BOOK OF ABSTRACTS

15TH INTERNATIONAL JOINT MEETING OF THE PORTUGUESE CARBOHYDRATE GROUP & GLYCOTWINNING

Welcome Letter

Dear Participants,

We are thrilled to welcome you to the 15th International Meeting of the Portuguese Carbohydrate Group – GLUPOR 15, which will take place from September 15 to 17, 2025 in the historical city of Coimbra, where the past and future converge, offering an inspiring backdrop for scientific exploration and collaboration. The meeting will be held at the historic Convento São Francisco, a unique venue steeped in cultural and architectural heritage, a perfect setting for meaningful discussions, innovative ideas, and collaborative opportunities in the Glycoscience field. Your participation will play a key role in making this event a truly enriching experience.

The Meeting is an initiative of the Portuguese Carbohydrate Group and the Portuguese Chemical Society (SPQ), aiming at bridging scientists working on different areas of carbohydrate research.

This year GLUPOR 15 will be joined with the International Network GLYCOTwinning: Building Networks to Excel in Glycoscience, bringing together national and international experts in transversal areas of glycoscience at the interface of biology, immunology, chemistry, biochemistry, biotechnology, material science, food science, medical science, and microbiology.

Known as the "City of Knowledge," Coimbra is home to the University of Coimbra, the oldest university in Portugal and a UNESCO World Heritage Site. Its iconic Joanina Library, a Baroque masterpiece, and the University Tower are symbols of centuries of academic excellence. As you immerse yourself in Coimbra's intellectual heritage, embark on a journey through its cultural and historical treasures. Discover the Criptopórtico de Aeminium, the Old Botanical Garden, Quinta das Lágrimas, and marvel at the majestic Monastery of Santa Clara-a-Velha. Experience the soul of Coimbra through the hauntingly beautiful melodies of Fado de Coimbra, a unique and emotive style of Portuguese fado, traditionally performed by students and alumni.

Whether you're exploring its ancient streets, enjoying the warmth of its people, or exchanging ideas with fellow scientists, Coimbra promises a unique and enriching experience. Join us in this extraordinary city, where the spirit of discovery thrives amid breathtaking history and culture.

We look forward to seeing you in Coimbra!

With our best regards,

Nuno Empadinhas

Nuno Empadinhas
CNC & CIBB, University of Coimbra
(Chair)

Elisabete Coelho

Elisabete Coelho
LAQV/REQUIMTE - University of Aveiro
(co-Chair)

Filipa Marcelo

Filipa Marcelo
UCIBIO-NOVA FCT
(GLYCOTwinning Chair)

Acknowledgments

The organizing committee gratefully acknowledges the following agencies, corporations and organizations, which have sponsored this meeting or contributed to its organization:

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Funding:

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- Nuno Empadinhas (CNC & CIBB , University of Coimbra) (Chair)
- Elisabete Coelho (LAQV/REQUIMTE - University of Aveiro) (co-Chair)
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- Ricardo Silva (UA)
- Sara Miguel Gonçalves (UC)

Secretariat & Local support

- Leonardo Mendes | SPQ

General Information

The GLUPOR15 will be held at Convento São Francisco, in Coimbra.

This remarkable venue, originally a 17th-century Franciscan convent, has been meticulously restored and transformed into a state-of-the-art cultural and congress center. With modern auditoriums, versatile meeting spaces, and top-tier facilities, it provides the perfect environment for engaging discussions, knowledge exchange, and networking. The venue seamlessly integrates its rich historical heritage with contemporary design, preserving the architectural beauty of its past while offering cutting-edge technology and comfort for international conferences and events.

Location Details and Contacts:

- Address: Avenida da Guarda Inglesa 1A, 3040-193 Coimbra, Portugal
- Phone: +351 239 857 190
- Email: geral@coimbraconvento.pt
- Website: www.coimbraconvento.pt
- GPS Coordinates: 40.2035° N, -8.4407° W

Nestled on the banks of the Mondego River, the Convento São Francisco offers stunning views of Coimbra’s historic skyline, including the iconic University of Coimbra, a UNESCO World Heritage Site (learn more). Its prime location provides easy access to the city’s cultural and academic landmarks, making it an inspiring setting for scholars, researchers, and professionals alike.

Beyond its role as a conference venue, the Convento São Francisco serves as a vibrant cultural hub, hosting concerts, exhibitions, and performances that celebrate Coimbra's artistic and intellectual legacy. Attendees will have the opportunity to experience the city's unique atmosphere, from its fado music tradition to its centuries-old academic customs.

For more information about the venue's history, visit: Convento São Francisco.

To explore the cultural program of the Convento, visit: Cultural Programme.

We look forward to welcoming you to Coimbra for an unforgettable conference experience, where history and innovation come together in a truly exceptional setting!

Parking

Free underground parking at Convento São Francisco is available.

Speakers

Speakers who have not sent their presentations to glupor15@chemistry.pt are kindly asked to upload their talks prior to their sessions by visiting the technician in the corresponding session room.

Poster Information

The posters should be displayed on the first day and kept throughout the meeting. Moreover, the posters should be placed on display after registering on the first day.

Posters will be presented in two separate sessions. Poster presenters are requested to be available by their posters during the networking breaks to address any queries regarding the posters.

Poster session 1

Monday, September 15th | 17h30-18h00

Poster session 2

Tuesday, September 16th | 17h30-18h00

Awards

The SPQ and the Scientific Committees of GLUPOR15 & GLYCOTwinning 2025 are thrilled to celebrate excellence in scientific communication with prestigious awards, generously sponsored by Pharmaceuticals, MDPI. Outstanding participants will be recognized in three categories:

Best Oral Communication (€300)

Best Poster Presentation (€200)

Best Flash Talk (€200)

Winners will be formally announced during the Closing Session, giving the glycosciences community the chance to celebrate their achievements and inspire future excellence.

Conference Dinner

Location: **Congress Dinner & Fado de Coimbra (Praxis Cervejeira Restaurante Beer Heritage)**

Praxis is a brewery-restaurant and microbrewery in Coimbra. It combines artisan beer production (you can even see part of the brewing process) with a menu of classic “cervejaria” dishes, steaks, francesinha, seafood, and assorted petiscos. The atmosphere is relaxed and friendly, with a spacious terrace overlooking the city.

It’s an excellent choice both for beer lovers and for anyone wanting to enjoy traditional Portuguese brewing paired with a hearty meal.

Where & How to Find It

Address: Rua António Gonçalves, 28/29, Quinta da Várzea, Santa Clara, 3040-375 Coimbra

Phone: +351 239 440 207

Parking: Available on site.

Directions from Convento São Francisco

From the Convent, walk to the roundabout in front, where the Portugal dos Pequenitos park is located.

At the roundabout, take Rua António Augusto Gonçalves.

Continue straight for about 600 m (7–8 minutes).

Enter Urbanização Quinta da Várzea.

Praxis will be on your left-hand side, clearly signposted.

It's less than 10 minutes on foot from the Convento São Francisco!

Directions from Coimbra City Center

From the University or Old Town area, head down to the river.

Cross the Mondego via Ponte de Santa Clara.

Continue along Rua António Augusto Gonçalves.

Follow signs to Urbanização Quinta da Várzea.

Praxis is at lote 28-29.

Meeting Point and hour:

For those that want to walk to the venue gather at 19h30 at Convento São Francisco entry, Tuesday, September 16th.

Communication

If you want to submit photos to the conference use the hashtag: **#Glupor15&GlycoTwinning**

More information:

For more information, please consult the website of the conference at: https://glupor15.events.chemistry.pt/

Content

Scientific program

	Day 1 – Monday, September 15th
08:00	**Registration**
09:00	**Opening & Welcome Session** Marta Piñeiro (Official Representative of the Portuguese Chemical Society – SPQ) Nuno Empadinhas, Elisabete Coelho, Filipa Marcelo, Angelina Palma (President of the Carbohydrate Group)
09:15	**KL1**: Salomé Pinho, i3S / IPATIMUP, University of Porto, Portugal *Glycans as master regulators of immune response in inflammation, autoimmunity and cancer: from disease prediction to therapeutic opportunities* Chaired by Nuno Empadinhas
	SESSION 1. Glycobiology in Health and Disease (Part 1)
	Chairs: Celso Reis & Yan Liu
10:00	**PL1**: Paula Videira, UCIBIO, NOVA University of Lisbon, Portugal *Glycosylation in Cancer and Rare Diseases: Integrating Mechanisms to Advance Precision Medicine*
10:30	**OC1**: Daniela Tomás, i4HB and UCIBIO, NOVA University of Lisbon, Portugal *From Hyposialylation to immune response: a possible mechanism for GNE Myopathy*
10:45	**OC2**: Marta Falcão, NOVA University of Lisbon, Portugal *Glycosylation Defects Disrupt Immunological Pathways in PMM2-CDG*
11:00	Coffee-Break
	SESSION 1. Glycobiology in Health and Disease (Part 2)
	Chairs: Helena Coelho & Ana Maranha
11:25	**IL1**: Joana Gomes, i3S, University of Porto, Portugal *The role of sialic acid in cancer therapy response and immunomodulation*
11:45	**OC3**: Carlos Lima, UCIBIO and i4HB, NOVA University of Lisbon, Portugal *MGL-Drug Conjugate: A Novel Glycan-Targeted Approach for Targeting Cancer Cells*
12:00	**OC4**: Gonçalo Trindade, iBET & ITQB, NOVA University of Lisbon, Portugal *Unveiling the Landscape of Tumor-Associated Myeloid Cells in Immunosuppressive Breast Cancer*
12:15	**OC5**: Inês Ribeiro, i4HB and UCIBIO, NOVA University of Lisbon, Portugal *Unveiling the role of Sialyl-Tn in pancreatic cancer progression and therapy*
12:30	**OC6**: Rafaela Abrantes, i3S and IPATIMUP, University of Porto, Portugal *Novel CAR T formulations targeting tumor-associated glycoepitopes: A new strategy for solid tumors*
12:45	**OC7**: Zélia Silva, UCIBIO and i4HB, NOVA University of Lisbon, Caparica, Portugal *Exploring the role of glycans in cancer therapy*
13:00	Lunch
	SESSION 2. Microbial Glycobiology and Microbe-Host Interactions (Part 1)
	Chairs: Elisabete Coelho & Nuno Empadinhas
14:30	**IL2**: Flaviana DiLorenzo, University of Naples Federico II, Italy *Lipopolysaccharides: Fantastic "structures" and where to find them*
14:50	**OC8**: Ana Maranha, CIBB, University of Coimbra, Portugal *Polymethylated polysaccharides in mycobacteria: Dissecting their roles in stress adaptation and pathogenesis*
15:05	**OC9**: Daniela Nunes-Costa, CIBB, University of Coimbra, Portugal *Structural studies of a highly thermostable GpgS reveal a cryptic aromatic binding pocket*
15:20	**OC10**: Benedita Pinheiro, UCIBIO & i4HB, NOVA University of Lisbon, Portugal *Decoding Mucin O-Glycan Recognition by Commensal Bacteroides: Structural and Functional Insights*
15:35	Coffee-Break
	SESSION 2. Microbial Glycobiology and Microbe-Host Interactions (Part 2)
	Chairs: Angelina Palma & Susana Alarico
16:00	**IL3**: Ron Cornellis Hokke, LUCID, Leiden University Medical Center, The Netherlands *Schistosome glycosylation as key factor in host-parasite biology*
16:20	**OC11**: Cátia Soares, UCIBIO and i4HB, NOVA University of Lisbon, Portugal *Structural Insights Reveal Contextual O-Glycan Cluster Recognition by the Mucin-Binding Module X409*
16:35	**OC12**: Helena Coelho, i4HB and UCIBIO, NOVA University of Lisbon, Portugal *Defining Host Glycan Ligands Targeted by the Giant Adhesin SiiE of Salmonella enterica*
16:50	**Flash Talks 1** (Presenters of **P01, P02, P04, P05, P06, P07, P08, P10, P11, P12, P28**)
17:30	**Poster Session**
18:00	**General Assembly GLUPOR**
19:00	Networking & Welcome Reception

	Day 2 – Tuesday, September 16th
	SESSION 3. (Bio)Synthesis, Structural Analysis, and Molecular Recognition (Part 1)
	Chairs: Paula Videira & Filipa Marcelo
09:00	**PL2**: Jesús Jiménez-Barbero, CIC bioGUNE, Basque Research & Technology Alliance, Spain *Breaking the Limits in Understanding Glycan Recognition by NMR*
09:30	**IL4**: Rita Ventura, ITQB NOVA University of Lisbon, Portugal *Synthesis and Application of Autoinducer-2 Sugar Derived Prodrugs*
09:50	**OC13**: Ana Gimeno, CIC bioGUNE, Basque Research and Technology Alliance, Spain *Glycan-Lectin Interactions in Context: New NMR Approaches*
10:05	**OC14**: Ana Sofia Grosso, UCIBIO and i4HB, NOVA University of Lisbon, Portugal *C1GalT1 and ST6GalNAc-I mechanistic insights in Mucin-1 O-Glycosylation*
10:20	**OC15**: Ana Ardá, CICbioGUNE, Basque Research and Technology Alliance, Spain *Molecular recognition properties of the tandem-repeats Galectin-4 and Galectin-9*
10:35	**OC16**: Pedro Mateus, ITQB NOVA and LS4FUTURE, NOVA University of Lisbon, Portugal *Arylamide glycofoldamers for targeting carbohydrate–protein interactions*
10:50	**OC17**: Catarina Maria, CQE-IMS, Universidade de Lisboa, Portugal *Design and Synthesis of Novel Carbohydrate-Based Antibacterial Agents to Address Antimicrobial Resistance*
11:05	Coffee-Break
	SESSION 3. (Bio)Synthesis, Structural Analysis, and Molecular Recognition (Part 2)
	Chairs: Manuel A. Coimbra & Olga Borges
11:30	**IL5**: Lígia Rodrigues, CEB, University of Minho, Portugal *Unravelling the potential of microbes in the biosynthesis of carbohydrates*
11:50	**OC18**: André Oliveira, LAQV-REQUIMTE & CICECO, University of Aveiro, Portugal *Carbohydrate-rich wastewater as a source of short-chain organic acids through acidogenic fermentation*
12:05	**OC19**: Ana Saldanha, CIMO, LA SusTEC, Polytechnic Institute of Bragança, Portugal *Structural modulation of Agaricus bisporus polysaccharides by lactic acid fermentation and its biofunctional implications*
12:20	**Flash Talks 2** (Presenters of **P15, P16, P17, P18, P20, P21, P23, P24, P25, P26, P32)**
13:00	Lunch
	SESSION 4. Carbohydrates in Food Science and Agriculture (Part 1)
	Chairs: Fernando Nunes & Miguel Cerqueira
14:30	**IL6**: Cláudia Nunes, CICECO, University of Aveiro, Portugal *Marine Polysaccharides: Functional Biomaterials for Biomedical and Food Applications*
14:50	**OC20**: Aida Moreira da Silva, ESAC, Polytechnic University of Coimbra, Portugal *Cyclodextrins in Food Systems: Technological Applications and Functional Perspectives*
15:05	**OC21**: Amélia Graça, LAQV-REQUIMTE, University of Aveiro, Portugal *Searching for sources of sweet-tasting oligosaccharides able to decrease sucrose content in foods*
15:20	**OC22**: Helena Laronha, LAQV-REQUIMTE, University of Aveiro, Portugal *The Role of Rhamnogalactan and Fucogalactan in the Cholesterol-Lowering Effects*
15:35	Coffee-Break
	SESSION 4. Carbohydrates in Food Science and Agriculture (Part 2)
	Chairs: Aida Moreira & Pedro Fernandes
16:00	**IL7**: Miguel Cerqueira, International Iberian Nanotechnology Laboratory, Braga, Portugal *Electrohydrodynamic processing for the design of micro and nanostructures based on polysaccharides*
16:20	**OC23**: Mário Bezerra, CQ-VR, University of Trás-os-Montes and Alto Douro, Portugal *Interactions Between Wine Polysaccharides and Bentonite: Implications for Protein Removal Efficiency in Model White Wine Solutions*
16:35	**OC24**: Mónica Jesus, LAQV-REQUIMTE, University of Porto, Portugal *Polysaccharides as modulators of procyanidins interactions with oral- cell models*
16:50	**OC25**: Paloma Lopes, LAQV-REQUIMTE, University of Aveiro, Portugal *Deciphering Raspberry seed-derived Biopolymers for Food Applications*
17:05	**OC26**: Tiago Durães, CQ-VR, University of Trás-os-Montes and Alto Douro, Portugal *Caloric reduction of sucrose-rich fruit products through an enzymatic approach*
17:30	**Poster Session**
18:00	**A Legacy of Excellence:** GLUPOR15 Launches Prestigious Prize for Young Scientists Honoring Portuguese Pioneer in Glycoscience: The GLUPOR Young Researcher **Amélia Pilar Rauter Award**
18:30	**GLYCOTwinning Meeting**
20:00	Congress Diner & Fado de Coimbra (Praxis)

	Day 3 – Wednesday, September 17th
	SESSION 5. Glyc(omics) and Biomarker Discovery
	Chairs: Amélia P. Rauter & Jesús Jiménez-Barbero
09:15	**PL3**: Peter Seeberger, Max Planck Institute of Colloids and Interfaces and CTC, Germany *Automated Glycan Assembly as Enabling Technology of Molecular Glycobiology*
09:45	**IL8**: Manfred Wuhrer, Leiden University Medical Center, The Netherlands *Human glycomics and glycoproteomics by mass spectrometry*
10:05	**IL9**: Yan Liu, Imperial College London, United Kingdom *Glycan microarray toolbox for microbiome research*
10:25	**OC27**: Luís Grilo, CIBB, University of Coimbra, Portugal Cardiac Molecular Analysis Reveals Aging-Associated Metabolic Alterations Promoting Glycosaminoglycans *Accumulation Via Hexosamine Biosynthetic Pathway*
10:40	**Sponsor:** Rui Rocha: Bruker, Portugal Bruker glycobiology solutions: *An integrated, comprehensive technology application suite making glycoscience accessible*
11:00	Coffee-Break
	SESSION 6. Carbohydrate-Based Materials and Applications in Medicine, Biotechnology, Industry, Heritage, and Environmental Sustainability
	Chairs: Ana Ardá & Manfred Wuhrer
11:30	**IL10**: Mara Braga, Green & Sustainable Processes Laboratory, University of Coimbra, Portugal *Polymer processing strategies for application in the environment and health areas*
11:50	**IL11**: José Gamelas, CIEPQPF, University of Coimbra, Portugal *Nanocelluloses and nanocellulose films. Opportunities in the conservation and restoration of historical documents*
12:10	**OC28**: Jan Vicha, Centre of Polymer Systems, Tomas Bata University in Zlín, Czech Republic *Dialdehyde Polysaccharides for the Sustainable Synthesis of Multifunctional Conductive Biomaterials*
12:25	**OC29**: Olga Borges, CIBB, University of Coimbra, Portugal *Administration of a Sugar-Functionalized Chitosan/DNA Complex Vaccine Elicits Protective Immunity Against SARS-CoV-2 in Mice*
12:40	**Awards Ceremony**
13:00	**Closing Session**

Flash Talks	
SESSION 1. Day 1 - Monday 16h50	**SESSION 2. Day 2 - Tuesday 12h20**
F1: Alexandra Couto Oliveira	**F11**: José Manuel Rojas-Marcos Hernáez
F2: Ana Luísa Benavente	**F12**: Lara Mingatos
F3: Daniela F. Barreira	**F13**: Alejandra Travecedo
F4: Inês Teodoro	**F14**: María Payá-García
F5: Inês Pinho	**F15**: Beatriz Thomas Metzner
F6: Henrique Sousa	**F16**: Bernardo A. C. Ferreira
F7: Marcello Mercogliano	**F17**: Filipe Coreta-Gomes
F8: Abdelrahman Farahat	**F18**: Ladislav Čurda
F9: Alícia Candeias	**F19**: Ramla Khiari
F10: Guilherme Oliveira	**F20**: Sara Gonçalves
F21: Joana Santinha	**F22**: Zdenka Víchová

Keynote Lecture

Glycans as master regulators of immune response in inflammation, autoimmunity and cancer: from disease prediction to therapeutic opportunities

Salomé S. Pinho[1,2,3]

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Glycans have been highlighted as essential determinants that integrate the regulatory networks that guide both innate and adaptive immune responses [1]. Glycans act as master regulators of the inflammatory response being fundamental molecular determinants for the discrimination between "self" and "non-self" [2]. Our results in Systemic Lupus Erythematosus (SLE), a classical autoimmune disease, revealed that patients with lupus nephritis exhibit at kidney cell surface a unique glycan signature characterized by an increased abundance and spatial distribution of unusual mannose-enriched glycans [3] that are recognized by specific glycans-recognizing receptors, expressed by gdT cells, culminating in the activation of pro-inflammatory pathways associated with autoimmunity [4]. Accordingly, our recent results in Inflammatory Bowel Disease (IBD), in which a series of preclinical serum samples were analyzed up to 6 years before IBD diagnosis, revealed the identification of a unique glycosylation signature on circulating antibodies (IgGs) characterized by lower galactosylation levels of IgG Fc domain that is detected many years before diagnosis. This specific IgG Fc glycan trait correlate with increased anti-microbial antibodies, specifically with anti-Saccharomyces cerevisiae antibody (ASCA) levels, pinpointing a glycome-ASCA hub, detected in serum, that predates by years the development of CD. Mechanistically, we demonstrated that this glycoform of ASCA IgG, elicits a pro-inflammatory immune pathway through the activation and reprogramming of innate immune cells (such as DCs and NK cells), via FcγR-dependent mechanism, triggering NF-kB and CARD9 signaling and leading to inflammasome activation. Adoptive transfer of ASCA IgG to recipient WT mice result in increased susceptibility to intestinal inflammation that is recovered in recipient FcγR KO mice [5]. Together these results unlock the identification of a pathogenic glyco-hub that may constitute a promising new serum biomarker for CD prediction and a potential target for disease prevention.

