GLUPOR 14

16-18 January 2023

UCIBIO/FCT-NOVA

Caparica - Portugal

14th International Meeting of the **Portuguese Carbohydrate Group**

Sociedade Portuguesa de Química (SPQ)

NOVA School of Science and Technology (FCT-NOVA)

Welcome Letter

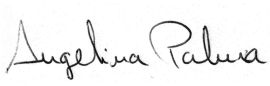
Dear Participants of the GLUPOR14,

On behalf of the organizing committee, the scientific committee, and the Grupo de Glúcidos da Sociedade Portuguesa de Química, it is a great pleasure to welcome you to the 14th International Meeting of the Portuguese Carbohydrate Group - GLUPOR 14, which will be held at the NOVA School of Science and Technology (FCT - NOVA) in Caparica, Lisbon.

The GLUPOR 14 program is dedicated to discussing scientific advances in carbohydrate research by displaying various and cross-disciplinary areas within the field of glycosciences, with a focus on basic and applied applications. This meeting provides a unique opportunity for the carbohydrate national and international community to form new collaborations and strengthen existing ones. This year, there will be 1 keynote lecture, 4 plenary lectures, 10 invited lectures, 6 invited oral communications, 14 oral communications, 17 flash presentations and 49 poster presentations from the following areas of glycosciences: **(i)** Carbohydrate synthesis, structure and molecular recognition; **(ii)** Glycobiology in health and disease; **(iii)** Glycomics, glycoproteomics and novel biomarkers; (iii) Carbohydrates in infection and microbial-host interations; **(iv)** Carbohydrates in food and agriculture; (v) Carbohydrates in biotechnology and other applications; and **(vi)** Carbohydrate-based biomaterials.

GLUPOR meetings have a long history, with the first taking place in Lisbon in 1995. Following the first meeting, it became a tradition to hold GLUPOR meetings every two years in different locations throughout Portugal, with GLUPOR 2 held in Porto in 1997, followed by GLUPOR 3 in Aveiro in 1999 also known as 1st Iberian Carbohydrate Meeting; GLUPOR 4 in Lisbon in 2001, recognized as the EuroCarb XI; GLUPOR 5 in Covilhã in 2003; GLUPOR 6 in Coimbra in 2005, the 3rd Iberian Carbohydrate Meeting; GLUPOR 7 in Oeiras in 2007; GLUPOR 8 in Braga in 2009; GLUPOR 9 in Vila Real in 2011, as the 5th Iberian Carbohydrate Meeting; GLUPOR 10 in Covilhã in 2013; GLUPOR 11 in Viseu in 2015; GLUPOR 12 in Aveiro in 2017, and finally, GLUPOR 13 in Porto in 2019. Since the last meeting, there has been a global pandemic, but this has not deterred us; in fact, significant advances in various fields of science, including glycosciences. Now the time has come to lay new foundation for future collaborations. It is with a great pleasure to host the GLUPOR 14 this year in Caparica, Lisbon.

On behalf of the organizing and scientific committees, I would like to wish you all a very productive GLUPOR meeting, and while it isn't summer, I hope you enjoy the Caparica sun and the delightful city of Lisbon.



Angelina S. Palma

Acknowledgments

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Institutional Support:

Supported by:

GLUPOR14 activities were also supported by the GLYCOTwinning project (grant agreement nº 101079417):

Funded by the European Union

Photograph, book of abstracts and Logo:

We acknowledge the cooperation of Daniela F. Barreira (MSc), and Pedro Granjo (BSc) for editing the book of abstracts, and Benedita Pinheiro (PhD) for the creation of the GLUPOR14 logo. The event symbol is inspired by the linear structure of some polysaccharides and the emblematic "Ponte 25 de Abril". We hope it inspires event attendees to build bridges within and outside of the Glycosciences community. The photograph from the “25th of April Bridge” was taken by Teresa Sequeira Carlos.

Scientific Committee

Amélia P. Rauter | University of Lisbon (President of the International Carbohydrate Organization)

Angelina S. Palma | UCIBIO, FCT-NOVA (Chair)

António Vicente | University of Minho

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Leonardo Mendes | SPQ

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General Information

The GLUPOR14 will be held in Building V – "Grande Auditório" at the FCT-NOVA – in NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal.

Registration and coffee breaks will occur in Building IV (next to main auditorium). Lunches and Poster sessions will be held in Sala Ágora, next to the campus' Library.

Parking

Free parking is available at the meeting venue, inside the faculty.

Speakers

Presenters are 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 three 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, January 16th | **12h45-15h30**

Poster session 2

Tuesday, January 17th | **12h55-15h**

Poster session 3

Wednesday, January 18th | **13h-15h**

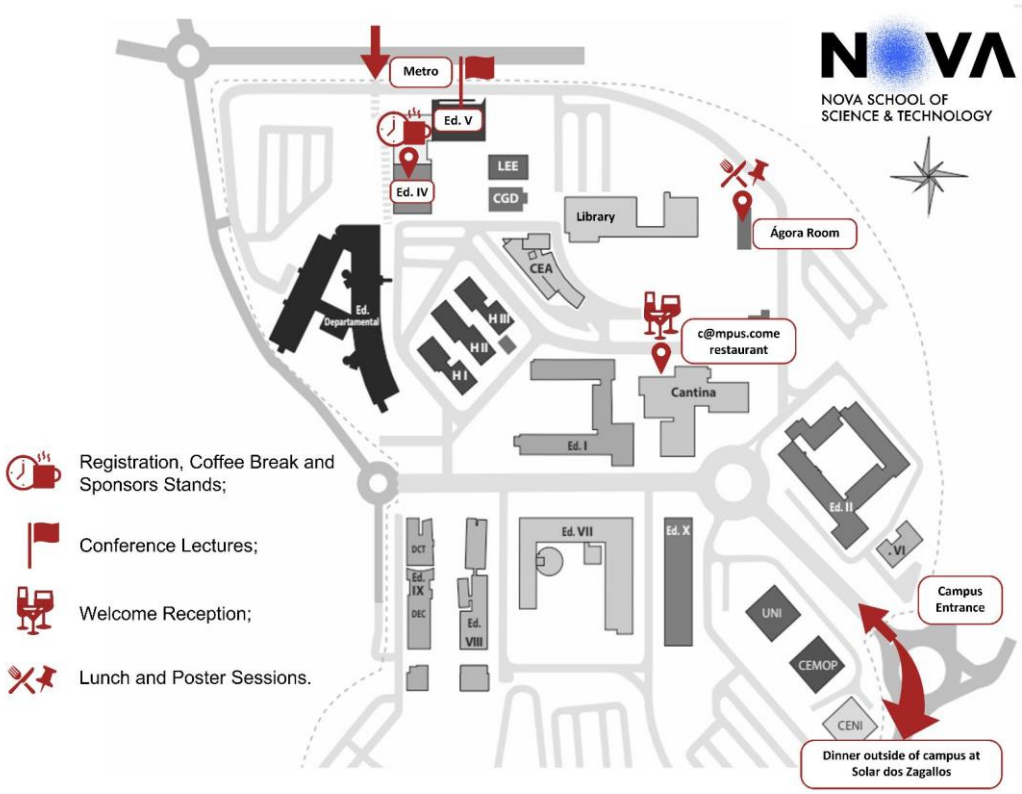
Awards

The SPQ and the scientific committee of the GLUPOR14 will award the 2 best oral communications and the 2 best poster presentations.

The award will be announced in the closing session of the meeting.

Conference Dinner

Meeting Point:
'Grande Auditório', Building V around 19:00. Includes transportation to the dinner location.



Content

Scientific program

Day 1 - Monday 16th		
8:30 – Registration (Building IV) and Poster Setup (Sala Ágora) **9:30 – Opening Session | Angelina S. Palma ('Grande Auditório', Building V)** *Dean of FCT-NOVA* | *Professor José Júlio Alferes* *Director of UCIBIO* | *Professor Maria João Romão* *President of the ICO* | *Professor Amelia Pilar Rauter* *President of SPQ Carbohydrate Group* | *Professor Celso Reis*		
Chaired by Angelina S. Palma (Grande Auditório)		
09:45	00:45	KL | Celso A. Reis, i3S University of Porto *Glycobiology in cancer: from the molecular mechanisms to the clinical applications*
10:30	00:30	Coffee break (Building IV)
SESSION 1 | Glycomics, glycoproteomics & novel biomarkers		
Chaired by Celso A. Reis & Hugo Santos		
11:00	00:30	PL1 | Júlia Costa, ITQB-NOVA *Challenges and perspectives in protein glycosylation analysis*
11:30	00:20	IL1 | Alexandre Ferreira, IPO, ICBAS University of Porto *Unravelling the cancer glycoproteome towards precision oncology and targeted therapeutics*
11:50	00:15	IOC1 | Ana Rita Grosso, UCIBIO FCT-NOVA *Decoding regulation of the glycosylation machinery in human health*
12:05	00:15	IOC2 | Ana Cristina Ribeiro, University of Lisbon *What Should be Called a Lectin? Explore Lectin Bioactivities*
12:20	00:15	OC1 | Mariangela Natale, CellmAbs *Strategies to develop anti-cancer therapies targeting truncated cancer glycan sialyl-Tn*
12:35	00:10	FP1 | Nuno F. Lopes *Profiling glycan epitopes in Triple Negative Breast Cancer to explore interactions with tumour-associated macrophages* FP2 | Filipa Trovão *Using Mucin Glycoprotein Microarray to Identify Novel Glycan Ligands Recognized by Human Gut Microbiota*
12:45		Lunch & Poster session (Sala Ágora)
SESSION 2 | Glycobiology in health and disease		
Chaired by Paula Videira & Alexandre Ferreira		
15:30	00:30	PL2 | Salomé Pinho, i3S University of Porto *A Bitter Sweet Symphony in Immune response: Glycans as key checkpoints at the frontiers of inflammation, autoimmunity and cancer.*
16:00	00:20	IL2 | Ana Magalhães, i3S University of Porto *Mind the GAGs in gastric cancer*
16:20	00:15	IOC3 | Dulce Quelhas, ICBAS, University of Porto *Congenital Disorders of Glycosylation in Portugal: 20 years in a nutshell*
16:35	00:30	Coffee break (Building IV)
17:05	00:15	IOC4 | Vanessa Ferreira, CDG & Allies *Patient and Public Involvement (PPI) in research led by Glycoscientists: A "How-to" guide that will shine light to your research*
17:25	00:15	OC2 | Catarina Marques, i3S University of Porto *Heparan Sulfate biosynthetic pathway: learning the GAGosylation road that leads to gastric cancer progression*
17:40	00:15	OC3 | Catarina Gomes, i3S University of Porto *High throughput screening of chemical compounds showed monensin as novel cancer drug by inhibiting SLeX expression and malignant properties in gastrointestinal cancer cells*
17:55	00:10	FP3 | Zélia Silva *Modulation of sialic acid content on monocyte derived dendritic cells: phenotypic and functional implications* FP4 | Mariana Nunes Barbosa *Amino sugar phosphate ManNAc-6-P as template molecule to modulate sialylation status in GNE myopathy*
18:05	01:00	***General assembly GLUPOR***
19:30		**Welcome Reception (Campus.come)**

Day 2 - Tuesday 17th		
SESSION 3 | Carbohydrate synthesis, structure & molecular recognition		
Chaired by Filipa Marcelo & Jesús Jimenez-Barbero		
9:00	00:20	IL3 | Rita Ventura, ITQB-NOVA *Synthesis of (oligo)arabinosides and development of a functional assay for the study of enzymes involved in mycobacteria cell wall biosynthesis*
9:20	00:20	IL4 | Nuno Xavier, University of Lisbon *Synthesis and Exploitation of the Biological Profile of Novel Triazole-Containing Xylofuranosyl Isonucleosides*
9:40	00:15	OC4 | Maria Moure, CICbioGUNE *Selective methods for the synthesis of repeating sugar oligomers to explain protein binding epitopes*
9:55	00:15	OC5 | Helena Coelho, UCIBIO FCT-NOVA *Molecular recognition of Mucin-1 O-glycosylation process by different enzymes: in cancer and bacterial infection*
10:10	00:15	OC6 | Benedita A. Pinheiro, UCIBIO FCT-NOVA *Mapping Molecular Recognition of β1,3-1,4-Glucans by a Surface Glycan-Binding Protein from the Human Gut Symbiont Bacteroides ovatus*
10:25	00:15	FP5| Cátia Soares *Molecular recognition of sTn glycostructures by immune-related receptors* FP6 | Catarina Caseiro *Structural and biochemical insights into glycoside hydrolase family 157 enzymes* FP7 | Luís Pinheiro *Synthesis of a phosphoglycoglycerol inspired from Nature*
10:40	00:30	Coffee break (Building IV)
SESSION 4 | Carbohydrate-based materials		
Chaired by Cláudia Nunes & Serge Perez		
11:10	00:30	PL3 | Iva Pashkuleva, I3Bs University of Minho *Hyaluronan-based materials for understanding and targeting breast cancer associated CD44 and RHAMM receptors*
11:40	00:20	IL5 | Filomena Freitas, UCIBIO FCT-NOVA *FucoPol, a multifunctional polysaccharide for high-value applications*
12:00	00:15	IOC5 | Helena Godinho, FCT-NOVA *Liquid crystalline phases to probe water cellulose nanocrystals (CNCs) interactions*
12:15	00:15	OC7 | Ana Barra, CICECO Aveiro Institute of Materials *Polysaccharide-based biocomposite films with electrical properties for food packaging applications*
12:30	00:15	OC8 | Tiago H. Silva, I3Bs University of Minho *Innovative compressive and absorption methodology for the production of hydrogels (CAMPH) based in marine biopolymers for tissue engineering*
12:45	00:15	FP8| Ana Silva *Carboxymethylcellulose-based dissolvable microneedle patches for rapid painless delivery of diclofenac* FP9 | Asiyah Esmail *Polymeric dressings based on Bacterial cellulose and FucoPol for advanced wound treatment* FP10 | Veronica Weng *Preparation and characterization of biodegradable films from purified arabinoxylan extract derived from corn fiber*
13:00		Lunch, Poster session & GLYCOTwinning mentoring (Sala Ágora)
SESSION 5 | Carbohydrates in infection & microbial-host interactions		
Chaired by Jaime Mota & Isabel Sá Nogueira		
15:30	00:20	IL6 | Nuno Empadinhas, CIBB University of Coimbra *Methylglucose vs methylmannose polysaccharides: biosynthesis, distribution, and proposed roles in mycobacterial adaptation to heat or cold*
15:50	00:20	IL7 | Agostinho Carvalho, ICVS/3B's University of Minho *Genetic determinants of C-type lectin receptor function in fungal infection*
16:10	00:15	IOC6 | Sérgio R. Filipe, UCIBIO FCT-NOVA *Coordination of the synthesis of two surface glycopolymers ensures the concealment and survival of pneumococcal bacteria*
16:25	00:30	Coffee break (Building IV)
16:55	00:15	OC9 | Viviana Correia, UCIBIO FCT-NOVA *High-throughput characterization of human gut microbiome glycan-protein binding interactions*
17:10	00:10	FP11 | Márcia Sousa *Challenging the virulence role of sialidases in Gardnerella spp.* FP12 | Raquel Costa *Molecular recognition of mucin O-glycans by family 32 carbohydrate-binding modules from Bacteroides thetaiotaomicron* FP13 | João Paquete Ferreira *Wall teichoic acids: a future target for antibiotics?*

17:20	01:30	*Round Table: "The Future of Glycoscience in Europe"* *Moderators - Amélia P. Rauter & Serge Perez*
19:30		**Conference Dinner (Solar dos Zagallos)**

Day 3 - Wednesday 18th

SESSION 6 | Carbohydrates in food and agriculture

Chaired by José Paulo Sampaio & Pedro Bule

9:00	00:30	PL4 | Manuel A. Coimbra, University of Aveiro *The clean label strategy that can be provided by food carbohydrates*
9:30	00:20	IL8 | Dulcineia Ferreira, Polytechnic Institute of Viseu *Carbohydrates in plant defense towards more sustainable agriculture*
9:50	00:15	OC10 | Filipe Gomes, University of Aveiro *Polysaccharides as hypocholesterolemic food ingredients*
10:05	00:15	OC11 | Pedro Fernandes, LAQV-REQUIMTE University of Aveiro *Tailoring the yacon carbohydrates towards sweetness and texture during syrup production*
10:20	00:15	OC12 | Marlene Duarte, University of Lisbon *Unconventional assembly of ruminal cellulosomes*
10:35	00:30	Coffee break (Building IV)

SESSION 7 | Carbohydrates in biotechnology & other applications

Chaired by Elisabete Coelho & Ana Luísa Carvalho

11:05	00:20	IL9 | Alexandra Castilho, University of Natural Resources and Life Sciences, Vienna *Plant Glyco-engineering: major achievements and troubleshooting*
11:25	00:20	IL10 | Pedro Bule, University of Lisbon *The dual-binding mode in the assembly of multi-enzyme cellulolytic complexes*
11:45	00:15	OC13 | Sónia S. Ferreira, University of Aveiro *Structural details of sulfated polysaccharides concentrated in salt pan waters*
12:00	00:15	OC14 | Rui Freitas, IPO, ICBAS University of Porto *Production and pre-clinical evaluation of immunogenic CD44 glycoconjugates: setting the molecular bases for novel multivalent anti-cancer glycovaccines*
12:15	00:20	FP14 | Vítor Martins *Enzymatic Deconstruction of Brewer's Spent Yeast Cell Wall Glucans and Their Structural Characterization* FP15 | João Pissarra *Insight into the role of RfbC and FucS on the composition of the released polysaccharides of the cyanobacterium Synechocystis sp. PCC 6803* FP16 | Ana Filipa Costa *Fluorinated GalNAc as a novel inhibitor of cancer-associated truncated O-glycans in gastrointestinal cancer* FP17 | Kleyde Ramos *Bioprospecting novel bacterial exopolysaccharide producers*
12:35		Lunch, Poster session & GLYCOTwinning mentoring (Sala Ágora)
14:30		***Awards & Closing session (Grande Auditório)***

GLYCOTwinning Satellite Meeting (Grande Auditório)

14:50 – Opening Session | Paula Videira, Angelina S. Palma & Filipa Marcelo

Vice-Dean of FCT-NOVA for Innovation and Research | *Professor Eurico Cabrita*

Director of UCIBIO | *Professor Maria João Romão*

Chaired by Paula Videira, Angelina S. Palma & Filipa Marcelo

15:00	00:15	"GLYCOTwinning: Building Networks to Excel in Glycoscience"
15:15	00:30	Jesús Jiménez Barbero – CICbioGUNE *Breaking the limits in understanding glycan recognition by NMR*
15:45	00:15	Coffee break (Building IV)
16:00	00:30	Ten Feizi - Imperial College London *Approaches to Unravelling Glycan Recognition Systems*
16:30	00:30	Manfred Wuhrer - Leiden University Medical Center *A look into the glycoanalytical toolbox*
17:00		***Closing Remarks***

Keynote Lecture

Glycobiology in cancer: from the molecular mechanisms to the clinical applications

Reis CA,[1,2,3,4]
1. Institute for Research and Innovation in Health, University of Porto (i3S), Rua Alfredo Allen, 208. 4200-135 Porto, Portugal; 2. Institute of Molecular Pathology and Immunology of the University of Porto (Ipatimup), Porto, Portugal; 3. Institute of Biomedical Sciences Abel Salazar of the University of Porto, Portugal; 4. Faculty of Medicine of the University of Porto, Portugal.

E-mail: celsor@ipatimup.pt

Glycan are key components of biological systems underlying a variety of essential structural and functional roles. Glycans control and define fundamental molecular, cellular, tissue, organ and systemic biological processes directing physiological functions and being involve in several human diseases, such as cancer [1,2].
Alterations of glycosylation are common molecular alterations during carcinogenesis [3] with major biological implications for cancer progression [1,2]. In this presentation the basis underlying the alterations of glycosylation that occur in cancer will be reported, such as the aberrant expression of the truncated O-glycan antigen Sialyl-Tn [4]. In addition, a particular focus will be given on the studies applying glycomic and glycoproteomic strategies in human cancer that provided novel information on the alterations of glycosylation impact the activation of oncogenic receptors in tumour samples, such as RON, MET, EGFR and HER2 (ErbB2) [5,6,7,8,9].
Given the fact that cancer is a heterogeneous disease that requires multidisciplinary treatment, and that current targeted therapy depends on patient stratification based on molecular features of the tumour. I will report on the glycoproteomic map of the HER2 and EGFR in cancer cells which disclosed a site-specific glycosylation profile of this receptor and how these protein glycosylation affects the biology of the receptor and the sensitivity of cancer cells to therapeutic humanized monoclonal antibodies used in the clinics [3,4].
The presentation is going to highlight the functional aspects of glycosylation modifications occurring in cancer [5,10] and their potential application as biomarkers for patient stratification, personalize medicine and for novel and improved therapeutic applications [1,8,9].

References
1. Mereiter S. et al., Cancer Cell, **2019**, *36(1)*, 6-16;
2. Pinho S, Reis CA. Nature Rev Cancer, **2015**, *15*, 540-555;
3. Magalhães et al., Biochim Biophys Acta, **2015**, *1852(9)*, 1928-39;
4. Marcos N et al.,Cancer Res, **2004**, *64(19)*, 7050-7;
5. Gomes C. et al., PLoS One, **2013**, *8(6)*:e66737;
6. Campos et al., Mol Cell Proteomics, **2015**, *14(6)*, 1616-29;
7. Mereiter S. et al., Biochim Biophys Acta, **2016**, *1860(8)*, 1795-808;
8. Duarte HO. et al., Oncogene, **2021**, *40(21)*, 3719-3733;
9. Rodrigues J, et al. Cell Oncol, **2021**, *44(4)*, 835-850;
10. Freitas D. et al., EBioMedicine, **2019**, *40*, 349-362.

Plenary Lectures

Challenges and perspectives in protein glycosylation analysis

Costa J
Instituto de Tecnologia Química e Biológica António Xavier, Avenida da República, 2780-157, Oeiras, Portugal

E-mail: jcosta@itqb.unl.pt

Glycosylation is a common post-translational modification of proteins with important structural and functional implications at cellular and organism level. Glycans play important roles in protein folding, intracellular targeting, cell recognition and adhesion events. Glycosylation is regulated by several factors including the set of glycosyltransferases and glycosidases expressed by the host cell, their localization along the secretory pathway, expression of nucleotide sugar transporters and substrate availability. Glycosylation patterns are characteristic of specific physiological conditions and are usually deregulated in disease; for example, changes in glycosylation are a hallmark of cancer and are used as biomarkers. In a biotechnological context, the glycosylation of recombinant therapeutic proteins impacts their pharmacokinetic as well as functional properties.
N- and O-glycosylation are the most widely studied types of protein glycosylation. N-glycans are covalently bound to asparagine residues within a consensus sequence whereas O-glycans may be bound to several amino acid residues, including serine and threonine. Structural diversity of glycans is high since several monosaccharides are bound in different linkages with two possible anomericities, and most structures are branched. Furthermore, a given protein exists as a set of glycoforms where an individual glycosylation site bears several glycans that are structurally related. Therefore, structural elucidation of glycans is challenging and requires complementary techniques for full elucidation. Glycan release from proteins may be accomplished enzymatically or chemically. Released glycans may be analysed in the native state or derivatized for detection or stabilization purposes. Techniques used for detailed structure analysis include liquid chromatography, mass spectrometry, exoglycosidase sequencing and NMR. Lectin-based techniques are commonly used and require lower amounts of protein.
Glycomics provides the set of glycan structures from a given protein, cell or organism. On the other hand, glycoproteomics provides information about the glycan structures and the site to which they are bound within the protein. Automated high-throughput technologies allow the recovery of large amounts of structural data concerning glycan structures from different biological systems and physiological conditions, including development, aging and disease. Efforts towards the establishment of standardized protocols and detailed description of procedures are of crucial importance to improve result comparability and quality. Furthermore, the field of glycoinformatics with the development of specific databases and software tools is essential for the study of carbohydrates and results integration.
In this lecture, specific examples illustrating methodologies and techniques used in protein N-glycosylation analysis are presented. These include: extracellular vesicles [1,2], SARS-CoV-2 Spike protein [3,4] and cerebrospinal fluid IgG [5].

References
1. J. Costa, BBA Rev Can, **2017**, *1868*, 157-166;
2. J. Costa et al., Anal Chem, **2018**, *90*, 7871-7879;
3. R. Castro et al., Biotech Bioengineer **2021**, *118*, 2202-2219;
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5. J. Costa et al., Molecular Neurobiol., **2019**, *56*, 5729–5739.

A Bitter sweet Symphony in Immune response: Glycans as key checkpoints at the frontiers of inflammation, autoimmunity and cancer

Salomé S. Pinho[1,2,3]
1. Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal; 2. Medical Faculty, University of Porto, Porto, Portugal; 3. ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal

E-mail: salomep@ipatimup.pt

The immune system is governed by a series of stimulatory and inhibitory pathways in which the disruption of the control of these molecular checks can lead to unpredictable autoimmune or cancer states. The mechanisms underlying the genesis of the loss of immunological tolerance in autoimmunity or the creation of immunosuppressive networks in cancer are still elusive. Glycans have been highlighted as essential determinants that integrate the regulatory networks that guide both innate and adaptive immune responses. Changes in protein glycosylation are a hallmark of immune-mediated diseases, in which glycans act as master regulators of the inflammatory response being fundamental molecular determinants for the discrimination between “self”/“non-self”. Our results in Systemic Lupus Erythematosus (SLE), a classical autoimmune disease, revealed a unique glycan signature characterized by an increased abundance and spatial distribution of unusual mannose-enriched glycans. This abnormal exposure of mannosylated glycans at the surface of kidney cells from patients with lupus nephritis (LN) was shown to promote an increased recognition by specific glycans-recognizing receptors, expressed by immune cells, potentially contributing to the immunopathogenesis of Lupus. This abnormal glycosignature of LN was demonstrated to be due to a deficient complex N-glycosylation and a proficient O-mannosylation pathway. Importantly, from the clinical standpoint, levels of mannosylation detected in kidney biopsies from LN patients at diagnosis were demonstrated to predict the development of chronic kidney disease (CKD) with 93% of specificity [1]. In line with this, our results on Inflammatory Bowel Disease (IBD) also point towards a role for complex N-glycans in the immunopathogenesis of IBD [2-3].
At the other pole of the immune response, in a cancer context, where immunosuppressive networks promote cancer progression, we also 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 [4]. In summary, our results demonstrate the regulatory power of glycans in governing both innate and adaptive immune responses with important roles in the pathogenesis of major diseases such as cancer and autoimmunity, pinpointing glycans as key checkpoints with promising clinical and therapeutic applications in autoimmune diseases and cancer.

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2. A Dias, et al., PNAS, **2018**;
3. X Verhelst, et al., Gastroenterology, **2020;**
4. M Silva, A Fernandes, et al., Cancer Immunol Res, **2020**.

