INNOGLY-ECI-WG3-2021
Young Glyco-Scientists on stage

September 27, 2021

In Memory of Prof. Hans-Joachim Gabius whose work and dedication to the GlycoScience will be of inspiration for the next generation of GlycoScientists.

Program

Starting time: 09:00 am (CEST)

Platform: zoom

Morning session 09:00 – 13:00 Chair: Giacomo Biagiotti

Welcome-room with introduction and help for the participants	08:45
Opening Ceremony. **Luigi Lay (INNOGLY Chair), Barbara Richichi and Sandra van Vliet (WG3 leaders)**	09:00
PL1: Francesca Micoli, PhD: "Professional growth in an international company working on polysaccharide based vaccines"	09:30
Young scientists stage 1 **Chair: Giacomo BIAGIOTTI**	**10:30 - 11:00**
OL1 Cristina Manuela Santi: *"Synthesis of an analogue of Neisseria meningitidis A capsular polysaccharide for the development of a glycoconjugate vaccine".*	10:30 - 10:45
OL2 Laura Marín Fernández: *"Metabolic functionalization applied to the production of complex polysaccharide of vaccine interest".*	10:45 - 11:00
Round table (moderated by Davide RET) and **Flash presentations**	**11:00 - 12:20**
EU Grant opportunities for ECI Denise Cuccia (INNOGLY Grant Manager)	11:00 - 11:15
FL1 Henrique Oliveira Duarte: *"Cracking the ErbB2 Sugar Code in Gastric Cancer – A Bitter Sweet Functional Target"*	11:15 - 11:20
FL2 Nadia Van der Meijs: *"Glycan-dependent signalling routes and transcriptional programs in human dendritic cells after triggering the C-type lectin receptor MGL".*	11:20 - 11:25
FL3 Cristina Di Carluccio: *"Molecular recognition of sialoglycans by streptococcal Siglec-like adhesins: toward the shape of specific inhibitors"*	11:25 - 11:30
FL4 Angela Marseglia: *"Interaction studies between bacterial glycans and host immune receptors"*	11:30 - 11:35
FL5 Celeste Abreu: *"Study of the glycosylation-dependent interaction between NK cell receptors and Galectin-1 by microscale thermophoresis".*	11:35 - 11:40
FL6 Ondrej Skorepa: *"Natural killer cell activation receptor NKp30 oligomerization depends on its N-glycosylation".*	11:45 - 11:50
FL7 Marina Milanesi: *"HIV-1 Tat/heparin interaction: translating new insight from molecular modelling to the comprehension of its biological functions".*	11:55 - 12:00
FL8 Laura Petrosilli: *"Towards a novel vaccine candidates against streptococcus pneumoniae 6a/6c"*	12:00 - 12:05
FL9 Katarzyna Durlik-Popińska: *"Cross reaction of antibodies against LPS P. mirabilis O3 with collagen type I, may mediated complement activation."*	12:05 - 12:10
FL10 Özge Ata: *"Extraction and characterization of chondroitin sulphate from chicken sternum".*	12:10 - 12:15
Opened discussion (moderated by Davide RET)	12:15 - 12:20
Young scientists stage 2 **Chair Elena Maria LOI**	**12:20 - 13:10**
OL3 Catarina Marques: *"Exostosin-like 2 and Exostosin-like 3 Modulate Cancer Cells' GAGosylation and Malignant Phenotype".*	12:20 - 12:35
OL4 Paola Chiodelli: *"HIV-1 Tat and heparan sulfate proteoglycans orchestrate the setup of in cis and in trans cell surface interactions functional*	12:35 - 12:50

to lymphocyte trans-endothelial migration".	
OL5 P. Perez Schmidt: *"A microfluidic approach to synthesize ultra-small glyco-gold nanoparticles".*	12:50 - 13:05

13:10-14:30 Lunch Break

Afternoon session 14:30 pm-17:00 pm

Young scientists stage 3 **Chair: Kristina ZLATINA and Cristiano CONCEIÇÃO**	**14:30 - 18:00**
PL2: Prof. Ryan A. Flynn, PhD: "Small RNAs are modified with N-glycans and displayed on the surface of living cells"	14:30 - 15:30
OL6 Anna-Kristin Ludwig: *"Influence of Modular Assembly on Galectin Activity"*	15:30 - 15:45
OL7 Mike Aoun: *"Mincle regulates T-cell responses in arthritis via ROS/MAPK pathway".*	15:45 - 16:00
OL8 Ferran Nieto-Fabregat: *DC-SIGN recognition of bacterial LPS".*	16:00 - 16:15
OL9 Mujtaba Hassan: *"Design, synthesis, and evaluation of a D-galactal derivative as a selective inhibitor of galectin-8 N-terminal domain"*	16:15 - 16:30
OL10 Filipa Trovão: *"Deciphering the complex molecular cross-talk of the gut microbiome with the human host".*	16:30 - 16:45
OL11 Vojtěch Hamala: *"Hybrid organometallic galectin inhibitors".*	16:45 - 17:00
OL12 Thomas Hicks: *"STD NMR spectroscopy reveals the structural basis of the interaction of the complex N-glycan G0 with fucosyltransferase FUT8".*	17:00 - 17:15
OL13 Mathieu Decloquement: *"Diversity of polysialylation machinery in fish species highlights exciting perspectives to generate original high therapeutic biomaterials"*	17:15 - 17:30
OL14 Irene van der Harr: *"Tumor Sialylation: a Master Regulator of anti-tumor Immunity in Colorectal Cancer".*	17:30 - 17:45
OL15 Y. Tepeli Büyüksünetçi: *"Development of electrochemical glycan biosensor including oxidase enzyme mimicking MOF structure".*	17:45 - 18:00
Closing Ceremony	18:00

Content overview

Plenary Lectures

PL1 Polysaccharide based vaccines to target neglected diseases in impoverished communities

Francesca Micoli, PhD
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CURRICULUM VITAE: PhD in industrial organic chemistry awarded at the Uniersity of Florence, Italy, in 2006. From 2007 working in GSK Vaccines Institute for Global Health (Siena, Italy), focusing her research on the development of effective and affordable vaccines for neglected diseases in impoverished communities. Involved in the development of vaccines against Salmonella Typhi, Salmonella Paratyphi A, non-typhoidal Salmonella, Shigella, Group A Streptococcus and Neisseria meningitidis. She has been Technology Platform Head at GVGH, working on two main technology platforms, glycoconjugation and Generalised Modules for Membrane Antigens (GMMA). From April 2020, Project Leader of the Shigella project and more recently also Director of the Innovation Academy, focused on innovative technologies for vaccine development. Author of more than 60 scientific publications and several patent applications, with many collaborations in place with academic and industrial partners. Board member of the MRC-funded BactiVac, the first bacterial vaccine network, with a LMIC-centric focus.

ABSTRACT: GSK Vaccines Institute for Global Health (GVGH) has the ambition to develop effective and affordable vaccines for neglected diseases in impoverished communities. Despite the success of vaccination in the last decades, there are still many infectious diseases in need of a vaccine, especially in LMICs, where multiple barriers, scientific, regulatory and economic, have so far prevented the effective deployment of vaccines that target even the most common infectious diseases.
The GVGH story of vaccine development for LMIC started with a glycoconjugate vaccine against *Salmonella* Typhi. In 2013, the vaccine technology was transferred under a licensing agreement to Biological E Ltd (BioE), a developing country vaccine manufacturer located in Hyderabad, India. Biological E's Typhoid Conjugate Vaccine (TYPHIBEV®) received Indian licensure and WHO prequalification in 2020.
Also, two different technologies, glycoconjugation and Generalized Modules for Membrane Antigens (GMMA) have been compared for the development of an O-antigen based vaccine against invasive nontyphoidal *Salmonella*, major cause of community-acquired bacteremia in Africa for which no vaccine is available. The O-antigen portion of *Salmonella* lipopolysaccharide has been implicated as a target of protective immunity and O-antigen-based vaccines are currently under development.
GMMA are outer membrane vesicles released by Gram-negative bacteria mutated to increase blebbing. *Salmonella* strains were genetically manipulated by deletion of tolR to induce GMMA production. O-antigen from corresponding wild type bacteria were extracted and conjugated to CRM_{197} as carrier protein.
When tested in mice, GMMA induced greater serum bactericidal activity and more diverse IgG subclass profile compared with equivalent quantities of glycoconjugate vaccine only inducing IgG1. Presence of Alhydrogel strongly increased the immune response induced by the conjugate, while no major effect was observed on the immune response induced by GMMA. Both GMMA and conjugate vaccines resulted in reduced bacterial colonization of spleens and livers of challenged mice.
Given the simplicity of production and induction of highly functional *in vivo* antibody responses, GMMA are a promising strategy for the development of affordable vaccines.
Technology platforms (e.g. glycoconjugate and GMMA) can facilitate vaccine development for their implementation in LMICs. Synergy of the different technologies currently available and their advancement will enable the tailored design of improved vaccines for existing and novel targets, expanding the number of available vaccines and tackling currently unmet medical needs.