At the other pole of the immune response, in a cancer context, where immunosuppressive networks promote cancer progression, we further demonstrated the immune-regulatory properties of glycans. We showed that complex branched N-glycans structures, typically overexpressed by cancer cells, are used by colorectal tumor cells to escape immune recognition, by instructing the creation of immunosuppressive pathways through inhibition of IFNγ production. The removal of this "glycan-mask" was found to expose immunogenic glycans that potentiate immune recognition through DC-SIGN-expressing immune cells resulting in an effective anti-tumor immune response [6]. More recently, we also revealed that branched N-glycosylation emerges in infiltrating T cells in premalignancy, imposing an exhausted and dysfunctional phenotype. We showed that targeting Mgat5-mediated branched N-glycans on CD8+ (CAR-) T cells prevent exhaustion, enhancing cytotoxicity and anti-tumor T cell activity [7].

In summary, glycans exert powerful immunoregulatory properties governing both innate and adaptive immune responses with important roles in the pathogenesis of major diseases such as cancer, inflammation and autoimmunity, pinpointing glycans as key checkpoints with promising clinical and therapeutic applications in autoimmune diseases and cancer.

References

1. Pinho SS, et al. Cell Mol Immunol. 2023 Oct;20(10):1101-1113.
2. Alves I, et al. FEBS Lett. 2022 Jun;596(12):1485-1502.
3. Alves I, et al. Arthritis Rheumatol. 2021 Nov;73(11):2069-2077.
4. Alves I, et al. Sci Transl Med. 2023 Mar 15;15(687):eabo1930.
5. Gaifem J, et al. Nat Immunol. 2024 Sep;25(9):1692-1703.
6. Silva MC, et al. Cancer Immunol Res. 2020 Nov;8(11):1407-1425.
7. Azevedo CM, et. al. Cancer Immunol Res. 2025 Aug 19. doi: 10.1158/2326-6066.CIR-25-0313

Plenary Lectures

Glycosylation in Cancer and Rare Diseases: Integrating Mechanisms to Advance Precision Medicine

PA Videira[1,2]

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Glycans play a fundamental role in all biological processes but their specific role in diseases characterised by altered glycosylation, such as cancer and congenital disorders of glycosylation (CDG), remains unclear. In cancer, the immune function is typically impaired, leading to a poor prognosis. Increased sialylation and truncated glycans, such as sialyl-Tn (STn), contribute to immunosuppression and poor prognosis in cancers like triple-negative breast cancer (TNBC). Genetic databases of TNBC patients have shown transcriptome alterations associated with sialyltransferase genes correlate with the infiltration of regulatory T cells and M2 macrophages, both of which are associated with a pro-tumoral and immunosuppressive environment. Ongoing studies with monoclonal antibodies against short sialylated O-glycans aim to test whether these can overcome immunosuppression and enhance the immune response, showing promise as anti-cancer therapeutics.

Interesting, congenital disorders of glycosylation (CDG) causing defects in the sialic acid biosynthesis, such as GNE myopathy (GNEM) have no clear clinical evidence of immune involvement. However, at the molecular level, critical immune modulators are altered in GNEM patient cells, and current work is ongoing to understand their impact. Patients with PMM2-CDG have impaired N-glycosylation and often experience immune-related clinical issues that worsen other symptoms. To further investigate this, we analyzed patients' cells upon an inflammatory stimulus. Our data indicate that immune-related gene expression in PMM2-CDG differs from controls. Furthermore, functional analysis showed deregulation in the MAPK signaling downstream to the TNF-α receptor, which is being further validated and may account for inefficient infection/inflammation control.

Our studies highlight the importance and complexity of glycans in immunopathology and identify potential therapeutic targets and biomarkers. Glycan-targeted therapies are promising in overcoming cancer immunosuppression and may have clinical applications.

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Breaking the Limits in Understanding Glycan Recognition by NMR

J. Jimenez-Barbero[1,2,3]

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Molecular recognition by specific targets is at the heart of the life processes. Carbohydrates (glycans, saccharides, sugars), prevalent on mammalian cells and in the extracellular matrix, collectively form the glycocalyx, impacting physical properties, regulating protein function, and acting as ligands for lectins (glycan-binding proteins). Indeed, dysregulation of glycosyltransferases results in tumor-associated carbohydrate antigens (TACAs), unique to tumor cells. TACAs play a key role in modulating immune responses within the tumor microenvironment through interactions with lectins. Similar interactions are observed in bacterial and viral-mediated infectious events. Therefore, the interactions between lectins, enzymes, antibodies and glycans mediate a broad range of biological activities, from fertilization and tissue maturation to pathological processes. In this context, the elucidation of the mechanisms that govern how sugars are accommodated in the binding sites of these receptors is currently a topic of interest. Thus, unravelling the structural, dynamic, and conformational factors that rule the interactions of these molecules is of paramount interest.

Solution NMR is unique in providing stereochemical, conformational, and dynamic information. Given the inherent flexibility and dynamic properties of glycans, we use NMR as key tool for deducing, at atomic resolution, molecular recognition events in which glycans are involved. Together with state-of-the-art NMR, we employ a combination of diverse and synergic methodologies, including chemical synthesis, protein biochemistry and molecular biology, biophysics (ITC, BLI), molecular modelling, and structural biology techniques (X-Ray crystallography and CryoEM). In this way, we investigate the interactions of glycans related to immune responses, from cancer to bacterial and viral infections.

This presentation focuses on the application of our NMR methodology, both from the ligand and receptor's perspective, to study conformation and dynamics of glycans and molecular recognition events with their receptors of biomedical interest. Recent examples will be shown, including new NMR avenues to unravel the interactions of glycans on intact glycoproteins and on cell surfaces.[1-7]

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Automated Glycan Assembly as Enabling Technology of Molecular Glycobiology

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Carbohydrates are the predominant biopolymer on earth as these molecules serve a variety of functions throughout biology [1]. Molecular glycobiology depends on access to defined, synthetic glycans as probes to study processes that involve glycans. With the development of Automated Glycan Assembly [2], we gained rapid access to oligosaccharides for biological studies. These synthetic glycans were placed on the surface of arrays to give rise to glycan microarrays [3] that were used to identify the glycan antigens recognized by antibodies present in bodily fluids as a result of infections [4] and may give rise to autoimmune diseases. [5] Synthetic glycans were used to prepare monoclonal antibodies recognizing the surface of bacteria such as tulerimia [6] and fungi [7] for the detection and treatment of infections with these agents.

For each ton of glycans on the surface of the earth, one ton of carbohydrates exists in the oceans. We initiated a program [8] aimed at understanding the myriad of enzymes involved in the synthesis and degradation of these marine glycans. [9,10]

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Invited Lectures

The role of sialic acid in cancer therapy response and immunomodulation

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Targeted therapies of colorectal cancer (CRC), including immune checkpoint inhibitors, have been applied to boost native anti-tumor responses and improve patient survival. However, most patients still face a dismal prognosis, highlighting the need to identify novel immunotherapeutic targets to optimize treatment outcomes. Glycosylation is a highly controlled cellular process that becomes deregulated in cancer cells, with aberrant expression of specific glycans such as terminal sialylated structures. These glycans are known to be recognized by immune cells through sialic acid binding-immunoglobulin-like lectins, Siglecs, inducing strong inhibitory signalling and therefore suppressing immune cell functions [1,2]. This study aims to investigate the role of ST6Gal1 in modulating antibody therapy response and crosstalk between CRC and immune cells.

We have disclosed the importance of terminal α2,6-sialylation of EGFR, a CRC targeted therapeutic antibody, in the antibody clinical performance and patient response to therapy, impacting cancer patients' clinical outcome [3]. Taking advantage of the developed genetic engineered cancer cell models with differential terminal sialylated glycan profiles, we identified a specific Siglec receptor as a binding partner for glycans synthesized by ST6GAL1 in cancer cells, which was also found to be expressed in subsets of tumor-associated myeloid cells, mainly macrophages. Analysis of the functional consequences of sialylated glycans on immune response have shown that terminal sialylation regulates macrophage-mediated phagocytosis of cancer cells and that this mechanism was dependent on a Siglec circuit activation.

These results uncover ST6Gal1 as a regulator of therapy response and tumor immune evasion in CRC, providing novel immunotherapeutic clinical targets and improving cancer patient stratification to optimize treatment outcomes.

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Lipopolysaccharides: Fantastic "structures" and where to find them

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A key aspect of host immune surveillance involves the recognition of microbial-associated molecular patterns, such as lipopolysaccharides (LPS), which are characteristic of Gram-negative bacteria. These molecules are present not only in pathogenic species but also in commensal and mutualistic bacteria residing in the intestines [1,2]. Due to their chemical composition, LPSs are widely recognized as powerful inducers of immune inflammatory responses in mammals and are often linked to harmful bacteria and adverse health effects. However, LPSs are also structural components of the outer membrane of beneficial Gram-negative bacteria within the gut microbiota. The mechanisms that allow LPSs from these commensal bacteria to be tolerated without triggering overt immune activation remain largely unexplored, representing a key frontier in our understanding of innate immunity [3]. Unraveling the chemical structure and immunological properties of LPSs from gut microbes, particularly those maintaining a neutral or beneficial relationship with the human host, is of critical importance for both fundamental biology and clinical research. A thorough investigation of LPS molecules from the gut microbiota will shed light on host-microbe interactions at both intestinal and systemic levels. This, in turn, will enhance our understanding of how gut bacteria influence immune responses through their LPS structures, ultimately expanding our knowledge of immune system dynamics. By analyzing microbiota-derived LPS, it will be possible to generate novel structural and functional insights that may drive innovation in biomedical research. These findings have the potential to contribute to the development of new immunotherapies and facilitate the identification of biomarkers for diagnosing, prognosticating, and predicting immune-mediated diseases.

In this communication, I will share recent and unprecedented findings on the structure and distinctive immunological characteristics of LPSs from specific commensals of the human gut. Additionally, I will show some important advancements in structure-to-function studies on gut microbiota LPS, building on our previously published work.

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Schistosome glycosylation as key factor in host-parasite biology

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Schistosomes are parasitic worms that infect over 200 million people, mainly in sub-Saharan Africa. Re-infection after treatment occurs rapidly in endemic areas due to lack of effective immunity, and a protective vaccine against schistosomes does not yet exist. Infection with these parasite induces a plethora of antibodies and cellular immune responses recognising a wide range of parasite antigens, including many different complex glycan antigens. The role of these anti-glycan responses in immunity remains poorly understood. Some are likely contribute to protective immunity or immunomodulatory effects, others may be irrelevant or give rise to an immunological smokescreen. Moreover, some glycan antigens are specific for schistosomes, but others are expressed also in subsets of other helminth species, microbes, plants, and (in)vertebrates including schistosome hosts. This raises additional questions regarding cross-reactivity of anti-glycan antibodies, both in the context of protective immunity and of their diagnostic potential. To address some of these questions we have carried out extensive glycomic analysis across the life cycle of the parasite, in combination with the application of glycan antigen microarrays to study antibody profiles in human and animal serum/plasma samples. Taking advantage of longitudinal sample sets from single and repeated controlled human *Schistosoma mansoni* infections, as well as samples from schistosome endemic areas, we have determined total IgG and subclasses to a wide range of glycans. Responses were analysed for associations with markers of tolerance, protection and infection. Particularly, a wide range of fucosylated glycans antigens in schistosomes elicit high antibody titres in humans after single of multiple exposures, as well as in partially protected animals. These antigens are highly expressed on the larval stage of the parasite, which in an *in vitro* model is sensitive to damaging effects of fucose-specific antibodies. Current work is aimed at engineering the glycosylation of live parasites through chemical or genetic approaches to allow evaluating the importance of schistosome glycosylation in *in vivo* models. Future plans on advancing glycan antigens as therapeutic or diagnostic targets will be discussed.

Synthesis and Application of Autoinducer-2 Sugar Derived Prodrugs

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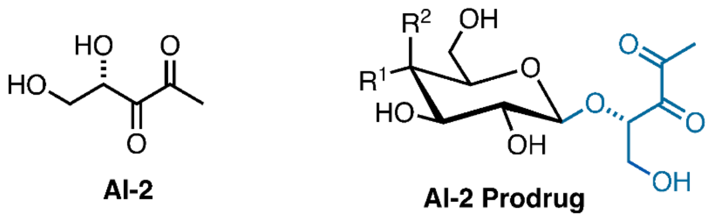
Bacteria are able to coordinate the behaviour of cell population by secreting and sensing small molecules called autoinducers. This phenomenon is known as quorum sensing (QS). Among the QS compounds, autoinducer-2 (AI-2, Figure 1) stands out as a potential "universal" bacterial signalling molecule for inter-species communication. [1,2,3] Understanding the molecular mechanisms that bacteria use to communicate and therefore regulate their group behaviours can lead to the development of new therapies to control bacterial infections.

AI-2 plays a crucial role in controlling the colonisation and homeostasis of the gut microflora. It has been shown that AI-2 can be used to mitigate the adverse effects caused by antibioticinduced microbiota imbalances in the gut.[3] Therefore, our hypothesis is that synthetic AI-2 can aid in restoring a healthy bacterial phyla ratio after antibiotic treatment.

We will present the synthetic strategies towards the preparation of AI-2 prodrugs to orally deliver intact AI-2 to the gut based in colon-specific drug delivery systems.[4] The AI-2 prodrugs consist of AI-2 linked to beta-glycosides (Figure 1) that will be specifically hydrolysed in the gut taking advantage of beta-glucosidases and beta-galactosidases produced by the gut microbiota. The enzymatic release of AI-2 from the new AI-2 prodrugs was demonstrated using commercial beta-glycosidases and mice gut extracts. Quantification of AI-2 released from the prodrugs was performed using a new GC-MS AI-2 quantification method developed by our group.[5]



AI-2

AI-2 Prodrug

Glucose: R1=OH, R2=H
Galactose: R1=H, R2=OH

Figure 1.

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Unravelling the potential of microbes in the biosynthesis of carbohydrates

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The development of sustainable bioprocesses is essential to meet the growing demand for functional ingredients while minimizing the environmental impact. Biocatalysts are central to this transition, enabling selective and effective biotransformations under mild conditions. The discovery of novel enzymes generally requires bioprospecting efforts, as many natural environments remain unexplored and offer a rich source of catalytic diversity. Additionally, identifying robust enzymes is crucial for the industrial converting carbohydrates into high-value prebiotics with well-recognized health benefits and growing market relevance. To address this challenge, multiple and complementary strategies can be employed: culture-dependent approaches (microbial screening), culture-independent methods (functional metagenomics), and synthetic biology tools for enhancing catalytic performance. Together, these strategies support the discovery, characterization, and optimization of biocatalysts to develop innovative and more sustainable bioprocesses for prebiotic production. These integrative approaches are expected to bridge the gap between scientific advances and their translation into cost-effective, market-ready solutions.

Marine Polysaccharides: Functional Biomaterials for Biomedical and Food Applications

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Polysaccharides derived from marine environments are emerging as a promising class of renewable and functional biomolecules. Microalgae and seaweed offer significant advantages for industrial-scale production due to their high growth rates, minimal resource requirements (no need for arable land or freshwater), and ease of cultivation and harvesting. As a result, microalgae and seaweed have the potential to complement or even replace terrestrial plants in polysaccharide production. Among marine-derived polysaccharides, unique to these ecosystems, hold significant potential for valorization due to their distinctive physical and biological properties. These compounds closely resemble mammalian glycosaminoglycans in both chemical structure and biological function, making them increasingly attractive for biomedical applications.

In the biomedical field, the demand for new biocompatible and biodegradable materials is rapidly increasing. Biopolymers, particularly polysaccharides, have garnered significant attention due to their biocompatibility, biodegradability, and abundance, as well as their functional groups that enable modification. Moreover, new functionalities can be integrated into these biomaterials, including antimicrobial properties, electrical conductivity, magnetic characteristics, and piezoelectricity, expanding their potential applications. As a result, these innovative materials address the growing need for durable and sustainable solutions, aligning with the global trend toward biobased and environmentally friendly alternatives. Beyond biomedicine, polysaccharide-based films have also gained attention as sustainable food packaging materials due to their biodegradability. The development of active and intelligent packaging is particularly critical, as it supports efforts to reduce plastic consumption and minimize food waste.

This study focuses on the structural analysis of polysaccharides derived from diverse marine sources, particularly seaweeds and microalgae, and investigates their relationship with biological activity. This approach highlights the considerable potential of marine environments as a rich source of bioactive polysaccharides, supporting the development of functional and sustainable biomaterials for targeted biomedical and food-related applications.

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Electrohydrodynamic processing for the design of micro and nanostructures based on polysaccharides

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The convergence of sustainable materials and advanced manufacturing technologies is opening new frontiers in the design of functional micro- and nanostructures. Among these, cellulose derivatives, biocompatible, biodegradable, and widely available, stand out as promising candidates for electrohydrodynamic processing (EHDP) and electrohydrodynamic jet printing. These techniques, which rely on electric fields to manipulate polymer solutions at the micro and nanoscale, enable the fabrication of highly tunable structures with applications ranging from encapsulation to smart packaging.

This talk explores the potential of cellulose-based systems, particularly hydroxypropyl methylcellulose (HPMC), in the development of micro- and nanostructures using EHDP. By tailoring solution properties such as viscosity, conductivity, and surface tension, and fine-tuning process parameters like voltage and flow rate, it is possible to define distinct processing regimes, electrospraying for particles and electrospinning for fibers. Beyond fabrication, the integration of bioactive compounds and the compatibility of these materials with emerging digital manufacturing approaches, such as e-printing, highlight their versatility. This talk will discuss the scientific principles, technological advances, and future directions for cellulose derivatives in EHDP, emphasizing their role in shaping the next generation of eco-friendly materials and devices.

