaving group manipulations, controlling anomeric stereoselectivity, separation, and analysis. The development of practical methods for the synthesis of building blocks, chemical glycosylation, and glycan assembly represent demanding areas of research.

At the core of this presentation is the development of new methods, strategies, and technologies for chemical synthesis of glycans. These tools will be discussed in light of recent results related to the development of new glycosylation reactions, methods for controlling the stereoselectivity, and HPLC-based automated synthesis. The effectiveness of methods developed will be illustrated by the synthesis of glycopharmaceuticals. This work has been generously supported by the National Institutes of Health and the National Science Foundation.

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Industrial aspects of the polysaccharides business

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Natural polysaccharides are part of the hydrocolloids mainly used in the food industry as additives.

In terms of chemistry, they are made of specific sugars and have complex structures that only nature can build. Many polysaccharides have specific properties – mostly in the texture domain – on top of natural and biodegradable claims explaining why they are so important in our daily life and why they are still growing on the market.

However, they face challenges at the market level but also on technological aspects. The processes to produce them, implement relatively similar concepts regardless of the nature and origin of the polysaccharides. And they finally come in the form of powders which physico-chemistry is complex.

Nowadays, the industry must overcome several challenges like the supply of raw materials for especially in terms of sustainability and the energy costs. It also appears that process operations and polysaccharide powders represent areas of study that are little explored.

The presentation will review all those aspects and will point out the needs for the future.

Stereoselective and visible-light mediated 1,2-cis-α-thio-glycosylation of 2-substituted glycals

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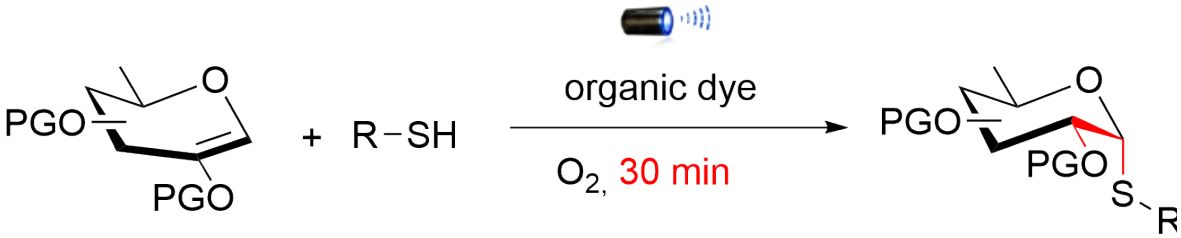
Due to their participation in various vital recognition processes in living systems, carbohydrates have received growing attention in drug[1] and vaccine[2] development. To enhance the drug-like characteristics of carbohydrates, several types of modifications have been introduced, leading to a group of compounds called glycomimetics.[3,4] *S*-Glycosides, in which the native *O*-glycosidic linkage is replaced by an *S*-glycosidic bond, are especially valuable glycomimetics because of their enhanced chemical stability and resistance to glycosidases.[5] One of the major complications inherent in the construction of an *S*-oligosaccharide/glycoside is control of the stereochemistry of a newly formed anomeric linkage.

Herein, we report the synthesis of various 1,2-*cis*-α-thioglycosides using a popular visible-light initiated thiol-en coupling reaction of 2-substituted glycals catalysed by an organic dye. The advantage of this photocatalyzed anti-Markovnikov hydrothiolation reaction initiated by visible-light is that this approach can be realised under mild conditions (low temperature, low loading of photocatalyst) with excellent regio- and stereoselectivity.

Acknowledgement: This work is supported by the Czech Science Foundation (grant No. 23-05805S) and the grant Specific University Research (grant No. A1_FPBT_2021_002).



- high regio- and stereoselectivity
- broad substrate scope
- metal-free
- mild conditions
- moderate to good yields
- fast reaction

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Isolation and total synthesis of microbial rhamnolipid-like surfactants

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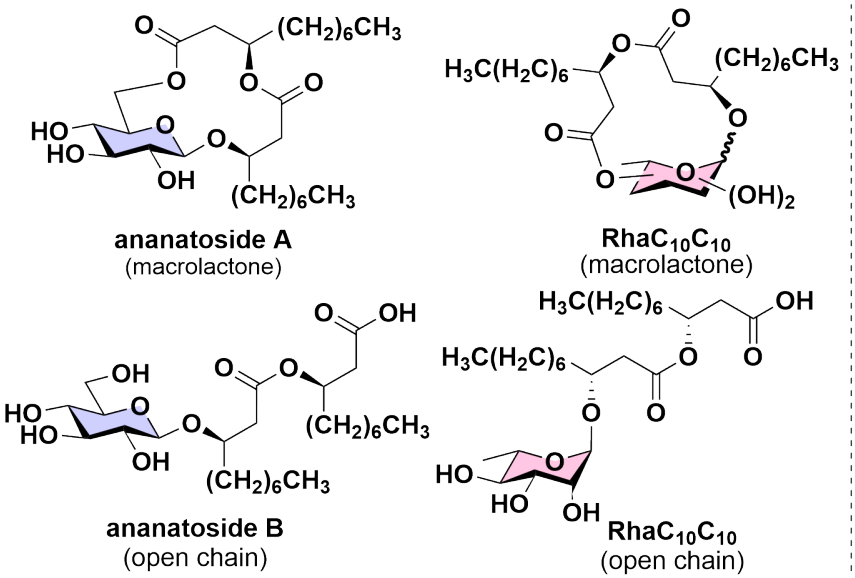
The microbial world represents an unparalleled source of carbohydrate-derived metabolites, showing astonishing structural diversity, biological functions, and therapeutic properties. The long-term goal of our research program is to develop innovative synthetic approaches enabling the total synthesis of structurally challenging and therapeutically relevant microbial glycans along with their mimics.[1] For the choice of our synthetic targets, we have been inspired by *Burkholderia* species, a group of Gram-negative bacteria that have important ecological and therapeutic implications.[2] In this keynote lecture, we will discuss on the isolation, total synthesis, tensioactive properties and biological activity of ananatosides, a novel family of microbial glycolipids that we have recently disclosed. Ananatoside A, a macrodilactone-containing glucolipid, was successfully synthesized through intramolecular glycosylation, and both chemical and enzymatic lactonizations. A series of diasteroisomerically pure, macrolactonized rhamnolipids were also synthesized through intramolecular glycosylation and their anomeric configurations as well as ring conformations were solved using molecular modeling in tandem with NMR studies. Our results suggest that the presence of the macrodilactone ring dramatically interferes with the physical and biological properties of rhamnolipid-like biosurfactants.[3]



- √ *Isolation*
- √ *Total synthesis*
- √ *Molecular modeling*
- √ *Surfactant properties*
- √ *Biological activities*

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Fingerprinting disease by mass spectrometry

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Most diseases affect glycosylation at the tissue as well as serum level. Glycosylation changes are modulating protein function in cancer, infection and immunity. Mass spectrometry is a powerful technique to unravel tissue- and protein-specific glycosylation changes as exemplified for disease- and antigen-specific antibody responses.

Antibodies have key roles in adaptive immune responses. They exert their function via antigen binding as well as effector functions mediated by Fc receptors. Antibody function is strongly influenced by glycosylation of the constant region as well as the antigen-binding region. In the case of immunoglobulin G (IgG), the absence of fucose on the highly conserved N-linked glycan in the IgG-Fc domain strongly enhances IgG binding to Fc gamma receptors and activation of myeloid and NK cells.

Recent studies have shown a massive skewing of antibody glycosylation in infectious diseases and vaccination towards low fucosylation in the case of plasma membrane-associated antigens. In malaria infections, antibodies against parasite-infected erythrocytes show markedly low levels of fucosylation which may be important for development of natural immunity to the parasite. In contrast, in COVID-19 afucosylated IgG responses may induce aggravated immunopathology.

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glAIcobiology

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Computational research on glycans is frequently hampered by the sparsity, complexity, and diversity of glycan-related data. This issue is further compounded by the realization that, despite the dawn of Big Data, most glycan-related projects only yield rather modest quantities of data, compared to other systems biology disciplines.

We thus present a new paradigm of AI-aided glycoinformatics, with the ultimate aim of dispensing with the need for large datasets for functional inference. The key insight of, now widely used, models in other fields, such as AlphaFold or ESM, was that large, pre-trained models can be used to distil meaning from small datasets, or even single sequences in the case of AlphaFold.

Similarly, we argue that large, pre-trained models in glycobiology will substantially advance our understanding of glycan function. Concretely, we will present examples of such large AI models to elucidate, at scale: glycan measurement, glycan biosynthesis, and glycan function. All these efforts are fully open-access and facilitated by our glycoinformatics platform glycowork as well as numerous curated datasets for these purposes.

Overall, these advances clearly demonstrate that the field already contains sufficient data, albeit in a very scattered manner, to prepare large, pre-trained AI models that can then be used to accelerate progress in glycobiology.

Developing biotherapeutic strategies to target glycosaminoglycans in immune-cell mediated diseases

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Glycosaminoglycans (GAGs) are major mediators of cell-cell communications in healthy and pathological processes. These carbohydrates exert their function typically by binding, localising and activating signalling molecules like growth factors and cytokines by which various cell types like immune and cancer cells, but also pathogens like viruses and bacteria, are directed to their place of action.

We are therapeutically targeting the interface of GAG-binding proteins by engineered chemokines, by monoclonal antibodies as well as by natural GAGs and GAG mimetics [1].

We will present data relating to the inhibitory potential of our biologics in the context of metastatis formation, solid tumor growth, and viral infection. A drug development point of view will be exploited by correlating *in vitro* pharmacology - like protein-drug affinities and GAG pattern recognition specificity [2] - with *in vivo* pharmacology, like serum halflife, exposure and bioavailability [3]. An outlook on the future of GAG drugability by biologics will be given.

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Catalytic approaches for a site-selective functionalization of sugars

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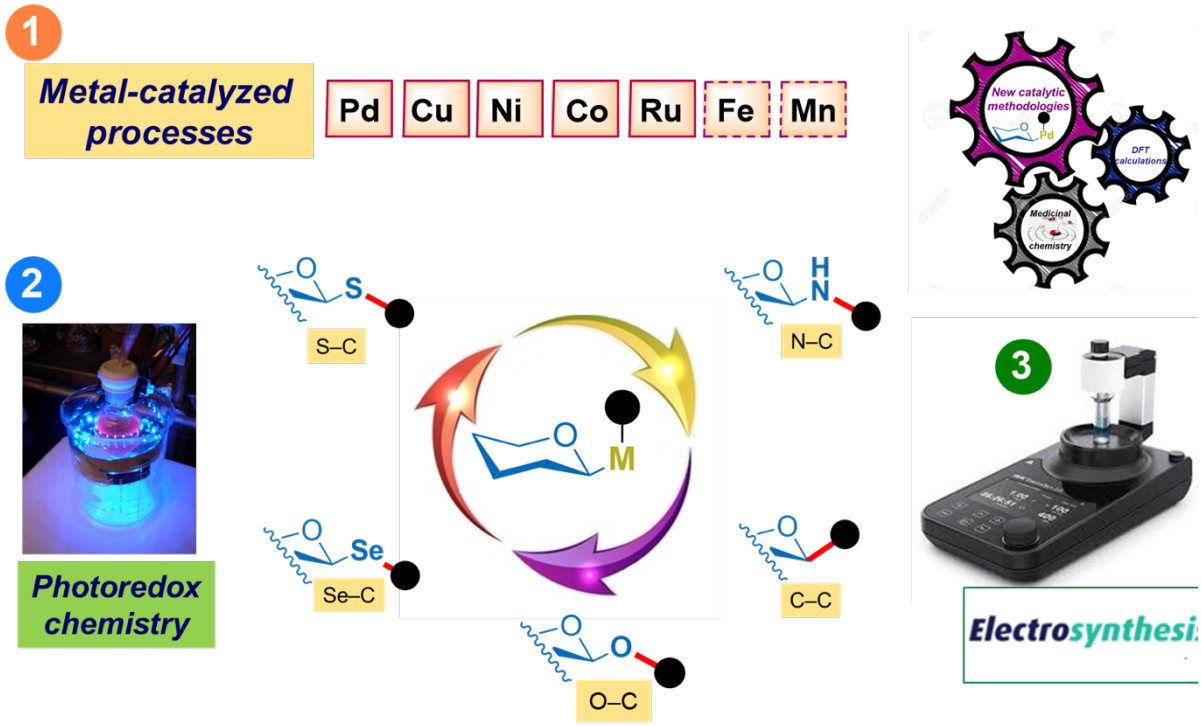
The glycosylation reaction is one of the most important and well known method to functionalize sugars. In this context, a significant research has been directed toward the development of new glycosylation methodologies. However, designing new transition metal-catalyzed transformations in glycochemistry is still one of the highly challenging task. In fact, the translation of scientifically well-established cross-coupling reactions to sugars often does not lead to the desired products.

Over the past few years, our laboratory has been embarked in the development of selective catalytic approaches to functionalize sugars regardless of the protecting groups and the nature of the sugar. To this end, different strategies were explored including (*i*) transition metal-catalyzed cross-coupling transformations, (*ii*) C-H activation processes with or without the use of a directing group, (*iii*) photoredox dual catalysis as well as (*iv*) electrocatalysis (Figure 1).

In this presentation, we will give an overview of our synthetic methodologies (1) related to C-C and C-heteroatom bond forming reactions, and we will discuss some applications in medicinal chemistry programs.



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Synthesis for Biomimesis: Modified Cyclodextrins as capsid proteins or molecular motors

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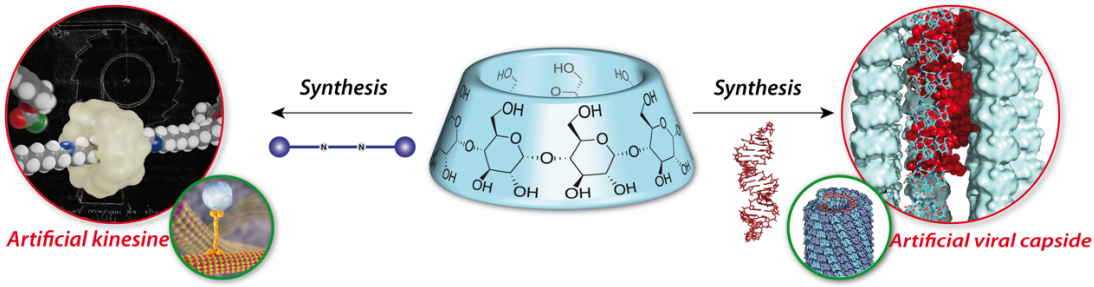
Cyclodextrins are cyclic oligosaccharides possessing a cavity used in our daily life as deodorants, excipients or in chiral stationary phases. In these applications, they are unfunctionalized or randomly functionalized. The concept of "artificial enzyme" has been proposed by Breslow using these cavitand molecules [1] assimilating their cavity to the active site of an enzyme.

However, a bottle-neck for their development was the lack of efficient functionalization. Over the years, we delineated several strategies to access poly-hetero-functionalized cyclodextrins.[2] The ability to place a function anywhere on a complex cavitand allowed us to mimic proteins. Hence we could add reactive center to imitate metallo-enzymes.[3] We also conferred the ability to self-assemble around DNA as capsid proteins[4] and we designed a kinesin-like molecular motor[5]...



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Structural studies of O-specific polysaccharides of the genera Pectobacterium and Dickeya bacteria

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Pectinolytic bacteria cause blackleg and soft rot diseases of vegetables, crops, and ornamental plants worldwide. Among the most damaging agents of these diseases are bacteria of the genera *Pectobacterium* and *Dickeya* belonging to the family *Pectobacteriaceae*.

The development of the mentioned diseases and the induction of their symptoms depend on several factors, including the production and secretion of plant cell wall-degrading enzymes, as well as the production of siderophore, biofilm and lipopolysaccharide (LPS). LPS is the major component of the outer membrane of Gram-negative bacteria. LPS consists of lipid A, a core oligosaccharide, and an O-specific polysaccharide (OPS). OPS is a polymer with highly structurally variable repeating oligosaccharide subunits located in the outermost part of the LPS molecule. As one of the most exposed elements of the cell wall, it plays an important role in the interaction of the bacterial cell with its environment.

The aim of the study was to investigate the structural variability of the OPSs of different strains of *Pectobacterium* and *Dickeya* spp [1-3].

Increasing our knowledge of the chemical structure of the OPS of different bacteria of the family Pectobacteriaceae may help to explain the details of the interaction between pathogen and plant host but can also be used for species identification.

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Antibody production in plants: advances and challenges in glycoengineering

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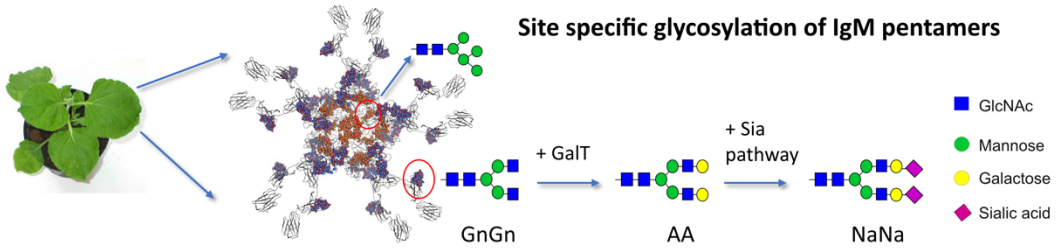
Monoclonal antibodies (mAbs) are amongst the most important biopharmaceutical products and demands are increasing. One reason for the need of large product quantities (several tons /year) is the generation of products with suboptimal efficacies. A possibility to approach this shortcoming is the expression of mAbs with optimized glycosylation profiles.

In line, recent serological profiling suggests that combatting infections requires a bouquet of Ab isotypes and subclasses, with glycosylation playing a central role1,2. Notably, while IgGs carry only one glycosite (GS), the glycosylation status of other Abs, like IgM and IgA is more complex, carrying up to 7 GSs and site specific glycosylation. This makes its controlled production difficult.

The ability of plants to produce mAbs with targeted glycosylation has been demonstrated in multiple studies3. A modular cloning and expression toolbox, which consists of single and multi gene expression vectors and glycoengineered production hosts, was developped3. The versatile approach enables (i) rapid Ab iso- and subtype switch and (ii) the generation of mAbs with engineered glycosylation profiles4,5.

The starting point is a glycoengineered tobacco related Nicotiana benthamiana line that generates GlcNAc-terminated N-glycan structures (GnGn), a conserved N-linked glycoform in higher eukaryotes. This structure is then subsequently modified by the coexpression of respective glycosylation enzymes, e.g. β1,4 galactosyltransferase (GalT) for the generation of galactosylated (AA), and the sialylation pathway (Sia) for sialylated (NaNa) structures. Advances and challenges in the expression of various mAbs, including multimeric IgA and IgM glyco-variants, are discussed.



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C-glucosylation, the miracle for preventing polyphenol pan-assay interference compound behavior

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Polyphenol health benefits are worldwide recognized [1] but many such products are Pan-Assay Interference Compounds (PAINS). They are bioactive but react non-specifically with various targets instead of acting on one specific target, thus giving false positive results in cell-based biological evaluations. We disclose now our results demonstrating that *C*-glucosylation is a synthetic tool generating compounds which do not induce changes in cell membrane dipole potential and are able to prevent the threat of polyphenol PAINS behavior, based on the established fact that altered membrane dipole potential translates into changes in transmembrane protein conformation and function. By investigating the planar lipophilic polyphenols resveratrol, genistein and phloretin, we synthesized their *C*-glucosyl derivatives and obtained polyphenols that are not able to modify dipole membrane, thus preventing their aglycone PAINS behavior.

We also discovered that *C*-glucosylation may increase polyphenol bioactivity and sometimes selectivity, as well as polyphenol bioavailability, as shown with antidiabetic glucosyldihydrochalcones, and with antioxidant glucosylflavones, some of which found to rescue neurons from β-amyloid (Aβ) toxicity, some block A_{β}-induced Fyn kinase activation and decrease derived Tau hyperphosphorylation, others disrupt Prion (PrP^{C})-Aβ oligomers interactions, which are key for the Aβ-induced neurodegeneration. We will also disclose their pharmacokinetic properties, in some of them ideal for further developments, aiming to reach new candidates for the treatment of Alzheimer's disease [2] which is unfortunately still uncurable.

We hope, with this presentation, to motivate glycoscientists to pursue methodologies for polyphenol *C*-glycosylation, a new technology towards liberating polyphenols from PAINS behavior, and generating more potent, selective, less toxic and more bioavailable polyphenols than their aglycones.

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Development of glycomimetics for targeting human immune lectins

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Lectins on the surface of human immune cells are a key element of the innate immune surveillance where they constantly sample potential antigens from their environment and present it to the adaptive immune system. Therefore, targeting certain C-type lectin receptors has been found to be a promising avenue for improving vaccine efficacy.

Certain pathogens have taken advantage of this internalization pathway to infect immune cells and tumor cells have developed mechanisms to suppress recognition by immune cells by overexpressing self-glycans that bind certain immune cells. Taken together immune lectins have become important target receptors for the development of vaccine adjuvants and cancer therapies and for the treatment of autoimmune diseases.

Here we report on our current strategies for the development of glycan mimetics to target several immune lectins with higher affinity and selectivity than natural glycans involving chemo enzymatic synthesis of glycan scaffolds, library generation via click chemistry, high throughput screening and determination of binding constants of selected hits.

Interdisciplinary Duo-Oral and Oral Lectures

Glow in the dark

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The field of chemical biology has been transformed thanks to the development of click and bioorthogonal chemistry. This particular branch of chemistry, where two partners specifically react together in complex biological media, allowed researchers to reach until now new biomolecules through the chemical reporter strategy, as exemplified by the emergence of the metabolic glycan engineering (MGE). The toolbox for *in vivo* imaging has never expanded wider and faster, but the cell compartment and the complexity of the whole organism aim to prevent the use of some strategies that would yet be suited for other targets. Over the past few years, our both teams have combined efforts to decipher the functions played by *O*-GlcNAcylation, a structurally very simple but functionally extremely complex post-translational modification (PTM). This difficult study is partly due to the fact that *O*-GlcNAcylation is very labile and occurs at all levels of cellular homeostasis. During this Interdisciplinary Duo Communication, we will present our most important results including: (i) the development of a biosensor to better understand the dynamics of *O*-GlcNAcylation on the proto-oncoprotein b-catenin, (ii) the importance of a reasoned choice of the chemical reporter, (iii) the great challenge for genetic code expansion for tailoring protein structure.

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Using large cohorts and CRISPR/dCas9 epitoolbox to identify & validate genes regulating IgG glycome

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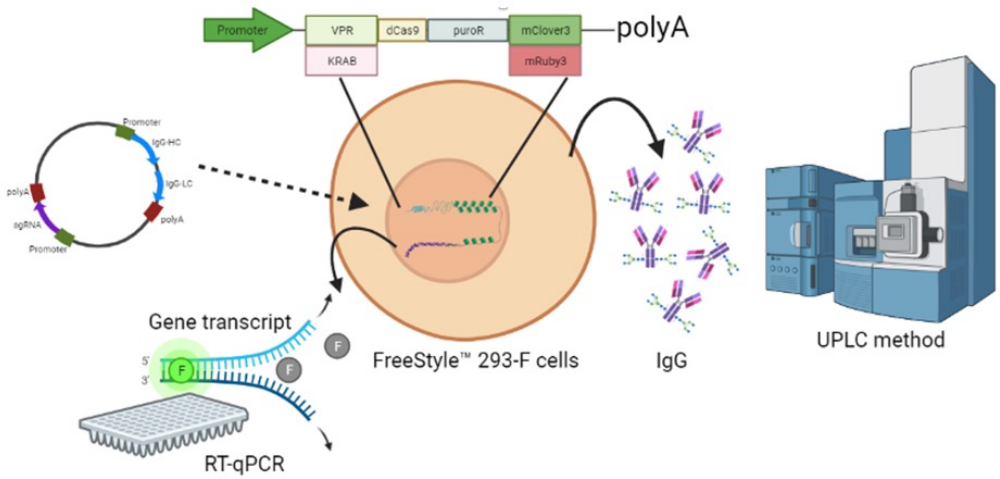
Glycosylation of IgG is an essential regulator of the immune system, but our understanding of this process is still limited. Significant efforts have been invested in studying the role of glycosyltransferases in IgG glycosylation, but today it is clear that enzyme expression is not the key regulatory mechanism. By performing large genome-wide association studies (GWAS) we mapped a network of over 40 genes that associate with IgG glycome composition and are in pleiotropy with different inflammatory diseases.

These includes many transcriptional factors, immune specific signalling proteins, chromatin regulators, solute carriers, cytoskeleton and Golgi and ER associated proteins. However, GWAS is just a hypothesis generating tool, thus GWAS hits need to be functionally validated. To approach the functional role of GWAS hits in IgG glycosylation we developed a transient expression system HEK293FreeStyle with stably integrated CRISPS/dCas9 system in the cell genome. The *in vitro*system enables manipulation of a gene of interest and subsequent analysis of the effect of gene activation/silencing on IgG glycan phenotype in a single experiment. We have also developed a new method to evaluate glycosylation of both Fab and Fc regions of recombinant IgG at the same time. Proof of principle for this approach was provided by analysing effects of several GWAS hits, including genes downstream of the oestrogen receptor, on glycosylation of IgG *in vitro*.



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Using Molecular Modelling to Rationalise Cross-Reactivity in the Development of Multivalent Vaccines

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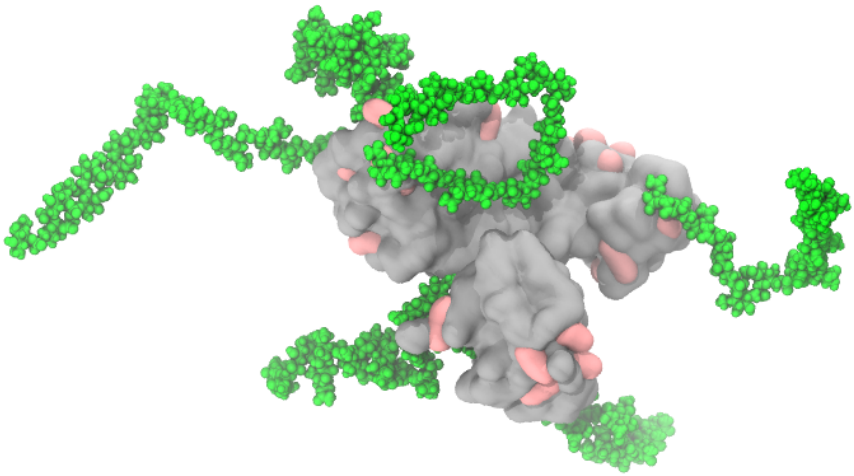
Licensed conjugate vaccines have demonstrated efficacy in preventing bacterial disease caused by *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* (Nm) and *Streptococcus pneumoniae* (Sp). The presence of multiple and emerging strains of bacteria has required the development of costly multivalent vaccines unless the antigens present elicit an immune response that provides cross-protection against infection by non-vaccine serotypes or serogroups. However, cross-protection is hard to predict, as structural similarity between carbohydrate antigens has not proven to be a reliable indicator of cross-protection. Over a series of studies, we have demonstrated that molecular modelling of structurally similar bacterial carbohydrate antigens can provide a mechanistic rationale for the existence (or absence) of cross-protection between them and thus may usefully inform the development of conjugate vaccines and further our understanding of carbohydrate immunogenicity.

The case studies comprise molecular modeling of the capsular polysaccharides for meningococcal serogroups (B and C, Y and W, A and X), key pneumococcal serotypes within serogroups 6, 15, 19 and 23 and *Haemophilus influenzae* (types a and b). Conformation as well as identification of key epitopes in the antigens, such as terminal residues and substituents, may aid in broadening vaccine coverage.



A model of CRM197 protein conjugated to 5 chains of Neisseria meningitidis serogroup A polysaccharide antigen

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How glycosidases operate: combining crystallography and quantum mechanics to uncover new mechanisms

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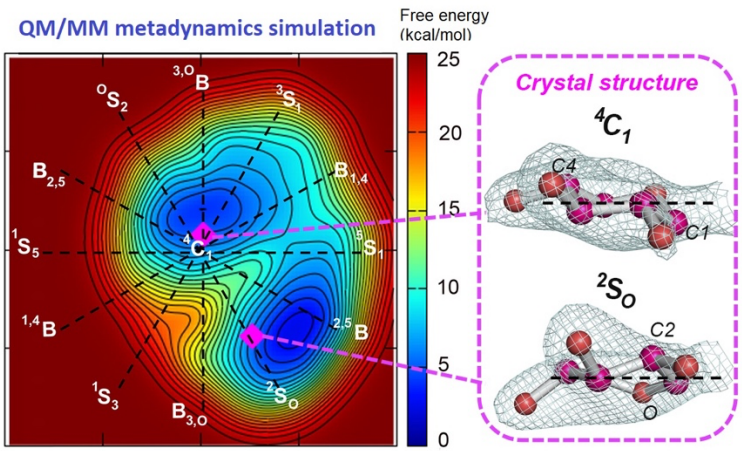
Glycosidases (GHs) are amongst the most proficient of enzymes, showing typical rate enhancements of up to 10^{17}-fold. Large efforts have been devoted since many decades to uncover their catalytic mechanisms. Most GHs follow the Koshland classical mechanisms for retaining and inverting glycosidases, in which two essential carboxylic acid residues catalyze the cleavage of the glycosidic bond. It is also recognized that GHs favor certain distorted sugar shapes or conformations upon binding to the enzyme [1,2]. However, there are notorious exceptions such as GHs following neighboring group participation and GHs with uncommon (e.g. Tyr, Cys) or yet uncharacterized nucleophiles, for which the molecular mechanisms remain elusive. Recently, we have uncovered the molecular mechanisms of GHs that either follow non-Koshland mechanisms or challenge the common view of the enzyme recognizing high energy substrate conformations. In this dual oral communication, we will bring our new discoveries which were based on both state-of-art experiments (discussion led by Mariana Morais) and theoretical calculations (discussion led by Carme Rovira).We will report the detailed molecular mechanism of two GHs with uncommon active site nucleophile residues [3,4], as well as exo-acting GHs that, unlike their endo-counterparts, do not require substrate distortion for catalysis [5,6]. We will also highlight how the enzymatic microenvironment can influence the substrate distortion [7], including new findings of GH families with distinct active-site topologies.



Conformations adopted by the xylopyranosyl ring at the −1 subsite of GH43 exo-oligoxylanase as showed by simulations and X-ray crystallography [8]

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Macromolecular glycan mimetics to fight virus infections

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Virus infections represent a major health threat and are associated with a significant socioeconomic burden. While vaccination is the most effective prevention strategy; cost and availability have limited their uptake in some instances.

One promising approach to fight viral infections is by targeting the entry into host cells, which is a critical step of the infection process. It is well-known, that this first step of infection is often mediated by pathogen engagement with host glycans, glycoconjugates, and polysaccharides. Host cell surfaces are decorated with a dense layer of carbohydrate structures known as the glycocalyx, thus offering a multitude of potential interaction partners for the pathogen. Hence, synthetic mimetics of the glycocalyx, such as glycopolymers or glycomacromolecules have gained increasing attention as molecular decoys that can block pathogen-host cell interactions via competitive inhibition.

Here, we present our recent work on the synthesis of defined mimetics of different components of the glycocalyx for pathogen targeting. Inspired by nature, we developed highly sulfated glycopolymers that act at glycosaminoglycan (GAG) mimetics and show efficient inhibition of viruses, such as Herpes Simplex Virus (HSV), Influenza A Virus (IAV), and Merkel Cell Polyomavirus (MCPyV) among others.[1,2] We have also used our established solid phase polymer synthesis, to design and synthesize sequence-defined glycooligomers mimicking matriglycan, a glycopolymer subunit of α-dystroglycan, which is a well-established receptor for arena viruses including the Lassa virus.

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Development of divalent LecA ligands as antivirulence agents against Pseudomonas aeruginosa

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The Gram-negative bacterium *Pseudomonas aeruginosa* is a critical threat for mankind. Chronic infections are characterized by biofilm formation, a major virulence factor of P. aeruginosa, which leads to extensive drug resistance. The tetrameric *P. aeruginosa* lectin LecA is a virulence factor and an anti-biofilm drug target. Increasing the overall binding affinity by multivalent presentation of binding epitopes can enhance the weak carbohydrate–ligand interactions. Low-nanomolar divalent LecA inhibitors with up to 260-fold valency-normalized potency boost and excellent selectivity over human galectin-1 were synthesized from D-galactose pentaacetate and benzaldehyde-based linkers in four linear steps. However, these molecules displayed an intrinsic pH-dependent chemical instability due to their design for dynamic combinatorial assembly, and furthermore, a very low aqueous solubility. Therefore, the acylhydrazone linking motif was isosterically replaced with a more stable amide bond and the linking unit between two galactosides was also varied. The resulting optimized divalent LecA ligands retained low-nanomolar binding affinities, showed improved metabolic stability and were up to 1000-fold more soluble. The lead compound inhibited LecA adhesion to lung cells, restored wound closure in a scratch assay and reduced *P. aeruginosa* invasiveness to host cells *in-vitro*.

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Unique “fluorine-codes” for glycan binding proteins in a multi-site fluorinated LewisX library

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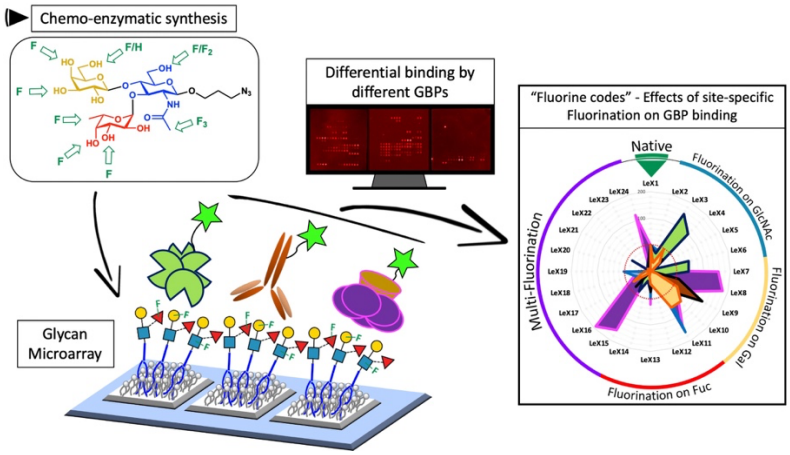
Glycans play many important biological roles, from cellular signalling to pathogen-host interactions. A glycan epitope can be recognized and shared by a diverse range of endogenous and exogenous glycan-binding proteins (GBPs) of humans and microbes. For example, the blood group-related LewisX (LeX) antigen was originally reported as a developmentally regulated embryonic antigen[1], but is also found in epithelia, blood cells and human milk, also on the surface of pathogens such as *Helicobacter pylori* where it is recognised by lectins of immune system and triggers immune responses[2]. Such “promiscuity” in glycan recognition raises the need for GBP-specific molecular probes that can target and intervene in specific glycan binding events.

Glycan fluorination offers great promise for introducing selectivity and distinction between proteins that recognise a particular glycan. With little change to overall glycan conformation, an OH to F replacement can have a range of attractive and repulsive effects on protein recognition[3]. Here we present a diversity-orientated chemo-enzymatic synthesis of a 150-member library of LeX trisaccharides with site-specific fluorination on each monosaccharide residue. A 24-member subset was converted to neoglycolipid probes[4] by a novel click-chemistry method for microarray screening studies with immune lectins, antibodies and bacterial toxins. Unique “fluorine-codes” have emerged for different LeX-binding proteins. The findings could potentially lead to designs of novel F-glycan-based diagnostic and therapeutic agents for bacterial infection.



This work was supported by BBSRC-Industrial Biotech. Catalyst Project “Chemo-enzymatic Production of Specialty Glycans” Wellcome Trust “Glycan microarrays for a new era of diagnostic tools” grants

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Evaluation of 8-azido-Kdo incorporation in LPS in gram-negative bacteria

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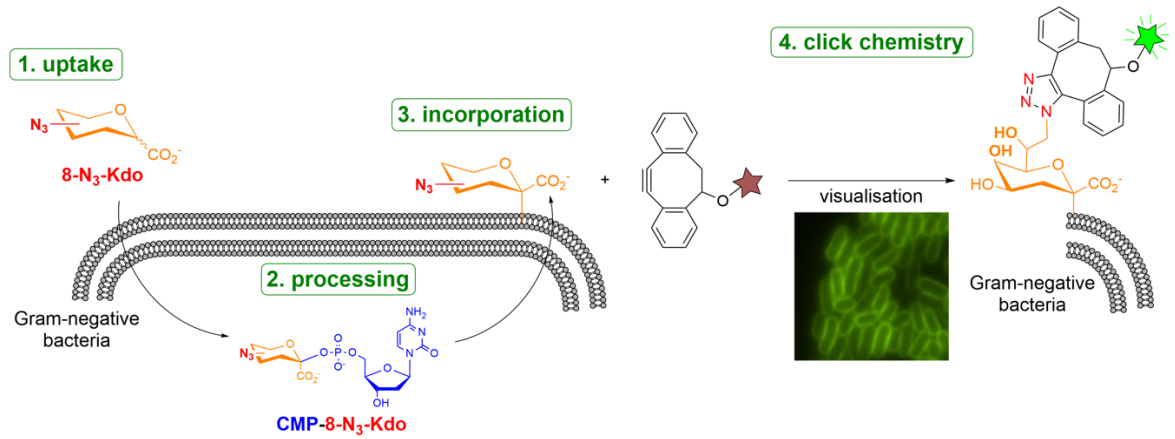
Metabolic Oligosaccharide Engineering[1] coupled with click-chemistry is a powerful and increasingly applied method to investigate cell components, including carbohydrates and glycoconjugates. Within this area, the 8-azido derivative of the bacterial sugar 3-deoxy-d-*manno*-oct-2-ulosonic acid (8-N_3-Kdo) has gained value in selective labeling of lipopolysaccharides (LPS) that are a key component of the outer membrane of Gram-negative bacteria. Several studies have reported that 8-N_3-Kdo is successful in labeling LPS of several Gram-negatives such as non-pathogenic and pathogenic *Escherichia coli* strains, *Salmonella typhimurium*, *Legionella pneumophila* and *Myxococcus xanthus*.[2,3]

Due to its increasing application in the investigation of LPS biosynthesis and cell surface labeling, we became interested in exploring the nature and efficiency of LPS labeling using 8-N_3-Kdo in a variety of Gram-negative bacteria. First, we optimized the synthesis of 8-N_3-Kdo, which was subsequently used to metabolically label Gram-negative bacteria, mainly focusing on non-pathogenic and pathogenic *E. coli* strains. Interestingly, different extents of labeling were observed, and the majority of labeled LPS appears to be the 'rough' LPS variant. In this communication, our optimized synthesis route of 8-N_3-Kdo, our findings on the extent of LPS labeling across species and a characterization of the labeled LPS structures will be presented.



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Full stereocontrol of alpha-glycosidations of sialic acid and Kdo by macrobicyclic glycosyl donors

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Sialic acid and Kdo (3-deoxy-D-*manno*-2-octulosonic acid) belong to the class of 3-deoxy-2-ketoaldonic acid and share common structural features, which make their stereoselective glycosidation difficult. The oxocarbenium ion intermediates of the both are unstable owing to the anomeric carboxyl group and vulnerable to decomposition via 1,2-elimination, which is enhanced by the 3-deoxy structure. The absence of a hydroxyl group at the position adjacent to the anomeric center prevents neighboring participation in the stereocontrol. Recently, we reported that macrobicyclic sialyl donors, which were tethered at the anomeric carboxyl group and the C5 amino group, enabled the fully alpha-selective sialylation that was unaffected by substrate structures and reaction conditions.[1] This method ensured the direct sialylation of oligosaccharides and glycolipids in high yields,[2] suggesting the potential of this method to rewrite the synthetic scheme of sialoglycans. Very recently, we demonstrated that macrobicyclic Kdo donors with alpha-configuration allowed for the full stereocontrol in the alpha-glycosidation.[3] This method facilitated the stereoselective synthesis of the dimeric and trimeric Kdos found in lipopolysaccharide of pathogenic bacteria.

In this presentation, I will share our recent results on the alpha-glycosidations of sialic acid and Kdo using bicyclic donors and their application to the synthesis of highly complex glycans and functionalized probes.

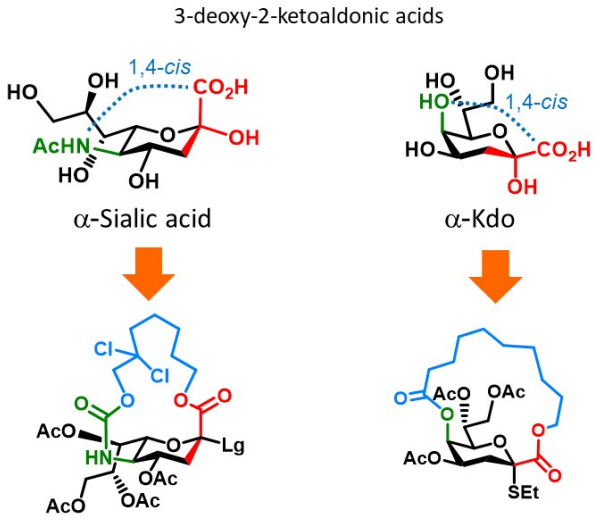

Fig. Macrobicyclic glycosyl donors of sialic acid and Kdo

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Competing reactive intermediates in stereoselective glycosylation reactions

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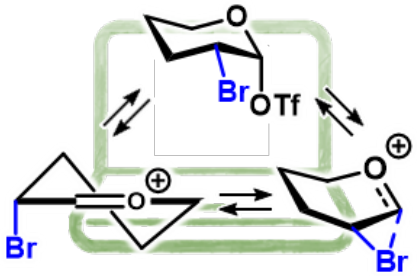
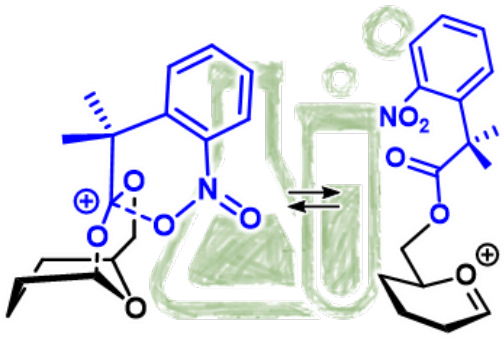
The stereoselectivity of the glycosylation reaction strongly depends on which reactive intermediates can form from the parent glycosyl donor.[1] These reactive intermediates generally can rapidly interconvert and each intermediate participates in a unique reaction pathway. The competition between the various pathways dictates the overall stereoselectivity of the glycosylation reaction.[2] We have studied these scenarios through a combinatory approach of experimental and computational chemistry. We will here present our recent results on the stereodirecting effect of C-2-halogens. Upon activation of 2-halo glycosyl donors oxocarbenium ions and halonium ions may form. The ratio between these cations depends on the hyperconjugative capabilities of the halogen and the ring strain in the halonium ions.[3] We also report on our studies on glycosyl donors bearing distal acyl groups. [4] These donors can form both oxocarbenium ions and bridged dioxolenium ions and the competition between these drives the stereoselectivity of the reaction.[5] The stereodirecting capacity of these groups can be further tuned through functionalization. The 2,2-dimethyl-2-(*ortho*-nitrophenyl)acetyl protecting group provides stereoselective reactions through the formation of dioxolenium ions stabilized by nitro-participation. These 'double participation' dioxolenium ions are in competition by direct stabilization of the oxocarbenium ion by the nitro-moiety.[6] The characterization of these competitive reaction pathways will enable the rational design of synthesis routes towards complex oligosaccharides.





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Study of $SnCl_4$ promoted anomerisation via endocyclic cleavage using LFERs and DFT calculations

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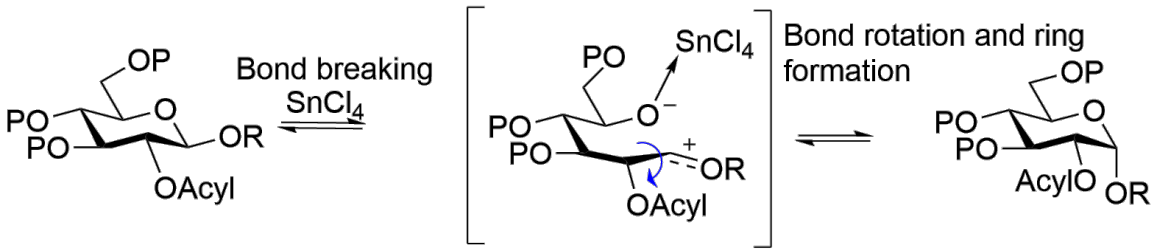
Anomerisation is used to convert equatorial glycosides to axial glycosides and the mechanism is proposed to involve endocyclic cleavage, followed by a bond rotation and ring reformation step. Rates of $SnCl_4$ promoted anomerisation were determined for 14 glucopyranosides, 12 glucuronic acid and 16 galacturonic acid derivatives to deduce the influence of 2-O-acyl groups on reactivity. Linear correlations for 2-O-benzoyl derivatives in plots of log k_f+k_r vs Hammett sigma$^+$ parameters were observed. Increasing the size of aliphatic 2-O-acyl groups led to an increase in rate for galactopyranosiduronic acid and glucopyranose, but not for glucopyranosiduronic acid. Plots of steric parameters such as Pavelich-Taft E_S or Verloop's Sterimol B1 vs log k_f+k_r indicate a correlation between size and rate. Taft's polarizability parameter sigma$_a$ was correlated with log k_f+k_r, for aliphatic substituents and may indicate that increased polarizability, which is related to the size of the aliphatic substituent is consistent with an increase in reactivity and could contribute. DFT study has been used to establish mechanistic pathways for the glucopyranosides and glucuronic acid. The pathways first involve carbocation intermediate formation followed by a high energy bond rotation step, likely to be rate determining. Contributions from 2-acyl group participation are evident but the protecting group role differs depending on the reacting glycoside. These data and mechanistic pathway will be discussed along with relative energies of intermediates and transition states.



Anomerisation of glucopyranoside

PM thanks Science Foundation Ireland for Investigator Award Funding and ICHEC for access to computing resources. We th
Elisa Fadda for preliminary calculations and discussion.

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CD44 glycoproteogenomics towards bladder cancer precision medicine and glycovaccines design

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Bladder cancer (BC) demands novel molecular targets for precision medicine. CD44 is a transmembrane glycoprotein linked to most cancer hallmarks. However, significant alternative splicing and multiple glycosylation generate a myriad of glycoproteoforms with potentially distinct functional roles, which remain mostly uncharacterized due to the lack of appropriate analytical tools. Transcriptome analysis of a large patient cohort showed remarkable CD44 isoforms heterogeneity and association between short CD44 standard splicing isoform (CD44s), invasion and poor prognosis. CD44 was also found carrying abnormal short-chain *O*-glycosylation not observed in healthy tissues. Glycoproteogenomics allowed, for the first time, the identification of clinically relevant glycoproteoforms by mass spectrometry. The link between abnormal CD44s glycosylation and invasion was confirmed in vitro, supporting findings from BC tissues. Building on glycoproteogenomics. we also enzymatically synthesized cancer specific CD44s-Tn glycopeptides that were covalently linked to immunogenic proteins generating an anti-cancer glycovaccine. Our glycovaccine was well tolerated *in vivo*, inducing both humoral and cellular immunity, including immunological memory. Generated antibodies exhibited specific reactivity against synthetic CD44s-Tn glycopeptides, CD44s-Tn glycoengineered cells and tumours. In summary, CD44s emerged as a biomarker of poor prognosis and CD44 carrying truncated *O*-glycans as promising molecular signatures for targeted interventions. A glycovaccine was prototyped for pre-clinical validation.

Acknowledgements: Portuguese Foundation for Science and Technology for assistant researcher position CEECIND/03186/2017 and projects REVERENT (2022.03621.PTDC) and RESOLVE (PTDC/MED-OUT/2512/2021)

Low-immunogenic glycoconjugate linkers yield improved anti-glycans antibodies for cancer treatment

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A general feature of cancer cells is the presence of aberrant carbohydrate surface structures that promote metastasis, angiogenesis or suppression of the immune system. [1, 2]. Several of these tumor-associated carbohydrate antigens (TACAs) have already been described several decades ago, but their utilization as targets for therapeutic antibodies remained a challenge due to low immunogenicity, high hydrophilicity and limited availability of these antigens. [3]

To improve antibody generation during immunization, targeted glycans are commonly coupled to carrier proteins which support T cell activation mandatory for efficient antibody affinity maturation. Several techniques for random or site-specific coupling are currently used that employ artificial linkers whose haptenic structures often represent the central target of an immune response. Glycans can also be coupled directly to carrier proteins but the required harsh conditions often lead to truncated glycan structures. [4]

To avoid these problems during the generation of anti-TACAs antibodies, we developed a platform that links the TACA structure with a hydrophilic, monosaccharide-like non-immunogenic linker to the carrier protein.

Data supporting the concept and superiority of this approach will be presented, that enables and facilitates the identification of target-specific high-affinity anti-glycan antibodies and their subsequent *in vitro*/*in vivo* characterization.

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Deciphering the molecular basis of the enigmatic macrophage galactose C-type lectin recognition

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Glycans have an important role on immunomodulation.[1] The human macrophage galactose C-type lectin (MGL) is the only C-type lectin present on immune cells with a marked sugar specificity for N-acetylgalactosamine (α- or β-GalNAc).[2] MGL recognizes GalNAc containing structures that can be present in pathogens, self-glycoproteins, and tumour cells, which makes MGL a modulator of distinct immune cell responses. Herein, our latest advances in unlocking the structural and dynamic features, behind the fine specificity, and molecular recognition of MGL, will be described. Through an integrative and multidisciplinary approach, we revealed that the carbohydrate recognition domain (CRD) of MGL is highly dynamic and is strongly dependent of the structure and presentation of the precise GalNAc-containing antigen, [3-5] which might explain the capacity of MGL to modulate tolerance versus immunity responses. Furthermore, the molecular recognition of distinct mucin-derived tumour-associated glycans, common in several tumours, by MGL was also investigated, and our data also pinpoints the ability of MGL to specifically discriminate different tumour-associated antigens.[6]

FCT-Portugal for PTDC/QUI-OUT/2586/2020, 2020.00233. CEECIND, UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/202(European Union for GLYCOTwinning (GA101079417) and GLYCONanoProbes (CA18132).

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[6] unpublished results

Quantifying molecular glycan-lectin binding parameters for efficient cross-presentation

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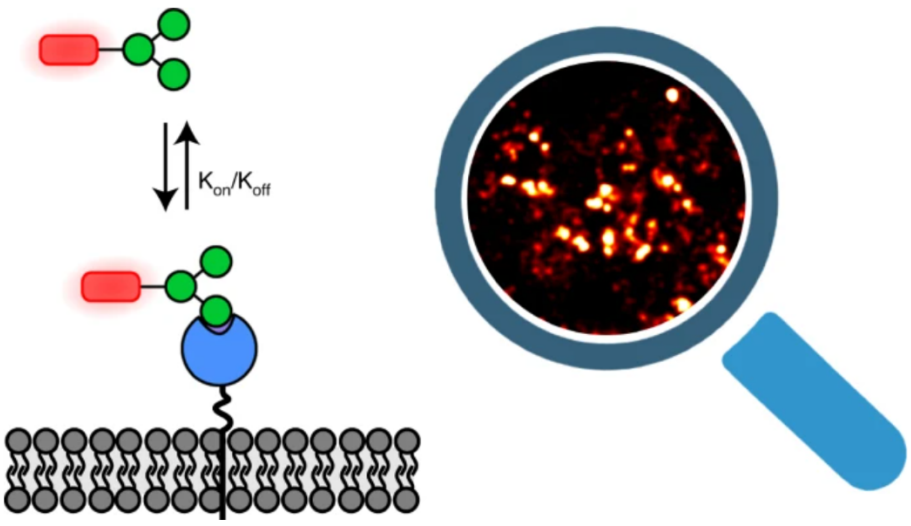
Obtaining glycan binding kinetics on living cells is hampered by their intrinsic weak lectin affinity and is therefore limited to *in vitro* techniques. To address this, we have developed the super-resolution microscopy technique Glyco-PAINT [1]. This novel methodology allows for the direct readout of single-molecule on and off-rates of labelled glycans on living cells and hereby provides the possibility for SAR studies on difficult-to-target lectins.

In this talk, I will showcase the Glyco-PAINT technique, including recent developments towards its use in studying the interaction of immune cells with glycans. In these studies, we investigate binding of high mannose and Lewis-type glycans to endocytic lectins such as the MR and DC-SIGN. Using a library of molecularly defined carbohydrate clusters we were able to obtain live-cell receptor density maps and correlate single-molecule binding events with glycan uptake. In a similar approach, these optimized glycan ligands were conjugated to synthetic long peptide (SLP) vaccines and evaluated for binding to primary murine immune cells. Here, a correlation of single-molecule binding parameters with the individual T cell cross-priming capabilities of the SLPs could be established obtaining crucial SAR insights. In conclusion, we present a novel method for measurement of live-cell binding of glycans that can be broadly applied to gain a further understanding of the efficacy of (synthetic) glyco-conjugates in many immunological synapses.



Graphical abstract of the GlycoPAINT technique

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Ferrier/aza-Wacker/epoxidation/glycosylation sequence to access 1,2-trans-3-amino-3-deoxyglycosides

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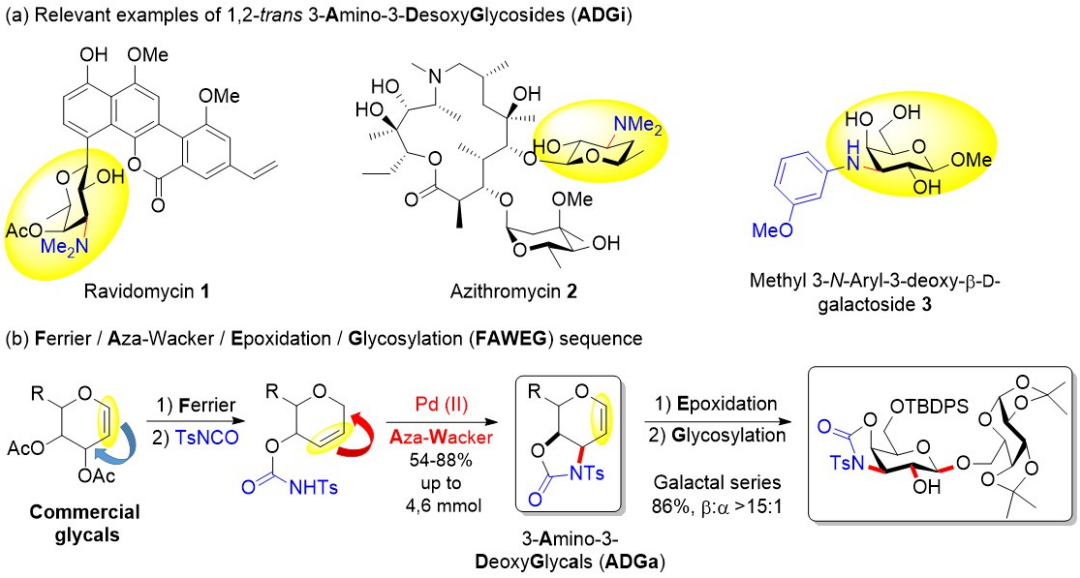
3-**A**mino-3-**D**eoxy**G**lycos**i**des (**ADGi**) constitute an essential class of glycosides found both on bacterial natural products and on a large number of bioactive compounds.[1] Among these structures, many important ADGi possess a 1,2-*trans* glycosidic linkage. As noteworthy examples, ADGi can be found in the structures of anticancer agents (ravidomycin **1**) or macrolide antibiotics (azithromycin **2**). More recently, Nilsson et *al.* also showed that 3-*N*-Aryl-3-deoxy-β-D-galactosides such as **3** are selective inhibitors of galectin-9C (illustration, (a)).[2] Owing to their biological importance, the development of an expedient stereoselective approach to synthesize effective glycosyl donors of ADGi giving rise to 1,2-*trans* glycosidic linkage is therefore an essential matter for the discovery of new drugs, vaccines and new biosynthetic pathways.[3]

In this work, we describe a new sequence, involving a Ferrier rearrangement, and subsequent aza-Wacker cyclization, allowing the rapid synthesis of orthogonally protected 3-**A**mino-3-**D**eoxy**G**lyca**l**s (**ADGa**). In order to obtain 1,2-*trans* ADGi, we made a proof of concept to evaluate the ability of these *N*-tosyl protected ADGa to act as competent glycosyl donors precursors in an epoxidation / glycosylation sequence. 3-Amino-3-deoxygalactal derivative was submitted to an epoxidation / glycosylation with high yield and great diastereoselectivity, highlighting **FAWEG** (**F**errier/**A**za-**W**acker/ **E**poxidation/**G**lycosylation) as a new approach to access 1,2-*trans* 3-amino-3-deoxyglycosides (illustration, (b)).[4]



(a) Context of the study, (b) Our work.

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Harnessing the reactivity of exo-glycals in iron-mediated hydrogen-atom transfer reactions

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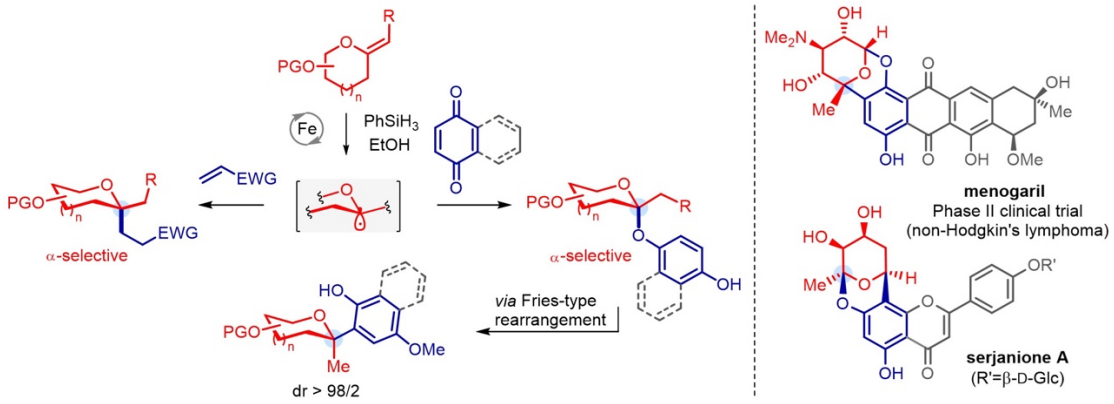
O- and *C*-aryl glycosides represent important classes of compounds of therapeutic interest [1]. Among them, serjanione A and menogaril, a clinically active antitumor drug derived from the natural product nogalamycin, are synthetically attractive [2]. In these unique structures, the sugar residue is joined to the aromatic moiety *via* both glycosidic and C-C bonds to form a benzoxocin ring system. In conjunction with our continuing studies on glycomimetics, we have recently reported a convenient strategy for the synthesis of *C*,*C*-glycosides from *exo*-glycals by way of Metal-hydride Hydrogen Atom Transfer (MHAT) [3]. The capture of the transient tertiary pseudoanomeric radicals by a range of Michael acceptors enables the stereocontrolled *C*-quaternization of the anomeric center. With the objective of developing a step-economical access to the benzoxocin core found in *C*,*O*-fused glycosyl (het)arenes such as serjanione A and nogalamycin, we envisioned the direct coupling of MHAT-generated glycosyl radicals with 1,4-quinones. This mild, one-step reaction which provides regiospecific access to phenolic *O*-ketosides may be viewed as a formal glycosylation of quinones, a transformation that has very few precedents [4].The synthesis of *C*-aryl ketosides *via*unprecedented Lewis acid-catalyzed *O*- to *C*- glycoside rearrangement was also demonstrated, opening the way to a unified strategy for the construction of *C*-glycoside motifs characterized by a stereodefined quaternary pseudoanomeric center bearing an exocyclic O- or C-aryl substituent.



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Automated monosaccharide building block synthesis and stereoselective glycosylations

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The first step in the chemical synthesis of oligosaccharides is the synthesis of monosaccharide building blocks with appropriate protecting groups. It typically involves multiple steps and chromatographic separations. Most chemists regard these as a necessary but uninteresting part of oligosaccharide synthesis. We contend humans should 'delegate' the effort involved to machines and focus on doing the interesting parts.[1] We will present our efforts to automate this process, removing the need for chromatography (with significant reduction of silica and solvent use). We have used Burke's TIDA-tags to enable facile access to building blocks.[2] The resulting building blocks have been applied in the synthesis of oligosaccharide targets.[3] In the second part of the talk we will present novel methods for alpha-glucosylation, and galactosylation that we have developed. The mechanism underpinning these highly selective glycosylations will be discussed.

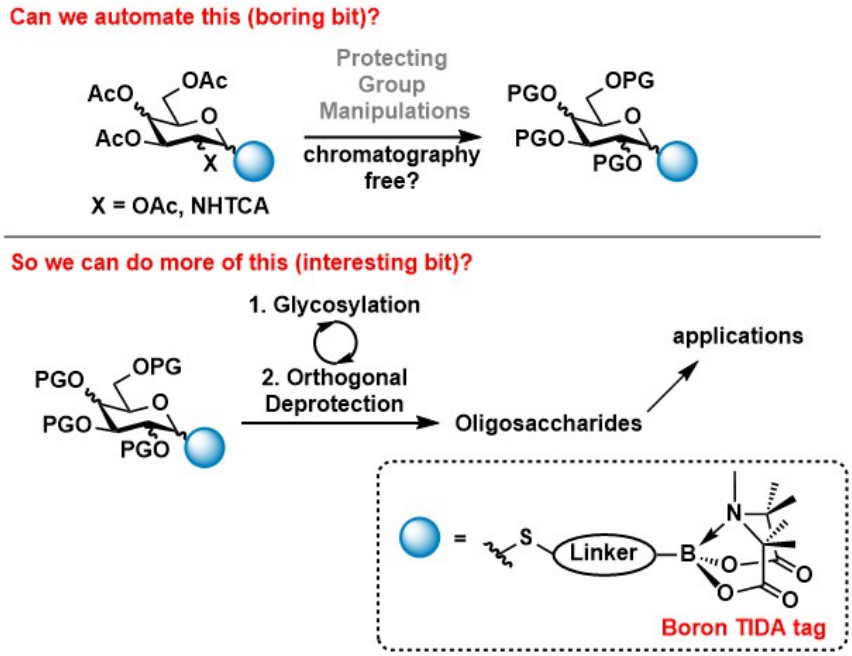

Figure 1: Proposal (part 1 of talk)

We thank SFI (CDA/15/3625) and IRC (GOIPG/2019/2747, GOIPG/2020/1129, GOIPD/2019/538) for funding.

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The algorithmic beauty of the starch granule

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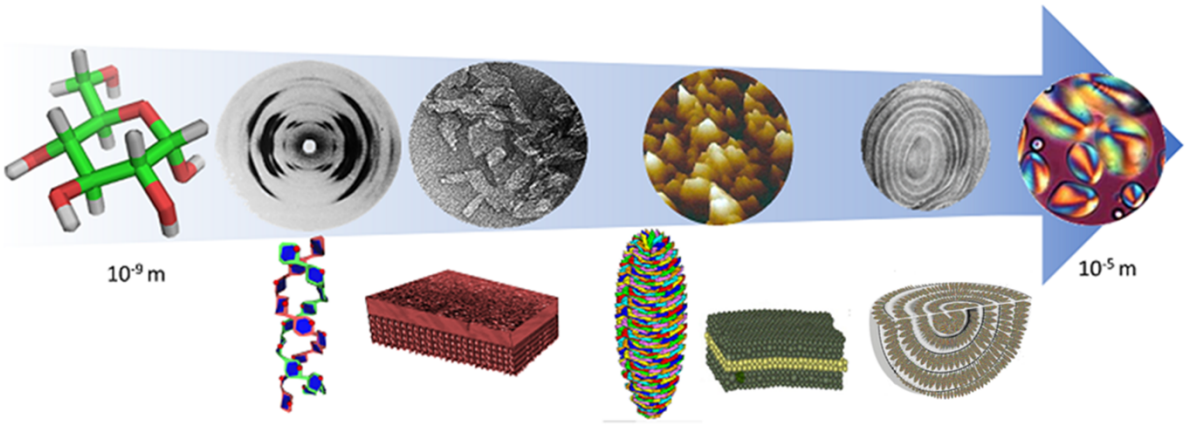
The starch granule is Nature's way of storing energy in green plants over long periods. Irrespective of their botanical origin and diversity, it is remarkable that the internal structures of starch granules share universal features, as indicated by the land-marked appearance of the Maltese cross when observed in polarized light. The construction requires the organization of structural elements over five orders of magnitude from the constituting glucose units to the complete granule. To reach a complete molecular description and understanding of such an architecture, we hypothesized that Nature retains hierarchical material structures at all levels and that some general rules control these structures' morphogenesis. We considered the occurrence of «phyllotaxis» like features that would develop at scales ranging from nano to micrometres and developed a novel geometric model to build complex structures from simple components. One among these structures is a golden spiral ellipsoid constructed with elements made up of parallel-stranded double-helices. Its shapes, sizes and high compactness would account for a macromolecular organization of about 10^8 Da, representing an amylopectin macromolecule.

While establishing a viable model of a consistent hierarchical organization, these results offer a new 3-dimensional vision to reconsider previously experimentally reported data and extend our understanding of the structures' complexity, where the underlying biosynthetic events are only the first steps of the construction which obey more general principles of spatial organization.



The structural levels of starch granule architecture

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Are unprotected glycosylhydroxylamines possible tools for glycoconjugation via SPANC?

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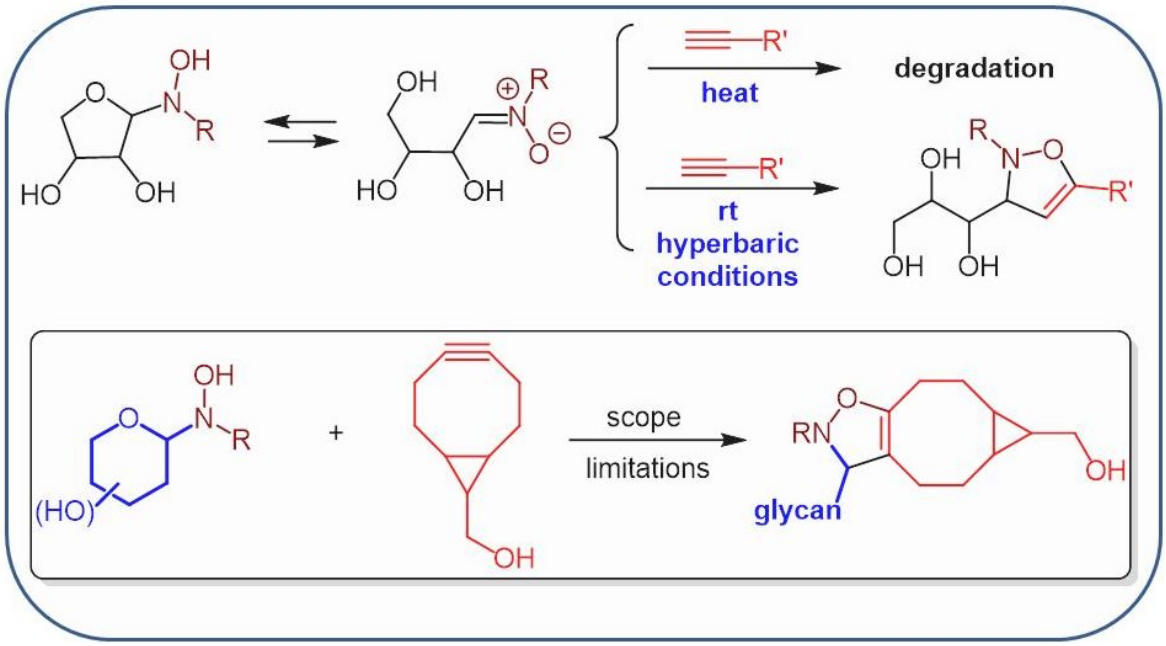
Conjugation of isolated glycans to biological counterparts by chemoselective ligation reactions is essential for unraveling physiological and pathological recognition events.[1] The peculiar reactivity of the aldehyde function of unprotected (reducing) glycans has found widespread application in the ligation with oxiamines or amines for example to prepare carbohydrate microarrays, fluorescently labeled carbohydrates or glycopeptide mimetics.[2] Recent focus of chemical ligation is shifting towards strain-promoted cycloaddition reactions with cyclooctynes which undergo rapid reaction with nitrones (SPANC).[3] The reaction of protecting-group free carbohydrate-derived glycosylhydroxylamines (as masked nitrones) with alkynes has been examined recently,[4] showing a high tendency of the formed isoxazolines to degradation under heat activation. However, hyperbaric conditions allowed the cycloaddition to occur with common alkynes, affording the expected products in good yields. Application of such a transformation to cyclooctynes proved efficient even under atmospheric pressure, which could afford an additional item in the toolbox of metal-free bioorthogonal reactions.



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A new non-incorporable metabolic inhibitor enables production of a fucosylated therapeutic antibodies

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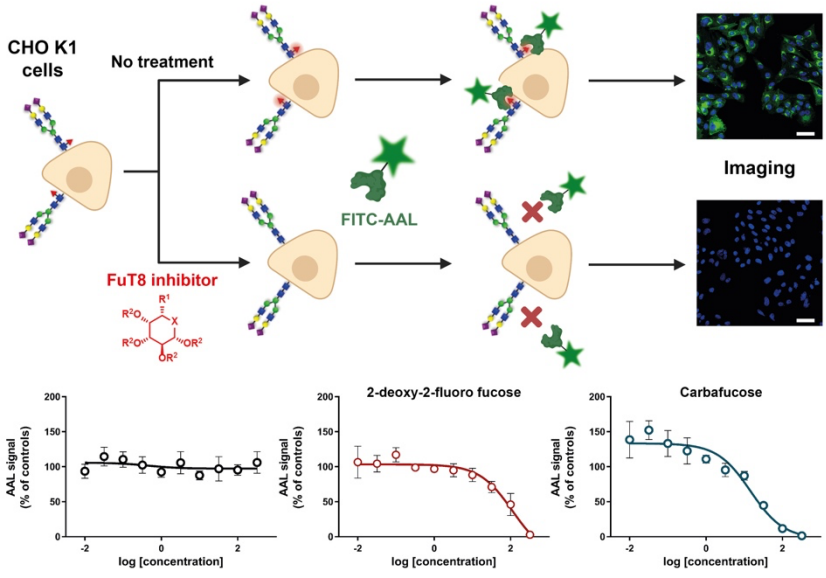
Glycosylation is a post-translational modification involved in many biological processes. This functional diversity is mirrored in a great structural diversity. Among the various monosaccharides is L-fucose, commonly found in terminal positions within glycans. Fucose residues mediate a range of biological effects, from regulation of leukocytes during inflammation to binding of IgG antibodies to Fc gamma receptors. Accordingly, there is great interest in developing tools to modulate cellular fucosylation. To date, the most successful approach pursued to block fucosylation has been the development of metabolic inhibitors: synthetic fucose mimetics able to hijack the biosynthetic pathway and to block the enzymes responsible for transfer of fucose onto glycans.[1] Existing metabolic inhibitors of fucosylation are limited in their application due to undesirable low-level incorporation into glycans which can raise concerns for industrial or therapeutic applications.[2] To address these concerns, we designed, synthesized, and used carbafucose as an efficient new metabolic fucosylation inhibitor that is assimilated into the *de novo* biosynthetic pathway and potently blocks cell fucosylation. We further demonstrated that carbafucose was not transferred onto resulting glycans and could, therefore, be used to produce afucosylated antibodies to enhance their efficacy for therapeutic applications. We expect that, more generally, carbafucose will find common use as a research tool to block cellular fucosylation and to enable fundamental studies into the physiological roles of fucosylation.



Lectin-based fucosylation assay in CHO K1 cells

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Glycosylation in cancer affects cellular receptor tyrosine kinases and regulates cancer cell biology

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Alterations of glycosylation in the tumour and its microenvironment are common molecular alterations with major biological implications for disease progression [1,2]. Cancer is a heterogeneous disease that requires multidisciplinary treatment. Current targeted therapy depends on patient stratification based on molecular features of the tumour. This presentation will report on the basis of alterations of glycosylation that occur in gastric cancer (GC). Recent results applying glycomic and glycoproteomic strategies have provided key information regarding the alterations of glycosylation occurring in cancer cells and their impact the activation of oncogenic receptors tyrosine kinase (RTK) in tumour samples, such as RON, MET, EGFR and HER2 (ErbB2) [3,4,5].

Our work demonstrates that ErbB2 is modified with both α2,6- and α2,3-sialylated glycan structures in GC clinical specimens. Glycomic and glycoproteomic analysis of ErbB2's ectodomain disclosed a site-specific glycosylation profile in GC cells, in which the sialyltransferase ST6Gal1 specifically targets ErbB2 N-glycosylation sites occurring within the receptor's binding domain of the therapeutic antibody approved and used in the clinics [3]. Abrogation of ST6Gal1 expression reshaped the cellular and ErbB2-specific glycosylation, expanded the cellular half-life of the ErbB2 receptor, and sensitized ErbB2-dependent GC cells to therapeutic antibody-induced cytotoxicity through the stabilization of ErbB dimers at the cell membrane, and the decreased activation of both ErbB2 and EGFR RTKs [3].

These results highlight the functional aspects of glycosylation modifications occurring in cancer and supports their potential application as biomarkers for patient stratification, personalize medicine and for novel and improved therapeutic applications [1,3].

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Novel glycosylation based diagnostic and prognostic for lyme disease

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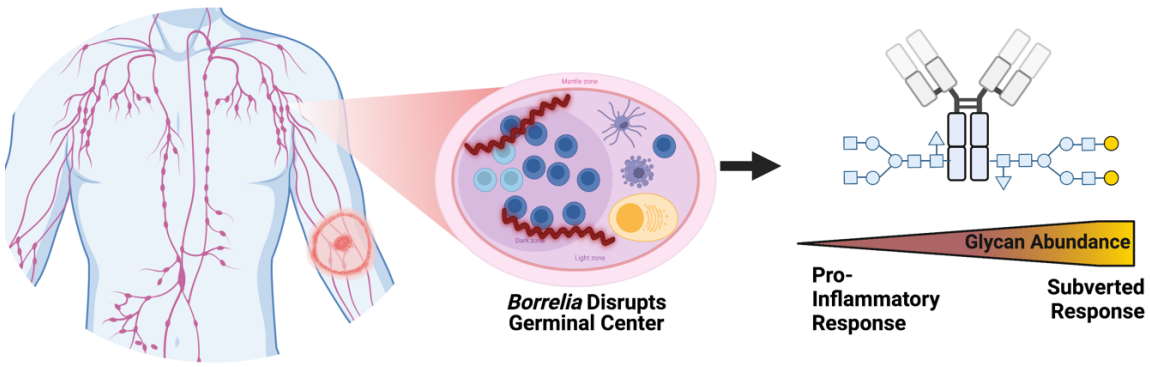
Current diagnostics for acute Lyme disease (LD) suffer from low sensitivity. As a result, patients with undetected cases of Lyme disease delay antibiotic treatment and risk bacterial dissemination to their brain, joints, and heart and causing irreversible damage. We present evidence that infection with the *Borrelia burgdorferi* spirochete alters the glycosylation of serum IgG and IgM in a manner specific to acute Lyme disease.

While inflammatory diseases canonically induce global IgG with increased agalactose content, we detect a marked decrease of agalactose during acute Lyme disease – below that of healthy control levels. Agalactosylated species are known to promote FcgR IIIA signaling on circulating lymphocytes to promote inflammation. Yet the IgG Fc fragment exhibits increased galactose and sialic acid content during acute Lyme disease. This finding suggests a novel immuno-modulation induced by acute *Borrelia burgdorferi* infection that permits evasion of adaptive immunity.

Moreover, we have detected acute LD-specific alterations of mannosylated and complex-type N-glycans in IgM. Recent reports suggest that rates of complement deposition and T-cell activation are partly controlled by the IgM glycosylation profile. Using machine learning, we determined our IgG and IgM N-glycan-based approach to be 72% sensitive and 100% specific for acute LD. In addition, the global IgG and IgM N-glycome were able to differentiate acute LD patients from diseases that present similarly. Our research indicates that glycosylation of IgG and IgM are useful biomarkers for acute Lyme disease and have diagnostic and prognostic potential.



Borrelia burgdorferi invasion of the lymph node can affect immunoglobulin glycosylation.

Glycans converted into functional fluorophores

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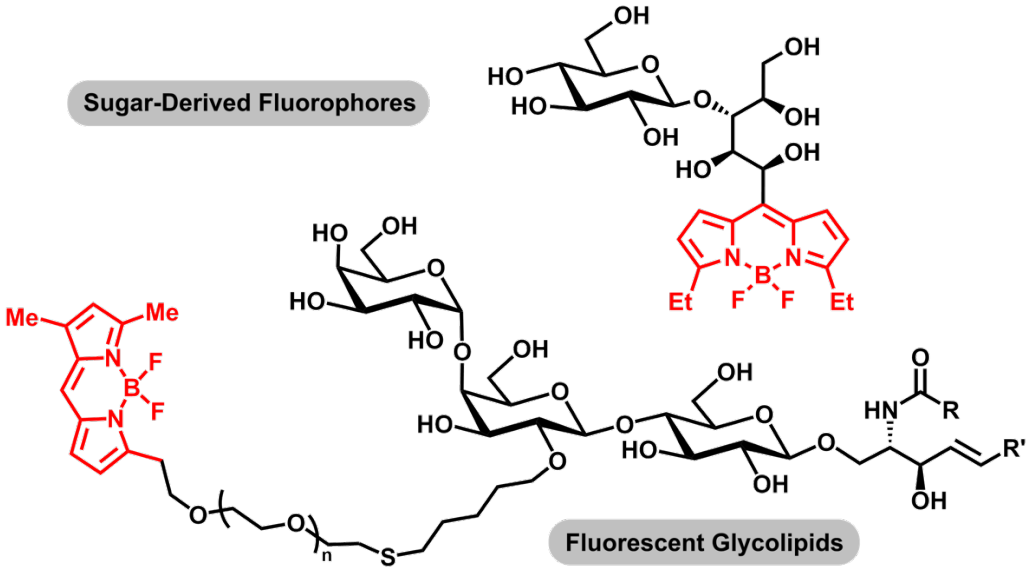
A major drawback to investigate the fate of glycans in biological environments is the fact that optical visualization is difficult because they are non-colored and non-fluorescent.

To investigate the influence of the fatty acid of the glycosphingolipid Gb_3 on its distribution in membranes, a modular synthetic route towards a set of fluorescently labeled Gb_3 glycosphingolipids with a BODIPY fluorophore attached to the head group was developed [1]. C_{24} fatty acids, saturated, unsaturated, α-hydroxylated derivatives and a combination thereof were attached to the sphingosine backbone. The fluorophore was attached in such a way that the binding properties of the carbohydrate head group stay intact. Using such constructs allows to study the effect of different fatty acids on the behavior of these glycolipids in membranes.

The synthesis of BODIPY itself uses an aldehyde as crucial component. Instead of common aromatic aldehydes sugars are employed which allows the facile construction of sugar-derived BODIPYs. Such fluorophores are water-soluble and enantiomerically pure. The use of different sugars differing in their stereochemical information paves the way to a distinct staining of cell organelles [2].



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Fluorescent probes that enable cell-based imaging of lysosomal enzyme activities and protein markers

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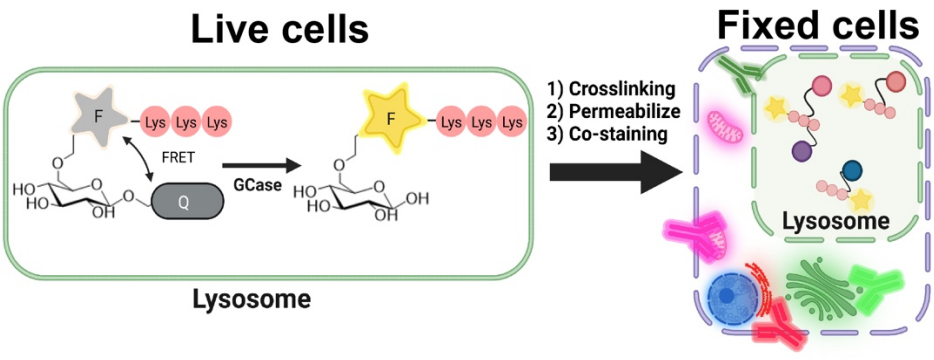
The ability to accurately quantify enzyme activity in live cells is of high interest both from an academic perspective as well as for translational research to enable new medicines, including gene therapies and next-generation enzyme replacement therapy[1,2]. The use of chemical tools such as fluorescence-quenched substrates is a promising approach to studying enzyme activity within live cells[3,4]. However, substrates for quantifying enzyme activity within fixed cells are lacking. Yet, such fixable substrates would permit the preservation of tissue samples for long-term storage as well as enable the concomitant use of other experimental methods such as immunofluorescence. Ideally, the fixing conditions used for such substrates would be the spatial distribution of protein factors of interest.

Here we present the first fixable substrate that enables quantitative analysis of enzyme activity within lysosomes of both live and fixed cells. We incorporated a small, fixable motif that enables the efficient chemical fixation of a fluorescence-quenched substrate for the Parkinson's Disease (PD) associated enzyme glucocerebrosidase (GCase) while preserving its fluorescence after fixation. Using fluorescence microscopy allows one to quantify the turnover of this probe, LysoFix-GBA, within both live and fixed cells. We demonstrate that LysoFix-GBA operates as a robust and selective tool to quantify chemical and genetic perturbations of lysosomal GCase activity. Furthermore, we apply LysoFix-GBA to multiplexed co-localization studies of GCase activity with various subcellular protein markers using immunocytochemistry. We expect that LysoFix-GBA will be broadly useful for compound screening and studying the role of GCase activity in PD, as well as for the development of new approaches targeting the GCase pathway for therapeutic benefits.



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Fluorescence quenched glycans enable visualisation and quantification of microbial carbon cycling

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Marine microalgae sequester as much CO_2 into carbohydrates as terrestrial plants. Bacteria are major players in the cycling of this carbon as they breakdown photosynthetic glycans. Identifying the rates and specificities of microbial carbon degraders on a molecular level remains challenging. Förster resonance energy transfer (FRET) is a powerful, simple and robust phenom that can be exploited to allow super-resolved optical measurements and provide real-time information about intra- and intermolecular distances on the nanometer level. Due to this it has widespread utility biomolecular research.[1] However, access and utility of fluorescence quenched glycan probes is not yet routine and a key bottleneck is the complexity and expertise required for glycan synthesis.[2–4] A toolbox of fluorescence quenched glycans would allow high-throughput, sensitive and quantitative means to discover microbes with differing abilities and feeding strategies to break down glycans. For these reasons, we have developed a general strategy to access these tools using automated solid-phase synthesis, enabling fast and reproducible access to these tools. Key in this method is the use of a terminal 6-amino 6-deoxy monosaccharide building block which allows assembly of bi-functional oligosaccharides, that once cleaved from the solid support can be derivatized into FRET probes. We then use these glycan-FRET probes as tools to visualize and quantify heterotrophic microbes that digest glycans in the ocean. Example one is an alpha-mannan fluorescence quenched probe, which is used to characterise *Salegentibacter sp* Hel_I_6, a microbe that possesses the rare ability to break down marine fungal glycans.[5] The second example details a beta-glucan based fluorescence quenched probe which we use to visualise laminarin digestion, a major molecule in the global carbon cycle.[6] These fluorescence quenched glycan tools allowed us to kinetically characterise microbial carbon digestion at the level of individual enzymes and image whole live cells. These tools offer a complementary approach to genomic based approaches with the advantage of being sensitive to changes in proteome expression.

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Synthesis of a fluorescent ganglioside probe using late-stage sialylation and its behavior analysis

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Gangliosides form functional domains (lipid rafts) with proteins in cell membranes. To study lipid rafts in detail, we developed fluorescently labeled gangliosides (ganglio-, and globo- series), and observed their behaviors by single-molecule imaging technique.[1] For inclusive understanding of ganglioside behaviors, this study focused on lacto-, and neolacto- series gangliosides, which have never been analysed.

For the synthesis of ganglioside probes in an efficient way, we designed a late-stage α-sialylation strategy of glycolipid acceptors using a fully stereoselective α-sialylation method.[2] To improve the aggregation property of glycolipid derivatives, we developed a glycolipid acceptor, which was multiply protected with TBBz groups.[3] As a result, α-sialylation of the glycolipid acceptor provided a ganglioside framework in high yield.[4]Based on this result, we next examined the synthesis of the lacto-series ganglioside probe. α-Sialylation of a Lc_4Cer acceptor by a C9-NHTFAc bicyclic sialyl donor provided the ganglioside framework successfully. Finally, global deprotection and fluorescent labeling of C9-NH_2 afforded $NeuLc_4Cer$ probe.[5] Similarly, Neolacto-series ganglioside probe was synthesized. The single-molecule imaging of the fluorescent $NeuLc_4Cer$ first revealed its colocalization with a major raft molecule CD59. Furthermore, $NeuLc_4Cer$ formed transient homodimers, which are commonly observed in other gangliosides examined previously. In conclusion, we have demonstrated the utility of the fluorescent ganglioside for studying their dynamic interactions on cell membranes.

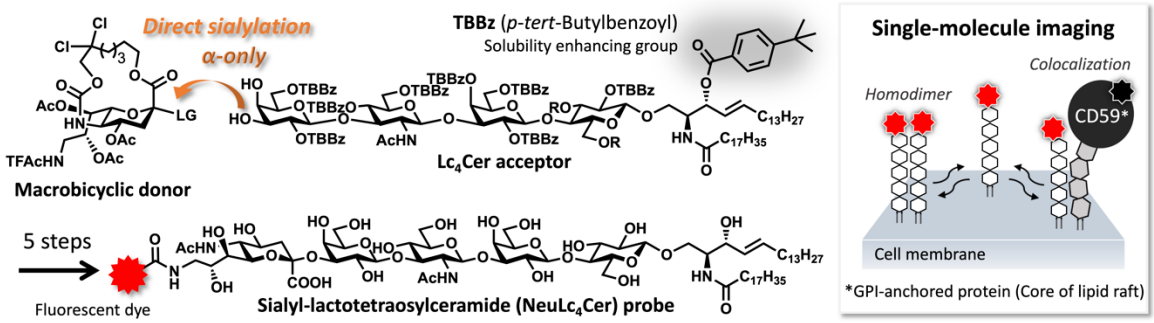

Figure. Chemical synthesis of the fluorescent ganglioside and its single-molecule imaging on living cells

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Identifying gut bacterial consumers of dietary glycans by metabolic labeling of microbiota samples

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Diet-derived polysaccharides are an important carbon source for gut bacteria and shape the human gut microbiome.[1] Despite recent advances in our understanding of glycan metabolism by human gut bacteria, we still need efficient methods to link glycans to their consuming bacteria. We used *ex-vivo* metabolic labeling of a human microbiota sample to identify and isolate gut bacteria that take-up fluorescent glycans. The method combines metabolic labeling using fluorescent oligosaccharides with fluorescence-activated cell sorting (FACS), followed by amplicon sequencing or culturomics (Fig. 1).[2] Using this method, bacteria consumers of various glycans were identified, including species not previously known to be consumer. In addition, we have used this method to reveal gut bacteria whose metabolism of maltodextrin is inhibited by α-amylases inhibitors used in the treatment of type 2 diabetes.[3] Acarbose, a compound used clinically was found to inhibit bacterial metabolism of maltodextrin in 4 species. In contrast, montbretin A, a new drug candidate for the treatment of type 2 diabetes, slowed the growth of only one bacterial species, supporting the fact that it is more selective. By linking bacteria to the glycans they consume, this approach increases our basic understanding of glycan metabolism by gut bacteria. Going forward, it could be used to provide insight into the mechanism of prebiotic approaches, where glycans are used to manipulate the gut microbiota composition.

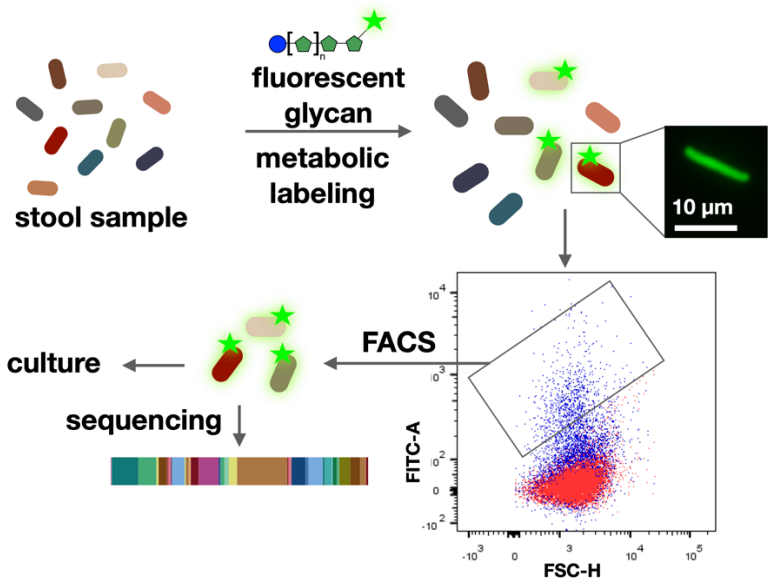

Figure 1. Metabolic labeling of human stool samples followed by fluorescence-activated cell sorting and amplicon sequencing or culturomics.

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A microfluidic photo-induced platform to synthesize ultrasmall glyco gold nanoparticles

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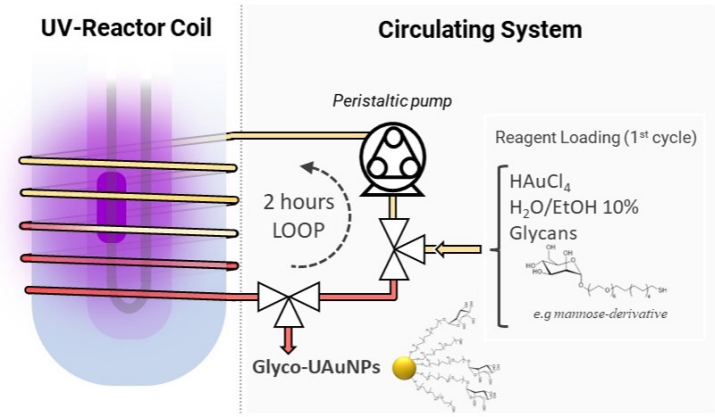
Ultra-small gold nanoparticles (UAuNPs) are a class of AuNPs with a diameter less than 5 nm, interesting for their peculiar physical-chemical properties which can be exploited for bio application and nanomedicine. In particular, UAuNPs are luminescent, have longer circulation time, improved biodistribution, better tissue penetration and efficient clearance pathways. UAuNPs engineered with glycans (Glyco-UAuNPs) emerged as excellent platforms for many applications since the displacement of multiple copies of glycans mimic the multivalent glycoside clusters effect which can overcome the low affinity of the individual ligands towards their receptors.[1,2] However, to fulfill the ambitious potentiality of these engineered UAuNPs, robust protocols for their synthesis, functionalization and characterization, are still needed and highly desirable. In the last years, microfluidic reactors have emerged as outstanding tools for synthesizing a wide range of NPs allowing for a fine control over particle size, morphology and reproducibility.[3,4] Herein, we show an innovative and straightforward synthesis of a library of Glyco-UAuNPs based on a reliable microfluidic approach coupled with a photo-induced reduction, that avoids the use of any further chemical reductant, templating agent or co-solvent.[5] Exploiting 1H-NMR spectroscopy, we showed that the amount of thiol-ligand exposed on the UAuNPs is linearly correlated to the ligand concentration in the initial mixture, paving the way towards the development of a programmable synthetic approach.



Synthesis of Mannose ultrasmall gold nanoparticles

Acknowledgements: Marie Skłodowska-Curie grant agreement No 814236
COST Action CA18103: INNOGLY: INNOvation with GLYcans, new frontiers from synthesis to new biological targets.

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Self-assembly of oligosaccharides: towards tunable hydrogels

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Self-assembly is a process commonly utilized in nature to build functional structures and has been extensively studied for biomolecules such as peptides and nucleic acids. In contrast, the mode of aggregation and types of intermolecular interactions of oligosaccharides remain unexplored due to synthetic and analytic challenges associated with carbohydrates. Recently, systematic studies on supramolecular organization of oligosaccharides were enabled by the advent of automated glycan assembly, allowing access to collections of oligosaccharides with well-defined length and composition. [1]

Here, we showed how insights into the self-assembling behavior of oligosaccharides inspired the creation of dynamic supramolecular hydrogels with tunable properties. We utilized cellulose oligomers to build the hydrogel backbone, as cellulose is the most abundant natural polysaccharide exhibiting a high tendency to form crystalline supramolecular assemblies. [2] The cellulose-based oligomers were functionalized with various carbohydrate solubilizing moieties [3], enabling the creation of dynamic supramolecular fibers capable of forming hydrogels (**Fig. 1**). These carbohydrate-based hydrogels mimic the mechanical properties of natural materials (e.g. extracellular matrix) while exposing biologically relevant carbohydrate epitopes. Future applications as mimics of biological environments are envisioned.

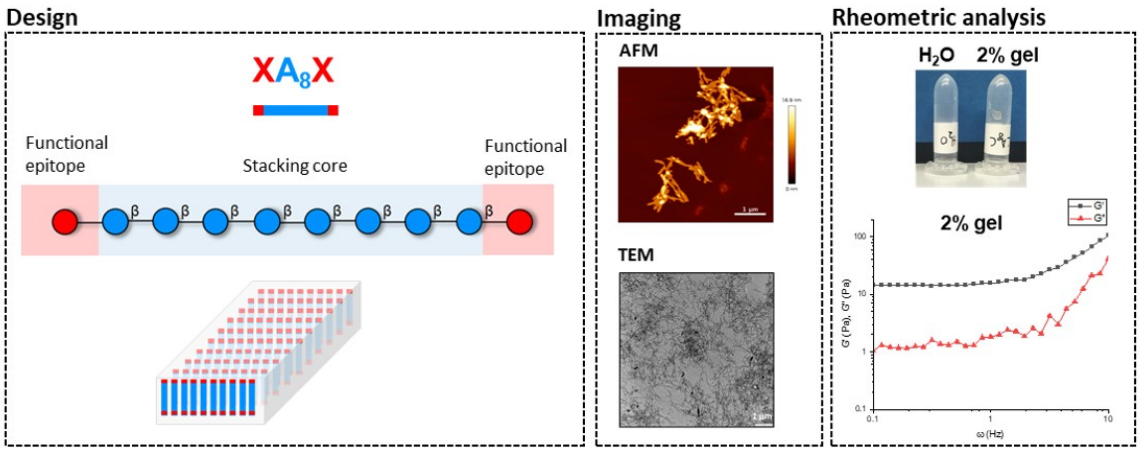

Figure 1: Design and analysis of synthetic carbohydrate-based supramolecular hydrogels.

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3D printing, wet spinning and cell culture with *N*-alkyl-D-galactonamide supramolecular hydrogels

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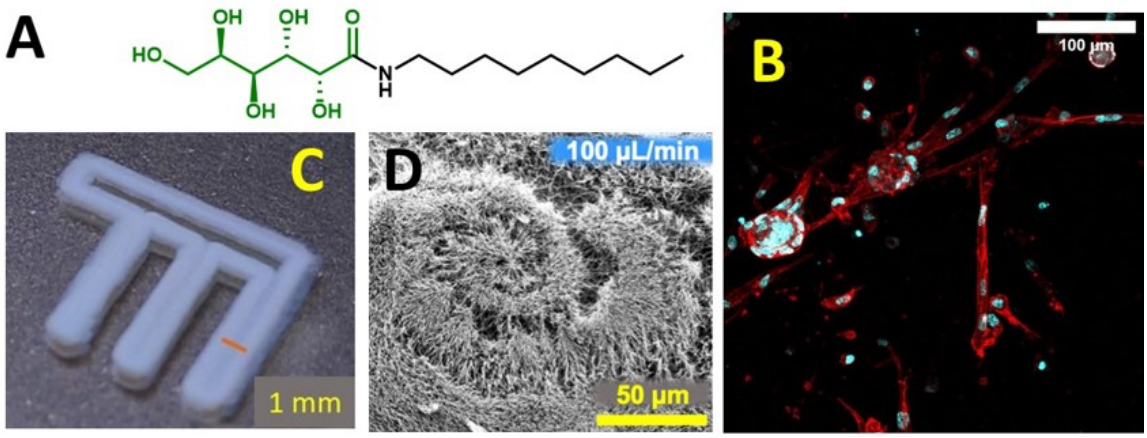
N-alkyl-D-galactonamides (Fig. A) are synthetic amphiphilic molecules that self-assemble in supramolecular fibers in water, giving hydrogels. These simple molecules are quite easy to synthetize in large quantity and high purity, which is suitable for biological applications. We showed that hydrogels of N-heptyl, N-octyl and N-nonyl-D-galactonamide are biocompatible and can be used as scaffold for 3D cell culture. On these hydrogels, neural stem cells, mesenchymal stem cells, fibroblasts or neural cell lines developed in 3D cell clusters linked together by cell extensions guided by the supramolecular fibers (Fig. B). Because the gels are fragile, specific methods have been implemented to observe the 3D cell organization after culture and immunostaining.

Methods to inject the hydrogels are also useful to make hydrogels in desired shapes and places. Because these hydrogels are not shear-thinning nor thixotropic, direct injection of the gels is not possible. It breaks irreversibly the gels. So, we developed a method in which the gelation is triggered in situ by liquid-liquid exchange. It has been applied to wet spinning and 3D printing. The very fast self-assembly of the gelator in contact with water provides well-resolved 3D printed patterns and well-shaped gel noodles (Fig. C). Also, by changing slightly the structure of molecular gelator, we got either sacrificial or persistent gels which can be imbricated by 3D printing. The spontaneous dissolution of the sacrificial gel gives supramolecular gel architectures with channels. In addition, at the microscopic level, in some conditions, the supramolecular fibers are radially organized highlighting diffusion and/or mixing phenomenon at the liquid-liquid interface (Fig. D).



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Glucoside-boronates behave as water-sensitive organogelators

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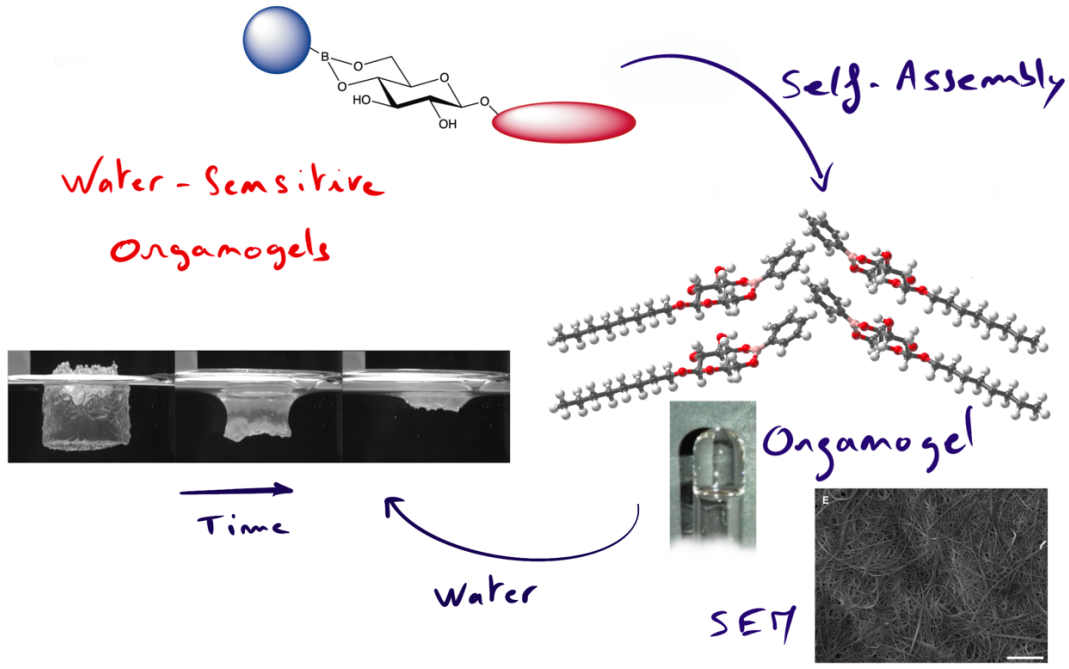
The preparation of gels with low-molecular-weight gelators is based on formation of a 3D-network responsible for the immobilisation of a solvent. The corresponding fibers are obtained through the self-assembly of those gelators thanks to van der Waals interactions, π–π interactions, hydrogen bonding or electrostatic interactions. It turns then aqueous or organic solutions into hydrogels or organogels, respectively. Sugar-based derivatives are already known for their potential as remarkable organogelators. Recently, we described an easy synthesis of a new class of organogelators obtained by esterification of a glucoside with aromatic or aliphatic boronic acid. These sugar-boronate derivatives permitted to investigate the impact of both the alkyl chain and the aromatic part on the gelation properties. Thanks to the boronate function, our organogels are water-sensitive and depending on the chemical structure, they showed different behavior upon hydrolysis. In addition, some members of this organogelator family bear fluorescent properties. We also fully characterized the gels by rheometry, electron microscopy (SEM) and X-ray diffraction to understand as much as possible the type of self-assembly involved during the formation of the organogels.



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Mechanisms of membrane fusion induced by re-engineered bacterial toxins

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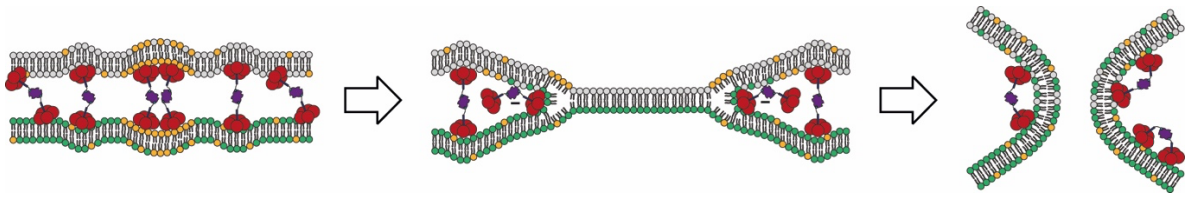
The cholera toxin B-subunit (CTB) is a pentameric lectin that binds to ganglioside GM1 in lipid bilayers, as a first step to achieve endocytic internalization of its associated toxic A-subunit. The multivalent nature of this interaction enhances the affinity of the toxin protein, but also causes the glycolipids to cluster and induce membrane curvature [1]. This process can be recreated in simple model systems such as Giant Unilamellar Vesicles (GUVs), which shows that CTB is both a sugar-binding and membrane bending protein. However, as CTB has all of its carbohydrate-binding sites on the same face of the pentamer, it does not naturally crosslink membranes unlike other lectins, e.g., LecA which has carbohydrate binding sites that face in opposing directions [2]. Recent we have shown that multimeric assemblies of streptavidin and a site-specifically biotinylated AB_5 complex of CTB can mediate GUV crosslinking and membrane fusion processes [3]. Further investigations of the mechanism of these unprecedented processes will also be presented, including the use of streptavidin mutants with defined valencies to identify the active species in the original study, and the use of parallel and antiparallel coiled-coil motifs to further control the arrangements of the CTB pentamers and their interactions with model membranes. Our results show that protein engineering strategies for controlling the architecture of multivalent lectins can allow repurposing of lectins as nano-scale building blocks and molecular machines for synthetic glycobiology.



Multimeric CTB assemblies crosslink and fuse membranes via a hemifusion diaphragm. Figure adapted from [3] under a Creative Commons CC-BY4.0 licence.

This research has received funding from the European Union's Horizon 2020 research and innovation programme under th Marie Skłodowska-Curie grant agreement number 814029 (synBIOcarb ITN).

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Probing the effects of glycosylation on peptide and protein activity through chemical synthesis

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Glycosylation is the most common co- and post-translational modification of polypeptides, with over 50% of human proteins predicted to display covalently bound glycans. Glycosylation has been shown to mediate an array of biological recognition events in all domains of life. Additionally, a number of recently approved biopharmaceuticals contain carbohydrate chains (or carbohydrate mimics) that are critical for activity and/or stability.[1] The non-templated enzymatic nature of the glycosylation process leads to heterogeneous mixtures of isoforms when glycopeptides and glycoproteins are isolated or produced in recombinant expression systems, thus hindering the ability to study how glycosylation influences function in a meaningful way. This has led to significant demand for new tools and technologies to facilitate access to homogeneous glycopeptides and glycoproteins to interrogate the role of individual carbohydrate modifications on structure and function.

Our lab has recently developed a number of synthetic technologies to access homogeneously glycosylated peptides and proteins for structure-function studies.[2,3] This talk will highlight the synthesis and evaluation of glycopeptide and glycoprotein hormones[4,5] and glycoproteins from Gram negative bacteria.

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Dissecting the hydration of glycans on proteins by using total chemical synthesis of glycoproteins

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The unique hydration property of glycans facilitates the interaction between glycoproteins with water molecules. Therefore, increases in the hydrophilicity of the protein moiety have been thought to be one of the functions of glycans on glycoproteins. We hypothesized that the dynamic behavior of water surrounding glycoproteins could be affected by glycans, resulting in the intrinsic functions of glycoproteins.

To address this hypothesis, we carried out the total chemical synthesis of glycoproteins and functional analysis. We have synthesized antifreeze glycoprotein (AFGP) having different sugar modifications.[1,2] AFGP is a highly *O*-glycosylated protein that inhibits the freezing of water. To shed light on the functional role of the O-glycans, we performed functional analysis including a hydrogen-deuterium exchanging (HDX) experiment by using homogeneous AFGPs prepared by chemical synthesis. The HDX experiment indicated that the sugar moiety of AFGP affected the dynamic behavior of surrounding water molecules. Together with other functional studies, it was suggested that sugar residues on AFGP form a unique dynamic water phase that disturbs the absorbance of water molecules onto the ice surface, thereby inhibiting freezing.[2]Upon this result, we further conducted the total chemical several *N*-glycoproteins, of which glycans are larger than those of AFGPs. The functional analysis of these molecules suggested that the unique hydration of N-glycans correlates with the biological function of proteins, such as protein-protein interaction, at a molecular level.

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Chemical Synthesis of homogeneous glycoforms of human Saposin A

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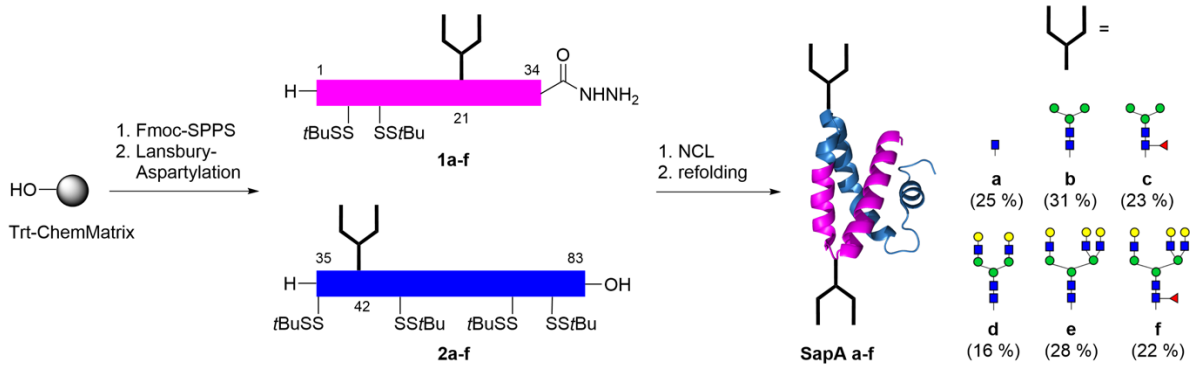
The four sphingolipid activator proteins saposin A-D are a family of small glycoproteins involved in the degradation of sphingolipids in the lysosome.[1] Saposin A (SapA) aids the degradation of galactosylceramide by β-galactocerebrosidase (GALC).[1] Its mechanism of action was revealed by a crystal structure of a dimeric $GALC_2SapA_2$ complex.[2] SapA is also known to form soluble lipid complexes (nanodiscs).[3] However, most studies were conducted with unglycosylated SapA and the role of its glycosylation is not yet well understood. The tendency of synthetic SapD glycoproteins to form soluble SapD-lipid complexes was found to be carbohydrate-dependent.[4] Here we show the synthesis of homogeneous glycoforms of human SapA. The solid phase synthesis of the two glycosylated SapA segments **1** and **2** was challenging requiring special conditions to achieve complete couplings. The glycopeptides (**1a-f** and **2a-f**) were obtained by pseudoproline-assisted Lansbury aspartylation using synthetic N-glycan azides[5] corresponding to the SapA glycans from Gaucher patients.[6] After thioesterification of **1a-f** both segments were ligated by native chemical ligation (NCL) and folded to the desired glycoforms (**SapA a-f**). Currently we are investigating the formation of supramolecular complexes of SapA glycoforms with glycosphingolipids.



Synthesis of SapA a-f glycoforms.

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Knowing and controlling the glycoprotein folding cycle by chemical synthesis

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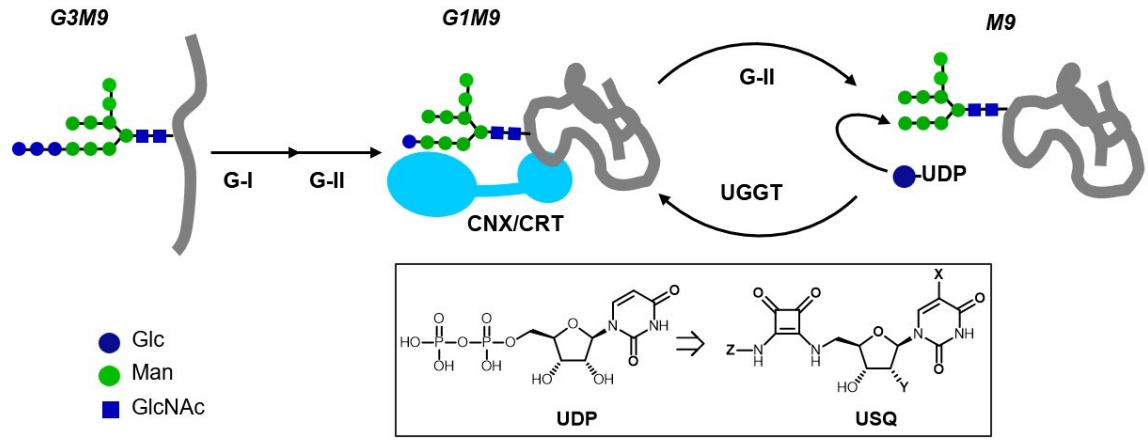
In eukaryotic cells, protein N-glycosylation occurs in the lumen of the ER, introducing the common precursor consisting of three glucose (Glc), nine mannose (Man), and two N-acetylglucosamine (GlcNAc) residues ($Glc_3Man_9GlcNAc_2$; G3M9). Subsequent digestion by glucosidase-I (G-I) and -II (G-II) sequentially removes terminal and penultimate Glc residues to produce the monoglucosylated glycoform (G1M9) which enters the N-glycosylation dependent folding cycle, consisting of lectin chaperones calnexin (CNX) or calreticulin (CRT), G-II, and UGGT.

In this cycle, glycoproteins captured by CNX or CRT are digested by G-II, removing the innermost Glc residue to generate the non-glucosylated glycoform ($Man_9GlcNAc_2$; M9). On the other hand, the glucosyltransferase UGGT functions as a folding sensor. When glycoproteins have failed to achieve correct folding, they are captured by UGGT which regenerates the G1M9 by glucosylation of the M9 using UDP-Glc as the donor substrate, thus allowing client glycoproteins to repeatedly interact with CNX/CRT. Using chemically synthesized glycans[1] and glycoproteins,[2]we have carried out systematic studies to clarify the properties of the components of the CNX/CRT cycle.[3] Molecules that perturb their activities would be valuable for knowing their roles. To that end, our recent effort aimed to develop UGGT inhibitors through structural modification of the enzyme's natural ligand UDP. The UDP analogs (USQ) having substituted squaryl group were shown to exhibit significant activity to inhibit UGGT.[4]



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Immobilized PNGase as a tool for studying the biological role of glycans: influence of the support

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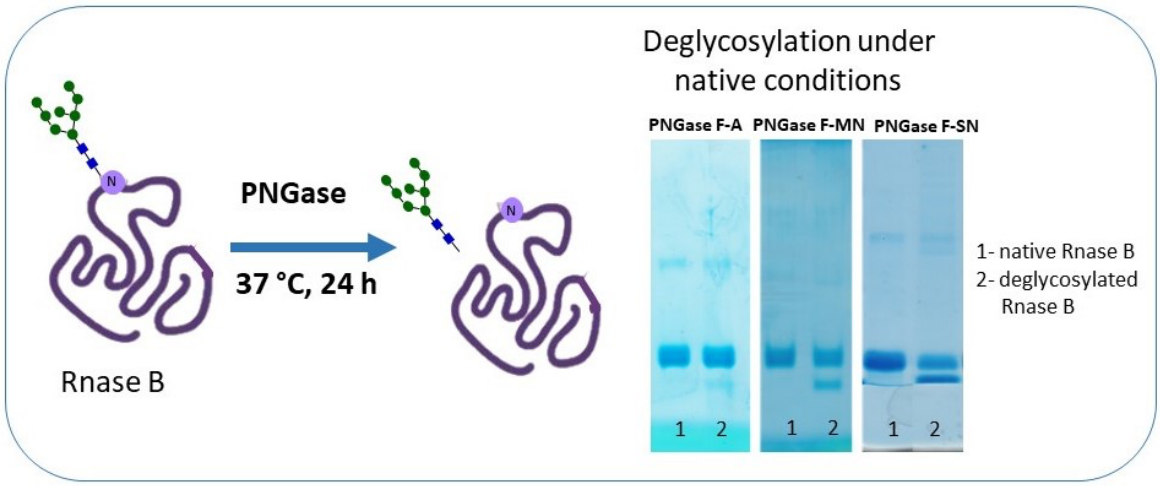
Carbohydrate-protein interaction between oligosaccharides from the cellular glycocalyx and protein receptors mediates several biological processes. Numerous technologies allow glycan structure elucidation, yet identification of their biological role is more complex. Enzymatic deglycosylation with immobilized PNGase is an interesting tool to confirm N-glycans role in a certain biological process. This enzyme catalyzes the hydrolysis of the bond between a glycoprotein asparagine and the internal GlcNAc. of the N-glycan. The fact that the enzyme is immobilized allow its removal upon deglycosylation so that changes in the biological function derived from the glycan removal can further be studied without its interference.

We immobilized PNGase F onto supports with different properties (agarose, magnetic and silica nanoparticles) using covalent strategies. In all cases immobilization yields above 80% were achieved with an expressed activity yield of 10%, allowing total removal of the N-glycan from RNase under denaturing conditions. Nevertheless N-glycan removal under native conditions, essential to perform further functional biological assays, was only possible with the enzyme immobilized onto nano-supports. Evaluation of N-deglycosylation of other model glycoproteins showed that magnetic nano-supports could not be used with iron containing proteins such as lactoferrin as they get stuck to the support. N-deglycosylation of epimastigote *Typanosoma Cruzi* was also evaluated. Successful results were obtained only when lysates were prepared in the presence of doxycholic acid.



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On-resin synthesis of glycolipopeptides towards self-adjuvanting and multivalent vaccines

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Glycolipopeptides are promising scaffolds for self-adjuvanting vaccines.[1] The synthesis of these constructs is challenging due to the demanding chemistry required for fusing lipids, carbohydrates and peptides. Merging solid-phase approaches of peptides (SPPS)[2] and automated glycan assembly (AGA)[3] will enable the fully automated on-resin procedure for synthesizing *O*-linked glycopeptides. Combinations of both techniques have not been developed yet. The use of partially or fully pre-assembled glycosylated amino acids while elongating the peptide by SPPS [4] enhances epimerization. Coupling glycans to serine or threonine is limited, due to their intrinsic low reactivity and the occurrence of β-elimination. We describe a chemically optimized amino acid residue designed to overcome these drawbacks and allow for bidirectional assembly of peptides and glycans. Incorporation of unnatural amino acid is the basis for the synthesis of glycolipopeptides on solid support. Developing automated methods of the synthesis of chimeric biomolecules could lead to several applications in vaccine development and biomaterial design.

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Towards self-adjuvanting cancer vaccines with synthetic TACA–αGalCer conjugates

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Immunotherapy is revolutionizing cancer therapy by harnessing the power of the innate and adaptive immune system against cancer cells and providing a more tumor-selective approach in assistance to traditional treatments. The identification of tumor-associated carbohydrate antigens (TACAs), aberrant glycans decorating the surface of tumor cells, has paved the way for the development of TACA–based cancer vaccines.

While significant progress has been made, TACA–based cancer vaccines have not yet reached the clinic and addressing some of the limitations that characterize classical approaches in carbohydrate cancer vaccine development can provide access to more effective candidates. In this context, iNKT cells are emerging as central players in cancer vaccine therapies. Indeed, recent reports have shown that iNKT cell-activating glycolipids, such as α-galactosylceramide (αGalCer),[1] can enhance the immune response against co-delivered cancer antigens by stimulating iNKT cells to serve as universal T helpers.[2,3] As this strategy appears to be well-suited to break the natural immunotolerance against TACAs,[3]here we present our synthetic efforts towards the preparation of novel ganglioside TACAs–αGalCer conjugates, their formulation in liposomes, and their immunological evaluation.[4] Furthermore, we illustrate their application as tool compounds for dissecting the molecular mechanisms driving the action of TACA–αGalCer conjugates.

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N-Glycosylation signatures of ovarian cancer tissues as defined by MALDI imaging mass spectrometry

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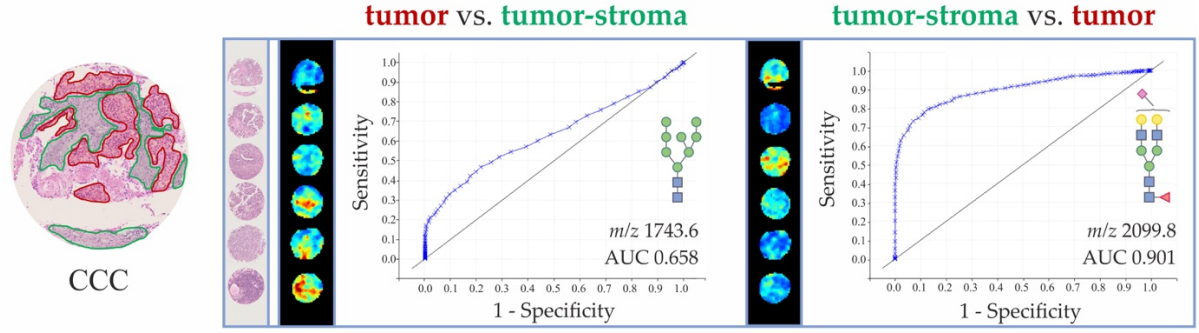
The particularly high mortality of epithelial ovarian cancer (EOC) is in part linked to limited understanding of its molecular signatures. We implemented MALDI mass spectrometry imaging (MALDI-MSI) in combination with sialic acid derivatization in formalin-fixed paraffin-embedded tissue microarray specimens of less common EOC histotypes, namely low-grade serous, clear cell (CCC), endometrioid, mucinous histotypes as well as non-malignant borderline ovarian tumor [1]. α2,6- and α2,3-sialylated N-glycans were enriched in tissue regions corresponding to tumor and adjacent tumor-stroma, respectively. Interestingly, analogous N-glycosylation patterns were observed in tissue cores of BOT, suggesting that regio-specific N-glycan distribution might occur already in non-malignant ovarian pathologies. All in all, our data provide proof that the combination of MALDI-MSI and sialic acid derivatization is suitable for delineating regio-specific N-glycan distribution in EOC and BOT tissues and might serve as a promising strategy for future glycosylation-based biomarker discovery studies.



ROC curves and MALDI-MSI pictures of the most discriminatory N-glycan structures as determined for tumor and tumor-stroma in CCC specimens.

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Improved *N*- and *O*-glycopeptide identification using FAIMS

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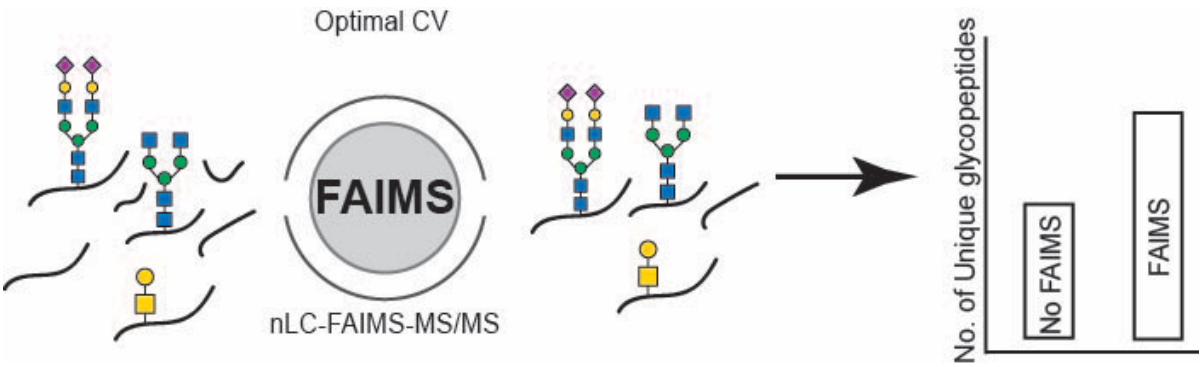
Mass spectrometry is the premier tool for identifying and quantifying site-specific protein glycosylation globally. Analysis of intact glycopeptides often requires an enrichment step, after which the samples remain highly complex and exhibit a broad dynamic range of abundance.

Here, we evaluated the analytical benefits of high-field asymmetric waveform ion mobility spectrometry (FAIMS) coupled to nano-liquid chromatography mass spectrometry (nLC-MS) for analyses of intact glycopeptide devoid of any enrichment step. We compared the effects of compensation voltage on the transmission of N- and O-glycopeptides derived from heterogeneous protein mixtures using two FAIMS devices. We comprehensively demonstrate the performance characteristics of the FAIMS device for glycopeptide analysis and recommend optimal electrode temperature and compensation voltage (CV) settings for N- and O-glycopeptide analysis.

Under optimal CV settings, FAIMS-assisted gas-phase fractionation in conjunction with chromatographic reverse phase separation resulted in a 31% increase in the detection of both N- and O-glycopeptide compared to control experiments without FAIMS. Overall, our results demonstrate that FAIMS provides an alternative means to access glycopeptides without any enrichment providing an unbiased global glycoproteome landscape. In addition, our work provides the framework to verify 'difficult-to-identify' glycopeptide features.



Genotype-dependent glycosylation affects histidine-rich glycoprotein activation by plasmin

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Histidine-rich glycoprotein (HRG) is an abundant plasma glycoprotein with 3 reported *N*-glycosylation sites, which integrates many biological processes, such as antiangiogenic activity[1,2], immune complex clearance[3], and pathogen clearance[4]. Importantly, the protein is known to have 5 genetic variants with minor allele frequencies of more than 0.1, meaning they exist with substantial frequency in the human population[2]. Among them, Pro204Ser can induce a new *N*-glycosylation site at Asn202[3]. Considerable research has been performed into the biological activity of HRG, while research on its glycosylation is rare[5]. To close this knowledge gap, we used C18-based nanoLC-MS/MS to investigate the glycosylation characteristics of HRG from human plasma, recombinant Chinese hamster ovary (CHO) cell lines and recombinant HEK293 cell lines with targeted mutations. Within endogenous plasma HRG, every *N*-glycosylation site proved dominant with N4H5S2 (**Figure 1a-d**). For the recombinant HRGs, on the other hand, glycans showed with different antennarities, sialylation and core-fucosylation, as well as the appearance of high-mannose glycans and antennary fucosylation. Furthermore, we discovered a previously unreported *O*-glycosylation site, Thr256, which showed an approximate 90% glycan occupancy in all HRG types (**Figure 1e**).To investigate the relevance of HRG glycosylation characteristics and its biological function, we set up an assay to study the plasmin cleavage of HRG under various conditions. In doing so, we showed that the sialylation of the new *O*-glycan, as well as the mutation dependent *N*-glycosylation, influence the plasmin cleavage of HRG significantly (**Figure 1f-g**).

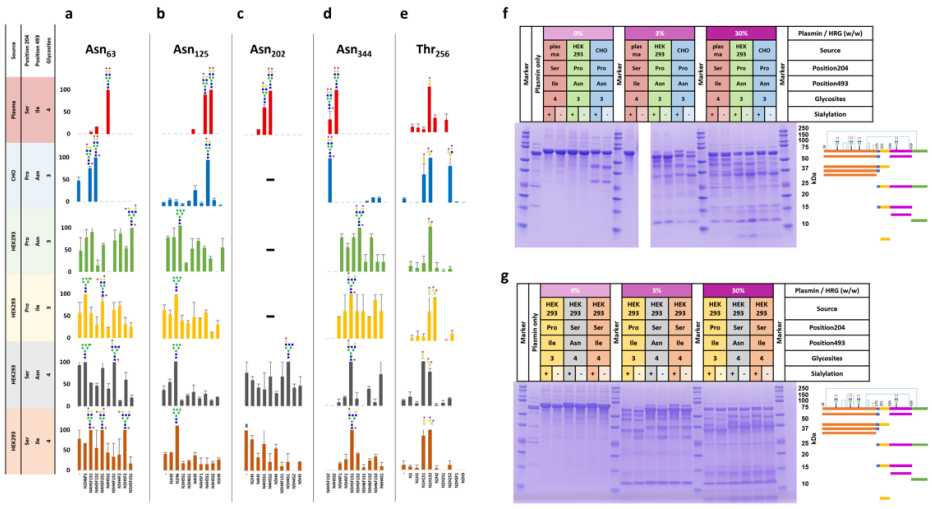

Figure1. Main N-glycan and O-glycan profile distribution of HRG from different sources and reducing gels of HRG proteins treated with plasmin.

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Underlying protein shape is a determinant of glycoform diversity

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It is frequently observed that a given glycoprotein can contain both predominantly highly-processed and under-processed glycans at different N-linked glycosylation sites. The differences occur despite these sites encountering the same environment while trafficking through the secretory pathways of the endoplastic reticulum and Golgi apparatus. Understanding the structural basis for such differences is important in fundamental processes, including innate and adaptive immune responses(1,2). Our group and others have proposed and demonstrated that accessibility of the glycosylation site correlates with the observed glycoforms at that site(2,3,4). Our molecular modeling efforts combined with experimental work predicted and confirmed that removal of an adjacent subdomain would trigger processing of a naturally under-processed site(4).

In this work we use molecular modeling to demonstrate that correctly combining the 3D structure of ERManI, an enzyme that acts early in the processing pathway, with molecular dynamics simulations of a target glycoprotein can be used to predict whether a given glycosite will be under-processed or not. We outline the subtleties required to successfully employ the technique and demonstrate its usefulness on Pdi1p, a well-characterized model glycoprotein, as well as the more challenging HIV Env SOSIP trimer. The work provides insights into enzymatic recognition of N-glycans and how underlying protein shape is the predominant determinant of glycoform diversity.

