Società Chimica Italiana

Gruppo Interdivisionale
Chimica dei Carboidrati

CONVEGNO-SCUOLA SULLA CHIMICA DEI CARBOIDRATI (XVIII CSCC 2023)

25-28 Giugno 2023

Certosa di Pontignano – Siena

Università di Pisa
Dipartimento di Farmacia

Università di Siena
Dipartimento di Biotecnologie, Chimica e Farmacia

Università di Firenze
Dipartimento di Chimica "U. Schiff"

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XVIII CSCC 2023 Program

	Sunday June 25th
11:00÷16:30	**Registration**
16:30÷17:00	**Opening**
	Chairman: FRANCESCO NICOTRA
17:00÷17:45	**Medaglia "G. Berti" Lecture** **Antonio Molinaro** ***(University of Napoli Federico II)*** **Glycoscience in microbial world: the power of sugars**
	Chairman: DOMENICO GAROZZO
17.45÷18.30	**PL-1: Todd L. Lowary** ***(Nangang, Taipei, Taiwan)*** **Synthesis of Complex Microbial Glycans**
18:30÷19:00	**KN-1: Serge Perez,** Olga Makshakova, Jesus Angulo, Emiliano Bedini, Antonella Bisio, Jose Luis de Paz, Elisa Fadda, Marco Guerrini, Michal Hricovini, Milos Hricovini, Frederique Lisacek, Pedro M. Nieto, Kevin Pagel, Giulia Paiardi, Ralf Richter, Sergey A. Samsonov, Romain R. Vives, Dragana Nikitovic, Sylvie Ricard Blum ***(University of Grenoble-Alpes, France)*** **Glycosaminoglycans: What Remains To Be Deciphered?**
19:30	**WELCOME COCKTAIL AT CERTOSA**
	Monday June 26th **Morning**
	Chairman: EMILIANO BEDINI
9:00÷9:45	**DL-1: Francesca Micoli** ***(GSK Vaccines S.r.l. Siena, Italy)*** **Carbohydrate based vaccines for low-middle income countries.**
9:45÷10:00	**OC-1: Rebecca Nappini,** Renzo Alfini, Paola Cescutti, Francesca Micoli, Carlo Giannelli ***(University of Trieste-GSK Vaccines S.r.l. Siena)*** **A Generic Conjugation Chemistry Supporting the Development of Multivalent Vaccines**
10:00÷10:15	**OC-2: Andrea Sodini,** Maria Salobehaj, Valerio Zullo, Linda Cerofolini, Daniela Eloisa Capialbi, Cristina Nativi, Marco Fragai ***(University of Firenze)*** **Rhamnosylated PD-1 mutant endowed with immunological activity.**
10:15÷10:30	**OC-3: Angela Marseglia,** Antonio Molinaro, Alba Silipo, Roberta Marchetti ***(University of Napoli FedericoII)*** **Recognition Mechanisms of Bacterial Glycans by Host Immune Receptors**
10:30÷10:45	**OC-4: Giuseppe Stefanetti,** Joon S. Park, Ximei Sun, Meng Wu, Dennis L. Kasper **(University of Urbino)** **Microbiota-mediated regulation of immune responses to vaccines in outbred mice**
10:45÷11:30	***COFFEE BREAK***
	Chairlady: LAURA RUSSO
11:30÷12:15	**PL-2:** Elita Montanari, Nicole Zoratto, Pietro Matricardi, **Chiara Di Meo** ***(Sapienza University of Roma)*** **Hyaluronan-Based Nanogels as Versatile Carriers of Bioactive Molecules**
12:15÷12:30	**OC-5:** Sabrina Bertini, Marco Guerrini, Giulio Bianchini, Stefano Elli, Emiliano Esposito, Minghong Ni, Tommaso Sisto, **Sofia Nizzolo** ***(IRCB-Ronzoni, Milano)*** **Chemical Modification and Structural Characterization of Lactosilated Hyaluronic Acid**

12:30÷12:45	**OC-6: Barbara Bellich,** Michele Cacioppo, Rita De Zorzi, Zois Syrgiannis, Paolo Bertoncin, I.A. Jou, J.W. Brady, Roberto Rizzo and Paola Cescutti ***(University of Trieste)*** **Morphological investigations of the polysaccharide extracted from biofilm produced by Burkholderia multivorans strain C1576.**
13:00÷14:30	***LUNCH AT CERTOSA***
	Monday June 26th **Afternoon**
	Chairman: MARCO GUERRINI
14:30÷14:45	**Premio intitolato alla memoria del Prof. Benito Casu**
14:45÷15:00	**OC-7: "B. Casu" award talk** **Sara Pollastri** ***(University of Milano)*** **Selective Glycomimetic Ligands of C-type Lectin Receptors**
15:00÷15:45	**PL-3: Romain Vives** ***(University Grenoble Alpes, France)*** **Post-synthetic editing of heparan sulfate sulfation pattern by the Sulfs**
15:45÷16:00	**OC-8: Simone Pepi,** Luigi Talarico, Gemma Leone, Marco Consumi, Stefania Lamponi, Marco Fragai, Marco Martinucci, Veronica Baldoneschi, Oscar Francesconi, Cristina Nativi, Agnese Magnani ***(University of Siena)*** **Viscosupplement based on a hyaluronan derivative grafted with a MMP inhibitor**
16:00÷17:45	**POSTER SESSION AND *COFFEE BREAK* (16.00-16.30)**
18:00	***Meeting of the Italian Group on Carbohydrate Chemistry (GICC)***
20:00	***DINNER AT CERTOSA***
	Chairlady: CRISTINA NATIVI
21:45-22:30	**Prof. Luigi Dei** ***(Dipartimento di Chimica, Università degli Studi di Firenze)*** **From Franz Schubert to Leonard Cohen: the chemical pleasure of listening to the voice in music.**
	Tuesday July 27th **Morning**
	Chairlady: PAOLA CESCUTTI
9:00÷9:45	**PL-4: Robert Field** ***(University of Manchester)*** **Understanding and exploiting CAZymes in glycan synthesis**
9:45÷10:00	**OC-9: Ferran Nieto-Fabregat,** Qian Zhu,b Diksha Rai, Corinne Vivès, Angela Marseglia, Michel Thépaut, Flaviana Di Lorenzo, Antonio Molinaro, Roberta Marchetti, Franck Fieschi, Suvarn S. Kulkarni, Biao Yu, Alba Silipo ***(University of Napoli FedericoII)*** **Lectin mediated surveillance of gut microbiota: DC-SIGN recognizes and binds selected epitopes within *Bacteroides vulgatus* LPS.**
10:00÷10:15	**OC-10: Francesco Milanesi,** Luca Unione, Ana Arda, Cristina Nativi, Jesús Jiménez-Barbero, Stefano Roelens, Oscar Francesconi ***(University of Firenze)*** **Recognition of the Core Disaccharide of *N*-Glycans by a Synthetic Receptor**