PL2 Small RNAs are modified with N-glycans and displayed on the surface of living cells

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CURRICULUM VITAE: Ryan completed his undergrad training at MIT in the lab of Phillip Sharp studying divergent transcription at eukaryotic promoters. He moved to Stanford to complete his MD and PhD, where he did his thesis work in the lab of Howard Chang. There, Ryan focused on developing techniques to assay RNA secondary structure as well as RNA-protein interactions. He subsequently switched fields in for his post-doctoral work towards chemical biology in the context of glycoscience in Carolyn Bertozzi's lab. Here Ryan made the surprising discovery of glycoRNA, the topic of his talk today and is the main focus of study for his independent lab, which he started in the beginning of this hear at Boston Children's Hospital and Harvard University.

ABSTRACT: Glycans modify lipids and proteins to mediate inter- and intramolecular interactions across all domains of life. RNA is not thought to be a major target of glycosylation. Here, we challenge this view with evidence that mammals use RNA as a third scaffold for glycosylation. Using a battery of chemical and biochemical approaches, we found that conserved small noncoding RNAs bear sialylated glycans. These ‘‘glycoRNAs’’ were present in multiple cell types and mammalian species, in cultured cells, and in vivo. GlycoRNA assembly depends on canonical N-glycan biosynthetic machinery and results in structures enriched in sialic acid and fucose. Analysis of living cells revealed that the majority of glycoRNAs were present on the cell surface and can interact with anti-dsRNA antibodies and members of the Siglec receptor family. Collectively, these findings suggest the existence of a direct interface between RNA biology and glycobiology, and an expanded role for RNA in extracellular biology.

Oral presentations

OL1 Synthesis of an analogue of Neisseria meningitidis A capsular polysaccharide for the development of a glycoconjugate vaccine

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KEYWORDS: Monosaccharides modification, Oligosaccharides synthesis, Synthetic analogues, Glycosylation, Phosphono analogues

ABSTRACT: *Neisseria meningitidis* A (MenA) had been for a long time the main cause of epidemics of meningococcal meningitis in the sub-Saharan Africa. Thanks to the introduction of MenAfriVac vaccine, serogroup A related infections have almost vanished in 2017; however, the World Health Organization pointed out the importance to persist with a strict vaccination program. Up to date, all the licensed vaccines targeting MenA are obtained from the extraction and size fragmentation of the capsular polysaccharide (CPS) from the bacterium. MenA CPS consists of (1→6)-linked-2-acetamido-2-deoxy-a-D-mannopyranosyl phosphate residues partially acetylated at C-3 and C-4. This structure, once isolated, is not stable in water due to the hydrolysis of the phosphodiester bond. Due to the instability issues, most of the licensed vaccines targeting MenA are distributed in a lyophilized form and the cold chain must be maintained during the entire process of distribution and storage. To achieve a more stable vaccine, which could be distributed in the more convenient liquid formulation, without the need of a strict temperature control, some, more stable, structural analogues have been developed. In particular, our group synthesized MenA CPS non-acetylated phosphonoester-linked oligomers up to the trimer [1]: these analogues showed good stability, however, they resulted to be poorly immunogenic [2], even after protein conjugation [3]. Since the acetylation was proven to have an important role in the immunogenicity of natural MenA CPS [4], our goal is the synthesis of the 3-O acetylated phosphonate analogue up to the trimer.
In this communication, the synthesis of the 3-O acetylated phosphonate analogue up to the trimer will be described.

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OL2 Metabolic functionalization applied to the production of complex polysaccharide of vaccine interest

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KEYWORDS: Metabolic functionalization, Synthetic biology, Lipopolysaccharides, Glycoconjugate vaccines, Lectin-based subunit vaccines

ABSTRACT: Infectious diseases remain the leading cause of death worldwide, and vaccination has had major positive impact on public health for more than a century. Many vaccines have been launched during the last three decades thanks to major technological breakthroughs such as the advent of glycoconjugate vaccines. This kind of vaccines makes use of bacterial surface polysaccharides, *e.g.* capsular polysaccharides, which are usually the target of host protective humoral immune response. However, their production relies on a complex chemistry-based manufacturing process. Manufacturing requires a multistep process of fermentation, purification, chemical processing, and cross-linking of the polysaccharide antigen with a bacterial carrier protein and a final purification step for the glycoprotein conjugate. In order to overcome one of the most challenging steps, the derivatization of the polysaccharide antigen, we develop an innovative approach using *Escherichia coli* and *Vibrio cholera*e O1 as a proof of concept.

This approach, the metabolic lipopolysaccharide labelling, relies on the plasticity of carbohydrate metabolic pathways to incorporate a chemical handle, or chemical reporter, within cell-surface glycans. Such a chemical reporter can be further exploited, *via* a bioorthogonal reaction, to introduce diverse types of molecular modules, including fluorophores or labels such as biotin, on the modified cell surface. In this study, we incorporate a modified monosaccharide derived from 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) to the LPS of Gram-negative bacteria, bearing an azido group that could be used for conjugation of the LPS to the carrier protein. In order to achieve this incorporation, it was necessary to construct mutants harbouring transporters or modified proteins of the biosynthetic pathway of LPS. Results showed an improved incorporation of the sugar to cell wall that would enable us to create a more efficient glycoconjugate vaccine.

ACKNOWLEDGEMENTS: This work was supported by the French National Research Agency (ANR-17-CE07-0019-02 IngéniOse)

OL3 Exostosin-like 2 and Exostosin-like 3 Modulate Cancer Cells' GAGosylation and Malignant Phenotype

Catarina Marques,[1,2,3]
Juliana Poças,[1,2,3] *Catarina Gomes,*[1,2] *Isabel Faria-Ramos,*[1,2] *Celso Reis,*[1,2,3,4] *Romain Vivès,*[5] *Ana Magalhães,*[1,2]*
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KEYWORDS: Glycosaminoglycans, Heparan Sulfate Biosynthesis, Glycosyltransferases, Biochemistry, Cancer

ABSTRACT: Heparan Sulfate ProteoGlycans (HSPGs) are essential components of cells' glycocalyx and ExtraCellular Matrix (ECM), which play key roles in cells' physiology and pathology. By binding to multiple biological ligands, via HS glycosaminoglycan (GAG) chains, HSPGs modulate cancer cells' interaction with ECM and signalling networks, ultimately controlling tumour microenvironment and disease development. This work aimed to disclose the regulatory mechanisms underlying HSPGs biosynthetic pathways in gastric cancer context. Furthermore, we evaluated the impact of cellular GAGs content in cancer cell signalling and motility features.
We developed glycoengineered gastric cancer cell models lacking either Exostosin-Like Glycosyltransferase 2 (EXTL2) and EXTL3, and revealed their regulatory roles in both HS and Chondroitin Sulfate (CS) biosynthesis. We showed that EXTL3 is key for initiating the synthesis of HS chains, in detriment of CS biosynthesis, while EXTL2 functions as a negative regulator of HS biosynthesis. Gene expression and protein levels of two major HS carriers were also determined and revealed that KO of *EXTL2* promoted significant alterations in HSPG core protein levels. In adition, we further demonstrated that this aberrant GAGosylation profile dysplayed by *EXTL2* KO gastric cancer cells promoted higher cellular motility and invasion, and altered the activation state of key cell surface receptors tyrosine kinase.
Overall, our results reveal the crucial roles of EXTL2 and EXTL3 in the modulation of proteoglycans expression and aberrant glycosylation profiles displayed by cancer cells, and the functional impact of these alterations on cells malignant behaviour, further supporting the clinical potential of HS biosynthetic machinery in cancer therapy.

ACKNOWLEDGEMENTS: This work was financed by FEDER funds through the COMPETE 2020, and through FCT in the framework of the projects POCI-01-0145-FEDER-007274 and POCI01-0145-FEDER-028489 (to AM). CM and JP are funded by FCT PhD scholarships 2020.06412.BD and SFRH/BD/137319/2018, respectively. The authors acknowledge the support of the CA18103 INNOGLY.