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Deep learning based prediction of protein-carbohydrate interfaces

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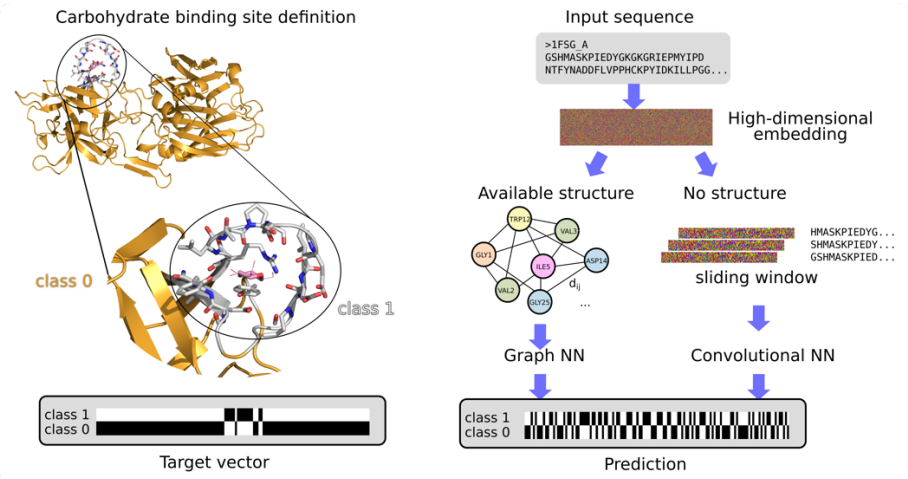
Protein-carbohydrate (PC) interactions are involved in a majority of crucial biological processes. Experimental resolution of 3D structures of PC complexes is particularly difficult due to chemical and structural variability of carbohydrates and weak affinities of PC interactions, leading to underrepresentation of carbohydrates in the Protein Data Bank. Data-driven methods have potential to indicate carbohydrate binding regions proteins with missing experimental information, but are much less developed as compared to protein-protein or drug-protein interaction prediction tools. In the current study, we propose for the first time two deep learning methods for carbohydrate binding site prediction. In the first model, we use embeddings derived from the pre-trained protein language model ESM-2 [1] for efficient encoding of sequence information, where we encode each residue using sliding windows. In the second model, if the experimental structure of the protein is available, we represent proteins as amino acid contact graphs and use the positional embeddings as node features. Both models are trained to predict residues in contact with carbohydrate ligands using convolutional neural networks as architecture for the sequence-based method and graph convolutional neural network for structure-based predictions in case the structure is available. Our models outperform the existing carbohydrate-specific [2] as well as non-specific binding site prediction tools [3] and successfully detect carbohydrate binding residues missed by other methods for proteins of biological interest. Therefore, the developed methods can have an important impact for understanding mechanisms of glycan recognition as well as for carbohydrate-based drug design.



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Structure based neural network predictions of protein carbohydrate interactions

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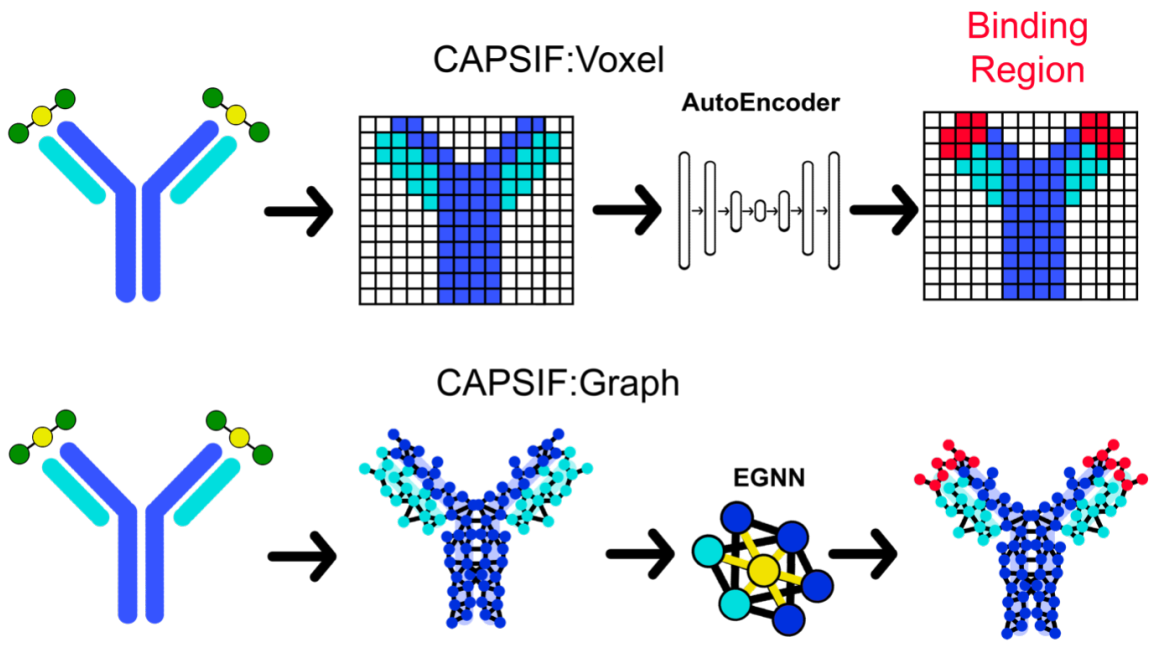
Carbohydrates dynamically and transiently interact with proteins for cell-cell recognition, cellular differentiation, immune response, and other cellular processes. Despite the molecular importance of these interactions, there are currently few reliable computational tools to predict potential carbohydrate binding sites on any given protein. Here, we present two deep learning (DL) models named CArbohydrate-Protein interaction Site IdentiFier (CAPSIF) that predicts carbohydrate binding sites on proteins: (1) a 3D-autoencoder voxel-based neural network model (CAPSIF:V) and (2) an equivariant graph neural network model (CAPSIF:G). We found CAPSIF:V performs better than CAPSIF:G, and both models outperform previous surrogate methods used for carbohydrate binding site prediction. CAPSIF also performs well when starting from computationally determined structures from AlphaFold2. CAPSIF models can be used in conjunction with local glycan-docking protocols, such as GlycanDock, to predict bound protein-carbohydrate structures. We will share how these tools can be used for *ab initio* predictions of bound oligosaccharide transferase-carbohydrate structures.



CArbohydrate Protein Site IdentiFier (CAPSIF) neural network architectures

Investigating the effect of the spatial topology of multimodular GHs on an insoluble substrate

Cédric Y. MONTANIER [1], Louise BADRUNA [1], Vincent BURLAT [2], Pierre ROBLIN [3], Michael J. O'DONOHUE [1], Claire DUMON [1], Antoine BOUCHOUX [1], Sarah BLOSSE [1,4], Paul DURU [4]

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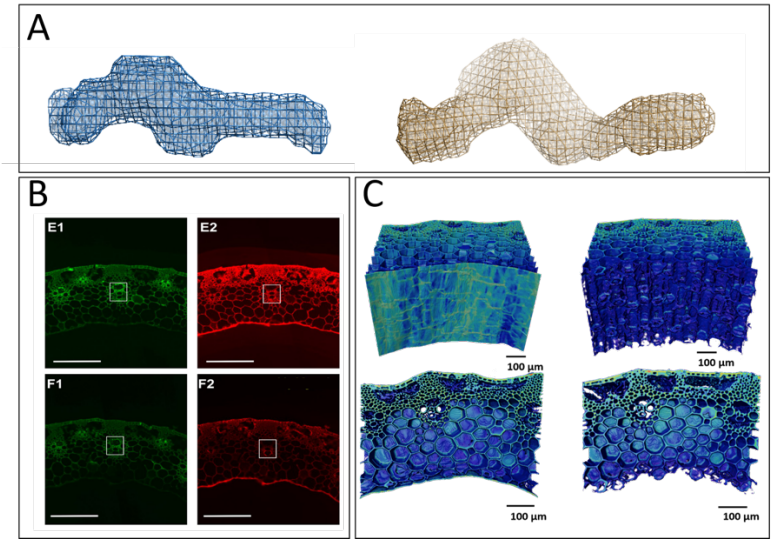
The wide diversity of carbohydrate-active enzymes (CAZymes) reflects the equally wide diversity in the composition and chemical bonds of the plant cell wall polysaccharides. This diversity is also reflected in the different strategies developed by microorganisms to circumvent the recalcitrance of these substrates to biological degradation. These aspects have been studied extensively for decades. CAZymes are also very diverse in terms of architecture, from the simplest to the most complex, such as the cellulosome. Such organisations have been shown to be important for catalytic function. However, the role of spatial topology and distances between the different domains of individual enzymes acting on a complex and insoluble substrate is underestimated. We propose a systematic approach to investigate this question, focusing on glycoside hydrolases (GHs). Our approach is based on two small proteins, Jo and In, which spontaneously form an intramolecular isopeptide bond and, incidentally, provide an original means of orienting GHs [1]. Chimeric multimodular GHs were produced and purified, and their structure in solution was solved by Small Angle X-rays Scattering (Fig.1A) [2]. In addition to their activity towards soluble and insoluble substrates, differences in the targeting of multimodular GHs in wheat straw were assessed *in situ*, monitored by immunological labelling (Fig.1B). We also demonstrated that X-ray microtomography is particularly well-suited to reveal the modification of the plant cell walls within the sample, at different stages of the enzymatic deconstruction (Fig.1C) [3].



A) SAXS model of chimeric GHs; B) Double immunofluorescence of paraffin-embedded wheat straw serial sections; C) Images from X-ray microtomographyA) S

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High-throughput analysis reveals miRNA up & down regulating α-2,6-sialic acid

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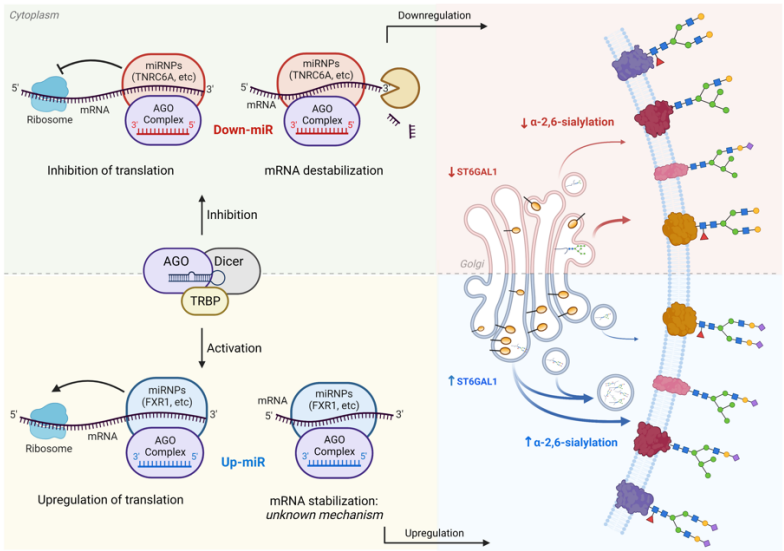
Chemical biology tools have increasingly revealed the importance of sialic acids as a major signal in physiology and disease. α-2,6-Linked sialic acids on galactose drive cancer development and metastasis, immunological recognition, and microglial phagocytosis (1). This modification is biosynthesized by two enzymes: ST6-β-galactoside-α-2,6-sialyl- tranferase-1 (ST6GAL1), and ST6GAL2. Although this modification is critical in both health and disease, the regulation and dysregulation of these enzymes and thus α-2,6-linked sialic acid are poorly understood.

microRNAs (miRNAs, miRs) are small non-coding RNA that tune protein expression through modulation of mRNA. The canonical view of miRNAs is that they are posttranscriptional repressors, binding to the 3'-UTR of mRNA within RISC complex and causing mRNA destabilization and/or loss of translation (2). Using our recently developed high-throughput fluorescence assay (miRFluR) (3), we comprehensively mapped the miRNA regulatory landscape of α-2,6-sialyltransferases ST6GAL1 and ST6GAL2. We found, contrary to expectations, the majority of miRNA upregulate ST6GAL1 and α-2,6-sialylation in a variety of cancer cells. In contrast, miRNAs that regulate ST6GAL2 were predominantly downregulatory (4). Mutational analysis identified direct binding sites in the 3'-untranslated region (UTR) responsible for upregulation, confirming it is a direct effect. The miRNA binding proteins AGO2 and FXR1 were required for upregulation. Our results upend common assumptions surrounding miRNA, arguing that upregulation by these non-coding RNA is common. Indeed, for some proteins, upregulation may be the dominant function of miRNA. Our work also suggests that upregulatory miRNA enhance expression of ST6GAL1 and α-2,6-sialylation, providing another potential pathway to explain their dysregulation observed in cancer and other disease states (4).



miRNA regulation of α-2,6-Sialic acids

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Synthesis, structure, modelling and interactions of GAG mimetics with proteins

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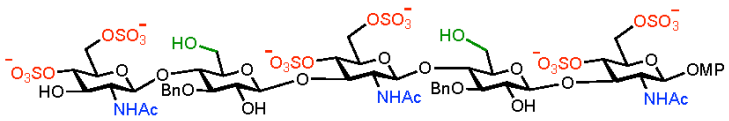
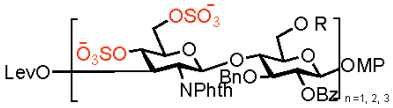
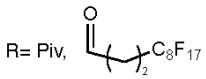
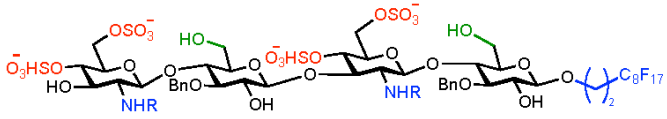
Midkine (MK), together with Pleiotrophine (PTN or HB-GAM), constitutes the family of neurite growth-promoting factors (NEGF). They are involved in early neural growth and other physiological actions related to mitogenesis or inflammation. They interact with extracellular glycosaminoglycans (GAG) that are fundamental for their activity through interaction with specific membrane receptors. We have been exploring the synthesis, binding properties, and structure of synthetic canonical Chondroitin Sulfate tetrasaccharides, [1] and now we showed our results with GAG mimetics with variations on the basic skeleton's (see figure). Variations were the substitution of GlcA by Glc;[2] or GalN by Glc and the introduction of fluorinated aliphatic chains to improve the isolation, both in the anomeric or in primary alcohol positions. We used NMR to study the 3D shape of these new compounds concluding that they still have the same 3D pattern that the GAG homologs. [2]

After our analysis combining NMR and modeling, we can conclude that the shape of the mimetics studied is similar to that of GAG. Therefore they can be considered good mimetics of them in spite of drastic changes such as removing a negative charge, per disaccharide.









R = Ac, Bz

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A novel heparin-like heparanase inhibitor hexasaccharide: synthesis and activity evaluation

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Heparanase (HPSE) is an β-endo-D-glucuronidase capable of cleaving the glycosaminoglycan chains of heparan sulfate (HS) at specific sites and thereby to modulate the biological function of this proteoglycan.

Deregulation of HPSE appeared in numerous pathological processes such as inflammation, metastasis, and angiogenesis [1], therefore, its inhibition is a good target for new therapeutic agents [2]. The development of non-anticoagulant heparin derivatives was a found promising strategy to develop HPSE inhibitors and among these compounds, Roneparstat was the first HPSE inhibitor reaching clinical trials [3]. It is obtained from heparin after N-desulfation, N-acetylation, periodate oxidation and borohydride reduction, originating the so called glyco-split residue (abbreviated as gs) in the heparin chain. It was later found that, when the primary alcohols of these gs units of heparin are replaced by carboxylic acids (gs/ox) heparanase inhibitory activity increased, thus resulting in a new drug (H1710), now in preclinical stage [4].

In the present work we synthesized a defined hexasaccharide containing a gs/ox uronic acid to check whether similar structural modifications on a hexasaccharide would result in similar effects. Surprisingly, we found that the synthetic derivative shows similar activity of Roneparstat (IC_{50} 70 nM), in spite its lower molecular weight.

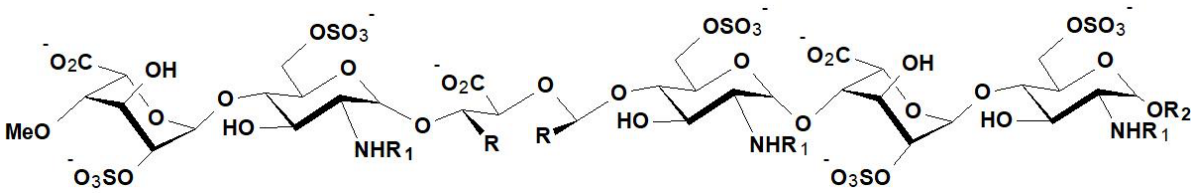

Figure 1, R = CH2OH, CO2H, R1 =Ac, SO3H, R2 = alkyl

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4. https://www1.hkexnews.hkhtt

Development of semi-synthetic methods to obtain glycosaminoglycans mimics from sustainable sources

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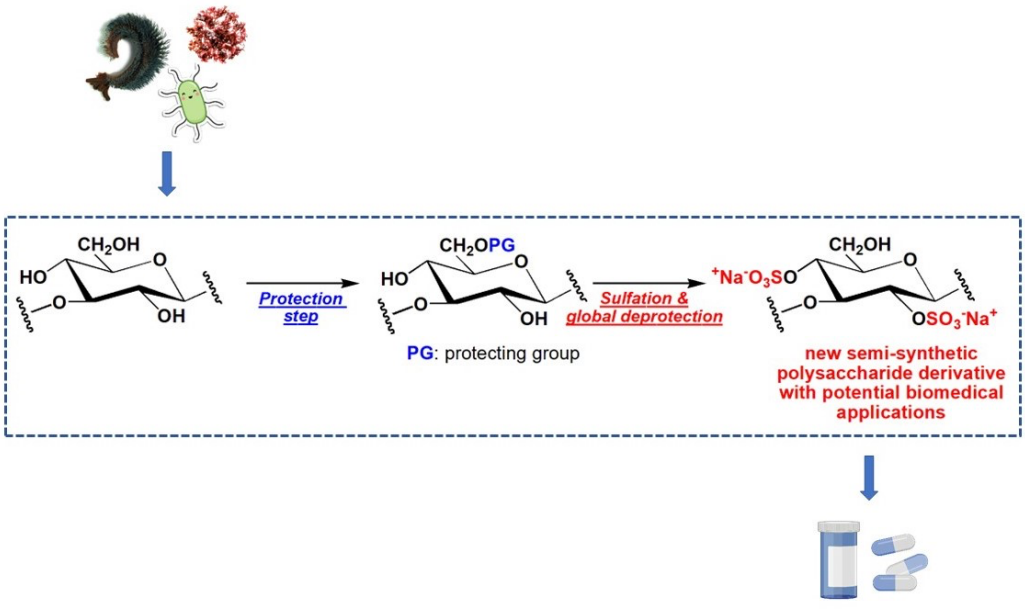
Polysaccharides are the most abundant biomacromolecules on our planet, possessing enormous structural diversity and functional versatility. They are currently employed for several purposes, both in their natural and structurally modified forms. Nonetheless, several polysaccharides used in pharmaceutical field are obtained from animal sources (*e.g.* glycosaminoglycans, GAGs) and this limits their use both for ethical and ecological reasons and for problems related to the possible contamination of the batches. However, sulfated polysaccharides can also be obtained in a semi-synthetic way: the introduction of sulfate groups into the backbones of natural unsulfated polysaccharides allows to endow them with bioactivities similar to sulfated GAGs but without risks derived from their typical animal sources.[1] In this frame, a special interest is focused on the sulfation of polysaccharides from eco-sustainable natural and/or biotech sources (algae, fungi, bacteria) already used in the biomedical and/or food fields, in order to improve their properties or to introduce new ones. Regioselective sulfation reactions can be conducted through multi-step strategies consisting in protection-sulfation-deprotection sequences.[2] In particular, the polysaccharides selected to this aim are M-rich alginic acid extracted from brown algae, curdlan from *Agrobacterium* strains,[3] and finally an exopolysaccharide (EPS)[4]from *Vibrio diabolicus* HE800 composed only of aminosugars and uronic acids. Their regioselective sulfation has been performed to obtain new derivatives acting as GAG mimics.



General scheme of semi-synthetic approaches towards glycosaminoglycans mimics

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Single molecule nanopore sensing of glycosaminoglycans

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Sequencing of polysaccharides is lagging behind compared to the very advanced situation for the two other major families of bio-polymers, nucleic acids and proteins, for which effective methods of structural analysis have been available for decades. The need for such methods is particularly felt for bioactive polysaccharides and among them glycosaminoglycans (GAGs).

GAGs are highly sulfated linear polysaccharides that play a dominant role in the communication of cells with their environment [1]. Comprising of disaccharide units, GAGs present an extraordinary structural complexity due to their non-template driven biosynthesis that results in their chemical heterogeneity and a broad diversification of structure.

Faced with this situation, a powerful solution is provided by the single-molecule detection and characterization based on the translocation through nanopores. This technique has been applied to GAGs by exploiting the confinement properties of the aerolysin nanopore. Heparin, chondroitin sulfate, dermatan sulfate, heparosan and hyaluronic acid saccharides were analyzed and distinguished, showing that aerolysin nanopore can detect and characterize GAGs with various sulfate patterns, osidic bonds and epimers of uronic acid residues [2].

The results of the study show for the first time the detection and resolution of different sequences of GAGs according to the different modifications distributed along the chains. The discrimination of the building blocks of GAGs is an essential step towards a sequencing pathway for these polysaccharides.

PB acknowledges post-doctoral fellowship from the program CHARMMMAT ANR-11-LABX-0039-grant. C.R. and M.Bilong acknowledge PhD fellowships from doctoral school SDSV (No. 577, Université Paris-Saclay).

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

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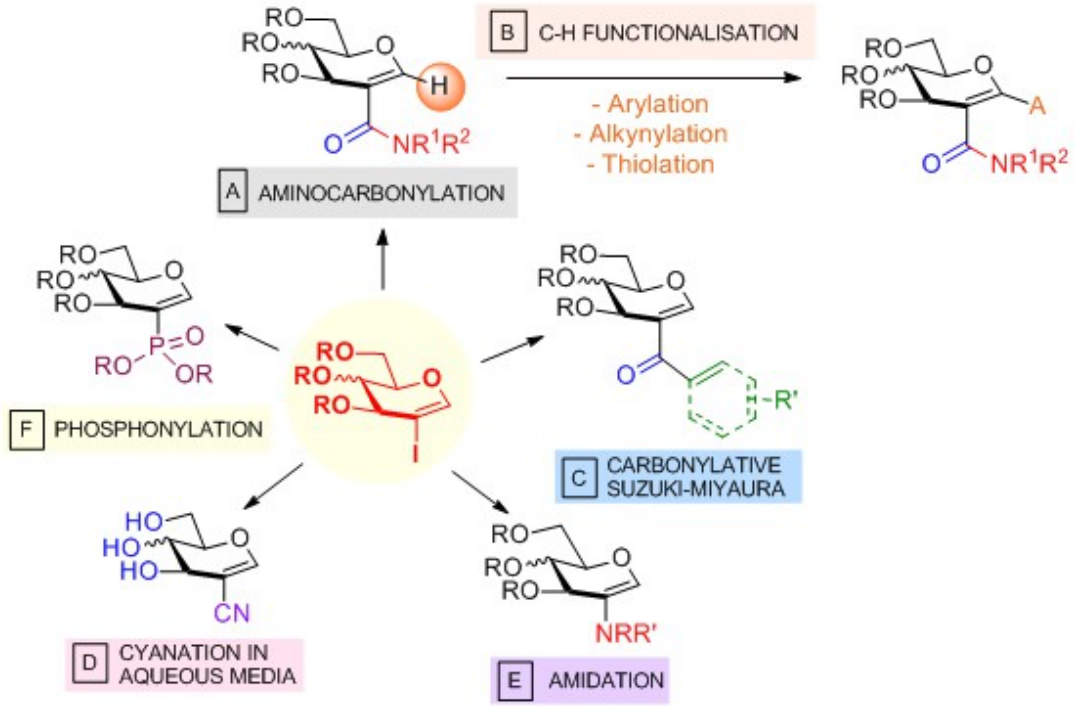
Development of new access to glycoconjugates has become of great interest in synthetic chemistry. In particular, glycoconjugates possessing an unnatural bond are largely studied due to their enzymatic and chemical stabilities towards natural links.

Our expertise deals with the metal-catalyzed functionalisation of glycal substrates using two different reactivities:

- Cross-coupling reactions on 2-iodoglycal starting compounds for the formation of C-C, C-N or C-P bonds: (A), (C), (D), (E) and (F).
- Directed C-H functionalisation reactions of the pseudo-anomeric position of C2-amidoglycals (B).



Developed metal-catalyzed functionalisation of glycals

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How does glucose affect the photoisomerisation of a quinazolinone-based glycoconjugate?

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Changes in molecular structures induced by UV or visible light play an important role in various biological processes [1]. This transformation, often accompanied by bond rotation, from a more energetically favourable conformation (*anti*-form) to a less energetically favourable conformation (*syn*-form) is called photoisomerisation. The photochemical process is very often associated with conjugated systems containing heteroatoms such as oxygen and nitrogen. There are a large number of nitrogen-containing heterocyclic compounds, including Schiff bases, which have highly conjugated systems with aromatic substituents bound to the aliphatic chain. Particular attention has been focussed on compounds possessing N=C and N=N bond systems [2].

We present a novel glycoconjugate consisting of two β-glucopyranoses attached to a quinazolinone-like structure, which has an interesting photochemical property. The new derivative exhibits photoisomerization around the −N−N= and =CH−C− bonds of the −N−N=CH−C− linkage simultaneously ("crankshaft rotation") upon exposure to UV light. Experimental high-resolution NMR spectroscopy, combined with DFT calculations, revealed that the attachment of carbohydrate residues to photoactive compounds [3,4] can significantly change the isomerization process, while the overall molecular structure remains virtually unchanged.

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O-antigen variability among clinical isolates of Klebsiella pneumoniae

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Klebsiella pneumoniae is a nosocomial pathogen, pointed out by the WHO as "critical" regarding the highly limited options for the treatment of infections.

Lipopolysaccharide (LPS, O-antigen) and capsular polysaccharide (K-antigen) are its virulence factors and surface antigens, determining O- and K-serotypes and encoded by O- or K-loci. They are promising targets for antibody-based therapies (vaccines and passive immunization) as an alternative to antibiotics. To make such immunotherapy effective, knowledge about O/K-antigen structures and distribution among clinical isolates is necessary. *K. pneumoniae O*-antigens seem to have limited variability *[1]*, however the presence of new O-serotypes was indicated by genetic analyses *[2]*.

Twelve nontypeable and drug-resistant clinical isolates of *K. pneumoniae* were analysed for *O*-antigen structure. Isolates were selected based on the lack of homology between isolates' O-loci and reference O-loci for already known O-antigens. Discrepancies for O2 serotyping between Kaptive-based predictions (O2 variant 2 serotype) and the actual phenotype (O2 variant 1) were explained for strains BIDMC 7B and ABC152 (presence of insertion sequences in O-loci) *[3,4]*. New O-antigens have been identified for isolates Kp175, Kp231, Kp254. Semi-rough character was found for Kp159 and Kp160 LPS. Isolates Kp164, Kp165, Kp166 were identified as the O4. Additionally O-antigen variability of New Delhi metallo-β-lactamase (NDM)-producing *K. pneumoniae* responsible for a countrywide outbreaks in Poland (2012-2018) is discussed.

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The Cryo-EM structure of human fucosidase FucA1 opens new avenues for the treatment of fucosidosis

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Enzymatic hydrolysis of α-L-fucose from fucosylated glycoconjugates is consequential in bacterial infections and the neurodegenerative lysosomal storage disorder fucosidosis. Understanding human α-L-fucosidase catalysis, in an effort toward drug design, has been hindered by the absence of three-dimensional structural data for any animal fucosidase. Here, we have used cryoelectron microscopy (cryo-EM) to determine the structure of human lysosomal α-L-fucosidase (FucA1) in both an unliganded state and in complex with the inhibitor deoxyfuconojirimycin. These structures, determined at 2.49 Å resolution, reveal the homotetrameric structure of FucA1, the architecture of the catalytic center, and the location of both natural population variations and disease-causing mutations. Furthermore, this work has conclusively identified the hitherto contentious identity of the catalytic acid/base, representing a shift from both the canonical glutamate acid/base residue and a previously proposed glutamate residue. These findings have furthered our understanding of how FucA1 functions in both health and disease.

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

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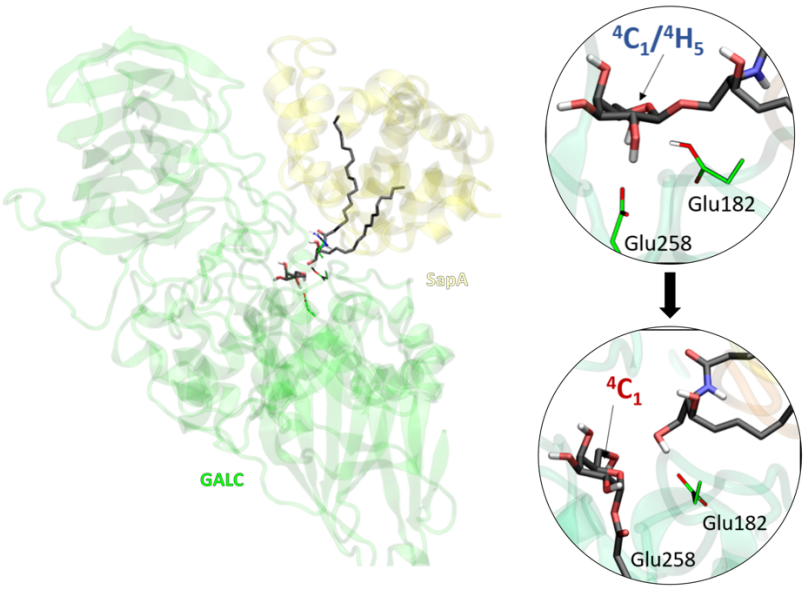
Krabbe disease is a neurodegenerative disorder related to misfunction of Saposin A or β-galactocerebrosidase (GALC), a glycosidase that catalyzes the cleavage of *β*-galactosidic bonds in glycosphingolipids [1]. Here we uncover the catalytic molecular mechanism of GALC in complex with Gal-β-*p*-nitrophenyl, a substrate analogue, using quantum mechanics/molecular mechanics (QM/MM) metadynamics simulations [2]. Our results clarify the unusual chair conformation of the substrate observed in the crystal structure [3] and show that catalysis can take place via two distinct conformational pathways with similar free energy barriers because of leaving group flexibility [4][5]. Moreover, we study the complex of the important agent for GALC activity *in vivo*, the lipid-transfer protein Saposin A (SapA) with GALC. SapA extracts the lipid substrate from the cell membrane, forming a soluble saposin-lipid complex that provides the lipid to GALC. In spite of the relevance of SapA for GALC function, the catalytic reaction mechanism of the GALC-SapA complex with its natural substrate has not been reported. Our simulations show that SapA not only acts as a transport agent but also it helps decreasing the hydrolysis energy barrier by stabilizing the reaction transition state [6]. This mechanistic insight can aid in the design of Krabbe diagnosis probes and GALC conformational chaperones and expand the knowledge of the importance of saposin domains and their interaction with lipid-degrading enzymes.



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Uncovering catalytic mechanisms of inverting glycosyltransferases involved in protein glycosylation

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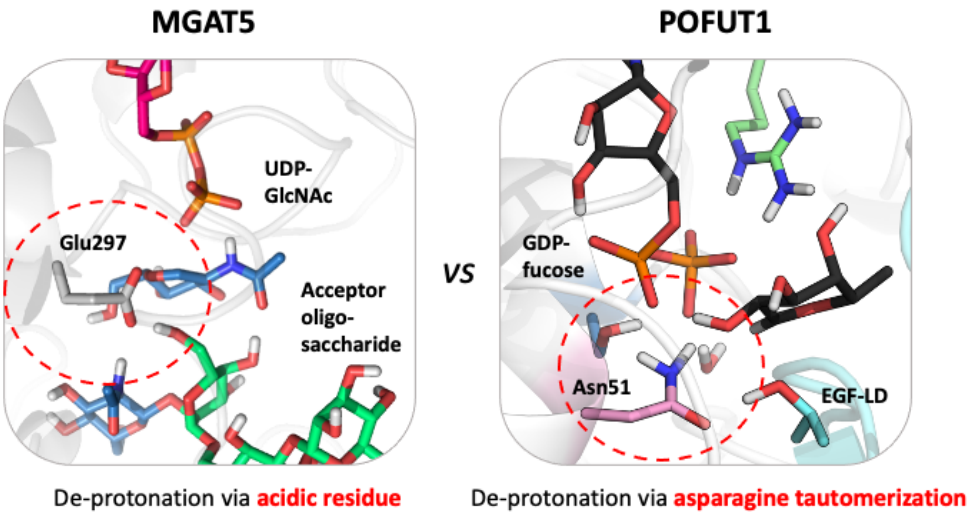
Post-translational protein modifications, such as N- and O-glycosylation, are essential to life. Glycosylation defects are the cause of several diseases and, therefore, there is a great interest in deciphering the mechanism of action of the enzymes responsible for the synthesis of protein glycoconjugates. Two of these enzymes are α-mannoside β-1,6-N-acetylglucosaminyltransferase V (MGAT5, also known as GnT-V) and protein O-fucosyltransferase 1 (POFUT1). MGAT5 catalyzes the transfer of an N-acetylglucosamine moiety to the core α-1,6-mannose of an N-glycan and its activity is connected to oncogenic behavior in cells. POFUT1 catalyzes the transfer of one moiety of L-fucose to a threonine or serine residue in the surface of epidermic growth factor-like domains (EGF-LD), small peptides involved in the Notch signaling cascade, and it is related to diseases such as colorectal cancer, leukemia and Dowling-Degos disease. We have recently uncovered the catalytic mechanism of these two enzymes by means of quantum mechanics/molecular mechanics (QM/MM) and metadynamics methods. Our simulations show that whereas MGAT5 follows a classical SN2 mechanism in which an acidic residue (Glu297) acts as the catalytic base [1], POFUT1 follows an unusual mechanism involving a proton shuttle through a conserved active site asparagine [2]. Site-directed mutagenesis experiments are in line with our results. These findings widen our understanding of these enzymes and will aid inhibitor development to correct disease associated O-glycosylation.



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Protecting group free transformations of reducing sugars in aqueous solution

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The development of new selective reactions of completely unprotected sugars, particularly in aqueous solution, has become an area of resurgent interest. Seminal studies reported by Shoda[1] and co-workers first introduced the dehydrating reagent 2-chloro-1,3-dimethylimidazolinium chloride (DMC) into the carbohydrate field, and revealed its remarkable ability to selectively activate the anomeric hydroxyl group of unprotected sugars in aqueous solution. A series of highly useful protecting group-free processes based on the use of DMC and analogues has since been developed.[2] I will discuss some of our recent work in this area,[3] building on the important developments of Shoda and others, focussing on the application of DMC and analogues for the direct conversion of unprotected sugars into a range of glycosides, glycoconjugates, and even (1-6)-linked disaccharides[4] without the need for any protecting group chemistry.

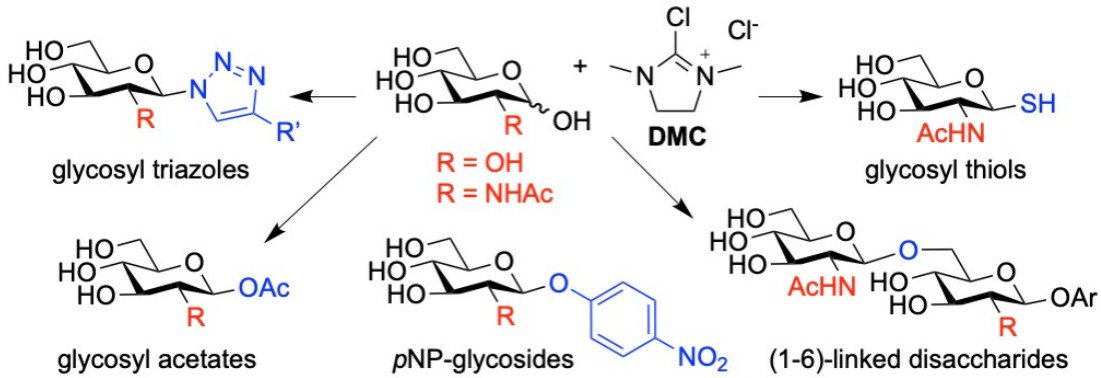

Figure 1: Structural concept of iminosugar based probes.

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Study of unprotected glycosyl cations in an enzymatic context

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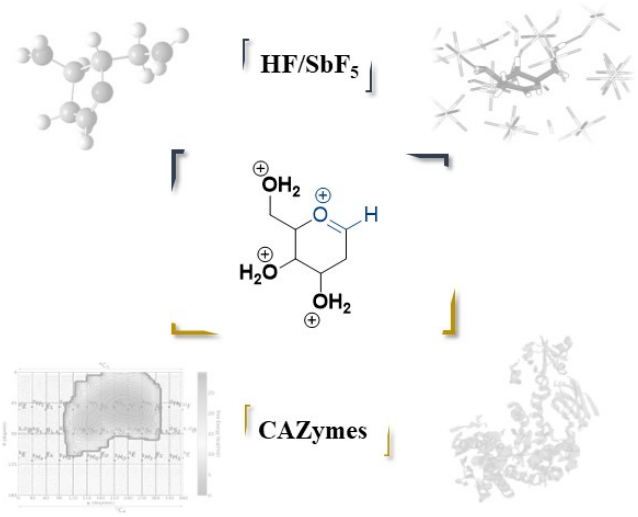
Malfunction Due to its extremely short lifetime, the study of glycosyl oxocarbenium ions is a real challenge in the field of glycosciences. The impact of protected glycosyl cations on the stereochemical outcome of glycosylation has been studied by various indirect methods[1], [2], [3]. This chemical species has been observed and characterized for the first time in a condensed phase using a superacid medium[4] and more recently using other spectroscopies[5], [6].

Essential intermediate in the glycosylation reaction, it has been also postulated in its unprotected form in enzymatic transformations performed by glycosidases and glycosyltransferases[7]. Therefore, its observation in a condensed phase is challenging and of high interest.

Using superacid chemistry, and in *situ* NMR analysis at low temperature supported by computation, the structure of these chemical species will be presented, and their relevance to enzymatic processes evaluated.



Study of the glycosyl cation in superacid and enzymatic medium

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Protecting-group-free phosphate cross-coupling enables efficient synthesis of ADP-ribose molecules

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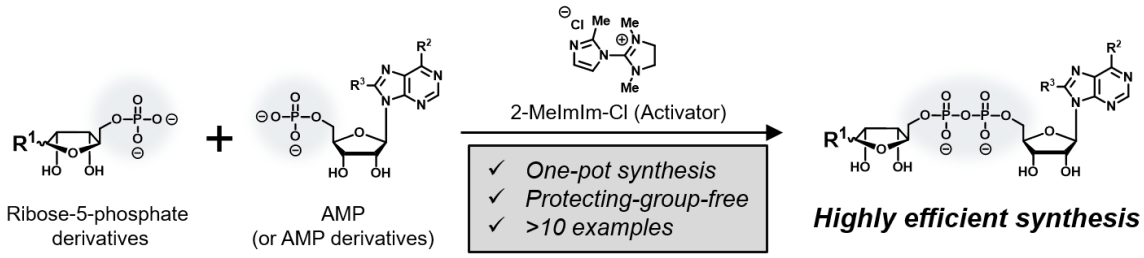
ADP-ribosylation, one of post-translational modifications (PTMs), is a reaction that transfers ADP-ribose moiety from NAD^+ to specific amino acid residues. This PTM plays an important role in a variety of cellular processes including DNA repair, signal transduction, transcriptional regulation, cell differentiation and apoptosis [1]. Its biological importance has widely been investigated but detailed functions of ADP-ribosylation remain poorly understood. The main difficulty in the molecular level research is to obtain homogeneous ADP-ribosylated samples from nature. Therefore, chemical synthesis is highly demanded.

In this study, we present an efficient method for chemical synthesis of diverse ADP-ribose derivatives through pyrophosphate formation by protecting-group-free phosphate cross-coupling reaction. We used 2-MeImIm-Cl, a hydroylsis stabilized ImIm-Cl [2], to activate a phosphate group by introducing an 2-methylimidazole leaving group for the phosphate cross-coupling reaction.

After chemoselective activation of the phosphate group of AMP (or derivatives) with 2-MeImIm-Cl, protecting-group-free phosphate cross-coupling reaction with various ribose-5-phosphate derivatives allowed for highly efficient synthesis of various ADP-ribose derivatives. Furthermore, we developed a method for the synthesis of ADP-ribose *N*-glycoside using stereoselective amidation reaction by traceless Staudinger ligation [3] with an azido analog of ADP-ribose.



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Block polysaccharides

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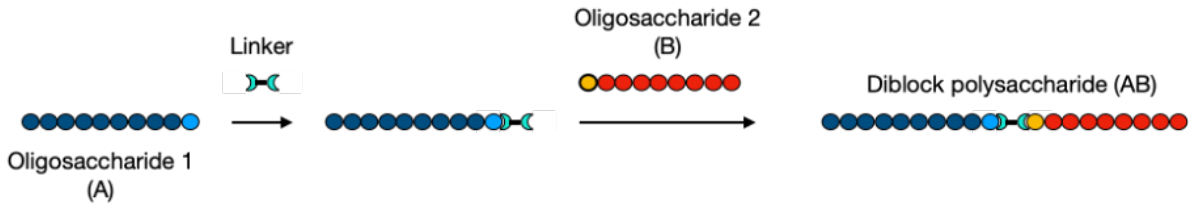
The conjugation at chain termini of two different polysaccharides provides diblock polysaccharides, a new class of precisely engineered polysaccharides. This architecture provides on one hand new solution and stimuli-responsive self-assembly properties, while retaining key properties such as biodegradability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

The first part of the presentation will focus on the preparation of blocks through dioxyamine linkers. The second part will focus on diblocks containing Ca-reactive oligoguluronates (derived from alginates) and their Ca-induced self-assembly studied by static and dynamic light scattering, SANS and SAXS.



General scheme for preparing diblock polysaccharides

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Mo, I. V.; Schatz, C.; Christensen, B. E. (2021) Carbohydr. Polym. (261), 118193.

One-pot and biomass-agnostic syntheses of biodegradable surfactants from snionic polysaccharides

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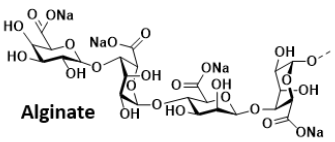
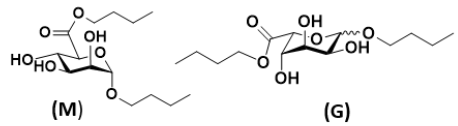
During past decades, thanks to the use of renewable resources in chemical production, the focus has shifted towards the development of fully bio-based surfactants. These surfactants have been widely studied due to their good biodegradability and low toxicity and ecotoxicity. The most common ones are the alkyl polyglucosides (APG) which are non-ionic compounds with a production of 90000 t/a. However, the examples of anionic surfactants derived from renewable resources are scarce and the use of novel building blocks derived from sustainable resources to obtain the targeted properties is a major challenge for the surfactant industry. Therefore, the ENSCR (Ecole Nationale Supérieure de Chimie de Rennes) has led researches on the development of novel surfactants and green/blue chemical processes, using biomass from terrestrial or marine origin. These researches allowed the development of One-pot and Biomass-Agnostic syntheses of anionic or non-ionic sugar- or furanic-based surfactants derived from algal polysaccharides (ulvans, alginates, agarose) and pectins.[1-3] Physicochemical studies of these original sugar-based molecules have been achieved and clearly highlight the potential of these original materials as surface-active agents and emulsifying products. In addition, the readily biodegradablity and the absence of aquatic ecotoxicty make these surfactants very promising for cosmetic or personal care applications. Some reaction intermediates have been identified as potential biosourced 'platform' molecules.



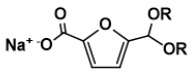
Alginate

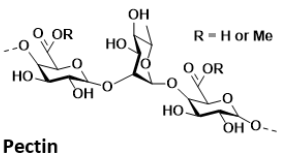
- 'One-pot' process
- Biodegradable acid catalyst
- Vegetable oils



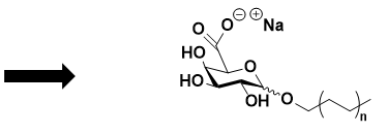
(M) **(G)**

Anionic surfactants D-mannunorate (M) and L-guluronate (G)





Pectin



Biodegradable and no Ecotoxicity

Anionic surfactant D-galacturonate

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“Azalevoglucosan”, a useful synthon in iminosugar chemistry

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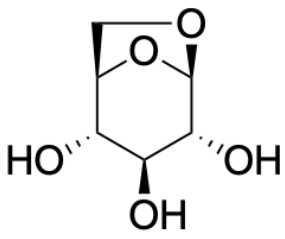
Levoglucosan **1** [1], 1,6-anhydroglucopyranose, has been extensively used in carbohydrate chemistry to access a wide range of glycosyl donors and acceptors, notably exploiting Cerny epoxide. The masked C1 and C6 positions in levoglucosan allow to modify the free hydroxyl groups at C2, C3 and C4 to access a wide range of derivatives.[2] In addition, ring opening of the 1,6-anhydro bridge is a convenient way to stereoselectively introduce functional groups at C1 while freeing the C6 position.[3]

Replacement of the oxygen atom in the 1,6 anhydro bridge by a nitrogen atom to produce “azalevoglucosan” **2** is scarce in the literature.[4] In the last decade, our group has exploited such scaffold to access various iminosugars through hemiaminal opening and skeletal rearrangement.[5]

Capitalizing on this chemistry, we will present our last results concerning the interconversion of the hydroxyl groups in azalevoglucosan **2** to access unprecedented iminosugars.



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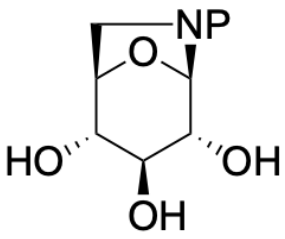

2

Bibliographic references:

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A new stereoselective approach to polyhydroxylated azepanes

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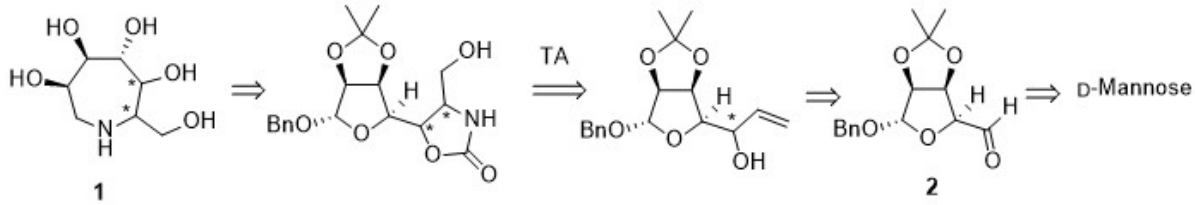
Iminosugars represent the most important class of glycomimetics, as they mimic the biological action of carbohydrates while circumventing their drawbacks. In particular, iminosugars are widely known as glycosidases and glycosyl transferases inhibitors [1].

The use of simple carbohydrates as starting materials for their preparation still offers many advantages. Compared to five- and six-membered iminosugars (e.g. polyhydroxylated pyrrolidines and piperidines), the synthesis of seven-membered analogues (polyhydroxylated azepanes) received little attention, albeit these compounds displayed interesting biological properties [2]. Therefore, the development of efficient and selective strategies for their preparation is of great interest.

In this context, we present a straightforward approach to pentahydroxylated azepanes **1** bearing five-contiguous stereocenters, three of which derive from the starting key intermediate **2**, a masked dialdehyde obtained from D-mannose.

We envisaged that the reductive amination reaction, widely employed for the preparation of polyhydroxylated piperidines [3], could be also useful in this case. The key step of our approach relied on a "tethered aminohydroxylation" (TA) reaction, introduced by Donohoe in 2001 [4], which we previously exploited for the stereoselective synthesis of 2- and 3-aminosugars from glycals [5]. This reaction, combined with the addition of a vinyl metal to aldehyde **2** [6], allowed the stereoselective introduction of the remaining two stereocenters.



This work was funded by Regione Toscana (Bando Salute 2018—project: Lysolate).

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Highly functionalized diaminocyclopentane inhibitors of protein-*O*-GlcNAcase

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The progression of Alzheimer's disease is characterized by abnormal structure and accumulation of amyloid and tau proteins leading to cell death.[1] The formation of neurofibrillary tangles, which are toxic to neurons, is a consequence of hyperphosphorylation of the tau protein.[2] This pathological phosphorylation can be prevented by selective inhibition of *O*-GlcNAcase, which is already being tested in clinical trials.[3] In fact, current treatment options for neurodegenerative Alzheimer's disease are very limited and ineffective.[4]

In this presentation, the design, synthesis, and biological activities of a new type of selective *O*-GlcNAcase inhibitors based on the structural features of a highly functionalized diaminocyclopentane (**1**, Figure 1) will be presented.[5]

Acknowledgement: Czech-Austrian project co-funded by the Austrian Science Fund (No. I5236) and Czech Science Foundation (No. GA21-01948L) is acknowledged.

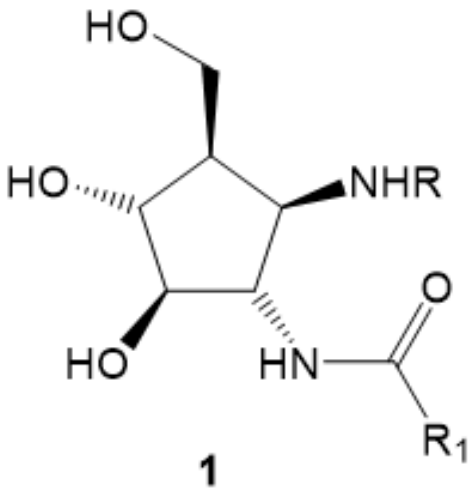

Figure 1. Diaminocyclopentanoid *O*-GlcNAcase inhibitors.

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Inhibitors of the phosphoribosyltransferases of protozoan parasites *P. falciparum* and *T. cruzi*.

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[1] Ferrier Research Institute, Victoria University of Wellington, New Zealand; [2] Department of Biochemistry, Albert Einstein College of Medicine, New York. [3] Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas.

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The protozoan parasites *P. falciparum* and *T. cruzi* are purine auxotrophs and require the salvage of purine bases from the host erythrocytes to perform nucleic acid synthesis. Purine salvage enzymes are attractive targets in the development of novel therapeutics for malaria and Chagas disease.

The design and synthesis of transition state analogue inhibitors will be presented that show good potency across all isozymes of both parasites.

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Y.V.T. Minnow, K. Suthagar, K. Clinch, R.G. Ducati, A. Ghosh, J.N. Buckler, R.K. Harijan, S.M. Cahill, P.C. Tyler, and V.L. Schramm (2022), ACS Chem. Biol. 17, 3407–3419.*

K. Glockzin, D. Kostomiris, Y.V.T. Minnow, K. Suthagar, K. Clinch, S. Gai, J.N. Buckler, V.L. Schramm, P.C. Tyler, T.D. Meek, A. Katzfuss, (2022), Biochemistry 61, (19), 2088–2105.

First-in-class selective nanomolar inhibitors of galectin-8 N-terminal domain

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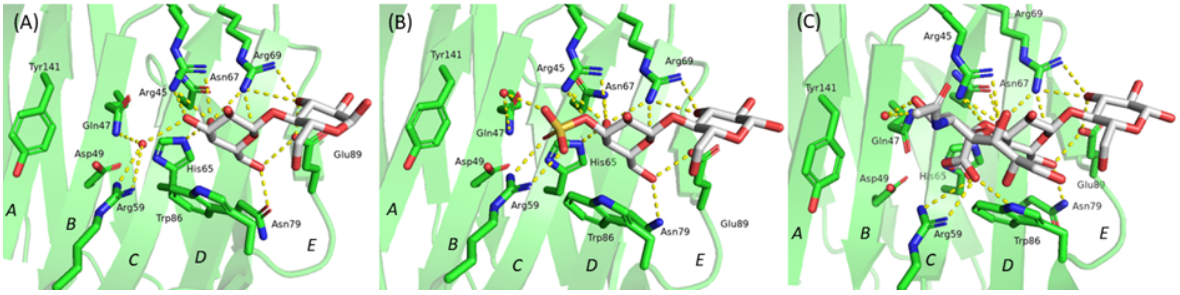
Galectins are found throughout the body and exhibit a variety of functions.[1] Among galectins, galectin-8, is nearly universal in both healthy tissues and tumours, and is found both intracellularly and extracellularly. Intracellularly, damaged and potentially pathogen-infected vesicles can be targeted for antibacterial autophagy by galectin-8 as a function of the immune system.[2] Extracellularly, carbohydrate recognition by galectin-8 modulates cell growth, death, and adhesion to influence lymphangiogenesis and tumour survival and metastasis.[3] In 2020 Cagnoni et al. stated: ˝The impact of specific galectin-8 CRD–glycan interactions in the biological functions of the full-length lectin is still a matter of controversy˝. [4] In order to allow advanced studies of these galectin-8-associated biological functions, potent and selective galectin-8 inhibitors are needed and exactly these were the overarching aim of our work.

Ligand selectivity among galectins has ever been a challenging objective. As we will present in this work, intrinsic differences between galectin binding sites allow a fine tuning of ligand selectivity and potency (Figure). Starting from our recently published series of benzimidazole-galactosides [5], we have designed a series of 2-substituted galactosides that exert sub-micromolar affinities for galectin-8 for the first time, while retaining promising selectivity versus related galectins. These selective compounds facilitate the study of galectin-8 biology and may have pharmaceutical relevance in the wide range of galectin-8 associated pathologies.



3D structure of Gal-8N in complex with A) lactose (PDB 3AP4); B) lactose 3'-sulfate (PDB 3AP6); C) 2,3'-sialyllactose (PDB 3AP7). A-E denote subsites.

This project has received funding from the European Union's Horizon 2020 under the Marie Skłodowska-Curie grant agreer No 765581. The authors kindly thank Barbro Kahl Knutson for FP assay.

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Exploring the dual role of proteins as carrier and protective antigen in glycoconjugate vaccines

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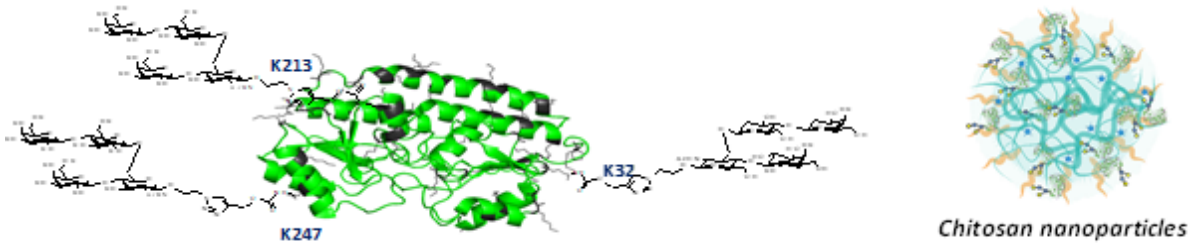
Bacterial capsular glycoconjugate vaccines have proven efficient, well tolerated and safe in humans including young children and immune-compromised population.[1] Current licensed glycoconjugate vaccines are made of poly/oligosaccharides conjugated to a carrier protein which triggers a T-cell dependant immune response to the glycan owing to the activation of the T-helper cells. However, to date only a handful of carrier proteins has been validated for use in humans, a situation which raises concerns regarding pre-exposure or co-exposure to a given carrier which can lead to immune interference and reduction of the anti-carbohydrate immune response.[2] Thus, it is tempting to explore the dual role of proteins from the pathogen against which we want to develop a vaccine as carrier and protective antigen to circumvent this issue. According to this strategy, is it possible to mount a protective humoral response against both B peptide epitopes of the protein and carbohydrate antigen which compete for the same limited number of T-helper epitopes?

Considering pneumococcal infection as a model disease, we studied structure/immunogenicity relationships of a panel of glycoconjugates obtained by random[3] or controlled conjugation. In the latter case, site selective mutagenesis or unnatural amino acid incorporation was applied to prepare homogeneous glycoconjugates.[4] We also demonstrate that glycoconjugate encapsulation into chitosan nanoparticles can improve humoral response by several order of magnitudes compared to glycoconjugate administered alone.[5]



Homogeneous pneumococcal glycoconjugate (left); nanoparticle glycoconjugate vaccine (right)

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Identification of common epitopes between different serotypes of *Streptococcus pneumoniae* group 19

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Vaccination with polysaccharide-based conjugate vaccines (PCVs) is recognized as one of the most successful strategy to prevent morbidity and mortality from pneumococcal diseases [1]. PCV vaccines contain capsular polysaccharide fragments from the *Streptococcus pneumoniae* (Sp) serotypes causing the majority of the diseases, covalently linked to a carrier protein. The most relevant limitation of PCV vaccines is caused by the large structural diversity of capsular polysaccharides: the protection offered by vaccination is serotype-specific and serotype prevalence is dynamic. Shifts in worldwide serotype distribution constitute a major challenge for eliminating pneumococcal infections, because commercial vaccines are unable to protect against serotypes not included in the vaccine [2]. This phenomenon is stimulating the search for a new generation of vaccines. Ideal candidates should be protective against a broader range of pneumococcal serotypes, with the possibility of the addition in the vaccine formulation of emerging new clinical isolates. In this framework, to simplify vaccine composition and to elicit a broader protection, we propose the identification of saccharide fragments containing chemical structures shared by different serotypes as cross-reactive and potentially cross-protective common antigens. In particular, we will present recent data on our ongoing work on the identification of common epitopes between different serotypes of Sp group 19 [3]. A small library of saccharides containing chemical structures shared by the 19F and 19A serotypes of S. pneumoniae has been synthesized and tested with a glycan array. The ability of the new compounds to be recognized by antibodies in reference group 19 antisera and factor reference antisera has been evaluated. Our study has shown that a phosphorylated simple disaccharide can be considered as a common carbohydrate epitope shared among different Sp 19 serotypes, setting the stage for exploring new common synthetic epitopes as potential candidates for a new generation of carbohydrate-based vaccines.

COST action CA18103 INNOGLY: INNOvation with GLYcans: new frontiers from synthesis to new biological targets.

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Design and evaluation of glycomimetics as ficolin antagonists

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As integral part of the host's antimicrobial defense, the complement system can mount a quick and effective response by causing inflammation and cell lysis and by inducing downstream immune processes [1]. The recognition of microbial carbohydrate signatures by pattern recognition receptors (PRRs) of the complement system's lectin pathway, including mannose-binding lectin, collectins, and ficolins plays a critical role in this process [2]. Moreover, as observed during severe cases of COVID-19, a fulminant activation of complement can do more harm than good by causing adverse thromboinflammatory states [3]. Therefore, there is an unmet need to elucidate this interaction network on a molecular level and to develop glycomimetic entities to inhibit (or enhance) such recognition events.

The three members of the human ficolin protein family (ficolin 1-3) are oligomeric lectins that bind mainly *N*-acetylated glycans such as Glc*N*Ac, Gal*N*Ac and Neu*N*Ac, although with different binding specificity [4]. Ficolins have been identified as PRRs for a wide range of disease-triggering pathogens, including eukaryotic protozoa [5], bacteria [6], and viruses [7]. In addition, ficolins play a major role in the pathogenesis of several autoimmune diseases [8].

We assess the binding of natural glycans to the protein's recombinantly expressed CRDs and use screening and rational design to generate glycomimetic ligands, which are subsequently characterized, optimized, and evaluated in relevant assays. These results will further improve our understanding of the ficolin's role in complement activation.

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Synthesis and functions of symbiotic bacterial lipid A for safe vaccine adjuvant development

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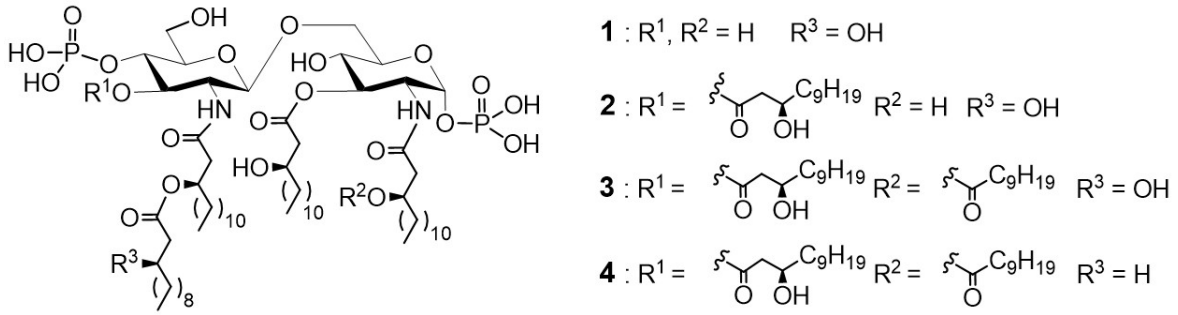
Lipopolysaccharide (LPS) is a major glycoconjugate in outer membrane of Gram-negative bacteria and canonical *Escherichia coli* LPS activate innate immunity to induce lethal strong inflammation. The terminal glycolipid lipid A is the active principle of LPS. Low inflammatory lipid A have been expected as adjuvants.

We hypothesized that co-evolved parasitic and symbiotic bacterial components should modulate host immunity moderately with low toxicity. We synthesized parasitic [1] and symbiotic [2] bacterial lipid A and elucidated the molecular basis of immunoregulation and developed safe and useful adjuvants. In this lecture, we introduce the structure determination, chemical synthesis, and structure-activity relationship studies of lipid A from *Alcaligenes faecalis* inhabiting gut-associated lymphoid-tissue (GALT) that is responsible for the mucosal immunity regulation. We synthesized *A. faecalis* lipids A **1**-**3** with diverse acyl group patterns and identified the active center as hexa-acylated **3** [2]. Lipid A **3** was confirmed to exhibit non-toxic but useful adjuvant function (enhancing antigen-specific IgA and IgG production) [3-6], and that vaccine model using **3** was found to be significantly protective against bacterial infection [4]. Since IgA is responsible for mucosal immune homeostasis, by focusing on GALT symbiotic bacteria, we found promising adjuvant that can safely regulate mucosal immunity. Furthermore, lipid A **4**, which lacks the hydroxy group in the acyl chain, was found to be less active than **3** [7], and the molecular basis of the adjuvant function is also becoming clear.



Chemical structure of gut-associated lymphoid-tissue (GALT) resident A. faecalis lipids A 1-3 and its derivative 4

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Synthesis of well-defined fragments of the *Staphylococcus aureus* type 8 capsular polysaccharide

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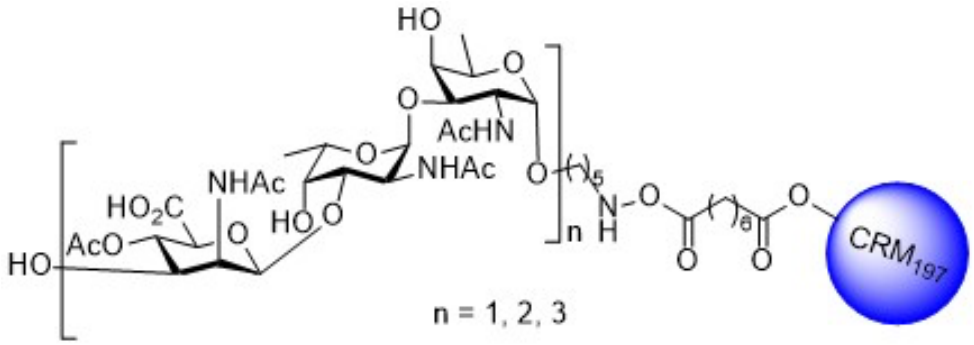
Staphylococcus aureus is a Gram-positive bacterium and it is found as an important hospital pathogen. Due to multi-drug resistant strains, which can pose significant health threats, a new course of treatment is needed. Therefore, much attention has been directed at the development of a vaccine. The cell wall of Staphylococcus aureus consists of capsular polysaccharides, wall teichoic acids and lipoteichoic acids, and all these components have been proposed as promising antigen candidates. 13 Different serotypes have been identified based on different capsular polysaccharide, of which type 5 and 8 are the most prominent. The CP8 polysaccharide is composed of trisaccharide repeating units that in turn are build up from *N*-acetyl β-D-mannosaminuronic acid, carrying a *C*-4-acetyl, *N*-acetyl-α-D-fucosamine and *N*-acetyl-α-L-fucosamine monosaccharides. The rare monosaccharides, cis-glycosidic linkages and *O*-acetylation represent significant challenges for the synthesis of CP8 fragments. Here the stereoselective assembly of well-defined type 8 capsular polysaccharide (CP8) fragments comprising a trimer, hexamer and nonamer carrying a linker, for conjugation to a carrier protein will be presented. This is the first time that fragments longer than a single repeating unit - which have been proven to be insufficient for antigenic activity - have been assembled. The fragments have been conjugated to the carrier protein CRM197 to generate conjugates that will be used for immunological studies.



This work is sponsored by Marie Skłodowska-Curie Innovative Training Networks under Grant Agreement No.: 861194.

Natural variations within the glycan shield of SARS-CoV-2 impact viral spike dynamics

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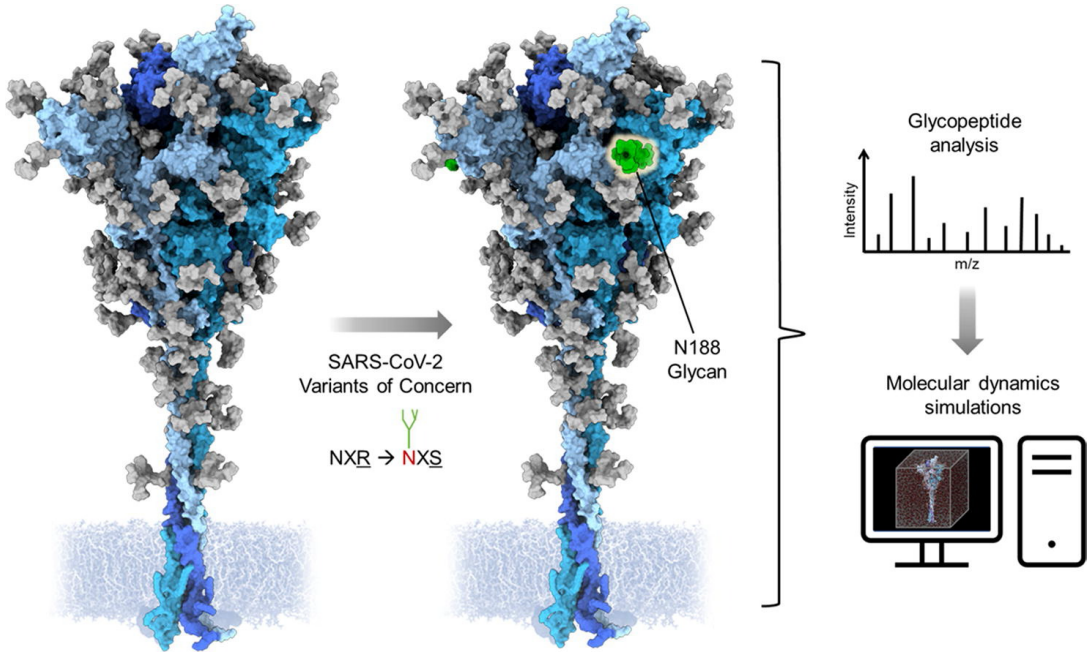
The effectiveness of current protection against SARS-CoV-2 infection whether acquired spontaneously or by vaccination, is altered by SARS-CoV-2 variants. Determining the effect of mutations on the antigenic surface is made easier by understanding the shape of the viral spike. One type of mutation which can drastically affect the antigenic surface is the introduction / deletion of glycosylation sites, which can affect the antigenic structure in ways that go beyond just shielding. We examine the glycosylation of a recombinant viral spike of the P.1 (Gamma) strain, which has three more N-linked glycan sites than the comparable the Wuhan strain. In this study, we ascertain the site-specific glycosylation of Gamma strain and determine the dynamics using molecular dynamic (MD) simulations. The N188 glycosylation site is observed to be novel in the gamma strain and the resulting Man 5 glycan occupies a cavity in the NTD, which affects the dynamics of this domain, according to structural modelling and molecular dynamics. These findings point to a mechanism by which mutations that change viral glycosylation sites influence the antigenic surface's structural composition.



International AIDS Vaccine Initiative (IAVI), Bill and Melinda Gates Foundation, The Irish Research Council and The Irish Cen for High-End Computing.

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M.L. Newby, et.al, Variations within the Glycan Shield of SARS-CoV-2 Impact Viral Spike Dynamics (2023), Journal of Molecular Biology (435,4), 167928

Aberrant cellular glycosylation may allow influenza virus to escape host immune responses

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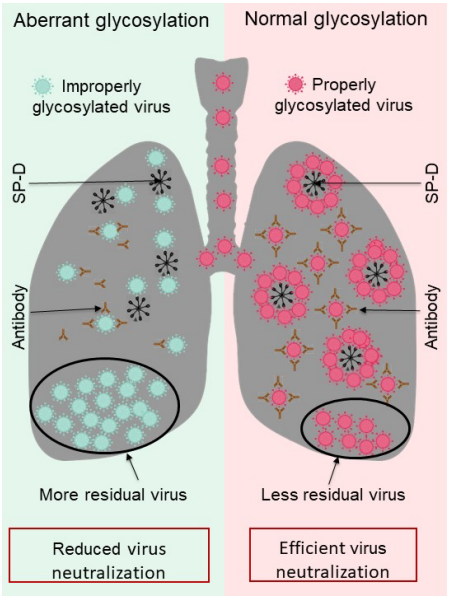
People with cancer, autoimmune disease, diabetes, or obesity often have metabolic dysregulation of cellular glycosylation, and also have tend to more severe influenza virus (IV) disease, with a poor immune response to the virus and low vaccine efficacy [1]. Host cells are responsible for glycosylation of the IV surface proteins, hemagglutinin (HA) and neuraminidase (NA), and glycosylation is important for interactions of these proteins with the immune system. To investigate the consequences of aberrant cellular glycosylation for the glycome, biology, and immune responses to IV, we moderately reduced N-linked glycosylation (NLG) in cultured cells with an oligosaccharyltransferase inhibitor, NGI-1. Treatment of cells with NGI-1 resulted in replication-competent virus with reduced NLG site occupancy of HA and NA. As with IV isolated from people with metabolic disorders, IV with an altered glycome did not show variations in genome and was able to efficiently infect cells that had normal glycosylation. However, glycome-altered IV required higher concentrations of the respiratory tract innate immune collectin surfactant protein D for virus neutralization than virus with normal glycan occupancy. It also generated lower total and protective antibody responses in mice than did IV with normal glycosylation. Thus, imbalanced cellular glycosylation can lead to sequence-neutral changes in the IV glycome, and these glycome-modified viruses may be less well recognized by the host innate and adaptive immune system resulting in more severe influenza disease and reduced IV vaccine efficacy.



Graphical abstract

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Development of multivalent glycopolymers for anti-viral applications

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Multivalent glycopolymers are widely used in the study of a variety of biological interactions, including host-virus early-stage binding. In our lab, glycopolymers are synthesized using a combination of green techniques such as: using minimal to no protecting groups, employing water as a solvent, using microwave-mediated reactions and incorporating a variety of chemoselective reactions. [1-4] The resultant glycopolymers are being designed as molecular mimics of host cell surface receptors to disrupt early-stage host-viral interactions, such that viral infections can be prevented. To evaluate the properties of the glycopolymers as anti-viral agents, we employ a variety of bioassays ranging from ELISA (enzyme-linked immunosorbent assay), MST (microscale thermophoresis) and live cell luciferase reporter gene assays. We have also incorporated molecular dynamics (MD) simulations of the glycopolymers with viral surface proteins to obtain more information about where specifically the glycopolymers interact with the surface of the proteins. Our results thus far have provided important information as to the overall glycopolymer size, degree of polymer branching, and sugar type/density needed for viral binding and inhibition of viruses such as HIV. This presentation will provide a report on our progress to date in this area. The long-term goals for this work entail the development of multivalent glycopolymers as topical anti-viral agents.

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Multivalent 9-O-Acetylated-sialic acid glycoclusters as potent inhibitors for SARS-CoV-2 infection

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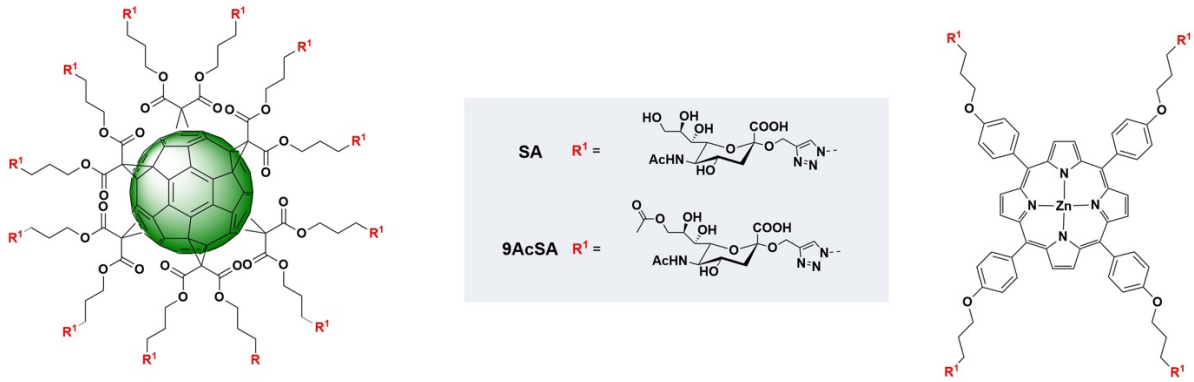
The recent emergence of highly transmissible SARS-CoV-2 variants illustrates the urgent need to better understand the molecular details of the virus binding to its host cell and to develop anti-viral strategies. While many studies focused on the role of the angiotensin-converting enzyme 2 receptor in the infection, others suggest the important role of cell attachment factors such as glycans.

The early binding events of the virus were studied using biophysical methods with the focus on the role of sialic acids (SA). We showed that SARS-CoV-2 binds specifically to (monomeric) 9-*O*-acetylated-SA with a moderate affinity, supporting its role as an attachment factor during virus landing to cell host surfaces. We demonstrated that 9-*O*-acetyl-sialic acid (9-AcSA) had a much stronger affinity towards the spike protein than sialic acid (SA) itself.

Four multivalent glycoclusters (with different topologies and valencies) presenting either SA or 9-AcSA were prepared. We identified 9-AcSA-derived porphyrin having high-binding inhibitory capacity (in the sub-µM range) both on purified receptors and on living cells. In addition, infection assays on living cells showed this molecule has a very promising neutralization potential.[1]



Sialic acid (SA) and 9-AcSA glycoclusters as potent SARS-CoV-2 ligands

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SugarFun2: carbohydrate functionalized carbohydrates

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The use of biopolymers from renewable resources for the fabrication of novel and sophisticated materials for technological as well as medicinal applications is an important research field today. As a matter of fact, carbohydrates are amongst the most abundant class of biomolecules, mostly biodegradable as well as non toxic which makes them highly versatile raw materials to be used in this respect. Derivatisation of polysaccharides is the most important path to impart further functionalities into the biopolymers and to tailor properties of the biopolymers.[1] In the field of cellulose chemistry, many derivatives have been developed and some are even produced in industrial scale.[2]. In order to tailor the properties of biobased materials and systems, chemical modification of oligo- and polysaccharides to introduce customised functionalisation is an expedient approach.

We have functionalized cellulose with different monosaccharides by azide-alkyne click chemistry approach. [3] Depending on the monosaccharide scaffold different properties can be introduced to the polymeric backbone. For example, if the decorating sugar entity presents the D-*manno* configuration, the obtained mannose-modified cellulose derivatives are potential ligands for *manno*-spezific lectins such as FimH of 1-fimbriated bacteria. Details on synthetic approaches and biological evaluation will be presented.

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

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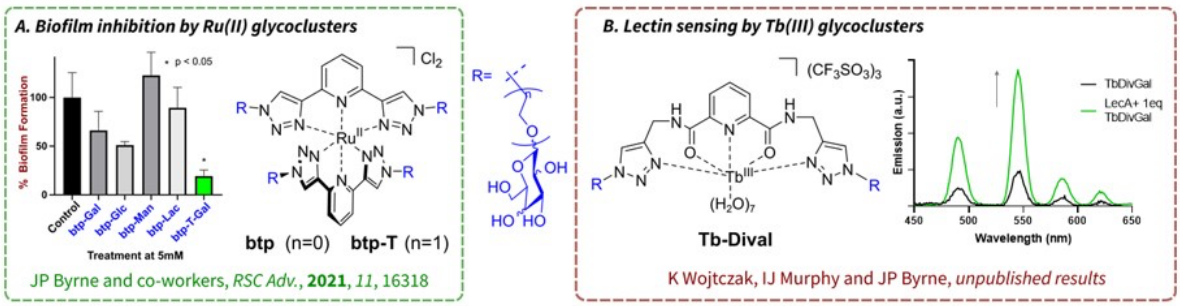
Carbohydrates can confer metal complexes with many properties beneficial for bioinorganic chemistry, including well-defined stereochemistry and water-solubility. Moreover, targeted selective interactions with biomolecules, such as carbohydrate-binding proteins, offer potential pathways for therapeutic and diagnostic applications. Carbohydrate–protein interactions are key to the pathology of many bacterial infections;[1] targeting carbohydrate-binding proteins (lectins) of *P. aeruginosa* has recently become an area of increasing interest in glycoconjugate chemistry.[2] While various multivalent glyconconjugate approaches are reported, use of metal coordination chemistry in design of lectin-targeting compounds is underexploited.

Carbohydrate-functionalised coordination-complexes allow properties of both carbohydrates and metals to be exploited to address healthcare challenges. We have synthesised Ru(II)-centred glycoclusters for targeting lectins, whose ability to inhibit *P. aeruginosa* biofilm formation was found to depend on the identity and presentation of the carbohydrate motif.[3] Building on this work, we also designed novel luminescent lanthanide(III)-centred glycoclusters, which detect lectins (including LecA from *P. aeruginosa*), aiming for diagnostic applications. These luminescent systems show 'switch-on' sensing behaviour in the presence of several lectins, with the selectivity of the lectin for different carbohydrate structures determining the response. Studies are ongoing with other lectins of different selectivities to establish the scope of this sensing paradigm.



Carbohydrate-functionalised metal complexes with therapeutic and diagnostic potential

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Efficient mannosylated calixarene-dendrimer ligands for uropathogenic *E. coli* FimH adhesin

Francesco SANSONE [1], Luca MORETTI [2], Laura LEGNANI [2], Martina POLLIOTTO [1], Carlo Alberto VEZZONI [1], Alessandro CASNATI [1], Alessandro PALMIOLI [2], Cristina AIROLDI [2]

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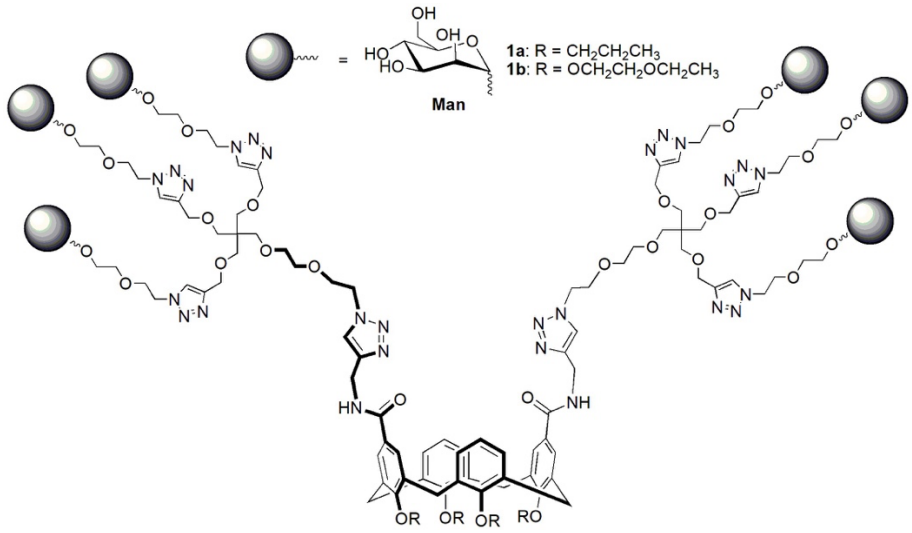
Uropathogenic *E. coli* (upEc) is an antibiotic resistant bacterium included in the priority list of WHO for which it is urgent to define innovative treatments [1]. It is one of the main responsible for urinary tract infections [2] and its ability of colonizing gut, bladder and kidney largely depends on adhesins [3]. Extracellular fibers, in particular type 1 pili, exploit these proteins to trigger the infection starting with a cell adhesion process [4]. Type 1 pili use FimH adhesin [5] that interacts with mannoside units of bladder epithelium glycoproteins. The inhibition of this recognition process can represent a therapeutic approach to prevent the upEc invasion. Calixarenes demonstrated to be versatile scaffolds for the preparation of polyglycosylated ligands that, thanks to multivalent effects, show high efficiency and selectivity in the interaction with different types of lectins [6]. In this work we designed calixarene-based dendrimers displaying multiple copies of α-mannoside and investigated their ability to interact with upEc and to inhibit its adhesion activity. Molecular Modelling studies shed light on the arrangement in the space of the ligand saccharide units, STD NMR experiments demonstrated the interaction and established the binding epitope map for the ligands while yeast agglutination assays evidenced the inhibition against upEc unequivocally due to the recognition between mannosides and FimH adhesin. Remarkably, the calixarene-based ligands showed potency significantly higher than simple dendrimers equipped with the same or a higher number of epitope units.



The studied multivalent mannosylated calixarene-dendrimer ligands.

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Dynamic combinatorial libraries of glycoclusters: When glycoclusters go dynamic

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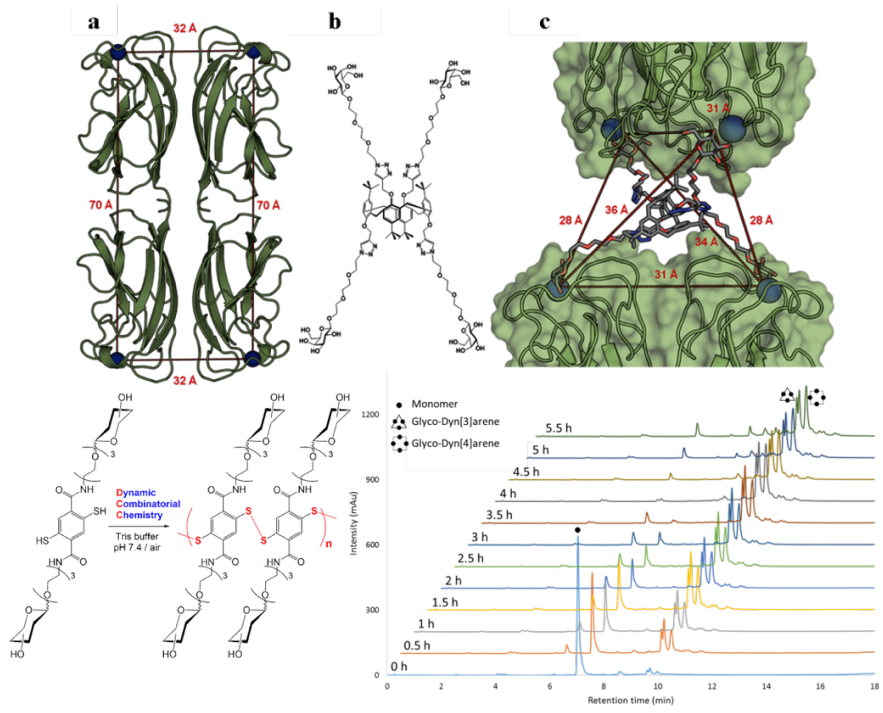
Pseudomonas aeruginosa is a well-known pathogen responsible for pulmonary infections among others. It is most notably found in hospitals as a cause for nosocomial infection. Anti-adhesive strategies are inhibiting the adhesion of bacteria to the host cells. Two soluble tetrameric lectins (LecA and LecB) have been identified in this process. LecA is known for its affinity for b-galactosides while LecB exhibit an affinity for a-fucosides. We have designed multivalent glycoclusters to inhibit these lectins with applications *in vivo* as potential therapeutic anti-infectious agents.[1] The calix[4]arene-based glycocluster (Figure top) displayed nanomolar affinity for LecA and provided protection against pulmonary infection in animal.[2] We have now developed self-assembling glycoclusters based on the concept of dynamic combinatorial chemistry (Figure bottom).[3] The building block is composed of an aromatic core, a spacer and a carbohydrate and will self-assemble in solution through disulfide bonds to generate a dynamic combinatorial library of glycoclusters. In this communication, we will detail the synthesis of the building-blocks and the results obtained during the dynamic combinatorial libraries. We can now evaluate simultenaously in a single experimental process, the binding affinities of the dynamic glycoclusters to several lectins of interest (LecA, LecB, ConA and AFL) from simple 1,4-dithiophenol building blocks.



Top: (a) LecA (b) glycocluster (c) complex / Bottom: (left) library of glycoclusters (right) equilibration.

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Chemoselective ligation reactions in the glycosciences

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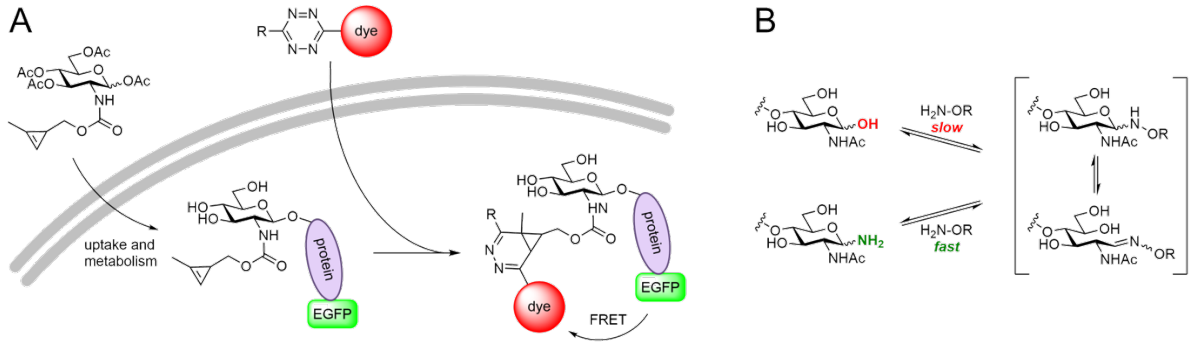
Carbohydrates are involved in a myriad of cellular functions. In the form of glycoconjugates, they modulate activity and properties of proteins and lipids, and they are themselves involved in molecular recognitions processes. To study the biological roles of carbohydrates, chemoselective ligation reactions are of increasing interest for the tracing of carbohydrates in living cells and organisms, the synthesis of glycoconjugates, and the labeling and immobilization of unprotected (reducing) carbohydrates. This lecture gives an overview of our group's activities in these fields. The inverse-electron-demand Diels-Alder (IEDDA) reaction was employed in metabolic glycoengineering [1] to visualize dienophile-labeled glycoconjugates in living cells [2] and the extracellular matrix [3] and in combination with copper-free click chemistry and the photoclick reaction to achieve a triple-orthogonal labeling of glycans. For the convergent synthesis of complex N-glycopeptides, we developed a method for the introduction of thiocarboxylic acids into peptides that subsequently can be ligated with glycosyl amines [4]. During these investigations, we discovered a novel side reaction that can lead to an efficient and site-selective peptide cleavage using thioacids (CUT). The oxime formation of reducing carbohydrates is an important ligation method for the attachment of fluorescent probes and the preparation of glycoconjugates. We found that this ligation is significantly accelerated (up to 500-fold) without the need for a catalyst, such as aniline, when starting with glycosyl amines [5].



A) Metabolic glycoengineering in living cells with the IEDDA reaction. B) Rapid glycoconjugation with glycosyl amines.

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Controlling the activity of bacterial sialidases with bioorthogonal chemical reporters

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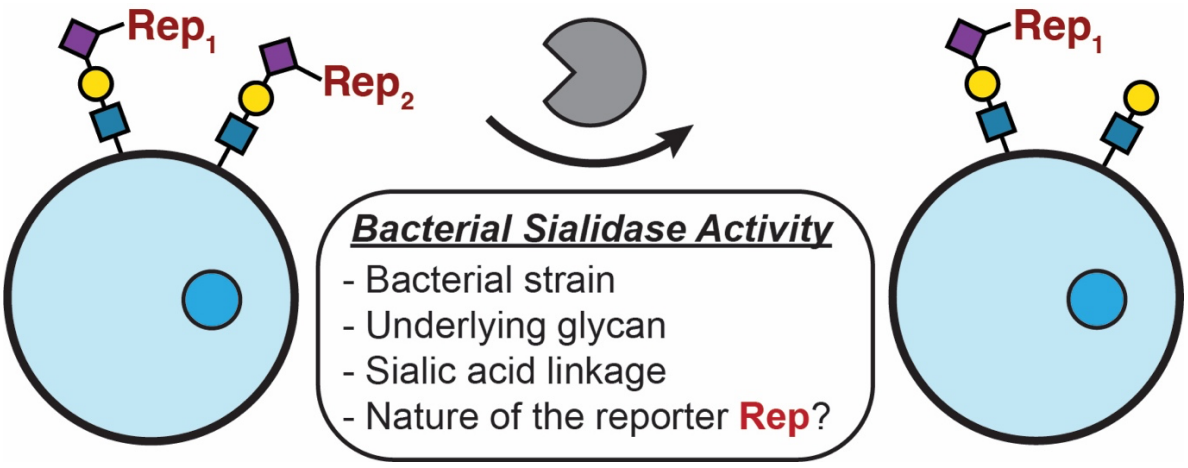
Sialic acids are anionic nine-carbon carbohydrates generally found as terminal sugars of mammalian cell-surface glycoproteins and glycolipids. Because of their distinct cellular location, sialo-glycoconjugates (also known as sialosides) are often key mediators of physiological and pathological events, including cell adhesion, host–pathogen interactions, and cancer progression.[1]

The bioorthogonal chemical reporter strategy, which elegantly combines the use of metabolically labeled azido-sugars and highly reactive cyclooctyne probes, is emerging as a versatile technology for labeling and visualizing sialosides.[2] This strategy relies on the fact that bioorthogonal chemical reporters are highly reactive species while being biologically noninvasive.

During this talk, I will present our recent efforts to show that chemical bioorthogonal reporters may actually impact sialosides processing enzymes activity.[3] More specifically, I will describe how bacterial sialidases may be significantly affected by the presence of bioorthogonal reporters on mammalian cell-surface sialosides, providing us with novel, more selective, chemical biology tools for studying the biological roles of cell-surface glycans.



Factors that can influence bacterial sialidases activity

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Cell-specific bioorthogonal tagging of glycoproteins

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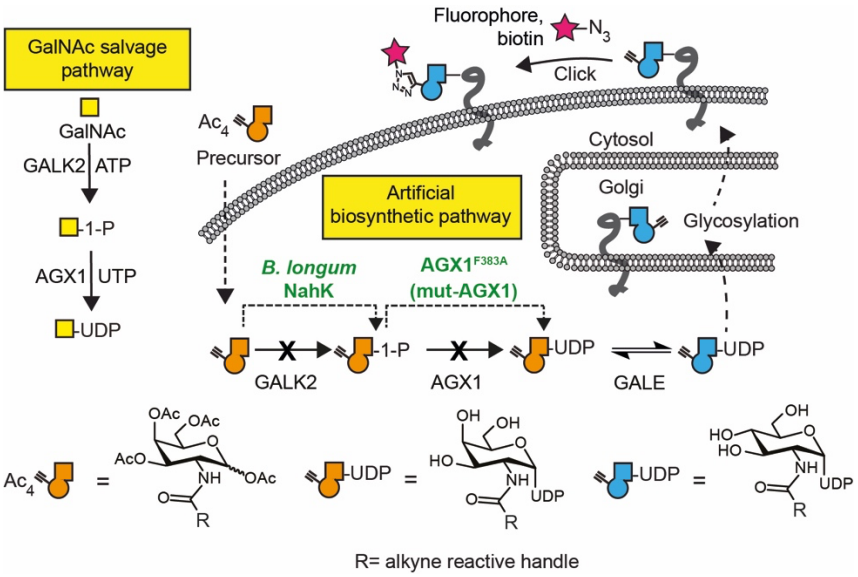
Altered glycoprotein expression is an undisputed corollary of cancer development. Some highly glycosylated proteins such as mucins are clinically recognised cancer biomarkers.[1,2] However, understanding the cancer-related alterations of glycoprotein expression is hampered by limitations in both glycan detection and cellular model systems. For instance, the intricate interactions between tumour and host cannot be adequately recapitulated in monoculture of tumour-derived cell lines. More complex co-culture models usually rely on sorting procedures for proteome analyses and rarely capture the details of protein glycosylation. Bioorthogonal ("clickable") monosaccharides have greatly contributed to our ability to reveal such details, but classically lack specificity for individual cell types.

Here, we develop a tactic to specifically study the cancer-derived glycoproteome in the presence of non-cancerous cells *in vitro* and *in vivo*.[3]Bio-Orthogonal Cell line-specific TAgging of Glycoproteins (BOCTAG) features an artificial biosynthetic pathway that transforms clickable sugars into the corresponding nucleotide-sugars. Only transfected cells incorporate clickable tags into glycoproteins in the presence of non-transfected cells. Modification with suitable reporter probes such as fluorophores or biotin allows for analysis of labelled glycoproteins.[4,5,6] We show that BOCTAG can be tuned to preferentially label either N- or O-GalNAc glycans through engineering of glycosyltransferases. We employ BOCTAG as an imaging technique and to annotate cell-specific glycosylation sites in mass spectrometry-glycoproteomics. Application in co-culture and mouse models allows for profiling of the glycoproteome as an important modulator of cellular function in cancer.



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Cell surfaces remodelling by tyrosine-click electrochemistry

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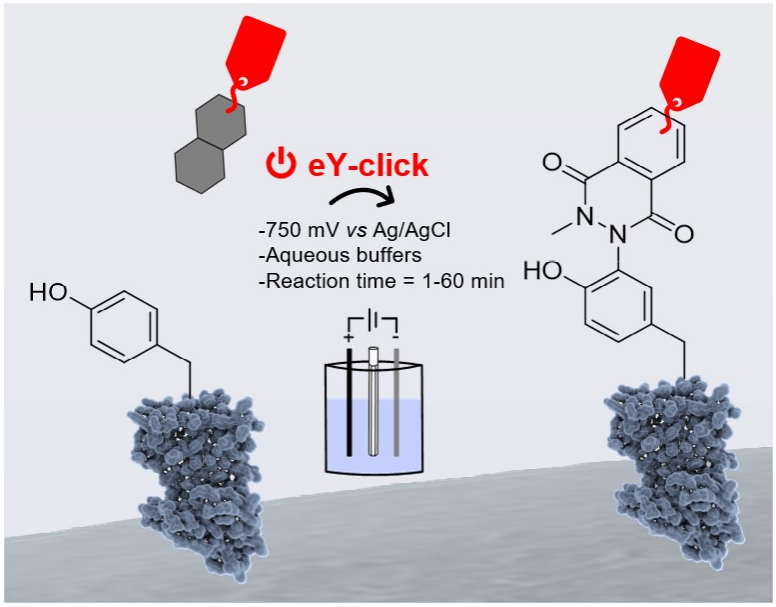
The chemo-selective modification of native proteins is of particular importance in chemical biology and for the development of therapeutic conjugates. Direct proteins modifications with chemical reagents are still mostly performed on nucleophilic lysine and cysteine, but much effort is now dedicated to selectively target less exploited amino acids.[1] Recently, we developed the first electrochemical method coined eY-click to functionalize tyrosine (Y) residues in biocompatible media.[2] Peptides, enzymes, and antibodies were labeled in aqueous buffers after dipping a three-electrode system, to selectively oxidize (activate) a functionalized diazodicarboxamide anchor *in situ*.[3]

Here, we used *N*-methylluminol, a fully selective Y anchoring group after one electron oxidation, for the electro-bioconjugation of cell surfaces from virus, bacteria and eukaryotic cells. The click-electrochemistry method was explored on therapeutic adeno-associated viruses (AAV2), *E. coli* (Gram-) and *S. epidermis* (Gram+) bacterial strains, and HEK293 and HeLa eukaryotic cell lines. Cell surfaces were decorated with azido-groups or carbohydrates in minutes. Surprisingly, living bacteria and cells fully conserved their ability to replicate, and a mannose decorated AAV2 its cell transduction efficiency, opening perspectives for studying complex cell surface process and to viral, bacterial and cell-based therapies.



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Activity-based protein profiling probes reveal the mode of action of the biofilm degrading PslG

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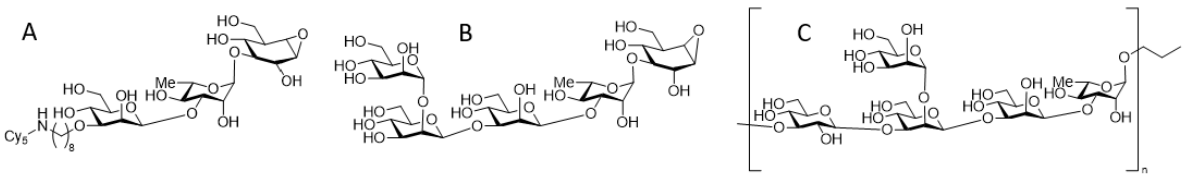
Pseudomonas aeruginosa are opportunistic bacteria causing chronic infections in cystic fibrosis and immune compromised patients.[1,2,3]Depending on the environment, *P. aeruginosa* can produce a biofilm that consists of a combination of DNA, proteins and polysaccharides.[4] This biofilm shields the bacteria from the host immune system and antibiotics. One of these polysaccharides is Psl, a polysaccharide built up from pentameric repeating units, consisting of mannose, rhamnose and glucose.[5] It has previously been shown that PslG can cleave Psl, making *P. aeruginosa* susceptible for antibiotics.[6] However, the mode of action of PslG remains enigmatic. We studied PslG using synthetic trisaccharide cyclophellitol-type probes and inhibitors based on different frameshifts of the Psl repeating unit. We show that PslG is actually an endo-glucosidase instead of a postulated[6] endo-mannosidase. This finding is further substantiated with hydrolytic experiments using synthetic pentameric and decameric Psl fragments. Structural studies are underway with the aim to prove the binding mode of inhibitor B, to unambiguously establish the endoglucosidase activity of PslG and provide structural insight into its hydrolytic mechanism. In all, our studies may pave the way for the design of new *P. aeruginosa*-targeting antibiotics.



A. One of the synthesized probes B. One of the synthesized inhibitors C. The synthesized Psl fragment, n = 1 or 2

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Selective labeling of glycoside hydrolases using ligand-directed protein profiling

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Small molecules selectively interacting with proteins and thereby modulating enzyme activity are of great interest for chemical biology and chemical medicine. Altering the intrinsic catalytic activity, either through enzyme activation or inhibition, leads to decisive effects on the biological system.[1]

With the development of activity-based protein profiling (ABPP), detection of actually active enzyme rather than the expression level of the protein became feasible. [2-3]

In ligand-directed chemistry (LDC), a variation of ABPP, profiling of enzymes without losing their intrinsic activity was achieved.[4-5] In this approach, a small molecule based probe features a reversible inhibitor as ligand (A) and a cleavable electrophilic group (B) which can be attacked by a nucleophilic amino acid residue, nearby, but outside the active site forming a covalent bond. Subsequently, the consequently truncated probe can depart from the ligand-binding site, thus the enzyme's activity is maintained (Figure 1).

Here we report the design, synthesis, and biological evaluations of iminosugar based probes for selective profiling of glycoside hydrolases, applying the ligand-directed chemistry (LDC) approach. Experimental details and results will be presented.

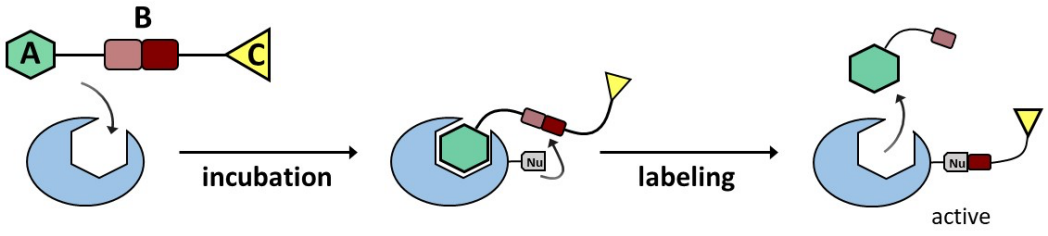

Figure 1: Principle of ligand-directed chemistry. (A) reversible inhibitor as ligand; (B) linker with electrophilic reactive group; (C) reporter tag.

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Chemoenzymatic synthesis of NDP sugars to explore the GDP-mannose dehydrogenase from *p. Aeruginosa*

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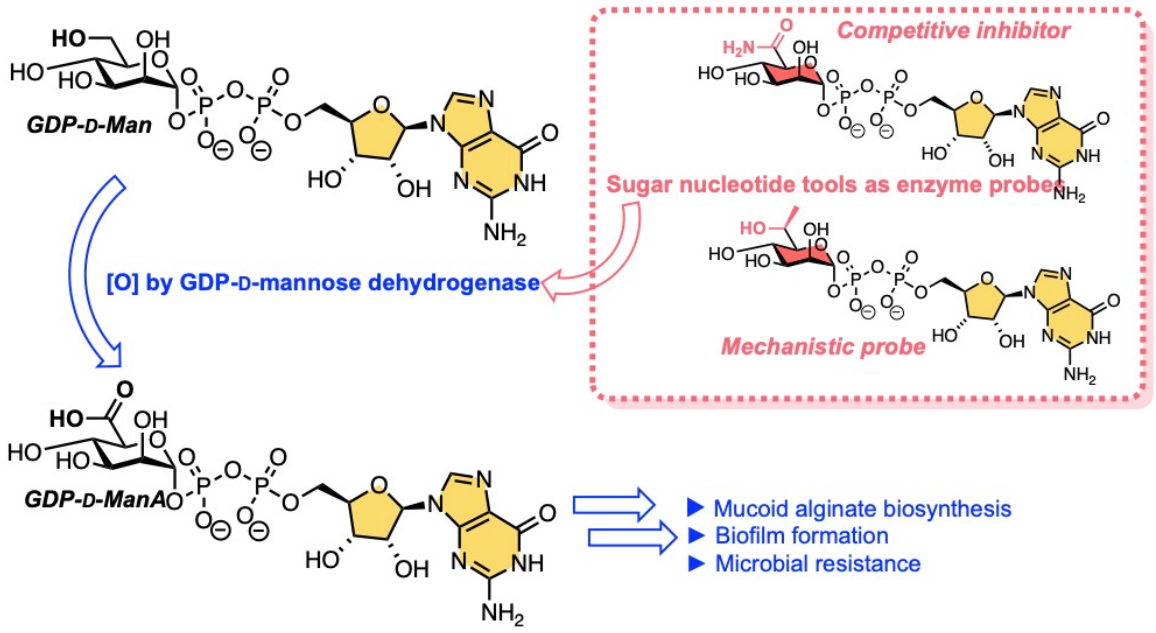
The opportunistic human pathogen *Pseudomonas aeruginosa* (PA) causes chronic bacterial infections in cystic fibrosis patients, contributing to a reduction in lung function and increased mortality rates. The lung environment induces a switch of *P. aeruginosa* to its mucoid phenotype, which is characterised by an overproduction of the exopolysaccharide alginate. Composed of β-D-mannuronic acid and its C5 epimer α-L-guluronic acid, alginate is a key component of the bacterial biofilm which increases persistence of the bacteria in the airways and retards antimicrobial treatments. Inspection of the PA biosynthetic pathway reveals a key enzyme involved in alginate production is GDP-mannose dehydrogenase (GMD), which catalyses an NAD^+-dependent oxidation of GDP-D-Man to GDP-D-ManA: the alginate feedstock monosaccharide. We have recently designed and synthesised a series of GDP-Man probes to interact with the GMD active site, providing mechanistic insight and identified a first sugar nucleotide inhibitor of GMD.



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Rutinosidase and other diglycosidases: Rising stars in biotechnology

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Diglycosidases are glycosidases catalyzing the cleavage of entire disaccharide moieties from the aglycone. Rutinosidases, main diglycosidase representatives, cleave rutinose (α-L-Rha-(1-6)-β-D-Glc) from rutin or other rutinosides (Fig. 1A). Some diglycosidases can be classified as monoglucosidases with extended substrate specificity. They also have distinct synthetic (transglycosylating) abilities. Rutinosidase from *A. niger*[1] and *A. oryzae* (GH5-23) can glycosylate various acceptors, including phenols, in a good yield using priceworthy rutin as a glycosyl donor. Surprisingly, they are able to glycosylate species such as inorganic azide to form β-rutinosyl azide [2] or carboxylic acids forming (anomeric) glycosyl esters [3], being a unique property of glycosidases. The variant of *A. niger* rutinosidase mutated at the catalytic nucleophile residue E319A is capable of generating α-rutinosyl azide [2]. It was found that rutinosidase is able to accept quercetin 3-β-glucopyranoside as a substrate and thus it is also able to transfer a β-glucosyl moiety [1]. This enzyme has a dual glycosylation activity, generating either rutinosides or glucopyranosides [4]. Its broad substrate specificity has also been demonstrated in the enzymatic cleavage of various 6''-acylated quercetin-3-*O*-β-glucopyranosides (Fig. 1B). Rhamnose-containing compounds (rutinose) are attracting attention due to their anti-cancer activity and as skin anti-aging agents [5]. Their easy availability through the action of rutinosidase opens a whole new avenue in cancer therapy, dermatology, and other fields.

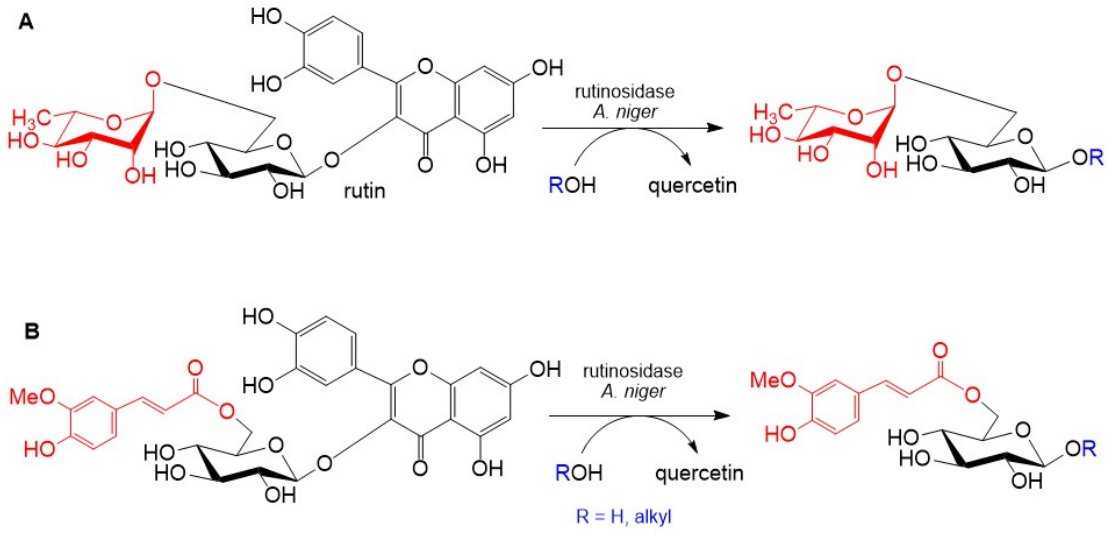

Fig.1 A Hydrolysis/transglycosylation by rutinosidase; B 6”-feruloyl quercetin 3-O-β-glucopyranoside

Acknowledgements

We acknowledge the support by the Czech Science Foundation project No. 22-00197K and by the COST Action CA18132.

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Glycosynthase-based synthesis of peptidoglycan oligosaccharides to decipher bacterial cell division

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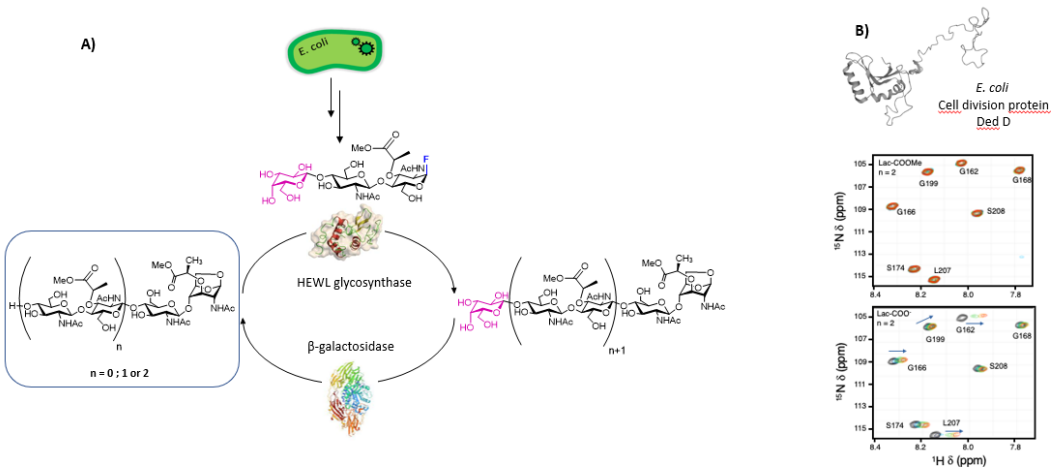
Most antibiotics used in human medicine inhibit the biosynthesis of peptidoglycan, an essential component of the bacterial wall. However, antibiotic resistance is rising to dangerously high levels, becoming one of the biggest threats to global health.1 Combating this burden therefore requires the detailed study of peptidoglycan metabolism and implies the need to produce well-defined molecular probes. 2,3

In this context we devised a glycosynthase-based chemo-enzymatic synthesis of peptidoglycan oligosaccharides using the D52S mutant of hen egg-white lysozyme (D52S HEWL).4 Size-control of the oligosaccharides during glycosylation was achieved using a non-polymerizable donor. A galactosyl group, whose introduction and deprotection can be done enzymatically was chosen as temporary protecting group. Trisaccharide Gal-GlcNAc-1,6-anhMurNAc was produced by metabolically engineering E. coli cells and chemically fluorinated at the reducing end to provide the target donor. Successive rounds of glycosylation using D52S HEWL followed by degalactosylation using a commercial β-galactosidase afforded the expected tetra-, hexa- and octasaccharides in 60-70% yields. These compounds were used to decipher the impact of the charge on the lactoyl group of MurNAc, of the anhydro at the reducing end of the oligosaccharide, and of the oligosaccharide chain length on the interaction with E. coli DedD, a SPOR-domain-containing protein involved in bacterial cell-division.5 The information retrieved using NMR spectroscopy in this study sheds a new light on the role of this protein.



A) Glycosynthase-based synthesis of peptidoglycan oligosaccharides; B) Characterization of E. coli Ded D-oligosaccharides interaction by NMR

This work was supported by the French National Research Agency (ANR) through Glyco_SWIM (ANR-20-CE07-0012-01)

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Chemoenzymatic synthesis of an unnatural Manβ1,4GlcNAc library using a glycoside phosphorylase

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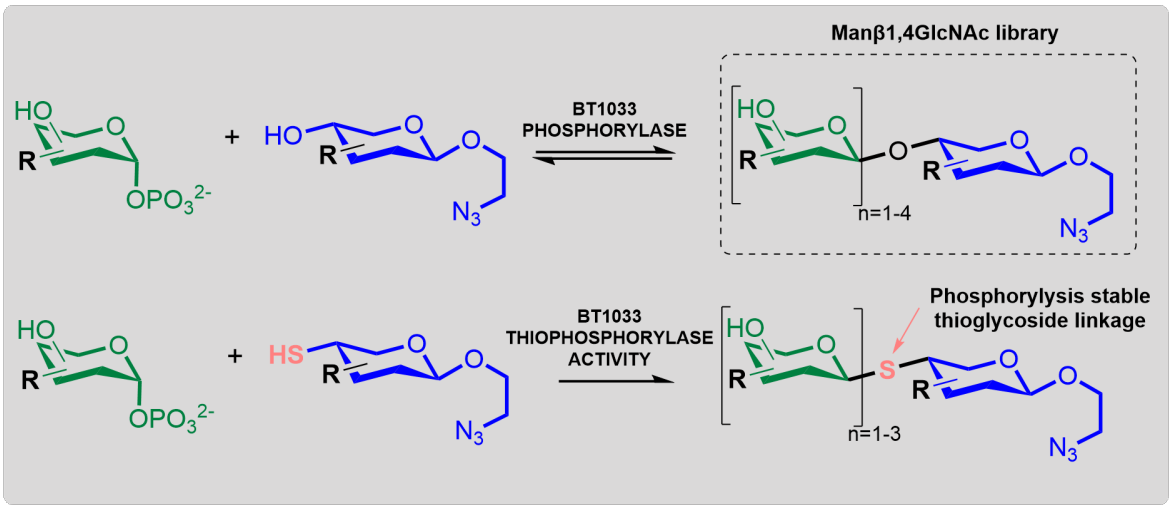
β-Mannosides are ubiquitous in nature, with diverse roles in many biological processes such as energy storage and cell wall biosynthesis. Notably Manβ1,4GlcNAc, a constituent of the core *N*-glycan in eukaryotes, was recently identified as a novel STING immune pathway activator, highlighting its potential for use in immunotherapy.[1] Yet, despite their biological significance, the synthesis of β-mannosidic linkages remains one of the major challenges in glycoscience. Here we present a chemoenzymatic strategy that affords a series of novel unnatural Manβ1,4GlcNAc analogues using the β-1,4-D-mannosyl-*N*-acetyl-D-glucosamine phosphorylase, BT1033. We incorporate unnatural functionality into the enzymatic building blocks through chemical synthesis and show that when fluorine is present in the GlcNAc acceptor, this facilitates further extension of Manβ1,4GlcNAc with Man producing longer β-mannan like glycans. We also pioneer a "reverse thiophosphorylase" enzymatic activity, favouring the synthesis of longer glycans by catalysing the formation of a phosphorylysis-stable thioglycoside linkage, an approach that may be generally applicable to other glycoside phosphorylases.



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Chemical and chemo-enzymatic synthesis of tandem ribitol phosphate scaffolding of matriglycan

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The glycosylation of proteins is an important post-translational modification. The core M3 *O*-mannosyl glycan (OMG) of α-dystroglycan was recently shown to play an important role in muscle and brain development. The complete structure of core M3 OMG was elucidated in 2016 [1,2]. The core M3 OMG is responsible for the link between the extracellular matrix and cytoskeleton that stabilizes muscle tissue. However, the underlying molecular mechanisms remain unclear because a sufficient amount of core M3 OMG cannot be purified from natural sources. To overcome this issue, sequentially extended partial structures of the core M3 OMG including a tandem ribitol phosphate (**1**~**6**) were synthesized (Figure 1). Rbo5P-3GalNAcβ with *p*-nitrophenyl at the aglycon (**2**) served as a substrate for ribitol phosphate transferase (FKRP, fukutin-related protein), and its product was glycosylated by the actions of a series of glycosyltransferases, namely, ribitol xylosyltransferase 1 (RXYLT1), β1,4-glucuronyltransferase 1 (B4GAT1), and like-acetyl-glucosaminyltransferase (LARGE). Rbo5P-3GalNAcβ equipped with an alkyne-type aglycon was also active for FKRP. The molecular information obtained on FKRP suggests that Rbo5P-3GalNAcβ derivatives are the minimal units required as the acceptor glycan for Rbo5P transfer and may serve as a precursor for the elongation of the core M3 OMG We propose the therapeutic potential of adopting versatile Rbo5P-3GalNAcβ units as glycan bridges bound to α-dystroglycan for patients with α-dystroglycanopathies, including Fukuyama congenital muscular dystrophy.

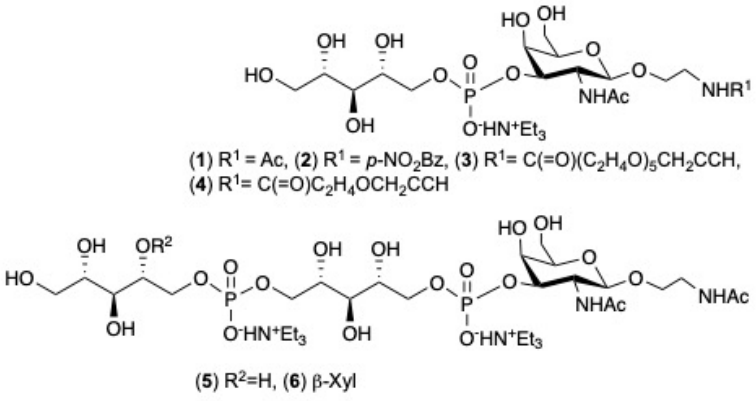

Figure 1

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Gram-scale chemoenzymatic synthesis of human milk oligosaccharides using crude cell lysate

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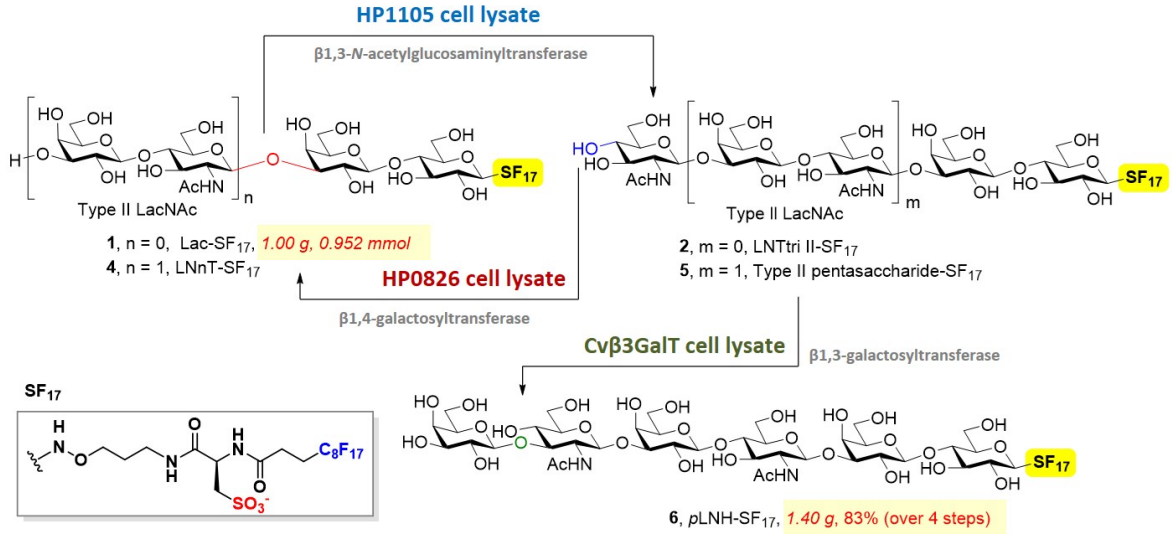
Human milk oligosaccharides (HMOs) are known to play an important role in living system such as prebiotic activity. The demand of pure materials for further studies rise approaches toward the synthesis of HMOs by chemical methods, microbial fermentations and enzymatic strategies. We recently reported a convenient enzymatic synthesis of fucosylated HMOs via appending a readily removable sulfo-fluorous affinity tag (**SF_{17}**) at the reducing end of glycan, which allows facile purification after enzymatic glycan extension.[1] Herein, we report a new function of the **SF_{17}**-tag that shows a glycosyl hydrolase-resistant property, which allow the glycosyltransferase-catalyzed reaction proceeding in the crude cell lysate without glycans decomposition. The preparative-scale synthesis (> 1 gram) of *para*-lacto-*N*-hexose (*p*LNH) and its derivatives including fucosyl *para*-lacto-*N*-hexose I, fucosyl *para*-lacto-*N*-hexose IV, difucosyl *para*-lacto-*N*-hexose I isomer, difucosyl *para*-lacto-*N*-hexose II and trifucosyl *para*-lacto-*N*-hexose I) were achieved, and so was the synthesis of A-antigen series HMOs (A antigen tetrasaccharide, hexasaccharide and heptasaccharide). The availability of these well-defined structures will provide valuable standards for the characterization and quantification of complex glycans isolated from nature, enables comprehensive screens for new prebiotic activities and the advances in exploring the HMO–gut microbiome relationship, those can significantly expedite the progress of tailor-made formula production in the future.



Gram-scale synthesis of para-human milk oligosaccharides by crude cell lysate of glycosyltransferases using the glycosyl hydrolase-resistant SF17-tagg

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Molecular details of α2–3-sialylated O-GalNAc glycan recognition by SLBR-N of *Streptococcus gordonii*

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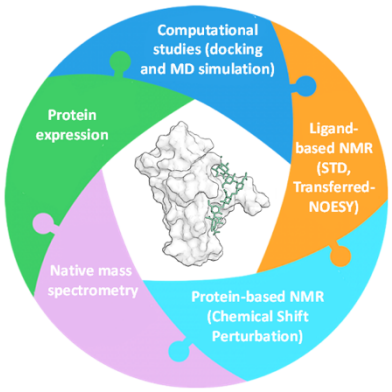
Siglec-like adhesins are streptococcal lectins associated with bloodstream infections and the progression of infective endocarditis (IE) [1]. Indeed, when Gram-positive bacteria such as *staphylococci*, *streptococci, enterococci* enter into the bloodstream, the adherence to and colonization of damaged cardiac valves is mediated by surface adhesins that interact with host proteins located on the valve surface. Among these, serine-rich repeat glycoproteins (SRRPs) recognize the terminal epitopes of O-GalNAc glycans on human salivary mucins and the glycoproteins of platelets have a major impact on pathogenesis [2,3]. Siglec-like adhesin SLBR-N has been recently considered as tool for identifying and enrich breast cancer stem cells (CSCs), due to its ability to recognize α2–3-linked sialic acid-containing glycans, such as disialyl core 2 O-glycans exposed on the tumor cell surface [4]. In the perspective of developing potential mimetics hindering IE progression and infections as well as understanding the potential implications of CSCs diagnosis, prognosis, and treatment, I am here reporting a comprehensive study of the molecular and biophysical interaction, recognition and binding process between SLBR-N and host O-glycans. A combination of multidisciplinary and complementary methods, including NMR spectroscopy (both ligand- and protein-based techniques), Native Mass spectrometry and computational approaches (as Docking studies and Molecular Dynamic simulations) provided the 3D features of the complexes, determining the preferred epitopes recognized by SLBR-N [5-7].



Schematic representation of the multidisciplinary approach to study the molecular interaction between Siglec-like adhesin SLBR-N and O-glycans.

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Recent developments in gas phase ion spectroscopy for structural analysis of sugars

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Oligosaccharides play vital roles in living organisms. Despite this vital importance, structural analysis of oligosaccharides suffers from a lack of a universal method to fully characterize them. Indeed, glycans have complex structures, especially due to the presence of numerous isomers, which complicates analyses. Ion vibrational spectroscopy coupled to mass spectrometry includes an ensemble of method initially developed to answer chemical-physics questions. These technics are increasingly used for the purpose of structural characterization of sugars, including the resolution of isomers and anomers[1,2].

Our spectroscopic scheme of choice is InfraRed Multiple Photon Dissociation (IRMPD) spectroscopy, which only requires minimal modifications of commercially available mass spectrometers as compared to other technics. The performance of this approach was demonstrated for a variety of sugars. Yet, the technique may suffer from several drawbacks, in particular the long acquisition time and the need for expertise in laser spectroscopy to acquire and interpret the data.

During this presentation, efforts and improvements made on our setup to counteract these drawbacks will be presented as well as results coming from these enhancements. We hope that these efforts will make IRMPD spectroscopy faster[3], easier to use, and a seamless extension of other analytical workflow[1,4], with the goal of facilitating sugar analyses.

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Probing interactions of carbohydrates with biomolecules and water by vibrational spectroscopy

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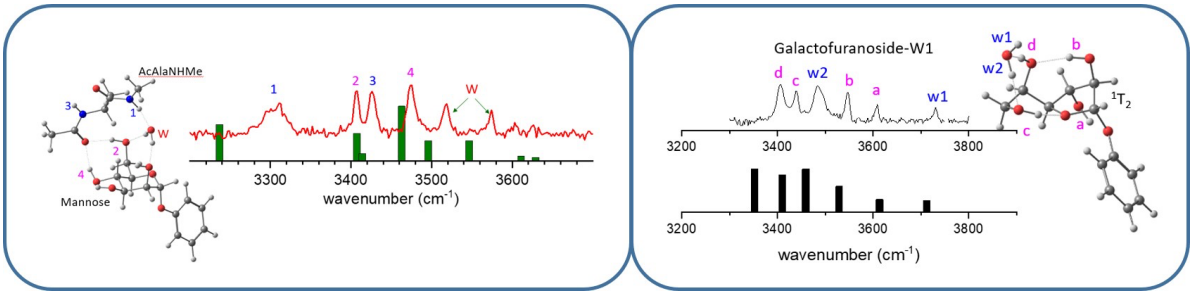
Carbohydrates are especially efficient as vectors of molecular information encoded in their structural and conformational diversity and flexibility that enables communicating selectively with other biomolecules. This molecular communication proceeds *via* subtle non-covalent interaction networks engaged by the carbohydrates. These networks are dominated by electrostatic Hydrogen Bonding and the full range of non-covalent interactions is at play, including polarization and dispersion. In the gas phase, applying to carbohydrates experimental mass resolved and conformer selective vibrational spectroscopy and modeling,[1] we have extensively studied the conformational and structural preferences of non-covalent complexes between carbohydrates molecules, water[2] and also peptide models.[3] The vibrational resolution achieved in cold gas phase measurements (Figure), allows identifying each molecular group involved in the interactions and evaluating their strength. It is possible to remove any solvent perturbation, before reintroducing water effects by studying size-controlled hydrated complexes.

In this presentation we will survey the most recent results obtained on the structural preferences of monosaccharides such as furanoside cycle stabilization with and without the perturbation of water molecules (on the right in the figure), or mannopryannosides engaged in direct interaction with peptide models in non-covalent complexes where water comes to play a cementing role, intercalated between the interacting molecules (on the left in the figure).



Vibrational spectra of complexes formed by one water molecule and (left) a mannoside and a peptide model, and (right) a Galactofuranoside.

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Role of Golgi vesicle tethering and fusion machinery in protein glycosylation in human cells

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Golgi is the central organelle in the secretory pathway. It hosts glycosylation machinery that modifies macromolecules passing through Golgi compartments. The identity of Golgi compartments and proper glycosylation is achieved by the retrograde flow of components of Golgi glycosylation machinery from TGN to cis-Golgi. This recycling is performed by vesicles formed at trans compartments by vesicle coats and then tethered and fused to cis compartments by vesicular tethers and SNARE molecules. We used gene editing, degron-assisted rapid protein degradation, TurboID proximal biotinylation, mass-spectrometry analysis, electron and superresolution microscopy to decipher the role of COG and GARP vesicle tethering complexes and SNAREs in Golgi glycosylation in human cells. We found that the TGN-located GARP complex is necessary for Golgi glycosylation, indicating that enzymes recycle beyond the Golgi stack and have to be retrieved from endosomal compartments in a GARP-regulated pathway. Detailed analysis of COG complex uncovers the essential role of all COG subunits in the recycling and stability of Golgi glycosylation machinery. Acute deletion of COG4 results in the accumulation of several distinct populations of vesicles carrying different Golgi glycosyltransferases and sugar transporters. Mass-spec analysis of Golgi SNAREs led to the discovery of two novel SNARE complexes and revealed the remarkable plasticity in the intra-Golgi SNARE-mediated fusion machinery, uncovering a novel response mechanism to the failure of "classical" intra-Golgi vesicle tethering/fusion machinery.

This work was supported by the National Institute of Health grant R01GM083144 for Vladimir Lupashin

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Targeting human langerin receptor for T cell response

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C-type lectins represent the largest and most complex family of mammalian carbohydrate-binding proteins. These lectins can have a distinct expression pattern on defined cellular subsets. For the last decades, these patterns have raised significant interest in targeting C-type lectins to enable cell specific delivery of therapeutics. C-type lectin receptors (CLRs) play a critical role in immunity, enabling internalization of foreign antigens via carbohydrate recognition. Nevertheless, traditional carbohydrate-based vaccines suffer from low specificity because most CLRs nonspecifically recognize similar antigenic glycans.