10:15÷10:30	**OC-11:** **Angelo Palmigiano,** Rita Barone, Luisa Sturiale, Renata Rizzo, Fabio Pettinato, Lara Cirnigliaro, Martina Randazzo, Angela Messina, Jessica Galli, Elisa Fazzi, Domenico Garozzo *(IPCB-CNR Catania)* **Glycomic analysis of Cohen syndrome suggests a sweet treatment.**
10:30÷10:45	**OC-12:** **Laura Russo,** F. Cadamuro, M. Ferrario, E. Ballarini, L. Crippa, L. Marongiu, F. Barbugian, M. Bracchi, A. Peanuti, Beatrice Sonzogni, L. Nespoli, N. Tamini, N. Zucchini, A. Ferramosca, G. Cavaletti, F. Previdi, F. Nicotra *(University of Milano-Bicocca)* **Glycosignature in Human Tissue Model Libraries Aided by Artificial Intelligence**
10:45÷11:30	*COFFEE BREAK*
	Chairman: OSCAR FRANCESCONI
11:30÷12:15	**DL-2: Marco Terreni** *(University of Pavia)* **Enzyme mediated synthesis of carbohydrate-based compounds. From the development of green processes to the design of engineered biotechnological products**
12:15÷12:30	**OC-13:** **Serena Traboni,** Emiliano Bedini, Alfonso Iadonisi *(University of Napoli FedericoII)* **Greener and Streamlined Approaches for the Synthetic Tranformations of Complex Organic Compounds: Focus on Glycochemistry**
12:30÷12:45	**OC-14:** **Fabiana Esposito,** Serena Traboni, Alfonso Iadonisi, Emiliano Bedini *(University of Napoli FedericoII)* **Regioselective sulfation of polysaccharides from sustainable sources**
13:00÷14:30	*LUNCH AT CERTOSA*
	Tuesday June 27th **Afternoon**
	Chairman: SERGE PEREZ
14:30-15:15	**PL-5:** Javier O. Cifuente, Julia Schulze, Andrea Bethe, Valerio Di Domenico, Christa Litschko, Insa Budde, Lukas Eidenberger, Hauke Thiesler, Isabel Ramón Roth, Monika Berger, Heike Claus, Cecilia D'Angelo, Alberto Marina, Rita Gerardy-Schahn, Mario Schubert, Marcelo E. Guerin, **Timm Fiebig** *(Medizinische Hochschule Hannover, Germany)* **Enzymatic strategies for the synthesis of vaccine antigens from Gram-negative pathogens**
15:15-16:15	**POSTER SESSION**
16:30	**Social Event & Social dinner at Monteriggioni (Siena)**
	Wednesday June 28th **Morning**
	Chairlady: ALBA SILIPO
9.30÷10.15	**PL-6: Federica Compostella** *(University of Milano)* **Synthetic saccharide epitopes in pneumococcal diseases: exploring multipresentation and multivalency.**
10.15÷10.30	**OC-15:** **Sebastiano Di Pietro,** Maria Chiara Santangelo, Lucilla Favero, Valeria Di Bussolo *(University of Pisa)* **Stereospecific One-Pot Reiterative Synthesis of Linear 1,4-oligosaccharides**

10.30÷10.45	**OC-16: Giulia Antonini,** Sarah Mazzotta, Riccardo Guerini, Annabelle Varrot, Anna Bernardi, Laura Belvisi ***(University of Milano)*** **Towards Covalent Ligands for the *N*-Terminal Domain of the Bacterial Lectin BC2L-C**
10.45÷11.00	**OC-17:** Carlo Antonini, Sabrina Bertini, Emiliano Esposito, Marco Guerrini, Marco Sansò, **Sabrina Ziliani** **(*University of Milano-Bicocca*)** **Synthesis and physio-chemical properties of sulphated tamarind (*Tamarindus indica L.)* seed polysaccharide**
11.00÷11.30	***COFFEE BREAK***
	Chairlady: Valeria Di Bussolo
11.30÷11.45	**OC-18: Maria Chiara Santangelo,** L. Bridoub, O. Maury, S. Di Pietro, V. Di Bussolo ***(University of Pisa)*** **Glycoconjugated Europium Complexes as Cancer Diagnostic Probes**
11.45÷12.00	**OC-19: Anna Notaro,** Antonio Molinaro, Abergel Chantal, Cristina De Castro ***(University of Napoli Federico II)*** **Biochemical Characterization of the First Pyruvyl Transferase Encoded by a Giant Virus.**
12.00÷12.15	**Poster awards and young scientists oral communication awards**
12.15÷12.30	***Closing remarks***
12.30	***Lunch at Certosa***

PL: Plenary Lecture; KN: keynote; DL: Didactic Lecture; OC: Oral Communication.