Hyaluronan-based materials for understanding and targeting breast cancer associated CD44 and RHAMM receptors

Iva Pashkuleva[1,2]
1. 3B's Research Group – Biomaterials, Biodegradable and Biomimetics, Avepark - Parque de Ciência e Tecnologia Zona Industrial da Gandra 4805-017 Barco GMR – Portugal; 2. ICVS/3B's – PT Government Associate Laboratory, University of Minho, Portugal

E-mail: pashkuleva@i3bs.uminho.pt

Hyaluronan (HA) is a non-sulfated glycosaminoglycan whose homeostasis is tightly controlled and regulated by several synthetases and hyaluronidases. During carcinogenesis, this process is altered and results in specific HA turnover profile within the tumor microenvironment: HA concentrations are usually higher in malignant tumors than in corresponding benign or normal tissues, and in patients with breast carcinomas, the increased HA concentration in the stroma is associated with low survival rates [1]. Besides these clinical evidences, the signaling pathways leading to this negative outcome are yet to be elucidated. Herein, we present several approaches that were developed by us recently and applied towards understanding of the crosstalk between HA and its main receptors - CD44 and RHAMM - in breast cancer. Keeping in mind the physiological presentation of HA, we established methods for its immobilization that preserves its bioactivity and demonstrated that immobilized HA induces amplification of signal transduction as compared with its soluble analogue [2-5]. This amplification depends on HA density and the expression level of CD44 and RHAMM [4]. We also evidenced the compensatory effect between CD44 and RHAMM in the signal transduction that is consistent with the concept of the molecular redundancy [2,5]. Apart from these mechanistic findings, we have developed several nanotherapeutics that target the HA-CD44 pathway and are based on end-on immobilized low molecular weight HA. These include stimuli-responsive nanoparticles that can encapsulate either hydrophilic or hydrophobic drugs and deliver these via CD44 selective targeting [3] and brush-like copolymers [6] that block the CD44 clustering and signal transduction.

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The clean label strategy that can be provided by food carbohydrates

Manuel A. Coimbra
LAQV-REQUIMTE - Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

E-mail: mac@ua.pt

Food carbohydrates are key components related with different properties such as sweetness, thickness or even preservation. Their structural features are not yet always understood. The arise of new methodologies and technologies permit their revisiting, allowing to explain and propose novel food functions and applications. This contributes to the new trends on demand by citizens to have clean label, tastier, and healthier foods. Examples of these challenges and solutions achieved so far will be presented focusing applications for the increment of soluble dietary fibre and oligosaccharides in fruit juices, formation of oligosaccharides by non-enzymatic transglycosylation reactions, reduction of the sucrose content in sweet formulations, and substitutes of food additives.

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Invited Lectures

Unravelling the cancer glycoproteome towards precision oncology and targeted therapeutics

Jose Alexandre Ferreira[1,2,3]

1. Experimental Pathology and Therapeutics Group, Research Center of IPO Porto, Portuguese Oncology Institute of Porto (IPO-Porto), 4200-072, Porto, Portugal; 2. Health Research Network (RISE), 4200-072, Porto, Portugal; 3. Porto Comprehensive Cancer Center (P.CCC), 4200-072, Porto Portugal.

E-mail: jose.a.ferreira@ipoporto.min-saude.pt

Malignant transformation and cancer progression are accompanied by profound alterations in protein glycosylation, contributing to all disease hallmarks [1,2]. Despite this sour side, changes in glycosylation also originate unique molecular signatures holding potential for clinical intervention [3,4,5]. Mass spectrometry has decisively contributed to comprehensively interrogate the cancer glycome and glycoproteome for prognosis and targetable glycoantigens. Showcasing some applications, we have demonstrated that advanced bladder tumours, circulating tumour cells, and metastases overexpress glycoproteins modified with short-chain sialylated *O*-GalNAc glycans [3,4,5,6,7]. These favour cancer cell invasion, immune escape, and metastasis, being associated with worst prognosis [3,4,5,8,9]. More importantly, immature Tn and sialyl-Tn *O*-glycans are absent from most healthy tissues, being only moderately expressed in specific cells across the respiratory and digestive tracts. Such observations paved the way for identification of cancer specific glycoproteoforms by targeted glycoproteomics. So far, we have identified MUC16 glycoforms associated with resistance to chemotherapy, holding potential for improved prognosis [8], and a panel of glycoproteins for targeted therapeutics [4,5]. Furthermore, we have unravelled the CD44 splicing code associated with bladder cancer invasion by glycoproteogenomics, exploiting RNAseq and glycomics-guided glycoprotein annotation by mass spectrometry [3], generating a knowledge bank for clinical intervention. Overall, glycomics and glycoproteomics have been decisive tools for identification of unique glycosignatures candidates for patient stratification and personalized therapeutics, contributing to the long-term goal of molecular-assisted precision oncology.

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Mind the GAGs in gastric cancer

Ana Magalhães[1,2,3]

1. i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, R. Alfredo Allen 208, 4200-135 Porto, Portugal; 2. IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, R. Júlio Amaral de Carvalho 45, 4200-135 Porto, Portugal; 3. ICBAS- Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.

E-mail: amagalhaes@ipatimup.pt

Glycans and glycoconjugates are essential players in the tumour microenvironment, with pivotal roles in extracellular matrix shaping and cancer cell communication [1,2]. Particularly, glycosaminoglycans (GAGs), which are linear polysaccharides built by disaccharide units of an amino sugar and an uronic acid, have been show to play important roles in cancer cell signalling with functional effects on tumour progression and therapy response [3].
We focused our studies on a particular class of GAGs, the Heparan Sulfate (HS) chains, that are assembled as N-acetylglucosamine (GlcNAc) and glucuronic acid (GlcA) disaccharides. HS is aberrantly expressed in different cancer models and may display altered structural features, including length and sulfation patterns that hold main implications in cellular signalling [4].
The profiling of gastric tissue samples revealed specific GAGosylation signatures along the gastric carcinogenesis pathway, which were associated with the patient's prognosis. To functionally address the role of specific glycosylation features, we have established glycoengineered gastric cancer cell models with defined GAGosylation profiles, either by modulating glycosyltransferases involved in HS biosynthetic pathway or by ablation of a main HS carrier. We demonstrated that increased HS expression, concomitant with upregulation of a major cell surface carrier of HS, promoted a more aggressive phenotype, characterized by higher invasion and altered cell surface receptor tyrosine kinase activation [5]. Moreover, we provided evidence that HS-glycosylated proteoglycans are packed on extracellular vesicles (EVs) that are secreted by gastric cancer cells. Noteworthy, the presence of HS-proteoglycans in EVs determined their uptake by recipient cells and modulated the tropism of gastric cancer cell-derived EVs to the organs that are the common metastatic sites of gastric cancer.
Our data discloses previously unappreciated roles of HS GAGs in gastric cancer biology and unveils their potential as tumour aggressiveness biomarkers and also as unexplored therapeutic targets to block cancer cell signalling and extracellular communication.

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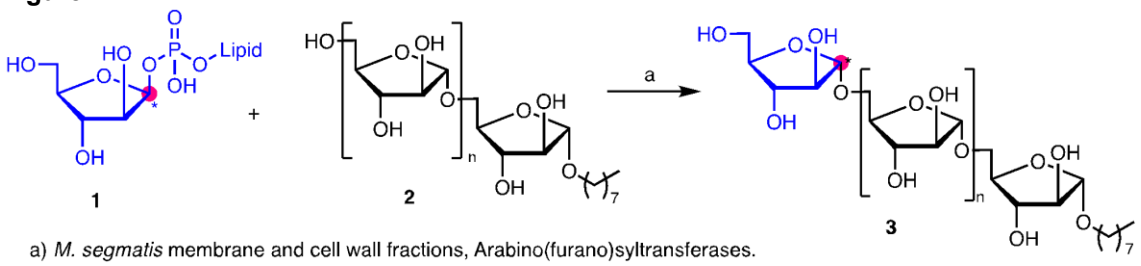
Synthesis of (oligo)arabinosides and development of a functional assay for the study of enzymes involved in mycobacteria cell wall biosynthesis

Conceição C A, Issoglio F, Almeida V T, Rodrigues J, Archer M, Ventura M R
Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), 2780-157 Oeiras, Portugal.

E-mail: rventura@itqb.unl.pt

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the primary causes of human death worldwide. The survival and pathogenicity of mycobacteria depends on the integrity of the cell wall, which contains two main polysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM). D-arabinofuranose (D-Araf) is present in these polysaccharides but not found in mammals, thus compounds that inhibit the enzymes essential for the building of these polysaccharides are potential antimycobacterial drugs[1]. Arabinofuranosyltransferases (AraT) use decaprenylphosphoryl-D-arabinofuranose (DPA) to donate an arabinofuranose residue to a saccharide acceptor and are essential for *M. tuberculosis* growth [2].
In this work, a multidisciplinary approach was used for the development of novel and efficient enzymatic assays for the characterisation of AraTs. Several linear and branched (oligo)arabinofuranoside acceptors were synthesised and their binding affinity with AraT was screened using differential scanning fluorimetry (nanoDSF) to select the best synthetic glycosyl acceptors. The total synthesis of [1]-^{13}C-labelled DPA analogues **1** (Fig. 1) was optimised achieving an overall yield of 38% and an excellent anomeric ratio of 31:1 (β:α). The total syntheses of several linear and branched arabinosyl acceptors for the enzymatic reactions were also efficiently accomplished. In order to study the protein conversions of the synthesised labelled donor with the acceptors a flexible NMR protocol was designed and implemented.

Figure 1



a) *M. segmatis* membrane and cell wall fractions, Arabino(furano)syltransferases.

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Synthesis and Exploitation of the Biological Profile of Novel Triazole-Containing Xylofuranosyl Isonucleosides

Nuno Manuel Xavier
Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa Ed. C8, 5° Piso, Campo Grande, 1749-016 Lisboa, Portugal

E-mail: nmxavier@fc.ul.pt

The development of structures analogous to nucleosides and nucleotides has attracted a significant interest in (bio)organic and in medicinal chemistry due to their propensity to interfere with nucleos(t)ide-dependent biological events that are crucial for life as well as for the progress of various diseases. The anticancer and antiviral efficacies of nucleos(t)ide analogs are demonstrated by the various examples of such compounds approved as anticancer and antiviral drugs,[1] while their antimicrobial potential has been well reported [2].
Strategies for the design of nucleos(t)ide analogs include simple modifications at purine, pyrimidine or at ribose/2-deoxyribose moieties, the use of other nitrogenous heteroaromatic systems or other glycosyl units, the installation of phosphate group mimetic motifs or modification on the type or location of the bond connecting nucleobase and sugar.
Herein, the synthesis and biological evaluation of a variety of 5′-isonucleosides constructed on xylofuranosyl templates and comprising a 1,2,3-triazole moiety is reported. The triazole unit was envisaged as a surrogate of a nucleobase or as a potential neutral and rather stable surrogate of a phosphate group when combined with other moieties such as phosphonate, phosphoramidate, or phosphate to establish new potential neutral diphosphate group mimetics. Molecules embodying a guanidine function were also accessed. The synthetic methodologies used azido xylofuranoses as precursors and employed key steps such as azide-alkyne 1,3-dipolar cycloaddition, phosphorylation, Arbuzov reaction, N-glycosylation, or guanidinylation.
Bioactivity assays revealed the therapeutic interest of some molecules, with compounds showing significant inhibition of acetylcholinesterase, potent antiproliferative activity in a breast cancer cell line or potent effects against the Gram-positive bacterial pathogen *Streptococcus pneumoniae*, with activities comparable to those of reference drugs.

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FucoPol, a multifunctional polysaccharide for high-value applications

Freitas F. [1,2,]
1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; 2. UCIBIO — Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal

E-mail: a4406@fct.unl.pt

FucoPol is a high molecular weight (1.7×10^6 - 5.8×10^6 Da) bacterial fucose-rich polysaccharide, with a fucose content of 32-36mol%, nearly equimolar contents of glucose (28-34mol%) and galactose (25-26mol%) and glucuronic acid (9-10 mol%) [1,2]. Its structure also comprises acetate (3.5-6.8wt.%), pyruvate (3.7-14wt.%), and succinate (0.6-3.0wt.%) as substituent groups. The presence of glucuronic acid, as well as the acyl substituents pyruvyl and succinyl, confer a polyanionic character to the biopolymer [3]. FucoPol's main chain is composed of a →4)-α-L-Fucp-(1→4)-α-L-Fucp-(1→3)-β-D-Glcp(1→ trimer repeating unit, and a trimer branch α-D-4,6-pyruvyl-Galp-(1→4)-β-D-GlcAp-(1→3)-α-D-Galp(1→ in the C-3 of the first fucose [2]. This polysaccharide possesses a range of valuable properties that include the ability to form viscous solutions with shear-thinning fluid behavior [4], film formation [5], bioflocculation [6], emulsion forming and stabilizing capacity [7], metal sequestration capacity [8], and the ability to reduce and stabilize metal nanoparticles [9]. Moreover, FucoPol has demonstrated biological properties, namely, antioxidant capacity [10], wound healing ability [9], photoprotection [11] and cryoprotection [2]. These features render FucoPol of great interest for the development of novel biotechnological high-value applications in the fields of cosmetics, pharmaceuticals, biomedicine and food.

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Methylglucose vs methylmannose polysaccharides: biosynthesis, distribution, and proposed roles in mycobacterial adaptation to heat or cold

Ana Maranha[1,2,3], Mafalda Costa[1], Jorge Ripoll-Rozada[4,5], José A. Manso[4,5], Vanessa Miranda[6], Vera M. Mendes[1,2], Bruno Manadas[1,2], Sandra Macedo-Ribeiro[4,5], M. Rita Ventura[6], Pedro José Barbosa Pereira[4,5], Nuno Empadinhas[1,2,3]

1. CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; 2. CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; 3. IIIUC - Institute of Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; 4. IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; 5. Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; 6. ITQB NOVA - Instituto de Tecnologia Química Biológica António Xavier, Universidade Nova de Lisboa, Portugal

E-mail: numenius@cnc.uc.pt

Some of the 200 known environmental nontuberculous mycobacteria (NTM) can be serious opportunistic human pathogens [1] and municipal water distribution systems are likely the main routes of dissemination. Considering the rising trend of lung infections, the poor therapeutic options available, and the progressively aged and chronically ill populations, NTM research lags far behind the risk they pose to public health. Mycobacteria possess a distinctive lipid-rich cell envelope that mediates interactions with their hosts and provides superior resistance to stress agents. These remarkable organisms also synthesize unique amphiphilic cytoplasmic polymethylated polysaccharides [2] implicated in fatty acid metabolism: the 6-O-methylglucose lipopolysaccharides (MGLP) isolated from all mycobacteria examined thus far, and the 3-O-methylmannose polysaccharides (MMP) that have so far only been isolated from rapidly growing NTM. We had previously identified the enzymes GpgS (EC 2.4.1.266), GpgP (EC 3.1.3.85), OctT (EC 2.3.1.273) and GgH (EC 3.2.1.208) for the synthesis, acylation and hydrolysis of glucosylglycerate (GG), which is primer of MGLP synthesis [3,4]. To further explore the biological functions of MGLP and MMP in mycobacteria, we here report the identification of the MMP biosynthetic genes and their wide distribution in NTM genomes. Combining MMP purification and chemical and enzymatic syntheses with enzyme characterization strategies, we unveiled the complete MMP biogenesis mechanism, which relies on novel enzyme activities, some of which are founders of new IUBMB families [5]. Disruption of this mechanism abolished MMP synthesis and affected mycobacterial growth at low temperatures, which suggested a role for MMP in cold adaptation, a condition to which NTM are often exposed in natural and artificial environments, from where they access humans and cause disease.

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Genetic determinants of C-type lectin receptor function in fungal infection

Agostinho Carvalho[1,2]
1. Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2. ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: agostinhocarvalho@med.uminho.pt

In recent years, advances in medical care have, paradoxically, promoted an increased prevalence of life-threatening susceptibility to severe forms of fungal disease, such as invasive pulmonary aspergillosis (IPA). There are currently no licensed vaccines, and despite improved diagnosis and therapy, management of IPA remains challenging, leading to unacceptable morbidity and mortality rates among immunocompromised hosts. Since the risk of infection varies considerably even among patients with comparable predisposing clinical factors and microbiological exposure, development of IPA is thought to rely largely on genetic predisposition. Protection against fungi is conferred mainly through phagocytes that recognize pathogen motifs through pattern recognition receptors, such as C-type lectin receptors (CLRs). CLRs comprise a large family of receptors that bind to carbohydrates in a calcium-dependent manner. The fungal cell wall is the main source of immunoactivating carbohydrates, which display a highly dynamic profile as the result of the structural properties of the cell wall according to morphotype, growth stage and environmental conditions. Over the past years, we have explored human genetic variation in C-type lectin receptors as potentially useful predictive markers for disease risk, and as a tool to dissect the molecular and cellular mechanisms that regulate the activation of antifungal immunity. Although the overall weight of the antifungal immune response results from adding effects of single genetic factors and their complex interactions with clinical immune dysfunctions, several genetic targets have been recently validated as robust markers of susceptibility to IPA across distinct disease settings. Relevant examples include the C-type lectin receptor Dectin-1, which recognizes β-1,3-glucan, and the MelLec receptor for DHN-melanin. In sum, the functional dissection of genetic variation in these carbohydrate-binding receptors has not only provided crucial insights into the pathogenesis of IPA but has contributed to establish the genetic profile of the host as an important clinical adjuvant for the personalized prognosis, diagnosis and treatment of IPA.

Carbohydrates in plant defense towards more sustainable agriculture

Dulcineia Ferreira Wessel
Polytechnic Institute of Viseu, 3500-606 Viseu, Portugal
LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: ferdulcineia@esav.ipv.pt

The targets to be achieved in the EU [1,2] by 2030 demand a 50% reduction in the use and risk of pesticides with ecological footprint, as well as a reduction of nutrient losses by at least 50% while ensuring that soil fertility does not deteriorate. These are the flagship proposals to follow the Biodiversity and Farm to Fork Strategies and will help ensure the resilience and security of food supply in the EU and across the world. For more sustainable agriculture and agroecological practices, it is necessary to explore alternatives to the application of presently used pesticides. While these pesticides have obvious advantages their use comprises several problems related to two main aspects: human health and environmental impact. Reducing pesticides will help mitigate the economic losses from declining soil health and pesticide-induced pollinator loss. Thus, scientific interest in the search of exogenous molecules with biological activity that stimulate plant resistance to diseases has been grown [3,4].
In this work, some strategies in the application of carbohydrates, and in particular polysaccharides, that can contribute to create sustainable alternatives for better resistance of plants to diseases will be presented. The biomass resources, the pathogen recognition, and signalling molecules will be approached in an attempt to demonstrate possible ways for a better understanding of carbohydrates in plant defense and their application in agriculture.

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Plant Glyco-engineering: major achievements and troubleshooting

Alexandra Castilho[1,2]
1. University of Natural Resources and Life Sciences; Department of Applied Genetics and Cell Biology (DAGZ); Institute of Plant Biotechnology and Cell Biology (IPBT); 2. Plant Glycan Engineering Group, Muthgasse 18, 1190 Vienna Austria

E-mail: Alexandra.castilho@boku.ac.at

Using plant-based platforms for the production of recombinant proteins, also referred to as Plant Molecular Farming, has been a promising concept since the 1980s, with the goal of making plant-produced pharmaceuticals an alternative for industrial and clinical applications [1,2]. During the past two decades, great progress has been made towards the development of plant-made therapeutic proteins, such as antibodies, vaccine antigens, hormones, to name a few. In particular, the tobacco relative, *Nicotiana benthamiana,* is well suited for transient expression of recombinant proteins via **'**agroinfiltration**'**, without the need for genetic transformation [3]. Despite several proposed advantages of plants over other production systems, the platform has mostly been confined to a few applications and not really progressed beyond academia. While their success has not yet led to a shift away from the common production platforms, plant glyco-engineering has carved an exciting research niche allowing the rapid generation of proteins with distinct glycan structures not mimicked by other systems [3]. Glycosylation is one of the most important post-translational modifications crucial to the clinical values of many pharmaceutical proteins.
The protein biosynthesis pathway is highly conserved between plants and mammals, but the maturation of *N*-glycans in the Golgi apparatus differs considerably, and plants do not carry out mucin-type *O*-glycosylation [4]. Major achievements to modulate plant *N*-glycosylation include the elimination of undesired glycan processing (core xylosyl- and α1,3-fucosylation), and the introduction of new ones: (i) core α1,6-fucosylation, (ii) β1,4-galactosylation, (iii) bisecting and branched N-glycans, (iv) Le^x epitopes and (iv) sialylated and polysialylated *N*-glycans [5,6].
We developed and optimized a massively multiplexed transient expression system for the synthesis and simultaneous glycoengineering of proteins in plants.
Here, I will describe step by step, our strategy to modulate the plant glycosylation pathway in order to produce recombinant proteins with "humanized" glycans showing the bottlenecks that we encounter along the way and how did we solve them.
Paradoxically, it seems that the limited glycosylation capacity of plant cells comes as an advantage for specific glycan manipulations. Plants have a small glycosylation repertoire and lack many mammalian glycosyltransferases and this can be used to facilitate a flexible stepwise overexpression of glycosyltransferases required for a specific glycan modification without interference from diverse mammalian glycosylation pathways. The possibility to produce proteins with customized/tailored glycans enables fundamental studies on the impact of glycosylation in protein-protein interaction (ex. viral infection and cancer progression). In addition, studies on the functional activity of glyco-optimized proteins are likely to provide major insights into glycan-dependent interactions that will help guide therapeutic applications.

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The dual-binding mode in the assembly of multi-enzyme cellulolytic complexes

Marlene Duarte,[1,2] Victor D. Alves,[1,2] Catarina Caseiro, [1,2], Ana Luísa Carvalho, 3,4 Shabir Najmudin,[5] Edward A. Bayer,[6] Carlos MGA Fontes,[7] Pedro Bule[1,2]*

1. CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal; 2. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal; 3. UCIBIO, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal: 4. Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 5. Randall Centre for Cell and Molecular Biophysics, 3rd Floor New Hunt's House, Faculty of Life Sciences and Medicine, King's College London, London SE1 1UL, UK; 6. Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot 76100, Israel; 7. NZYTech Genes & Enzymes, 1649-038 Lisbon, Portugal;

E-mail: pedrobule@fmv.ulisboa.pt

The cellulosome is an elaborate multi-enzyme structure secreted by many anaerobic microorganisms for the efficient degradation of lignocellulosic substrates. It is composed of multiple catalytic and non-catalytic components that are assembled through high-affinity protein-protein interactions between the enzyme-borne dockerin (Doc) modules and the repeated cohesin (Coh) modules present in non-catalytic protein scaffolds [1-3]. The Coh-Doc interactions constitute the primary driving force for cellulosomal assembly and are amongst nature's strongest protein-protein interactions. Also, due to a distinctive two-fold internal symmetry, Doc modules can potentially bind their cohesin partner in two different orientations. This is known as a dual binding mode, as opposed to a single binding mode that occurs when only one of the Doc interfaces supports the formation of the Coh-Doc complex [4]. A sequence-based classification of Cohs and Docs distinguishes type I and type II interactions as major categories. In the archetypal cellulosome of *Clostridium thermocellum*, the assembly of the different enzymes into the main scaffoldins is mediated by type I Coh-Doc interactions with a dual-binding mode, whereas the anchoring of the scaffoldin to the bacterial cell wall is achieved through type II single-binding mode Coh-Doc interactions. Initially, this was considered the rule, but as more cellulosome systems were described, more exceptions started emerging. For example, in the cellulosome of *Bacteroides cellulosolvens,* there is a role reversal in the Coh - Doc types, with type I complexes working in scaffoldin assembling and type II in enzyme recruiting [5], both with dual-binding mode, while in *Ruminococcus flavefaciens,* a new type of complexes (type III) is responsible for both cellulosome assembly and cell wall tethering, all through single-binding mode interactions [6-8]. As such, there is still a lot of uncertainty surrounding the biological relevance of the dual-binding mode. By combining structural characterization through X-ray crystallography with affinity studies, we have uncovered the molecular mechanisms governing the dual-binding mode in multiple cellulosome producing species and realized that the single vs dual binding mode is not related either to Coh-Doc type or function, but rather with cellulosome size, with larger cellulosomes seemingly making a more extensive use of the dual-binding mode. Furthermore, we have concluded that this mode of binding is present in all studied systems and can even be selectively incorporated in some.

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Invited Oral Communications

Decoding regulation of the glycosylation machinery in human health

Ana Rita Grosso[1,2]

1. Associate Laboratory i4HB-Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2. UCIBIO-Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.

E-mail: ar.grosso@fct.unl.pt

Glycosylation is a fundamental cellular process affecting human development and health. Complex machinery establishes the glycan structures whose heterogeneity provides greater structural diversity than other post-translational modifications. Still, many steps remain largely uncharacterized, hindered by the inherent complexities of glycosylation. Large consortia have been generating a wide diversity of molecular profiles, but they have not yet been comprehensively examined to elucidate the role of glycosylation factors. Here, we combined genome and transcriptome profiles of healthy and diseased tissues to uncover novel insights into the complex role of glycosylation in humans [1]. Our data showed that glycosylation factors constitute an ancient family of genes, where evolutionary constraints suppressed large gene duplications, except for genes involved in O-linked and lipid glycosylation. The transcriptome profiles of 30 healthy human tissues revealed tissue-specific expression patterns preserved across mammals. In addition, clusters of tightly co-expressed genes suggest a glycosylation code underlying tissue identity. Interestingly, several glycosylation factors showed tissue-specific profiles varying with age, suggesting a role in ageing-related disorders. In cancer, our analysis revealed that glycosylation factors are highly perturbed, at the genome and transcriptome levels, with a strong predominance of copy number alterations. Moreover, glycosylation factor dysregulation was associated with distinct cellular compositions of the tumor microenvironment, reinforcing the impact of glycosylation in modulating the immune system. Currently, we are exploring how such dysregulation can impact glycosylation patterns and tumor progression. Overall, this work provides genome-wide evidence that the glycosylation machinery is tightly regulated in healthy tissues and impaired in ageing and tumorigenesis, unveiling novel potential roles as prognostic biomarkers or therapeutic targets.

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What Should Be Called a Lectin? Explore Lectin Bioactivities

Oliveira, I.,[1,2] Grácio, M., Silva, I.,[1,2] Rocha, J.,[3]Borralho, P.,[3] Nunes, A.,[1,2] Rodrigues, C.,[3] Ferreira,R.B.,[2]Ribeiro, A.C.[1,2]

1. Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal; 2. Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal; 3. Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-Lisboa, Portugal;

E-mail: acribeiro@ff.ulisboa.pt

From bacteria to men, the plasma membrane of each cell contains glycoproteins and glycolipids with an array of oligosaccharide side chains projecting outward were that structures have been considered a code for which no template is believed to exist. For that a decoding system is required. This role is, at least in part, fulfilled by lectins, a large protein family, defined as glycan-specific receptors, distinguished from antibodies, from enzymes using carbohydrates as substrates, and from transporters of free saccharides. A large number of lectins remain to be discovered as well as new bioactivities as lectins display a great versatility in what concerns to its biological application [1-3].

Objectives: To show the binding capacity of lectins from different botanic sources to glycome cell membranes of, HT29 colon cancer cells line and Mia PaCa -2 pancreatic cancer cell line and HepG2 hepatic cells line, with analysis of the arising bioactivities. For this purpose, we conducted multiple assays: proteomic analysis (2D electrophoresis), Lectin activity detection (haemagglutination assay), Glycoproteins detection by con-A method and chemiluminescence, protein purification (DEAE-cellulose; anionic and cationic exchange-chromatography), Cell membranes purification, Wound healing, flow cytometry and *in vivo* assays.

Results: Experiments conducted *in vitro* and *in vivo*, as well as preclinical trials for hypoglycemic therapeutics, support the hypoglycemic properties of the lectin γ-conglutin from *Lupinus albus* seeds, by a mechanism that has not yet been clarified. Structural studies established that binding of γ-conglutin, in native and denatured form, to insulin occurs by a strong binding that resists rupture by 0.4 M galactose. Studies on binding of γ-conglutin to HepG2 membrane glycosylated receptors reveal that only the native form of γ-conglutin with lectin activity is capable of binding to these receptors. *In vivo* assays with male mice (CD-1) indicated that both native and denatured γ-conglutin display anti-hyperglycemic effect. Measurement of organ injury/function biomarkers (hepatic, pancreatic, renal and lipidic profile) revealed results comparable or better with the metformin exposure, in terms of safety endpoints (pancreatic and hepatic biomarkers) [4]. Concerning HT29 cells, a screening of antitumor activity on HT29 cells, based on polypeptide characterization and specific lectin binding to HT29 cells membrane receptors as well cell viability and inhibitory activities, was performed in order to assess the bioactivities present in four Mediterranean plant species: *Juniperus oxycedrus* (subsp. oxycedrus, and subsp. badia), *Arbutus unedo* and *Corema album*. All species revealed the presence of proteins with affinity to HT29 cell glycosylated receptors, being the two most promising species, JO, subsp. badia and A. unedo [5]. Also, Mia PaCa-2 cells exhibit modifications concerning to its migration, viability and cell death, when treated with A. unedo extracts. The affinity binding of the lectins extracted to MIA PaCa-2 cells to glycosylated receptors was studied where several polypeptide bands were identified [6].