Along these lines, a modified heparin glycomimetic, N-tosylglucosamine, recently developed [1] is specific for one of those C-type receptors, human langerin, also improving affinity compared to natural glycans, such as mannose. This glycomimetic, presented onto proteins or liposomes, promotes highly specific antigen internalization by Langerhans cells (LCs).[2,3]

On this work, the beforementioned glycomimetic has been used as a human langerin targeting ligand onto different vaccine scaffolds such as peptides, lipid nanoparticles and liposomes to study the cellular immune response they elicit. The ultimate desired outcome is the transcutaneous administration of the formulations, as an advantageous alternative to classic intramuscular vaccination methods. Herein we show how to utilize CLRs for specific targeting LCs.

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Importance of receptor geometry for signalling initiation by the mincle family receptors

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The mincle receptor family consists of the structurally similar C-type lectins, mincle, dectin-2 and BDCA-2. Constellation of amino acids around the primary Ca^{2+}-binding site of these receptors confers affinity for mannose, glucose and *N*-acetylglucosamine. Via association with FcRγ, mincle and dectin-2 stimulate macrophages in response to pathogen glycans, while BDCA-2 tapers production of type I interferon caused by TLR signalling followed by pathogen infection or cell death in autoimmunity. In this work, the oligomeric states of these receptors and the orientations of their CRDs have been investigated to elucidate how extracellular ligand binding initiates cytosolic signalling. We show that dimers of mincle are stabilized by disulfide bonds between cysteine residues in the neck sequence. BDCA2 forms noncovalent dimers, although a naturally occurring variant can form an interchain disulfide bond. Cysteine residues in the transmembrane portions of these receptors are not required for dimer formation or association with FcRγ, but may facilitate trafficking to the cell surface. We investigated how CRDs are positioned in receptor dimers using fusion protein of receptor extracellular domains and N-terminal dimerization domains. Analysis of these constructs showed limited interaction of the CRDs in the dimers, but interactions can be stabilized by the presence of the neck region. The resulting orientation of sugar-binding sites in the dimers would favour crosslinking of multiple dimers by oligosaccharide ligands, causing clustering of FcRγ to initiate signalling.

ST3GAL5-catalyzed gangliosides inhibit TGF-β-induced epithelial-mesenchymal transition

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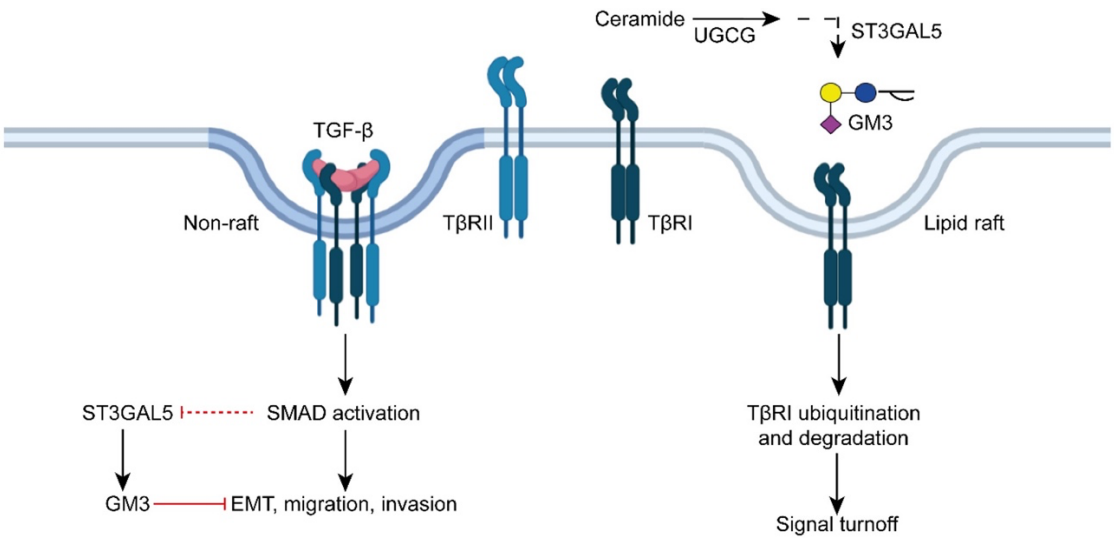
Epithelial-mesenchymal transition (EMT) is of pivotal importance in the development and initiation of cancer cell metastasis. We observed that abundance glycosphingolipids (GSLs), especially the gangliosides subtype, strikingly decreased during the TGF-β-induced EMT of mouse NMuMG cells and human lung A549 adenocarcinoma cells using porous graphitized carbon chromatography coupled to tandem mass spectrometry (PGC nano-LC-MS^2). Transcriptional profiling showed that the TGF-β/SMAD response genes and EMT signatures are strongly enriched in NMuMG cells depleted of UDP-glucose ceramide glucosyltransferase (Ugcg), which catalyses the initial step in GSL biosynthesis. Consistent with this notion, the genetic or pharmacological inhibition of UGCG promoted TGF-β signalling and TGF-β-induced EMT. The inhibition of UGCG stimulated A549 cell migration, extravasation in the zebrafish xenograft model and metastasis in mice. Mechanistically, GSLs inhibited TGF-β signalling by promoting the TGF-β type I receptor (TβRI) localisation into lipid rafts and by increasing TβRI ubiquitination and degradation. Importantly, we identified ST3GAL5-synthesised *a*-series gangliosides as the main branch of GSLs involved in the inhibition of TGF-β signalling and TGF-β-induced EMT in A549 cells. Notably, ST3GAL5 is weakly expressed in lung cancer tissues compared to adjacent normal tissues, and its expression correlated with good prognosis.

This study identifies plasma membrane GSLs composition and related biosynthesis enzymes as key suppressors of dynamic EMT and malignant transformation in human epithelia.



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https://youtu.be/ytcVqWTXz7A

Novel β-*C* and β-*N* fucosides as first synthetic ligands for BC2L-C N-term lectin from *B. cenocepacia*

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Burkholderia cenocepacia is an opportunistic Gram-negative bacterium, which causes infections in immuno-compromised individuals, mainly in cystic fibrosis patients. Several *B. cenocepacia* strains were found to be insensitive to many classes of antibiotics, making the treatment of related diseases very difficult.[1] The establishment of an infection by *B. cenocepacia* requires adhesion to host cells through carbohydrate/protein interactions. The BC2L lectins mediate this process and represent potential targets for antiadhesion antimicrobial therapy. Among the group of BC2L, BC2L-C presents an *N*-terminal trimeric domain with fucose-binding activity (BC2L-C-Nt) and a *C*-terminal domain, which recognises mannose (BC2L-C-Ct).[2]

This work aims at developing novel fucose-based glycomimetics able to interfere with the carbohydrate–lectin recognition of BC2L-C-Nt. A modular fragment-based library of *C*- and *N*-fucosides was designed and synthesized, starting from virtual screening of a fragment library.[3,4]The synthesized compounds were tested for their affinity towards BC2L-C-Nt through different biophysical techniques, including saturation transfer difference NMR spectroscopy (STD-NMR), isothermal titration calorimetry (ITC) and crystallographic studies. This study allowed to identify hit compounds with increased affinity compared to the monosaccharide parent structure, up to one order of magnitude.[4,5] These initial structure-activity relationships data will be used to develop high affinity ligands to be tested in the disruption of *B. cenocepacia* biofilm.

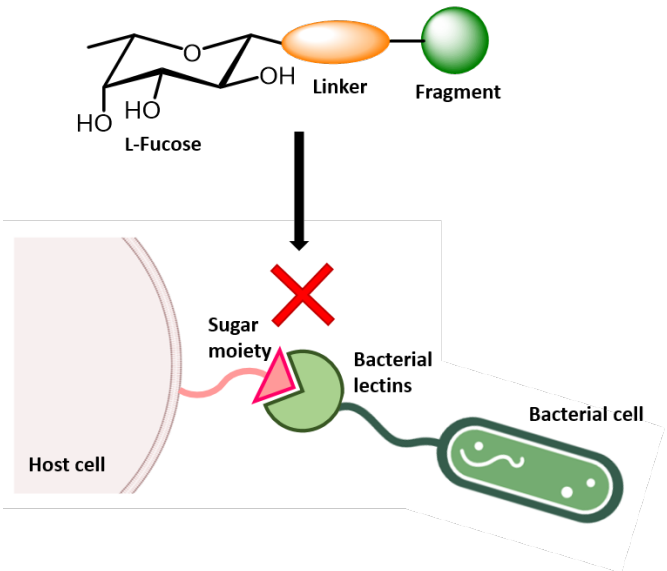

Figure 1. General structure of new BC2L-C ligands.

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Synthesis of constrained C-glycosidic analogues of Tn antigen

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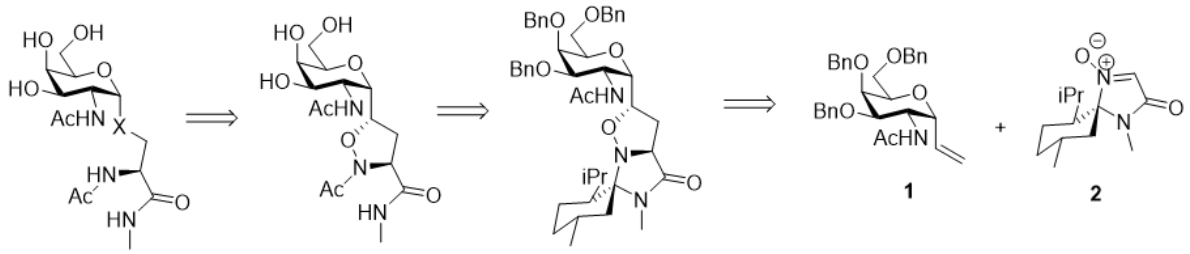
Tumor associated carbohydrate antigens (TACAs) are highly present on cancer cells, but almost no detectable on normal cells. Therefore, these components are of great interest as therapeutics targets, particularly in anticancer vaccines. By incorporating such structures into this kind of vaccine, the immune system should produce a more efficient response against cancer cells. However, the chemical instability of the *O*-glycosidic link in biological system towards glycosidases presents a major downside of their use in this purpose.[1] The replacement of the glycosidic bond is well known to afford more stable analogues such as *C*-glycosidic ones. Furthermore, constrained analogues of Tn antigen in vaccine could improve the anticancer immune response.[2]

Very recently we focus our attention on the synthesis of original constrained *C*-glycosidic analogues of the Tn antigen. For that, a synthetic strategy based on a [3+2] cycloaddition between a *C*-vinylGalNAc **1** and a chiral cyclic nitrone **2** has been developed (scheme 1). This key step provided access to constrained *C*-glycosidic analogues stereoselectively and regioselectively controlled[3]. Moreover, the cleavage of N-O bond of the isoxasoline ring and the post-functionnalization will allow the access to several original C-glycosides analogues of Tn antigen. Conformational study of constrained analogues, using DFT calculations and NMR experiences, has been performed. The global synthesis will be presented as well as the conformational study.



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C-glycosyl compounds: multifunctional scaffolds for the development of dual imaging tools

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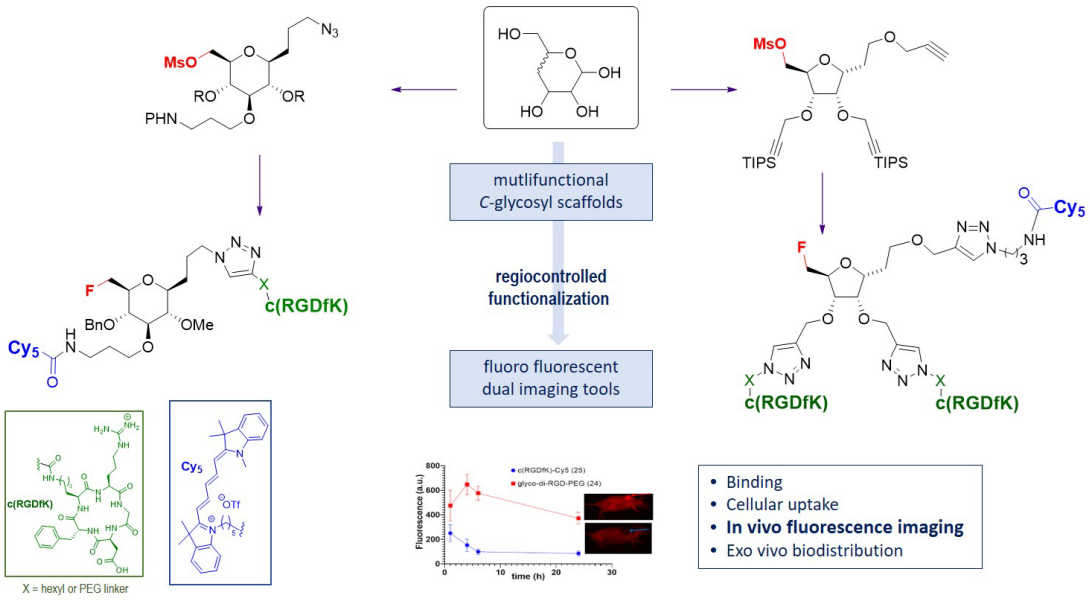
Close analogs of *O*-glycosides, *C*-glycosyl compounds display chemical and biological stabilities toward enzymatic hydrolysis and are thus used to build bioactive compounds, peptidomimetics and more complex sugars. In this work, *C*-glycosyl compounds are selected as multifunctional scaffolds for the development of imaging tools. More precisely, we focused here on bimodal molecular imaging, a current trend which combined two complementary modalities: PET (Positron Emission Tomography) and NIRF (Near Infra-Red Fluorescence).[1] Two *C*-glycosyl scaffolds are thus functionalized in a regiocontrolled manner in order to introduce the key elements at different stages: a fluorescent cyanine derivative for NIRF, a fluorine-18 atom for PET and one or two c(RGDfK) peptides targeting integrins overexpressed in some malignant tumours. The copper-catalyzed alkyne-azide cycloaddition (CuAAC) was used for the introduction of the fluorophore and for the bioconjugation step with peptides. *In vitro* and *in vivo* evaluations by fluorescence imaging and the resection of the tumor demonstrated the potential of the conjugates in glioblastoma cancer diagnosis and image-guided surgery.[2,3]



Dual imaging agents based on C-glycosyl scaffolds

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Efficient synthetic methodology for pseudo-glycans with C-glycoside linkage and their biological pot

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Our research group has been developing glycoconjugate analogs with the aim of contributing to glycobiology and drug discovery research. Our interest is to develop glycoconjugate analogues (pseudo-glycoconjugates or pseudo-glycans) with enhanced or different biological functions, while preserving their structures as much as possible. In this talk we will present the development of pseudo-glycans with a C-glycosidic linkage that are not degraded by glycoside hydrolases in cells.

Most carbohydrates exist as glycans or glycoconjugates and regulate biological events by interacting with various biomolecules in cells. However, the exact function of carbohydrates remains poorly understood and their biological potency is usually moderate. These drawbacks are partly attributed to the hydrolytic cleavage of carbohydrate or glycoconjugate structures by enzymes in cells. The C-glycoside analogues of native O-glycosides are expected to be useful molecular tools as glycoside hydrolase-resistant carbohydrate analogues. We have previously developed the sialidase-resistant ganglioside GM3 analogues with C-sialoside linkage.[1)] In this case, the simple replacement of an O-sialoside linkage with a CH_2-linkage proved to be non-ideal, and we proposed the introduction of a fluorine atom into the C-sialoside linkage, namely CHF-glycoside analogues. In fact, the (*S*)-CHF-linked GM3 analogue has shown the potent biological activity compared to native, CH_2-linked, CF_2-linked, or (*R*)-CHF-linked analogues. Therefore, we are currently investigating the application of this molecular design concept to other glycans and glycoconjugates.

Although various synthetic methods of C-glycosides have been reported, reports on the synthesis of glycan or glycoconjugate analogues with the C-glycoside linkage are still limited due to the complexity of their synthesis. We believe that, as with standard O-glycosylation, a C-glycosylation reaction capable of directly linking stable donor and acceptor building blocks (direct C-glycosylation) would facilitate the preparation of a variety of glycoconjugate analogs that would be useful in biological studies. In this context, we have recently established an efficient method to stereoselectively obtain C-glycoside analogues by controlling anomeric radical species generated by a photoredox catalytic system.[2,3)] In this talk, we will present the detail of this methodologies, their application to the synthesis of glycan or glycolipid analogues with the CH_2- or CHF-glycoside linkage, and their biological activities.

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Novel multifunctional glycan probes for glycan recognition studies on microarrays and cells

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Glycans mediate key biological processes through specific glycan-protein interactions. Glycan microarrays have revolutionized the elucidation of glycan ligands in diverse recognition systems. Two major slide-based array systems are covalent arrays with amino-terminating glycans immobilized on amine-reactive glass[1,2] and noncovalent neoglycolipid (NGL)-based arrays with lipid-linked glycans immobilized on nitrocellulose-coated glass[3,4]. There are few reports on cross-platform comparisons, and the data are largely with covalent arrays and proteins that give robust binding signals[5,6]. Here we present the design of a novel tri-functional Fmoc-amino-azido (FAA) linker which enables efficient derivatization of free reducing glycans and their conversion into amino-terminating or lipid-tagged probes for constructing covalent arrays and NGL-based noncovalent arrays, respectively. This provides a unique opportunity for a close comparison of the two array platforms with a variety of glycan recognition systems. Whilst similar results are obtained with most plant lectins and antibodies investigated, a striking difference is observed with several viral adhesins and Siglecs which gave binding to low affinity sialyl glycan ligands only in the NGL platform. Our findings highlight that the mode of display and clustering of glycans can dramatically affect microarray readout with certain recognition systems, an important consideration for the glycan recognition knowledgebase. The FAA glycan probes can moreover be rendered fluorescent for detecting glycan binding by proteins on cell surfaces.

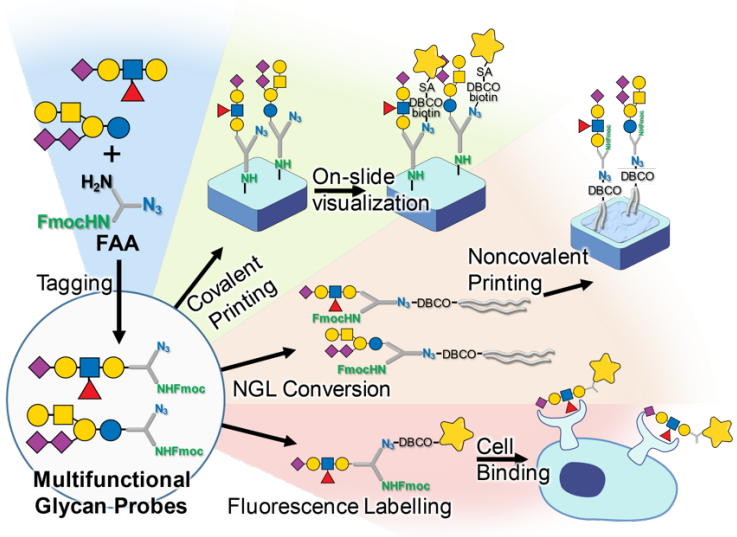

Figure. Schematic representation of the design and applications of FAA linked glycan probes.

Supported by UK Medical Research Council grant (MR/R010757/1) and Wellcome Trust Biomedical Resource grant (218304/Z/19/Z).

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Synthetic glycans as tools for studying plant arabinogalactan biosynthesis

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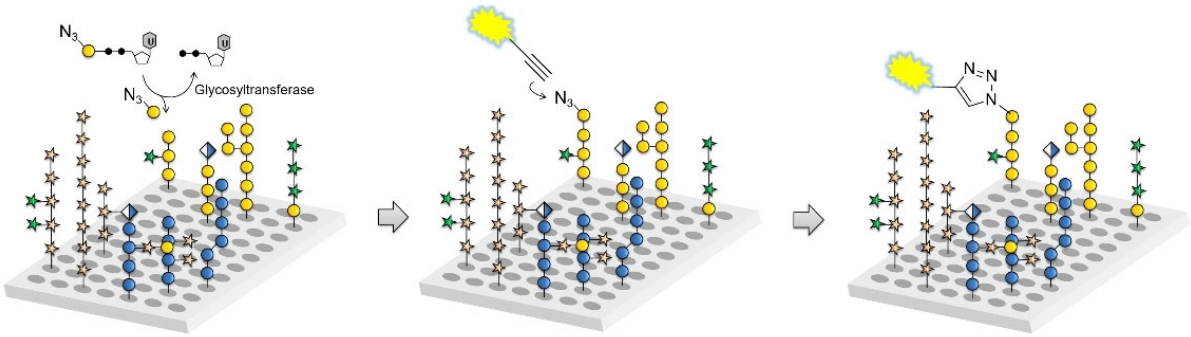
Plant cells are surrounded by a polysaccharide-rich matrix that constitutes the cell wall of all higher plants and provides the richest available resource of fermentable carbohydrates and bio-based materials. Optimal exploitation of this resource requires investigations into the biosynthesis of cell wall glycans, which are aided by well-defined and pure glycan samples obtained through chemical synthesis. We have prepared oligosaccharides derived from different classes of cell wall glycans and printed them as microarrays to generate a tool for characterization of cell wall glycan-directed antibodies, plant immune receptors, and glycosyltransferases that are involved in plant cell wall biosynthesis. The glycan arrays were for example incubated with azido-functionalized sugar nucleotide donors and putative glycosyltransferases to enable product detection “on chip” via click reaction with an alkynyl-modified dye [1]. Using this assay, a new glycosyltransferase involved in plant arabinogalactan synthesis was recently identified and functionally characterized.



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Developing synthetic and analytical tools to study glycans on binding preferences

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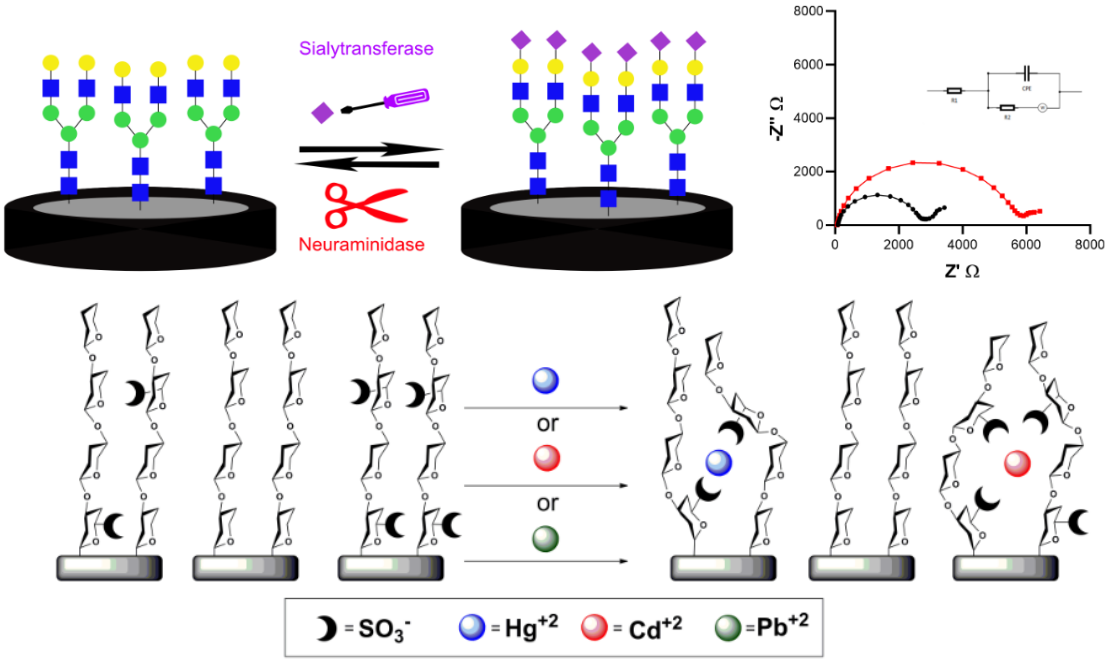
Modifications of complex glycans govern their binding preferences and biological activities. Evaluating the effect of sulfation and sialylation on glycans interaction on binding preferences is not easy for two main reasons. First, obtaining libraries of modified complex glycans in sufficient quantity is hampered by synthetic hurdles. Second, many of the interactions are too weak to quantify using standard bioanalytical tools.

I will present our advances towards the development of new methods to synthesize complex glycan and present our approach for expediting their preparation. I will show that label-free electrochemical tools provide insight to the biology of those unique modified glycans. I will highlight how the combination between new synthetic methods and analytical approached enable us to study the effect of glycan modifications on interaction preferences with metal ions, proteins and metal-ion mediated protein interactions.



Optimized and accelerated Oligosaccharide synthesis enabled the development of electrochemical tools to study glycan interactions

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Recent advances in the sequencing of rare lichenic carbo-hydrates using advanced mass spectrometry

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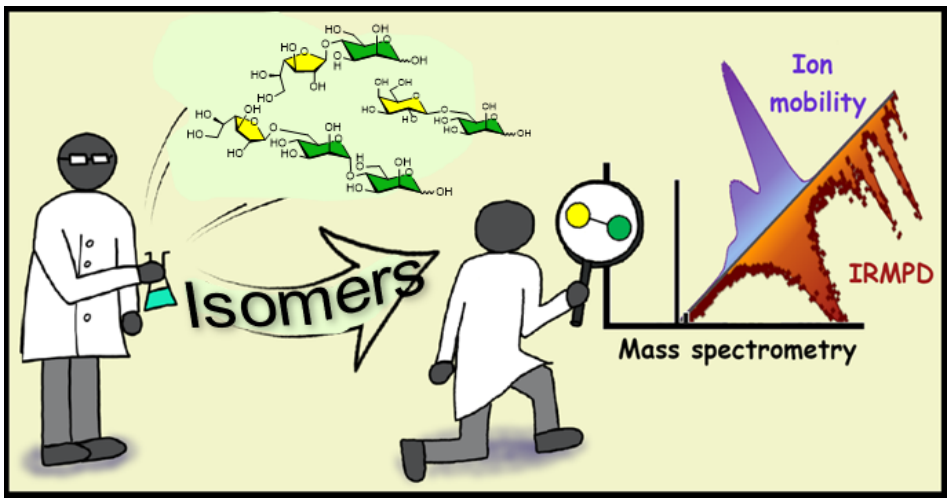
Lichens constitutes a unique symbiotic system of microalgae and a fungus. Till now, the lichen chemistry is mainly focused on secondary metabolites that represent 3 to 20% of the lichen dried weight while polysaccharides represent about 60% of the biomass. Interestingly, among identified polysaccharides, the galactomannans have attracted attention either as chemotaxonomic character to classify lichens or as bioactive compounds. The galactomannans are constituted mainly of a mannan backbone with different decorations depending of the symbiotic partners. One of the main one is the branching of a galactofuranose. Sugars in their furanose form are not usual in nature but of high interest as this motif can be found in numerous pathogenic microorganisms. Nevertheless, the detection of galactofuranose in Lichen's polysaccharides remains cumbersome, principally because of the sensitivity of galactofuranosides to mild hydrolysis conditions. To circumvent this problem, we have synthesized a library of di- and trisaccharides of galactomannans where the galactose adopted either the pyranose or the furanose form. This library served as standard for the implementation of a unique analytical flowpath relying on both Ion-mobility-Mass spectrometry analysis and Infra-Red Multiple-Photon Dissociation technics. It allowed to identify without any ambiguity the presence of the galactofuranose isomery as well as its regioisomery. Correlations between both techniques was established by molecular modeling to better understand the impact of conformational changes on physicochemical properties.



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From SF2a-TT15 to a synthetic glycan-based quadrivalent *Shigella* vaccine candidate

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The burden caused by shigellosis, a major diarrheal disease, calls for a *Shigella* vaccine that would induce broad serotype protection especially in children under five from low and middle income countries. Protective immunity is believed to be achieved to a large extent by antibodies specific for the O-antigen (O-Ag) part of the *Shigella* lipopolysaccharide. Aside the use of glycans of biological origin, vaccine candidates encompassing synthetic glycans mimicking the putative protective determinants carried by the O-Ag of selected *Shigella* serotypes was undertaken at Institut Pasteur.[1] SF2a-TT15, a semi-synthetic glycoconjugate designed to help protect against *S. flexneri* 2a[2] was well tolerated and immunogenic in healthy adult participants in a first-in-human phase 1 clinical trial.[3]

The concept of synthetic O-Ag functional mimics,[4] that serve as pillar of a vaccine candidate providing broad coverage against circulating *Shigella* strains, will be introduced. The design and optimization of monovalent glycoconjugates, combined to SF2a-TT15, pave the way to such a vaccine candidate.

Epitope mapping will underline the molecular features governing functional O-Ag mimicry by short well-defined glycans. The possible influence of chain length, end-chain residue and non-stoichiometric acetylation will be examined.

Immunogenicity data for various combinations of monovalent components will be discussed together with selection criteria to achieve a promising *Shigella* vaccine candidate featuring the best compromise between synthesis feasibility, conjugation chemistry and immunogenicity.

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Some general insights in the scale up for a multigram synthesis under GMP of one oligosaccharide

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Subject proposed will deal with the development and production work to produce an oligosaccharide planned to be conjugated to a protein. This final conjugated oligosaccharide is intended to be used to initiate preclinical and phase I/II clinical studies of a vaccine drug.

Presentation will focus on scale-up for a kg scale production of several synthesis intermediates as well as gram scale of last steps synthesis, which were done under GMP to deliver gram scale of this oligosaccharide. Emphasis will be done on how development was conducted to avoid problems or concerns during scale up, starting from medicinal chemistry lab procedures.

Semi-synthetic O25B-CRM197 conjugate vaccines give rise to functional antibodies in murine model

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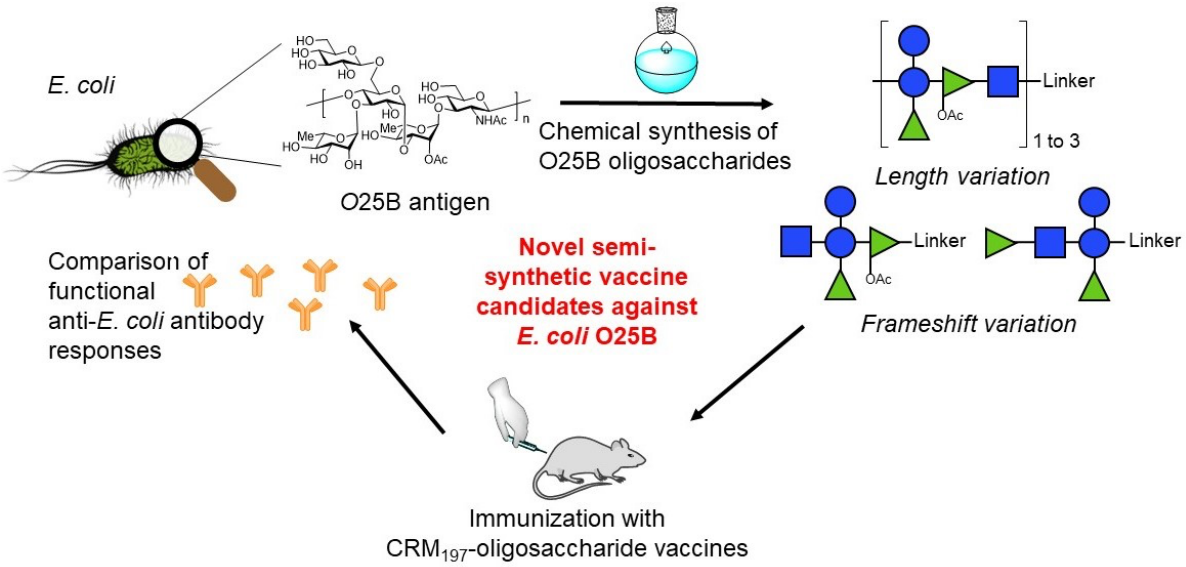
Pathogenic, multidrug resistant *E.Coli* strains causing enteric/diarrheal, urinary tract infections, sepsis and meningitis are of increasing concern worldwide. A major serotype of extraintestinal pathogenic *E.Coli* (ExPEC) is O25B, expressing an unique *O*-antigen consisting of a branched five monosaccharide repeating unit. The chemical synthesis of five fragments of various length or frameshift oligosaccharides gave access to a set of well-defined semi-synthetic glycoconjugate vaccine candidates targeting the *O*-antigen of *E.Coli* O25B serotype. The semi-synthetic glycoconjugate vaccines induced similar levels of functional IgG antibodies with opsonophagocytic activity against *E.Coli*O25B in mice as the conventional polysaccharide vaccine candidate prepared with native O25B *O*-antigen. Furthermore, it was shown that our synthetic O25B antigens can give rise to antibodies with nanomolar affinity. Moreover, we found that acetylation of a rhamnose residue as it occurs in the natural polysaccharide most likely does not influence the immunogenicity of the antigens as also our deacylated antigen elicited a strong functional IgG response. Overall, the direct comparison of the immunogenicity of a glycoconjugate vaccine prepared with isolated *O*-antigen to those prepared with chemically synthesized *O*-antigens allows a more comprehensive analysis of the binding epitopes and lays foundation for rationally designed chemically synthesized oligosaccharide-based vaccines.



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Loop size and dynamics modulate substrate specificity in chitin deacetylases

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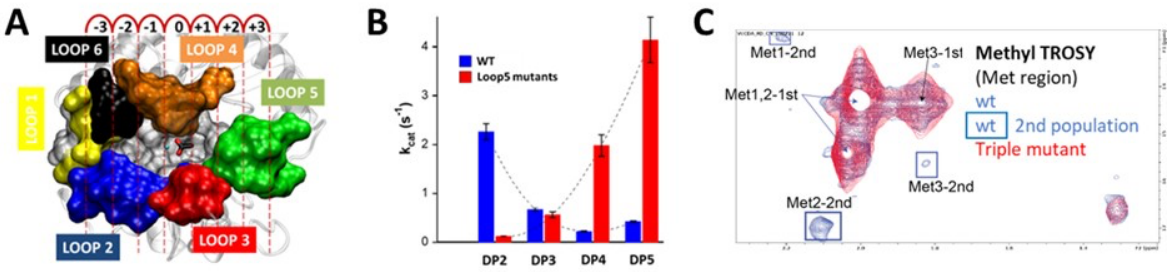
Chitin deacetylases (CDA) are members of family 4 carbohydrate esterases (CE4) which deacetylate chitooligosaccharides (COS) with different deacetylation patterns [1]. We are interested in understanding the structural bases of substrate specificity by CE4 enzymes and the use of engineered variants as biocatalysts [2]. Because of the influence of different acetylation patterns in signaling events (*i.e.* pathogenic fungi-host interactions), the availability of a panel of CDAs with defined specificities will provide sequence-defined partially deacetylated COS with applications in biomedicine and biotechnology.

Most CDAs follow a multiple attach mechanism but few are specific for deacetylating a single position of COS. The 3D structure of *Vibrio cholerae* CDA in complex with COS substrates showed six surface loops that shape the binding site cleft and guide deacetylation to a single position of short COS [3]. We proposed the "subsite capping model" to rationalize the differential accessibility of substrates to the binding cleft mediated by these non-conserved loops within CE4 enzymes [3,2]. After identifying Loop5 in *Vc*CDA as the structural element controlling specificity for short substrates [4], here we report on a rational design to modify specificity towards larger substrates. By biochemical, NMR side-chain (CH_3) relaxation and MD studies, we show that loops dynamics are coupled and their modulation strongly alters specificity. The mutations shift ligand binding from a conformational selection to an induce fit mechanism, with a large impact in specificity and activity.



A) Loops of VcCDA that shape the binding site. B) Specificity change from wt to a triple mutant at Loop5. C) NMR methyl TOSY of wt and triple mutant.

Work funded by Grant PID2019-104350RB-I00 from MICINN, Spain

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Family GH157 endo-β-1,3(4)-glucanases exhibit exo-hydrolytic activity

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β-glucans are a class of polysaccharides whose hydrolysis is catalysed by β-glucanases. Due to the complex relationship between the molecular structure and functional profile of β-glucans, there is a continuous need for specific β-glucanases that can fully reveal β-glucans' potential applications [1]. Here we report the first biochemical and structural characterization of two β-glucanases from the novel glycoside hydrolase family 157 (GH157) and investigate their molecular basis for substrate hydrolysis.

Genes encoding the GH157s from the human gut bacteria *Bacteroides cellulosilyticus* and psychrophilic bacteria *Labilibaculum antarcticum*were expressed and structurally characterized by X-ray crystallography. Their specificity and activity were analyzed with reducing sugar assays, enzyme kinetics and product analysis by HPAEC-PAD and LC-MS.

Specificity screening revealed that the enzymes show a preference for mixed-linkage glucans. HPAEC-PAD and LC-MS on hydrolysis products revealed that both enzymes display an endo mode of action, capable of cleaving β1-3 and β1-4-linked glucoses, when preceded by a β1-3 linkage. *La*GH157 structure showed a $(\beta/\alpha)_8$ barrel fold and a retaining mechanism of hydrolysis, with two glutamates serving as the catalytic residues.

This study provides the first characterization of GH157 members, identifying them as retaining endo-β-1,3(4)-glucanases, with exo-hydrolytic activity in the case of *La*GH157. This provides insight into β-glucan deconstruction in the human gut and marine biomes, while identifying potential β-glucan catalysts.

Acknowledgements: We acknowledge FCT, through the grants: UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); and 2022.07903.PTDC. We also acknowledge ANI through the grant LISBOA-01-0247-FEDER-047033 [GlycoMed] and the Gilead GÉNESE program through the project 17805. Moreover, we thank the Royal Society (Ken Murray Research Professorship to GJD), the BBSRC (grant BB/R001162/1 to GJD) and the European Research Council ERC-2020-SyG-951231 "Carbocentre" to GJD. We also thank Rachael Hallam and Dr. Leonardo Gomez for skilled maintenance and provision of access to HPAEC-PAD instrumentation. C.Caseiro is funded by an individual PhD scholarship from FCT (SFRH/BD/147152/2019).

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Structural and mechanistic insights into the cleavage of clustered *O*-glycan by mucinases

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Mucinases of human gut bacteria cleave peptide bonds in mucins strictly depending on the presence of a neighboring *O*-glycan. The *Akkermansia muciniphila* AM0627 cleaves specifically in between contiguous (bis) *O*-glycans of defined structure, suggesting that this enzyme may recognize clustered *O*-glycan patches. Here, we report the structure and molecular mechanism of AM0627 in complex with a glycopeptide containing a bis-T (Gal-GalNAc) *O*-glycan, revealing that AM0627 recognizes both the sugar moieties and the peptide sequence. Interestingly, AM0627 prefers bis-T over bis-Tn (GalNAc) *O*-glycopeptide substrates, with the first GalNAc residue being critical for cleavage, and follows a mechanism relying on a nucleophilic water molecule and a catalytic base Glu residue. Structural comparison among mucinases identifies a conserved Tyr, engaged in sugar-p interactions in both AM0627 and the *Bacteroides thetaiotaomicron* BT4244 mucinase, as responsible for the common activity of these two mucinases with bis-T/Tn substrates. Our work illustrates how mucinases, through tremendous flexibility, adapt to the diversity in *O*-glycan distribution in mucins.

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Structural basis for donor sugar specificity in a plant galactolipid synthase

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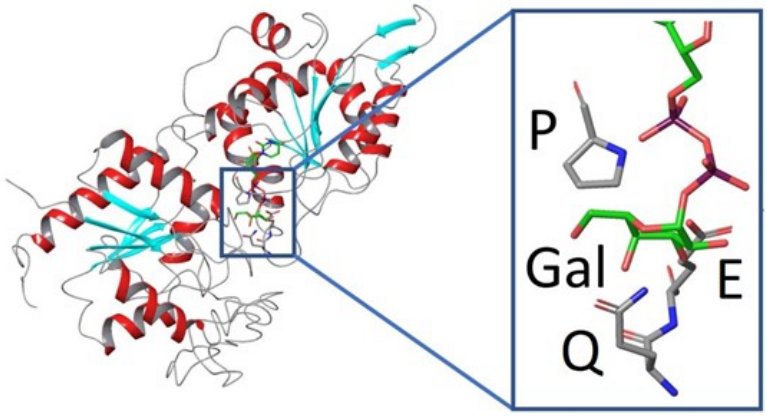
Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are essential galactolipids for the biogenesis of plastids and the functioning of the photosynthetic apparatus, and they comprise around 80% of the lipid content of photosynthetic membranes. In Arabidopsis, MGD1 is the major enzyme that initiates galactolipid synthesis. MGD1 is a monotopic membrane protein located in the inner envelope membrane of chloroplasts, which catalyzes the transfer of galactose from UDP-Galactose to diacylglycerol (DAG) to form MGDG.

It remains unclear why galactose (and not the more abundant glucose) was selected and conserved during the evolution of photosynthetic organisms, from cyanobacteria to plants. It was, therefore tempting to modify the substrate specificity of MGD1 so that it could use UDP-glucose (present in the cytosol but also in the stroma of chloroplasts) and to evaluate its impact on the different cellular functions of galactolipids in planta.

The crystal structure of MGD1 was previously solved [1], identifying three key residues that determine donor specificity: P433, Q455 and E456, also named the PQE motif. Several mutants were designed based on rational protein design and comparison of the equivalent 'PQE' motif in closely related glucosyltransferase. The P433 residue and its environment were key for donor specificity. The presence of a GPG motif seems to be a signature of a galactosyltransferase activity, whereas a GGX (X being an aliphatic residue) is indicative of a glucosyltransferase activity.



PQE motif of MGD1

BM and DJ: equal contributors

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Substrates promiscuities of bacterial glycosyltransferases enable site-specific fucosylation

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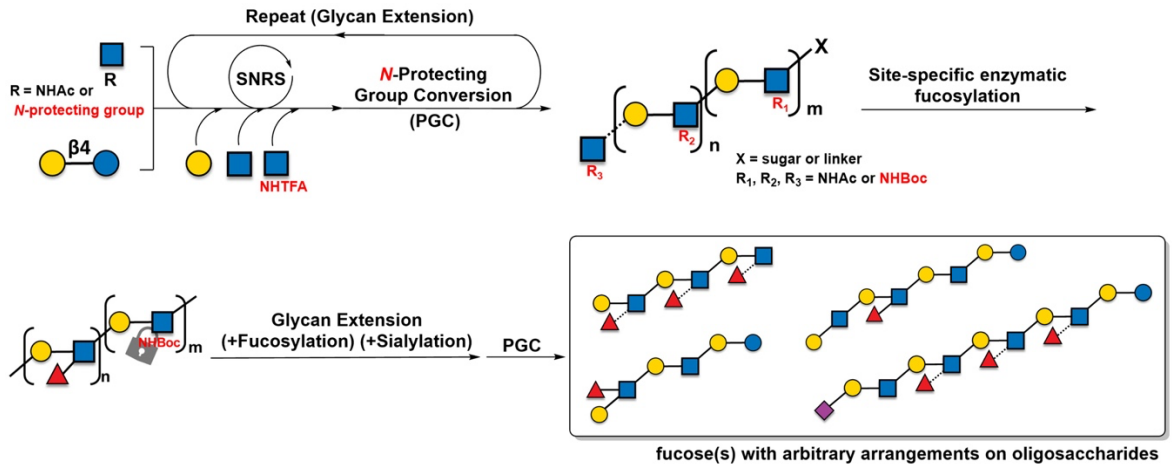
Fucosylated glycans are frequently existing in the *N*-glycans, *O*-glycans, glycosphingolipids (GSLs) and human milk oligosaccharides (HMOs), and involved in unique biological activities of organisms. Fucoses are assembled at multiple sites of *N*-acetyllactosamine (LN) and/or lacto-*N*-biose (LNB) which are also known as Lewis antigen. The diversity of Lewis antigens on the complex glycans causes their chemical synthesis difficulty due to the lower stereoselectivity and regioselectivity. The enzymatic fucosylation of oligosaccharide backbones is a prosperous strategy owing to its stereoselectivity and higher synthetic efficiency. However, it also leads to heterogeneous glycan determinants resulting in time-consuming and difficult purification steps. Herein, we developed a general strategy for site-specific fucosylation on poly LN or LN/LNB hybrid backbones. The promiscuous bacterial glycosyltransferases can assemble *N*-modified glucosamines into oligo-LN and LNB, respectively. The regioselectivity of fucosylation can be controlled by the amino protecting groups. Although various site-specific fucosylation strategies have been reported,[1]-[3] our strategy makes the purification and reaction monitoring easier. Importantly, the synthetic scale and efficiency can be raised by coupling with sugar nucleotide regeneration system (SNRS). The robust strategy was demonstrated by the facile synthesis of myeloglycans, dodecasaccharides, and internal/terminal fucosylated HMOs. Our method features the feasible applications on other complex carbohydrates.



Schematic illustration of site-specific fucosylation strategy in this work

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Elucidation of the exquisite reaction selectivity of human GDP-fucose synthase

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L-Fucose is found in a variety of important glycoconjugates in mammalian cells[1]. GDP-fucose (GDP-Fuc) is biosynthesized in two enzymatic steps catalyzed by GDP-mannose-4,6-dehydratase[2] and GDP-Fuc synthase (GFS)[3]. GFS catalyzes the conversion of GDP-4''-keto-6''-deoxy-mannose (GDP-4k6d-Man) into GDP-Fuc using three reactions within a single active site: epimerizations at C-3'' and C-5'', and a NADPH-dependent reduction at C-4''[3]. The mechanism that controls conformational changes is currently not understood. We demonstrate evidence for stereochemical control of GFS by employing a multidisciplinary approach of structural, biochemical and computational analysis.

Solvent derived deuterium incorporation into GDP-4k6d-Man and GDP-Fuc assessed the timing of the epimerization steps, revealing that the first epimerization occurs at C-3" and second one at C-5". C116 acts as a base and H186 as an acid responsible for epimerization. Y143 is the proton donor involved in the final reduction. Crystal structures of hGFS in complex with GDP-Fuc or GDP-4k6d-Man show the deoxy-hexose moiety well positioned for epimerization. However, the conformation is incompatible with C-4" reduction indicating a need for substrate repositioning. QM/MM simulations showed that the sugar conformation switch during catalysis is coupled to a change in protonation states of C116, H186. For each catalytic step a distinct substrate conformation is adopted which is strictly controlled by GFS. Together these results fully resolve reaction mechanism and elucidate the underlying mechanism of hGFS reaction selectivity.

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Carbohydrate foldamers and assemblies

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Natural biopolymers have inspired the development of synthetic analogues – i.e. foldamers – capable of adopting defined conformations and forming programmable three-dimensional architectures. These compounds are mainly based on peptides and nucleic acids, that are well understood at the molecular level. In contrast, the complexity of carbohydrate synthesis and structural analysis have prevented access to synthetic carbohydrates capable of adopting defined geometries. In the Delbianco group, we prepare well-defined carbohydrates to understand how the primary sequence affects the carbohydrate conformation.1 With multiple analytical techniques, we study the conformation of single carbohydrate chains2 and explore how several carbohydrate molecules aggregate to form a material3.

Building on this fundamental knowledge, we present the rational design and synthesis of a glycan adopting a stable secondary structure, challenging the common belief that glycans are not capable of folding due to their flexibility. By combining natural glycan motifs, stabilized by a non-conventional hydrogen bond and hydrophobic interactions, we have designed a glycan hairpin, a secondary structure not present in nature. Automated glycan assembly enabled rapid access to synthetic analogs, including site-specific 13C-labelled ones, for NMR conformational analysis. Long-range inter-residue nuclear Overhauser effects (NOEs) unequivocally confirmed the folded conformation of the synthetic glycan hairpin. The ability to control the conformation of glycans could lead to the generation of 3-D architectures, with applications in catalysis and nanotechnology.

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Synthetic chitin nanocrystals and their assemblies

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Nanochitin, an extraction product of the most prevalent biopolymer in the ocean (chitin),[1] has appealing qualities as biocompatible and biodegradable material.[2] Although its outstanding material properties, it is still one of the least utilized biomass resources. The common top-down approaches to extract and isolate nanochitin from natural precursors often result in undefined chemical compositions making it nearly impossible to establish rational correlations between three-dimensional structures and macroscopic properties.[3]

Following a bottom-up approach,[4] we used automated glycan assembly (AGA) to prepare well-defined chitin oligomers as tools to understand the transfer of chirality from the single oligomer to supramolecular assemblies. Simple oligomers self-assembled into synthetic chitin nanocrystals that we characterized at the molecular level with electron and atomic force microscopy. These nanocrystals further assembled into bundles with intrinsic chiral features. We discovered the profound impact of water on the assembling process, enabling us to control and fine-tune the nano-scale morphology. This knowledge will expand our understanding of chitin, providing essential guidelines for the generation of well-defined chitin-based materials.

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Phosphorylase-catalyzed bottom-up synthesis of cello-oligosaccharides and property-tunable materials

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Cellulose-based materials are produced industrially via top-down processing from natural lignocellulose biomass, while challenges involved in this approach has promoted the development of bottom-up synthesis. Building up a cellulose chain via bottom-up approach is promising since it offers higher synthetic precision than the top-down processing.[1]

Glycoside phosphorylases (GPs) are carbohydrate-active enzymes involved in the formation/cleavage of glycosidic bond. The primarily relevant GP for biosynthesis of cellulose is cellodextrin phosphorylase (CdP, EC 2.4.1.49). It catalyses iterative β-1,4-glycosylation from α-D-glucose 1-phosphate to elongate a diversity of acceptors including cellobiose and a range of synthetic glycosides with non-sugar aglycons.1 Due to iterative glycosylation leading to different degrees of polymerization (DP), soluble cello-oligosaccharides (COS) or insoluble cellulosic materials are formed.[2] Here, we present an approach using CdP as bio-catalyst for the bottom-up synthesis of functional cellulose materials. The synthetized soluble COS (DP ≤ 6) exhibited a selective prebiotic effect.[3] The synthetic cellulose with chains DP ≥ 9 form as insoluble materials in a sheet-like crystalline structures of cellulose allomorph II. Reducing-end functionalized (e.g., thiol-) materials thus obtained can expand cellulose applications towards the fields that are difficult to access via top-down approach.[4,5] With solvent condition set, bottom-up synthesis also enables the possibility to generate composite materials which can be useful as functional hydrogels.[6]

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Developing self-assembling cyclodextrins to tackle the cytopathic activity of SARS-CoV-2

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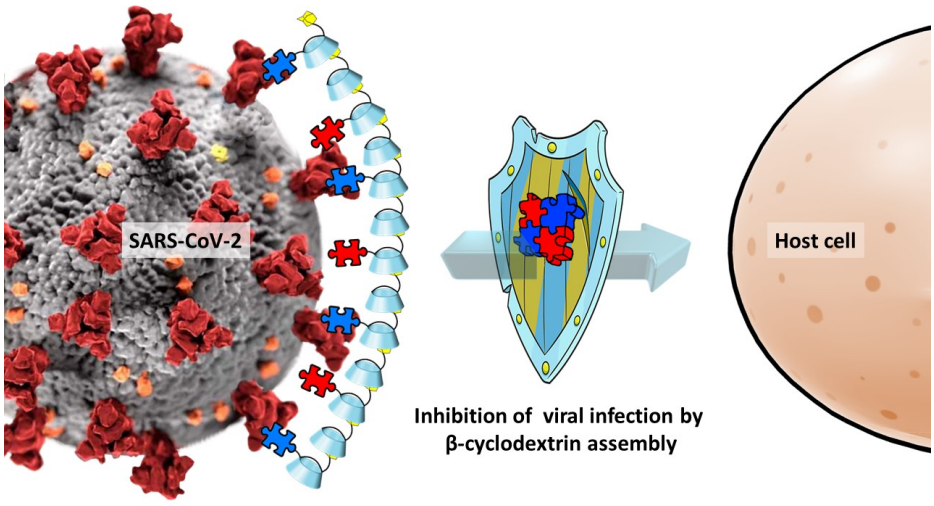
The global pandemic caused by SARS-CoV-2 has arisen awareness about the importance of the development of rapid tools to fight the transmission of viruses. However, there is still a lack of over-the-counter products to be used by the public as preventive systems against covid-19. Efforts have been made to inhibit the infection by the virus targeting the SARS-CoV-2 spike protein and its affinity for different ligands including carbohydrates[1] and peptides.[2] To target the different binding domains of the spike protein, a library of ligands comprising sialic acids with various linkages and substitutions, heparan sulphate mimics, peptides and small molecule protein-protein interactions inhibitors has been synthesised. However, the multivalency of the spike protein needs to be addressed for the design of viral infection inhibitors. Cyclodextrin-adamantane conjugates have been proved useful in the past years to generate thermodynamically self-assembling systems.[3] Furthermore, it was found that the co-assembly was favoured in the presence of a molecular template such as DNA to form fibres.[4] To investigate whether the surface receptors of a pathogen can function as a biological template for the self-assembly of our cyclodextrin-based system, a library of ligands will be applied to study the cytopathic effect inhibition of SARS-CoV-2 to protect cells. These results will inform the development of novel self-assembling cyclodextrin system as preventive treatment against viral infection.



Adamantane-promoted inclusion assembly of functionalised β-cyclodextrins grants protection to host cells against the infection by SARS-CoV-2.

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Multichromophoric carbohydrates as fluorescent & reversible photoswitches for optical applications

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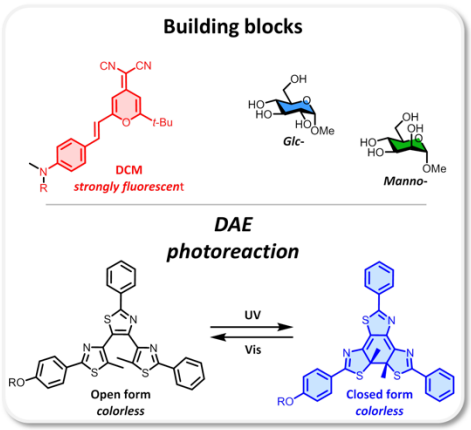
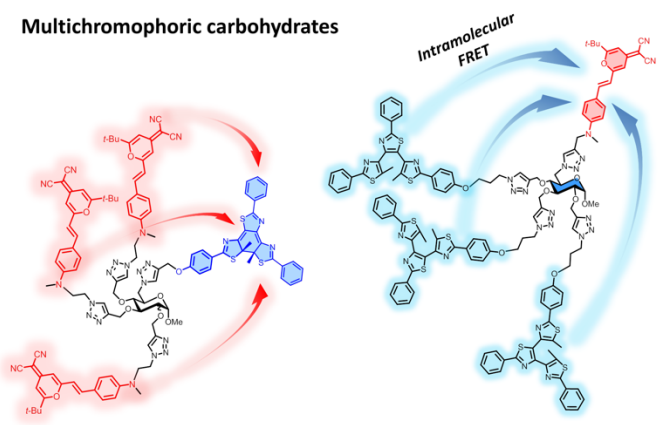
The development of photosensitive molecular systems attracts considerable interest in the growing fields of the photopharmacology and the photoresponsive nanotechnologies (optical memories and super-resolution imaging).[1],[2]

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

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

Synthesis of these photoswitchable multichromophoric carbohydrates as well as their photochemical and photophysical properties by absorption and fluorescence under light illumination will be presented.





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A synthetic receptor for the biomimetic recognition of N-glycans

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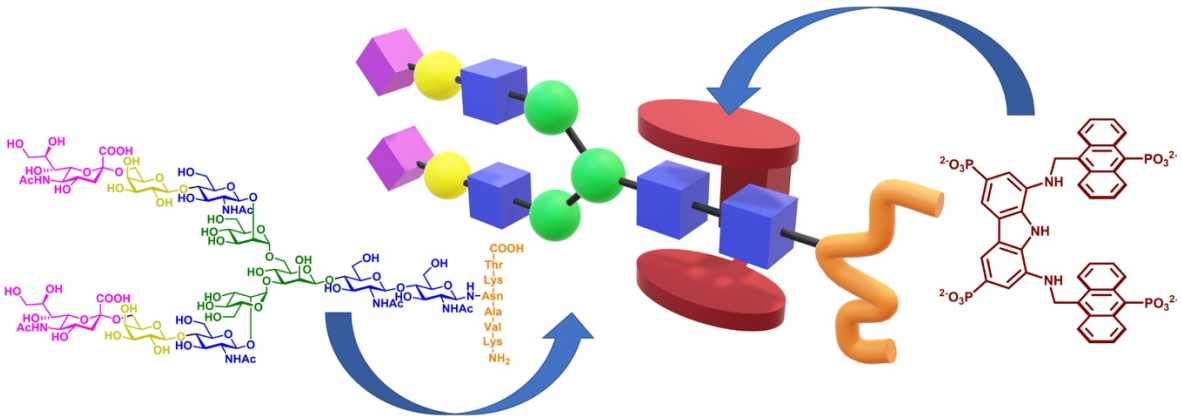
N-glycosylation is one of the most common post-translational modification of proteins in eukaryotic cells. *N*-glycans are linked to proteins through their highly conserved core disaccharide $GlcNAc_2$.[1] *N*-glycans have pervasive roles in biological systems, including protein folding, stability, solubility and resistance to proteolysis, self/non self discrimination by the immune system and pathogen adhesion and infection. Selective recognition of glycans by biomimetic receptors, to interfere with physio- and pathological processes mediated by carbohydrate recognition, represents a major challenge of the current research.[2] Because water is a strong competitor for recognition of polar molecules such as carbohydrates, most of the literature on the topic is confined to organic media, and examples of biomimetic receptors effective in water are sporadic and mainly based on appropriately sized macrocyclic architectures.[3] Recently, we have presented a simple biomimetic receptor, based on an acyclic structure, which exhibits a marked selectivity for the methyl-β-glycoside of $GlcNAc_2$ in water, showing an unprecedented affinity of 160 mM.[4] In this communication we describe the most recent advances of using this tweezers-shaped architecture to target the core $GlcNAc_2$ disaccharide of *N*-glycans.[5]



Schematic representation of the tweezers-shaped receptor binding to the GlcNAc2 disaccharide at the stem of sialoglycopeptide SGP.

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Synthesis and binding interactions of secondary cell wall polysaccharide fragments of paenibacillus

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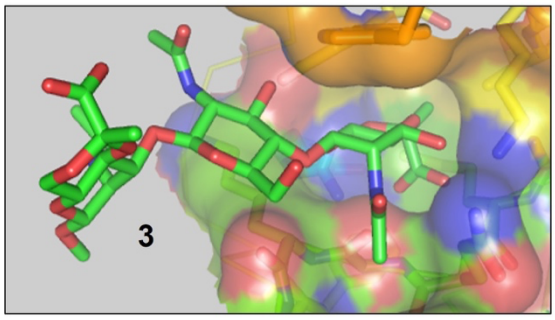
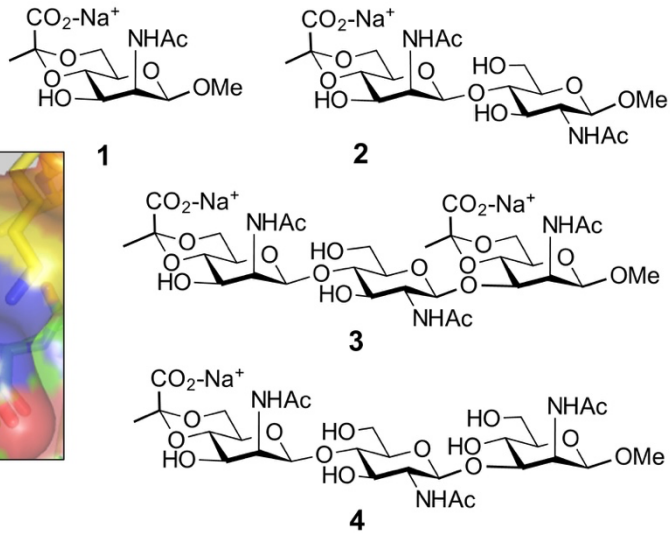
Surface (S-) layer proteins of *Paenibacillus alvei* are non-covalently bound through the pyruvyl anchor of its secondary cell wall polysaccharide (SCWP) [-3)[4,6-*O*-pyruvyl]-β-D-ManNAc-(1-4)-β-D-GlNAc-(1-. Aiming at an in-depth study of SCWP biosynthesis and the molecular details of the binding interaction with the N-terminal S-layer homology (SLH) domain trimer, we have set out to synthesize defined oligomeric ligands to be used for crystallographic and ITC binding studies of the wild-type SLH domain trimer as well as select mutants. For the preparation of the challenging β-D-ManNAc linkage, two approaches have been studied.

Inversion at C-2 of a β-linked glucopyranosyl unit followed by azide introduction and conversion into acetamido groups was used for the assembly of ligands **1**-**3**.[1,2] For the synthesis of a tetrasaccharide ligand, hydrogen bond mediated aglycon delivery[3] was employed using a 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-picoloyl-protected mannosyl thioglycoside donor. Binding of ligands (in a nanomolar K_D range) was mainly driven by the terminal pyruvylated ManNAc residue as shown for trisaccharide **3** (pdb 7sv4), thus opening future perspectives to inhibit bacterial cell wall assembly.[4]





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Biochemical characterization of Mimivirus L143 enzyme: the first pyruvyl transferase from a virus

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Pyruvylation consists in the transfer of a pyruvate moiety to the monosaccharide target in enol or ketal form, where the ketal-pyruvylation, the most widespread in nature, is found in bacteria, yeasts, and algae [1]. Interestingly, the chemical characterization of the mimivirus glycans [2] has extended this type of sugar modification to the viral glycans. Indeed, in one of the two polysaccharides covering mimivirus fibrils, the repeating unit is an N-acetyl-glucosamine modified with a pyruvic acid linked as a ketal to the hydroxy function 4 and 6. Since the sugar pyruvylation is absent in amoeba, the host of mimivirus, it is likely that mimivirus encodes its own pyruvyl transferase enzyme. Bioinformatic studies have identified L143 as a good candidate [3] and the object of this work was to assess the function of L143 enzyme by chemical and spectroscopic studies. Biochemical assays identified the phosphoenolpyruvate as a donor of the pyruvic acid, for which the enzyme presents a high affinity (Km < 1). Therefore, we demonstrated that the substrate of this reaction is the N-acetyl-glucosamine monosaccharide (Figure 1), the enzyme being unable to work at the level of the nucleotide sugar (UDP-N-acetyl-glucosamine). These results suggested that *in vivo* the reaction substrate could be the full polysaccharide or the disaccharide precursor of the repeating unit, prior the assembly of the polysaccharide.

Sugar pyruvylation plays key biological functions [1], and in the case of mimivirus could be involved in the adhesion process on the amoeba host membrane.

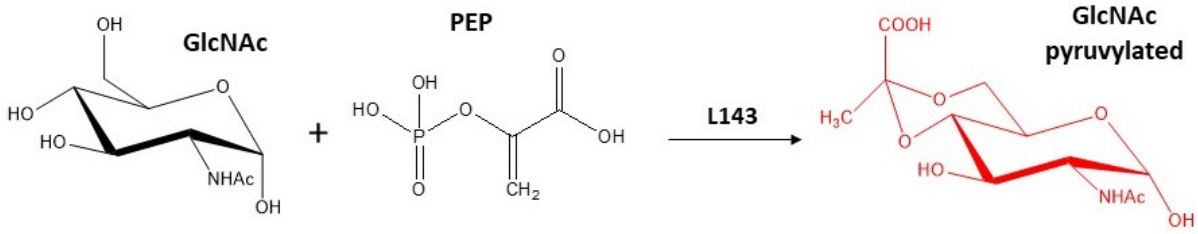

Figure 1. L143 enzyme is the viral pyruvyl transferase that links a pyruvic acid as a ketal to the hydroxyl functions 4 and 6 of GlcNAc.

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Synthesis of the *B. adolescentis* EPS repeat containing cis- and trans-linked 6-deoxy-talose

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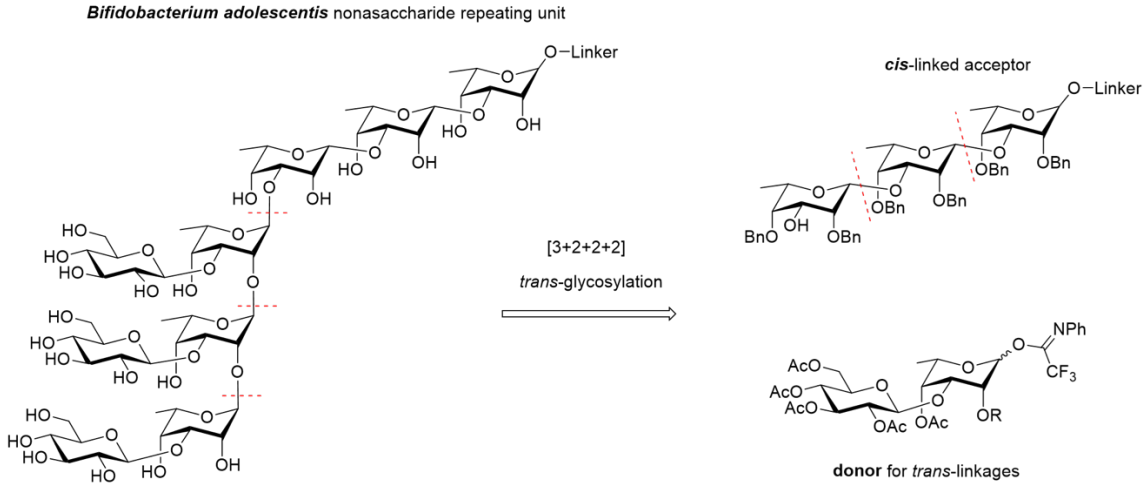
Exopolysaccharides (EPS) are present on the outside of bacteria, where they can be loosely attached to the cell wall or secreted in the environment. EPS of lactobacilli and bifidobacteria demonstrate several beneficial effects such as antitumor activity, and they serve as prebiotic or as immune modulators.[1]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

To develop a robust method to couple 6dTal units through *cis*- and *trans*-linkages, we set out to develop an efficient protecting group strategy, and performed a thorough study of the reactivity and selectivity of the resulting 6dTal donors and acceptors. The best suitable 6dTal building blocks were further applied in the successful nonasaccharide assembly.



Scheme 1. Nonasaccharide repeating unit of the B. adolescentis exopolysaccharide, and the synthetic strategy

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Dissection of sulfoglycolysis pathways in nature

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Sulfoquinovose (SQ or 6-deoxy-6-sulfoglucose) is a sulfonated sugar present within plant sulfolipids (SQ-diglyceride SQDG and its metabolites) found embedded in the thylakoid membranes of photosynthetic plants and lower organisms such as cyanobacteria. SQ locked up in SQDG is a major source of biosulfur on earth and constitutes an important arm of the biogeochemical sulphur cycle with its estimated production amounting to 10 billion tonnes annually [1]. In Nature, dedicated SQDG degradation pathways exist that enable certain microorganisms to utilize this sulfoglucose as energy molecule, which were found to be analogous to the classical glycolytic Embden-Meyerhof-Parnas (EMP) and Entner-Duodoroff (ED) pathways [2-3], however the structural and biochemical characterization of core enzymes involved in these pathways is lacking. Here we present, structural studies of sulfoglycolysis enzymes, in complex with their proposed intermediates, as well as the kinetic studies to shed light on their mechanisms, the determinants of sulfo-sugar specificity and their selectivity over glycolysis intermediates [4-7]. The sulfonate recognition sequences and motifs thus identified will inform our search for sulfoglycolysis pathways in different environmental niches.

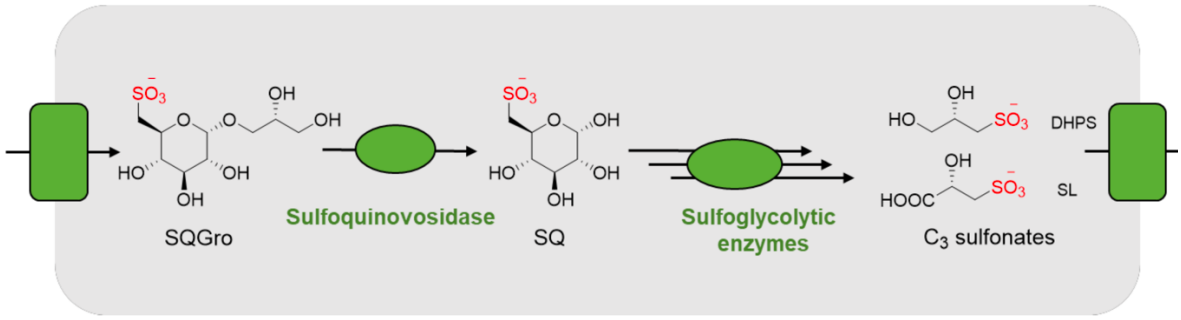

Figure 1. Schematic summary of sulfoglycolytic pathways prevalent in Nature.

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Synthesis of the matriglycan, -3Xylα1-3GlcAβ1- oligomer and its interaction with laminin

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Matriglycan, a polysaccharide that is fundamental as a part of core M3 *O*-mannosyl glycan (OMG) composed of the repeating disaccharide -3Xylα1-3GlcAβ1-, interacts with laminin to stabilize muscle tissue (Fig. 1) [1]. Defects in the genes encoding processing enzymes associated with the formation of the core M3 OMG cause a form of muscular dystrophy. For example, FKTN, one of the processing enzymes to form OMG is inactive in patients of fukuyama congenital muscular dystrophy. Supply of the synthesized matriglycan from outside of the tissue may improve the conditions of muscular dystrophy. Recently, Boons and his co-workers reported the chemo-enzymatic synthesis of the matriglycan oligosaccharides [2]. We herein report the chemical synthesis of matriglycan-repeating oligosaccharides equipped with an alkyne linker to form glycoconjugates [3]. The key step in the formation of an α-linked xylosyl glycoside was resolved by solvent-specific separation from an anomeric mixture. Successful glycan elongation was regio- and stereoselectively executed to obtain $(\text{-3Xyl}\alpha\text{1-3GlcA}\beta\text{1})_n\text{-O}(C_2H_4O)_3CH_2C{\equiv}CH$ and the biotin conjugate. We investigated interactions between matriglycan oligosaccharides and laminin-G-like domains 4 and 5 of laminin-α2 using saturation transfer difference-NMR. The dissociation constant obtained from bio-layer interferometry was estimated to be 7.5×10^{-8} M in case of hexasaccharide. We also modified the matriglycan-conjugate to effectively interact with laminin. These results indicate that a chemical approach may be applied to the reconstruction of muscle tissue.

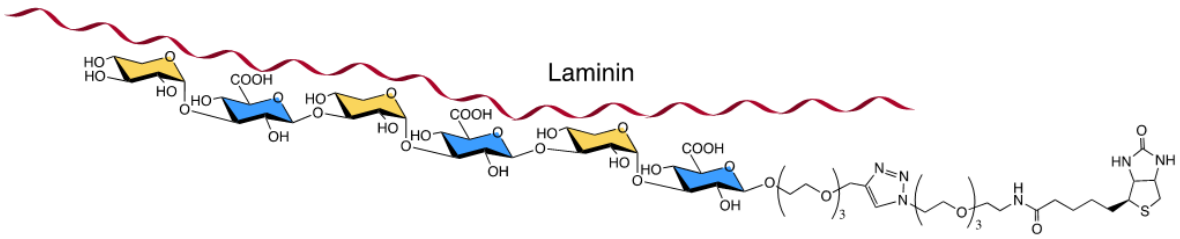

Fig. 1. Biotin-conjugated matriglycan hexasaccharide which interacts with laminin

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Structural and mechanistic characterization of heparan sulfate *N*-deacetylase-*N*-sulfotransferase 1

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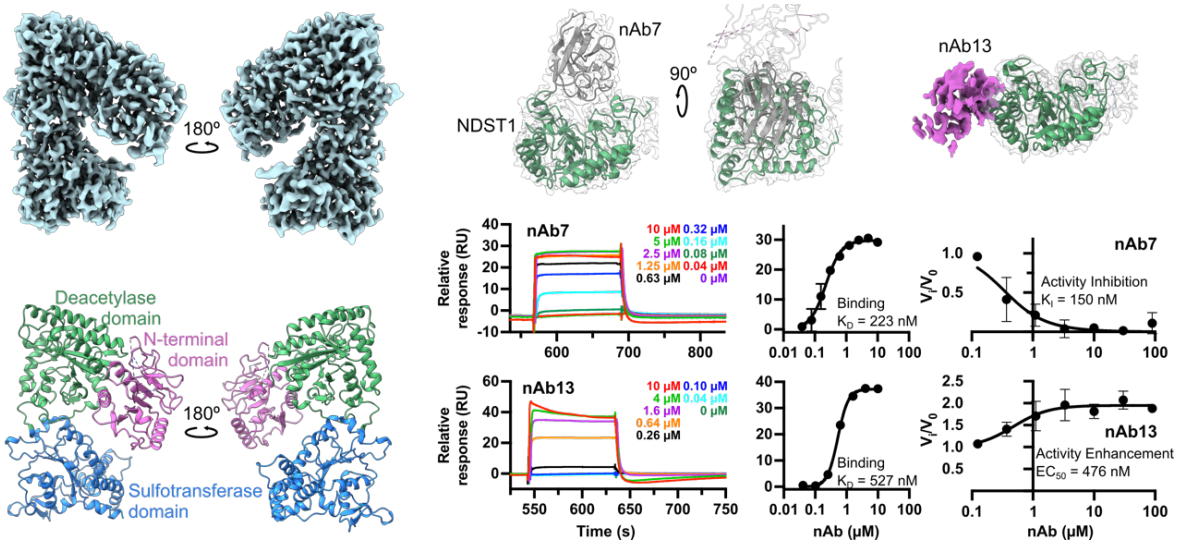
Mammalian heparan sulfate (HS) biosynthesis is a complex non-templated process, mediated by multiple enzyme activities in the Golgi complex (polymerases, sulfotransferases, epimerase etc). The interplay between enzymes and substrates involved in HS construction creates diverse polysaccharide sequences, which are essential for key biological processes, including cell adhesion, cytokine signalling and host-pathogen binding.

We have recently investigated the structure and function of heparan sulfate N-deacetylase-N-sulfotransferase (NDST)1, the first enzyme that acts on nascent HS after its polymerization. NDST1 possesses bifunctional deacetylase and sulfotransferase activity and converts N-acetyl-glucosamines in HS to N-sulfo-glucosamines. Whilst a crystal structure of the NDST1 sulfotransferase domain was reported in 1999, the molecular details of the NDST1 deacetylase domain, and how it works alongside the sulfotransferase domain, remain unknown.

Here, we report cryo-EM structures of full length bifunctional NDST1. Our structures show an unusual back-to-back arrangement of the enzyme domains, which imposes strong steric constraints on functional cooperativity. Aided by novel activity modulating nanobodies, we also carried out biochemical and biophysical analysis of NDST1 function. Our results suggest non-catalytic binding must operate alongside catalytic turnover to mediate cooperativity in the bifunctional enzyme. These data shed light on the molecular basis of function



Fluorinated natural epitope Man9 as chemical probe for DC-SIGN molecular recognition studies

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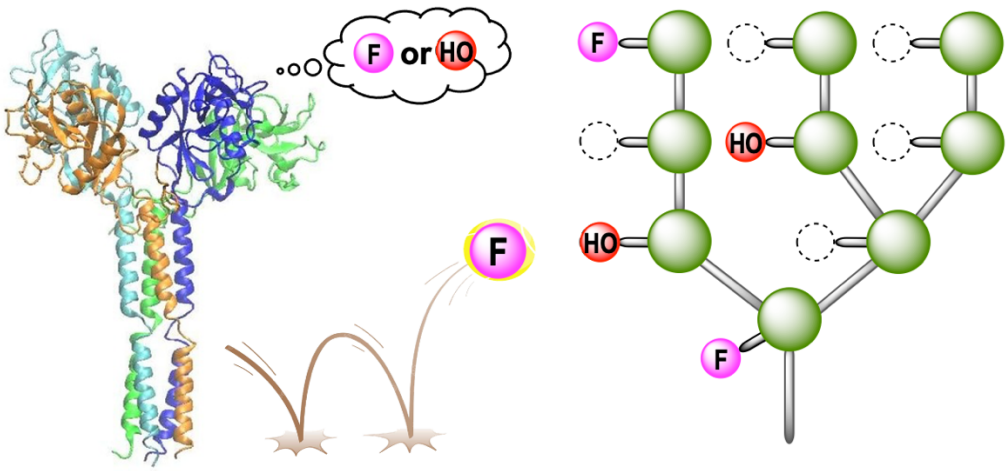
DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) is a C-type lectin that plays a key role in many biological events (including viral infections, cancer, inflammation, etc.). The main carbohydrate ligand recognized by DC-SIGN is the high-mannose glycan, $(Man)_9(GlcNAc)_2$, with the mannosyl nonasaccharide $(Man)_9$ as the epitope to interact with this receptor.[1]

Recently, our research group reported a convergent, fast, straightforward, high yield and large amount accessible synthesis of Man_9 epitope.[2] In spite of this advance, the complexity of Man_9 itself has impeded a deep analysis of its interaction with DC-SIGN. In this sense, fluorination of carbohydrates is becoming one of the most used strategies in glycoscience to investigate protein-carbohydrate interactions at the molecular level owing to favourable NMR properties of the ^{19}F nucleus.[3]

Herein, we proposed the convergent, and straightforward synthesis of fluorinated Man_9 as chemical probe for DC-SIGN molecular recognition studies. In particular, using ^{19}F STD NMR experiments, we could gain insights at molecular level of the binding of the natural epitope to the carbohydrate recognition domains of DC-SIGN.



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Thermodynamic stabilization of conformations in Lewis Antigens

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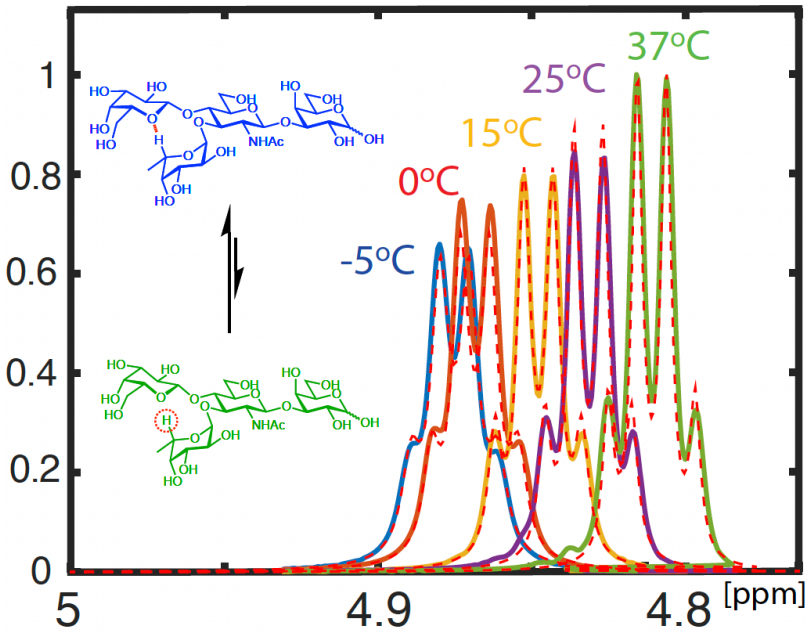
The Lewis antigens are a well-known family of fucosylated glycans whose structures were thought to be conformationally inflexible, until recently. In this presentation, we present evidence for conformational flexibility between hydrogen bonded conformations and non-hydrogen bonded ones. We show here that the formation of a C-H→O non-conventional hydrogen bond from the H5 of fucose III or II to the pyranose oxygen of galactose II or III, respectively, in Lewis A, Lewis B, Lewis X and Lewis Y, partially stabilizes a "compact structure" of these antigens. This creates NMR spectral conditions that allowed us to analyze Lewis antigen spectra by using the partial stabilization in NMR lineshape analysis. We analyzed temperature dependent spectra in the aggregate together with slow-exchange chemical shifts obtained from fucose monomer. These analyses led us to determine that the ΔG^{o} values for the hydrogen bonded conformers in the Lewis Antigens studied here, range from -1.5 to -1.0 kcal/mol. Lineshape analysis also yielded rate constants which we used to determine the free energy barriers to breaking these hydrogen bonds.

In Lewis Antigen analogs where a rhamnose residue replaces the fucose, the ΔG^{o} values are comparable to those containing a fucose, suggesting that this type of non-conventional hydrogen bond is general and may be used in design of vaccines or drugs to stabilize or destabilize desired conformations.



Molecular basis for mucin O-glycan recognition by human gut microbiota

Angelina S. PALMA [1,2,4] Viviana G. CORREIA [1,2], Raquel L. COSTA [1,2], Filipa TROVÃO [1,2], Benedita A. PINHEIRO [1,2], Alicia CANDEIAS [1,2], Sandra BEHREN [3], Antonio DI MAIO [4], Ulrika WESTERLIND [3], Ten FEIZI [4], Wengang CHAI [4], Yan LIU [4], Ana Luísa CARVALHO [1,2]

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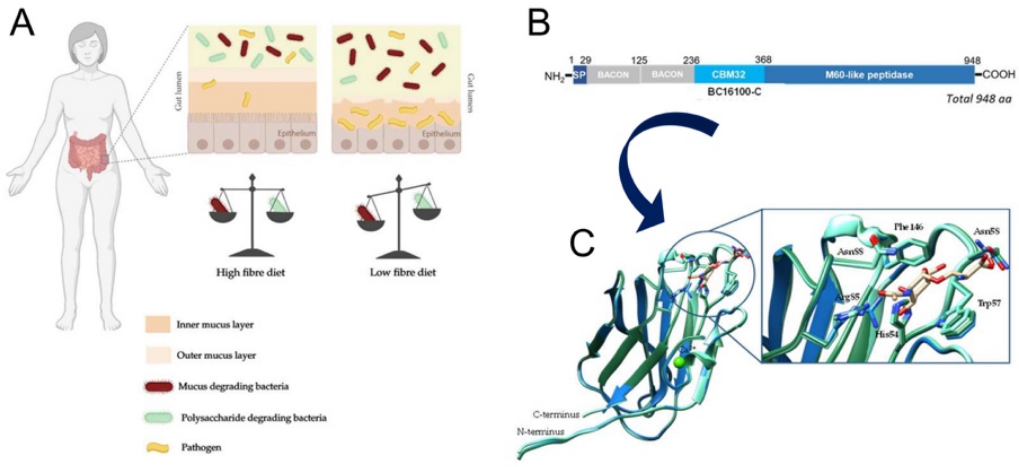
Mucins are extensively *O*-glycosylated proteins, present in the mucus layer of the intestinal epithelial cells [1]. How mucin O-glycans are differentially exploited by intestinal commensal or pathogenic bacteria and influence the crosstalk with the human host largely remains to be elucidated at the molecular level. *Bacteroides thetaiotaomicron* and *B. caccae* are prominent commensal bacteria with increased activity on mucin *O*-glycans in conditions of a low-fiber diet and are implicated in susceptibility to infection [2,3]. In this communication, we will report the characterization of newly identified proteins (glycan-binding proteins and enzymes) from these bacteria, which are encoded by sets of co-regulated genes termed polysaccharide utilization loci systems (PULs) that target a specific glycan structure [2]. We combined i) bioinformatic analysis of bacterial genomes and high-throughput production of putative glycan binding proteins with ii) ligand discovery using microarrays of human mucin-type glycoproteins, glycopeptides and sequence-defined glycans [4-7], and iii) structural characterization of protein-glycan complexes by X-ray crystallography [8]. We will highlight the molecular basis for the unique specificities of proteins targeting mucin *O*-GalNAc-Thr/Ser cores and fucosylated Lewis A structures. Uncovering the molecular determinants for mucin O-glycan recognition by the bacterial proteins can be used to understand the role of commensal bacteria in gut health and to design new therapeutic and diagnostic strategies.



A) Impact of diet on gut microbiota; B) Molecular architecture of B. caccae PUL-53; C) Ribbon representation of BC16100-C in complex with GalNAcα-Ser.

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Synthesis of dimeric Lewis X fragments and mapping of mAbs SH2 and 1G5F6

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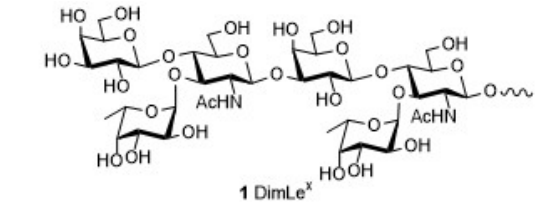
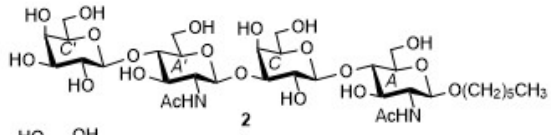
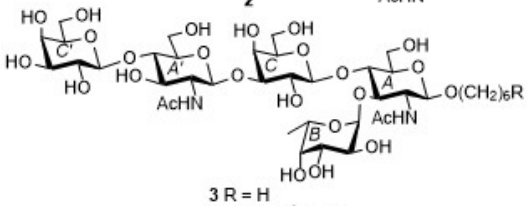
Aberrant glycosylation of tumor cell surface oligosaccharides is a universal hallmark of cancer pathogenesis. Indeed, several clinical and preclinical studies have shown that antibodies raised in response to tumor-associated carbohydrate antigens (TACAs) can eliminate tumor cells. One such TACA of interest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



1 DimLe^x



2



3 R = H
4 R = NH_3^+, AcO^-

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Adventures in anomeric reactivity

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Glycosylation reactions constitute a class of reactions that fuse separate sugar units forming glycosides, oligosaccharides and ultimately highly complex polysaccharides via glycosidic bonds. The configuration of the glycosidic linkages determine saccharides' shape and thus the properties including their biological function to a great degree. Efficient access to structurally defined saccharides or derivatives thereof is highly desired in order to study their properties and effects in different fields of research. The glycochemistry synthetic toolbox has a rich history of successful syntheses of highly complex target structures, yet many of these strategies depend on laborious protecting group modifications.

The question that we sought to answer was whether a novel reagent-based approach borrowing aspects of different modes of glycosylation would be feasible. With a broad potential substrate scope in mind, we envisioned a novel auxiliary-mediated glycosylation, which relies on spatial tethering of donor and acceptor. The tethered linker structures do not only provide spatial orientation to enhance diastereoselectivity, but should also simultaneously serve to activate both donor and acceptor in the presence of a suitable promoter.

Au-catalysed stereoselective synthesis of deoxyglycoside analogues as diagnostic probes

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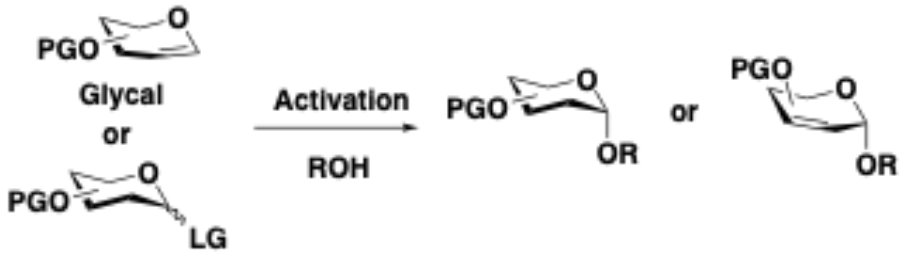
The stereoselective synthesis of glycosides remains one of the biggest challenges in carbohydrate chemistry.1 The chemical synthesis of complex carbohydrates generally involves the coupling of a fully protected glycosyl donor bearing a leaving group at its anomeric centre, with a suitably protected glycosyl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

In recent years, our group has endeavoured to develop catalytic and stereoselective methods to address this important synthetic challenge.2 Recent years have seen a steady increase in the application of transition metal catalysis applied to oligosaccharide synthesis,3 since the reaction conditions are mild and the careful choice of catalyst can offer significant improvements over traditional methods in terms of atom economy, high yields and control of anomeric selectivity.

Herein, we will report the latest applications of Au-catalysis for the a,a-stereoselective synthesis of trehalose derivates and their application as probes of microbial detection.4



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Interrupted Pummerer reaction mediated glycosylations

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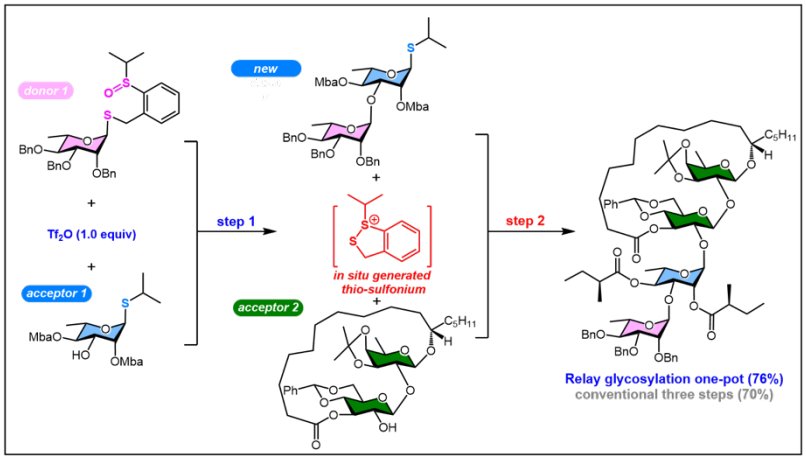
Recently, we have developed two interrupted Pummerer reaction mediated (IPRm) glycosylations. These methods are convenient and efficient in synthesizing complex glycosides due to the allowance of employing latent-active glycosylation strategy. In the IPRm glycosylations, O/S-2-(2-propylthio)benzyl (O/S-PTB) glycosides were introduced as "latent" glycosyl donors, which are quite stable under most of glycosylation and many protection/deprotection conditions.

The latent O/S-PTB glycosides can be conveniently oxidized to their "active" counterparts, O/S-2-(2-propylsulfinyl)benzyl (O/S-PSB) glycosides to perform satisfying reactivity in the glycosylation process via an interrupted Pummerer reaction mechanism. In these reactions, the anomeric S-2-(2-propylsulfinyl)benzyl (SPSB) group was activated to form a cyclic-thiosulfonium ion which was able to active thioglycosides. Based on these observations, we developed a relay glycosylation strategy for the assembly of oligosaccharides.



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Self-supported solution synthesis of oligosaccharides using thioglycosides donors

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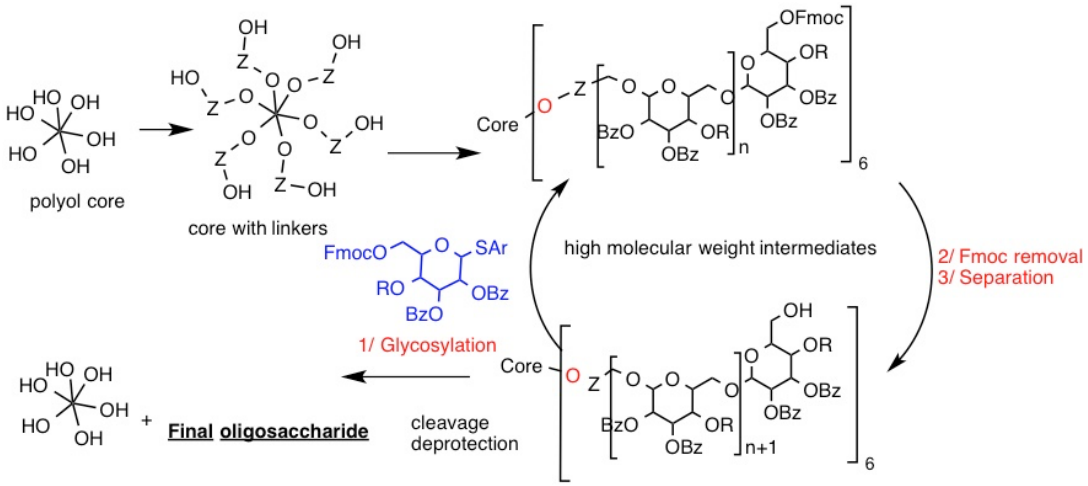
Phagocytosis of pathogens by macrophage is initiated by 3 activation pathways, antibody recognition, complement activation and the lectin pathway. In the latter case, pathogen-associated molecular patterns (PAMPs) present on the surface of the microbe bind to several macrophage lectins (mannose-binding lectin, dectins, Mincle, etc.) leading to the adhesion and internalization. In order to study this process, we recently developed glycosylated oil microdroplets as a microbial model using fluorescent mannolipids. (1). To further explore the specificity of sugars, small libraries of oligosaccharides should be prepared.

The chemical synthesis of oligosaccharides proceeds by repeating the sequence of glycosylation/deprotection steps and the solution synthesis of oligosaccharides generally requires separation and purification at each step, which is tedious and time-consuming. Solid phase alternatives have been successfully developed and are suitable for the rapid preparation of small amounts of oligosaccharides. In parallel, methods based on a soluble support have been proposed to overcome certain limitations of the solid phase approach (larger scale, use of less reactive donors, etc.). The most effective are the methods using fluorinated chains and ionic groups (2) We propose here a different strategy for the synthesis of oligosaccharides based on a multi-glycosylation of polyols. This method was exemplified by the successful preparation of a beta (1-6) tetraglucoside and a alpha(1-2) trimannoside using thioglycosyl donors. Compared to the reported syntheses, the separation here is not based on a specific marker (that transfers its solubility properties to whole molecules) but on the increase in molecular mass (from 1500 to 15000), allowing a simple separation on a steric exclusion column (Sephadex LH - 20). Moreover, the proposed protocol does not require any aqueous treatment and uses a yellow tracer to visually follow the separation without TLC or detector. (3)



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New purine nucleosides as copper chelators and cholinesterase inhibitors for Alzheimer's disease

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Alzheimer's disease (AD), despite being the most common form of dementia, is a multifactorial neurodegenerative disease that has no efficient treatment yet. Metal dyshomeostasis, especially copper dyshomeostasis, and the progressive decline of the level of the neurotransmitter acetylcholine (ACh) are two factors that contribute to the pathology, the first being related to several AD features such as the oxidative stress, the Aβ aggregation and the τ-protein hyperphosphorylation. ACh may be hydrolyzed by two enzymes: acetyl- and butyrylcholinesterase (AChE and BChE, respectively) that, in addition to this role, may form toxic complexes with Aβ and/or contribute to its aggregation and accumulation.

Nucleosides have been widely known for their therapeutic properties and the mannosylpurine nucleosides previously synthesized in our group showed a potent BChE inhibition. In this context, we now present the synthesis of new rhamnosyl- and mannosylpurine nucleosides by two different methods for the coupling of N^6-benzoyladenine with glycosyl donors. Catalysis by trimethylsilyl triflate under microwave irradiation or by iodine under conventional heating gave the target molecules with exclusive formation of the N^9 isomers with the latter methodology. Compounds' structure was confirmed by computational studies. Moreover, their metal chelation ability was evaluated, the chelation site disclosed, and the cholinesterase inhibition determined. To conclude, in this work it was possible to obtain the first nucleoside-based molecules with potential to become dual-target drugs against AD.

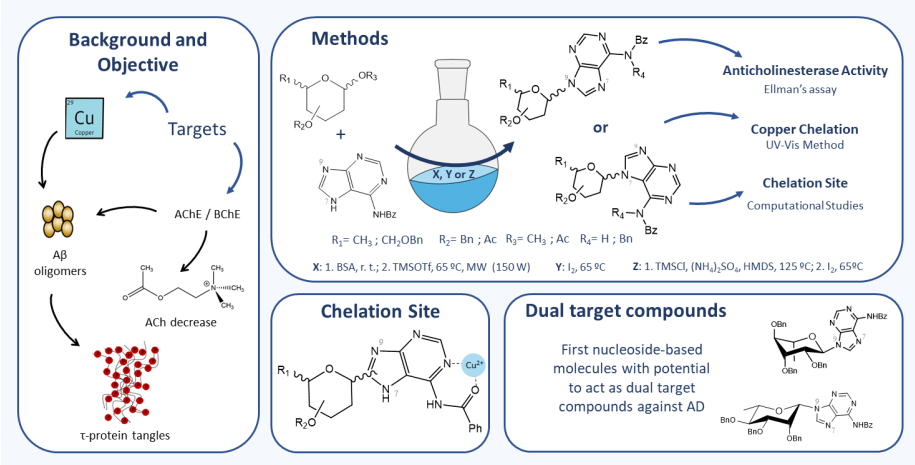

Illustration of this work's objectives, methods and results

Fundação para a Ciência e Tecnologia is acknowledged for supporting Centro de Química Estrutural through project UIDB/00100/2020 and Institute of Molecular Sciences through project LA/P/0056/2020.

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N-Glycosylation with Sulfoxide Donors for the Synthesis of Peptidonucleosides

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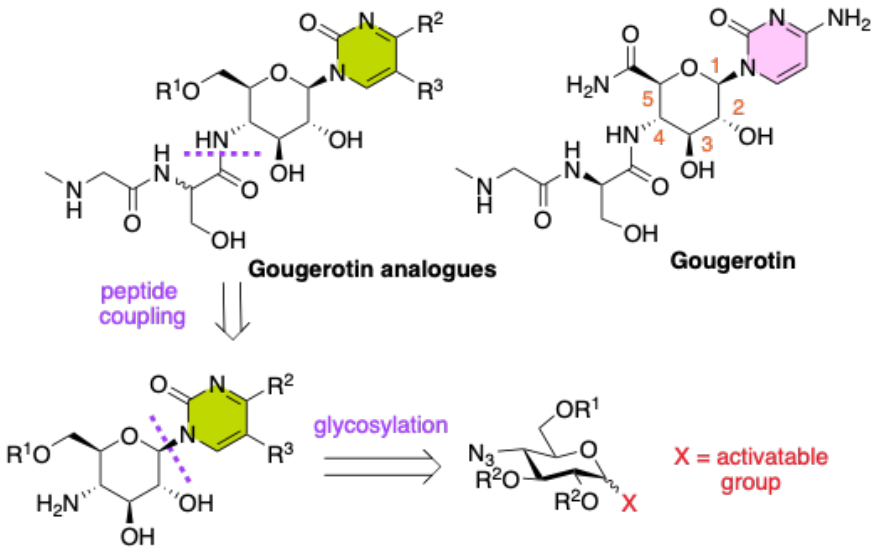
Natural products remain an important source of inspiration for the discovery of new active molecules. Among them gougerotin, isolated for the first time in 1962 from strains of *Streptomyces gougerotii*, caught the attention of researchers (1). This peptidylnucleoside has a very broad spectrum of biological activities: antiviral (2), antifungal (1), antiparasitic and antibacterial (3) by inhibiting protein synthesis in procaryotic and eucaryotic systems. It is active on several varieties of plants whether in preventive or curative tests but its phytotoxicity limits its direct use on plants (4). In order to optimize its crop specificity, we were interested in the preparation of a few gougerotin analogues.

The main modifications relate to the replacement of the natural nucleic base by other pyrimidine bases and the replacement of the carboxamide function at C5 by an hydroxymethyl group (Figure 1). To access these compounds, different pyrimidine nucleobases were glycosylated with donors carrying an azide group at the C4 position. A methodological study involving different anomeric leaving groups (acetate, phenylsulfoxide and *ortho*-hexynylbenzoate) showed that a sulfoxide donor in combination with trimethylsilyl triflate as the promoter led to the best yields (5).



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Synthetic study on plant glycosphingolipid GIPC

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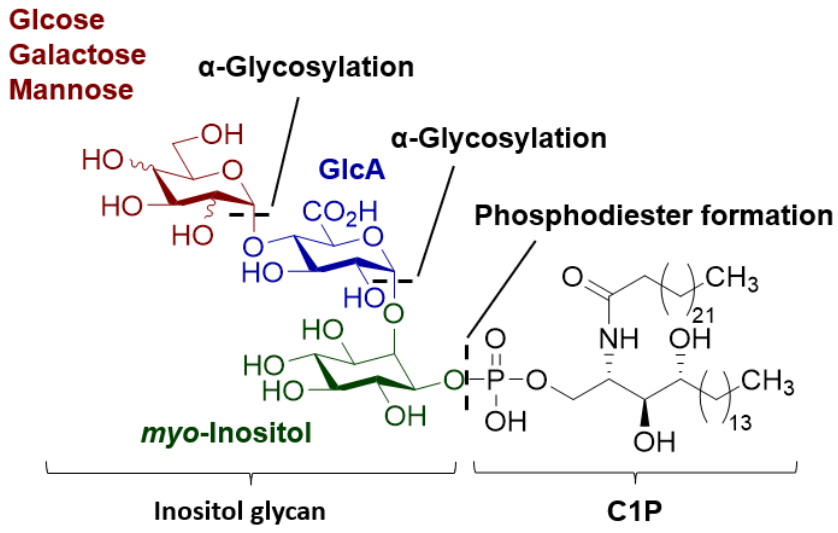
Glycosyl inositol phosphoceramides (GIPCs), consisting of an inositol glycan headgroup and a ceramide 1-phosphate tail, are the major sphingolipids in plants and present on the outer leaflet of the plasma membrane. The inositol glycan has a common structure of glucuronic acid (GlcA) linked to inositol at the 2 position with α-glycosidic bond. In contrast, the monosaccharide attached to GlcA differs depending on plant species and cell tissues. Recent studies have demonstrated that GIPCs behave as lipid raft molecules like mammalian gangliosides and play an important role in biological processes such as signal transduction [1]. For instance, GIPCs have been identified as a receptor for a toxin from the plant pathogen *Phytophthora infestans* [2] and as a salt sensor for the plasma membrane Ca^{2+} influx channel [3]. However, molecular-level insights into the relationship between their chemical structures and functions have not been intensively studied because of the difficulty in obtaining homogeneous GIPCs from nature. Chemical synthesis enables providing structurally well-defined GIPCs to elucidate their detailed functions at the molecular level. Herein, we report our synthetic efforts toward three GIPCs that differ in the monosaccharide at the non-reducing end. By utilizing temporary boronate ester protecting groups for both both glycosyl donor [4] and acceptor [5], the inositol glycan frameworks have successfully been synthesized. Currently, we have been working on the first total synthesis of GIPCs.



Chemical structure of GIPCs

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Synthesis of mannoside probes for the study of PIMs biosynthesis

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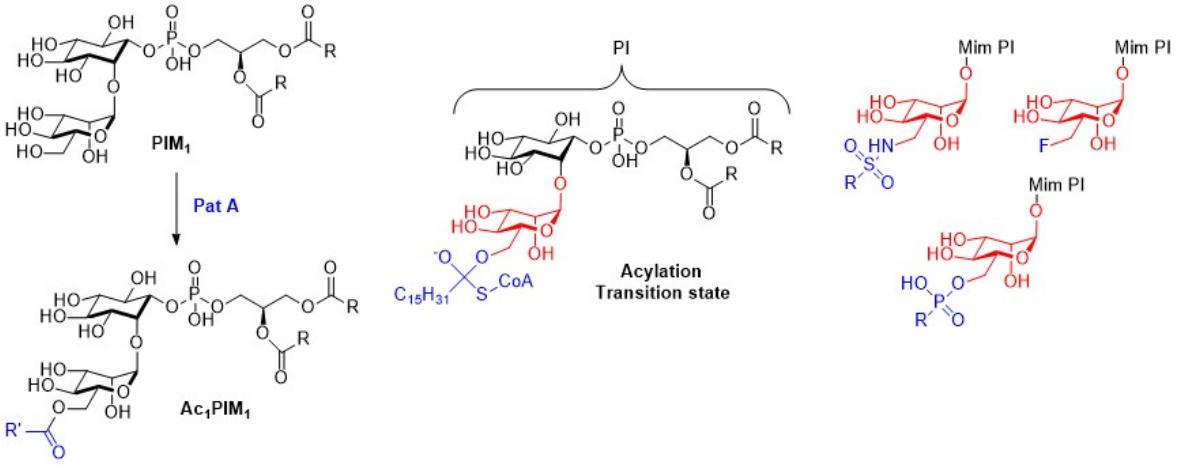
Mycobacterium tuberculosis is the second most deadly infectious agent in the world after COVID-19. Drug treatment require daily dosage of two to four drugs over six months, and compliance is poor. Most recently, there has been an alarming rise of multi-drug resistant and extensively drug resistant tuberculose (TB), making discovery of new drugs crucial. Current anti-TB drugs are targeting diverse biological processes[1] but no molecules are designed to target PIMs biosynthesis. PIMs (Phosphatidyl-*myo*-Inositol Mannosides) are the precursors of two major lipoglycans implicated in host-mycobacteria interactions. According to the currently accepted model, the biosynthesis starts with the transfer by essential mannosyltransferases PimA and PimB of a mannopyranosyl residue to the 2 and 6-position of the inositol ring of PI leading to PIM_1 and then PIM_2. The acyltransferase PatA catalyzes the transfer of a palmitoyl moiety to the 6-position of the mannose ring linked to the 2-position of inositol in PIM_1 or PIM_2, to obtain Ac_1PIM_1 or Ac_1PIM_2.[2] Docking studies of PatA gave useful information for inhibitors design.[3]

We are therefore focused on the synthesis of a panel of molecules with mannopyranosyl scaffold with the aim to develop new PatA inhibitors. Structures present different aglycones to mimic the PI part and different groups at the 6-position of mannose mimicking the acylation tetrahedral transition state. The following step will be to study the molecule/enzyme interactions, to determine the inhibitory activities and to test the best molecules on *Mycobacterium tuberculosis.*



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First total synthesis of ganglioside SJG-2 and evaluation of its neurite outgrowth activity

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SJG-2, a ganglioside found from the sea cucumber *Stichopus japonicus*, exhibits the most potent neurite outgrowth activity toward PC12 cells among gangliosides ever evaluated [1,2]. SJG-2 displays a unique sialic acid-congested glycan structure, which poses a highest-level challenge in the chemical synthesis (Fig. 1). In this study, we report the first total synthesis of ganglioside SJG-2. In the chemical synthesis of SJG-2, the most challenging subject is to install three sialic acids (Neus) at the vicinal 3,4-diol and the anomeric position of the outer Gal residue. First, to install Neus to the 3,4-diol, we have developed a Neuα(2,3)Gal with a free hydroxyl group at the C4 of Gal as a glycosyl acceptor. We found that the conformational fixation of the Neu residue by 1,5-lactamization and cyclic protection boosted the reactivity of the C4-OH of the Gal. By glycosylation using a macrobicyclic sialyl donor developed by our group [3], the disialyl Gal was produced in excellent yield with perfect α-selectivity. Next, the disialyl Gal was successfully combined at the C8-OH of 1,5-lactamized Neu residue in a trisaccharide acceptor, producing a sialic acid-congested hexasaccharide. The hexasaccharide was converted into a glycosyl donor via 13 steps, which was then coupled with Glc-Cer cassette to afford the ganglioside framework. Finally, global deprotection delivered ganglioside SJG-2. The synthesized SJG-2 and its glycan part were subjected to biological evaluation, which revealed that both SJG-2 and the glycan part promoted neurite extension toward primary neurons.

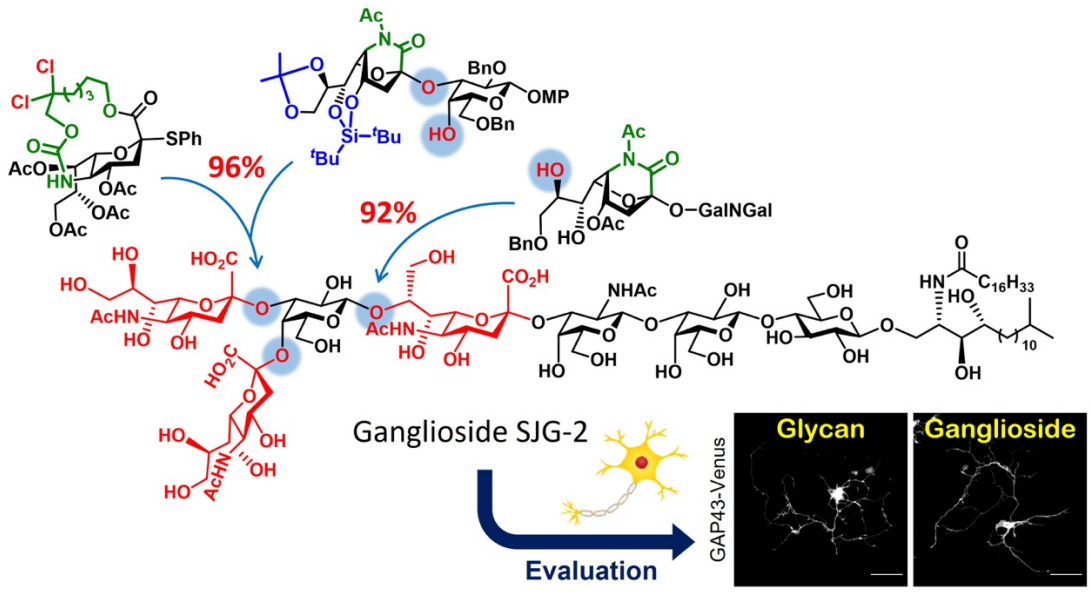

Figure 1. Chemical synthesis of ganglioside SJG-2 and evaluation of its neurite outgrowth activity

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Synthesis and immunomodulatory functions of microbial and endogenous glycoconjugates

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Various glycoconjugates of cell membrane activate the immune system via interaction with host's innate immune receptors, lipid antigen presenting proteins, and antibodies. We have established the synthetic methods for glycolipid compound library with structural variation of lipid moiety, including glycosylphosphatidylinositol (GPI) and sphingoglycolipids (SGL).

Based on our previously developed methods of GPI related structures [1], we performed the synthesis of mannosyl inositol phospholipids such as Ac_1PIM_1, a potential biosynthetic intermediate for phosphatidylinositol mannosides (PIMs) from *Mycobacterium tuberculosis*, and then demonstrated that the compounds is the key entity of a DCAR (a member of C-type lectin receptors) agonist among PIM molecules [2]. As for the SGLs, based on our previous convergent synthesis of GalCer derivatives [3], we have synthesized various related SGL structures, and analyzed the immunomodulation via Mincle and also via lipid antigen presentation.

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Lipopolysaccharides from gut microbiota: is it time for a paradigm shift?

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Gut Microbiota is crucial in the immune response modulation. Perception of microbial-associated molecular patterns, such as lipopolysaccharides (LPS), represents the first line of the host immune defense.[1] Due to their chemical structure, LPS are considered potent elicitors of immune inflammatory reactions, being usually associated to perilous bacteria and detrimental outcomes for human health.[2] Nevertheless, LPS also decorate the membrane of harmless Gram-negatives composing our gut microbiota. How LPS is tolerated and remains (apparently) silent in the gut is a major unsolved question representing a frontier in our understanding of innate immunity.

Deciphering the structure and immunological properties of LPS from "good" gut microbes is of paramount importance, with tremendous repercussions for basic and clinical domains of biomedicine. A detailed structure to function study of LPS from gut microbiota will give insights in the mechanisms at the basis of host-microbes crosstalk. This will provide priceless information about how gut microbiota modulate immune response through their LPS, thus resulting in an unprecedented improvement of the knowledge of the immune system.[2]

In this communication, I will show some recent results about the chemical structure and immunological properties of LPS from some beneficial gut bacteria that displayed unique features. I will show the potential of these glycomolecules in the perspective of a future design of novel inflammation-silencing drugs as an alternative therapeutic approach for the treatment of immune inflammatory disorders.

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Biomimetic LPS nanoparticles for interaction studies at the surface of Gram-negative bacteria

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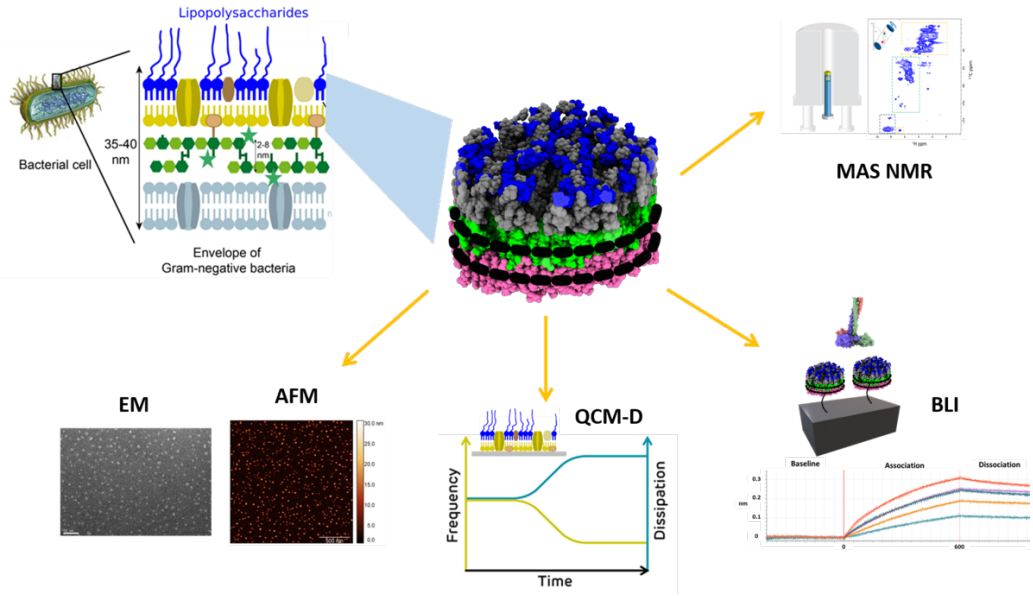
The envelope of Gram-negative bacteria is decorated with Lipopolysaccharides (LPSs) representing the main lipid component of its surface. They form an impermeable barrier and are key in antimicrobial resistance and virulence[1]. Ample studies have been conducted on LPSs' chemistry and biology, however, their interactions ruling the immune response modulation and antimicrobial resistance are not fully understood. In this context, there is a clear need for new methodologies to study intact LPS in a membrane-like environment. We have exploited the amphipathic copolymer nanodiscs technology[2] to form nanodiscs from purified LPSs or directly from bacterial outer membranes of pathogenic and non-pathogenic strains. This cell surface mimetic model was studied and validated by several biophysical methods; LPS nanodiscs can be obtained from a wide range of *E. coli* strains and form stable objects. Their size distribution and thickness were assessed by Dynamic Light Scattering and Atomic Force Microscopy. The different components of bacterial outer membranes can be observed at atomic scale, including phospholipids and the different LPS moieties, by solid-state NMR. LPS nanodiscs were also successfully used to monitor interactions with immunity C-type lectins and antibiotics by Quartz Crystal Microbalance and BioLayer Interferometry. LPS nanodiscs constitute a promising approach for the study of structure and interactions of LPSs. They could serve as an important tool in biomedical applications[3] and be used for high-throughput screening of gram-negative outer membrane binding profiles.



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The glyco-barcode of micro-algae

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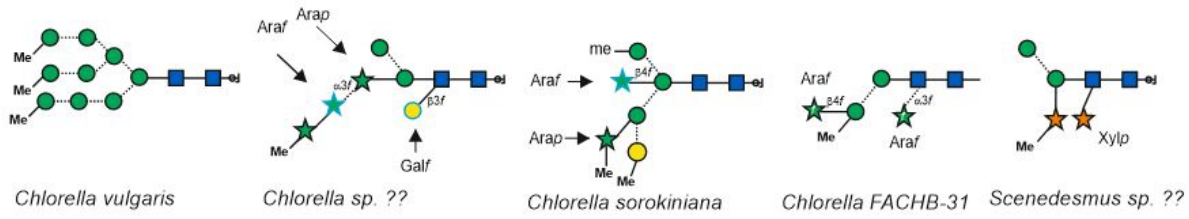
All land plants, more exactly, all tracheal plants, come to do with a highly conserved set of N-glycan features that is found in ferns, needle trees, grasses, fruits and flowers. Only two of the few glyco-genes required for decoration of complex-type N-glycans are unique to plants. In striking contrast, (micro)-algae present a fascinating variety of N-glycan structures – as if they wanted to compensate for their often inconspicuous appearance. A dozen or more of unique structure patterns occur in commercial samples of *Chlorella*-clade algae, many more unique structures in members of the geni *Scenedesmus, Acutodesmus, Desmodesmus* and the well-known *Chlamydomonas* accompanied by again unique patterns and in the red alga *Porphyromonas sp.*. The example of the *Chlorella*-clade indicates that glycan patterns provide a much clearer distinction of strains or species than the inherently continuous changes of DNA sequence. The data to be shown was initially acquired by MALDI-TOF MS. In several cases, LC-ESI-MS with porous graphitic carbon (PGC) as the stationary phase allowed further distinction of isobaric N-glycans. While full structural elucidation of this ever-growing number of novel glycans is elusive, MALDI-TOF MS collection with retention-time normalized PGC chromatography data appears as a promising tool for unambiguous strain characterization. This is a necessity for novel food regulations, technological research and last but not least, consistent taxonomy.



Examples of N-glycan structures from Chlorella-like microalgae

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LEctPROFILE® kits, a relevant tools to study glycosylation pattern of glyco-molecules

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Glycosylation is one of the most important post-translational modifications of proteins: it affects folding, stability, molecular recognition and functionality. According to the regulatory agencies, glycosylation is considered as a critical quality attribute (CQA) regarding the safety and functionality of a biotherapeutic. Indeed, presence of Galα1,3Gal motif and/or NeuGc coming from production in non-human mammalian cell lines can make therapeutic glycoprotein unsafe, triggering an immune response from the human organism. Moreover, it's well established, that glycosylation alterations occur in a large range of diseases such as cancer.

In this context, the understanding, the identification andthe monitoring of glycans signatures of glycoproteins is quite relevant. We have therefore developed dedicated lectin arrays based on the GLYcoDiag's GLYcoPROFILE® technology. Indeed, a relevant selection of lectins canintended either for: 1)detection of desirable or undesirable glycans during the early stages of pre-clinical development but also clinical and QC of biotherapeutics, 2) identification of glyco-biomarkers related to cells behaviour and/or pathology[1], 3) analyse and characterize the glycosylation level of glycoproteins contained in biological fluids[2] or target the sialylation status of relevant serum biomarkers in relationship with 4) research of antagonists for lectins of interest well-known to be involved in particular disorder[3] or in biological mechanisms.

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Siglec-8 complex structures with a therapeutic antibody and a high-affinity sialoside analog

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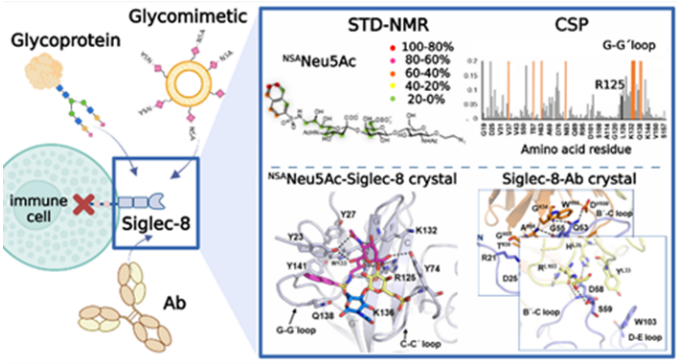
Siglec-8 is an inhibitory receptor that induces eosinophil apoptosis and inhibits mast cell degranulation when bound by monoclonal antibodies (mAbs) or sialylated ligands. Consequently, Siglec-8 has emerged as a crucial negative regulator of inflammatory responses in various diseases, including allergic airway inflammation [1]. Herein, the molecular recognition features of the interaction of Siglec-8 with the monoclonal antibody (mAb) lirentelimab (2C4) and a sialoside mimic with the potential to reduce mast cell degranulation have been deciphered [2]. The X-ray crystallographic solution of the structure of Siglec-8 and the fragment antigen-binding (Fab) component of 2C4 shows that the mAb binds close to the carbohydrate recognition domain on Siglec-8. Additionally, the STD-NMR experiment demonstrates the inhibition of the binding between Siglec-8 and natural ligand in the presence of 2C4. Moreover, using a combination of NMR spectroscopy and X-ray crystallography, we have also deduced the binding mechanism of a high-affinity analog of its sialic acid ligand (9-N-napthylsufonimide-Neu5Ac, ^{NSA}NeuAc). Our data demonstrate that ^{NSA}NeuAc's sialoside ring binds to the classic sialyl binding pocket of the Siglec receptor family, with the high affinity resulting from the accommodation of the NSA aromatic group in a contiguous hydrophobic patch provided by the N-terminal tail and the unique G-G' loop (Figure 1). These results provide pointers for the rational design of the next generation of Siglec-8 inhibitors and explain the foundation for this ligand's observed high affinity [2].



The binding of a sialic acid mimetic with a 9-N aromatic substituent, and antibody with therapeutic potential to Siglec-8 are revealed by a synergic c

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Shining a light on bacteria: lanthanide-based glycoconjugate molecular sensors for lectins

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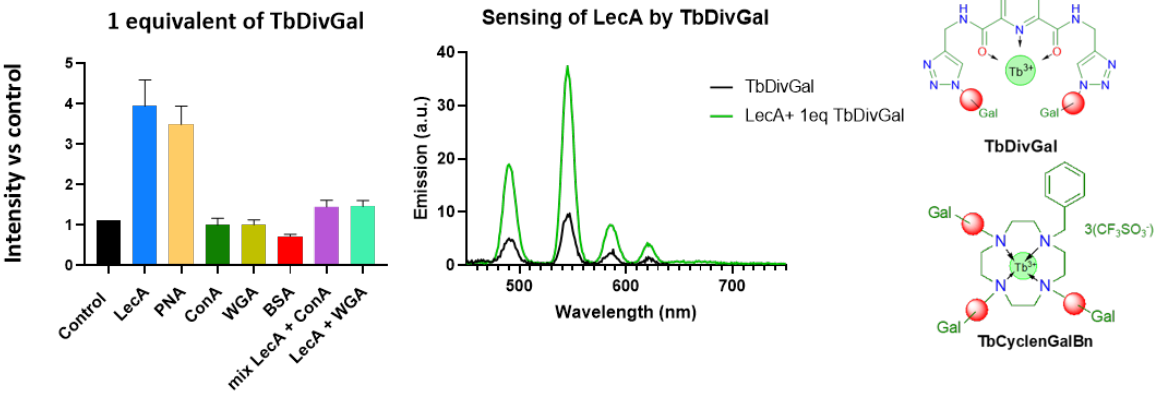
Lanthanide probes are advantageous for sensing applications due to their characteristic and time-resolved emission spectra, which can be easily distinguished from background fluorescence of biological samples.[1] Many pathogenic bacteria such as *Pseudomonas aeruginosa* (PA) produce lectins which present viable targets for detection, as well as for new therapies. Diagnostic methods for bacterial infections rely on cell culture, leading to delays in targeted treatment. New detection methods are needed in the fight against antimicrobial resistance. This project aims to use the selective nature of carbohydrate-lectin interactions to develop visually responsive glycoconjugate probes, which would be suitable for diagnosis of infections, such as by PA, a bacterium classified as a Priority 1 pathogen by the WHO.[2] LecA and LecB are lectins on the surface of PA with high affinity for galactoside and fucoside glycans respectively.[3] Many approaches have been developed to inhibit the binding of these lectins to human tissue cells by the development of inhibitory glycoconjugates with varying degrees of success.[4] Here we report two generations of multivalent glycoconjugate lanthanide complexes, featuring different architectures, which demonstrate enhanced emission in the presence of relevant lectins as divalent and tetravalent systems. Integration of these systems into smart materials has potential for application in the medical devices industry. We also present a series of transition-metal glycoconjugates which have been tested for biological activity against PA and *Candida albicans*.



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Molecular details of host glycans recognition from *M. genitalium* and *M. pneumoniae* cytoadhesins

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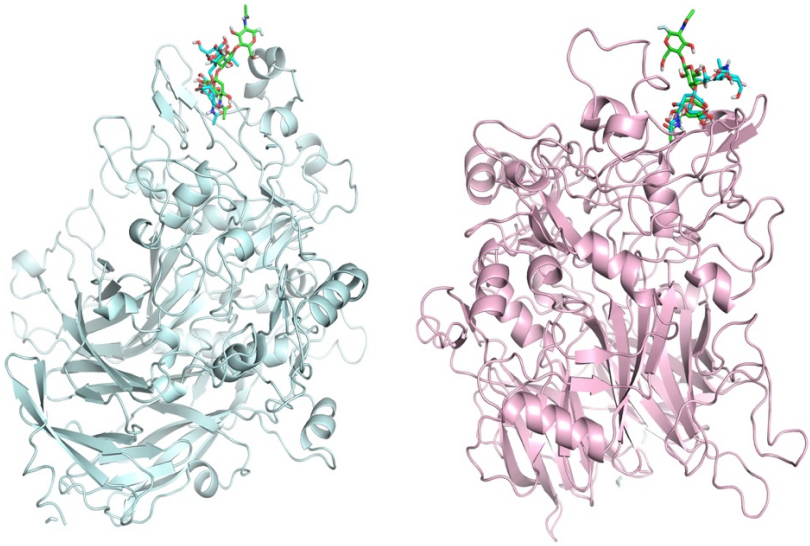
Mycoplasma pneumoniae (*Mpn*) and *Mycoplasma genitalium* (*Mge*) are two closely related human pathogens associated with respiratory tract and urogenital infections, respectively. *Mpn* is responsible for up to 40% of community-acquired pneumonias in persons of all ages [1]; *Mge* is instead implicated in several urogenital pathologies such as urethritis in men and cervicitis and pelvic inflammatory disease in women [2]. Additionally, this sexually transmitted bacterium has been associated with preterm birth, spontaneous abortion and HIV acquisition. Both pathogens express cytoadhesins that mediate the attachment to host sialylated glycans, favoring the bacterial infection. Remarkably, in contrast to other important respiratory pathogens, a vaccine for *Mpn* is not yet available. Moreover, the rapid emergence of antibiotic resistance documented for both patoghens, Mpn and Mge, emphasizes the urgency for the development of alternative therapeutic strategies [3]. In this perspective, a deep knowledge of the molecular details of host glycans recognition by bacterial cytoadhesins is strongly needed. Thus, in this project, we used NMR techniques [4] and computational methods to establish the molecular basis for sialoglycans recognition and ligand specificity of both Mpn and Mge cytoadhesins. The information obtained provided the 3D features of the complexes, determining the preferred epitopes recognized by each cytoadhesin and could be used to identify competitive binding inhibitors



3D complexes of ctytoadhesins from Mpn (left) and from Mge (right) interacting with host sialylated glycans (3'SLN in green and 6'SLN in cyan).

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Akkermansia muciniphila sialidases' roles in growth on mucin and nutrition sharing in the human gut

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Akkermansia muciniphila is a core human gut microbiome member, strongly associated with host metabolic health[1]. Despite being a mucolytic specialist, *A. muciniphila* contributes to a balanced mucin turnover in healthy humans. In the lower gut, mucin *O*-glycans are heavily capped with fucose, sialic acid and sulphate units. The capping confers resistance to microbial attack and provides nutrients and adhesion site for distinct bacteria adapted to the mucus biogeography. Despite the relevance of mucin glycosylation in host-microbe symbiosis/pathogenesis, the initial steps of mucin glycan breakdown by *A. muciniphila* remain unexplored. Here, we described the molecular preferences of *A. muciniphila* sialidases on mucin-conjugated and free *O*-glycans including those from colonic mucins. While GH33 sialidases displayed broad, albeit variable, preferences to different sialyl-motifs, we discovered the defining member of a novel inverting sialidase family with strict *O*-glycan specificity to the sialyl-T antigen. This enzyme exhibited unique structural features consistent with its strict specificity. Finally, we showed that the sialic acid released by *A. muciniphila* sialidases was not utilized, but shared with other health-beneficial mucus-associated bacteria *in vitro*. These findings bring novel insights into the initiation of mucin *O*-glycan degradation by *A. muciniphila* and the contribution of sialidases to syntrophy with the mucus-associated microbial community.

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Flash Lectures and Poster Presentations

Targeting Siglec10-CD24 in feline mammary carcinoma – a glimpse of a new immunotherapy approach

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Immunotherapies have revolutionized the fight against cancer yet response rates are still low, dictating the need for new therapeutic targets. The interaction of tumoral sialosides with immune cell sialic-acid-binding Ig-like lectins (Siglecs) has emerged as a key immune response modulator. Recently, the Siglec10-CD24 axis was proposed as a major immune-checkpoint in human breast cancer [1]. Given the similarities between this cancer and feline mammary carcinoma (FMC) we sought to describe this axis in FMC and develop a new immunotherapy based on the targeted desialylation of Siglec-10 ligands.