Poster Communication (XVIII CSCC 2023)

PC-1	**Adele Vanacore,** Maria Concetta Forgione, Domenico Cavasso, Ha Ngoc Anh Nguyen, Antonio Molinaro, James P. Saenz, Gerardino D'Errico, Luigi Paduano, Roberta Marchetti, Alba Silipo. *(University of Napoli Federico II)* **Role of EPS in Mitigation of Plant Abiotic Stress: The Case of Methylobacterium Extorquens PA1**
PC-2	Fabiana Esposito, Serena Traboni, Alfonso Iadonisi, **Emiliano Bedini** *(University of Napoli FedericoII)* **Towards the Semi-Synthesis of Phosphorylated Glycosaminoglycans**
PC-3	**Antonio Lembo,** M. Aurilia, G. L. Sardone, F. Berti, C. De Castro, A. Molinaro, M. Biagini *(University of Napoli Federico II)* **Development of a platform method for the analysis of glycosylation in recombinant proteins by Mass Spectrometry**
PC-4	**Roberta Cirella,** Alba Silipo, Antonio Molinaro, Flaviana Di Lorenzo *(University of Napoli Federico II)* **Lipopolysaccharides (LPS): the importance of the structure in the extreme environment and in the human gut**
PC-5	Carlo Alberto Vezzoni, David Goyard, **Maria Cremonini**, Laura Baldini, Alessandro Casnati, Olivier Renaudet, Francesco Sansone *(University of Parma)* **Fucosylated calix[4]arene-based ligands for microbial lectins**
PC-6	**Michela Zaro,** Barbara Bellich, Cristina Lagatolla, Demi Vattovaz, Greta Ponsecchi, Rita De Zorzi, Marco Maria D'Andrea, Paola Cescutti *(University of Trieste)* **Exploiting bacteriophage enzymes against K. pneumoniae infections**
PC-7	**Andrea Iovine,** Giulia Bisson, Immacolata Speciale, Anna Notaro, Marilena Marino, Cristina De Castro *(University of Napoli FedericoII)* **Structural Elucidation of Polysaccharides from *Lactobacillus plantarum***
PC-8	**Marco Abbate,** Massimiliano Cordaro, Giulia Neri, Antonino Mazzaglia, Angela Scala, Anna Piperno *(University of Messina)* **Innovative Hybrid Nano-Architectures based on Cyclodextrin-Hyaluronic Acid Bioconjugate and Metal Nanoparticles**
PC-9	**Luca Paoletti,** Nicole Zoratto, Laura Forcina, Antonio Musarò, Roberto Matassa, Giuseppe Familiari, Luciana Mosca, Maurizio Mattei, Chiara Di Meo, Pietro Matricardi *(Università di Roma-La Sapienza)* **Hyaluronan-cholesterol nanogels for the enhancement of the ocular delivery of therapeutics**
PC-10	Maria Michela Corsaro, Angela Casillo, Rosanna Papa, **Raffaele D'amico** *(University of Napoli Federico II)* **Investigating the Role of Glycans in Antibiotic Resistance of Biofilm-Associated Infections**
PC-11	Maria Michela Corsaro, Angela Casillo, Daria Monti, Davide Liberti**, Silvia Fanina** *(University of Napoli Federico II)* **Polysaccharides from the Red Microalga *Porphyridium Cruentum***
PC-12	**Giorgia Bray,** Giulia Galgani, Valentina Citi, Vincenzo Calderone *(University of Pisa)* **Protective Effect of Tamarind-Seed Polysaccharide in a Human 3D in Vitro Model of Dry Eye Desease**

PC-13	**Sara Pavone,** Camilla Matassini, Francesca Clemente, Costanza Cicchi, Simone Luti, Francesca Cardona ***(University of Firenze)*** **Multivalent Iminosugars Inhibit the Levansucrase Enzyme: A New Plant Protection Strategy Against Kiwifruit Canker?**
PC-14	**Francesca Milano,** Camilla Matassini, Francesca Cardona, Marco Marradi and Andrea Goti ***(University of Firenze)*** **Theranostic Iminoglyco-NPs targeting the Blood Brain Barrier**
PC-15	**Roberta Di Benedetto,** Gianmarco Gasperini, Martina Carducci, Luisa Massai, Olimpia Pitirollo, Francesca Mancini, Pedro Henriques, Francesca Necchi, Omar Rossi, Luigi Lay, Roberto Adamo, Francesco Berlanda Scorza, Danilo Gomes Moriel, Francesca Micoli ***(GSK Vaccines, Siena)*** **Design of an effective glycoconjugate vaccine against Group A *Streptococcus***
PC-16	**Elena Palmieri,** Paola Cescutti, Francesca Micoli, Gianmarco Gasperini ***(University of Trieste, GSK Vaccines, Siena)*** **Investigation of Alternative Technologies for the Development of Polysaccharide-Based Vaccines**
PC-17	Linda Cerofolini, Kristian Vasa, Elisa Bianconi, **Maria Salobehaj,** Giulia Cappelli, Alice Bonciani, Giulia Licciardi, Anna Pérez-Ràfols, Luis Padilla-Cortés, Sabrina Antonacci, Domenico Rizzo, Enrico Ravera, Caterina Viglianisi, Vito Calderone, Giacomo Parigi, Claudio Luchinat, Antonio Macchiarulo, Stefano Menichetti, Marco Fragai ***(University of Firenze)*** **Combining Solid-State NMR with Structural and Biophysical Techniques to Design Challenging Protein-Drug Conjugates**
PC-18	**Martina Quaglia,** Marco Zuccolo, Cristina Corno, Nives Carenini, Paola Perego, Diego Colombo ***(University of Milano)*** **Antitumor Platinum(II) Hybrid Compounds Based on a Glucosylglycerol Scaffold**
PC-19	Cristina Manuela Santi, Alessia Izzo, Laura Petrosilli, Luigi Lay, **Giuseppe D'Orazio** ***(University of Milano)*** **Synthesis of a Small Library of Compounds Inspired by *Bacteroides fragilis* Lipid A as Potential Vaccine Adjuvants**
PC-20	**Andrea Sodini**, Laura Cheti Baldaccini, Silvia Fallarini, Francesco Papi, Federico Licciardi, Francesca Natali, Grazia Lombardi, Francesca Maestrelli, Cristina Nativi, ***(University of Firenze)*** **Multivalent glycosidic vectors for the modulation of the immune system**
PC-21	**Angelica Mero,** Andrea Mezzetta, Felicia D'Andrea, Lorenzo Guazzelli ***(University of Pisa)*** **Sustainable valorization of polysaccharide fraction in food industry biomass wastes**
PC-22	**Davide Rubes,** Massimo Serra, Marco Filice, Marco Terreni ***(University of Pavia)*** **Mannosylated Liposomes as Advanced Delivery Vehicles for Selective Dendritic Cells Targeting**

Glycoscience in microbial world: the power of sugars

Antonio Molinaro

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Department of Chemistry, School of Science, Osaka University, JAPAN

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

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

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

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

References

[1]. F. Di Lorenzo, K.A: Duda, R. Lanzetta, A. Silipo, C. De Castro, A. Molinaro (2022), Chem. Rev., (122) 15767-15821.
[2]. I. Speciale, A. Notaro, C. Abergel, R. Lanzetta, T.L. Lowary, A. Molinaro, M. Tonetti, J. L. Van Etten, C. De Castro (2022), Chem. Rev. (122) 15717-15766

Glycosaminoglycans: What Remains To Be Deciphered?