Conclusion: Extracted lectins from different plant species revealed affinity binding to the cell glycome of HepG2, HT29 and Mia PaCa -2. It was performed viability and cytotoxicity studies in a way to relate the lectin binding to cells with hypoglycaemic and antitumor activities.

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Congenital Disorders of Glycosylation in Portugal: 20 years in a nutshell

Dulce Quelhas
1. Unidade de Bioquímica Genética; 2. Centro de Genética Médica Doutor Jacinto de Magalhães;
3. Centro Hospitalar e Universitário do Porto, Unidade Multidisciplinar de Investigação Biomédica – UMIB, ICBAS-UP

E-mail: dulcequelhas.cgm@chporto.min-saude.pt

First described in 1980 by Jaak Jaeken, CDG, initially defined as defects in N-glycosylation, followed a classification according to glycan isoform patterns seen in transferrin protein analysis.
The journey of CDG diagnosis in Portugal began in 1999. Over these last 20 years, diagnostic approach contributed to discovering new and rare N-glycosylation defects. In the early 2000s, besides the transferrin pattern, lipid-linked oligosaccharide analysis by HPLC and glycan analysis by mass spectrometry were in the biochemical examination of aberrant glycosylation. Together they enabled an oriented investigation of the genetic basis of patient defects. This approach allowed the diagnosis of over sixty individuals, covering a wide spectrum of CDG. An intense effort to raise awareness for CDG in the clinical community, together with selective phenotype screening programs, lead to the identification of 15 distinct CDG, besides the more common PMM2-CDG. *ATP6AP1, ATP6AP2, ATP6V0A2, CCDC115, COG1, COG4, DPAGT1, MAN1B1, SRD5A3*, *SLC37A4* and *RFT1* have a rare and distinct phenotype, and intense collaborative efforts paved the road to diagnosis. This demonstrates how studies on CDG have discovered new genes and pathways for glycosylation.
The introduction of Whole Exome Sequencing (WES) in current clinical practice, which allows the untargeted examination of affected patients, together with deep phenotyping, has been crucial in opening a fast track to discover new CDG. Although CDG are mostly multisystem disorders often associated with neurologic impairment, milder phenotypes have recently widened the clinical spectrum of these disorders. These recently reported "unexpected CDG," did not exhibit the classic CDG phenotype but later proven to have a pathophysiology linked to altered glycosylation or pathogenic variants in a gene previously related to glycosylation. The discovery of heterozygous and, consequently types of CDG due to dominant mutations in genes previously related with an autosomal recessive pattern of inheritance, is an additional noteworthy surprise.
Although WES has helped in the diagnostic odyssey, novel molecular pathways of disease must be considered for patients who remain without a final genetic diagnosis.

Patient and Public Involvement (PPI) in research led by Glycoscientists: A "How-to" guide that will shine light to your research

Vanessa dos Reis Ferreira

1. UCIBIO – Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB -Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 3. CDG & Allies-Professionals and Patient Associations International Network, 2829-516 Caparica, Portugal

E-mail: vmr.ferreira@fct.unl.pt

Patient and Public Involvement (PPI) in research, is defined as research carried out 'with' or 'by' patients and those who have experience of a condition, rather than 'for', 'to' or 'about' them. Whilst engagement and participation are important ways of interacting with people living with a certain condition, yet this does not include the earliest stages of laboratory-based basic research, where limited evidence of PPI has been found to date. This is despite a growing swell of evidence that involving people living with a certain condition provides very influential and meaningful insight that is essential to anyone aspiring to improve the quality of life of those people.
Involving people "as early as possible" is highly recommended and improve research outcomes. This challenges you to ask the question "how relevant is my research to patient?". Understanding this creates an impact in the mindset, focusing research where is needed and you will have your work on a trajectory toward future tailored patient benefits. Moreover, people living with a certain condition cut through jargon and ask simple but important questions that researchers rarely do.
Research in glycobiology, is a key field with impact across many diseases such as Congenital Disorders of Glycosylation (CDG), cancer, Rheumatoid Arthritis (RA), Inflammatory Bowel Disease (IBD), Systemic Lupus Erythematosus (SLE), granulomatosis with polyangiitis (GPA; formerly called Wegener granulomatosis), diabetes mellitus, and so forth. Thus, PPI is considered relevant in the field of Glycobiology.

This talk is designed to share tips on "How" researchers from Glycobiology can lead in an easy, systematic and successful manner PPI within their research cycle.

Liquid crystalline phases to probe water cellulose nanocrystals (CNCs) interactions

Rafaela R. da Rosa[1], Pedro E. S. Silva[1], Diogo V. Saraiva[1], Anant Kumar[2], António P. Mendes de Sousa[3], Pedro Sebastião[2], Susete N. Fernandes[1] and Maria Helena Godinho[1]
1. i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, 2829-516 Caparica, Portugal; 2. Centro de Física e Engenharia de Materiais Avançados, Departamento de Física, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal; 3. RAIZ - Forest and Paper Research Institute, Aveiro, Portugal.

E-mail: mhg@fct.unl.pt

Colloidal suspensions of aqueous cellulose nanocrystals (CNCs) are well known to form a liquid crystalline phase above a certain critical concentration. As noted by Onsager in the 1940, this transition can be explained by using entropic arguments [1]. The stability of the nematic phase depends on the fact that, at higher particle concentrations, the loss of orientational entropy associated with particle alignment is balanced by the gain in configurational entropy. The critical concentration and the stability of the anisotropic phase were found to depend upon the dimensions, size polydispersity and surface charge of the CNCs. In this work we demonstrate that a nematic low-density phase can develop at the isotropic-nematic transition in CNCs liquid crystal colloidal aqueous suspensions [2]. The CNCs were isolated by acid hydrolyses from different sources and under different conditions. The low-density liquid crystalline phase was obtained from never dried Bleached Eucalyptus Kraft Pulp (BEKP). We also show the existence of atactoids decorating the low- density liquid crystalline phase. The water present in the never dried Bleached Eucalyptus Kraft Pulp (BEKP), before extracting the CNCs, and a small change in the CNCs acid hydrolyses chemical procedure were found crucial for the anomalous behaviour observed. Water/CNCs interactions are discussed taking into account the formation of the LC phase.

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Coordination of the synthesis of two surface glycopolymers ensures the concealment and survival of pneumococcal bacteria

Joana Figueiredo[1,3], Mafalda Xavier Henriques[2], Maria João Catalão[2], Sara Pinheiro[1,3], Ana Rita Narciso[2], Francisco Mesquita[5], Bruno Manuel Saraiva[6], Madalena Carido[2], Didier Cabanes[5], Mariana Gomes Pinho[6], Sérgio Raposo Filipe[1,3]*

1. Laboratory of Bacterial Cell Surfaces and Pathogenesis, UCIBIO-Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade Nova de Lisboa, Monte da Caparica, Portugal; 2. Laboratory of Bacterial Cell Surfaces and Pathogenesis, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal; 3. Associate Laboratory i4HB—Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, Monte da Caparica, Portugal; 4. Host-Pathogen Interaction Unit, Faculty of Pharmacy, Research Institute for Medicines, iMed-ULisboa, Universidade de Lisboa, Lisboa, Portugal; 5. Group of Molecular Microbiology, Instituto de Investigaçãoo e Inovaçãoo em Saúde (i3S), Universidade do Porto, Porto, Portugal; 6. Laboratory of Bacterial Cell Biology, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

E-mail: numenius@cnc.uc.pt

The capsular polysaccharide is a major virulence factor for many pathogenic bacteria and it is required for bacterial survival within the infected host. In Streptococcus pneumoniae, the presence of this polysaccharide at the division septum is ensured by the interaction and septal co-localization of Wze, an autophosphorylating tyrosine kinase, and Wzd, a membrane protein required for Wze autophosphorylation.

To determine how bacteria regulate capsule synthesis in S. pneumoniae, we looked for proteins that interact with Wzd and Wze using bacterial two hybrid assays and fluorescence microscopy. We found that Wzd interacts with Wzg, the putative ligase that attaches a capsule to the bacterial cell wall, and recruits it to the septal area.

Wzd mutants, which lack capsule at midcell, are better recognized by secreted pneumococcal peptidoglycan LytA hydrolase, are more susceptible to LytA-induced lysis, and are less virulent in a zebrafish embryo infection model.

We propose that the Wzd/Wze pair guarantees full encapsulation of pneumococcal bacteria by recruiting Wzg to the division septum, ensuring that capsule attachment is coordinated with peptidoglycan synthesis. Impairing the encapsulation process, at localized subcellular sites, may facilitate elimination of bacteria by strategies that target the pneumococcal peptidoglycan.

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Oral Communications

Strategies to develop anti-cancer therapies targeting truncated cancer glycan sialyl-Tn

Mariangela Natale,[1] *Nuno P. Ramos*[1,2], *Filipa Trovão*[3,4], *Benedita A. Pinheiro*[3,4], *Wengang Chai*[5], *Angelina S. Palma*[3,4], *Paula Videira*[1,2,4]

1. CellmAbs Biopharmaceuticals, Parque Tecnológico de Óbidos, Rua da Criatividade, Edifícios Centrais, 2510-216 Obidos, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal; 3. UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal; 4. Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal; 5. Glycosciences Laboratory, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, United Kingdom

E-mail: mnatale@cellmabs.com

OBJECTIVE: Aberrant glycosylation process is a well-established hallmark of cancer, leading to the generation of different types of tumour-associated carbohydrate antigens (TACAs). Truncated O- glycans represent one subset of many TACAs, including Tn, TF and sialyl-Tn (sTn) antigens. STn has been reported to be highly overexpressed in different tumour types, and involved in cancer development and progression, in some cases leading to immunomodulation of certain immune cells. In addition, sTn expression has been previously reported primarily in cancer tissues, leaving normal healthy organs free or rare of its expression, thus representing a relevant target for selective cancer- therapy. At CellmAbs we characterized humanized antibodies with increased binding and affinity to sTn, suitable for the development of different target-therapy strategies.

METHODOLOGY: To increase antibody similarity to those normally produced in humans, we performed antibody humanization assay, using the original murine anti-sTn, followed by affinity maturation processes to improve binding to the target. Antibody binding and affinity to sTn were defined using different tools including glycan microarray, EC50 analysis and antibody internalization profiling. Finally, proof-of-concept in vivo models were selected and described based on distinct antibody features and their relevance in clinical treatments.

RESULTS: Data obtained in this study revealed retained specificity of binding to sTn after antibody humanization process, underling enhanced binding and affinity to the target, after antibody affinity maturation. In addition, proof of concept *in vivo* data underlined safety and efficacy of antibody treatments, further suggesting the relevance of this antibody-based therapy.

CONCLUSIONS: In this study we generated a set of anti-cancer antibodies, with distinct features, representing a tool for the development of antibody-based therapy strategy. Considering the promising results from the data obtained at CellmAbs, and the urgent need to find tumour therapies that work for those unmet cancer patients, further IND enabling studies are ongoing, with the intention to recruit patients for human clinical trials.

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Heparan Sulfate biosynthetic pathway: learning the GAGosylation roadthat leads to gastric cancer progression

Marques C.[1,2,3], *Poças J.*[1,2,4], *Gomes C.*[1,2], *Ribeiro A. R.*[1,2], *Faria-Ramos I.*[1,2], *Reis C. A.*[1,2,4,5], *Vivès R. R.* [6], *Magalhães A.*[1,2,4,*]

1. i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, R. Alfredo Allen 208, 4200-135 Porto, Portugal; 2. iPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, R. Júlio Amaral de Carvalho 45, 4200-135 Porto, Portugal; 3. Programa Doutoral em Biologia Molecular e Celular (MCbiology), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal; 4. ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal; 5. FMUP - Faculdade de Medicina da Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal; 6. Univ. Grenoble Alpes, CNRS, CEA, IBS, 71 Av. des Martyrs, 38000 Grenoble, France

E-mail: amagalhaes@ipatimup.pt (*)

Heparan Sulfate Proteoglycans (HSPGs) are important cell surface and Extracellular Matrix (ECM) components that orchestrate crucial events in physiology and pathology. These glycoconjugates bind to multiple molecules, through their HS glycosaminoglycan (GAG) chains, functioning as scaffolds for protein-protein interactions and reservoirs of extracellular ligands. Aberrant expression of HSPGs and HS biosynthesis enzymes was reported in different types of cancer, and it was shown to modulate tumour cell-ECM interaction and signalling networks [1,2]. However, the roles of specific glycosyltransferases in the deregulated biosynthesis of HSPGs are not fully understood. This work aimed to study the roles of HS in cancer cell signalling and motility, and to unravel the impact of deregulated HS biosynthetic pathways in gastric cancer.

We established glycoengineered gastric cancer cell models lacking specific glycosyltransferases, Exostosin-Like Glycosyltransferase 2 (EXTL2) and EXTL3 and revealed their regulatory roles in HS and Chondroitin Sulfate (CS) biosynthesis [3]. GAG disaccharide structural analysis and transcriptomic data revealed that knock-out of *EXTL2* leads to overproduction of HS chains with altered structural features, and upregulation of Syndecan-4, a major cell surface HSPG. Further, we demonstrated that this aberrant GAGosylation profile increased cell motility and invasion and impaired the activation of Ephrin type-A 4 cell surface receptor tyrosine kinase. On the other hand, *EXTL3* abrogation resulted in impaired HS biosynthesis and led to the production of HSPGs abnormally glycosylated with CS chains, suggesting that a shift in the EXTL2/EXTL3 ratio impacts cellular overall GAG profiles. Validation of the aberrant GAGosylation signatures in gastric tumour samples was also performed.

In conclusion, our findings enlighten the roles of HS biosynthetic machinery in the modulation of cancer cell GAGosylation and the impact of aberrant HS/CS balance in cellular malignant features, further supporting the clinical potential of HS biosynthetic machinery in cancer therapy.

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High throughput screening of chemical compounds showed monensin as novel cancer drug by inhibiting SLeX expression and malignant properties in gastrointestinal cancer cells

Ana Filipa Costa[1,2,3], Emanuel Senra[1,2], Diana Campos[1,2], Joana Gomes[1,2], Marta T Pinto[1,2], André Maia[1,4], António Pombinho[1,4], Celso A. Reis[1,2,3,5], Catarina Gomes[1,4]

1. i3S–Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; 2. IPATIMUP–Institute of Molecular Pathology and Immunology, University of Porto, Portugal; 3. ICBAS-School of Medicine and Biomedical Sciences University of Porto, Portugal; 4. IBMC-Institute of Molecular and Cell Biology, University of Porto, Portugal; 5. Faculty of Medicine, University of Porto, Portugal

E-mail: cgomes@ipatimup.pt

Cancer is a world major concern, with most cancer-related deaths deeply associated to metastatic dissemination of cancer cells. Aberrant expression of glycans are active molecular drivers in this process, and Sialyl LewisX (SLeX, NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc) has been described as a major player in metastasis formation. Thus, our goal was to develop a novel strategy to identify molecules with potential to inhibit the expression of SLeX, impacting cancer cell invasive and metastatic properties. We developed a fluorescent high throughput screening to search for inhibitors of SLeX expressed in cancer cells. Using the colon cell line COLO205 as SLeX expressing model, we screened 7836 chemical compounds. From our screening assay, we identified monensin as the most promising SLeX inhibitor. The reduction in SLeX expression at cell surface was further confirmed through a dose-response analysis and flow cytometry, and validated in a gastric cell line KATOIII. Biologically, we observed that monensin impacts cell metabolism, proliferation, and death mostly in gastric cells, and completely abrogates motility and invasive properties in both cell lines. Using *in vivo* models, we showed that monensin reduce tumor size and tumor invasion. Morphologically, monensin treated cells present a larger vacuolar cell phenotype and an increase cells' aggregation. By TEM analysis, we observed increased presence of autophagic and Golgi associated vacuoles suggesting ER and Golgi stress and altered mitochondria in gastric cell line. These altered processes were validated by transcriptomic analysis.

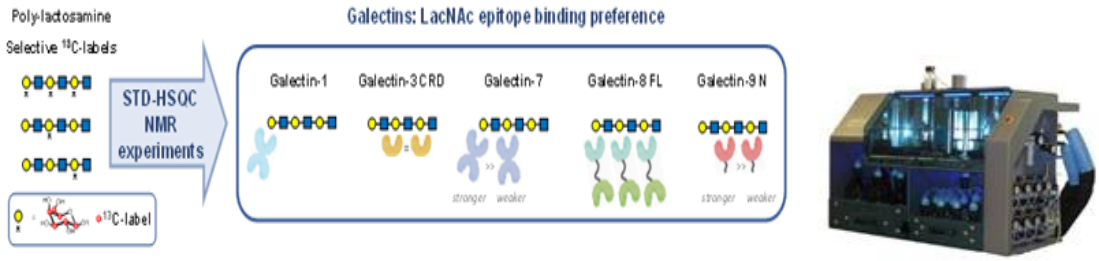
Altogether, our results show the potential of monensin as novel cancer drug by inhibiting SLeX expression and impairing cancer cell motility and invasion.

SELECTIVE METHODS FOR THE SYNTHESIS OF REPEATING SUGAR OLIGOMERS TO EXPLAIN PROTEIN BINDING EPITOPES

M. J. Moure, A. Gimeno, S. Delgado, T. Diercks, G.J. Boons, A. Ardá, J. Jimenéz-Barbero
Chemical Glycobiology lab, CIC bioGUNE, Basque Research & Technology Alliance (BRTA), Derio, Spain

E-mail: mmoure@cicbiogune.es

An automated and chemical synthesis/NMR-based methodology is presented to establish the specific binding epitope within repeating sugar oligomers when binding to protein receptors [1,2]. The idea is based on the inclusion of 13C-labels at specific monosaccharide units, selected within a repeating glycan oligomeric structure [3,4]. Thus, since no new chemical tags are added, the chemical entity remains the same, while the presence of the 13C-label breaks the NMR chemical shift degeneracy that occurs in the non-labeled compound and allows the unique recognition of the different components of the oligomer. The success of the proof of concept has been already presented for the interaction of a polylactosamine with five different galectins that display distinct binding preferences for these entities [5]. New advances in automated glycan assembly (AGA) [6,7] will be discussed in this presentation.



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Molecular recognition of Mucin-1 O-glycosylation process by different enzymes: in cancer and bacterial infection

Grosso A.S.,[1,2] González-Ramírez A.,[3] Gomes J.,[4] García-Martín F.,[5] Reis C.,[4] Hurtado-Guerrero R.,[3] Corzana F.,[5] Coelho H.,[1,2] and Marcelo F.[1,2]*
1.UCIBIO, REQUIMTE, Departamento de Química, NOVA School of Science and Technology, Universidade Nova de Lisboa, Caparica, Portugal, 2. I4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, Caparica, Portugal, 3. BIFI-IQFR (CSIC), Universidad de Zaragoza, Zaragoza, Spain, 4. i3S/Ipatimup, Universidade do Porto, Porto, Portugal, 5. Department of Chemistry, Universidad de La Rioja, Logroño, Spain.

E-mail: h.coelho@fct.unl.pt, filipa.marcelo@fct.unl.pt (*)

Mucin-type O-glycosylation is a complex mechanism regulated by the coordinated activity of specific glycosyltransferases (GTs) [1]. Altered regulation of several GTs is a common feature of cancer that yields tumour-associated carbohydrate antigens (TACAs) [1].
Mucin-1 glycoprotein (MUC1) is overexpressed in many tumour tissues and tends to carry simple oligosaccharides, which allow for the presentation of different TACAs, such as the Tn-TF- or sTn antigens (GalNAc-α-1-*O*-Thr/Ser, Galβ1-3GalNAcα1-*O*-Ser/Thr and Neu5Acα2-6GalNAcα1-*O*-Ser/Thr, respectively). Tn-antigen is built by a large polypeptide GalNAc-transferase (GalNAc-Ts) family that catalyses the transfer of GalNAc from a sugar donor UDP-GalNAc to Ser/Thr side chains of many cell-surface proteins [2]. After the synthesis of the Tn antigen by the GalNAc-Ts, core extension takes place by different glycosyltransferases (GTs) as inverting C1GalT1 enzyme, which transfers a Gal residue from the sugar donor UDP-α-Gal to GalNAc-containing mucin peptides [3]. Also, the inverting ST6GalNAc-I enzyme, can adds a Neu5Ac residue from CMP-β-Neu5Ac sugar donor to GalNAc-mucin peptides in cancer cells [4].
Mucins are an important target for some microorganisms (commensal and pathogenic) of the human microbiome. During infection, invading microbes interact with host mucins lining the glandular epithelial cells and trigger inflammation. MUC1, in particular, plays an important role in defence against invasive bacterial pathogens such as *Helicobacter pylori* and *Campylobacter jejuni* [5,6]. However, in the case of Salmonella infections, transmembrane mucin MUC1 is required for Salmonella SiiE-mediated entry. The giant adhesin SiiE is the adhesin responsible for engaging MUC1 and the interaction is mediated by glycans on MUC1. In this communication it will be reported the application of NMR methods to follow the glycosylation process of MUC1 glycoprotein, unveiling new structural, conformational, and dynamic insights at atomic level of this biological event. Also, a perspective on how these homogeneous and controlled mucins can be used to prevent Salmonella infections.

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Mapping Molecular Recognition of β1,3-1,4-Glucans by a Surface Glycan-Binding Protein from the Human Gut Symbiont Bacteroides ovatus

Viviana G. Correia[1,2], Filipa Trovão[1,2], Benedita A. Pinheiro[1,2], Lisete M. Silva[3], Yan Liu[3], Ten Feizi[3], Barbara Mulloy[3], Wengang Chai[3], Ana Luísa Carvalho[1,2], Angelina S. Palma[1,2]
1. UCIBIO, Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; 2. Associate Laboratory i4HB—Institute for Healthand Bioeconomy, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; 3. Glycosciences Laboratory, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, United Kingdom

E-mail: b.pinheiro@fct.unl.pt

A multigene polysaccharide utilization locus (PUL) encoding enzymes and surface carbohydrate (glycan)-binding proteins (SGBPs) was recently identified in prominent members of Bacteroidetes in the human gut and characterized in *Bacteroides ovatus* [1]. This PUL-encoded system specifically targets mixed-linkage β1,3-1,4-glucans, a group of diet-derived carbohydrates that promote a healthy microbiota and have potential as prebiotics [2]. The BoSGBPMLG-A proteinencoded by the BACOVA_2743 gene is a SusD-like protein that plays a key role in the PUL's specificity and functionality. Here, we perform a detailed analysis of the molecular determinants underlying carbohydrate binding by BoSGBPMLG-A, combining carbohydrate microarray technologywith quantitative affinity studies and a high-resolution X-ray crystallography structure of the complex of BoSGBPMLG-A with a β1,3-1,4-nonasaccharide. We demonstrate its unique binding specificity toward β1,3-1,4-gluco-oligosaccharides, with increasing binding affinities up to the octasaccharide and dependency on the number and position of β1,3 linkages [3].
The interaction is defined by a 41-Å-long extended binding site that accommodates the oligosaccharide in a mode distinct from that of previously described bacterial β1,3-1,4-glucan-binding proteins. In addition to the shape complementarity mediated by CH-π interactions, a complex hydrogen bonding network complemented by a high number of key ordered water molecules establishes additional specific interactions with the oligosaccharide. These support the twisted conformation of the b-glucan backbone imposed by the β 1,3 linkages and explain the dependency on the oligosaccharide chain length. We propose that the specificity of the PUL conferred by BoSGBPMLG-A to import long β1,3-1,4-glucan oligosaccharides to the bacterial periplasm allows Bacteroidetes to outcompete bacteria that lack this PUL for utilization of β1,3-1,4-glucans [3].
Understanding at the molecular level how commensal bacteria, such as prominent members of Bacteroidetes, can differentially utilize dietary carbohydrates with potential prebiotic activities willshed light on possible ways to modulate the microbiome to promote human health.

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Polysaccharide-based biocomposite films with electrical properties for food packaging applications

Ana Barra,[1,2] *Nuno M. Ferreira,*[3] *Eduardo Ruiz-Hitzky,*[2] *Cláudia Nunes,*[1] *Paula Ferreira,*[1]
1. CICECO – Aveiro Institute of Materials, Department of Materials & Ceramic Engineering, University of Aveiro, Aveiro, 3810-193, Portugal; 2. ICMM – Instituto de Ciencia de Materiales de Madrid, CSIC, c/ Sor Juana Inés de la Cruz 3, Madrid, 28049, Spain; 3. I3N, Department of Physics, University of Aveiro, 3810-193, Aveiro, Portugal

E-mail: abarra@ua.pt

The consumers demand for sustainable and healthy food is driving the adoption of emerging non-thermal food processing technologies. The pulsed electric field (PEF) is a promising technique, where microbial inactivation is accomplished through electroporation phenomena. The food is processed inside a treatment chamber with the application of short high voltage pulses during microseconds. However, the PEF treatment directly in the food, before the packaging, represents a risk of recontamination [1]. In addition, it requires aseptic lines and sterile packaging with extra costs associated. The PEF treatment in-pack, the packaging step before the PEF treatment, is a strategy to eliminate these drawbacks. However, this concept relies on a flexible and electrically conductive food packaging that should meet sustainability and food compatibility standards. In this context, polysaccharides are eco-friendlier alternatives to synthetic polymers and excellent matrices to develop functional composite materials for this application [2].
Herein, alginate-based biocomposites containing graphitized carbon supported on natural sepiolite clay as conductive filler were developed which was prepared following a modified procedure under N_2 flow for 1 h at 550 or 800 ºC [3]. The graphitic material obtained after the thermal treatment was analyzed. The Solid state ^{13}C NMR and Raman spectroscopy confirm the successful graphitization of carbon by the thermal treatments, being the highest temperature the most effective. The SEM images reveal sepiolite fibers well homogenized with the graphitized carbon. The XRD analysis shows the maintenance of sepiolite structure after the thermal treatment at 550 ºC but lost at 800 ºC. The carbon/sepiolite material with the highest electrical conductivity was selected as filler to prepare the biocomposite films. The alginate solution was blended with corn zein to improve the matrix hydrophobicity and homogenized with 0-70% carbon/sepiolite filler. The films were prepared by solvent casting and their mechanical and electrical properties were investigated. The mechanical properties of the composite materials were affected by the high loads of filler, presenting a reduction of tensile strength and elasticity. The film containing 70% of carbon/sepiolite achieved an electrical conductivity of 329 and 6 µS/cm in-plane and through-plane directions, respectively. The alginate-based films with electric properties are promising sustainable materials to be used as food packaging for PEF-in pack.