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Human glycomics and glycoproteomics by mass spectrometry

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Virtually all diseases – including various infectious diseases and malignancies, inflammatory bowel disease, rheumatoid arthritis, and type 2 diabetes – are associated with changes in cellular and molecular programs including glycosylation changes. These changes manifest in a cell-type and tissue-specific manner and can be revealed using laser capture microdissection in combination with high-sensitivity mass spectrometry N- and O-glycomics. Alternatively, mass spectrometry imaging approaches are evolving that allow the sequential analysis of lipids, metabolites and N-glycans from a single tissue section revealing spatial, cell type-specific integrated molecular programs. Next to showing specific glycosylation programs in tissues, diseases also affect systemic glycosylation of blood proteins. Of particular interest are antibodies that are often produced in inflamed tissues but can be retrieved from the circulation as a liquid biopt providing insights into tissue- and disease-specific immune responses, with potential for diagnosis, patient stratification and treatment monitoring.

Glycan microarray toolbox for microbiome research

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The interactions of glycans with their binding partners, governed by an intricate "glyco-code," regulate numerous fundamental biological processes. As key host cell surface molecules, glycans mediate cell adhesion, trigger signalling, and serve as attachment sites for microbes, playing critical roles in colonization and infection. At the same time, microbial glycans are sensed by host lectins and immune receptors, activating innate and adaptive immune pathways and shaping inflammatory responses. Moreover, glycans derived from the host or its diet, both digestible and non-digestible, provide nutrients for the host and its microbial community, establishing a reciprocal relationship that benefits the physiology of both and supports host's mucosal and systemic health.

Glycan microarrays have emerged as a powerful tool that has revolutionized the field of glycobiology over the past two decades. Microarrays of host glycans continue to drive discoveries of cell-, tissue-, and host-tropisms of diverse microbes. Meanwhile, microarrays of plant- and microbial-derived polysaccharides are increasingly recognized as essential tools for elucidating the complex, multidirectional glycan-mediated interactions between hosts and their microbiota. In this short communication, I will highlight recent advances at the Imperial College Carbohydrate Microarray Facility in the development and application of glycan microarray tools to explore host–microbiome interactions.

Polymer processing strategies for application in the environment and health areas

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A large number of polymer processing strategies have been developed and applied in the laboratory and on industrial scales. Despite the supercritical fluids (SCF) not being new, in recent years, processing using supercritical carbon dioxide ($scCO_2$), in particular, has attracted great attention from the pharmaceutical industry, mostly due to the several well-known advantageous technical features, namely their green, sustainable, safe and environmentally friendly intrinsic characteristics. From nanoparticle formation to aerogel drying, this technology can be adapted to be used with materials soluble or insoluble in $scCO_2$. Some of those methods, such as supercritical CO_2 drying (SD) and the supercritical CO_2 foaming method (SFM), have been successfully used to produce porous polymer matrices, with applications in healthcare and environmental fields. In addition to these processes, the matrices can also be decontaminated and sterilised with supercritical carbon dioxide, making this process attractive for the natural polymer, avoiding the physicochemical degradation common in other methods. These methods will be presented with examples developed in scientific research.

Nanocelluloses and nanocellulose films. Opportunities in the conservation and restoration of historical documents

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Nanocelluloses have emerged as a new class of materials with a wide range of applications, ranging from packaging, through biomedicine to transparent electronics. These materials can be obtained from cellulose using appropriate chemical (or enzymatic) and mechanical treatments. They are composed of nanofibers with diameters of a few nanometres (2-50 nm) and lengths typically higher than 1 μm [1,2]. Nanocelluloses are usually obtained in the form of hydrogels, and from the hydrogels, films or aerogels can be prepared under optimized conditions of water removal and drying. Nanocelluloses enable the production of strong, transparent and stiff films with high barrier to oxygen. Due to these properties, nanocelluloses and nanocellulose films have potential to be used in the conservation and restoration of cellulosic objects like historical paper documents. In this presentation, nanocellulose films will be compared with Japanese Paper typically used for the mending of old paper documents [3]. Some results of the application of nanocellulose films on rag paper written with iron gall ink (IGI) will be presented, as well as preliminary results of the intervention on real old paper documents written with IGI. Advantages and limitations of the use of nanocellulose films in conservation and restoration will be presented.

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Oral Communications

From Hyposialylation to immune response: a possible mechanism for GNE Myopathy

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GNE Myopathy (GNEM) is an ultra-rare autosomal recessive disorder with an estimated worldwide prevalence of 11.00–87 individuals per million [1]. It results from mutations in *GNE*, that encodes for the bifunctional enzyme UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase, crucial in the sialic acid (SA) biosynthesis [2]. GNEM mainly affects young adults, leading to progressive skeletal muscle weakness [2]. Although hyposialylation is considered the main hallmark of GNEM, the cause of the muscle phenotype remains unclear. We aim to understand if GNEM has underlying immune implications, as some patients' muscle biopsies presented immune infiltrations [3]. Moreover, it has been shown that lower SA content leads to an upregulated Major Histocompatibility Complex Class-I (MHC-I) signal at cell surface [4]. Studies by our group using HEK-293 WT and *GNE* KO cells showed an overall higher MHC-I expression in the latter, as assessed by immunofluorescence [5] and western blot (WB) (*unpublished*). A decay assay indicated slower MHC-I turnover on *GNE* KO cells at 1h. In addition, a higher MHC-I mRNA expression on *GNE* KO cells was confirmed by RT-qPCR. For further validation, the expression of the MHC-I chaperone Erp57 was evaluated by WB, and there was no significant difference. Using GNEM patient-derived fibroblasts alongside healthy controls, we observed that only one patient presented significant differences in MHC-I cell surface expression, coincidently the one with lower sialylation levels. No difference in MHC-I and sialylation levels were found after 24 h incubation with a prodrug developed by the ProDGNE project to treat GNEM (*unpublished*). However, more assays need to be performed with longer incubation times. Overall, SA expression is intimately related to MHC-I cell surface levels. MHC-I seems like a possible biomarker to study immune involvement in GNEM, but more cell lines need to be studied to corroborate these results.

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Glycosylation Defects Disrupt Immunological Pathways in PMM2-CDG

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Phosphomannomutase 2-Congenital Disorder of Glycosylation (PMM2-CDG) is the most common subtype of congenital disorders of glycosylation, a group of rare genetic diseases caused by defects in glycan biosynthesis. Individuals with PMM2-CDG often present with immune-related complications, including recurrent infections, yet the mechanisms underlying immune dysfunction remain poorly defined [1, 2]. Previous work demonstrated that skin fibroblasts respond to inflammatory stimulus such as TNF-alpha by upregulating proinflammatory cytokines, chemokines, and key signalling pathways including NF-κB and MAPK cascades. These features underscore their relevance as a physiologically pertinent in vitro model for dissecting inflammation-related molecular mechanisms in PMM2-CDG [3].

Given the critical role of glycosylation in immune regulation, we explored how glycosylation defects impair immunological pathways in PMM2-CDG. We applied a comprehensive approach combining transcriptomics, glycomics, and immune assays in patient-derived fibroblasts and blood cells. Our results demonstrate that PMM2-CDG fibroblasts exhibit altered glycosylation patterns. Furthermore, we identified structural and functional abnormalities in the TNFRI receptor, which may lead to defective receptor processing and impaired downstream signaling. These molecular changes were accompanied by altered gene expression profiles enriched for immune and inflammatory pathway dysregulation, such as NF-Kb.

Immune phenotyping of blood samples from PMM2-CDG individuals further revealed broad alterations in immune cell sub-populations, such as $CD3^{+}CD4^{+}CD69^{+}$ and $CD3^{+}CD4^{+}CD274(PD\text{-}L1)^{+}$ and cytokine expression, such as TNF-alpha, IL-6 and IL-10, consistent with general immune impairment. Ongoing work is focused on profiling glycosylation patterns of immune receptors to further uncover mechanisms of dysfunction.

Overall, our findings suggest that glycosylation defects disrupt immunological pathways, contributing to immune dysregulation in PMM2-CDG. This study advances our understanding of the disease's immunopathology and highlights new potential avenues for targeted therapeutic development.

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MGL-Drug Conjugate: A Novel Glycan-Targeted Approach for Targeting Cancer Cells

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Cancer continues to pose a significant global health challenge and remains one of the leading causes of death. Despite notable advancements, many existing treatments still fall short in effectiveness and are often accompanied by difficult side effects. [1]. Antibody-Drug Conjugates (ADCs) are effective therapeutic tools, however, they often target protein receptors that are also found on normal cells, limiting their true cancer-specific delivery. Lectins offer a promising strategy for selectively targeting cancer cells while sparing healthy tissues. The human macrophage galactose-type lectin (MGL), expressed on antigen-presenting cells like dendritic cells and macrophages, stands out for its specific recognition of N-acetylgalactosamine (GalNAc) residues in tumour-associated glycans [2, 3, 4]. MGL has the distinct ability to bind both the Tn-antigen (GalNAcα1-*O*-Ser/Thr) and its sialylated derivative, sTn (Neu5Acα2–6GalNAcα1-*O*-Ser/Thr), which are characteristic of cancer cells and are absent on normal, healthy cells.

In this presentation, we share our latest progress in developing innovative MGL-drug conjugate. We selected MMAE, a FDA-approved antimitotic agent in ADCs [5], and incorporated a cleavable linker to enable controlled release. For precise drug attachment, we engineered an MGL construct with a C-terminal cysteine residue [6]. Conjugation reactions were monitored by mass spectrometry, while NMR spectroscopy and biophysical techniques confirmed that the protein retained its structure, stability and binding ability. Preliminary studies, using glycoengineered cancer cell models, have also allowed us to thoroughly evaluate the binding selectivity and toxicity of the MGL-drug conjugate.

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Unveilling the Landscape of Tumor-Associated Myeloid Cells in Immunosuppressive Breast Cancer

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Breast Cancer (BC) subtypes with poor prognosis are associated with tumor microenvironments (TMEs) rich in myeloid immune cells, known to promote pro-tumorigenic immunosuppressive (IS) TMEs. Immunotherapies targeting immune suppression, typically cytokine signaling, have been proposed. Yet they faced challenges from signaling redundancy and compensatory mechanisms, hampering clinical success.

Myeloid lectins, immune-modulating carbohydrate receptors that recognize aberrant tumor glycans, help sustain IS TMEs and are potential immunotherapy targets. However, systematic characterization of their expression pattern remains underexplored. Here, we profiled the lectin landscape of BC myeloid cells to identify targets for blocking myeloid-driven immunosuppression. We analyzed an RNA-Seq dataset (3207 patients) [1] classifying samples as IS (44%) and Non-IS, using a classifier gene panel [2]. HER2-overexpressing and triple negative tumors were overexpressed in the IS group, associated with decreased overall survival. We identified 13 lectins of interest by combining differential gene expression analysis (IS vs. Non-IS) with the top 25% overexpressed lectins in myeloid cells from a single-cell RNA-Seq cohort [3].

For biological validation we employed a BC human heterotypic 3D cell model (3D-3) using alginate microencapsulation and agitation-based cultures [4]. Stromal (fibroblast) and immune components (blood-derived monocytes) were co-cultured with spheroids of BC cell lines MDA-MB-231, HCC1806 and BT474. After 1-week culture, an IS TME was acquired featuring cancer-associated fibroblasts and tumor-associated macrophages (TAM) phenotype. Probed lectins showed differential protein expression upon TAM polarization (CD163+CD206+). Biological validation of hit lectins in paraffin- fixed patient tumor samples by immunohistochemistry was performed and correlated with clinical outcomes. Functional validation of hit lectins by CRISPR gene knockout and evaluation of the impact on TAM polarization, phagocytosis and T cell activation is ongoing.

In sum, our findings provide a comprehensive lectin profile of myeloid cells within the BC IS TME, identifying potential targets to tackle immunosuppression, and enhance immunotherapy response.

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Unveiling the role of Sialyl-Tn in pancreatic cancer progression and therapy

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Pancreatic cancer (PC) is the 6th leading cause of cancer-related deaths, being Pancreatic Ductal Adenocarcinoma (PDAC) the most common and aggressive form [1]. Understanding PDAC pathogenesis is crucial to develop targeted therapeutics.

Aberrant *O*-glycosylation is a cancer hallmark. Sialyl-Tn (STn), a truncated *O*-glycan absent in healthy tissues [1], but expressed in PDAC [2], is linked to immunosuppression, poor survival and metastasis in other cancers [3]. Our study assesses STn and its main carrier protein's role in PC progression, clinical correlation, and immunotherapeutic potential.

We analyzed STn expression in 185 formalin-fixed paraffin-embedded (FFPE) samples (precursor lesions, PDAC and metastases) by immunohistochemistry (IHC) using L2A5 antibody [4]. STn quantification was conducted via H-score. IHC results showed that STn expression was tumor-specific and increased significantly with disease progression. High STn levels correlated with elevated recurrence risk and reduced patient survival. Bioinformatic analysis of The Cancer Genome Atlas (TCGA) PDAC dataset revealed that high expression of ST6GALNAC1, the key enzyme responsible for STn biosynthesis, was independently associated with reduced overall survival, supporting its clinical relevance in PDAC progression. *In vitro*, PANC1 cells transduced with *ST6GALNAC1* (PANC1 STn) showed to overexpress STn as compared to control cells that carried an empty vector (PANC1 MOCK) when analysed by Flow Cytometry and Western Blot. MUC1, a potential key STn carrier, was identified as selectively expressed in PANC1 STn cells. PANC1 STn cells showed similar proliferation rates to control cells, but demonstrated enhanced wound healing capacity in scratch assays, suggesting a role for STn in promoting metastasis.

In summary, this study highlights the tumor-specific expression and clinical significance of STn in PDAC. By integrating patient tissue analysis, bioinformatics, and functional assays, we aim to better understand the role of STn in disease progression and evaluate its potential as a target for novel immunotherapeutic strategies.

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Novel CAR T formulations targeting tumor-associated glycoepitopes: A new strategy for solid tumors

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The accurate targeting of tumor-specific antigens is behind the success of any Chimeric Antigen Receptor (CAR) T cell therapy [1]. While cell surface antigens are rarely exclusively cancer-specific, their post-translational modifications (PTMs) offer a promising alternative. Indeed, the carbohydrate coat present on the surface of every living cell, which is altered in cancer, offers a variety of potential targets for immunotherapeutic approaches [2,3]. Among these, truncated *O*-glycans are potential candidates given their prevalent presence in various epithelial tumors and rare detection in healthy tissues [4,5].

In this study, we developed a novel short *O*-glycan-targeting monoclonal antibody (mAb) with unprecedent binding profile. This novel mAb specifically binds to epithelial tumors expressing the target antigen without reacting with healthy tissues. We identified the coding sequence of the novel mAb and engineered its corresponding single-chain variable fragment (scFv) into a second-generation CAR scaffold. The resulting CAR T cells effectively killed various solid cancer cell models in the in vitro setting. Additionally, these CAR T cells also eliminated patient-derived organoids (PDOs) from gastric cancer while sparing normal gastric mucosa-derived organoids. Notably, the novel CAR T formulation demonstrated robust control over various human cancer xenografts in mouse models, underscoring its efficacy in targeting and managing complex solid tumors.

Overall, our study introduces a novel, powerful, and precise glycan-directed CAR T cell therapy designed to target a broad spectrum of carcinomas, combining exceptional specificity and efficacy with a strong safety profile.

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Exploring the role of glycans in cancer therapy

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Immune checkpoints, key regulators of immune responses, play a crucial role in cancer evasion mechanisms. The discovery of immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 has revolutionised cancer treatment, with monoclonal antibodies (mAbs) becoming widely prescribed. Yet, challenges related to clinical efficacy and the high cost of mAb production drive the search for alternatives. Recognising that glycans expressed on cancer cells can also function as (glyco)immune checkpoints has sparked interest in these glycans as potential new targets in cancer therapy.

Aiming to explore new glycan-based mechanisms and potential new treatment interventions, we have 1) tested how sialylation interferes with the interaction of PD-1 to PDL-1, 2) surveyed small molecules among approved drugs that are commercially available as potential blockers of PD-1/PDL-1, and 3) assessed the effect of targeting cancer glycans with natural marine lectins.

1) Resorting to recombinant PD-L1 molecules produced in plants in two formats that differed in their glycan decoration: a sialylated molecule (PD-L1 NaNa) and a non-sialylated molecule (PD-L1 GnGn), we have evaluated the effects of sialylation on the ligation of PD-L1 to PD-1. We concluded that sialic acid had no effect on PD-1/PD-L1 binding.
2) To expedite drug discovery for PD-1/PD-L1 axis inhibition, we employed an integrated approach combining computer-aided drug design (CADD) tools. These tools include QSAR modelling, drug repurposing, and molecular docking. As a result of this approach, sonidegib was selected and subsequently validated for modulating PD-1/PD-L1 binding in vitro using ELISA and flow cytometry.
3) To target high-mannose N-glycans in cancer cells, we used lectins from the marine algae *Solieria filiformis* (SfL), Meristiella echinocarpa (MEL), and Amansia multifida (AML). All have shown potential anti-cancer activity through the induction of apoptosis as tested in the lung cancer cell line A549.

These findings underscore the pertinence of investigating a broader range of molecules to contribute to the ongoing efforts to advance cancer therapy.

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Polymethylated polysaccharides in mycobacteria: Dissecting their roles in stress adaptation and pathogenesis

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The genus *Mycobacterium* includes both obligate pathogens, such as *Mycobacterium tuberculosis* (Mtb), and nontuberculous mycobacteria (NTM), ubiquitous environmental organisms capable of causing opportunistic infections, particularly in immunocompromised individuals [1]. Their remarkable resilience is largely attributed to a distinctive lipid-rich cell envelope. The synthesis of fatty acid envelope precursors are influenced by cytoplasmic polymethylated polysaccharides (PMPS), namely 6-*O*-methylglucose lipopolysaccharides (MGLP) and 3-*O*-methylmannose polysaccharides (MMP) [2, 3]. While MGLP is conserved across all mycobacterial species, MMP is absent from pathogens such as Mtb and *M. abscessus*, but present in several other species, including *M. avium*, which can synthesize both polysaccharides [4].

Recently, we discovered that MMP biosynthesis operates through a self-recycling mechanism involving a novel α-endomannosidase (MO), which generates mannooligosaccharide primers, and an α-(1→4)-mannosyltransferase for their extension. Disruption of this pathway impairs MMP production and affects growth under cold stress, suggesting a role for MMP in cold adaptation [4]. Conversely, MGLP-deficient mutants show sensitivity to heat stress, implicating MGLP in heat responses [5, 6]. Decades of research have elucidated most genes involved in the biosynthesis of MGLP, enabled by the generation Mtb mutants [7]. Notably, neither MMP nor MGLP are essential for *in vitro* growth, despite longstanding assumptions about the essentiality of MGLP biosynthetic enzymes and their proposed involvement in fatty acid metabolism [4, 7].

We have constructed a double knockout mutant of *M. smegmatis* lacking both polysaccharides, creating a unique model system to investigate their hypothesized isofunctional and potentially synergistic roles. Our ongoing studies are focused on elucidating whether they are essential for survival under specific environmental stress conditions, persistence during infection, or the establishment of virulence. The outcomes of this research are expected to shed new light on the mechanisms underlying mycobacterial stress adaptation. Ultimately, these insights may facilitate the identification of novel molecular targets for strategies aimed at controlling both environmentally resilient and clinically significant mycobacteria.