CD24 and Siglec10 expression was studied by IHC on tumoral samples from FMC patients, revealing marked Siglec10 and CD24 staining on TAMs and poorly differentiated neoplastic cells, respectively. A panel of putative novel sialidases was designed, expressed in *E. coli* and purified. Enzyme activity was tested using a fluorogenic substrate. Desialylation of mammary carcinoma cell lines with selected sialidases was analyzed by flow cytometry. A total of 74 proteins were identified and characterized, 10 of them with high expression yield and sialolytic activity. The enzymes' ability to remove MALII and SNA ligands were tested in cell lines, revealing their potential to degrade α2-3 and α2-6 linked sialic acids.

The present results suggest the presence of a Siglec10-CD24 axis in FMC and identified a set of sialidases with ability to degrade sialosides. In the future, these enzymes will be tested as part of an antibody-sialidase conjugate to assess their potential anti-tumoral effect.

Acknowledgements

We acknowledge FCT, through the grants: UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); and 2022.07903.PTDC. We also acknowledge ANI through the grant LISBOA-01-0247-FEDER-047033 (GlycoMed) and the Gilead GÉNESE program through the project 17805. Alexandra Couto Oliveira is funded by an individual PhD scholarship from FCT (UI/BD/153068/2022)

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Synthesis of fluorinated oligomannosides for DC-SIGN recognition

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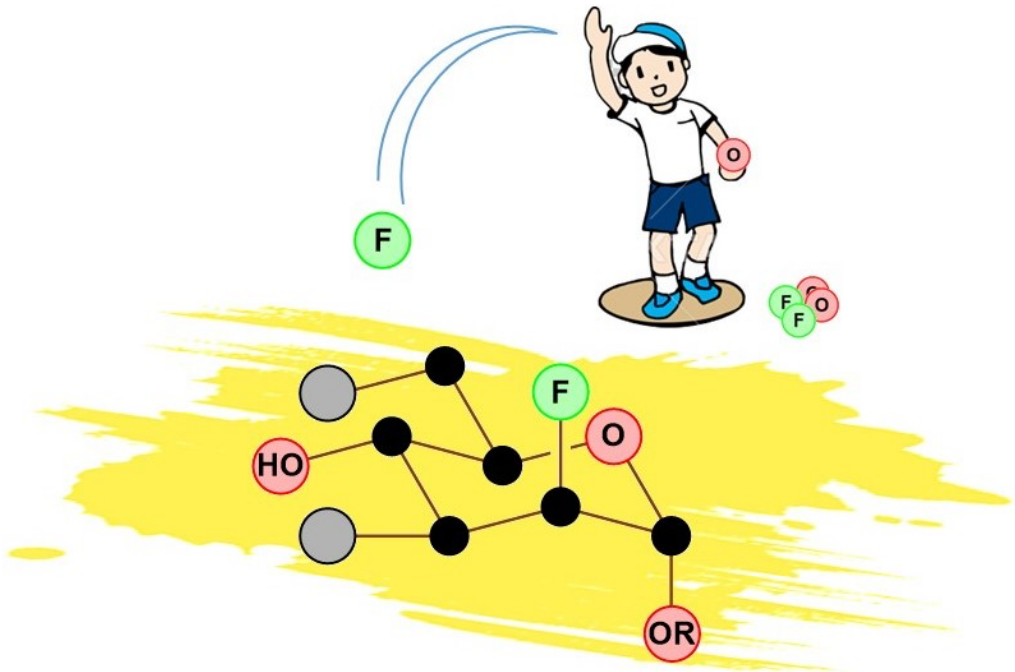
Addressing fluorinated carbohydrates has become a priority in glycoscience.[1] Fluorination provides enhanced features, as increased lypophilicity, metabolic resistance, or favourable NMR properties, leading to applications involving PET, MRI, or the development of glycomimetics.[2] Therefore, novel, straightforward and high-yielding strategies are crucial to access more complex fluorinated carbohydrates, and further exploit their beneficial properties.

Herein, based on our previous experience with the synthesis of complex oligomannosides (both linear and branched), including Man_9[3,4], the natural epitope of DC-SIGN receptor, we report the synthesis of ^{19}F-labelled oligomannosides in different positions based on Man_9 architecture to gain more insights at molecular level of the binding of these compounds to DC-SIGN.



General representation of the fluorine-containing oligomannosides.

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The first orthogonal photoswitchable azobenzene glycocluster: synthesis and photochromic properties

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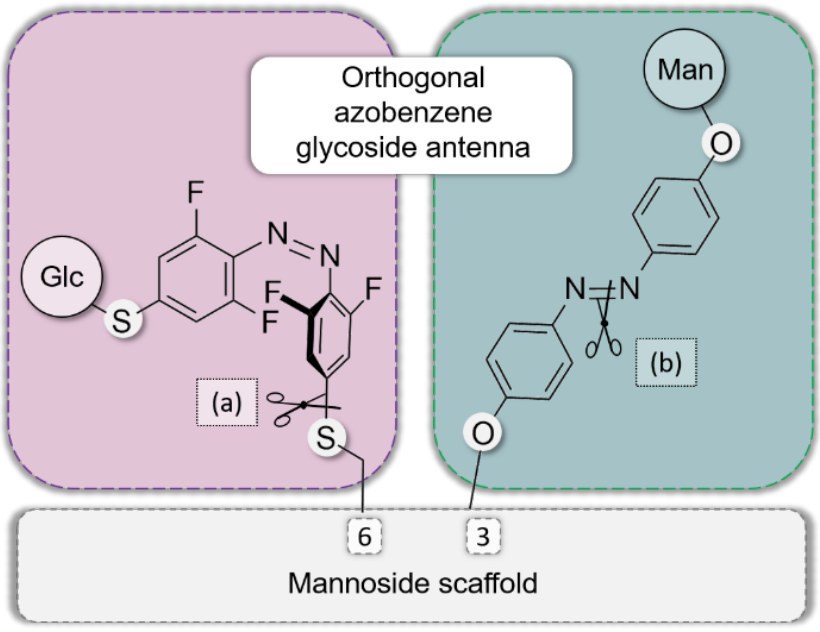
The glycocalyx of eukaryotic cells consists of a dense disordered layer of sugars conjugated to proteins and lipids. These cell surface glycoconjugates mediate diverse cell recognition processes including the adhesion of pathogens such as bacteria.[1] For the study of bacterial adhesion and the molecular details of carbohydrate recognition, photoswitchable azobenzene glycoclusters are privileged tools,[2] in particular to investigate the biological meaning of the relative orientation of sugar ligands in a 3D environment.[3]

Multivalent photoswitchable glycoconjugates also serve for the investigation of (hetero)multivalence effects in carbohydrate recognition.[4] In this project, orthogonally photoswitchable glycoazobenzene units were conjugated to a glycoside scaffold in order to widen the scope of this class of functional glycomimetics. A mannoside scaffold was functionalized at positions 3 and 6 according to typical linkages found in *N*-glycans. The employed conjugation chemistry restricts the degree of conformational flexibility of the linkages, allowing for the effective switching of ligand orientation by light (Fig. 1). The photochromic properties of the new orthogonal glycophotoswitch were analyzed and the kinetics of its thermal relaxation is described as well as its ligand properties with the lectins FimH and ConA, respectively.



Concept of the orthogonal control of carbohydrate orientation in a multifunctional glycocluster.

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Conservation of Allosteric Secondary Binding Sites Across Human C-type Lectins

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Due to their integral role in a wide range of physiological processes, C-type lectins (CTLs) represent attractive targets for diverse therapeutic interventions [1]. Yet, owing to their often shallow and polar carbohydrate binding site, the number of selective chemical probes remains limited [2]. In this context, previous studies indicated the presence of distal allosteric secondary sites in several CTLs with the potential of increased druggability and selectivity while simultaneously modulating the receptors activity [3–5].

Here, we computationally predict and characterize allosteric secondary sites across human CTLs. For this, we integrated established tools into a Python-based workflow that features (i) structural alignment of available CTL structures, (ii) pocket prediction, (iii) evaluation of allosteric potential of each pocket using an elastic network model (ENM)-based method and (iv) a sequence-based co-evolutionary approach. Density-based clustering of the resulting pocket ensemble enabled identification of 20 small, but structurally conserved pocket locations, of which at least four were predicted to possess allosteric potential. Additionally, comparison with experimental data showed overlap of several clusters with binding sites for endogenous and synthetic ligands as well as protein-protein interaction interfaces. While experimental validation will be necessary, these observations point towards an evolutionary conserved function of allosteric pockets across CTLs that could be leveraged as a new modality for targeting this protein family.

Acknowledgements

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A Genome-Wide CRISPR Screen Identifies SORT1 as a Mediator of Galectin-1 Intracellular Trafficking

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Despite their clear importance regulating critical cellular functions such as cell migration, a detailed understanding of the mechanistic biology of the galectin family of glycan-binding proteins remains elusive. Along with the biochemical challenges inherent to the study of lectin-glycan interactions, investigations into the mechanisms of galectin function are complicated by the fact that several members of this family do not exclusively function via glycan-binding activity. Galectin-1, for example, is secreted and internalized via unconventional and poorly-understood mechanisms, and is known to exhibit both extracellular activity, dominated by its interaction with cell-surface glycans, and intracellular activity, where it is believed to be involved in RNA processing and maturation.

Here, we present a genome-wide CRISPR screen that investigates the mechanism of galectin-1 internalization and demonstrate that its internalization is mediated in part by the trafficking receptor sortilin. We identify a point mutant deficient in sortilin-binding to facilitate separate study of galectin-1's glycan-dependent and glycan-independent activity and highlight the potential applicability of this endogenous protein-internalization machinery to the intracellular delivery of large biomolecules.

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The Newly Discovered EclA Lectin: An Emerging player in Enterobacter cloacae Infections

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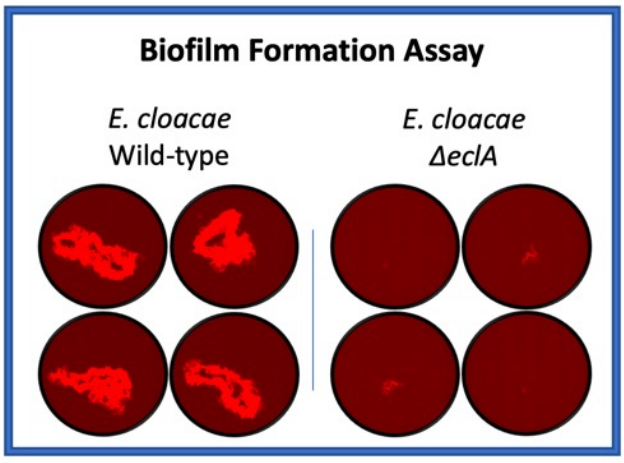
Lectins are carbohydrate-binding proteins that exist in many pathogenic bacteria and are important for infection (Meiers *et al.*, 2019). These virulence factors can mediate cell attachment and biofilm formation, thereby decreasing the capacity of the host to clear the infection and the treatment options of the clinician. The inhibition of these lectins can disrupt the infection process and ultimately reduce pathogenesis without the risk of developing antimicrobial resistance (Leusmann *et al.*, 2023).

Recently, the fucophilic lectin EclA was discovered from the ESKAPE pathogen *Enterobacter cloacae* (Beshr *et al.*, unpublished). Interestingly, EclA is an orthologue of the galactophilic lectin LecA of *Pseudomonas aeruginosa* involved in biofilm formation (Gilboa-Garber *et al.*, 1982). Glycan specificity arrays demonstrated the affinity of EclA to the Lewis^a antigen present in most newborns and to the blood group antigen O (Beshr *et al.*, unpublished).

We hypothesize that *E. cloacae* utilizes EclA for cell attachment, biofilm formation, and pathogenesis. In this work, an *E. cloacae eclA* knockout mutant was generated by allelic exchange. The expression of EclA was studied by Western blot analyses and showed a prominent and constant expression, supporting its putative importance for the bacterium.

In vitro biofilm formation activity observed under fluorescence microscopy was greatly decreased in the *eclA* deletion mutant, validating the role EclA plays in biofilm formation. EclA showed hemagglutination potential in human red blood cells, further validating its role as a virulence factor. Lewis^a and blood group O-antigen adhesion assays are still to be performed to provide insights into whether EclA also mediates cell attachment, making it a strong therapeutic target for the treatment of *Enterobacter cloacae* infections.



The knockout of eclA in E. cloacae significantly reduces biofilm formation

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Exploring the synthesis and the biological profile of Novel D-Glucopyranuronamide-base Nucleosides

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Synthetic nucleos(t)ides are important groups of molecules in medicinal chemistry, due to their ability to interfere with nucleos(t)ide-dependent biological events and act as anticancer and antiviral agents. [1] Their antimicrobial potential is also well reported. [2] However, some limitations are associated with their clinical use, such as low oral bioavailability and chemotherapeutic resistance.[1] Therefore, the development of bioactive nucleos(t)ides-like structures that may overcome such issues and act through alternative mechanisms of action is of significant interest.

In this context, the synthesis and biological evaluation of novel nucleosides based on D-glucuronamide units will be presented. The inclusion of this glycosyl moiety was motivated by the known biological profile of D-glucuronamide derivatives.[3] Moreover, it allows structural variations in a *gluco*-configured template at C-6 via N-substitution, which can be tuned for attaining better bioactivities.

Differently *N*-substituted glucuronamide-based purine and uracil nucleosides were accessed as well as nucleotide sugar mimetics based on a pseudodisaccharidic skeleton and containing a (triazolyl)methyl amide linkage as a potential neutral and rather stable surrogate of a diphosphate group.

The biological evaluation of the compounds included the study of their antiproliferative activities in cancer cells and of their antibacterial effects. Some molecules showed a potent anticancer activity with GI_{50} values similar or lower than those of standard drugs, turning them prospective lead molecules for further studies.

Acknowledgements

We thank FCT for funding: grant CEECIND/03881/2018, projects EXPL/MED-QUI/1017/2021, UIDB/00100/2020, UIDP/00100/2020 (CQE), LA/P/0056/2020 (IMS).

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Total Syntheses of Trisaccharide Repeating Units of Staphylococcus aureus Type 5 & 8 (CP5 & CP8)

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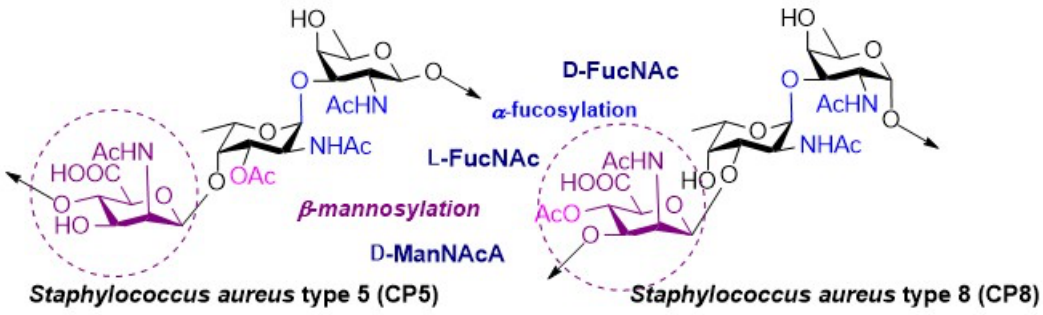
Polysaccharides isolated from pathogenic bacteria are endowed with unique immunological properties and are emerging as immunotherapeutic agents as well as vaccine carriers. Reported herein is a total synthesis of trisaccharide repeating unit of *Staphylococcus aureus* type 5 & 8 (CP5 & CP8). *Staphylococcus aureus* is listed as a "high priority" pathogen by WHO[1] which is a major cause of serious nosocomial infections such as bacteraemia, sepsis, and endocarditis. Owing to their ability to adapt resistance to almost any antibiotics, vaccines against these pathogens are urgently required, therefore synthesis of these urgently required oligosaccharides of S. aureus CP5 and CP8 has been attempted by various group.[2]

These pathogens express structurally unique and densely functionalized glycans on their surfaces which are absent on the host cells. Such carbohydrate antigens are valuable targets for the development of glycoconjugate vaccines and diagnostics. Herein, we report a highly efficient total synthesis of *Staphylococcus aureus* type 5 & 8 (CP5 & CP8) trisaccharide repeating unit in a lesser number of steps and high stereoselectivity.[3,4] These complex trisaccharides contain rare amino sugars, viz., D-fucosamine, L-fucosamine, and 2-acetamido D-mannuronic acid. The preparation of rare sugar building blocks and installation of consecutive sterically hindered 1,2-*cis* glycosidic linkages, especially *β*-mannosylation, is the key challenge in the synthesis.



- Rare deoxy amino sugars
- Consecutive 1,2-*cis* glycosylations
- Orthogonal groups at connecting points
- Direct *β*-mannosylation

Structure of Trisaccharide Repeating Units of Staphylococcus aureus Type 5 & 8 (CP5 & CP8)

Acknowledgements

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Effect of FMT on blood serum *N*-glycome in patients with fulminant Clostridium difficile infection

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Clostridium difficile infection (CDI) mainly occurs in hospital environment. [1] Patients with CDI in general have a good response to antibiotics therapy, but a subset of patients develop antibiotic-refractory severe or fulminant CDI (SFCDI) for witch a different therapeutic approach is necessary.[2] An effective treatment for SFCDI is fecal microbiota transplantation (FMT) from healthy donors. [3] From the studies it is apparent that gut homeostasis is reconstituted after FMT, but the effect of the FMT on metabolic state of a person is yet unknown. Therefore, in this study we aimed to elucidate the effect of FMT in SFCDI patients on blood serum N-glycome as well on blood serum isolated immunoglobulin G (IgG) N-glycome. The study enrolled four patients longitudinally followed in five time points. In three patients the FMT procedure was successful, while one patient was non-responsive. Temporal changes in blood serum and IgG N-glycome were observed after a successful FMT procedure. Specifically, we detected higher relative abundance of monosialylated and digalactosylated N-glycans in blood serum following successful FMT. Furthermore, the non-responsive patient has higher abundancies of circulating IgG subclass 4 with agalactosylated, bisected and core-fucosylated N-glycans. These findings suggest that changes in gut microbiome have an impact on the metabolic state of a person. However, no broad generalisation can be drawn due to the small sample size. Therefore, a replication study with a larger number of patients is required.

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Accelerated Solid Phase Glycan Synthesis ASGS

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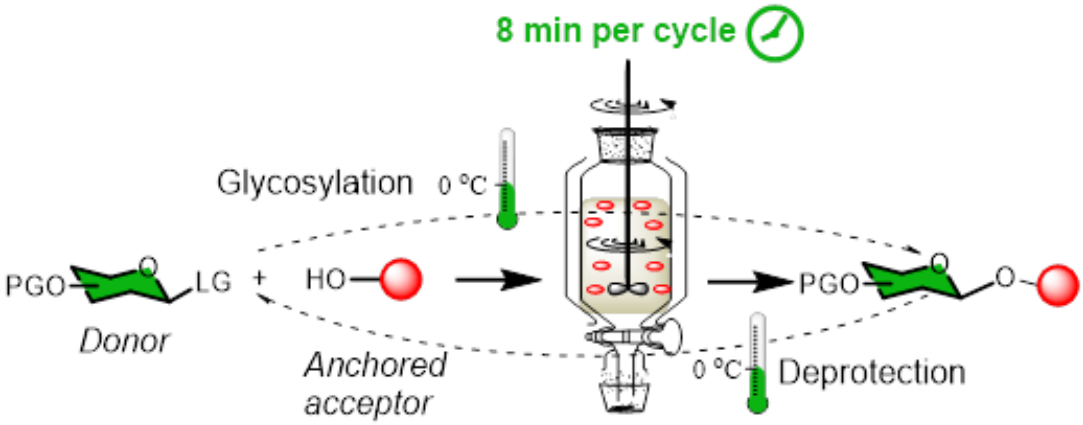
Solid phase oligosaccharide synthesis is a complicated process. Each step of the assembly is done at different temperatures and under unique reaction conditions. Glycosylation on solid support suffers from poor mixing conditions which leads to diffusion-independent hydrolysis of the activated donors and results in excessive use of expensive glycosyl donors. Sophisticated machinery is used to control the temperature and reaction conditions in order to maximize the efficiency of the process but these manipulations require prolonged reaction times.

In this work, we established a new strategy that utilizes high shear stirring to accelerate solid phase oligosaccharides synthesis. By applying efficient mixing, we show that reactive intermediates can diffuse faster to the solid support thereby increase the kinetics of the reactions. We show that the efficient mixing eliminates the need in using high excess of glycosyl donors and enabled performing extremely fast glycosylation cycles in ambient atmosphere.



Accelerated solid phase glycan synthesis (ASGS), the new approach relies on high shear mixing, it is continuous, performed under constant temperature

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Bakhatan Y, Alshanski I, Chan C-K, Lo W-C, Lu P-W, Liao P-H, et al. Accelerated Solid Phase Glycan Synthesis ASGS . ChemRxiv..

Galactosamine and sialic acid glycosystems based on nanosized MOFs for therapeutic strategies

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Multivalent carbohydrate systems, involving synthetic constructs featuring multiple carbohydrate units attached to a central core/scaffold, have shown exceptional potential in inhibiting cell binding and infectivity, thus opening new possibilities for the treatment of infections and cancers. Among these glycosystems, those based on carbohydrates linked to metal-organic frameworks (GlyconanoMOFs) have emerged as ideal contenders for drug diagnostic and therapeutic (theranostic) strategies, albeit still relatively limited in their development [1,2]. Of special interest are glycosystems derived from biocompatible Zr-MOFs referred to as "PCNs" (porous coordination networks), such as PCN-222, PCN-224, and PCN-225, composed of Zr(IV) nodes interconnected by porphyrinic ligands, showing large surface areas and chemical stability [3]. This study reports the design, synthesis, characterization, and evaluation of water-dispersible PCNs, carefully tailored to possess well-defined morphology and size-controlled structures (<100 nm).

These PCNs were modified with specific directional sulphate-PEG ligands functionalized with N-acetyl galactosamine (GalNAc-PEG-SO_4) and sialic acid derivatives (SA-PEG-SO_4) through the coordination of the sulphate group with the Zr. The incorporation of GalNAc aimed at exploiting its specificity towards the asialoglycoprotein receptor (ASGPR), overexpressed on hepatic cancer cells [4]. Additionally, the incorporation of SA derivatives, including 9-O-acetylated-SA, was targeted at developing anti-viral strategies, such as combating SARS-CoV-2 infections [5].

Acknowledgements

PAIDI 2020 programme 54A, PY20_00882 and CV20-04221 from the Andalusian Government and PID2020-119949RB-I00 from the Spanish MICINN.

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Carbohydrate – Aromatic interactions in Carbohydrate Foldamers

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T Cyclodextrins (CDs), consisting of sugar molecules bound together to form different sizes ring, are regarded as the only homogenous, well-defined carbohydrate-based architecture capable of adopting a stable 3-D conformation.[1] While CDs can host hydrophobic guests in their cavity, the over-rigid structure limit its applicability in supramolecular chemistry and biology. Expanding the scope of carbohydrate-based architectures capable of selectively recognizing, binding and hosting aromatic subtracts will be significant for the design of protein ligand and drugs.

Recently, we reported the synthesis of a carbohydrate foldamer, that self-organize into a rigid hairpin conformation.[2] Here, we adjusted the 3-D conformation of the carbohydrate hairpin and tuned its electron density in order to promote the interaction between the carbohydrate foldamer and aromatic subtracts *via* CH/π interactions[3]. Automated glycan assembly (AGA) allowed for the fast synthesis of a series of carbohydrate foldamer analogues. Lateral chemical modifications including phosphorylation, sulfation, and amination allowed for the introduction of ionic residues to tune the electronic properties of the hairpins. The interaction between the modified carbohydrate foldamer and a series of aromatic subtracts was studied by multiple NMR techniques. Specific CH/π interactions were identified, suggesting that carbohydrate foldamers could be designed to bind polypeptides and proteins.

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Synthetic study of sialoglycans found in capsular polysaccharide of *Neisseria meningitidis* W135

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Bacterial meningitis is a serious infection associated with high mortality and morbidity. Because of the serious and very rapid spread of infection, development of vaccine is important for its control. *Neisseria meningitidis* can be classified into different serogroups (eg. A, B, C, W135, X and Y) based on the chemical structures of their capsular polysaccharides. For the elucidation of their infection mechanism and the development of vaccines, pure and structurally well-defined capsular polysaccharides are highly demanded. In this study, we focused on the chemical synthesis of polysaccharides present in W135 serogroup, which consist of disaccharide repeating unit [-6)-Galα(1-4)Neu5Acα(2-] [1,2].

To approach the targeted polysaccharides, we envisioned a 2^n elongation through fully stereoselective α-sialylation [3] at the C6 hydroxyl group of the external Gal residue linked to Neu residue with an α(1-4)-glycosidic bond. First, a C4-hydroxy macrobicyclic sialyl acceptor was glycosylated with a 4,6-DTBS-protected galactosyl donor [4] to give a Galα(1-4)Neu derivative with perfect α-selectivity. Next, the disaccharide was converted into a phosphate donor and a 4,6-diol acceptor by the removal of DTBS group in the Gal moiety. Then, we performed a [2+2] coupling in the presence of TMSOTf in CH_2Cl_2 at −80 ˚C, providing a tetrasaccharide derivative in a high yield with complete α-stereoselectivity. Furthermore, [4+4] and subsequent [8+8] elongations were successfully carried out to produce a hexadecasaccharide for the first time. These results indicate the utility of the 2^n elongation strategy employing the fully stereoselective α-sialylation.

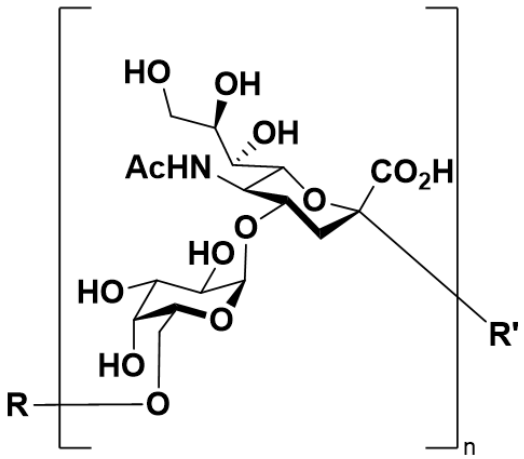

Fig 1. Structure of polysaccharides derived from Neisseria meningitidis W135

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Revealing the Contribution of Glycan Structures to Galectin-dependent Glycoprotein Dynamics

Ayane MIURA [1], Shuto Miyake [1], Yoshiyuki Manabe [1,2], Asuka Shirakawa [1], Hiroki Shomura [1], Toshiyuki Yamaji [3], Kenichi G. N. Suzuki [4], Kazuya Kabayama [1,2], Koichi Fukase [1,2]

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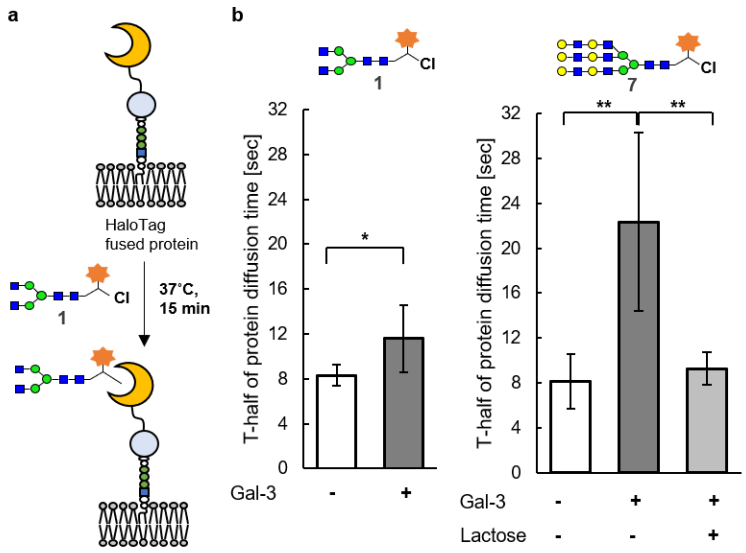
Glycans regulate the function of membrane molecules via interaction networks with various molecules. The temporal and spatial structural diversity of glycans makes it extremely difficult to correlate the glycan structures with the function of membrane molecules. Therefore, we have developed a method to display synthetic homogeneous glycans to membrane proteins on the cell surface via HaloTag protein. Furthermore, we applied this system to analyze the regulation of protein dynamics by the interaction between glycans and Galectin-3 (Gal-3).

Gal-3 forms a network structure called galectin lattice on the membrane via glycoprotein recognition.[1)] Although the galectin lattice has been thought to suppress the dynamics of membrane proteins and regulate their function, precise verification of galectin lattice functions dependent on glycan structures has been difficult. Therefore, we measured the lateral diffusion rate of the protein with the homogeneous synthetic glycans by several live cell imaging methods. These analyses showed that the lateral diffusion of target protein with Gal-3 high-affinity glycan ligands (Ligand 7) was significantly suppressed by Gal-3. Furthermore, the Gal-3 was able to divide the lateral diffusion of the target proteins into two major groups according to the degree of the inhibition. This result suggested that the percentage of proteins which were trapped by the galectin lattice depends on the Gal-3 affinity of glycans.

In conclusion, we have succeeded in analyzing the function of galectin lattice for each glycan structure.



The method to display synthetic homogeneous glycans to membrane proteins via HaloTag (a). The lateral diffusion analysis of HaloTag fused protein (b).

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Application of Flow Chemistry in the Synthesis of Iminosugars

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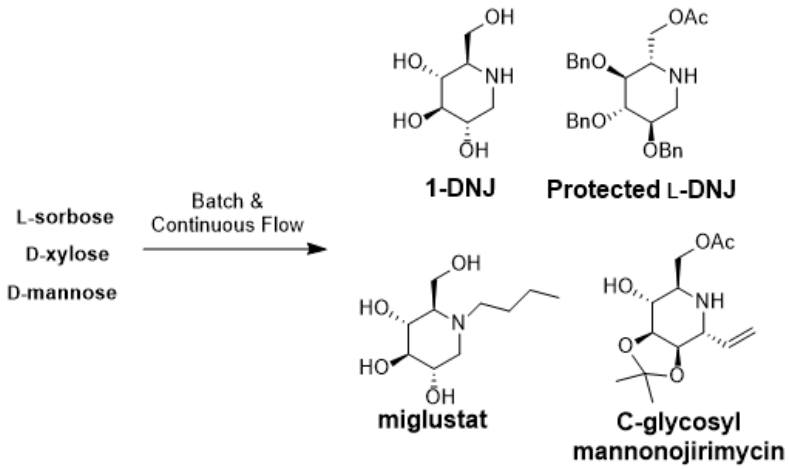
Iminosugars are a subset of glycomimetics in which the ring oxygen of a sugar is replaced with nitrogen. These mono-/bicyclic structures are potent inhibitors of many glycoprocessing enzymes, particularly glycosidases, and they are currently marketed for treatment of Type 2 diabetes, Gaucher disease and Fabry disease.[1] In addition to their current therapeutic uses, they have demonstrated potential as antivirals.[2]

Continuous flow chemistry involves the mixing of reagents in continuous streams through defined reactor zones (e.g., coiled tube reactors), as opposed to the traditional batch methods of carrying out reactions in flasks/vessels.[3] Flow chemistry has received much attention in recent years in both research and industrial laboratories for its potential advantages over batch processes, such as improved mixing, greater heat transfer, potential for automation, ease of scale-up and improved safety.[4]

My research is focused on improving the synthesis of valuable iminosugars (such as 1-DNJ) from renewable sugars through batch and flow techniques. The primary aim is to investigate the potential for flow chemistry in key steps to produce improvements in yield, reaction time and safety, along with creating greener processes.



Overview of Iminosugars Synthesised Using Batch & Continuous Flow Techniques

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Process development for gram-scale synthesis of nucleotide sugars using in-vitro enzyme cascades

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Large-scale enzymatic synthesis of functional oligosaccharides and glycoconjugates such as human milk oligosaccharides and glycoproteins using Leloir glycosyltransferases is still limited by the price and availability of their building blocks, nucleotide sugars. We have successfully developed in-vitro multi-enzyme cascades consisting of up to six recombinant enzymes to produce the nucleotide sugars UDP-N-acetylglucosamine (UDP-GlcNAc), UDP-galactose (UDP-Gal) and UDP-N-acetylgalactosamine (UDP-GalNAc) from inexpensive precursors, i.e. uridine or UMP, polyphosphate and GlcNAc, Gal and GalNAc, respectively. For further cost reduction, the relatively expensive co-substrate ATP is in situ regenerated from polyphosphate. Utilizing a set of duet vectors, each cascade is expressed in one single E. coli strain.

Using cell lysate as biocatalyst formulation, UDP-GlcNAc was produced with a conversion yield approaching 100 % with respect to 100 mM UMP in a batch process at 100 mL scale within 24 h. The final product concentration was 61 g/L. Using the same set-up, UDP-Gal can be produced with a conversion yield of 90 % and a final product titer of 51 g/L. For the synthesis of UDP-GalNAc, a conversion yield of 86 % with respect to 50 mM uridine and a final product titer of 26 g/L were obtained within 24 h in a 50 mL batch process.

As an initial purification step, ultrafiltration with a cut-off of 10 kDa can be applied for protein removal. To further improve product purity, a scalable anion exchange chromatography protocol was established using HiTrap Q HP columns – a strong anion exchange resin. With a dynamic binding capacity of approximately 24 mgnucleotide/mLresin, milligram-scale purification per run utilizing mL-scale columns is possible.

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Biotechnological production of sialylated solid lipid microparticles as Influenza virus inhibitors

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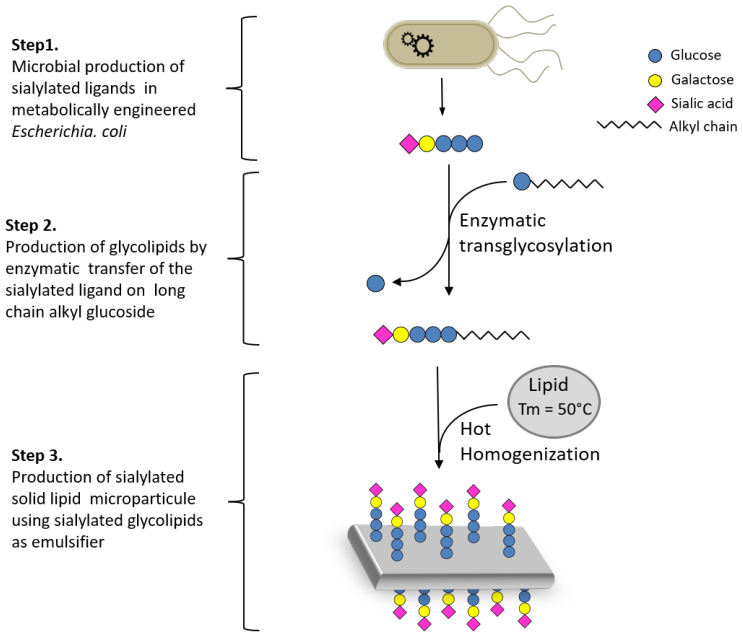
Influenza viruses bind to their target through a multivalent interaction of their hemagglutinins with sialosides at the host cell surface[1]. To fight the virus, one therapeutic approach consists in developing sialylated multivalent structures that can saturate the virus hemagglutinins and prevent the binding to host cells[2,3]. We describe herein the biotechnological production of sialylated solid lipid microparticles (SSLMs) in three steps: (i) a microbiological step leading to the large-scale production of sialylated maltodextrins by metabolic engineering of an *Escherichia coli* strain, (ii) a new *in vitro* glycosylation process using the amylomaltase MalQ, based on the transglycosylation of the terminal sialoside ligand of the sialylated maltodextrin onto a long-chain alkyl glucoside, and (iii) the formulation of the final SSLMs presenting a multivalent sialic acid. We also describe the morphology and structure of the SSLMs and demonstrate their very promising properties as influenza virus inhibitors using hemagglutination inhibition and microneutralization assays on the human A/H1N1 pdm09 virus.



Acknowledgements

The NanoBio-ICMG platform is acknowledged for mass spectrometry (A. Durand, L. Fort, and R. Gueret), NMR spectroscopy, and electron microscopy.

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Chemoenzymatic Approaches to Unsymmetric *N*-Glycans

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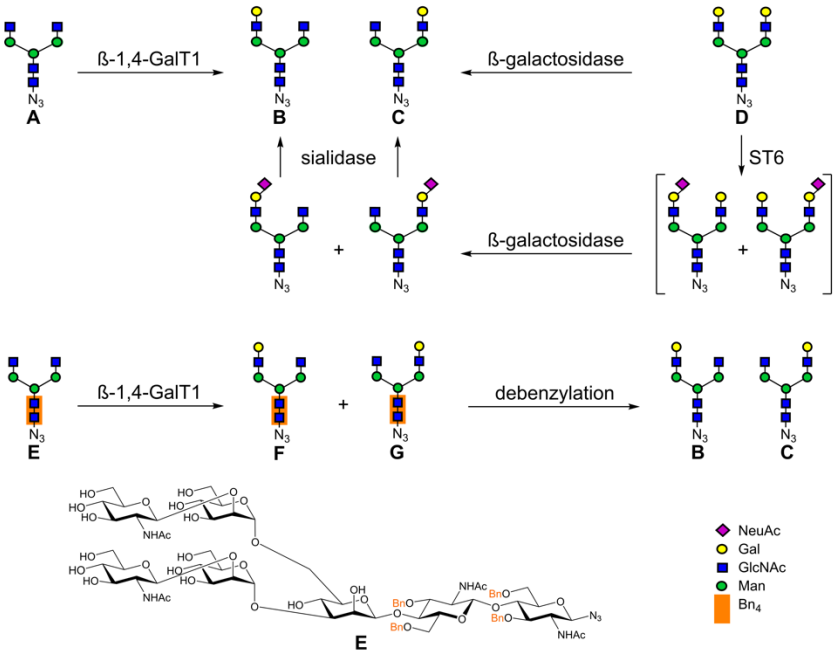
The presence of *N*-glycans can greatly influence the biological properties of *N*-glycoproteins.[1] To evaluate the biological recognition of natural *N*-glycans via glycan-microarrays, *N*-glycans with symmetric and unsymmetric[2,3,4,5] substitution patterns are needed. Unsymmetric *N*-glycans can be obtained by enzymatic conversions of the antennae of biantennary heptasaccharide azide **A**. The incomplete galactosylation of **A** led to the isomeric octasaccharides **B** and **C**. However, the ratio of both isomers is unfavourable (**B**:**C** = 10:1) and the regioisomers separate poorly even over a porous graphitic HPLC-column (PGC). Digestion of galactosylated nonasaccharide **D** with ß-galactosidase improves the ratio of **B**:**C** to 4:1. An alternative route via monosialylation of **D** with a bacterial 2,6-sialyltransferase (PdST6) followed by digestion with ß-galactosidase and desialylation provided pure unsymmetric *N*-glycan azides **B** and **C**. The monosialylation improved the separation of unsymmetric *N*-glycans. In contrast, the partial galactosylation of benzyl-protected *N*-glycan **E** yielded the monogalactosylated compounds **F** and **G** in a ratio of nearly 1:1. Additionally, the HPLC-separation of the isomers was markedly improved. Since the oxidative debenzylation of the protected azides **F** and **G** with bromine radicals[6,7] was not feasible, a photochemical debenzylation using riboflavintetraacetate was developed.[8]



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Bioinspired formulation of Lignocellulosic composites for preparation of bio-based materials

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Climate change hazards have a significant impact on the composition of lignocellulosic materials, altering the chemical distribution of the primary carbon biopolymers in straw and affecting the yield and quality of agronomic systems. To mitigate climate change, bio-based construction materials are a viable option for achieving neutral carbon emissions by emulating natural processes.

Natural lignocellulosic fibers such as cellulose, hemicellulose, and lignin, are considered as the model fibers due to their exceptional mechanical properties. However, the use of lignocellulosic byproducts presents a disadvantage due to the size and non-uniform distribution of fibers. One solution to improve the mechanical properties and structure of bio-based materials is to incorporate natural additives such as carbohydrates and lignans to simulate plant structures. Green chemistry processes can also be applied to modify the surface of materials and reinforcements to enhance durability and meet construction standards.

Overall, these findings suggest that utilizing bio-based materials can be a viable solution to mitigate the impact of climate change on agronomic systems and promote sustainable construction practices. In this study, a wheat field trial was conducted under high carbon dioxide and drought conditions. The straw compounds (lignin, cellulose and hemicellulose) have been quantified to determine the effect of drought and CO_2 uptake that may have affected wheat growth on the compound's distribution and the formation of new agrocomposites. Lignosulfonate, polysaccharides, and wheat fibers were used as the matrix and the reinforcement, respectively, to produce bio-based composites. The mechanical properties of the composites were validated, and surface modifications were performed using chemo-enzymatic processes to enhance mechanical strength and permeability.

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New insights into *Ruminococcus flavefaciens* cellulosome assembly

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The cellulosome is an elaborate multi-enzyme structure secreted by anaerobic microorganisms for the degradation of lignocellulosic substrates. Its assembly is mediated by high-affinity interactions between enzyme-borne dockerin (Doc) modules and repeated cohesin (Coh) modules present in non-catalytic scaffoldins. The cellulosome of *Ruminococcus flavefaciens* is one of the most intricate described to date and its structure is assembled exclusively through single-binding-mode Coh-Doc interactions [1-3]. However, a set of R. *flavefaciens* Docs exhibits certain features associated with the classic dual-binding mode (DBM) [4-6].

To investigate DBM in ruminal cellulosomes, we have solved the structures of two Coh-Doc complexes through X-ray crystallography, one involving the Doc of the monovalent adaptor scaffoldin ScaH and another involving an atypical truncated dockerin with a single calcium-binding repeat. Our finding revealed that the DBM can be selectively incorporated into R. *flavefaciens* cellulosome through ScaH and that the single-repeat Docs can interact in three distinct conformations, including one with two Docs binding to a single Coh, a feature never previously reported.

These results suggest the existence of adaptor scaffoldins with the sole purpose of improving cellulosomal spatial conformation and of naturally occurring atypical dockerins with distinct binding mechanisms. We were also able to improve Coh-Doc affinity through structure-informed protein engineering, a key feature for the design of affinity-based technologies using tailored Coh-Doc interactions.

Acknowledgements

We acknowledge FCT, through the grants: UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); and 2022.07903.PTDC. We also acknowledge ANI through the grant LISBOA-01-0247-FEDER-047033 [GlycoMed] and the Gilead GÉNESE program through the project 17805. M. Duarte is funded by an individual PhD scholarship from FCT (SFRH/BD/146965/2019)

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Interaction study of sugammadex with phytotoxins – new indications for an old antidote?

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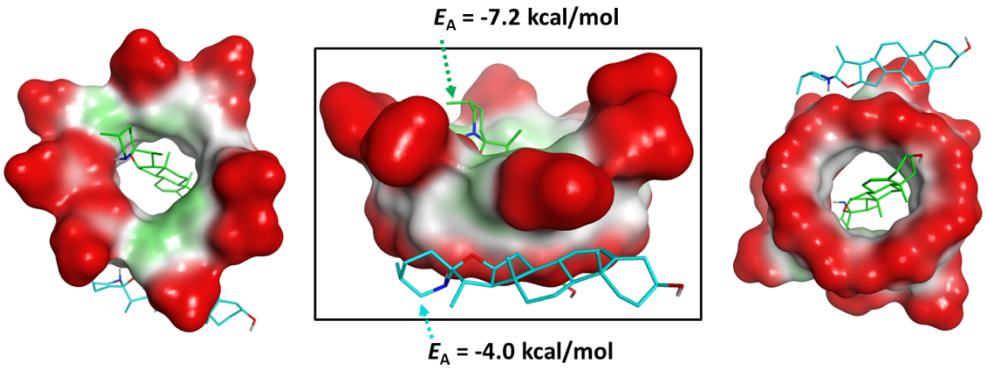
Sugammadex (SGM), - an octacarboxylic acid derivative of the native gamma-cyclodextrin - is used in the clinical practice to reverse the neuromuscular blockade of aminosteroid anesthetics. The selective binding is enabled by the formation of a particularly strong host-guest complex, driven by the ionic interactions with the cyclodextrin side chains and the tight fit of the steroid backbone into the cyclodextrin cavity [1].

Toxic glycoalkaloids with similar aminosteroid structures are abundant in nature. Various species of the *Solanaceae* family accumulate toxic glycoalkaloids during ripening. Consumption of part of these species (e.g., immature or sprouted potatoes, berries of *Solanum nigrum*) leads to poisoning, manifesting in gastrointestinal and nervous system symptoms, in extreme cases with fatal outcome. To date, the intoxication is only treated by supportive therapy as there is no known antidote available for these phytotoxins.

Considering the structural similarities between the anesthetics and the toxins of the *Solanaceae* family, we investigated the interactions of SGM with two phytotoxin aglycons, solasodine and solanidine, aiming to find a potentially applicable antidote. Using NMR spectroscopy, particularly stable host-guest complexes were identified between both solasodine and solanidine and SGM. A slow-exchange system in the NMR timescale was recognized, indicating particularly stable supramolecular binding. Through-space magnetic dipole correlation methods were used to identify the structure of the complexes and computational methods were also performed to support the identified structure.



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New approach to glycoamphiphiles integrated in a LC-based biosensor for pathogen lectin detection

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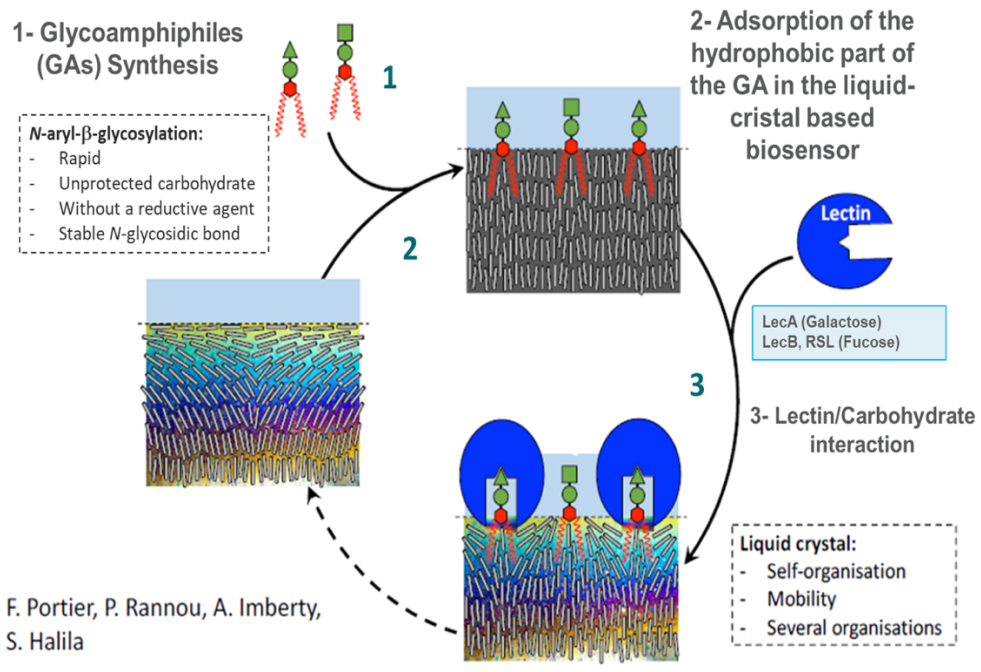
Glycoconjugates present on membrane cell surfaces play critical roles in a wide variety of pathological processes acting as signaling, recognition, and bacterial adhesion. Consequently, major scientific and biotechnological interests in glycoconjugates derive from their use as probes for biological research, and lead compounds for diagnostic tools[1].

The project of this research aims at developing a modular access to GlycoAmphiphiles (GAs) for the liquid crystal-based optical detection[2] of carbohydrates interacting with lectins coming from opportunistic pathogens such as *Pseudomonas aeruginosa*[3].

This poster will discuss about the synthesis of GAs by *N*-aryl-glycosylation of unprotected carbohydrates. The recognition between the carbohydrates part of our GAs and their specific pathogenic lectins has been studied using isothermal titration calorimetry(ITC). The stability to chemical hydrolysis of the *N*-glycosidic bond was also analysed using HPLC.



Acknowledgements

This work was supported by CNRS (Centre National de le Recherche Scientifique), ANR (Agence Nationale de la Recherche) and the doctoral school EDCSV.

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Using activity-based probes to understand α-amylase substrate specificity

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α-amylases are useful industrial tools for the commercial processing of starches and related carbohydrates. These glycosyl hydrolases, of the GH13 class, break down a-1,4 bonds and are essential to the production of high-fructose corn syrups, modern detergents, and starch-derived biofuels. The discovery and engineering of GH13 enzymes which can withstand the harsh conditions often utilised in these processes is of high market value. These harsh conditions often involve extreme pH and temperature, and of particular interest in this work, a lack of calcium. Many GH13 enzymes are calcium-dependent.

In this work, we present the application of activity based probes based on an a-1,4-linked disaccharide for the study of GH13 activities. An epoxide group is installed to trap the covalent intermediate. These probes are used to profile a panel of a-amylases against a range of different conditions to understand more about their applicability to different industrial processes. These profiling assays aim to demonstrate the ease of using activity based probes to identify suitable enzymes for a particular set of process conditions.

The development of a-1,6 branched probes is also presented. These branched probes are used to profile GH13 a-amylases in human gut bacteria which may accommodate a branch in a particular position in the substrate grove. These insights are used to explain the ability of some of these bacteria to grow on complex carbohydrates.

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Synthesis and Biological Evaluation of C-Linked α-Galactosylceramide Analogs

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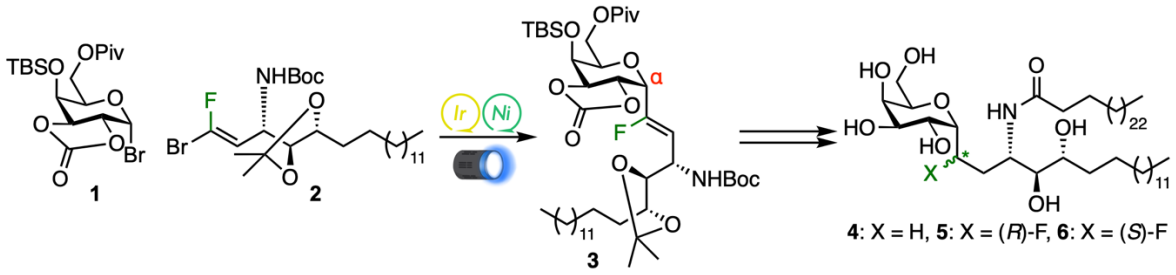
α-Galactosylceramide (α-GalCer) is a potent immunostimulatory glycolipid with therapeutic potential for cancer and infectious diseases.[1] According to previous SAR studies,[2] CH_2-linked analog of α-GalCer stimulates an enhanced Th1-type response. Here, we developed new synthetic methods for *C*-glycoside analogs of α-GalCer, including CH_2-, (*S*)-CHF-, and (*R*)-CHF-linked analogs that would adopt different conformations around glycoside bonds due to stereoelectronic effect of fluorine atom, and evaluated their biological activities.

In our previous work, we developed a direct cross-coupling method for glycosyl xanthate and terminal olefin using atom-transfer radical addition.[3] This method resulted in the formation of *C*-glycosides in an α-selective fashion. In particular, coupling a galactose-type donor with a sphingosine-type acceptor led to the production of α-*CH_2*-galactosylsphingosine, which was then further transformed into a *CH_2*-linked analog of α-GalCer.[4] Unfortunately, the method could not produce *CHF*-glycosides analogs due to the unreactive nature of the fluoroalkene.

This work synthesized CH_2- and CHF-glycoside analogs using a direct cross-coupling reaction of glycosyl bromide donor **1** and BrF-olefin acceptor **2** in the presence of Ir-photocatalyst and Ni-catalyst under blue LED irradiation, affording the fluoroalkene **3** in an α-selective manner. The chemo- and stereo-selective hydrogenation of **3** followed by deprotection and acylation successfully afforded CH_2-, (*S*)-CHF-, and (*R*)-CHF analogs of α-GalCer (**4**, **5**, **6**). In this presentation, we present the detail of the synthetic method and biological activity of the synthesized α-GalCer analog.



Synthesis and biological evaluation of C-linked analogs of α-Galactosylceramide

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Lipopolysaccharides: the importance of the structure in the extreme environment and in human gut

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Lipopolysaccharide (LPS) is a crucial constituent of the outer membrane of Gram-negative bacteria, playing a fundamental role in the protection of bacteria from environmental stress factors, in drug resistance, in pathogenesis, and in symbiosis. The LPS is an amphiphilic molecule composed of three regions: a conserved phosphoglycolipid (the lipid A), an oligosaccharide chain (core OS region), and a surface-exposed O-polysaccharide (O-antigen).[1] One of the most important LPS functions relies in its structure-dependent capability of eliciting an immune response in infected hosts, i.e. depending on its chemical features, an LPS is able to potently activate, poorly activate or not activate an inflammatory response, or even activate an anti-inflammatory one.[2] To investigate how modifications in the structure of this glycomolecule can influence the elicitation of the immune response, the determination of LPS chemistry is a first but crucial step. In this communication I will focus on LPS deriving from both environmental and human intestinal bacteria. On one hand I will show that enviromental bacteria can survive in extreme habitats thanks to to the development of peculiar modifications of their LPS component.[3] On the other hand, I will show results about the the structure of LPS derived from key gut commensal bacteria, and their ability to modulate the immune response [4].

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First Total Synthesis of Tetrasaccharide Repeating Unit of Vibrio Cholera 043

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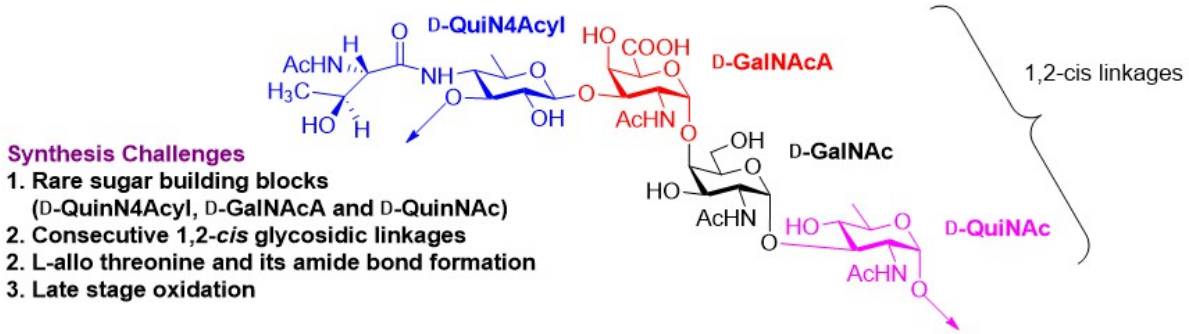
Cholera is a well-known deadly disease causing acute watery diarrhoea and severe dehydration. According to WHO fact sheets, it is estimated that every year about 1.3 to 4.0 million people get infected due to cholera, and 21,000 to 1,43,000 deaths are reported worldwide.[1] *Vibrio cholera*, a causative agent of deadly pandemic-cholera is a facultative, anaerobic, Gram negative, marine bacterium having both human and environmental stages in its life cycle.

This deadly pathogen is a threat for many developing countries, predominately in Asia and Africa.

Therefore, in order to curb the spread of the disease, development of vaccines is an absolute essential. It is noteworthy that the glycans present on the surface of dense bacterial cell wall are different than the monosaccharides on human glycome. This distinguishing information is the gateway for the development of vaccines and inhibitors. Our lab established an efficient, one-pot methodology of sequential displacements of triflates by suitable nucleophiles for the synthesis of various orthogonally functionalised rare D- and L-deoxy amino sugars.

These building blocks can can be assembled to get access to biologically important and structurally complex glycoconjugates. Herein, we report the first total synthesis of the tetrasaccharide repeating unit (RU) of OPS of *Vibrio cholerae* O43. The structure has a novel D-viosamine (Quip4NAcyl) unit attached to L-threonine amino acid.[2] The significant challenges are: (a) synthesis of functionalized rare monosaccharide building blocks i.e D-quinosamine (D- QuipNAc), D-viosamine (Quip4NAcyl), D-galacturonic acid moiety (D-GalpNAcA) and D- galactosamine (D-GalpNAc) (b) Installation of 1,2-cis glycosidic linkages, (b) amide bond formation (b) late stage functional group interconversion as well as global deprotection.



Tetrasachharide Repeating unit of O-antigen of *Vibrio cholera* O43

Acknowledgements

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Development of Ligand for Targeted Delivery to Murine Langerin

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Multivalent ligand-based targeted delivery can improve selectivity and efficacy of drugs or vaccines. Here, we develop targeting ligands that could specifically bind murine Langerin, a carbohydrate binding protein mainly expressed on Langerhans cells (LCs). LCs are skin-resident dendritic cells responsible for antigen presentation. Since many vaccines are delivered via skin, conjugating these ligands on nanoparticles could therefore reduce the side-effect and required dose. To develop molecules into targeting ligand,

(I): SAR study were carried out based on the HSQC data. The SAR analysis provided information about optimal linker sites and replacements to improve solubility and affinity.

(II): their binding sites were validated through solvent Paramagnetic Relaxation Enhancement (sPRE) NMR.

(III): the problem that hydrophobic ligands are inserting into the lipid bilayer of the liposome was addressed by conjugating a hydrophilic peptide spacer, which is composed of lysine and aspartic acid.

The outlook on this project is combining these ligands including the carbohydrate ligand that we developed based on the previous reported structure on the nanoparticle which yield multivalency that could provide better selectivity and affinity.

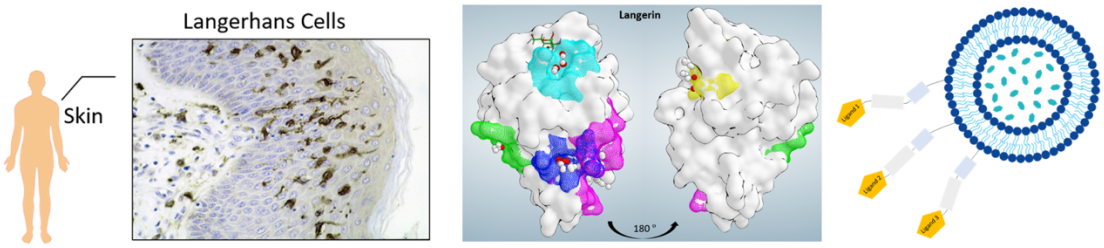

Figure 1. (a) Neoglycolipids. (b) Supramolecular characterization. (c) Heteromultivalency.

Acknowledgements

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Synthesis of Alkyne-tagged Ribitol-5-phosphate as Metabolic Labelling Tools

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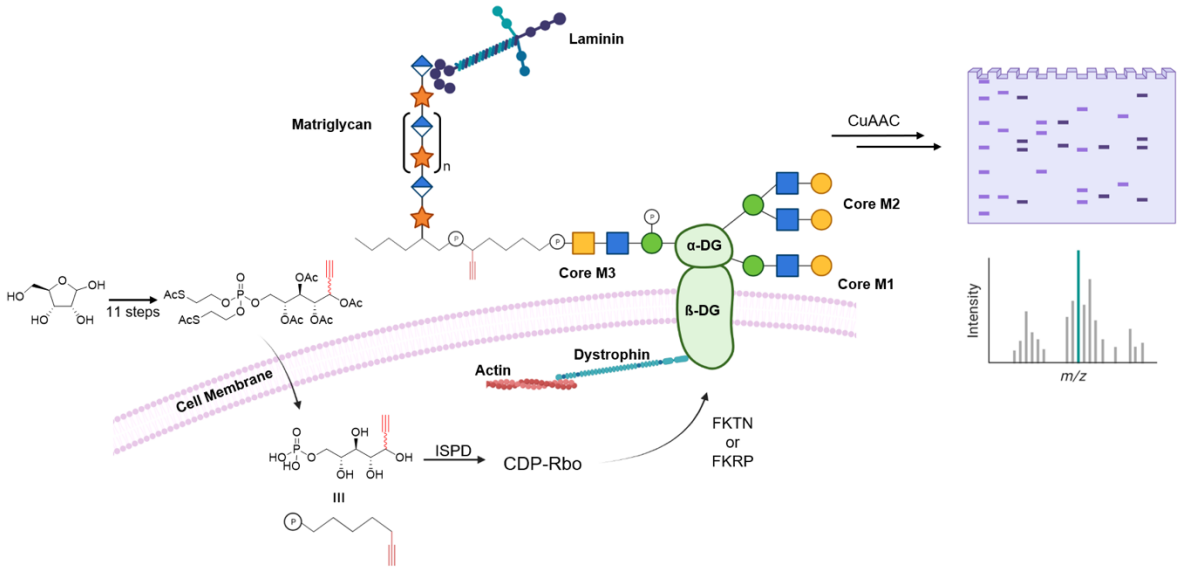
α-Dystrogylcan (α-DG) is a cell surface protein present on the cell membrane in peripheral nerve, skeletal muscle, and brain tissues. α-DG is heavily glycosylated and one of its glycans (core M3) is essential for interactions between the cell and laminins in the extracellular matrix.[1]Failures in the regular biosynthesis of this glycan result in a range of congenital muscular dystrophies, sub categorised as α-dystroglycanopathies.[2]

In 2016, two groups independently elucidated the structure of a linker region consisting of a tandem ribitol-5-phosphate (Rbo5P) responsible for maintaining the extracellular interactions. This was first, and to date only, discovery of Rbo5P in mammalian tissue.[3,4]

This makes Rbo5P a unique target to label α-DG. We therefore aim to build a library of ribitol and Rbo5P derived metabolic labelling tools.

The work presented herein, describes the development of a challenging synthesis towards a range of alkyne-tagged ribitol and Rbo5P derivatives. We employ these derivatives in mammalian cells and use bioorthogonal reactions with azide containing fluorophores or biotin to analyse the glycoprotein. These tools also enable us to potentially identify new Rbo5P containing glycoproteins.

We also report the development of a new methodology for the installation of a cell liable protected phosphotriester without the use of hazardous phosphoramidate intermediates.



Incorporation of an alkyne-Rbo5P probe into α-dystroglycan

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Synthesis of β-D-mannopyranosyl derivatives from a novel 2,3;4,6-Di-O-isopropylidene-D-glucal

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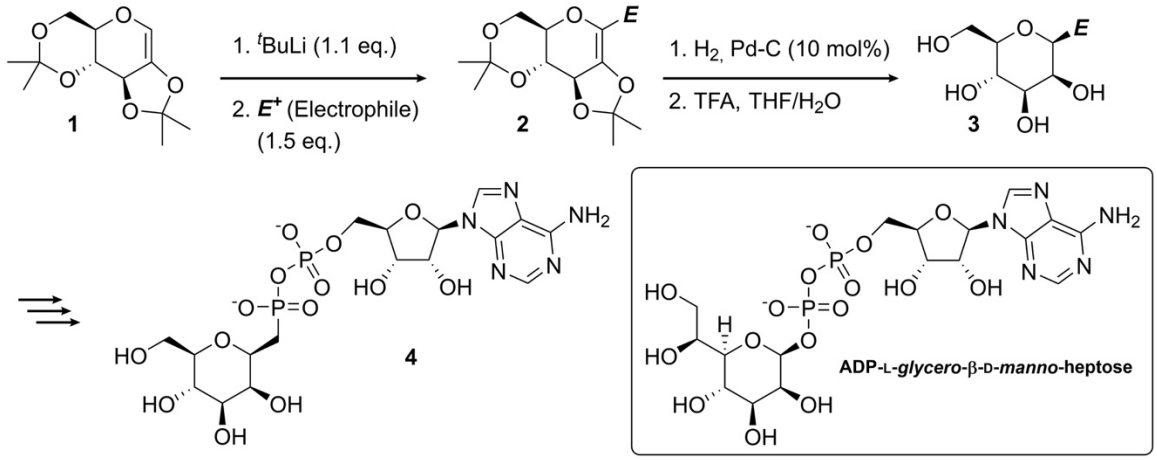
Carbohydrates, as the electrophilic species in glycosylation reactions, are usually limited by the variability of aglycones that can be introduced into the sugar moiety.[1] Therefore, glycals (1,2-unsaturated derivatives of carbohydrates) were designed to enable α-metalation of their vinyl ether functionality.[2] However, the application of these C-1 metalated glycals is complicated by the subsequent stereoselective hydroxylation of C-2 resulting in a fully hydroxylated glycosyl derivative.[3-5] To avoid this step of oxidation, synthesis[6-7] and C-1 metalation of 2,3:4,6-di-*O*-isopropylidene-D-glucal (**1**), as an innovative protected 2-hydroxyglycal, have been optimized. We have proved that this C-1 lithiated intermediate can be efficiently used for the high-yield introduction of various electrophiles under relatively mild conditions. Furthermore, we have also shown that the obtained C-1 substituted protected 2-hydroxyglycals **2** can be easily transformed into the most challenging β-D-mannopyranosyl derivatives **3** with exclusive β-stereoselectivity. Using the approach mentioned above, the synthesis of ADP-l-*glycero*-β-d-*manno*-heptose analogue **4** as a potential inhibitor of bacterial heptosyltransferase was optimized.



Use of 2,3:4,6-di-*O*-isopropylidene-D-glucal (**1**).

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Mucin Glycoprotein Microarray towards Glycan Ligand discovery for CBMs of Human Gut Microbiota

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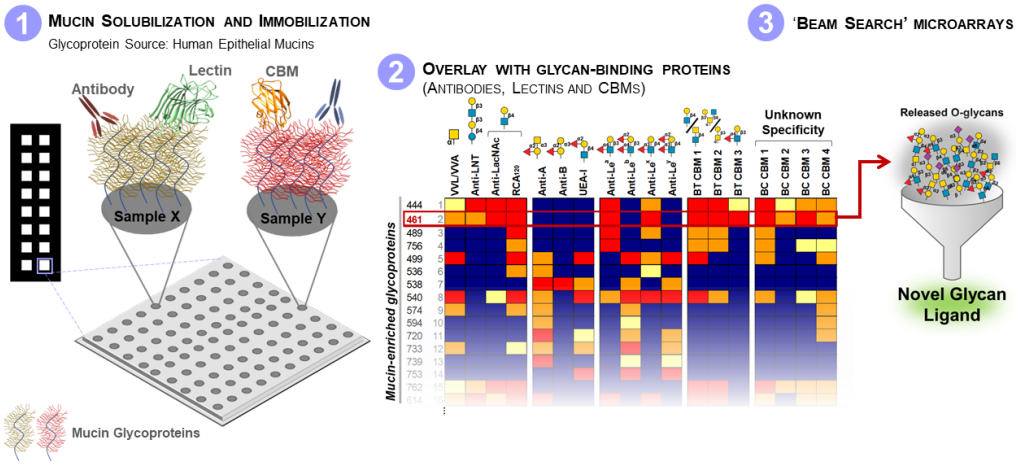
The mucus layer of intestinal epithelium contains extensively *O*-glycosylated proteins, mucins. How mucin O-glycans are differentially exploited by the microbiota and influence the crosstalk with the human host largely remains to be elucidated at the molecular level [1].

The commensal gut microbiota *Bacteroides caccae* is implicated in the digestion of the colonic mucus layer in low fibre diet conditions [2]. During growth on mucin-type glycans, *B. caccae* showed an increased expression of modular enzymes, comprising appended non-catalytic carbohydrate-binding modules (CBMs). The hypothesis is that these CBMs facilitate mucin foraging by the bacteria and thereby render the intestinal epithelium susceptible to pathogen infection, promoting states of dysbiosis [1, 2].

To identify glycan ligands targeted by the bacterial CBMs, we developed microarrays containing mucin-enriched glycoprotein samples that originated from diverse human epithelial cell types as found in the teratomatous tissues of ovarian cystadenomas. They present structurally diverse and complex O-glycans that are representative of the human O-glycome [3].

In initial screening analyses the CBMs showed differential binding to microarrays of various mucin-enriched cystadenoma samples. However, some CBMs did not show binding in our existing sequence-defined glycan microarrays suggesting the recognition of novel O-glycan ligands. Thus, these mucin glycoproteins are an important starting point for the development of mucin O-glycome 'Beam Search' microarrays [4] for discoveries of novel glycan ligands.



Mucin Glycoprotein Microarray Workflow for Glycan Ligand Discovery

Acknowledgements

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In planta engineering of KDNylated glycoproteins

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The 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a seldom studied sugar residue in the family of sialic acids [1]. Unique characteristics of KDN are resistance to sialidase, termination of polysialic acid chain length, and its presence as free sugar in certain tumors. In contrast to Neu5Ac, the biological function and immunogenic profiles of KDN carrying glycoconjugates are largely unknown. The KDN and Neu5Ac biosynthetic pathways overlap at different levels, making it highly challenging to produce KDN in mammalian cells. To address this challenge, we considered advantage of plants lacking sialic acid biosynthesis pathway in engineering approaches.

For in planta engineering a donor substrate for KDNylation, two proteins involved in the KDN biosynthetic pathway are co-expressed, the human N-acetyl neuraminic acid synthetase (hNANS) synthesizing KDN and the CMP-sialic acid synthase 2 from Zebra fish (ZfCMAS2) to activate the KDN to CMP-KDN [2]. Quantitative analysis showed a measurable amount of free KDN and CMP-KDN, indicating the ability of plants in synthesizing CMP-KDN. To synthesize KDNylated glycoproteins, we co-expressed a reporter glycoprotein along with 2 proteins required for CMP-KDN synthesis and 3 proteins involved in the mammalian sialylation pathway.

The mass spectrometry analyses of reporter highlights the ability of plants to synthesize KDNylated proteins. However, they also point to the need for optimization of the engineering strategies to obtain quantitatively KDNylated proteins for further functional studies.

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Lectins on Beads: Selection of Glycopeptide Ligands for Galectin 1 Using OBOC Libraries

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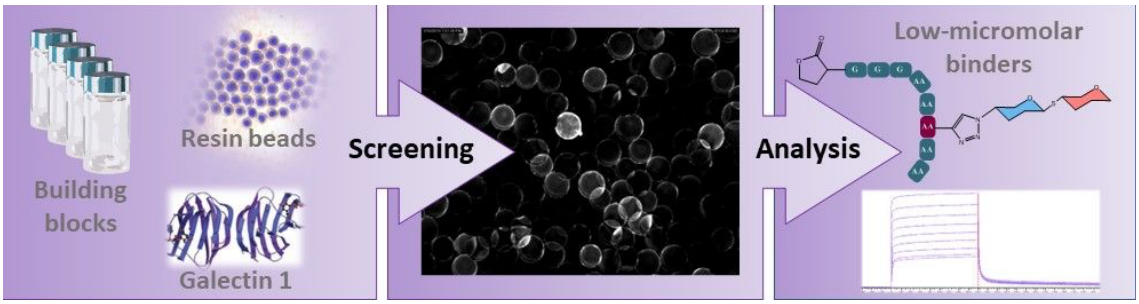
Combinatorial chemistry is an elegant concept, based on a combination of simple motifs to provide complex mixtures of target compounds – combinatorial libraries. We exploited diversity present in those libraries to create a very simplified, fractioned version of cell surface. By a combination of two powerful methods – SPPS and CuAAC [1,2] - we prepared libraries of glycosylated peptides and used them as a tool to study behavior of protein Galectin 1.

Galectin 1 is a small lectin (25 KDa) with many functions in human body [3]. Among others, Galectin 1 is expressed in cancer cells to help the tumor grow and escape our immune system, its expression is also correlated with obesity and diabetes [4]. A member of the Galectin family, Galectin 1 is a soluble protein known to bind both saccharides (Galβ1, lactose, Galβ1-4GlcNAc on cell surface glycoconjugates) and proteins.

Glycopeptide library consisting of peptides modified with S-Lactose (Galβ1-S-4Glc) was designed and screened against recombinant Galectin 1. The two-phase screening process and subsequent control experiments selected two low-micromolar binders of Galectin 1. The fact that their affinity to Galectin 1 is higher than that of S-Lactose alone confirms the role of the underlying peptide in the binding interaction.



Acknowledgements

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Synthesis of new iminosugar-sugar disaccharides-Access to sp² iminosugars through a one-pot reaction

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Iminosugars are potent glycomimetics in which the endocyclic oxygen is replaced by a nitrogen atom. This characteristic gives them unique inhibition properties towards carbohydrate active enzymes. Many syntheses have been reported and some iminosugars are already commercialized as drugs like miglitol (type 2 diabetes) and miglustat (Gaucher disease). [1]

Although potent, they sometimes lack selectivity thus leading to side effects. The development of iminosugar-sugar disaccharides aims at building new efficient and more selective inhibitors. In the literature, numerous examples of disaccharides with the iminosugar at the reducing end have been reported, as well as disaccharides with the iminosugar at the non-reducing end, but with a carbon glycosidic bond. [2]

In this communication, we present the synthesis of iminosugar-sugar disaccharides having an oxygen glycosidic bond between the iminosugar and the sugar as well as a new one pot reaction to obtain sp² iminosugars via a cyclisation/deprotection/glycosylation one-pot reaction. It is a very versatile scaffold allowing many modifications to design enzyme specific molecules and other probes. [3]

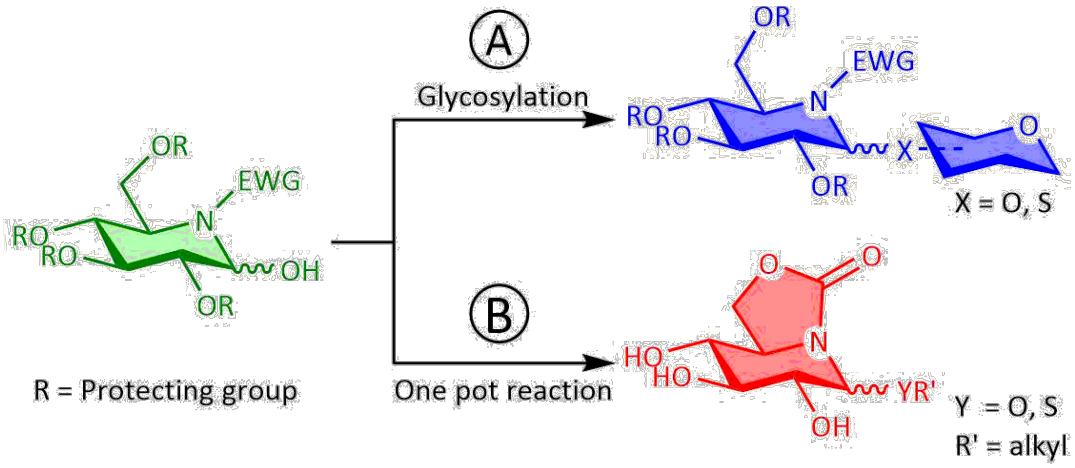

Figure 1: (A) New iminosugar-sugar disaccharides (B) Original one-pot access to sp² iminosugars

Acknowledgements

I would like to thank Region Centre-Val de Loire for funding my thesis.

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Exploration of long range participation in 3,4-diacetylated glycosyl donors

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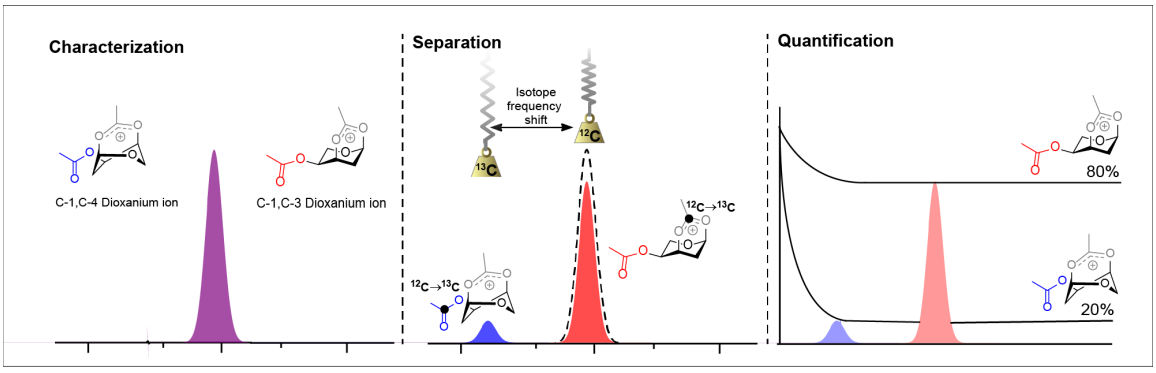
The key challenge in chemical oligosaccharide synthesis is the stereoselective installation of glycosidic bonds. Each glycosidic linkage has two stereo-outcomes, α/β or *cis*/*trans* and is affected by the reaction mechanism operating on a SN1-SN2 continuum. One of the most established approaches to reliably install 1,2-*trans* glycosidic bonds is neighboring group participation (NGP) mediated by a 2-*O*-acyl group. Through formation of a transient bicyclic intermediate, the C-1,C-2 dioxolenium ion, effectively one face of attack is shielded which enhances reaction stereoselectivity. The extension of this intramolecular stabilization to nucleophilic groups located at more remote positions has also been suggested but remains a controversial topic and is poorly understood. However, to improve the stereoselective synthesis of glycans, characterizing these highly-reactive intermediates and understanding their role in glycosylation mechanisms is crucial. Previously, we employed infrared ion spectroscopy to characterize the molecular ions of monoacetylated sugar donors and showed how the strength of the stabilizing effect depends on the position of the participating ester group on the glycosyl donor ring as well as its relative stereochemistry. In this work, we extended this workflow towards diacetylated glycosyl donors. This study highlights the potential of isotope labelling in resolving spectra of compounds bearing multiple structurally similar functional groups. In combination with population analysis a powerful combination is created that allows one to disentangle isomeric mixtures and establish the relative contribution of individual isomers.



Acknowledgements

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Incorporation of non-canonical amino acids toward the engineering of artificial metalloenzymes

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Metals are present in almost half of all enzymes to fulfill structural and/or catalytic functions. Metalloenzymes have been successfully engineered for biocatalysis, often by modifying the substrate or metal specificity of a natural metalloenzyme [1]. An alternative to these enzymes consists in the insertion of a metal complex into a versatile protein scaffold able to accommodate various substrates and/or reactions [2]. In this study, the goal is to preserve the natural enzyme's substrate specificity while adapting new-to-nature metal-based reactivity through active site remodelling. However, designing a metal-binding site is not trivial as it often requires a proper positioning of several amino acid residues (usually a histidine brace motif, plus an additional residue) to ensure an efficient coordination of both the metal ion and the *ad hoc* substrate. To circumvent this obstacle, bipyridyl-alanine (BpyA), a metal-chelating non-canonical amino acid (ncAA), was used.

NcAA incorporation can be achieved by reassigning a nonsense codon to a ncAA by the use of an orthogonal amino-acyl tRNA synthetase (aaRS)/tRNA pair [3]. Despite some achievements over the last 15 years, this approach still suffers from highly variable and often low incorporation efficiencies, which can be deterrent.

Based on the historical and widely used pEVOL incorporation system [4], we developed the pINS systems by altering the tRNA and aaRS expression levels. Several amino acid positions identified by molecular modelling were selected to incorporate BpyA in the vicinity of the catalytic site of our model enzyme. Using the pINS system, both BpyA incorporation efficiency and protein production were improved with success, reaching production levels comparable to the wild-type enzyme. For each targeted position, the proper incorporation of BpyA was confirmed by mass spectrometry and X-ray crystallography. Interestingly, the reorientation of a neighboring amino acid side chain was observed to obtain a complete metal ion coordination in the absence of a bound substrate. Moreover, the overall 3D structure and thermal stability of the mutants were found to be poorly affected by the introduction of BpyA. Future experiments will investigate the substrate-binding properties of the BpyA mutants and their catalytic potential.

Acknowledgements

This work was performed in the framework of the Carnot 3BCAR project NeoZYM (2021-2023).

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Structural and functional diversity of the *N*-acetyl hexosaminidases from *Akkermansia muciniphila*

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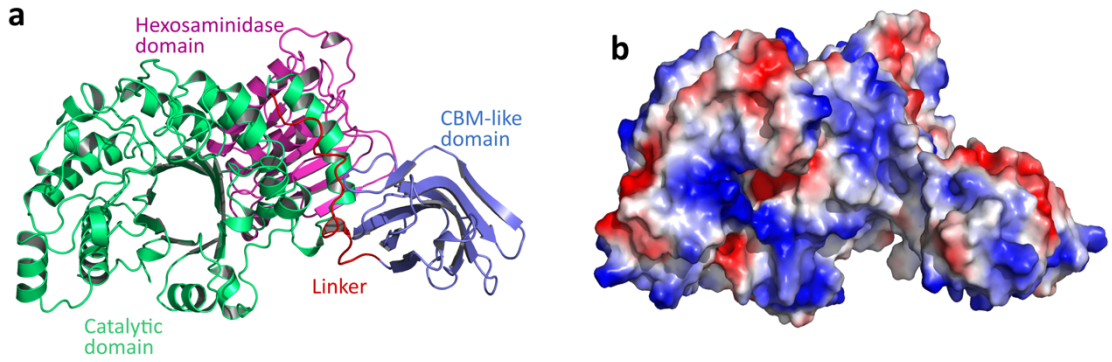
Akkermansia muciniphila is an atypical metabolic specialist of the human Gut Microbiota (HGM), which colonizes the outer layer of gastrointestinal mucus [1]. *A. muciniphila* specializes in the degradation of mucin, a heavily *O*-glycosylated glycoprotein that form the major component of mucus [2].

The most represented family of glycoside hydrolases (GHs) in the genome of *A. muciniphila* is family 20, comprising 11 enzymes. Here, we screen the specificity of the aglycone (-1) subsites of the aforementioned enzymes, using *para*-nitrophenyl β-d-*N*-acetylglucosamine/galactosamine (*p*NP-β-GlcNAc/*p*NP-β-GalNAc) to shed light on their functional diversity. The GH20 enzymes displayed mostly dual HexNAc activity, but some displayed a strong preference for one of these two HexNAc components of mucin.

We also report the structure of a previously uncharacterized GH20 from *A. muciniphila*. Comparison of the new structure to previously characterized counterparts from the same bacterium, revealed commonalities and differences, most notably, the presence of a C-terminal uncharacterized and structurally unique domain, which may resemble a new binding domain, and unique loop conformations around the active site. These findings that promote our understanding of the exoglycosidases machinery that *A. muciniphila* deploys to harness mucin *O*-glycans for growth will be presented.



(A) Overview of GH20 domains (B) Electrostatic surface potential

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Glycoproteomics-compatible MS/MS-based quantification of glycopeptide isomers

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Glycosylation is an essential protein modification occurring on the majority of extracellular human proteins, mass spectrometry (MS) being an indispensable tool for its analysis. Not only can MS determine glycan compositions, but also position the glycan at specific sites via glycoproteomics. However, glycans are complex branching structures with monosaccharides interconnected in a variety of biologically relevant linkages such as α2,3- and α2,6-linked sialylation (1,2) - isomeric properties which are invisible when the readout is mass alone. We developed an LC-MS/MS-based workflow for determining glycopeptide isomer ratios. Making use of isomerically-defined glyco(peptide) standards, facilitated by methods of chemoenzymatic synthesis (3), we observed marked differences in fragmentation behavior between isomer pairs when subjected to collision energy gradients, specifically in terms of galactosylation/sialylation branching and linkage (figure 1). These behaviors were developed into component variables that allowed relative quantification of isomerism within mixtures. Importantly, at least for small peptides, the isomer quantification appeared largely independent from the peptide portion of the conjugate, allowing broad application of the method. If this broad application is successful, MS-based differentiation between structural glycan isomers would not only revolutionize the field of glycoproteomics, but also, for example, open up new insights into the mechanisms behind diseases and enable better therapeutical antibody design and quality control.

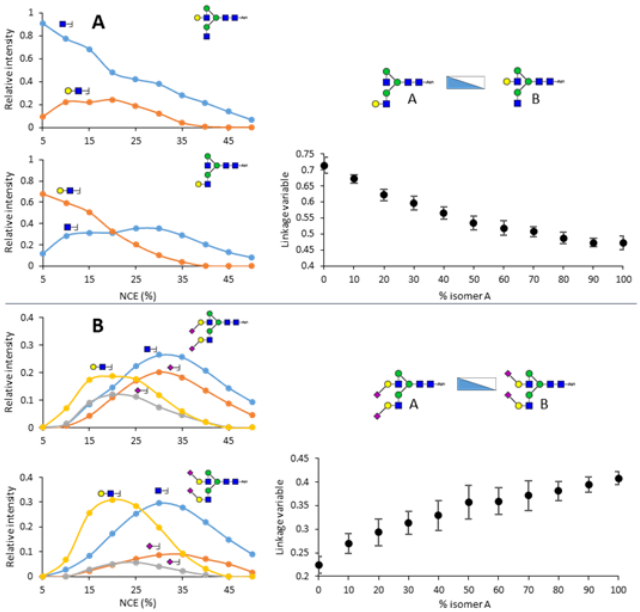

Figure 1: HCD-MS/MS patterns and average linkage variables with standard deviations for galactose branching (A) and sialic acid linkage (B) isomers.

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Structural characterization of immunoglobulin G epitope (N-glycans) by MS-IR

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Mass spectrometry (MS) has become an essential part of the glycomics toolbox, allowing for instance to characterize N-glycans cores. N-glycosylation is a post-translational modification which may be involved in many diseases including rheumatoid arthritis (RA). However, MS is insufficient to describe entirely the terminal sugars of N-glycans (known as epitopes). Epitopes are not well understood because they present many isomers and they may carry diverse functional modifications introducing a lot of heterogeneity.

To characterize epitopes, we propose an original approach based on the coupling of tandem MS and infrared ion spectroscopy: InfraRed Multiple Photon Dissociation spectroscopy[1]. A mass spectrometer was modified to introduce a tunable IR laser inside the ion trap allowing to obtain simultaneously a mass spectrum and an IR fingerprint. These fingerprints will be helpful to characterize the isomerization of the compound.

Considering the importance of sialylated extremities in RA, we currently focused our research on this epitope present on immunoglobulin G. In this case, sialylated extremities will be present in two forms: sialic acid will be linked to a galactose via an α2.3 or an α2.6 linkage. Our strategy allows to determine their ratio. They can also present a rare modification: an O-acetylated group. Preliminary data on the position of O-acetylated groups are promising because regioisomers show distinctive IR fingerprints. Therefore, our approach could be a helpful tool for an early diagnostic of RA, using these rare glycome anomalies as biomarkers.

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Synthesis of isotopically labelled substrates for KIE studies of human GDP-L-fucose synthase

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Human GDP-l-fucose synthase (hGFS)[1] converts GDP-4-keto-6-deoxy-d-mannose into GDP-l-fucose in three distinctive steps: two epimerisations at C3" and C5" positions of the l-fucose sugar ring followed by NADPH-dependent reduction at C4". Currently, the identity, and the precise roles in catalysis, of active-site residues of hGFS are not fully understood. In this research, therefore, we decided to probe the hGFS reaction by kinetic isotope effects (KIEs), in order to dissect the multistep catalytic mechanism of the enzyme[2, 3].

For kinetic isotope effect measurements, we first show enzymatic synthesis and isolation of site-selectively deuterium labeled substrates: (4S)-[^{2}H]-NADPH, [3"-^{2}H]- and [5"-^{2}H]-GDP-4-keto-6-deoxy-d-mannose. Then, we used a combination of steady state and stopped flow kinetic measurements to obtain KIEs on the turnover number (k_{cat}) and the first-order rate constant of the kinetc transient. Using the wildtype hGFS, KIEs due to [3"-^{2}H] and [5"-^{2}H] are not different from unity, indicating that the proton abstractions for epimerisation at the C3" and the C5" are not rate-determining at steady state. The reduction appears to be only partially rate-determining due to (4S)-[^{2}H]-NADPH (KIE $\simeq$ 1.4). Stopped flow experiments reveal a rapid burst of NADPH consumption, suggesting that the k_{cat} is limited by release of the product. Measured KIEs for several active site mutants support the notion that Y143 is involved in hydride transfer for reduction and the pair of general-base and general-acid catalytic residue for epimerisations is represented by C116 and H186.

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Computer-assisted protein enginerring of glycoside hydrolases with BINDSCAN

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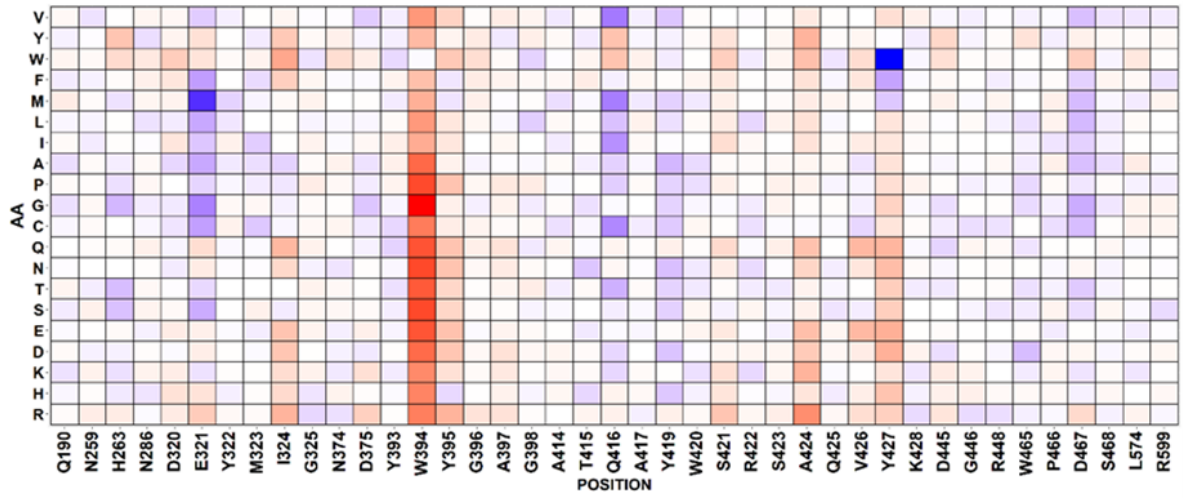
BINDSCAN is an in-house computational protocol aimed at assisting in the design of biocatalysts. It simulates and predicts mutational effects on a protein-substrate complex with the objective of modulating different enzymatic properties such as substrate specificity, enzymatic reactivity or stability in the context of biocatalysis. BINDSCAN can be used for either enhancing protein's performance or to redesign its function towards novel substrate specificities. It is especially useful when it is necessary to expand the range of chemical structures on which a specific enzyme is bioactive. The protocol is composed of three separate modules: 1) generation of the enzyme mutant library, 2) modelling the 3D structure of the protein-substrate complex and 3) evaluation of specificity and reactivity metrics. Mutational effects on the target property can then be analyzed with different selection criteria and graphical representations

BINDSCAN has been applied to different CAZYmes in our group including glucosidases, fucosidases, hexosaminidases, xylosidases and transglycosidases among others [1, 2]. Here we will present the recent advances incorporated to the protocol and its validation against massive experimental data on the hydrolytic activity of mutants of Spodoptera frugiperda β-glucosidase (O61594, GH1) [3]. Results showed that BINDSCAN can effectively be used for the identification of mutation sites to redesign enzymatic activity. Statistical analysis comparing experimental data with BINDSCAN results using different metrics will be presented and the method sensitivity and specificity discussed.



BINDSCAN example heatmap. Color-intensive spots are to be considered for experimental protein design

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Investigating Hydrogen bond donating ability of deoxygenated and deoxyfluorinated carbohydrates

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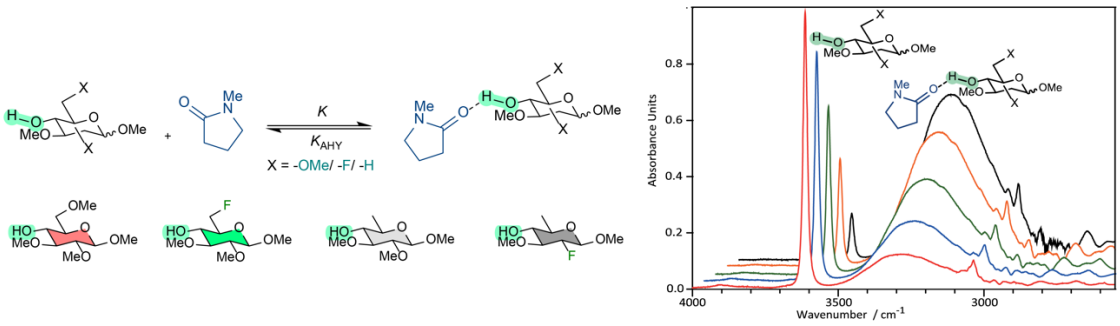
The Hydrogen bond (HB) is one of the main interactions between carbohydrates and proteins. There is little detailed knowledge of HB properties of individual alcohol groups in carbohydrates. Based on our previous work on HB donating (HBD) properties of alcohol groups in model compounds [1], this work aims to chart the HBD properties of alcohols in important sugars, and how this is influenced by substitutions such as deoxygenations and deoxyfluorinations.

The HBD capacity is measured as the equilibrium constant between an alcohol and a standard acceptor (*N*-methylpyrrolidinone, NMP), and achieved by FTIR through the absorption bands of the free and hydrogen-bonded O–H bonds. [2]

This IR-based method is only suitable for monohydroxylated compounds. Hence, initial series of model substrates have been synthesised with the 4-OH group unprotected. For some compounds, both anomers were investigated. Furthermore, the influence of deoxygenation and deoxyfluorination at the 6- and 2-positions was investigated.

The data show that despite the presence of intramolecular OH•••OMe hydrogen bonding, the alcohol groups of carbohydrates display a hydrogen bond donating capacity similar to that of isolated alcohols, and that there are marked differences upon changing stereochemistry or introducing remote deoxygenation and deoxyfluorinaton. These results will be valuable for the interpretation of binding data of carbohydrates and glycomimetics to proteins.



Determination ohydrogen-bond (HB) donating ability of an alcohol by infrared (IR) spectroscopy

Acknowledgements

MN thanks FWO [Odysseus] for funding

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Method for the identification of novel glycoproteins modified with ribitol-5-phosphate

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Alpha-dystroglycan (αDG) is an O-mannosylated protein, part of the dystrophin-glycoprotein complex present on the membrane of mammalian cells, whose function is to give rigidity to tissue through binding laminin in the extracellular matrix[1]. αDG has a unique O-glycan, essential for its function, made of a core M3 elongated with two ribitol-5-phosphate (Rbo5P) residues. Terminal Rbo5P acts as a linker to a long chain of alternating xylose and a glucuronic acid named matriglycan. Rbo5P is found in mammalian cells uniquely on αDG[2-3]. Enzymes responsible for the synthesis of the alditol donor CDP-Rbo and for Rbo5P transfer onto the O-mannosyl glycans have been identified[4]. A whole pathway is dedicated to this modification therefore we hypothesize that other mammalian proteins might have glycans with Rbo5P and share a similar function to αDG. Novel glycoconjugates carrying Rbo5P will be identified through the chemical tagging of Rbo5P. In particular, Rbo5P is susceptible to oxidation with sodium periodate which generates an aldehyde on the glycoprotein.

The aldehyde can be tagged using a bioorthogonal conjugation with a biotin probe and purified with a streptavidin column. Glycoproteins carrying Rbo5P enriched this way can then be digested into peptides which are identified by mass spectrometry.

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Mucin-like glycocalyx modules for creatingcomplex artificial glycocalyxes

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The glycocalyx on the outer surface of cells is made of glycoproteins, glycolipids, and proteoglycans, and accomplishes many crucial functions in the communication of the cell with its environment.[1] Cholera toxin (CT), a lectin secreted by *Vibrio cholerae* that causes life-threating diarrhea, targets Lewis^y and Lewis^x carbohydrate antigens in this layer in addition to ganglioside GM1 in the cell membrane.[2,3] Shiga toxin (ST), a lectin secreted by *Shigella dysenteriae* type 1 and some strains of *Escherichia coli*, binds to the Gb_3 glycosphingolipid on the cell membrane and causes haemorrhagic colitis and hemolytic uremic syndrome.[4–6]

Even though the general mechanisms of infection for both CT and ST are well known, how these proteins interact with the glycocalyx en route to infecting the host cell remains poorly understood. The complexity of the glycocalyx, and the scarcity of tools to control and analyse glycocalyx composition and organisation, make mechanistic studies *in vivo* and *in vitro* challenging.

We aim to generate well-defined glycocalyx models to understand how CT and ST interact with the cell surface. To this end, hyaluronan (HA) and Lewis^x or Gb_3 oligosaccharides were prepared with appropriate bio-orthogonal reactive groups to allow their conjugation, followed by the incorporation of a biotin group on the reducing end of the HA backbone, to build a well-defined mucin-like molecular structure suitable for anchorage to cell membrane models, and to perform quantitative binding studies using quartz crystal microbalance or spectroscopic ellipsometry.

Acknowledgements

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Synthesis of a potential decasaccharide vaccine-candidate against Cryptococcus neoformans infections

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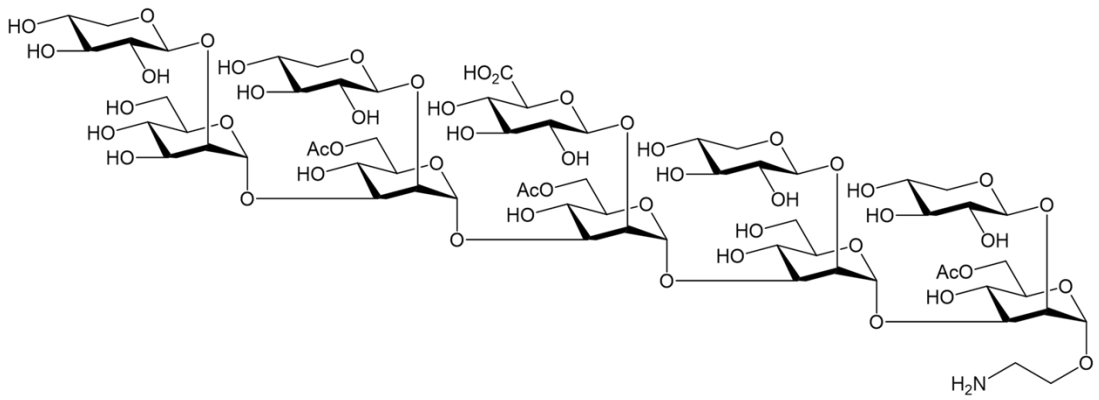
Cryptococcus neoformans causes around a million infections yearly resulting in death for more than half of the patients. [1,2] This environmental yeast is particularly deadly to immunosuppressed patients and current therapies are confronted by the emergence of resistance mechanisms. Therefore, new tools such as an efficient vaccine against the prevalent serotypes, that are type A and D, are needed. Synthetic carbohydrate-based vaccines have shown a rise of interest these past decades after the success of the *Haemophilus influenza type b* vaccine based on the use of capsular polysaccharide (CPS) conjugated to a protein carrier in 2003 (QuimiHib).[3]

Recently, Oscarson and co-workers screened 26 synthetic mono- to octodecasaccharides mimicking capsular glucuronoxylomannan (GXM) of all four serotypes of *C. neoformans* against anti-GXM monoclonal antibodies (mAbs).[4,5] It interestingly showed serotype A decasaccharide and bigger motives being of interest for vaccine development. However, it has been reported that some mAbs were protective while others were not. Same study suggested the acetylation pattern could be of importance regarding the efficiency of recognition by mAbs.

In that respect, we're developing a robust and efficient synthetic pathway towards the desired decasaccharide. The latter is aimed to be printed on microarrays for further mAbs recognition screening. Also, crystallisation and co-crystallisation with specific anti-GXM mAbs studies are underway. It may allow us to decipher the link between GXM structures and protective and unprotective properties of anti-GXM mAbs.



Targeted serotype A GXM decasaccharide motif bearing an amino linker allowing further conjugation

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Design Synthesis of Sialyl Triazoles with the 2-Naphthylsulfonyl C-9 Substituent as Siglec-8 Ligands

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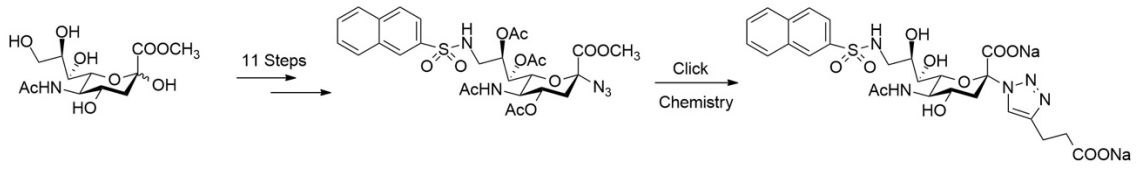
Siglecs are sialic acid-binding immunoglobulin [Ig]-like lectins, a family of single-pass transmembrane cell surface proteins in humans [1]. Siglec-8 is a human immune-inhibitory receptor is expressed by human eosinophils, basophils and mast cells [2]. Siglec-8 upon binding with antibodies or glycan ligands results in apoptosis in human eosinophils and inhibits the release of mediators from human mast cells without affecting their stability. Thus, potential glycan ligands may be ideally used as inhibitors for treatment of eosinophil and mast cell-related diseases, such as asthma, chronic rhinosinusitis, chronic urticaria, hypereosinophilic syndromes, mast cell and eosinophil malignancies and eosinophilic gastrointestinal disorders by targeting Siglec-8.[3].

In a previous study, mimetics of 6'-sulfo-sLe^x, a specific glycan ligand for siglec-8, were identified. Keeping the neuraminic acid (sialic acid), while replacing the galactopyranose with a cyclohexyl derivative led to a higher affinity ligand[4] with significantly reduced carbohydrate character. Here, we will present the design and synthesis of 2-naphthyl sulfonyl C-9 based sialic acid ligands with a triazole ring having carboxylate and sulphate group as a replacement for the sulphated galactose residue. The triazole moiety has predicted additional interactions with siglec-8 based on glide-docking.



Scheme of Azide Intermediate

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Molecular cloning and characterisation of two novel mollusc T-synthases

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Glycoprotein-N-acetylgalactosamine β1,3-galactosyltransferase (T-synthase, EC 2.4.1.122) catalyses the transfer of the monosaccharide galactose from UDP-Gal to GalNAc-Ser/Thr, synthesising the core 1 O-glycan structure (T-antigen) of mucin-type O-glycans. These structures play very important role in animal development, immune response and recognition processes.

Molluscs are the largest and most successful groups of animals on the planet and can be found in a wide range of environments, including freshwater, marine, and terrestrial habitats. They play a significant role in many ecosystems as they are important filter feeders, decomposers but also pests in agriculture and intermediate hosts of many human and cattle parasites. Their ability to produce complex glycans is a fundamental aspect of their biology. The first mollusc T-synthase is already characterised [1] and identification of novel carbohydrate active enzymes will give a better understanding of their glycosylation abilities and help in elucidating their successful adaptation and survival abilities.

The sequences of the enzymes were identified by homology search using the *B.glabrata* T-synthase sequence (QXN57605.1) as a template. The genes code for transmembrane proteins with two putative N-glycosylation sites for *Pomacea canaliculata* and one putative N-glycosylation site for *Crassostrea gigas*. The coding sequences were synthesised and expressed in Sf9 cells. The expression product of the putative enzymes displayed core 1 β1,3-galactosyltransferase activity using pNP-α- GalNAc as the substrate. These enzymes showed similar biochemical and structural parameters with previously characterised T-synthases from other species

In this study, we present the identification, cloning, expression and characterisation of the glycoprotein-N-acetylgalactosamine β1,3-galactosyltransferase from *Pomacea canaliculata* and *Crassostrea gigas* in comparison with the previously characterised one from *B. glabrata.*

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Acknowledgements

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

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Characterization of Rhamnosyl Dioxanium Ions and Their Application in Oligosaccharide Synthesis

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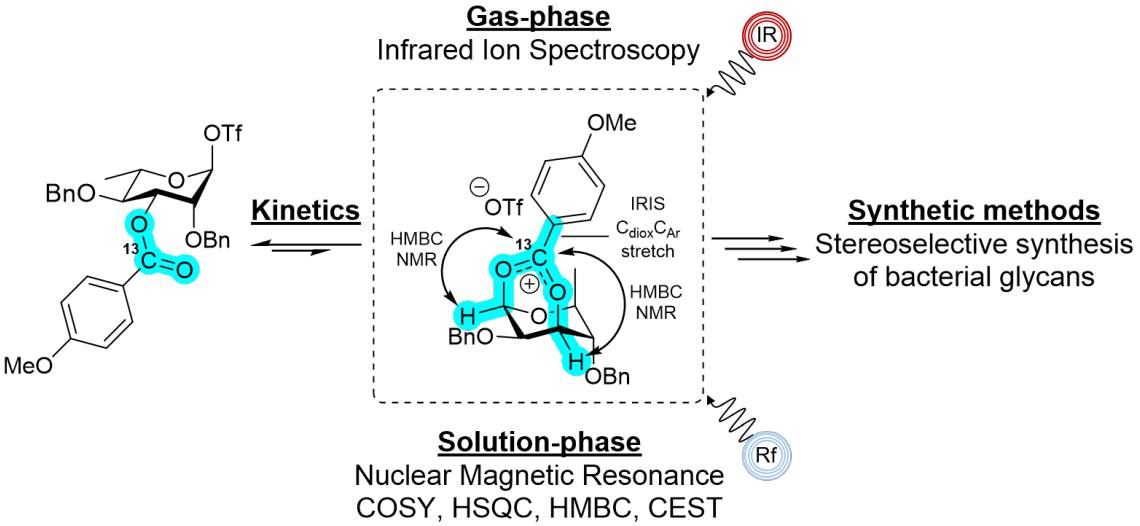
Attaining complete anomeric control remains to be one of the biggest challenges in carbohydrate chemistry. Glycosyl cations such as oxocarbenium and dioxanium ions are key intermediates of glycosylation reactions. Characterizing these highly-reactive intermediates and understanding their glycosylation mechanisms is essential in the stereoselective synthesis of complex carbohydrates. Although C-2 acyl neighbouring-group participation (NGP) has been well-studied, remote participation remains a more elusive process and is difficult to study. Herein, we report the consecutive characterization of rhamnosyl 1,3-bridged dioxanium ions derived from C-3 p-anisoyl ester rhamnosyl donors. First, we used a combination of quantum chemical calculations and infrared ion spectroscopy (IRIS) to determine the preferred glycosylation intermediate in the gas-phase. In addition, we established the structure and exchange kinetics of highly-reactive, low-populated species in the solution-phase using chemical exchange saturation transfer (CEST), COSY, HSQC and HMBC NMR spectroscopy. Finally, we applied this methodology to the synthesis of complex bacterial oligosaccharides. This combination of answering fundamental chemical questions using stated methods and applying them in organic synthesis provides a robust basis for developing stereoselective glycosylation tools and reactions.



Selective conformational inhibitors for glycosidases using cyclic sulfates and sulfamidates

Ken KOK [1], Alexandra MALES [2], Alba NIN-HILL [c], Jeroen D. C. CODÉE [4], Herman S. OVERKLEEFT [4], Carme ROVIRA [3], Gideon J. DAVIES [2], Marta ARTOLA [1]

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Selective inhibition of glycosidases by small molecule inhibitors has proven to be an essential tool to study glycosidases, their mechanisms and their role in complex biochemical pathways and on a larger scale, diseases. These enzymes can usually be divided into two groups based on their reaction mechanism. These mechanisms go through various conformational itineraries to navigate through any problems that might arise from any steric or electronic features. Mimicry of these conformational itineraries has been used to develop inhibitors for various glycosidases including GH47-α-mannosidases. This group of enzymes is essential for the maturation and quality control of glycoproteins in the secretory pathway. Previously, *cis*-cyclic sulfates electrophiles have shown to be covalent nanomolar inhibitors for α-glucosidases by mimicking there $^4C_1$Michaelis conformation (Figure 1).[1] A similar strategy has been applied to develop *cis*-cyclic sulfamidates which by introducing a nitrogen atom at the pseudo-anomeric position were able to function as selective competitive inhibitors for α-galactosidase and glucosidase.[2,3] In this study we describe the development of *trans*-cyclic sulfates and sulfamidates, which by virtue of their *trans*-stereochemistry switch to a $^1C_4$ chair conformation. Since GH47-α-Mannosidases follow a unusual $^3S_1$ (Michaelis complex) $^3H_4$ (transition state) $^1C_4$ (product) conformational itinerary, we capitalized on the $^1C_4$ trans-cyclic sulfates and sulfamidates to develop new selective inhibitors for GH47-α-Mannosidases through a bump and hole strategy (Figure 1).[4]

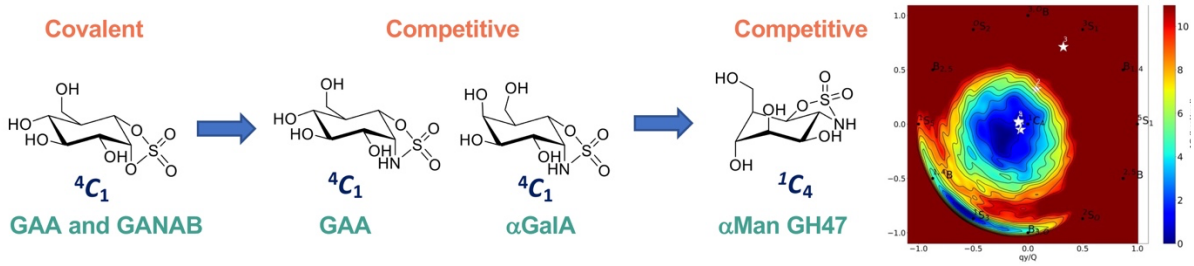

Figure 1. Cyclosulfate and cyclosulfamidate-based cyclitols as selective glycosidase inhibitors.

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In vivo evaluation of Antibody Recruiting Glycodemdrimers (ARGs) for targeted cancer immunotheraphy

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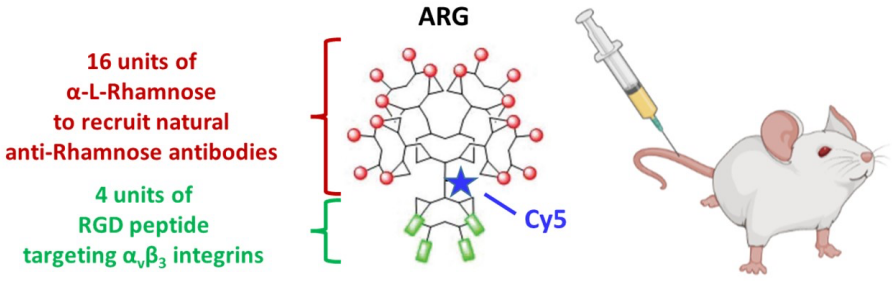
Cancer is a major cause of mortality worldwide and it is estimated that 40% of the population will be diagnosed with cancer within their lifetime. Currently, immunotheraphy with monoclonal antibodies (Abs) has become a promising strategy to fight cancer. However, its use is difficult to standardize, expensive and can lead to intolerable toxicity events. Targeted immunotherapy is an ideal choice to improve cancer treatment.

Over the last fifteen years, synthetic chemistry has allowed the development of bifunctional molecules called Antibody Recruiting Molecules (ARMs). ARMs are composed by a target binding terminus (TBT) able to bind to specific receptors on cancer cells, and by an antibody binding terminus (ABT) able to gather endogenous antibodies. The ternary complex cancer cell-ARG-Abs leads to the activation of different immunological mechanisms and, consequently, to cancer cell clearance without need for previous immunization [1].

In our group, by using supramolecular chemistry, molecular engineering, biochemistry, immunochemistry and glycoscience approaches, we have designed Antibody Recruiting Glycodendrimers (ARGs) bearing four copies of RGD peptide as TBT and sixteen copies of rhamnose as ABT. These ARGs selectively target overexpressed integrins on tumor surface and recruit the natural anti-rhamnose Abs present in human serum [2]. Furthermore, they have also been proved to promote up to 60% of selective cytotoxicity towards cancer cells in vitro [3].

In order to investigate the commercial feasibility and the *in vivo* properties of our lead compounds, we have further investigated ARGs serum stability and blood compatibility *in vitro*, as well as their kinetics, biodistribution and cytotoxic activity *in vivo*. The set of results obtained with these studies would facilitate the transfer of technology and the initiation of clinical development.



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Mechanistic Insight into the SN1 side of Benzylidene-Directed Glycosylations by Cryo-IR Spectroscopy

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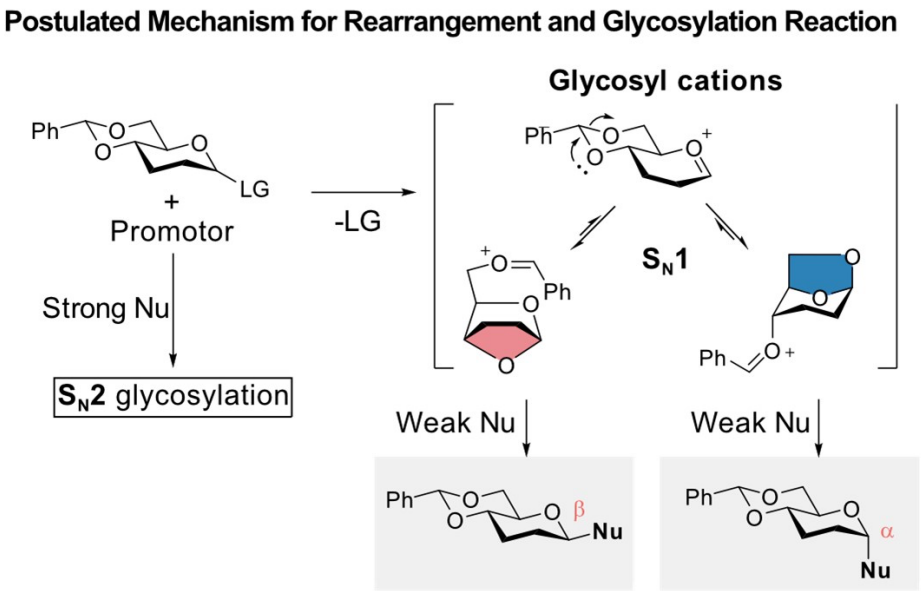
4,6-O-benzylidene directed glycosylation is one of the most promising approaches to construct 1,2-cis glycosidic bonds. However, the S_N1-side is mechanistically poorly understood due to its instability and extremely short lifetime of its intermediate – the glycosyl cation. Herein, we report an integrated strategy of cryogenic ion infrared spectroscopy and computational calculation to unravel the structure of glycosyl cations. Our results first confirm an unexpected intermediate rearrangement on 4,6-O-benzylidene glycosyl donors that leads to an energetically more stable anhydro cations. The structure of the glycosyl cation correlates with the stereoselective outcome in glycosylations via a dissociative (S_N1) mechanism. These discoveries illustrate a brand-new pathway in reaction mechanisms and provide crucial insight to origin 1,2-cis glycosylation.



Postulated Mechanism for Rearrangement and Glycosylation Reaction

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Synthesis of inhibitor probes against GH116 D-arabinofuranosidase

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D-Arabinan is found in the cell wall component of mycobacteria such as mycolyl arabinogalactan-peptidoglycan complex and lipoarabinomannan. The D-arabinan is a branched polysaccharide with linear alpha-(1,5) linkages of D-arabinofuranoside (Ara*f*) in the backbone, alpha-(1,3) branching linkages and beta-(1,2) linkages at non-reducing terminal. D-Arabinofuranosidases (Ara*f*ases) have been found in*Microbacterium* sp. [1,2] to cleave D-arabinan. As a substrate to study one of the enzymes, beta-D-Ara*f*ase (GH116), *para*-nitrophenyl (*p*NP) beta-D-Ara*f* was synthesized stereoselectively according to the synthesis of *p*NP beta-L-Ara*f*.[3] By enzymatic reactions of the substrate suggested the retention mechanism of action. For further analysis of the enzyme, the inhibitor candidates were prepared for both competitive and covalent inhibitions and were used for the inhibition study.

In the structure of the inhibitor, a substrate motif, D-Ara*f*, was included for the binding efficiency with the enzyme. Hydroxymolactone derivative [4], which can competitively interact with both carboxy groups of two acidic amino acid for acid-base catalytic and nucleophilic residues, and 2-deoxy-2-fluoro derivative that stabilize covalently bonded enzyme-substrate complexes reacted by nucleophilic residues, were designed. Hydroxymolactone derivative was synthesized by applying the synthetic procedure [5] when investigating inhibitors of beta-L-Ara*f*ase previously, and 2-deoxy-2-fluoro derivative was synthesizedfrom a known 2-deoxy-2-fluoro-D-aranbinofuranose derivative.[6] We also would like to report their inhibitory activities against the enzyme.

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Base catalyzed acylation of sterically crowded conformationally locked galactosides

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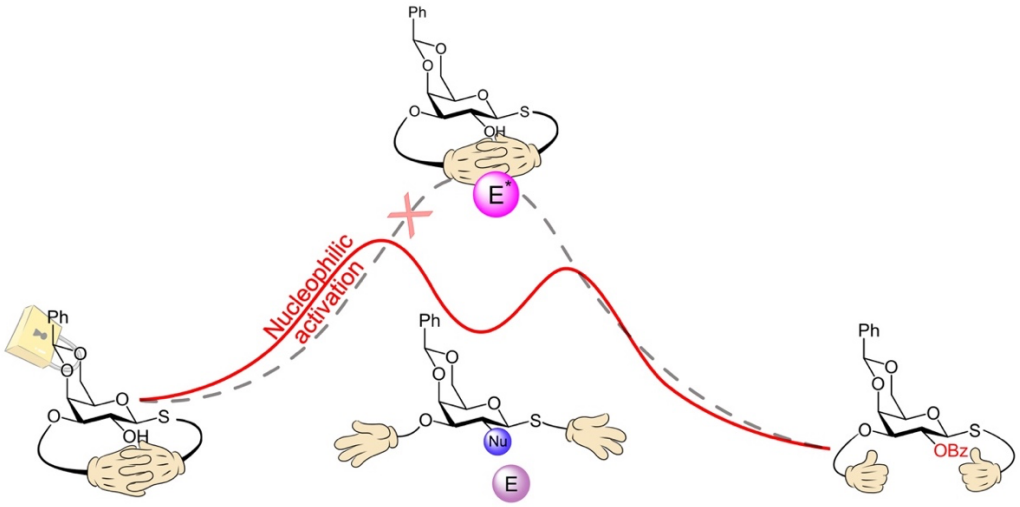
Access to fully protected glycoside monomeric building blocks is essential for the bottom-up synthesis of well-defined oligosaccharides. Alongside introduction of novel glycosylation strategies, development of reliable and efficient protocols for regioselective installation of protecting groups is equally crucial and challenging. The control of protecting group installation on partially protected monosaccharides is often unpredictable and depends on the electronic, the steric and the conformational effects of other substituents. We uncovered an O-2 masking effect in Lewis base catalyzed acylation of the valuable conformationally locked 4,6-O-benzylidene thiogalactoside intermediate that is highly dependent on the 3-O-ether substituent. A combination of crystallographic data, investigation of analogous O-3 ether protected systems and computational efforts revealed previously overlooked steric effects on the accessibility of the nucleophilic species. Characterization of the O-2 masking effect allowed for development of an alternative protecting group installation strategy *via* nucleophilic activation. This insight facilitated access to the target galactoside building block whilst preserving the envisioned stepwise strategy and protecting group hierarchy. Adapting this strategy for obtaining a diverse set of orthogonally protected galactoside monomeric building blocks will be valuable in the synthesis of complex oligosaccharides.



Nucleophilic activation of sterically crowded O-2 in a conformationally locked system allows for an alternative facile acylation strategy

Protected Tn antigen: an efficient gram-scale synthesis

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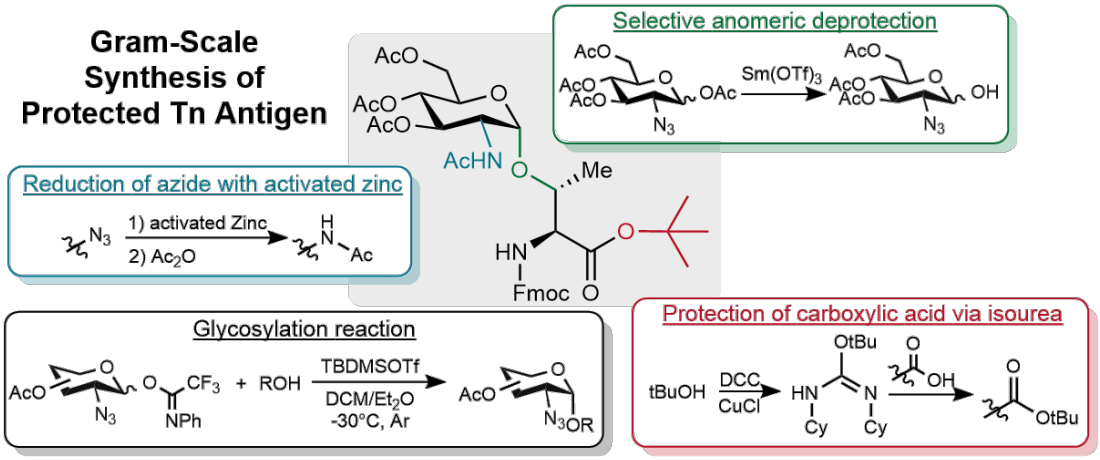
T antigens (T, Tn or sTn) were reported at the surface of numerous tumour cells and constitute an important target for cancer research. As such, an efficient method to access the molecule at a large scale is required. Although many syntheses have been reported [1,2,3], some steps fail to be reproduced or necessitate complex strategies.

Herein is proposed an efficient gram-scale synthesis of protected Tn antigen (*N*-acetyl-*O*-threonine-galactosamine), that will be introduced afterwards in a supported peptide synthesis. This seven steps synthesis utilises L-threonine and galactosamine as starting materials with an overall yield of 34%. The key glycosylation relies on coupling 2-azido-2-deoxy-galactose with the protected threonine using optimised conditions to yield the alpha anomer. Proper selection of the leaving group for the glycosylation was also undertaken to maximise the yield of this step.

Important steps were optimised for large-scale and time-efficient synthesis. Particularly, the selective protection of the carboxylic acid of threonine via an isourea was performed at a shorter reaction time (1 day) compared to previously reported methods (5 days) [3]. A replicable protocol for zinc activation was optimized to reduce the azide and add an acetyl group in a one-pot reaction. Additionally, condition screening was performed to adapt selective anomeric deprotection of the 2-azido-2-deoxy-galactosamine moiety using lanthanide triflate [4]. Overall, this strategy afforded gram-scale quantities of the protected antigen.



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A generic conjugation chemistry supporting the development of multivalent vaccines

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Glycoconjugation is a well-established technology for vaccines development as linkage of the polysaccharide (PS) antigen to an appropriate carrier protein makes them effective in infants and provides immunological memory. Glycoconjugates have been successful in reducing the burden of different diseases, however many diseases still remain to be controlled and alarming concern is emerging toward antibiotic resistant bacteria. Considering the variety of PS antigens displayed on the surface of the pathogens for which vaccines are not available yet, high-valency glycoconjugates need to be developed.

CDAP chemistry was identified with the aim to develop a generic conjugation chemistry that can be applied to PS having different structures. This chemistry works with hydroxyl groups on the PS and amino groups on the protein[1,2]. Starting from published procedure[3,4], reaction conditions were extensively investigated. The resulting protocol has been successfully applied to a broad range of bacterial PS from different pathogens like *Klebsiella pneumoniae*, *Salmonella* Paratyphi A, *Salmonella* Enteritidis and Typhimurium, *Haemophilus influenzae* type B, *Shigella sonnei* and *flexneri*. Furthermore, new statistical tools were applied in order to understand the impact of the reaction conditions on critical quality attributes of the resulting glycoconjugates, using *Salmonella* Paratyphi A O-antigen as model.

This work will support generation of a large number of conjugates in very short time and the development of multivalent vaccines, meeting unmet medical needs.

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Towards applying paramagnetic NMR to study the conformation of Heparin/Heparan Sulfate

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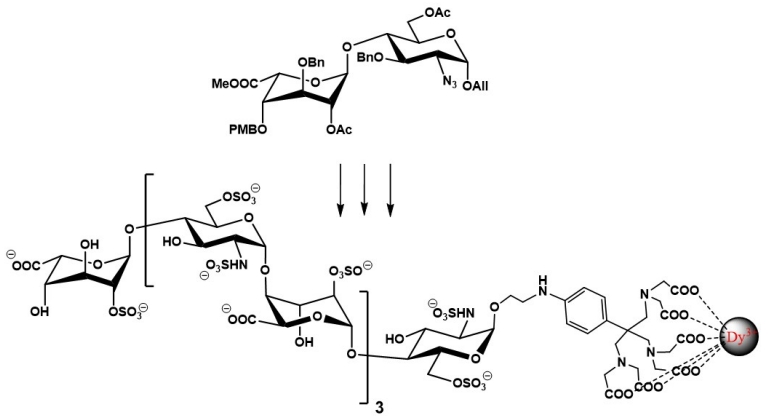
Heparin (HP) and Heparan sulfate (HS) are linear sulfated polysaccharides found in the extracellular enviroment. They are involved in multiple biological functions, such as cell growth and adhesion, virus recognition, and cancer metastasis. These multifaceted biological impacts arise from their molecular diversity, conformational flexibility and sulfatation pattern.

Many HP/HS-protein interactions have been described from affinity-based procedures and X-ray crystallography. Moreover, different approaches have been employed to assess their conformations and interactions in solution by NMR. Indeed, it is evident that these molecules display conformational flexibility at different levels, from the L-IdoA residues to their overall shape. Despite fundamental advances, the NMR-based interpretation of their conformational, dynamic, and interactions features remain a challenging process, especially for long saccharides. The intrinsic homogeneity of these chains, which display numerous disaccharide repeating units, makes the full and unambiguous NMR assignment a rather exigent task, even at very high field. We herein propose the use of paramagnetic NMR; in particular, pseudo-contact shifts (PCSs), to elucidate the conformation and interactions of these molecules. On this basis, a new synthetic approach has been developed to conjugate the target HP/HS to a paramagnetic probe, which allows measuring PCS. These results expand the applications of paramagnetic NMR to chemically synthetized HP/HS and pave the way for further analysis of their interactions with proteins.



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Towards the Chemical Synthesis of S-linked Heparan Sulfate Oligosaccharides

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Heparan Sulfate (HS) is a sulfated linear carbohydrate that decorates the cell surface and extracellular matrix and is a key regulator of biological activities.[1] An example of a glycosaminoglycan, HS is composed of L-iduronic acid (IdoA) or its C-5 epimer D-glucuronic acid (GlcA), (β-1→4)-linked to D-glucosamine. Its microstructure is diverse: the amino sugar can be *N*-sulfated (D-GlcNS) or *N*-acetylated (D-GlcNAc), while D-GlcA and L-IdoA are variably substituted with *O*-sulfate groups at the C2. D-GlcN is commonly sulfated at C6 and occasionally at C3 (**Figure 1A**).

Due to HS chemical heterogeneity, structure-to-function correlations with HS binding proteins has largely been impaired. However, the use of native proteins and non-natural oligosaccharides (glycomimetics) have proven successful in probing carbohydrate-protein interactions in the past.[1,2] Such a move towards preparing HS glycomimetics, may enhance our understanding of these interactions. One glycomimetic in particular, *S*-linked glycosides, where the glycosidic linkage oxygen is replaced with sulfur, offers the exciting possibility of studying unique conformational preferences about the thioglycosidic and aglyconic bonds.[3] Subsequent comparison to native sequences will improve our understanding and capability to perturb HS structure-to-function relationships.

Previously, Kovensky and co-workers synthesised *S*-linked disaccharide **1** (**Figure 1B**), a HS mimic of the (β-1→4) linkage, to be incorporated into chemical tools to study HS-protein interactions.[2,3] Our work involves synthesising *S*-linked disaccharide **2** (**Figure 1C**), mimicking the (α-1→4) linkage found in HS, from simple monosaccharide building blocks. Following this, deprotections of **2**, followed by similar SAR studies will be carried to further unmask HS biological function.