Serge Perez,* Olga Makshakova, Jesus Angulo, Emiliano Bedini, Antonella Bisio, Jose Luis de Paz, Elisa Fadda, Marco Guerrini, Michal Hricovini, Milos Hricovini, Frederique Lisacek, Pedro M. Nieto, Kevin Pagel, Giulia Paiardi, Ralf Richter, Sergey A. Samsonov, Romain R. Vives, Dragana Nikitovic, and Sylvie Ricard Blum

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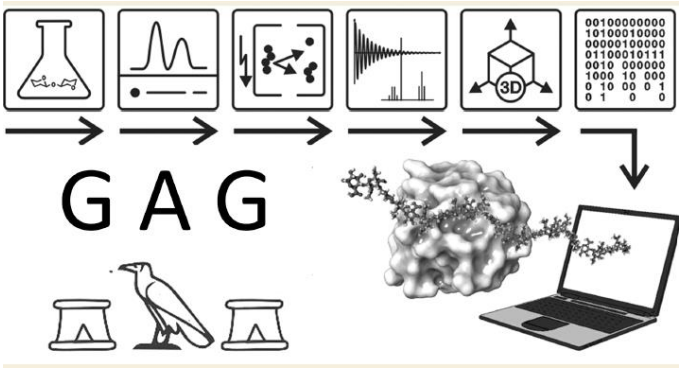
Glycosaminoglycans (GAGs) are complex polysaccharides exhibiting a vast structural diversity and fulfilling various functions mediated by thousands of interactions in the extracellular matrix, at the cell surface, and within the cells where they have been detected in the nucleus. The chemical groups attached to GAGs and GAG conformations comprise "glycocodes" that are not yet fully deciphered.



The molecular context also matters for GAG structures and functions, and the influence of the structure and functions of the proteoglycan core proteins on sulfated GAGs and vice versa warrants further investigation. The lack of dedicated bioinformatic tools for mining GAG data sets contributes to partially characterising the structural and functional landscape and interactions of GAGs. These pending issues will benefit from the development of new approaches reviewed here, namely (i) the synthesis of GAG oligosaccharides to build large and diverse GAG libraries, (ii) GAG analysis and sequencing by mass spectrometry (*e.g.*, ion mobility-mass spectrometry), gas-phase infrared spectroscopy, recognition tunnelling nanopores, and molecular modeling to identify bioactive GAG sequences, biophysical methods to investigate binding interfaces and to expand our knowledge and understanding of glycocodes governing GAG molecular recognition, and (iii) artificial intelligence for in-depth investigation of GAGomic data sets and their integration with proteomics.

References

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SYNTHESIS OF COMPLEX MICROBIAL GLYCANS

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Synthetic glycoconjugates are important biological probes. This seminar will describe ongoing investigations from the group focused on synthesizing different classes of complex glycan probes. A particular focus is those from microbial systems. Targets of interest include phenolic glycolipids, lipooligosaccharides and lipoarabinomanans from mycobacteria, glycosylphophoprenols from gram-negative bacteria and highly-branched glycans from chlororviruses.

HYALURONAN-BASED NANOGELS AS VERSATILE CARRIERS OF BIOACTIVE MOLECULES

Elita Montanari, Nicole Zoratto, Pietro Matricardi, **Chiara Di Meo**

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Self-assembling nanohydrogels or *nanogels* (NHs) are nanosized structures that couple the features of both nanoparticles, as nano-dimension, and hydrogels, such as soft consistency, high amount of water, and biocompatibility. Amphiphilic polymers consisting of hydrophilic polysaccharides derivatized with hydrophobic moieties are able to self-assemble in aqueous media in NHs allowing the formation of a hydrophilic shell that faces towards the solvent and inner hydrophobic domains with minimal interactions with the aqueous medium. As such, a variety of self-assembled NHs based on hyaluronic acid (HA) derivatized with different hydrophobic molecules has been developed[1-3] for potential applications as drugs carriers, able to increase the therapeutic efficacy of the drugs and to reduce their side effects.

A new method to simultaneously obtain the formation, the sterilization and the drug loading into HA-based NHs was also developed and patented[4]. Moreover, the biocompatible and highly hydrated structure of NHs makes these systems suitable as carriers of therapeutic proteins and natural substances. All NHs formulations were tested for their cytocompatibility on a number of cell lines, showing a complete safety up to high concentrations. Several NHs formulations with model drugs, such as antibiotics[5,6], anti-inflammatory[7,8] and anticancer drugs[9], were prepared, their activities were tested *in vitro* and *in vivo*, and in all cases the improvement of the therapeutic activity was observed.

References

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Post-synthetic editing of heparan sulfate sulfation pattern by the Sulfs

Romain Vivès

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Heparan sulfate (HS) are complex polysaccharides abundantly found in extracellular matrices and cell surfaces. These polysaccharides participate to major cellular processes through their ability to bind and modulate a wide array of signalling proteins. HS/ligands interactions occur through saccharide domains (termed S-domains) of specific sulfation pattern, present within the polysaccharide. Assembly of such functional domains is orchestrated by a complex biosynthesis machinery and their structure is further regulated at the cell surface by post-synthetic modifying enzymes, including extracellular sulfatases of the Sulf family. Sulfs specifically target HS S-domains and catalyze the selective removal of 6-O-sulfate groups, which are required for the recognition of many proteins. Although structurally subtle, these modifications have great functional consequences, and Sulfs have emerged as critical regulators of HS activity, in physiological processes such as embryogenesis and tissue regeneration, and in diseases such as cancer. However, and despite increasing interest, Sulfs remain highly elusive enzymes and little is known about their structure, substrate specificities, or catalytic mechanism. Over the recent years, our group has focused on this challenging issue. We have developed an efficient expression and purification system of recombinant HSulf-2 in mammalian HEK293 cells, showed that the enzyme catalyzed the 6-O-desulfation of HS following an orientated and processive mechanism, initiated an exhaustive structural analysis on the protein and its post-translational modifications, and investigated the contribution of its sub-domains in the enzyme biological activity. Altogether, our data provide critical insights into this original regulatory mechanism of HS functions.