OL4 HIV-1 Tat and heparan sulfate proteoglycans orchestrate the setup of in *cis* and in *trans* cell surface interactions functional to lymphocyte trans-endothelial migration

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KEYWORDS: HSPGs, sialic acid, angiogenesis, cell biology, surface plasmon resonance

ABSTRACT: The HIV-1 transactivating factor Tat is released by infected leukocytes and, in the extracellular environment, has the tendency to form dimers. Extracellular, dimeric Tat (ExD-Tat) activates the same producing leukocytes and endothelial cells (ECs), triggering some pathological processes such as inflammation, angiogenesis and leukocyte extravasation that, in turn, contribute to the arise of AIDS-associated pathologies, the dissemination of HIV-1 and hence the progression of the disease.
To induce these effects, ExD-Tat engages several receptors including integrins, VEGFR2 and heparan sulfate proteoglycans (HSPGs) expressed on leukocytes and ECs. In particular, the dimeric nature of ExD-Tat allows it to bind two HSPGs expressed on the surface of facing leukocytes and ECs, acting as a bridge between the two cell types that contribute to the above mentioned pathological processes [1-3].
ExD-Tat has the tendency to tether to the HSPGs of the extracellular matrix or of the endothelial surface favoring leukocyte adhesion. Accordingly, we demonstrated that plastic-immobilized Tat induces leukocyte adhesion but also their spreading, by which leukocytes increase the area and the strength of their adhesion, functional to their chemiotactic migration and extravasation during virus infection and not only related inflammation. By using B-lymphoid cells specifically overexpressing two different HSPGs (the GPI-anchored Glypican-1 and the signaling Syndecan-1) we demonstrated that while leukocyte adhesion is a simple mechanical process that, as such, can be mediated by both the HSPGs, the subsequent spreading is instead an active process that can be mediated only by the signaling HSPG Syndecan-1 upon its engagement and activation by surface-immobilized Tat.
By means of a panel of biochemical activation assays and specific synthetic inhibitors of various receptors and second messengers, we characterized the signaling transduced in lymphocytes adherent to substrate-immobilized Tat, that consists in the activation of Syndecan-1, integrin $\alpha_v\beta_3$, FAK, src and $ERK_{1/2}$.
It has been demonstrated that, once release by HIV-1 infected lymphocytes, ExD-Tat has the tendency to remain associated to the HSPGs of the same producing cells. We observed that, when loaded onto Syndecan-1 expressed on lymphocytes, Tat triggers the *in cis* activation of lymphocytes with the same signal transduction pathway described above. Also, Tat-loaded leukocytes undergo adhesion and spreading onto an EC monolayer.
We finally wonder if, due to its dimeric configuration, ExD-Tat “presented” by leukocytes to ECs can induce the *in trans* activation of the latter cell type. In effect, when exposed to Tat-presenting, but not naïve leukocyte, ECs undergo a reorganization of VE-chaderin at the cell junctions and consequent permeabilization, resulting in an increased extravasation of Tat-presenting lymphocytes. ECs permeabilization depends on the engagement and activation of VEGFR2 by Tat presented by leukocytes, that in turn recruits integrin $\alpha_v\beta_3$ and induces the phosphorylation of src and $ERK_{1/2}$.
In conclusion, the Syndecan-1/Tat complex orchestrates the setting up of a series of *in cis* and *in trans* interactions responsible of a “two-way” stimulation of lymphocytes and ECs by ExD-Tat that leads to an increase of leukocyte extravasation.

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OL5 A MICROFLUIDIC APPROACH TO SYNTHESIZE ULTRA-SMALL GLYCO-GOLD NANOPARTICLES

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KEYWORDS: Nanoparticles, Monosaccharides, Microfluidic system, Nobel metals, Nanomedicine

ABSTRACT: Gold nanoparticles are a platform of interest with a broad range of applications, and they are emerging as a powerful tool in nanomedicine[1]. The surface chemistry based on the soft-soft interaction among gold and sulfur allows a reliable coating of the nanoparticles, paving the way to numbers of bio-applications. In particular, the multivalent presentation of carbohydrates can trigger a cluster effect which can overcome the low affinity of the individual ligands towards their receptors[2]. Moreover, the glycans surface modification can improve the gold nanoparticle circulation time in blood, tuning the formation of the "bio-corona"[3] and therefore preserving their active targeting[4]. In the last years, great efforts have been addressed to the synthesis and characterization of glyco-coated gold nanoparticles, in order to develop reliable and robust nanosystems which can be employed in many fields, from drug delivery to diagnosis[5].
Herein, we propose a new photo-induced one-pot synthesis based on a microfluidic approach to obtain a library of ultra-small glyco-gold nanoparticles (GAuNPs). GAuNPs were synthesized without the addition of template or reducing agents, affording fully characterized and size-controlled functionalized nanoparticles.

ACKNOWLEDGEMENTS: In collaboration with: Dr. John Porter, Dr. Africa G.Barrientos, Prof. Luigi Lay, Prof. Cristina Lenardi,Dr. Laura Ragona, Dr. Katiuscia Pagano

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OL6 Influence of Modular Assembly on Galectin Activity

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KEYWORDS: protein engineering, galectins, glycosylation, EVs, protein purification

ABSTRACT: A distinct feature of galectins is its architecture to present carbohydrate recognition domain (CRD). This is maybe one of the principles of structure-activity relationships that contribute to the specificity of recognition and ensuing post-binding effects. The CRD(s) of wild-type galectins (Gal) are typically organized in a proto-type, chimera-type or tandem-repeat-type displays. To identify the physiological meaning(s) of these presentations of CRDs we used growth/adhesion-regulatory Gal-1/-7 and the multifunctional Gal-3 as models to generate variants with different modular assembly by genetic engineering. Different stable (covalently linked) linked homo- and heterodimers were purified by affinity chromatography, its sugar-binding activity was tested by ITC and its fine specificities by glycan array. Preferentially, both in tested wild-type Gals and variants binding to LacNAc oligomers and blood group epitopes was detected. The study of carbohydrate-inhibitable binding of the test panel disclosed up to qualitative cell-type-dependent differences in sections of fixed murine epididymis and especially jejunum. By probing topological aspects of binding, the susceptibility to inhibition by a tetravalent glycocluster was markedly different for the wild-type vs. variant proteins. The result teaches the salient lesson that protein design matters: the type of CRD presentation have a profound bearing on whether basically suited oligosaccharides will become binding partners in situ. Testing (ga)lectin variants will thus be useful to determine the impact of altering protein assembly, besides potential applications of the novel galectin forms could be of interest from a biomedical point of view.

OL7 Mincle regulates T-cell responses in arthritis via ROS/MAPK pathway

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KEYWORDS: Flow cytometry, Antigen-specific T cells, C-type lectin receptors, Autoimmune arthritis, Reactive oxygen species

ABSTRACT: Pattern recognition receptors (PRRs) are vital for clearing infections and resolving tissue damage, nevertheless their role in autoimmune arthritis remain nebulous. Herein, we unravel a novel role for a C-type lectin receptor (CLR) Mincle in regulating collagen type-II (Col2)-induced arthritis, a mouse model for rheumatoid arthritis. Abrogation of Mincle in arthritis-susceptible mice exhibited a reduced arthritis severity. In vivo and In vitro uptake experiments revealed a crucial role for macrophages in processing and presenting Col2 via Mincle to antigen-specific T cells. Consistently, evaluation of Col2-specific T cells shows a Th-17 skewing bias regulated exclusively by Mincle. Using a di-mannosylated version of an arthritis-associated T cell epitope we succeeded in harnessing Mincle's activity in modifying Col2-specific T cell responses and disease phenotype. Proteomic analysis of macrophages activated by a Mincle-specific ligand identified MAPK as a potential downstream signalling pathway that may be regulated by reactive oxygen species. Our data support a direct implication of a CLR in priming antigen-specific T cells and specific targeting of Mincle may potentially regulate autoimmune arthritis.