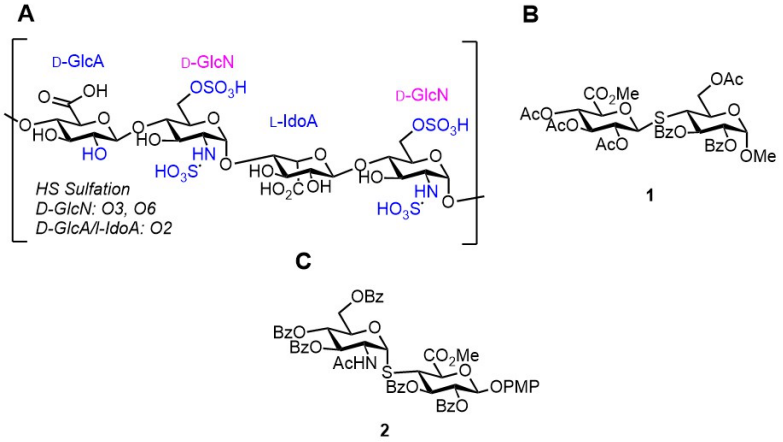

Figure 1: A) HS structure, B) Kovensky's S-disaccharide 1, C) Target Disaccharide 2

Acknowledgements

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Dendritic Heparin and Heparan Sulfate Mimetics for Therapeutic applications

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Heparan sulfate (HS) and heparin are structurally related sulfated glycosaminoglycans produced within the human body, with HS expressed by virtually every human cell. The structural intricacy, negative charge and multivalent nature of HS facilitate its interaction with hundreds of naturally occurring proteins. These interactions and its ubiquitous presence mean that HS plays numerous roles in physiological processes. Consequently, HS–protein interactions have been implicated in a number of pathologies, including viral infection, neurodegenerative diseases and numerous types of cancer.

Many of these pathologies have been linked to aberrant expression of HS and HS binding proteins, alongside the dysregulation of HS biosynthesis. As such, protein–HS interactions have come to be considered valid and interesting therapeutic targets for a range of pathologies. Our work focuses on the synthesis and design of novel dendritic glycosaminoglycan mimetics for application towards cancer and multiple-sclerosis treatment.

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Heteromultivalent Carbohydrate-Based Supramolecular Hydrogels to Mimic the Extracellular Matrix

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The pursuit of designing and synthesizing complex systems that closely resemble the highly glycosylated extracellular matrix (ECM) has gained significant interest in various fields: 3D cultures, drug delivery and tissue engineering [1]. Supramolecular hydrogels hold great potential for achieving this goal. However, the development of these hydrogels has been hindered by a lack of knowledge behind the fundamental parameters governing their hierarchical self-assembly. Furthermore, the limited examples of carbohydrate-based hydrogels in the literature predominantly involve homomultivalent presentation of a single carbohydrate, which falls short of replicating the complex heteromultivalent nature of the ECM [2].

To address these challenges, this project aims to synthesize biocompatible supramolecular hydrogels that emulate the highly and heterogeneously glycosylated ECM through a hierarchical supramolecular self-assembly approach, by employing rationally designed neoglycolipids. Various photopolymerizable neoglycolipids with distinct sugar headgroups, such as α-D-mannose, β-D-galactose, β-D-glucose, and β-lactose, have been synthesized. The hierarchical self-organization of these neoglycolipids into different hydrogel structures has been thoroughly characterized. Importantly, the resulting hydrogels exhibit multiple interactions with fluorescent lectins specific to the exposed sugars, highlighting their heteromultivalency.

In addition, hybrids of hydrogels and glyconanoring-coated carbon nanotubes have been developed to enhance the mechanical properties of the constructs.

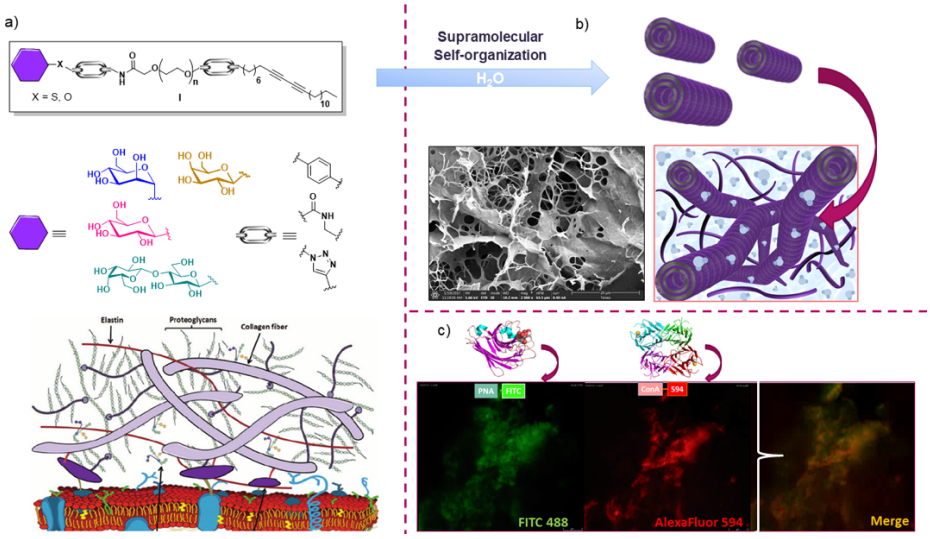

Figure 1. (a) Neoglycolipids. (b) Supramolecular characterization. (c) Heteromultivalency.

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Synthesis and in vivo evaluation of MUC1-carbon dot conjugates as cancer vaccines

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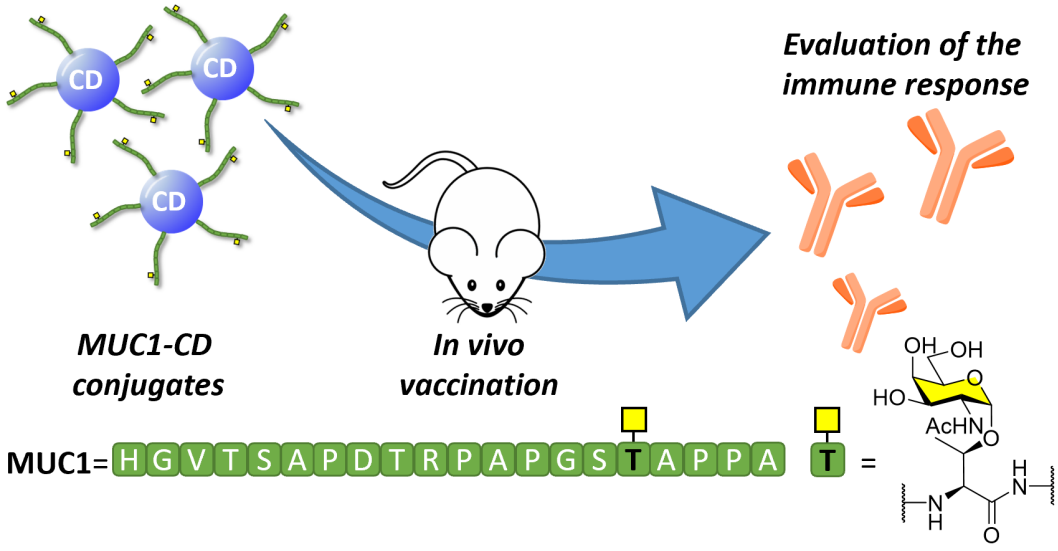
Carbon dots (CDs) are an emerging class of carbon-based nanoparticles which possess inherent immunostimulant properties [1]. Several types of CDs have been synthesized from different precursors, and conjugated with the MUC1 antigen. MUC1 is highly glycosylated glycoprotein expressed on the surface of epithelial cells. In cancer cells, it is overexpressed and presents truncated carbohydrate residues, such as the Tn antigen (α-*O*-GalNAc-Ser/Thr), which can be recognized by the immune system [2]. Unfortunately, MUC1 has low *in vivo* stability and low immunogenicity. Therefore, the conjugation of MUC1 on the surface of carrier proteins or nanoparticles such as CDs is essential to elicit a strong immune response [3].

Herein we report the preliminary results of a novel set of MUC1-CD conjugates. We show that the immunostimulant properties of CDs depend on the nature of the CD precursors, and that CD nanoparticles constitute promising scaffolds for the synthesis of novel self-adjuvant cancer vaccines.



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Chemical tools for functional studies of tumor-associated glycans of MUC1

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One of the main barriers to explaining the functional significance of glycan-based changes in cancer is the natural epitope heterogeneity found on the surface of cancer cells. To help address this knowledge gap, we synthesized for the first time a MUC1-derived positional scanning synthetic glycopeptide combinatorial library (PS-SGCL) that vary in number and location of cancer-associated α-GalNAc (Tn antigen). This focused combinatorial library with defined structural complexity allowed us to evaluate the effect of neighboring residue glycosylation, glycan density, and/or the presence of unique patterns of *O*-glycan clusters on binding to lectins, thus helping us understand the multivalent carbohydrate-lectin recognition processes at the molecular level. Glycopeptide library was prepared by "tea-bag" approach using standard Fmoc-SPPS. Enzyme-linked lectin assay (ELLA) was used to screen PS-SGCL against two plant lectins, *Glycine max soybean agglutinin* (SBA) and *Vicia villosa* (VVA). Results revealed a carbohydrate density-dependent affinity trend and site-specific glycosylation requirements for high affinity binding to these lectins. The Tn antigen on Thr^{9} in the PDTR epitope of MUC1 showed the highest affinity for SBA, followed by $Thr^{16}$and Ser^{15}, and lastly, Ser^{5} and Thr^{4}, therefore, suggesting that interaction depends not only on the carbohydrate moiety but also on the peptide region surrounding the glycan site of attachment. In conclusion, PS-SGCLs provide a platform to systematically elucidate MUC1-lectin binding specificities, which in long term may provide a rational design for novel inhibitors of MUC1-lectin interactions involved in tumor spread and glycopeptide-based cancer vaccines.

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Recognition mechanisms of bacterial glycans by host immune system receptors

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Glycans act as an interface between the outer environment and the cell membrane of all living organisms. They exhibit broad structural diversity and are involved in fundamental biomolecular mechanisms. Particularly, glycans are the main actors in the interaction mechanisms of bacteria with eukaryotic host, serving as counter receptors for different proteins, including lectins[1]. These are exposed on the surface of innate immune cells and represent an important class of Pathogen Recognition Receptors (PRRs) characterized by their ability to recognize glycans. These PRRs may contribute to initial recognition of bacterial glycans, thus providing an early defense mechanism against bacterial infections, but some of them may also be exploited by bacteria to escape immune responses. Several human pathogens have indeed developed the capability to cover their surfaces with glycans mimicking eukaryotic SAMPs (Self Associated Molecular Patterns) structures, able to interact with inhibitory host receptors, thus eluding host immune responses and promoting infections. Among them, the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* species) pathogens exhibit multidrug resistance and represent a global threat to human health[2].

Within this frame, we elucidated the chemical structure of the capsular polisaccharide extracted from an *A. baumannii* clinical isolate with the aim to investigate its recognition by inhibitory host receptors.

To achieve our goal, a multidisciplinary approach, relying on different biophysical techniques including NMR spectroscopy, Mass Spectrometry and computational methods, have been applied.

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Microbial glycans immune recognition by human lectins

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In the past decade, there has been a rising interest in investigating the role of bacterial glycoconjugates in the interaction between the human gut microbiota and the immune system. Lipopolysaccharides (LPS) composed by the lipid A, the core oligosaccharide and the O-antigen polysaccharide (Figure 1A), are potent MAMPs (microbe-associated molecular patterns) due to their immunostimulant properties. Although historically known for the endotoxic activity of the lipid A, recent studies unveiled the capacity of LPS to act as weak agonists or antagonists, positioning them as interesting potential drugs to target dysregulated immune response.{1}-{3}

Here I will report the findings of our studies focusing on the interactions between LPS from beneficial and harmful bacteria and lectins, CTL{4} and Siglecs,{5} expressed on immune cells using NMR{6} and biophysical and computational approaches (Figure 1B). We showed that: i) DC-SIGN is able to recognize E. coli R1 OPS, being the outer core pentasaccharide the one that acts as cross-linker between two different tetrameric proteins, ii) B. vulgats mpk LPS{7}{8} is recognized by formation of a heterobivalent interaction with tetrameric DC-SIGN by recognition of the terminal moiety (tMan-Rha-Man) in a length independent manner and the core Fuc residue and iii) Siglec-7 recognized two F. nucleatum strains, ATCC 10953 and 51191,{9} highlighting the recognition of strain 51191 due to the absence of a sialic acid unit.

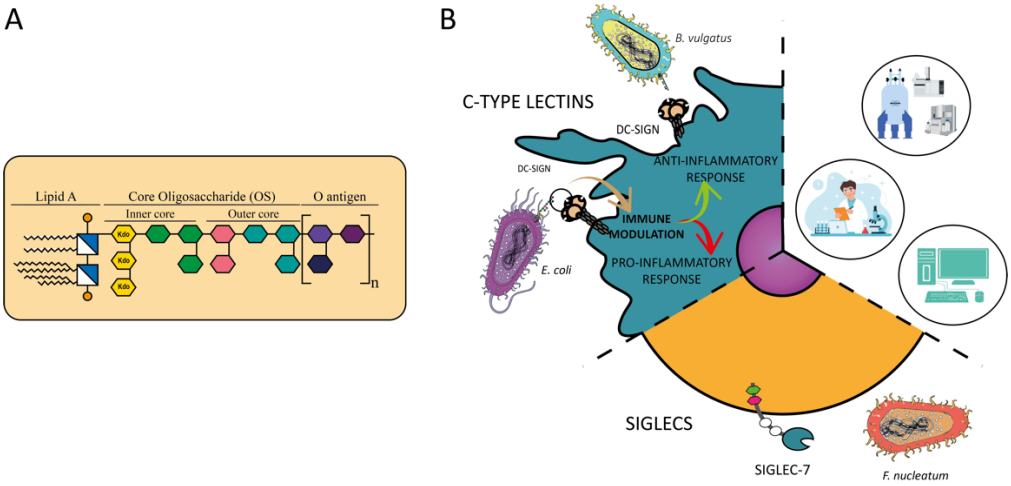

Figure 1. A) LPS schematic representation. B) Bacterial glycoconjugates recognition by lectins studied applying a multidisciplinary approach.

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Isomeric separation of glycopeptides by HILIC LC coupled to MS/MS

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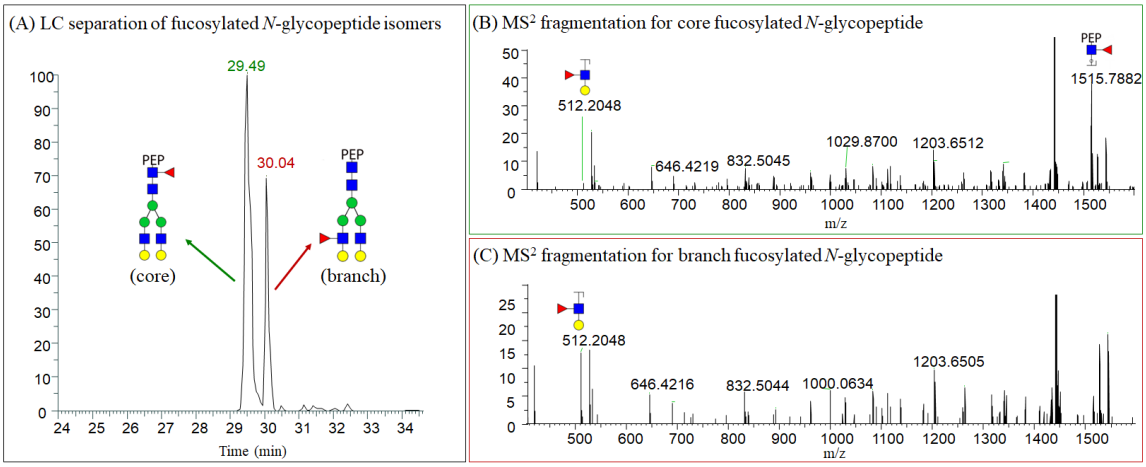
Glycosylation is a critical biological process that can profoundly influence the function of proteins. The structurally different glycans attached on specific site of proteins have been shown to be involved in a variety of disease progressions. Despite the recent advances in LC-MS/MS methodologies, the profiling of site-specific glycosylation is still a challenge due to the glycan isomeric structures attached on the same peptide backbones. Hydrophilic interaction liquid chromatography (HILIC) is a powerful technique for the separation of glycopeptides, as it has the ability to retain highly hydrophilic analytes [1][2]. Additionally, long column length can increase the absolute column efficiency and separation ability.

In this study, we focused on the isomeric separation of N-glycopeptides using a 75 µm × 350 mm HILIC column with 5-hydroxyl groups under a nanoflow rate. To achieve better resolutions on glycopeptide isomers, different LC conditions were optimized using standard glycoprotein digests, including hemopexin, immunoglobulin G (trastuzumab) and haptoglobin.

Many glycopeptide isomers were successfully separated, including core and branch-fucosylated N-glycopeptide isomers (Figure 1) and sialylated N-glycopeptide isomers. In addition, the comparison of C18 and HILIC trap columns coupled with HILIC analytical columns was evaluated in detail. Finally, we applied this approach to separate glycopeptide isomers derived from complex biological samples, including plasma and cell lysates. HILIC not only proved to be a useful tool for the comprehensive characterization of glycoproteins and their isomers, but also a good potential alternative to C18-based glycoproteomics workflows.



PEP, MVSHHN[184]LTTGATLINEQWLLTTAK.

Figure 1. LC-MS and MS2 of core- and branch- fucosylated N-glycopeptide isomers from haptoglobin digests.

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Revised Structure of the Capsular Polysaccharide from Streptococcus Pneumoniae Serotype 7C

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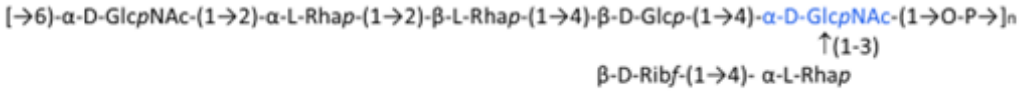
The polysaccharide capsule of Streptococcus *pneumoniae* is recognized as the most important virulence factor of pneumococci and the capsular polysaccharides are widely used as the antigens in pneumococcal vaccines. The structure of the polysaccharide from Streptococcus *pneumoniae* serotype 7C was published in 2018 by Christian Kjeldsen et al based on NMR spectroscopy.

A discrepancy in the composition of this polysaccharide was found from both composition and linkage analysis. No galactosamine was detected by HPAEC-PAD from the hydrolysate of 7C polysaccharide, and an extra glucosamine was found instead. The extra glucosamine residue was determined to be in a 3,4-linkage by GC/MS analysis of the partially methylated alditol acetates. The presence of 3, 4-Glc*p*NAc was further confirmed by NMR analysis. While the ^{1}H NMR and 2D NMR (COSY, HSQC and HMBC) spectra are comparable with the data published, the large ^{3}J coupling constant (~9.6 ppm) between both H-4 and H-3 ($J_{3,4}$) and H-4 and H-5 ($J_{4,5}$) of the 3,4-linked Hex*p*NAc from a selective NOE experiment suggested that the H-4 is in an axial position, and the 3,4-Hex*p*NAc should be 3, 4-Glc*p*NAc instead of 3, 4-Gal*p*NAc in the published structure. The same conclusion is supported by the strong NOE between the H-4 of 3, 4-Glc*p*NAc and the H-1 of the glucose link to 3, 4-Glc*p*NAc through O-4.

In conclusion, the revised structure of the polysaccharide from Streptococcus *pneumoniae* serotype 7C is:



Revised structure of SP 7C

Acknowledgements

GSK Vaccines

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Promiscuities in Sialic Acid Biosynthesis in Vertebrates and Invertebrates

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N-acetylneuraminic acid (Neu5Ac) is synthesized in nature by a class of enzymes called Neu5Ac aldolases (NPLs), which catalyze the aldol addition of ManNAc and pyruvate. Given the close homology between Neu5Ac aldolases in bacteria and animals, we examined if recombinant NPLs from mammalian origin (i.e. from human, hNPL), avian origin (i.e. from chicken, chNPL), and from lower animals (i.e. from snail or oyster, respectively, sNPL and oNPL) showed similar substrate promiscuities. The results and implications of these findings will be presented.

In contrast to the mammalian and avian NPLs, which can synthesize and degrade Neu5Ac, sNPL and oNPL were not able to catalyze the cleavage of N-acetylneuraminic acid into N-acetylmannosamine and pyruvate. Interestingly, the NPLs from higher animals showed contrasting C4(*R*)/(*S*) diastereoselectivity towards the substrates D-mannose and D-galactose in the presence of pyruvate compared to the NPLs from lower animals. In addition, sNPL was able to synthesize a series of 4-hydroxy-2-oxoates using the corresponding aliphatic aldehyde substrates in the presence of pyruvate, which could not be achieved by the mammalian or avian NPLs.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant numbers 31671854, 31871793, and 31871754) to JV and LL.

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The chemical structure of O-polysaccharide isolated from Pectobacterium versatile CFBP6051T(IFB5636)

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Numerous bacteria from the *Pectobacterium* spp. cause symptoms of soft rot and blackleg, thus causing serious losses in the world production of potatoes, vegetables and ornamental plants. One of the new species was named *P. versatile* due to the wide diversity of its habitats. These bacteria were isolated from diverse plants and water streams. *P. versatile* produces many virulence factors, among others: plant cell wall degrading enzymes and extracellular lipopolysaccharides (LPSs). LPSs participate in bacterial adhesion to plant tissue, take part in the efficient colonisation of plant tissues and overcome the host defence mechanisms.

The LPS of *P. versatile* strain CFBP6051^T (IFB5636) [1] was isolated from dry bacterial cells using phenol-water extraction, then purified by enzymatic digestion and dialyzes. The obtained LPS was hydrolyzed by mild hydrolysis with 1% acetic acid. The lipid A was centrifuged, and the sugar fraction was separated by size-exclusion chromatography.

The O-polysaccharide isolated from the LPS of *P. versatile* strain CFBP6051^T (IFB5636) was structurally characterised using spectroscopic techniques and chemical methods. The analyses revealed that the polysaccharide repeating unit consists of Gal, two Man and two residues of an unusual monosaccharide called erwiniose (3,6,8-trideoxy-4-C-(R-1-hydroxyethyl)-d-gulo-octose; Erw).

Knowledge of the structure of polysaccharides may help to explain the mechanisms of the bacterium-plant interaction.

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Optimalization of NADES-based polysaccharide extraction from carrot pomace

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Poland, as one of the leading producers of carrots in Europe, generates a huge amount of carrot pomace as a result of juice production. Until today it was treated as waste but in terms of sustainable development this type of waste can be used as a valuable raw material for other processes of obtaining of fine chemicals [1].

Carrot pomace has prebiotic properties due to its high pectin content. This type of polysaccharide with high amount of uronic acids can be a food source for the symbiotic human intestinal microflora, such as Bacteroidetes and Firmicutes. Both groups of bacteria provide a set of enzymes needed not only to depolymerize complex polysaccharides structures, but also play a role in the fermentation of monosaccharides into short-chain fatty acids (SCFAs). SCFAs are used by numerous organs of the human body to produce beneficial chemicals, e.g. the liver utilizes SCFAs to produce cholesterol or to synthesize glutamine and glutamate [2].

In the presented study, response surface methodology (RSM) was used as a tool to optimize the process of obtaining polysaccharides from industrial carrot root pomace. The independent variables were: (i) extraction time and (ii) the molar ratio of components forming the natural deep eutectic solvent (NADES), i.e. choline chloride, glucose, and citric acid. NADES as an environmentally friendly alternative can successfully replace strong acids that are used to extract pectins on an industrial scale. The product obtained in the optimized process parameters was analyzed using spectrophotometric and chromatographic methods.

Acknowledgements

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Synthesis and immune function of acetic acid bacteria Acetobacter pasteurianus lipid A

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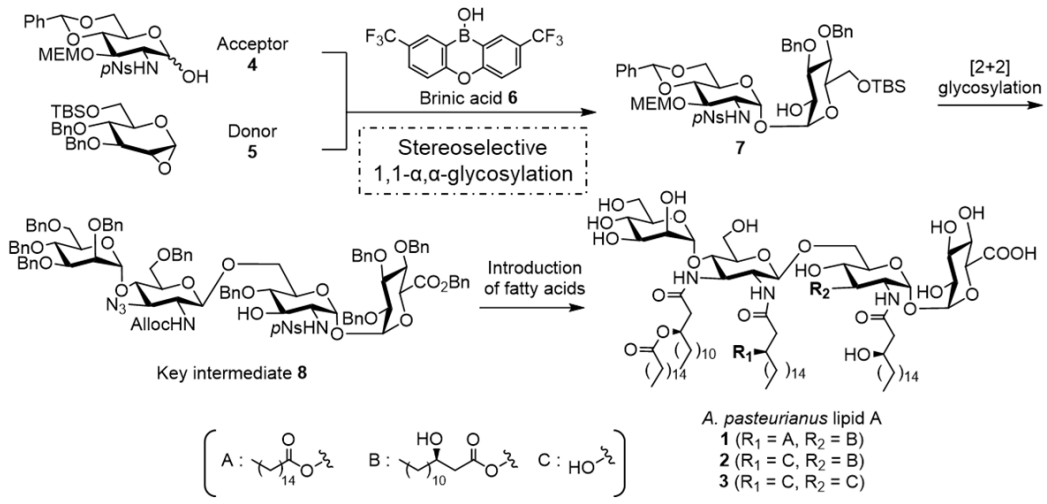
Lipopolysaccharide (LPS), one of the cell surface components of Gram-negative bacteria, activates innate immunity. LPS and its active entity lipid A have potential as adjuvants that enhance the vaccine efficacy. However, canonical Escherichia coli LPS has highly inflammatory effect and exhibits lethal toxicity, necessitating the need to control its toxicity for application in adjuvants.

In this study, we focused on an acetic acid bacteria Acetobacter pasteurianus lipid A 1, which is expected to be safe due to its use in food, as a low-toxicity adjuvant. A. pasteurianus lipid A contains three types of lipid A 1-3 (Scheme 1) with different fatty acid patterns, which possess a unique tetrasaccharide backbone for which chemical synthesis has not yet been achieved. We investigated the efficient construction of 1,1-α,α-glycosidic linkage of 7 and found that 1,2-cis-glycosylation between 4 and 5, catalyzed by borinic acid 6,2 afforded 7 in high yield and stereoselectivity. We used [2+2] glycosylation to synthesize the key intermediate 8 with an orthogonal protecting group pattern. After introduction of various fatty acids into appropriate positions of 8, all protecting groups were removed by catalytic hydrogenolysis to achieve the first chemical syntheses of 1-3. We evaluated their immunostimulatory activities and found that 1 is most active among 1-3, identifying 1 as the active entity of A. pasteurianus LPS. The adjuvant activity of 1 is currently under investigation.



Scheme 1. Syntheses of A. pasteurianus lipid A 1-3

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Synthetic study of Sialyl *N*-glycan using efficient glycosylation by controlling aggregation

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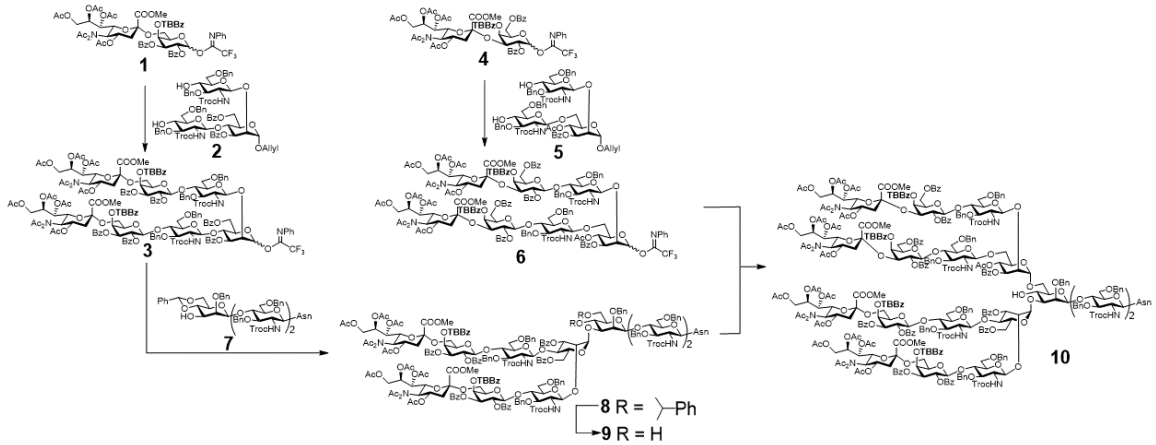
Asparagine-linked glycans (*N*-glycans) play important roles in various biological events. For example, sialyl *N*-glycans interact with sialic acid-binding immunoglobulin-like lectins (Siglec) and are involved in regulation of the immune responses.

We have reported the synthesis fully sialylated tetraantennary *N*-glycan possessing siaα(2,6)gal linkages. In this synthesis, we used diacetyl strategy in which NHAc is protected as NAc_2 to improve the reactivity by preventing intermolecular hydrogen bonds. [1] Nevertheless, sialic acid containing fragments used here had low solubility to decrease the reactivity in glycosylation. In order to increase the solubility, we employed *p-tert*-butyl benzoyl (TBBz) group as a protecting group of sialyl fragments. [2]

In this study, we investigated the synthesis of the tetraantennary sialyl *N*-glycan possessing siaα(2,3)gal and siaα(2,6)gal linkages. Heptasaccharides **3** and **6** were synthesized by the glycosylation using siaα(2,6)gal **1** and siaα(2,3)gal **4**, having a TBBz group. The [3+7] glycosylation between **7** and **3** afforded decasaccharide **8**. After the cleavage of benzylidene group, the glycosylation between **9** and **6** is under investigation.



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Morphology and carbohydrate surface antigens of Flavonifractor plautii PCM 3108

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The human digestive tract is one of the most complex microbial ecosystems. Microbiota has a great influence on the maintenance of homeostasis or the development of several diseases, such as inflammatory bowel diseases (IBD). IBD include Crohn's disease (CD) and ulcerative colitis (UC), which pathology is characterized by chronic inflammation of the digestive tract. It is known that patients with IBD have a greater amount of certain specific species of bacteria compared to healthy individuals [1].

Flavonifractor plautii is a strictly anaerobic, Gram-variable bacterium belonging to the Clostridiales [2,3]. It is a component of the human gut microbiome and is well known for its ability to metabolize a wide range of flavonoids, however, that is the only one clearly defined property of this species. *Flavonifractor plautii* is characterized by slow and minimal growth, thus its phenotypic identification is a challenge for microbiologists. Until now, three cases of infections have been described [4]. The enrichment of the *F. plautii* has been indicated in patients with colorectal carcinoma, IBD and other gastrointestinal tract disorders [5].

The purpose of this study was to further analyze the biological properties of clinical isolate *F. plautii* PCM 3108. The complete structure of the exopolysaccharide was determined using NMR spectroscopy as the repeating unit of →2)-Rha*f*-(1→4)-Rha*f*-(1→. Bacterial cultures were also analyzed by transmission electron microscopy showing the bacterial cells morphology and structure of the cell wall. The ability to produce membrane vesicles was also demonstrated.

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Synthetic Glycans as Vaccine Candidates and Diagnostic Tool for Candida Infections

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Fungal diseases affect more than one billion people and claim around 1.5 million lives worldwide every year - more than prostate or breast cancer. The fungal species *Candida* is responsible for the majority of cases. The World Health Organization (WHO) classifies *Candida albicans*and *Candida auris* as pathogens of the "critical priority group" because they show increasing resistance to antifungal drugs. Therefore, the development of an effective vaccine against and an early diagnostic tool for Candida infections is highly desirable. We synthesized a series of beta-glucans and mannans, which are essential components of the cell wall of *Candida*, with diagnostic and preventive potential for *Candida*infections.

We screened sera from infected patients and mice for antibodies to these structures on glycan arrays and used the structures against which the strongest immune response occurred to produce different conjugate vaccines. We immunized mice with the alum adjuvanted conjugate vaccines, and they developed robust serum levels of specific IgG and showed efficient killing of the fungus in *in vitro* experiments.

Acknowledgements

We thank Dr. R. Sprute and Prof. O. Cornely (University Hospital Cologne, Germany) and Dr. M. Bruno and Prof. M. Netea (Radboud University Medical Center, The Netherlands) for providing serum samples.

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Synthesis of BODIPY-based fluorescent analogues of the molecular adjuvants Sulfavants

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The sea represents a huge source of biologically active compounds characterized by a wide structural variety and a great potential for therapeutic uses.[1] During the ongoing exploration of the immunomodulatory potential of marine natural products, the work of recent years has been focused on the identification of novel chemical candidates able to stimulate dendritic cells (DCs), a specific type of antigen-presenting cells (APCs) that operate as master regulators for the adaptive immune response.

Inspired by the natural immunomodulators α-6'-sulfoquinovosyldiacylglycerols (α-SQDGs), a synthetic sulfolipid, named Sulfavant A, has been described as the progenitor of a novel class of molecular adjuvants able to stimulate an unconventional maturation of human dendritic cells (hDCs) via the Triggering Receptor Expressed on Myeloid cells-2 (TREM2).[2] Crucial step for the pharmacological development is the elucidation of the biological mechanism and sub-cellular localization of the cellular targets. For this reason, in this communication, the synthesis of fluorescent analogues (**1-3**) of Sulfavant A, bearing the 4,4-difluoro-1,3,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene moiety (Me_3-BODIPY), is provided (**Figure 1**).[3]

The fluorescent probe **1**, showing unaltered *in vitro* activity compared to that of Sulfavant A, has been used for both live cell imaging experiment in presence of hDCs and biodistribution studies in Zebrafish animal model, while the other two fluorescent derivatives (**2-3**) were prepared to investigate emission wavelengths closer to the IR region, in order to overcome the overlapping problem with basic natural fluorescence.[4] The development of a general synthetic strategy for the preparation of fluorescent analogues of this new family of immunomodulators and their *in vitro* and *in vivo* applications open the way for more in-depth studies on the interaction with cellular targets and biological behaviour.

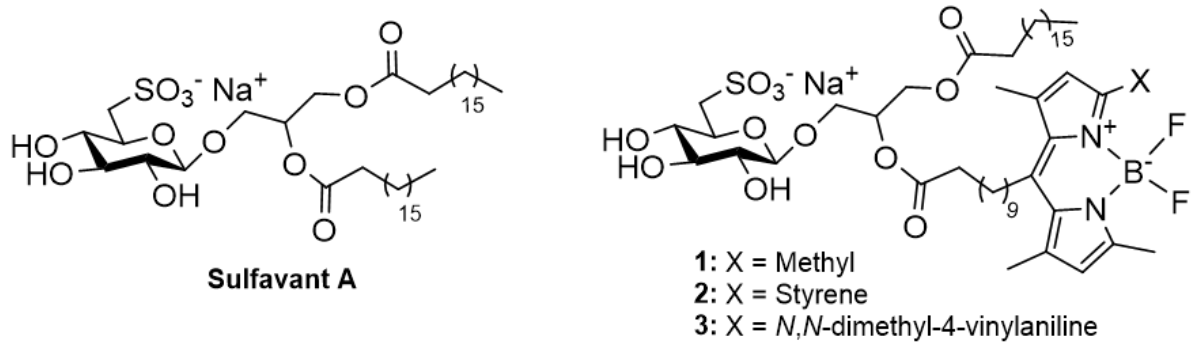

Figure 1: Sulfavant A and its fluorescent analogues.

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Posters

Session 1: Monday 10th

15h30 - 17h30

Even numbers

Session 2: Tuesday 11th

15h30 - 17h30

Odd numbers

Salle 151: P1 to P112

Salle 251: P113 to P224

Role of EPS in mitigation of plant abiotic stress: The case of *Methylobacterium extorquens* PA1

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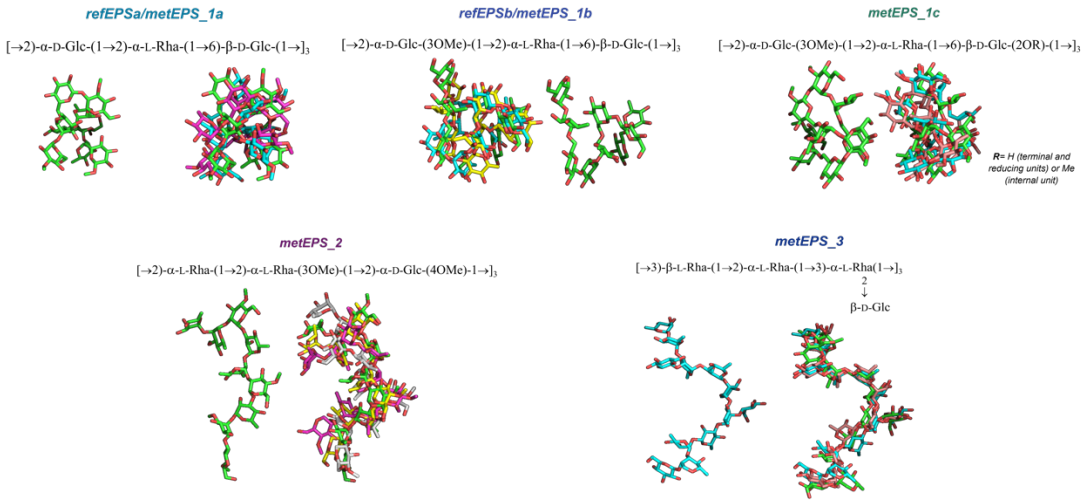
Methylobacterium extorquens is a facultative methylotrophic Gram-negative bacterium, often associated with plants [1], that exhibits a unique ability to grow in the presence of high methanol concentrations, which serves as a single carbon energy source [2].

We found that *M. extorquens strain PA1* secretes a mixture of different exopolysaccharides (EPSs) when grown in reference medium or in presence of methanol, that induces the secretion of a peculiar and heterogenous mixture of EPSs, with different structure, composition, repeating units, bulk and a variable degree of methylation [3]. These factors influenced 3D structure and supramolecular assets, diffusion properties and hydrodynamic radius, and likely contribute to increase methanol tolerance and cell stability.

No direct methanol involvement in the EPSs solvation shell was detected, indicating that the polymer exposure to methanol is water mediated. The presence of methanol induces no changes in size and shape of the polymer chains, highlighting how water-methanol mixtures are a good solvent for *refEPS* and *metEPS*.



M. extorquens PA1 RefEPS and MetEPS conformational behavior.

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Application of Synthetic Glycans to orchestrate animal gut health

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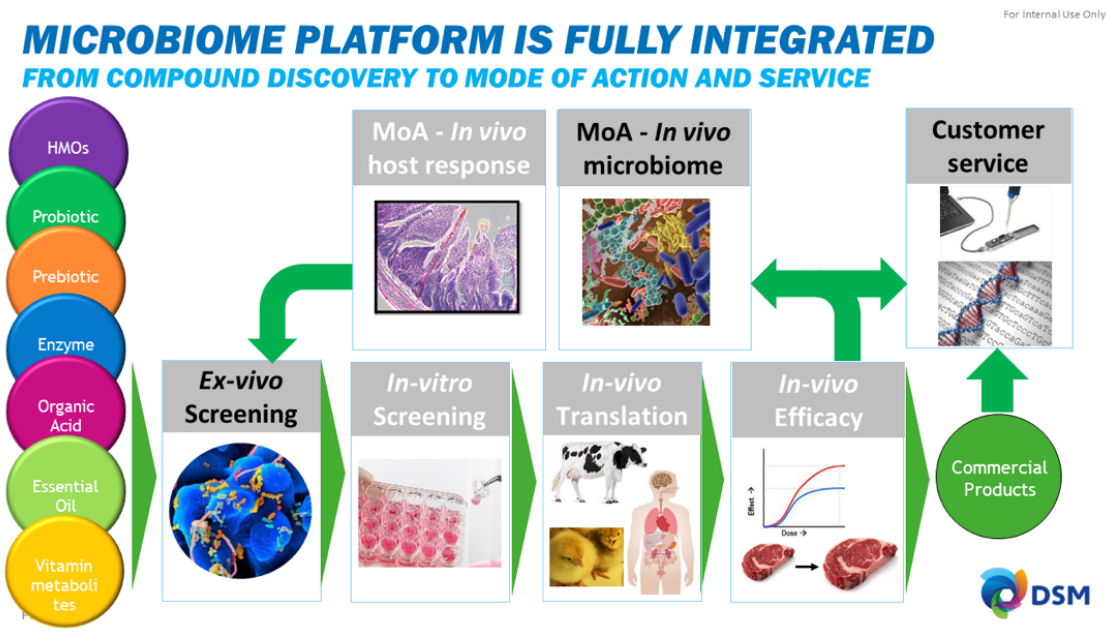
The role of glycans in modulating microbiome composition and promoting gut health is well-established. Specifically, glycans in the form of complex polysaccharides that resist human and animal digestion have been shown to have a significant positive impact on the gut microbiome by changing the relative abundances of bacterial species. In this study, we investigated the potential of using a diverse range of synthetic glycans to modulate the microbiome of poultry. Our results demonstrate that these glycans can promote cooperation amongst existing microbes, direct metabolite production by the microbiome, and optimize resilience to enteric stress, improve nutrient utilization, and reduce emissions. Importantly, these glycans also improved the welfare and productivity of the birds. In this presentation, we will present the results of our screening and characterization studies with these synthetic glycans, as well as the effects of optimized glycan mixtures on animal trials.



DSM-firmenich microbiome platform applied for complex glycans

Palladium catalyzed Sonogashira and Heck coupling reactions of 2-iodo-1-C-substituted glycals

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Carbohydrates play an important role in the biological processes of living organisms [1], however the *O*-glycosidic bond of natural glycosides shows low stability against chemical and enzymatic hydrolysis. Glycomimetics can mimic the structure and biological function of natural carbohydrate derivatives and at the same time the stability of the molecule could be much higher [2].

C-2 branched carbohydrate derivatives and 1,2-annulated sugars can be potential antibiotics, or glycomimetics of 2-*N*-acetyl sugars and inhibitors of lipid biosynthesis [3].

1-C-Substituted glycals are unsaturated carbohydrate derivatives bearing endocyclic double bond and carbon substituents on the anomeric carbon. We have elaborated synthetic methods for the synthesis of 1-C-acceptor-substituted (CN, $CONH_2$, $COOCH_3$) glycals starting from anhydro-aldonic acid and (ulosylbromide)onic acid derivatives [3-5].

In this presentation we will represent the synthesis of 2-iodoglycals starting from glycals using *N*-iodoimides. During our research we optimized the Sonogashira cross coupling reaction between 2-iodoglycals and phenylacetylene or TMS-acetylene in details. From the TMS protected acetylene derivatives we synthetized 2-ethynylglycals, which were reacted with glycosyl azides under CuAAc conditions.

The 2-iodo 1-C-acceptor glycals could be the substrates for the Heck coupling reactions. The palladium catalyzed Heck coupling of 2-iodoglycal derivatives were studied in detail, we optimized the reaction conditions and examined the substrate scope of the coupling.

Acknowledgements

Research was supported by the Hungarian National Research, Development, and Innovation Office (FK 128766) and the Gedeon Richter Talentum Foundation.

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Synthesis of potentially biologically active carbohydrates: transformation of anhydro-aldose oximes

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Candida albicans is a yeast which can exist in a commensal state in the mucosae and gastrointestinal tract. *C. albicans* becomes pathogenic in immunocompromised patients under various conditions, like superficial and systemic infections. The high pathogenicity of *Candida* species may be related to their adherence to the host organism [1]. The cell surface glycans are important receptors for *C. albicans* and warrant the development of anti-adherence ligands that can mimic them, thus disrupting *C. albicans* - epithelial cell interactions. Mono- and disaccharideswere found to inhibit the adhesion of C. albicans,among these carbohydrate derivatives the bivalent galactoside derivativecontaining triazole units has been shown one of themost effective [2].

Our group have elaborated synthetic procedures for the preparation of anhydro-aldoximes by the transimination reaction of anhydro-aldose semicarbazones [3]. Oximes can be applied to *in situ* generation of nitrile oxides, these intermediates can be further transformed in various ways.

Based on these preliminaries, we aim systematically study the reactions of anhydro-aldose oximes derived nitrile oxides to synthesize glycopyranosylidene-spiro isoxazolines as galectin and glycogen phosphorylase inhibitors, and C-glycosyl-isoxazoles, -isoxazolines and 1,2,4-oxadiazoles, which may be potential anti-adhesion agents of C. albicans as these heterocycle units are bioisosteres of the triazole [2] which were prepared and tested in Maynooth University.

Acknowledgements

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Characterization of the host glycan specificity and recognition by Neisseria meningitadis T4P

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Neisseria meningitidis is a Gram-negative bacterium responsible for causing life-threatening meningococcal infections, which are traditionally treated with antibiotics. However, recent reports indicate the emergence of antibiotic-resistant strains, calling for new approaches to combat the infection. The bacterium\'s ability to adhere to host cells through long, thin protein assemblies called type IV pili (T4P) is a key factor to its virulence.

In this study, we aim to provide a molecular framework for PilE adhesion mechanism through the characterization of its glycan specificity and recognition using docking and molecular dynamics. We will also reconstruct a fully atomistic model of T4P to provide a 3D structural understanding of pili stability and dynamics within a substrate recognition context.

Our results will provide a rationale for glycan specificity and highlight essential information to screen and validate the binding poses identified through docking/MD. This work is expected to provide new insight into the mechanism of meningococcal infection and have a major impact on our understanding of Neisseria host-pathogen interactions.

Rendering of multi subunit T4P (pdb: 5KUA), visualised using Pymol

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Studies on anomeric phosphorylation under modified Mitsunobu reaction conditions

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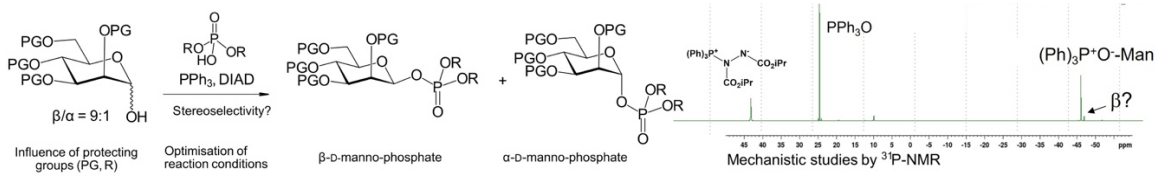
Glycosyl phosphates are important biomolecules involved in essential biological processes such as metabolism and oligosaccharide/phosphoglycan biosynthesis which requires the development of efficient approaches for the stereoselective synthesis of anomerically pure glycosyl phosphates as substrates or synthetic intermediates for NDP-sugars. The stereoselectivity of phosphorylation at the anomeric center generally reflects the anomeric ratio in the lactol precursors and, therefore, requires the preparation of anomerically enriched hemiacetals, which should then be directly converted to phosphates using P(III) or P(V) chemistry. For some pyranoses, however, the preferred anomeric configuration in lactols is the opposite of that required for the synthesis of biologically important glycosyl phosphates: D-Man and L-Ara4N are some of examples.[1] Also, D-manno-heptose, a precursor of ADP-(L/D)-glycero-β-D-manno-heptose and β-heptose 1,7-bisphosphate which serve as substrates for alpha-kinase 1, a recently discovered pattern recognition receptor that plays a key role in Gram-negative inflammation,[2] also shows a preferred α-configuration of the anomeric lactol. Here we investigated the feasibility of stereocontrolled synthesis of glycosyl phosphates by inversion of the anomeric configuration using modified Mitsunobu reaction conditions.[3,4] We performed mechanistic analysis by *in situ* ^{31}P-NMR spectroscopy using variably protected D-Man and examined the influence of protecting groups in both coupling partners on the efficiency and the stereoselectivity of the phosphorylation reaction.



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Unveiling the interaction siglecs-sialylated glycans to understand diseases of the immune system

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Carbohydrates are key mediators in fundamental biological events, taking an active part in cell-cell and cell-matrix interactions. Sialic acids are monosaccharides expressed on the terminal residues of glycolipids and glycoproteins that are extremely heterogeneous, counting more than 60 structural modifications [1]. Sialic acid can be overexpressed Cancer cells and on the cell envelope of feared microbes and pathogens. Sialylated glycans are recognized by several proteins, like Siglecs (Sialic acids immunoglobulin-like lectins).

By means of these interactions, they can evade the immune system and promote infection and cancer [2-3]. Siglecs recognise highly abundant sialylated glycans on the surface of tumor cells, inhibiting immunosurveillance and mediating immune evasion of tumor cells. Siglecs are also targets of human pathogens, such as viruses and bacteria, which are used to evade host immunity, promoting bacterial colonization [4-6]. For this reason, it is interesting to investigate the molecular basis involved in this process, to understand the progression of specific types of tumors.

To better understand the etiology of immune system diseases, it is certainly important to clarify how Siglecs and sialylated glycans interact, so that diagnostic/therapeutic tools for the treatment of these diseases can also be developed.

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Synthesis of chemical inhibitors to monitor OGA activity

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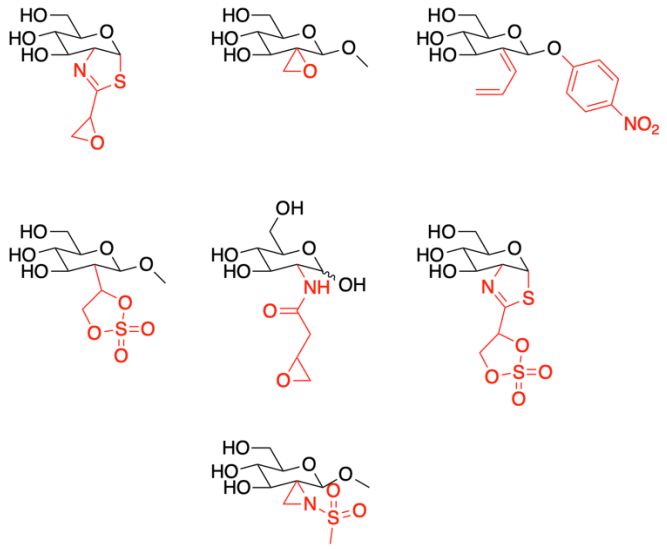
O-linked N-acetylglucosamine (O-GlcNAc) is a dynamic post-translational modification applied to serine and threonine residues on hundreds of intracellular proteins. O-GlcNAcylation is believed to play a role in several diseases, such as Alzheimer's disease (AD).[1] The modification is controlled by two enzymes: the O-GlcNAc transferase, OGT, and the O-GlcNAc hydrolase, OGA. OGA catalyses the breakdown of the GlcNAc-serine/threonine bond on a diverse number of substrates. Inhibiton of OGA has become of significant interest as target for therapeutic agents due to its role in disease, however the detection of enzyme activity and inhibition within a cellular context remains challenging. In this work, we aim to develop novel activity-based probes that allow us to monitor active enzyme levels in cells. Similarities between the active site of OGA and other hexosaminidases (HexA/HexB) alongside the lack of a covalent intermediate in the substrate assisted mechanism create difficulty in the generation of specific high affinity probes.[2, 3] We aim to use a variety of electrophilic warheads placed strategically to interact with the catalytic base of OGA (Asp174) to generate covalent enzyme inhibitors that will form the basis for the synthesis of activity-based probes. In addition to this we aim to use a natural pocket in the active site of OGA as a way to gain selectivity over HexA/HexB where this pocket is absent. We show the results of molecular dynamics studies on transition state mimics, and the



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Targeting the lectin LecA with high-affinity ligands enables the imaging of Biofilms and Infections

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Pseudomonas aeruginosa is one of the most common nosocomial pathogens and often causes life-threatening acute and chronic infections. To date, no fast and pathogen-specific diagnostic tools for *P. aeruginosa* infections are available. The two lectins of *P. aeruginosa,* LecA and LecB, are involved in biofilm formation, a hallmark of chronic infections. Here, we present the detection *of P. aeruginosa* by LecA- and LecB-targeted fluorescent probes as pathogen-specific diagnostic tools. To this end, several probes were developed to target these extracellular lectins and demonstrated to stain *P. aeruginosa* biofilms *in vitro*. For LecA an activity boost to low-nanomolar affinity could be achieved by multivalency. *In vitro*, the divalent LecA-targeting imaging probe accumulated effectively in biofilms under flow conditions. Investigation of divalent LecA- and monovalent LecB-targeting imaging probes in an *in vivo* murine lung infection model revealed elevated probe accumulation in lungs of infected animals. These findings demonstrate the use of LecA- and LecB-targeting probes for the imaging of *P. aeruginosa* infections and suggest their potential as pathogen-specific diagnostics.

Glyco-tagging of native proteins for developing improved therapeutics

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Proteins are increasingly used for drug development and in clinical practice,[1] and improving their physical, chemical, and pharmacological properties is an area of intensive research. In addition to mediating cell-cell and cell-matrix interactions, glycosylation exerts a major influence on protein folding, stability and pharmacokinetic properties.[2] Inspired by nature and aiming to recapitulate these important features, glycosylation is a strategy to enhance the therapeutic properties of peptide and protein drugs.[3] Here, we describe a novel chemoenzymatic methodology for the glyco-conversion of native proteins without introducing immunogenic modifications offering a streamlined approach to accelerate the discovery and improvement of biotherapeutics.[4]

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Multivalent presentation of glycosidic antigen mimetics for the modulation of the immune system

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Mucins (MUCs) are heavily glycosylated proteins excreted by epithelial cells. Transmembrane mucin type 1 (MUC-1) is the most abundant MUC in humans. MUC-1 external domain is rich in serine (Ser) and threonine (Thr) residues. In tumor cells these residues are *O*-glycosylated with an *N*-acetyl galactosamine (GalNAc) through α-*O*-glycosidic bonds. This fragment is rarely exposed on healthy cell surfaces and it is known as Tn antigen. Indeed, in cancer cells, glycosylation mechanisms are limited and simple carbohydrate units are exposed to the immune system. These shortened residues, including Tn antigen, are overexpressed on many cancer cells and are known as Tumor-Associated Carbohydrate Antigens (TACAs).

In cancer events, immune response is mediated by Macrophages and Dendric Cells (DCs). Human Macrophage Galactose-type Lectin (MGL) is a C-type lectin expressed on macrophage and DCs surfaces and specifically recognize galactose and GalNAc. Lectins like MGL, poorly interact with carbohydrate monomers while multimeric sugar ligands generally confer much stronger binding and shall be preferred to the corresponding monomeric ligand.

We will discuss the effects of the multivalent presentation of sulfor-containing MUC1 mimetics on the binding *vs.* DCs and on immunogenicity.

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Bacterial vesicles as new drug-delivery tool: the role of glycans

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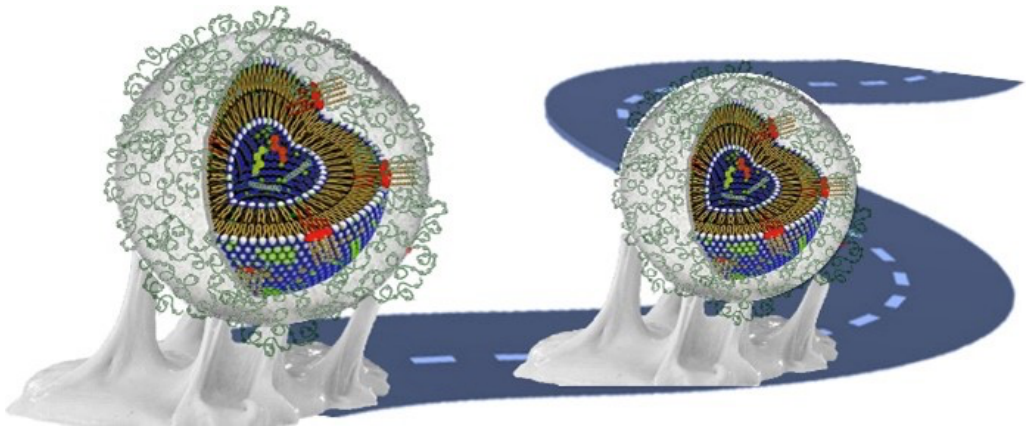
The treatment and the rapid detection of antimicrobial resistance diseases pose a formidable challenge in medicine. Nowadays, it is well established that antibiotics are not the only solution to treat these infections since microbes, especially bacteria, can adapt and develop resistance due to the selective pressure induced by the massive administration of prophylactic pharmaceuticals [1]. The translation from acute to chronic infections can cause the point of no return in their treatment [2]. The persistence of chronic infection is related to the bacteria's ability to form biofilms and to the lack of efficient detection techniques.

Biofilm is defined as communities of microorganisms embedded within a matrix, which consists of water, polysaccharides, extracellular vesicles, proteins, lipids, and nucleic acids [3]. A crucial role in promoting the emergence of multi-drug resistant (MDR) bacteria is also played by Extracellular Membrane Vesicles (EMVs), small vesicles (20–250 nm) formed by bacterial lipid-bilayer membranes comprising lipopolysaccharides (LPSs), proteins, peptidoglycans, DNA and RNA [4]. EMVs play different roles in the physiology and pathogenicity of bacteria: biofilm formation, delivery of toxins, antibiotic resistance, immunomodulation, stress response, horizontal gene transfer, and communication among cells and species [4]. By taking inspiration from the structural features of EMVs it is possible to design nanoparticles to be used for the prevention and diagnosis of biofilm infections. Recently, we described the polysaccharide "corona" from the EMVs of *Shewanella vesiculosa* HM13, demonstrating its role in the adhesion on polystyrene nanoparticles [5].

Here we described the structural characterization and determination of the physicochemical properties of glycans isolated from the Antarctic Gram-negative bacterium EMVs producer, as an innovative tool to deliver antibiofilm compound against *Staphylococcus epidermidis*.



Polysaccharide corona decorating the EMVs from Shewanella vesiculosa HM 13.

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Structural studies of *Plesiomonas shigelloides* CNCTC 70/89 lipopolysaccharide

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Plesiomonas shigelloides is a Gram-negative rod belonging to the *Enterobacteriaceae* family. *P. shigelloides* is associated with episodes of intestinal infections and outbreaks of diarrhoea in humans. Foreign travel, particularly to Latin America, the Caribbean, and South and Southeast Asia, is a second major risk factor associated with *Plesiomonas* infections in humans [1]. The extra-intestinal infections caused by this bacterium, *e.g.* meningitidis, bacteraemia and septicaemia, usually have gastrointestinal origin and serious course. Lipopolysaccharide (LPS, endotoxin), the main component of the outer membrane of the cell envelope of Gram-negative bacteria, is built of an O-specific polysaccharide and core oligosaccharide covalently linked to lipid A.

Despite the rising knowledge of *P. shigelloides* LPS structures over the past two decades, complete or partial LPS structures have been elucidated only for 15 strains out of 102 identified O-serotypes [2].

The structure of *P. shigelloides* O5 LPS was determined by chemical analysis, mass spectrometry and NMR spectroscopy. The O-specific polysaccharide of *P. shigelloides* O5 has the following structure: →4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*NAc-(1→. The same structure was identified before in the O-specific polysaccharide of *Hafnia alvei* strain 38 [3]. Furthermore, a new core oligosaccharide was described, which shares *P. shigelloides* common feature, that is the presence of uronic acids. The lipid A of *P. shigelloides* O5 LPS is identical with lipid A of the *P. shigelloides* serotype O74 [4].

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9-Anthracenemethyl glycosides as chiral supramolecular synthons and bio-imaging agents

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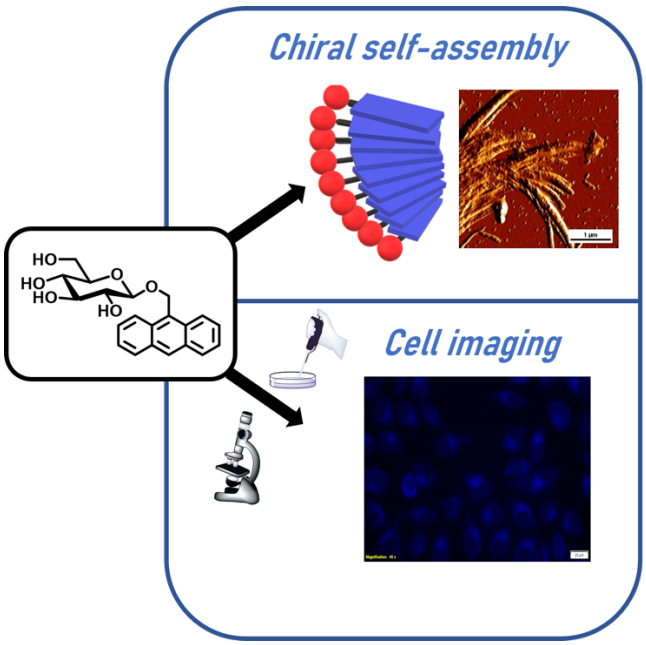
Self-assembly of amphiphiles, leading to the formation of supramolecular structures, occurs in biology and is studied in material chemistry. Chiral centers-rich carbohydrate moieties provide a rich source in the study of self-assembly, due to the feasibility of securing chiral supramolecular structures. Design of the monomers is critical in order to realize the chiral self-assembly of such synthons. 9-Anthracenemethyl glycosides were undertaken for the study covering (i) the formation of chiral supramolecular structures and (ii) the applicability of the photophysical properties of anthracene moiety at the biological interface. 9-Anthracenemethyl O-glycosides, installed with mono- and disaccharides, are studied for their self-assembly properties. Emerging chiral structures in aq. solutions follow the configuration of the attached sugar moiety. Monosaccharides D- and L-glucopyranosides alternate between left- and right-handed chiral structures, respectively. Whereas, a disaccharide-containing derivative does not exhibit chirality, even when the self-assembly occurred. A photochemical [4π+4π] cycloaddition occurs in the self-assembled structure in aq. solution, disrupting the chirality of the supramolecular structure. Anthracene as a probe to investigate bio-imaging properties of the newly-formed anthracenemethyl glycosides was studied. Cell viability assay using HeLa cells shows 80% cell viability, in the presence of 50 mM of 9-anthracenemethyl D-glucopyranoside. Significant uptake of this derivative occurred, whereas the corresponding D-galactopyranoside and L-glucopyranoside containing derivatives showed a relatively weaker cellular uptake. Bio-imaging was facilitated through fluorescence occurring at the perinuclear region of the cells, indicating an active transport of the glycosides, through the cell membrane. Results of the study will be presented.



We thank SERB-DST, New Delhi, for a financial support. AG acknowledges the CSIR, New Delhi, for a research fellowship.

Synthesis of boronated nucleotidyl sugar analogues as glycosyltransferase inhibitors

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Nucleotide sugars are high-energy donor substrates that consist of a monosaccharide and a nucleoside mono- or diphosphate moiety. As glycosyl and phosphoglycosyl donors, nucleotide sugars are essential in the biosynthesis of glycans and glycoconjugates in all living organisms. These biosynthetic products play crucial roles in many biological processes, including cell growth and development, cell signaling, and host-pathogen interactions, while alterations in sugar nucleotide metabolism have been implicated in various diseases [1]. Hence, nucleotide sugar-utilizing enzymes are promising targets for drug development, and nucleotide sugar analogues present potential inhibitors. Replacement of the non-bridging oxygen of the phosphate with a borane group (BH_3) furnishes phosphate analogues closely resembling the natural (pyro)phosphate with regard to charge, bond angles, and acidity and may considerably increase lipophilicity and enzymatic stability. Preparation of boranophosphate analogues has been described in-depth for nucleoside (poly)phosphates containing the borane either in α, β, or in γ-position [2,3]. We herein describe the synthesis of various nucleotide-P-boranodiphospho sugars through a phosphoramidite approach. These boronated natural sugar nucleotide analogues could be employed as molecular probes to investigate the stereochemistry and mechanisms of relevant enzymes such as glycosyltransferases, phosphoglycosyltransferases, and nucleotide sugar pyrophosphorylases, or in therapeutic applications, including boron neutron capture therapy.

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A new synthetic pathway to 3-Amino-3-deoxyglycals: selective deprotections and glycosylation

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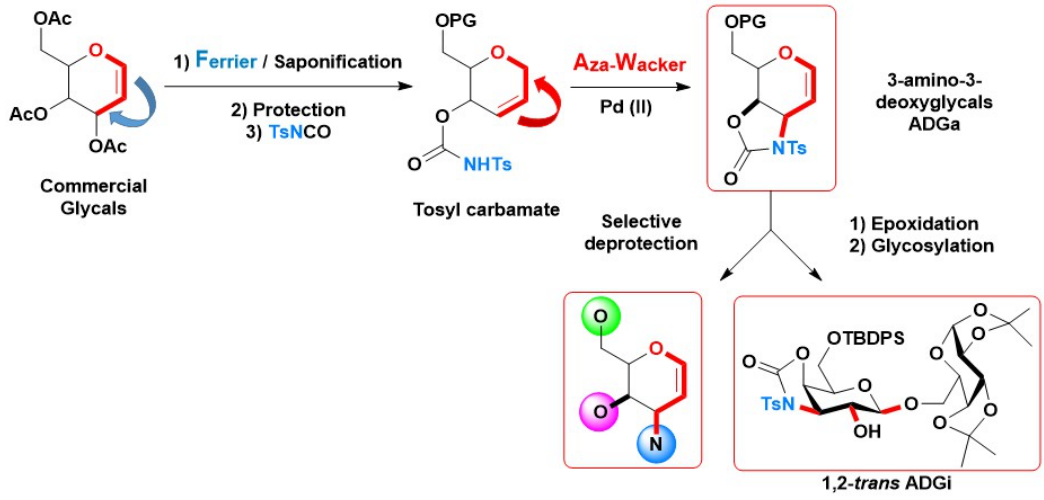
Among the most significant sugars in drug synthesis, 3-**A**mino-3-**D**eoxy**G**lycosides (**ADGi**) are bioactive compounds with applications in different domains.[1] They can either be found in oncology as anticancer agents or involved in fighting infectious diseases as antibiotics or antifungal medicines. The synthesis of efficient donors of these sugars is still a hot topic.

Fortunately, 3-**A**mino-3-**D**eoxy**G**lyc**a**ls (ADGa) could lead the way as key building blocks to access ADGi. In glycochemistry, glycals are well known for being efficient glycosyl donors (or precursors) for the synthesis of 2-deoxy and 1,2-*trans* glycosides.[2] The synthesis and reactivity of ADGa have been poorly studied. Most of the reported methods for their synthesis are either limited in terms of diversity or have an excessive number of steps, low yields or modest stereoselectivities.[3]

Here we describe a new sequence involving a type I Ferrier rearrangement and an aza-Wacker cyclization starting from commercially available glycals.[4] This new synthetic strategy allows rapid, gram-scale, and diastereoselective synthesis of orthogonally protected ADGa. These glycoplateforms can be selectively deprotected and engaged in an epoxidation / glycosylation sequence to obtain 1,2-*trans* ADGi.



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A simplified method for the synthesis of robust oligomers of sialic acid (ROSAs)

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The COVID-19 pandemic was predicted by countless experts and generally accepted to be the first of many more viral outbreaks to come. Despite the quick response to develop a vaccine, there still exists a void in the development of antiviral prophylactics. The goal of the current project is the development of a new synthetic pathway for oligosaccharides that forgoes many of the traditional and challenging methods by which carbohydrate chemistry is executed, which includes the use of protecting groups.

The carbohydrate products presented here are derivatives of N-acetylneuraminic acid (sialic acid) which have been shown to be prime candidates as preventative agents against multiple viruses, such as HIV-1, SARS-CoV-2, and HPV, to name a few. While sialic acids are believed to be effective in preventing viral infections, they are naturally subjected to enzymatic and acidic degradation. Here we present the synthesis of robust oligomers of sialic acid (ROSAs) which are anticipated to be resistant in environments often destructive to sialic acid oligomers. In addition to this, we are using green chemistry methods that provide higher yields than those commonly seen in carbohydrate chemistry. This research has the potential to address the need for preventative measures against viral infection in addition to other possible applications in carbohydrate chemistry.

Classification of protein-carbohydrate interfaces using unsupervised machine learning

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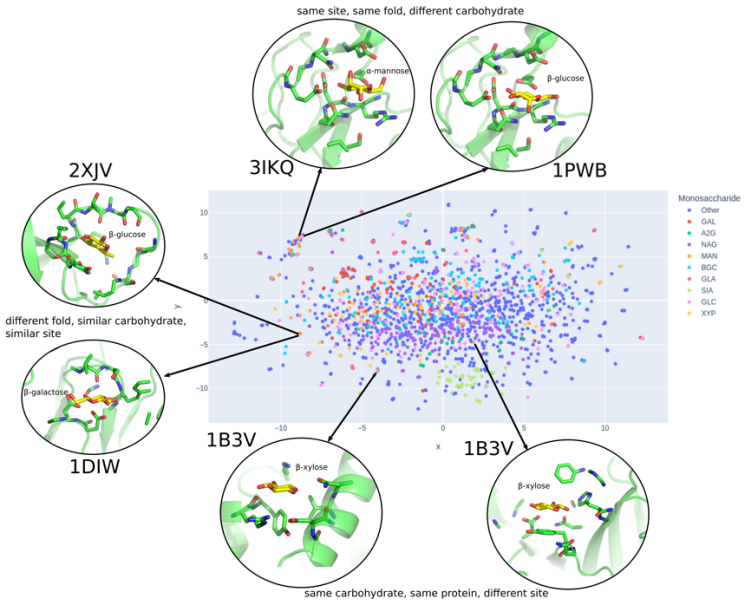
Numerous essential biochemical pathways are mediated by protein-carbohydrate (PC) interactions. However, their experimental characterization remains a technical challenge and the structural data on PC interfaces is scarce and underrepresented. Moreover, carbohydrate binding sites' (BS) diversity is very peculiar. Indeed, some similar protein regions can bind to different carbohydrates, while other BS targeting specific carbohydrates are found in proteins with very different folds (several examples are displayed in the figure attached). The goal of the current study was to provide a generalized view on different types of PC interfaces and perform their classification using unsupervised machine learning (ML) methods. We have extracted information on all the available BS present in Protein Data Bank (more than 20k) and performed a pairwise comparison of the most representative BS using a graph-based score evaluating local distance distortion between different atom types[1]. Then, we performed clustering for the obtained affinity score matrix and defined hierarchical classification of carbohydrate BS by their similarity. We show that most carbohydrate BS can be assigned to just a few categories: some of them being protein- or ligand-specific and others being more general, e.g. including proteins with very low homology relations. Our result paves the way to the development of the carbohydrate BS computational prediction tools, which have the potential to significantly impact understanding of fundamental biological processes and provide new drug design strategies.



t-SNE projection of representative carbohydrate binding sites present in the Protein Data Bank colored according to the carbohydrate name.

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Use of glycans metabolic engineering for studying plant cell wall biosynthesis and functions

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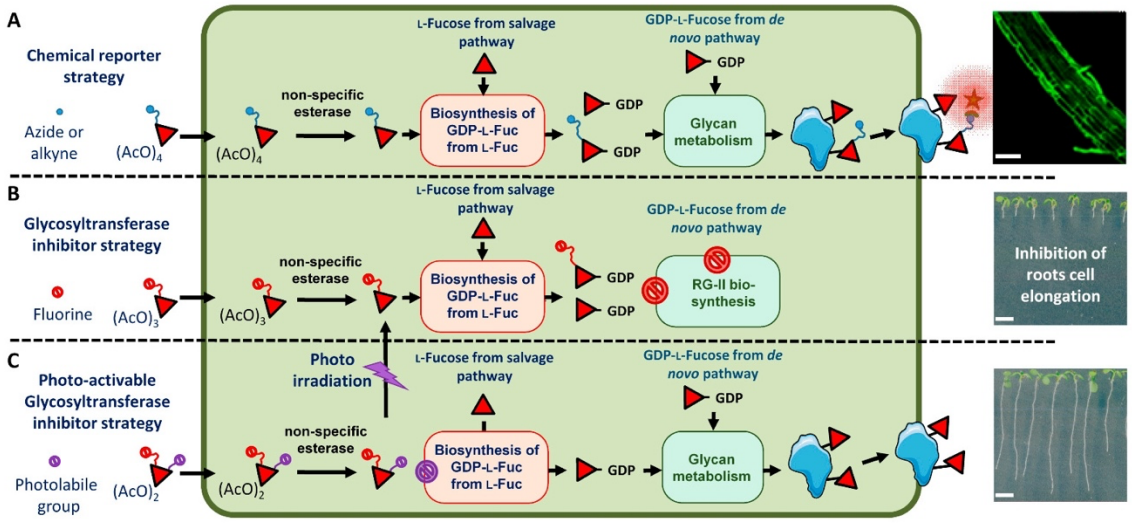
Glycans metabolic engineering is a powerful tool for studying the glycosylation of living cells. The use of modified monosaccharides, such as deoxy or fluorinated sugars, has been reported as a powerful pharmacological approach for studying carbohydrate metabolism and plant cell wall biosynthesis. Moreover, non-natural metabolite derivatives that carry functions enabling bio-orthogonal ligations are now widely used for glycomolecules imaging in living organism. In both cases, these derivatives must cross the cell membrane and be accepted by the biosynthetic machinery of the cell to produce nucleotide-sugars that will be taken in charge by the enzymatic machinery to build complex glycomolecule such as cell wall polysaccharides in plant.

Here, we illustrate the use of glycan metabolic engineering, photo release of caged monosaccharide derivative and click-chemistry, for studying the plant cell wall biosynthesis and function with an emphasis of the pectic domain rhamnogalacturonan II.



Glycan metabolic engineering in plant cells using fucose derivatives.

Design synthesis and biological evaluation of *C* and *S*-glycoside based galectins inhibitors

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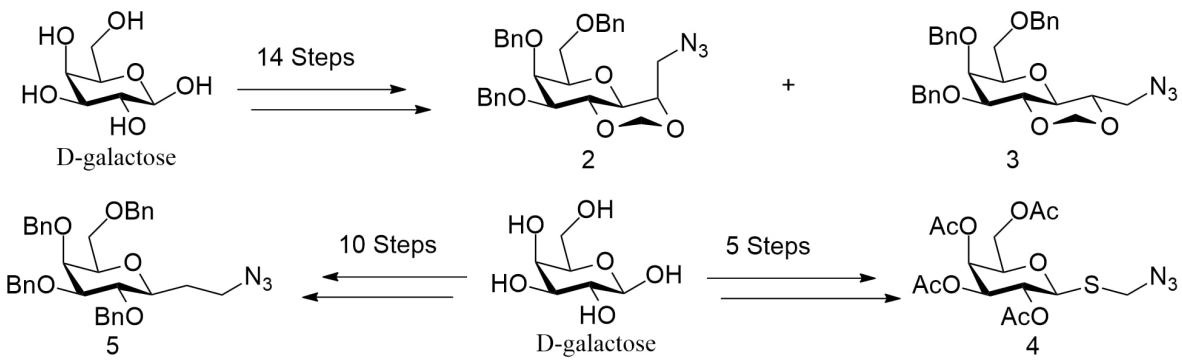
The galactoside binding specific subclass of lectins, galectins, are classified into three subfamilies based on the structure and number of carbohydrate recognition domains (CRD). Galectins involve in several cellular activities, certain cancers, infections, inflammations, fibrosis, HIV, and many other wide range of biological processes. The molecular basis for the selectivity of galectins is well documented and revolves around appropriate interactions of glycans with amino acid residues. The selectivity for galactose moiety stems largely from the hydrogen bonds (HBs) between histidine/arginine and the axial hydroxyl (-OH) at the 4-position. This axial hydroxyl is equatorial oriented in analogous O-linked β-glucosides. Mimetics of glycan ligands of galectins (glycomimetics) have been of interest.

We have synthesized "the clickable" intermediates (2, 3, 4 and 5) from β-D-galactose and current focus is given to its modification at the C1 and C3 position with the suitable pharmacophore to increase the affinity for galectins. For example, Nilsson et al[1] reported that galactose C3-modification via click chemistry stacked above the Arg144 sidechain, which in turn forms a water-mediated salt-bridge with Asp-148.

We will present the synthesis and biophysical evaluation of some b-C-glycosides derivatives, conformationally constrained C-glycosides, and an S-glycoside mimetic derived from 2-5. The compounds were tested as inhibitors of gal-1, 3, 8C, 8N, 9C, 9N and 4C. The synthesized compounds provided a basis of further modification and evaluation of their properties in search for better galectin inhibitors which is currently ongoing in the laboratory.



Synthesis Scheme of key intermediates (Fig: Synthesis of 2, 3, 4 and 5)

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Synthesis of glycosylated schweinfurthins

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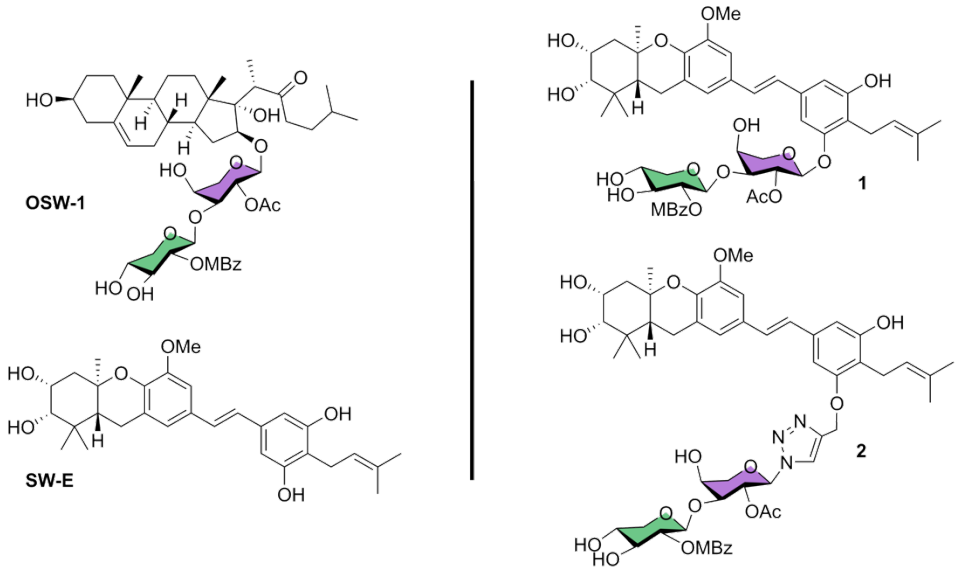
Schweinfurthins (SW) are a family of compounds comprising a stilbene motif associated to a hexahydroxanthene (HHX) moiety. The first derivative of this family was isolated from the leaves of Macaranga vedeliana in 1992.[1] These compounds were later found to exhibit a promising cytotoxicity preferentially towards cancer cells, based on screening on HL-60 cancer cells of NCI.[2] Even more remarkably, the pharmacological profile of these compounds bears no resemblance to the profiles of the compounds used so far in chemotherapy but is similar to other natural compounds (including OSW-1) which indicates that they act via a new common biological target. This common target is the protein OSBP which transports cholesterol in cells between the endoplasmic reticulum and the golgi. Our objective is to synthesize hybrid molecules between SW and OSW-1 while retaining the two pharmacophores essential to their biological activity: the HHX motif for SW and the glycosidic part for OSW-1.

As these two pharmacophores are positioned a priori in different places of the target protein, these hybrid molecules should have a better affinity. We are therefore interested in the preparation of compounds such as 1 and 2 which can be obtained either by glycosylation of SW-E, or by a Huigen-type cycloaddition reaction between a propargylated schweinfurthin and a disaccharide comprising an azide in the anomeric position (Figure 1).



OSW-1, SW-E and glycosylated SW-E hybrids

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Synthesis of trifluoromethyl-substituted MEP analogues for the study of the non mevalonate pathway

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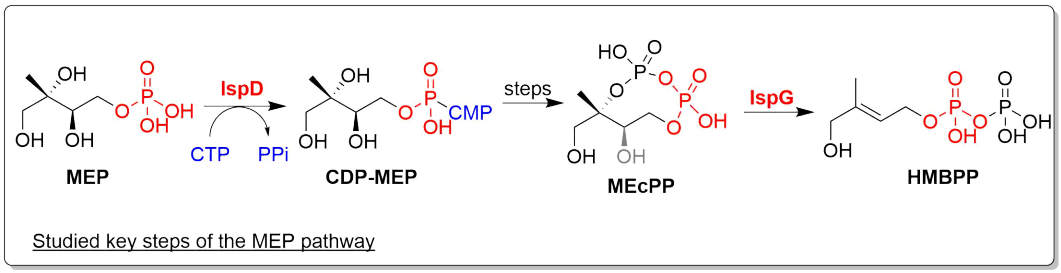
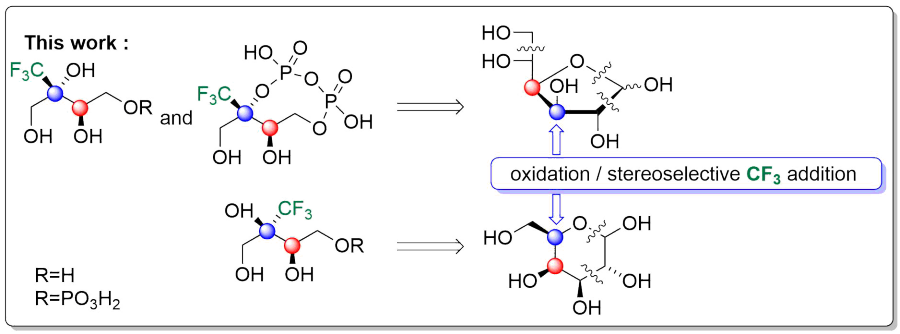
The 2-*C*-methyl-d-erythritol 4-phosphate (MEP) pathway is an essential biological process for numerous pathogenic bacteria species, while being absent from mammal metabolism (1). Its seven consequential enzymes have been reported as targets of interest for development of novel antibiotic drugs families (2). We focus on IspD (YgbP), a transferase catalyzing the transformation of 2-*C*-methyl-d-erythritol 4-phosphate (MEP) into 4-diphosphocytidyl-2-*C*-methyl-d-erythritol (CDP-MEP), and IspG (GcpE), a metalloenzyme catalyzing ring opening and reductive dehydration of 2-*C*-methyl-d-erythritol 2,4-cyclodiphosphate (MEcPP) into 4-hydroxy-3-methylbut-2-enyle 4-diphosphate (HMBPP).

Here, we present linear multistep stereoselective synthesis of 2-*C*-trifluoromethyl-d-erythritol in its carbohydrate, phosphate and cyclodiphosphate forms starting from d-glucose. We also present another strategy for the synthesis of 2-*C*-trifluoromethyl-d-threitol in its carbohydrate and phosphate forms. This structural modification is supposed to impair the enzymatic mechanism of MEP pathway enzymes by steric hindrance or destabilizing the activated complex, leading to inactivation or a low turnover (3). Some preliminary biological results will be presented.





Studied key steps of the MEP pathway and targets of the synthesis

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Nucleation and growth of silver nanoparticles on reducing supramolecular glyco-nanofiber

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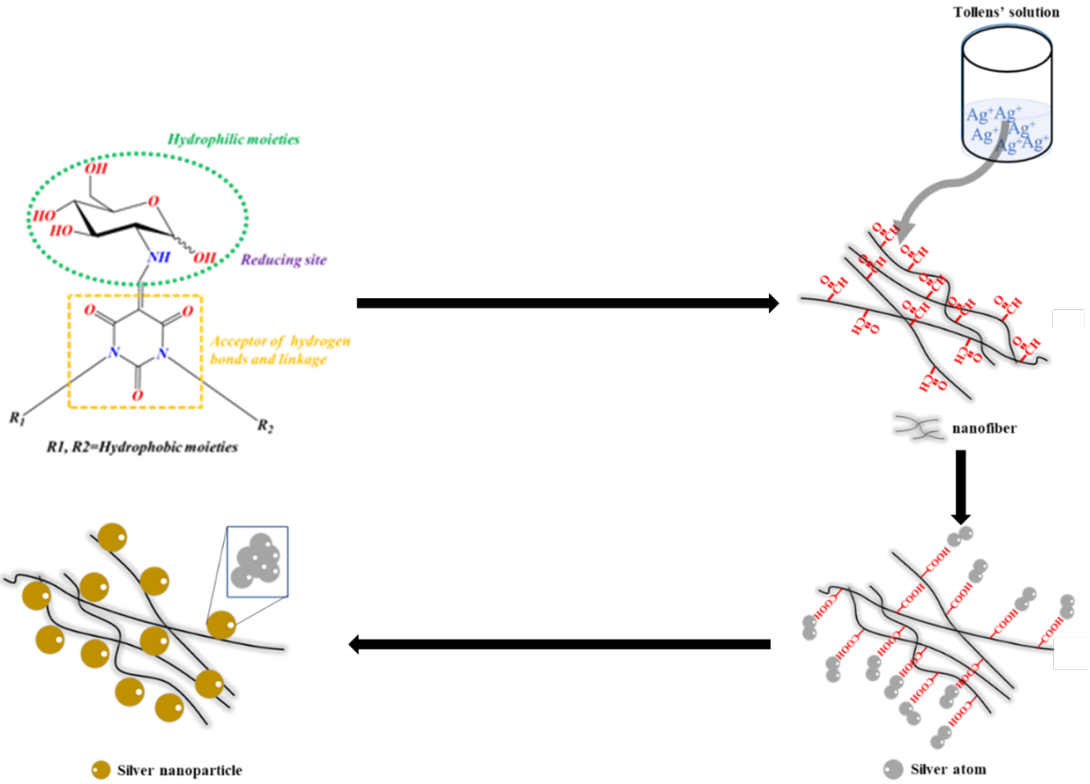
Among metallic nanoparticles, silver nanoparticles (AgNPs) are particularly interesting as a plasmonic nanoparticle for sensing and imaging, as well as for use as a catalyst or antimicrobial agent[1] . Carbohydrate hydrogel with the native reducing end aldehyde could form AgNPs, obviating the requirement for external reducing agents, stabilizers, or nucleation control measures.

The aim of this study is to devise a glyco-nanofiber consisting of reducing carbohydrate derivatives that exhibit intrinsic aldehyde functionality, thus facilitating the reduction of silver ions and subsequent formation of AgNPs on the surface of the glyco-nanofibers in aqueous media. This poster will present the synthesis of glyco-nanofibers by reacting between *N,N'*-substituted barbituric acid derivatives and the amino functionality of D-(+)-glucosamine hydrocholoride, allowing us to comprehensively assess its gelation properties. The formation of AgNPs on the surface of nanofiber has been studied using TEM and UV-vis spectrum.



Acknowledgements

this work was supported by POLYNAT CARBOGEL, Centre national de la recherche scientifique (CNRS) and Guangzhou Elite Scholarship Coucil(GESC) .

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GlycoShape3D: a database and toolbox for structural glycomics

Callum IVES [1], Ojas SINGH [1], Carl FOGARTY [1], Aoife HARBISON [2], Akash SATHEESAN [1], Silvia D'ANDREA [1], Beatrice TROPEA [1], Elisa FADDA [1]

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To understand the architecture of glycan-mediated interactions, a detailed atomistic understanding of glycan structure is necessary. However, determining the 3D structure of glycans is difficult due to their intrinsic flexibility and micro/macro-heterogeneity. As a result, glycans represented within the Protein Data Bank (www.rscb.org) are often incomplete or presented in questionable and/or highly distorted conformations.

The release of the AlphaFold (AF) Protein Structure Database (alphafold.ebi.ac.uk) has dramatically increased the availability of protein structure data, yet this information is incomplete as it does not include co- and post-translational modifications, metals and cofactors that are often essential to protein structure and function. Some of us found that AF predicted glycoprotein regions where glycans should have been present are preserved in these models. [1] This facilitates the direct grafting of glycans onto these models, providing that the structure of the glycan is known [1].

Here we will present the design and principles of GlycoShape3D, a unique online open-access repository of structural data on free (unbound) glycans from equilibrium MD simulations [2-4]. GlycoShape3D will deliver complete and consistent structural information on glycan structure in a format that is accessible to glycobiology experts and non-experts alike, with a demonstration of some of the key potential applications and innovations of this novel repository.

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Stimuli responsive glycopolymers for the detection of bacterial lectins

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Prompt diagnosis of infectious disease is vital in combatting the spread of infection and improving patient prognosis. A potential biomarker for point of care (POC) testing is the carbohydrate binding proteins, or lectins, that are associated with a variety of pathogens such as bacteria and fungi. Lectins selectively bind to glycans displayed on almost all cell surfaces and play a key role in cellular recognition during pathogenesis. Inspired by natures glycocalyx, work toward a biosensor for the detection of bacterial lectins is reported. This biosensor will feature an array of fluorescently labelled glycopolymers on graphite electrodes that aims to rapidly identify bacterial lectins in complex samples via a thermal- and optical-detection dual sensing approach. These involve the monitoring of both thermal wave transport through functionalised interfaces and the fluorescent emission of the glycopolymers. [1]

Fluorescent glycopolymers that can mimic lectin-glycan interactions were synthesised; first, masked acyl hydrazide monomers were prepared and used to produce a library of polymers by reversible addition-fragmentation chain transfer (RAFT) polymerisation. *N*-Isopropylacrylamide (NIPAM) was also integrated into the polymer backbone to impart thermal responsiveness that will release a bound analyte when heated above the lower critical solution temperature (LCST) and facilitate the reuse of the sensor. [2] Two solvatochromic fluorophores were then conjugated to the polymers end groups using thiol-maleimide coupling, generating differing emission profiles based on their local environment. A variety of unmodified reducing sugars have been appended to the polymer backbone to produce glycopolymers. Graphite electrodes were also modified using electrochemical techniques to afford amine functionalised surfaces that were used to conjugate glycopolymers. Ongoing work aims to determine response and limits of detection using TWTA.

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Investigating the role of carbohydrate sulfotransferases in the green seaweed ulva

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Polysaccharides are the major component in seaweed cell walls. Many of these polysaccharides are subject to extensive modifications, the two major modifications being acetylation and sulfation, carried out by carbohydrate acetyltransferases and sulfotransferases respectively. Interest in seaweed derived polysaccharides has grown in recent years and highly sulfated polysaccharides are of particular interest because their unique properties could benefit a variety of commercial and biomedical applications (1). Despite this, the physiological role of these polysaccharides and the enzymes that catalyse their modifications remain poorly understood.

My work looks at the Ulva genus of green seaweed whose species can be found in abundance along coastlines all across the globe (2). A large proportion of their cell wall is made up of ulvan, large heterogeneous polysaccharides that exhibit a high degree of sulfation. The genome for Ulva mutabalis/compressa was sequenced in 2018 and was the first green seaweed genome to be published, opening up new opportunities for research (3).

Using a homology-based approach, we have identified at least 19 putative carbohydrate sulfotransferase genes, suggesting a relatively recent gene expansion. However, based on previously collected RNAseq data, only four genes appear to be expressed under normal conditions. To investigate this further, I will be using FTIR to measure changes in levels of sulfation in response to different physiological stresses. This will be followed with qPCR to determine the link between expression, level of sulfation and stress.

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Generation of galectin inhibitors from green sources: deglycosilación of lactoferrin

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Galectin 1 and 3 are over-expressed in cancer cells and are involved in tumour progression and metastasis. Thus they are an interesting target for cancer therapy and their inhibitors potential anti-tumour agents. Particularly, galactosides are potential inhibitors as they can bind to their carbohydrate recognition domain [1-2].

Lactoferrin (LF), one of the most abundant glycoproteins in serum milk, is rich in N-glycans with potential anti-galectin activity. Thus, their glycans could be an economic and green source of galactosides[3-4].

Here we describe the release of LF glycans by enzymatic and chemical approaches focusing on the comparison of these methodologies, both in terms of yield and N-glycan profile. Even though these strategies are used for sample preparation for analytical glycomics, they have not been applied for the generation of glycan at preparative scale.

Enzymatic deglycosylation (DG) was carried out using the enzyme PNGase F under denaturing conditions and chemical DG was achieved using NaClO solution. The released glycans were purified and their profile was evaluated by SDS-PAGE, HPLC and TLC. Then, graphitized carbon SPE-column was used to separate mannosylated from sialylated glycans and further characterized by NMR.

The N-glycan profile was slightly different for enzymatic and chemical release, nevertheless higher yields were obtained with chemical DG.

LF deglycosylation proved to be an interesting tool for high scale glycan generation. We are now working in their biological evaluation as potential galectin inhibitors both in vitro and in silico.

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Exploring the synthetic application of *akkermansia muciniphila* α1,3-fucosyltransferase AkkFT

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Human milk oligosaccharides (HMOs), which are the third abundant solid component in breast milk, can be grouped into neutral, fucosylated, and sialylated glycans. Amongst, a majority of HMOs are fucosylated glycans. Studies showed that these glycans reduced the risk of infection in infants by acting as the soluble decoy receptors to the pathogens or viruses which potentially would bind to the surface of epithelial cells in the guts. Enzymatic catalysis is one of the powerful strategies to produce these valuable biomolecules. However, the majority of key enzymes for fucosylated glycans production, the fucosyltransferases (FucT), are recombinant enzymes from *Helicobacter pylori* and *Bacteroides fragilis*. Here we report a novel FucT named AkkFT which was isolated from *Akkermancia muciniphila* and demonstrated its synthetic application for human milk glycans production for the first time. The recombinant AkkFT was successfully expressed in *E. coli* BL21(DE3) with 3.68 mg/L yield. Biochemical characterization revealed that AkkFT exhibited a broad range of catalytic pH (from 6 to 8.5), with the highest activity observed at pH 7. AkkFT showed a 41% increase in FucT activity when magnesium ions were used as cofactors. Additionally, with the broad substrate tolerance, recombinant AkkFT was able to catalyze the α1,3-fucosylation on various human milk glycans including lactose, lacto-*N*-tetraose (LNT), and lacto-*N*-neotetraose (LNnT), to produce 3'fucosyllactose, Lacto-*N*-fucopentaose V (LNFP V) and Lacto-*N*-neofucopentaose (LNnFP V), respectively. The results indicated that AkkFT showed high regioselectivity on α1,3-fucosylating the reducing end of the lactose unit on a given glycans. These finding suggests that AkkFT would provide a promising new approach for human milk glycans synthesis.

Acknowledgements

National Science and Technology Council, TAIWAN

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Dimerization strategy towards higher carbon sugar alcohols as potential phase change materials

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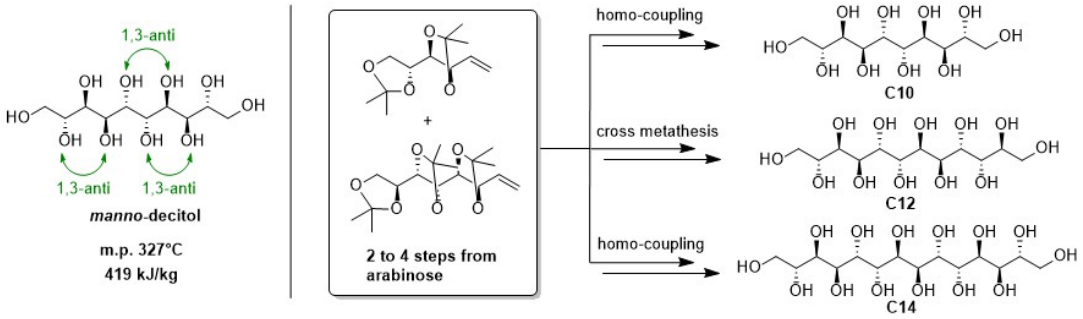
Sugar alcohols are interesting candidates for phase change materials (PCMs). Their strong hydrogen bonding tendency gives them high thermal storage potentials. Recently, specific sugar alcohols with extended chain length have been predicted by computational calculations to possess remarkably high values of latent heat making them promising PCMs [1] (Scheme left). An even number of carbon atoms, no branching and all hydroxy groups in 1,3-*anti* orientation have been identified as prerequisite. However, experimental validation of these predictions is yet lacking as these materials are not readily available.

We aim to make these PCM candidates accessible for thermal evaluation. In order to obtain higher sugar alcohols of C10 and longer chain length, we apply a dimerization approach [2]. Linking two sugar-derived building blocks by metathesis allows us to directly and efficiently translate the natural stereochemistry of the carbohydrates into our target materials.

We have already succeeded in the homo-coupling of a D-arabinose derived building block to the D-*manno*-D-*manno*-decitol (*manno*-decitol, C10). Next, by combining differently sized precursors even higher sugar alcohols (C12, C14) are accessible via (cross) metathesis. The synthesized compounds are being investigated for their thermal properties and compared to their computationally estimated potentials.



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Heteromultivalent s

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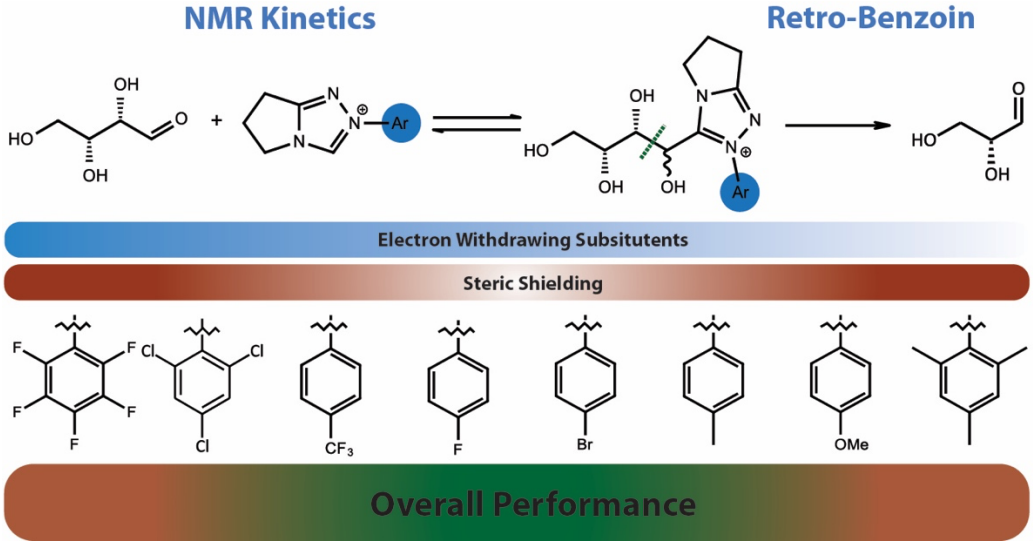
Classical carbohydrate chemistry often focuses on selectivity issues inherent to their polyhydroxy functions and the glycosidic linkage. Interestingly, their most reactive function – the aldehyde – receives comparably little attention. In this light, we study the interactions of aldoses' aldehyde moiety with aldehyde-selective *N*-heterocyclic carbene (NHC) catalysts, aiming to increase the use of carbohydrates as chiral pool materials in organic chemistry.

Recently, we demonstrated a selective dehomologation of selected carbohydrates using an NHC-catalysed retro-benzoin reaction in good yields. In this approach, a Stetter reaction with chalcone as substrate was used as the catalyst recycling step. However, applicability remains limited to particularly suitable substrates. Towards the ultimate goal of a general methodology, we are further elaborating the complex mechanism of this reaction from two points of view:

First, NMR-based kinetic measurements enabled us to understand the kinetics of the initial adduct formation between a specific NHC and an aldose, giving us new insights into catalyst requirements for their efficient carbohydrate activation. Second, we were indirectly probing for the successive retro-benzoin step, by monitoring the rate of formylation of chalcone, thereby recycling the catalyst.

Our investigations have hinted at a sweetspot between the efficiency of the retro-benzoin and Stetter reactions, which needs to be finely balanced.



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Enzymatic synthesis of 0-, a-, b-, and c-series gangliosides

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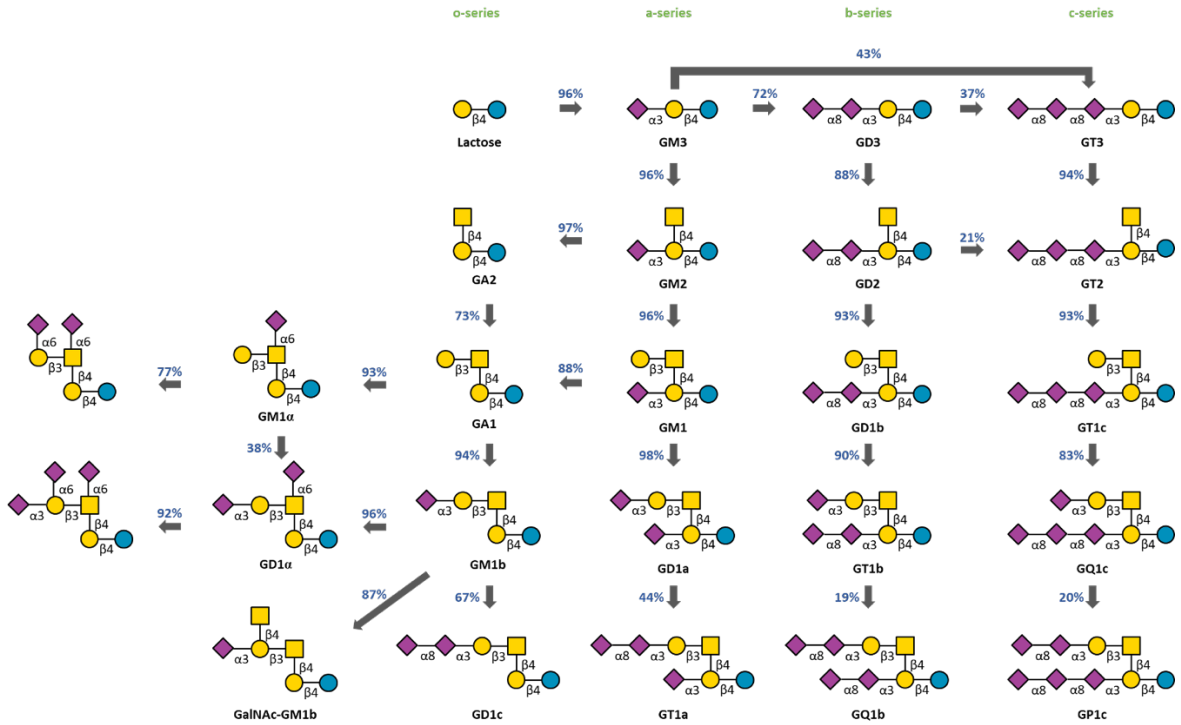
Gangliosides are sialic acid-containing glycosphingolipids. They are primarily localized in the outer leaflets of plasma membranes, participating in cell-cell recognition, adhesion, and signal transduction.[1]

In past decades, many groups had developed different chemical or enzymatic approaches to synthesize gangliosides. However, the chemical synthetic pathways encounter challenges not only precisely controlling regio- and stereo-selectivities in sialylation, but also complicated protection and deprotection are unavoidable. By contrast, enzymatic is more feasible to access these complicated glycans. In order to obtain pure and sufficient amount of these compounds, we started with the chemically synthesized lactose which contains an azide linker for further application in microarray.

Multiple enzymes were applied on the enzymatic synthesis of glycans of 0-, a-, b- and c-series gangliosides. Complicated gangliosides, including GT1a, GQ1b, and GP1c glycans, were synthesized by sequential one-pot enzymatic or regeneration synthesis.



Total synthesis of *Campylobacter jejuni* NCTC11168 CPS assisted with iMAP 1,6-anhydrous strategy

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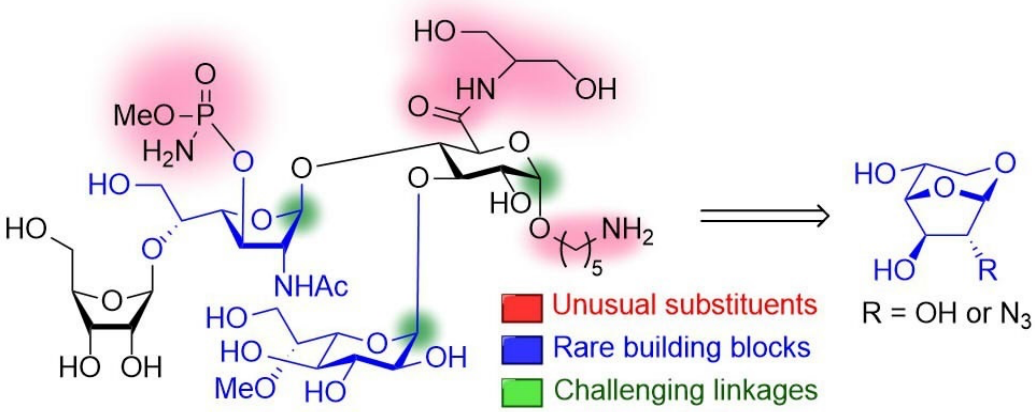
Campylobacter jejuni, a general gastrointestinal pathogen, causes severe diarrhea or Guillain-Balre´ Syndrome (GBS) and threatens human lives. W.H.O. has announced that *C. jejuni* ranks in high-priority of alternative therapy development due to the gradually increasing antibiotic resistance.[1] The pathogenicity of *C. jejuni* is highly related to capsular polysaccharide (CPS) with a regular structure. Therefore, these feature structures can provide a suitable antigen for vaccine development. Here, we report a total synthesis of *C. jejuni* NCTC11168 CPS repeating unit.

The synthesis utilized the strategy of intramolecular anomeric protection (iMAP), which generates a 1,6-anhydro-furanoside sugar residue in a one-pot manner from free-sugar, to concise the building block synthesis.[2] Accordingly, the synthesis of heptose building block from free-galactose in 6 steps, and the galactosamine building block from free-galactosamine in 2 steps. Consequently, merely 28 steps were used to synthesize product 1, including all building blocks synthesis from free-sugar. After structuring the tetrasaccharide skeleton with [2+1+1] glycosylation and completing the required modification, the desired product 1 shows a 13.46 ppm resonance on the ^{31}P NMR spectrum, similar to the native CPS chemical shift of 13.6 ppm.[3] In addition, this product 1 containing linker moiety on the glucose part can conjugate to carrier protein for further vaccination development and is undergoing.



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Development of glycomimetic ligands blocking SARS-CoV-2 binding to C-type lectin receptor L-SIGN

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C-type lectin receptors (CLRs) are a class of Pathogen Recognition Receptors involved at the surface of dendritic cells in pathogen detection. They are dedicated to the recognition of carbohydrate-based molecular patterns associated to pathogens. [1]

Some of those pathogens have found strategies to bypass the role of CLRs in immunity activation and even hijack CLRs for their benefit during infection process. Thus, subversion of CLRs has been reported as alternate receptors or attachment factors, notably by HIV, Ebola virus as well as SARS-CoV virus, responsible of the severe acute respiratory syndrome (SARS) in 2002. [2]

In the context of the world-scale coronavirus outbreak, we demonstrated that two CLRs, DC-SIGN and L-SIGN, are used by SARS-CoV-2 to enhance its infection by trans-infection [3]. This enhancement can be blocked by glycomimetic. L-SIGN is present on airway epithelium cells while DC-SIGN is present on immune dendritic cells.

Our main issue focuses on the development of antagonists with a selectivity towards L-SIGN, as interesting tools to compete with SARS-CoV-2 anchoring. Being able to block virus attachment on airway epithelia (targeting L-SIGN) without activating immune systems (via DC-SIGN binding) is attractive in the SARS-CoV-2 physiopathological context.

Optimized and selected glycomimetic molecules through diverse biophysical studies will be presented as well as development of high affinity multivalent systems. Preliminary data of the use of these candidate-compounds in competition experiment within a cellular model of infection will also be presented.

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Length-controlled glycopolymers from a poly(norbornenyl azlactone) platform for lectin recognition

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Glycopolymers are synthetic backbones with pendant sugars that mimic natural polysaccharides. While the interaction between a single sugar molecule and the complementary carbohydrate-binding protein (lectin) is rather weak in a number of important biological processes, the simultaneous binding of numerous sugar groups of a multivalent polymer can result in a strong conjugation.[1] To prepare such structures, several synthetic strategies have been developed, and among them, click post-polymerization modification (PPM) of functionalized polymer platforms with appropriate sugar derivatives offers a rapid route to generate glycopolymer libraries with a wide range of carbohydrate structures, without requiring tedious protection-deprotection sequences. In this context, ring-opening polymerization metathesis (ROMP) has been widely used as a versatile and controlled method to synthesize functional polymers. In addition, the presence of carbon-carbon double bonds and cyclopentane along the polymer scaffold increases the rigidity of the structure, avoiding conformational entropy penalty and promoting target specificity.[2] The azlactone moiety has emerged as a powerful anchoring group able to react with amino-terminated functional moieties. Indeed, this group shows a high reactivity, full atom economy in a broad range of organic solvents as well as in aqueous solution at room temperature without generating by-products.[3] Moreover, the azlactone functionality is compatible with ruthenium-based catalysts, enabling the fast design of glycopolymers.[4] We report herein the ROMP of the *endo*-and *exo*-(norbornenyl azlactone) diastereoisomers with ruthenium-based third generation Grubbs catalyst in order to access a library of poly(norbornenyl azlactone) (PNBAzl) scaffolds with a wide range of number-average degree of polymerization (*DPn*) ranging from 10 to 1,000. The subsequent PPM with amine-functionalized mannoside and glucosyl ligands led to length-controlled glycopolymers. The mannose analogs were functionalized with a hydrophilic triethyleneglycol (TEG) spacer or hydrophobic heptyl chain. Finally, a TEG-glucosyl was also prepared as a mismatched sugar reference for lectin assays. The binding inhibition of the resulting glucose- and mannose-functionalized PNBAzl was evaluated against a set of models, and therapeutically relevant lectins, using a "lectin profiling" technology. We selected five mannose-binding lectins (ConA, FimH, Bc2L-A, DC-SIGN and langerin) for their biological relevance and marked sensitivity to multivalency.

Scheme 1. Strategy for the synthesis of glycopolymers from a poly(norbornenyl azlactone) platform

Acknowledgements

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Oligogalacturonide (OG): new prospects for plant disease biocontrol and plant health

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The current work is part of the ECOPHYTO II+ project, to find safer alternatives to harmful phytosanitary products by developing bio-based molecules that can activate plant defenses. One promising approach is the use of oligogalacturonides (OGs) derived from plant cell wall pectins via the action of polygalacturonases (PGs). While OGs have been shown to elicit plant defenses, the diversity of tested OGs has been limited[1,2].

In this study, we investigated the structure, dynamics, enzymatic kinetics, hydrolysis products of various plant PGs. When, pectins from commercial sources or agriculture waste were digested by various plant PGs, to product differents OGs pools. LC-MS analysis of these OGs showed that the average degree of polymerization (DP) depended on the substrate's origin and the enzyme used.

We then applied concentrated OGs (5g/L) under controlled conditions to wheat two days before infection with *Blumeria graminis f.* sp. *Tritici (Bgt)*, the fungal causal pathogen of powdery mildew. The results showed that wheat protection against powdery mildew ranged from 25% to 55% depending on the pectin-enzyme pair used.

Our findings demonstrate the importance of characterizing OGs and highlight new ways of inducing defense mechanisms in wheat. By creating bio-based molecules with original structures, we can work towards gradually replacing harmful phytosanitary products and promoting a healthier and more sustainable environment.

OGs from sugar beet and digested by PGs were able to induce wheat resistance against powdery mildew

Acknowledgements

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Functional carbohydrates for health and nutrition

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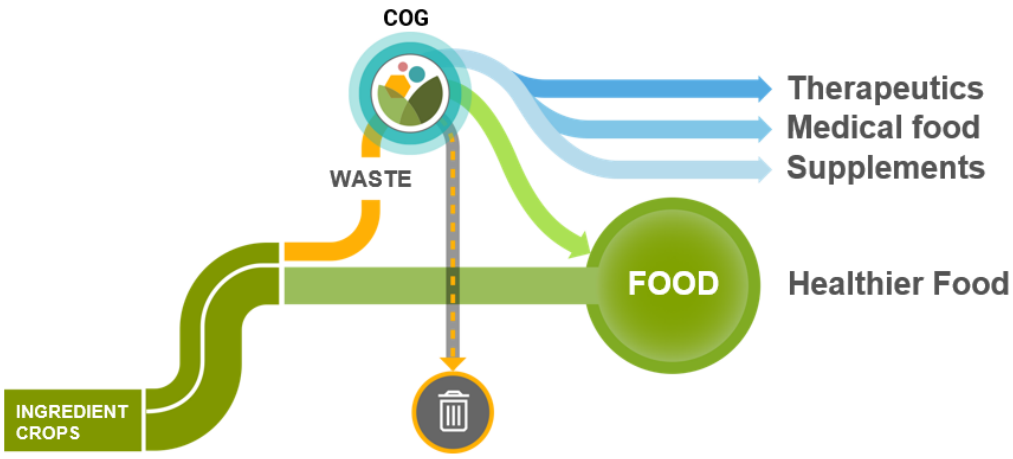
While gut microbiome research continues to uncover new associations with human health and disease, strategies for modulating the underlying bacterial communities have not kept up. Purely bacterial based interventions have received much attention but have not shown to be effective in community redesign. Alternatively, prebiotics have been shown to be more drastic drivers on gut microbiome modulation but are currently limited to just a few structurally diverse products with limited functionality. New strategies for creating novel and structurally diverse prebiotic oligosaccharides would enable new opportunities for impacting health. We have developed a Fenton oxidation-based approach for depolymerizing practically any polysaccharide-rich source material into pools of oligosaccharides with unique and diverse structures. To date, BCD Bioscience has employed this food-grade reaction on over fifty unique food processing waste streams, which has allowed us to generate the largest catalogue of structurally diverse oligosaccharides that can be mined for potential bioactivity. By combining extensive in-house LC/MS-based structural analysis and simulated gut microbiome fermentation assays, we have demonstrated microbial taxa modulation and have linked it to the defined carbohydrate structural features. By shaping the gut microbiome and thus microbial-derived metabolite production, we are exploiting fiber structure-function relationships to develop ingredients with bioactive potential for human health, as food ingredients, medical foods, and pharmaceutical products.



BCD Bioscience ingredient creation flowchart, through our patented COG (creative oligosaccharide generation) process

Synthesis of sugar-functionalised micro-beads for *S. aureus* detection

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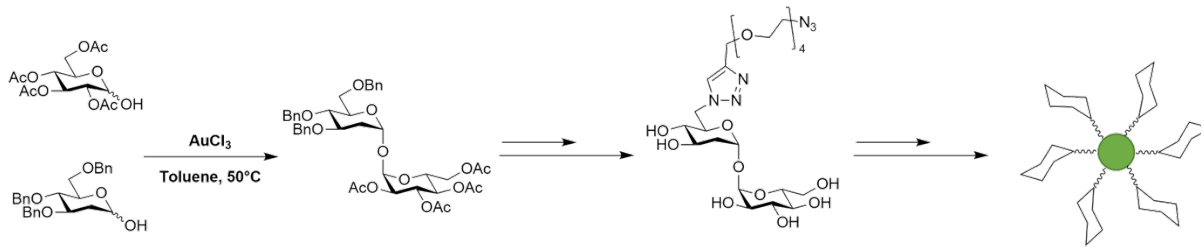
Staphylococcus aureus is a bacterial human pathogen responsible for a wide variety of diseases in both community and hospital-acquired settings. Its widespread occurrence, combined with the emergence of multi-drug resistant strains, requires the development of fast detection platforms for its early detection to maintaining effective antibiotic stewardship and avoiding the unnecessary prescription of broad-spectrum agents.

Bacteria surface ability to interact with specific sugars on the surface of cells can be exploited to specifically target bacteria[1]. Our team recently developed a mannose-based agglutination assay based on computer-aided cluster analysis for *E. coli* detection[2]. Herein, we expand the capabilities of this novel strategy and describe the preparation of trehalose-based microprobes and their evaluation for the cheap and fast detection of *S. aureus*. For this purpose, a small library of trehalose analogues was synthesised using a $AuCl_3$-catalysed glycosylation protocol previously established in our group[3]. The synthetic disaccharides were conjugated to polystyrene micro-beads via click chemistry, and the functionalised microparticles agglutination in the presence of bacteria was evaluated using cluster analysis revealing the key glycan features needed for selective binding to *S. aureus*. Using this platform, we could detect bacteria at 10^8 cfu/ml concentrations. Moreover, we further demonstrated this strategy can be used as a tool to investigate sugars-bacteria interactions which can aid the development of new detection probes and therapies.



Example of platform preparation

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3 R. Jeanneret, C. Walz, M. van Meerbeek, S. Coppock, M. C. Galan (2022), Org. Lett. (34) 6304–6309

Stereo-controlled synthesis of 2-deoxy-C-glycosides with β-glycosyl boronate

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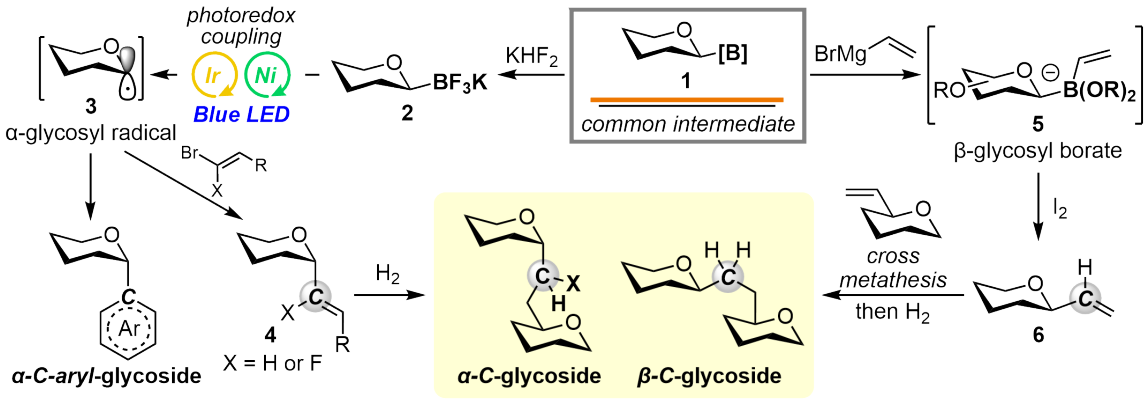
Native glycans are involved in a variety of biological phenomena as polysaccharides or glycoconjugates. Avoiding the enzymatic cleavage of cleavable *O*-glycoside bonds and maintaining the original glycan structures is one of the important issues to analyze their exact functions. *CH_2*-glycoside analogues, in which the oxygen atom of O-glycoside bond is replaced with the methylene group, are expected to be useful tools because of their stability against glycoside hydrolases. However, synthesis of glycan analogues (pseudo-glycans) with a *C*-glycoside linkage is generally complicated, which prevents their application to biological studies or investigation of their therapeutic agents. We envisioned β-glycosyl boronate **1** as the common intermediate for psudo-glycans with both α- and β-C-glycoside linkage [1, 2].

Single electron oxidation [3] of **2** generates α-glycosyl radical **3** stabilized by anomeric effect. Then, cross-coupling reaction with aryl or vinyl halide catalyzed by Ni-complex proceeded to give *C*-aryl-glycosides or α-*C*-vinyl-glycosides (**4**) in a high α-selectivity. Hydrogenation of **4** successfully gave the α-*CH_2*-linked 2'-deoxy-disaccharide analogues. On the other hand, nucleophilic addition to pinacol boronate **1** followed by oxidative 1,2-migration gave β-C-glycoside **6** stereospecifically [4]. Cross-metathesis reaction between **6** and olefins followed by hydrogenation enabled synthesis of β-*CH_2*-linked disaccharide analogues.



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Kinetic *S*-glycosylation by photocatalytic thiol-ene reaction with high stereocontrol

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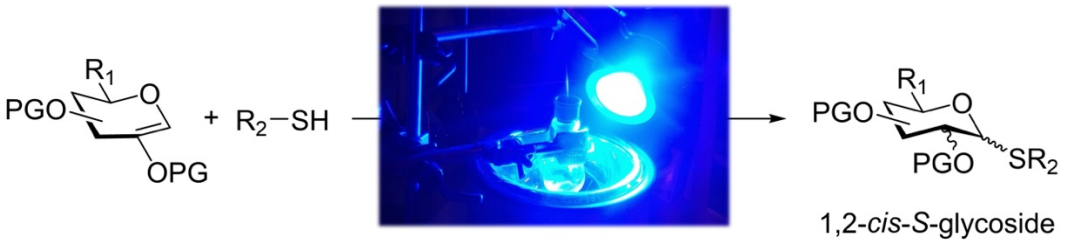
S-Glycosides, isosteres of the most common *O*-glycosides with higher hydrolytic stability, have become interesting for their potential in the development of novel therapeutics in recent decades [1]. However, most of the conventional methods for their synthesis lead to the formation of thermodynamic products with 1,2-*trans* configuration in particular. There are only a few approaches studying the kinetic *S*-glycosylation in the literature, but most of them suffer from use of expensive or toxic mediators composed of transition metals, or UV-light irradiation, which requires specialised laboratory equipment.

Therefore, the synthesis of *S*-glycosides with 1,2-*cis* configuration still remains challenging. In this work, we deal with the regio- and stereoselective synthesis of these *S*-glycosides by thiol-ene reaction, which has become popular in the last decades [2]. Our improved methodology utilises only a catalytic amount of a nontoxic organic initiator excited by environmentally and economically favourable blue visible light. This radical addition on 2-substituted glycals (1,2-unsaturated carbohydrates) provides a wide range of products in good yields with exclusive stereo- and regioselectivity within only 30 minutes.



PG = protecting group
R_1 = H, CH_3, CH_2OH
R_2 comes from EtSH, glycosyl thiols, 4-thioglycosides, 6-thioglycosides, or L-cysteine

Acknowledgements

This work was financially supported from the specific university research (grant No. A1_FPBT_2022_002).

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Serum *N*-glycosylation RPLC-FD-MS assay to assess colorectal cancer surgical interventions

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A newly developed analytical strategy was applied to profile the total serum N-glycome of 65 colorectal cancer (CRC) patients before and after surgical intervention. In this cohort it was previously found that serum N-glycome alterations in CRC associated with patient survival. Here, fluorescent labeling of plasma N-glycans was applied using procainamide and followed by sialic acid derivatization specific for α2,6- and α2,3-linkage types via ethyl esterification and amidation, respectively. This strategy allowed efficient separation of specific positional isomers on reversed-phase liquid chromatography–fluorescence detection–mass spectrometry (RPLC-FD-MS) and complemented the previous glycomics data based on matrix-assisted laser desorption/ionization (MALDI)-MS that did not include such separations. The results from comparing pre-operative CRC to post-operative samples were in agreement with studies that identified a decrease of di-antennary structures with core fucosylation and an increase in sialylated tri- and tetra-antennary N-glycans in CRC patient sera. Pre-operative abundances of N-glycans showed good performance for the classification of adenocarcinoma and led to the revisit of the previous MALDI-MS dataset with regard to histological and clinical data.

This strategy has potential to monitor patient profiles before, during and after clinical events such as treatment, therapy or surgery and should also be further explored.

Acknowledgements

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Calixarene-based cluster for immunostimulation against *Streptococcus Pneumoniae* 19F

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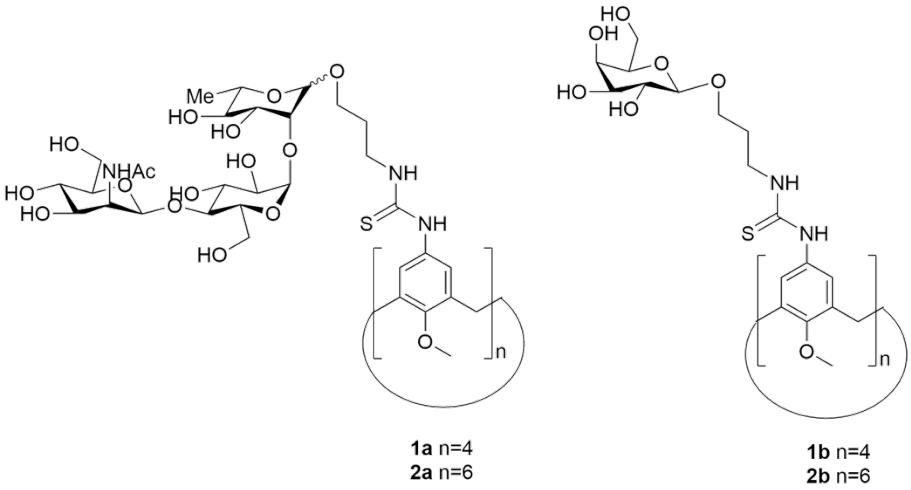
Streptococcus pneumoniae (SP) is one of the most diffused encapsulated bacteria and the principal cause of bacterial infections in young children [1]. Among virulence factors, capsular polysaccharide (CPS) is the most relevant. Serotype 19F is the most virulent. Its CPS structure is formed by β-D-ManpNAc-(1→4)-α-D-Glcp-(1→2)-α-L-Rhap repeating units linked through phosphodiester bridges. The chain length effect of the pneumococcal polysaccharide on immunogenicity has been demonstrated [2]. To trigger an immune response and, then, to develop, in perspective, potential vaccines, the use of molecularly well-defined and monodispersed polyglycosylated cores exposing multiple copies of the simple repeating unit could be exploited in alternative to disomogeneus poly(oligo)saccharide chains. Calixarenes are versatile platforms for the preparation of multivalent glycoclusters [3]. The possibility to regulate valency and geometry allowed to produce efficient and selective calixarene-based ligands, the glycocalixarenes, for carbohydrate recognition proteins (lectins) [3]. In a previous work, the different presentations of multiple copies of β-D-ManpNAc and 19F trisaccharide using calixarenes as scaffold were studied, evidencing the ability to interact with specific anti SP-19F antibodies [4]. Starting from these results, we selected the conformationally mobile methoxycalix[4]- and calix[6]arenes as scaffolds to continue our investigation in using them to develop immunostimulants. We will present the synthesis of the calixarenes and their functionalization with the SP-19F trisaccharide repeating unit (**1a** and **2a**) and with galactose as negative controls (**1b** and **2b**). The preliminary data relative to the biological properties and the immunization experiments will be also illustrated.



1a n=4
2a n=6

1b n=4
2b n=6

Glycocalixarenes respectively for immunostimulation and as negative control

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Chemical synthesis of *A. faecalis* monophosphoryl Lipid A and a conjugate of Lipid A with Tn antigen

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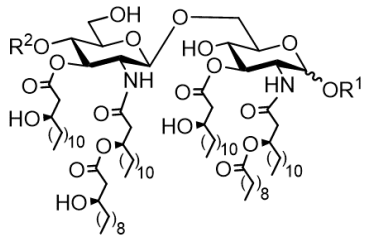
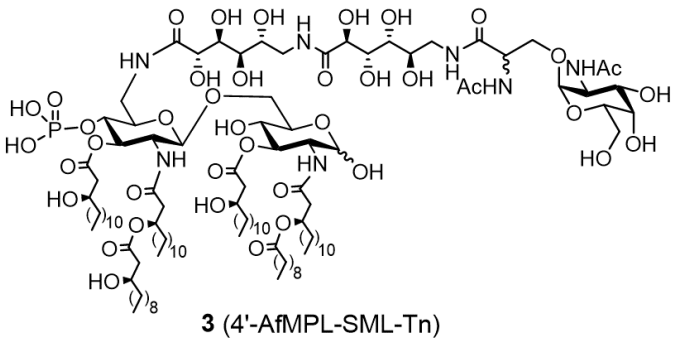
Lipopolysaccharide (LPS), a glycoconjugate constituting the outer membrane of Gram-negative bacteria, is known as a representative innate immunostimulant. LPS and its active principle lipid A have potential to function as adjuvants that enhance vaccine efficacy. While the canonical *E. coli* LPS is toxic, monophosphoryl lipid A (MPL), lacking one phosphate group was found to be an attenuated immunostimulant hence attractive for adjuvant applications. GlaxoSmithKline also developed an MPL analog, 3D-MPL[1], which was approved as a safe adjuvant for practical use in vaccines. Alternatively, our group revealed that symbiotic *Alcaligenes faecalis* lipid A (AfLA)[2], diphosphate type, showed useful adjuvant activity without toxicity[3]. Here we synthesized *A. faecalis* MPLs (AfMPLs) **1** and **2**. Both AfMPLs induced weaker IL-6 cytokine induction than AfLA. Their adjuvant activity is now under investigation. Meanwhile, self-adjuvanting vaccine strategies in which antigen and adjuvant are covalently linked have recently been well studied especially in the development of carbohydrate-based vaccines[4,5]. However, there are few reports of lipid A-based self-adjuvanting vaccines[5,6] because structural modification often inactivates lipid A. Here we successfully synthesized conjugate **3** consisting of 4'-AfMPL **2** covalently linked to a tumor-associated carbohydrate Tn antigen via a sugar mimic linker (SML). Conjugate **3** significantly induced IL-6 cytokine; about 10 times weaker than unmodified **2**, and now in *vivo* assay is underway.