Understanding and Exploiting Cazymes in Glycan Synthesis

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"Carbohydrates in molecular biology are like dark matter in the universe.....poorly studied yet crucial to a full understanding of how things actually work" (Ajit Varki, UCSD, 2012). This presentation will address some of the issues in identifying carbohydrate-active enzymes and understanding their specificity, with implications for how they may be used in glycan synthesis and/or deployed in a synthetic biology era.

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Enzymatic strategies for the synthesis of vaccine antigens from Gram-negative pathogens

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Capsular polysaccharides (CPS) are important virulence factors that protect bacterial pathogens from the host immune system. They are structurally diverse and can consist exclusively of saccharide units, or sugars alternating with phosphate or polyol-phosphate moieties. CPS can be used as antigens in highly effective glycoconjugate vaccines, in which they are coupled to a carrier protein to induce a T-cell dependent immune response. The manufacturing of glycoconjugate vaccines includes the purification of CPS from pathogen culture. To reduce biohazard and costs, chemical and enzymatic synthesis have been extensively studied as alternatives for CPS production. Our research focusses on the biochemical and structural characterization of capsule biosynthesis enzymes and the development of enzyme-based synthesis cascades for the provision of CPS. The enzymes required for these protocols include (i) nucleotide-sugar epimerases and nucleotidyltransferases to generate substrates like nucleotide sugars and polyols, (ii) capsule polymerases using said substrates to polymerize the CPS, and (iii) CPS-modifying transferases that add glycosyl- or O-acetyl groups to e.g. distinguish between serotypes.
Here, we present the biochemical and mechanistic characterization as well as the biotechnological exploitation of capsule biosynthesis enzymes required for the generation and modification of phosphate-containing CPS from the Gram-negative pathogens *Neisseria meningitidis*[1], *Haemophilus influenzae*[2,3] and *Actinobacillus pleuropneumoniae*[4,5].

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SYNTHETIC SACCHARIDE EPITOPES IN PNEUMOCOCCAL DISEASES: EXPLORING MULTIPRESENTATION AND MULTIVALENCY

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Capsular polysaccharides (CPSs) of encapsulated bacteria are critical determinants of bacterial virulence and have been proven to stimulate protective immunity against infectious diseases. This sets the stage for the development of antibacterial vaccines.[1] CPSs are cell-surface polymers consisting of oligosaccharide repeating units and able to simultaneously display a large number of densely arranged epitopes. The resulting arrangement favors clustered multivalent interactions with specific antibodies.

In this framework, the rationally designed chemical synthesis of structurally defined CPS fragments is important for both fundamental and practical reasons. From the fundamental point of view, this will improve understanding of the structural requirements of antibody glycan recognition. From the practical point of view, this will open the possibility to design neo-structures with improved properties.

In this lecture, I will show our studies on *Streptococcus pneumoniae* (Sp) serotypes 19F and 19A as model systems to address the identification of the shortest polysaccharide fragment able to be recognized by the natural antibody, the role of the multiple presentation of short CPS fragments mediated by calixarenes to increase binding efficacy,[2] and our recent preliminary studies on the identification of common epitopes between different serotypes of Sp group 19 to increase vaccine coverage.[3]

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Carbohydrate based vaccines for low-middle income countries

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Infectious diseases are still a threat for human populations, especially in low and middle - income countries (LMICs), and over the last years antimicrobial resistance (AMR) has become an endemic and widespread problem disproportionally affecting people living in LMICs. Vaccines can play a major role to fight such diseases and development of novel technologies is key to accelerate the design, development and implementation of affordable and effective vaccines. Cell surface carbohydrates have been proven optimal targets for vaccine development and glycoconjugate vaccines represent a successful strategy to prevent infectious diseases.

Here we report examples on the development of traditional glycoconjugates and more recent technologies (e.g. glycoengineering and use of alternative carrier systems like GMMA) to develop polysaccharide based vaccines against diseases like *Salmonella*, *Shigella*, Group A Streptococcus, major causes of morbidity and mortality in LMICs. The characteristics of the different types of polysaccharide-based vaccines and the attributes that majorly can affect their immunogenicity will be highlighted.

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Enzyme mediated synthesis of carbohydrate-based compounds. From the development of green processes to the design of engineered biotechnological products.

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The design of new carbohydrate-containing drugs has attracted great attention in the recent years as means to target several diseases. In this topic the search for sustainable and efficient processes is crucial. Therefore, the use of enzymes in reactions leading to the production of glyco-pharmaceuticals has increased exponentially in the last years [1]. In glycochemistry and glycobiology, enzymes belonging to the families of glycosidases, glycosyltransferases, lipases and, in the case of nucleosides and nucleotides analogues, also nucleoside phosphorylases (NPs) are the preferred choices as catalysts.
The development of tailor-made immobilisation techniques for the enzymes was critical in the development of efficient bioprocess for the synthesis of glyco-products ranging from nucleoside and nucleotides to complex glycoconjugate derivatives and engineered biotechnological drugs.

Thus, efficient syntheses of antiviral and anticancer nucleosides were performed using immobilized enzymes, such as NPs, in transglycosylation reaction. In addition, the use of deoxyribonucleoside kinases for the phosphorylation of the sugar moiety of nucleosides was investigated.

Immobilized lipases and glycosidases were considered for the chemo-enzymatic synthesis of different oligosaccharides. For example, lipases allowed the regioselective hydrolysis of acetylated monosaccharides and disaccharides producing sugar building blokes bearing free hydroxyl groups in the desired positions, that were then assembled to obtain complex oligosaccharides in few steps, and only using the acetyl as protecting group. Alternatively, the use of glycosidases allowed the preparation of disaccharides avoiding completely the use of protecting groups.
These approaches have been utilised for the preparation of semi-synthetic glycolipids. Thus, sialylated di and trisaccharide have been prepared and conjugated to lipid moieties to obtain analogous of GM3 with different anticancer activity.