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Structural studies of a highly thermostable GpgS reveal a cryptic aromatic binding pocket

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Mycobacteria have long posed a threat to human health, being responsible for a range of hard-to- treat infections such as tuberculosis, leprosy, and atypical mycobacterioses. Their intrinsic resistance to antibiotics and resilience when exposed to environmental stress complicate treatment and underscore the need for discovering novel drug targets and developing new therapeutic strategies [1]. *Mycobacterium hassiacum*, a thermophilic mycobacterium, is a promising source of stable proteins suitable for detailed structural analysis [2]. In this study, we investigated its glucosyl-3-phosphoglycerate synthase (GpgS), an enzyme essential for *M. tuberculosis* growth [3] which is implicated in responses to nitrogen starvation and thermal stress in different mycobacterial species [1]. We confirmed the potential of *M. hassiacum* as a source of enzymes amenable to structural studies, since its GpgS orthologue yielded not only the first mycobacterial GpgS X-ray three-dimensional structure at near atomic resolution (1.11 Å), but also multiple high-resolution structures under different pH conditions and in complex with different ligands. These structural insights revealed a previously unrecognized aromatic binding pocket, suggesting possible links between GpgS activity and broader metabolic pathways in mycobacteria. Our findings establish *M. hassiacum* as a valuable model for structural enzymology in mycobacteria and highlight GpgS as a potential drug target. The detailed structural data provided here offer a foundation for rational drug design, opening new avenues for the development of inhibitors targeting this essential enzyme.

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Decoding Mucin O-Glycan Recognition by Commensal Bacteroides: Structural and Functional Insights

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The mucus layer covering intestinal epithelial cells is rich in heavily O-glycosylated proteins known as mucins [1]. Both commensal and pathogenic bacteria in the gut exploit these mucin O-glycans in distinct ways. However, the molecular mechanisms by which O-glycans modulate the interaction between bacteria and the human host need elucidation. Notably, the commensal species *Bacteroides thetaiotaomicron* and *B. caccae* exhibit increased mucin O-glycan-degrading activity under low-fibre dietary conditions, which has been linked to an increased susceptibility to infection [2,3]. In this study, we present the characterisation of newly identified glycan-binding proteins from modular enzymes from these bacteria. These proteins are encoded by co-regulated gene clusters known as polysaccharide utilisation loci (PULs), which specifically target defined glycan structures [2]. Using sequence and structural bioinformatics analyses, we identified several candidate glycan-binding proteins for high-throughput expression and ligand screening. Structural studies using X- ray crystallography, combined with microarrays of human mucin-type glycoproteins and sequence-defined glycans [4–6], have revealed the molecular basis for recognising mucin O-glycan structures, including truncated core O-glycans, which are often overexpressed in cancer. Following these discoveries, we are characterising the associated catalytic domains for determining their activities as mucinases with novel O-glycan and peptide selectivities. Overall, this work offers new insights into the interaction between commensal bacteria and host glycans, with potential applications in cancer research.

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Structural Insights Reveal Contextual O-Glycan Cluster Recognition by the Mucin-Binding Module X409

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The mucus barrier covering wet epithelial surfaces is formed by densely O-glycosylated mucins, which modulate host-microbiota interactions by shaping microbial colonization and suppressing virulence [1,2]. However, the molecular basis by which microbial glycan-binding proteins recognize mucins remains poorly understood. Here, we provide structural and mechanistic insights into how X409 - a mucin-binding module from the enterohemorrhagic *E. coli* StcE mucinase - recognizes mucin-specific glycopeptidic epitopes [3]. Single molecule mass photometry shows that X409 selective binds O-glycan clusters. Crystal structures revealed that X409 recognizes α-GalNAc clusters on the conserved STTT motif of MUC5Ac, favouring residues S1, T3, and T4. NMR titration experiments using ^{15}N-labeled X409 revealed slow-exchange chemical shift perturbations and ligand-induced stabilization of a dynamic loop, supporting a strong and specific interaction. In contrast, a TTTT variant failed to elicit similar effects, underscoring the importance of the GalNAc–Ser linkage and glycan presentation. Molecular dynamics simulations further confirmed key residue interactions, enhanced stability with extended glycans, and a defined conformational ensemble compatible with binding. Our findings define X409 as a mucin-binding module that recognizes contextual glycan motifs - clustered saccharide patches comprised of rows of inner monosaccharides in adjacent O-glycans, unique to mucins. These findings reveal a distinct mechanism for mucin recognition involving contextual glycopeptidic epitopes and suggest how microbial proteins can retain mucin binding despite O-glycan degradation during colonization.

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OC12

Defining Host Glycan Ligands Targeted by the Giant Adhesin SiiE of *Salmonella entérica*

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Mucin-type *O*-glycans are complex glycoconjugates that form a key structural and functional component of the intestinal mucosal barrier, mediating host–microbe interactions and influencing microbial colonization patterns. These glycan structures are normally involved in shielding epithelial cells from pathogen invasion by acting as traps. However, certain enteric pathogens, including *Salmonella enterica* serovar Typhimurium, have evolved specific mechanisms to recognize and bind to these *O*-glycan motifs, facilitating epithelial attachment and subsequent invasion, a process that exacerbates infection severity and contributes to the persistence of gastrointestinal diseases, particularly in the context of rising antibiotic resistance [1,2].

This study investigates the molecular interactions between *Salmonella* and host-*O*-glycans, focusing on the role of the giant non-fimbrial adhesin SiiE in recognizing sialylated *O*-glycan structures of the membrane-bound mucin MUC1[3]. Through the integration of glycan microarray screening, and high-resolution Nuclear Magnetic Resonance (NMR) spectroscopy, we explore the binding specificity of the SiiE-glycan interaction.

Our findings underscore the potential of targeting SiiE-mucin interactions to develop novel anti-adhesion therapies aimed at preventing *Salmonella* colonization. By identifying structural motifs essential for glycan recognition, this study will contribute to the rational design of glycomimetic inhibitors that disrupt pathogen adhesion without exerting selective pressure for resistance.

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Glycan-Lectin Interactions in Context: New NMR Approaches

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The molecular recognition of glycans is at the heart of numerous biological processes within living organisms and plays crucial roles in health and disease. Understanding these interactions at the molecular/atomic level is of paramount importance for developing novel therapeutic approaches, improving disease diagnostics and/or advancing in biotechnological applications [1].

Given the dynamic nature of glycans, NMR has been extensively used to study glycan-lectin interactions and remains at the forefront of existing approaches [2]. In this context, 19F-labelled glycan probes have been exploited in diverse applications [3] and fluorine NMR has emerged as a powerful strategy to interrogate biological systems in complex environments closely resembling native conditions [4].

Herein, we describe the NMR analysis of the molecular recognition of complex glycoconjugates, even in complex environments such as the cell, by the exploitation of 19F-NMR spectroscopy. This novel approach allowed easily detecting complex glycans, monitoring binding events and/or profiling the glycosylation of intact cells.

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C1GalT1 and ST6GalNAc-I mechanistic insights in Mucin-1 *O*-Glycosylation

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Mucin-type O-glycosylation is a complex mechanism regulated by the coordinated activity of specific glycosyltransferases (GTs) [1]. Misregulation of GTs is a common feature of cancer that yields tumor- associated carbohydrate antigens (TACAs) [1]. Two of the most common TACAs are the T-(Galβ1- 3GalNAcα1-*O*-Ser/Thr) and sTn (Neu5Acα2-6GalNAcα1-*O*-Ser/Thr) antigens. The T-antigen is naturally formed during the *O*-glycosylation process by the inverting enzyme C1GalT1. This enzyme transfers a Gal residue from the sugar donor UDP-α-Gal to the GalNAc in a β1-3 linkage [2]. In normal cells, this antigen is known as the core 1 structure and is the precursor and a hidden part of more complex *O*-glycans; however, truncation of *O*-glycans, in cancer cells, exposes the T-epitopes in malignancy [1]. The sTn antigen, mostly found in cancer cells, is generated by the inverting enzyme ST6GalNAc-I, which adds a Neu5Ac residue from the CMP-β-Neu5Ac sugar donor to GalNAc-mucin peptides in a α2-6 linkage [3]. Despite the biological significance of C1GalT1 and ST6GalNAc-I in health and disease, their molecular mechanisms of recognition and catalysis remain elusive.

Understanding the molecular mechanism of catalysis of these antigens in complex substrates and recognition of GalNAc-containing mucin peptides by these enzymes are of extreme importance to develop strategies targeting C1GalT1 and ST6GalNAc-I. Herein, new structural and conformational insights on the molecular mechanism of mucin-1 *O*-glycosylation by these enzymes will be highlighted through a multidisciplinary approach focused on NMR spectroscopy and molecular dynamic simulations. C1GalT1 and ST6GalNAc-I glycosylation preferences will be described and compared towards different complex acceptor substrates and the impact of glycosylation in the backbone of mucin-1 conformation will also be highlighted.

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Molecular recognition properties of the tandem-repeats Galectin-4 and Galectin-9

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Galectins are a family of β-galactoside binding lectins that have attracted significant attention as targets for therapeutic intervention because of their roles in cancer progression and prognostic implications. [1] Within the 3 existing subfamilies, that of tandem-repeat Galectins (TR-Gals) is characterised by having two different Carbohydrate Recognition Domains (CRD), which have different binding preference towards short galactose containing saccharides, and are covalently linked through a short peptide. This domain architecture endows TR-Gals with an enhanced ability to cross-link and agglutinate multivalently-presenting-glycan binding partners (i.e., glycoproteins). This is fundamental for the mechanisms through which Galectins are thought to start downstream signalling pathways.

The main objective of this work is the characterization of the binding properties of the TR-Gals, Galectin-4 [2] and Galectin-9, towards different ligands, including oligosaccharides, glycoproteins, and Lipopolysaccharides, with a special focus is on the structural and mechanistic details.

For that we have employed a combination of biophysical techniques, with a special emphasis on Nuclear Magnetic Resonance (NMR) methods.

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Arylamide glycofoldamers for targeting carbohydrate–protein interactions

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Multivalency plays a central role in carbohydrate-mediated recognition processes, where weak monovalent interactions are transformed into high-affinity binding through the simultaneous engagement of multiple ligands [1]. This principle underlies a broad spectrum of carbohydrate–protein interactions in biology, including those involved in cell–cell communication, immune recognition, and pathogen adhesion [2]. Inhibiting such interactions, or studying them in detail, requires synthetic scaffolds capable of displaying carbohydrates with defined valency, spacing, and orientation—features that strongly influence binding strength and selectivity [3].

Arylamide foldamers are bioinspired synthetic oligomers that, like peptides or nucleic acids, fold into well-defined conformations in solution [4]. When made from quinoline subunits, their predictable helicity, tunability, and ease of synthesis make them ideal platforms for the precise multivalent presentation of carbohydrates [5]. In addition, they can incorporate proteinogenic side chains to mimic protein surfaces, which further enhances their biomimetic potential.[6]

This presentation will outline our recent efforts to develop quinoline-derived arylamide glycofoldamers as modular and structurally defined platforms for the multivalent display of carbohydrates, highlighting their application in the inhibition of bacterial adhesion and in the profiling of carbohydrate-binding interactions.

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Design and Synthesis of Novel Carbohydrate-Based Antibacterial Agents to Address Antimicrobial Resistance

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Antimicrobial Resistance (AMR) is recognized as one of the top ten health threats by the World Health Organization (WHO), with bacterial AMR being particularly alarming due to the rapid emergence of multidrug-resistant strains [1,2]. In 2024, the WHO published the list of priority pathogens divided into three groups, with the most critical group being exclusively composed of resistant Gram-negative bacterial (GNB) strains [3]. In the last two decades, our group has been focused on the development of new antibacterial carbohydrate-based agents, obtaining promising results with dodecyl deoxyglycosides against Gram-positive bacteria (GPB), namely *Bacillus cereus* and *Bacillus anthracis* [4-7]. The mode of action of these compounds was determined to be related to the interaction of the glycosides with phosphatidylethanolamine (PE) lipids present in the cytoplasmic membranes of the tested GPB. In GNB, although these compounds showed potential to target their inner membrane (IM), their outer membrane (OM) prevented them from exhibiting antibacterial effects [7]. In this context, our efforts have recently shifted towards modifying these glycosides to target GNB. In 2024, a strategy relying on the combination of these compounds with sub-therapeutical concentrations of polymyxins was developed, presenting promising results against several multidrug-resistant GNB strains [8]. Moreover, a prodrug approach is currently being developed. This approach relies on the synthesis of disaccharide mimetics of the GNB cell wall *N*-acetyl muramic acid-β-1,4-*N*-acetylglucosamine repeat units, comprising a dodecyl deoxyglycoside linked to a MurNAc unit through a β-1,4 bond. Hopefully, these analogues will be able to cross the OM, entering the periplasmic space where their β-1,4 bond will be hydrolyzed by peptidoglycan glycosidases. Upon hydrolysis, the active glycoside is expected to interact with the PE lipids of the IM, promoting cell lysis.

With these new strategies we aim at contributing to the research in the field of overcoming the concerning phenomenon of AMR.

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Carbohydrate-rich wastewater as a source of short-chain organic acids through acidogenic fermentation

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Annually, several tons of food frying waste are generated from household and restaurant kitchens and industrial processes potentiating environmental burden due to incorrect management or disposal. This waste require transportation to the treatment facilities and the cleaning of the transport tanks generate high volumes of wastewater (TTWW), which are difficult to treat due to their complex composition. This study aims to address the challenge of treating TTWW through its biological valorization by applying acidogenic fermentation (AF) to produce short-chain organic acids (SCOAs) [1]. The mixtures of SCOAs obtained have been studied for added-value applications, including in bioplastics [2]. TTWW was found to be constituted by ca. 65% of carbohydrates, with glucose from starch as the main constituent, and lower amounts of fructose and arabinose, possibly derived from fruits and vegetables frying, and traces of lipids. The total amount of organic matter corresponded to a chemical oxygen demand (COD) of 90.04 g COD/L. Four microbial inocula collected in two different municipal wastewater treatment plants (WWTP), two aerobic (AES1 and AES2) and two anaerobic (ANS1 and ANS2). were tested with different food to microorganism (F/M) ratios of 1:1, 1:2, and 2:1 g COD/g volatile suspended solids (VSS). All assays resulted in significant SCOAs production and total consumption of the carbohydrate-rich substrate. The AES2 allowed to obtain the highest acidification degree (AD) and, globally, the highest SCOAs diversity, particularly of acetic (54%), butyric (18%) and propionic (18%) acids. This study revealed the potential of TTWW to produce SCOAs, that in the future can be used for the production of polyhydroxyalkanoates (PHAs) by mixed microbial cultures. This is a process that follows the circular economy concept and leads to a sustainable valorization of food industry waste.

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Structural modulation of *Agaricus bisporus* polysaccharides by lactic acid fermentation and its biofunctional implications

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Edible mushroom fermentation by lactic acid bacteria is gaining relevance to enhance bioavailability and nutritional value of foods, improve their digestibility, enhance sensory quality, extended shelf life and food safety, and promote probiotic and prebiotic benefits, allowing the development of functional foods and beverages, as well as the valorization of by-products [1,2]. In this study, aiming to evaluate the feasibility of using *Agaricus bisporus* polysaccharides as substrates for lactic acid bacteria fermentation, fresh residues (300 g; ~39 g dry matter) were fermented and the polysaccharide fraction was recovered from the alcohol-insoluble residue (AIR) and analysed by GC-DIF using 2-deoxyglucose as an internal standard after derivatization to alditol acetates. The unfermented AIR contained 18.9% of polysaccharides, mainly glucose (74 mol%), galactose (8%), and xylose (4%). After fermentation, the amount of AIR decreased to 50% when compared to the fresh residue, although with a similar amount of polysaccharides (19.3%). The content of glucose decreased to 66%, galactose to 5%, and xylose increased to 11%. The dialyzed fermented aqueous liquid, although accounting only 0.17% of the unfermented AIR, was rich in polysaccharides (53,6%), of which 75% was galactose. Linkage analysis of the partially methylated alditol acetates showed the release of soluble 1,6-linked galactans with branches at O-2 and the presence of thermibally linked fucose and xylose residues. The data suggest that lactic acid bacteria uses the mushroom glucan for fermentation, allowing the release of soluble galactans into the aqueous medium. These galactans are increasingly recognized for their potential health benefits [3].

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Cyclodextrins in Food Systems: Technological Applications and Functional Perspectives

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Cyclodextrins (CDs), cyclic oligosaccharides composed of α-1,4-linked D-glucopyranose units, have become essential tools in food science due to their ability to form inclusion complexes with a wide array of guest molecules. Originally discovered over a century ago, their relevance has expanded from pharmaceutical and chemical applications to food processing and nutraceuticals, supported by more than 48,000 scientific publications and over 53,000 patents involving food-related uses [1,2].

The present paper explores the biological synthesis and structural-functional properties of native α, β, and γ-cyclodextrins, with particular attention to their encapsulation capabilities. These complexes improve the solubility, stability, and bioavailability of sensitive food ingredients such as flavors, vitamins, unsaturated fatty acids, and phytochemicals [3].

CDs offer significant technological advantages in food systems: reducing bitterness and cholesterol, modifying the glycaemic index of bakery products, stabilizing hydrocolloids, and serving as dietary fibers. They are also integrated into nanosensors for detecting bioactives and play a role in active and intelligent food packaging systems (Fig. 1) with antimicrobial and aroma-control functionalities [4].

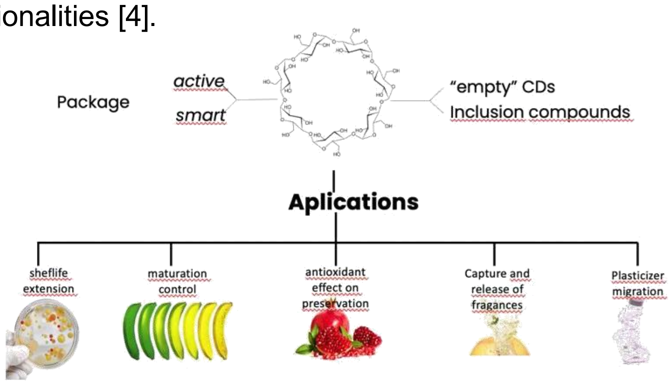

Figure 1: Cyclodextrins in food packing

Furthermore, functional food ingredients based on CDs show promise for large-scale applications. Studies include encapsulation of bioactive compounds from halophyte extracts and essential oils with antioxidant, antimicrobial, and anti-inflammatory properties [5]. Future directions suggest the use of CD-based metal-organic frameworks (MOFs), nanosponges, and nanofibers for targeted delivery and smart food systems [6].

This work underlines the versatility of cyclodextrins at the intersection of supramolecular chemistry, nanotechnology, and food engineering, offering innovative solutions for food quality, safety, and health promotion.

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Searching for sources of sweet-tasting oligosaccharides able to decrease sucrose content in foods

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Oligosaccharides can be naturally found in foods or produced through the depolymerization of polysaccharides. Besides acting as prebiotics, some oligosaccharides possess sweet-tasting properties, allowing their potential application for decreasing sucrose content in foods [1]. This study aimed to isolate and produce manno-, galacto-, and fructooligosaccharides from different plant sources and test their potential as sweet-tasting and prebiotic ingredients. Mannooligosaccharide-rich fractions were produced from konjac glucomannan, following enzymatic treatments with cellulase and 1,4-β-mannanase. Galactooligosaccharides were obtained by solid-phase extraction of chickpea soaking water, while fructooligosaccharides were isolated from yacon syrup by 80% ethanol precipitation. These oligosaccharides were characterized by high-performance anion exchange chromatography with pulsed amperometric detection, and carbohydrate composition by alditol acetates analysis using gas chromatography with flame ionization detection. Their prebiotic potential was determined by faecal *in vitro* fermentation trials.