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Innovative compressive and absorption methodology for the production of hydrogels (CAMPH) based in marine biopolymers for tissue engineering

Duarte Nuno Carvalho[1,2], Cristiana Gonçalves[1,2], David S. Williams[3], Carmen G. Sotelo[4], Ricardo I. Pérez-Martín[4], Andrew Mearns-Spragg[3], J. Miguel Oliveira[1,2], Rui L. Reis[1,2], Tiago H. Silva[1,2]

1. 3B´s Research Group, I3Bs – Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4805-017 Barco, Guimarães, Portugal; 2. ICVS/3B´s – PT Government Associate Laboratory, Braga/Guimarães, Portugal; 3. Jellagen Limited, Unit G6, Capital Business Park, Parkway, St Mellons, Cardiff, CF3 2PY, United Kingdom; 4. Group of Food Biochemistry, Instituto de Investigaciones Marinas (IIM-CSIC), C/Eduardo Cabello 6, Vigo, Pontevedra, Spain

E-mail: duarte.carvalho@i3bs.uminho.pt

In the last decades, marine-origin products have been widely used in several sectors due to the extensive diversity of chemical and biological products offered by aquatic resources. Many of them are considered a great alternative to land mammal sources, namely marine biopolymers studied as building block materials for tissue engineering scaffolding concepts. Besides their similarities with proteins and polysaccharides present in human extracellular matrix (ECM), they are considered safe as presenting low risk of zoonosis and overcoming social/religious-related constraints [1]. In this work, several formulations of marine collagen-chitosan-fucoidan hydrogel were processed using an innovative compression and absorption methodology to produce hydrogels (CAMPH) [2,3]. Firstly, solutions of 5% collagen from jellyfish and/or 3% collagen from blue shark skin were blended with solutions of 3% chitosan from squid pens and 10% fucoidan from brown algae, at different ratios. Then, CAMPH device was applied, promoting compaction forces on the biopolymers solution while part of the solvent (namely water) was removed by absorption, yielding a jelly-like material. The efficiency of this fast and cheap methodology was herein demonstrated with marine origin biopolymers, but can be extended to other components, namely other polyelectrolytes or polymers undergoing significant intermolecular interactions. The physicochemical properties of the developed hydrogels were assessed by rheology, weight variation by water uptake and degradation, and SEM. The main results revealed polymeric matrices exhibiting a strong viscoelastic character and structural stability, particularly the ones encompassing the 3 different biopolymers. Among them, the more cohesive formulation is highlighted, resulting from the blending of 5% jellyfish collagen, 3% shark skin collagen, 3% chitosan, and 10% fucoidan. Furthermore, biological assessments were performed using encapsulated chondrocyte-like cells (ATDC5), which revealed a non-cytotoxic behavior for all hydrogel formulations. Likewise, Live/Dead and Phalloidin/DAPI staining were further performed to assess the cell viability, proliferation, and cytoskeletal organization, proving that the proposed hydrogels could provide a suitable microenvironment with appropriate signals for cell culture, supporting its proliferation and suggesting applicability towards the regeneration of articular cartilage tissue.

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High-throughput characterization of human gut microbiome glycan-protein binding interactions

Viviana G. Correia[1,2], *Raquel L. Costa*[1,2], *Benedita A. Pinheiro*[1,2], *Joana L.A. Brás*[3], *Lisete M. Silva*[4,5], *Maria João Romão*[1,2], *Yan Liu*[4], *Wengang Chai*[4], *Ten Feizi*[4], *Carlos M.G.A. Fontes*[3,6], *Ana Luísa Carvalho*[1,2] *and Angelina S. Palma*[1,2,4]

1. UCIBIO Applied Molecular Biosciences Unit, Department of Chemistry/Department of Life Sciences, School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal; 2. Associate Laboratory i4HB, Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal; 3. NZYTech genes & enzymes, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal; 4. Glycosciences Laboratory, Department of Metabolism, Digestion and Reproduction, Imperial College London, W12 0NN, United Kingdom; 5. REQUIMTE-LAQV, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 6. CIISA, Faculty of Veterinary Medicine, University of Lisbon, Pólo Universitário do Alto da Ajuda, Av. Universidade Técnica, 1300-477 Lisbon, Portugal;

E-mail: vivianacorreia@campus.fct.unl.pt

To thrive in the human gut, the microbial community known collectively as the microbiome, has evolved to readily target and utilize the great diversity of glycans derived from diet and intestinal mucus-layer, influencing host nutrition, immunity and susceptibility to infection by pathogens [1].
Prominent gut bacteria, such as *Bacteroides* species, exhibit extensive sets of co-localised substrate-specific genes designated as polysaccharide utilization loci (PULs), which allow bacteria to cope with nutrient fluctuation [1]. Each set encodes all the necessary elements for the recognition and degradation of a specific glycan, including modular carbohydrate-active enzymes (CAZymes) with appended carbohydrate-binding modules (CBMs) and other non-catalytic glycan-binding proteins displayed at the cell surface, that often reflect the PULs specificity [2]. In the post-genomic Era, there is an urgent need to develop high-throughput approaches to study these recognition systems at a functional and structural level [1].
In this communication, we report the successful implementation of an integrative strategy to characterize novel PUL glycan-recognition systems of *Bacteroides* species by combining i) bioinformatic analysis of bacterial genomes and high-throughput production of putative glycan binding proteins [3] with ii) ligand discovery using carbohydrate microarray technology [4] and iii) structural characterization of new protein-ligand complexes by X-ray crystallography [5]. Diverse microarray platforms featuring mammalian-, plant- or microbial derived glycans were used aiming to cover, in a miniaturized format, the glycan structural diversity that bacteria may encounter in the gut. In total, a “microbiome protein library” comprising 84 different purified proteins was successfully produced and 50 were screened for carbohydrate binding. We will highlight our findings on newly identified glycan-binding proteins from *Bacteroides thetaiotaomicron* that enabled identification of a PUL targeting host-mucin *O*-GalNAc glycan cores.
Our strategy can be extended to other microbial strains to help unravel the holistic effect of glycan recognition in the gut and pinpoint pre- and probiotics for biopharmaceutical solutions.

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Polysaccharides as hypocholesterolemic food ingredients

Fernanda Machado,[1] Cláudia Nunes,[2] Manuel A. Coimbra,[1] Filipe Coreta Gomes[1,3]
1. LAQV-REQUIMTE, Chemistry Department, University of Aveiro, 3810-193, Aveiro, Portugal; 2. CICECO-Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193, Aveiro, Portugal; 3. Coimbra Chemistry Center - Institute of Molecular Sciences (CQC-IMS), Department of Chemistry, University of Coimbra, 3004-535, Coimbra, Portugal

E-mail: filipecoreta@ua.pt

Carbohydrates are within the most prevalent nutrients in foods, being mostly present as polysaccharides. Depending on sugar chemical composition and ramification, these high molecular weight compounds can have distinct bioactivities, namely hypocholesterolemic activity [1]. In this work, polysaccharides such as arabinogalactans, galactomannans, β-glucans, fucoidans and chitooligosaccharides, from different food sources such as coffee, mushrooms, algae, and shrimp by-products, were extracted and chemically characterized. Their hypocholesterolemic potential was assessed measuring their effect on bile salt sequestration and cholesterol accessibility using an *in vitro* intestinal simplified model. Different degrees of coffee roasting were shown to affect hypocholesterolemic properties of arabinogalactans and galactomannans. The increase of galactomannans degree of branching was shown to decrease cholesterol accessibility. β-glucans extracted from mushrooms didn't show any effect on bile salt sequestration or cholesterol accessibility. Algae fucoidans which are negatively charged polysaccharides were also shown to affect cholesterol accessibility being this effect higher for less charged polysaccharides. The positively charged chitooligosaccharides were shown to be effective on bile salt sequestration and cholesterol accessibility. This was promoted both by electrostatic and hydrophobic interactions. This work reflects that polysaccharide's chemical structure diversity and ramification can be used to optimize their hypocholesterolemic properties, highlighting that charge as well as hydrophobic interactions should be considered in the development of innovative hypocholesterolemic food ingredients based on carbohydrates.

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Tailoring the yacon carbohydrates towards sweetness and texture during syrup production

Pedro A. R. Fernandes[1,2], *Bruna Antunes*[1], *Sónia S. Ferreira*[1,2], *Vitor Alves*[3], *Adriana Silva*[3], *Cláudia Nunes*[4], *Elisabete Coelho*[1], *Manuel A. Coimbra*[1]

1. LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 2. CICECO, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 3. Frulact, 4475-150 Gemunde – Maia, Portugal; 4. CICECO, Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: pedroantonio@ua.pt

Fructose, glucose, fructooligosaccharides (FOS) and inulin account the most prevalent carbohydrates in yacon. They can be obtained as sweet tasting syrups where the extraction and processing approaches tailor the syrups carbohydrate proportion, and thus, the sweetening power. In this sense, this work aimed to determine the fate of yacon carbohydrates when performing syrup production by taking advantage of water evaporation by boiling at atmospheric or reduced pressure. These experiments were performed in the presence of citric acid (0.1-0.5% w/v) as acidulant, and/or ascorbic acid (0.08% w/v) as antioxidant. The obtained syrups, with 73 ºBrix, were analysed regarding their carbohydrate composition by high performance anionic exchange chromatography with pulsed amperometric detection (HPAEC-PAD), as well as texture profile, measured by texture analysis.

The inulin degree of polymerization (DP), initially of 50 sugar residues, decreased as the amount of added citric acid increased, reaching the lowest detected maximum DP of 27 using 0.5% citric acid and water evaporation at atmospheric pressure. This depolymerization was found to yield new FOS structures, differing on their DP (2-9) and sugar composition, and to increase the amount of free Fru and Glc, resulting in syrups of higher sweetness. Besides that, the extent of inulin acid depolymerization was found to yield syrups of lower stickiness and stringiness. The amount of Fru and Glc also increased, although at lower extent, when combining citric and ascorbic acids in both atmospheric and reduced pressure water evaporation approaches. This suggested their possible side reactions with ascorbic acid, leading to syrups of lower predicted sweetness than those produced with only citric acid aqueous solutions. In this sense, it can be concluded that besides hydrolysis, yacon carbohydrates undergo other side reactions that tailor syrups final sweetness and texture. These comprehend features that need to be considered for tailored application of yacon syrups for sugar replacement.

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Unconventional assembly of ruminal cellulosomes

Marlene Duarte,[1,2] *Victor D. Alves*,[1,2] *Catarina Caseiro*,[1,2] *Luís M. A. Ferreira*,[1,2] *Ana Luísa Carvalho*,[3,4] *Shabir Najmudin*,[5] *Edward A. Bayer*,[6] *Carlos MGA Fontes*,[7] *Pedro Bule*[1,2]*

1. CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal; 2. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal; 3. UCIBIO, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal; 4. Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 5. Randall Centre for Cell and Molecular Biophysics, 3rd Floor New Hunt's House, Faculty of Life Sciences and Medicine, King's College London, London SE1 1UL, UK; 6. Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot 76100, Israel: 7. NZYTech Genes & Enzymes, 1649-038 Lisbon, Portugal;

E-mail: pedrobule@fmv.ulisboa.pt (*)

Plant biomass is a highly abundant renewable resource, which can be converted into many value-added products. It is also at the foundation of the ruminant production system as its main constituent, cellulose, is a major source of energy for both ruminal microflora and its host. Because of its structural complexity, plant-cell wall is highly recalcitrant requiring many different catalysts for its complete digestion. In the rumen, plant biomass hydrolysis is carried out by many cellulolytic microorganisms, with the bacteria *Ruminococcus flavefaciens* being one of the most important. Unlike free-enzyme secreting microorganisms, *R. flavefaciens* owes its highly cellulolytic capacity to an elaborate multi-enzyme structure, termed cellulosome, which centralizes the efforts of many polysaccharide degrading enzymes. It is an elaborate multi-enzyme structure composed of multiple pieces assembled through high-affinity interactions between enzyme-borne dockerin (Doc) modules and repeated cohesin (Coh) modules present in non-catalytic scaffoldins [1-3]. The cellulosome *R. flavefaciens* is one of the most intricate described to date and its elaborate architecture is assembled exclusively through single-binding-mode Coh-Doc interactions. However, a set of R. *flavefaciens* Docs revealed a remarkable structural symmetry capable of supporting two 180◦-related conformations, an associated feature of the classic dual-binding mode [4-6]. To investigate the dual- binding mode in ruminal cellulosomes, we have solved the structure of the Coh-Doc complex involving the Doc of a monovalent adaptor scaffoldin without specificity switch (DocScaH) and the Coh from anchoring scaffoldin ScaE (CohScaE), through X-ray crystallography. The interactions were then further characterized through site-directed mutagenesis and isothermal titration calorimetry. Unlike previously characterized R. *flavefaciens* Docs, DocScaH can interact with its cognate Cohs in a dual-binding mode, which is thought to promote conformational flexibility, avoiding steric hindrance between the several cellulosomal components in periods of intense cellulolytic activity [7, 8]. These results suggest the existence of adaptor scaffoldins with the sole purpose of improving cellulosomal spatial conformation and of naturally occurring atypical dockerins with distinct binding mechanisms. A better understanding of cellulosomal assembly will provide tools to design tailored Coh-Doc interactions that may be applied to improve fibre digestion, leading to better animal productivity and wellbeing, as well as to reduce methane emissions.

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Structural details of sulfated polysaccharides concentrated in salt pan waters

Sónia S. Ferreira[1,2], *Cláudia Nunes*[3], *Manuel A. Coimbra*[2]
1. CICECO - Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 2. LAQV-REQUIMTE - Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 3. CICECO - Department of Materials and Ceramic Engineering, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

E-mail: soniasferreira@ua.pt

Marine environments encompass a variety of novel bioactive compounds prone to be explored [1-3]. Salt pans are man-made systems where seawater gives rise to sea salt due to its evaporation by wind and sunlight. Seawater is a source of highly heterogeneous sulfated polysaccharides that are excreted by marine organisms, being accumulated in salt pan brine water. These polysaccharides have been shown to improve the immune system [4]. Therefore, in this study polymeric material and polysaccharides, obtained by dialysis, were analysed along salt production in the evaporation ponds and in the crystallizer water. Along salt production, polymeric material of seawater (13 mg/L) was accumulated in the evaporation ponds (9-73 mg/L) and in the crystallizer (133-144 mg/L). This polymeric material was composed by 29% of sulfated polysaccharides, with 45 mol% of sulfate esters, 23 mol% of uronic acids, 12 mol% of galactose, and 1 to 6 mol% of glucose, mannose, xylose, fucose, rhamnose, arabinose, and ribose. The uronic acid pattern was analysed by high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) [5]. Galacturonic (57%) and glucuronic acids (43%) were identified, whereas guluronic, mannuronic, and iduronic acids, common in some marine polysaccharides, were not detected. These results detailed the heterogeneity of these sulfated polysaccharides and highlight salt pan water as a worth exploring source of highly sulphated polysaccharides for biomedical and/or functional food applications.

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Production and pre-clinical evaluation of immunogenic CD44 glycoconjugates: setting the molecular bases for novel multivalent anti-cancer glycovaccines

Rui Freitas[1,2,3,4,6,7], *Andreia Miranda*[1,2,3], *Dylan Ferreira*[1,2,3,4,6,7], *Cristiana Gaiteiro*[1,2,3,4], *Eduardo Ferreira*[1,2,3,4,5], *Marta Relvas-Santos*[1,2,3,4,6,7,8], *Janine Soares*[1,2,3,4,5], *Sofia Cotton*[1,2,3,4], *Andreia Peixoto*[1,2,3,4,6,7], *Carlos Palmeira*[1,2,3,9,10], *Flávia Castro*[6,7], *Maria José Oliveira*[6,7], *Bruno Sarmento*[6,7], *André M. N. Silva*[8,11], *Lúcio Lara Santos*[1,2,3,4,9,11,12], *José Alexandre Ferreira*[1,2,3,4,11]

1. Experimental Pathology and Therapeutics Group, IPO Porto Research Center (CI-IPOP), Portuguese Oncology Institute (IPO Porto), 4200-072 Porto, Portugal; 2. RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto), 4200-072 Porto, Portugal; 3. Porto Comprehensive Cancer Center (P.ccc), 4200-072 Porto, Portugal; 4. Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal; 5. REQUIMTE-LAQV, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 6. Institute for Research and Innovation in Health (i3S), University of Porto, 4200-135 Porto, Portugal; 7. Institute for Biomedical Engineering (INEB), University of Porto, 4200-135 Porto, Portugal; 8. REQUIMTE-LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences of the University of Porto, 4169-007 Porto, Portugal; 9. FP-I3ID, University Fernando Pessoa, 4249-004 Porto, Portugal; 10. Immunology Department, Portuguese Oncology Institute (IPO Porto), 4200-072 Porto, Portugal; 11. GlycoMatters Biotech, 4500-162 Espinho, Portugal; 12. Department of Surgical Oncology, Portuguese Oncology Institute (IPO Porto), 4200-072 Porto, Portugal.

E-mail: rui.freitas@ipoporto.min-saude.pt

Cancer remains one of the deadliest diseases worldwide urging the need for more efficient and safer therapies. Advances in glycomics, glycoproteomics and bioinformatic approaches have allowed the identification of abnormally glycosylated proteins at tumours cell surface, caring the short chain *O*-glycans Tn and STn holding potential for immunotherapeutic settings [1]. The identification of these cancer-associated molecular fingerprints has boosted the construction of antitumour glycovaccines that have shown capability to promote effective anti-cancer immune responses at pre-clinical stages. However, the immunosuppressive character of tumour-associated glycans have been a major concern, delaying the establishment of anti-cancer vaccines. To overcome this drawback, several strategies have been developed in order to increase the immunogenicity of tumour-associated/specific antigens. Herein, we have enzymatically synthesized a cancer specific CD44s-Tn glycopeptide, previously identified by us in advanced staged bladder tumours [2], and we combined it with the immunogenic protein keyhole limpet hemocyanin (KLH) to construct a multivalent anti-cancer glycovaccine. Toxicity and immunological evaluation of KLH-CD44s-Tn alone or co-adjuvated with monophosphoryl lipid A (MPLA) were accessed both *in vitro* and *in vivo*. The glycovaccine showed no toxicity *in vitro* and it was able to stimulate dendritic cells in the presence of the MPLA. In addition, the glycovaccine was well-tolerated *in vivo*, and the immunological evaluation revealed the production of IgG and IgM antibodies and immune responses mediated by CD8+ T cells, B-cells, dendritic cells, type 1 macrophages and memory CD4 and CD8+ T-cells. Moreover, purified IgG and IgM antibodies exhibited specific reactivity against synthetic CD44s-Tn glycopeptides and CD44s-Tn based constructs with low or no recognition of the non-glycosylated CD44s peptide. Furthermore, isolated IgG antibodies could recognize a bladder cancer cell line model overexpressing the Tn antigen. In conclusion, we described the production and pre-clinical evaluation of a multivalent glycovaccine based on the CD44s-Tn cancer-specific molecular signature. Our glycovaccine was proven to be safe and immunological evaluation showed its capability to elicit humoral and pro-inflammatory cellular responses including immunological memory.

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Posters

*Underlined are the posters selected for Flash presentations

Structural insights behind the recognition of sialosides by siglec-7

Alejandra Travecedo,[1,2] Cátia Oliveira Soares,[1,2] Helena Coelho,[1,2] Mario Schubert,[3] Ana Sofia Grosso,[1,2] Francisco Corzana,[4] Jesús Jimenez-Barbero,[5,6] June Ereño-Orbea [5,6] Filipa Marcelo[1,2]*

1. UCIBIO, Department of Chemistry, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 3. Department of Biosciences, University of Salzburg, Hellbrunnel Str. 34, 5020 Salzburg, Austria; 4. Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, 26006 Logroño, Spain; 5. CIC bioGUNE, Basque Research and Technology Alliance, Bizkaia Technology Park, Ed. 800. E-48160, Derio, Spain; 6. Ikerbasque, Basque Foundation for Science, Euskadi Plaza 5, 48009 Bilbao, Bizkaia, Spain

E-mail: m.travecedo@campus.fct.unl.pt; filipa.marcelo@fct.unl.pt (*)

Sialosides play important roles in cell-cell interactions, both in healthy and abnormal cells. Hypersialylation is a hallmark of cancer with major impact on immune evasion [1], increased metastasis [2] and chemotherapy and radiotherapy resistance [3].
The human Siglec (sialic acid–binding immunoglobulin-like lectins) family recognizes siologlycans through their N-terminal V-set immunoglobulin (Ig)-like domain [4]. Upon the recognition of sialosides expressed by the host cell, the immunoreceptor tyrosine-based inhibitory motif (ITIM) transmits immune inhibitory signals creating tolerance by increasing the threshold of immune activation, mechanism behind the recognition of self-glycans by Siglecs [4].
Siglec-7 binds both α(2 → 3) and α(2 → 6) sialic acid glycoconjugates primarily expressed in Natural Killer (NK) cells where it has an important role to inactivate them in tumour escape [5,6] As well Siglec 7 binds disialyl motifs found in GD3, a ganglioside that contains an α(2 → 8) disialic acid, where it inhibits NK cell cytotoxicity as an immune evasion mechanism [5].

To understand the molecular determinants behind the recognition of different sialosides by Siglec-7, NMR binding experiments from protein and ligand viewpoint were carried out. For the point of view of the protein, ^{15}N-Siglec-7 was recombinantly expressed and purified for a ^{1}H,^{15}N-HSQC based titration with GD3 to elucidate the main residues involved in the interaction. For the point of view of the ligand, STD-NMR experiments were performed to obtain the STD-derived epitope map of GD3. The binding site of Siglec-7 and the epitope of GD3 will be presented. This information can be further used to rational design sialic acids' mimics that may disrupt the interaction of this complex.

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Evaluation of the Siglec10-CD24 axis as new immunotherapy target for feline mammary carcinoma

Alexandra C. Oliveira,[1,2] Paula Videira,[3] Jorge Correia,[1,2] Fernando Ferreira,[1,2] Carlos Fontes,[1,4] Pedro Bule[1,2]

1.CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisbon, Portugal; 2. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS); 3. UCIBIO, Departamento Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Lisbon, Portugal; 4. NZYTech, Genes & Enzymes, Campus do Lumiar, Estrada do Paço do Lumiar, 1649-038 Lisbon, Portugal;

E-mail: alexandraoliveira@fmv.ulisboa.pt

The interaction between tumoral cell-surface sialoglycans and immune cell sialic-acid-binding immunoglobulin-like lectin (Siglec) receptors have emerged as a key immune response modulator, as some of these contain immunoreceptor tyrosine-based inhibitory motifs. Recently, the Siglec-10- CD24 axis was proposed as the major immune checkpoint in human breast cancer, promoting immune evasion by preventing macrophage dependent phagocytosis of tumoral cells.[1] Due to the similarities between feline mammary carcinoma (FMC) and human breast cancer we sought to identify and characterize the Siglec-10-CD24 axis in FMC, with the aim of developing a novel immunotherapeutic approach based on targeted tumour cell desialylation.

Expression of CD24 and Siglec-10 was evaluated using immunohistochemistry on tumoral samples from 17 FMC patients. To develop the ideal enzyme for the targeted desialylation strategy, 74 putative sialidase genes were selected from the Cazy.org database, expressed in *Escherichia coli* and purified in a high-throughput platform. The enzymatic activity of the sialidases was evaluated using the 4-methylumbelliferyl-α-N-acetylneuraminic acid.

Immunohistochemistry analysis showed marked Siglec-10 and CD24 staining in tumour associated macrophages and in poorly differentiated neoplastic cells, respectively. All selected recombinant sialidases were soluble and approximately 20 displayed a good yield-activity combination.

High expression of Siglec-10 was observed in the macrophages infiltrating the tumour samples. Furthermore, CD24 expression in FMC cells appears to be correlated with poor differentiation. This preliminary study suggests the presence of Siglec-10-CD24 signaling in FMC, likely leading to immune evasion, similarly to what is observed in human breast cancer. Finally, a group of promising novel sialidases was identified, which will be further characterized and have their cell desialylation potential assessed.

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The 3D structure of BC16100-C, a novel protein from *Bacteroides caccae*, in complex with the Tn antigen reveals the molecular determinants that allow this bacterium to recognise host glycoproteins

Alícia Candeias,[1,2] *Filipa Trovão*,[1,2] *Benedita A. Pinheiro*,[1,2] *Angelina S. Palma*,[1,2] *Ana Luísa Carvalho*[1,2]

1. UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

E-mail: ai.candeias@campus.fct.unl.pt

The human gastrointestinal tract harbors a diverse community of commensal bacteria, named gut microbiota. These microorganisms are beneficial to the human gut, due to breaking down dietary polysaccharides, such as starch, which cannot be digested. However, in a low-fiber diet, host carbohydrates provided by the intestinal mucus, which is mainly composed of *O*-glycosylated glycoproteins, are used as an alternative source of energy. Therefore, in these conditions, the human gut microbiota has been associated with susceptibility to pathogens and the progression of intestinal diseases, owing to the destruction of the colonic mucous layer (Figure 1A) [1]. Thus, the study of these microorganisms and their binding to the host glycans may elucidate the role of the gut microbiota in these diseases. The commensal bacteria *Bacteroides caccae* has been reported to express different polysaccharide utilization loci (PUL), which encode for all the genes necessary for the breakdown and uptake of carbohydrates, such as those that code for M60-like metallopeptidases (Pept_MA) and their appended non-catalytic ancillary carbohydrate-binding modules of family 32 (CBM32) [2]. We have solved the individual crystal structures of BC16100-C (a member of the CBM32 family appended to a Pept_MA from the *B. caccae* PUL 53) in its free form, bound to GalNAc and bound to the Tn antigen (GalNAcα-Ser) (Figure 1B). The 3D structures reveal the molecular determinants that allow *B. caccae* to recognise human *O*-glycosylated glycoproteins from the gut mucous layer.

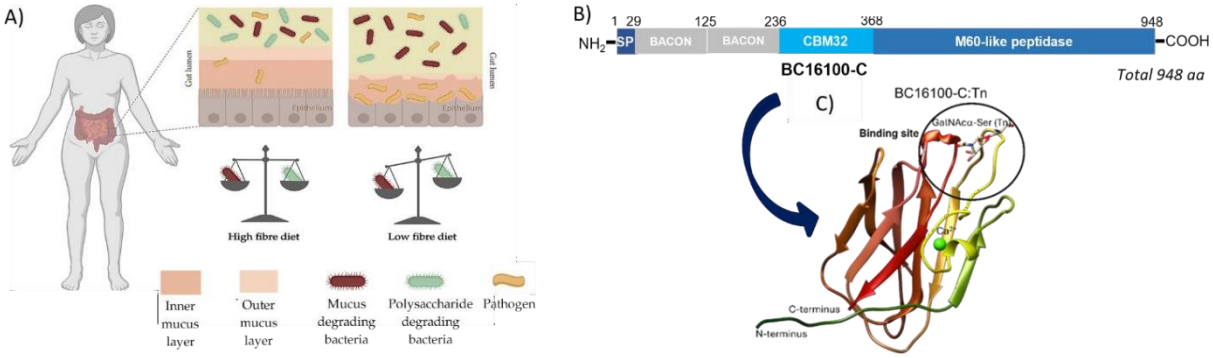

Figure 1 – A) Impact of diet on the gut microbiota; B) Modular organization of PUL-53 from *B. caccae*; C) Ribbon representation of BC16100-C in complex with the Tn antigen (GalNAcα-Ser), colored from the N-terminus to the C-terminus. The Tn binding site is indicated by the black circle and the calcium ion is represented by the green sphere. The bound GalNAc is shown as sticks.