In addition, the synthesis of sugars bearing at the anomeric position a reactive linker, as required for the chemical glycosylation of proteins by selective reaction with lysines, has been studied for the preparation of different *neo*glycoproteins. Therefore, the conjugation of protein antigens from *Mycobacterium tuberculosis* with immunogenic and/or antigenic oligosaccharides has been investigated for the development of new potential glyco-conjugated vaccine candidates.

Finally, we have recently considered the use of Endo Glycosidases for the glyco-engineering of monoclonal antibodies. These enzymes naturally catalyse the cleavage of the chitobiose core of *N*-glycans between two GluNAc, but are also able to catalyse a glycosylation reaction, linking synthetic glycan bearing an anomeric GluNAc to a protein glycated with *N*-linked GluNAc residues; sugars activated as oxazolines are the best donor substrates.

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From Franz Schubert to Leonard Cohen: the chemical pleasure of listening to the voice in music

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In 2011 some Canadian scientists published in *Nature Neuroscience* an article entitled "Anatomically distinct dopamine release during anticipation and experience of peak emotion to music". Their results indicated that the intense pleasure in response to music can lead to dopamine release in the striatal system. Moreover, the anticipation of an abstract reward can result in dopamine release in an anatomical pathway distinct from that associated with the peak pleasure itself. These findings have been known since long time for biological stimuli as hunger, sex, and fear which are associated to the instinct of survival and reproduction of the species and connected to the so-called reward circuits – expectation and reward – within the mesolimbic system, but it was the first time they were observed for a cultural stimulus as music is. This extraordinary discovery helps to explain why music is of such high value across all human societies and also suggests an intriguing consideration about music as a "biological" art, probably due to its relationship with the irreversibility of time. The lecture-performance proposed aims to describe in a popularised way these scientific results accompanying the audience in a promenade that visits maybe the most fascinating music, that is that involving human voices singing with instrumental accompaniment. The lecture explores the physics of the singing voice, its extension in frequency, its timbre, its capability to give to words apparently meaningless an incredible pathos, making the audience listening to many different voices – bass, baritone, soprano, rock and jazz singers, song-writer singers – and equally several "songs" – De André, Rossini, Saint-Saëns, Springsteen, Mozart, Marlen Dietrich, Giordano, Puccini, Lennon, Verdi, Stratos, Armstrong, Schubert, Cohen – and trying to make the audience to experience what the Canadian scientists found about chills, respiratory and heart rate, intense pleasure sensations. The message in bottle the lecture-performance would like to offer is the power of chemistry to play a fundamental role in some activities the conventional wisdom judges absolutely very far from it. Finally, the lecture-performance could also be considered as an excellent and low-cost drug to be taken after dinner for the following indications: it fights stress, malaise, bad mood, intolerance, annoyances, and whatever else makes life poor in joy; it deactivates the 72 muscles used to have the sulk, activates the 12 of them to have a smile, and distracts us from daily worries by providing us with pleasure.

A GENERIC CONJUGATION CHEMISTRY SUPPORTING THE DEVELOPMENT OF MULTIVALENT VACCINES

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Glycoconjugation is a well-established technology for bacterial vaccines as binding of polysaccharide (PS) antigens to carrier proteins makes them effective in infants and provides immunological memory. Despite the success of glycoconjugate vaccines, many infectious diseases still remain to be controlled, and alarming concern is emerging toward antibiotic resistant bacteria (AMR). Considering the variety of PS antigens displayed on the surface of the pathogens for which vaccines are not available yet, high-valency glycoconjugates need to be developed.

Here, CDAP chemistry has been identified with the aim to develop a generic conjugation approach that can be applied to PS having different structures. This chemistry exploits hydroxyl groups and amino groups commonly present on PS and proteins respectively [1,2]. Starting from published procedures[3,4], reaction conditions were extensively investigated. The resulting protocol was successfully applied to a broad range of PS from different pathogens like *Klebsiella pneumoniae*, *Salmonella* Paratyphi A, *Salmonella* Enteritidis and Typhimurium, *Hemophilus influenzae* type b, *Shigella sonnei* and *flexneri*. Statistical tools were used in order to understand the impact of conjugation parameters on potential critical quality attributes of the resulting conjugates, using *S.* Paratyphi A *O*-Antigen (O:2) conjugated to CRM_{197} as model. Animal studies are ongoing to assess the impact of O:2-CRM_{197} structural features on immunogenicity and identify optimal vaccine design. CDAP chemistry was also succesfully used for the conjugation of O:2 to alternative carriers, like Generalized Modules for Membrane Antigens (GMMA). The conjugation chemistry identified can be rapidly extended to a large number of PS in short time supproting the development of multivalent vaccines, meeting unmet medical needs.

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Rhamnosylated PD-1 mutant endowed with immunological activity

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T-cells are essential for the immune system's adaptive response against pathogens and unhealthy cells, including cancer cells.[1] Programmed cell death protein-1 (PD-1) and its ligands (PD-L1) are key checkpoints that modulate T-cells' functions. Cancer cells overexpressing PD-L1 can inhibit T-cells becoming free to replicate and metastasize.

Currently, PD-L1 is a target for cancer therapy and several monoclonal antibody designed to bind the ectodomain of this transmembrane protein have been approved for clinical use.

Alternatively, recombinant PD-1 ectodomain can inhibit the PD-1/PD-L1 axis and can also be employed as a vector to deliver therapeutic compounds, probes, or toxins to cancer cells that are overexpressing PD-L1.[2] Particularly attracting is the possibility to employ recombinant PD-1 as carrier to address cancer cells with immune stimulating agents.

In this context, L-rhamnose conjugated to peptides or proteins proved effective in inducing an immune response through the generation of anti-rhamnose antibodies, activating macrophages and lymphocytes.[3]

Herein, we report on the design, biophysical characterization and site-selective rhamnosylation of a novel mutant of PD-1, presenting a nanomolar affinity towards PD-L1 and immunomodulatory properties.