Mannooligosaccharides (DP2-DP6), composed of 48 mol% mannose and 41 mol% glucose were obtained by hydrolysis of konjac glucomannan using cellulase and mannanase yielded a similar oligosaccharide profile, with a lower proportion of polymeric material due to a higher extension of hydrolysis. Ciceritol (Gal-(α1→6)-Gal-*O*-methyl-*myo*-inositol), raffinose (Gal-(α1→6)-Glc-(α1→β2)-Fru) and stachyose ([Gal-(α1→6)]$_2$-Glc-(α1→ β2)-Fru), and were obtained as part of the hydrophilic material of chickpea soaking water (331 g/kg), resulting in an ingredient with a relative sweetness of 0.22 compared to sucrose. Kestotriose ([Fru-(β2→1)]$_3$-α-Glc), kestotetraose ([Fru-(β2→1)]$_4$-α-Glc), and kestopentaose ([Fru-(β2→1)]$_5$-α-Glc) were obtained as part of ethanol-soluble material of yacon syrup. Besides presenting a relative sweetness of 0.48, these oligosaccharides promoted the growth of Actinobacteriota and Firmicutes. These results evidence that plant-derived oligosaccharides can serve as dual-function ingredients, offering moderate sweetness and prebiotic benefits, supporting industry goals for sugar reduction and functional food innovation.

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The Role of Rhamnogalactan and Fucogalactan in the Cholesterol-Lowering Effects

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Polysaccharides, the main component of mushrooms' dry matter, have been described with bioactive properties. Several *in vitro* and *in vivo* studies demonstrated the cholesterol-lowering properties [1], however, the structure-function relationship between this effect and polysaccharides characteristics are still undisclosed.

In this work, water-soluble polysaccharides present in *L. deliciosus* and *M. procera* were extracted and fractionated with graded ethanol precipitation. Polysaccharides – rich fractions (93–32%), insoluble in 50%, 60%, 70% and 80% of ethanol, are mainly composed of galactose and glucose in three species, and rhamnose in *L. deliciosus* or fucose in *M. procera.* The glycosidic linkages analysis of the partially methylated alditol acetates showed the presence of linear (1→6)-liked galactose and (1→2,6)-linked galactose. The presence of rhamnogalactan and glycogen-like structure in the *L. deliciosus* fractions was demonstrated with terminally-linked rhamnose, (1→4)-liked glucose and (1→4,6)-linked glucose, respectively. To remove the glycogen-like structure, the Et50 fraction *from L. deliciosus* was hydrolyzed by α-amylase, obtaining a fraction Et50> 12 kDa. The *M. procera* fractions showed the presence of fucogalactan, with terminally-linked fucose. The average polymerization degree of rhamnogalactan and fucogalatan varies between 129 – 34 and 56 – 49, respectively.

Assessment of cholesterol lowering activity of fractions from *L. deliciosus* were tested using an *in vitro* intestinal simplified model [2]. The solubility of cholesterol films was measured by GC-MS and bile salts sequestration capacity was quantified using ^{1}H NMR. Cholesterol solubility decreased between 90 – 73% and bile salts sequestration showed a decreased of 38 – 10%, depending on the average polymerization degree. The cholesterol-lowering effect is greater in rhamnogalactan present in *L. deliciosus* with lower average polymerization degree, by interaction with cholesterol and bile salts sequestration, demonstrating the structure-function relationship between mushroom polysaccharide and hypocholesterolemic properties.

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Interactions Between Wine Polysaccharides and Bentonite: Implications for Protein Removal Efficiency in Model White Wine Solutions

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To understand how various wine components affect bentonite's ability to remove proteins, a model white wine solution was prepared (12% v/v ethanol, 4 g/L tartaric acid, pH 3.2). Bovine Serum Albumin (BSA) was used as a model protein at a maximum concentration of 500 mg/L [1], while sodium-activated bentonite [2] was selected as the fining agent. All experiments were carried out with a 24-hour reaction period, after which the remaining protein content was determined using the Bradford assay [2] and polysaccharide content was measured via the phenol-sulfuric method [3]. To assess the influence of polysaccharides on bentonite performance, naturally occurring wine polysaccharides— arabinogalactans, mannoproteins, and pectin—were added to the model white wine solution at concentrations equivalent to the sum of their monosaccharide units [2]. The presence of these polysaccharides led to a decrease in bentonite efficiency at lower concentrations, attributed to polysaccharide-bentonite interactions, where the polysaccharides occupy adsorption sites on the bentonite surface. Furthermore, arabinogalactans and pectin themselves demonstrated direct interactions with BSA proteins. To further characterize the adsorption process, isotherm adsorption models were performed by maintaining a constant bentonite concentration (20 g/hL) while varying the concentrations of individual polysaccharides and BSA protein from 500 to 1 mg/L. Apart from pectin, that showed no adsorption due to its neutral charge, the Freundlich isotherm model accurately described the data. This model indicates heterogeneous adsorption surfaces and potential interactions among adsorbed molecules [4]. BSA showed more favourable adsorption onto bentonite, whereas polysaccharides exhibited (near) irreversible adsorption ($n \approx 1$) leading to the suggestion that arabinogalactans and mannoproteins may be absorbed by their protein fraction. These findings highlight the complex colloidal nature of wine, providing insights into the interactions among proteins, polysaccharides, and bentonite. Understanding these interactions is essential to improving the predictability and efficiency of bentonite fining in the wine industry.

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Polysaccharides as modulators of procyanidins interactions with oral-cell models

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Polysaccharides play a pivotal role in modulating mouthfeel by attenuating astringency in plant-based foods (rich in polyphenols). Through two principal mechanisms - (i) competitive interaction with polyphenols to prevent their binding to salivary proteins, and (ii) ternary complex formation between polyphenol–protein-polysaccharide aggregates to reduce precipitation - these polymers offer promising sensory benefits. In this study, we evaluated five structurally diverse polysaccharides – arabinogalactan (arabic gum), glucuronomannoglucan (xanthan gum), arabinoxylan, xyloglucan, and mannoprotein - for their capacity to inhibit the binding of grape-seed procyanidins onto an oral epithelial cell model. Procyanidins were incubated with each polysaccharide to mimic their co-existence in foods before being consumed. The mixtures were then applied to oral models constituted by epithelial cells (HSC-3 human tongue squamous carcinoma cell line), HSC3 and mucins and other salivary proteins, or only salivary proteins (SP). Bound procyanidin were quantified by colorimetric assay and analysed by HPLC in the presence and absence of polysaccharides, enabling assessment of each polymer's efficacy. Additionally, it was explored how polysaccharide molecular weight, branching pattern and other structural features could influence their interaction with procyanidins. Results demonstrated that xanthan gum and arabinoxylan significantly reduced procyanidin binding. For xanthan gum fractions, chain length was a key determinant in the epithelial model (HSC3), while side-chain were more relevant in salivary protein-containing models. The interplay of backbone linearity, substitution patterns, and ester-linked phenolics governs the specificity and efficacy of each arabinoxylan fraction.

These findings underscore the importance of polysaccharide structure in designing plant-based formulations with improved sensory profiles and highlight xanthan gum and arabinoxylan as leading candidates for mitigating astringency in procyanidins-rich food applications.

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Deciphering Raspberry seed-derived Biopolymers for Food Applications

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Approximately 97% of raspberry harvest is directed into industrial processing to create products like juices, jams and desserts due to raspberries' high perishability. At the same time, this process generates *ca.* 110k ton of raspberry seeds (RS) as byproducts from pressing operations. In turn, RS have no food-related applications and are often simply discarded, raising environmental concerns associated with landfills, for example. Furthermore, the detailed composition of the majority of RS, that is, its polymeric constituents like polysaccharides and proteins, remains underexplored [1]. In this work, the polymeric material of defatted RS was recovered by microwave-assisted extraction (MAE) [2], employing a design of experiments to optimize the temperature (T), operation time (t), and solvent quantity (0-0.6% acetic acid aqueous solution) parameters. The defatted RS allowed to obtain up to 14% (w/w) of water-soluble extracts made up majorly of carbohydrates rich in arabinose, glucose, and xylose (up to 45%). MAE also allowed to enhance up to fourfold the original RS protein content (up to 31%). The MAE soluble extracts showed antioxidant (up to IC_{50} 65 μg/mL) and antidiabetic (up to 84% α-glucosidase inhibition) activity. Selected MAE extracts also showed promising hypocholesterolemic activity (up to 43% increase in insoluble cholesterol). The best performing MAE extract, considering a compromise of yield and bioactivity, was incorporated (2.5% and 5% w/w to its flour content) to develop bioactive biscuits with improved antioxidant activity (up to IC_{50} 0.35 mg/mg biscuit) correlated with an increment of activity up to fivefold in relation to control. In sum, RS-derived biopolymers represent a promising renewable source of biomolecules for innovative food applications, adding value to this agri-food byproduct.

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Caloric reduction of sucrose-rich fruit products through an enzymatic approach

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Sugar consumption is a major concern in modern developed societies. Although essential for human metabolism, excessive intake has been linked to diseases development, including dental caries, obesity, Type-2 Diabetes Mellitus, cardiovascular diseases and metabolic dysregulation [1]. Fruit is a common dietary source of sugar. However, contrary to whole-fruits, sugars from fruit-derived juices and concentrates are rapidly absorbed. When consumed directly or incorporated into products, these concentrates can significantly increase total sugar concentration of foods. The growing consumer interest in Clean Label foods which emphasize health, sustainability, and environmental responsibility has driven demand for minimally processed, organic, "natural" foods, free from artificial ingredients and with the shortest possible ingredient list [2]. Considering this, our objective was to reduce sugar content of sucrose-rich fruit concentrates by using invertase and fructosyltransferase enzymes. These convert sucrose into fructooligosaccharides(FOS), which are low calorie sweeteners. FOS also contribute to satiety and body weight control, have a low glycaemic index, are non-carcinogenic, and well-known prebiotics [3]. As enzymatic treatments are generally classified as processing aid, as they do not remain active in the final products, typically do not need to be labelled. In this study, enzymes were applied at a concentration of 12 U/g of initial sucrose under the samples' endogenous pH, at two temperatures (10 and 35 ºC) and over various reaction times (0,2,4,6,24 h).The results showed that both enzymes are capable of reducing caloric content by at least 50%, primarily through conversion of sucrose into FOS. The highest conversion rates were observed in early stages of reaction, with a gradual slowdown over-time. Interestingly, enzymatic activity occurred efficiently at low temperature and at endogenous pH of sample, with no significantly pH alteration. These results present a promising Clean Label strategy for reducing sugar content in sucrose-rich fruit products and lay a strong foundation for future research and industrial applications.

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OC27

Cardiac Molecular Analysis Reveals Aging-Associated Metabolic Alterations Promoting Glycosaminoglycans Accumulation Via Hexosamine Biosynthetic Pathway

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Age is a prominent risk factor for cardiometabolic disease, often leading to heart structural and functional changes. However, precise molecular mechanisms underlying cardiac remodeling and dysfunction exclusively resulting from physiological aging remain elusive. Previous research demonstrated age-related functional alterations in baboons, analogous to humans. The goal of this study is to identify early cardiac molecular alterations preceding functional adaptations, shedding light on the regulation of age-associated changes.

Unbiased transcriptomics of left ventricle samples are performed from female baboons aged 7.5–22.1 years (human equivalent ≈30–88 years). Weighted-gene correlation network and pathway enrichment analyses are performed, with histological validation.

Modules of transcripts negatively correlated with age implicated declined metabolism-oxidative phosphorylation, tricarboxylic acid cycle, glycolysis, and fatty-acid β-oxidation. Transcripts positively correlated with age suggested a metabolic shift toward glucose-dependent anabolic pathways, including hexosamine biosynthetic pathway (HBP). This shift is associated with increased glycosaminoglycan synthesis, modification, precursor synthesis via HBP, and extracellular matrix accumulation, verified histologically. Upregulated extracellular matrix-induced signaling coincided with glycosaminoglycan accumulation, followed by cardiac hypertrophy-related pathways.

Overall, these findings revealed a transcriptional shift in metabolism favoring glycosaminoglycan accumulation through HBP before cardiac hypertrophy. Unveiling this metabolic shift provides potential targets for age-related cardiac diseases, offering novel insights into early age-related mechanisms.

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Dialdehyde Polysaccharides for the Sustainable Synthesis of Multifunctional Conductive Biomaterials

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The advancement of bioelectronics and tissue engineering relies on developing sustainable and biocompatible conductive materials. While conductive polymers like polypyrrole (PPy) show promise, their traditional synthesis methods are often using highly toxic and environmentally harmful reagents.

Here, we describe a novel, green synthesis of conductive biomaterials, based on dialdehyde polysaccharides (DAPs), serving as both the template and chemical initiator for pyrrole polymerization [1,2]. The core of the method lies in the newly discovered spontaneous aldol condensation reaction between DAPs and pyrrole that occurs in water at ambient conditions [3].

In the first step, this versatile method allows for the efficient functionalization of various DAPs (derived e.g. from cellulose, hyaluronate, alginate, or dextran) with pyrrole and its derivatives. The reaction is highly efficient, allowing straightforward immobilization of pyrrole cycles for modification of surface properties or immobilization of pyrrole-based drugs. Moreover, the intrinsic polyaldehydic structure of DAPs can be used to initiate the spontaneous polymerization of pyrrole, resulting in covalently bonded DAP/PPy composites with good conductivity and greatly improved durability. DAP also acts as a template, allowing the preparation of various conductive materials, from nanoparticles and nanofibers to hydrogels, textiles, and 3D hierarchical structures.

Comprehensive characterization using FTIR, NMR, SEM, TEM, and electrochemical methods was performed to clarify the reaction mechanism and properties of the prepared materials. Furthermore, biological assays demonstrated excellent cytocompatibility along with significant antioxidative, anti- inflammatory, and immunomodulatory effects and wound healing acceleration.

To summarize, this work establishes dialdehyde polysaccharides as a versatile and sustainable platform for the eco-friendly synthesis of advanced conductive biomaterials, opening new avenues for applications in biosensors and advanced wound dressings, with potential uses also in wearable electronics and energy storage.

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OC29

Administration of a Sugar-Functionalized Chitosan/DNA Complex Vaccine Elicits Protective Immunity Against SARS-CoV-2 in Mice

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The emergence of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and its devastating global impact emphasise the urgent need for effective, non-invasive vaccines. While current mRNA and adenoviral vaccines have mitigated severe disease, they face challenges in preventing mucosal transmission and addressing the emergence of new viral variants. This study investigated an intranasal DNA vaccine encoding the SARS-CoV-2 spike protein. The underlying hypothesis of this study is that an optimal DNA vaccine delivery system should combine mucoadhesive properties with targeted delivery capabilities. Chitosan was selected due to its intrinsic mucoadhesiveness and its ability to form stable complexes with DNA. Furthermore, its functionalization with ligands such as lactobionic acid and mannose aims to promote receptor-mediated uptake, via C-type lectin receptors [1], by antigen-presenting cells, thereby enhancing transfection efficiency and immunogenicity. The study aimed to develop DNA complexes based on chitosan functionalized with either lactobionic acid or mannose. These complexes were evaluated *in vitro* through transfection studies to identify the most efficient formulations, which were subsequently selected for further investigation in in vitro studies and vaccination experiments. *In vitro* experiments using human primary cells demonstrated that these chitosan/DNA complexes polarised macrophages toward an M1 phenotype, promoted dendritic cell maturation and migration, and stimulated lymphocyte differentiation toward a Th1-biased response. Intranasal vaccination in mice elicited robust systemic and mucosal humoral immunity, accompanied by a strong cellular response characterised by heightened cytolytic activity and a balanced Th1, Th2, and Th17 cytokine production in the spleen and lungs of the mice. These findings underscore the immunostimulatory potential of functionalized chitosan as a mucosal adjuvant, highlighting its promise for enhancing vaccine efficacy and guiding the development of next-generation SARS-CoV-2 vaccines.

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Bruker Glycobiology Solutions – an integrated, comprehensive technology application suite making glycoscience accessible

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Posters and Flash Presentations

Targeting tumour sialylation: a novel therapeutic strategy for feline mammary carcinoma

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Despite major advances brought by cancer immunotherapies, many patients still fail to respond, emphasizing the need for novel therapeutic strategies. Aberrant glycosylation has been described as a key mechanism of immune evasion in cancer. One of the most common alterations is the overexpression of sialylated glycans [1]. Sialoglycans can interact with sialic acid-binding immunoglobulin-like lectins (Siglecs) on immune cells, triggering inhibitory signalling pathways resulting in the suppression of antitumoral response. In human breast cancer (HBC), several Siglec receptors have been linked to immune escape mechanisms. The similarities between feline mammary carcinoma (FMC) and HBC position the feline model as a valuable platform for the development of immunotherapeutic strategies with translational potential. Therefore, our main goal is to develop a novel immunotherapeutic strategy for FMC, with possible application to HBC, based on tumour-specific desialylation using a neuraminidase–antibody conjugate.

A feline neuraminidase was conjugated to an anti-Trop2 single chain fragment variable (scFv) antibody - fNeu-scFv. Following expression and purification, the conjugate was characterized regarding its enzymatic activity and target-binding capacity. Its ability to remove cell surface sialic acids and specifically recognize Trop2 was evaluated using flow cytometry, confocal microscopy and western blot. fNeu-scFv conjugate retained sialolytic activity comparable to the unconjugated form of the neuraminidase while exhibiting specific Trop2 binding in both HBC and FMC cell lines.

These results suggest that antibody-mediated tumour desialylation using fNeu-scFv conjugate holds promise as an immunotherapeutic strategy for feline mammary carcinoma (FMC), with potential translational application in HBC. This strategy is consistent with the "One Health" framework, highlighting its cross-species therapeutic value. Future studies will evaluate the conjugate's capacity to enhance anti-tumour responses by PBMCs and macrophages *in vitro*.

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Exploring recognition of host glycans by *Fusobacterium nucleatum* Fap2 adhesin for applications in cancer research

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Mucin-type O-glycosylation is a modification of cell-surface and secreted proteins, playing essential roles in cell development and differentiation. Alterations of O-glycosylation modulate aggressive colorectal cancer (CRC) phenotypes, and tumor associated O-glycans are crucial molecular targets for therapy and as clinical biomarkers. These glycans also shape the mucosal-associated bacteria, for they contain recognition sites for adhesive bacterial glycan-binding proteins [1]. Research on the human microbiome uncovered a dysbiosis state with an abundance of certain pathogenic bacteria, including *Fusobacterium nucleatum (Fn)*, which may lead to a pro- inflammatory *milieu* and tumor growth) [1],[2]. One of its most malignant adhesins, Fap2, has been identified as a potential glycan-binding protein targeting aberrant O-glycans differentially expressed in CRC, hinting at the role of this adhesin in the pro-oncongenic process [2] [3]. Yet the glycan structures preferentially targeted by this adhesin are lacking.

Here, we will present initial results of the characterization of Fap2 glycan-binding specificities at molecular and cellular levels by combining structural analysis, glycan microarrays, and *in vitro* binding to CRC cell lines using flow cytometry. We will detail our strategy to obtain functional fragments of Fap2 adhesin and produce these recombinantly to be analysed side by side with the full-length adhesin. Different glycan microarray platforms were screened with the recombinant Fap2 proteins, which comprised structurally diverse mammalian glycans, mucins from human epithelial cell types and non-mucin glycoproteins, sequence-defined mucin O-glycans and mucin-1 glycopeptides. The integrated glycan microarray data showed as initial hits some truncated O- glycans (T and Tn antigens), and fucosylated/sialylated glycan structures, overlapping with recent studies [4]. Consistently, the *in vitro* studies conducted with CRC cells showed a higher binding of Fap2 to cell lines with heterogenous glycan profiles, enriched in the aforementioned structures.

These findings reveal the interplay between tumor cell glycosylation and the microbiome, further highlighting Fap2 molecular interaction with glycans as a promising therapeutic target to modulate tumor progression. and demonstrating how exploiting cancer-associated glycans can advance personalized therapies by improving targeting specificity and drug delivery to cancer cells.