1 (1-AfMPL)
R^1 = -P(=O)(OH)$_2$, (only α form), R^2 = H

2 (4'-AfMPL)
R^1 = H, R^2 = -P(=O)(OH)$_2$



3 (4'-AfMPL-SML-Tn)

Bibliographic references:

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Gram scale synthesis of three regioisomeric tetrasaccharides composed of alpha-glucoside units

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Oligosaccharides are very common natural compounds composed of monosaccharides conjugated together via glycosidic bonds through exceptional diversity.[1] Their composition and structural diversity is as varied as their biological or physico-chemical properties. The tailor-made synthesis of complex glycans is an everlasting challenge of carbohydrate chemistry that can be tackled through multi-step organic synthesis[2]and/or chemo-enzymatic synthesis[3] in solution and/or through solid phase synthesis.[4] Stereoselective glycosylation is the key to such complex oligosaccharides synthesis, with a particular additional challenge to prepare 1,2-*cis* glycosides with high stereocontrol.[5] We focused here on the multi-step synthesis of three regioisomeric tetrasaccharides composed of glucose with exclusively 1,2-*cis* linkages, the four glucose units being connected through either alpha-1,4 or alpha-1,6 linkages, linear or branched (**Figure 1**). The first tetrasaccharides **A** was obtained in 9 steps from benzylated maltotriose with a key [3+1] glycosylation using a glucosyl *N*-phenyl trifluoroacetimidate donor according to Codée's protocol for primary alcohol acceptors.[6] Tetrasaccharide **B** was synthesized in a [2+2] glycosylation strategy from two maltose building blocks, using a *N*-phenyl trifluoroacetimidate donor. The third tetrasaccharides **C** could be accessed through two different [2+1+1] glycosylation strategies from (1) a maltosyl acceptor with either both 4'- and 6'-positions free to be glucosylated simultaneously or through (2) a sequential 6'- then 4'-glucosylation of a 4',6'-naphthylidene maltoside.

Figure 1

A detailed description of each strategy and synthetic steps will be provided with focus on the regio- and stereoselective access to each of the three tetrasaccharides in convergent and efficient synthetic schemes. Each tetrasaccharide could be obtained in a multi-gram scale from commercially available monosaccharides and commonly available reagents.

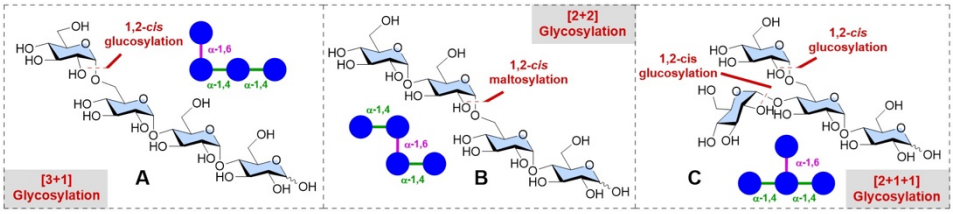

Figure 1 : Structure and general strategy towards the three regioisomeric tetrasaccharides

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Development of novel D-glucuronamide-based nucleoside analogs containing 1,2,3-triazole units as potential anticancer agents

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The synthesis of D-glucuronamide-containing molecules has attracted increasing attention in the context of the search for new bioactive compounds, which is motivated by the biological profile reported for either natural or synthetic derivatives containing this glycosyl unit.[1]

Within our continuous interest in the synthesis of new potentially bioactive D-glucuronamide-based compounds, among them nucleoside analogs [2,3], in this communication we report on the synthesis of innovative nucleoside analogs constructed on D-glucuronamide scaffolds containing 1,2,3-triazole units. The synthesized molecules included [*N*-(glucuronamidyl)triazolyl]methyl phosphonates as potential sugar diphosphate mimetics, in which the (triazolyl)methyl phosphonate system was planned as a prospective neutral diphosphate mimetic, and glucuronamide-based (purinyl)methyl triazole nucleosides. For their access, D-glucofuranuronolactone was used as starting material and key synthetic steps included amidation, furanose to pyranose isomerization, anomeric azidation, azide-alkyne 1,3-dipolar cycloaddition or Arbuzov reaction. The biological evaluation demonstrated that some of the synthesized compounds have significant antiproliferative activities in cancer cells.

Acknowledgements

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Epitope mapping of the PA surface polysaccharide Pel

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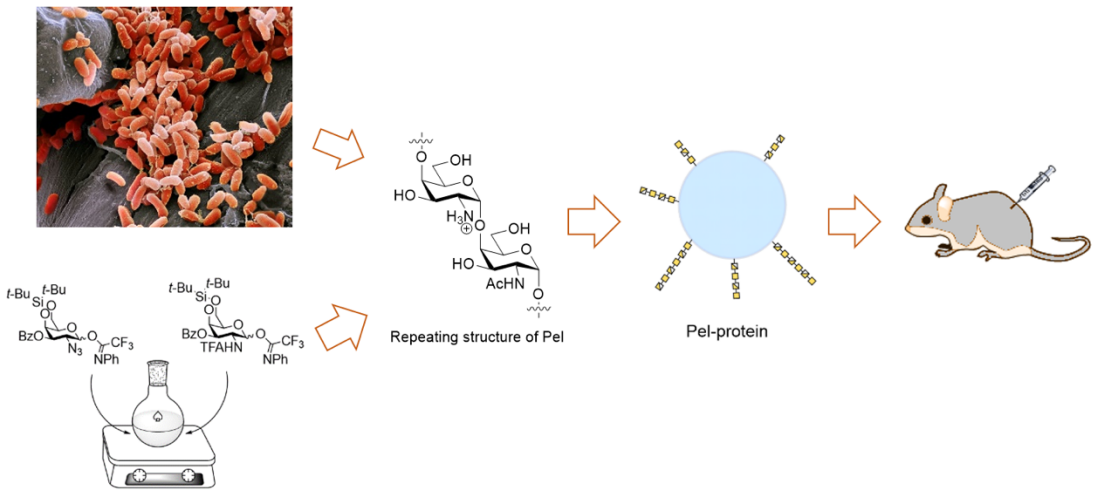
Pseudomonas aeruginosa (PA) is an antimicrobial resistant pathogen responsible for serious infections and it belongs to the ESKAPE pathogens, which are the main cause of nosocomial infections and a growing concern for antibiotic resistance. Developing a vaccine against *P. aeruginosa* is therefore a promising approach. Three exopolysaccharides, which are considered potential targets for bacterial vaccine development, have been identified in the *P. aeruginosa* biofilm: alginate, Psl, and Pel.

The structure of exopolysaccharide Pel has recently been elucidated and is thought to be composed of 1,4-alpha-linked galactosamine and N-acetyl galactosamine[1-2]. Well-defined Pel fragments have been synthesized and will be used as standards for structure elucidation studies, as well as being conjugated to CRM and BSA for use in *in vivo* studies. Meanwhile, natural Pel will be purified from the *P. aeruginosa* strain for vaccine generation and structural analysis experiments.



Acknowledgements

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A new solid-phase method for the chemoenzymatic synthesis of heparan sulfate and chondroitin sulfate

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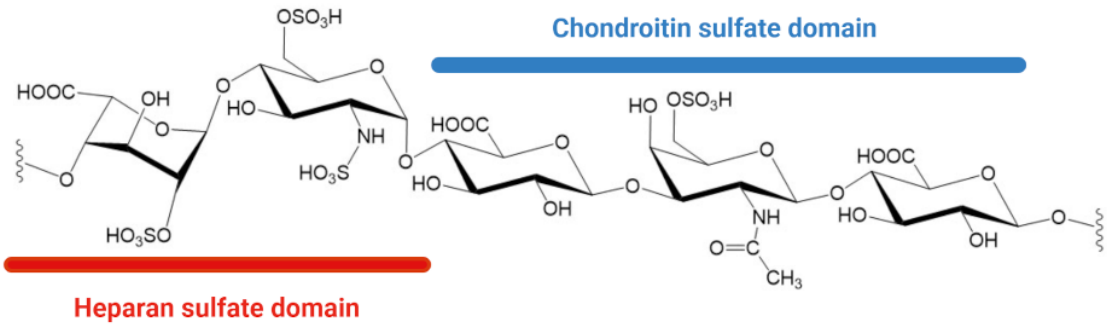
Heparan sulfate (HS) and chondroitin sulfate (CS) are two structurally distinct natural polysaccharides that play important roles in a variety of biological processes. Here, we report the development of a solid-phase enzymatic synthesis approach for the synthesis of HS and CS chimeras. Our approach utilizes specialized linkers that are covalently attached to a solid support. The linkers are then used to enzymatically synthesize HS or CS chains, which are subsequently released from the support by enzymatic digestion.

We also have successfully synthesized a library of seven structurally homogeneous HS and CS chimeric dodecasaccharides (12-mers). The chimeras contain a CS domain on the reducing end and a HS domain on the nonreducing end. The synthesized chimeras display anticoagulant activity as measured by both in vitro and ex vivo experiments. Furthermore, the anticoagulant activity of H/C 12-mer 5 is reversible by protamine. Our findings demonstrate the synthesis of unnatural HS-CS chimeric oligosaccharides using natural biosynthetic enzymes, offering a new class of glycan molecules for biological research.



The molecular basis for inhibition of heparanases and β-glucuronidases by Siastatin B.

Elisha MORAN [1], Yurong CHEN [1], Rian van den NIEUWENDIJK [1], Liang WU [1], Foteini SKOULIKOPOULOU [1], Vera Van RIET [1], Hermen S. OVERKLEEFT [1], Gideon J. DAVIES [1], Zachary ARMSTRONG [1]

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The sulfated glycosaminoglycan polysaccharide known as heparan sulfate (HS) is a highly heterogeneous molecule that's central to biological and pathological processes including its role as a ubiquitous component of the extracellular matrix [1] [2]. HS regulation is modulated by continual breakdown and biosynthesis. Heparanases are GH79 endo-b-glucuronidases that are involved in the breakdown of HS [2]. The human heparanase (HPSE) is involved in the cleavage of HS at GlcA residues. HPSE is a promising target for anti-cancer drugs due to its enzymatic; it is highly involved in cancer biology. Aberrant HPSE expression has been linked to the development of larger and highly metastatic tumours consequently leading to poor prognosis [3]. Multiple compounds that inhibit heparanase have significant effects in tumour models and have progressed to clinical trials [3].

The iminosugar siastatin B is a known inhibitor of neuraminidases, N-acetyl-glucosaminidases and b-D-glucuronidases [4]. However, structures of HPSE with inhibitors and substrates suggest that the active site would be unable to accommodate siastatin B due to the steric clashes of the N-acetyl group at the 2-position [5]. Here we show that the inhibition of HPSE (and related enzymes) reflects not the parent compound but a pair of breakdown products; a galacturonic-noeuromycin or 3-geminal diol derivative of galacturonic-isofagomine. This new class of glycosidase inhibitors have been subsequently re-synthesized and their co-crystal structures with the bacterial b-glucuronidase AcGH79 and HPSE were investigated.

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Expression of α1,2-mannosidases impacts the protein *N*-glycan profiles in *Chlamydomonas reinhardtii*

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The green microalga *Chlamydomonas reinhardtii* is a model for studying cellular biological processes including synthesis of glycoproteins (**Mathieu-Rivet et al., 2020**). *N*-glycosylation represents a post-translational modification of major importance since it impacts the biological function of the expressed proteins. Structural analysis of glycans *N*-linked to *C. reinhardtii* proteins has demonstrated that mainly oligomannoside structures are synthesized, the main one being a non-canonical $Man_5GlcNAc_2$ (**Vanier et al., 2017**). In the context of bioproduction of recombinant proteins, the glyco-engineering of *C. reinhardtii* oligomannosides into humanized *N*-glycans requires the accumulation of the chassis $Man_3GlcNAc_2$. Indeed, this structure is a prerequisite since it allows the transfer of terminal *N*-acetylglucosamine and then decorations such as galactose or fucose to give human-like *N*-glycans. Thus, the accumulation of $Man_3GlcNAc_2$ in *C. reinhardtii*implies to remove the two α1,2-linked Man from $Man_5GlcNAc_2$. Towards this objective, we expressed in *C. reinhardtii,* the α1,2- mannosidases either from *Aspergillus saito* (AsMAN) or from *Arabidopsis thaliana* (AtMNS1). In the transformed microalgae expressing either AsMAN or AtMNS1, $Man_3GlcNAc_2$ was strongly increased to the detriment of $Man_5GlcNAc_2$. These results demonstrated that the glyco-engineering of the *C. reinhardtii N*-glycosylation pathway can be performed by expression of heterologous glycoenzymes and further confirm that the targeting mechanisms of glycoenzymes into the secretory system is conserved between microalga and plants.

Acknowledgements

The authors are thankful to the Region Normandie for funding this work through the SweetTrip RIN RECHERCHE 2021, and the SweetBioPharm RIN XLChem prog

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Synthesis of multivalent chondroitin sulfate (CS) oligosaccharides to study CS-Cat K interactions

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Chondroitin sulfates (CS) are polysaccharides which belong to a family of linear polyanionic polymers named glycosaminoglycans (GAGs). They consist of a repeating dimeric unit composed of a D-glucuronic acid (D-GlcA) linked to a 2-acetamido-2-deoxy-D-galactose (D-GalNAc) and contain sulfate groups at various positions. The most common sulfated positions are position 4 (CS-A) and/or position 6 (CS-E/CS-C) of the D-GalNAc moiety.[1] CS play an important role in different physiological and pathological processes[2] through their interaction with numerous proteins such as lectins, cathepsins or midkines. As an example, CS are involved in osteoporosis by forming a complex with the exosites of cathepsin K (Cat K) that is involved in the excessive degradation of collagen.

In order to better understand and to inhibit natural linear CS-Cat K interactions, we are particularly interested in the design and synthesis of variously sulfated multivalent oligosaccharides of CS. Indeed, multivalency is widely observed in Nature because it improves activity and affinity towards the ligands. Very few synthetic multivalent GAGs mimetics have been reported and the field of CS remains largely unexplored.[3]

Our strategy relies on the ligation of new CS oligosaccharides fragments bearing an azido group on the aglycone and various platforms with a terminal alkyne to build new multivalent CS oligosaccharides which will be assessed on Cat K. We will present here our recent results towards their synthesis.[4]

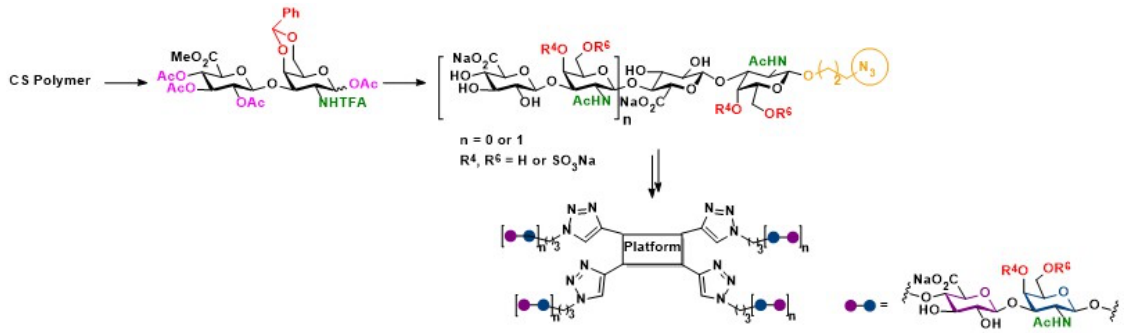

Figure 1: Synthesis of multivalent oligosaccharides

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Glycoengineering lipooligosaccharides on bacterial pathogens

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Bacterial glycans play an important role in the interaction with their host. Lipooligosaccharides (LOS) are the most abundant cell surface glycoconjugates on the outer membrane of most Gram-negative bacteria. They contribute to the rigidity and impermeability of the cell wall and can stimulate the host immune system. Some bacteria display LOS capped with sialic acid as terminal epitopes to mimic host glycans. This phenomenon, known as molecular mimicry, can help bacteria hijack host biological mechanisms to facilitate infection and evade the host's immune system. To further investigate the functional role of sialic acid-capped LOS at the molecular level, it is important to have tools readily accessible for the detection and manipulation of Neu5Ac on glycoconjugates of live bacteria.

Here we report a novel strategy to incorporate Neu5Ac with a reporter group onto the lipooligosaccharides of a selection of Gram-negative bacteria. We show that the native sialyltransferases of several pathogens are able to accept extracellular unnatural sugar nucleotides, thereby introducing unnatural sialosides onto their LOS. This new technique, Labeling via Bacterial Native Sialyltransferases, is an efficient and rapid way to screen for bacteria that can decorate their glycoconjugates with exogenous sialic acid. This strategy complements other glycoengineering techniques, such as Metabolic Oligosaccharide Engineering (MOE) and Selective Exo-Enzymatic Labelling (SEEL), and can help to dissect host-bacterial glycan interactions.

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Phosphates as traceless assisting groups in glycan synthesis

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Many new recent hardware and chemical developments in the instruments and protocols utilized for automated glycan assembly have expanded the possibilities of glycans achievable. Additionally, new protocols have been developed for the expedited synthesis of oligosaccharides containing either an anionic phosphate or sulfate moiety.

These phosphate motifs were then utilized to overcome glycan synthesis limitations including increasing solubility of cellulose oligosaccharide chains to ease purification and also used as a protecting group for regioselective enzymatic sialylation. The phosphate motifs could advantageously be removed in a traceless fashion in a controlled manner under mild conditions with phosphatase to allow for natural sialyated structures and controlled cellulose platelet formation.

Optimization and chemical characterization of pectins from blackcurrant fruit pomace obtained by NADES extraction

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Nowadays, one of the main goals of the industry is to proceed efficient sustainable processes that result in high-quality products at low cost. For this reason, known procedures are modified to meet new requirements. Improvements are possible thanks to the optimization of technological processes, and thus the modifications of products or the course of individual processes. The changes result from the implementation of the principles of green chemistry and include environmentally friendly methods.

The fruit processing is an important part of the food industry in Poland, and juice production especially. Every year tons of fruit pomace are generated. These wastes are usually rich in polysaccharides, mainly pectin, which present a wide range of applications, i.e. as gelling agents, thickeners, emulsifiers, stabilizers, etc. [1,2]. Commercially, pectin isolation process is carried out using conventional methods based on mineral strong acid solutions and toxic organic solvents [3]. The synergy of the management of wastes from fruit processing for pectin extraction with clean separation methods is an attractive direction in the development of sustainable technologies. One of the most promising, green methods is the use of natural deep eutectic solvents (NADES).

The experimental model of pectins extraction from black currant pomace using NADES systems was developed. NADES were made from different molar ratios of choline chloride (HBA) and citric acid (HBD). The optimization was performed with response surface methodology (RSM). The products with the most favorable results were analyzed using spectroscopic and chromatographic methods.

Acknowledgements

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Anti-toxin agents for the treatment of *Clostridium difficile* infections

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Clostridium difficile (*C. diff*) is the most common nosocomial infection in western countries with symptoms ranging from mild diarrhoea to a life-threatening inflammation of the bowel. *C. diff* is a spore-forming gram-positive bacteria, which usually lives harmlessly in the gut of about 1 in every 30 healthy adults. However, antibiotic use can unbalance the gut microbiota and facilitate *C. diff* proliferation. *C. diff* produces two toxins, **TcdA** and **TcdB**, which are responsible for the disease pathogenesis. TcdA and TcdB are glucosyltransferases that use UDP-glucose to glucosylate and inactivate host Rho GTPases, resulting in cytoskeletal changes causing cell rounding and loss of intestinal integrity (Figure 1). Developing a selective and potent anti-toxin agent would offer a new *C. diff* therapy while minimising damage to the colon. Importantly, the human gut microbiota barrier would be spared, which is critical to prevent *C. diff* recurrence.

Transition-state analysis of TcdA and TcdB has allowed the design of transition state analogue inhibitors of the toxins which demonstrate therapeutic potential.[a] Here we will present our latest results.

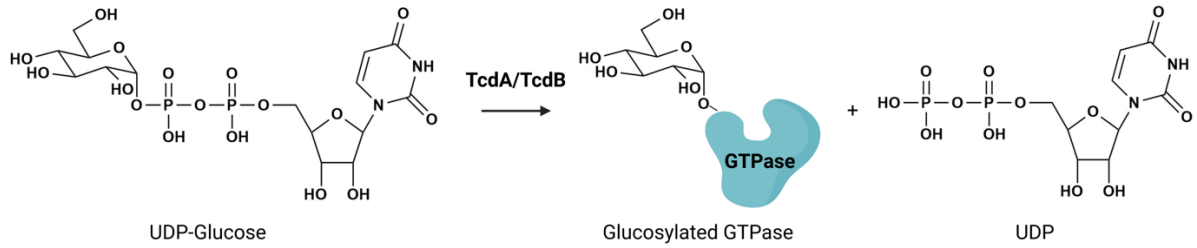

Figure 1: TcdA and TcdB catalysed glycosyltransferase reaction.

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Rational design of gold(III)-glycoconjugates as antiviral agents against SARS-CoV-2

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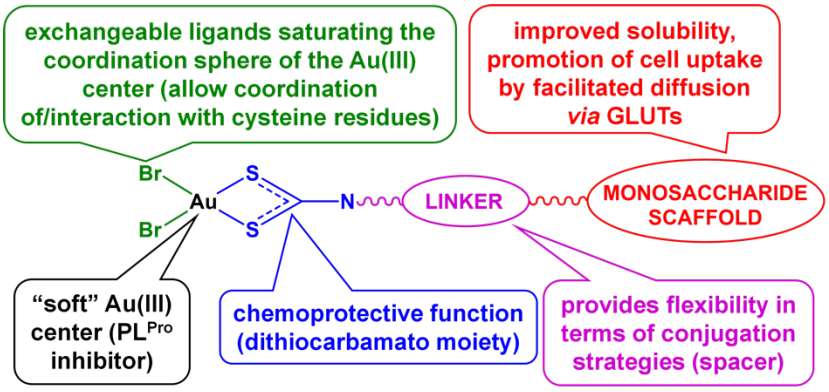
First identified in Wuhan (China) in December 2019, the Coronavirus Disease 2019 (COVID-19) pandemic has been causing a major global health (and, subsequently, socio-economic) emergency which undoubtedly exposed the fragility of the current globalized society. Remarkably, it was the third recent coronavirus-related outbreak after SARS-CoV (2002) and MERS-CoV (2012), thus highlighting the urgent need for dedicated antiviral therapeutics,[1] not an easy task given their extremely low approval rates.[2]

Notwithstanding the recent development of state-of-the-art vaccines has considerably reduced the spread of the virus and the associated risk of hospitalization and fatalities, infection figures still pose a significant threat to patients (especially unvaccinated and vulnerable), and are still putting the healthcare systems under major pressure.[3] Although substantial efforts in drug design and repurposing have been undertaken, to date there are only a few drugs (which were already marketed) have been officially approved for the treatment of COVID-19 infection, mostly recommended to treat patients at high risk.[4] In this context, metal derivatives are generally under-represented in the compound libraries used for screening in drug discovery campaigns, despite of the growing evidence of their role in medicinal chemistry. On account of the aforementioned considerations, based on solid encouraging preliminary results recently obtained in our group,[5] we here report on the design of monosaccharide-containing gold(III)-based derivatives as antiviral agents against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The goal is to generate non-toxic metal-glycoconjugates to be taken up by coronavirus-infected cells and, once internalized, capable of acting as potent inhibitors of specific SARS-CoV-2 target proteins, such as the Papain-like Protease (PL^{Pro}), with a view to preventing the replication of the virus and its subsequent spread to other host cells.[6]



General designing strategy to the target metal-glycoconjugates against SARS-CoV-2.

Acknowledgements

Financial support from the University of Galway (Postgraduate Scholarship) and scientific support from Prof. Paul Murphy are gratefully acknowledged.

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Bypassing cotton cellulose biosynthesis to design fibers with tailored properties

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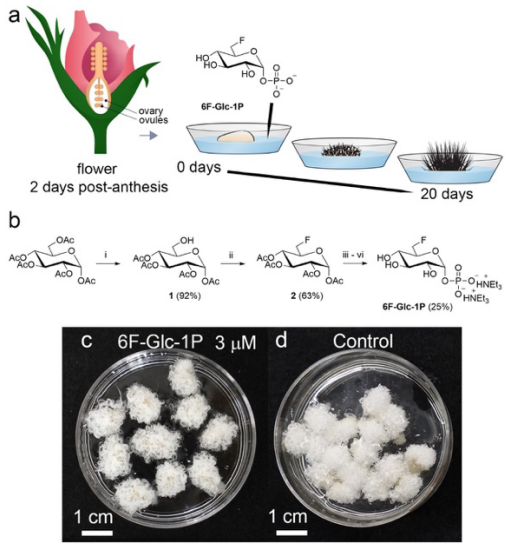
Current manufacturing strategies for cotton fiber post-processing generate large amounts of chemical waste, endangering the environment and the livelihoods of millions of people. Understanding and harnessing cotton's underlying mechanisms to produce fibers with tailored properties is a significant step toward future sustainable alternatives. In this study, we demonstrate that a chemosynthesized phosphorylated glucose derivative carrying a fluorine atom at position C6 of the glucose moiety - 6-deoxy-6-fluoro-glucose-1-phosphate - is biologically incorporated into cotton fibers in cotton ovule in vitro cultures. The resulting modified fibers showed increased tensile and water-retaining properties, as a result of an alteration of the cellulose structure. Finally, our study demonstrates yet another successful example of the advantages of biological fabrication of cellulose-based materials with novel emerging properties that surpass their natural counterparts toward the design of future sustainable materials and the implementation of a true bio-based economy and the concept of material farming.



Feeding cotton in vitro ovules with chemosynthesized 6F-Glc-1P to bypass cotton biosynthesis

Acknowledgements

This research was supported by the European Union, ERC Consolidator project" BIOMATFAB" (Project #101045466), GIF and MINERVA Stiftung.

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Promiscuity in glycosyltransferases: chitin nanofibrils formation by cellulose synthase

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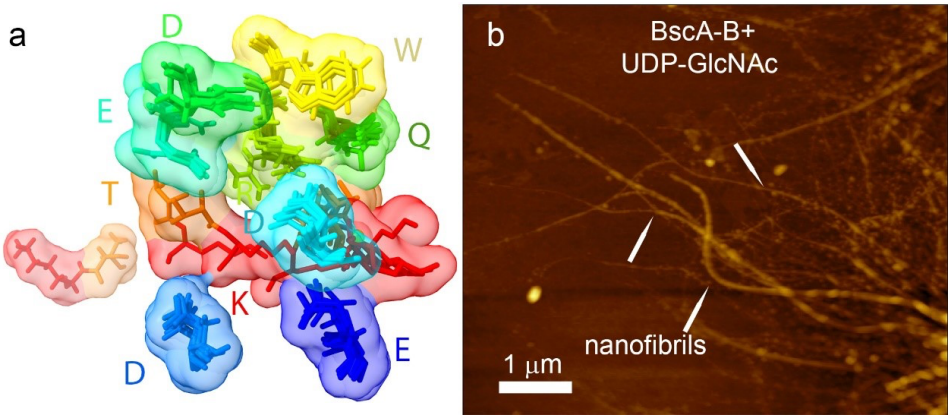
Glycosyltransferases (GTs) play essential roles in various biological processes and exhibit promiscuity in substrate specificity, allowing the synthesis of diverse glycoconjugates, crucial for the adaptation and survival of organisms. Cellulose and chitin synthases are examples of processive GTs involved in cellulose and chitin biosynthesis, respectively. Organisms that produced chitin do not produce cellulose and vice-versa. Recently, we have demonstrated that bacterial cellulose and chitin synthases share highly similar active site architecture despite low global amino acid sequence and 3D structure similarities [1], laying a theoretical framework for experimental validation. Here we demonstrate that recombinant bacterial cellulose synthase from *Rhodobacter sphaeroides*(BscA-BscB) catalyzes the cleavage of uridine diphosphate N-acetylglucosamine (UDP-α-D-GlcNAc), resulting in the formation of chitin nanofibrils in vitro glucose. BscA-BscB has a similar substrate affinity between UDP-α-D-GlcNAc and its natural substrate uridine diphosphate glucose (UDP-α-D-GlcN) but less efficient toward UDP-α-D-GlcNAc. *R. sphaeroides* grown in the presence of UDP-α-D-GlcNAc resulted in the formation of chitin oligomers.

This study highlights the catalytic promiscuity of bacterial cellulose synthase, providing insights into the formation of chitin nanofibrils and expanding our understanding of GTs' functional versatility. The findings contribute to the broader field of glycobiology and have implications for biotechnological applications involving glycoconjugate synthesis.



a, Conservation of bacterial cellulose and chitin active sites [1]. b, AFM of chitin fibrils

Acknowledgements

This research was supported by the European Union, ERC Consolidator project" BIOMATFAB", GIF and Minerva Foundation

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Molecular basis for substrate recognition and septum cleavage by AtlA from *Entercoccus faecalis*

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The cleavage of septal peptidoglycan at the end of cell division facilitates the separation of the two daughter cells. The hydrolases involved in this process (called autolysins) are potentially lethal enzymes that can cause cell death; their activity, therefore, must be tightly controlled during cell growth. In Enterococcus faecalis, the N-acetylglucosaminidase AtlA plays a predominant role in cell separation. atlA mutants form long cell chains and are significantly less virulent in the zebrafish infection model. The attenuated virulence of atlA mutants is underpinned by limited dissemination of bacterial chains in the host organism and more efficient uptake by phagocytes that clear the infection.

AtlA has structural homologs in other important pathogens, such as Listeria monocytogenes and Salmonella typhimurium, and therefore represents an attractive model for designing new inhibitors of bacterial pathogenesis. Here, we provide the crystal structure of the E. faecalis AtlA catalytic domain and a catalytic mechanism for the hydrolysis of the glycosidic bond. A model of the AtlA–substrate complex helped us identify key residues critical for substrate recognition and septum cleavage during bacterial growth. We propose this work will provide helpful information for the rational design of specific inhibitors targeting this enterococcal virulence factor and its orthologs in other pathogens.

Acknowledgements

We thank Laurent Hoffer (Cancer Research Center of Marseille, Marseille, France) for his help with the docking experiment and Gerlind Sulzenbacher fo

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Design and synthesis of covalent inhibitors for inverting α-glucosidases

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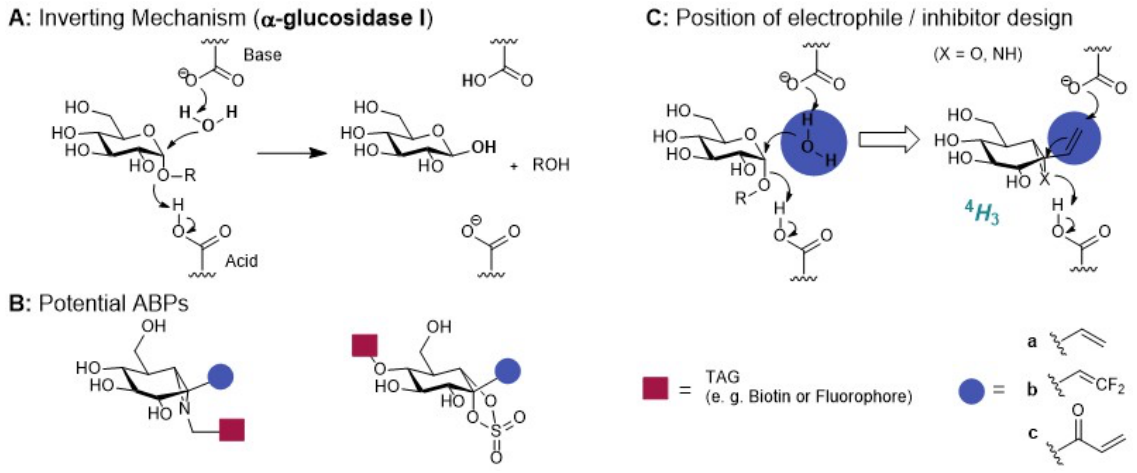
Inverting glycosidases are an important and widespread class of enzymes (Fig. 1 A). As for retaining glycosidases, these enzymes can be promising targets in biomedicine and biotechnology. Inverting α-glucosidases are of particular interest as they play an important role in cellular functions, such as the correct folding of proteins in the endoplasmic reticulum. However, selective inverting α-glucosidase inhibitors are scarce, and chemical probes that selectively report on their activity in biological samples do not exist.

To overcome the absence of inhibitors we sought to apply activity-based protein profiling (ABPP) to this class of enzymes. This powerful method depends heavily on the availability of mechanism-based enzyme inhibitors to develop affinity-based probes (ABPs, Fig. 1B). Herein we present the rational design and synthesis of potential covalently binding inhibitors targeting inverting α-glucosidases. The designs are based on the use of carbaglucose scaffold, carrying an epoxide, mimicking the $^4H_3$ conformation of the natural substrate in the transition state of the hydrolysis reaction. To enable the formation of a covalent bond between the inhibitor and the enzyme, a suitable electrophile is introduced that can take up the space normally occupied by the water molecule involved in the hydrolysis reaction (Fig. 1C). In addition, a specially developed assay was implemented and used for biological evaluation.



Design approach for covalently binding inverting α-glucosidase inhibitors in ABPP.

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En route to the synthesis of mannosidase-resistant oligomannoside mimetics as anti-HIV vaccine

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Despite the development of highly efficient treatments preventing HIV contagion, 1.46 billion people still get infected every year. Some surface proteins of HIV-1 envelope are found under a glycosylated form, bonded with specific oligomannans. As theses oligomannans are targeted by several neutralizing antibodies, they present an interest in the development of anti-HIV vaccines.[1] Classic approaches of vaccination with mannosidic glycosides were unsuccessful. One hypothesis is that mannosidase-type enzymes present in blood trimmer oligomannosides into shorter chains during immunization process, making neutralizing antibodies non-specific to the molecules used for vaccination.[2] To validate our concept, we have focused on the synthesis of non-hydrolyzable mannans: thiomannans. We assume that a mannan bearing *S*- rather than *O*-glycosidic bonds could present a better tolerance towards blood mannosidases. Moreover, to make the mannan more distinct than others, our target will be an analog based on the LPS of *rhizobium radiobacter* Rv3 [3], which is recognized as a functional glycomimetic of the D1 arm of mannans expressed by HIV. To this end, we designed the synthesis of heptamannoside **1**. The retrosynthetic scheme presented in Fig. 1 consists in the synthesis of five building blocks. The synthesis of derivative **3** is crucial as the introduction of the thioacetyl moiety allows us to do stereocontrolled glycosylation (due the participation of the acetyl group). The most challenging reaction is the synthesis of the thioglycosidic bond as it represents the key of this project.

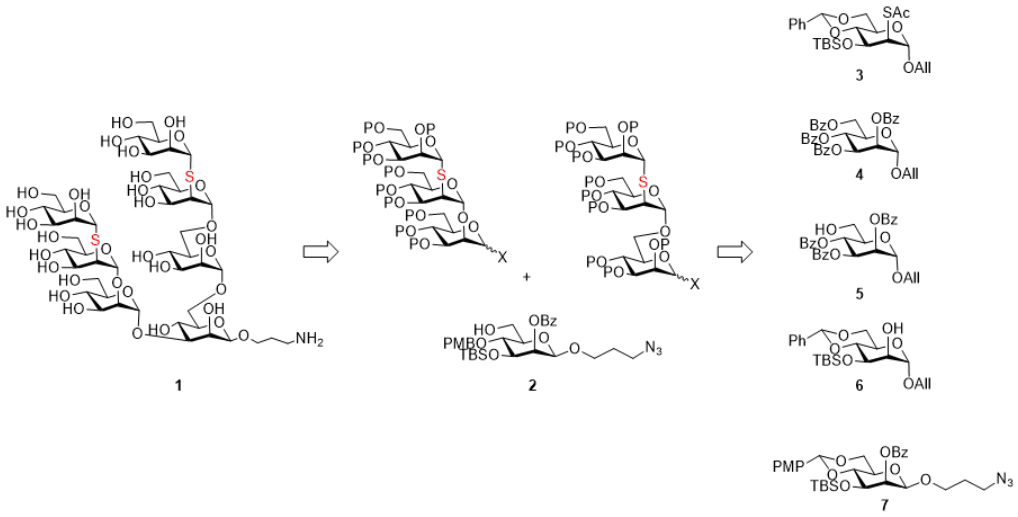

Figure 1: retrosynthetic scheme for the synthesis of 1

Acknowledgements

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Design and synthesis of inhibitors for sialic acid esterases

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Human cells are coated with a dense array of glycoproteins and glycolipids called the glycocalyx [1]. Sialic acids are nine-carbon monosaccharides that are mostly found as the terminal residues of glycans forming the glycocalyx with N-acetyl-5-neuraminic acid (Neu5Ac) being the most common member of this family [2]. A common post translational modification of glycoproteins, is the O-acetylation of the hydroxyl at C-4, 7, 8, and/or 9 on Neu5Ac which is important in cell-cell signaling, autoimmunity and viral infection [3,4].

O-acetylated-Neu5Ac is a receptor for Influenza C and type 2a coronaviruses and toroviruses [5]. These viruses bind host cell surface O-acetylated-Neu5Ac-glycoproteins via hemagglutinin-esterases (HEs) or a spike protein followed by cleavage of the acetyl-group, leading to the destruction of the receptor. Viruses bearing non-functional HEs have reduced infectivity, highlighting their potential anti-viral targets [6].

The goal of this project is to design and synthesize covalent inhibitors targeting viral sialic acid esterases (SAEs). These inhibitors will be tested for their efficacy against recombinant 9-O-SAEs.

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Characterization of a panel of isolates to inform vaccine design against *Klebsiella pneumoniae*

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Klebsiella pneumoniae (Kp) is a leading cause of neonatal sepsis in low- and middle-income countries (LMICs). In neonates, Kp is the major infectious disease killer globally and 87% of deaths are AMR-related[1].Two types of polysaccharides are expressed on Kp cell surface and have been proposed as key vaccine antigens: capsular polysaccharides or K-antigens (K-Ag) and O-antigens (O-Ag). Kp has been classified historically by capsule serotyping and of the 141 distinct K-Ag identified to date, only 79 have been classified[2]. In contrast, 11 distinct OAg serotypes have been described[2]. Considering their relevance for the design of Kp effective vaccines, the main goal of this work is to characterize 165 isolates enrolled to the BARNARDS study which aimed to identify the cause and burden of AMR in neonatal sepsis for LMICs.

Surface polysaccharides have been isolated from all the strains and both K- and O-Ag have been characterized in terms of expression levels, molecular weight and monosaccharide composition. Through a synergistic approach involving Nuclear Magnetic Resonance and Gas-Liquid Chromatography Mass Spectrometry, new molecular structures have been determined for unknown K-Ag belonging to the most epidemiological relevant Kp antigens associated neonatal sepsis.

Structural analysis/chemotyping of K- and O-Ag from clinical isolates is key to elucidate the sero-epidemiology specific to neonatal sepsis in LMICs and will contribute to define the design of an optimal vaccine composition for a maternal immunization strategy to prevent neonatal sepsis caused by *Kp* infections.

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Synthesis of nonglycosaminoglycan-type heparinoid trisaccharides with potential biological activity

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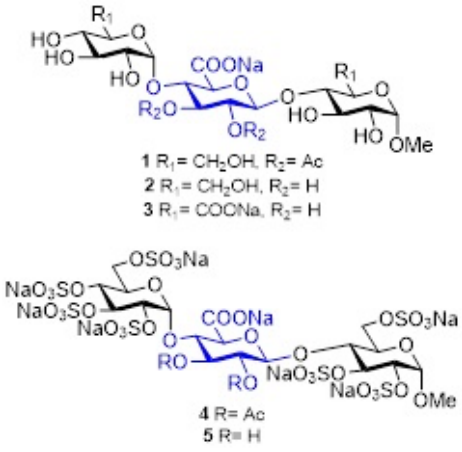
Heparin and heparan sulfate are linear anionic polysaccharides which are the members of glycosaminoglycans. Both are built up from alternating D-glucosamine and hexuronic acid units, but there are some structural differences between their saccharide sequences and sulfation degree.[1] Heparin has been employed in the medical practice as a blood-anticoagulant, but heparin and its analogues have many other biological effects such as growth factor inhibitiory, anti-inflammatory and cell growth inhibitory activity.[2] Heparin and its derivatives are also being investigated for the treatment of a number of disorders, including cancer.[3] Based on the aforementioned, non-glycosaminoglycan analogues of heparin might be important structures in the development of anticancer agents. Our research group has long been working on the synthesis of heparin-analogue oligosaccharides.[4] Moreover in our Department two D-glucuronate-containing trisaccharides were synthesized, which showed significant and selective inhibitory effects on the growth of tumor cells.[5] We supposed that, the newly synthesized trisaccharide fragments of heparin (Scheme 1., 1-5) might also display cell growth inhibitory activity. Based on the mentioned results we performed the efficient synthesis of four Glc-GlcA-Glc and a GlcA-GlcA-GlcA sequenced non-glycosaminoglycan, heparin-related trisaccharides with various sulfation, and acetylation patterns. The cell growth inhibitory effects of the compounds will be investigated against cancerous human cell lines and non-cancerous cell lines.



Scheme 1.: Structures of the planned trisaccharides (1-5)

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Insight of the molecular mechanisms of *A. oryzae* β-galactosidase transgalactosylation system

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The transgalactosylation system of Aspergillus oryzae's β-galactosidase has been successfully used for the synthesis of galactosides. To carry out this reaction it is necessary the presence of both a galactose donor (lactose/ONPG) and an acceptor molecule with a free hydroxyl group available for reaction. However, the chemical nature of the acceptor molecule determines the interactions with the enzyme's active site and therefore the specificity and efficiency of the reaction.

To provide a better understanding of the molecular mechanisms of this transgalactosylation system, we propose a combined approach of in silico and experimental studies, using acceptor molecules with differentiated structural characteristics.

Five molecules were selected: benzyl alcohol, tyrosine, dopamine (all with aromatic character), as well as lactic acid and ethyl lactate. The synthesis conditions (reaction time, temperature, acceptor, donor, and enzyme concentrations) were optimized, each galactoside was purified and its structure was determined by NMR. The enzymatic synthesis of the galactosides was successful only for benzyl- and ethyl lactate-galactosides, indicating that the phenolic hydroxyl groups are not appropriate acceptors, while the esterification of lactic acid is necessary for the reaction to occur. The galactosyl acceptors were docked into the active site of the enzyme covalently bound to galactose, followed by molecular dynamics experiments.

Results obtained with complementary in silico and experimental strategies, provide some insights to understand the transgalactosylation reaction.

Acknowledgements

This work was supported by Programa de Desarrollo de las Ciencias Básicas (PEDECIBA, Uruguay) and Agencia Nacional de Investigación (ANII, Uruguay)

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Glycosyltransferase engineering to dissect *N*-linked protein glycosylation

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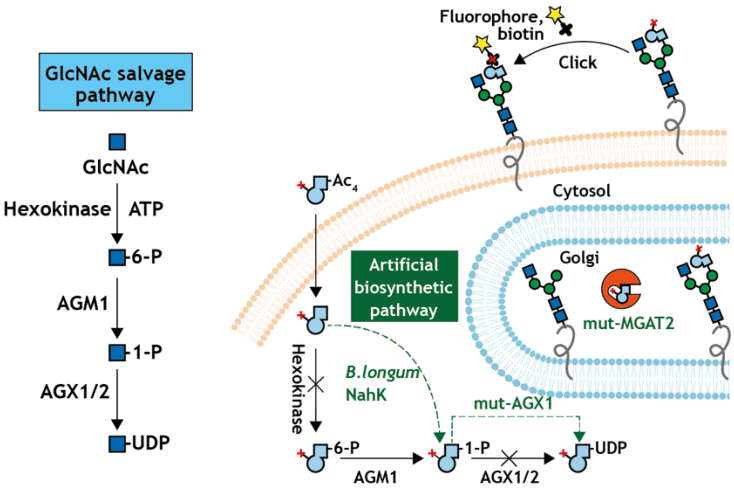
The pursuit of designing and synthesizing complex systems that closely resemble the highly glycosylated extracellular matrix (ECM) has gained significant interest in various fields: 3D cultures, drug delivery and tissue engineering [1]. Supramolecular hydrogels hold great potential for achieving this goal. However, the development of these hydrogels has been hindered by a lack of knowledge behind the fundamental parameters governing their hierarchical self-assembly. Furthermore, the limited examples of carbohydrate-based hydrogels in the literature predominantly involve homomultivalent presentation of a single carbohydrate, which falls short of replicating the complex heteromultivalent nature of the ECM [2].

To address these challenges, this project aims to synthesize biocompatible supramolecular hydrogels that emulate the highly and heterogeneously glycosylated ECM through a hierarchical supramolecular self-assembly approach, by employing rationally designed neoglycolipids. Various photopolymerizable neoglycolipids with distinct sugar headgroups, such as α-D-mannose, β-D-galactose, β-D-glucose, and β-lactose, have been synthesized. The hierarchical self-organization of these neoglycolipids into different hydrogel structures has been thoroughly characterized. Importantly, the resulting hydrogels exhibit multiple interactions with fluorescent lectins specific to the exposed sugars, highlighting their heteromultivalency.

In addition, hybrids of hydrogels and glyconanoring-coated carbon nanotubes have been developed to enhance the mechanical properties of the constructs.



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We thank Richard Meek and Gideon Davies for providing MGAT5 enzymes. We thank Kevin Breummer and Carolyn Bertozz for sharing with us MGAT1 plasmids.

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Synthesis of photoswitchable glycosides as chiral dopants for liquid crystals,

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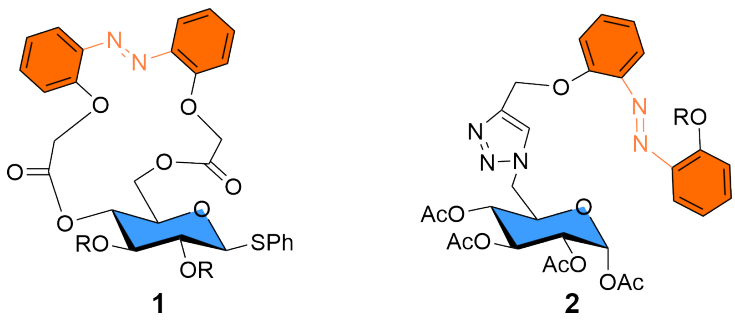
Carbohydrates and derivatives are of great interest because of their natural existence, their interesting biological and physicochemical properties, which offer potential applications in various fields.[1] One of the actual approaches to control molecular properties is to use the light as external stimulus by using photoswitchable species, especially photochromes such as azobenzene which can be reversibly isomerized by light into isomers featuring different structural and electronic properties.[2]

Since several years, we are developing in the group new photoresponsive molecules, such as photoswitchable glycomacrocycles and their acyclic analogues. Particularly for photoswitchable glycomacrocycles, we have shown their interesting photochemical and supramolecular properties like their ability to chirality transfer, organo-gel formation and their possibility to be used as chiral dopants for liquid crystals (LC).[3,4,5]

In this project, we designed and synthesized two new series (macrocyclic, acyclic) of photoswitchable glycosides, containing azobenzene and natural carbohydrates, in order to explore their potential as future chiral dopants for LC. Under light irradiation, it is possible to modulate or switch the orientation of LC molecules to achieve desired optical and mechanical properties of the system remotely and selectively. Also, adding a chiral dopant into a commercially available achiral nematic liquid crystal host, at the appropriate concentration, represents a convenient and economical approach to develop chiral LC. [5,6]

Macrocyclic serie **Acyclic serie**



1 **2**

R = C_6H_{13}, 4-CN-biphenyl-4'-OC_6H_{12}

Structure of target compounds.

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Chemical synthesis of 4’-modified nucleoside analogues

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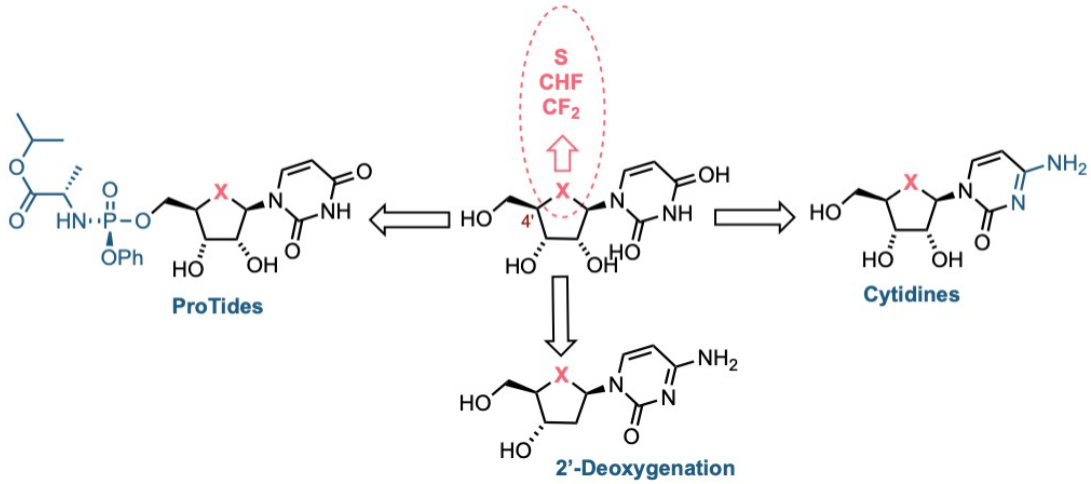
Nucleosides are the building blocks of DNA and RNA. Due to their involvement in a myriad of biological processes, nucleoside analogues represent an ideal starting point for the discovery of new drug candidates and have formed a cornerstone of treatment for viral infections and cancers. Notwithstanding their rich history in successful drug development, therapeutic intervention using nucleoside analogues is often limited by poor cellular uptake, low conversion to the active triphosphate metabolite, rapid degradation or clearance and development of resistance profiles in certain cell types. Consequently, research activity in this field continues to develop syntheses for next generations of biologically active nucleoside analogues that can overcome these limitations and provide new therapeutic options. Within this context, 4’-postion modification of the ribose ring represents an important structural motif for exploration. We have developed synthetic methods to chemcially synthesise 4’-thionucleosides, alongside replacing the furanosyl oxygen with CHF and CF_2 groups.

Exploring 4’-ring modification of pyrimidine nucleosides



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Development of potential new vaccine adjuvants inspired by Lipid A structure of *Bacteroides fragilis*

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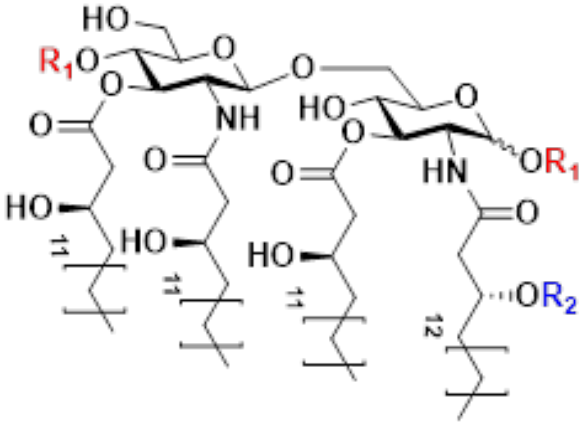
Adjuvants are components of vaccine formulations widely used to enhance their overall efficacy, providing an enhancement in the response towards antigens and allowing for the use of smaller doses of vaccine active ingredient[i]. Up to date, only few adjuvants are licensed and, only recently, their mechanism of action started to be understood. Hence, new efforts are needed for the research of novel chemical entities to be developed as adjuvants.

Bacteroides fragilis is a Gram-negative bacterium belonging to the human gastrointestinal microbiota, involved in the homeostasis of host immune system. Recently, new Lipid A moieties of this bacterium have been isolated, showing promising immunomodulatory activities[ii]. This feature, coupled with their probable low toxicity, makes them interesting candidates for the development of new vaccine adjuvants. In fact, some low toxicity Lipid A-based compounds can be used in vaccine formulations, as demonstrated by the authorization for the clinical use of "Monophosphoryl Lipid A", the first TLR-agonist vaccine adjuvant[iii].

The glycolipid mixture isolated from *B. fragilis* is only partially characterized and their exact structures is still not completely established. In this context, our project is focused on the chemical synthesis of a small library of Lipid A structures (Figure 1), based on the available information, with the aim to define and clarify their chemical structures and their immunological properties, as well as to investigate their potential application as vaccine adjuvants.



R_1 = H, $PO(OH)_2$ or $PO(ONa)_2$

R_2 = H or $CO(CH_2)_{13}CH_3$

Figure 1. Schematic representation of the designed lipid A library.

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Predicting glycan structure from tandem mass spectrometry via deep learning

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Glycans constitute the most complicated post-translational modification, modulating protein activity in health and disease. However, structural annotation from tandem mass spectrometry data is a bottleneck in glycomics, preventing high-throughput endeavors and relegating glycomics to a few experts. Trained on a newly curated set of 300,000 annotated MS/MS spectra, we present CandyCrunch, a dilated residual neural network predicting glycan structure from raw LC-MS/MS data in seconds (Top1 Accuracy: 87.7%). We developed an open-access Python-based workflow of raw data conversion and prediction, followed by automated curation and fragment annotation, with predictions recapitulating and extending expert annotation. We demonstrate that this can be used for *de novo* annotation, diagnostic fragment identification, and high-throughput glycomics.

For maximum impact, this entire pipeline is tightly interlaced with our glycowork platform and can be easily tested at https://colab.research.google.com/github/BojarLab/CandyCrunch/blob/main/CandyCrunch.ipynb.

We envision CandyCrunch to democratize structural glycomics and the elucidation of biological roles of glycans.

Acknowledgements

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Chromatography-free deprotection of benzylidene acetals and p-methoxybenzyl ethers

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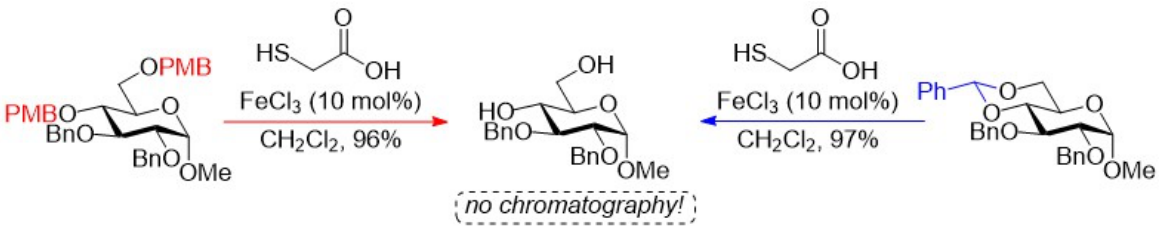
We demonstrate a new and high-yielding method for removal of benzylidene acetals and para-methoxybenzyl ethers under catalytic conditions with mercaptoacetic acid as a scavenger. The reaction co-products are converted to water-soluble molecules, which can be removed by aqueous extraction thereby bypassing the need for chromatographic purification. The reaction was demonstrated on both multi-milligram and multigram scale.



Acknowledgements

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Preparation and surface properties of fluorinated nanocomposites containing gluconamide units

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Much attention has been focused on the synthetic sugar-containing polymers (glycopolymers), of whose polymers possess the pendent gluconic residues (pentaol units), owing to their role as biomimetic analogues and their applications in biomedical and technological fields such as paints and cosmetics [1]. These glycopolymers also have high potential use as polymeric surfactants [2]. Therefore, the studies on the preparation of the novel gluconamide units-containing polymers bearing longer fluoroalkyl groups are of particular interest, because these polymers would exhibit not only the unique characteristics related to the gluconamide units but also the surface active characteristic imparted by longer fluoroalkyl groups.

Here we report that two fluoroalkyl end-capped vinyltrimethoxysilane oligomer [3] can undergo the sol-gel reaction in the presence of *N*-(3-triethoxysilylpropyl)gluconamide under alkaline conditions to afford the corresponding fluorinated oligomeric silica nanocomposites containing gluconamide units. The modified surfaces treated with these obtained nanocomposites can provide the unique wettability such as superamphiphobic, highly oleophobic/superhydrophilic and superoleophilic/superhydrophobic characteristics. These results will be demonstrated in this conference.

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Chemoenzymatic approach to produce rhamnoxylolipids

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Rhamnolipids (RLs) are a class of biosurfactants consisting of one or two rhamnose units linked to β-hydroxylated fatty acid chains. They are value-rich molecules because their chemical-physical properties and biological activities [1] are promising for the use in several fields, such as: detergents [2]; active agents in skin re-epithelialization; additives in the cosmetics industry [3]; as antibacterial, antifungal, antiviral and antitumoral agents in biomedicine. However, rhamnolipids are essentially obtained by fermentation of opportunistic bacteria, namely strains of *Burkholderia*, *Acinetobacter* or, mainly, of *Pseudomonas* [1]. Moreover, any microbial fermentation produces a mixture of rhamnolipid congeners with variations in the chain length and in the degree of unsaturation of the fatty acid chains, as well as differences in the number of rhamnose molecules, with the overall consequence that different production batches do not have the same composition. To overcome these troubles, new approaches have recently been pursued: a fully synthetic strategy [4] or their production in a cell-free (or in vitro) system [1]. Here, we propose a new chemoenzymatic strategy to produce a new class of RLs, the **rhamnoxylolipids** (RXL), that differ in the presence of a xylose monosaccharide as linker between rhamnose and lipids (Figure 1).

Therefore, the aim of our research activity is to create a library of compounds, obtained with a safe and targeted production, which could be excellent substitutes for rhamnolipids in dermatology industry.

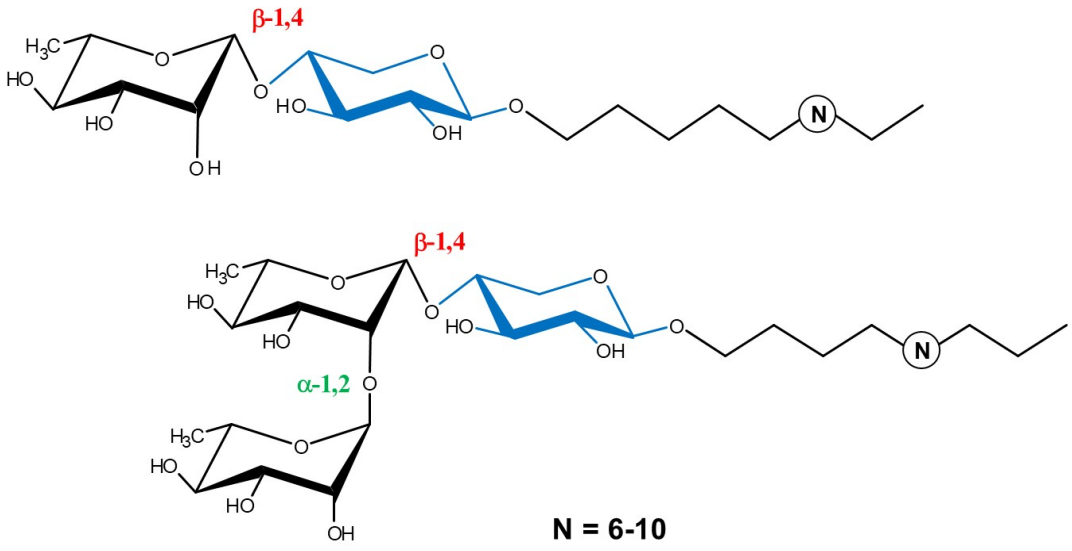

Figure 1. Examples of mono- and di-rhamnoxylolipids.

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Synthesis of Man/Glc glycoclusters for systematic variation of glycoligand presentation in 3D space

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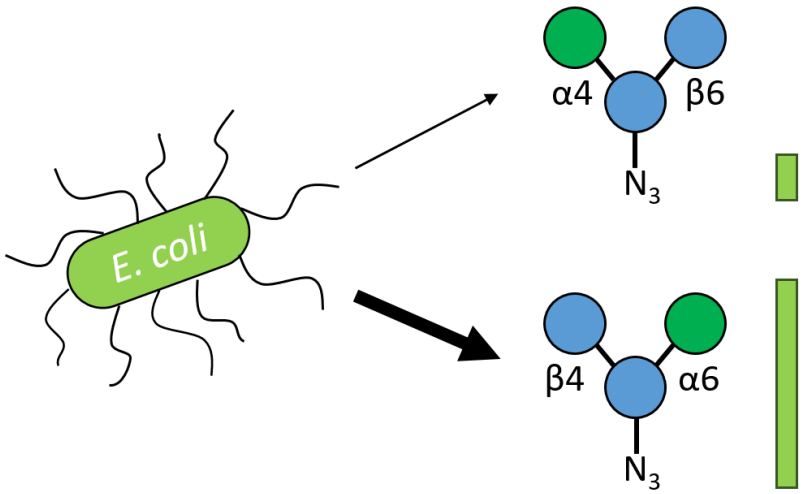
Carbohydrate recognition plays a fundamental role in biological processes like signal transduction, molecule transport, cell-cell recognition or cell adhesion.[1] These are complex processes comprising the interplay of different functional aspects such as, e.g., multivalency and heteromultivalency effects.[1] Furthermore, carbohydrate recognition is apparently regulated by the presentation mode of glycoligands in three-dimensional (3D) space. We have investigated the effect of 3D ligand presentation using synthetic glycomimetics allowing for the precise control of the spatial relation between a mannoside ligand (Man) and a glucoside moiety (Glc).[2,3]

Biological testing of these synthetic model systems in an adhesion inhibition assay[4] employing mannose-specific live *E. coli* bacteria revealed that different ligand presentation results in a significant difference in the inhibition of mannose-specific *E. coli* adhesion. Rationalisation of these results was obtained by computer-aided molecular docking of the bacterial lectin FimH and the synthetic regioisomeric glycoligands.

Future investigations will aim at a more complex testing system utilizing metabolic oligosaccharide engineering and human cells decorated with tailor-made synthetic glycoligands.



Regioisomeric trisaccharides demonstrate varying inhibition strengths against E. coli adhesion.

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Rational design of picomolar sp^2-iminosugar-based mannobioside ligands of DC-sign

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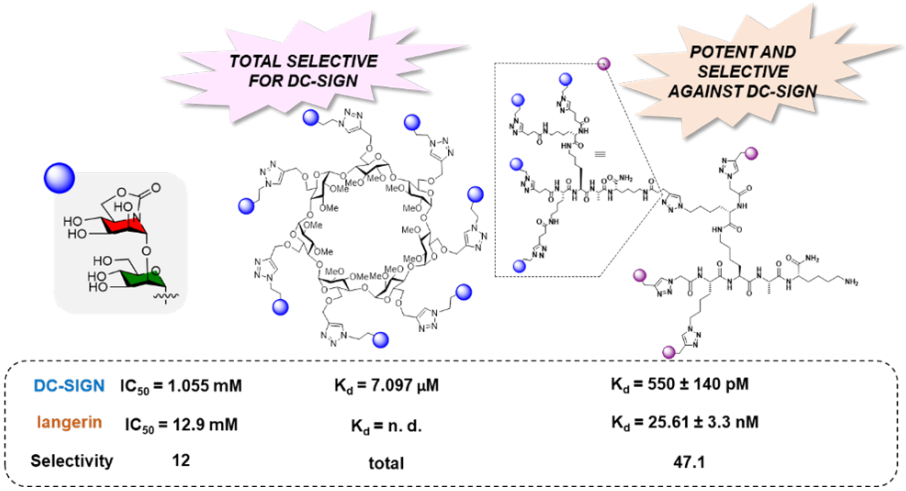
C-Type lectin receptors (CLRs) are present at the surface of antigen-presenting cells (APCs), notably in dendritic cells (DCs), allowing the recognition of carbohydrate-based PAMPS or DAMPS (pathogen- or danger-associated molecular patterns, respectively) and initiating adaptive immune responses. DC-SIGN and langerin are CLR expressed in DCs that recognize high mannose oligosaccharides (HMOs). Binding predominantly involves HMO terminal mannobioside residues (Manα1,2Man) for both lectins, making it difficult the design of ligands with discrimination capabilities [1]. Here we present a general strategy to target DC-SIGN preventing langerin binding that relies on manobioside mimetics where the reducing, non-reducing, or both mannose subunits have been replaced by sp^2-iminosugar motifs [2].

A comparative binding affinity study of monovalent ligands was first conducted using a range of techniques (tr-NOESY and STD NMR, computational methods and SPR) [3]. The most promising candidates were further "clicked" onto different scaffolds (β-cyclodextrin, RAFT cyclopeptides, polylysine dendron, and cyclotriphosphazene) to obtain 4-, 6-, 7-, 14-, 16- and 24-valent species. The impact of valency, structure, and size of the conjugates on DC-SIGN and langerin affinity have been investigated using SPR, and selective and potent multivalent ligands for DC-SIGN were identified. These results validate the hypothesis that sp^2-iminosugar-based glycoligand mimetics enable customizing the lectin binding profile in a structure- and valency-dependent manner. From a general perspective, these results open new opportunities to modulate the interaction with lectins involved in the immune response.



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Chemical synthesis of sugar nucleotide donors for the investigation of plant glycosyltransferases

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Knowledge of the structures and biosynthesis of plant cell wall polysaccharides is essential in the search for alternative, renewable resources for the production of fuels, chemicals and bio-based materials. Glycosyltransferases (GTs), the enzymes responsible for the construction of cell wall glycans, thereby play a key role, as they determine cell wall structure and properties. [1]

We report the chemical synthesis of sugar nucleotide donors related to the biosynthesis of plant cell wall glycans for the glycan array-based search for new biosynthetic GTs. [2] Oligosaccharide acceptors are immobilized as microarrays and incubated with putative GTs and the synthesized sugar nucleotide donors, which are chemically modified with an azido group, enabling detection of the products by click chemistry with a fluorescent dye.

UDP-N_3-galactose, *UDP-N_3-arabinofuranose*, *UDP-N_3-rhamnose* and *UDP-N_3-apiofuranose* have been chosen as the initial synthetic targets, as many unknown activities are to be discovered for GTs transferring these donors within AGP biosynthesis. We also report examples for the use of the synthesized sugar nucleotides in the aforementioned glycan array assay.

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Development of glycomimetics as a novel herpesviral glycoprotein UL141 antagonists

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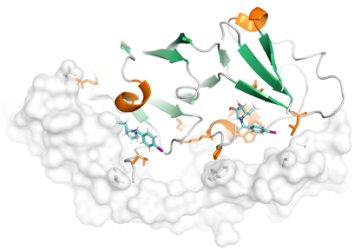
The worldwide seroprevalence of human cytomegalovirus (HCMV, a beta herpesvirus) is estimated to 83% (a global CMV seroprevalence) in the general population [1]. Usually, it is controlled by a vigorous immune response so that infections are asymptomatic or symptoms are mild. However, if the immune system is compromised, HCMV can replicate to high levels and cause serious end organ disease [2]. Although HCMV represents a complex target, the rounds of iterative studies could potentially bring this important and under-recognized human pathogen under control. Over four receptor-binding patches (RBP) of the glycosylated UL141 protein is capable of binding to human TRAIL death receptor 2 (TRAIL-R2) [3] and trigger the NK signaling pathway to benefit viral fitness [4, 5, 6]. Hence, it is rational to inhibit the RBP activity of the UL141 protein by blocking the RBP interaction with TRAIL-R2, which makes the glycosylated UL141 protein a potential target for designing and developing antiviral agents. In this study, the molecular features of the UL141 of HCMV are highlighted, such as the structure, functions, and interactions of the UL141 and TRAIL-R2. Furthermore, the development of glycomimetic structures by computational design and biochemical testing is reported. The aim is to develop the short peptide or synthetic compound (UL141 antagonist) based on our recent crystal structure and computational design that would specifically bind viral UL141 to block receptor binding thus prevent the viral action. This is relevant, as the UL141 is also the most abundant HCMV protein on plasma membrane and it is also a component of the virion. Based on our computational screening of iminosugars the 'hit' structure was selected. We test a small library of synthetized compounds (potential UL141 antagonists) that would block the receptor binding *in vitro*, on the cell or virion surface. Series of compounds that have been tested are of glycomimetics structures consisting of various saccharide units linked with non-saccharide. In particular; non-ionic glycolipids, 'click'-conjugates or iminosugars. The ELISA-like TMB assay has been used in combination with dynabeads™ coating to test whether the compound could block the TRAIL-R2 binding. Five most promising compounds out of 23 tested have proven the ability to block UL141/TRAIL-R2 complex formation. SPR kinetics analysis was then used to determine the binding constants (K_D). The affinities to UL141 were determined in low micromolar scale. The successful compounds will be further optimized by using *in silico* methods to target particular epitope on viral glycoprotein UL141 derived from our structural analysis and tested *in vivo* for HCMV inhibition.



UL141 (cartoon) and TRAIL-R2 (surface) in complex. The binding of UL141 antagonist (cyan molecules).

Acknowledgements

This research was funded by the Slovak Research and Development Agency (APVV-19-0376) and Scientific Grant Agency (VEGA 02/0026/22 and 02/0060/21).

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Neu5Ac and Neu5,9Ac$_2$ in human plasma: potential biomarkers of cardiovascular disease

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CVD is characterized by high levels of inflammation which has been linked to a rise in the concentration of *N*-acetyl neuraminic acid (Neu5Ac) in blood. [1] Another sialic acid, 9-*O*-Acetyl-*N*-acetyl-neuraminic acid (Neu5,9Ac$_2$), has been of interest as a biomarker for diseases such as breast cancer but has not been studied in the case of CVD. Neu5Ac and Neu5,9Ac$_2$ concentrations were determined by quantitative analysis using liquid chromatography in plasma obtained from both patients with CVD and healthy controls. [2] Mean concentrations of Neu5Ac and Neu5,9Ac$_2$ were significantly elevated between the two sample groups (Neu5Ac: $P < 0.001$; Neu5,9Ac2: $P < 0.04$). Receiver operator curve analysis (ROC) further revealed the predictive power (AUC) of the two markers (Neu5Ac: 0.86; Neu5,9Ac2: 0.71).

A combined Neu5Ac/Neu5,9Ac$_2$ marker exhibited an AUC of 0.93. The sensitivity and specificity of each marker was then assessed, with the combined marker performing best overall. Neu5Ac appears to have good discriminatory power for CVD. Combining the two markers together may offer a better biomarker than either of the markers individually. Further analysis was undertaken to determine the *N*-glycan profile, percentage galactosylation of *N*-glycans and c-reactive protein concentration of each sample. ROC analysis was performed and AUC values were compared with the data for Neu5Ac and Neu5,9Ac$_2$. The samples were also analysed via nanoparticle prefractionation (*U.K. Patent Application Number 2117557.5*). [3] This allowed for the extraction of fibrinogen and analysis of fibrinogen derived glycans.

Acknowledgements

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Biolayer interferometry – a soft technique for tough analysis of carbohydrate-lectin interactions

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Biolayer interferometry (BLI) is a label-free method used to detect biomolecular interactions by analyzing the interference pattern of reflected white light from two sensor surfaces (**Figure 1A**). Due to its speed, ability, and sensitivity, it enables to readily detect analytes even in complex matrices such as blood serum [1], as its response depends solely on the thickness of the bio-layer and not on the optical properties of the sample environment. BLI provides more reproducible data than other commonly used assays like ELISA [1], may require fewer samples and less specific antibodies for detection. Therefore, it is an emerging complementary method for characterizing protein-carbohydrate interactions, which is essential for designing better diagnostic and therapeutic tools.

This study demonstrates various aspects of using BLI for determining the kinetics of interactions between a library of galectins and various glycoconjugates. We compared two labels for protein immobilization: His-tag for complexation with nickel ions or *in vivo* monobiotinylated Avi-tag, which binds to streptavidin-coated sensors (**Figure 1B**). Advantageously, the biotin-streptavidin binding achieves a low equilibrium dissociation constant K_D and is highly selective for the biotin epitope. In addition, the blocking of the sensor surface with biocytin (lysine conjugated with biotin) reduced the non-specific interaction for assessing interactions in complex matrices such as blood serum. Thus measurement of high-affinity glycoconjugates [2] showed no significant effects between K_D values measured in blood serum and PBS buffer.

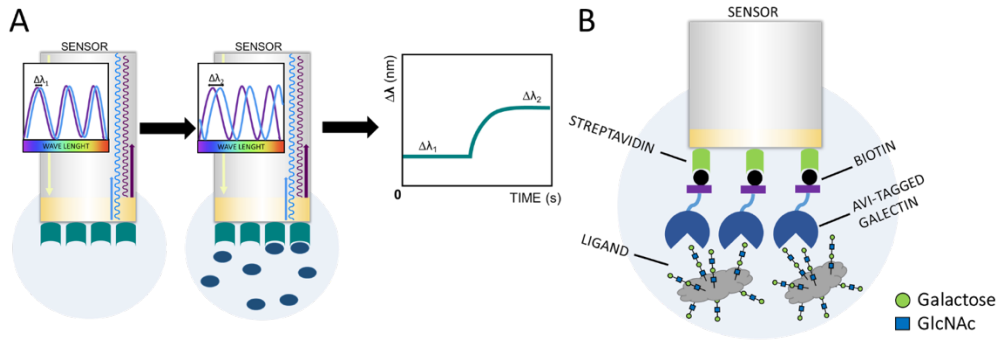

Figure 1. 1A – principle of biolayer interferometry; 1B Streptavidin coated sensor with Avi-tagged G

Acknowledgements

This study was supported by the project 22-00262S of the Czech Science Foundation.

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Stable electron-poor glycosylamides and their utilization

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Glycosylation has many roles in biochemical processes, cell-communication through lectin-carbohydrate interactions, and is a key factor in solubilization of lipophilic active compounds. The downside of glycosides often being the ease of cleavage of glycosidic bonds by glycosidases. Substitution of the glycosidic oxygen for other atoms or motifs can be a solution to this disadvantage.

Copying the natural *N*-glycosylation of peptide residues and building on 100 years old literature procedures, we have revived the methods for preparation of electron-poor glycosylamines and developed a better method of their acylation. This opened up a path to novel, hydrolytically stable scaffolds with potential use as lectin-ligands and drug-carriers.

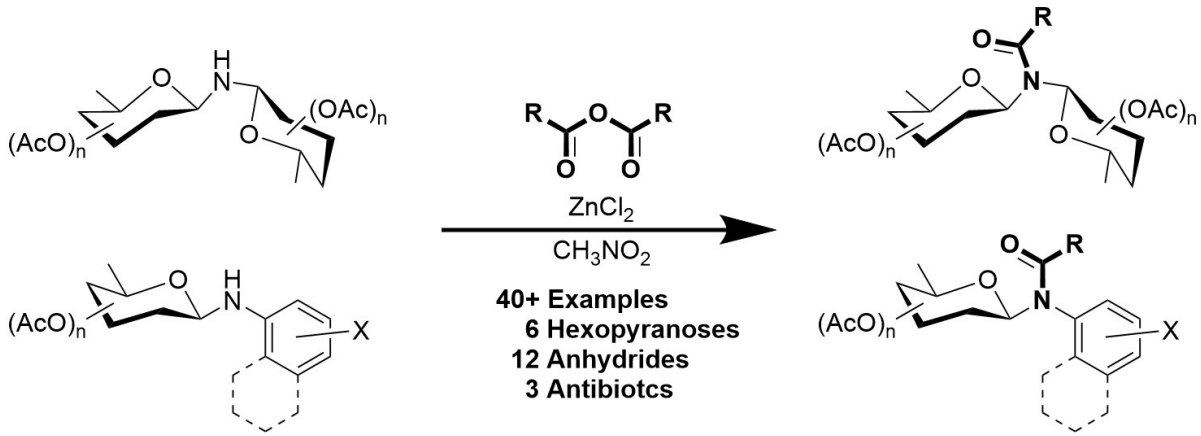

Figure 1: (A) HS repeating unit. (B) HPSE covalent inhibition. (C) Stabilized inhibitors.

This work was supported by the Czech Science Foundation (project 22-17586S) and grant of Specific University Research (grant No. A2_FPBT_2019_004).

Probing aberrant glycosylation using lectin-based glycoprotein microarrays

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To detect aberrant glycosylation, various bioanalytical methods can be employed. We focus on the development and application of affinity techniques based on glycan-binding molecules on a microarray platform. Typically, lectins are used as biorecognition elements, which are proteins recognizing glycan structures enabling glycoprofiling of proteins, cells, and tissues. Our group develops lectin-based glycoprotein microarray assays in the reverse-phase format, the technique defined as a high-throughput approach enabling simultaneous and rapid analysis of a large cohort of samples by a set of lectins without the need for the separation of glycans from proteins [1,2]. The microarray biochip is prepared by spotting of tens to hundreds of samples on the microarray substrate which is then allowed to interact with a panel of lectins. This method provides effective glycoprofiling of samples and screening/analysis of glycan biomarkers having huge applications in biomedicine, biology, and biotechnology.

We have applied our microarray platform for the study of glycan changes in a number of various cases, e.g. cancer, gestational diabetes mellitus, kidney diseases, COVID-19, congenital disorder of glycosylation (CDG), attention-deficit hyperactivity disorder (ADHD), age-related glycosylation changes, or glycostructure of therapeutic proteins. Herein, we present our approach and some of the obtained results.

Acknowledgements

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Deciphering glycosaminoglycan structures using biological based nanopores

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Glycosaminoglycans (GAGs) are linear, highly complex, and heterogeneous polysaccharides mainly located in the extracellular matrix (ECM) and at the cell surface. Their polysaccharide backbones contain repeating disaccharide blocks composed of (a) one N-acetylhexosamine or hexosamine residue, linked to (b) one uronic acid or galactose residue. GAGs biosynthesis includes further modifications by sulfation, deacetylation, or epimerization, which lead to their vast structural diversity. Both their localization and diversity allow GAGs to interact with various extracellular proteins, and they are involved in different physiological and pathological events [1].

Despite of a great interest in studying GAGs structure-to-function relationships, structural characterization of GAGs is challenging due to their high complexity and polydispersity. Also, commonly employed analytical methods for GAG structural analysis are time-consuming and often fail to provide information on minor structural patterns [2]. On the other hand, recent developments in nanopore-based analysis demonstrated the potential of this method for in-depth structural characterization of GAGs and detection of their low abundant but crucial structural motifs. This method was shown to successfully determine the degrees of polymerization (DP), detecting differences in osidic bonds, sulfation patterns, and epimers of uronic acid residues of GAG oligosaccharides on a single molecule level [3]. Another advantage of biological nanopores is that they can be modified at the Å-level precision by protein-engineering techniques for improving fine structural discrimination of analytes. A replacement of a single amino acid residue in the case of aerolysin (AeL) nanopore showed promising results in analyzing structures of peptides [4].

The present study employs the wild-type AeL and its mutant R220S to determine the impact of this single-point mutation within the pore lumen on the nanopore structural analysis of GAG oligosaccharides. Ongoing work is as well focused on creating a specific signal database for known GAG structures. Preliminary results suggest that this approach can be used for fast and reliable fine structural characterization of different GAGs. Among our future goals is to employ this approach for analyzing fine structural differences between GAGs from pathological and non-pathological samples and further our knowledge on relations between GAG structures and function.

Acknowledgements

This research is financed by French National Research Agency (Agence Nationale de la Recherche, ANR) as a part of the GAGS-NanoSensor project AAPG2021

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Sialic acid determinations by HPAE-PAD choosing the appropriate method

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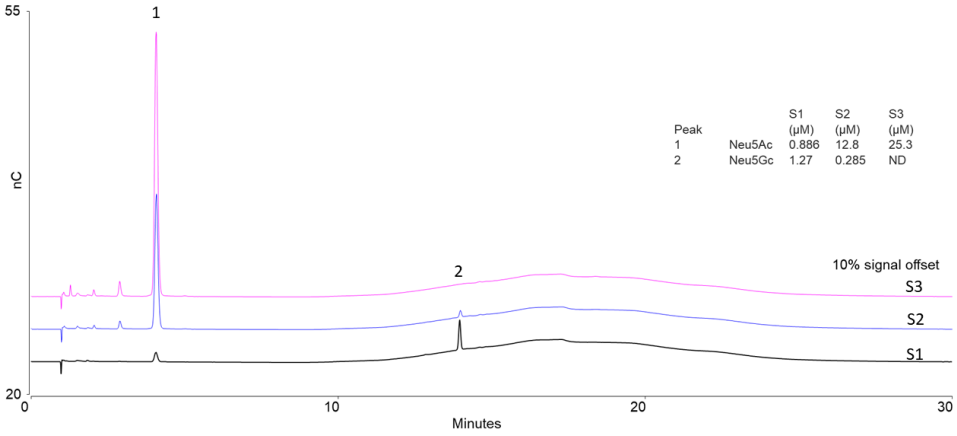
High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) was introduced over 40 years ago for the sensitive direct determination (no analyte derivatization) of carbohydrates. Typically, the carbohydrates are separated in high pH mobile phases which is also the alkaline environment needed for the electrochemical detection of carbohydrates. Shortly after the introduction of HPAE-PAD, it was applied to the determination of the sialic acids N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) from glycoproteins and glycolipids. Since then, multiple methods have been developed for a variety of samples and analytical goals.

These applications include the determination of sialic acids in glycoprotein drug products, the rapid determination of sialic acids to screen the efficacy of cell lines, the determination of sialic acids in milk and milk-containing products, the determination of sialic acids in serum, and the recent application of technology that enables online generation of the mobile phases needed for HPAE-PAD to sialic acid determinations. This poster will review these methods and include recommendations for choosing the method that is appropriate for the desired analytical outcome.



Determination of the Sialic Acid Content of Glycoproteins by HPAE-PAD with Eluent Generation

Acknowledgements

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Association of C3 glycosylation with the complement pathway proteins

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Complement is a central component of the innate immune system. It plays a key role in maintaining homeostasis and defense against pathogens. Activation of the complement system is possible through three different pathways: the classical, the alternative and the lectin pathways. Plasma proteins, mainly derived from the liver and membrane proteins expressed on the cell surface make up the complement system. The most abundant protein of the complement is C3. Like most plasma proteins, C3 is glycosylated. It has three potential N-glycosylation sites, but only two of them are occupied by glycans, exclusively the high-mannose type. In this study, we analyzed C3 glycosylation in a site-specific manner by nano LC-MS. C3 was enriched with concanavalin A lectin affinity matrix from plasma. This method was applied to plasma samples of four different groups. In addition, peptides of various complement proteins were measured in these samples. The C3a protein, released from C3 by C3-convertase and C5a, cleaved from C5 by C5-convertase were included in this study. Furthermore, components of the alternative pathway included in the analysis were Ba and Bb. C4a as a component of the classical and lectin pathways and SC5b-9 as a component of the terminal pathway were measured in each group. Preliminary statistical analysis showed a significant correlation between C3 glycopeptides and peptide levels of the complement system. The significant correlation points in the same direction in all four groups.

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Synthesis of potentially anticancer azido/guanidino nucleosides

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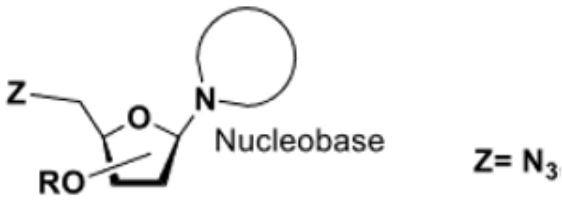
The synthesis of nucleosides, nucleotides as well as their analogs/mimetics have attracted much attention in organic and medicinal chemistry, due to their propensity to exhibit a variety of biological properties. Numerous examples of nucleoside and nucleotide analogs achieved clinical application as anticancer or antiviral drugs, acting through interference with nucleic acid biosynthesis [1].

The ability of these types of molecules to show antimicrobial effects [2] or to inhibit cholinesterases [3] has also been reported. The search for new structures of nucleos(t)ide analogs that may potentiate innovative mechanisms of action and open new therapeutic opportunities remain of interest.

In this context, in this communication we report on the synthesis of novel 5-azido/guanidino nucleosides based on a xylofuranose template. The synthetic pathway employed diacetone- D-glucose as precursor and involved the access to an acetylated 5-azidoglycosyl donor and its further N-glycosydation with uracil or with a purine derivative. Conversion of the azido nucleosides into their guanidino derivatives was then exploited. An interesting result during the synthesis of the glycosyl donor precursor was the access to an imino sugar via an intramolecular Boyer reaction. Herein our results will be presented and discussed.



General structure of the synthesized nucleosides.

Acknowledgements

We thank FCT for funding through grant CEECIND/03881/2018, project EXPL/MED-QUI/1017/2021, and project UIDB/00100/2020, UIDP/00100/2020.

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Multivalent 9-*O*-Acetylated-sialic acidglycoclusters as potent inhibitors for SARS-CoV-2 infection

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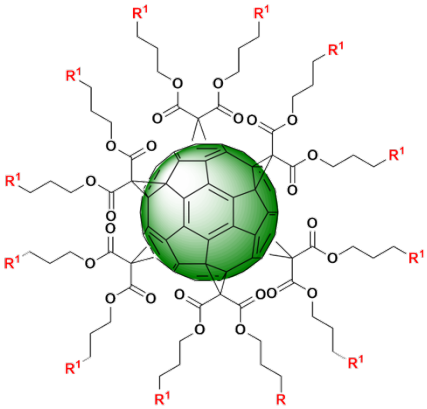
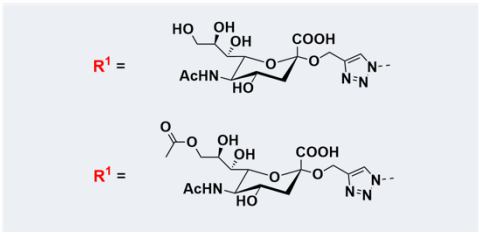
The recent emergence of highly transmissible SARS-CoV-2 variants illustrates the urgent need to better understand the molecular details of the virus binding to its host cell and to develop anti-viral strategies. While many studies focused on the role of the angiotensin-converting enzyme 2 receptor in the infection, others suggest the important role of cell attachment factors such as glycans. We first synthesized a series of glycoclusters based on various central scaffolds and a controlled number of sialic acids, and we use atomic force microscopy to study these early binding events with the focus on the role of sialic acids (SA). We show that SARS-CoV-2 binds specifically to 9-O-acetylated-SA with a moderate affinity, supporting its role as an attachment factor during virus landing to cell host surfaces.

For therapeutic purposes and based on this finding, we have designed novel blocking molecules with various topologies and carrying a 4 to 12 SA residues, enhancing affinity through a multivalent effect. Inhibition assays show that the AcSA-derived glycoclusters are potent inhibitors of cell binding and infectivity, offering new perspectives in the treatment of SARS-CoV-2 infection.





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Stereoselective access to iminosugar *C,C*-glycosides

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Naturally occurring six-membered 1-deoxyiminosugars have been extensively used as non-covalent probes to decipher the mechanism of glycosidases by virtue of their mimicry of the oxocarbenium character of the enzyme transition state.[1,2] In the search for other therapeutic candidates, mimicking the aglycon moiety of the enzymatic substrate with the introduction of a pseudo-anomeric substituent has been pursued, leading to a vast array of so-called iminosugar *C*-glycosides.[3] Within this family, introduction of an extra carbon substituent at the pseudoanomeric position to yield iminosugar *C,C*-glycosides is worth investigating as it might lead to new potent and selective glycosidase inhibitors. In contrast with the broad palette of methods allowing access to iminosugar *C*-glycosides,[4] syntheses of iminosugar *C,C*-glycosides are scarce illustrating the challenging introduction of a quaternary carbon atom alpha to a nitrogen atom.

In this context, we have developped a robust route to original iminosugar *C,C*-glycosides exploiting a Staudinger aza-Wittig (SAW) reaction applied to 6-azidoketosugars.[5] The last developments of our strategy will be presented.

Target azepane and piperidine C,C-glycosides

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Reductive Opening of 4,6-*O*-Benzylidene with $PhBCl_2/Et_3SiH$: how to prevent unreported side-reactions

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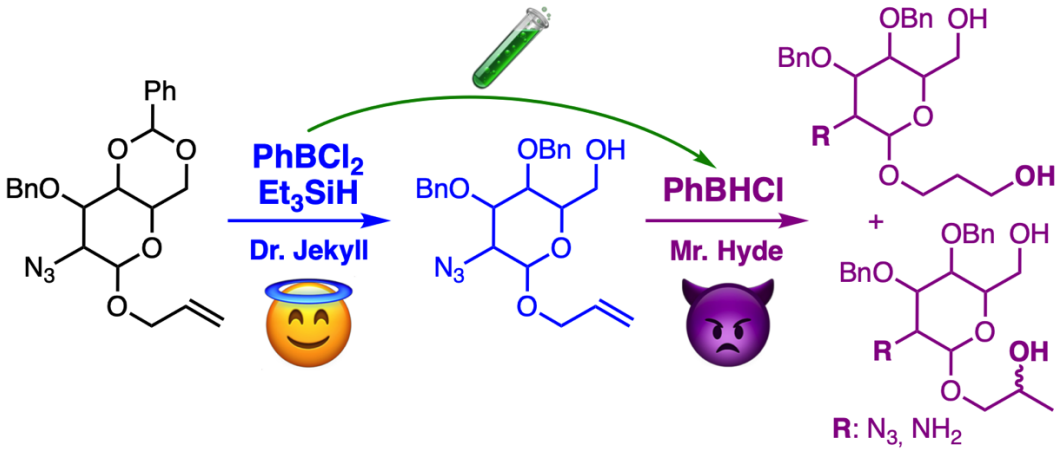
Arylidene acetals are widely used protecting groups, not only for the high regioselectivity of their introduction, but also for the possibility to perform further regioselective reductive opening in the presence of a hydride donor and acid catalyst. In this context, the $Et_3SiH/PhBCl_2$ system present several advantages: silanes are efficient, environmentally benign, and user-friendly hydride donors, while $PhBCl_2$ open the way to unique regioselectivity with regards to all other Brønsted or Lewis acids used with silanes. This system has been extensively used by several groups and we have demonstrated its high regioselectivity in the reductive opening of 4,6 and 2,4-*O*-*p*-methoxybenzylidene moieties in protected disaccharides.

Surprisingly, its use on the 4,6-*O*-benzylidene containing also *O*-allyl or azide moieties led to unreproducible yields due to the unexpected formation of several side products. Their characterizations allowed us to identify different pitfall potentially affecting the outcome of reductive opening of arylidenes with the $Et_3SiH/PhBCl_2$ reagent system: alkene hydroboration, azide reduction and/or Lewis acid promoted cleavage of the arylidene. With this knowledge, we optimized reproducible and high yielding reaction conditions that secure and extend the scope of the $Et_3SiH/PhBCl_2$ system as reagent for the regioselective opening of arylidenes in complex and multifunctional molecules.



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TheFrench MESR for P. QUELLIER's one.

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Clustering of mycobacterial ligands and DC-SIGN are key determinants for pathogen recognition

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The bacterial pathogen *Mycobacterium tuberculosis* binds to the C-type lectin DC-SIGN on dendritic cells to evade the immune system. While DC-SIGN glycoconjugate ligands are ubiquitous among mycobacterial species, the receptor selectively binds pathogenic species from the *M. tuberculosis* complex (*MTBC*). Here, we unravel the molecular mechanism behind this intriguing selective recognition by means of a multidisciplinary approach combining single-molecule atomic force microscopy with Förster resonance energy transfer and bioassays.

Molecular recognition imaging of mycobacteria demonstrates that the distribution of DC-SIGN ligands markedly differs between *Mycobacterium bovis* BCG (model *MTBC* species) and *Mycobacterium smegmatis* (non-*MTBC* species), the ligands being concentrated into dense nanodomains on *M. bovis* BCG. Upon bacteria-host cell adhesion, ligand nanodomains induce the recruitment and clustering of DC-SIGN. Our study highlights the key role of clustering of both ligands on *MTBC* species and DC-SIGN host receptors in pathogen recognition, a mechanism that might be widespread in host-pathogen interactions.

Structural analysis of *N*-glycans and their quantities in bovine submaxillary mucin by LC-MS/MS

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Bovine submaxillary mucin (BSM) is a heavily-glycosylated macromolecular (approximately 4MDa) protein that is used in various biomaterial applications in light of its high viscosity and biocompatibility, in addition to use as a biochemical substrate or inhibitor as a result of its abundant *O*-glycans. Although *N*-glycosylation has been reported to provides stability of human mucins, most BSM research has been focused on its *O*-glycans, while *N*-glycans have not been reported to date.

In this study, a common *N*-glycan core component was detected by monosaccharide analysis of BSM, and the structures and relative quantities of the *N*-glycans were determined by liquid chromatography–tandem mass spectrometry.

Seventeen *N*-glycans comprising ten complex-type [$Fucose_{0\sim2}Hexose_{3\sim4}$*N*-$acetylhexosamine_{1\sim6}Sulfate_{0\sim1}$; 61.1% (the sum of the relative quantities of each *N*-glycan out of the total *N*-glycans)], two high-mannose-type ($Hexose_{5\sim6}$*N*-$acetylhexosamine_2$; 12.0%), and five paucimannose type ($Fucose_{0\sim1}Hexose_{3\sim4}$*N*-$acetylhexosamine_{2\sim3}$; 26.9%) were identified, but no hybrid-type or sialylated *N*-glycans were found. Additionally, these are less-branched structures compared to human mucins. Of these, ten glycans (77.2%), including two sulfated glycans (8.0%), were core fucosylated, which confer unique biological functions to glycoproteins. These *N*-glycans are less-branched structures compared to human mucins. This is the first study to confirm *N*-glycosylation of BSM, and these results support further expansion of the biological function of non-human mucin.

Proposed structure[a]	LC-ESI-HCD-MS/MS				
	Mass (m/z)		Charge	Mass error (ppm)[b]	Relative quantity (%)[c]
	Theoretical $[M+H]^+$	Observed $[M+H]^+$			
	1583.6205	1583.6221	1	1.0	23.9
	1380.5411	1380.5435	1	1.7	12.2
	1786.6998 (893.8536)[e]	893.8536	2	0.0	10.8
	1177.4617	1177.4630	1	1.1	9.0
	1355.5095	1355.5111	1	1.2	7.1
	1745.6733 (873.3403)[e]	873.3414	2	1.3	5.3
	1866.6566 (933.8320)[e]	933.8330	2	1.1	5.2
	1517.5623	1517.5636	1	0.9	4.9
	1437.5625	1437.5631	1	0.4	3.6
	1989.7792 (995.3932)[e]	995.3940	2	0.7	3.2
	1234.4832	1234.4836	1	0.4	3.0
	2069.7360 (1035.3717)[e]	1035.3734	2	1.7	2.8
	1932.7577 (966.8825)[e]	966.8837	2	1.2	2.6
	1948.7527 (974.8800)[e]	974.8807	2	0.7	2.2
	1031.4038	1031.4049	1	1.1	1.8
	1843.7213 (922.3643)[e]	922.3638	2	0.6	1.5
	1396.5360	1396.5380	1	1.4	0.9

N-glycan analysis of BSM for determination of the structure and the relative quantity.

Synthesis of difluoromethylene bisphosphonate mimics as probes for bacterial capsular polysaccharide

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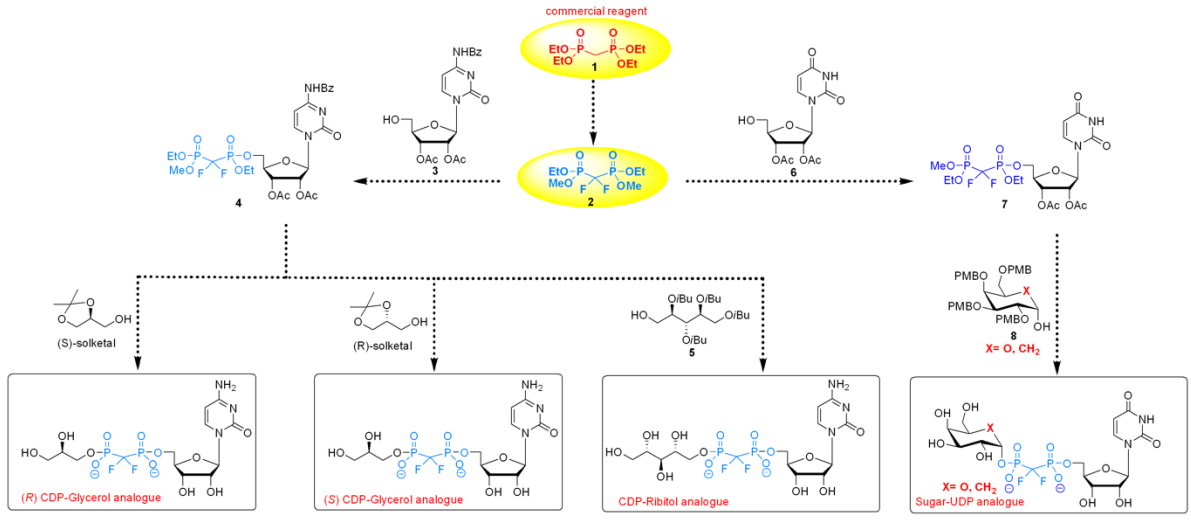
Poly(glycosylpolyol phosphate) polymerases and glycosyltransferases are significant enzymes for the biosynthesis of virulence-associated polysaccharides of several pathogenic Gram-negative bacteria. These enzymes construct polysaccharide chains using precursors that contain a pyrophosphate diester structural motif, such as CDP-glycerol, CDP-ribitol and Gal-UDP[1]. The sensitivity of the P-O-P for both enzymatic and chemical hydrolysis offers a unique opportunity for the design and synthesis of stable non-hydrolysable inhibitors. In particular, difluoromethylene bisphosphonate-linked (P-CF_2-P) analogues are highly sought after as versatile biochemical tools.

These analogues closely resemble the natural pyrophosphate, in terms of pKa-value[2] (as the electron-withdrawing effect of fluorine atoms increase the acidity of the phosphonates), as well as the bond angles and lengths. We will present a synthetic method to access P-CF_2-P-substituted analogues of several nucleotide phosphate donors, designed to study the interactions between these molecules and their cognate enzymes, to advance our understanding of the biosynthesis of components of bacterial cell wall and contribute to the development of novel therapeutic agents, and antimicrobial strategies.



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Identification and quantification of sialylated and core-fucosylated *N*-glycans in human transferrin

Jieun KIM [1], Mijung JIN [1], Jihye KIM [1], Chi Soo PARK [1], Chulmin MOON [1], Mirae KIM [1], Subin YANG [1], Leeseul JANG [1], Ji Yeon JANG [1], Chang MYEONG JEONG [1], Ha Hyung KIM [1]

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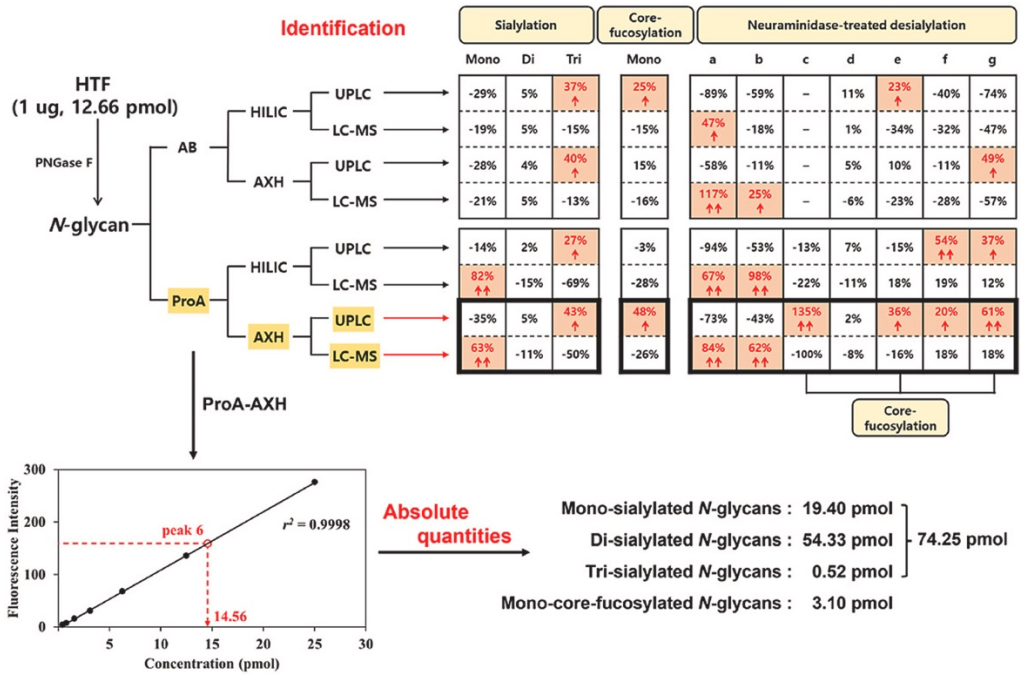
Sialylated and core-fucosylated *N*-glycans in human transferrin (HTF) are used as glycan biomarkers due to their increased or decreased characteristics in certain diseases. However, efficient identification and quantification of *N*-glycans in HTF remain unclear.

In this study, *N*-glycans of HTF were identified by UPLC and LC-MS/MS using combinations of fluorescence tags [2-aminobenzamide (AB) and procainamide (ProA)] and columns [HILIC and anion exchange chromatography-HILIC (AXH)].

The structures of 14 (including five core-fucosylated) *N*-glycans in total comprising two non-, six mono-, four di-, and two tri-sialylated *N*-glycans were identified. The quantities (%) of each *N*-glycan relative to the total *N*-glycans (100%) were obtained. HILIC and AXH were better for peak identification and separability except for desialylation, respectively. Specifically, sialylated (in ProA-HILIC and ProA-AXH by UPLC or LC-MS/MS) and core-fucosylated (in AB-HILIC and ProA-AXH by UPLC) *N*-glycans were efficiently identified. Seven neuraminidase-treated (including three core-fucosylated) *N*-glycans were efficiently identified in ProA-AXH, even with their poor separation. Additionally, ProA-AXH was more efficient for the estimation of the absolute quantities of *N*-glycans from the results of fluorescence intensity (by UPLC) and relative quantity (by LC-MS/MS).

These results first demonstrate that ProA is useful for identifying and quantifying sialylated, core-fucosylated, and neuraminidase-treated desialylated *N*-glycans in HTF using AXH by UPLC and LC/MS.



Efficient combination to identify and quantify N-glycans in HTF.

Acknowledgements

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Substrate promiscuities of a bacterial galactokinase and a glucose-1-phosphate uridyltransferase enable xylose salvaging

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Galactokinases (GalKs) catalyse the transfer of a phosphate group from adenosine triphosphate (ATP) to galactose. Here we investigate the promiscuity of a bacterial galactokinase originating from the soil bacterium *Solitalea canadensis* (*Sc*GalK) for other monosaccharides than for the synthesis of diverse sugar-1-phosphates, which than can be further converted into sugar nucleotides to provide donors.

Together a previously uncharacterized glucose-1-phosphate uridyltransferase from the same soil bacterium (*Sc*GPUT), *Sc*GalK was able to convert D-xylose into uridine diphosphate xylose (UDP-Xylose) in the presence of ATP and uridine triphosphate (UTP), therefore showing the feasibility of a novel intrinsic salvage pathway for xylose in *Solitalea canadensis*.

Acknowledgements

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Combating DC-SIGN-mediated SARS-CoV-2 Dissemination by Glycan-mimicking Polymers

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The C-type lectin receptor DC-SIGN is a promiscuous attachment factor for pathogenic viruses on innate immune cells. In the context of the recent SARS-CoV-2 pandemic, DC-SIGN-mediated virus dissemination has been identified as a mechanism of immune dysfunction that contributes to severe COVID-19.[1] Carbohydrate-based compounds can efficiently interfere in the interaction between viral surface glycoproteins and DC-SIGN and, thus, prevent virus binding to innate immune cells.[2-4] As a consequence, glycan-mimicking molecules represent a host-directed strategy to combat the spread of epidemic and pandemic viruses. A recent study identified preferred oligomannose fragments of DC-SIGN in high-mannose glycans.[5] Here, we aim to leverage this insight in order to enhance the activity of polyvalent DC-SIGN ligands. For this, poly-l-lysine polymers were functionalized with selected mono-, or oligosaccharide epitopes and evaluated for their ability to bind DC-SIGN. Hydrodynamic properties and multivalent interaction thermodynamics were characterized in biophysical assays and correlated with polymer activity in SARS-CoV-2 *trans*-infection studies.

Oligosaccharide epitopes containing a-d-Man-(1→2)-a-d-Man motifs displayed particularly high activity in the low nanomolar range. The optimized oligomannose glycopolymers identified here represent highly active and fully biocompatible lead candidates to enable a rapid host-directed response to known and newly emerging viral pathogens.

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Efficient construction of homogenous glycoproteins

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Glycoproteins in cells are produced in a highly heterogeneous form. Thus, it is difficult to elucidate glycoprotein-mediated biological processes and to discover therapeutic agents. Therefore, general and efficient methods to obtain homogeneous glycoproteins are greatly demanded. We developed a general method for the efficient preparation of homogeneous glycoproteins that employs a combination of genetic code expansion and chemoselective ligation techniques. In this method, an alkyne tag-containing protein, that were produced by genetic encoding of an alkynylated unnatural amino acid, was quantitatively coupled via click chemistry to various azide-appended glycans.

The glycoproteins generated by this strategy were found to recognize mammalian cell-surface lectins and to enter the cells via the lectin-mediated endocytosis. In addition, the glycoprotein possessing multiple mannose-6-phosphate residues entered diseased cells lacking specific lysosomal glycosidases by binding to the cell-surface M6P receptor, and subsequently migrated to lysosomes for efficient degradation of stored glycosphingolipids.

Characterization of an *N*-Acetylglucosaminyltransferase I (GnT-I) from *Crassostrea gigas*

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The biosynthesis of hybrid and complex type N-glycans takes place in the medial-Golgi and requires the prior action of the α-1,3-mannosyl-glycoprotein 2-β-N-acetylglucosaminyltransferase I, GnT-I (encoded by *Mgat1*, EC 2.4.1.101). It catalyzes the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine (UDP-GlcNAc) to the Man-alpha-1-3 arm of Man5GlcNAc2 (Man5), producing a suitable substrate for the further enzymes, such as alpha-mannosidase-II and subsequently GnT-II.

Although the enzyme has been cloned and characterized from several plants and animals, the GnT-I from invertebrates, especially from mollusks, remains rather undiscovered.

In this study, we present the expression of a ~50 kDa truncated GnT-I from the Pacific oyster, *C. gigas* (ΔR2-M24 GnT-I, NCBI Ref. Nr.: XP_034321804.1), in Sf9 insect cells and its characterization with 2-aminopyridin labelled Man5 N-glycan (Man5-PA) as the substrate.

The GnT-I shows highest activity at neutral environment, pH 7.0, and at a temperature of 30 °C. The addition of divalent cations such as Mn^{2+}, Co^{2+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, and Ni^{2+} [24 mM] increases activity, with highest activity at 40 mM Mn^{2+}, while the addition of EDTA or Cu^{2+} abolishes the enzyme's activity completely. The activity is also negatively influenced by the addition of UMP, UDP, UTP or galactose [0.1 %]. Moreover, the GnT-I enzyme is sensitive towards storage in methanol (20 % v/v), acetonitrile (10 % v/v) or glycerol (10 % v/v).

Acknowledgements

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Practical use of analytical tools for glycan structures for glycoproteins and cells

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We've tried new glycan engineering with the synergistic effects of chemical synthesis technology for oligosaccharides, development for anti-glycan antibodies, and practical use of endoglycosidase. As a technology related to the antibody drug field, we synthesized series of the structure-defined *N*-type glycans found on Fc region of antibody drug and achieved the separative production of 3-G1 and 6-G1 glycan isomers. We have also realized the development of antibodies for the non-human type of glycan epitopes using high-purity chemical synthetic glycans containing αGal and NeuGc which may cause anaphylactic symptoms as antigens, and the developed antibodies are capable of detection of antigenic antibody drugs using sandwich ELISA and western blotting. It was also confirmed that the antibody drug having this non-human type antigen can be captured by a recombinant lectin-agarose immobilized with high concentration by preferential affinity to αGal.

In addition, this lectin-immobilized resin can capture an artificial antibody possessing homogenous glycan structure produced by the transglycosylation reaction of endoglycosidase (Endo-M) with glycopeptides as a glycan donor. We have also succeeded in obtaining an antibody with a highly pharmacologically active glycosylation with a terminal galactose without core fucose.

Based on this technology base, we developed various types of glyco-conjugates in which chemically synthesized oligosaccharides are attached to proteins and chemically modified functional polysaccharides. Furthermore we attempt to develop unique tools and analytical techniques useful for functional research toward the glycocalyx of cells and microvesicles.

Chemoenzymatic synthesis of ^{13}C enriched sialic acids

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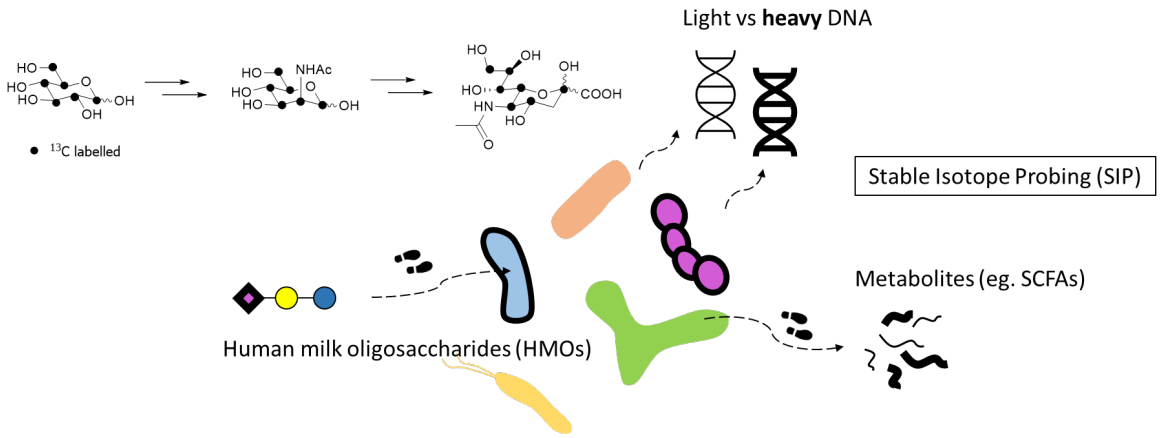
Sialic acid or N-acetylneuraminic acid (Neu5Ac) is a ubiquitous sugar residue found in the gut mucus layer. It is commonly found at the terminal location of sugar chains, and its prior removal is necessary for gut microbiota to gain access to the rich underlying sugar residues.

Metabolism of glycans by gut bacteria leading to the production of short chain fatty acids (SCFAs) is of great interest because of their known health benefits to human hosts. Through Stable Isotope Probing (SIP), 13C labelling of Neu5Ac can identify gut microbiota able to metabolise Neu5Ac and enable clear distinction of metabolites generated in the Neu5Ac metabolism pathway in the presence of other sugars.

To obtain these 13C enriched sugars, we have developed a facile chemoenzymatic synthesis route from the cheap and commercially available [U-13C]glucose. Chemical synthesis yielded the key intermediate, peracetylated N-acetyl mannosamine ([13C6]Ac4ManNAc) on a gram scale at 21% yield. Subsequently, enzymatic condensation of [13C6]ManNAc with [2-13C]pyruvate gave asymmetrically labelled [13C7]Neu5Ac, which can be used to prepare 3'-SL and 6'-SL through a facile one-pot enzymatic reaction using CMP-sialic acid synthetase and sialyltransferase. Synthesis of other forms of sialic acids are also possible as demonstrated by the synthesis of 2,7-anhydro Neu5Ac.



Acknowledgements

SweetCrosstalk ITN is funded by EU H2020 programme under Grant Agreement No. 814102

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Transition-state analogue Inhibitors of *P. falciparum*, *T. Cruzi* and *T. Brucei* PPRTases

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The pathogenic protozoans such as *Plasmodium falciparum*, *Trypanosoma Cruzi* and *Trypanosoma Brucei* are responsible for malaria, Chagas disease and sleeping sickness respectively. These protozoan parasites are unable to synthesize purines *de novo* and rely on salvage of purine from the host. Purine salvage enzymes purine phosphoribosyltransferases (PPRTases) are critical for purine salvage and are potential targets for novel therapeutic development.

Transition states of N-ribosyl transferase enzymes have been characterized with all having some ribooxacarbenium ion characteristics. We have reported immucillin-H 5'-phosphate (ImmHP), immucillin-G 5'-phosphate (ImmGP), and some acyclic nucleoside phosphonates (AIP) as transition state analogue inhibitors of the *Plasmodium falciparum* PPRTase.

We have synthesised a range of cyclic and acyclic aza-*C*-nucleoside structural homologues (phosphates and phosphonates) as transition state analogue inhibitors of *Plasmodium* and *Trypanosoma* PPRTases and shown that some are selective and potent nanomolar inhibitors.

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Pyridoneimine-promoted aqueous anomeric functionalization of unprotected carbohydrates

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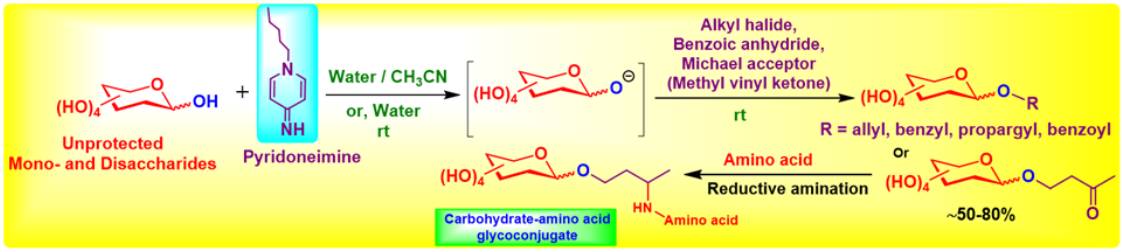
Anomeric functionalization of unprotected carbohydrates in water is ambitious, due to the presence of competing hydroxy moieties in a monosaccharide. As a result, the most often practiced route is to install required protecting groups that permit selective functionalization at the desired hydroxy functionality, including the anomeric lactol moiety. There is a reasonable basis to differentiate the anomeric lactol moiety from that of remaining hydroxy functionalities, factoring the pK_a differences. Difference in the p*K*a value of ~12.5 *vs* 16-18 for the anomeric and the remaining hydroxy groups, respectively, prompts that such a difference is available for the selective functionalization of anomeric lactol in carbohydrates.

With this basis, we have uncovered the potential of pyridoneimine as a suitable and selective base for deprotonation of the hemiacetal and subsequent reaction of the corresponding hemiacetal anion. Reactions of the resulting hemiacetal anion with alkyl halides, acid anhydrides and Michael acceptors lead to facile formation of the corresponding anomeric functionalized derivatives. Unprotected mono- and disaccharides of varied constitutions are subjected to the reactions and the selective functionalization at the anomeric carbon achieved, in aq. solutions. The anomeric oxa-Michael addition-derived keto-glycosides are resourceful for further glycoconjugations with amino acids, through reductive amination. The presentation will include the development of pyridoneimine as a base for selective anomeric functionalization of mono- and disaccharides, fulfilling the solubilities of carbohydrates in water, in a complete site-selective fashion in such a multi-functional synthons.



Hemiacetal deprotonation followed by further alkylation, acylation and oxa-Michael addition reaction

Acknowledgements

1.Science and Engineering Research Board, Department of Science and Technology, New Delhi, for a research support.
2. CSIR for research fellowship

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A chemoenzymatic approach to the synthesis of dolichol pyrophosphate linked oligosaccharide

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Dolichol pyrophosphate linked oligosaccharides (DLOs) are precursors of *N*-glycans. The DLOs are biosynthesized on the cytoplasmic side of the endoplasmic reticulum (ER) membrane with the construction of $Man_5GlcNAc_2$ structures. It is acknowledged that the $Man_5GlcNAc_2$-PP-Dol flips into the ER luminal side. However, the catalytic mechanism remains unclear [1,2].

Thus, we are aiming to reveal such a catalytic mechanism and develop a practical construction method of ER-mimicking liposomes having pure DLOs into the surfaces. In this research, we attempted to promote a chemoenzymatic approach by utilizing the activity of *N*-acetylglucosamine-phosphotransferase (DPAGT1) with the chemically synthesized UDP-GlcNAc probes, as well as endo-β-*N*-acetylglucosaminidase (ENGase) to the phosphorylated GlcNAc residues.

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Synthesis of a tetrasaccharide donor for the synthesis of rhamnogalacturonan II side chain A

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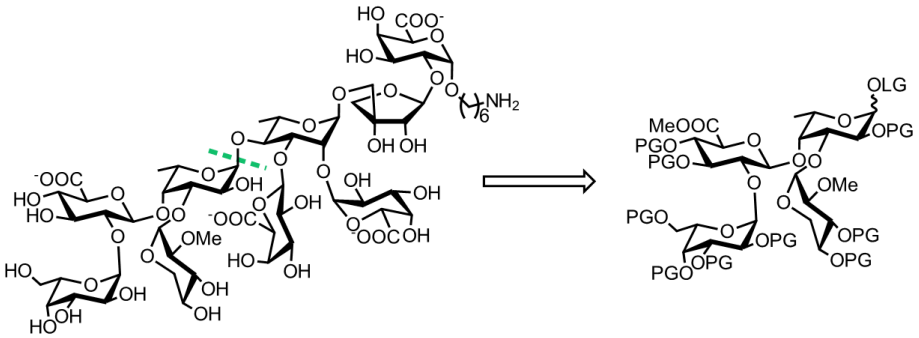
The plant cell wall contains three types of polysaccharides: cellulose, hemicellulose and pectin. Pectin is an acidic polysaccharide consisting of different domains with either homogalacturonan or rhamnogalacturonan backbone. The most complex part of pectin is rhamnogalacturonan-II (RG-II).[1] It mostly occurs in dimers, which are covalently linked by a borate diester which has been shown to be crucial for plant growth.[2]

For further investigations into the biosynthesis, structure and biological function of RG-II, oligosaccharide fragments of RG-II will be highly valuable. Here we present the synthesis of a tetrasaccharide donor (**2**) for the synthesis of the full side chain A nonasaccharide of RG-II (**1**) as well as different side A fragments. Glycosyl donor **2** is a branched tetrasaccharide, consisting of a fucose carrying an α-1,3-linked 2-*O*-methyl xylose a β-1,4-linked glucuronic acid, which in turn is substituted with an a-1,2-linked L-galactose. The glycosylation reactions for constructing tetrasaccharide **2** were mostly conducted using thioglycoside donors activated by *N*-iodosuccinimide and silver triflate. Importantly, a highly orthogonal set of protecting groups was chosen to achieve the branched structure of donor (**2**). Tetrasaccharide donor **2** will be reacted in a [4+5]-glycosylation reaction with a suitable pentasaccharide acceptor to provide full side chain A. Synthetic side chain A and fragments thereof generated using donor **2** will serve as substrates for glycosyltransferases, glycosyl hydrolases, and as ligands for carbohydrate receptors.[3]



Full Side Chain A (1) **Tetrasaccharide Donor (2)**

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Towards the synthesis of bicyclic constrained carbacycles as competitive α-galactosidase inhibitors

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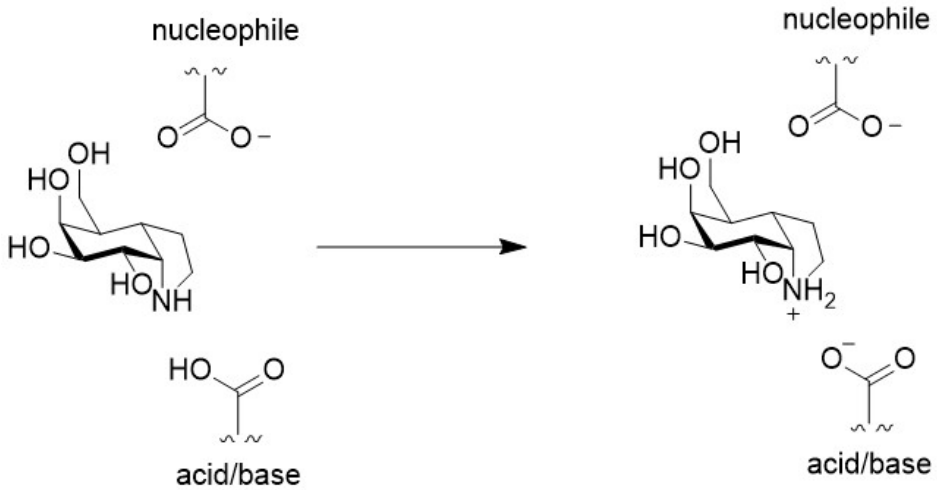
Fabry disease is an inherited lysosomal storage disorder caused by difficiency of α-galactosidase A (α-gal A). α-Gal A catalyses the hydrolysis of terminal, non-reducing α-galactose residues from globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Mutations in the GLA gene encoding for α-gal A result in decreased or lack of activity of α-gal A leading to toxic accumulation of the glycosphingolipids Gb3 and lyso-Gb3. Current treatment is enzyme replacement therapy with a recombinant enzyme or pharmacological chaperone (PC) therapy, however both therapies have limited efficacy. Therefore, the joint administration of a recombinant enzyme with a pharmacological chaperone holds promise for a more effective treatment of Fabry disease.

Previous research for a new class of inhibitors showed that α-D-galactose-configured cyclosulfamidate stabilizes recombinant human a-D-galactosidase (agalsidase beta, Fabrazyme®) effectively. This cyclosulfamide is conformationally constrained and thereby mimics the Michaelis complex conformation. In a search for more potent and selective competitive inhibitors targeting α-gal A, it is thought that instead of the sulfamidate a nitrogen containing carbacycle, either exo- or endocyclic, might increase the potency through ionic interactions, while still keeping the selectivity. Here the synthesis towards these bicyclic contrained carbacycles is described.



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Regioselective and Stereospecific β-Arabinofuranosylation Using a Boron-Mediated Aglycon Delivery

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β-Arabinofuranoside (β-Arb*f*)-containing glycans have garnered significant attention across numerous research fields due to their intriguing biological activities. However, achieving stereoselective construction of β-Arb*f* linkages has proven challenging due to the absence of neighboring-group participation, the unfavorable anomeric effect, and the steric hindrance of the substituent at the C2 position. Moreover, S_N1 pathway is preferential for furanoside formation compared to pyranoside formation owing to its structural and electronic properties, resulting in diminished stereoselectivity. With this in mind, we directed our attention towards our boron-mediated aglycon delivery (BMAD) method, which is capable of constructing 1,2-*cis* pyranosides with exceptional regio- and stereoselectivities and explored its applicability in β-arabinofuranosylation.

As a result of the investigation, it was found that the glycosylations of 1,2-anhydroarabinofuranoses and several sugar diols in the presence of boronic acid catalyst proceeded smoothly to provide corresponding β-Arb*f*s with complete β-stereoselectivities and high regioselectivities. In addition, a variety of diols, triols, and unprotected sugar acceptors could be employed for this present arabinofuranosylation, and the regioselectivity was completely reversed depending on the optical isomerism of the donor used and was predictable a priori using predictive models. Furthermore, the chemical synthesis of arabinogalactan fragments from Timothy grass demonstrated the usefulness of this glycosylation method.

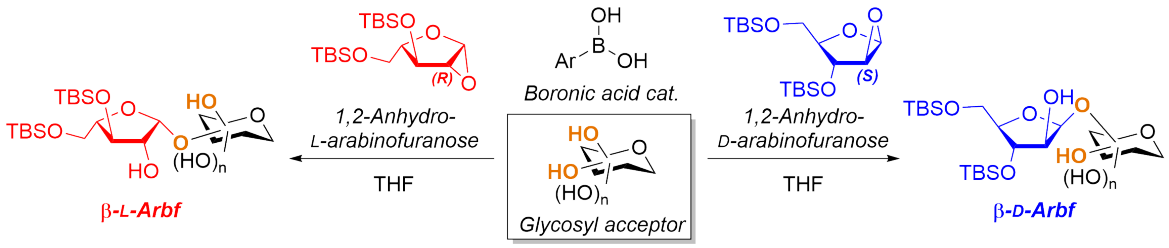

Figure 1. (a) Neoglycolipids. (b) Supramolecular characterization. (c) Heteromultivalency.

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Synthesis and immunofunctional analysis of monoglycosphingolipids

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Monoglycosphingolipids such as GlcCer and GalCer are widely found in living organisms, and it has been reported that some of these glycosphingolipids modulate the immune system via innate immune receptors (eg. Mincle)[1] or lipid antigen-presenting molecules (CD1d).[2]There are many structural variations of lipid moiety as well as glycan part of glycosphingolipids, and the ratio of lipid length and structure varies depending on the type of organ or cell present,[3] as well as enzyme dysfunctions and other factors.[4] These structural transformations may affect the immunomodulatory function, but the details have not been clarified.

We therefore developed the synthetic strategy of diverse glycosphingolipids of GlcCers and GalCers having various lipids for building their compound library for further understanding of their immunomodulatory activities. We used allyl-type protecting group strategy to enable the synthesis of various GlcCers and GalCers containing lipid moieties with unsaturated bonds including regular sphingosine ceramide.[5] Firstly, allyl-protected glycosyl donor and sphingosine acceptor were prepared and used for the glycosylation to prepare the glycolipid backbone. After deprotection of the allyl-type protecting groups, subsequent selective amidation with various fatty acids gave GlcCers and GalcCers, containing a variety of lipids. We then evaluated biological activities of the synthesized glycosphingolipids, and characteristic immunological functions were observed.

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Analysis of the interactions between ganglioside GM3 and insulin receptor transmembrane peptide

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Biochemical and cell biological experiments have shown that GM3, a cell membrane glycolipid, inhibits the function of insulin receptor (IR). However, due to the heterogeneity of the cell membrane, it has been difficult to analyze the interaction between GM3 and IR in detail. In this study, we synthesized a fluorescently labeled transmembrane peptide (NBD-IR-TM) and incorporated it into liposomes to construct a simplified model system for interaction analysis.

The localization of NBD-IR-TM in phase separated GUVs containing glycolipids was analyzed (Figure 1). The co-localization of NBD-IR-TM with GM3 was significantly increased compared to that of lactosylceramide (LacCer), a GM3 precursor without sialic acid. Furthermore, the fluidity of NBD-IR-TM in GUVs was significantly reduced by the addition of GM3; in MLVs, the association of NBD-IR-TM was inhibited by GM3, but not by LacCer. Finally, we analyzed the aggregation state of two model peptides with and without charge and found that only the association of the positively charged peptide was inhibited by GM3.

These results indicate that the basic amino acids and acidic sugars of each molecule are important for the interaction between transmembrane peptides and GM3, and that this electrostatic interaction also occurs between the insulin receptor and GM3.