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Recognition Mechanisms of Bacterial Glycans by Host Immune Receptors

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Glycans act as an interface between the outer environment and the cell membrane of all living organisms. They exhibit broad structural diversity and are involved in fundamental biomolecular mechanisms. Particularly, glycans are the main actors in the interaction mechanisms of bacteria with eukaryotic host, serving as counter receptors for different proteins, including lectins [1]. These are exposed on the surface of innate immune cells and represent an important class of Pathogen Recognition Receptors (PRRs) characterized by their ability to recognize glycans. These PRRs may contribute to initial recognition of bacterial glycans, thus providing an early defense mechanism against bacterial infections, but some of them may also be exploited by bacteria to escape immune responses.

Several human pathogens have indeed developed the capability to cover their surfaces with glycans mimicking eukaryotic SAMPs (Self Associated Molecular Patterns) structures, able to interact with inhibitory host receptors, thus eluding host immune responses and promoting infections.

Among them, the ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species) pathogens exhibit multidrug resistance and represent a global threat to human health [2].

Thus, given their therapeutic relevance, we aim to elucidate the chemical structure of capsular polisaccharide extracted from A. baumannii isolate, and the recognition mechanisms by inhibitory host receptors.

To achieve this, a multidisciplinary approach is applied, relying on different biophysical techniques, mainly NMR spectroscopy and Gas Chromatography – Mass Spectrometry, together with computational studies, in order to validate experimental results.

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Microbiota-mediated regulation of immune responses to vaccines in outbred mice

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Vaccine-induced immune responses exhibit significant variability among individuals and populations, with the underlying reasons still not fully elucidated. Growing evidence suggests that the gut microbiota, along with other interacting factors, plays a role in modulating both humoral and cellular responses to infection and vaccination[1,2]. In this study, we aimed to investigate this connection by analyzing the antibody and cellular immune responses of outbred and inbred mice to several vaccines using different microbiota models.

Swiss Webster (SW) (outbred) and C57BL/6 (inbred) mice, respectively maintained as specific pathogen-free (SPF), antibiotic-treated or germ-free (GF), were vaccinated with glycoconjugate or protein antigens in alum adjuvant. We monitored antigen-specific antibody responses by ELISA and characterized cellular immune responses by flow cytometry, examining dendritic cells, T cells and B cells in various lymphoid organs. The impact of the microbiota on the antibody response was also assessed by analyzing the gut microbial community structure using 16S rRNA amplicon sequencing.

After vaccination, SPF SW mice showed significantly higher antigen-specific IgG responses than GF or antibiotic-treated mice, regardless of the antigen tested. In SW SPF mice, we observed enhanced germinal center B cell development and T cell activation, while SW GF mice exhibited a more pronounced regulatory/co-inhibitory environment. Cohousing of SW germ-free mice revealed the transmissible and ampicillin-sensitive nature of the microorganisms responsible for the enhanced response to vaccination. Taxonomic analysis demonstrated an association between increased IgG responses in SW mice and high Firmicutes abundance and decreased Bacteroidetes levels at the phylum level. In contrast, the influence of microbiota on vaccine response in C57BL/6 mice was less pronounced[3].

These results underline the regulatory role of specific microbiota compositions in the modulation of humoral and cellular responses to vaccines in outbred mice providing an important contribution to a better understanding of the microbiota-immune response crosstalk after vaccination.

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Chemical Modification and Structural Characterization of Lactosilated Hyaluronic Acid

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Hyaluronic acid (HA) is a polysaccharide which exhibits anti-inflammatory and anti-angiogenic properties, it's involved in cellular activity and promotes tissue reconstruction [1-2]. The modification of HA with an amino lactitol derivatives (LAC-NH_2) produces a new biopolymer named HYLACH® [3], the introduced β-galactose provide potential interaction with Galectins. Galectin3 in particular is found to be overly expressed in fibrotic diseases [4]. The aim of functionalizing HA with β-galactose residues is to couple the biocompatible and anti-inflammatory properties of HA with the capability of subtracting Gal3 from the pro-fibrotic environment. The HYLACH® products are characterized by NMR, Size exclusion chromatography and their stability towards enzymatic activity is investigated. HYLACH® molecules showed molecular weights in the range of 80-280kDa, and degree of substitution (DS) up to 45%, according to synthetic conditions. Interestingly, the DS determination by heteronuclear 2D-NMR was performed both on hydrolysed and un-hydrolysed compounds and the results compared and investigated. Increase of stability towards enzyme degradation is observed in HYLACH® respect to native HA. The conformational and structural behaviour are evaluated by molecular dynamics simulation, where simulation predicts for HYLACH® a less extended helical conformation than HA, and that the β-galactose is accessible to molecular recognition by proteins. Interactions with Galectin3 (Gal3), studied by isothermal titration calorimetry and circular dichroism resulted to be entropically and exothermic favoured with affinity in the range of μM.

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Morphological investigations of the polysaccharide extracted from biofilm produced by *Burkholderia multivorans* strain C1576

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Microbial communities often experience a collective way of life, known as biofilm, where bacterial cells are embedded in a gel-like matrix that confers protection against external threats. It is generally accepted that polysaccharides constitute the main structural component of the biofilm matrix. Due to the huge variety of repeating unit composing the bacterial polysaccharides, different 3D biofilm matrix can be generated. The structural properties of the exopolysaccharide (Epol) recovered from the biofilm matrix formed by *Burkholderia multivorans* strain C1576 were investigated. The repeating unit of the Epol C1576 (1) is characterized by the presence of Rha, a 6-deoxy sugar, which displays a less polar character than other common monosaccharides. In addition, 25% of the Rha residues are methylated, thus producing less polar segments in the polysaccharide backbone.

The experimental investigations, carried out by using AFM, both in dried and liquid state, and TEM showed that the Epol C1576 forms compact structures characterized by a globular morphology, confirming the ability of Epol C1576 to self-aggregate (2). Computer modelling of Epol C1576 3D conformation was carried out to assess the flexibility of the polymer backbone and it demonstrated that a polysaccharide segment was able to collapse into a compact structure which remained almost constant during the simulation time. This study demonstrated the polysaccharide ability to form folded compact structures that are expected to affect the overall biofilm matrix architecture.