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O-glycan-selective metallopeptidases from the human microbiome: mucin-degrading activity relevant to cancer research

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Altered mucin O-glycosylation — a hallmark of gastrointestinal (GIT) cancers — plays a critical role in tumour initiation, progression, and response to therapy [1]. Microbial O-glycopeptidases, which selectively recognise and degrade mucin O-glycans, are emerging as valuable molecular tools to characterise aberrant glycosylation patterns in cancer [2]. The interaction between mucins and the gut microbiota influences bacterial colonisation and metabolism, with dysbiosis increasingly implicated in GIT cancer pathogenesis [3]. BC16100 is a modular putative O-glycopeptidase identified in the *Bacteroides caccae* genome, a prevalent human gut commensal. Previous studies have identified the specificity of the carbohydrate-binding module (BC16100-CBM) towards GalNAcα-terminating glycans with a clear preference for GalNAcα−Ser/ Thr (Tn antigen). Here, we will present our initial studies on elucidating glycan and peptide specificity of the catalytic module, which belongs to the peptidase family M60 (BC16100-M60). We evaluated the wild type protein and the catalytic mutant (E690A) for binding and degrading activity against model mucin O-glycoproteins: bovine submaxillary mucin (BSM) and porcine stomach mucin (PSM), performing a parallel comparison with the characterised M66 StcE mucinase from *Escherichia coli* [2]. Binding was assessed by ELISA and mucin glycoprotein microarrays. Enzymatic degradation was evaluated by substrate digestion assays, with glycoprotein cleavage confirmed by SDS-PAGE with periodic acid–Schiff staining. The results showed binding and activity preferentially on BSM mucin after removing the terminal sialic acid using a pan specific sialidase. As next steps, we aim to further characterise the full modular enzyme and determine the cleavage specificity using sequence-defined O-glycopeptides, with cleavage products analysed by LC-MS. Different O-glycopeptidase constructs will be evaluated in GIT cancer cell models for their potential as tools in O-glycomics and O-glycoproteomics workflows.

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The impact of sialylation as viral entry mediating agent of SARS-CoV-2

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Five years after the onset of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic, the molecular mechanisms that influence differential patient response, remain poorly understood. Sialylation, the addition of sialic acid to glycans, plays a critical role in numerous cellular functions and has been implicated in modulating viral infections, including coronaviruses [1]. Therefore, this study aimed to evaluate the impact of sialylation as a viral entry mediating agent.

For this purpose, we used a cell line with disrupted sialic acid biosynthesis by *GNE* knockout (KO) [2], and developed an ACE2+ cell line, in which glycosylation profile was analysed by lectin staining and glycomics and infection assays were conducted. The *N*-glycomics' results confirmed the cellular hyposialylation found at the cell surface of *GNE* KO cells by lectin staining and unveiled an increase in oligomannose content in this cell model.

The infection assays revealed that SARS-CoV-2 infection is increased by hyposialylation. Moreover, it also shows that SARS-CoV-2 infection can be altered upon supplementation with *N*-acetylmannosamine (ManNAc), an investigational drug and precursor of the sialic acid pathway.

These findings provide, for the first time, an ACE2+ cell model for evaluating the impact of sialylation in infectivity by ACE-dependent viruses. It also enlightens the possible susceptibility of patients with conditions associated with sialic acid defects, including cancer and rare diseases, to SARS-CoV-2 infection.

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Restoring Phosphomannomutase 2 Function: Pharmacological Chaperone Treatment in Patient Fibroblasts

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Congenital disorders of glycosylation (CDG) are a group of ~190 rare genetic diseases [1], characterized by defects in the glycosylation pathway, often leading to multisystemic involvement [2]. Phosphomannomutase 2 (PMM2)-CDG was the first N-glycosylation defect identified, and it is the most common type of CDG, with approximately 1000 patients worldwide [3]. This disorder is caused by recessive mutations in the *PMM2* gene, which encodes the enzyme responsible for converting mannose-6-phosphate to mannose-1-phosphate, a crucial step in the synthesis of N-glycans [4]. Despite efforts, there's currently no cure or approved therapies for patients with PMM2-CDG, only preventive and symptomatic treatments are available [3]. PCs have emerged as potential therapeutics to assist protein folding and stabilize mutated PMM2 [3][5], but none have reached clinical use. To explore this potential, we tested PC candidates on PMM2-CDG fibroblasts (GM27386), focusing on restoring glycosylation, comparing the results with those of healthy controls (GM03349). These candidates were selected from a library of FDA-approved drugs using chemoinformatic tools (Machine Learning and Molecular Docking), which identified four lead compounds with high predicted interaction with human PMM2. To achieve this, we started by conducting viability and cytotoxicity assays on GM27386 cells treated with different concentrations of each compound. Afterwards, both cell lines were incubated with the selected compounds for 72 and 120 hours. Glycosylation effects were assessed by immunoblot techniques, using Concanavalin A (ConA) for overall N-glycosylation and antibodies against ICAM-1, a glycoprotein dependent on proper glycosylation. Treatment with two of the PC (PC1 and PC3) for 72h led to a statistically significant increase in high-mannose N-glycans in PMM2-CDG, detected by ConA. However, no statistical difference was observed in ICAM-1 expression by patient fibroblasts. These findings suggest that PCs may offer a promising therapeutic strategy for PMM2-CDG, although further validation in other cell lines with different mutations is required.

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Characterization of glycosyltransferase GLT8D1 disease- associated variants

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Glycosylation is a fundamental post-translational modification involved in maintaining cellular and organismal homeostasis, often disrupted in pathological conditions such as cancer and neurodegeneration. Variants of the glycosyltransferase 8 domain-containing protein 1 (GLT8D1) were identified in amyotrophic lateral sclerosis (ALS) [1] and soft tissue sarcoma [2]. Our previous studies indicated that GLT8D1 is a GT-A fold enzyme with galactosyltransferase activity [2].

This study aims to further characterize GLT8D1 properties and how they are impacted by selected disease-associated mutations.

Recombinant forms of GLT8D1 including the full-length form, as well as soluble secreted forms containing the catalytic domain wild-type or disease-associated mutants, were produced in HEK293 cells, as previously described [4]. Enzymatic assay was performed using the UDP-Glo glycosyltransferase assay. GLT8D1 was analyzed by reducing and non-reducing SDS-PAGE and detected by immunoblotting. The glycosylation profile of GLT8D1 was established based on sensitivity to peptide N-glycosidase F and endoglycosidase H digestion.

Sugar-nucleotide donor screening confirmed that secretory and full-length GLT8D1 use UDP-Gal as donor. The activity was divalent cation-dependent with Mn^{2+} being a preferential cofactor in comparison with Mg^{2+} and Ca^{2+}. Full-length enzyme required the presence of the detergent Igepal CA-630 for optimal activity. Several potential acceptors were tested and a preference for N-acetylgalactosamine was confirmed for both full-length and secretory forms.

Secreted GLT8D1 ALS-associated variants were poorly expressed in comparison with the wild- type counterpart; however, this was not due to intracellular accumulation. By contrast, the sarcoma variant was efficiently expressed. Non-reducing SDS-PAGE analysis revealed a small amount of high molecular mass aggregates for some of the ALS variants, suggesting potential misfolding propensity. On the other hand, preliminary results indicated that none of the mutations tested impaired enzyme activity.

These findings expand our biochemical understanding of GLT8D1 and its potential implications in disease.

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Understanding the role of short truncated O-glycans in colorectal cancer

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Despite a recent decline in global incidence, colorectal cancer (CRC) remains the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide. [1] CRC is classified into four consensus molecular subtypes (CMS): CMS1 (immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal), which differ in their biological features and clinical outcomes. [2] Altered gly cosylation, particularly the increased expression of truncated glycans such as Tn and Sialyl-Tn (STn), is a hallmark of many cancers, including CRC. [3] Overexpression of STn is associated with immune evasion and tumor aggressiveness. [4] STn is aberrantly expressed in the majority of carcinomas, potentially driven by the sialyltransferase ST6GALNAC1, whose overexpression has been linked to tumor progression. [5]

This study investigated STn antigen expression and ST6GALNAC1 regulation across CRC subtypes, with a focus on CMS3. Tumor tissues from 208 CRC patients were analyzed using immunohistochemistry, transcriptomics, and bioinformatics. Expression data were integrated with clinical parameters and validated using The Cancer Genome Atlas (TCGA). In addition, functional assays were performed using the CMS3 cell line LS174T and the CMS4 cell line SW620 under high- and low-glucose conditions, with glycan expression assessed by flow cytometry.

STn expression was highly prevalent and positively associated with microsatellite instability (MSI). A strong correlation was observed between STn and ST6GALNAC1 expression, with significantly higher levels of ST6GALNAC1 in CMS3 tumors. This finding was validated using TCGA datasets. However, *in vitro* assays revealed no consistent STn expression across CMS-representative cell lines, despite detectable ST6GALNAC1 transcripts and protein in both LS174T and SW620. Notably, STn was undetectable in both cell lines by flow cytometry, while the Tn antigen was consistently detected across all conditions. These results suggest that STn biosynthesis may require additional regulatory mechanisms beyond ST6GALNAC1 expression or glucose availability.

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Structural heterogeneity and immunomodulatory potential of Lipopolysaccharides of marine Gram-negative bacteria

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Lipopolysaccharides (LPSs) are among the most extensively studied bacterial molecules, particularly due to their high potential in immunological and biomedical fields, as they are recognized by several immune receptors, most notably the TLR4/MD-2 receptor complex .[1,2] In this communication, I will describe how the variable structure of LPS of two marine Gram-negative bacteria can impact biophysical and immunological aspects.

Marine bacteria are a promising source of LPSs with TLR4-inhibitory activity and the capacity to modulate pro-inflammatory responses. I will present the first structural characterization of lipid A and a preliminary immunological evaluation of LPS from the marine bacterium *Rheinheimera japonica* KMM 9513^{T}.[3] Analysis by MALDI-TOF MS and tandem MS revealed high structural heterogeneity, with fatty acids varying in length, saturation, branching, and position. Functionally, this LPS did not activated TLR4 but instead acted as an antagonist, inhibiting TLR4 activation induced by potent inflammatory *Escherichia coli* LPS. These findings identify *R. japonica* LPS as an atypical marine LPS with potential as a model for developing synthetic derivatives capable of eliciting a controlled immunological response.

Pseudomonas is one of the most versatile genus able to adapt to extreme environmental conditions therefore, it is an ideal research model for studying how environmental factors affect the chemical and physical properties of the cell envelope.[4] In this communication I will describe the LPS structure of the *Pseudomonas* strain ESM#7, isolated from Enigma Lake in Antarctica characterized by unique extreme environmental conditions.[5] LPS was isolated from this bacterium grown at 0.4 °C and 20

°C and the chemical structure of both LPS was compared and investigated by chemical analyses, NMR and MALDI-TOF MS experiments. This structural study was then complemented by morphological analysis by Cryo-EM and SAXS which together with molecular coarse-grained MD simulations provided insights into the role of the lipid A component in membrane stability.

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Arylamide glycofoldamers for bacterial adhesin inhibition

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The WHO has placed antimicrobial resistance among the top global public health threats [1]. Antivirulence agents promise to circumvent resistance by disarming the pathogen as opposed to affecting growth or viability [2]. A common strategy consists in interfering with adhesion, which is mediated by proteins that bind multiple carbohydrates displayed on the host cell surface [3]. To inhibit such interactions, many molecular scaffolds have been devised for the multivalent presentation of carbohydrates [4]. However, few allow a precise control of number, orientation, and distance between the sugars, which is fundamental to maximize biological activity.

Arylamide foldamers are bioinspired synthetic oligomers that, like peptides or nucleic acids, fold into well-defined conformations [5]. They feature key properties which make them ideal to build materials for multivalent presentation of carbohydrates: they adopt stable helical conformations in solution, whose predictability, tunability, and ease of synthesis render them particularly suitable to allow precise control of number, nature, and orientation of carbohydrate ligands [6]. In addition, they can feature proteinogenic side chains to allow binding to protein surfaces [7].

Herein we describe our current efforts towards developing chemically defined glycofoldamers functionalised with galactose or mannose residues to selectively target the lectins LecA and LecB of Pseudomonas aeruginosa, two key virulence factors involved in adhesion and biofilm formation.[8]

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Deciphering tumour-associated mucin *O*-glycans recognition by a human gut microbiome-associated bacteria: a novel binding module from *Bacteroides caccae*

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The gastrointestinal tract harbors a diverse community of commensal bacteria that impacts on human health. In a low-fiber diet, some commensal bacteria can switch to host mucin *O*-glycoproteins as alternative substrates. In this context, the commensal *Bacteroides caccae* was implicated in the deconstruction of the colonic mucous layer, increasing susceptibility to pathogen infection and progression of intestinal diseases [1,2]. During growth of *B. caccae* on mucin *O*-glycans, an increase of the expression of modular enzymes, including modular M60-like metallopeptidases (also known as mucinases), with appended non-catalytic carbohydrate-binding modules of family 32 (CBM32), is observed [2]. These enzymes are organized in gene clusters, termed polysaccharide utilization loci (PUL), which encode all the genes necessary for the breakdown and uptake of a given glycan.

Herein, we describe our research advancement using an integrative approach to uncover glycan recognition by a novel CBM32 (BC16100-CBM) appended to a putative mucinase of PUL-53. Analysis using MUC1 *O*-glycopeptide microarrays, combined with affinity studies, showed a remarkable specificity towards GalNAcα-terminating glycans with a clear preference for GalNAcα−Ser/ Thr (Tn antigen). The molecular determinants of Tn-antigen recognition by BC16100-CBM were elucidated combining NMR and X-ray Crystallography. Furthermore, 2D NMR titration experiments demonstrated differential recognition by the CBM for GalNAcα−glycosylation sites within the MUC1 peptide sequence.

This data shows the first clues of complex molecular mechanisms behind the recognition of tumour- associated antigens and utilization of mucin substrates by *B. caccae*.

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Towards Structural Understanding of Host-Microbe Recognition by Studying CBM32-Glycan Interactions in Bacteroides spp.

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The human gut harbors a diverse community of commensal bacteria that contribute to the breakdown of dietary polysaccharides indigestible by humans but also can adapt to thrive in host-derived glycans. [1] This microbial ecosystem, referred to as the gut microbiota, includes members of the Bacteroides spp. That express a wide range of carbohydrate-active enzymes (CAZymes). These CAZymes are often modular and function in conjunction with non-catalytic carbohydrate-binding modules (CBMs) [2], which recognize specific carbohydrate structures and direct the catalytic domains of CAZymes to their substrates. Despite their importance, many CBMs remain structurally and functionally uncharacterized, limiting our understanding of their molecular interactions.

This study aims to structurally characterize family 32 CBMs from two prevalent Bacteroides members of the gut microbiota: BT0865-CBM from *Bacteroides thetaiotaomicron* and BC07580-CBM from *Bacteroides caccae.*

Here, we will show integration of glycan microarray data showing preferential binding of BT0865- CBM to O-glycans presenting LacDiNAc (GalNAcβ1–4GlcNAc) over LacNAc (Galβ1–4GlcNAc) sequences. ITC and MST data further corroborated glycan microarray data. We will also present protein characterization by CD and TSA, as well as the predictive structural analysis using AlphaFold. Upon identifying relevant glycan-CBM interactions, co-crystallization trials were performed to solve the 3D structures of the resulting complexes. High-throughput crystallization screens have been conducted for both CBMs, but no crystals have been obtained so far. For the BC07580-CBM, partial removal of the N-terminal region using Tobacco Etch Virus (TEV) protease was attempted to improve protein stability. Ongoing efforts are focused on optimizing crystallization and co-crystallization strategies to enable structural characterization of these CBMs and their interactions with preferred glycan ligands.

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Targeting *Pseudomonas aeruginosa* Lectins: Assessing Arylamide Foldamers as Inhibitors of LecA and LecB

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Pseudomonas aeruginosa is a significant opportunistic pathogen responsible for various acute and chronic infections, with limited treatment options. Central to its pathogenicity is its ability to adhere to host tissues, which is essential for initiating the infection process. This adhesion is mediated by recognising glycan patterns on the host cell surface through two soluble lectins found in the bacterium's outer membrane, LecA (PA-IL) and LecB (PA-IIL), which specifically bind galactose- and fucose-terminating oligosaccharides, respectively [1,2,3]. Limited conventional therapeutic options necessitate alternative strategies, such as anti-adhesion therapy, an emerging approach that targets these lectins [4]. The binding of LecA and LecB may be impeded by compounds that can competitively inhibit attachment by mimicking host cell binding partners. Arylamide foldamers are promising candidates for LecA and LecB inhibitors, as these bio-inspired synthetic oligomers fold into well-defined conformations [5], enabling them to display proteinogenic side chains to mimic protein surfaces and, most importantly, carbohydrates in a multivalent manner.

In this study, our primary goal was to assess the potential of foldamers as inhibitors of LecA and LecB by analysing their interactions, thereby providing valuable insights for foldamer design and synthesis. LecA and LecB produced in-house were successfully crystallised, and preferential binding was demonstrated in a mucin microarray and ELISA with various immobilized carbohydrates. Interaction studies of the lectins with various foldamers, including microscale thermophoresis (MST) and thermal shift assay (TSA), indicated non-specific binding. Co-crystallisation of the lectins with the foldamers was unsuccessful, but initial results from a customised microarray with immobilised foldamers showed more promising outcomes.

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CBMcarb-DB: A Curated Database for Structural and Functional Insights into Carbohydrate Binding Modules and Their Ligands

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Carbohydrate Binding Modules (CBMs) are a diverse class of carbohydrate-binding proteins, often found as non-catalytic domains enhancing enzyme efficiency, or functioning independently. CBMs are classified and annotated in the CAZY (Carbohydrate-Active enZYmes) database [1]. They exhibit broad binding specificities, recognizing various carbohydrates including non-crystalline cellulose, chitin, hemicellulosic substrates [1, 3], and mucins [2]. CBMs serve as an excellent model for studying protein-carbohydrate interactions.

Addressing a long-standing need for an integrative database, we developed CBMcarb-DB, a novel curated platform for displaying and analyzing 3D structures of CBM-carbohydrate complexes [4, 5, 6]. CBMcarb-DB provides meticulously curated structural data on binding interactions, carbohydrate conformation, and functional specificity. Integrated with PDB, UniProtKB, and GlyTouCan, CBMcarb- DB promotes discoverability, accessibility, interoperability, and reusability (FAIR principles). Our rigorous curation workflow, supported by experimental data and validation, ensures high data quality and completeness.

CBMcarb-DB is poised to be an invaluable resource for the scientific community, particularly for researchers involved in glycoscience and biotechnology. By promoting the optimized reuse of high- quality data, CBMcarb-DB facilitates the exploitation of CBMs for various biotechnological applications, including the rational design and generation of engineered CBMs with novel and diverse functional properties. Future developments include continuous updates, integration of experimental molecular recognition data like carbohydrate microarray specificity, and structural glycobioinformatics tools. We also plan a workflow to integrate AI 3D structure predictions and curated database information with Machine Learning-based bioinformatics tools for comprehensive structure-function studies.

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Unravelling the molecular recognition of CD44V6 through nmr analysis

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Altered protein glycosylation is a hallmark of cancer and offers promising avenues for improving cancer diagnosis, predicting disease outcomes and developing tailored treatment strategies [1,2]. In this line, deciphering how tumour-associated glycoproteins influence the communications between cancer cells and the host environment (and thereby regulate cellular behaviour) is critically important for advancing cancer research and therapy [3,4].