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Differential protein and glycan packaging into extracellular vesicles by 3D gastric cancer cells

Álvaro M Martins[1,2,3], *Tânia Lopes* [1,2], *Francisca Diniz* [1,2], *Hugo Osório*[1,2,4], *Filipe Pinto*[1,2], *Daniela Freitas*[1,2], *Celso A Reis*[1,2,3,4*]

1. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto; 2. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto; 3. ICBAS – Instituto Ciências Biomédicas Abel Salazar; 4 FMUP – Faculdade de Medicina da Universidade do Porto

E-mail: celsor@ipatimup.pt (*)

Alterations in the glycosylation machinery are common events associated with cancer progression that lead to the synthesis of aberrant glycan structures [1]. These are involved in cancer hallmarks affecting patient prognosis and survival. It is reported that extracellular vesicles (EVs) shed by cancer cells carry several functional molecules, such as miRNAs, proteins, and glycans, holding modulatory potential on the recipient cell to promote tumour progression [2]. Our group has previously identified the presence of aberrant glycan structures in EVs released by cancer cell models [3]. Current 3D cell culture methods have been shown to better mimic cancer in vivo phenotypes compared to monolayer cell culture. However, it remains to be clarified the effects of 3D cell culture conditions on EV glycosylation. In this study, we evaluate the glycosylation profile and the proteomic content of EVs derived from established glycoengineered cancer cell models applying 3D and 2D monolayer cell culture. Isolated EVs from these conditions were characterized, and their glycosylation profile was assessed using specific a glycan-binding antibody, and lectins. Moreover, the EV proteome was analysed using mass-spectrometry. Interestingly, our results revealed that 3D cellular architecture not only promoted EV secretion but also led to specific distinct glycosignatures of the derived EVs, for instance, affecting the expression of the tumour-associated glycan STn, and other terminal glycan structures. This study highlights the importance of cell culture methodologies when studying EVs and their glycosylation profile, along with offering insight on glycosylation alterations found in EVs derived from 3D cell culture that could resemble the tumour microenvironment.

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One-pot sustainable synthesis of valuable nitrogen compounds from biomass resources

M. Carolina A. Carreira, M. Conceição Oliveira, Ana C. Fernandes[*]
Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.

E-mail: anacristinafernandes@tecnico.ulisboa.pt (*)

The sustainable synthesis of nitrogen compounds is currently a topic of great interest to academics and industry to reduce the production of these compounds from fossil resources. In this context, the search for cost-efficient processes for the preparation of nitrogen compounds from biomass resources is highly desirable.
Among biomass resources, carbohydrates form by far the largest natural source of carbon and are considered the ideal feedstock for the production of a platform of valuable nitrogen compounds.
In continuation of our work on the sustainable synthesis of value-added nitrogen compounds from carbohydrates,[1-2] in this communication we describe a new one-pot process for the synthesis of 2-furanylquinazolines and 2-furfurylidene derivatives from xylose, fructose and xylan, with moderate overall yields, catalyzed by perrhenic acid.[3]

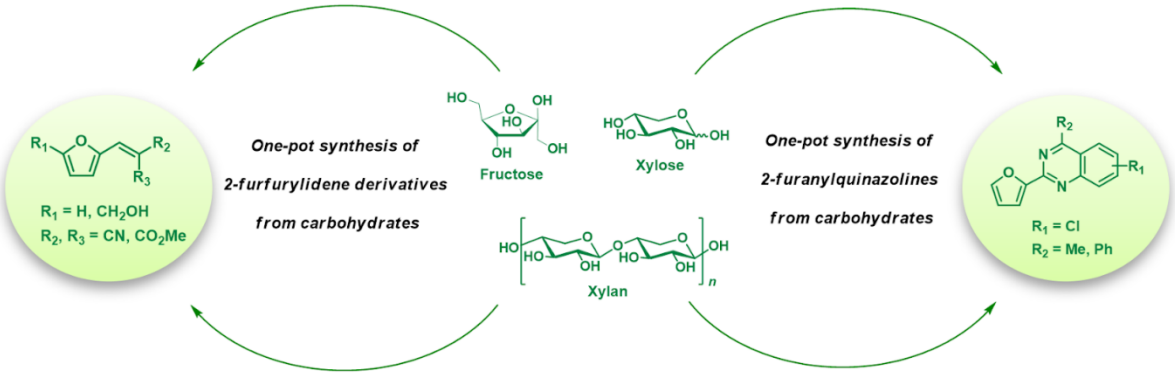

Figure 1 – One-pot sustainable synthesis of nitrogen compounds from carbohydrates.

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Carboxymethylcellulose-based dissolvable microneedle patches for rapid painless delivery of diclofenac

Ana C. Q. Silva,[1] Bárbara Pereira,[1] Nicole S. Lameirinhas,[1] Paulo C. Costa,[2] Isabel F. Almeida,[2] Patrícia Dias-Pereira,[3] Inês Correia-Sá,[4] Helena Oliveira,[5] Armando J. D. Silvestre,[1] Carla Vilela,[1] Carmen S. R. Freire [1]

1. CICECO – Aveiro Institute of Materials and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, MedTech – Laboratory of Pharmaceutical Technology & Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; 3. Institute of Biomedical Sciences Abel Salazar, ICBAS –UPorto, University of Porto, 4050-313 Porto, Portugal; 4. Department of Plastic, Aesthetic, Reconstructive and Aesthetic Surgery, Centro Hospitalar de S. João, 4200-319 Porto, Portugal; 5. Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: ana.cristina.silva@ua.pt

Nonsteroidal anti-inflammatory drugs (NSAIDs) are over-the-counter pharmaceuticals, advised as the first step in pain management owing to their analgesic and anti-inflammatory properties [1]. Given the risks of long-term systemic use, dermal application is one of the primary targets for NSAID administration in the treatment of acute pain disorders. Nonetheless, the protective skin barrier (*stratum corneum*) is known to hinder the permeation of these medications, reducing their therapeutic efficacy. Microneedles (MNs) are systems composed of an array of needle-like projections, ranging in length from 25 to 2000 µm, which are capable of disrupting the *stratum corneum* and creating microconducts that enable molecules to pass through the skin in a painless, noninvasive, and self-administrable fashion, promoting patient compliance [2]. In this work, we investigated the fabrication of dissolvable carboxymethylcellulose (CMC) microneedles loaded with diclofenac sodium salt (DCF) aiming at its single-step transdermal administration, envisaging rapid pain relief [3]. The MNs patches were fabricated using a simple and environmentally friendly micromoulding technique. The obtained pyramidal-shaped microneedles display adequate morphology and an average height of 456 µm. Mechanical tests reveal that the MNs have the necessary force for skin insertion (> 0.15 N. needle^{-1}), reaching a maximum of 0.75 N needle^{-1} for the drug-loaded MN patches. Experiments in a skin model and in *ex vivo* abdominal human skin demonstrate the capacity of these MNs for skin insertion, reaching depths up to 401 µm. The rapid release profile in saline buffer (maximum of 98% of DCF after 40 min) and dissolution ability (ca. 10 min, in a skin simulant), allied with the noncytotoxic behavior towards human keratinocytes, highlights the promising nature of these systems for quick pain alleviation.

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Fluorinated GalNAc as a novel inhibitor of cancer-associated truncated O-glycans in gastrointestinal cancer

Ana Filipa Costa[1,2,3,*], *Emanuel Senra*[1,2,*], *Rafaela Abrantes*[1,2,3], *Celso A. Reis*[1,2,3,4], *Jindřich Karban*[5], *Catarina Gomes*[1,2]

1. Instituto de Investigação e Inovação em Saúde (I3S), University of Porto, Porto, Portugal; 2. Institute of Molecular Pathology and Immunology, University of Porto (IPATIMUP), Porto, Portugal; 3. School of Medicine and Biomedical Sciences (ICBAS) University of Porto, Porto, Portugal; 4. Faculty of Medicine, Universidade do Porto, Porto, Portugal; 5. Institute of Chemical Process Fundamentals, Prague, Czechia
* Both authors contributed equally to this work

E-mail: afscosta@ipatimup.pt

The dysregulation of the normal process of cellular glycosylation is a hallmark among various types of cancer, with a subsequent expression of aberrant glycosylated antigens. One of the most common alterations is the expression of the truncated *O*-glycans Tn (GalNAcα-O-Ser/Thr) and Sialyl-Tn (STn-Neu5Acα-2,6GalNAcα-O-Ser/Thr). The STn antigen, for instance, is expressed in around 80% of tumours, and its expression is associated with increase tumours' aggressiveness and poor patients' prognosis. Thus, targeting truncated *O*-glycans biosynthesis is a strategy with great potential for the development of new effective cancer therapies. Recent studies have shown the efficacy of various fluorinated monosaccharide analogues (F-MAs) in the inhibition of glycans biosynthesis. Therefore, the use of F-MAs could represent a clever strategy to impair the biosynthesis of Tn and STn, opening opportunities for translational studies. Hence, this work aims to evaluate the impact of different fluorinated analogues of *N*-acetylgalactosamine (GalNAc) on biosynthesis of the truncated O-glycans (Tn and STn) in gastrointestinal (GI) cancer cells. With this in mind, we first tested the capacity of three different GalNAc fluorinated at the positions-3, -4, and -6 (3F-, 4F- and 6F-GalNAc) in a STn positive-glycoengineered HEK293T cell line. We observed that only 4F-GalNAc impairs STn expression, by Western Blot, compared to DMSO-treated cells. To validate this result in GI cancer cells, two cell lines (MKN45 and COLO205) were selected according to the higher expression levels of ST6GalNAcI, the enzyme that catalyses the addition of sialic acid (Neu5Ac) to Tn antigen forming STn. The KnockOut (KO) of the C1GalT1, the enzyme responsible for Core1 biosynthesis in O-glycans, was performed in the selected GI cancer cell lines, and expression of STn validated by Western Blot, Immunofluorescence and Flow Cytometry. The engineering of the different cell lines was an essential step in this study due to the lack of suitable cancer cellular models to study truncated O-glycans. The obtain results showed that 4F-GalNAc was able to impair both STn and Tn antigens biosynthesis in both established cellular models. Preliminary results show that 4F-GalNAc reduces cell proliferation when compared to DMSO-treated cells, especially in MKN45 KO cells. Additionally, transcriptomic analysis results highlight the effect of 4F-GalNAc in the Core1 biosynthetic pathway, along with alterations in protein processing and trafficking.

Altogether, we successfully established two different models of GI cancer cell lines that express both Tn and STn antigens, opening the opportunity to perform numerous studies in the glycobiology in cancer field. Moreover, the fluorinated analogue 4F-GalNAc constitutes a very promising approach for the development of a new therapy against cancer by preventing aberrant Tn and STn antigens biosynthesis.

Repurposing Alkyl Deoxyglycosides for Use Against MDR Gram-negative Bacteria: Lead Synthesis Optimization and Biological Evaluation in the Presence of Adjuvant Agents

A. Marta de Matos,[1,] Patrícia Calado,[1] Amélia P. Rauter,[1] Vera Manageiro,[2] Manuela Caniça[2]*

1. Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências da Universidade de Lisboa, 1749-016, Lisboa, Portugal; 2. Laboratório Nacional de Referência de Resistência aos Antibióticos e IACS, Instituto Nacional de Saúde Doutor Ricardo Jorge, 1649-016, Lisboa, Portugal.

E-mail: amamatos@fc.ul.pt (*)

Antimicrobial resistance (AMR) is a major public health concern. Estimates indicate that, in 2019 alone, over 1.27 million deaths worldwide were directly attributable to infections caused by multidrug-resistant (MDR) pathogens [1]. Gram-negative bacteria, which are particularly difficult to treat due to the outer membrane (OM) present in their celluar envelope, are the top priority of the World Health Organization (WHO) when it comes to the development of new antimicrobial therapies [2]. In this study, we aimed to repurpose alkyl deoxyglycosides, a class of compounds previously shown to have antimicrobial activity against Gram-positive, but not Gram-negative bacteria [3], for use against the latter. To achieve this, we combined alkyl deoxyglycosides with adjuvant agents known to be effective at permeating the OM of Gram-negative bacteria, specifically polymyxin B (PMB) and colistin (polymyxin E) — two last-resort antibiotics approved for use in clinical practice. In this perspective, we have optimized the synthesis of *O*- and *C*- alkyl deoxyglycoside leads and evaluated their antimicrobial activity in combination with different concentrations of PMB and colistin against a panel of MDR Gram-negative (MDRGN) bacteria. In addition, we conducted cytotoxicity assays in HEK-293T and Caco-2 cells to assess the safety profile of these combinations. Our results show that, in the presence of a subtherapeutic concentration of PMB or colistin, four out of five tested sugar-based antibiotics were active against several MDRGN strains, with low MIC values and acceptable cytotoxicity in cell cultures. These findings suggest that these alkyl deoxyglycoside-polymyxin combinations have the potential to be developed as a new treatment for infections caused by MDRGN bacteria.

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Consolidate starch with inorganic additives as a strategy to replace the white food colorant titanium dioxide

Andreia S. Ferreira[1], Elisabete Coelho[1], Cláudia Nunes[2], Manuel A. Coimbra[1]
1. LAQV-REQUIMTE - Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 2. CICECO - Department of Materials and Ceramic Engineering, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

E-mail: a39493@ua.pt

Titanium dioxide (TiO_2) is a ubiquitous white and opacifying agent, widely used in several areas, mainly in pharmaceutical, cosmetic, and food industries [1]. In Europe, TiO_2 is used as a food colour additive (E171), with the technological function of making food more visually appealing, providing a white colour to foods that otherwise could be colourless, or to shade other pigments, namely in sauces, icings, chewing gums, and candies. However, in 2022 European Food Safety Authority (EFSA) determined that TiO_2 is no longer authorized as a food additive, due to its potentially harmful effects to human health [2]. Consequently, European food industries are seeking for alternatives to TiO_2. Thus, the aim of this study was to develop alternatives to the white TiO_2 powder to be used in food stuffs. The strategy used was the consolidation of starch with inorganic material, that consisted in swelling the polysaccharides of starch granules, and, consequently, decreasing the distance between inorganic particles, bringing them into contact to form a solid network [3]. Thus, firstly, rice starch was consolidated with different inorganic additives approved by EFSA, namely, calcium carbonate (E170), calcium phosphate (E341), silicon dioxide (E551), and calcium silicate (E552), by gelatinization at 90 ºC during 30 min. The resulting white powders were then milled and sieved. As a showcase, the different white powders were applied in the recipe of commercial candies ("Flocos de Neve", Vieira de Castro – Produtos Alimentares, S.A.). The measurement of colour by CIELab system (L*a*b* coordinates) revealed that candies produced with the addition of rice starch consolidated with silicon dioxide are more promised (L*=69), following by the mixture of rice starch with calcium silicate (L*=61), with calcium phosphate (L*=53), and with calcium carbonate (L*=48). In conclusion, the use of carbohydrate polymers, namely the consolidation of rice starch with inorganic compounds revealed to be a good strategy as white food colour additives to replace TiO_2.

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Polymeric dressings based on Bacterial cellulose and FucoPol for advanced wound treatment

A. Esmail [1, 2], *A. C. Marques* [3], *M. A. M. Reis* [1, 2], *F. Freitas* [1, 2]

1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, 2829-516, Caparica, Portugal; 2.UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, 2829-516, Caparica, Portugal; 3. CENIMAT|i3N, Department of Materials Science, School of Science and Technology, NOVA University Lisbon and CEMOP/UNINOVA, 2829-516, Caparica, Portugal

E-mail: a.esmail@campus.fct.unl.pt

Wound care is a vital part of medicine, where wound dressings play a major role in protecting the wound from bacterial infection while also promoting healing [1]. Wound dressings should fill a few requirements to serve their purpose, including providing and maintaining a moist environment, improving epidermal migration, allowing gas exchanges, offering thermal insulation, and presenting wound adherence. Their material must also be biocompatible, non-toxic, and non-allergic [2]. Although they are many dressings from synthetic polymers due to their higher reproducibility and availability on the demand, natural polymers offer higher biocompatibility and biodegradability [2].

Bacterial cellulose is a glucose homopolymer that is naturally biosynthesized as a hydrogel-like porous three-dimensional structure. It presents several qualities, such as strong mechanical performance, water absorption capacity, and biocompatibility [3]. Though it lacks bioactivity, it can be resolved by functionalization with other compounds. FucoPol is a fucose-rich exopolysaccharide that has been proven to have wound-healing abilities, as well as bioactive, adhesive, and photoprotective properties [4-6].

In this work we propose the utilization of two bacterial polysaccharides, bacterial cellulose, and FucoPol, to deliver a functional composite intended for wound dressing applications that takes advantage of each polymer's attributes. For this effect, different techniques were tested, specifically *in-situ* strategies that incorporated FucoPol during BC synthesis, and *ex-situ* modification where FucoPol was impregnated into already produced BC pellicles. The presence of FucoPol in the culture medium hindered *in-situ* biocomposite formation, however, *ex-situ* impregnation using a 2.5 g/L Fe^{3+} solution as a gelation agent yielded an increase in fucose content by 15% without influence on chemical structure and a slight rise in degradation temperature from 350 ºC to 360 ºC.

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Evaluation of alginate hydrolysates as clean label substitutes of phosphates in cooked ham formulation

Bernardo A. C. Ferreira[1], *Andreia S. Ferreira*[1], *Sónia S. Ferreira*[2], *Cláudia P. Passos*[1], *Elisabete Coelho*[1], *Cláudia Nunes*[3], *Manuel A. Coimbra*[1]

1. *LAQV-REQUIMTE - Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 2. CICECO - Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 3. CICECO - Department of Materials and Ceramic Engineering, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*

E-mail: bacferreira@ua.pt

Phosphates, namely tripolyphosphate, are food additives used for the retention of water in several protein-rich products, enhancing texture, tenderness, succulence, and production yield in processed meats [1]. However, high values of phosphate intake has been associated to cardiovascular comorbidity, as well as chronic kidney disease, justifying the search for clean label substitutes [2]. Alginate is an anionic polysaccharide from brown seaweed constituted by D-mannuronic and L-guluronic acids. Due to its anionic properties, soluble oligosaccharides and low molecular weight polysaccharides from alginate are potential clean label alternatives to tripolyphosphates in meat products, mimicking their size and charge. Therefore, in this study alginate was depolymerised by microwave assisted partial hydrolysis, at 120 °C (MW120) and at 150 °C (MW150). No uronic acid degradation was observed under microwave assisted partial hydrolysis. Furthermore, the MW150 hydrolysate contained a higher content in monosaccharides, oligosaccharides, and low molecular weight polysaccharides in comparison with the MW120 hydrolysate as observed by analysis of the chromatograms from the high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) [3]. Due to its carbohydrate composition, MW150 hydrolysate was incorporated in the cooked ham formulation. For comparison purposes, hams were also prepared without additive addition (negative control), with the addition of sodium tripolyphosphate (positive control), and with commercial clean label additives. All hams had higher moisture (72 – 79 %) than the ham without phosphate (67 – 69 %). Textural analysis showed that ham with clean label substitutes achieved less hardness (21 – 42 %) than ham without phosphate (49 – 65 %). Nevertheless, moisture determination and texture profile analysis of cooked hams showed that MW150 alginate addition possessed similar moisture and hardness in comparison with the cooked hams with the commercial additives. Colour analysis showed that in comparison with hams with phosphate (positive control) all other formulations showed perceived differences. These results show that alginate hydrolysates, due to its oligosaccharide profile and anionic character, are promising clean label substitutes of synthetic phosphates additives in cooked ham formulations.

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Targeting human macrophage galactose C-type with a multivalent GalNAc mimetic

Carlos D. L. Lima[1,2], Simon E. Johnson[3], Ana Sofia Grosso[1,2], Jorge S. Dias[1,2], Eurico Cabrita[1,2], Francisco Corzana,[4] Carmen Galan[3], Filipa Marcelo[1,2]*

1. UCIBIO, Department of Chemistry, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 3. School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, United Kingdom; 4. Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, 26006 Logroño, Spain

E-mail: cd.lima@campus.fct.unl.pt; filipa.marcelo@fct.unl.pt (*)

Multivalent presentation of the lectins and glycans is usually a requisite to achieve physiologically relevant selectivity and binding affinities [1]. In this context, most of the natural ligands or non-natural ligands (glycomimetics) to efficiently target lectins should be displayed in a multivalent presentation.

Human macrophage galactose-type lectin (MGL) receptor expressed by antigen presenting cells, such as the macrophages and dendritic cells, is the only lectin present on immune cells able to recognize αGalNAc-Ser/Thr epitopes (Tn-antigen) displayed in clusters of mucins glycoproteins [2]. Through this interaction, a suppressive anti-tumour immune response seems to be produced [3-5].

Biophysical methods have demonstrated that the extracellular domain of MGL, constituted by the neck region and the CRD domain, is a trimeric structure, mainly stabilized by the helical coiled-coil neck region and where the CRDs act as independent domains [6]. In addition, we have recently provided new clues, from a structural perspective, to understanding the MGL-glycan recognition process [7-9].

In this communication, the molecular view of the recognition of GalNAc mimetic [10] in multivalent presentation by the carbohydrate recognition domain of MGL (MGL-CRD) deduced by NMR spectroscopy in tandem with molecular modelling will be presented.

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Structural and biochemical insights into glycoside hydrolase family 157 enzymes

Caseiro C[1,2], McGregor N[3], Correia M[4,5], Carvalho AL[4,5], Davies G[3], Fontes CMGA[6], Bule P[1,2]
1. CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Av. Universidade Técnica, 1300-477 Lisbon, Portugal; 2. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Av. Universidade Técnica, 1300- 477 Lisbon, Portugal; 3. York Structural Biology Laboratory, Department of Chemistry, The University of York, Heslington, York YO10 5DD, United Kingdom; 4. UCIBIO, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516, Caparica, Portugal; 5. Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516, Caparica, Portugal; 6. NZYTech Genes & Enzymes, Campus do Lumiar, Edifício E, Estr. Paço do Lumiar 2nd floor, 1649-038 Lisbon, Portugal

E-mail: ccaseiro@fmv.ulisboa.pt

β-glucans are a class of polysaccharides whose hydrolysis is catalysed by β-glucanases. Due to the complex relationship between the molecular structure and functional profile of β-glucans, there is a continuous need for highly specific β-glucanases that can fully reveal β-glucans' potential applications [1]. Here we report the first biochemical and structural characterization of two β glucanases from the recently founded glycoside hydrolase family 157 (GH157) and investigate their molecular basis for substrate recognition and hydrolysis.
Genes encoding the GH157s from the human gut bacteria *Bacteroides cellulosilyticus* and psychrophilic bacteria *Labilibaculum antarcticum* were cloned, expressed and structurally characterized by X-ray crystallography. Their specificity and activity were analyzed with reducing sugar assays, enzyme kinetics and product analysis by HPAEC-PAD and LC-MS.
Specificity screening revealed that both enzymes are active on β-1,3-glucans and mixed-linkage glucans. Temperature catalytic dependency reflected the psychrophilic nature of *La*GH157 (4- 32.5°C), with maximum activity being observed at 25°C. *Bc*GH157 was stable in a broader range of temperatures (4-49ºC) with optimal temperature of 48ºC. Both enzymes exhibited the highest activity at pH 6.5. HPAEC-PAD and LC-MS on hydrolysis products revealed that both enzymes display an endo mode of action, capable of cleaving β1-3 and β1-4-linked glucoses, when preceded by a β1-3 linkage. The structure of *La*GH157 revealed a classic TIM-barrel fold and suggested a retaining mechanism of hydrolysis, with two glutamates serving as the catalytic residues, confirmed by site directed mutagenesis.
We have performed the first structural and biochemical characterization of GH157 members, which were found to be retaining endo-β-1,3(4)-glucanases. This provides insight into β-glucan deconstruction in the human gut and identifies novel catalysts for the production of value-added products through β-glucan depolymerization.

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New purine nucleosides as potential chelating and anticholinesterase agents for Alzheimer's disease

Maria C,[1,2] Barros J,[1] Nuno M X,[1] Karina S,[1]Freitas A A,[1] Ferreira M J,[1]Oliveira M C,[1] Schino I,[3] Cantore M,[3] de Candia M,[3] Colabufo N,[3] Canongia Lopes J N,[1] Rauter A P[1]

1. Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa and Institute Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal; 2. NOVA University of Lisbon, Department of Chemistry, 2829-519 Caparica, Portugal; 3. Università degli Studi di Bari, Dipartimento di Farmacia-Science del Farmaco, Bari, Italy

E-mail: c.maria@campus.fct.unl.pt

Alzheimer's disease (AD), the most common form of dementia, is a neurodegenerative pathology that is caused by multiple factors, including the progressive decline of the level of acetylcholine, [1] and the deregulation of metal homeostasis, such as copper, iron and zinc. [2]

Acetylcholine, an important neurotransmitter, may be hydrolysed by two enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). [3] Currently, the therapeutic strategies are mainly based on disease symptomatic relief by treating patients with AChE and BChE inhibitors, however it was already shown that the long-term use of these drugs may lead to disease modifying benefits. [4]

Metal dyshomeostasis has been related to the induction of oxidative stress, Aβ aggregation and tau protein hyperphosphorylation, three main features of AD. [2]

Hence, the discovery of multitarget drugs against this disease is an interesting challenge towards finding a disease modifying therapy. In this context, we now present the synthesis of mannosyl- and rhamnosylpurine nucleosides and the evaluation of their biological properties as metal chelators and cholinesterase inhibitors. These compounds were synthesized following two different *N*-glycosylation procedures for the coupling of N^6-benzoyladenine with different glycosyl donors, one using microwave irradiation and trimethylsilyl triflate as the catalyst and the second using a reflux reaction and iodine. Nucleoside metal chelation properties were evaluated with the UV-Visible method and the cholinesterase inhibition was determined by Ellman's colorimetric assay with some modifications. [6,7] Moreover, computational studies were performed to know where the metal binding site was located.

In conclusion, this work discloses the structure of the first nucleoside-based molecules with potential to become dual-target drugs against AD.

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Molecular recognition of sTn glycostructures by immune-related receptors

Cátia Oliveira Soares,[1,2] *Ana Diniz,*[1,2] *Alejandra Travecedo,*[1,2] *Ana Sofia Grosso,*[1,2] *Helena Coelho,*[1,2] *Daniela F. Barreira,*[2,3] *Paula A. Videira,*[2,3,4] *June Ereño-Orbea,*[5,6] *Jesús Jimenez-Barbero,*[5,6] *Francisco Corzana,*[7] *Filipa Marcelo*[1,2]

1. UCIBIO, Department of Chemistry, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 3. UCIBIO, Department of Life Sciences, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 4. CellmAbs Biopharmaceuticals, Parque Tecnológico de Óbidos, Rua da Criatividade, Edifícios Centrais, 2510-216 Óbidos, Portugal; 5. CIC bioGUNE, Basque Research and Technology Alliance, Bizkaia Technology Park, Ed. 800. E-48160, Derio, Spain; 6. Ikerbasque, Basque Foundation for Science, Euskadi Plaza 5, 48009 Bilbao, Bizkaia, Spain; 7. Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, 26006 Logroño, Spain.

E-mail: ca.soares@campus.fct.unl.pt

Abnormal glycosylation is a universal feature of cancer cells, which can be manifested through the occurrence of shortened *O*-glycans and increased sialylation [1]. The sialyl-Tn (sTn) antigen (Neu5Acα2-6GalNAcα1-Ser/Thr) is a tumour-associated carbohydrate antigen that arises from premature sialylation caused by the overexpression of ST6GalNAc-I, which disrupts the elongation of the *O*-glycan [1]. Since the sTn-antigen is tumour-specific and overexpressed by more than 80% of human carcinomas [1], it becomes relevant to understand how it is recognized by immune-related receptors, namely lectins expressed by immune cells and antibodies. It is known that the immune system-lectins Siglec-15, Siglec-7 and Macrophage Galactose C-type Lectin (MGL) recognize the sTn antigen, inducing the suppression of anti-tumour immune responses [2-5].
Therefore, strategies to interrupt these aberrant interactions, such as, the design of glycostructures containing sialic acid mimics, and lectin or anti-sTn blocking antibodies, have been developed to alleviate suppressive anti-tumour immune responses [6]. However, for a smart and rational design of better-targeted approaches targeting sTn recognition is of paramount importance to decipher the molecular determinants behind the sTn-antigen recognition by immune-related receptors. In this context, herein it will be presented our latest results relying on the recognition of different sTn glycostructures (sTn-Thr and sTn-PDT*RP) by the human lectins Siglec-7/15 and MGL, through an integrative approach mainly focused on NMR binding methods combined with molecular dynamics simulations. As well, the latest structural insights to uncover the molecular basis of sTn recognition by the anti-STn monoclonal antibody L2A5, previously demonstrated to specifically recognize sTn- antigen [7], will be described.