ACKNOWLEDGEMENTS: In collaboration with Jacopo Tricomi and Barbara Richichi

OL8 DC-SIGN recognition of bacterial LPS

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KEYWORDS: Lectins, LPS, Glycochemistry, NMR, Computational chemistry

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

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OL9 Design, synthesis, and evaluation of a D-galactal derivative as a selective inhibitor of galectin-8 N-terminal domain

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KEYWORDS: Monosaccharides modifications, NMR, Molecular modelling, Glycosylation, Cell assays

ABSTRACT: Galectins are a family of proteins that bind β-D-galactopyranoside-containing glycoproteins through their carbohydrate recognition domains (CRD) to form lattices.1 These lattices modulate the cellular mechanisms and the immune system.2,3 Galectin-8 is a member of the galectins family with two CRDs in tandem joined by a peptide linker. It plays a key role in autophagy,4 modulation of the innate and adaptive immunity,5,6 and pathological lymphangiogenesis, which is implicated in tumor growth and metastasis, and inflammatory conditions.7 Thus galectin-8 represents a promising target for the discovery of antiinflammatory and antitumor drugs.
We have designed and synthesized a library of C-3 substituted D-galactal derivatives. Evaluation of the compounds as inhibitors of the human galectin-1, 2, 3, 4N (N-terminal domain), 4C (C-terminal domain), 7, 8N, 8C, 9N, and 9C has led to the discovery of a D-galactal-benzimidazole hybrid that represents the most selective galectin-8N inhibitor to date 15 folds selectivity over galectin-3. X-ray crystallography followed by molecular dynamics simulation and quantum mechanical calculations has revealed that the molecular orbitals interaction between the LUMO Arg45 and the HOMO of the olefine of as well as the HOMO of O4 of the D-galactal are responsible for the high affinity and selectivity compared to the galactose derivatives. Treating the triple-negative breast cancer cells MDA-MB-231 cells with the D-galactal-benzimidazole compound reduced the secretion of the proinflammatory cytokines IL-6 and IL-8 in a dose-dependent manner. Evaluation of the cytotoxicity of the D-galactal-benzimidazole compound on a panel of cell lines in MTS assay has shown that this compound does not affect the cell viability at the tested concentrations. Therefore, the D-galactal-benzimidazole compound is a safe and promising lead compound for the design and synthesis of potent and selective galectin-8 inhibitors.

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OL10 Deciphering the complex molecular cross-talk of the gut microbiome with the human host

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KEYWORDS: Human gut microbiome, Glycan binding proteins, O-glycans, Glycan microarrays, X-ray Crystallography

ABSTRACT: The human gut microbiome houses a densely populated microbial community with a broad capacity to utilize host-derived and dietary glycans as nutrients, reflecting a complex cross-talk with the human host. How the structural diversity of host glycans is exploited by bacteria and how it influences commensal vs pathogenic interactions is largely unknown at the molecular level. Gut colonization by commensals promotes health benefits for the host and can prevent infection by enteric pathogens. However, an imbalance in the human microbiome community (dysbiosis) affects the host health, causing idiopathic diseases, such as inflammatory bowel disease and colorectal cancer, and promotes pathogen infection [1], [2].
The intestinal epithelial cells have a mucous layer composed of extensively O-glycosylated proteins, mucins. A paradigm in human microbiome research relates to the ability of some commensals to forage on mucin O-glycans, using these as nutrients and attachment sites, enhancing their competitiveness to colonise the mucus layer [3]. The commensal *Bacteroides caccae* was recently implicated in the destruction of the colonic mucous layer in low fibre carbohydrate diet conditions. During growth on mucin O-glycans, *B. caccae* showed an increased expression of different polysaccharide utilization loci (PULs) that code for modular enzymes, such as glycoside-hydrolases (GHs) with appended non-catalytic carbohydrate-binding modules of family 32 (CBM32) [1], [4]. My project focuses on the study of CBM32 modules from different PULs of *B. caccae* known to be overexpressed in the presence of mucin glycans. The hypothesis is that these proteins may facilitate mucin foraging of *B. caccae* and consequently enable other bacteria/pathogens to access specific glycan structures. We will use an integrative strategy combining glycan microarrays with structural studies, to identify and characterise at molecular level novel CBM32-glycan interactions. These contributions will unravel glycan structures as host-specific mediators of commensal/pathogenic interactions impacting human health.

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OL11 Hybrid organometallic galectin inhibitors

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KEYWORDS: synthesis of glycomimetics, chemical glycosylation, galectin inhibitors, organometallic compounds, fluorinated saccharides

ABSTRACT: Galectins are lectins defined by their ability to bind β-galactosides. There are at least 13 known human galectins. The most studied are galectin-1 (Gal-1) and galectin-3 (Gal-3), both involved in tumor progression. Elevated Gal-1 levels are reported for many tumor tissues and Gal-1 contributes to tumor progression including cell migration and tumor-immune escape. Gal-3 is characteristically overexpressed in many cancers and an elevated Gal-3 level is associated with increased invasiveness, metastatic spreading, immunosuppression and angiogenesis. Galectins are also implicated in progression of serious diseases other than cancer e.g. cardiovascular, diabetes mellitus, fibrosis, and others. These properties make them therapeutic targets of great potential.[1]
Natural ligands of galectins are galactose, lactose, *N*-acetyllactosamine and their glycosylated and sulfated forms. Modification of these carbohydrate ligands can create glycomimetics with enhanced binding to galectins, thus acting as their potent inhibitors. For example, introducing an aromatic substituent at hydroxyl positions not involved in binding resulted in an increased binding affinity to galectins.[2]
In this project, I focus on replacing planar aromatic substituents in known galectin inhibitors with arene-like or arene-containing organometallic structure – ferrocenes[3], ruthenium arenes[4] and ruthenium tetrazenes,[5] which all possess known antitumor or antimetastatic properties. This creates hybrid molecules that can act both as galectin inhibitors and cytotoxic and/or antimetastatic agents. The electroactivity of the attached organometallic component also allows to detect interaction of the hybrid molecules with their biological targets.

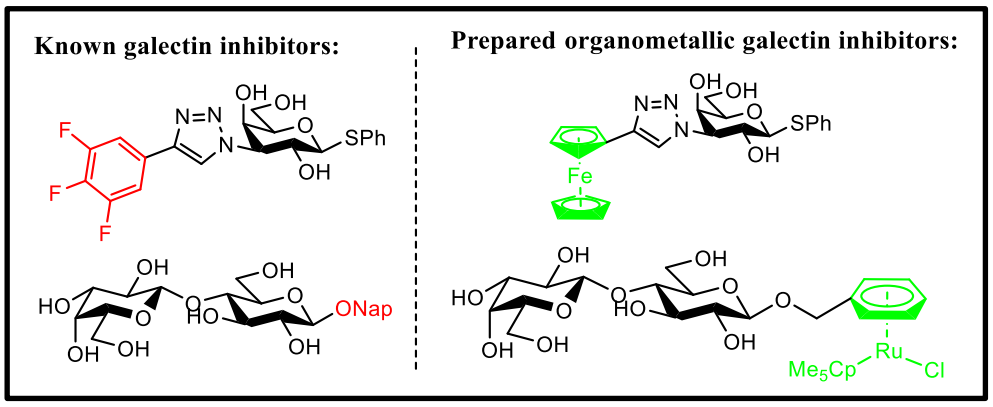

Fig. 1: *Examples of syntetic galectin inhibitors and selected derivates of prepared hybrid organometallic structures.*

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OL12 STD NMR spectroscopy reveals the structural basis of the interaction of the complex N-glycan G0 with fucosyltransferase FUT8

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KEYWORDS: NMR spectroscopy, Protein-Carbohydrate Interactions, Glycosyltransferases, Molecular dynamics, Carbohydrate structure

ABSTRACT: FUT8 catalyses the transfer of a fucose residue from GDP-Fuc to core GlcNAc moieties of N-Glycans via an α1,6-linkage [1], and notably its up-regulation is characteristic of many cancer types [2]. In vitro, FUT8 fucosyl transfer occurs most often on the complex N-glycan oligosaccharide, G0 [3], and is able to fucosylate high mannose N-glycopeptides without GlcNAc residues. Curiously, it appears that the inclusion of a short chain peptide (KVANKT) is what allows for this fucosylation [4]. This work sought to understand the role of the peptide in recognition and fucosyl transfer, using a series of ligands prepared by enzymatic synthesis, including G0 and its peptide conjugate G0-peptide, as well as the high mannose N-glycan Man3 and its peptide conjugate Man-3 peptide. From a multidisciplinary approach, including ITC, X-Ray crystallography and molecular dynamics it was shown that the active substrates for FUT8 include G0, G0-peptide and Man3-peptide.
To explore the structural rationale of these observations and understand the role of the peptide conjugate, we resorted to saturation transfer difference (STD) NMR spectroscopy. STD NMR provides structural information on how a ligand is recognised by a protein identifying those parts of the ligand intimately recognised by the protein, determining the crucial molecular features allowing recognition (ligand binding epitope mapping). We studied the binding of these complex ligands both in the presence and absence of GDP. We elucidated the binding epitope of G0 and G0-peptide whereas this was not possible for Man3 and Man3-peptide as they showed very low binding affinity to FUT8, outside the observable range for STD NMR. For G0-peptide the STD NMR epitope qualitatively matched the previously published crystal structure for FUT8-GDP-G0. Furthermore, we deduced the role of the peptide indicating that, although it has minimal effect on the binding epitope of the glycan, it is itself recognised by FUT8. Finally, a clear difference in epitopes in the presence and absence of GDP was observed implying that GDP may have a pre-organising role in the active site which alters substrate recognition. [5]