We are focused on CD44, a specific transmembrane glycoprotein that is overexpressed in a wide range of tumours and plays a key role in cancer stem cell properties such as regeneration, tumour initiation, metastasis and chemoresistance [5]. In addition, CD44 can interact with a wide range of carbohydrate-binding proteins, like Siglecs, selectins, or galectins [4]. Herein, we have examined the glycosylation characteristics and interaction dynamics of CD44v6, an isoform that is thought to be expressed and to display increased tumorigenicity in human tumors [6]. This isoform was expressed in HEK293F cells and its interaction with lectins has been scrutinized at the molecular level using NMR as major tool, in order to eventually elucidate how glycosylation alterations could drive cancer development and progression.

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Arylamide foldamers as new platforms for glycan microarray technology

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Glycan microarrays are powerful tools to investigate the carbohydrate binding specificity of proteins and antibodies, identify potential drug targets, and detect the presence of carbohydrate-binding biomarkers that may indicate disease states [1].

These arrays typically feature glycan sequences attached to the array surface using a linker appended to the glycan's reducing end. However, the spatial arrangement of glycans on a cell surface is different from that on a synthetic glycoarray. Glycoconjugate mimetics that spatially position glycans similarly to natural glycoproteins can be integrated into arrays to create a more physiologically authentic platform for probing glycan-binding proteins [2]. However, few allow a precise control of number, orientation, and distance between glycans, parameters that greatly alter the avidity and specificity of glycan–protein interactions.

Arylamide foldamers are synthetic oligomers composed of aromatic amide units that adopt stable and predictable conformations in solution through the establishment of intramolecular non-covalent interactions [3]. Quinoline-based variants, in particular, display a well-defined helical geometry and can be readily synthesized and modified,[4] offering a robust platform for the precise multivalent presentation of carbohydrates.

Herein we present our recent efforts to develop quinoline-derived arylamide foldamers as new platforms for the well-defined display of carbohydrates, and to validate their compatibility with current glycoarray technologies.

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NMR and Molecular Recognition: The Interaction of the Histo Blood Antigens with Human Galectin-2

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Galectins are a family of β-galactoside-binding proteins involved in diverse physiological and pathological processes. Among them, Galectin-2 plays critical roles in inflammation, immune regulation, and apoptosis [1]. Interestingly, Galectin-2 exhibits a dual, context-dependent, effect in disease progression [2]: its overexpression is associated with anti-inflammatory outcomes and favourable prognosis in cancers like oral squamous cell carcinoma and breast cancer [3,4], whereas in atherosclerosis, antibody-mediated inhibition of Galectin-2 reduces inflammation and slows disease progression [5]. Despite its seemingly contradictory roles across different diseases, these findings support the potential of Galectin-2 as a therapeutic target, emphasizing the need for a deeper understanding of its molecular recognition mechanisms.

To address this, we are investigating the recognition features of Galectin-2 towards the histo-blood group antigens, known as its preferred ligands. A combination of saturation transfer difference NMR (STD-NMR), isothermal titration calorimetry (ITC), and molecular dynamics simulations have been used to decipher the thermodynamics of the interaction and the binding events at atomic resolution.

Our findings indicate that Galectin-2 recognizes histo-blood group antigens in a consistent manner with other galectins, where the central β-galactose (β-Gal) moiety plays a critical role in ligand binding. Furthermore, additional decorations such as α-galactose (α-Gal), N-acetylgalactosamine(α- GalNAc), and α-L-fucose (α-L-Fuc) enhance the binding affinity.

These insights will deepen our understanding of the ligand specificity of Galectin-2 to guide the rational design of targeted therapeutic strategies.

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Molecular mechanisms of glycosaminoglycan recognition in biological systems: an NMR perspective

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Glycosaminoglycans (GAGs) are major components of the glycocalyx and the extracellular matrix (ECM), where they can be found in their free form or being part of proteoglycans. This family of linear and negatively charged polysaccharides display mainly electrostatically-driven interactions with their receptors, to provide essential functions for a wide variety of biological processes related to health and disease [1]. GAGs share a common structure composed of repeating disaccharide units consisting of an amino-substituted sugar, either GlcNAc or GalNAc, and an uronic acid, namely GlcA or IdoA [2]. However, frequent modifications on the sugar residues, particularly sulfation of the amino groups, together with their high conformational plasticity render GAGs structurally complex molecules. Investigation of their molecular recognition processes is therefore challenging.

Herein, we present NMR-based interaction studies of diverse glycosaminoglycans with FGFR2 (Fibroblast Growth Factor Receptor 2). A variety of GAGs have been employed, ranging from well-defined idose-based heparan sulfate (HS) mimetics, prepared by chemical synthesis, to complex native proteoglycans, such as CD44 glycoprotein. Notably, the crosstalk between FGFR2 and CD44 has been suggested as a regulatory circuit governing cancer stemness [3]. The results highlight the use of NMR as a powerful tool to decipher the interacting epitopes of the glycan, not only when free in solution, but also when presented in an intact glycoprotein.

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GlycoToolBox - essential tools for glycoscience research

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Herein, the GlycoToolbox service, a technological platform developed under the GLYCOTwinning network - *Building Networks to Excel in Glycoscience* - will be presented. GLYCOTwinning is an European funded project led by UCIBIO–NOVA (Portugal), networking with the European partners Imperial College (United Kingdom), LUMC (Netherlands) and CICbioGUNE (Spain), with the aim to integrate technologies and advance research in glycoscience. The GlycoToolbox platform, as an output of this project, is designed to combine complementary advanced glycoscience methodologies and expertise into a comprehensive toolkit for investigating glycans and their interactions with proteins across various biological contexts.

Leveraging complementary and integrative technologies, which include glycan microarrays, high-resolution mass spectrometry, biophysical affinity techniques (ITC, MST), nuclear magnetic resonance (NMR), X-ray crystallography, molecular modelling, fluorescence microscopy, flow cytometry, and heterologous expression of glycan-binding proteins (GBPs) in different host-systems, the GlycoToolbox enables: 1) Identification of glycan structures and discovery of ligands, 2) Structural elucidation of glycan–protein complexes, 3) Rational design of smart glycans and GBPs, and 4) Functional analysis of glycans within cellular environments .

The platform allows to identify specific glycan motifs/ligands that interact with glycan-binding proteins, and it facilitates atomic- and molecular-level characterization of these interactions, offering insights into their roles in cellular processes. By providing standardized, multi-technology workflows, the GlycoToolbox service accelerates glycoscience research, enhances scientific visibility, supports pre-clinical evaluation in disease models and the development of therapeutic strategies for cancer, infectious diseases, and congenital glycosylation disorders. Ultimately, the platform mission is to empower the scientific community by advancing knowledge, providing advanced training and consulting, and fostering collaboration between academia and industry.

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Floridoside and isofloridoside profiling in *Porphyra dioica*: towards sustainable prebiotic sources

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Floridoside (2-α-*O*-D-galactopyranosylglycerol) and isofloridoside (1-α-*O*-D-galactopyranosylglycerol) are glycerol galactosides predominantly synthesized by red algae as osmoprotective metabolites. These carbohydrates have drawn increased attention due to their prebiotic effects, particularly in promoting the growth of *Bifidobacterium* and *Lactobacillus* bacteria, which highlights their promising applications in functional foods and health-related industries [1]. Among red algae, *Porphyra* is a well-known edible seaweed that is traditionally consumed for its nutritional value, including the sulphated polysaccharide porphyran, so it represents a sustainable biomass source for the production of bioactive carbohydrates [2].

The objective of this study was to assess the potential of *Porphyra dioica* as a source of glycerol galactosides. Accordingly, seasonal variations in floridoside and isofloridoside content were determined in biomass cultivated throughout the year. Samples were acetylated with acetic anhydride, and floridoside and isofloridoside were quantified by GC-MS using 2-deoxyglucose (1 mg/mL) and mannitol (1 mg/mL) as internal standards. In spring and summer, total glycerol galactoside levels reached 16.2% and 8.4%, respectively, while in autumn and winter, levels dropped to 1.1% and 0.4%. Isofloridoside predominated in spring and winter (9.6% and 0.3%, respectively), while floridoside was higher in summer and autumn (6.0% and 0.6%, respectively). These findings confirm the metabolic adaptability of *P. dioica* in response to seasonal environmental changes. They reveal the potential of selecting biomass to optimize the yield of bioactive glycerol galactosides. The higher glycerol galactoside content in spring samples suggests that targeted harvesting could enhance the use of *P. dioica* as a natural source of these prebiotic carbohydrates for functional food applications.

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Applicability of Carrageenan Hydrocolloid Structures in Protein Rich Food Products

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Carrageenan is a family of sulfated galactans derived from red algae. These polysaccharides are widely used to improve the texture of meat products and their plant-based alternatives. In meat substitutes, achieving the desired firmness and texture typically relies on protein gelation or the use of hydrocolloids. While vegetarian formulations may include animal-derived ingredients like egg whites for their gelling abilities, vegan versions must rely entirely on alternative proteins and carbohydrate-based hydrocolloids capable of forming robust gels.

This study investigated the functionality of carrageenans in two distinct protein-rich food systems: cooked ham and a meat-free analogue. In the cooked ham model, a meat industry carrageenan was used to enhance water-holding capacity through gel formation, which helped bind water, reduce shrinkage, and increase yield. The carrageenan had a total carbohydrate content of 34% (w/w) and consisted of 36 mol% galactose and 19 mol% 3,6-anhydrogalactose, with sulfation level of 40 mol%. Texture Profile Analysis showed that carrageenan significantly improved product texture, increasing hardness from 83.1 N to 97.0 N and chewiness from 2474 N to 3239 N compared to the carrageenan- free product, which is consistent with enhanced gel structure and improved sliceability.

To form a sliceable gel-emulsion matrix in meat analogue, it was necessary to use a carrageenan- rich commercial ingredient containing 65% (w/w) carbohydrates (39 mol% galactose, 18 mol% 3,6- anhydrogalactose, and 20 mol% glucose) and a sulfation level of 22 mol%. The resulting formulation yielded a firm, well-textured product, comparable to a vegetarian analogue using egg white as the gelling agent.

In conclusion, carrageenans seem to have the potential to play an ubiquitous role in meat products and analogues by providing the textural properties commercially recognized for these commodities.

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Exploring hypocholesterolemic polysaccharides potential: from structure towards activity

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Polysaccharides chemical diversity, given by their monomer composition, glycosidic linkages, branching degree, within others, can have impact on their functional properties, within these the hypocholesterolemic potential. Polysaccharides such as galactomannans and arabinogalactans extracted from coffee have shown to sequestrate bile salts, leading to a decrease on cholesterol bioaccessibility and its precipitation. [1] Regarding charged polysaccharides, both positively and negatively charged, chitooligosaccharides (shrimp) and fucoidans (algae), respectively were shown to affect bile salt sequestration, showing that aside electrostatic interactions, non-ionic interaction can also contribute for their effect on cholesterol bioaccessibility. [2,3] Moreover, the fermentation profile of arabinogalactans-rich fractions were shown to have distinctive profile of short chain fatty acids, affecting acetate/propionate ratio which is known to impact cholesterol synthesis as well as on secondary/ primary bile acids which influences cholesterol bioaccessibility and bioavailability. [4] The understanding of the interplay between chemical structure of polysaccharides and hypocholesterolemic potential may aid on the development of improved cholesterol lowering functional ingredients.

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Preparation of galactooligosaccharides and their application in a yogurt-type product

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This work is focused on the preparation of galactooligosaccharides (GOS) and the evaluation of chemical, microbiological, and sensory parameters during their application in a fermented yogurt-type product with probiotics. GOS were made from a solution of dried whey and skim milk containing 25% (w/w) lactose using a new enzyme with high transgalactosylation activity Nurica (Danisco A/S). The saccharides were analysed by HPLC – the monosaccharides were determined on ion exclusion H+ Polymer column using a refractive index detector. HILIC column and an evaporative light scattering detector were used for the oligosaccharide analysis. The high initial lactose content enables a high yield of 56% GOS (i.e. conversion of lactose to GOS) to be achieved. Most of the galactose was incorporated into GOS - the residual galactose (0.5%) was 10 times lower than the residual glucose. The degree of polymerization of the GOS was in the range from 2 to 5. The obtained GOS were applied to yogurt with a probiotic culture of *Bifidobacterium animalis* ssp. *lactis* in amounts of 0, 2, 10, and 20% (w/w). The yogurts were stored and monitored for 9 weeks. Bifidobacteria showed counts higher than 10^6 CFU/g throughout the storage period. GOS were not significantly utilized during fermentation and their changes during storage were also insignificant. The sensory analysis revealed the overall acceptability of the prepared yogurts, the dose of 20% (w/w) GOSP led to a sweeter taste.

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Unveiling the glycosidic linkage diversity of fig water-soluble polysaccharides

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Recent research trends show that soluble polysaccharides non-digestible by human gut enzymes are of great interest for their health benefits and natural nutraceutical value [1]. Despite extensive research on the nutritional quality of figs [2,3], the structural features of their water-soluble polysaccharides remain largely uncharacterized. Thus, this study explores the glycosidic linkage diversity of fig water-soluble polysaccharides, thereby elucidating their potential as a source of soluble dietary fiber (SDF).

Polysaccharides from ripe Pingo de Mel fig (*Ficus carica* L.) were extracted by alcoholic extraction (80%). After that, the alcohol-insoluble residue (AIR) was subjected to cold-water (CW) extraction, followed by freeze-drying. Subsequently, CW-AIR extract was fractionated by re-extraction with cold water and graded ethanol precipitation at 50% and 70%, yielding four distinct polysaccharide samples: cold water precipitate (CWpp), 50% ethanol precipitate (Et50), 70% ethanol precipitate (Et70), and ethanol supernatant (EtSn). The glycosidic linkages of these fractions were profiled using methylation followed by gas chromatography-mass spectrometry (GC-MS).

The results revealed that the CWpp and EtSn fractions were characterized by the presence of notable amounts of (1→4)-Glc (26.2 and 16.2 mol%, respectively) while Et50 and Et70 fractions were differentiated by the presence of (1→2,3,5)-Ara*f* (22.5 mol%) and (1→2,3,6)-Gal (13.6 mol%), respectively, suggesting variations in polysaccharide architecture among fractions with high branching of the arabinogalactans in Et50 and Et70. Moreover, considerable amounts of terminally-linked-Ara*f*, (1→5)-Ara*f*, (1→4)-Xyl, (1→3,6)-Gal, and (1→4)-Gal glycosidic linkages, were detected in the different fractions (ranging from 6.7 to 17.7 mol%) indicating the presence of mainly arabinogalactan, in addition to xylans, and galactans derived from pectic polysaccharides. These findings unveil the remarkable glycosidic linkage diversity of Pingo de Mel fig water-soluble polysaccharides, highlighting fresh figs as source of SDF due to the branching patterns across pectic polysaccharide fractions, that underscore potential functional properties as prebiotic food.

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Modulation of Sugar and Dietary Fibre Partitioning in Fruit Juices and Pulps through Centrifugal Decantation

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Carbohydrates are essential contributors to fruit juice sweetness and texture. Dietary fibre, particularly soluble fibre and oligosaccharides, enhances nutritional quality and may provide prebiotic effects [1, 2]. Juice processing typically removes most dietary fibre, reducing the final product's nutritional value [2]. Fruit processing co-products such as pulp are rich in fibre and present valuable opportunities for integrating these beneficial components into food products [3].

This work quantified sugar and dietary fibre contents of apple and pear juices and their respective pulps, produced under two extreme speeds of centrifugal decantation, to evaluate their impact on component distribution.

The centrifugal decantation of apple and pear purees at differential/bowl speeds of 10/4400 rpm had higher yields of apple and pear juices (79-84% and 75-83%, respectively) than at 20/3800 rpm (apple: 58-75%; pear: 54-63%). Apple and pear juices exhibited water contents of 85-86% and 87- 89%, respectively. Pulps showed lower moisture (apple: 80-81%; pear: 77-85%), especially at 10/4400 rpm speeds. Free sugar content was higher in apple juices (12.7-14.8%) than in pear juices (10.1-10.2%), with similar trends in pulps (apple: 11.2-12.6%; pear: 8.6-10.7%). Total dietary fibre (TDF) in apple juice ranged from 0.28-0.32 g/100 g and in pear juice from 0.26-0.35 g/100 g, with soluble dietary fibre (SDF) as the predominant fraction (apple: 71-85%; pear: 87-100%). Pulps were significantly richer in TDF (apple: 4.65-7.18 g/100 g; pear: 6.13-14.21 g/100 g) than juices. In pulps, SDF represented 32-38% of TDF in apple and 18-27% in pear, with higher proportions at 20/3800 rpm speeds, where lower yields of juice were observed and less soluble fibre was extracted.

In conclusion, centrifugal decantation speeds influenced the yield of juices and the dietary fibre composition of pulps, without affecting the overall composition of juices. Therefore, adjusting these speeds can optimize juice yield and tailor pulp fibre content for specific food applications.

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Circular bioeconomy pathways for *Gelidium corneum* residue after agar extraction

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Agar is a sulfated polysaccharide primarily obtained from *Gelidium* species and used for its gelling and thickening properties. The extraction process is incomplete, leaving substantial amounts of agar in the solid residue. Although the agar industry generates large quantities of this carbohydrate-rich byproduct, its composition and potential applications remain unknown [1].

This study aimed to characterize the insoluble biomass residue from *Gelidium corneum* after agar extraction (provided by Iberagar, S.A.) and to recover sugars to evaluate its valorization potential. The waste biomass consisted of 31% carbohydrates (16% glucans and 14% agar), 33% ash, 14% protein, and 9% moisture. Hot water extractions were performed at time intervals ranging from 1 to 4 h to evaluate the effect of extraction duration. The results showed that a two-hour extraction was sufficient. The extracted fraction contained 70% of the total sugars, mainly galactose and 3,6-anhydrogalactose, the main components of agarose (the gelling fraction of agar). In addition, the extract contained 6% sulfates, indicating the presence of agaropectin, a more soluble and less gelling component. The total phenolic content, determined by the Folin–Ciocalteu method, was 28 µg GAE/mL in the aqueous extract, a value seven times higher than that observed for agar (4 µg GAE/mL). Consequently, the extract exhibited a higher antioxidant potential (evaluated using the ABTS assay) with an IC_{50} of 0.8 mg/mL, whereas commercial agar showed no inhibition at the highest concentration tested (2 mg/mL).

These results underscore the potential of *Gelidium corneum* residues as a valuable source of functional hydrocolloids, supporting their application in the food industry and contributing to sustainable resource utilization within a circular economy framework.

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Investigation of glycomic profiles and their implications for Alzheimer's disease biomarker discovery

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Alzheimer's disease (AD) is the most prevalent form of dementia and the number of patients with the disorder is increasing due to ageing of the population [1]. Deregulated molecules from the cerebrospinal fluid (CSF), such as Abeta42 and tau protein, constitute important markers of the disease to support diagnosis. However, they do not reflect all the complexity of AD pathogenesis and the identification of new biomarkers would be of value. Recent research has revealed significant alterations of glycosignatures from the brain and the CSF in AD patients (reviewed in [2]).

In this work, we aim at investigating glycosylation changes of glycoproteins from the CSF associated with AD to unveil new insights into disease mechanisms and potential diagnostic and therapeutic targets.

The CSF from 10 patients with Mild Cognitive Impairment due to AD – high likelihood (MCIAD) [3] and 10 control MCI patients, that is, Aβ-negative and neurodegenerative-negative (MCIcontrol) was collected via lumbar puncture. Glycomic analysis of CSF glycoproteins was performed by lectin blotting with lectins to detect specific glycan structures, including GNA, E-PHA, MAL II, SNA and AAL. For glycoproteomic analysis, peptide mapping of tryptic peptides from the CSF was performed by LC-MS using a ZenoTOF 7600 mass spectrometer. Protein searches used MSFragger and glycan assignement used GlycomeDB.

Lectin blotting revealed differences in glycosylation between MCIAD and MCIcontrol patients, including in sialic acid and bisecting N-acetylglucosamine. N-glycosylation site occupancy of major CSF glycoproteins (e.g., beta-trace protein) and corresponding glycan structures were elucidated. Comparison of glycosignatures between MCIAD and MCIcontrol patients is currently under investigation.