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Valorization of agro-industrial wastes/by-products into high value bacterial cellulose production

Cátia V. Gil,[1,2] *Filomena Freitas*,[1,2] *Maria A. M. Reis*,[1,2] *Cristiana A. V. Torres*,[1,2]
1. Laboratory i4HB — Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, 1099-085 Caparica, Portugal; 2. UCIBIO — Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, 2829–516 Caparica, Portugal

E-mail: cv.gil@campus.fct.unl.pt

Bacterial cellulose (BC) is a prime natural extracellular polysaccharide composed of repeating glucose units linked by β (1–4) glycosidic linkages. Since each glucose monomer has six carbon atoms with three hydroxyl groups, BC provides an appealing matrix for functionalization [1]. Moreover, BC membranes present several remarkable physicochemical properties, in particular high-water absorption and tensile strength, polymerization degree, crystallinity, which, allied to straightforward purification techniques, make it a versatile material for several biotechnological purposes in various sectors, including cosmetics [2], textile [3], pharmaceutical/biomedical [4], electronics [5], food and packing industries [6]. Despite the superior properties over natural plant cellulose and its increasing demand, industrial implementation is still limited by the low-yield production and high production costs, mainly due to the culture media [7]. In a bid to valorize agro-industrial wastes and to reduce BC production costs, several agro-industrial wastes, namely, apple pulp waste (AP), stale bread waste (BD), banana (BP) peels and brewer's spent grain (BSG) were explored, with no nutrients' supplementation, as alternative culture media for production of BC by *Gluconacetobacter xylinus* DSM2004 strain. AP and BP led to the highest BC production, almost identical to the yield obtained using the reference glucose-containing HS medium, demonstrating the feasibility and cost-effectiveness of replacing conventional media by such feedstocks, thus contributing to circular bioeconomic values.

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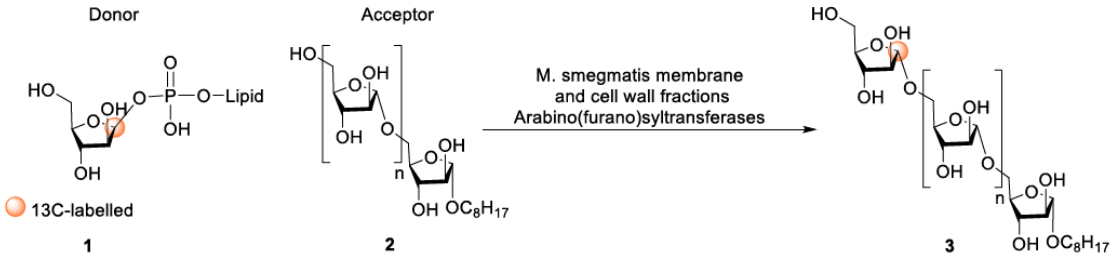
New functional assay to study potential anti-tuberculosis drugs targeting arabinofuranosyltransferases

Cristiano A. Conceição, Vanessa T. Almeida, Federico Issoglio, Margarida Archer, M. Rita Ventura
Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), 2780-157 Oeiras, Portugal

E-mail: cconceicao@itqb.unl.pt

The increasing prevalence of drug-resistant bacteria represents a significant global health problem. *Mycobacterium tuberculosis* remains the leading cause of mortality from a single infectious organism. The development of novel antimicrobials is a major approach to overcome drug resistance problems since new compounds can offer a unique mechanism of action to which target pathogens are susceptible.[1] The cell envelope of *M. tuberculosis* is a common antibiotic target and has a unique structure comprising covalently linked peptidoglycan (PG), branched heteropolysaccharide arabinogalactan (AG) and long chain mycolic acids, termed the mycolyl-arabinogalactan- peptidoglycan (mAGP) complex. Arabinofuranosyltransferases (AraT)[2] use decaprenylphosphoryl- D-arabinofuranose (DPA) to donate an arabinofuranose residue to mAGP and are essential for *M. tuberculosis* growth [3].
In this work, a multidisciplinary approach was used for the development of enzymatic assays for AraT targets. Several linear and branched synthetic arabinofuranoside acceptors were synthesised and their binding affinity with AraT was screened using Saturation-Transfer Difference (STD) NMR to select the best synthetic glycosyl acceptors. The total synthesis of chemical anomeric $_{13}$C-labelled decaprenylphosphoryl arabinofuranose analogue **1** was optimised and well-characterised achieving an overall yield of 38% and an excellent anomeric ratio up to 31:1 (β:α). In order to study the protein conversions of the synthesised labelled donor with the acceptors or either in *in-vivo* bacterial studies, a flexible NMR protocol was designed and implemented.



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Anti-STn antibodies' molecular characterization for precision oncology

Daniela F. Barreira,[1,2] Nuno P. Ramos,[3] Helena Coelho,[2,4] Filipa Trovão,[2,4] Benedita A. Pinheiro,[2,4] Wengang Chai,[5] Cátia Soares,[2,4] Angelina S. Palma,[2,4] Filipa Marcelo,[2,4] Paula A. Videira[1,2,3]

1. UCIBIO – Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal; 3. CellmAbs Biopharmaceuticals, Parque Tecnológico de Óbidos, Rua da Criatividade, Edifícios Centrais, 2510-216 Obidos, Portugal; 4. UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal; 5. Glycosciences Laboratory, Department of Medicine, Imperial College London, W12 0NN, London, United Kingdom.

E-mail: ds.barreira@campus.fct.unl.pt

Cancer remains one of the leading causes of mortality due to its complex nature and lack of efficient therapies [1]. Current efforts are focused on medicines targeted against molecules only expressed by cancer cells to maximise efficacy an reduce adverse effects. Such molecules include tumour- associated carbohydrates antigens (TACAs), such as the sialyl-Tn (STn), often associated with poor prognosis, reduced survival and immunosuppressive environment and not expressed in normal tissue. To target this TACA, we developed a monoclonal antibody (mAb) of murine origin that has great affinity for the STn [2]. To be used in human trials, the mAb was reformatted, having undergone species change and humanization processes, resulting in multiple humanized variants. To assure these humanized mAbs maintained their affinity and specificity for STn, multiple and complementary assays were conducted, such as: Glycan Microarray, Surface Plasmon Resonance (SPR), Flow cytometry, Western Blotting, Enzyme-linked immunosorbent assay (ELISA) and Saturation transfer difference (STD) Nuclear Magnetic Resonance (NMR).

The glycan microarray and SPR assays confirmed that the humanized mAbs maintained their STn specificity and high binding, in comparison with the original murine mAb. Flow cytometry and western blot analysis were performed using an MDA-MB231 cell line overexpressing STn in its proteins. These results demonstrated that our mAbs presented high cellular binding to STn-expressing cell line. Moreover, in the western blot the MDA cells were also submitted to enzymatic treatment by sialidase, which weakened the mAbs binding, as expected. Overall, these results confirm the specificity and high binding of our mAbs to STn and hint the sialic acid residue in STn as the preferred binding site for the mAbs.

Furthermore, since post-translation modifications of molecules can affect the binding of therapeutics antibodies, we decided to evaluate the impact of sialic acid O-acetylation in our mAbs binding. Using BSM as the STn carrier protein, we chemically removed its O-acetyl groups from the sialic acids'. The ELISA preliminary data indicated that the mAbs can bind to STn with its sialic acid either acetylated or deacetylated, contrary to the tested commercially available mAbs. Therefore, our mAbs are highly versatile and specific for the target antigen, not being affected by post-translation modifications. In parallel, to understand how the mAb-STn interaction occurs and the critical mAb binding sites in the antigen, we conducted a STD-NMR experiments. This assay revealed most of the critical interacting sites are in the terminal sialic acid of STn, besides some additional contacts involving GalNAc moiety.

Hence, anti-STn mAbs bring promise for further development as target therapy and may show biological activity of interest.

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Novel hydrogel membranes based on the bacterial polysaccharide FucoPol: Design and characterization

Diana Araújo[1,2], *Patrícia Concórdio-Reis*[1,2], *Matilde Martins*[1,2], *Vitor Alves*[3], *Filomena Freitas*[1,*]

1. Associate Laboratory i4HB – Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; 3. LEAF – Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

E-mail: df.araujo@campus.fct.unl.pt

Hydrogel membranes are polymeric structures that combine the dynamic mechanical properties and the water absorption characteristics of the hydrogels with the porous morphology and permeability properties of the thin membranes. These unique properties make them suitable for being used in several biomedical applications such as tissue engineering, drug delivery and wound care management [1]. This study reported, for the first time, the use of FucoPol, a fucose-rich polyanionic polysaccharide, for the preparation of hydrogel membranes. Recently, the cation-mediated gelation of FucoPol has been demonstrated [2]. In this study, the impact of FucoPol (0.69 – 2.81 %) and Fe^{3+} concentration (0.05 – 9.95 g/L) on the hydrogel membrane strength were evaluated through a response surface methodology (RSM). The results demonstrate that Fe^{3+} concentration had a lower influence on the hydrogel membrane strength, and the main differences were achieved by varying the FucoPol concentration. Therefore, three different FucoPol concentrations (1, 1.75 and 2.5 %) were combined with Fe^{3+} (1.5 g/L) and the resulting structures with around 2 mm of thickness were physically and chemically characterized. The FucoPol hydrogel membranes prepared had a water content above 97 wt% and their stability, morphology, mechanical and viscoelastic properties were influenced by the concentration of FucoPol used.

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Synthesis of Novel D-Glucuronamide-based Nucleoside Analogs Containing 1,2,3-Triazole Units

Domingos M. Manuel, Joana Rosa, Rafael Nunes, Nuno M. Xavier
Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências,Universidade de Lisboa Ed. C8, 5° Piso, Campo Grande, 1749-016 Lisboa, Portugal

E-mail: moraisbwill@gmail.com; nmxavier@fc.ul.pt (*)

The synthesis of D-glucuronamide-containing molecules has attracted increasing attention in the context of the search for new bioactive compounds, which is motivated by the biological profile reported for either natural or synthetic derivatives containing this saccharidic unit.[1]
Moreover, the synthesis of D-glucuronamide derivatives offers opportunities for performing structural variations in a regioselective manner at C-6 on a *gluco*-configured template by varying the N-substitution, which enables bioactivity tuning. Anomeric functionalization of N-substituted D-glucuronamides can also be easily achieved using common methods in carbohydrate chemistry.
Within our continuous interest in the synthesis of new potentially bioactive D-glucuronamide-based compounds, among them nucleoside analogs,[2,3] in this communication we report on the synthesis of innovative nucleoside analogs constructed on D-glucuronamide scaffolds and containing 1,2,3-triazole units. The synthesized molecules included [*N*-(glucuronamidyl)triazolyl]methyl phosphonates as potential sugar diphosphate mimetics, in which the (triazolyl)methyl phosphonate system was planned as a prospective neutral diphosphate mimetic, and glucuronamide-based (purinyl)methyl triazole nucleosides.
For their access, D-glucofuranuronolactone was used as starting material and key synthetic steps included amidation, furanose to pyranose isomerization, anomeric azidation, azide-alkyne 1,3-dipolar cycloaddition or Arbuzov reaction.

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Synthesis of novel glucofuranuronamide-based nucleosides

Euclydes P. Neto, Nuno M. Xavier
Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, 5° Piso, Campo Grande, 1749-016 Lisboa, Portugal

E-mail: iameuclydes@gmail.com

Over the last years the synthesis of new nucleosides and analogs has been of growing interest in the field of medicinal chemistry, being able to greatly fulfill demanding roles from antibacterial agents to anticancer or antiviral medication [1,2]. The research work presented in this communication was motivated by previous studies from our group that showed potent anticancer activities of *N*-dodecyl-containing glucuronamide nucleosides [3,4], particularly furanosyl derivates [4]. It aims to synthesize, and to analyze novel glucofuranuronamide-based compounds comprising different nucleobases and a *N*-propargyl group (Figure 1a) for further evaluation of their biological profile, namely their antiproliferative and antibacterial potential. Further synthetic work will focus the coupling of these molecules to azido pyranoses via triazole formation [5], towards novel pseudodisaccharide nucleosides (Figure 1b).

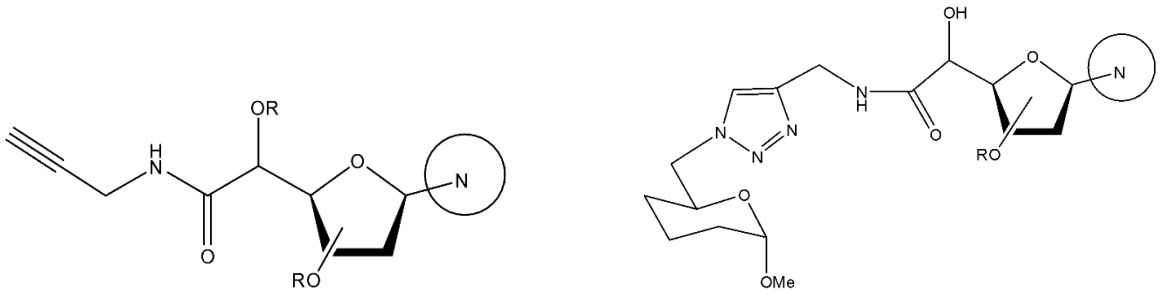

Figure 1 – a) General Structure of Synthesized Nucleosides; b) (Triazolyl)methyl amide-linked Pseudodisaccharide nucleosides.

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Using Mucin Glycoprotein Microarray to Identify Novel Glycan Ligands Recognized by Human Gut Microbiota

Filipa Trovão,[1,2,] Jodie Abrahams,[3] Antonio Di Maio,[3] Jin Yu,[3] Wengang Chai,[3] Ana Luísa Carvalho,[1,2] Ten Feizi,[3] Yan Liu,[3] Angelina S. Palma[1,2*]*

1. UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, Universidade NOVA de Lisboa, 2819-516 Caparica, Portugal; 2. Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, Universidade NOVA de Lisboa, 2819-516 Caparica, Portugal; 3. Glycosciences Laboratory, Department of Metabolism, Digestion and Reproduction, Imperial College London, W12 0NN, London, United Kingdom
*equal contribution

E-mail: f.trovao@campus.fct.unl.pt

The human intestinal microbiota houses a densely populated bacterial community, with a broad capacity to utilize dietary and host-derived glycans as nutrients. This promotes a complex crosstalk with the human host that impacts on nutrition, immune system regulation and mechanisms of pathology. How the intestinal mucus glycans are differentially exploited by commensal or pathogenic bacteria and influence the crosstalk with the human host largely remains to be elucidated at the molecular level [1].

Mucins are extensively *O*-glycosylated proteins, present in the mucus layer of the intestinal epithelial cells. *Bacteroides caccae* is considered a model of commensal intestinal bacterium that shows increased activity on mammalian-type glycans, particularly on mucin *O*-glycans, in conditions of low-fibre diet, and is implicated in susceptibility to infection, promoting states of dysbiosis [2]. During growth on mucin *O*-glycans, *B. caccae* was reported to show an increase expression of modular glycosyl hydrolases and metallopeptidases, which contain appended non-catalytic carbohydrate binding modules of family 32 (CBM32) [1], [2]. The hypothesis is that these modular proteins may facilitate mucin foraging by commensal bacteria and thereby enable pathogenic microorganisms to have access to glycan structures on the epithelial cell surface [2].

Here we will present the development of microarrays containing a broad repertoire of human mucin type glycoproteins for their application to identify the glycan ligands targeted by CBMs of human gut bacteria including *B. caccae*. These glycoproteins originated from diverse human epithelial cell types as found in the teratomatous tissues of ovarian cystadenomas. They present structurally diverse and complex O-glycans that are representative of the human O-glycome yet to be exploited [3].

The results obtained from the initial glycoprotein microarray screening analyses show differential binding by the CBMs to various cystadenoma samples, suggesting the expression of 'promising' glycan epitopes. These results are an important starting point for the development of mucin O-glycome 'Beam Search' microarrays generated from glycans of ligand-bearing human epithelial mucins [4]. These novel mucin glycoprotein arrays are invaluable tools for glycan ligand discovery by unravelling glycan structures as host-specific mediators of interactions of commensal and pathogenic bacteria with impact on human health.

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Extraction and characterization of polysaccharides present in wild endogenous mushroom species

Helena Laronha[1], *Filipe Coreta-Gomes*[1,2], *Manuel A. Coimbra*[1], *Elisabete Coelho*[1]
1. LAQV-REQUIMTE – Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 2. Coimbra Chemistry Center – Institute of Molecular Sciences (CQC-IMS), Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal

E-mail: h.laronha@ua.pt

Mushrooms are mostly composed by carbohydrates (50-65%) [1], namely polysaccharides, which have been described with bioactive properties. However, a detailed characterization of monosaccharides composition and glycosidic linkages of mushrooms polysaccharides are not well known, restricting the understanding of their structure-function relationship [2].
In this work, polysaccharide extraction and chemical characterization from four Portuguese endogenous mushroom species with gastronomic importance were studied – *Hydnum spp*, *Lactarius spp*, *Macrolepiota spp* and *Tricholoma spp*, as well as *Ganoderma spp*, used in Chinese traditional medicine. The free sugars were determined by gas chromatography and the mushrooms with gastronomic interest have a higher mannitol content, mainly the *Lactarius spp*. The aqueous extracts from all mushroom species presented proteins (8 – 44 %) and polysaccharides (26 – 78%). Mushrooms polysaccharides species with gastronomic interest were shown to be rich in glucose (34 – 71 mol%), galactose (10 – 22 mol%) and mannose (2 – 13 mol%) whereas aqueous extracts from *Ganoderma spp* were rich in glucose (58 – 65 mol%), mannose (6 – 12 mol%) and galactose (5 – 11 mol%). In both mushroom classes, after the extraction of water-soluble polysaccharides a residue richer in glucans (72 - 88 mol%). *Lactarius spp* aqueous extract was further fractionated with ethanol precipitation, using 50%, 80% and 90% of ethanol. The monosaccharides composition of polysaccharides presents in the different precipitates (Et_{50}, Et_{80} and Et_{90}) and supernatant (EtSn) showed that Et_{50} had the highest concentration of sugars, mainly glucose (84%) and galactose (8%). The glycosidic linkage analysis of Et_{50} was determined by gas chromatography and mass spectrometry of the partially methylated alditol acetates showed the presence of linear (1→4)-linked glucose (62%), terminally-linked glucose (13%), (1→4,6)-linked glucose (9%), indicative of glycogen-like structures. Also, it was observed the presence of (1→6) galactose (6%), (1→2,6)-linked galactose (4%) and (1→3)-linked galactose (3%), indicative of the presence of a galactan. This information will be used to relate the anticancer bioactivity and hypocholesterolemic effects of mushroom polysaccharides [2,3]. The aqueous extraction and ethanolic precipitation allowed to obtain mushroom polysaccharide with their structural features.

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Synthesis of novel 5′-azido/guanidino xylofuranosyl nucleosides

Jennifer Szilagyi,[1] *Rafael Nunes,*[1,2] *Nuno M. Xavier* [1]
1. Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, 5° Piso, Campo Grande, 1749-016 Lisboa, Portugal; 2. Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa

E-mail: fc52881@alunos.ul.pt

Synthetic nucleosides, nucleotides and their analogs/mimetics have attracted much attention in organic and in medicinal chemistry, owing to their ability to exhibit a variety of biological properties of therapeutic interest. Various examples of nucleoside and nucleotide analogs achieved clinical application as anticancer or antiviral drugs, acting through interference with nucleic acid biosynthesis [1,2]. The propensity of these types of molecules to show antimicrobial effects [3] or to inhibit cholinesterases [4,5] has also been reported. The search for new structures of nucleos(t)ide analogs that may potentiate innovative mechanisms of action and open new therapeutic opportunities remains of interest.

In this context, in this communication we report on the synthesis of novel 5-azido/guanidino nucleosides based on a xylofuranose template. The synthetic pathway employed diacetone-D-glucose as precursor and involved the access to an acetylated 5-azidoglycosyl donor and its further N-glycosydation with uracil or with a purine derivative. Conversion of the azido nucleosides into their guanidino derivatives was then exploited. An interesting result during the synthesis of the glycosyl donor precursor was the access to an imino sugar via an intramolecular Boyer reaction. Herein our results will be presented and discussed.

Figure 1. General structures of the synthesized nucleosides

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Highly porous magnetic chitosan-based scaffolds with potential for cancer treatment and bone regeneration

Joana Gonçalves,[1] *Helena Oliveira*,[2] *Claudia Nunes*[1], *Paula Ferreira*[1]
1. CICECO - Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 2. CESAM & Department of Biology, CICECO, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

E-mail: joanadfgoncalves@ua.pt

The development of new strategies to treat malignant bone tumours and simultaneously to regenerate bones defects produced by tumour resection is pivotal. In this work, it was developed magnetic chitosan-based scaffolds, through freeze-drying, with potential to simultaneously promote bone regeneration and kill residual cancer cells by magnetic hyperthermia. Chitosan was used as main matrix due to its suitable properties for bone tissue engineering, such as biocompatibility and antibacterial behaviour. There is, nonetheless, a drawback related to its poor mechanical properties, and low bioactivity [1]. Co-precipitated spherical magnetite nanoparticles (Fe_3O_4 NP) with sizes around 9 nm, and with a required thermal efficiency (Specific Loss Power of 98 W/g) were incorporated in the chitosan matrix at different quantities (10 and 20% w/w) aiming to increase scaffold mechanical properties and to be used as heat mediators in magnetic hyperthermia therapy (MHT). In addition, nano-hydroxyapatite (n-HA) at a 60/40% w/w chitosan n-HA ratio was added to improve their bioactivity.
The incorporation of the fillers enhanced the scaffolds mechanical properties, with an increase in the compressive modulus and strength from 1.3 and 0.1 MPa, for pristine chitosan scaffold, to 4.7 and 0.4 MPa, respectively, for the scaffolds with 20% w/w of Fe_3O_4 NP and n-HA. This was achieved without compromising the degree of scaffolds porosity (>80%) that is fundamental for cells proliferation. After 24 h of immersion in the culture medium, all the scaffolds exhibited a high swelling capability (> 800%), which increases the pore size, promoting cell attachment and growth. After 21 days of immersion in PBS with lysozyme, pristine chitosan has a degradation rate that seems to be exponential with a mass loss around 70%. Although, the incorporation of the fillers retarded their degradation, 10% of mass loss was observed for the scaffold with 20% w/w of Fe_3O_4 NP and n-HA. In addition, the potential cytotoxicity of the scaffolds was evaluated on the osteosarcoma cell line MG-63 using an indirect MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. A cell viability higher that 80% was found, revealing biocompatibility of the composites.
Overall, the produced scaffolds exhibited a highly porous structure that is essential for cells proliferation. The addition of magnetic Fe_3O_4 NP allowed to produce biocompatible scaffolds more robust and with lower degradation rate in cellular media. Therefore, these highly porous magnetic chitosan-based scaffolds could have a great potential to address, simultaneously, the regeneration of bone defect and eradicate residual cancer cells by thermal hyperthermia.

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Wall teichoic acids: a future target for antibiotics?

João Paquete-Ferreira[1,2], *Sérgio F. Sousa*[1,3], *Maria João Romão*[1,2], *Alexandra R. Fernandes*[1,4], *Teresa Santos-Silva*[1,2]*

1. Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2. UCIBIO, Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 3. UCIBIO, Applied Molecular Biosciences Unit, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernani Monteiro, 4200-319 Porto, Portugal; 4. UCIBIO, Applied Molecular Biosciences Unit, Life Sciences Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

E-mail: tsss@fct.unl.pt (*)

Bacterial biofilms represent a serious health threat worldwide as they are often associated with the failure of indwelling medical devices and persistent and chronic infections. To date, there are no drugs available to address this problem, making the development of new therapeutic strategies of pivotal importance. [1]

Wall teichoic acids (WTAs) are important components of the gram-positive cell wall and are crucial for bacterial survival and adherence to surfaces. Depleting bacteria from WTAs impairs their growth and division, increasing their susceptibility to antibiotic drugs and, hence, contributing to fighting infections. The biosynthesis pathway of WTAs is quite complex; however, the last step in this pathway occurs outside the cell and is accomplished by the LytR. CpsA-Psr - LCP - family of proteins, making these very interesting targets. [2,3] The goal of this work was to structurally and functionally characterize two LCP proteins, LytR and Lcp2, from *Streptococcus dysgalactiae subsp. dysgalactiae* (SDSD), a bovine pathogen. Using X-ray crystal diffraction, we determined the 3D structure of the LytR LCP domain at 2.80 a resolution.

Among the conserved residues, four histidines have been proposed to be responsible for stabilizing the physiological substrate of the enzyme at the active site. Site-directed mutagenesis was used to mutate these residues to alanine. Differences in the catalytic activity were evaluated using the malachite green assay. The results revealed that none of the mutants completely abolished pyrophosphatase activity, but all showed a significant decrease when compared to the wild-type protein (20% lower activity).

These results put us in a good position to identify the enzyme's molecular determinants, identify molecules that act as inhibitors, and help in the development of new antibiotics and antibiofilm drugs.

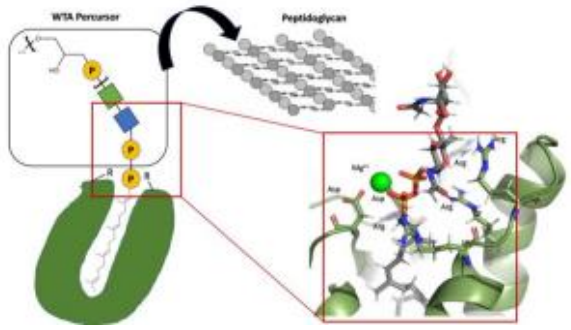

Figure 1 – Crystallographic structure of SDSD LytR at 2.80 Å resolution with *in silico* modeled LIIa WTA.

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Insight into the role of RfbC and FucS on the composition of the released polysaccharides of the cyanobacterium *Synechocystis* sp. PCC 6803

João Pissarra,[1,2,3] Marina Santos,[1] Sónia S. Ferreira,[4,5] Cláudia Nunes,[6] Manuel A. Coimbra,[5] Paula Tamagnini,[1,3] Rita Mota[7]

1. i3S - Instituto de Investigação e Inovação em Saúde, IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, R. Alfredo Allen 208, 4200-135, Porto, Portugal; 2. ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313, Porto, Portugal; 3. Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4169-007, Porto, Portugal; 4. CICECO, Departamento de Química, Universidade de Aveiro, Campus de Santiago, 3810-193, Aveiro, Portugal; 5. LAQV-REQUIMTE, Departamento de Química, Universidade de Aveiro, Campus de Santiago, 3810-193, Aveiro, Portugal; 6. CICECO, Departamento de Engenharia de Materiais e Cerâmica, Universidade de Aveiro, Campus de Santiago, 3810-193, Aveiro, Portugal; 7. acib GmbH - Austrian Centre of Industrial Biotechnology, Konrad-Lorenz-Straße 20, 3430 Tulln, Austria

E-mail: joao.pissarra@ibmc.up.pt

Cyanobacteria are photosynthetic prokaryotes that can produce extracellular polymeric substances (EPS) that can remain attached to the cell (CPS) or be released to the extracellular environment (RPS). These EPS are mainly composed by heteropolysaccharides with an unusually high diversity of monosaccharides, including acetyl-, methyl-, and amino-sugars, peptide moieties, and sulphate groups, conferring them characteristics that make these polymers attractive for several biotechnological and biomedical applications [1]. However, despite recent efforts [2, 3], their full potential remains locked behind the lack of knowledge on the intricate mechanisms involved in the cyanobacterial EPS production and export. Aiming at better understanding how to control the production and/or to fine-tune the characteristics of these polymers, the model cyanobacterium *Synechocystis* sp. PCC 6803 was used to generate two knockout mutants on genes encoding proteins putatively involved in the biosynthesis of the deoxyhexoses dTDP-L-rhamnose and GDP-L-fucose: *rfbC* (*slr0985*) and *fucS* (*sll1213*). Our results indicate that both mutants display a clumping phenotype at low cell densities, and that the *fucS* mutant grows slower than the wild type. Regarding the RPS, the *rfbC* mutant produces significantly less than the wild type but its monosaccharide composition showed no major differences compared to wild type. Interestingly, in this mutant the transcription of another putative copy of *rfbC* (*slr1933*) seem to be upregulated. In contrast, the evaluation of the monosaccharide composition of the RPS from the *fucS* mutant showed no detectable levels of fucose and rhamnose, suggesting that the presence of a fucose residue is necessary for the incorporation of a rhamnose. Further studies, namely the generation of multiple mutants are required to clarify various steps of these biosynthetic pathways.