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OL13 Diversity of polysialylation machinery in fish species highlights exciting perspectives to generate original high therapeutic biomaterials

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ABSTRACT: Polysialic acids (polySia) are biopolymers known for their repulsive properties in the developing brain [1]. They have drawn attention recently because of their attractive properties. These charged polymers are able to bind and modulate the function of biomolecules like the BDNF, histones and anti-microbial substances in the nervous [2] and in the immune [3] systems. In contrast to human tissues that show a single type of polymer of α2,8-linked N-acetylneuraminic acid (Neu5Ac), fishes generate a wide range of heteropolymers made of various sialic acids including Neu5Gc, Neu5Ac or KDN [4]. Moreover, polySia quantity and nature are much more important and diverse in fish serum than in humans [5]. However, nothing is known about the origin of these polySia and how are they synthesized and the fish biosynthetic machinery is currently not known. Therefore, we started the study of the evolutionary history of the α2,8-sialyltransferase (ST8Sia) family in vertebrates. This phylogenetic study revealed a particular distribution of fish polysialyltransferases compared to their human homologs [6]. Furthermore, amino-acids changes were described in PBR and PSTD polysialyltransferase specific-domain suggesting the existence of novel enzyme activities [7]. Indeed, the whitefish *Coregonus maraena* of the salmonid family exhibits three polysialyltransferase genes: 2 ST8Sia II and 1 ST8Sia IV paralogs. We engineered soluble chimeric polysialyltransferases with a *N*-terminal FLAG tag and produced these recombinant proteins in the cell culture medium of transiently transfected mammalian cells. We also chemo-enzymatically synthetized natural (CMP-Neu5Ac, CMP-Neu5Gc, CMP-KDN) and unnatural (CMP-SiaNAl) donor substrates. CMP-SiaNAl is a functionalized donor with a small alkyne group allowing bioorthogonal click-chemistry approaches. Using this highly specific biosynthetic tools and the recently developed MicroPlate Sialyltransferase Assay (MPSA) [8], we optimized sialylation assay conditions (temperature, pH, time course, substrates concentrations). We then studied the enzymatic activity and kinetic parameters of ST8Sia IV and demonstrated that this enzyme is active preferentially on an α2,3-sialylated acceptor and has a polysialylation activity. Our preliminary data also indicate that unlike human enzyme, the fish ST8Sia IV synthetizes polySia chains using CMP-Neu5Gc or CMP-KDN and DMB-acceptors. To analyze the nature and the composition of polySia, we used HPLC/MS and detected polySia chains of various nature. These strategies enabled us to highlight distinct activities between a fish polysialyltransferase and its human counterpart and will help us generating novel polySia-based biomaterials with high therapeutic potential.

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OL14 Tumor Sialylation: a Master Regulator of anti-tumor Immunity in Colorectal Cancer

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KEYWORDS: sialic acids, immunology, gangliosides, CRISPR/Cas9, Colorectal cancer

ABSTRACT: Abnormal levels of sialylation are observed in many types of cancer, including colorectal cancer (CRC), where they are associated with increased invasion and metastasis. Tumor-exposed glycans, especially sialic acids, are thought to play a key role in immunosuppression, but how they impact immune responses in CRC remains unclear. Previously, we generated a murine MC38 CRC glycovariant without sialic acids using CRISPR/Cas9 technology. Unexpectedly, abrogation of sialic acid expression on MC38 cells resulted in significantly increased *in vivo* tumor growth and increased $CD8^+$ T cell apoptosis in the tumor microenvironment (TME) [1]. Thus, we hypothesized that the level of sialic acids governs the effectiveness of the anti-tumor immune response, whereby high sialylation results in immune evasion due to an increase in regulatory T cells in the TME [2], and complete removal of sialic acids induces immune cell death.
Our aim is to uncover how sialylation affects anti-tumor immunity in CRC by generating syngeneic CRC glycovariants (MC38 and CT26 cells) with different levels of sialylation. So far, we successfully generated sialic acid knock-out tumor cells and hypersialylated MC38 cells. Once all the cell lines are validated, we will characterize the *in vivo* immune landscape of the different sialo-variants and evaluate their responsiveness to immunotherapy. Our findings will be also confirmed using metastatic tumor samples of CRC patients that have different sialo-immunoscores.

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OL15 Development of electrochemical glycan biosensor including oxidase enzyme mimicking MOF structure

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KEYWORDS: sialic acid, glycan, biomarker, metal organic framework, enzyme mimicking

ABSTRACT: Sialic acid (SA), is a kind of monosaccharide that is found at the end of the glycan chain. It also acts as a biomarker for many diseases including cancer. Increased serum concentrations of SA have been detected in several tumors, such as pancreatic cancer, skin squamous cell carcinoma, lung, prostate, breast, ovary, colon, and thyroid cancers[1].
In this work, we aim to develop an electrochemical biosensor including N-acetylneuroaminic Acid Aldolase (NANA-Aldolases) and oxidase enzyme mimicking metal-organic framework (MOF) structure for SA detection. For this purpose, Co/2Fe MOF was used as an oxidase enzyme mimicking material. The bimetallic Co/2Fe MOF was synthesized with Teflon lined stainless steel autoclave by a hydrothermal method [2]. The morphologies and atomic composition of the synthesized Co/2Fe MOF structure were characterized by X-ray diffraction scanning electron microscopy (SEM), SEM-mapping and Energy-Dispersive X-Ray Spectroscopy. For the preparation of the electrochemical sialic acid biosensor, first gold screen-printed electrode (AuSPE) was modified with the synthesized Co/2Fe bimetallic MOF. Then, NANA-Aldolase enzyme was also attached onto it (Fig. 1). This enzyme converts the free SA into pyruvate and N-acetyl-D-mannosamine. After this conversion, oxidase mimicking Co/2Fe bimetallic MOF converts pyruvate into acetylphosphate and oxygen into H_2O_2. The consumption of O_2 was monitored amperometrically approximately at -0.7 V. After the optimization of experimental parameters analytical characteristics studies were examined for the developed SA biosensor.

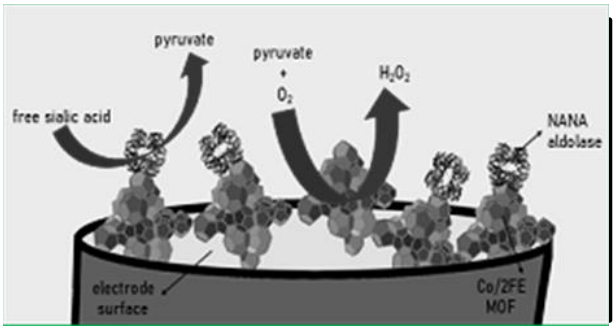

Fig. 1: *Schematic representation of the developed SA biosensor*

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Flash presentations

FL1 Cracking the ErbB2 Sugar Code in Gastric Cancer – A Bitter Sweet Functional Target

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KEYWORDS: Glycosylation, Cancer Biology, Biomarker, Targeted therapy, Glycomics and Glycoproteomics

ABSTRACT: The clinical performance of the therapeutic monoclonal antibody trastuzumab in the treatment of ErbB2-positive unresectable gastric cancer (GC) is severely hampered by the emergence of molecular resistance. Trastuzumab's target epitope is localized within the extracellular domain of the oncogenic cell surface receptor tyrosine kinase (RTK) ErbB2, which is known to undergo extensive *N*-linked glycosylation. However, the site-specific glycan repertoire of ErbB2, as well as the detailed molecular mechanisms through which specific aberrant glycan signatures functionally impact the malignant features of ErbB2-addicted GC cells, including the acquisition of trastuzumab resistance, remain elusive. Here, we demonstrate that ErbB2 carries both α2,6- and α2,3-sialylated glycan structures in GC tissue clinical specimens. In-depth mass spectrometry-based glycomic and glycoproteomic analysis of ErbB2's ectodomain disclosed a site-specific glycosylation profile in GC cells, in which the ST6Gal1 sialyltransferase specifically targets ErbB2 *N*-glycosylation sites occurring within the receptor's trastuzumab-binding domain (Fig. 1). Abrogation of ST6Gal1 expression through precise genome editing reshaped the cellular and ErbB2-specific glycomes, expanded the cellular half-life of the ErbB2 receptor, and sensitized ErbB2-dependent GC cells to trastuzumab-induced cytotoxicity through the stabilization of ErbB dimers at the cell membrane, and the decreased activation of both ErbB2 and EGFR RTKs. Overall, our data demonstrates that ST6Gal1-mediated aberrant α2,6-sialylation actively tunes the resistance of ErbB2-driven GC cells to trastuzumab [1].