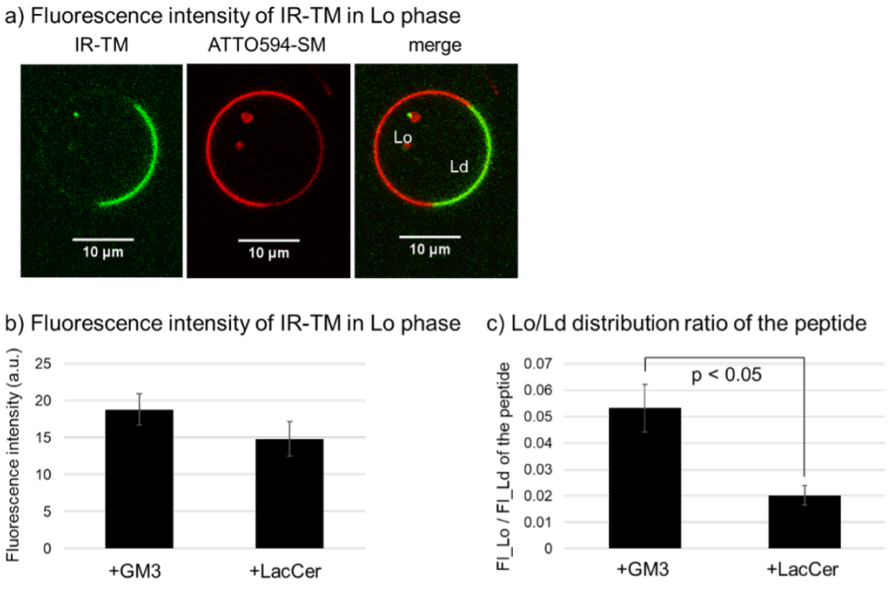

Figure 1 Localization analysis of IR-TM in phase-separated GUVs. GUVs were prepared with molar ratios of DOPC / sphingomyelin / GM3 or LacCer / cho

NMR Chemical Shift Prediction of Aglycone-linked Oligosaccharides using CASPER

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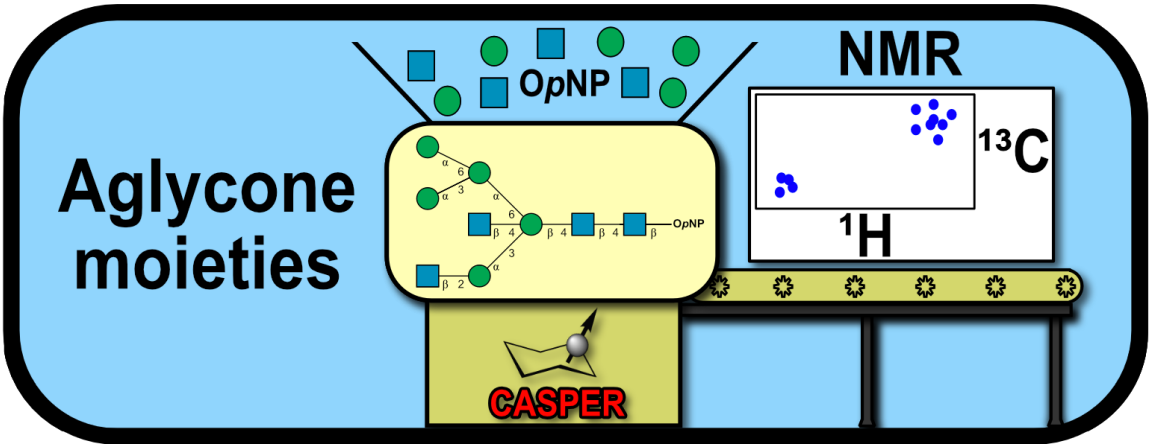
Advances in the structural characterization of carbohydrates: Carbohydrates play important roles in a plethora of biological processes.[1] Highly complex carbohydrate structures can be found in nature and isolated, though usually in small quantities. Elucidation of aforementioned structures with nuclear magnetic resonance (NMR) spectroscopy as the primary tool is a time-consuming and error-prone task.[2] In order to facilitate and partially automate this process dedicated software, such as CASPER (**C**omputer **A**ssisted **Sp**ectrum **E**valuation of **R**egular Polysaccharides), has been developed.[3]

With the developments of carbohydrate chemistry during the last decades, more complex structures can be obtained in a highly pure form via chemical/chemoenzymatic synthesis.[1,4] Often times, these synthetized structures are carrying an aglycone at the reducing end, making it suitable for e.g. conjugation to proteins or immobilization on microarrays. CASPER is now being developed to accommodate aglycones, such as aminoalkyl and p-nitrophenol substituents at the reducing end, both for NMR chemical shifts prediction of a user defined structure, as well as for automated structural elucidation of a structure based on unassigned NMR data.

This development provides a valuable tool not only for researchers focusing on elucidation of unknown structures and interaction studies by NMR, but furthermore for synthetic chemists seeking structural verification of their synthesized products, as well as tentative NMR chemical shift assignment.



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Glycosylation of Polyfluorinated Carbohydrates

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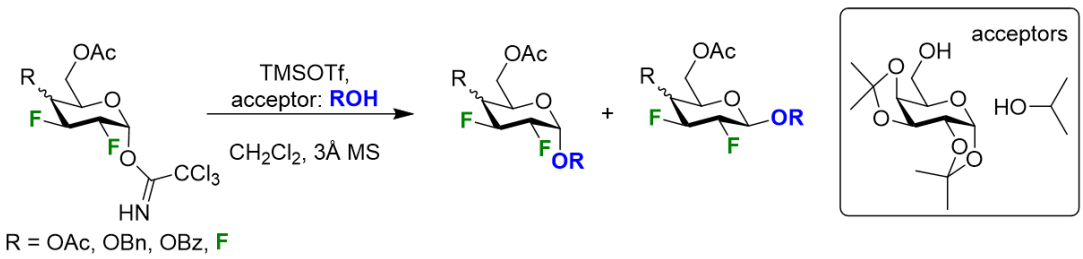
Glycosylation is a ubiquitous process in nature, where glycans are involved in metabolic pathways and recognition events. Thus, fluorinated glycans can act as probes in the investigation of protein-glycan interactions and epitome mapping studies. Currently, monofluorinated glycans can be made by enzymatic glycosylation of monofluorinated donors. There are no enzymatic glycosylations described with sugar donors having >1 fluorine atom in their ring. In carbohydrates, fluorination was shown to increase chemical stability and reduce their hydrophilicity.

Chemical glycosylation of deoxyfluorinated carbohydrates is challenging because the fluorine electron withdrawing effect destabilizes the transition states of anomeric C–O bond forming reactions. The effect is pronounced when the number of fluorine atoms increases and when they are located adjacent to the anomeric position. To date, there is limited precedence for the chemical glycosylation of polyfluorinated carbohydrates. Most of the glycosylation methods developed involve anomeric alkylation which results in inversion of a stereogenic centre on the acceptor. Most of the methods developed are glycosidation, i.e., involve non carbohydrate acceptors. This poster will describe our efforts to achieve conventional chemical glycosidation and glycosylation of 2,3-difluoro- and 2,3,4-trifluorinated glucose and galactose donors using trichloroacetimidate pre-activation. Two distinct goals were established: being able to reach full conversion and increasing the anomeric selectivity of the reaction.



Glycosylation of polyfluorinated carbohydrates through trichloroacetimidate pre-activation

Acknowledgements

K.H. and B.L. thank the University of Southampton for funding

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Biofunctional analysis of α-GalCer derivatives with CD1d-ligand complex stability modulation

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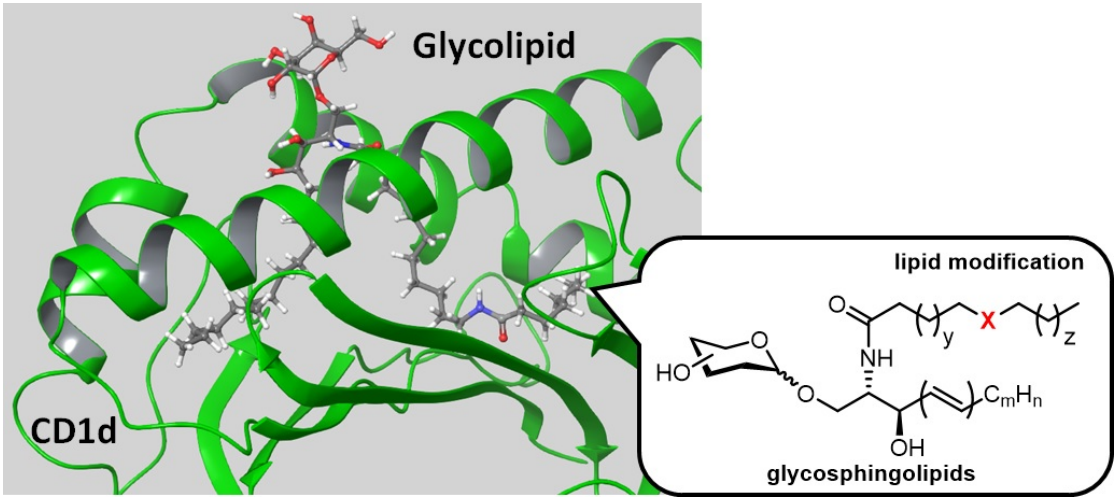
Glycolipid antigens play an important function in immune modulation by activating NKT cells through the formation of glycolipid-CD1d complexes on antigen presenting cells to secrete various cytokines. Examples of the antigens include glycolipids such as α-GalCer (KRN7000), bacterial α-GalCer$_{Bf}$, endogenous β-GalCer, and other complex lipids. We have previously shown that modification of the lipid moiety of α-GalCer (KRN7000) by polar functional groups strongly affects binding affinity and immune activity balance, but the mechanisms of these phenomena have been only partially investigated. Therefore, to elucidate the mechanism of immunomodulation by structural differences in CD1d ligands, we conducted synthesis and biofunctional evaluation of various glycolipids, and analysed the stability of CD1d-antigen complexes both experimentally and computationally. The synthesis of glycolipids used key intermediates to facilitate the introduction of various lipid structures. The desired glycolipids were obtained by global deprotection of these key intermediates followed by selective amidation. The biological functions of the obtained glycolipids were evaluated by measuring their binding affinity to CD1d. Modification of the lipid structure had a significant effect on the binding affinity. The thermal stability of the glycolipid-CD1d complex was also examined, combined with computational studies utilizing MD simulations. Details of the above experimental results will be further discussed in the poster presentation.



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Design and synthesis of 5-thio-glucose based endo-glycosidase inhibitors

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Glycomimetics are molecules that are structurally similar to carbohydrates but differ in their architecture around the glycosidic linkage.[1] By altering this key feature, these molecules are able to mimic the behaviour of natural substrates while being customizable in reactivity and stability.[1] One way of increasing the stability of the glycosidic linkage is by substituting its endo-cyclic oxygen atom with a sulfur atom.[2] Known as thiosugars, the increased glycosidic stability of these compounds makes them useful tools in chemical biology, as thiosugar-linked substrates are known to act as inhibitors for endo-specific glycosidases.[3]

A prominent member of the endo-glycosidase family is heparanase (HPSE), an endo-β-D-glucuronidase that degrades the linear polysaccharide known as heparan sulfate (HS) (Fig. 1A).[4] Recently, the cyclophellitol derivative **1**, a mimic of the repeating disaccharide present in HS, was found to be a potent and covalent inhibitor of HPSE (Fig. 1B).[5] With this in mind, mimics featuring a thiosugar could potentially stabilize such an inhibitor by preventing exo-glycosidase cleavage of the glycosidic linkage, thereby increasing selectivity (Fig. 1C). Here, the development of such 5-thio-D-glucopyranoside linked inhibitors is presented.

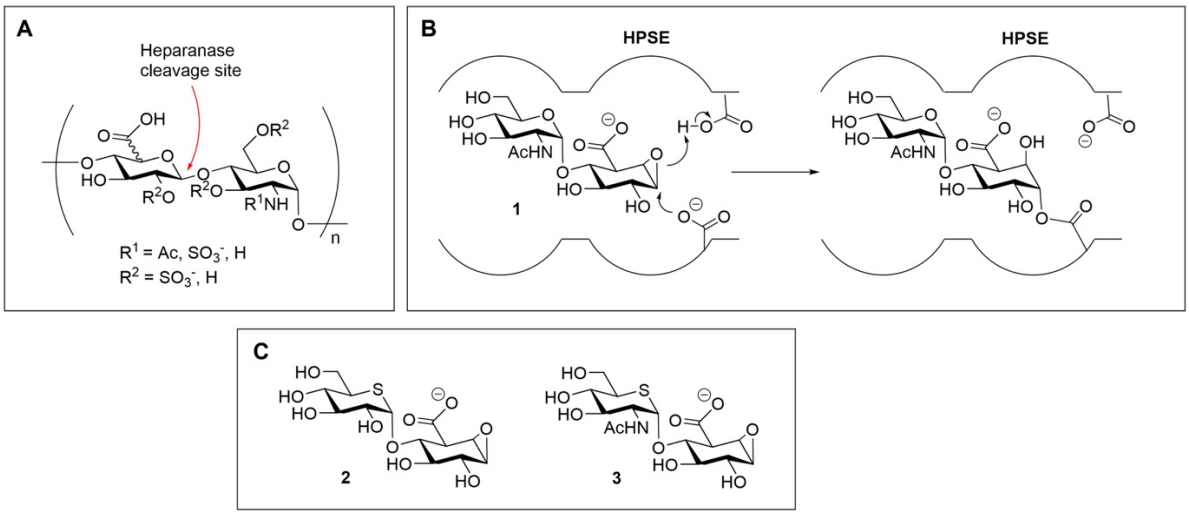

Figure 1: (A) HS repeating unit. (B) HPSE covalent inhibition. (C) Stabilized inhibitors.

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Rapid and stereoselective synthesis of novel azasugars as glycosyltransferase inhibitors

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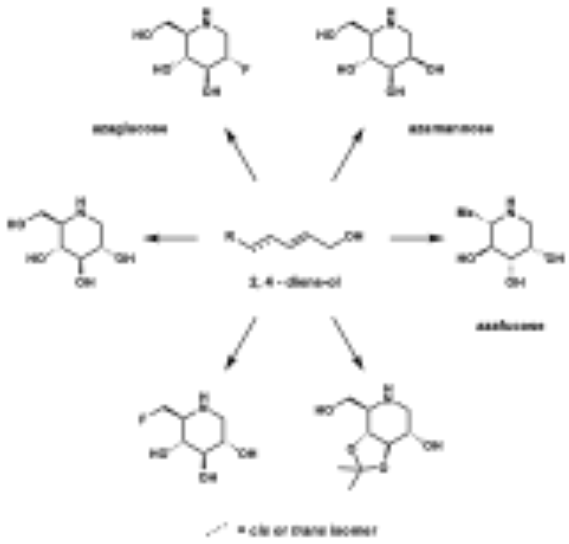
Glycosylation is known to play an essential role in cell signaling, immunity, and disease progression. Glycomimetics, such as azasugars, have proven to be effective therapeutics for both congenital disorders of glycosylation as well as viral infections. However, synthetic strategies for the synthesis of azasugars has remained extremely limited. We present a rapid, stereoselective, and diversifiable synthetic strategy to access a variety of valuable azasugar scaffolds.

Our collection of novel compounds are tested for biological efficacy as inhibitors of 1,2- and 1,3-fucosyltransferases, enzymes known to take part in essential cellular differentiation, gut cell health, and disease management. We utilize a uniquely synthesized FucT small molecule inhibitor in a novel assay to elucidate azasugars' ability to selectively target key glycoenzymes.



Diversification of an Azasugar Scaffold from A Common Starting Material

New supramolecular multivalent anti-adhesive agents against SARS-CoV-2

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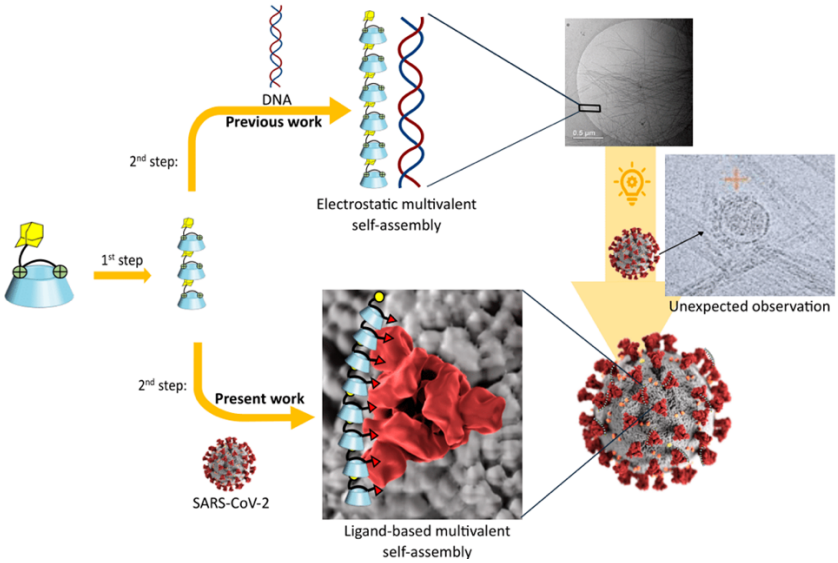
In 2020, during a CRYO-EM experiment, we unexpectedly observed a SARS-CoV-2 particle near self-assembled cyclodextrins fibers. We thus wondered if we could bring specific interactions between them and trigger this assembly on purpose. Indeed, our team previously showed that it was possible to form fibers from small oligomers of DNA and cyclodextrins functionalized by an adamantane and ammonium. The hydrophobic effect between cyclodextrins and adamantanes allows the formation of small supramolecular polymers by self-assembly (1st step). Then, through multivalent electrostatic interactions between the monomers and DNA, the co-assembly becomes a lot bigger (2nd step).[1]

The surprising observation encouraged us to explore the ability of cyclodextrins assemblies to cooperatively interact with SARS-CoV-2 particles and use them as anti-adhesive agents to potentially inhibit cell infection by this virus. We therefore changed non-specific electrostatic interactions into more specific ones, using sugar-based ligands allowing multivalent effect. For that, we functionalized cyclodextrins with an adamantane and the targeted ligand on the side. We are now studying their self-assemblies (1st step), their multivalent ability to interact with several receptors at the surface of SARS-CoV-2 and potentially observe cooperative assembly.



From CDs interactions with DNA to multivalent anti-adhesive agents against SARS-CoV-2

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Combined experimental and computational study for the characterization of *Pseudoarthrobacter siccito*

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Here, we present the characterization of *Ps*P2Ox, a *C*-glycoside-3-oxidase from *Pseudoarthrobacter siccitolerans,* carried out by a combination of experimental and computational approaches [1].

C-glycosides are natural products that present important biological activities and chemical properties, such as high stability against degradation. Glycoside 3-oxidases (G3Oxs) are newly identified FAD-dependent bacterial enzymes capable of oxidizing *C*-glycosides with the concomitant reduction of O_2 to H_2O_2. Interestingly, soil and gut microorganisms have different oxidative enzymes. This oxidation is then followed by C-C acid/base-assisted bond cleavage in two-step *C*-deglycosylation pathways.

Our results show that *Ps*GO3x oxidizes the glucose moiety of mangiferin to 3-keto-mangiferin with 50,000-fold higher specificity (k_{cat}/K_m) than free D-glucose to 2-keto-glucose. Analysis of *Ps*G3Ox crystal structures (without and with substrates bound), combined with mutagenesis, molecular dockings and molecular dynamics simulations, reveal functional, structural and dynamical details that explain these enzymes' ability to bind and oxidize larger glycoside substrates (Fig 1).

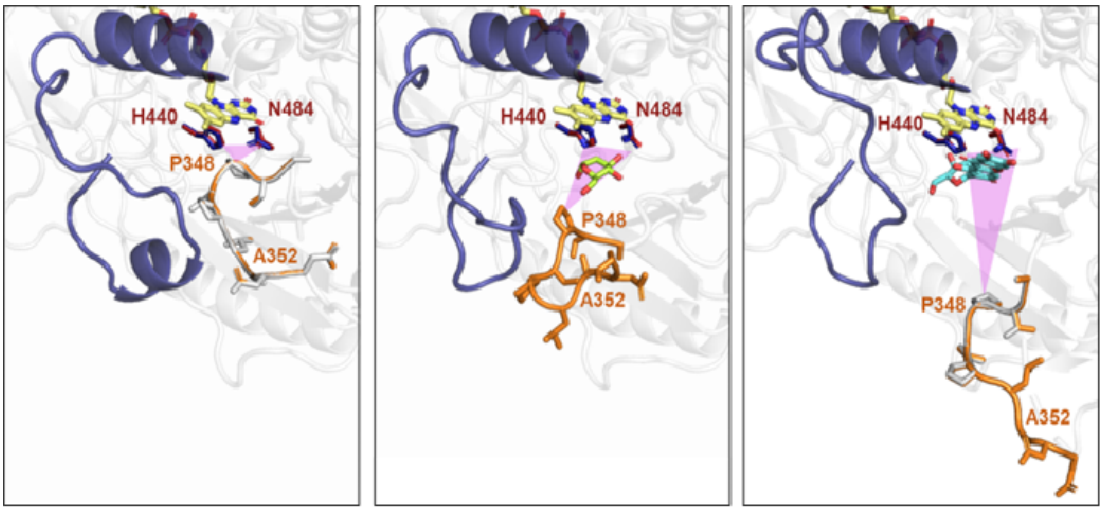

Fig 1. PsP2Ox modelled structure for the substrate free, the glucose-bound and the mangiferin bound

Acknowledgements

This work was supported by the Fundação para a Ciência e Tecnologia, Portugal, and the Spanish MCIN/AEI/10.13039/501100011033/ FEDER, UE.

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Synthesis of *Streptococcus pneumoniae* 6A/6C capsular polysaccharide fragments

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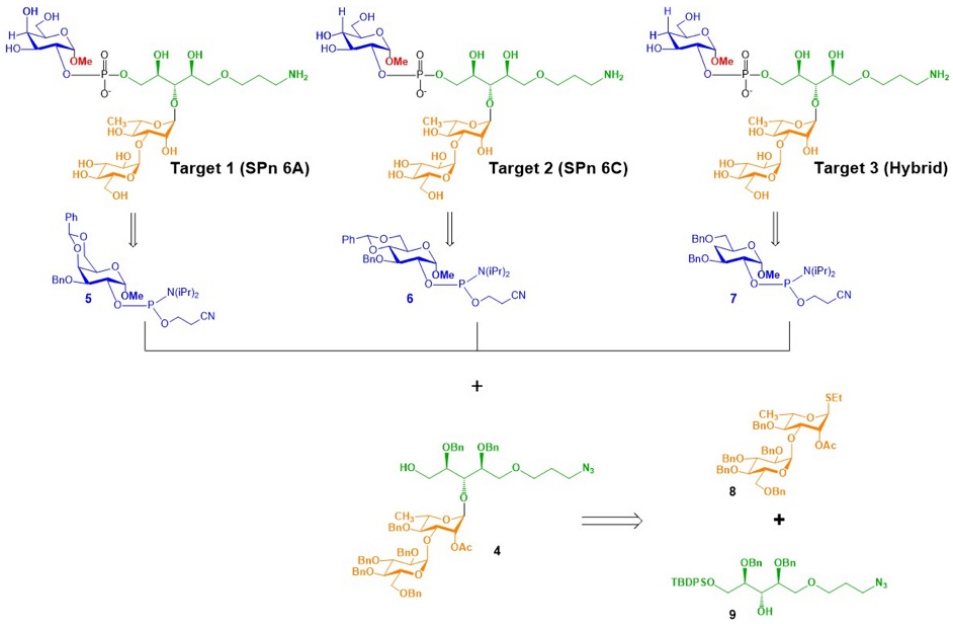
Streptococcus pneumoniae (SPn) is a significant cause of otitis media, pneumonia, and meningitis. Just 7 out of the approximately 100 serotypes were initially included in the pneumococcal polysaccharide conjugate vaccine in 2000, and this number has been increased in subsequent years. A significant rise of infections from non-vaccine serotypes SPn 6C was recorded following the widespread administration of pneumococcal vaccines containing serogroups 6A and 6B, a phenomenon usually referred to as serotype replacement1. Thus, the identification of novel antigens able to provide protection against more than one serotype is key to the future development of novel and more effective vaccines. The polysaccharide repeating units of SPn 6 serotypes have minimal structural differences: 6A and 6C have the same glycosidic linkages, but they differ in their monosaccharide composition, with glucose replacing galactose in 6C2. The tetrasaccharide fragments of SPn 6A (Target 1) and 6C (Target 2) serotypes are the primary synthetic goals of this project (Scheme 1). In addition, we designed the 4-deoxy derivative of both tetrasaccharides (Target 3), where the only structural difference between 6A and 6C CPS is removed. According to the retrosynthetic approach, compound **4** was synthesized from a suitable rhamnose derivative, which can be used both as an acceptor for the synthesis of disaccharide **8** and as a donor for the glycosylation of ribitol **9**. Finally, target tetrasaccharides **1**, **2** and **3** have been assembled using the phosphoramidite derivatives **5**, **6** and **7**. The synthetic tetrasaccharides will be eventually conjugated to a carrier protein, and the glycoconjugates obtained will be subjected to in-depth immunological studies. The results will help to identify protective epitopes to be employed for the development of an effective anti-SPn 6A/6C glycoconjugate vaccine.



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Procainamide-labeled *N*-glycans and their structural role in mucin from bovine submaxillary glands

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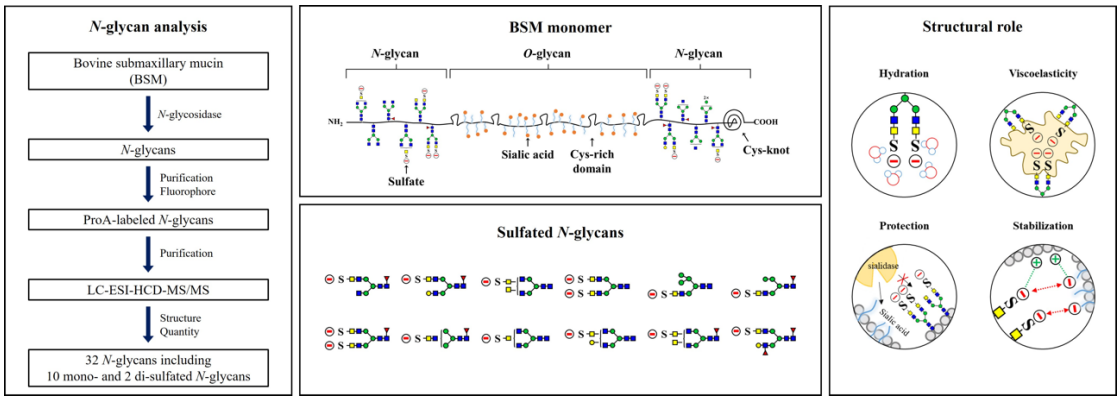
Bovine submaxillary mucin (BSM) is a heavily-glycosylated macromolecular (approximately 4MDa) protein that is used in various biomaterial applications in light of its high viscosity and biocompatibility, in addition to use as a biochemical substrate or inhibitor as a result of its abundant *O*-glycans. Although *N*-glycosylation has been reported to provides stability of human mucins, most BSM research has been focused on its *O*-glycans, while *N*-glycans have not been reported to date.

In this study, a common *N*-glycan core component was detected by monosaccharide analysis of BSM, and the structures and relative quantities of the *N*-glycans were determined by liquid chromatography–tandem mass spectrometry.

Seventeen *N*-glycans comprising ten complex-type [$Fucose_{0\sim2}Hexose_{3\sim4}$*N*-$acetylhexosamine_{1\sim6}Sulfate_{0\sim1}$; 61.1% (the sum of the relative quantities of each *N*-glycan out of the total *N*-glycans)], two high-mannose-type ($Hexose_{5\sim6}$*N*-$acetylhexosamine_2$; 12.0%), and five paucimannose type ($Fucose_{0\sim1}Hexose_{3\sim4}$*N*-$acetylhexosamine_{2\sim3}$; 26.9%) were identified, but no hybrid-type or sialylated *N*-glycans were found. Additionally, these are less-branched structures compared to human mucins. Of these, ten glycans (77.2%), including two sulfated glycans (8.0%), were core fucosylated, which confer unique biological functions to glycoproteins. These *N*-glycans are less-branched structures compared to human mucins. This is the first study to confirm *N*-glycosylation of BSM, and these results support further expansion of the biological function of non-human mucin.



Workflow of N-glycan analysis, BSM monomer, sulfated N-glycans, and their structural role.

Acknowledgements

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Development of a 2,3-difluorosialic acid based covalent neuraminidase probe

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Neuraminidase (NA), one of the major surface glycoproteins of influenza A virus, is an important diagnostic biomarker and antiviral therapeutic target. Probing NA provides important information on influenza virus biology, that can monitor the emergence of drug-resistant strains and guide the development of novel drugs and vaccines. However, there is still a lack of covalent NA probes with enhanced specificity and higher stability and this limits the in-depth exploration of NA.

Based on the covalent NA inactivators, 2,3-difluoro sialic acids (DFSA) synthesized by Wennekes and colleagues1, we modified DFSA with an azide mini-tag to converted it into a covalent probe. In this study, we chemically synthesized 5*N*-azidoacetyl-2,3- difluoro sialic acid as a covalent probe. The reactivation assay had shown the probe binding to NA without cleavage in 6 hours. The NA protein inhibited by the DFSA probe could be labeled with a biotin reporter via the CuAAC reaction, which also proved the covalent binding of the probe to the NA protein. Besides, the probe also kept an inhibition activity on NA with an IC50 value from 50 to 60 µM. With these properties, we expect the DFSA probes to be promising tools in labeling, visualizing, and mobilizing NA proteins and Influenza virus particles.

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Investigation of anomeric selectivity in NIS/TfOH promoted glycosylation reactions

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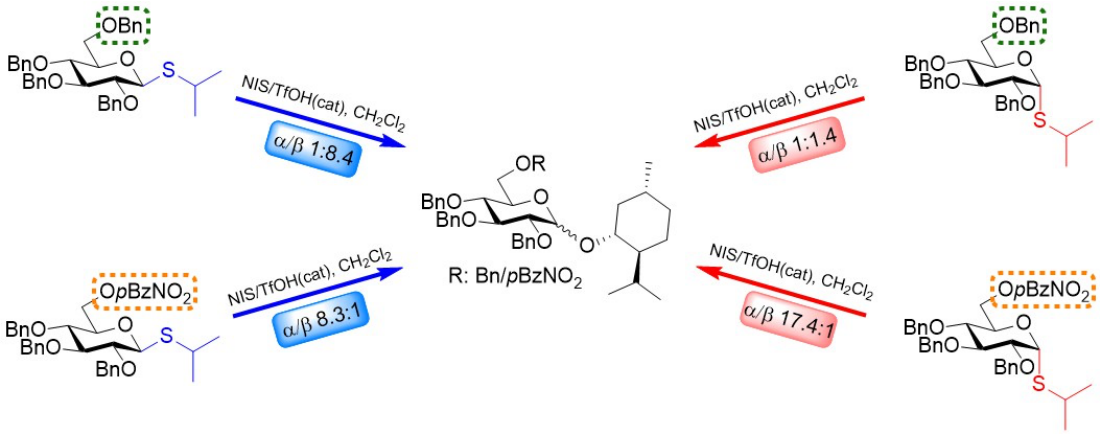
The reaction of a series of anomeric thioglycosides with various glycosyl acceptors and *N*-iodosuccinimide/triflic acid has been investigated with respect to reactivity and anomeric selectivity.[1] In general, beta-configured donors were found to give a more beta-selective reaction outcome compared to their alpha-configured counterparts.

Furthermore, a systematic study of various 6-*O*-acyls effect on anomeric selectivity in glucosylations with thioglucoside donors was conducted.[2] All eight different esters were found to induce moderate-to-high alpha-selectivity in glucosylation with L-Menthol as acceptor, with the best being 6-*O*-*p*-nitrobenzoyl. The alpha-directing effect was found to be general across various glucosyl acceptors, glucosyl donors, and modes of activation. No evidence was found in favor of distal participation.



Acknowledgements

We are grateful to Independent Research Fund Denmark and the Novo Nordisk Foundation for financial support.

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Pharmacological chaperone therapy treat krabbe disease

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Krabbe disease (KD) is a rare and fatal neurodegenerative lysosomal storage disorder (LSD) caused by a deficiency of the lysosomal enzyme β-galactosylceramidase (GALC).[1] As a consequence the glycolipids galactosylceramides and psychosine accumulates (**Figure 1A**).[2] A deficiency of this enzyme leads to the build-up of galactosylceramide and then, through the action of acid ceramidase (AC), leads to the accumulation of psychosine, also a substrate of GALC. This psychosine metabolite is cytotoxic to neuronal cells, which give rise to KD.[2] Unfortunately, there is no cure for this neurodegenerative disease, ultimately leading to death within the first two years of life.

Pharmacological chaperone therapy (**Figure 1B**) is an innovative approach to treating protein misfolded diseases like KD. This therapy uses small molecules to act as molecular scaffolds to help stabilise and therefore rescue partially defective enzymes from premature degradation by the cell's quality control process.[3] Counterintuitively, competitive inhibitors are often utilised as pharmacological chaperones, to selectively bind to the active site of the mutated enzyme, facilitating its folding and proper trafficking.[1] Once at its site of utility, the pharmacological chaperone is displaced, and the mutated enzyme can break down the accumulated substrates. This presentation will cover the design and synthesis of new carbohydrate-based compounds that can act as pharmacological chaperone for the treatment of KD.

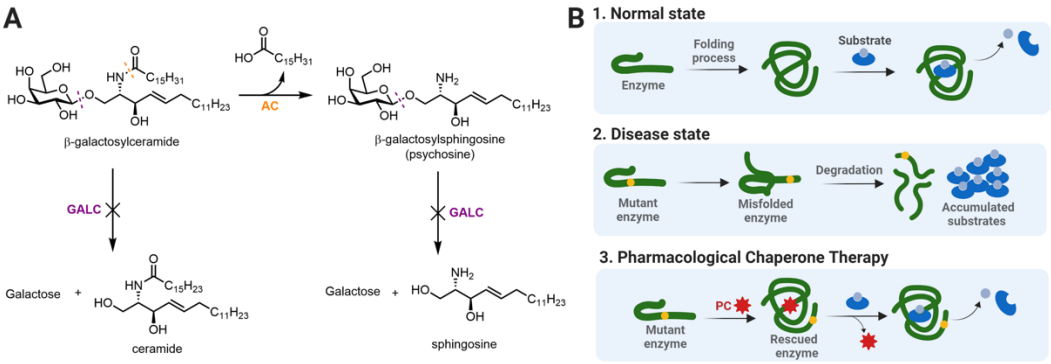

Figure 1: A) Enzymatic reaction of the hydrolysis of β-galactosylceramide by AC. B) Schematic representation of the proposed mechanism of PC action.

Acknowledgements

We would like to acknowledge the support of Research for Life for funding this travel and the Ministry of Business Innovation and employment for funding this research.

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3. J. Q. Fan (2008), Biol Chem (389), 1, 1-11.

Synthesis of open-chain carbohydrate amides, computational and bioactivity studies

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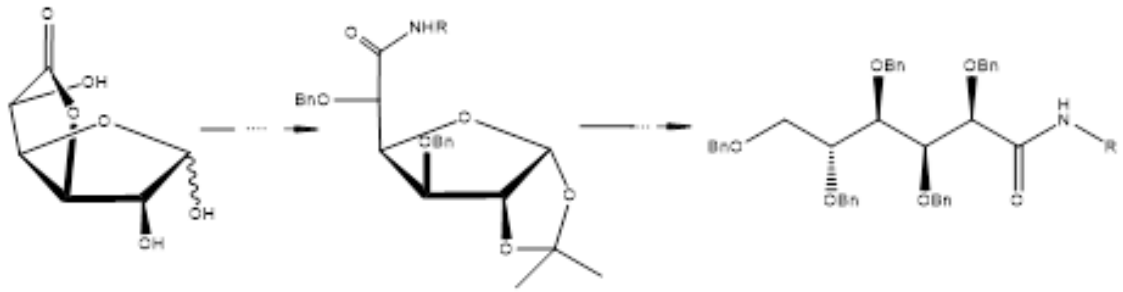
Alzheimer\'s disease (AD) is a severe neurodegenerative disorder characterized by progressive dementia with memory and cognition loss, ultimately leading to death [1,2]. It is considered an urgent public health problem [3,4], being the third leading cause of death after cancer and heart disease [2]. According to the 2020 update of the World Alzheimer Report 2015 [5,6], there are over 50 million people worldwide living with dementia. This number will almost double every 20 years, reaching 152 million in 2050. AChE inhibitors are the mainstay drugs for early disease stages [1].

Recently, we reported the synthesis of open-chain sugar amides which have some potential as AChE inhibitors [7]. Computational studies have shown that aromatic groups and the H-bond donor and acceptor groups, together with the molecule flexibility of these open-chain sugars, are responsible for their activity as the hydrophobic and H-bond interactions with the residues at the AChE gorge are mandatory for recognition and/or to block the action of the catalytic triad, and thus for the inhibitory activity. In this communication, we report the development of a new synthetic route to yield open-chain sugar amides from the commercially available glucuronolactone. The amide sugar derivatives were obtained in good yield (70 – 90%) (Scheme 1). The evaluation of acetylcholinesterase inhibition was carried out and the results will be disclosed.



Fundação para a Ciência e Tecnologia is acknoweldged for supporting Centro de Quimica Estrutural through the project UIBD/00100/2020 and Institute of Molecular Sciences through project LA/P/0056/2020

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New glycolipid synthase for alpha-galactosylceramide synthesis

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In an effort to achieve sustainable and efficient synthetic routes to glycoconjugates, glycobioengineering has focused to find out the biosynthetic catalysts present in nature, redesign and apply these enzymes in *in vitro* or/and *in vivo* biotechnological platforms.

Glycolipids are complex molecules that play important roles in cellular processes, and one such molecule, the glycosphingolipid alpha-galactosylceramide, has been found to have immunostimulatory properties, making it of interest in biomedicine [1,2]. Most glycolipids present beta glycosidic linkage but for clinical applications, the alpha configuration is especially important. To synthesize this molecule, we look for ceramide glycosyltransferase enzymes in natural bacterial producers [3,4].

Several GT4 enzymes from *Bacteroides fragilis* were evaluated, but only one, BF9343_3149, exhibited glycolipid synthase activity [5,6]. This non-processive glycosyltransferase prefers UDP-Gal as a donor substrate, and its maximum activity was observed at pH 7.3 and around 30-35°C [6]. Unlike other GT4 enzymes, it does not require metal cations for activity, but Zn^{2+} can inactivate it. The enzyme works best when the ceramide lipid acceptor is solubilized with BSA, but not in mixed micelles, and the presence of anionic lipids does not increase activity, as in other membrane-associated glycolipid synthases [6].

Acknowledgements

Work supported by MINECO grants BFU2016-77427-C2-1-R (GLYCO2ZYMES). M. Caballé acknowledges a predoctoral contract fromAGAUR, Generalitat de Catalunya

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A novel enzyme collection as a potent tool for bacterial biofilm exopolysaccharides degradation

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Bacterial biofilm infections represent a major threat in medical care with conventional therapies failing due to low penetration of antibiotics into the biofilm. Biofilms are bacteria aggregates surrounded by a polysaccharide rich extracellular matrix. As such, enzymatic polysaccharide degradation is a potential strategy to enhance antibiotic biofilm penetration and bacteria eradication. Thus, a library of 135 carbohydrate active enzymes (CAZymes) with the potential to digest the main exopolysaccharides produced by *Staphylococcus aureus* (PNAG) and *Pseudomonas aeruginosa* (Pel, Psl and alginate) was explored [1,2].

The genes encoding the selected potential CAZymes were chemically synthesized, cloned, expressed and purified in a high-throughput platform. The enzymatic activity of the purified proteins was tested using chromogenic substrates and biofilm biomass evaluation.

All expressed enzymes were soluble and 30 presented high production yields. Four enzymes were able to efficiently depolymerize PNAG, Psl, Pel and alginate, respectively. One alginate lyase, in particular, was able to reduce the biofilm biomass of a mucoid *P. aeruginosa* strain by 70%, after 6.5h of incubation. Two family 20 glycoside hydrolases were also able to promote a 50-60% biofilm formation inhibition on a *S. aureus*strain after 6h of incubation. These enzymes will be further tested in combination with antibiotics to evaluate their synergistic potential towards bacterial elimination. Overall, this study allowed the identification of novel enzymes capable of *P. aeruginosa*and *S. aureus* biofilm degradation.

We acknowledge FCT, through the grants: UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); and 2022.07903.PTDC. We also acknowledge ANI through the grant LISBOA-01-0247-FEDER-047033 [GlycoMed] and the Gilead GÉNESE program through the project 17805.

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Production of lacto-*N*-biose I using the extract of bifidobacterial cells

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Lacto-*N*-biose I (LNB) is the building block of the major type I oligosaccharides contained in human milk oligosaccharides. Bifidobacteria, particularly those present in the intestine of infant, have a unique metabolic system that selectively and efficiently utilizes type I oligosaccharides to increase the intestinal occupancy in breast-fed infants. Furthermore, the bifidobacteria-promoting effect of LNB has drawn attention as a prebiotic, hence, its use as a food ingredient has expected.

We succeeded in producing 200 g/L of LNB using sucrose and *N*-acetylglucosamine in a multi-enzyme reaction that takes advantage of the mechanism by which bifidobacteria utilize LNB to produce LNB. However, the commercialization of LNB production has not progressed due to the low consumer acceptance of recombinant enzymes prepared from genetically modified *E. coli*. Therefore, we developed a method to produce LNB using wild-type enzymes present in the bifidobacterial extract.

In addition to the four enzymes necessary for LNB production, the bifidobacteria extract contains other components. Among these, adenosine triphosphate, phosphoglucomutase, fructose 6-phosphate phosphoketolase, and glycogen phosphorylase are the inhibitors of LNB production. Therefore, we investigated methods such as membrane filtration, pancreatin treatment, and glucoamylase treatment to remove or suppress these inhibitors. Thereafter, all treated extracts were used for LNB production. The LNB concentration reached up to 288 mM in a 100 mL reaction, a 15-fold increase in LNB productivity compared with untreated extracts.

Acknowledgements

The authors appreciate Morinaga Milk Industry Co., Ltd. for the donation of the cells of Bifidobacterium species containing the enzymes.

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Exploration of human gut microbiota enzymes targeting glycosaminoglycans

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The human gut microbiota is a complex community of microorganisms that inhabit the gastro intestinal tract and play major roles in our health. In particular, specific gut bacteria degrade the complex polysaccharides present at the surface of the epithelium, among which glycosaminoglycans (GAGs).

The catabolism of GAGs is an important process that leads to the production of short-chain fatty acids, which contribute locally to the health of colonocytes and also have systemic beneficial effects, especially on the central nervous system. To efficiently degrade GAGs, gut bacteria rely on Carbohydrate-Active enZymes (CAZymes) with complementary activities to completely break down the polysaccharides. These enzymes, primarily Glycoside Hydrolases and Polysaccharide Lyases, are classified in the CAZy database [1].

In the previous years, several new families of Polysaccharide Lyases have been identified which exhibited activities on GAGs [2]. Here, we present the characterization of several enzymes belonging to family PL35 and originating from gut bacteria, with specificities towards a variety of glycosaminoglycans.

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The Myrosinase-Glucosinolate reaction as a bioconjugational tool to prepare neoglycoproteins

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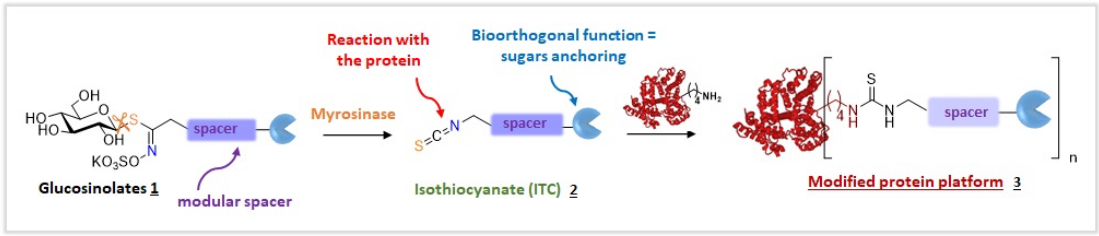
Multivalency is widely observed in Nature in glycan-proteins recognition to counterbalance the rather weak association constants of carbohydrate with their receptors (lectins for example). This strategy has been exploited by chemists to design and synthesize novel efficient carbohydrate-based ligands. Indeed, multivalent presentation of the ligands usually leads to significant enhancements in terms of affinity and selectivity and also induces specific supramolecular arrangement on the cell surface which may be critical for recognition.[1]

The central platform, from which multiple carbohydrate elements are displayed, influences the size and the shape of the multivalent ligand. Proteins, which have the advantages of being water-soluble, are commonly used as carriers for the multivalent presentation of glycans with valencies around 15 to 20. The resulting (semi)synthetic ligands are called *neo*glycoproteins, with respect to naturally occurring glycoproteins, and have been used for many years as probes for carbohydrate-proteins interactions.[2] We have recently developed a safe and biocompatible isothiocyanate-based conjugation process that relies on the *in situ* enzymatic generation of the reactive isothiocyanate species **2** from stable water-soluble synthetic glucosinolates precursors **1** by action of a highly specific β-thioglucoside hydrolase, namely myrosinase.[3]

The functionalized proteins **3** can then serve as platforms to anchor well-defined monosaccharides or synthetic oligosaccharides fragments to build high valency *neo*glycoproteins with a specific macromolecular architecture.[4] Thus, we would like to present here our recent results around the chemical modification of native proteins using an enzymatically triggered bioconjugation process.



Functionalization of native proteins using the myrosinase-glucosinolate reaction

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Structural heterogeneity in *Streptococcus suis* serotype 9 capsular polysaccharide

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Streptococcus suis, an encapsulated bacterium, is an important swine pathogen and zoonotic agent. The capsular polysaccharide (CPS) of serotype 9 contains D-Gal, L-Rha, Glc-ol6*P*, and a labile keto sugar, D-6d*xyl*HexNAc-4-ulo [1]. The function of genes responsible for its biosynthesis by the Wzy pathway has been putatively assigned [1].

The CPS obtained from Ss2to9, a serotype-switched mutant from a serotype 2 strain that expresses the serotype 9 CPS, had different ^{1}H NMR data than published initially for *S. suis* serotype 9 [1,2]. First, doubling of signals was observed in the anomeric region, attributed to the *ribo*isomer of 2-acetamido-2,6-dideoxyhexos-4-ulose (Sug) formed by keto-enol tautomerization during the purification procedure. Second, signals corresponding to one residue were shifted from their original position, and indeed glucose instead of galactose was found in the side chain of the mutant CPS.

Molecular models of oligosaccharides comprising either the *xylo* or the *ribo* isomer of the keto sugar were constructed to support the structure of the new isomer and explain its formation.

Because the donor strain for the construction of the mutant was different from the strain used for CPS structure determination, glycosyltransferase genes in the different *S. suis* serotype 9 strains were examined to find mutations potentially responsible for the replacement of Gal by Glc.

Finally, a comparison was made with various CPS structures having a single Gal/Glc difference in serotypes of *Streptococcus pneumoniae*within the same serogroup, where their genetic basis has been examined.

$$\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-Glc-ol-}(6\rightarrow P\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Sug}p\text{-}(1\rightarrow \\ \alpha\text{-D-Glc}p\text{-}(1\rightarrow 2)\lrcorner$$

Ss2to9 mutant CPS repeating unit structure

Acknowledgements

This research was supported by the International Development Research Centre (IDRC) and GlycoNet.

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A building block tetrasaccharide as precursor to the RUs of prevalent *Shigella flexneri* serotypes

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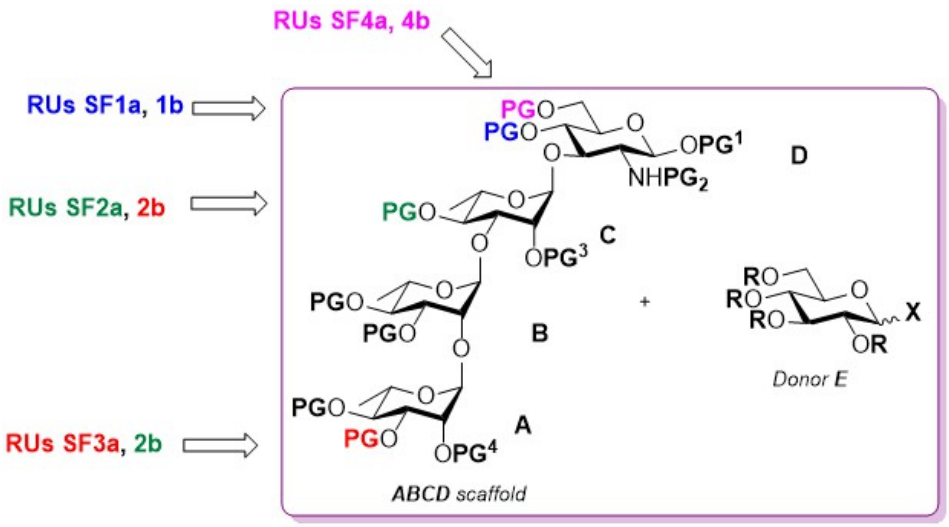
S. flexneri, a Gram-negative enterobacterium, is the main causative agent of the endemic form of shigellosis, a diarrheal disease of high prevalence in children under five in developing countries and a prime target for vaccine development. The O-antigen (O-Ag) part of the bacterial lipopolysaccharide (LPS) is considered to be the major target of the immune response against reinfection. Most *S. flexneri* serotypes exhibit closely related O-Ags. They share a common backbone featuring a unique repeating unit (RU) made of a *N*-acetyl-D-glucosamine and three L-rhamnose residues (**ABCD**) linked to one another by 1,2-*trans* glycosidic linkages. Structural diversity reflecting serotype specificity derives from the site-selective O-acetylation and α-D-glucosylation of the **ABCD** tetrasaccharide.

The identification of oligosaccharides acting as functional mimics of the O-Ags characterizing the most predominant *S. flexneri* serotypes (1a, 1b, 2a, 2b and 3a) is of interest as part of ongoing development toward a broad coverage synthetic carbohydrate-based *Shigella* vaccine. Going beyond original achievement, this communication reports an original concept whereby key pentasaccharide building blocks featuring serotype-specific substitutions are built from a single orthogonally protected tetrasaccharide. A three-step strategy was implemented : 1) synthesis of a fine-tuned core **ABCD** building block featuring suitable orthogonal protecting groups at all substitution sites ; 2) selective unmasking, on demand, of selected hydroxyl groups to provide ready-for-modification well-designated tetrasaccharide acceptors ; 3) controlled 1,2-*cis*chemical glucosylation of the resulting **ABCD** acceptors to provide fully protected pentasaccharides representative of the RUs of the selected *S. flexneri* O-Ags. Chemical chain elongation at either end post α-D-glucosylation will generate the required panel of serotype-specific oligosaccharides as potential haptens for vaccine design against shigellosis.



Coupling of Surface Plasmon Resonance with Mass Spectrometry to study protein glycosylation

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Lectins are widely appreciated, in the fields of hematology and immunology, due to their specific binding to carbohydrate structures. Lectins are commonly present on the cell surface or elsewhere; they are also known as specific markers for membrane glycoprotein structures. This study is focused on the use of Jacalin lectin (Artocarpus integrifolia) (AIA), which belongs to the family of galactose-binding lectins and is used here as a model of lectin. The lack of sensitivity in MS detection hinders the development of approaches using Surface Plasmon Resonance (SPR) coupled with Mass Spectrometry (MS). Among the factors affecting sensitivity, the amount of ligands retained on the biochip surface is crucial [1]. Thanks to their smaller size and multimerism, lectins offer an attractive receptor alternative to usual antibodies for increasing ligand capture, which is particularly interesting in glycoconjugates analysis.

Our project aims to develop a multiplex SPR biochip with immobilized lectins to afford the coupling between SPR Imaging and MS (SPR_i-MS) to determine protein glycosylation and further study of unknown lectins and their interactions with *N*-glycans. This coupling allows the real-time monitoring of interactions and the determination of their kinetics and thermodynamics parameters, together with the structural identification of the glycoconjugates captured from a complex mixture [1], [2], [3].

Fetuin is a plasma glycoprotein containing *N*-acetylglucosamine (GlcNac) and *N*-acetylgalactosamine (GalNac) in its structure, which is commonly used as a model for *N*- and *O*-glycans analysis. Our SPR_i analysis confirmed significant interactions between AIA and fetuin. The sensorgrams showed considerable variations in reflectivity accompanied by the lighting of the spots upon the injection of increasing concentrations of fetuin. Detection of the captured fetuin was successfully achieved by MALDI-TOF MS directly on the lectin biochip.

Moreover, a protocol for the on-chip spraying of the MALDI matrix is currently in development in the laboratory. This mode of matrix deposit will be combined with a direct on-chip MALDI-TOF MS imaging (MS_i) experiment to evaluate the impact on both the sensitivity and the selectivity of detection of fetuin by a SPR_i-MS_i approach.

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Effects of sialylation on human serum AAG-drug interactions assessed by ITC

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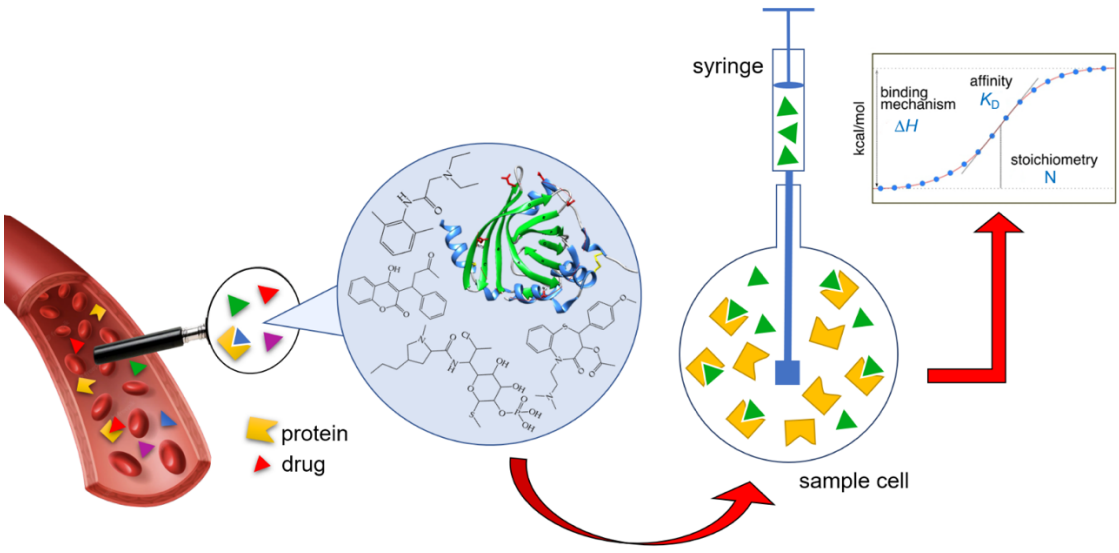
Human serum alpha-1 acid glycoprotein is an acute-phase plasma protein involved in the binding and transport of many drugs, especially basic and lipophilic substances. It has been reported that the sialic acid groups that terminate the N–glycan chains of alpha-1 acid glycoprotein change in response to certain health conditions and may have a major impact on drug binding to alpha-1 acid glycoprotein. The interaction between native or desialylated alpha-1 acid glycoprotein and four representative drugs-clindamycin, diltiazem, lidocaine, and warfarin-was quantitatively evaluated using isothermal titration calorimetry. The calorimetry assay used here is a convenient and widely used approach to directly measure the amount of heat released or absorbed during the association processes of biomolecules in solution and to quantitatively estimate the thermodynamics of the interaction.

The results showed that the binding of drugs with alpha-1 acid glycoprotein were enthalpy-driven exothermic interactions, and the binding affinity was in the range of 10^{-5} – 10^{-6} M. Desialylated alpha-1 acid glycoprotein showed significantly different binding with diltiazem, lidocaine, and warfarin compared with native AAG, whereas clindamycin showed no significant difference. Therefore, different degree of sialylation may result in different binding affinities, and the clinical significance of changes in sialylation or glycosylation of alpha-1 acid glycoprotein in general should not be neglected.



Potential clinical significance of protein sialylation on drug binding could contribute to the development of personalized medicine.

Synthesis of diversely *O*-acetylated segments from the *Shigella flexneri* 6 surface polysaccharide

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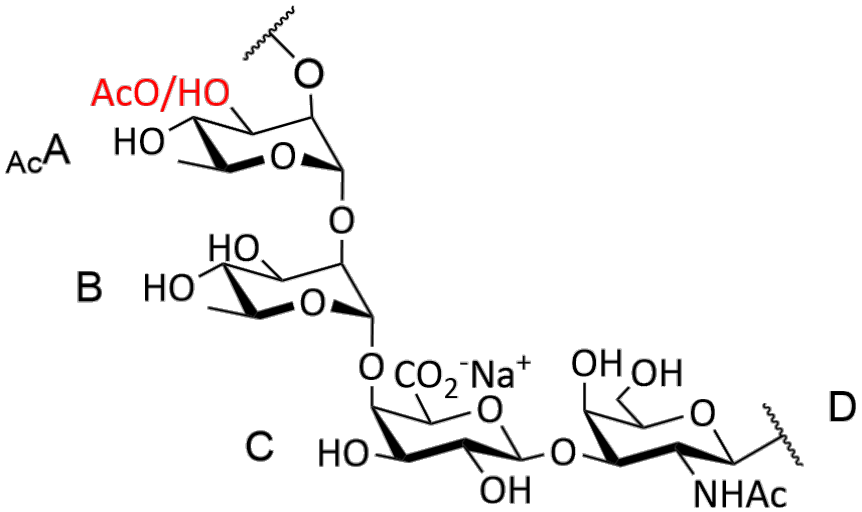
Shigella are gram-negative enteroinvasive bacteria causing shigellosis, a major diarrheal disease, especially in low and middle income countries. Shigellosis is responsible for a high burden and is a prime cause of mortality due to diarrhea in young children.[1]

The O-antigen (O-Ag) part of the *Shigella* lipopolysaccharide (LPS) is a major target of protection induced by natural infection. Numerous *Shigella* vaccine candidates aimed at inducing an immune response against the LPS were proposed. In this context, our group has investigated synthetic O-Ag surrogates as alternatives to antigens purified from biological extracts. A *S. flexneri* 2a synthetic glycan-based vaccine candidate was found immunogenic in a first-in-human phase 1 clinical trial.[2]

Focus is here on the identification of surrogates of the O-Ag from *S. flexneri* 6 (SF6), another prevalent serotype.[3] The SF6 O-Ag is defined by a partially *O*-acetylated linear tetrasaccharide repeat ($_{Ac}$ABCD).[4]

The strategy to ready-for-conjugation oligosaccharides (up to 4 repeats) acetylated in a non-stoichiometric fashion at OH-3_A is described. Emphasis is on the design and multi-step synthesis of tetrasaccharide blocks obtained in multi-gram amounts. Their combination and chemical diversification into sets of oligomers is discussed. Conditions enabling the final deprotection of the fully protected intermediates into fine-tuned linker-equipped SF6 O-Ag segments are presented.

These oligosaccharides will serve as molecular probes to decipher the importance of chain length and *O*-acetylation for O-Ag functional mimicry.



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Linker, loading, reaction scale - influence on automated glycan assembly

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Automated glycan assembly (AGA) allows for fast synthesis of well-defined oligo- and polysaccharides.[1] Recently, the implementation of new synthetic strategies[2] as well as technological improvements[3] permitted access to highly complex carbohydrates.[4] Still, variations in yields are not always ascribable to the AGA process, with structures assembled in high purity, but isolated in relatively low yields. Herein we analyzed how parameters connected to the solid support (i.e. linker type, resin loading, reaction scale) affect the productivity of AGA (Fig.1). While loading and reaction scale did not significantly influence the AGA outcome, the chemical nature of the linker dramatically altered the isolated yields. This systematic study identified that the major determinants of AGA yields are cleavage from the solid support and post-AGA purification steps. Future efforts need to focus on the development of new linkers and the implementation of post-AGA manipulation steps on resin.

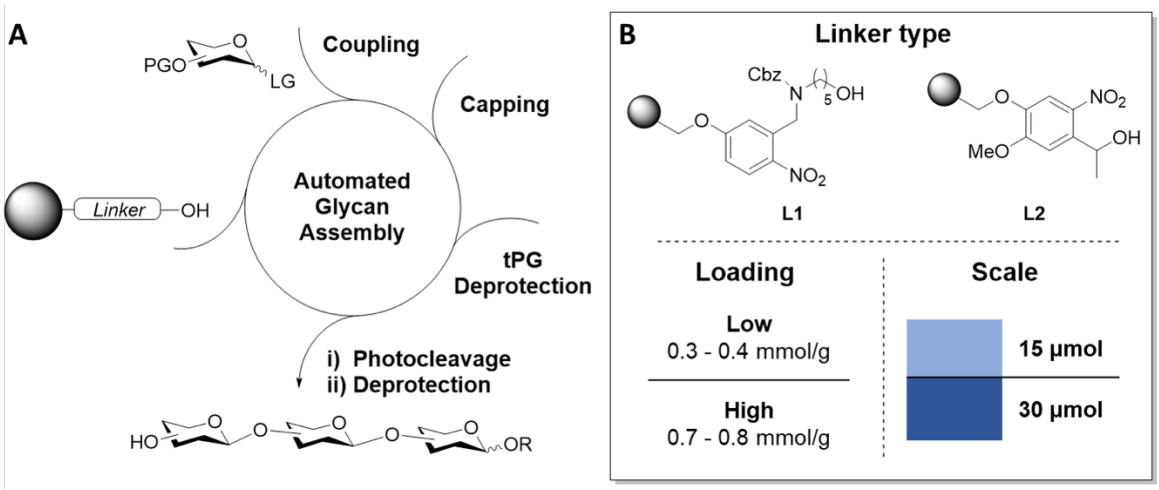

Figure 1. Schematic representation of the AGA process (A). Variables that can affect the AGA outcome

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Structure and properties of bacterial envelope components

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Human pathogens, including viruses and bacteria, have evolved their ability to activate or evade the host immune response by using the glycans of their envelopes. Bacterial lipopolysaccharides (LPSs) are the main components of the outer membranes of Gram-negative bacteria, they have an important role in the activation of the immune response as they act as microbe-associated molecular patterns" (MAMPs). They are microbes that mimic host glycans, to engage immune proteins and evade the host immune response, successfully promoting bacterial colonization and tolerance.[1] Some bacteria decorate their cell envelope with sialic acids, a group of 9-carbon backbone nonulosonic acids, found primarily at the termini of glycans displayed on vertebrate cell surfaces and therefore ideal candidates to be used as "self-associated molecular patterns" (SAMPs) for self/non-self discrimination by the host immune system. Siglecs (sialic acid-binding immunoglobulin-like lectins) are immune proteins with the ability to preferentially recognize and bind exposed sialylated glycans on cell surfaces, acting as regulators of a variety of critical cellular mechanisms.[2,3]

I will here present the structure and activity of bacteria envelope glycans (*Odoribacter splanchnicus*, *Acinetobacter baumannii…)* involved in the host immune recognition. Understanding the role of Glycans and glycosylation in immunity is critical to understanding the etiology and progression of immune-related diseases. Therefore, the structure and function of the bacterial envelope components will be evaluated.[4]

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A short synthetic approach towards isoiminosugars

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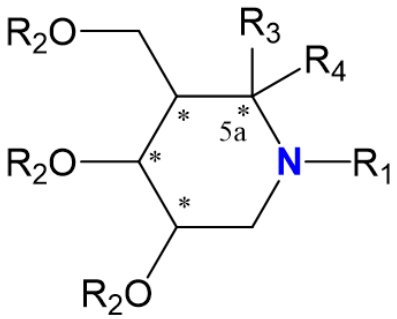
Isoiminosugars (**A**) are sugar analogues in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group. In general, these compounds are selective and highly potent inhibitors of their corresponding β-glycosidases. Moreover, selected *C*-5a-chain branched derivatives of this compound class, e.g. *C*-5a-chain extended derivatives of 4-*epi*-isofagomie (**1**) have been proven as highly potential pharmacological chaperones for the treatment of GM1 gangliosidosis [1,2], clearly rivalling any chemical chaperon published to date. As a matter of fact, the indicated structural characteristics remain to challenge the synthesis of isoiminosugars (**A**). However, valuable synthetic strategies towards this compound class have been reported [3-6].

In context with our interest in the advanced design and synthesis of such structures, we have found a novel, efficient and concise synthetic approach towards isoiminosugars (**A**). This method can be applied on different configurations and allows variations in the reaction sequence, opening the avenue to various modifications in the substitution pattern. Herein, synthetic details as well as the scope and limitations of this approach will be presented.

ISOIMINOSUGARS (**A**)



4-*epi*-isofagomine (**1**):
D-*galacto* configurated,
$R_1 = R_2 = R_3 = R_4 = H$

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[1] S. Front, O.R. Martin, et al. (2017), Eur. J. of Med. Chem. (126) 160-170.
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Chemoselective enzymatic deacetylation of protected phenylpropanoid saccharides

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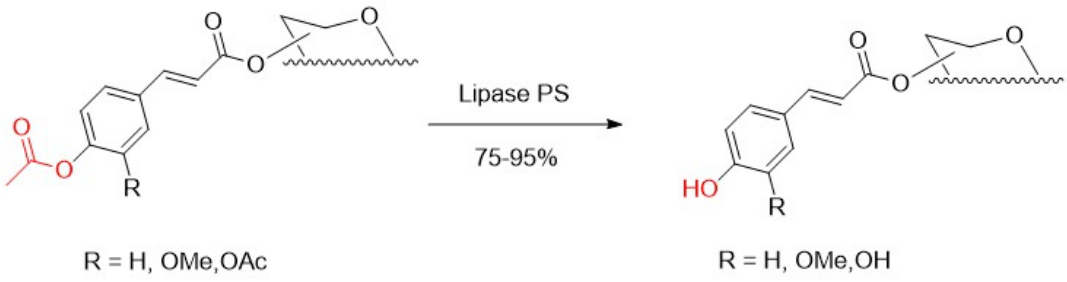
Glycophenolics represent a class of naturally occurring compounds with a wide range of biological activities, especially their antioxidant activity, from which they develop secondary anti-inflammatory, antiviral, cardioprotective, neuroprotective and anticancer properties [1]. These glycophenolics are divided into several subcategories, from which one of them are phenylpropanoid esters of carbohydrates, which consist of saccharide moiety decorated with hydroxycinnamic acids. Chemical synthesis of these compounds usually involves the use of acetyl protecting group on saccharides and on phenolic hydroxyls as well. However, free phenolic hydroxyl is the key factor in antioxidant activity of these glycophenolics and to evaluate biological activities of synthetic analouges of these natural products, chemoselective deacetylation of phenolic hydroxyls is essential. Common chemical methods of deacetylation usually lead to several different side products, and it is difficult to achieve desired chemoselectivity.

During our work on the synthesis of substrates for feruloyl esterases, we found that Lipase PS (lipase from *Burkholderia cepacia*) selectively deacetylates esters of 4-*O*-acetylferulic acid [2]. Therefore, we investigated the possibility of enzymatic deacetylation of protected phenylpropanoid saccharides with Lipase PS to deacetylate phenolic hydroxyls. Reaction proceeded in two-phase system at neutral pH and with only mild heating (37 °C). We achieved excellent chemoselectivity towards phenolic acetates with yields 75-95%, while other ester bonds remained intact.



Acknowledgements

Grant Agency for Science VEGA (grant 2/0111/22), COST Action CA18103 (INNOGLY), supported by COST (European Cooperation in Science and Technology)

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From glycals to carbohydrate-tethered 2,5-disubstituted pyrazines as potential hypoglycemic agents

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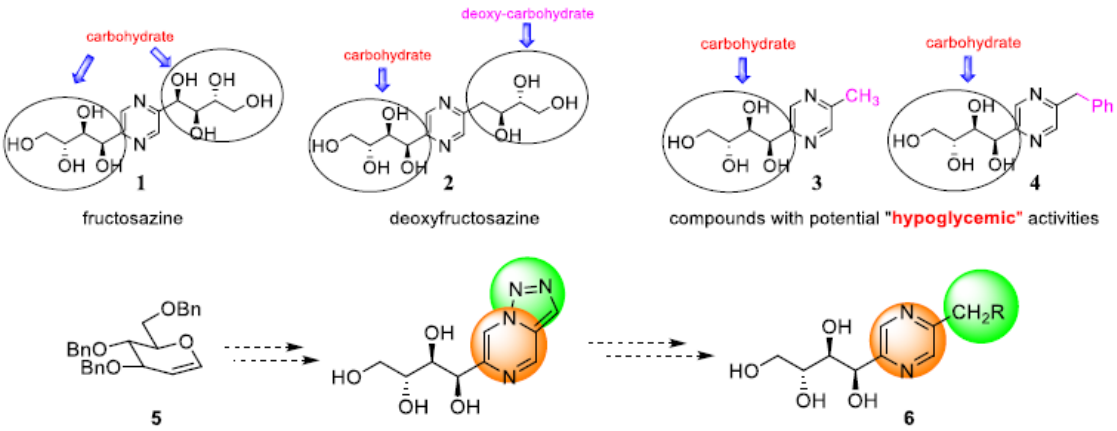
Fructosazine (FZ) **1** and deoxyfructosazine (DOF) **2** belong to the class of molecules containing a pyrazine structure tethered to a sugar in its open chain form and are very useful chemicals with varied applications, such as flavours in tobacco industry, treatment and prevention of diabetes (type II), resistance of cancers, treatment of immunological and inflammatory disease, reagents for DNA strand cleavage. Certain derivatives of pyrazines such as **3** and **4** have been identified as molecules with potential hypoglycemic activities. Though quite a few procedures are available for the synthesis of FZ and DOF, synthetic methods for making derivatives such as **3** and **4** are limited. To our knowledge, there is only one synthetic route available which involves the deoxygenation of the corresponding *N*-oxides. As of now, no synthetic strategy that provides access to a library of 2,5-disubstituted fructosazine containing a sugar moiety is available in literature.

Recently we have developed a practical access to the synthesis of a variety of carbohydrate-tethered 2,5-disubstututed pyrazines **6** through a sequence of novel organic transformations starting from readily available 3,4,6-tri-*O*-benzyl-D-glucal **5**. The details of the work will be presented.



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Swainsonine and mannostation based analogues: targeting selective inhibition of Golgi α-mannosidase

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An obligatory step in the formation of complex *N*-glycans involves the removal of two mannosyl linkages in $GlcNAcMan_5GlcNAc_2$.[1] Golgi *α*-mannosidase (GMII) is a glycoside hydrolase that catalyses the removal of terminal *α*-1,3- and 1,6-linked mannoses from $GlcNAcMan_5GlcNAc_2$.[1] Dysregulated synthesis or alteration of *N*-glycans is often linked to the manifestation of various diseases.[2]

Therefore, GMII and its inhibition, is a promising candidate for the development of various therapeutics such as anticancer drugs. Swainsonine is a highly characterised inhibitor of GMII and can be used during the treatment of various cancers.[3] Nevertheless, swainsonine administration can elicit undesirable side effects such as mannosidosis due to its off-target inhibition of lysosomal *α*-mannosidase.[4]

As a result, there is a pressing need to find potent, yet selective, inhibitors towards GMII. Discussed herein, are the structural and biochemical based findings for the potential inhibition of GMII by employing a range of swainsonine and mannostatin based analogues.

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β-Galactosidase BgaD isoforms: comparison of the structure and functions

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β-Galactosidases catalyze the cleavage of lactose, but they can also use lactose as a glycosyl donor and acceptor and synthesize galactooligosaccharides. β-Galactosidase from *B. circulans* ATCC 31382 (BgaD) is unique due to its high synthetic potential. Four isoforms of this enzyme are formed by truncation of the C-terminal peptide by endogenous proteases [1]. The isoforms differ in size (A – 189 kDa, B- 155 kDa, C – 135 kDa, D – 92 kDa), structure, and transgalactosylation activity. In our previous study, recombinant β-galactosidase from *Bacillus circulans* isoform A (BgaD-A; EC 3.2.1.23) was produced and its synthetic potential was studied through mutagenesis [2]. To further explain the behavior, we crystallized the BgaD-A isoform. So far, only isoform D has been crystallized by Ishikawa et al [3]. Structural analysis of BgaD-A isoform by cryo-EM revealed new insights that are important for understanding the catalytic properties. The major difference between BgaD-A and BgaD-D is the flexible Big-4 domain, which is absent in BgaD-D. This domain is adjacent to the active site and hinders the binding of longer glycosyl substrates in BgaD-A. We confirmed this hypothesis by reactions with both isoforms under the same conditions, using lactose as donor and acceptor. BgaD-D produced higher amounts of more diverse mixtures of galactooligosacharides.

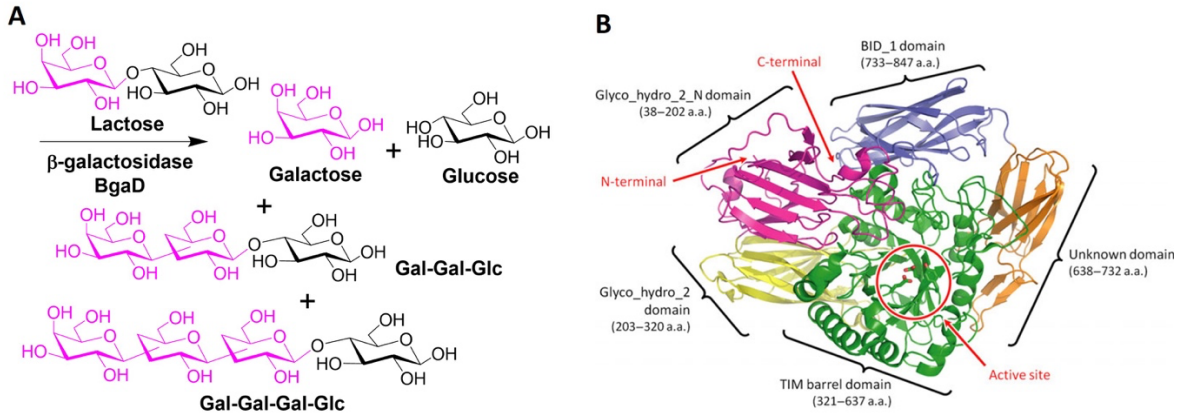

Figure. A. Synthesis of galactooligosacharides. B. Crystal structure of the β-galactosidase from B. circulans isoform D (BgaD-D), PDB code: 4YPJ [3].

Support from the grant projects 23-05146S and 22-00197K by the Czech Science Foundation is gratefully acknowledged.

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The role of substitution pattern on molecular structure and NMR parameters in sulphated saccharides

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Biological properties of sulphated saccharides are surprisingly large and include angiogenesis, anti-inflammatory, antiviral, anticoagulant properties, cell adhesion, growth and differentiation, etc. These manifold properties originate from the structural arrangement of these saccharide derivatives. The present contribution discusses applications of quantum chemical DFT methods, combined with NMR spectroscopy, for description of molecular properties of heparan sulphate, dermatan sulphate, chondroitin sulphate and iota-carrageenan. Theoretical methods have been used for examination of 3D molecular structure and NMR parameters taking into account explicit solvent molecules. The data showed that formation of a complex hydrogen bond network and strong ionic interactions influence the first hydration shell and play an important role in shaping the 3D saccharide molecules.

The DFT analysis of $^{3}J_{H–C–C–H}$ coupling constants indicated that the oxygen lone pairs of neighbouring oxygen atoms could significantly contribute to the magnitudes of coupling constants, mainly Fermi-contact terms. The computed $^{3}J_{H–C–C–H}$ data, together with theoretical analysis of molecular structure highlight the need of appropriate quantum-chemical calculations for detailed understanding NMR parameters as well as solution properties of sulphated oligosaccharides.

Acknowledgements

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N-glycan profiles of acid alpha-glucosidases produced in transgenic rice cell suspension cultures

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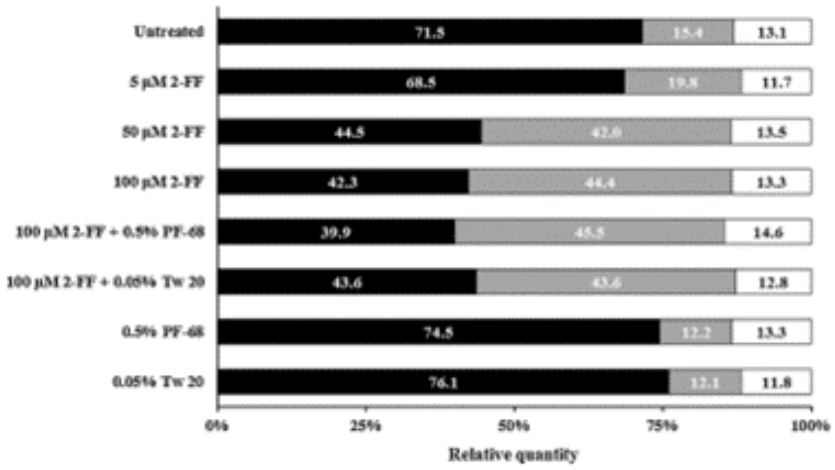
Recombinant human acid alpha-glucosidase (rhGAA) from Chinese hamster ovary cells is the only approved treatment for patients with Pompe disease.

In this study, rhGAAs were produced in transgenic rice cell suspension cultures under eight different conditions; untreated, 5 µM of 2-fluoro-L-fucose (2-FF), 50 µM of 2-FF, 100 µM of 2-FF, 100 µM of 2-FF + 0.5% Pluronic F-68 (PF-68), 100 µM of 2-FF + 0.05% Tween 20 (Tw 20), 0.5% PF-68, and 0.05% Tw 20. The *N*-glycans of eight rhGAAs were analyzed using liquid chromatography and tandem mass spectrometry. The relative quantity (%) of each glycan was obtained from the corresponding UPLC peak area per the sum (100%) of individual UPLC peak area. Fifteen *N*-glycans, comprising seven core-fucosylated glycans (71.5%, sum of each relative quantities) that have immunogenicity-inducing potential, three de-core-fucosylated glycans (15.4%), and five non-core-fucosylated glycans (13.1%), were characterized with high mass accuracy and glycan-generated fragment ions. The increases or decreases of relative quantities of each glycan from seven rhGAAs were compared with those of untreated control. These results indicate that the relative quantity of each glycan of rhGAA produced in rice cell suspension cultures is significantly affected by their culture condition.

This study performed the comparison of the *N*-glycan profiles of rice cell-derived rhGAA to identify the core-fucosylated glycans using tandem mass spectrometry and will provide useful insights for the development of plant-derived biotherapeutics focused on *N*-glycans.



The sum of relative quantities of the N-glycans of rhGAA.

New perspectives for aging research of an extremely long fertile life in a highly social termite

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[1] iEES-Paris UMR CNRS 7618 France, (9) INSERM Paris France, [2] University of Münster Germany, [3] LEEC France, [4] University of Rennes ECOBIO France; [5] UTC France; [6] University of California Berkeley USA, [7] University of Pretoria South Africa

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Aging is characterized by a progressive loss of physiological integrity, leading to impaired function such as reduction of reproductive performance and increased physiological vulnerability to death. Over recent years, aging research has discovered that the rate of aging is controlled by genetic pathways and biochemical processes conserved in evolution of many species. I will present our results obtained from a novel natural aging system, the long-lived and highly fertile *Macrotermes natalensis* termite queen that overcomes simultaneously several well-known hallmarks of aging while receiving a prolonging carbohydrate-rich diet.

Open royal chamber with one physogastric queen, one king, and several workers and soldiers of Macrotermes natalensis. Credit: M. Vasseur-Cognet

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Synthesis and biological evaluation of novel xylofuranosyl nucleoside phosphonates

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Nucleoside and nucleotide analogs are important group of molecules in medicinal chemistry owing to their ability to display a variety of biological activities. Their best-known therapeutic applications are in the field of anticancer and antiviral drug research, with several approved molecules approved as drugs acting as nucleic acid antimetabolites.[1,2] Their antimicrobial potential has also been reported in the literature, indicating that nucleos(t)ide-like structures may target various microbe cellular processes. and therefore be exploited towards new agents with unique mechanisms of action to circumvent antimicrobial resistance.[3]

Within our interest in the development of novel nucleos(t)ide analogs of potential therapeutic interest, [4,5] in this communication we report on the development of novel potentially bioactive nucleotide analogs based on an 3-*O*-dodecyl xylofuranyl unit and containing a phosphonate group. Motivation for their synthesis arose from the significant antiproliferative activities exhibited by previously reported related azido nucleosides from our group.[4] For their access, diacetone-D-glucose was used as starting material and key synthetic steps included sugar iodination, Arbuzov reaction or purine/pyrimidine N-glycosylation. The compounds were further studied for their antiproliferative effects on a panel of cancer cells and for their antibacterial activities on Gram-positive and negative bacterial pathogens.

Herein both the results of the synthetic work and those of the bioactivity screening will be disclosed.

Acknowledgements

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Specificity of a MurNAc/GlcNAc peptidoglycan deacetylase acting on chitooligosaccharides

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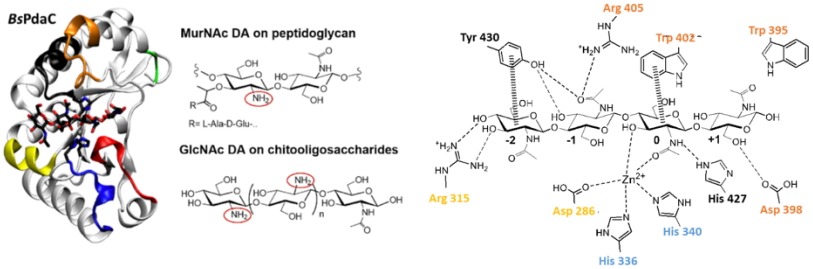
The bacterial cell wall peptidoglycan (PGN or murein) is an heteropolymer composed of a linear glycan chain of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) units linked by β-1,4 glycosidic bonds. Moreover, the lactyl group of the MurNAc units are substituted by short (stem) peptides that are crosslinked to form a mesh-like structure. In addition to variations in polymer length, many bacteria subsequently modify the sugar structures. One of the most common modification of the glycan backbone is the de-N-acetylation of either one or both sugars and pathogenic bacteria utilize those deacetylations to evade detection by the innate immune system. PGN deacetylases (PGN DAs) are members of family 4 carbohydrate esterases (CE4 enzymes) which operate by a metal-assisted general acid/base catalytic mechanism [1][2]. Whereas PGN GlcNAc DAs are specific for GlcNAc residues of the PGN glycan backbone and confer resistance to lysozyme hydrolysis, PGN MurNAc DAs are specific for MurNAc residues and are involved in sporulation. Both subfamilies have mutually exclusive specificities, but currently no sequence or structural signatures can be assigned to each enzyme class to predict specificity and function. PdaC from Bacillus subtilis was recently discovered as a novel MurNAc DA. It is unique in that it acts on intact PGN, it is not involved in sporulation and it is also active on chitooligosaccharide (COS), an activity that was thought to be restricted to GlcNAc deacetylases [3]. Moreover, BsPdaC shares higher sequence similarity with the peptidoglycan GlcNAc deacetylase SpPgdaA than with other MurNAc deacetylases.

Computational dockings with (GlcNAc)4 substrate confirmed the presence of two binding modes (BM) with equal energy which explain the experimental first deacetylation pattern at the two central GlcNAc units. The goal is to gain insight into the substrate specificity of BsPdaC by generating mutants in certain positions of the catalytic cleft and evaluate the changes in activity and specificity compared to the wild type enzyme. Thus, new information about the interactions of the residues surrounding the active site can be inferred, aimed at understanding the specificity of PGN DAs as therapeutic targets for the design of novel antimicrobials.



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Cu(I) catalyzed stereoselective synthesis of deoxy glycosides on electron-poor glycal systems

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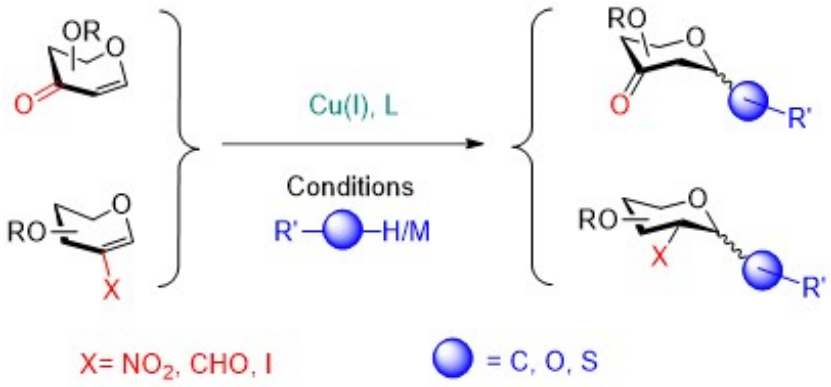
Deoxysugars play a crucial role in as key components of many natural products, such as glycoproteins, bacterial endotoxins, and secondary metabolites. Research into the functions of deoxysugars has revealed a fascinating array of diverse roles for these compounds.

Glycals have been identified as an effective starting material for synthesizing deoxyglycosides[1]., various direct glycosylation techniques have been developed that utilize glycals to produce 2-deoxy and 2-substituted-deoxy sugars. More recently modified glycals containing electron withdrawing groups, such as 2-deoxy nitro or 2-deoxy aldehyde or 2-deoxy sulfonyl or 3-keto moieties (scheme 1) have been identified as interesting scaffolds for the synthesis of glycoside analogues. To carry out glycosylation on these deactivated enol ether-containing systems, various methods have been explored, including lewis acid or base catalyzed glycosylation, transition metal catalysed or organocatalyzed glycosylations[2].

In this report, we describe a Cu(I)-catalyzed strategy that enables the use of modified electron-deficient glycal systems to generate C-, O-, and S-glycosylated products with moderate to high yields and high to complete stereoselectivity. The novel deoxy-analogues can be further modified upon removal of the electron withdrawing groups or their further functionalization to generate a range of naturally occurring glycans or glycomimetics that can be used to study biological pathways or even as potential drug candidates.



Graphical Abstract

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Photocleavable ABPs for a native enrichment assay

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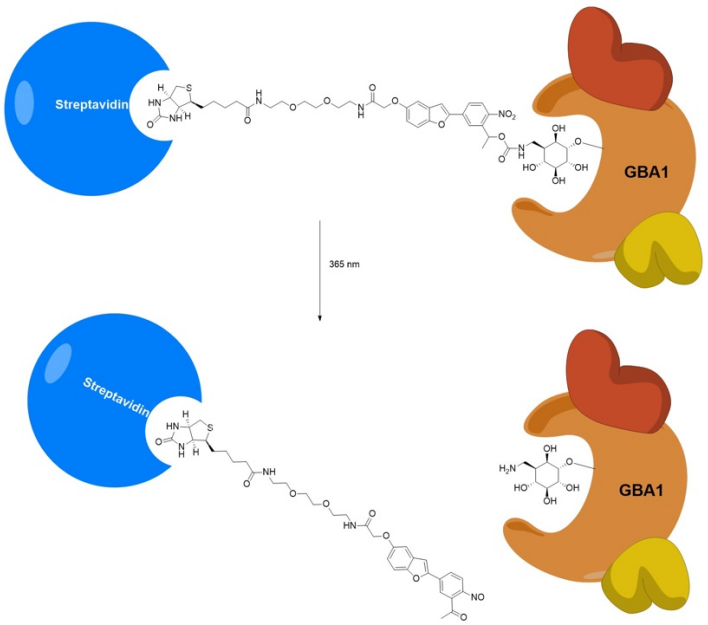
Lysosomal storage diseases Gaucher is caused by the accumulation of metabolite glucosylceramide in the lysosome due to impairment of its degrading enzyme Glucocerebrosidase. For a better understanding of the disease, the affected enzyme and its biological environment are investigated extensively. Identifying proteins that are interacting with the enzyme, for example activating or stabilizing it, could give insight in the enzyme's mechanisms and activated downstream pathways. In this work we aim to identify and characterize these interacting proteins via a native enrichment assay using photo-cleavable activity-based probes.



Photocleavable ABP bound to streptavidin-coated beads and GBA, before and after photocleavage.

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Neolectins, towards new tools for selective sugar targeting

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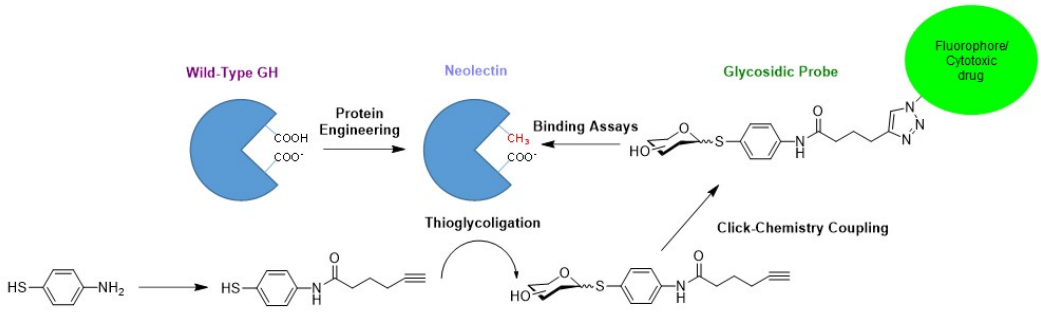
Lectins are carbohydrate-binding proteins specific for sugar molecules incorporated in glycosides or glycans at cell surface, playing a critical role in recognition processes at cellular and molecular levels. Engineering of lectins has been a challenge for many years. In our group, a synthetic lectin has recently been developed by engineering a galactofuranosidase, able to recognize and hydrolyse the galactofuranose entity, by removing its catalytic activity and retaining its sugar binding ability, resulting in a "neolectin".[1] Based on this result, we aim at developing a library of neolectins, by selecting glycoside hydrolase selective for targeted carbohydrate structures and turning them into corresponding "neolectin" by site-directed mutagenesis. However, to determine their binding affinity and sugar selectivity, a matching library of glycosides has to be synthesized to serve as chemical probes for *in-vitro* assays. In this context, an original biocatalytic approach has been chosen as the engineered neolectins are also thioglycoligase able to catalyze the formation of the corresponding *S*-glycosides.[2,3] Thus, the neolectins to be assayed as sugars receptors will also serve as biocatalysts to generate their own dedicated chemical probes. Several examples of chemo-enzymatic synthesis of such *S*-glycosides bearing either a fluorescent moiety or bioorthogonal functions for subsequent coupling will be presented.



Acknowledgements

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Niosomes functionalized with a synthetic carbohydrate binding agent for mannose-targeted doxorubicin

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Niosomes are self-assembled nanometer sized-vesicles, which represent a versatile drug delivery system (DDS) for several therapeutic applications.[1] Their excellent ability to encapsulate antitumoral drugs as well as the possibility to easily functionalize their surface, make these nanocarriers particularly suitable for the development of active targeted DDS in cancer therapy.

In the last decades, our research group has been involved in the development of synthetic carbohydrate binding agent (CBA), small-molecules selectively able to target saccharides of biological interest.[2] Recently, a diaminopyrrolic synthetic receptor has been developed, which is effectively able to recognize the mannosidic residues of high-mannose-type glycans, overexpressed on the surface of various cancer cells.

In this project doxorubicin-based niosomes were functionalized with the CBA effectively recognizing mannosides.[3] Several formulations and different preparation methods were taken into consideration to obtain functionalized nanovesicles suitable for parental administration, which resulted stable for over six months, and able to encapsulate up to 85% of antitumoral drug. Cell viability, evaluated on a triple-negative cancer cells (MDA-MB-231), showed an increase of apoptosis for the functionalized DOXO-based niosomes, whereas comparison studies, carried out with H9C2 normal cells, confirmed the protective role of niosomal formulations on rat cardiomyocytes by a reduction of cytotoxicity.

The encouraging results obtained may open the way to further in vivo investigations.

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Exosomes as potential biomarkers for prostate cancer diagnosis

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Cancer is among leading causes of deaths worldwide. Prostate cancer (PCa) is one of the most common type of cancer diseases in men and one of the most frequently diagnosed cancer in more than half of the countries of the world [1]. Early diagnosis remains one of the most important factors of a successful treatment. Since PCa diagnostics lacks sensitivity and specificity, liquid biopsy is taking on an increasingly important role in early diagnosis research, detecting cancer-specific biomarkers in body fluids. Aberrant glycans attached to these markers are a frequent sign of tumour progression. Several predictive and prognostic biomarkers are already known today, and their serum levels can be analysed. The problem is elevated serum levels of these biomarkers lack sensitivity. Extracellular vesicles, especially exosomes, are considered as a potential biomarker for cancer diagnostics [2]. Exosomes, as naturally produced nanoparticles, are constantly released by the cells and tissues into various body fluids. They have the same topology as the parental cell and are enriched in different proteins, lipids, glycoconjugate and nucleic acids [3]. This work is focused on the analysis of exosomes isolated from benign prostate epithelial *RWPE1* cell line and cancerous prostate epithelial *22Rv1* cell line. The storage conditions, stability, production rate and mode of analysis of isolated exosomes are investigated in the experiments.

The authors wish to acknowledge the financial support received from the Slovak Research and Development Agency APVV 21-0329.

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Edition of Heparan sulfate by human endosulfatases monitored by several analytical techniques

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[1] Univ. Paris-Saclay, CNRS, Univ Evry, LAMBE, UMR 8587, Evry-Courcouronnes, FRANCE ; [2] Univ. Paris-Saclay, ICSN, CNRS, FRANCE, [3] Univ. Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, Lille, FRANCE, [4] Univ. Grenoble Alpes, CNRS, CEA, IBS, Grenoble, FRANCE.

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Heparan sulfate (HS) is a sulfated polysaccharide involved in cellular processes through its ability to bind and modulate various proteins. These interactions are controlled by the saccharide domains with rich sulfation patterns whose distribution is orchestrated by highly regulated biosynthetic machinery. In addition, at a different level of structural control, HS remodeling occurs at the cell surface. These modifications are ensured by editing enzymes involved in the post-synthetic sequencing of HS, including the extracellular sulfatases HSulf-1 and HSulf-2, which regioselectively remove 6-O-sulfate groups from HS. HSulf-1 and HSulf-2 are unique endosulfatases that target the functional sulfated domains of HS, alter ligand binding properties, and modulate a variety of signaling pathways [1]. Studies have shown that HSulfs are associated with many diseases, including cancer. Although they are closely related enzymes, HSulf-1 is most often largely reported as having a tumor-suppressive activity, whereas HSulf-2 has a pro-oncogenic activity and is consistently overexpressed in tumors.

Our work aims to characterize and understand these enzymes at the structural and functional levels [2]. Currently, conventional methods for monitoring and measuring the activity of these enzymes use synthetic sulfated substrates that are not carbohydrates in nature (e.g., p-catechol sulfate) and therefore do not accurately approach the appropriate true enzyme endolytic activity. To gain new insights into the structural features of the oligosaccharide substrates of these enzymes, we investigated the development of robust and resolving analytical methods that allow enzymatic monitoring of the natural sulfated substrates. First, kinetic studies of HSulf-2 using Hp oligosaccharides as substrates were performed by HILIC-MS [3]. We observed the sequential hydrolysis of 6-O-sulfate groups along the sulfated oligosaccharides; the activity of HSulf-2 was then compared with that of HSulf-1. We investigated whether these two enzymes also differ in their catalytic properties. Their desulfation reaction on various natural and synthetic Hp-oligosaccharide substrates was followed using several techniques. Overall, the use of IM-MS in combination with HILIC-MS, for example, can provide a powerful tool for the structural and functional characterization of HSulfs and their interactions with HS and other biomolecules.

Exploration of the functional specificities of each HSulf and their correlation to the structural differences will be discussed in detail.

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

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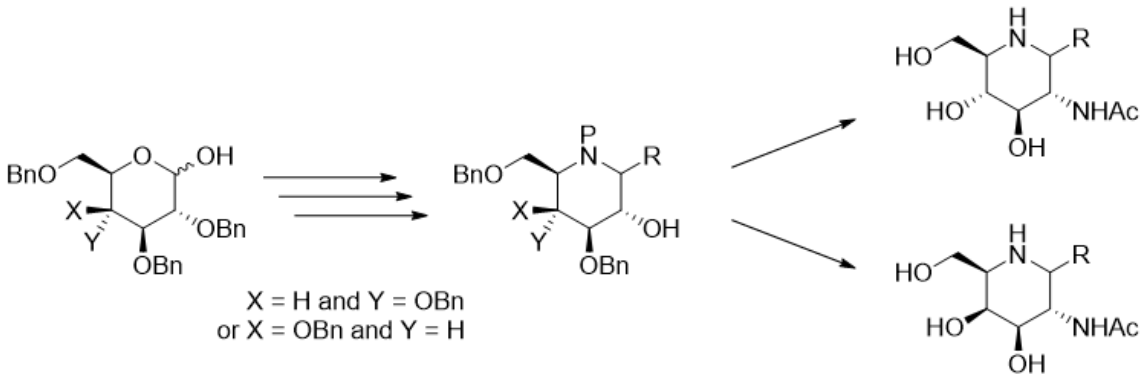
Whereas iminosugars are ranking among the most powerful glycosidase inhibitors *[1]*, their promising *C*-glycoside derivatives have not yet shown their full potential as glycosidase or glycosyltransferase inhibitors.

One of the main drawbacks associated with iminosugar-*C*-glycosides is their multistep sequence synthesis. This is even more obvious with 2-acetamido-2-deoxy-D-iminosugars, due to the presence of the sensitive NHAc moiety.

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

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



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

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Synthesis towards diversity of potential SGLT−2 inhibitors for the treatment of type 2 diabetes

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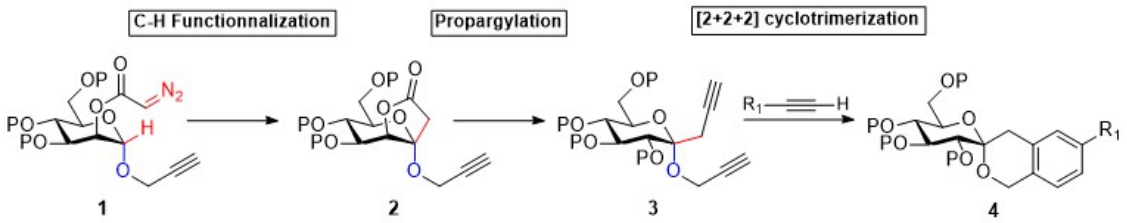
450 million people around the world are suffering from type 2 diabete, and its prevalence is increasing each year. In this context, gliflozines like Dapagliflozin have been marketed in 2013 as a new class of anti-diabetic agents acting as inhibitors of the sodium-glucose co-transporter SGLT−2. Among them, spiro-bicyclic compounds like Tofogliflozin showed great promises, but they have been underestimated because of synthetic approaches that only give rises to [6,5]-spiroacetals having an α configuration.

In this context, we wish to report herein a new approach towards potential inhibitors of SGLT-2, where a carbene-mediated functionalization of the anomeric C-H bond of carbohydrate would first give rise, on demand, to quaternary sugars of α- or β-configuration. Starting from mannose, the α-propargyl 2-diazoacetyl mannoside was first prepared and engaged in a Rh(II)-catalyzed 1,5-C-H insertion to yield γ-lactone. After conversion into the key *C,O*-bis propargyl glycoside, a [2+2+2] cyclotrimerization gave rise to [6,6]-spiroacetals fused to an aromatic ring.

This new approach allowing introduction of key pharmacophores in a late stage of the synthetic sequence provide a fast entry towards a large molecular diversity of unprecedented potential inhibitors of SGLT-2.



Divergent synthetic approach towards original [6,6] spirobicyclic potential inhibitors of SGLT-2.

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Pneumococcal serogroup 10 CPS: conformational rationalization of immunological cross-reactivity

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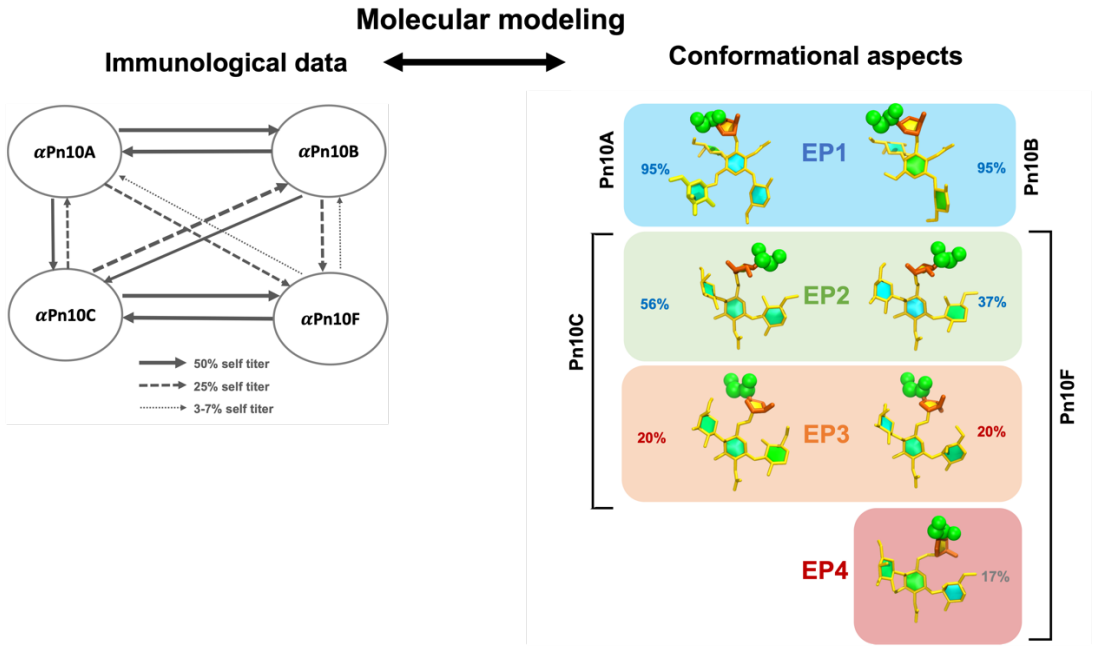
Molecular modeling can be used to correlate the conformational features of carbohydrate antigens with data on serotype cross-protection produced by immunological studies, revealing the important key epitopes for serotype cross-protection. *Streptococcus pneumoniae* is an encapsulated gram-negative bacterium and an important human pathogen responsible for significant disease and mortality in children under five. The streptococcal capsular polysaccharide (CPS) is essential for virulence and an important target antigen for vaccines. CPS structures of *S. pneumoniae* serogroup 10 are highly conserved, differing only in a 2- or 4-linked Rib-ol-5P backbone linkage and the presence/location of βDGal*f* and βDGal*p* side groups on a branching βDGalNAc residue. However, despite this similarity, serological data from immunological studies report complex, asymmetrical cross-reactivity between the four main constituents of the serogroup: Pn10A, Pn10B, Pn10C and Pn10F. Our conformational modelling of serogroup 10 identified four distinct conformational epitopes focused on an immunodominant βDGal*f* side group. These distinct epitopes provide a rationalization of the observed complex asymmetric cross-reactivity, and thus inform the design of the next generation of vaccine.



S. pneumoniae serogroup 10 rabbit antisera (α) cross-reactivity trends and representative conformational epitopes

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Structure of the *Listeria innocua* ŽM39 cell wall teichoic acid and analysis of its biosynthesis gene

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Wall teichoic acids (WTA) make up the majority of cell wall carbohydrates of *Listeria*. They play vital physiological roles and are involved in the interaction with the hosts and the environment. They are composed of ribitol phosphate polymers that are differentially glycosylated. Most is known about the WTA of the foodborne pathogen *Listeria monocytogenes*. Since the non-pathogenic *Listeria innocua* is its close relative, it has been proposed as a surrogate organism for the foodborne opportunistic pathogen, especially for determining the efficacy of antimicrobial strategies against *L. monocytogenes* because it is safer to work with. Since there were few reports of WTA structures of *L. innocua*, we set out to determine the structure of native WTA, for the food isolate strain ŽM39, by 1D and 2D NMR spectroscopy. We report the complete structure of the WTA of *L. innocua* ŽM39, which consists of [→4)-β-GlcNAc*p*-(1→4)-Rbo-(1P→] backbone with an α-GlcNAc*p* bound to C3 of the β-GlcNAc*p* in stoichiometric amount. No acetyl groups were detected.

According to the current classification of *L. monocytogenes* WTA, the *L. innocua* ŽM39 WTA can be assigned to type II, since the β-GlcNAc residue is part of the backbone. This structure is similar to type II WTA serovar 6a which however contains a β-GlcNAc substituted in non-stoichiometric amounts with Gal, α-GlcNAc and acetyl groups. In addition, we sequenced the genome of this *L. innocua* strain and deciphered most of the putative WTA synthesis genes.

Acknowledgements

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N-glycoproteomics at the cell surface

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Cell surface glycans are essential in establishing cell communication, adhesion, and migration. Interestingly, glycosylation changes in cancer cells, making carbohydrate antigens compelling targets for cancer diagnosis and therapy. However, because it remains challenging to obtain cell surface-specific information on glycoconjugate structures, it is unclear to what degree the observed cancer-associated glycan changes are related to cell surface glycans or rather biosynthetic intermediates present inside cells. Obtaining this information is essential for unraveling the functional role of glycans and for exploiting them as clinical targets.

To specifically analyze the N-glycoprotein forms expressed at the cell surface, we developed a mass spectrometry (MS)-based method for the sensitive analysis of cell surface enriched glycoproteins. Using human skin keratinocytes as a model system, we identified and quantified the site-specific N-glycosylation of hundreds of surface glycoproteins. This approach allowed us to study the glycoforms present at the functional relevant cell surface, omitting immaturely glycosylated proteins present in the secretory pathway. Furthermore, natural simplification of the sample was obtained by excluding non-glycosylated proteins from e.g., the nucleus and cytoplasm, allowing a deeper investigation of the glycoproteome.

With this approach, we also compared N-glycosylation sites of proteins expressed both on the cell surface and in a total cell lysate. The analysis showed profound differences in glycosylation between the two subcellular components.

Evaluating the substrate specificity of ENGases using fluorogenic glycan probes

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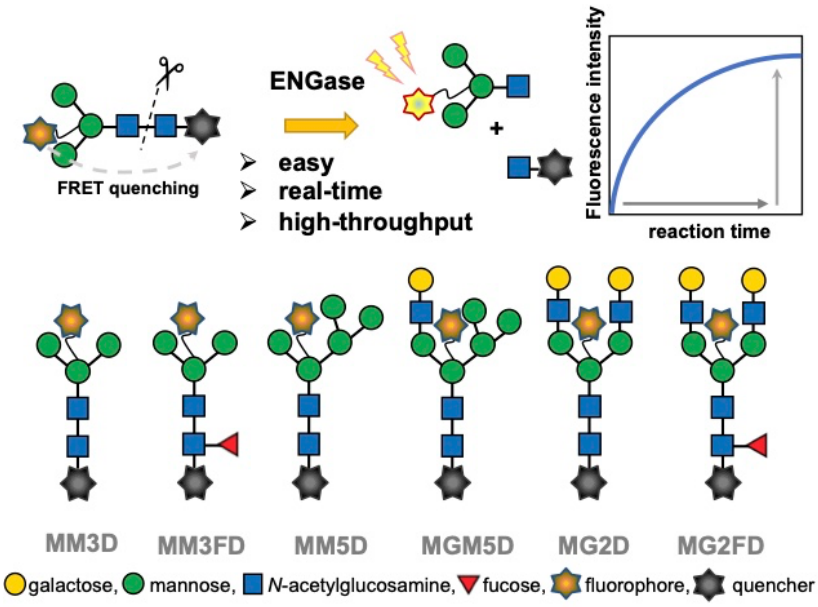
Endo-β-*N*-acetylglucosaminidases (ENGases) are glycosyl hydrolases widely used for glycan analysis of glycoproteins. ENGases hydrolase β1-4 glycosidic bond in the *N*,*N*-diacetylchitobiose core of *N*-glycan, release the glycan from glycoproteins and leave a single *N*-acetylglucosamine on proteins. ENGases are widely distributed in bacteria, fungi, and higher-order species, and each enzyme exhibits different substrate specificities.

To conveniently detect ENGase activity and evaluate the substrate specificity of ENGases, we have developed Förster resonance energy transfer (FRET) type substrates with *N*-glycans structure carrying a fluorescent group and a quenching group [1]. For a systematic understanding of substrate specificity of ENGases, we synthesized glycan probes with different structures of N-glycan, i.e., core-fucosylated hexasaccharide, biantennary asialocomplex type nonasaccharide, fucosylated complex type decasaccharide, oligomannose type heptasaccharide, and hybrid type nonasaccharide.

In this presentation, we will show the systematic synthesis of the FRET-based glycan probes and the results of hydrolytic reactions of these glycan probes by several ENGases and discuss the specificity of these ENGases.



Detection of ENGase activity using fluorogenic glycan probes

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Novel bioactive triazole- and guanidine-containing xylofuranosyl isonucleoside analogs

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Nucleoside and nucleotide analogs occupy a distinctive place in (bio)organic and in medicinal chemistry due to their propensity to interfere with nucleos(t)ide-dependent biological events that are crucial for life as well as for the progress of various diseases. Their therapeutic interest is demonstrated by the various examples of such compounds approved as anticancer and antiviral drugs [1], while their antimicrobial potential has been well reported [2].

Approaches for the design of nucleos(t)ide analogs include simple modifications at purine, pyrimidine or at ribose/2-deoxyribose moieties, the use of other nitrogenous heteroaromatic systems or other glycosyl units, the inclusion of phosphate group mimetic motifs or modification on the type or location of the bond connecting nucleobase and sugar.

In this communication the synthesis and biological evaluation of a variety of 5'-isonucleoside analogs constructed on xylofuranosyl templates and comprising a 1,2,3-triazole moiety and/or a guanidine group is reported. The triazole motif was envisaged as a surrogate of a nucleobase and was also connected to a phosphonate, phosphoramidate, or a phosphate moiety to establish new potential and rather stable neutral mimetics of the diphosphate system. The synthetic methodologies used azido xylofuranoses as precursors and employed key steps such as azide-alkyne 1,3-dipolar cycloaddition, phosphorylation, Arbuzov reaction, N-glycosylation, or guanidinylation.

From the molecules subjected to biological assays, some showed significant inhibition of acetylcholinesterase, potent antiproliferative activity in a breast cancer cell line or potent effects against the Gram-positive bacterial pathogen *Streptococcus pneumonia*, with activities comparable to those of reference drugs.

We thank FCT for funding: grant CEECIND/03881/2018, project EXPL/MED-QUI/1017/2021, projects UIDB/00100/2020, UIDP/00100/2020 and LA/P/0056/2020, UIDP/04378/2020, UIDB/04378/2020 and LA/P/0140/2020.

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Complement C3 *N*-glycoprofiling in diagnosis of type 1 diabetes

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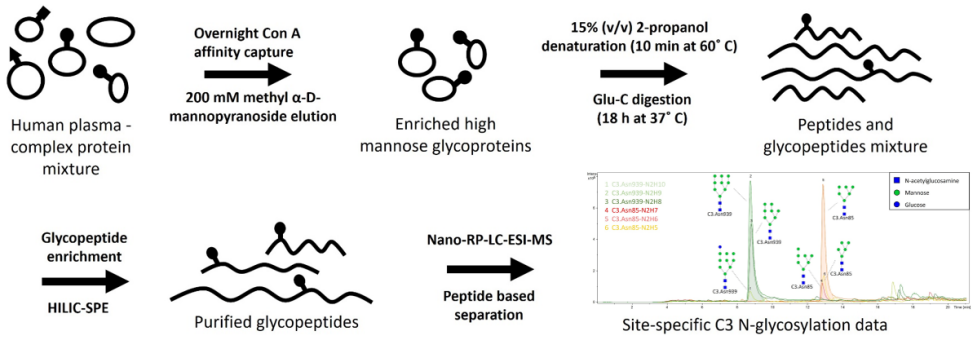
Recently, we showed that children at the onset of type 1 diabetes (T1D) have a higher proportion of oligomannose glycans on their total plasma proteins compared to their healthy siblings and identified these levels to be associated with the C3 gene. Complement component 3 (C3) contains two N-glycosylation sites occupied exclusively by oligomannose glycans. In order to evaluate the diagnostic potential of C3 N-glycosylation in T1D, we developed a high-throughput workflow for C3 concanavalin A lectin affinity enrichment and subsequent LC-MS glycopeptide analysis which enables protein-specific N-glycosylation profiling. Plasma samples of 61 children newly diagnosed with T1D and 84 of their unaffected siblings were analyzed. Significant changes in C3 N-glycome were found. T1D was associated with an increase in glycan structures with more mannose units. A regression model including C3 N-glycans showed considerable discriminative power (AUC = 0.879). Further, we analyzed serum samples from 189 adults with T1D concerning the most frequent complications of this disease. C3 N-glycome changed significantly among different stages of the albuminuria, as well as among hypertension status. All except one of the C3 glycopeptides proved to be associated with HbA1c levels. One of the glycoforms was also shown to be changed in retinopathy, in addition to elevated fasting glucose levels. Our study implies that the glycosylation of C3 could be a valuable diagnostic tool in assessing T1D risk in children as well as disease complications in adults.



F Schematic workflow for a high-throughput and site-specific C3 N-glycosylation LC-MS analysis

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Multivalent glycosidic vectors for the modulation of the immune system

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Among the different breast cancer types, the triple negative breast cancer (TNBC) is the most difficult to treat and to recover from [1]. Cancer immunotherapy is nowadays a consolidated strategy and Tumor-Associated Carbohydrate Antigens (TACAs) are used to develop therapeutic cancer vaccines (CVs). In designing a potential TACA-based CV it must be considered that saccharidic antigens suffer from a reduced metabolic stability *in vivo* and a poor T cells-dependent immunogenicity, which compromise a strong immune response, crucial for a promising CV. To overcome these issues TACA analogues can be designed to mimic the native antigens and to ensure a better stability and immunogenicity [2].

Over the past two decades, saccharidic structures mimicking the well-established MUC-1 TACAs, were successfully developed in our group.[3] To overcome an intrinsic low immunogenicity TACAs are generally covalently linked to immunogenic proteins; novel immunogenic vectors have recently been exploited in vaccine assembling to deliver and efficiently present TACAs. In this communication, we present the preliminary results obtained in the development of TNBC vaccine candidates which relies on a carrier-adjuvant conjugated with structurally immunogenic TACAs mimetics, specifically Tn and STn mimetics (Tn-mim and STn-mim in **Figure 1**).

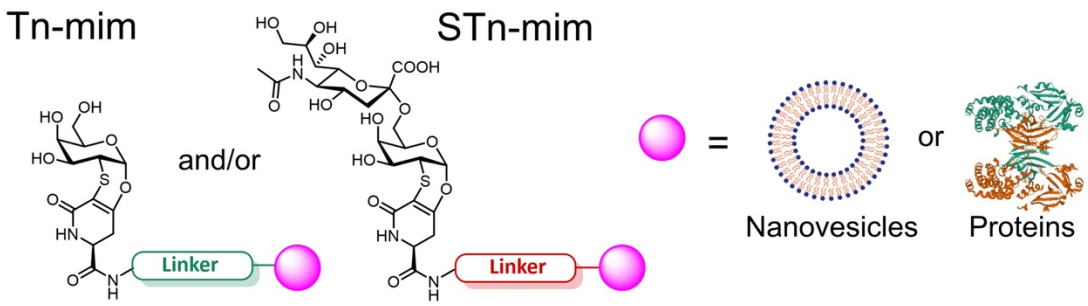

Figure 1: Carrier-adjuvant conjugated to Tn and STn mimetics.

Acknowledgements

The research leading to these results has received funding from AIRC under IG 2021 - ID. 25762 project – P.I. Nativi Cristina.

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The *N*-glycosylation of the SARS-CoV-2 SPIKE virus-like particles produced in tobacco plants

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The coronavirus SARS-CoV-2 emerged in China in late 2019 and led to the COVID-19 pandemic. Of the major structural proteins encoded by the SARS-CoV-2 genome, the SPIKE protein has attracted considerable research interest because of the central role it plays in entry into host cells. SARS-CoV-2 SPIKE is highly glycosylated with 22 predicted *N*-glycosylation sites, as well as numerous mucin-type *O*-glycosylation site. It is well documented that the protein glycans are mainly exposed at the surface and form a shield masking specific epitopes to escape the virus antigenic recognition.

Moreover, the analysis of SARS-CoV-2 SPIKE from coronavirus variants has revealed significant variations in their glycan profiles. Virus-like particles (VLPs) of the SARS-CoV-2 SPIKE were produced in *Nicotiana benthamiana* and its *N*-glycosylation was investigated by a glycoproteomic approach.

As demonstrated for the viral protein produced in mammalian cells, we show that 20 among the 22 *N*-glycosylation sites are dominated by complex plant *N*-glycans and one is occupied by oligomannosides. This suggests that the CoV-2 SPIKE protein produced in tobacco plants adopt an overall 3D structure similar to the one of recombinant homologues produced in mammalian cells [1].

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New glycocalix[4]arenes for targeting galectins and their biodistribution

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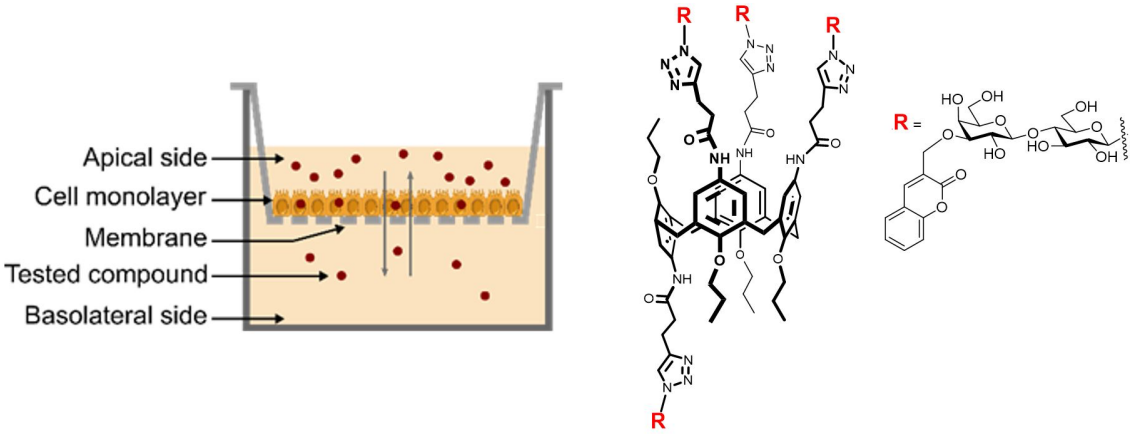
Galectins are carbohydrate-binding lectins that modulate important extra- and intracellular biological processes such as cell proliferation, adhesion, or migration. Elevated levels of galectins in serum and affected tissues are associated with various diseases, e.g., inflammation, carcinogenesis, fibrosis, and metabolic disorders [1]. Inhibition of galectins by high-affinity carbohydrate ligands, especially glycomimetics, opens new therapeutic routes. We show the synthesis of a library of glycocalix[4]arenes as ligands of prototype, chimeric and tandem-repeat galectins. The affinity of glycocalix[4]arenes to galectins was strongly steered by the linker and core structure. Moreover, they were able to induce supramolecular clustering of galectins [2]. A *partial cone* calix[4]arene carrying a novel glycomimetic, 3-*O*-coumaryllactose, proved to be promising for absorption into intestinal cells as shown in an established model of Caco-2 cell monolayer. Penetration into the intracellular compartment, otherwise hardly accessible for hydrophilic carbohydrates, may allow the interaction of glycocalix[4]arenes with intracellular targets such as galectin-3. Thus, the present glycocalix[4]arenes represent prospective tools for biomedical research.



Coumaryl-derived glycocalix[4]arene and its penetration through the monolayer of human colon epithelial cells Caco-2.

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The dual-binding mode in cellulosome assembly: size over type and function

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The cellulosome is one of nature's most intricate macromolecular protein complexes, which centralizes the cellulolytic efforts of many anaerobic microorganisms, promoting enzyme synergy and stability. Its components are assembled through high-affinity protein-protein interactions between enzyme-borne dockerin (Doc) modules and repeated cohesin (Coh) modules present in non-catalytic scaffold proteins [1-3]. Typically, a sequence and function-based classification distinguishes between two types of Coh-Doc modules. Thus, type I Coh-Doc complexes are usually responsible for enzyme integration, while type II complexes tether the cellulosome to the bacterial wall [4]. Contrastingly, in *Bacteroides cellulosolvens,* Coh types are reversed in all scaffoldins, with type II Cohs located on the enzyme-recruiting primary scaffoldin and type I Cohs located on the anchoring scaffoldins [5]. It has been previously reported that type I *B. cellulosolvens* interactions possess a dual-binding mode (DBM) that adds flexibility to scaffoldin assembly.

Here, by combining structural characterization with affinity studies we have uncovered the mechanisms governing enzyme recruitment into *B. cellulosolvens'* cellulosome and identified the molecular determinants of its type II Coh-Doc interactions. The results indicate that, unlike type II complexes from other species, these possess a DBM, similarly to type I. Therefore, the plasticity of DBM interactions seems to play a pivotal role in the assembly of *B. cellulosolvens'* cellulosome, which is consistent with its unmatched complexity and size.

Acknowledgements

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Selective involvement of UGGT2 in lipid glucosylation

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The ER is the primary site facilitating the biosynthesis of membrane-bound and secretory proteins. In addition, a large number of lipid classes are also biosynthesized at the ER. ER stress and UPR activation have been reported to be induced not only by accumulation of misfolding proteins but also by ER membrane lipid perturbation, with the latter playing a fundamental role in the pathogenesis of Alzheimer's disease, type 2 diabetes and cardiovascular disease. As a key component of the protein quality control system (PQC), Uridine diphosphate glucose:glycoprotein glucosyltransferase 1 (UGGT1) re-glucosylates misfolded glycoproteins to promote re-entry in the protein-folding cycle and curtails aggregation of misfolded glycoproteins [1,2]. The biological function of UGGT2, a UGGT1 paralogue, remains poorly understood [3,4], but has recently been proposed to be involved in the maturation of lysosomal proteins [5].

Our findings suggest that UGGT2 is largely unnecessary for efficient protein biogenesis and demonstrate that ER stress inducing phosphatidic acid derivatives with saturated fatty acyl chains are one of the physiological substrates of UGGT2, yielding lipid-raft resident phosphatidyl-β-D-glucoside. UGGT2 but not UGGT1 was protective during hypoxic stress by positively modulating autophagy and mitigating PERK-C/EBP homologous Protein (CHOP)-mediated apoptosis. Our findings are not limited to basic lipid biochemistry but provide a novel perspective on lipid induced ER stress and the up to now elusive "lipid quality control" (LQC) system.

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Diglycosidases as a new synthetic tool in biocatalysis

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Diglycosidases are becoming a promissing group of endoglycosidases enabling fast and simple syntheses of oligosaccharides and their glycosides without formation of glycon isomers. Screening and application of diglycosidases is however hampered by limited availability of appropriate substrates. We have tested three types o diglycosidase activities of various origin in glycosylation of tyrosol.

1. Rutinosidase-comprising plant materials, flower buds from Japanese sophora (*Sophora japonica*) [1] and seed meal from tartary buckwheat (*Fagopyrum tartaricum*) rutinosylated tyrosol exclusively on the primary hydroxyl with conversions above 60 %, providing after isolation solely the 2-(4-hydroxyphenyl) ethyl β-rutinoside in 24 and 35 %, respectively. Both catalysts are highly specific towards rutin as the substrate.
2. On the other side, transrobinobiosylation from robinin to tyrosol catalyzed by seed meal from common buckthorm (*Rhamnus cathartica*) proceeded with lower chemoselectivity, providing after isolation 23 % of a mixture of both theoretical tyrosol β-robinobioside isomers 2-(4-hydroxyphenyl)ethyl β-robinobioside and 4-(2-hydroxyethyl)phenyl β-robinobioside in ratio ca. 8:1 in favor of glycosylation of the primary hydroxyl.
3. Aromase H2, a commercial mixture of glycosidases and diglycosidases, catalyzed transacuminosylation from 4-nitrophenyl β-acuminoside to tyrosol, providing after isolation approximately equal mixture of 2-(4-hydroxyphenyl)ethyl β-acuminoside and 4-(2-hydroxyethyl)phenyl β-robinobioside [2].

Acknowledgements

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The first synthesis of autoinducer-2 prodrugs

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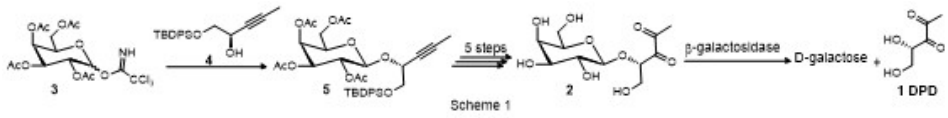
Bacteria are able to coordinate the behaviour of cell population by secreting and sensing small molecules called autoinducers.[1] This phenomenon is called quorum sensing (QS). Among the QS compounds, autoinducer-2 (AI-2) stands out, proposed to be a "universal" bacterial signalling molecule in inter-species communication. AI-2 plays an important role in controlling the colonisation and homeostasis of the gut microflora. There is an evidence that AI-2 can be used to ameliorate the effect caused by antibiotic-induced microbiota imbalances in the gut.[2] Thus, our premise is that synthetic AI-2 can help in the recovery of a healthy bacterial phyla ratio after antibiotic treatment.

To study the mechanisms involved in the response of the gut microbiota to the AI-2, it is necessary to synthesise a suitable chemical tool to deliver AI-2 unaltered to the gut. For this reason, we decided to follow the strategy of colon-specific drug delivery systems.[3] In this contribution we will discuss chemoenzymatic strategies towards linking **DPD 1** (the uncyclised precursor of AI-2) to a monosaccharide to create a prodrug **2** (Scheme 1). This prodrug will deliver **DPD** to intestine where it will be liberated by beta-D-galactosidases produced by the gut microbiota.

To verify the plausibility of this experimental approach, the development of an *in vitro* method for enzymatic hydrolysis of the glycosidic bond between the sugar and **DPD** using commercial beta-D-glycosidase will be presented together with subsequent quantification of AI-2 released using *Vibrio harveyi* bioluminescence assay.



Scheme 1

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Using the nitrogen dimension in NMR spectroscopy to resolve individual residues in chitin oligomers

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Chitin is the second most abundant biopolymer in nature after cellulose and is composed of *N*-acetylglucosamine (GlcNAc) connected via β(1→4)-glycosidic bonds. Due to its high abundance, the interaction with other biomolecules, such as enzymes during degradation or synthesis, is of a central role in the world´s carbohydrate turnover. Additionally, chitin is used as biomaterial in pharmaceutical applications.[1] Despite its prominence, there is still a need to develop methods to study structure and function of chitin and its corresponding oligomers and their interactions with other biomolecules. Efforts have been made to analyse chitin oligomers by NMR spectroscopy, but spectral overlap has prevented any differentiation between the interior residues. For glycosaminoglycans, such as hyaluronan, ^{15}N NMR has been utilized on amide and sulfamate groups to resolve individual residues in oligomers.[2-4] Herein, we present a similar approach on chitin oligomers.

Chitin oligomers up to hexaose with natural abundance of ^{15}N were analysed with NMR spectroscopy in H_2O:D_2O (9:1) solution. Under the present conditions ^{1}H,^{15}N-HSQC allowed for individual residues in the oligomer to be resolved in the nitrogen dimension. Additionally, all oligomers were analysed for the presence of the *cis* amide form. According to previous studies on GlcNAc, the *trans* conformation is predominant, but about 1% of *cis* form is present.[5] It is still unknown whether the *cis* amide form plays a significant role in the conformation of polysaccharides containing *N*-acetyl groups, or if it has any biological function.

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Conformational space of glucose mono- and di-saccharides: comparison of GLYCAM06j and CHARMM36

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Carbohydrates are an important class of biomolecules, which are involved in a huge diversity of biological roles in living organisms. Typically, the pyranose ring adopts stable 4C1 or 1C4 conformations, but less predominant conformations also significantly influence the biological activity of carbohydrate-containing systems. The conformational analyses of carbohydrate molecules is of great importance to uncover the role in the related biological events.

Molecular dynamics is an important technique to study carbohydrates at atomic level. So far, the widely used and developed all-atom additive force fields for carbohydrates are GLYCAM06j and CHARMM36, with parameters available for most pyranoses and furanoses in eukaryotic. However, it is well-known that both of the two force fields over stabilize the chair conformation of pyranose molecules.

In this study, we use molecular dynamics with enhanced sampling at both molecular mechanics and quantum mechanics levels to address the force field issues. This study will be useful for future carbohydrates force fields development.

Mucosal glycans as novel antimicrobial agents

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The mucosal barrier is well-established to play an important role in microbiota development and as a first line of host defence. Although this has traditionally been attributed to the physicochemical properties of mucus, recent reports indicate that mucin glycoproteins and their associated glycans can regulate gene expression and are capable of attenuating virulence in diverse, cross-kingdom pathogens, including Gram-positive bacteria, Gram-negative bacteria, and fungi.

With mucins displaying several hundred distinct glycan structures, we sought to identify discrete glycan structures responsible for this novel gene regulation. Individual mucin O-glycan structures are not commercially available, are not yet amenable to automated synthesis, and given their overlapping physical and chemical properties cannot be isolated as pure compounds from natural sources using current technologies.

Therefore, through a multi-centre collaborative effort we have been actively: (i) characterizing complex mucin O-glycan pools to identify structures most likely to display biological activity; (ii) developing a synthetic approach to obtain individual mucin O-glycans in sufficient quantity for functional analysis [2]; and (iii) assessing the virulence attenuating capabilities of individual glycans in diverse pathogens [1,3]. Within this framework, we have successfully identified specific structures that suppress virulence phenotypes in the fungal pathogen *Candida albicans* (e.g., filamentation, biofilm formation), and regulate pathogenicity in *Vibrio cholerae* (e.g., reduced cholera toxin production), with potency comparable to native mucin glycan pools.