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Bioprospecting novel bacterial exopolysaccharide producers

Kleyde Ramos,[1] *Cristiana Torres*,[1] *Filomena Freitas*[1]
Chemistry Department, NOVA School of Sciences and Technology, University NOVA of Lisbon, Campus da Caparica, 2825-519 Caparica, Portugal

E-mail: kd.ramos@campus.fct.unl.pt

Context: Extracellular polysaccharides (EPS) have a wide range of physicochemical properties, being considered promising polymers with several applications in the industrial sector. [1] Polysaccharides are produced and secreted by a variety of microorganisms, including bacteria, cyanobacteria, fungi, microalgae, and are also found in plants (e.g. starch and pectins), algae (e.g. alginate and agar), and animal sources (e.g. chitosan).[2,3]

Aims: The work is part of the PROMICON project (101000733 - PROMICON - H2020-FNR-2020 / H2020-FNR-2020-2), and the main objective was to isolate novel bacteria from different microbiomes existing in nature and to develop innovative EPS production processes.

Methodology: Samples collected from different habitats, namely: soil (Mata Medos, Caparica; Adrenal Bussaco, Aveiro), river sediments (Porto Brandão and Sapal Corroios, Setúbal) and plant roots (*Dicksonia antartica*, *Phaseolus lunatus* and *Carpobrotus edulis*). Then, isolated colonies were selected for testing EPS production in liquid medium. The inoculum was then prepared by placing an isolated colony from the agar plate in 25 mL of LB medium. At the end of the assay, the broth was centrifuged, and the supernatant was dialyzed and lyophilized. The sugar composition of the samples was determined by high performance liquid chromatography (HPLC). In addition, assays were also carried out in a mineral medium (Medium E), supplemented with glycerol. The produced EPS were extracted and analysed to determine their composition.

Results: Initially, 38 cultures were isolated from the agar plates, out of which 11 isolates were selected based on their ability to produce EPS, as well as on the biopolymers' sugar composition, particularly their content in rare sugar monomers (e.g., rhamnose, fucose, glucosamine, ribose). The selected isolates were: two cultures from Adrenal Bussaco (AB-2 and AB-5), one from Mata Medos (MM-1), one from Porto Brandrão (PB-2), two cultures from Sapal Corroios (SC-3 and SC-4), two from *Carpobrotus edulis* roots (RD-1 and RD-5), one from the *Phaseolus lunatus* roots (RL 3) and two from the *Dicksonia antartica* roots (FB-1 and FB-5), whose performance in terms of EPS secretion was evaluated in bioreactor cultivation. The produced EPS were characterized for their physical-chemical and functional properties, envisaging their future use in specific applications.

Conclusion: Nature is composed of a large and diverse microbial community, capable of producing a variety of EPS with distinct properties, which may have great potential and be promising in applications in the industrial sector.

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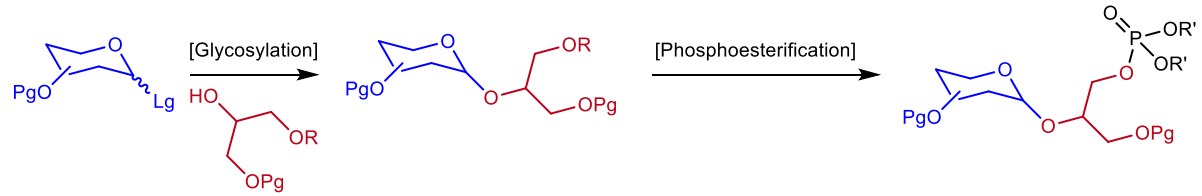
Synthesis of a phosphoglycoglycerol inspired from Nature

Luís Pinheiro,[1] *Patrícia Máximo, Paula S. Branco*[1*]
1. REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516, Caparica, Portugal

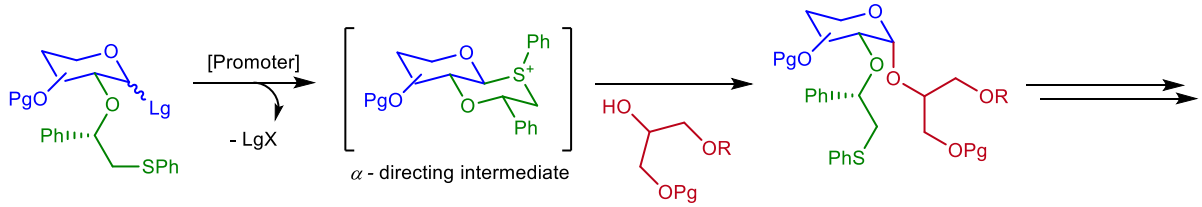
E-mail: paula.branco@fct.unl.pt(*)

Phosphoglycoglycerols (PGG) are constituents of cell walls and membranes and have a variety of roles in an organisms' metabolism.[1] Natural products of this kind are reported to have a multitude of bioactivities, namely anti-inflammatory activity.[2,3] In this work, we present a synthetic pathway to attain a PGG using as lead a natural product isolated by our group. A 1,3-diprotected glycerol is reacted with a galactosyl donor in the presence of NIS/TfOH. The acetyl of the glycerol moiety is removed and the galactoglycerol is reacted with $POCl_3$ and octanol in a one-pot reaction to achieve the PGG derivative. Alternatively, a stereospecific pathway is being developed to achieve solely the α anomer of the PGG. This route employs a chiral auxiliary moiety that is coupled to the C-2 galactosyl position. After activating the anomeric carbon, the chiral auxiliary forms a 6-membered ring intermediate through an in situ β attack in C-1 and directing the glycosyl acceptor towards the α position.[4]

Present work



Ongoing work



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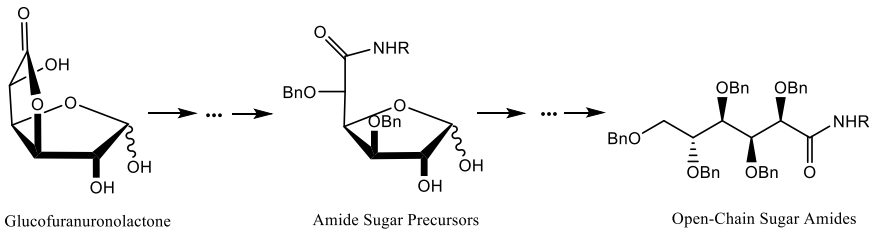
Synthesis of Open-Chain carbohydrate amides as cholinesterase inhibitors for Alzheimer's disease

Ismael M.I[1,2], *de Matos A. M.*[2], *Rauter A. P.* [2]
1. Dep Química, Unidade I&D FibEnTech, Universidade da Beira Interior, Av. Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal; 2. Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

Email: miismael@fc.ul.pt

Alzheimer's disease (AD) is the most common cause of dementia among elderly people. This severe neurodegenerative disorder is characterized by progressive memory and cognition loss, leading to disability and, eventually, to death [1-4]. According to the 2020 update of the World Alzheimer Report, there are over 50 million people worldwide living with dementia — a number that is expected to double every 20 years, reaching 152 million in 2050 [5]. Because of the well-established degeneration of cholinergic neurons in early AD, acetylcholinesterase (AChE) inhibitors are still to this day used as the first-line treatment for AD. These drugs are able to provide symptomatic relief to AD patients through the increase of acetylcholine brain levels [6].
Following our recent report of new open-chain sugar amide derivatives with good biological activities as AChE inhibitors [7], in this communication we disclose the development of a novel synthetic route for this class of compounds starting from the commercially available glucuronolactone (Scheme 1). Several structurally diverse amide sugar precursors have already been accessed in good yields over 4 steps. Furanose ring opening and benzylation gives the target open-chain carbohydrate amides for the AChE inhibition assays.



Scheme 1. Synthetic pathway towards new carbohydrate-based AChE inhibitors

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A novel enzyme collection as a biofilm degradation strategy for *Pseudomonas aeruginosa* and *Staphylococcus aureus* infection therapies

Magda Ferreira,[1,2] *Alexandra C. Oliveira,*[1,2] *Catarina Caseiro,*[1,2] *Joana L.A. Brás,*[1,3] *Carlos M.G.A. Fontes,*[1,3] *Pedro Bule,*[1,2]

1. CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisbon, Portugal; 2. Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS); 3. NZYTech, Genes & Enzymes, Campus do Lumiar, Estrada do Paço do Lumiar, 1649-038 Lisbon, Portugal.

E-mail: magdaferreira@fmv.ulisboa.pt

Biofilm-associated infections are a major concern in medical care. The current therapies are hampered by the low penetration of antimicrobials in biofilms. Biofilms are bacterial communities surrounded by an extracellular matrix, which has exopolysaccharides as one of its major components. As such, enzymatic polysaccharide degradation could pose an alternative strategy to increase the antimicrobial penetration and consequently bacteria eradication. In this context, we constructed a library of highly specific carbohydrate active enzymes (CAZymes) with potential to promote biofilm degradation by digesting the main exopolysaccharides produced by *S. aureus* (PNAG) and *P. aeruginosa* (Pel, Psl and alginate).

A library of 135 CAZymes was established through a bioinformatic analysis. Genes encoding the enzyme library were chemically synthesized, cloned, expressed and purified in a high-throughput platform. Enzyme activity was tested using chromogenic substrates and biofilms biomass assessment.

All proteins were soluble and 30 had high production yields. A preliminary analysis of the enzymes' efficacy allowed selecting 4 enzymes capable of efficiently depolymerize PNAG, Psl, Pel and alginate, respectively, promoting bacterial biofilm degradation. Three enzymes were also found to be able to effectively inhibit PNAG, Pel or Psl-based biofilm formation. One alginate lyase in particular, was capable of promoting a biofilm biomass reduction of around 70% after 6.5h of incubation with 24h old biofilms of a mucoid *P. aeruginosa* strain.

This study allowed the identification of novel enzymes with the potential for *P. aeruginosa* and *S. aureus* biofilm degradation. Overall, we expect that these enzymes, in combination with antimicrobials, can constitute a promising alternative approach for the treatment biofilm-associated infections.

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Challenging the virulence role of sialidases in *Gardnerella* spp.

Márcia Sousa[1,2], *Filipa Grosso,*[1,2] *Magdalena Ksiezarek,*[1,2] *Teresa Gonçalves Ribeiro,*[1,2] *Luísa Peixe*[1,2*]

1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

E-mail: lpeixe@ff.up.pt(*)

Sialidases, a virulence factor of Gardnerella spp. encoded by the nanH2 and nanH3 genes, are enzymes that cleave α-2-3 and α-2-6–linked sialic acids present in N-linked and O-linked glycan substrates [1,2], relevant in mucosa of urogenital, gastrointestinal and airways tracts [3]. In pathogenic conditions, such as the bacterial vaginosis typically attributed to Gardnerella vaginalis [4], this enzymatic activity results in the degradation of vaginal mucus and subsequent bacterial adhesion and biofilm formation, being also associated with local immunoglobulin A (IgA) degradation. Gardnerella has been isolated also in the urogenital tract of healthy women, although the production and diversity of sialidases among this genus is barely characterized in Gardnerella of both healthy and unhealthy microbiota. In this study, we aim to evaluate sialidase production and molecular structure of NanH amongst a collection of Gardnerella isolates obtained from the urogenital tract of healthy women (HW) and women diagnosed with overactive bladder (OAB).

Thirteen Gardnerella isolates (Gardnerella vaginalis, n=5; Gardnerella genomic species 3, n=7; Gardnerella genomic species 8, n=1), obtained from urine and vaginal swabs from HW (n= 11) and OAB (n= 2), were subjected to Whole Genome Sequencing (Illumina). Sialidase activity was screened by filter paper spot test, adding lysed bacterial pellet to neuraminic acid fluorogenic substrate (4-MU-N-acetylneuraminic acid) [5]. Nucleotide and aminoacidic sequences of nanH clusters were detected using the MyDbFinder 2.0 tool (Center for Genomic and Epidemiology) and compared with available sequences retrieved from NCBI (Gardnerella piotti strain JCP8151B, accession number ATJH01.1).

We detected the nanH3 gene in 11 isolates (from HW and OAB), but only 10 displayed sialidase activity. The exception corresponded to one isolate (from 1 OAB-donor) that presented a sialidase domain with 18 deletions, and genes coding for 4 hypothetical proteins of unknown function surrounding it. In all the others isolates, the complete nanH3 cluster (comprising the signal peptide, sialidase domain and transmembrane domain) was present, although nucleotide (94.07 - 99.63% identity to JCP8151B) and aminoacidic (95.14 - 99.51% identity to JCP8151B) diversity was observed.

Our findings demonstrate the limitations of using only the nanH genes detection to infer the presence of sialidase activity. Moreover, we demonstrated the presence of sialidase activity in urine and vaginal isolates of both HW and OAB. The aminoacidic diversity observed may suggest the existence of sialidases with activity towards different sialo glycoconjugates, conditioning bacterial tropism and/or adaptation to host's particular features. Future studies are necessary to clarify the specificities of sialidases' activity in the context of the healthy urogenital tract, and their influence in disease development.

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Altered expression of sialyl Lewis X in experimental models of Parkinson's disease

Maria João Nunes[1], Andreia Neves Carvalho[1], Alexandra I. Rosa[1], Paula A. Videira[2,3], Maria João Gama[1], Elsa Rodrigues[1], Margarida Castro-Caldas[1,2]

1. Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal; 2. UCIBIO, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 3. CDG & Allies - Professionals and Patient Associations International Network (CDG & Allies - PPAIN), NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

E-mail: mcastrocaldas@ff.ulisboa.pt

The cellular mechanisms underlying neurodegeneration in Parkinson's disease (PD) are still not fully understood, but impaired proteostasis, oxidative stress, and neuroinflammation are key events in PD pathology.
Glycosylation is an important post-translational modification that affects protein function, cell-cell contacts, and the inflammatory response, and can be modified in pathologic conditions. Although the involvement of aberrant glycosylation has been proposed for PD, the knowledge of the heterogeneity and functions of glycans in PD is minimal.
Sialyl Lewis x (sLeX) is a sialylated and fucosylated tetrasaccharide mainly expressed in leukocytes, with an essential role in cell-to-cell recognition processes. Pathological conditions and pro-inflammatory mediators can up-regulate sLeX expression on cell surfaces, which has an important role in intracellular signalling and immune function. However, the expression of this glycan in PD models and, in particular, its importance in PD is still completely unknown. Here, we show that in the brain of mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin-based model of sporadic PD, there is activation of glycation related pathways, triggered by oxidative stress, and involved in pathogenic mechanisms underlying neurodegeneration in PD. Importantly, our results show expression of glycoproteins decorated with sLeX and its rate-limiting fucosyltransferase enzyme in mice brains, and that sLeX is expressed in neurons and microglia and up-regulated upon MPTP treatment. However, the underlying mechanism that drives increased sLeX epitopes, the nature of the glycoprotein scaffolds and their functional importance in PD are still unknown. Yet, our data indicate for the first time that sLeX is expressed in the brain, especially after damage, suggesting a role in neuronal signalling and immunomodulation.

Amino sugar phosphate ManNAc-6-P as template molecule to modulate sialylation status in GNE myopathy

Barbosa M,[1,2,3*] *Pereira B,*[1,2,3] *Cunha J,*[1,2,3] *Videira PA.* [1,2,3]

1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 3. CDG & Allies – Professionals and Patient Associations International Network (CDG & Allies – PPAIN), Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.

E-mail: mn.barbosa@fct.unl.pt (*)

GNE myopathy (GNEM) is an ultra-rare disease that manifests in early adulthood causing progressive distal muscle atrophy and weakness. GNEM results from mutations in the *GNE* gene, leading to decreased sialic acid (Sia) production [1]. Although there are still no approved therapies for GNEM, sialylation-increasing therapies such as *N*-acetyl-D-mannosamine (ManNAc) has been proposed as a promising approach. ManNAc, an uncharged monosaccharide, has indeed reached the phase 2 of clinical trials (ClinicalTrials.gov NCT04231266); however, severe gastrointestinal adverse effects have been reported [2]. ProDGNE project (EJPRD/0001/2020) arose then to answer the unmet needs of GNEM patients, by fostering the pre-clinical development of an innovative class of drugs, designed to deliver intracellularly ManNAc-6-P that bypasses the defective GNE enzyme.

In this work, we aimed to evaluate the effect of ManNAc and ManNAc-6-P supplementation in the sialophenotype of GNEM cell model, and to assess whether these intermediates of the Sia biosynthesis pathway alter the immune response, by flow cytometry.

Although supplementation with non-cytotoxic concentrations of ManNAc and ManNAc-6-P (0.1 mM) did not show a significant increase in cell sialylation, a decrease of the expression of the major histocompatibility complex (MHC)-I in GNEM cell model was observed. After supplementation with ManNAc and ManNAc-6-P, the levels of interleukin (IL)-6 measured in the GNEM cell supernatant rose significantly compared to the basal levels, being even similar to those observed for normal cells.

Overall, and even though no restoring of the Sia content was observed with both compounds, the effect upon immune-related molecules suggests an improvement in an altered immune function and the potential involvement of pathways other than sialylation. Still, there is room for improvement, especially due to the high polarity of these compounds that hinders their bioavailability. Further work with newly synthesised prodrugs, using ManNAc-6-P as template molecule will be conducted to help guiding the selection of lead candidates that are expected to compensate for the defective GNE enzyme, restore Sia cellular expression, and rehabilitate physiological function both *in vitro* and *in vivo* GNEM models.

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Salmonella/host-glycans crosstalk: Structural perspective

Marlene Leite,[1,2] Cátia Oliveira Soares,[1,2] Angelina Sá Palma,[1,2] Jorge Dias,[1,2] Filipa Marcelo,[1,2] Helena Coelho[1,2]*

1. UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Largo da Torre, 2829-516 Caparica, Portugal

E-mail: mp.leite@campus.fct.unl.pt ; h.coelho@fct.unl.pt (*)

Salmonellosis, a disease caused by *Salmonella* bacteria, is a global health issue and is frequently the main factor in food-borne diseases [1]. According to the Centers for Disease Control and Prevention, there are roughly 1.35 million cases of salmonellosis reported a year in the U.S., along with 26,500 hospitalizations and 420 deaths [2].
The starting point of bacterial infection is the adhesion to the host cells. The adhesion of the bacteria is ensured by specific interactions between adhesins from the pathogen and receptors from the host cells, which can be proteins, lipids, or glycans from glycoproteins [3].
Nowadays, there is limited information about *Salmonella* adhesins. There is two different types of adhesins that can mediate the attachment between the bacteria and host cells, the fimbrial and afimbrial adhesins. The fimbrial adhesins are long filaments that stick out from the bacterial surface and they are composed of many subunits, while the afimbrial adhesins are giant proteins with different domains attached to the membrane [3,4].
Until now, various *Salmonella* fimbrial gene clusters were identified, however, the binding specificities are not well known. Still, it is reported that three of them bind glycans: Pef, StdD and FimH. Pef binds the Lewis X blood group antigen, StdD binds to α (1,2)-fucose and FimH recognize mannose residues, erythrocytes and glycoprotein-2 [3].
Afimbrial adhesins important for the interaction with polarized epithelial cells is the SiiE. This adhesin facilitates the initial interaction by binding to N-acetyl-glucosamine and/or α2,3-linked sialic acid, and permits further invasion [4].
SiiE is a giant adhesin constituted of 53 repeats of bacterial immunoglobulin (BIg) domains, followed by an N-terminal domain with β-sheet and coiled-coil repeats. Also, SiiE present two Ca^{2+} binding sites with a conserved aspartate or glutamate residues per BIg domain [4].
Thus, in order to discover new targets and tactics to obstruct the *Salmonella* infection process, the aim is to characterize the structure of the adhesins from *Salmonella* by Nuclear Magnetic Resonance techniques and study the recognition process of host glycans by these adhesins.

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Carbohydrate characterization of coffee pulp produced in Azores

Miguel F. Galrinho[1], *Cláudia P. Passos*[1], *Rita C. Alves*[2], *Anabela Costa*[2], *M. Beatriz P. P. Oliveira*[2], *Sónia S. Ferreira*[1], *Manuel A. Coimbra*[1]

1. LAQV/REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 2. LAQV/REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy of the University of Porto, 4050-313 Porto, Portugal

E-mail: miguel.fgalrinho@ua.pt

Coffee is one of the most popular beverages, consumed every day by millions of people. However, during the coffee processing, by-products are discarded despite the presence of many compounds of interest such as polyphenols and flavonoids (antioxidant properties), or oligosaccharides (prebiotic effects) (1-2). These compounds can help reducing the risk of metabolic syndrome, which comprises conditions such as diabetes, and high blood pressure, and high triglycerides increasing the risk of strokes and blood vessels damage (3). Coffee pulp stabilization can be achieved by drying, and this work aimed to compare the influence of two different methods of water removal (freeze-drying and 40°C drying) on the pulp composition.
Mature coffee cherries were manually collected from *Coffea arabica* plants ("Caturra Vermelho" cultivar) produced in S. Miguel, Azores. The fruits were washed with tap water, dried with blotting paper, and manually depulped. The pulp was then divided in two groups, freeze-died and dried at 40°C in an oven. An analysis by the dinitrosalicylic acid (DNS) method allowed to quantify the reducing sugars in these pulps, followed by an ethanolic extraction (85%, v/v) at 80°C for 10 min. The insoluble material recovered was then subjected to another extraction but with hot water at 80°C for 2h. Each initial sample, supernatant (ethanol and water), and residues, were then characterized in terms of carbohydrate composition using GC-FID derivatization. To complement the analysis, uronic acids were also quantified.
The colorimetric DNS method allowed to quantify the reducing sugars, showing a percentage of 29.6±0.4% for the freeze-dried pulp and 26.4±1.6% for the 40°C dried, correspondent to ~10% losses when oven dried. A more complete characterization by a chromatographic approach for sugar analysis, revealed that the pulps contained 43.9+3.6% (freeze-dried) and 29.9+5.8% (40°C dried), respectively, as total sugar content, most of it corresponding to free sugars (glucose, fructose, and arabinose). After the ethanolic extraction process that recovered free sugars and an oligomeric fraction, the hot water supernatants showed a high content of uronic acids (~40%) due to the pectin extracted.
This characterization showed how different extraction methodologies can influence the coffee pulp-deriving composition, allowing to enrich extracts with different carbohydrates. Azores pulp can be a sustainable source of pectin, but also arabinose, a trait that can be explored due to the ability of this monomer to improve conditions in the gut, and reducing blood glucose levels (4).

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Taste properties modulation in the development of food products: the match between phenolic compounds and polysaccharides

Mónica Jesus,[1]*Elisabete Coelho*[2], *Bras, Natercia F.*[1], *Nuno Mateus*[1], *Victor de Freitas*[1], *Susana Soares*[1]

1. REQUIMTE, LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal; 2. REQUIMTE-LAQV, Department of Chemistry,University of Aveiro, Aveiro, 3810-193, Portugal

E-mail: up201405882@up.pt

Food industry is developing healthier and more sustainable food products. This include (re)designing foods to require less processing while still being tasty and healthful. This trend highlights plant-based foods and so on the phenolic compounds (PCs). PCs are responsible for key organoleptic properties, i.e. colour and taste [1], and they have several health benefits. Astringency (AST) and bitterness are both sensory qualities associated with PCs that, when strong, are unpleasant [2]. At the end, no matter the health benefits, taste is the key to food choices by consumers. The mechanism widely accepted for AST is the precipitation of salivary proteins (SPs) by PCs [3]. Among others, polysaccharides (POL) are a promising tool for the AST modulation. POL are known to reduce astringency by two mechanisms: by the interaction with PCs and that limit additional binding to SPs and/or other oral constituents (competition mechanism); or by binding to PCs-SPs complexes and reducing their precipitation (ternary mechanism). In this framework, a gap is to establish a structure-effect relationship to reach a targeted modulation. In this study and focusing on the competition mechanism, a pre- screening using molecular docking was performed to assess the binding affinities of several POL and PCs compounds. The Autodock 4.2 software was used to dock various oligosaccharides with different structural features to several families of PCs. All solutions were ranked by the binding free energies, and the best docking solutions were analyzed using the Visual Molecular Dynamics (VMD) program. The preferred binding sites on each oligosaccharide as well as the main type of intermolecular interactions were taken into consideration to select the promising POL-PCs complexes. Among the examined POL, the examples that showed higher affinity with a greater number of PCs were the arabinogalactan-proteins, arabinoxylans, xyloglucans, mannoproteins and pectic polysaccharides with higher neutral sugar content. In general, side chains and residues at terminal position appears to be significant structural features. Neutral sugars in pectic polysaccharides appear to increase the affinity with PCs. In mannoproteins, *N*-acetylgalactosamine residues and the contiguous branched mannose residues was also preferred binding site. These approaches will be used to select the POL structures able to modulate astringency. This will be followed by *in vitro* studies to verify the *in silico* results and efficacy in AST modulation.

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Nanofibrillated cellulose/gellan gum hydrogel based bioinks for 3D bioprinting of skin cells

Nicole S. Lameirinhas,[1] Maria C. Teixeira,[1] João P. F. Carvalho,[1] Bruno F. A. Valente,[1] Ricardo J. B. Pinto,[1] Helena Oliveira,[2] Jorge L. Luís,[3] Liliana Pires,[3] José M. Oliveira,[3] Carla Vilela,[1] and Carmen S. R. Freire[1]

1. CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 2. CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal; 3. School of Design, Management and Production Technologies Northern Aveiro, ESAN, Portugal

E-mail: nicoleslameirinhas@ua.pt

Three-dimensional (3D) bioprinting is promoting a great advance in many biomedical fields, including diseases research and drugs investigation, among others [1]. Nowadays, there are many synthetic and natural polymeric materials that can be used to develop bioinks, particularly hydrogel-based bioinks. Natural polymers, such as gellan gum (GG), are particularly interesting due to their gelling ability, crosslinking mechanism, and biocompatibility [2]. Nonetheless, GG hydrogels have poor printability and structural stability. One strategy to overcome this limitation is the development of nanocomposite hydrogels using, for example, nanofibrillated cellulose (NFC) [3]. Here, we combined NFC with GG in four different NFC:GG mass proportions, namely 90:10, 80:20, 70:30, and 60:40, to develop bioinks with different compositions for 3D bioprinting applications. All the obtained hydrogels showed a shear-thinning behavior, with shear viscosities decreasing with increasing shear rate. Also, the increasing content of GG, as well as its combination with NFC, improved the rheological properties (increasing both storage (G') and loss (G'') moduli) and the G' recovery of the hydrogels (up to 82.63 ± 1.21% for the hydrogel 60:40). Mechanical properties were also enhanced, with an increase in both compressive stiffness and stress (up to 337.16 ± 34.03 Pa and 47.17 ± 3.59 kPa, respectively, for the hydrogel 60:40). Moreover, these hydrogels were non-cytotoxic towards human keratinocyte (HaCaT) cells, with cell viabilities above the 70% threshold for up to 72 h. The hydrogel 60:40 was loaded with 3×10^6 cells mL^{-1} of HaCaT cells and bioprinted. Until day 7 after bioprinting, the cell viability was kept very high (90 ± 3%). This way, cell-laden NFC:GG bioinks are promising alternatives for 3D bioprinting applications.