In conclusion, our results provided a detailed characterization of the CSF glycoprotein glycosylation. Moreover, the alterations detected in AD patients at initial stages of the disease open perspectives towards biomarker discovery reflecting novel aspects of AD pathogenesis.

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Impact of Drying Methods on the Encapsulation Efficiency of Oils Using Polysaccharides

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Vegetable oils are commonly used as carrier oils to dilute and protect essential oils, enhancing their stability and usability [1]. Microencapsulation is an effective technique that protects these oils and enables their incorporation into a variety of applications in the food industry, nutraceuticals, and cosmetics [2]. It could be performed using polysaccharides through two physical methods: spray drying and freeze drying [3].

In this study, oil-in-water (O/W) emulsions were prepared with 7.5% sunflower oil and 4.5% modified starch (O/W1), or 7.5% sunflower oil, 1.5% modified starch, and 3% dextrin (O/W2). Emulsification was carried out using either an high-shear (Ultraturrax) or moderate-shear (Thermomix) homogeneizer for 2 min followed by sonication (20 kHz for 1 min). Ultraturrax emulsions were freeze- dried (O/W-FD), while Thermomix emulsions were spray-dried (O/W-SD). The dried products were blended (1:1) with either modified starch or microcrystalline cellulose, as excipient, and compressed using a tablet press (500 kg) to assess oil retention.

Before drying, all emulsions showed similar polydispersity indices (0.52-0.58) except for O/W1-SD (0.16 ± 0.03). Droplet sizes were smaller in O/W2 emulsions (O/W1-FD 2953 ± 51 nm; O/W1-SD 2312 ± 65 nm; O/W2-FD 1574 ± 23 nm; O/W2-SD 1569 ± 46 nm). Zeta potential values remained similar before and after drying, approximately –25 mV for O/W1-FD; –5 mV for O/W1-SD; –20 mV for O/W2-FD and -18 mV for O/W2-SD. Tablets from spray dried emulsions allowed to retain more oil (91.9%-95.4%) than the freeze-dried ones (70.9%–75.6%).

Overall, the tablets resultant from the spray dried products retained higher amounts of oil indicating that this drying method provided better oil encapsulation compared to freeze drying. Additionally, as no consistent differences were observed between the O/W1 and O/W2 formulations within each drying method, the formulation composition did not appear to influence encapsulation efficiency under the tested conditions.

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Encapsulation of bioactive compounds from *Moringa oleifera* leaves using sodium alginate and gelatine for functional food fortification

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Moringa oleifera leaves are widely recognised for their high nutritional and nutraceutical value, being rich in bioactive compounds such as phenolics, flavonoids, and antioxidants [1]. However, stability, taste, or bioavailability challenges can limit their direct incorporation into food products. Encapsulation technologies offer promising strategies to protect these sensitive compounds and ensure their controlled release, enhancing the development of functional foods with added health benefits [2]. This study aimed to produce microcapsules containing *Moringa oleifera* leaves extracts using sodium alginate and gelatine as encapsulating agents. The phenolic-rich extract was first obtained through ethanol-based extraction and purified by membrane separation (ultrafiltration and nanofiltration) to concentrate its bioactive fraction. Encapsulation was carried out via extrusion, employing calcium chloride to crosslink the alginate and form stable microbeads [3]. Six different formulations were tested to optimise the encapsulation efficiency of phenolic compounds, particle size, and controlled release properties. The 3% alginate formulation showed the highest encapsulation efficiency (68%) and the highest retention of total phenolics (81.6 mg GAE/g d.b.) and flavonoids (37.5 mg CE/g d.b.). However, due to its high viscosity and poor extrusion performance, the 1.25% alginate + 1.25% gelatine formulation was selected as optimal, balancing processability (61% encapsulation efficiency) with high bioactive content and antioxidant activity. Microparticles displayed spherical morphology with average diameters between 1.6 and 2.0 mm, and smooth surfaces depending on polymer concentration. When incorporated into a gelatine matrix at 1%, the fortified product maintained good visual appeal and acceptable texture. Rheological and texture profile analysis showed that fortification slightly reduced hardness and chewiness while maintaining springiness and cohesiveness. These modifications suggest improved mouthfeel without compromising structure. This work highlights the potential of using *Moringa oleifera* leaves extracts in functional food applications, demonstrating that sodium alginate-gelatine microcapsules can effectively encapsulate and deliver bioactive compounds while maintaining desirable food properties.

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Versatile Polysaccharide Hydrogels for Tissue Healing

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The development of biomaterials capable of supporting tissue regeneration while minimizing inflammation is a major goal in regenerative medicine [1]. In this study, we present injectable, self-healing chitosan-based hydrogels crosslinked with dialdehyde cellulose (DAC) and incorporating polypyrrole (PPy) nanoparticles. These hydrogels are based on water-soluble, half-acetylated chitosan (SCN), prepared without synthetic modification, ensuring biocompatibility and solubility at physiological pH [2]. DAC enables both reversible Schiff base crosslinking and covalent anchoring of PPy colloids, resulting in a stable, shear-thinning hydrogel network [3].

The materials were designed for application in wound healing across different tissue types, including bone and skin. Rheological analyses confirmed excellent injectability and rapid recovery after mechanical disruption, suitable for minimally invasive application. The hydrogels maintained cytocompatibility with fibroblasts and macrophages and exhibited consistent structural homogeneity.

In the skin-related context, PPy-containing hydrogels promoted fibroblast migration *in vitro* and demonstrated strong anti-inflammatory properties by significantly reducing nitric oxide and interleukin-6 production in LPS-stimulated macrophages.

For bone regeneration, BMP-2-loaded microspheres were incorporated into the hydrogel, enhancing osteogenic differentiation and repair in critical-size calvarial defects *in vivo,* while the presence of PPy reduced inflammation. *In vivo* implantation studies in mice demonstrated effective modulation of immune response and substantial bone regeneration, with the combination of PPy and BMP-2 yielding the highest bone repair percentages.

These results demonstrate that the developed hydrogels represent a versatile platform for tissue healing, with potential applications in both orthopedic and dermal contexts. Their injectability, self- healing capability, anti-inflammatory action, and adaptability to tissue-specific modifications make them strong candidates for advanced wound healing therapies.

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Ohmic Heating vs. Conventional Extraction of Immunomodulatory Polysaccharides from *Chlorella vulgaris*

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Microalgae are photosynthetic microorganisms rich in bioactive compounds with diverse applications [1]. Among such compounds, soluble polysaccharides, such as glucans, can be responsible for immunomodulatory activities [2]. The aim of this work was to obtain β-glucans from microalgae *Chlorella vulgaris* using two different methodologies and to compare their performance. The physicochemical properties of the extracts were characterized by Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), Nuclear Magnetic Resonance (NMR), and determination of carbohydrate content using sulfuric acid/UV spectrophotometry method [3]. Two extraction methods, conventional hot-water extraction and ohmic heating (using 100% seawater and 50% seawater), were applied to biomass suspensions at three concentrations (25, 50, and 75 g L^{-1}). The results revealed that ohmic heating produced higher extraction yields (18-32%, w/w), compared to conventional extraction (6%, w/w), regardless of seawater concentration. However, carbohydrate content of these extracts was lower, suggesting that ohmic heating may extract a broader range of compounds in addition to the interesting β-glucans. ATR-FTIR results confirm the presence of polysaccharides by the existence of signature peaks: 1150–1000 cm^{-1}, for glycosidic linkages (particularly β-1,3 and β-1,6 bonds). However, peaks specific to β-glucans, 890 ± 10 cm^{-1} [4], were not observed. The different composition of the extracts was determined by NMR spectroscopy. Future work involves a purification step, such as dialysis, to remove interferences that could be affecting the results, and evaluation of the extracts' immunomodulatory effects in aquaculture.

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Multifunctional Encapsulation of Sunflower Oil Using Modified Starch and Mannitol: Comparison Between Drying Methods

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Cannabis Oil from *Cannabis sativa* has been successfully used for therapeutic purposes. In particular, the cannabinoids Tetrahydrocannabinol (THC) and Cannabidiol (CBD) have gained renewed interest due to their anti-cancer properties. Targeted delivery of cannabis oil to the colon presents a promising approach for enhancing the therapeutic efficacy of cannabinoids in the treatment of colorectal cancer. However, oral administration often results in low bioavailability due to degradation in the gastrointestinal tract. Colon-specific delivery systems are a promising strategy to overcome these limitations by protecting cannabinoids during gastrointestinal tract and ensuring localized release at the tumor site. [1–4]

With this purpose, the aim of the present work is to use polysaccharides to encapsulate cannabis oil, protecting the oil from gastrointestinal degradation. To test different encapsulation strategies, an oil-in-water (o/w) emulsion was developed using food emulsifier modified starch (E1422) and sunflower oil, due to the documented emulsifying properties. High-shear (Ultraturrax) and moderate- shear (Thermomix) homogenizers were used for mechanical stirring, followed by sonication. An emulsion capable of incorporating up to 7.5% of sunflower oil was successfully formulated. Mannitol was also added as cryopreservative. Emulsions were either dried through spray drying or freeze drying. The dried powders were blended on a 1:1 proportion with the excipients modified starch and microcrystalline cellulose and compressed to assess oil retention and mimic a pill, a possible oral delivery system. Pills from freeze-dried emulsions allowed to retain less oil (73,0% – 83.0%) than the spray-dried ones (83,5% – 98,0%). Polydispersity indices and droplet size of emulsions were measured throughout the processes but remained similar and independent of the drying method.

Therefore, spray-drying seems to be a better oil encapsulation approach in comparison with freeze- drying, once it retained higher oil amounts.

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Influence of growth conditions on the carbohydrates profile of brown algae

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Brown algae play crucial roles in marine ecosystems and are rich in bioactive compounds, as carbohydrate, having considerable importance in fields as nutrition, pharmaceuticals, and biomedicine (1). Environmental conditions during seaweed cultivation play a critical role in the yield and composition of bioactive compounds. The objective of this work was to study the carbohydrate profile of the brown algae, *Saccorhiza polyschides*, *Saccharina latissima*, and *Laminaria ochroleuca*, with different deployment dates, growing conditions and from two hatcheries (CIIMAR and S2AQUA). The three seaweeds contain between 20 and 35 % (w/w) of total sugars, with *Saccorhiza polyschides* showing the lowest content. The overall monosaccharides profile is similar across all samples with mannose, uronic acids, and glucose as the main sugars, and lower amounts of fucose and galactose. It is known that, in brown algae, glucose mainly comes from laminarans, uronic acids mostly from alginates, fucose and galactose from fucose-containing sulphated polysaccharides, and mannose from free mannitol (2). The main difference between species is that *Saccharina latissima* showed more than the double quantity of glucose when compared to the other algae. Results have also shown that *Laminaria ochroleuca* samples from S2AQUA presented a higher quantity of mannose, when compared to the samples from CIIMAR. Additionally, when considering the same deployment date at CIIMAR, harvesting the sample one month later did not significantly change the carbohydrate content and composition. However, when the samples were deployed three months apart and harvested at the same time, mannose and galactose content slightly decreased, while uronic acids increased. The samples of *Saccorhiza polyschides* had the same deployment date and hatchery but the quantity of their carbohydrates were distinct, with the sample harvested in July presenting much more mannose and less uronic acids, when compared to the one harvested in June, which could be related with the seasonal conditions of growing.

In conclusion, the growing conditions of brown algae significantly influence their carbohydrate content and composition. This knowledge is essential for selecting optimal cultivation conditions and harvest timing based on the target polysaccharides.

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Chemical characterization of polysaccharides from *Agaricus bisporus* by-products

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In Portugal, mushroom consumption has been increased 10-15% per year, mostly due to their organoleptic properties, and the rising consumer demand for healthy, vegan and high protein food[1]. Annual national production is 5.3 kt, from which 5 to 10% are by-products currently used as fertilizer or animal feed, with low added commercial value[2]. However, these by-products are a rich source of polysaccharides, such as β-glucans, which have been associated with potential hypocholesterolemic effects[3,4], and proteins.

In this work, extraction and characterization of different fractions containing polysaccharides and proteins from *Agaricus bisporus* by-products was addressed.

Ethanolic, aqueous and alkaline extracts of *Agaricus bisporus* by-products resulted in low yield of soluble (29%) and high yield of insoluble (54%) polysaccharides. The fractions are rich in sugars (37-83%) with glucose as a major component (44-96%mol). After washing with water, the residue (WIR) (53% carbohydrates; 69%mol glucose; 19%mol glucosamine) yielded 39% of initial mushroom contained an insoluble glucan with similar quantities of (1→4)-Glc (30%) and (1→6)-Glc (23%). Its insolubility could be due to the presence of (1→4)-GlcNAc (11%) from chitin and 28% of protein. After alkali extraction, it's possible to solubilize 16% of glucans (Sn.1M_NaOH) (83% carbohydrates; 96%mol glucose) with a main chain of (1→4)-Glc (43%) and (1→6)-Glc (26%) and an average polymerization degree of 7 residues. The presence of (1→3,4)-Glc (14%) shows a branched degree of 14%. The insoluble residue (R_1M.NaOH) (60% carbohydrates; 53%mol glucose; 42%mol glucosamine) yielded 12% of initial mushroom contained an insoluble glucan, with (1→3)-Glc (5%), (1→4)-Glc (6%) and (1→6)-Glc (12%), in complex with chitin in the same proportion due to the presence of (1→4)-GlcNAc (25%).

The amount of β-glucans, chitin and protein may be promising for development of functional food and ingredients with hypocholesterolemic potential or nutritional and organoleptic claims, which will contribute for the valorisation of mushroom by-products.

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Glycosaminoglycans extraction and characterization from porcine cartilage towards food applications

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Glycosaminoglycans comprehend high value polysaccharides founds in cartilages, especially as by- product of meat industry. Structurally, glycosaminoglycans are negatively charged polysaccharides constituted by a disaccharide repetitive unit of galactosamine or glucosamine and L-iduronic acid or D-glucuronic acid. These polysaccharides often present a protein core, forming proteoglycans. Depending on sugar composition and sulphate pattern, glycosaminoglycans are classified as heparin, chondroitin sulphate, dermatan sulphate, keratan sulphate, and hyaluronic acid.

So, the aim of this study is to obtain and disclose the structural details of glycosaminoglycans present in porcine cartilage and evaluate their potential as food ingredients for meat product applications. To achieve this. Neutral sugars and hexosamine, as alditol acetates, were analysed by GC-FID and GC-MS, respectively, uronic acids by m-phenylphenol colorimetric method, protein content by BSA protein assay, and amino acid content by HPAEC-PAD. The defatted cartilage accounted for 53% of the initial mass. Glycosaminoglycan extraction using papain followed by ethanol precipitation resulted in an extract that accounted for 10% of the mass of the defatted extract. This extract presented a carbohydrate content of 60 % (w/w), where the molar proportion of uronic acids and hexosamine was about 1:1. Also, the extract presented a protein content of 14% (w/w). These features are indicative that the ethanol insoluble material obtained after papain hydrolysis contains glycosaminoglycans at high level of purity. The fact that glycosaminoglycans probably still contain covalently linked amino acids, together with the occurrence of negative charges, provide to the glycosaminoglycan-rich material not only amphipathic features useful for emulsification, but also chelating, antioxidant, and retention of water properties promising for development of new food technological ingredients from porcine cartilage.

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Soluble and insoluble polysaccharides partitioning in apple juices and pulps through modulation of centrifugal decantation speeds

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Apple polysaccharides are essential contributors to dietary fibre intake and may provide prebiotic effects [1]. As most apples are consumed as juice, the modulation of juice processing to increment the dietary fibre is of utmost importance. In this work, apple juices and pulps produced under two speeds of centrifugal decantation from two purees with different particle sizes (<2 mm and <0.25 mm) were characterized for their polysaccharides [2] to evaluate the impact of these processing parameters.

Soluble polysaccharides (SP) in juices accounted for 0.10-0.12% of fresh weight (%fw), while insoluble polysaccharides (IP) accounted for 0.03-0.07%fw. Juices prepared at differential/bowl speeds of 20/3800 rpm had lower ratio of SP to IP (1.4 from puree with <3mm and 2.9 from puree with <0.2mm) than juices prepared at speeds of 10/4400 rpm (3.8 from puree with <3mm and 3.9 from puree with <0.2mm). SP in juices were mainly composed by uronic acids (UA, 62-65 mol%), followed by Glc (12-15 mol%), Gal (8.8-11 mol%), Ara (8.6-9.1 mol%), and Xyl (1.3-1.4 mol%) while IP were mainly composed by Glc (29-42 mol%), followed by UA (23-42 mol%), Gal (7.8-13 mol%), Ara (6.5-11 mol%), and Xyl (0.7-25 mol%). Pulps contained 1.9-3.7 times more of SP (0.18-0.45%fw) and 58-182 of IP (2.9-4.9%fw) than juices. Pulps obtained at speeds of 20/3800 rpm had lower amount of IP (2.9-4.0 %fw) than the ones produced at 10/4400 rpm (4.9 %fw). The processing of purees with <0.2 mm at 20/3800 rpm resulted in pulps with 1.4 to 1.7 lower amounts of IP than the other pulps. SP in pulps were also mainly composed by UA (55-63 mol%) when comparing with SP in juices but had lower amount of Glc (5.8-8.1 mol%) and Gal (6.1-9.9 mol%) and had higher amount of Ara (17-27 mol%) and Xyl (1.9-4.6 mol%).

In conclusion, the ratio of SP to IP in juices can be modulated by centrifugal decantation speeds, without affecting SP composition. Partitioning of galactans and glucans into SP of juices was observed, while arabinans and xyloglucans remained in pulps. Therefore, adjusting centrifugal decantation speeds can be a useful strategy to tailor juice and pulp fibre content for specific applications.

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Development of Pectic Polysaccharide Microarrays for Structural and Functional Profiling of Pectins

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Pectins, key components of dietary fiber, have beneficial functions such as regulating gut microbiota, supporting weight control, and lowering the risk of chronic diseases [1]. Deciphering their complex structures and associated biological functions is fundamental for understanding their health-promoting properties and for guiding the rational design of functional foods. As part of a collaboration between Zhejiang University and the Carbohydrate Microarray Facility at Imperial College London, pectin microarrays are being developed using a unique and comprehensive library of over 300 pectins derived from fruits, vegetables, and medicinal herbs. Using advanced analytical techniques, such as LC-MS/MS and HPSEC-MALLS, structural data on monosaccharide composition, linkages, and molecular weights were obtained. Based on these insights, 30 representative pectins were selected to construct pilot microarrays using covalent and non-covalent immobilization strategies.

This presentation highlights the design and preliminary findings from the pilot pectin arrays, which were probed using a panel of plant-specific monoclonal antibodies and archetypal carbohydrate binding modules (CBMs) from bacterial species residing in different ecological niches and that use different molecular organizations to efficiently target plant carbohydrates. These included: the human gut commensal *Bacteroides thetaiotaomicron*, with well characterized *polysaccharide utilization loci* targeting pectins [2], and two other fibrolytic bacteria, *Clostridium thermocellum* predominant in the soil and *Ruminococcus flavefaciens* in the rumen, which use a highly organized enzyme system - the cellulosome - to target different polysaccharides, but are less well characterised for their pectin-targeting capacity. Among interesting findings are the difference in antibody binding profiles towards okra pectin, *Citrus reticulata* (chenpi) pectin, and berry-derived polysaccharides, suggesting their unique structural features. The distinct binding preferences identified for the different CBMs toward pectins with varying neutral side chains highlight the potential of the approach for CBM-guided in-depth analysis of pectin recognition and utilization mechanisms. Further data analysis integrating detailed structural information is currently underway.

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BOOK OF ABSTRACTS

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