The authors gratefully acknowledge Innosuisse (104.462 IP-LS), the Swiss National Science Foundation (CRSK-3_196773), and the University of Basel for financial support.

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Chemical synthesis of oligosaccharides related to plant rhamnogalacturonan-I

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The plant cell wall is the outer fibre composite layer which plays an important role in plant growth as well as in plant–microbe interactions, such as the defence response against pathogens. It is a complex network to a large part composed of structural polysaccharides such as cellulose, hemicellulose and pectin. [1] Pectin comprises the domains homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II). The RG-I backbone is made up of alternating units of (1→2)-α-L-rhamnopyranose and (1→4)-α-D-galactopyranuronic acid, substituted with various L-arabinan, D-galactan, and arabinogalactan side chains. [2] Oligosaccharide fragments of RG-I represent important research tools for studying RG-I biosynthesis as well as its potential involvement in plant immune responses.

Towards the chemical synthesis of RG-I fragments, we employ a post-assembly-oxidation strategy using galactose building blocks that are later oxidized to the corresponding galacturonic acids. [3] The backbone is constructed using thioglycoside donors activated with NIS and AgOTf. Fluorenylmethoxycarbonyl (Fmoc) serves as a temporary protecting group for chain elongation, and benzoyl (Bz) protecting groups at C-6 of the galactose units provide remote participation to facilitate α-selective glycosylations before they are selectively removed and oxidized after backbone assembly. [4] The synthetic RG-I fragments will be used for glycan array-based studies with glycosyltransferases and plant immune receptors.

Acknowledgements

This work was supported by the Austrian Science Fund (FWF, grant P35404 to FP)

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A simplified analytical approach for Glycoconjugate content (potency) quantification

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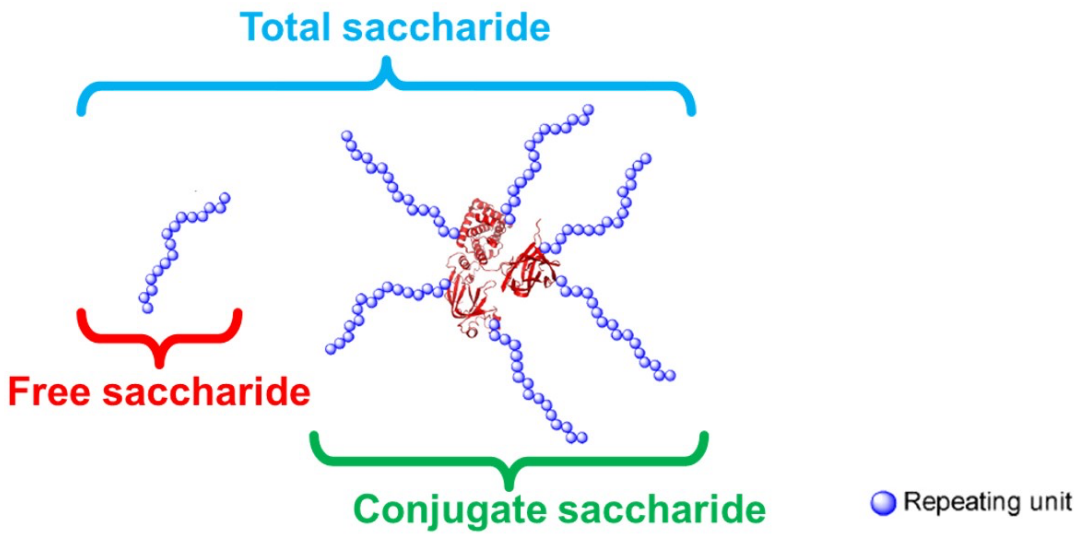
The use of glycoconjugate vaccines is a critical weapon in fighting bacterial infections, and several glycoconjugate vaccines have been licensed through times or are currently in clinical development. This work proposes a new approach to the glycoconjugates vaccine antigen quantification (vaccine potency). According to Pharmacopeia chapter <1234> to assess glycoconjugate vaccine potency, it is requested to measure both the Total amount of Saccharide content TS (conjugated and unconjugated saccharide) and the Free unconjugated amount of saccharide FS (which is not immunogenic), see Figure 1. With those two attributes (TS/FS), it is possible to estimate the amount of Conjugated Saccharide CS (which is the active ingredient). Here, we illustrate a new method to quantitatively purify the CS moiety from the sample matrix and from the FS, enabling, in one single test, the direct accurate quantification of the active ingredient[1]. The assay has been developed and validated for a quadrivalent conjugate vaccine containing meningococcal serogroup A, C, W and Y polysaccharides each coupled with CRM197 protein. Method validation results showed high linearity, precision, accuracy, repeatability, and specificity. In addition, the assay demonstrates to be stability indicating, meaning that if during drug product stability, a decrease of conjugated saccharide occurs it can be quantitatively and accurately measured. The CS test implementation as release test replacing the classical TS and FS assays has been agreed with FDA and has been successfully implemented in Quality control facility.



Total Free and Conjugated Saccharide graphical representation

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Synthetic study of glycosyl-stem cell factor using β-mercapto norleucine

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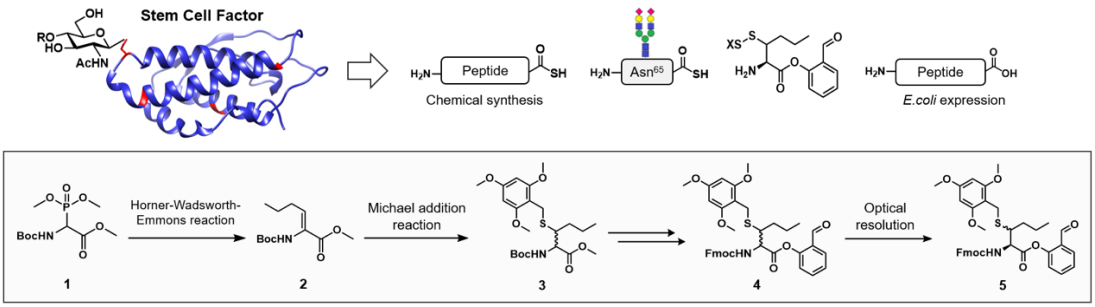
Stem Cell Factor (SCF) is a glycoprotein hormone and play important roles in hematopoiesis. SCF is known to dimerize and have asparagine-linked (N-) glycans near the dimeric interface. However, the function of these glycans has not yet been investigated. Therefore, we have synthesized homogeneous glycosyl SCF.

For the synthesis of glycosyl SCF by the convergent synthesis, we designed four building blocks: N-terminal peptide thioacid, Asn-(sialyloligosaccharide)-thioacid, β-mercaptonorleucine derivative, and C-terminal recombinant peptide. These compounds could be sequentially coupled by diacyl disulfide coupling [1], Ser/Thr ligation [2], and thioacid capture ligation [3].

First, we carried out the synthesis of β-mercaptonorleucine derivative, which can expand the choice of ligation sites. Glycylphosphonate was used for Horner-Wadsworth-Emmons reaction with butylaldehyde. The following Michael addition with a thiol derivative and protecting group manipulations were then performed. In addition, the optical resolution by chiral column successfully yielded the desired norleucine derivative having a thiol functionality at the β-position.

Furthermore, three peptide building blocks were successfully prepared by Boc solid phase peptide synthesis (SPPS) and E. coli expression system. Since all building blocks were already prepared, we examined the peptide coupling reactions to obtain the full-length glycosylated polypeptide. This presentation will describe these experiments in detail.



Strategy for the synthesis of β-mercaptonorleucine derivative

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PROTACs as new modality for targeting glycosidases and glycosyltransferases

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Glycosidases and glycosyltransferases are carbohydrate processing enzymes that are involved in a wide variety of diseases. Despite extensive research, discovery of effective drugs targeting glycosidases and especially glycosyltransferases has proven rather challenging. To overcome this problem, paradigm-shifting approaches are needed to target these enzymes in a clinical setting. We propose application of proteolysis-targeting chimeras (PROTACs) technology as a potential solution. PROTACs are bifunctional molecules that consist of a ligand of the protein of interest (POI), a linker and an E3 ligase ligand. Upon binding both the POI and the E3 ligase, the POI and E3 ligase are brought in close proximity causing ubiquitinylation of the POI, followed by downstream protein degradation. The mode of action of PROTACs provides several advantages over traditional occupancy-driven inhibitors, including a catalytic degradation mechanism and enhanced selectivity due to the requirement of efficient protein-protein (POI-E3) interactions. As model cytosolic glycosyltransferase and glycosidase targets we chose glycosylceramide synthase (GCS) and non-lysosomal glucocerebrosidase (GBA2) respectively, with implied relevance in Gaucher disease and Parkinson's disease. For this purpose, we selected a dual GCS- and GBA2 iminosugar ligand and functionalized the ligand both at the ring nitrogen atom and at the C1 position in β-configuration. Based on these designs, both GCS-selective and GBA2 selective PROTACs capitalizing on CRBN and VHL E3 ligands are currently under development.

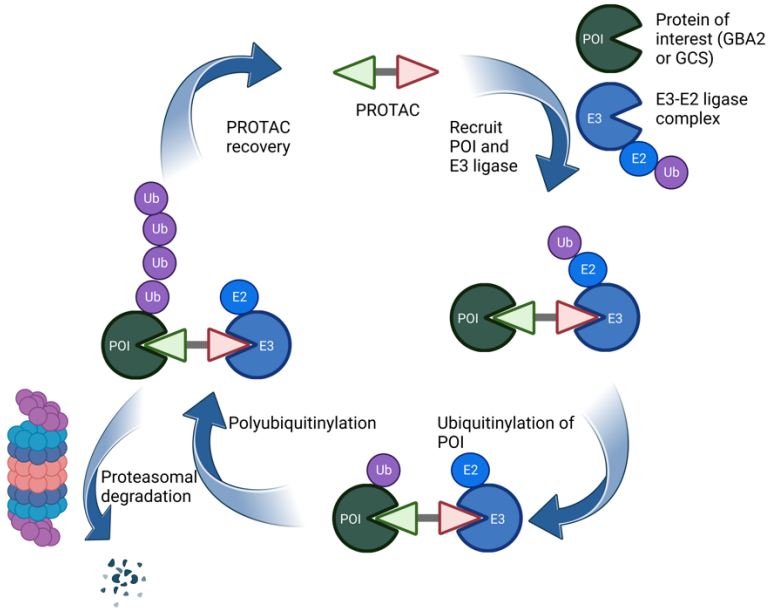

Figure 1. Mechanism of protein degradation caused by PROTACs. Created with BioRender.com.

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Synthesis of β-(1→3)-glucans as a possible candidate for vaccines against *Cryptococcus neoformans*

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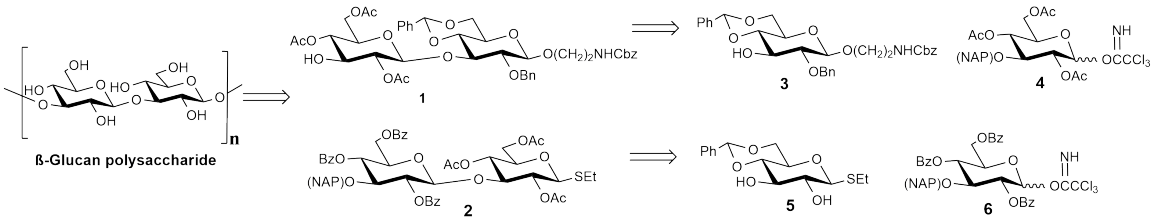
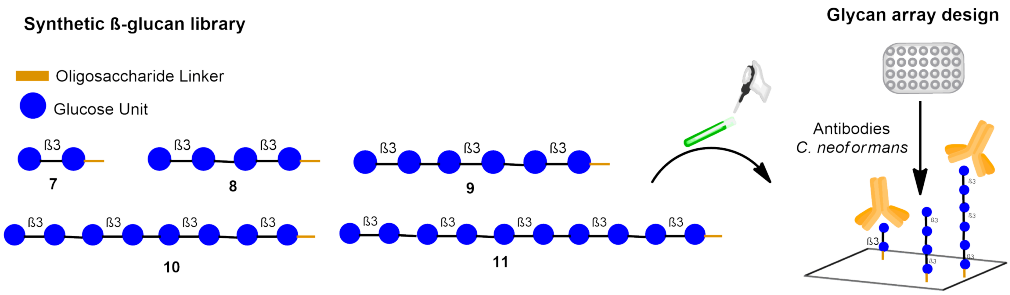
Cryptococcus neoformans, an opportunistic fungal pathogen that is ubiquitous in the environment, can cause systemic infection in the immunocompromised and it is estimated that 1 million infections occur annually [1]. *C. neoformans* is unique among pathogenic fungi in that its polysaccharide capsule is essential for virulence in mammals. The capsule and cell wall is composed of several constituents, including mannoproteins, β-glucans, galactoxylomannan (GalXM) and glucuronoxylomannan (GXM) [2]. In this context, β-glucan, which is functionally necessary for fungi and immunologically active, is an attractive target antigen [3].

That is why we proposed the synthesis of disaccharides acceptor **1** and donor **2** with the aim of obtaining different polysaccharides units of β-(1→3)-glucan, for immunological study. We synthesized disaccharide **1** derived with a spacer arm with an amino group, from precursors **3** and **4**. Donor disaccharide **2** was obtained with a similar strategy, using precursors **5** and **6**. Donors **4** and **6** were prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose.

The library of obtained β-glucan oligosaccharides of different length (**7**-**11**), will be printed to produce a glycan microarray that will be screened with sera from *Cryptococcus* infected patients to select for possible vaccine candidates.





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C-glycosyl compounds, key intermediates for the synthesis of anti-proliferative [3.3.0]furofuranone

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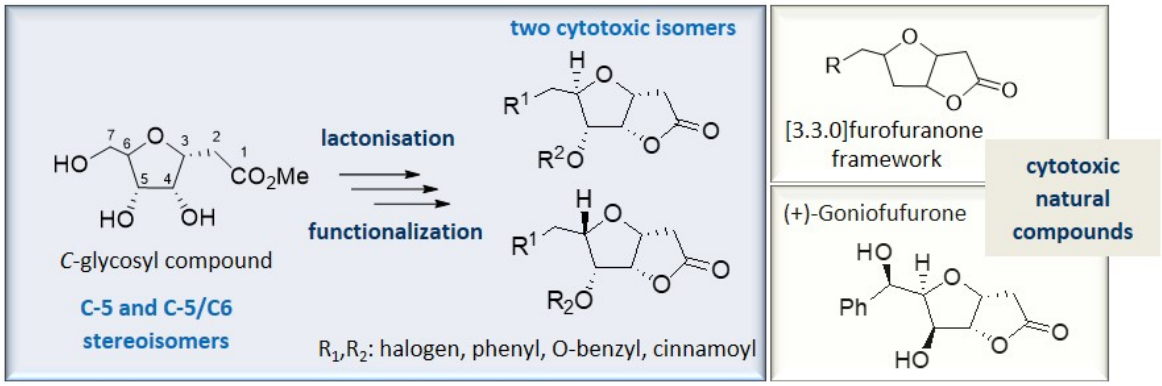
The [3.3.0]furofuranone structure is found in numerous families of biologically active natural products. We took advantage of the stereodiversity afforded by carbohydrate derivatives, and more precisely *C*-glycosyl compounds displaying chemical and biological stabilities,[1] to prepare several compounds structurally similar to goniofufurone and crassalactones which are natural cytotoxic agents.[2,3]

We designed and synthesized several stereoisomers of these natural compounds via lactonization of *C*-glycosyl compounds bearing a hydroxyl on position 4 and a methyl ester on the pseudo-anomeric position. The reactivity of this bicyclic moiety was explored through etherification of hydroxyls in position 5 and 7 and various substituents such as halogen, phenyl, benzyl and cinnamoyl were introduced. The anti-proliferative properties of these mimics were then evaluated on various cancer cell lines and the results highlighted that the stereochemistry of the furofuranone moiety is a crucial point to ensure cellular activity. Interestingly, two compounds demonstrated promising IC_{50} value of 1.34 µM (U251) and 7.60 µM (U87).[4]



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Towards the synthesis of defined carbohydrate-based ligands for the C-type lectin clec-2

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C-type lectin-like receptor 2 (CLEC-2) is a receptor expressed on human platelets. Platelets play an important role in several biological events such as hemostasis, inflammation, infection and immunity. CLEC-2 is activated by podoplanin, a mucin-type protein, causing platelet aggregation.[1] Another ligand that was reported to act as an agonist of CLEC-2 is fucoidan, a sulfated polysaccharide from the algae *Fucus vesiculosus*.[2]

We report on our research towards the chemical synthesis of podoplanin- and fucoidan-based ligands for studying CLEC-2 biology. The sialyl Tn (STn) disaccharide moiety of the glycoprotein ligand podoplanin interacts most closely with CLEC-2.[3] Therefore, aminoalkyl-linker-functionalized STn was prepared by enzymatic 2,6-selective sialylation of linker-functionalized *N*-acetyl-galactosamine with CMP-sialic acid. Podoplanin-based glycopeptide-ligands will be synthesized as well, starting from a galactosylated threonine building block. In this case, both the sugar and the peptide moiety will contribute to binding to the receptor.[4]

Fucoidan-based hexasaccharide ligands with alternating α1,3- and α1,4-linkages will be prepared through several rounds of glycosylation reactions using two different L-fucose thioglycoside building blocks followed by selective sulfation. The choice of the positions for sulfations are guided by the sulfation pattern of the natural fucoidan ligand for CLEC-2 from *Fucus vesiculosus*.[5] The binding between the ligands and CLEC-2 will be studied by biophysical techniques.

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Towards the use of *Chlamydomonas reinhardtii* as biopharmaceuticals expression system[...]

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For the biopharmaceutical production, the green microalga *C. reinhardtii* benefits from numerous molecular and biochemical tools.. However, most of the biopharmaceutical products are glycoproteins and it is now well-established that glycans harbored by proteins are implicated in their life time, activity and immunogenicity (Lingg et al., 2012, van Beers and Bardor, 2012). To date, the genes encoding enzymes responsible for the *N*-glycosylation steps occurring in the endoplasmic reticulum and Golgi apparatus have been identified in *C. reinhardtii* (Mathieu-Rivet *et al*, 2013). This microalga synthesizes non canonical oligomannosidic *N*-glycans ranging from $Man_5GlcNAc_2$ to $Man_3GlcNAc_2$ and bearing specific residues such as β(1,2), β(1,4)-xylose and α(1,3)-fucose that are absent in mammalian glycoproteins (Lucas *et al*, 2020; Oltmanns *et al*, 2019; Schulze *et al*, 2018). Therefore, in the purpose to use *C. reinhardtii* for the expression of glycosylated biopharmaceuticals suitable for human therapy, the engineering of its *N*-glycosylation pathways represents a major issue. Crossings of insertional mutants for the genes encoding the characterised xylosyltransferases (XTA, XTB) and fucosyltransferase (FucT) lead to a glycoengineered strain with a higher amount of $Man_3GlcNAc_2$ *N*-glycan that represent the basic structure for further glycoengineering.

However, analysis of this strain suggests that an additional xylosyltransferase is involved in the xylosylation process in *C. reinhardtii*. Additional putative candidates have been identified and inactivated in this triple mutant by CRISPR/Cas9. On another hand, the expression level of recombinant proteins remains modest in Chlamydomonas current strains. Recently, it has been shown that mutations in a gene encoding for Sir2-like NAD(+) dependent protein deacetylase may allow a better insertion of DNA that newly enters the cells with no silencing and a better expression level (Neupert *et al*, 2020). The inactivation of this protein in a glycoengineered strain is also a step forward that we performed.The output of this work would help in a near future the heterologous expression of glycosylation enzymes to complement the N-glycosylation pathway in *C. reinhardtii* and increase the biologics production yield to compete with the current expression systems of biopharmaceuticals for human therapy.

Acknowledgements

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+4 others

One-pot biosynthesis of fluorescence lectins using a cell-free transcription-translation system

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Mushroom lectins harboring carbohydrate binding specificity are powerful tools to detect glycoconjugates with N-linked or O-linked glycans for diagnosis. However, the conventional preparation for these lectins with a chemical labeling are still a bottleneck. Here, we report one-pot biosynthesis of recombinant fluorescent lectins using a cell free transcription system with *Escherichia coli* extract. Codon-optimized genes encoding the eight different types of mushroom lectins were successfully expressed as green or red fluorescent protein-fused forms in a cell-free protein synthesis system. Although the production yields of the recombinant proteins were depended on their coding genes, the resulting recombinant fluorescent lectins are apparent homo-multicomplex proteins with different molecular sizes and pI values. In hemagglutination inhibition assay, the lectins demonstrated agglutination activities towards various glycoconjugates.

Moreover, the fluorescent lectins can be applicable to detect glycan binding specifies on using a glycan microarray. The glycan binding specificities of the florescent lectins are also useful to analyze glycan epitopes expressed in different cancer cell lines. Taken together, these results provide an efficient and optimized procedure for the high-throughput synthesis and screening of lectin coding gene products based on in vitro production of the recombinant fluorescent lectins.

Acknowledgements

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Enzymatic transglycosylation for synthesis of novel glycosides from renewable galacto- and glucomann

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Hemicellulose, such as softwood galactoglucomannan is promising renewable bioresource [1]. Degradation is possible with a combination of Glycoside Hydrolases (GHs), e.g family GH27 α-galactosidase and GH5 β-mannanase in cooperation. These enzymes can also synthesize new glycosidic bonds during the catalysis in the presence of other acceptor molecules besides water, referred to as *transglycosylation*. In our work, we have demonstrated synergy between α-galactosidase and β-mannanase in formation of transglycosylation products using galactomannan as donor substrate through covalent fusion of mannose or galactose units to allyl alcohol. We have shown that using HPLC for product screening in conjunction with NMR for structural determination of novel glycosides is a powerful approach for monitoring this reaction. Coincubation of GHs with complimentary activities has potential to result in improved substrate conversion and increased synthesis yields of allyl glycosides [2]. These reactive glycosides could be utilized for downstream applications such as production of biomaterials and for surface modification [3]. Furthermore, an engineered subsite +1/+2 variant of *Trichoderma reesei* b-mannanase *Tr*Man5A showed enhanced allyl mannoside synthesis capacity [2], when compared to the wild-type. This application allows for generation of numerous different novel glycosides such as using allyl- or propargyl alcohol [4] as the acceptor, allowing for downstream application in thiol-ene or -yne click chemistry respectively [5].

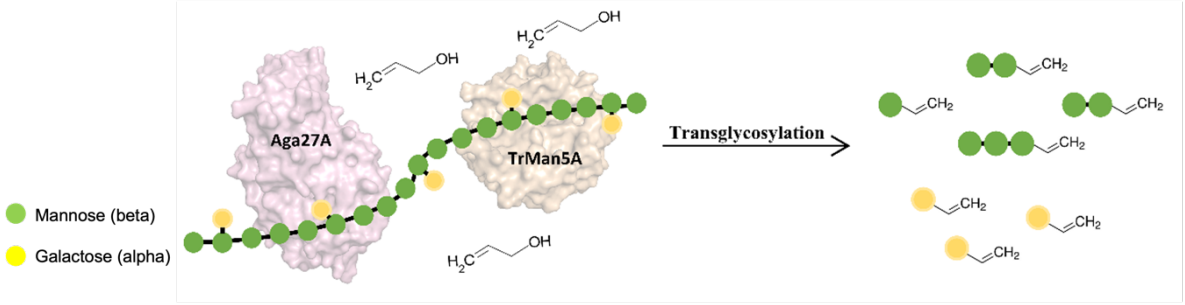

Figure 1. Schematic representation of synergistic approach to enzymatic synthesis of allyl-glycosides using locust bean gum galactomannan as substrate

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Biorefining of hemicellulosic beta-mannans and their utility as glycan donors in enzymatic synthesis

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beta-Mannans are abundant plant polysaccharides which today are underutilized. Pilot-scale recovery and purification of the main softwood hemicellulose galactoglucomannan (GGM) from a pulping stream were developed [1]. Lignin removal enhanced the yield of enzymatic GGM-conversion using Trichoderma reesei beta-mannanase TrMan5A. Several potential enzyme-generated products were demonstrated, e.g. monosugars, prebiotic oligomers [2] and activated beta-mannosides and alpha-galactosides, applicable in surface and material chemistry as monomers for synthesis of novel glyco-polymers [3-6]. We investigated the transglycosylation capacity of family GH5 beta-mannanases for the synthesis of surfactants (alkyl mannosides) and activated acrylate- or allyl- (galacto)mannosides using beta-mannans (galactomannan or GGM) as glycan donors and different alcohols or acrylates as glycan acceptors [3]. Product- and reaction-screening using mass-spectrometry, HPLC and NMR were set up. Bioinformatics and protein engineering approaches generated improved enzyme-variants [3, 4]. When using galactomannan as donor, enzyme synergy (combining TrMan5A with an alpha-galactosidase) gave improved yield [3] and demonstrated enzymatic synthesis of novel allyl- and propargyl-functionalised glycosides with applicability in further “click-chemistry” coupling [5]. Transglycosylation products were purified and analysed by NMR. Novel thermoresponsive mannoside-acrylamide co-polymers were generated from enzymatically synthesised mannoside-acrylates and acrylamide and their properties characterized [6].

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Optimisation of a practical synthesis of Lactosamine from Lactulose using a Heyns Rearrangement

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Derivatives of *N*-acetyl-D-lactosamine play an important role in a variety of biological systems and are often found as a component of lipooligosaccharide in bacteria, as well as glycoconjugates. Literature suggests that one of the more practical synthetic methods for preparing *N*-acetyl-D-lactosamine is by employing a Heyns rearrangement on commercially available lactulose [1,2].

Following the published methods for converting lactulose into *N*-acetyl-D-lactosamine (Figure 1), we encountered challenges in the purification of *N*-benzyl-lactosamine, as well as poor yields due to a variety of side-reactions. This presentation will describe our modifications to the published methods in an attempt to improve the efficiency of compound purification and optimise the yield of *N*-acetyl-D-lactosamine from lactulose.

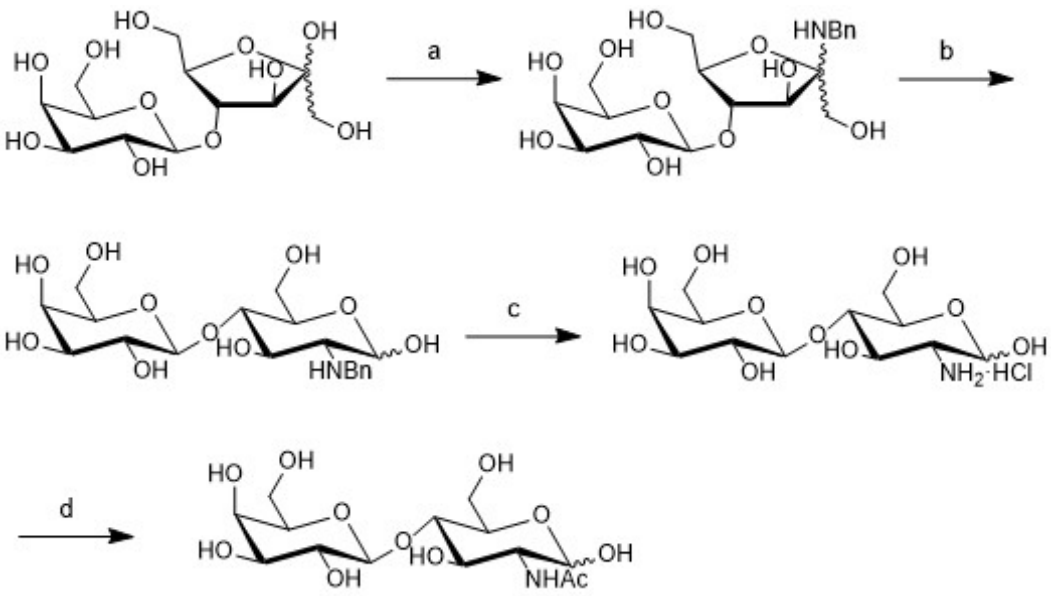

Figure 1. Conversion of lactulose into N-acetyl-D-lactosamine via a Heyns rearrangement

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In silico virtual screening of small molecules targeting GlfT2

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Galactofuranosyltransferase 2 (GlfT 2) from *Mycobacterium tuberculosis* is a glycosyltransferase with homotetrameric structure catalyzing transfer of Gal*f* residue from UDP-Gal*f* donor to growing Gal*f* chain while creating β-(1→5) and β-(1→6) bonds between Gal*f* residues. Since GlfT2 is absent in human cells, it is an ideal candidate for development of novel tuberculosis drugs. The aim of our work was to find possible inhibitors of GlfT2 using methods of computational chemistry. Ligand structures were selected in cooperation with Dr. Sean Ekins (Collaborations Pharmaceuticals, Inc.) by virtual screening of Chembridge database. Selected 92 structures were prepared using LigPrep [1] tool and optimized by Jaguar [1,2] using DFT B3LYP-D3 method and 6-31G** basis set. Subsequently the optimized structures were docked using Glide [1,3] by Standard Precision protocol into model of GlfT2 without acceptor substrate as well as model with acceptor in position allowing transfer of galactofuranose to O5 oxygen of acceptor galactofuranose residue and second model with acceptor in position suitable for transfer targeting O6 oxygen. Best ligands were selected based on their binding affinity evaluated by means of docking score as well as other parameters including interaction with Mg^{2+} ion, catalytic Asp372 and other aspartates located in binding site.

Acknowledgements

This work was supported by the Slovak Grant Agency of Science (VEGA 2/0137/20) and the Slovak Research an Developmental Agency (APVV-20-0230).

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New 6-*O*-functionalized β-glucoside substrates

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Human cells and tissues contain retaining β-glucosidases that catalyze the removal of glucose from substrates, including the ubiquitous glycosphinglipid glucosylceramide (GlcCer).[1] The lysosomal β-glucosidase, aka glucocerebrosidase (GBA1), is deficient in Gaucher disease (GD), a common lysosomal storage disorder. Mutations in the GBA1 gene cause GD, a disorder manifesting with a great variation in severity and nature of symptoms. Disfunctional GBA1 leads to an accumulation of GlcCer within the macrophages of cell lysosomes, producing irregular and metabolically activated "Gaucher cells".

The various pathophysiological mechanisms are still poorly understood as is the increased risk for Parkinsonism in GD carriers.[2] In plants steryl-β-glucosides (sterolins) occur and some of these 6-*O*-acylated structures are also present.[3] The existence of steryl glycosides (SGs) in animals and humans is poorly understood and the sterol glycosyltransferases that are responsible for the biosynthesis of SGs in plants, fungi, and bacteria have not been identified in mammals. It has recently become apparent that lysosomal GBA1 and its cytosolic counterpart β-glucosidase named GBA2 not only cleave GlcCer to glucose, but also may act as a transglucosidase, by transferring a glucose to a metabolite acceptor.

The physiological relevance is well documented for glucosylated cholesterol (GlcChol).[4] This research aims to study the ability of human GBA1 to hydrolyze 6-*O*-acylated 4MU-β-glucose and close analogues and its ability to generate SGs. More stable variants of this ester linked compound towards esterases are desired as deacylation was observed in cell lysate samples. Five different analogues of this ester linked substrate were synthesized containing different chemical linkages to their acyl or alkyl chain. These substrates were tested for their activity against pure recombinant GBA1 (Cerezyme®) in a 4MU assay. Subsequently the transglucosylation of NBD-cholesterol, a fluorogenic analogue of cholesterol, with the most promising ether linked substrate was investigated by HPTLC analysis.

These results provide the first evidence that GBA1 is able to hydrolyse and transglucosylate 6-alkylated 4-MU-β-glc substrates to metabolite acceptors such as cholesterol. This new substrate toolbox will shed some light on the relevance and scope of glycosylation by GBA1 in humans.

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Lead generation for catechols as glycomimetic LecA inhibitors of *Pseudomonas aeruginosa*

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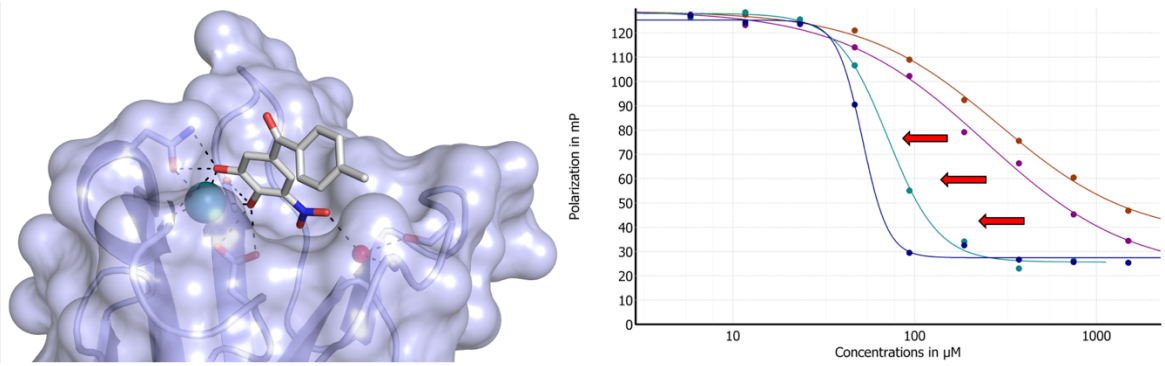
LecA is one of the lectins of *Pseudomonas aeruginosa* employed for host cell adhesion and invasion as well as biofilm formation.[1] Inhibitors of LecA therefore hold promise to synergize with antibiotics and aid in the treatment of pseudomonal infections.[1],[2]

A virtual screening followed by rigorous experimental hit conformation yielded catechols as novel glycomimetic binders of calcium-dependent lectins.[3] Here, we report on the on-going lead generation towards LecA.

Starting from the initial, fragment-like hit compounds, possessing excellent ligand efficiencies, a new frontrunner was identified (IC_{50} = 238 µM). The glycomimetic binding mode of this molecule – tolcapone, a licensed Parkinson drug marketed by Roche – was confirmed by x–ray crystallography. To gain deeper insights into the structure-activity relationship more than 3500 catechol and tolcapone derivatives of the Roche in-house library were tested experimentally at three concentrations against LecA in a competitive binding assay based on fluorescence polarization. Based on this data affinity cliffs were revealed and a set of 48 compounds was selected for further in-depth studies. The best compounds of this set reach low micromolar affinities, a 20-fold improvement compared to the initial screening hits.

This substantial increase in affinity provides a promising basis for further development of catechols as glycomimetic antivirulence drugs in light of the antibiotic resistance crisis.



Catechols are glycomimetic binders of LecA with drug-like properties

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Synthesis, conformational analysis and GalNAc-Lectin interactions of constrained C-glycosidic analog

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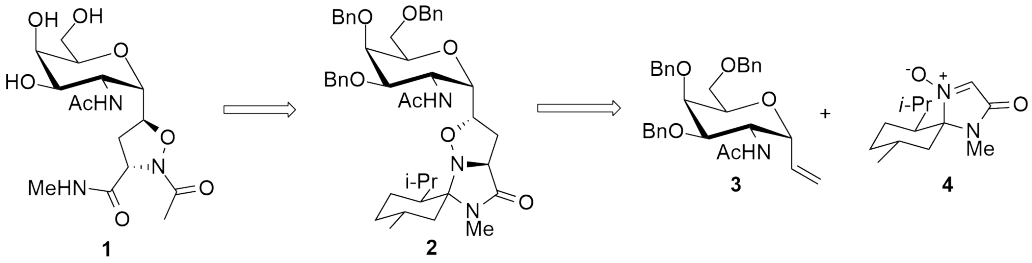
Tumor-associated carbohydrate antigens (TACAs) are highly present on cancer cells, but almost no detectable on normal cells. Such structures like T_N antigen represent interesting therapeutics targets, particularly by incorporating them in anticancer vaccines. However, glycoconjugate vaccines displaying *O*-glycoside could suffer from the lability of the *O*-glycosidic link [1] leading to a loss of the saccharidic moiety which would have a direct impact on first, the recognition and then on the immune response. The replacement of the glycosidic bond is well known to afford more stable analogues such as *C*-glycosidic ones. Incorporation of analogues containing non-natural carbohydrates in vaccine could also improve immune response of such vaccines. [2,3]

Recently, we focused our attention on the synthesis of constrained *C*-glycosidic analogues of Tn antigen, based on a [3+2] cycloaddition strategy between a *C*-vinyl-GalNAc **3** and a chiral cyclic nitrone **4** (Scheme 1). This key step provided access to cycloadduct **2** [4], precursor of analogue **1**. Conformational analysis of constrained analogue **1** and its molecular recognition with Gal-NAc-specific lectins have been studied and will be presented.



Scheme 1. Synthetic strategy of constrained C-glycoside analogue of Tn antigen

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Unreported sialylated *N*-glycans modified with *O*-acetylation of recombinant human acid α-glucosidase

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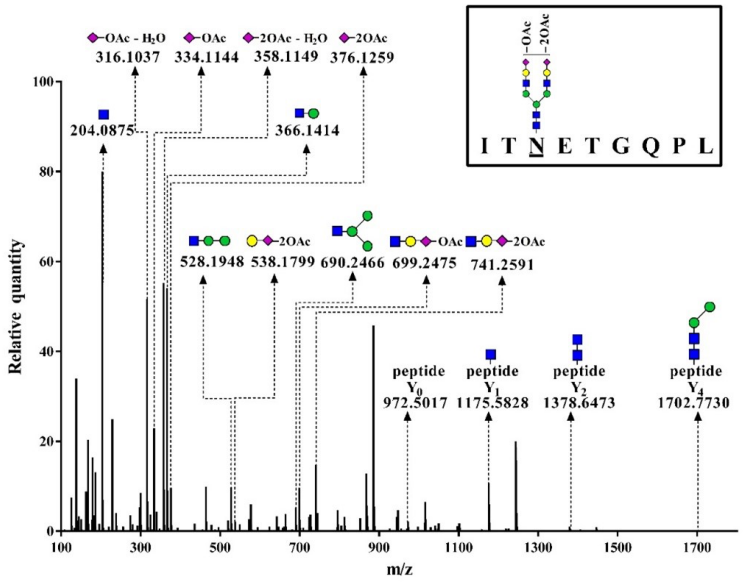
O-acetylated sialic acid (SA) attached to the *N*-glycans of therapeutic glycoproteins reportedly inhibit sialidase activity, increase protein half-life, decrease protein antigenicity, and stabilize protein conformation. Recombinant human acid α-glucosidase, Myozyme, is the only drug approved by the United States Food and Drug Administration for the treatment of Pompe disease.

In this study, structural analysis of *N*-glycans in Myozyme and the relative quantities of glycans with a focus on unreported sialylated *N*-glycans modified with *O*-acetylation were investigated using liquid chromatography (LC)-electrospray ionization (ESI)-high-energy collisional dissociation (HCD) tandem mass spectrometry (MS/MS).

The 17 *N*-glycans (6.4% of total glycans) containing mono-, di-, mono/di-, and di/di-*O*-acetylated *N*-acetylneuraminic acid (Neu5Ac) were identified. The analysis of peptides containing mono- and/or di-*O*-acetylated Neu5Ac ions sorted from all peptides using nano-LC-ESI-HCD-MS/MS confirmed six *O*-acetylation sites (Asn 140, Asn 233, Asn 390, Asn 470, Asn 652, and Asn 882), at least five of which (Asn 140, Asn 233, Asn 390, Asn 470, and Asn 652) could contribute to the drug efficacy or cellular uptake of Myozyme.

This is the first study to identify *N*-glycans containing *O*-acetylated Neu5Ac and *O*-acetylation sites in Myozyme. The results could be useful for the development of biobetter versions or glycoengineered α-glucosidase with enhanced drug efficacy or cellular uptake compared with that of the original Myozyme.



Nano-LC-ESI-HCD-MS/MS spectrum of O-acetylated glycopeptide obtained from human acid α-glucosidase.

Acknowledgements

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Synthesis of glycopolymers via Click2 Chemistry for Viral Inhibition

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Heparan sulfate proteoglycans, or HSPGs, are ubiquitous molecules found on host cell surfaces. They are implicated in the binding and fusion of many viruses such as HIV or SARS-CoV-2. The McReynolds lab is developing HSPG mimics to prevent the initial binding of the virus to the host cells, which could minimize transmission of viral pathogens, or lessen/shorten the severity or length of illness in those infected. My project focuses on synthesizing a sulfated carbohydrate compound that will be clickable to a polymer to make glycopolymers that will serve as viral-binding HSPG mimics.

A crucial step in my project is the attachment of a sulfated carbohydrate compound to a star-shaped polymer that utilizes the click chemical reaction SPAAC, or strain-promoted alkyne-azide cyclization, yielding the final sulfated glycopolymer. This reaction is under the scope of green chemistry, utilizing reagents and conditions that are environmentally friendly. Other important green aspects of this chemistry that make it attractive are the total atom economy, simple purification methods, and high resultant yields. The glycopolymer products will undergo binding affinity assays to the HIV glycoprotein, gp120, and the SARS-CoV-2 spike (S) protein. The overall goal of this project is the development of a broad spectrum anti-viral drug to combat a variety of viruses.

Synthesis of various pseudo-glycans with a *C*-glycoside linkage by metallophotoredox cross-coupling

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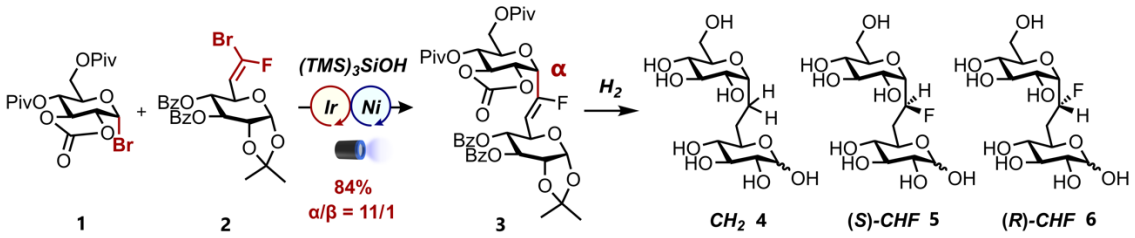
Glycan analogues with a C-glycoside linkage have resistance against glycoside hydrolases and different conformational properties from native glycans. Native O-glycoside linkage regulates glycan conformation by both steric and stereoelectronic effects (exo-anomeric effect). The simplest CH_2-linked analogues lose conformational control because of the lack of stereoelectronic effect, making their conformation more flexible than native glycans. In contrast, the conformation of CHF-linked analogues should be regulated to some extent by the gauche effect of a fluorine atom. Thus, the synthesis of three types of analogues with CH_2-, (*R*)-CHF-, and (*S*)-CHF-glycoside linkages would enable the creation of pseudo-glycans with different conformational properties as well as glycoside hydrolase-resistance, which we expected results in enhanced or altered biological activity to parent native glycans. Previously, we demonstrated that (*S*)-CHF-ganglioside GM3 analogue has exhibited superior biological activity to the native, CH_2-linked, or (*R*)-CHF-linked GM3[1]. However, the lack of a versatile synthetic method for CHF-glycosides made it difficult to apply this molecular design concept to other glycans or glycoconjugates.

In this study, we have developed a direct C-glycosylation reaction toward the efficient synthesis of CHF and CH_2-glycoside analogues. We employed the reductive cross-coupling of glycosyl bromide **1** and bromofluoroolefin **2** to furnish the disaccharide analogues **3** with fluoroalkene C-glycosides. As a result of several optimizations, we established the conditions to give **3** in high yield and stereoselectivity, by the metallophotoredox cross-coupling reaction developed by Macmillan and coworkers.[2] Furthermore, stereo- and chemoselective hydrogenation after the coupling reaction successfully produced CH_2, (*S*)-CHF, and (*R*)-CHF-linked disaccharide analogues (**4-6**). This methodology was found to be capable of synthesizing a variety of pseudo-disaccharides and pseudo-glycolipids.



✓ **α-selective C-glycosylation**
✓ **Applicable to disaccharides and glycolipids**
✓ **Stereo-and chemoselective hydrogenation**
✓ **Efficient synthesis of CH_2-, (S)-CHF- and (R)-CHF-glycosides**

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Synthesis and immunomodulatory functions of the glycolipid ligands of C-type lectin receptor Mincle

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The macrophage-inducible C-type lectin (Mincle, Clec4e, ClecSf9) recognizes various glycolipids, including trehalose dimycolate (TDM) from *Mycobacterium tuberculosis*, and activates the innate immune system.[1] Regarding the ligand recognition mechanism of Mincle, the polar head groups of the ligands (eg. trehalose moiety in TDM) are generally considered to interact with Ca ion /its proximal site around Mincle surface. Although several types of ligand structures have been reported, the detail recognition of the hydrophobic part of the ligands was not really understood. The intracellular behavior of Mincle and the ligands has not been explored either.

In order to understand the detailed structure-activity relationships, we firstly focused on b-mannosyloxymannitol glycolipid “44-2”, which consists of L-mannitol modified with b-mannosylated triacyl moieties at the 1,3,4-position showing a potent murine Mincle-mediated signaling activity. We established an efficient synthetic method for both enantiomers of 10-hydroxy stearic acid and achieved the synthesis of the diastereomeric pair of b-mannosyloxymannitol glycolipids and their analogues.[2] With using similarly modified lipid moiety, design and synthesis of trehalose diester analogues were achieved. The ligand structure–Mincle-mediated signaling activity relationships were then analyzed.[3] Based on the established synthetic method, we also synthesized molecular probes and utilized them for observation of the intracellular behavior of Mincle and the ligand.

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A glycochemical approach toward an active substance for fruit-body induction in *Pleurotus ostreatus*

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The fruit-body development of wood-rotting fungi is still unexplained. The hormones or external signal molecules, even though they are omnipresent in nature, have been not identified. In our trials to find chemical or natural substances that effectively stimulate the fruiting of *Pleurotus ostreatus* (an oyster mushroom) on agar medium [1-3], several effective compounds were found, including the sucrose ester of fatty acids (SE), synthetic triterpenoid glycoside (saponin), 3-*O*-alkyl-D-glucose, some glyceroglycolipid analogous compounds of GlcDAG, Glc_2DAG and ManDAG, and a commercially available glucosyl ceramide (GlcCer) of fungal origin, with methyl group at C-9 and the 4E double bond in the sphingoid base moiety.

With above findings as a background, we are now trying to isolate an endogenous active substance from *P. ostreatus*. We have successfully isolated the fungal type GlcCer from *P. ostreatus* by chromatographic fractionations of crude acetone extracts of the mycelium using silica-gel, suggested the possibility of inducing fruit-body formation in *P. ostreatus* at significantly low concentrations from the results of preliminary assay using paper disk. The GlcCer fractions were extracted from the three different development stages of *P. ostreatus*: the mycelium, primordia and matured fruit body, and most abundantly contained at the stage of primordia, just when fruit-body formation was started.

In this conference, we will discuss the potent biological role of GlcCer in terms of fruit-body formation in mushroom, referencing the first report on *Schizophyllum commune* [4].

Acknowledgements

This work was supported by JSPS KAKENHI Grant Numbers 18K19241 and 20H03051

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Synthesis of erythropoietin with tetraantennary *N*-Glycans

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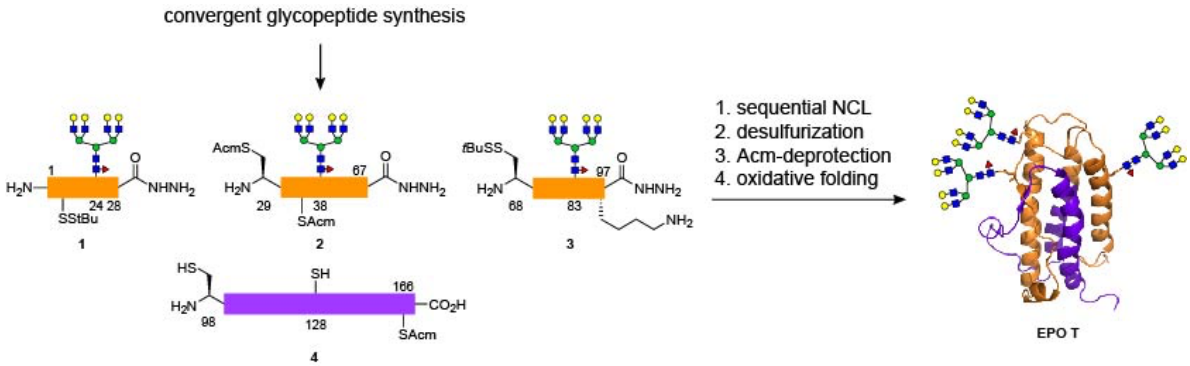
Erythropoietin (EPO) is an *N,O*-glycosylated cytokine that plays an important role in the formation of erythrocytes. Recombinantly expressed human EPO is thus used for the treatment of anaemia in kidney and cancer patients. EPO obtained from Chinese hamster ovary cells shows a broad spectrum of different glycoforms.[1] To determine glycan-related bioactivities of EPO, homogenous glycoforms are needed. This can be accomplished by chemical synthesis of glycoproteins and chemoenzymatic modification.[2,3]

Erythropoietin bearing three *N*-glycans can be synthesized by sequential native chemical ligation.[4,5] Here we show the synthesis of EPO carrying three of the typical tetraantennary core-fucosylated *N*-glycans.[1,6] The sialylation of the galactosylated tetraantennary glycopeptide segments **1**-**3** turned out to be difficult. Thus, the non-sialylated segments **1**-**4** were sequentially ligated followed by an intermediate desulfurization of the non-native cysteines to alanine and removal of the Acm groups. The homogenous glycoprotein **EPO T** was successfully obtained after oxidative folding (22 %). In contrast to the glycopeptide segments **1**-**3**, folded **EPO T** was more readily modified by α-2,6-sialyltransferase.[5]



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Naked-eye monitoring systems for carbohydrate-carbohydrate interactions

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Glycosphingolipids (GSLs) on cell surfaces aggregate laterally to form GSL-enriched microdomains. Since carbohydrate units of GSLs are prominently exposed on cell surfaces, GSL-enriched microdomains thus have densely packed carbohydrate clusters (glycoclusters) on their surfaces. It has been recognised that such glycoclusters on cell surfaces interact with other glycoclusters located at the neighbouring cell surfaces in intercellular fashions and induce various bioprocesses such as embryonic compactions and cancer metastases. We recently prepared bacterial cellulose hydrogels carrying Le^x trisaccharides, GM3 trisaccharides and Gg3 trisaccharides from commercially available *nata de coco* via $NaIO_4$ oxidation, reductive amination using propargylamine and Cu^+-catalyzed click coupling with the corresponding glycosyl azides.

Since these hydrogels have a visible scale, their homo- and hetero-assembly could be readily monitored in aqueous media without devices. This is the first example of the naked-eye monitoring system for Le^x-Le^x and GM3-Gg3 interactions, which are associated with embryonic compactions and cancer metastases. Reference experiments using similar bacterial cellulose hydrogels carrying *N*-acetyl-lactosaminides, *N*-acetyl-glucosaminides, lactosides or glucosides revealed the contributions of each carbohydrate subunit of Le^x, GM3 and Gg3 trisaccharides to their homo and hetero bindings.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP21K85298.

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Biochemical approach to the development of novel covalent inhibitors of viral 9-I-acetylesterases

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Sialic acids are a diverse family of nine carbon α-keto sugars, found at the termini of glycoproteins and glycolipids [1]. Many different pathogens use terminal sialic acids of sialoglycans as primary or accessory receptors, often showing specificity for a type of sialic acid [2]. The most prevalent post translational modification of sialic acids is the acetylation of the C9 hydroxyl group [3]. These 9-O-acetylated sialic acids are exploited by influenza C (ICV) and human coronaviruses (HCoV), specifically the HKU1 and OC43 strains [4,5]. Inhibition of haemagglutinin-esterase (HE) in recombinant HCoV-OC43 with a neutralizing antibody identified viral esterases as valid anti-viral targets [6].

The goal of this project is to identify and characterize small molecule inhibitors of viral sialic acid esterases, to generate a new type of anti-viral molecule. To achieve this goal, the HEs of HKU1, OC43, and the hemagglutinin esterase fusion (HEF) of ICV was purified. After obtaining pure protein samples, a high-throughput screen was developed to identify active small molecule inhibitor scaffolds. An active scaffold will lead to a hit-optimization study and eventually result in a novel viral esterase inhibitor.

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Serum *N*-glycans analysis by LC-MS allows the prediction of patients' response to treatment for CD

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Crohn's disease (CD) is a type of inflammatory bowel disease that affects millions of people worldwide. As a lifelong condition, it is imperative for patients to have access to the right treatment as quickly as possible. Therefore, there is a need for reliable biomarkers to predict whether an individual patient will respond to a treatment. In this study, a comparison of serum *N*-glycan profiles between responders and non-responders to Vedolizumab (VDZ), one of the treatments for CD, was used to detect potential biomarkers for the response.

Serum samples from 58 CD patients were taken before (t1) and after (t2) initiation of VDZ treatment. After enzymatic release, the glycans were fluorescently labelled and analysed by HILIC-MS. From the relative abundances of each individual glycan, derived glycan traits were calculated by combining direct traits with shared structural similarities. Correcting for age and sex, the direct and derived traits were tested across treatment responses using logistic regression and cross-validation to determine which traits at t1 are predictive of response and traits at t2 are markers of response.

Two glycans, MAN6 (0.65 AUC) and FA2G2S2 (0.7 AUC) were higher in the responders prior to treatment and moderate predictors of response, with no improvement in a combined model. In accordance with previous studies, galactosylation levels were found to increase in responders after treatment. Overall, 17 direct traits and 4 derived traits were observed as markers of response after treatment, and collectively proved to be good predictors of response (0.80 AUC).

Characterization and engineering PsGalOx, a new bacterial galactose oxidase

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Galactose oxidases (GalOxs) are highly attractive enzymes because they show high specificity and regioselectivity toward carbohydrate substrates efficiently combined with reduction of oxygen to hydrogen peroxide.[1] These metalloenzymes have a copper ion as a cofactor and are secreted by various filamentous fungi, with GalOx from *Fusarium graminearum* being the best-studied representative. These enzymes' potential include biotechnological applications in small molecule synthesis, oxygen removal, biosensors, and cell surface glycoprotein modification.[2] While galactose oxidases from fungal origins have been vastly studied, there is a growing interest in GalOxs from bacterial origin due to its ease of production and engineering.

In this work we have investigated *Ps*GalOx, an enzyme from the bacterium *Pseudoarthrobacter siccitolerans*. We have unraveled its biophysical and kinetic characteristic while tailoring its catalytic properties through enzyme engineering approaches. To accomplish this, directed evolution was applied recurring to random mutagenesis through error-prone PCR (epPCR). The workflow was developed with two high-throughput screening approaches, 'activity-on-plate' and 96-well plate liquid screening, which were successfully optimized and validated. Multiple rounds of engineering led to the identification of a variant which shows close to 20-fold higher protein production yields and 10-fold increased catalytic efficiency (kcat/Km) for D-galactose as compared with the wild-type. We are currently evolving further *Ps*GalOx to enhance its catalytic activity for aromatic alcohols and further exploring its applications to carbohydrates. These findings will contribute to a better understanding of GalOx, revealing novel or improved properties to be explored in various biotechnological fields.

Acknowledgements

Fundação para a Ciência e Tecnologia (FCT), Portugal
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Mapping the miRNA Regulation of -1,2 Fucosylation

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MicroRNAs (miRNAs) are short segments of non-coding RNAs that regulate the translation of messenger RNA (mRNA) into protein. Glycogenes, i.e. the genes that control glycosylation, are highly regulated by miRNAs. The glycan motif *α*-1,2 fucose is a crucial component of Lewis^{b} and Lewis^{y} and is the defining feature of the blood group H antigen.

FUT1 and *FUT2* genes encode a Golgi stack enzyme α-1,2-fucosyltransferase that catalyzes the transfer of 1,2-linked fucose to galactose residue of glycans.

In this project, we aim to generate high throughput data that shows the regulation of *FUT1* by miRNA using miRFluR high-throughput assay. This assay utilized genetically encoded dual-colour fluorescence reporters to identify regulatory miRNAs thus generating a comprehensive map of the regulation of *FUT1* by approximately 2700 human miRNAs.

pMIR-3' UTR sensor of *FUT1* has been developed and by utilizing our high throughput assay we have identified 76 regulatory miRNAs for *FUT1*. We have validated the regulation of *FUT1* gene by these miRNAs at a protein and glycan level via western blot and lectin staining experiments, respectively in multiple cell lines. The dominant paradigm is that miRNA binding to mRNA represses protein expression. Nonetheless, this work and other ongoing and recently published work from our lab showed miRNA –mediated up-regulation of glycogenes via direct interaction. Our work overturns the current thinking of miRNA upon the regulation of glycogenes.

Catching E. coli: targeting FimH with *C*-glycosidic α-mannoside modified cellulose

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C-Glycosides, where the glycosidic linkage consists of a carbon-carbon bond, are stable against most chemical reactions as well as hydrolysis in biological systems, compared to the naturally occurring *O*- and *N*-glycosides.[1] This stability makes *C*-glycosides interesting candidates for therapeutics against various diseases. Different ligation handles, such as azides, alkynes, amines or aldehydes, can be introduced, which makes the linkage to e.g. polysaccharides via orthogonal conjugation methods possible.[2] Thus, specific biological properties of the polysaccharide backbone can be introduced by target specific choice of the sugar based small molecule entity.

For example, uropathogenic *E. coli* (UPEC) uses lectin FimH to bind to D-mannose residues on the surface of human cells, causing urinary tract infections (**Figure 1A**). D-Mannose can therefore be used in an antiadhesion therapy against UPEC. To increase the effectivity of such therapies, different potent FimH inhibitors have already been synthesized.[3] In our approach, we synthesized *C*-glycosidic D-mannose derivatives followed by linkage to an azido modified cellulose matrix (**Figure 1B**). Synthetic details and results will be presented.

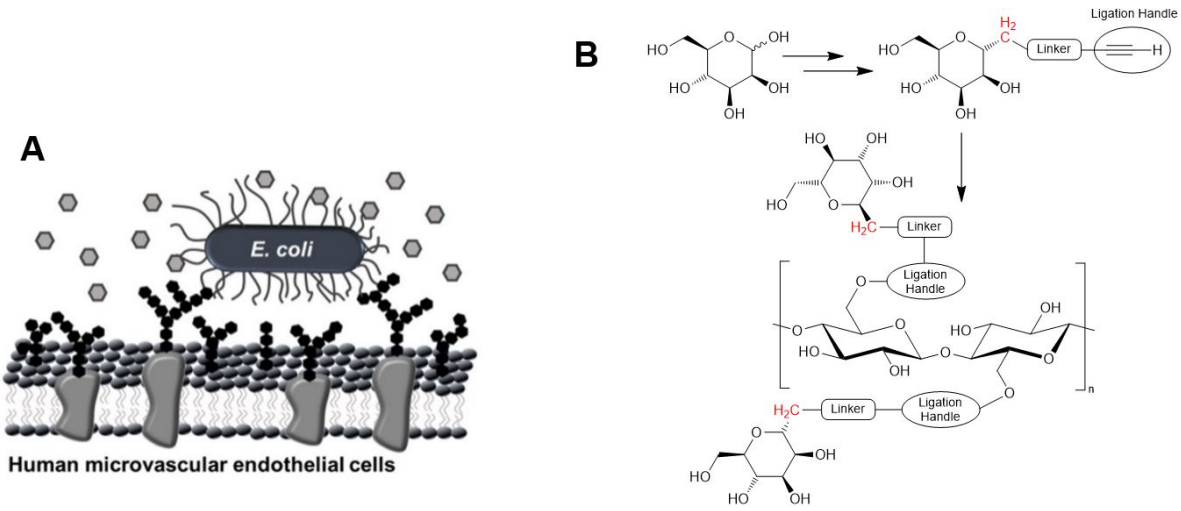

Figure 1: A) E. coli binds to human cells using FimH.[3] B) Synthesis of C-glycosidic D-mannose derivatives for a covalent linkage to cellulose.

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Variability of human alpha-1-acid glycoprotein *N*-glycome

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Alpha-1-acid glycoprotein (AGP) is a heavily glycosylated protein in human plasma and one of the most abundant acute phase proteins in humans. Glycosylation plays a crucial role in its biological functions, and alterations in AGP N-glycome have been associated with various diseases and inflammatory conditions. Still, large scale studies of AGP N-glycosylation in general population are lacking.

Using recently developed high-throughput glycoproteomic workflow for site-specific AGP N-glycosylation analysis, 803 individuals from Croatian island of Korcula were analysed and their AGP N-glycome data associated with biochemical and physiological traits.

Regression analysis revealed that 61 out of 79 AGP glycopeptides were significantly associated with sex, where the largest differences between males and females were observed in the levels of fucosylation and sialylation. While 38 out of 79 AGP glycopeptides were significantly associated with age, the strongest associations were not as strong as associations observed between AGP N-glycome and sex and the patterns of changes associated with age were not similar across different glycosylation sites.

Furthermore, regression analysis with age and sex included as covariates was performed on available biochemical and physiological traits. The results revealed strong associations between multiple AGP N-glycome traits and biochemical (triglycerides, uric acid, glucose, fibrinogen etc.) and clinical (height, weight, waist, and brachial circumference, etc.) parameters as well as smoking status.

Synthesis of fully desymmetrized trehalose building blocks

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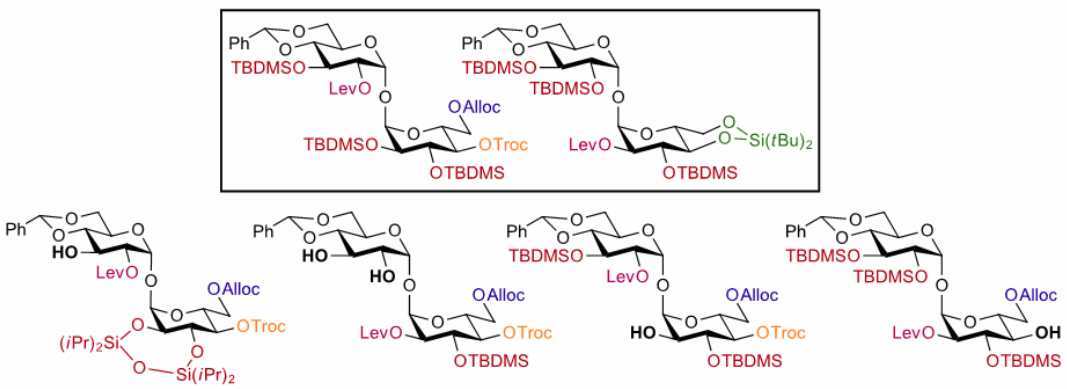
Trehalose-containing glycans found in bacteria (*Mycobacteria, Corynebacteria*), fungi (*Fusarium*) and worms (*Caenorhabditic elegans*) play an essential role in host-pathogen interaction, cell signaling and bacterial pathogenesis and are therefore important synthetic targets. The chemical synthesis of trehalose derivatives as part of glycoconjugates and parasitic glycans is challenging as it requires either stereoselective 1,1´-glycosylation or desymmetrization of eight hydroxyl groups in a C_2-symmetric nonreducing disaccharide with the aim of attaching different functionalities at a specific position. We aimed to produce a collection of differently and variably protected non-symmetrical trehalose building blocks ready for a final chemical derivatization with functional or reporter groups.

We developed an efficient and scalable (7-step, multigram scale) reaction sequence leading to a set of desymmetrized trehaloses as a starting point for a library of fully orthogonally protected trehalose derivatives. Using a site-specific protection strategy and regioselective functionalization, we created a pool of trehalose-derived building blocks with one or two free hydroxyl groups at specific positions. Such synthetically advanced trehalose derivatives can find wide application in the synthesis of sulfolipids[3], derivatized trehalose molecules ready for biorthogonal chemistry to track pathogenic *Mycobacterium* strains or to label the mycobacterial membrane; or for the development of probes to explore mycobacterial transporter proteins.[4]



Fully orthogonally protected trehalose building blocks (box), selection of desymmetrised trehaloses

Acknowledgements

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Enzymatic glycosylation catalyzed by GH84 O-GlcNAcase using an oxazoline derivative

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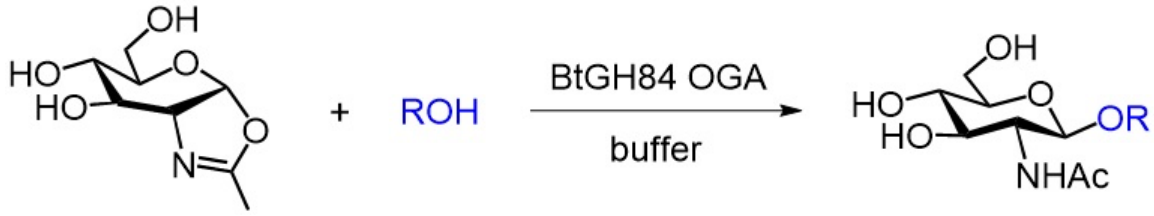
O-Glycosylated *N*-acetyl-β-D-glucosamine-selective *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.169) (OGA), belonging to glycoside hydrolase family 84 (GH84), has been found in various organisms ranging from humans and bacteria and catalyzes the elimination of *N*-acetyl-D-glucosamine (GlcNAc) from serine and threonine residues on proteins in humans. Bacterial OGA also belongs to GH84 and is a retaining glycosidase via a substrate-assisted hydrolysis mechanism through a sugar 1,2-oxazoline derivative as an intermediate. It is well known that retaining glycosidases have potential enzymatic transglycosylation. However, as far as the authors know, no transglycosylation catalyzed by GH84 OGA has been reported. In this study, we report the first enzymatic transglycosylation catalyzed by the GH84 OGA from *Bacteroides thetaiotaomicron* (BtGH84 OGA) [1].

The BtGH84 OGA-catalyzed transglycosylation using 1,2-oxazoline derivative of GlcNAc (GlcNAc-oxa) as a glycosyl donor substrate was examined. The transglycosylation product was observed in case of excess feeding of *N*-(2-hydroxyethyl)acrylamide as an acceptor substrate in a buffer pH 7.0. No glycosylation product was observed when pNP-GlcNAc was used as a glycosyl donor substrate under the same condition as that using GlcNAc-oxa. When the GlcNAc derivative with a triazole-linked acrylamide group were used as an acceptor substrate, b1,6-linked disaccharide of GlcNAc was obtained. Molecular docking simulation of the transglycosylation products for BtGH84 OGA suggests the substrate recognition around the catalytic site of the enzyme.



Scheme. Enzymatic transglycosylation catalyzed by BtGH84 OGA using GlcNAc-oxa.

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Towards specific small molecule ligands for the C-type lectin-like receptor 2 (CLEC-2)

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Cardiovascular diseases are the primary cause of death worldwide [1]. One treatment option for acute coronary syndromes, stroke, and peripheral arterial disease are antiplatelet drugs that inhibit platelet aggregation and activation [2]. In this context, the glycoprotein receptor C-type lectin-like receptor 2 (CLEC-2) emerged as a promising therapeutic target due to its critical involvement in thrombocyte-associated pathologies and alongside predicted minimal disruption of homeostasis [3].

Here we present our efforts towards the development of specific ligands for CLEC-2 through a multifaceted, biophysical approach. Initially, we evaluated a repertoire of known small molecule ligands for CLEC-2 using a range of biophysical assays (thermal shift assay, nuclear magnetic resonance & grating-coupled interferometry). Further, we applied a fragment-based screening approach to discover novel ligands for CLEC-2.

Surprisingly, our experimental efforts to reproduce previously reported evidence of interaction between CLEC-2 and the available small molecule ligands yielded inconsistent results. This discrepancy emphasises the necessity for comprehensive and careful evaluation of ligand-receptor interactions. Ligands derived from the identified fragments hold the potential to modulate CLEC-2 and platelet activity, which could open up new avenues to tackle the significant burden imposed by cardiovascular diseases.

Acknowledgements

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Elucidating mucinase and bacteria toxin binding specificities towards mucin *O*-glycopeptides

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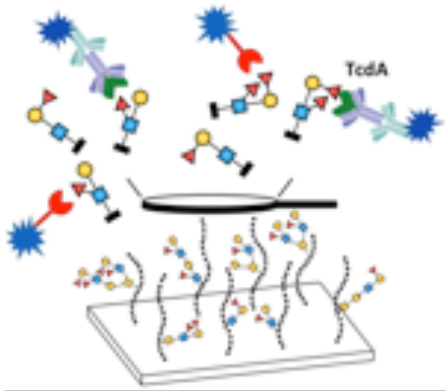
Mucins are densely *O*-glycosylated membrane-bound or secreted proteins ubiquitously found on the epithelial cell surface.[1] They are part of the innate immune system and play major roles as protective barriers to defend the host against invading pathogens.[2] However, bacteria and viruses have co-evolved with the human host and developed strategies to promote virulence for instance by adhering to carbohydrate ligands on the host cell-surface via pathogenic lectins. In order to improve our understanding of the pathogenic adhesion processes on a molecular level, we study the interactions between bacteria adhesion proteins and *O*-glycans presented on mucin tandem repeat peptide backbones. Through the use of carbohydrate binding bacteria toxins such as Toxin A, interactions with glycans at the epithelial cell-surface, results in internalization of enzymes that promote virulence by modulation of the intracellular host GTPases. To enable penetration of the mucus layer and make the epithelial cell-surface accessible bacteria further secrete glycosidases and mucinases. In recent years we have through a chemoenzymatic approach prepared extensive libraries of mucin tandem repeat glycopeptides consisting of different O-glycan core structures modified with LacNAc, LacdiNAc, sialylation and fucosylation.[3-5] On Eurocarb we will present our latest results elucidating binding interactions between mucin *O*-glycopeptides and mucinase glycan binding domains as well as binding studies of toxin A originating from different pathogenic bacteria strains.



Elucidating Mucin O-glycopeptide binding partners.

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Synthesis of oligosaccharides related to Rhamnogalacturonan-II

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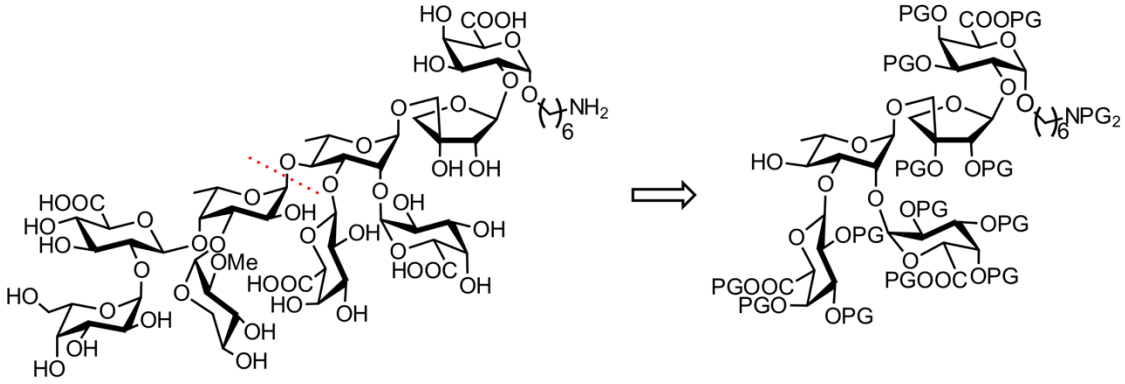
Pectin is one of the most complex, yet poorly understood polysaccharide structures in nature, with irreplaceable relevance for the human diet.[1] The pectin domain rhamnogalacturonan-II (RG-II) is the most heterogenic, structurally diverse part of pectin and crucial for vascular plant growth and survival.[2] We present here our work towards a chemical total synthesis of linker-functionalized RG-II side chain A, including the retrosynthesis and the synthesis of a pentasaccharide acceptor fragment.

Key features of the target structure are seven different monosaccharides including the rare sugar apiose, three branches, five 1,2-*cis*-glycosidic linkages, three galacturonic and one glucuronic acid and a methyl ether. The total synthetic approach relies on carefully chosen orthogonal protective group patterns and various glycosylation methods to tackle this highly branched and unusually complex nonasaccharide.



Acknowledgements

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Towards catalytic aminoglycoside: probing the modification of kanamycin B at 3'- and 4'-positions

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The ongoing emergence of multidrug-resistant pathogens has become a severe clinical problem for the treatment of infectious diseases worldwide. To address this issue, we have recently set out to explore the concept of "catalytic antibiotics" as a new paradigm in antibiotics research.

Concerning the catalytic aminoglycoside, we synthesized a series of new derivatives of neomycin B in which the catalytic warhead, 1,2-diamine appendage was selectively attached at the 4'-position of ring I as a single arm to catalytically cleave the scissile phosphodiester bond of rRNA by general acid/general base catalysis. The new derivatives exhibited comparable antibacterial activity to the parent neomycin B against wild-type bacteria and were especially potent against resistant and pathogenic bacteria. However, all attempts to demonstrate cleavage of the scissile phosphodiester bond of rRNA have been unsuccessful. By using molecular dynamics simulations, we found that the designed single-arm ethylenediamine appendage was unable to efficiently cleave the scissile phosphodiester bond of rRNA.

To overcome this limitation, we decided to install the general base and general acid appendages on the aminoglycoside scaffold as two separate arms to allow efficient catalysis. Herein, we describe our efforts in probing potential sites of kanamycin B (KanB) scaffold to introduce the desired two catalytic arms. We initially selected the 4'- and 3'-positions of ring I as the modification sites and show the synthesis and evaluation of the new derivatives of KanB, compounds **2**-**5** (Fig. 1).

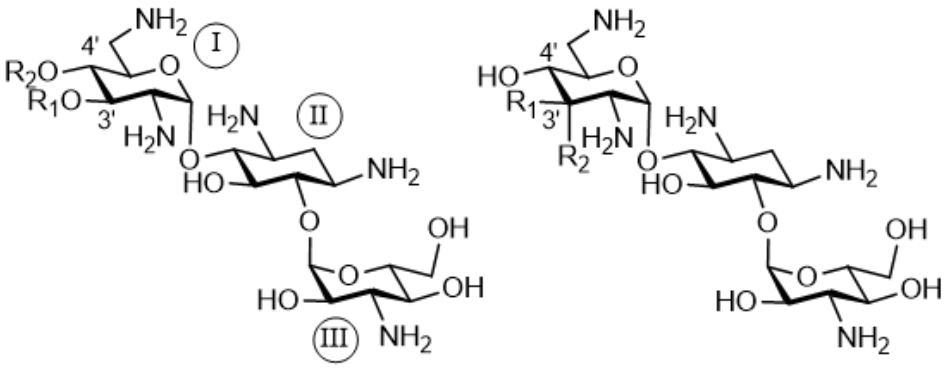

Figure 1. Kanamycin B and its new derivatives studied.

Active material from Bulgarian fenugreek polysaccharides

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Trigonella foenum graecum L. (Fenugreek) is a dicotyledonous medicinal plant which belongs to the Fabaceae family. Its seeds are used as a traditional remedy for decreasing blood sugar levels, lactation stimulant and kidney problems. These therapeutic applications are attributed to various bioactive compounds present in the seeds, such as saponins, flavonoids, alkaloids [1]. They also contain carbohydrates and, in particular, galactomannans which could be used for bone tissue engineering [2]. However, there is limited information on the determination of the composition of Bulgarian fenugreek polysaccharides and their use for hydrogel preparation. In the present study, isolation and purification of fenugreek seeds polysaccharides was carried out. Galactomannan composition and structural characterization were determined by GC-MS, HPAEC, SEC-MALLS, NMR and FTIR.

The obtained galactomannan was regio-selective oxidized using TEMPO chemistry to generate new family of polyglycuronic acid. Antioxidant and antimicrobial activities were investigated and compared with the natural Fenugreek galactomannan. The polyglycuronic acid derivative was investigated for reticulation using $CaCl_2$ as cross-linker to generate stable and homogenous hydrogels. This new anionic-biomolecule constitute an innovative active polysaccharides-based hydrogel as a bioink for 3D-bioprinting in the tissue engineering field.

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Genetic Engineering and Chemical Approaches for Enhancing Siglec-3 Binding in “HASLECs"

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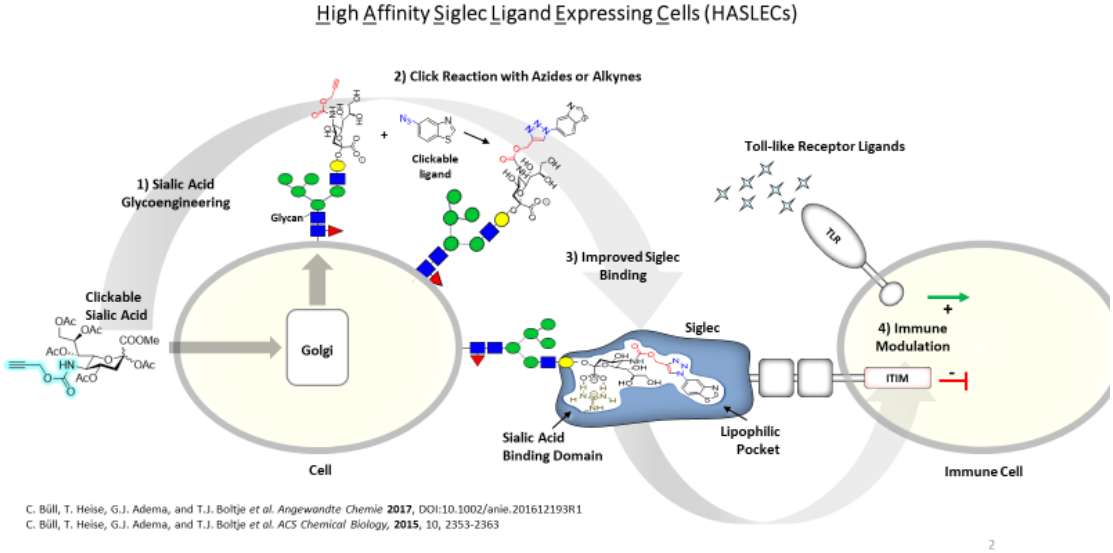
Sialic acids or N-acetylneuraminic acids (Neu5Ac) are a diverse family of 9-carbon monosaccharides synthesized in mammals and some prokaryotes, present at the outermost end of N-linked and O-linked carbohydrate chains and in lipid-associated glycoconjugates. Sialic acid sugars form the ligands for the sialic acid binding immunoglobulin-like lectin (Siglec) family, which are immunomodulatory receptors expressed by immune cells. Interactions between sialic acid and Siglecs regulate the immune system, and aberrations contribute to pathologies like autoimmunity and cancer. Sialic acid/Siglec interactions between living cells are difficult to study owing to a lack of specific tools.

We combined metabolic labeling using unnatural sialic acid derivatives in cells with combinatorial loss/gain of individual sialyltransferase/suflotransferase genes to optimize Siglec-3 binding. Using bioorthogonal chemistry, we introduced chemical modification of alkyne and azide containing sialic acid derivatives to afford High Affinity Siglec Ligand Expressing Cells (HASLECs). Combining this chemical editing of sialic acids with the genetic engineering of the sialyltransferases that underpins their biosynthesis, we identified very potent Siglec-3 binding HASLECs.



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F-labelled 2-aminobenzamidoximes as probes for ^{19}F-NMR analysis of aldoses

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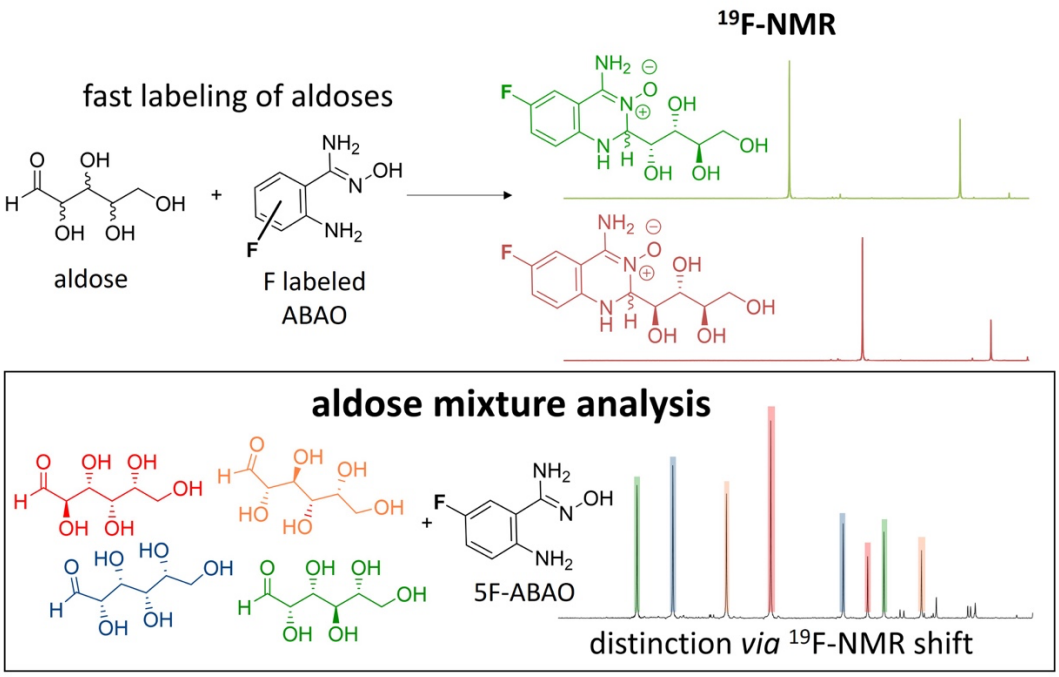
Aldoses are structurally composed of a reactive aldehyde group and multiple hydroxy groups. Each specific sugar exhibits a defined stereochemistry of these alcoholic moieties. Being constitutional isomers, the differentiation between sugars, especially in mixtures, is challenging. Previously, we have described a photometric kinetic assay to determine the open-chain content of aldoses utilizing the selective reaction with 2-aminobenzamidoxime (ABAO) [1].

Herein, we have taken further advantage of this aldehyde-selective reaction, using it for a qualitative aldose assay based on ^{19}F-NMR, a highly sensitive and fast method. The influence of the position of the F-label on the adduct formation rates was determined and derivatives of selected aldoses were prepared and the deviation of chemical shifts in ^{19}F-NMR of the products were compared. While all four tested F-ABAO derivatives showed differences in shifts between the tested aldoses, one derivative was especially promising for the application in a qualitative sugar assay and the scope was expanded to a larger set of aldoses for this derivative.



Acknowledgements

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beta-Stereoselective *C*-aryl glycosylation through intramolecular « aryl » delivery (IArD)

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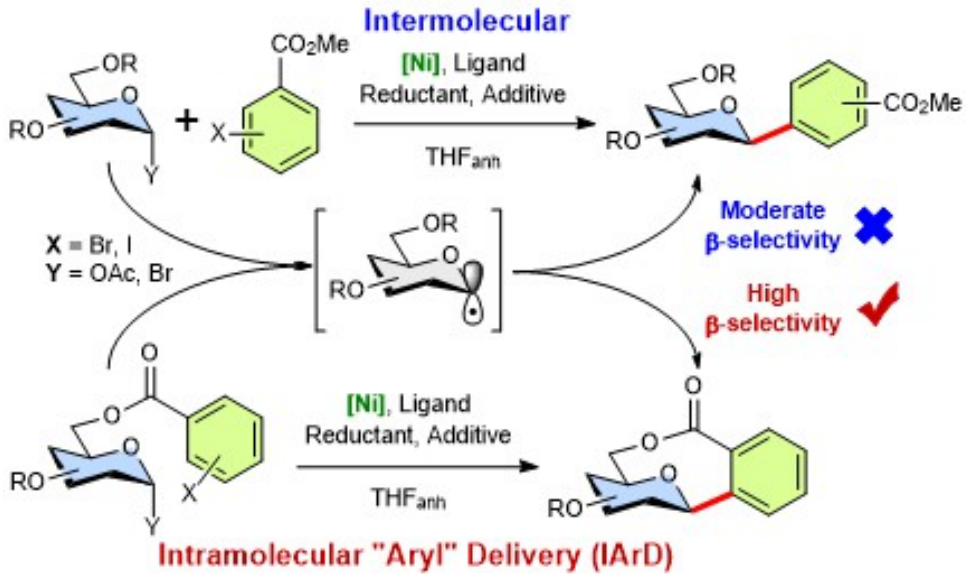
C-Aryl glycosides are an important class of natural products with various biological activities against cancer, bacteria or hypoglycemic agents in the context of type 2 diabetes. The control of glycaemia can be achieved by the inhibition of enzymes such as glycogen phosphorylase (GP) or the sodium/glucose co-transporter type 2 (SGLT2) for which the best ligands identified and marketed are *C*-aryl glycosides.[1] A particular attention must be paid to the α/β-stereoselectivity of the *C*-glycosylation since stereoelectronic effects favor the axial configuration of the anomeric radical thus leading to *C*-aryl αlpha-glycosides. Very few and recent methodologies propose alternatives with improved beta-selectivities in an intermolecular *C*-glycosylation under Ni(0) catalysis.[2] We investigate herein (1) the intermolecular approach towards such *C*-aryl b-glycosides using nickel catalysis, but also (2) the application of intramolecular "aryl" delivery[3] (**IArD**) for an exclusive beta-stereoselectivity in the *C*-glycosylation of hexopyranosides.

Conditions were initially tested under intermolecular coupling conditions using an aryl aglycon activated with an iodide (X = I) and glycosidic donors (Y = OAc, Br) in the presence of nickel ($Ni(acac)_2$), a terpyridine ligand, a reductant (Mn or Zn) and additive ($ZnCl_2$, NaI, $MgCl_2$). The results obtained showed an influence of several parameters such as additive and the reductant. The optimized conditions were then tested in the **IArD** strategy and provided, so far, limited yields of the macrocyclic *C*-aryl beta-glycoside. A discussion will be provided with additional data and a better understanding of the parameters governing both the yield of the *C*-glycosylation as well as its stereoselective outcome.

The *C*-aryl beta-glycosides will be synthesized in the glucose series towards GP or SGLT2 ligands and applications in the context of type 2 diabetes,[1] through functional diversification at the benzoic acid moiety. Furthermore, application into the galactose or fucose series will also be investigated to give access to potential ligands of lectins with application in glycobiology.[4]



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Libraries of Functionalized Human Milk Oligosaccharides

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Human milk oligosaccharides (HM-OS) were converted into glycine-spacered derivatives [1] to give rise to a library of over 50 amino functionalized oligosaccharides. These included core structures (lactose, N-acetyllactosamine, lacto-N-tetraose, lacto-N-neotetraose, lacto-N-hexaose, lacto-N-neohexaose, para-lacto-N-hexaose, lacto-N-iso-octaose), fucosylated (2'/3-fucosyllactoses, difucosyllactose, lacto-N-fucopentaoses I,II,III,IV,V, lacto-N-difucohexaoses I,II, lacto-N-neodifucohexaose I, monofucosyllacto-N-hexaoses I,II,III, monofucosyllacto-N-neohexaoses I,II, monofucosyl-para-lacto-N-hexaose I,IV, difucosyllacto-N-hexaoses a,b,c, difucosyllacto-N-neohexaose I,II, difucosyl-para-lacto-N-hexaose, difucosyl-para-lacto-N-neohexaose, trifucosyllacto-N-hexaoses I,II, monofucosyllacto-N-iso-octaoses I,II, difucosyllacto-N-iso-octaoses I,II, trifucosyllacto-N-iso-octaoses I,II, sialated (3'/6'-sialyllactoses, sialyllacto-N-tetraoses a,b,c, disialyllacto-N-tetraose, monosialyllacto-N-hexaose, monosialyllacto-N-neohexaose, disialyllacto-N-hexaose), and sialated/fucosylated (3'-sialyl,3-fucosyllactose, sialyllacto-N-fucopentaose, monosialyl,monofucosyllacto-N-hexaose, monosialyl,monofucosyllacto-N-neohexaose, disialyl,monofucosyllacto-N-hexaose) HM-OS.

These derivatives may be used for plasmon resonance studies [2], preparation of neoglycoconjugates [3] and nanoparticles [4] but most promising is their application for glycoarray preparation via amino [5] or aldehyde [6] group, or by click addition after modification with 4-pentinoic acid.

Acknowledgements

This work was supported by the Ministry of Science and Higher Education of the Russian Federation (Contract No. 075-03-2023-642)

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Rutinosidase and other diglycosidases: rising stars in biotechnology

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Diglycosidases are glycosidases catalyzing the cleavage of entire disaccharide moieties from the aglycone. Rutinosidases, main diglycosidase representatives, cleave rutinose (α-L-Rha-(1-6)-β-D-Glc) from rutin or other rutinosides (Fig. 1A). Some diglycosidases can be classified as monoglucosidases with extended substrate specificity. They also have distinct synthetic (transglycosylating) abilities. Rutinosidase from *A. niger*[1] and *A. oryzae* (GH5-23) can glycosylate various acceptors, including phenols, in a good yield using priceworthy rutin as a glycosyl donor. Surprisingly, they are able to glycosylate species such as inorganic azide to form β-rutinosyl azide [2] or carboxylic acids forming (anomeric) glycosyl esters [3], being a unique property of glycosidases. The variant of *A. niger* rutinosidase mutated at the catalytic nucleophile residue E319A is capable of generating α-rutinosyl azide [2]. It was found that rutinosidase is able to accept quercetin 3-β-glucopyranoside as a substrate and thus it is also able to transfer a β-glucosyl moiety [1]. This enzyme has a dual glycosylation activity, generating either rutinosides or glucopyranosides [4]. Its broad substrate specificity has also been demonstrated in the enzymatic cleavage of various 6"-acylated quercetin-3-*O*-β-glucopyranosides (Fig. 1B). Rhamnose-containing compounds (rutinose) are attracting attention due to their anti-cancer activity and as skin anti-aging agents [5]. Their easy availability through the action of rutinosidase opens a whole new avenue in cancer therapy, dermatology, and other fields.

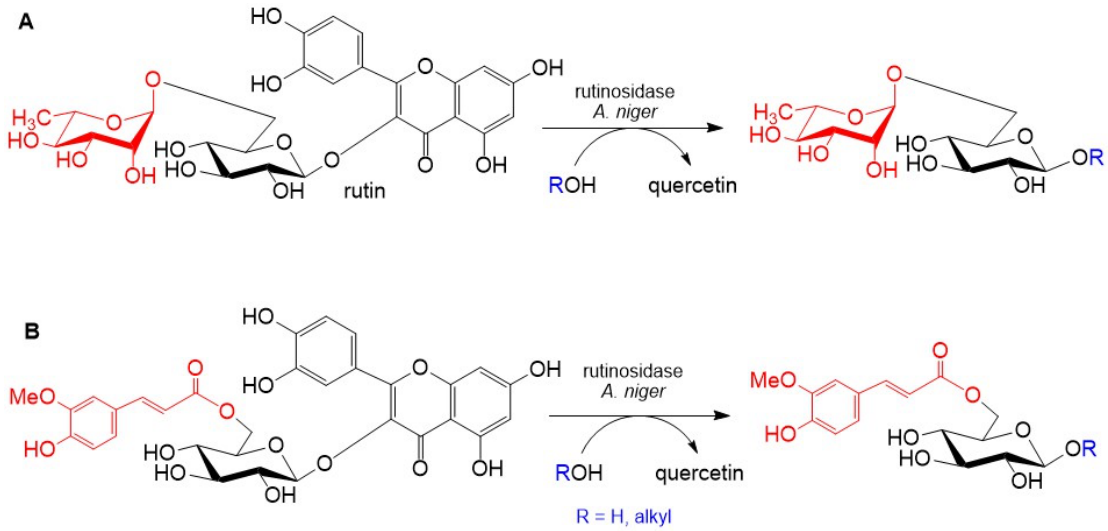

Fig.1 A Hydrolysis/transglycosylation by rutinosidase; B 6”-feruloyl quercetin 3-O-β-glucopyranoside

Acknowledgements

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Multiscale computational approaches to investigate the solution structure of saccharides

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Saccharides exhibit a high degree of conformational heterogeneity which makes studying their dynamic solution structure a demanding task. In addition to well-established experimental methods (e.g. the NMR spectroscopy) a series of theoretical approaches can be used either as independent or complementary techniques. One of the most popular one are the explicit-solvent, classical molecular dynamics (MD) simulations. However, the more advanced, quantum-mechanical (QM)-based techniques allow, at cost of reduced computational efficiency, to determine a series properties relying on electronic structure of molecule. There is a growing number of reports, indicating the QM-based calculations should not be limited to only a single saccharide structure (even if this structure has the lowest possible energy) but, instead, they should consider a broad set of diverse structures, accounting for conformational diversity of saccharides in solution systems.

We are going to present and discuss the computational approach that combines the most beneficial features of classical MD simulations and high-level QM computations. According to this method, designed, tested and validated in the context of small saccharide molecules, the structural data from classical MD simulations are rationally subsampled and passed to further, more advanced calculations, carried out at the QM level of theory. In particular, such approach can be used for studying the conformational properties of non-standard saccharides (e.g. conformers of glycosidic linkage and other rotatable groups), problematic degrees of freedom (e.g. ring distortions in both furanoses and pyranoses) as well as anomeric and tautomeric properties of monosaccharides. In particular, the use of QM-based methodology allows for eliminating the uncertainties related to the accuracy of the classical force fields, and, more importantly, for obtaining the spectroscopic data that can directly be referred to experiment (e.g. *J*-coupling constants or chemical shifts).

The applicability and generality of our approach will be demonstrated by presenting the calculation results related either to well-studied systems (D-glucopyranose, L-iduronate) or to rare sugars (tautomers of D-allosamine). Moreover, we will show how our theoretical predictions may contribute to obtaining valuable information on the saccharides that are either synthesized compounds or isolated from natural sources.

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Synthesis of advanced sialylated glycans libraries by automated glycan assembly

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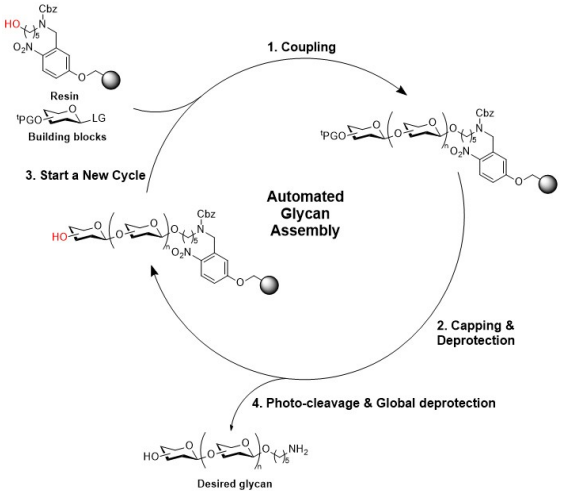
The pursuit of designing and synthesizing complex systems that closely resemble the highly glycosylated extracellular matrix (ECM) has gained significant interest in various fields: 3D cultures, drug delivery and tissue engineering [1]. Supramolecular hydrogels hold great potential for achieving this goal. However, the development of these hydrogels has been hindered by a lack of knowledge behind the fundamental parameters governing their hierarchical self-assembly. Furthermore, the limited examples of carbohydrate-based hydrogels in the literature predominantly involve homomultivalent presentation of a single carbohydrate, which falls short of replicating the complex heteromultivalent nature of the ECM [2].

To address these challenges, this project aims to synthesize biocompatible supramolecular hydrogels that emulate the highly and heterogeneously glycosylated ECM through a hierarchical supramolecular self-assembly approach, by employing rationally designed neoglycolipids. Various photopolymerizable neoglycolipids with distinct sugar headgroups, such as α-D-mannose, β-D-galactose, β-D-glucose, and β-lactose, have been synthesized. The hierarchical self-organization of these neoglycolipids into different hydrogel structures has been thoroughly characterized. Importantly, the resulting hydrogels exhibit multiple interactions with fluorescent lectins specific to the exposed sugars, highlighting their heteromultivalency.

In addition, hybrids of hydrogels and glyconanoring-coated carbon nanotubes have been developed to enhance the mechanical properties of the constructs.



Acknowledgements

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Semi-synthetic study of glycoprotein interleukin-6 (IL-6)

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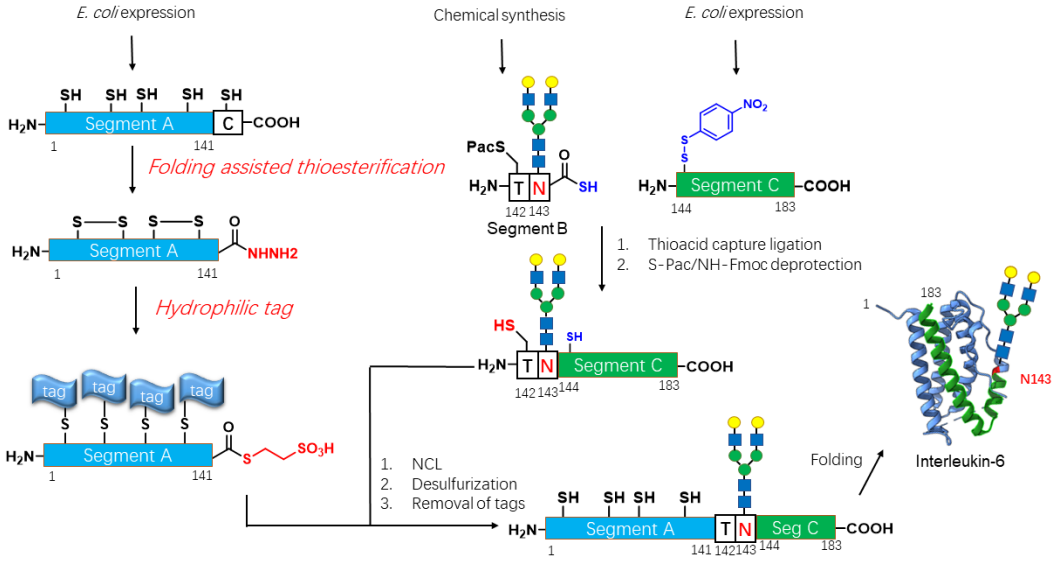
As a ubiquitous post-translational modification on protein, *N*-glycosylation performs various functions which are closely related with its structure. The elucidation of such structure-function relationship requires preparation of homogenous glycoprotein. However, mammalian cell expression only produce protein with heterogenous *N*-glycan structure, while total chemical synthesis using solid-phase peptide synthesis (SPPS) is usually laborious and time-consuming.

In this research, to investigate human interleukin-6 (IL-6) bearing *N*-glycan at N143, we established a novel semi-synthetic strategy to obtain homogeneous glycoprotein within few chemical conversion steps. In this strategy, a key building block: glycodipeptide (T142-N143) bearing asialo *N*-glycan was chemically synthesized, while non-glycosylated fragments were prepared by *E. coli* expression. Notably, the challenging expressed peptide thioesterification of N-terminal fragment containing 141 residues was achieved by utilizing a novel "folding-assisted thioesterification" method. In addition, the problems derived from hydrophobicity of IL-6 were solved by using a novel hydrophilic tag, which could improve the solubility of peptide and impede aggregation.

Finally, we succeeded in folding of N143-glycosylated IL-6 after two steps of ligation and removal of hydrophilic tag. We suppose that these newly developed methods could greatly facilitate semi-synthesis of homogeneous glycoprotein, especially challenging synthetic target, for elucidation of *N*-glycan functions.



Semi-synthetic strategy of N143-glycosylated interleukin-6

Deoxygenation as a useful tool for tuning the selectivity of glycosidase-targeted ABPs

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Glycosidases are enzymes abundant in nature that hydrolise glycosidic linkages. Inhibitors and activity-based probes (ABPs) of glycosidases are valuable tools to study these enzymes and enable therapeutic and biotechnological applications. [1]

One widespread class of glycosidase ABPs is the cyclophellitol aziridines. These usually are structural mimetics of natural sugars and bear an electrophilic aziridine warhead that facilitates irreversible binding with the enzyme. Because of the high degree of resemblance of "fully oxygenated" cyclophellitol-type inhibitors to their natural carbohydrate counterparts, high target selectivity can be achieved. However, the many hydroxyl groups present in these molecules prohibit broad-spectrum activity-based profiling studies and make these compounds less attractive from a medicinal chemistry point of view. Cyclophellitol aziridines with modified structures can ameliorate these issues.

Here we present the design and synthesis - using both chiral pool and *de novo* approaches – of a comprehensive library of deoxygenated cyclophellitol aziridines (24 compounds in total) that have been tested against therapeutically relevant GBA, GBA2 and GBA3 enzymes to provide insight into the contribution of each hydroxy group to binding affinity and deliver highly selective inhibitors. We demonstrate that deoxygenation of sugar-mimicking ABPs in certain positions can tune their reactivity and drastically change selectivity patterns among enzymes with similar natural substrates.

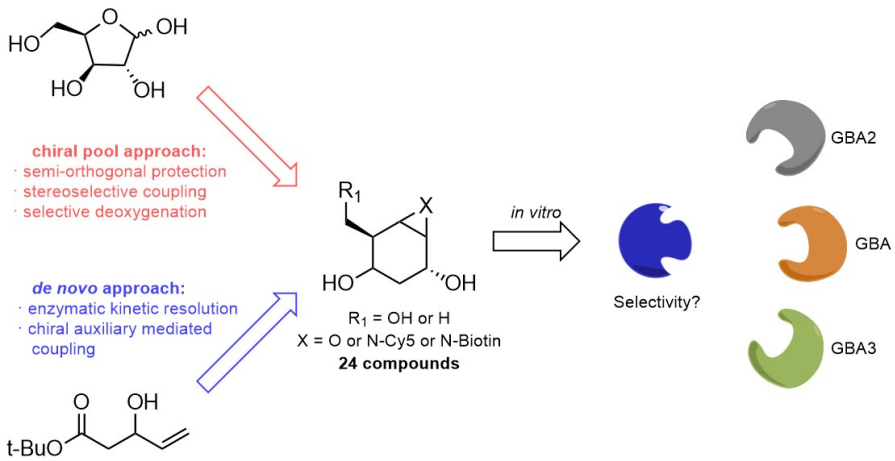

Figure 1: Structural concept of iminosugar based probes.

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Analysis of selenoprotein F binding to UDP-glucose:glycoprotein glucosyltransferase (UGGT)

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UDP-glucose: Glycoprotein glucosyltransferase 1 (UGGT1) glycosylates non-glucosylated glycoproteins in an incompletely folded state. The mono-glucosylated glycans generated by the action of UGGT1 bind to the ER-resident lectin chaperones, calnexin and calreticulin, which in turn promote folding of the bound glycoproteins in collaboration with ERp57. UGGT1 is also known to form a 1:1 complex with selenoprotein F (SelenoF). However, the function of SelenoF associated with UGGT1 is not clear.

Furthermore, the binding position of SelenoF to UGGT1 has also been unclear. In this study, we aimed to investigate the binding region of human UGGT to recombinant SelenoF with Sec replaced by Cys by co-immunoprecipitation analysis using FLAG-tagged UGGT1 and c-myc-tagged SelenoF. The results show that SelenoF binds to the TRXL2 domain of UGGT1. In addition, a newly developed photoaffinity crosslinker was selectively introduced into the cysteine residues of SelenoF to determine the spatial orientation of SelenoF to UGGT1.

Acknowledgements

Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (JSPS KAKENHI), grant numbers JP16H0629 (YI), and JP21K05282 (YT).,

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Synthesis and study of photoswitchable glycosides for biological applications

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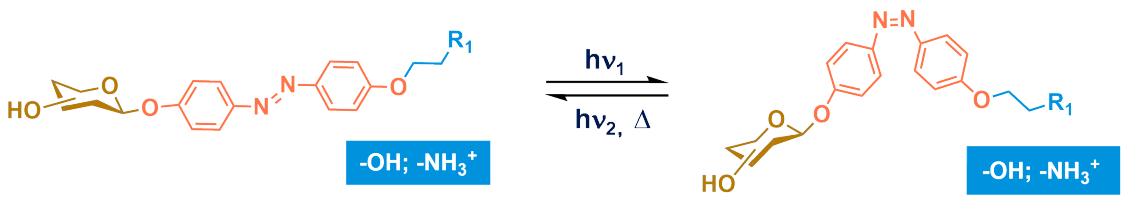
Photochromic molecules are bistable molecules featuring different structural and/or electronic properties which may be reversibly isomerized by light, with the possibility to cycle up to one million "round trips".[1] They offer numerous opportunities for reversibly photomodulating chemical, biological or pharmacological activities or proprieties.[2] Light is generally noninvasive and orthogonal toward most elements of living systems. It can be easily and precisely controlled in time, location, wavelength and intensity, thus enabling the precise activation and deactivation of biological function.

Carbohydrate-lectin interactions are crucially involved in the modulation of myriads of physiological and pathological events.[3] As a continuing interest in the development of photoswitchable glycosides,[4] we decided to prepare galactosyl and fucosyl azobenzene derivatives (Scheme 1) targeting Lectins LecA and LecB which are essential for the bacterial adhesion, biofilm formation and host cell invasion of *Pseudomonas aeruginosa*, a bacterium classified as a Priority 1 pathogen by the WHO.[5]

Photoisomerization of the azobenzene moiety could induce large conformational changes, which provides a clean, fast and easy way to control the geometry and spatial orientation of glycosides so as to reversibly modulate carbohydrate-lectin interaction by light. Synthesis of photoswitchable and their photoswitching properties will be presented.



Scheme 1: Structure and photoisomerisation property of azobenzene-functionalized glycosides.

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Probing Glycosaminoglycan Interactome using a 'Designer' GAG oligosaccharide microarray

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Glycosaminoglycans (GAGs) have important roles in numerous biological systems through interactions with diverse proteins[1]. Due to their structural heterogeneity, defining the specificity of GAG–protein interactions is a challenge. GAG microarrays are much sought-after for detailed GAG recognition studies. A number of microarrays with synthetic heparan sulfate (HS) oligosaccharides have emerged as valuable tools in the field.[2,3] The GAG oligosaccharide microarray based on neoglycolipid (NGL) technology[4] is highly complementary to these, in that it covers size-defined oligosaccharides obtained from natural GAG polysaccharides, including hyaluronic acid, chondroitin sulfate (CS) A and C, dermatan sulfate, heparin, HS and keratan sulfate (KS). The NGL-based system has given insights into the GAG binding specificities of human papillomavirus 16[5], adenovirus 37[6], Chikungunya virus[7], Complement Factor H and Related Protein 5[8], and has led to the assignment of a KS antigen on induced pluripotent stem cells[9].

Here we describe the further development of the NGL-based system towards a 'Designer' GAG screening array platform. Among newly prepared probes are NGLs of CSD, HS, variously desulfated heparins, and heparins from different biological sources with distinct structural features as shown by HPLC disaccharide-compositional and NMR analyses. Binding profiles of a collection of GAG-specific antibodies, endogenous proteins, and pathogens will be presented. These contribute to our understanding of the GAG Interactome networks and potentially lead to novel therapeutic opportunities.

Acknowledgements

This work is supported by Imperial College London-China Scholarship Council (CSC) scholarship programme and Wellcome Trust Biomedical Resource Grant (218304/Z/19/Z).

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An activity-based probe, near-infrared (NIR) fluorogenic probe to detect *O*-GlcNAcase in mitochondria

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O-GlcNAcase (OGA), which promotes the cleavage of the β-O-GlcNAc monosaccharide from serine (Ser) and threonine (Thr) residues of proteins, is associated with the dynamic cycling of protein O-GlcNAcylation in conjunction with the action of O-GlcNAc transferase, which catalyzes the attachment of GlcNAc to Ser and Thr side chains. Dysregulation of protein O-GlcNAcylation is closely related to the pathogenesis of diverse human diseases.

Two isoforms (short and long) of OGA in the nucleus and cytosol have been characterized. Although OGA was found to be present in mitochondria, its function has not been well studied owing to the lack of suitable tools to detect this enzyme. We designed and synthesized an activity-based, near-infrared (NIR) fluorogenic probe that selectively responded to and captured mitochondrial OGA. The probe consisted of (1) a NIR fluorophore, (2) an ethyl carbamate group as a reactive group to covalently capture OGA, and (3) a triphenylphosphonium moiety as a mitochondria-targeting motif.

The intact probe displayed weak fluorescence. Addition of OGA to the probe in aqueous buffer led to label the protein concomitant with an increase in NIR fluorescence. The probe was utilized successfully for imaging of mitochondrial OGA and identify the protein. Details will be described in the presentation.

Efficient synthesis of glycopeptides and glycoproteins by flow-based peptide synthesis

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Glycosylation of peptides and proteins is important for their stability and biological activity. For instance, the glycosylation pattern changes hematopoietic activity of erythropoietin [1] and the artificial glycosylation of insulin inhibits fibril formation.[2] Chemical synthesis of glycopeptides and glycoproteins has enabled us to investigate the functions of glycosylation. However, preparation of glycopeptides usually requires long synthetic time because the traditional solid-phase peptide synthesis (SPPS) is time-consuming and 30-60 minutes are needed for each amino acid elongation step.

In this work, we examined flow-based SPPS for the efficient synthesis of glycopeptides. The flow-based peptide synthesis enabled more rapid reactions at heated temperatures compared to the traditional SPPS, and each elongation step was performed within only 3 minutes. [3,4] The insulin B-chain was rapidly synthesized and the glycosyl asparagine was intentionally introduced at the N-terminal. The obtained glycosylated B-chain was ligated with the insulin A-chain by stepwise disulfide bond formation, resulting in the folded glycoinsulin. In addition, we have been investigating synthesis of native glycoproteins by the efficient flow-based peptide synthesis. In this presentation, we would like to present a detail of our efficient glycopeptide and glycoprotein synthesis.

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Monitoring the chemical sensitivity of carbohydrate-based furanic platforms: clean, mild and atom-ec

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In the field of the use of biomass as a renewable resource for chemistry, carbohydrate-based furanic platform molecules offer original building blocks for the design of novel chemical architectures, like original novel furanic surfactants or nitrogen-containing targets obtained by Morita-Baylis-Hillman, Biginelli, Kabachnik-Fields products, nitrone dipolar cycloaddition or oxazole C-H arylation reactions [1-4]. A key issue is to find the appropriate conditions which are compatible with 5-HMF specific reactivity and known sensitivity to harsh conditions.

We now report new atom-economical reactions forming nitrogen containing cyclic structures such as 1,5-benzodiazepines from 5-HMF, o-phenylenediamines and alkynones and 1,4-dihydropyridines, under remarkably mild and clean conditions. The study included careful optimization of the reaction conditions and their application to a wide scope of substrates, leading to a library of variously substituted products [5].

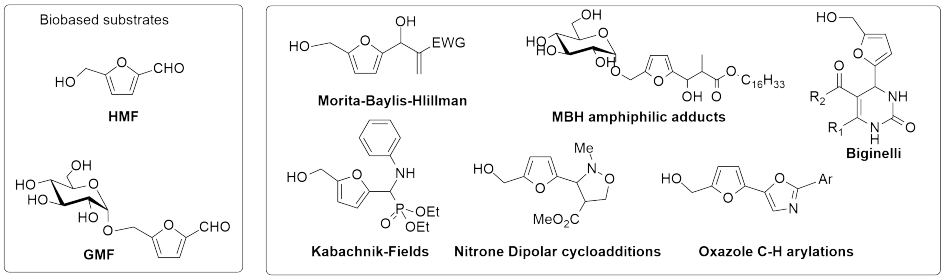
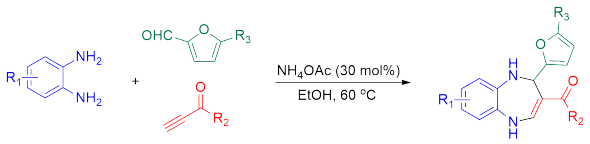


Fig. 1: Examples of fine chemicals derived from HMF and analogs



23 examples, up to 74% yield

√ ***One-pot MCR strategy, clean solvent, mild and new catalyst***
√ ***First access to 1,5-benzodiazepines involving 5-HMF***

Scheme 1: Multicomponent access to 5-HMF-containing 1,5-benzodiazepines

Acknowledgements

Financial support from CNRS and MESRI is gratefully acknowledged. We also thank the Chinese Scholarship Council for a PhD grant to JJ (CSC UT-INSA).

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Dual inhibitors of *Pseudomonas aeruginosa* virulence factors lectin LecA and elastase LasB

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The rise of antimicrobial resistance crisis has put us in a race against time to develop new antibiotics. Over the years, the antivirulence therapy and the polypharmacology research fields have made remarkable progress in drug discovery.[1] Extracellular virulence factors from *Pseudomonas aeruginosa*, lectin LecA and elastase LasB are considered as valuable targets to develop inhibitors.[2]

Combining these in multitarget polypharmacological drugs led to the selective and non-toxic thiol inhibitors.[3] Here, we present an expansion of the structure-activity relationship of these agents. Furthermore, the dimerization of thiol-based inhibitors under physiological conditions affords divalent inhibitors of LecA with 1000-fold increase in potency. The study will pave a way for a systematic exploration of polypharmacology in the development of anti-virulence agents in the aim of treating of resistant *Pseudomonas aeruginosa*-derived infections.

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One-pot synthesis of glycosyl azobenzenes and study of their photoswitching properties in water

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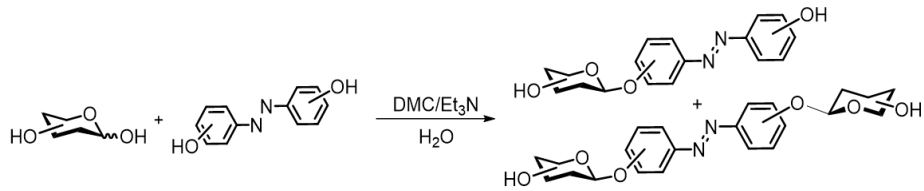
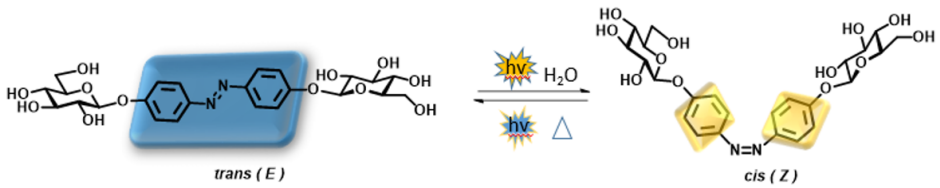
Molecular photoswitches with photoswitching ability in aqueous medium are highly demanded for biological applications and photopharmacology. *[1]* However, the common developed photoswitches like azobenzenes, diarylethenes are barely soluble in water. Linking carbohydrates to a photoswitching unit is an interesting approach to obtain water-soluble photochromic compounds.

Since several years, azobenzene-functionalized photoswitchable glycoconjugates have been developed for light-controlled carbohydrate-protein interactions, cell adhesion, enzyme inhibitors and glycolipid mimics, *[2]* and so on. Consequently, a rapid access to the target compounds with unprotected sugars in aqueous media is of interest. Recently, DMC (2-chloro-1,3-dimethylimidazolinium chloride) mediated glycosylation of unprotected sugars with phenols in aqueous medium has shown promising results. *[3]* As a continuing interest in the development of photoswitchable carbohydrates, *[4]* we are developing the DMC-mediated glycosylation of hydroxyazobenzene with unprotected sugars (Scheme **1**). After optimization of reaction conditions, we are able to prepare a series of water-soluble glycosyl azobenzenes. Furthermore, the synthesized glycosyl azobenzenes displayed remarkable photoswitching behavior in water (Scheme **2**). *[5]*These new results will be presented.



Scheme **1**: DMC-mediated glycosylation of hydroxy-azobenzene derivatives.



Scheme **2**: Isomerization of glycosyl azobenzene upon light and heat.

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Stereoselective synthesis of 'superarmed' thioglycoside donors from 1,2-orthoesters

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Bovine submaxillary mucin (BSM) is a natural polymer used in biomaterial applications for its viscoelasticity, lubricity, biocompatibility, and biodegradability. *N*-glycans are important for the stability and function of mucins, but unlike *O*-glycans, their structures are not fully elucidated.

In this study, *N*-glycans labeled with procainamide of BSM were identified using UPLC and LC-ESI-HCD-MS/MS. The microheterogeneous structures of 32 *N*-glycans were identified, and the quantities (%) of each *N*-glycan relative to total *N*-glycans (100%) were obtained. The terminal *N*-acetylgalactosamines in 12 *N*-glycans (sum of relative quantities; 27.9%) were modified with mono- (10 glycans) and di- (2 glycans) sulfations. Total concentration of all sulfated *N*-glycans was 6.1 pmol in BSM (20 µg), corresponding to 25.3% of all negatively charged glycans (sum of present *N*-glycans and reported *O*-glycans). No *N*-glycans with sialylated or phosphorylated forms were identified, and sulfate modification ions were the only negative charges in BSM *N*-glycans. Mucin structures, including sulfated *N*-glycans located in the hydrophobic terminal regions, were indicated.

This is the first study to structurally characterize and quantify 12 microheterogeneous sulfate modifications in *N*-glycans of natural mucins. These sulfations play important structural roles in hydration, viscoelasticity control, protection from bacterial sialidases, and polymer stabilization to support the functionality of BSM via electrostatic interactions.

Acknowledgements

The authors wish to thank BiOrbic Research centre and Science Foundation Ireland for funding this project

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Breaking down complex isomeric HMO structures with ^{1}H-^{15}N NMR m⍰ethods

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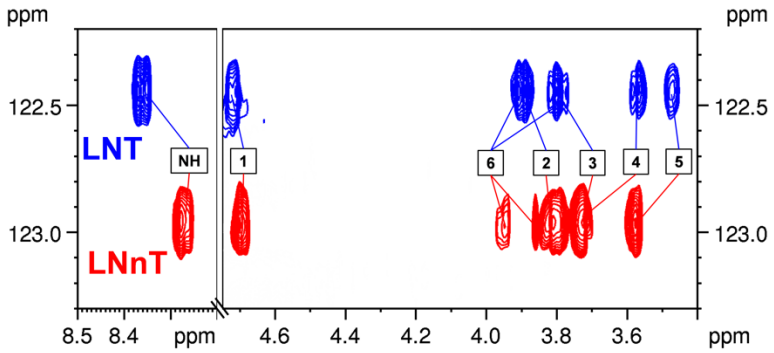
Human milk oligosaccharides (HMOs) are complex glycans that offer numerous health benefits to neonates. However, analytical methods for their routine investigation are still incomplete. NMR spectroscopy provides detailed structural information that can be used to indicate subtle structural differences, particularly for isomeric carbohydrates.

As most HMOs contain the NMR active nucleus ^{15}N incorporated into *N*-acetylglucosamine (GlcNAc) and/or *N*-acetylneuraminic acid (Neu5Ac) moieties, we aimed to introduce the ^{15}N and ^{1}H NMR chemical shifts of GlcNAc or Neu5Ac building blocks into the characterization of HMOs to facilitate the identification of isomeric structures: LNT/ LNnT, 3'-SL/6'-SL, LNFP II/LNFP III and LSTa/ LSTb.

Complete ^{1}H, ^{13}C, and ^{15}N NMR resonance assignments were achieved for the investigated HMOs. The isomeric HMO pairs showed remarkably different ^{15}N NMR resonances (obtained from the ^{1}H-^{15}N HSQC experiment). The highest chemical shift perturbation was observed between the tetrasaccharides LNT and LNnT bearing a GlcNAc unit, while the ^{15}N NMR chemical shifts of the Neu5Ac moiety in 3'SL and 6'SL trisaccharides were less responsive to the minor structural difference. When considering both ^{1}H and ^{15}N NMR chemical shifts of the GlcNAc and/or Neu5Ac moieties (obtained from the ^{1}H-^{15}N HSQC-TOCSY experiment), the distinction of the structural isomers was unambiguous.

This NMR-based method offers a straightforward approach for the identification of common HMOs, which may contribute to the analytical investigation of HMO standards, HMO products, or even human milk.



Overlaid 1H-15N HSQC-TOCSY spectra of LNT and LNnT with the 1H NMR assignment of their GlcNAc moiety

Acknowledgements

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Unnatural gallotannins: powerful antioxidants, antibacterials and antibiofilm agents

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Gallotannins (GTs) are secondary metabolites of higher plants and represent a large class of biologically active polyphenols. GTs play an important role in protection of plants against free radicals, toxins, microbial pathogens and as response to various abiotic stress conditions [1]. Moreover, numerous plant polyphenols exhibited strong antibacterial and antibiofilm activity against staphylococci [2]. Among the staphylococci, *Staphylococcus aureus* is of most clinical concern. Undesirable bacterial *S. aureus* biofilm layers are formed on indwelling medical devices or food processing contact-surfaces, resulting in microbial community more resistant to the traditional disinfectants [3].

GTs from various species have been extensively studied as they exhibit multiple biological activities. As a general rule, the antioxidant activity of GTs is dependent on the number and position of galloyl groups, but the type of the sugar moiety also displays an important role. Many studies have been done with galloylated glucopyranose core, penta-1,2,3,4,6-*O*-galloyl-d-glucose (PGG) [5], but only few galloylated furanoses have been investigated. Thus, a series of new unnatural GTs derived from d-lyxose, d-ribose, l-rhamnose, d-mannose, d-glucose and d-fructose have been designed, synthesized and characterized in order to study the protective and antibacterial effects of synthetic polyphenols that are structurally related with plant-derived compounds. Based on the experimental results obtained in this study it can be concluded that studied GTs are excellent antioxidants and radical-scavenging agents.

Compounds exhibited only moderate activity against gram-positive pathogens *S. aureus* and *E. faecalis*. However, studied compounds proved to be potent inhibitors and disruptors of *S. aureus* biofilms at concentrations much lower than the MIC values. Moreover, they are non-toxic to eukaryotic cells, thus are promising candidates for further studies. Overall, these findings suggest that synthetic GTs could be considered as promising alternatives for biomedical, consumer products, and food industry applications.

Acknowledgements

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Optimalization of lung tissue *N*-glycoprofile analysis

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Altered glycosylation is a common hallmark of a number of diseases, such as metabolic disorders, oncological diseases, and other pathological states. Therefore, a development of reliable method with the highest informative value about the N-glycan structures is an essential tool that can be utilized in biomarker discovery. In presented study, fresh lung resections were homogenized and proteins were extracted to be subjected to PNG-ase F treatment. Released glycans were isolated and analysed by MALDI TOF mass spectrometry in their unlabelled form, after permethylation and after linkage-specific derivatization of sialic acids. The appropriate composition of buffers used for homogenization and protein extraction, as well as the various introduced solid phase extractions with the focus on preserving the labile terminal sialic acid and increasing the obtained signal intensities were optimized.

By the established protocol, even larger tetra-sialylated or tetra-antennary N-glycan structures with *m/z* up to 4,938 (Hex9HexNAc8Fuc2NeuAc2) were observed in substantial relative intensities after the permethylation. Linkage-specific derivatization of sialic acids led to the characterization of signals with *m/z* up to 3,870 (Hex7HexNAc6Fuc2NeuAc4); however, larger structures were identified in low relative intensities. After the efficient glycan isolation from lung tissue and clean-up, both derivatization methods led to the successful detection of a plethora of hybrid, high-mannose and complex N-glycan structures.

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Metallic cation removal with quercus ilex acorn biosorbents

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Acorn pericarps of the Algerian holm oak (*Quercus ilex*) constitute a largely underexploited forestry co-product. In the aim of valorization, adsorption efficiencies of crude, parietal, and lignocellulosic fractions of acorn pericarps were evaluated towards lead, cadmium, nickel, and copper ions. The results were modeled using Langmuir and Freundlich isotherms. The best results were obtained with the lignocellulosic fraction towards lead and cadmium with q_{max} values of 370.37 and 303.03 mg.g^{-1}, respectively. On the contrary, crude and parietal fractions showed the highest capacities for nickel and copper, with q_{max} values of 200 and 303.03 mg.g^{-1}, respectively.

This work thus provides the first trial of acorn pericarps of oaks growing in northwest Algeria as an efficient biosorbent for the removal of metallic cations from aqueous solutions, whose adsorption capacities surpass most of the previously described biosorbents.

Synthesis and in vivo evaluation of MUC1-carbon dot conjugates as cancer vaccines

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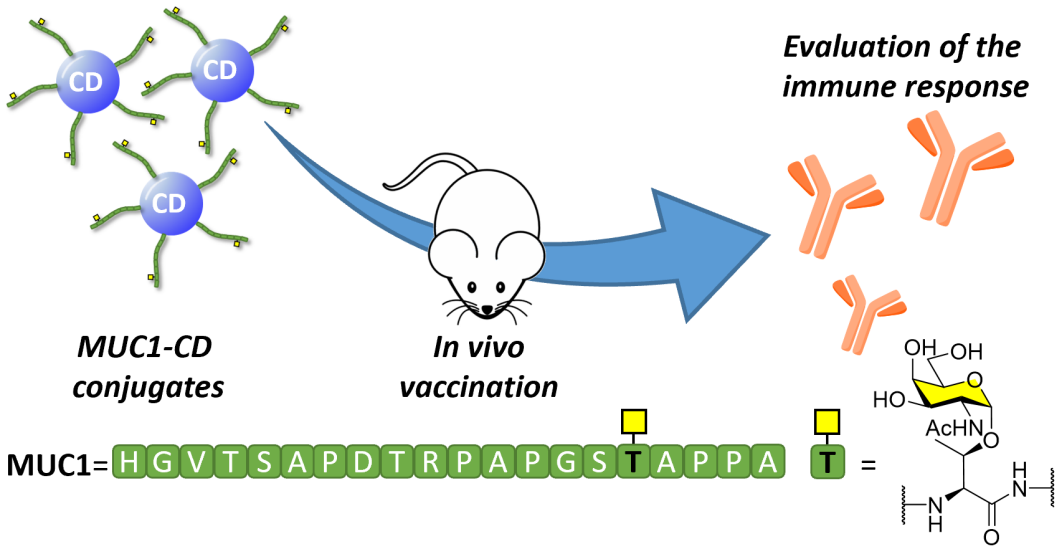
Carbon dots (CDs) are an emerging class of carbon-based nanoparticles which possess inherent immunostimulant properties [1]. Several types of CDs have been synthesized from different precursors, and conjugated with the MUC1 antigen. MUC1 is highly glycosylated glycoprotein expressed on the surface of epithelial cells. In cancer cells, it is overexpressed and presents truncated carbohydrate residues, such as the Tn antigen (α-*O*-GalNAc-Ser/Thr), which can be recognized by the immune system [2]. Unfortunately, MUC1 has low *in vivo* stability and low immunogenicity. Therefore, the conjugation of MUC1 on the surface of carrier proteins or nanoparticles such as CDs is essential to elicit a strong immune response [3].

Herein we report the preliminary results of a novel set of MUC1-CD conjugates. We show that the immunostimulant properties of CDs depend on the nature of the CD precursors, and that CD nanoparticles constitute promising scaffolds for the synthesis of novel self-adjuvant cancer vaccines.



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Physico-chemical characteristics of pectin fractions from Flaxseed

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Flaxseed is a rich source of pectins with unique physico-chemical properties that make it a valuable component of various food products. Moreover, flaxseed pectins have potential health benefits due to their ability to form a gel-like matrix in the gastrointestinal tract, which can slow down nutrient absorption and promote satiety. These polysaccharides may also have prebiotic effects by promoting the growth of beneficial gut bacteria [1].

Natural deep eutectic solvents (NADES) are not only more environmentally safe, but also are a preferred medium for the extraction of polysaccharides. NADES have several advantages over strong inorganic acids, traditionally used in pectin separation, but aggressive and problematic. From the point of view of disposal NADES have several advantages over strong inorganic acids, i.e. show low toxicity and biodegradability [2,3].

The optimization for the proper combination of NADES components for flaxseed pectins extraction, using response surface methodology (RSM) and the I-optimal method, was applied. A mixture of choline chloride and citric acid in the appropriate combination, diluted in water to reduce viscosity, has been shown to be an extremely effective solvent in obtaining dietary fiber. Uronic acid-rich polysaccharides were obtained and subjected to GPC analysis to estimate their molecular weight. The degree of methylation of carboxyl residues was assessed by FT-IR, and the content of neutral sugars by GC-MS.

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