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Profiling glycan epitopes in Triple Negative Breast Cancer to explore interactions with tumour-associated macrophages

Lopes, NF[1,2,3,4], Trindade, G[1,2], Pinheiro, BA[3,4], Palma, AS[3,4], Brito, C[1,2]**
1. iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901, Oeiras, Portugal; 2. Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Av. da República, 2780-157, Oeiras, Portugal; 3. UCIBIO, Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, 2859-516, Caparica, Portugal; 4. Associate Laboratory i4HB-Institute for Health and Bioeconomy, School of Science and Technology, NOVA University of Lisbon, 2819-516, Caparica, Portugal
* author's contribution was equally important

E-mail: nlopes@ibet.pt

The scarce availability of therapeutic options in triple negative breast cancer (TNBC) lead to research targeting the suppression of the immune system cytotoxic action by tumour-associated macrophages (TAMs) for novel therapies [1]. Still, there remains a high number of refractory patients to current TAM-targeting therapies, with increasing evidence highlighting knowledge on the cell-cell signalling axes underlying TAM differentiation is required [1]. Aberrant glycans overexpressed at the surface of TNBC cells have been recently highlighted to bind to specific lectins at the surface of TAM, inducing an immunosuppressive TAM phenotype [2]. A research challenge to study novel therapies is to develop experimental models that accurately mimic key events of TNBC, facilitating the translation of therapies into a clinical setting [3]. This work was hence focused on developing and employing breast cancer (BC)-based cell models to explore the axes between glycans from TNBC and lectins from TAM, which remain widely unknown. We aim to identify glycan-based epitopes expressed at the surface of TNBC cells and possible modulators of TAM differentiation.
Cell lines of different BC subtypes were expanded in monolayers and aggregated into multicellular spheroids. BC spheroids were encapsulated within alginate along with human primary monocytes to establish co-cultures. Our results showed larger TAM populations in models of TNBC, compared to those of the less aggressive luminal BC subtype (control). We also detected high expression of anti-inflammatory markers at the surface of TAM induced by TNBC, supporting their immunosuppressive potential. The profiling of released cytokines from TNBC co-cultures further showed increased detection of CCL2, IL-8, IL-10 and lactate, described to modulate the microenvironment towards immunosuppression and induce TAM differentiation. The models representing different BC subtypes were then profiled for glycan epitopes on tumour cells. For initial studies, the protein extracts from BC, from monolayer and spheroid cultures, were probed using a lectin microarray with 95 immobilised lectins which showed an increase of detection with lectins with specificity for core fucosylated N-glycans, and for Lewis B and N-acetyl-lactosamine structures, in both luminal and TNBC cells. Different glycan binding profiles were observed distinguishing BC subtypes, with a higher detection with lectins targeting B and H antigens and Gal/GalNAc-containing glycans for TNBC cells. Reverse phase microarrays with immobilised protein extracts of BC models were also applied to detect binding of immune-cell receptors. Binding of SIGLEC-9 and -15 to TNBC glycoproteins was detected with a higher intensity, compared to extracts from luminal BC models. These results support the interactions between TNBC epitopes and TAM lectins, and further highlight the potential of this work to unveil novel markers of TAM differentiation.

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Boosting insights on the immunopathology of PMM2-CDG

Pedro Granjo,[1,2,3] *Carlota Pascoal*,[1,2,3], *Patrícia Mexia*,[1,2,3] *Ana Rita Grosso*,[1,2] *Vanessa dos Reis Ferreira*,[1,2,3] *Paula A. Videira*[1,2,3]

1. UCIBIO – Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2. Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 3. CDG & Allies-Professionals and Patient Associations International Network, 2829-516 Caparica, Portugal

E-mail: p.granjo@campus.fct.unl.pt

Background: Congenital Disorders of Glycosylation (CDG) are a group of ~170 rare diseases characterised by anomalies in the glycosylation pathways, mostly resulting in a multi-systemic involvement [1]. For some CDG, immune system abnormalities have a significant impact on the patients' quality of life [2]. In PMM2-CDG, the most common CDG, recurrent and severe infections account for nearly 20 % of mortality in the first year of life [3]. Recently, we have identified a correlation between inflammation and severe clinical phenotypes [4]. Yet the underlying mechanisms are still unclear.
Objectives: We aimed to decipher the cellular and molecular mechanisms behind the immunological (dys)function in PMM2-CDG patients, using patient-derived fibroblasts submitted to TNF-α as a model of a proinflammatory insult.
Methodology: Three patient-derived fibroblasts from healthy (WT) and three PMM2-CDG individuals were stimulated with TNF-α (n = 3), followed by RNA sequencing. We identified the differentially expressed genes (DEGs) in response to the stimulus using R statistical analysis and performed a functional enrichment analysis. To validate the *in silico* results, *in vitro* assays were carried out.
Results and Discussion: Differential expression analysis depicted 305 and 222 altered genes in WT and PMM2-CDG samples upon stimulus, respectively. From these, 122 genes responded exclusively in WT, whereas only 39 were specific for PMM2-CDG samples. Functional analysis revealed that transcriptome alterations were associated with immunological processes, enclosing TNF-α-induced pathways like MAPK, ERK1/2, and JNK, and IL-6 cytokine production and regulation. Upon stimulus, western blot assays showed lowered levels of JNK-2 expression in PMM2-CDG compared to WT. This kinase is linked to the activation of the AP-1 complex which is required for the expression of genes following TNF-α stimulation. This defect might partially explain the deregulation in PMM2-CDG gene expression.
Conclusion: This study might guide us toward the identification of possible defects in biological pathways, which might be novel biomarkers and/or potential therapeutic targets to ameliorate immune-related symptoms in PMM2-CDG.

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Chemoenzymatic strategy towards new autoinducer-2 prodrugs

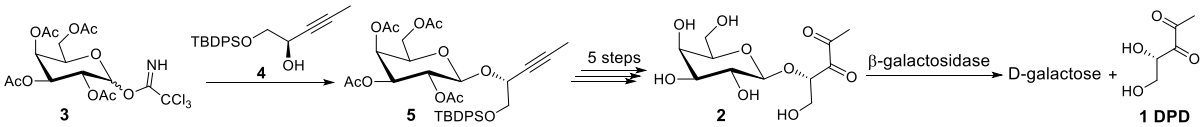
Kis P,[1,2] Miranda V,[1] Rodrigues V M,[1] Xavier B K,[3] Ventura, M R[1]
1. Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal; 2. Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia; 3. Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal

E-mail: peter.kis@itqb.unl.pt

Bacteria are able to coordinate the behaviour of cell populations by secreting and sensing small molecules called autoinducers.[1] This phenomenon is called quorum sensing (QS). Among the QS compounds, autoinducer-2 (AI-2) stands out, proposed to be a “universal” bacterial signalling molecule in inter-species communication. AI-2 plays an important role in controlling the colonisation and homeostasis of the gut microflora. There is evidence that AI-2 can be used to ameliorate the effect caused by antibiotic-induced microbiota imbalances in the gut.[2] Thus, our premise is that synthetic AI-2 can help in the recovery of a healthy bacterial phyla ratio after antibiotic treatment.

To study the mechanisms involved in the response of the gut microbiota to AI-2, it is necessary to synthesise a suitable chemical tool to deliver AI-2 unaltered to the gut. For this purpose, we decided to follow the strategy of colon-specific drug delivery systems.[3] In this contribution we will discuss chemoenzymatic strategies towards linking **DPD 1** (the uncyclised precursor of AI-2) to a monosaccharide to create a prodrug **2** (Scheme 1). This prodrug will deliver **DPD** once it reaches the intestines where it will be liberated by beta-D-galactosidases produced by the gut microbiota.

To verify the success of this experimental approach, the development of an *in vitro* method for enzymatic hydrolysis of the glycosidic bond between the sugar and **DPD** using commercial beta-D-glycosidase will be presented together with subsequent quantification of AI-2 released using *Vibrio harveyi* bioluminescence assay.



Scheme 1

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In silico studies and synthesis of halogenated sugar mimetics as potential modulators of protein-carbohydrate recognition

Rafael Nunes,[1,2] *Paulo J. Costa,*[2] *Nuno M. Xavier*[1]
1. Centro de Química Estrutural – Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; 2. Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

E-mail: rsnunes@fc.ul.pt

The design of glycomimetic structures is a common strategy in chemical glycobiology and carbohydrate-based drug discovery given the ubiquitous role of carbohydrates in biological recognition processes and hence their medicinal interest [1]. In this context, a variety of functionalities are often incorporated in carbohydrate templates whereas the utility of heavier halogens (X = Cl, Br, I) has been largely neglected. These species are able to establish specific interactions with Lewis bases, known as halogen bonds (XBs), through an electrophilic region at the tip of the halogen, known as sigma–hole [2]. These interactions have found varied applications across the chemical sciences, including in medicinal chemistry [3].
In this work, we synthetized a series of glycosides bearing halogen substituents as novel chemical probes to investigate the potential of XBs to act as surrogates for intermolecular interactions commonly involved in the molecular recognition of carbohydrates by proteins. Target structures were rationally designed taking advantage of DFT calculations in order to establish their relative potency as XB donors. These glycomimetic entities will serve as tools to study protein–carbohydrate binding and will ultimately be screened as modulators of sugar-binding proteins of therapeutic interest.

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Molecular Recognition of Mucin O-glycans by Human Gut Microbiota

Raquel L. Costa[1,2], Viviana G. Correia[1,2], Benedita A. Pinheiro[1,2], Yan Liu[3], Maria João Romão[1,2],Wengang Chai[3], Ten Feizi[3], Ana Luísa Carvalho[1,2] & Angelina S. Palma[1,2]
1. UCIBIO, Department of Chemistry, FCT-NOVA, 2829-516, Caparica, Portugal; 2. Associate Laboratory i4HB - Institute for Health and Bioeconomy, FCT-NOVA, 2829-516 Caparica, Portugal; 3. Glycosciences Laboratory, Department of Medicine, Imperial College London, W12 0NN, London, United Kingdom.

E-mail: rl.costa@campus.fct.unl.pt

Bacteroides thetaiotaomicron is a prominent gut commensal bacterium that can switch its metabolic preferences according to the glycan sources that are available in the human gut [1]. To orchestrate the breakdown of complex glycans, this species holds highly efficient polysaccharide utilization loci (PUL) systems, which consist of sets of co-regulated genes encoding different proteins that target and degrade a specific glycan structure, including glycan-binding proteins (e.g. CBMs, non-catalytic carbohydrate-binding modules) and enzymes [2]. Considering the CBMs, B. Thetaiotaomicron contains a relatively high number of putative CBMs belonging to family 32, of which characterised members are known to target mammalian-type glycans [3]. Family 32 CBMs are known to be expressed by human gut and animal pathogens and some are associated with enzymes responsible for the host infection [4]. However, little is known about the function of this CBM family in human gut commensals. The aim of this work was to elucidate, at molecular level, the glycan-binding specificities of family 32 CBMs from different PUL of B. thetaiotaomicron known to be upregulated upon bacterial growth on mucin O-glycosylated substrates. For this purpose, we applied an integrative strategy of ligand discovery using glycan microarrays and structural characterization of protein and protein-glycan interactions using X-ray crystallography [5]. In the case where the structures of the protein-glycan complexes were not attained, site-directed mutagenesis was employed to map key protein residues for binding and understand the molecular basis of the different glycan-binding specificities. Glycan microarray screening analysis using sequence-defined glycans identified unique specificities for 3 family 32 CBMs: the core 1-Thr and core 2-Thr for BT3015C (PUL 45), Lewis A antigens for BT4040 (PUL 73) and N-acetyllactosamine motifs for BT0865 (PUL 12). Structural characterization by X-ray crystallography revealed a canonical binding site that recognizes the galactose moiety. The fine specificity of BT3015C and BT4040 is characterized by different amino acids in the adjacent loops. In the case of BT3015C, the Aspartate147 is responsible for the recognition of the N-acetylgalactosamine moiety. For BT4040, information retrieved from site-directed mutagenesis allowed to identify Glutamate42 and Glutamate73 as key residues for binding with Lewis A.
Uncovering the molecular recognition of family 32 CBMs with mucin O-glycans is essential to understand their function in B. thetaiotaomicron and consequently to give hints about their role in commensals. Also, as human gut diseases, like intestinal bowel disease, are increasing, this knowledge can be used to design new strategies (prebiotics, probiotics) to improve gut health.

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Unveiling the Sialyl-Tn antigen role in triple negative breast cancer

Rita Adubeiro Lourenço[1,2,3], Daniela F Barreira[1,2,3], Carla Lopes[4], Ana Sofia Rodrigues[1,2,3], Pedro Granjo[1,2,3], Zélia Silva[1,2,3], Manuela Martins[4], Ana Rita Grosso[1,2], Paula A Videira[1,2,3]

1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, Largo da Torre, 2829-516, Caparica, Portugal; 2. UCIBIO, Departamento Ciências da Vida, NOVA School of Science and Technology, NOVA University of Lisbon, 2829-516, Caparica, Portugal; 3. CDG & Allies – Professionals and Patient Associations International Network (CDG & Allies – PPAIN), 2829-516, Caparica, Portugal; 4. Centro Hospitalar de Lisboa Central, EPE e Serviço de Anatomia Patológica, 1150-199, Lisbon, Portugal.

E-mail: rad.lourenco@campus.fct.unl.pt

The cell-surface carbohydrate antigen sialyl-Tn (STn) is expressed in cancer while absent in healthy tissues [1]. It has been associated with poor cancer prognosis in several types, such as breast cancer (BC) [2,3]. One of the most aggressive types of BC is the triple negative breast cancer (TNBC), which accounts for 10–20% of all BC cases, and due to its lack of targetable biomarkers patients have been deprived of the current targeted therapies [4]. Thus, to better understand the role of STn in TNBC, we analysed 128 TNBC tissues by tissue microarray immunohistochemistry for the expression of STn and epithelial-mesenchymal transition and stemness biomarkers. The STn was expressed in 30 (23.4%) tumours and associated with reduced overall survival and increased patients' age. Nonetheless, the tumour grade and the c-Myc and Ki67 biomarkers expression were significantly higher in the STn-. Moreover, the STn antigen correlated negatively with the c-Myc and marginally significantly with the Ki67 (p=0.07). We have also analysed a TNBC *in vitro*, the MDA-MB-231 cell line overexpressing the *ST6GALNAC1* gene (STn+), which codes for the enzyme responsible for the STn biosynthesis [5]. The MDA-MB-231 STn+ cell line presented lower c-Myc protein expression by western blot, and higher proliferation by CFSE dilution assay, compared to the WT cell line. In addition, TNBC genetic data set from The Cancer Genome Atlas (TCGA) further supports prior findings through the statistically significant negative correlation between the *ST6GALNAC1* gene, and the c-Myc and Oct4 associated genes (*MYC* and *POU5F1* genes, respectively). When these TNBC samples were divided based on the median of *ST6GALNAC1* expression, the TNBC Low *ST6GALNAC1* expression group showed statistically significant higher expression of *MYC* and *POU5F1* as well as lower expression of *SOX2* in comparison to the High expression group. Functional enrichment analysis between the two groups further enlightens the potential molecular mechanisms behind the high proliferation in the TNBC STn+ cells, non-dependent on c-Myc. It revealed that the transcriptome alterations were associated with proliferation related biological processes, namely the PLC-activating GPCR and the PKA signalling pathways. Moreover, several immune related pathways were enriched, implying a potential involvement of this system in the pathology of this cancer subgroup. The frequency of immune cells was then accessed and showed a positive significant correlation with Treg cells, macrophages M2, CD4 T and B cells, and negative significant correlation with monocytes. Overall, our findings point to novel (yet unidentified) roles of STn and its relevance as a biomarker in TNBC stratification and as a therapeutic target, which could be exploited to address the enormous unmet clinical need in this aggressive cancer.

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Developing a computer-aided drug design approach to discover lead-like Phosphomannomutase 2 pharmaco-chaperones for congenital disorders of glycosylation (CDG) therapy

Salvador C.M. Magrinho,[1,2,3] *Sandra Brasil*,[2,3,4] *Giuseppina Andreotti*,[5] *Paula A. Videira*,[2,3,4] *Florbela Pereira* [1,4]

1. LAQV-Requimte, NOVA University of Lisbon - School of Science and Technology, Caparica, Portugal; 2 Associate Lab i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology; 3. UCIBIO Applied Molecular Biosciences Unit, NOVA School of Science and Technology; 4. CDG & Allies PPAIN, NOVA School of Science and Technology, Caparica, Portugal; 5. Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Naples, Italy

E-mail: s.magrinho@campus.fct.unl.pt

Congenital disorders of glycosylation (CDG) are a group of rare diseases caused by inherited defects in cellular glycosylation mechanisms, ultimately leading to a range of multisystem disease phenotypes. The most common, PMM2-CDG, with >1000 reported cases [1], is characterised by genetic defects in phosphomannomutase 2 (PMM2), which catalyses the conversion of mannose 6-P to mannose 1-P, a substrate for posttranslational *N*-glycosylation. While no effective treatment is yet available, previous functional characterisation studies in patient-observed mutations raised the possibility of designing pharmaco-chaperone (PC) therapies to stabilize PMM2 structure and partially rescue its activity [2].

Using an experimentally-validated dataset containing over 10.000 compounds whose interaction with human PMM2, namely thermal stability and IC_{50} (half maximal inhibitory concentration), had been previously assayed, we developed two machine learning strategies to unveil possible PCs for hPMM2 activation: 1) A quantitative structure-activity relationship (QSAR) classification model to predict the interaction of submitted molecules with PMM2 and 2) a QSAR regression model to estimate a theoretical IC_{50} value. These QSAR models served as computational tools to perform a virtual drug screen to search and select molecules with the desired PC profile. Compounds with interesting results were submitted to docking studies to further explore possible protein-ligand interactions.

Promising hits include quinolone derivates, phenylpropanoic acid derivates, vitamins, amongst others. Some of these compounds were acquired and we are currently conducting *in vitro* experiments in patient-derived fibroblast cell lines to analyse their effect on mutant PMM2 activity from cell extracts using different molecular biology techniques. This experimental work allows to elaborate on the therapeutic potential of selected hits and will provide proof-of-concept for the applied methodology, defining the models' predictive capabilities for such complex structure-biological activity relationships.

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Synthesis of Novel Potentially Bioactive D-Glucuronamide-based Nucleosides and (Triazolyl)methyl Amide-linked Pseudodisaccharide Derivatives

Tânia Moreira[1], *Rita G. Pereira*[1], *Rafael Nunes*[1],
Maria João Frias[2], *Sérgio Filipe*[2], *Radek Jorda*[3] *and Nuno M. Xavier*[1*]
1. *Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências,Universidade de Lisboa* Ed. C8, 5° Piso, Campo Grande, 1749-016 Lisboa, Portugal; 2. *Laboratory of Bacterial Cell Surfaces and Pathogenesis, UCIBIO-Applied Molecular Biosciences Unit - Associate Laboratory i4HB, Faculdade de Ciências e Tecnologia,Universidade Nova de Lisboa, 2825-149 Caparica; 3. Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 78371 Olomouc, Czech Republic*

E-mail: taniamelizia1998@gmail.com; nmxavier@fc.ul.pt (*)

Synthetic nucleos(t)ides and their analogs constitute important groups of molecules in medicinal chemistry due to their propensity to display a variety of bioactivities. Their ability to interfere with nucleos(t)ide-dependent biological processes has been exploited and various examples of such compounds are established anticancer or antiviral agents.[1] The antimicrobial potential of synthetic and natural nucleos(t)ides has also been well reported.[2] However, some limitations are associated with their clinical use, such as low oral bioavailability and the emergence of chemotherapeutic resistance.[1] Therefore, the development of novel bioactive nucleoside/nucleotide-like structures that may overcome such issues and act through distinctive mechanisms of action is of significant interest.

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

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

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

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Preparation and characterization of biodegradable films from purified arabinoxylan extract derived from corn fiber

Weng Verónica[1], *Alves Vítor*[2], *Coelhoso Isabel*[1], *Brazinha Carla*[1]
1. LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, FCT NOVA, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; 2. LEAF – Linking Landscape, Environment, Agriculture and Food, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

E-mail: v.weng@campus.fct.unl.pt

The corn starch industry produces various by-products in vast amounts, including corn fiber, which is mainly used in animal feed applications. This raw material is very rich in arabinoxylan, a hydrophilic polysaccharide with film-forming properties. In this work, arabinoxylan was extracted from corn fiber with a mild alkaline solution [1] and further purified with membranes processes, more precisely, a pre-concentration step by ultrafiltration [2] followed by purification with ultrafiltration in diafiltration mode [3]. The purified arabinoxylan extract was used as the basis for the production of films for packaging applications [4].
The crude and purified extracts were characterized in terms of bioactivity (cytotoxicity and antiproliferative activity), showing no toxicity to Caco-2 cells (IC50>10mg/mL), which potentiates their application in the production of edible films, and some antiproliferative activity to HT29 cells (the lowest being EC50=0.12 ± 0.02mg/mL), related to a potential anticancer effect [5].
The resulting purified aqueous extract presented an intense brown color. Therefore, decolorization was attempted, for a more appealing appearance, employing activated charcoal or hydrogen peroxide. Decolorization with activated charcoal was not successful, however, partial decolorization was achieved with hydrogen peroxide, resulting in a light-yellow solution [4].
Films were formulated with 30% (w/wdry basis) glycerol as plasticizer. Though presenting a high solubility in water, they showed promising properties to be used as wrapping materials. Decolorized films still presented significant antioxidant activity ((3.21 ± 0.40)·10-5mmol Trolox/mg film), and water vapor permeability values ((2.94 ± 0.49)·10-11mol·m/m2·s·Pa) similar to that of non-decolorized films and other polysaccharides. In addition, they showed good mechanical properties under perforation (Tension of Perforation = (1.22 ± 0.41) MPa and Deformation = (53.0 ± 1.7)%, meaning that the decolorization process did not alter significantly the properties of the films. These results show that these films have promising properties for food packaging applications, especially for lower moisture content food products [4].

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Enzymatic Deconstruction of Brewer's Spent Yeast Cell Wall Glucans and Their Structural Characterization

Vítor J. Martins, Manuel A. Coimbra, Elisabete Coelho
LAQV-REQUIMTE, Departamento de Química, Campus Universitário de Santiago, 3910-193, Aveiro, Portugal

E-mail: vitorjmartins@ua.pt

Brewer's Spent Yeast (BSY) is a major by-product of the brewing industry with a large potential to be purposed for food and biomedical applications. This large potential for reutilization is due to its cell wall composed of polysaccharides with biological activities, namely β-glucans [1]. Yeast β-glucan preparations, such as Zymosan, have long been recognised for their immunomodulatory activities such as the activation of various immune receptors, including Dectin-1 [2, 3].

Saccharomyces yeast cell wall glucans are generally described in literature as long (β1→3)-linked chains with (β1→6)-linked branches, which can be linked to glycogen [4]. (β1→4)-Linked glucans were also found for the first time on *S. pastorianus*, which increase with further brewing reuse [5]. In order to purpose novel BSY biomedical applications, the structural characterization of its β-glucans is key to properly establish structure-function relationships between these and immune receptors such as Dectin-1.

In this work, we aim to give further insight into the glucan structures present in insoluble residues retrieved after strong alkali (4M KOH) extraction of BSY. Analysis of both soluble and insoluble fractions obtained after partial enzymatic hydrolysis with zymolyase, a (β1→3)-endoglucanase, was performed. The high molecular weight soluble material (MW > 10 kDa) was further fractionated by gradual ethanol precipitation up to 85%.

Glycosidic linkage analysis of both solubilized (31-43% (w/w) of initial insoluble residue) and insoluble fractions of the enzymatic hydrolyses show a partial hydrolysis of (β1→3)-glucans. Soluble high molecular weight material and insoluble residues composed of 8-15% and 24-41% of (β1→3)- linked glucose residues were obtained, respectively. Gradual ethanol precipitation of the soluble high-molecular weight material reveals the presence of three distinct pools of glucan structures containing (1→4), (β1→3), and (β1→6) linked residues, comprising respectively 36-61% (w/w), 9- 19% (w/w) and 4-16% (w/w) of initial soluble high-molecular weight material. (β1→3)-Glucans and (1→4)-glucans are mostly linear, with a degree of branching (DB) of 0.16-0.21 whereas (β1→6)- glucans were more branched DB = 0.35-0.55 and were more soluble in ethanol solutions. These results give further insight into the glucan structures present in the brewer's spent yeast (BSY) cell wall, enabling future glycan-protein interaction studies using Dectin-1 proteoliposomes, ultimately aiding in the development of novel biomedical applications based on BSY, such as using the yeast cell wall capsule as a drug delivery system.

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Modulation of sialic acid content on monocyte derived dendritic cells: phenotypic and functional implications

Zélia Silva,[1,2] *Vanessa Luz,*[1,2] *João A Rabaça,*[1,2] *Paula Videira* [1,2,3]

1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 3. CDG & Allies – Professionals and Patient Associations International Network (CDG & Allies – PPAIN), Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.

E-mail: zm.silva@fct.unl.pt

Dendritic cells (DCs) play a very important role in immune response, especially because they are unique professional antigen-presenting cells endowed with the capacity of generating immunologic memory. Sialic acid content at the cell surface is tightly regulated, differing among cell types, and varying markedly according to cell differentiation and stimuli. Our previous work showed that sialic acid at the cell surface plays an immunomodulatory role on monocyte-derived DCs (Mo-DCs), contributing to dampening maturation and down-regulating their ability to activate T cells. Sialic acid removal from the cell surface of Mo-DCs by sialidases induces maturation, increased antigen-cross presenting, and co-stimulatory abilities, resulting in higher polarization of T cells towards a Th1 phenotype and improves the ability to specifically activate autologous T cells toward tumor cell killing [1,2]. In this context, sialic acid content manipulation is a promising immunomodulation strategy. **Objectives:** 1) compare the phenotypic and functional characteristics of sialidase treated Mo-DCs with those subjected to a cytokine maturation cocktail used in clinical trials and 2) characterize and compare the glycosylation changes of Mo-DCs subjected to different treatments and 3) study the impact of sialidase treatment on a distinct family of MHC Class I-like proteins - the CD1 lipid presenting molecules. **Results:** The maturation profile of sialidase treated Mo-DCs is markedly different from the maturation with cytokine cocktail. While sialidase removal leads to overexpression of MHC-I, MHC-II and CD40, cytokine cocktail treated Mo-DCs also highly upregulate the immune checkpoints PD-L1. Both treatments lead to higher secretion of IL-12 cytokine, but it is 8.5 times more pronounced for the cytokine cocktail. Lectin staining was used to evaluate the α2,3 and α2,6 sialic acid content and it was observed that, unlike sialidase treatment, the cytokine cocktail did not significantly alter the sialic acid profile. Regarding antigen presenting molecules, in Mo-DCs, CD1a, CD1b and CD1c, is not stably altered, unlike MHC-I and MHC-II which keep a stable higher expression at the cell surface for up to 48h after sialidase treatment. These effects on CD1 molecules were corroborated in C1R cells expressing CD1a, CD1b or CD1c, when treated with sialidase, showing that sialic modulation of immune receptors is transversal to different cells. **Conclusion**: 1) Sialic acid manipulation generates Mo-DCs with a unique maturation profile 2) Sialic acid removal improves the stabilization of the MHC molecules presenting peptides but not CD1 molecules presenting lipids.

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