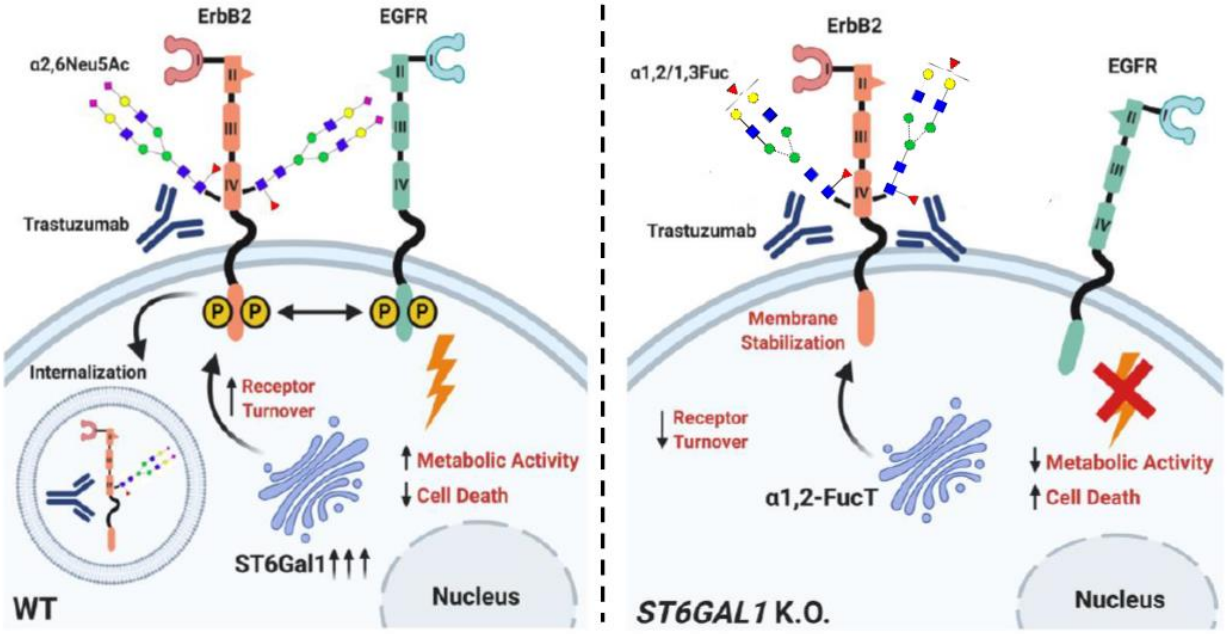

Fig. 1: *Proposed molecular mechanism underlying the differential sensitivity of Wild-Type and ST6GAL1 K.O. ErbB2-addicted gastric cancer cells to trastuzumab-induced cytotoxicity*

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FL2 Glycan-dependent signalling routes and transcriptional programs in human dendritic cells after triggering the C-type lectin receptor MGL

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KEYWORDS: Glycodendrimers, Macrophage galactose-type lectin (MGL), Dendritic cells, Flow cytometry, ELISA

ABSTRACT: C-type lectin receptors on dendritic cells, recognizing glycans, can shape the immune response to bacteria, viruses and tumor cells. The macrophage galactose-type lectin (MGL) binds terminal N-Acetylgalactosamine residues and signaling through this receptor reduces the glycolytic capacity of monocyte-derived dendritic cells (moDCs) [1] and increases their IL-10 and TNFα production, which stimulates a Th2 and Tr1 response. Recently, several MGL ligands were discovered to induce different conformations of the MGL carbohydrate-recognition domain [2], but the effect on moDC maturation and functionality was only investigated for two of these MGL ligands. [1] We coupled five different MGL ligands to dendrimers to increase their polyvalency. Our aim is to characterize the transcriptional programs that are induced in response to these MGL ligands and determine which cytokines, co-stimulatory and inhibitory receptors, and signaling molecules are associated with the different transcriptional programs. Up to now, MGL stimulation resulted in a higher CD11c and CD14 co-expression by moDCs. The five MGL ligands vary in their affinity to MGL and also displayed a different capacity to increase IL-10 secretion. In the future, MGL-mediated ligand-specific transcriptional programs could be exploited to manipulate immune responses in human DCs.

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FL3 Molecular recognition of sialoglycans by streptococcal Siglec-like adhesins: toward the shape of specific inhibitors

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KEYWORDS: glycoconjugates, sialic acid, recombinant proteins, NMR, molecular modelling

ABSTRACT: *Streptococcus gordonii and Streptococcus mitis*, commensal species among the normal oral microbiota, become opportunistic pathogens that can cause infective endocarditis (IE) when they enter the bloodstream [1]. The presence on the microbial surface of "Siglec-like" serine-rich repeat adhesins may increase the propensity of streptococci to cause IE. These adhesins contain Siglec-like binding regions (SLBRs) that recognize α2-3 sialylated glycan structures, especially O-linked glycans displayed on salivary MUC7, platelet GPIb and several mucin-like plasma proteins [2]. The SLBRs from different strains recognize different repertoires of sialoglycans, with some displaying selectivity for a single structure and others showing broader specificity [3].
We here explored the recognition and binding process of different Siglec-like serine-rich repeat adhesins, including those from *S. gordonii* SLBR-B (M99 strain), highly selective, and SLBR-H (Challis strain), instead showing broader specificity, with different sialoglycans. In particular, the binding modes of 3'-sialylactosamine, sialyl-T-antigen and GM1b ganglioside into the Siglec-like adhesins binding sites were described by a combination of NMR ligand-based methods and computational approaches [4,5]. Thus, unveiling the molecular mechanism of host glycans recognition by Siglec-like adhesins could provide strategies for the development of potential glycomimetics to counteract IE disease. Moreover, despite a similar fold and conserved F-strand Arg, the pattern of interactions established by the SLBRs are mechanistically very different from those reported for mammalian Siglecs [6]. Therefore, the more detailed understanding of SLBR-Siglec differences may enable the design of tailored inhibitors that do not interfere with important Siglec interactions.

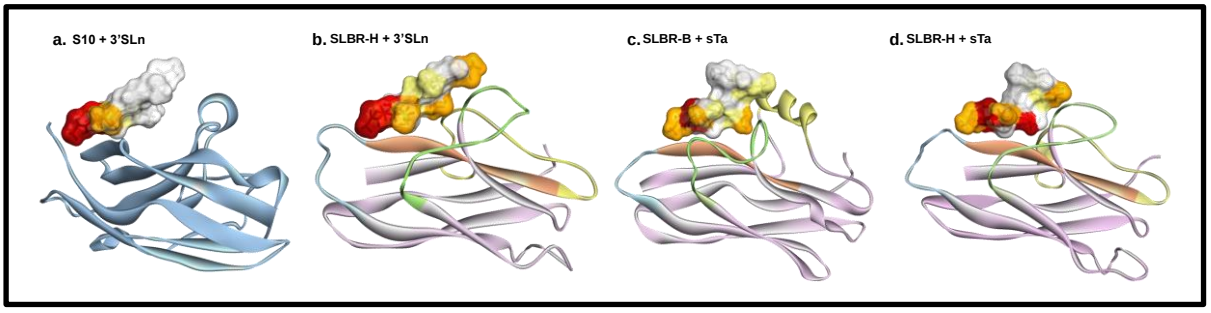

Fig. 1: *Comparison of the Siglec and Siglec-like adhesin binding sites. Molecular view of 3D complexes: Siglec-10 – 3'-Sialylactosamine (a) SLBR-H – 3'-Sialylactosamine (b); SLBR-B – sialyl-T-antigen (c); SLBR-H – sialyl-T-antigen (d).*

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FL4 Interaction studies between bacterial glycans and host immune receptors

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KEYWORDS: glycans, molecular recognition, immune receptors, NMR spectroscopy,

ABSTRACT: All cells of living organisms are covered by a layer of glycans that acts as an interface between the outer environment and the cell membrane. Glycans exhibit broad structural diversity and are involved in fundamental molecular and biological mechanisms, including protein folding, cell adhesion, signal transduction, receptor activity modulation and immunological and pathological processes. Particularly, glycans are involved in the interaction mechanisms of bacteria with eukaryotic host. Glycans serve as counter receptors for different proteins, including lectins [1]. These are exposed on the surface of innate immune cells and represent an important class of Pathogen Recognition Receptors (PRRs) characterized by their ability to recognize glycans.
These PRRs may contribute to initial recognition of bacterial glycans, thus providing an early defense mechanism against bacterial infections, but some of them may also be exploited by bacteria to escape immune responses.
Several human pathogens have indeed developed the capability to cover their surface with glycans mimicking eukaryotic SAMPs (Self Associated Molecular Patterns) structures, able to interact with inhibitory host receptors, thus eluding host immune responses and promoting infections.
Among them, the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* species) pathogens exhibit multidrug resistance and virulence and represent a global threat to human health. [2].
Thus, given their therapeutic relevance, we aim to elucidate, at a molecular level, the recognition of glycoconjugates isolated from Gram-negative bacteria, such as capsular polysaccharides from *A. baumannii*, by inhibitory host receptors, as Siglec-10.
In order to dissect the fine details of the recognition of feared pathogens from immune response, we use a multidisciplinary approach based on different and advanced biophysical techniques, mainly NMR spectroscopy, combined with computational studies.

ACKNOWLEDGEMENTS: FSE, PON Ricerca e Innovazione 2014–2020, Azione I.1 "Dottorati Innovativi con caratterizzazione Industriale" is acknowledged for funding the PhD grant to Angela Marseglia.

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FL5 Study of the glycosylation-dependent interaction between NK cell receptors and Galectin-1 by microscale thermophoresis

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KEYWORDS: C-type Lectin-like Receptors, N-glycosylation, Glycosylation in NK cell receptors, NK cell receptors, Galectin-1

ABSTRACT: CD69, an early activation marker constitutively expressed by several subsets of immune cells, is a disulfide-linked homodimer with two extracellular N-glycosylation sites. Although its precise involvement in immunity remains to be fully elucidated, progress has been made in the identification of putative protein ligands. Galectin-1, a prototypical lectin characterized by a common structural fold
and conserved CRD with affinity for β-galactosides, has been demonstrated as a ligand of CD69 in a N-glycosylation-dependent manner [1].
Using microscale thermophoresis we independently confirmed the interaction of CD69 with Galectin-1, as well as further investigated its dependency on varying N-glycosylation moieties. Interestingly, Galectin-1 was additionally shown to interact with similar or stronger affinity to other C-type lectin-like receptors (e.g., human LLT1 and KACL), thus revealing that the interaction is not as specific as initially assumed. These findings suggest the possibility of a structurally conserved interaction between Galectin-1 and NK cell receptors with a CTLD fold. Further efforts in the characterization of these interactions in a biophysical and structural manner is pivotal for a better understanding of the precise role of Galectin-1 in the context of NK cells.

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FL6 Natural killer cell activation receptor NKp30 oligomerization depends on its N-glycosylation

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KEYWORDS: Mammalian protein expression, Natural Killer cells, Protein crystallization, NKp30, Oligomerization

ABSTRACT: NKp30 is one of the main human natural killer cell activating receptors used in directed immunotherapy. The oligomerization of the NKp30 ligand binding domain depends on the length of the stalk region, but our structural knowledge of NKp30 oligomerization and its role in signal transduction remains limited. Moreover, ligand binding of NKp30 is affected by the presence and type of N-glycosylation. We assessed whether NKp30 oligomerization depends on its N-glycosylation. Our results show that NKp30 forms oligomers when expressed in HEK293SGnTI$^-$ cell lines with simple N-glycans. However, NKp30 was detected only as monomers after enzymatic deglycosylation. We characterized the interaction between NKp30 and its ligand, B7-H6, with respect to glycosylation and oligomerization, and we solved the crystal structure of this complex with glycosylated NKp30, revealing a new glycosylation-induced mode of NKp30 dimerization. Our study provides new insights into the structural basis of NKp30 oligomerization and explains how the stalk region and glycosylation of NKp30 affect its ligand affinity. This furthers our understanding of the molecular mechanisms involved in NK cell activation, which is crucial for the design of novel NK cell-based targeted immunotherapeutics.

FL7 HIV-1 Tat/heparin interaction: translating new insight from molecular modelling to the comprehension of its biological functions

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KEYWORDS: glycosaminoglycans modelling, glycosaminoglycans-protein docking, glycosaminoglycans molecular dynamics, surface plasmon resonance, heparan sulfate proteoglycans

ABSTRACT: HIV is the ethological agent of AIDS. HIV^+ lymphocytes (LCs) release the transactivating factor (Tat) which, in its dimeric form, bind to different host cell receptors including heparan sulfate proteoglycans (HSPGs), vascular endothelial receptor 2 (VEGFR2) and integrins, mediating a variety of biological effects involved in the onset of AIDS. Accordingly, various functional domains of Tat have been identified: the cysteine rich domain (aa 22-37) for dimerization, the "basic domains" (aa 48-57) for HSPGs and VEGFR2 binding and a RGD motif (aa 78-80) for integrins binding. Once released by HIV^+ LC, Tat dimers associate to HSPGs of the same cell, favoring the formation of an *in-cis* trimeric complex with integrins that stimulates LC migration. Also, due to its dimeric nature, Tat associated to LC's HSPGs retains the ability to bind to HSPGs on facing endothelial cells (ECs), forming an *in-trans* HSPG/Tat-Tat/HSPG quaternary complexes that promotes LC extravasation. Tat bound to LC's HSPGs also retains the ability to bind *in trans* VEGFR2 and integrins of ECs, inducing their inflammatory activation and vessel permeability. Thus, the HSPGs/Tat complex orchestrates the recruitment of signaling receptors and regulate biological processes relevant to AIDS pathogenesis. It is therefore important to characterize at a molecular level the structure of the HSPGs/Tat complex.
To this aim, here we have firstly modelled the monomeric Tat using the MODELLER program. The lowest energy model was selected from the conformations obtained. To model the Tat dimer, the automatic protein-protein docking ClusPro web-server was used; the resulting best complexes were filtered by visual inspection. Blind docking simulations were performed by ClusPro using 4-mer heparin as ligand option to identify heparin-binding regions on Tat that were then filtered by best score, cluster size, visual inspection and finally positioned onto Tat to achieve a traced heparin path.
The 4-mer heparin probe was used in local docking simulation along the traced heparin path in Tat by Autodock 4. The "sliding window method" was set up to create a sequence of overlapping sliding grids. Local docking poses were filtered for free energy of binding, clusters size and correct orientation. The aligned 4-mer heparin probes were joined by 1→ 4 glycosidic linkages using Pymol. Gasteiger-Hückel charges were assigned to the sugar and then minimized by Chimera, obtaining heparin chains of increasing length. The two previous methods have been developed and applied in heparin protein interaction studies.
A first model has been obtained with a 10-mer heparin chain that binds several regions of monomeric Tat, including the heparin binding but not the cysteine rich motif, suggesting that Tat heparin binding does not impaired protein dimerization.
A second model has been obtained using two Tat monomers to predict its dimeric form in which the heparin regions remains solvent exposed and available to interact with 10-mer heparin chain.
In the Tat monomer but not in the dimer model, the integrin-binding RGD motif remains fully accessible. MDs of the Tat monomer and dimer are ongoing to evaluate their stability. Stable complexes will be used to model the biological relevant heparin/Tat complexes and their further interaction with the other Tat signaling receptors VEGFR2 and integrins. Molecular dynamic simulations are ongoing for both Tat monomer and dimer complexed to 10-mer heparin using

Amber18 package.

FL8 TOWARDS A NOVEL VACCINE CANDIDATES AGAINST STREPTOCOCCUS PNEUMONIAE 6A/6C

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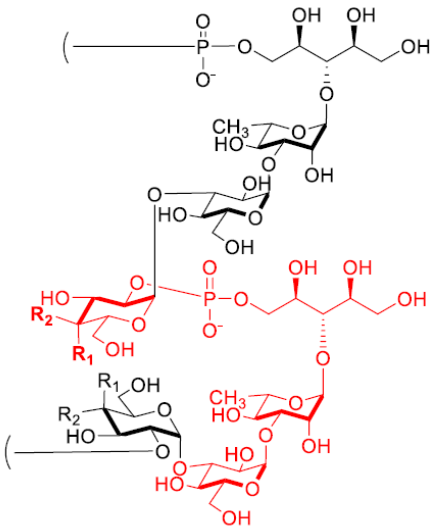
KEYWORDS: monosaccharides modification, synthesis oligosaccharides, glycosylation, synthesis of analogues, NMR analysis on carbohydrates

ABSTRACT: Carbohydrates are amongst the most widespread organic molecules in nature. They are essential constituents of all living organisms playing a key role in the communication between cells and the outside world. For instance, encapsulated bacteria present an external coating constituted by specific polysaccharides sequences (capsular polysaccharides, CPS).



R_1 = OH, R_2 = H (Gal): SPn 6A
R_1 = H, R_2 = OH (Glc): SPn 6C
R_1 = R_2= H: common epitope SPn 6A/6C
Fig.1*: Repeating units as synthetic aims.*

Steptococcus pneumoniae (SPn) is a Gram-positive encapsulated bacterium characterized by more than 90 serotypes with a different CPS and different virulence. Currently SPn is the responsible of at least half of pneumonia diseases worldwide and almost 19% of children under 5 years old deaths. Because of the high antibiotic resistance of this bacterium, the use of vaccines is the most efficient method to prevent pneumococcal infection in children. For children below 5 years old there are 3 efficient glycoconjugate vaccines on the market: PCV7, PCV10 and PCV13, covering the 13 most virulent serotypes. These vaccines led to a substantial decrease of infections. However, an increase of infections caused serotypes not included in the vaccines was recorded over the last years (serotype replacement phenomenon). Accordingly, new polyvalent vaccines able to confer protection against a higher number of SPn serotypes are needed. Serogroup 6 includes 4 serotypes: 6A, 6B, 6C, 6D, but only 6A and 6B are comprised in the main PCV vaccines commercially available. However in 2012, due to the “serotype replacement”, 3.6% of SPn infections were caused by serotype 6C. Given the CPS structure similarities between serogroup 6 (Fig.1), we aim to the preparation of tetrasaccharide repeating units of serotypes 6A/6C. In addition, we designed a glycomimetic of 6A/6C repeating units potentially able to raise cross-protection against both serotypes. In this communication, the most recent results of our synthetic effort will be reported.

FL9 Cross reaction of antibodies against LPS P. mirabilis O3 with collagen type I, may mediated complement activation

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KEYWORDS: Lipopolysaccharide, cross reactive antibodies, rheumatoid arthritis, Proteus mirabilis, synthetic lipopolysaccharide fragments

ABSTRACT: Rheumatoid arthritis (RA) is one of the most common autoimmune diseases in worldwide population. As most of autoimmune diseases, etiology of RA is complex and not fully understood. There are suggestions that infections agents may be a one of the factors considered to the disease development. A number of publications showed a relationship between *Proteus mirabilis* bacteria and RA development.
The objective of this study was to investigate the cross reactivity potential of antibodies binding to lysine containing LPS *P. mirabilis* O3.
Results of antibodies isolation obtained by affinity chromatography showed that patients possess more antibodies binding to LPS *P. mirabilis* O3. Analysis of isolated antibodies reaction with other lysine containing LPS of P*. mirabilis* showed a cross-reactivity dependent on a tested serogroup. Moreover, based on ELISA assay, the cross reaction of isolated antibodies with collagen type I was shown. The reaction with collagen type I was correlated with reaction against LPS P. mirabilis O3. Analysis of complement activation results show that isolated antibodies bound to collagen type I, activated complement more efficiently than the antibodies bound to LPS. Due to information suggest a role of lysine epitope in cross reactivity between P. mirabilis lipopolysaccharides and collagen type I, on the next step reaction with synthetic Lys-GalA, and other haptens was investigated. Samples of isolated antibodies presented individual pattern of reaction with panel of synthetic haptens being LPS non-carbohydrate fragments contain amino acids (lysine, alanine, serine and threonine). The reactions were dependent from the type of amino acid, type of monosaccharide and bond.
Results obtained by isolated antibodies reaction indicate antibodies binding with LPS O3 may show a variable of specificity with respect to the antigen. Moreover, complement activation via antibodies binding to bacterial LPS with collagen suggest a role of bacterial antigens with autoimmune disease development.

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FL10 Extraction and characterization of chondroitin sulphate from chicken sternum

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KEYWORDS: chicken sternum, glycosaminoglycans, chondroitin sulfate, extraction, osteoarthritis treatment

ABSTRACT: Glycosaminoglycans (GAGs) are one of the basic extracellular components and have wide application prospects in the field of pharmaceutical, cosmetic, food industries. CS is one of the GAGs types and widely used for osteoarthritis treatment. Clinical studies have shown that orally taken chondroitin sulfate improves joint function and reduces pain in osteoarthritis patients (Garnjanagoonchorn, Wongekalak, and Engkagul 2007). In this study, glycosaminoglycans (GAGs) including chondroitin sulfate were extracted and characterized from chicken sternum. GAGs were obtained by proteolysis with papain at 65°C, for 24 h, precipitated with 10% TCA and 80 % ethanol, dialyzed and finally lyophilized to dry powder. Dimethylmethylene blue assay was performed to estimate the quantity of CS extracted. Identification of types of GAGs through FTIR spectroscopy was carried out. The CS concentration extracted from chicken sternum was 87,46 %. Khan et al. (2013) reported the chondroitin sulfate content of GAG samples extracted from chicken sternum as 70.77%. These findings indicate that an effective extraction method has been developed. Comparative FTIR analysis result of chondroitin sulfate standard (Sigma C9819) against GAG extract obtained from chicken sternum is shown in the graph in Figure 1 (correlation value 0.6882). FTIR specta of CS exhibited the characteristic C –O –S peaks were observed at about 850 cm^{-1} for chondroitin sulfate standard and GAG extract (Sundaresan et al, 2018). It has been shown in this study that chicken sternum is the one of the promising potential sources of chondroitin sulfate for applications especially in the treatment of osteoarthritis.

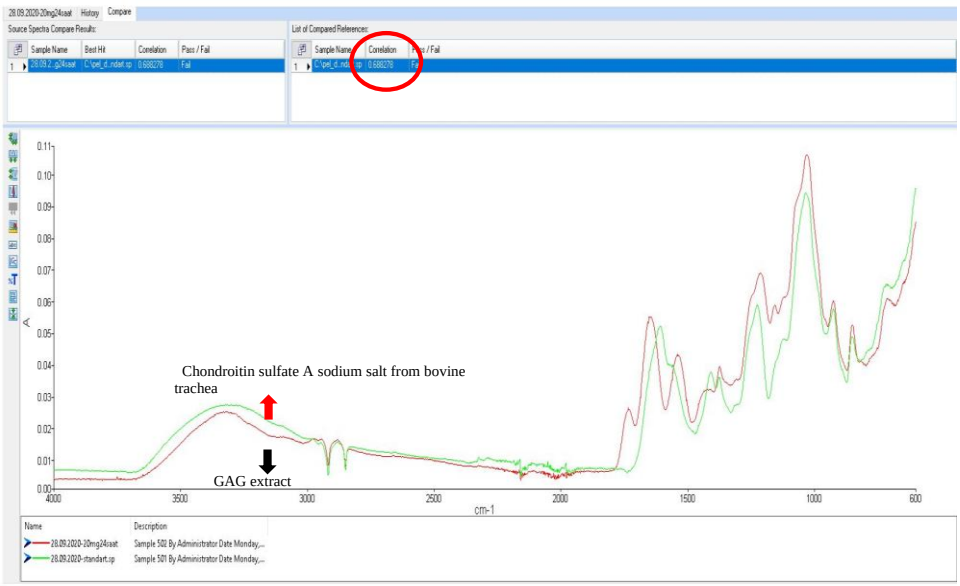

Figure 1. *FTIR spectra of standard chondroitin sulfate and GAG extract*

ACKNOWLEDGEMENTS: This research was supported by the Lezita/Abalıoğlu Feed-Soybean Industry, İzmir,TURKEY.

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