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Selective Glycomimetic Ligands of C-type Lectin Receptors

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C-type lectin receptors (CLRs) are calcium-dependent proteins dedicated to sensing glycan motifs, including those expressed on the surface of invading pathogens. Although this recognition event usually leads to induction of an immune response, many pathogens have evolved mechanisms to exploit lectins to promote infection. [1] A number of viruses of major impact on public health, such as HIV-1, Ebola and SARS-CoV-2,[2] adopt this strategy to subvert host defence responses and enhance the infectious process. Selective inhibitors of lectins have thus high potential as therapeutic agents, but their use is limited by the intrinsic low affinity and selectivity of carbohydrate-based molecules towards CLRs. These limitations can generally be overcome through the use of chemically modified analogues of sugars that emulate carbohydrate activities but presents improved drug-like properties.

In my presentation I will discuss a glycomimetic approach to selectively target the C-type lectin DC-SIGN and its homologue L-SIGN, which we have developed together with the group of Franck Fieschi at IBS in Grenoble. A library of monovalent mannose-based glycomimetic ligands bearing a triazole-functionalization in position 2 has been synthesized by varying the nature of the substituent and the aglycon unit (**Figure 1**, left). Ligand affinity towards target lectin receptors has been evaluated through SPR (Surface Plasmon Resonance) competition assays. [3,4] The most promising ligands were selected for construction of glycoconjugates of higher valency, to further increase ligand affinity and selectivity. [4] Multivalent constructs were built on different platforms (**Figure 1**, right) and the synthesized ligands were evaluated as DC/L-SIGN antagonists via SPR direct interaction assays.

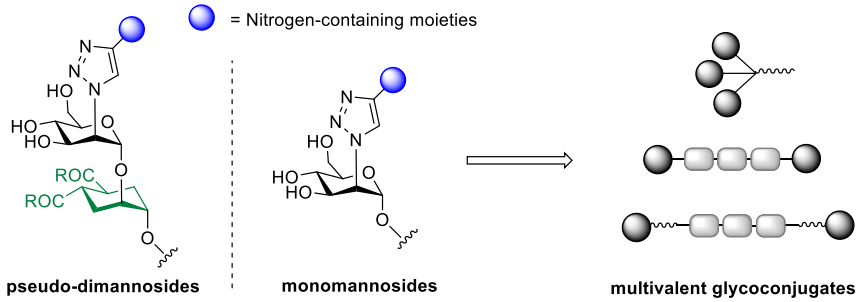

Figure 1 *General structure of C2-triazole modified monovalent mannose-based ligands (left) and schematic representation of synthesized multivalent glycoconjugates.*

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Viscosupplement based on a hyaluronan derivative grafted with a MMP inhibitor

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MMPs are a family of Zn peptidases, that, if over expressed, lead to the uncontrolled degradation of specific protein substrates. Among the MMPs, MMP3, MMP12 and MMP13 attack and cleave the peptide bonds of collagen and gelatin present in the connective tissue. Hyaluronic Acid (HA) is a ubiquitous linear polysaccharide, and it plays mainly structural and hydration roles, in the human body. The HA molecular weight (MW) affects the HA functions and have good or bad impacts in its location: low MW (<100kDa) can induce either inflammatory reactions or immuno-stimulatory and angiogenic effects, high molecular weight HA is characterized by anti-inflammatory and anti-angiogenic properties. (2-((N-(6-aminohexyl)-4-methoxyphenyl)sulfonamido)-N-hydroxyacetamide) (MMPI), having inhibition activity against membrane metalloproteins (MMPs) involved in inflammatory processes, in particular the MMP12, was grafted to a high MW sodium hyaluronate (NaHA). The covalent bonded derivative (HA-MMPI) was characterized in terms of structure, thermal and rheological behavior (viscosity and viscoelastic properties in shear). Furthermore, in vitro cytocompatibility, sulphated glycosaminoglycans (sGAG) and hydroxyproline (HYP) content, were evaluated. The inhibition activity of MMPI and HA-MMPI versus MMP12 catalytic domain was tested to demonstrate that the MMPI maintains its function as MMP12 inhibitor. The final product demonstrates viscoelastic properties close to those of healthy human synovial fluid, cytocompatibility towards human chondrocytes and nanomolar affinity towards MMP12 [1].

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Lectin mediated surveillance of gut microbiota: DC-SIGN recognizes and binds selected epitopes within Bacteroides vulgatus LPS

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The gut microbiota (GM) in the human gastrointestinal tract plays a vital role in immune system development, nutrient processing, and protection against pathogens.[1] Lipopolysaccharide (LPS), composed by the Lipid A, the core oligosaccharide and the O-Antigen moieties, is a component of Gram-negative bacteria that can have both beneficial and detrimental effects on host-microbe interactions.[2] Lectin receptors, particularly C-type lectins (CTLs), are involved in recognizing glycans on microbial surfaces and modulating immune responses.[3] DC-SIGN (CD209), a member of the CTL family, interacts with bacterial LPS and plays a crucial role in immune recognition. Thus, here is tackled the interaction between DC-SIGN and B. *vulgatus* mpk (BVMPK) LPS, which exhibits a protective role and selective affinity for DC-SIGN,[4] using a multidisciplinary strategy by combining synthetic, spectroscopic, biophysical and computational approaches to evaluate the contribution of different regions of the LPS structure to binding. The study (under submission) points out the importance of glycan complexity and identity in host recognition of BVMPK LPS by DC-SIGN highlighting the involvement of both O-antigen and core regions in the host immune response as being recognized the terminal trisaccharide of the OPS, being mandatory a terminal mannose residue, and the core region where the fuccose residue acts as anchor for the recognition. Therefore, the findings propose an heterobivalent 3D complex of the DC-SIGN and BVMPK LPS interaction.

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Recognition of the Core Disaccharide of *N*-Glycans by a Synthetic Receptor

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Recognition of specific saccharidic epitopes on cells surfaces is well known to regulate several biological and pathological processes.[1] Molecules able to modulate these processes are potentially useful in medicine and in the diagnostic field. Among the classes of compounds capable of recognizing a carbohydrate in solution, those of synthetic receptors have been deeply investigated in the last two decades. Synthetic receptors are small, abiotic compounds capable of recognizing carbohydrates by non-covalent interactions under physiological conditions. Recognition of monosaccharides is a largely explored research field, while recognition of oligosaccharides and glycans have only recently been exploited due to the complexity of these guests. In 2021 our research group proposed a new acyclic and adaptable receptor[2] for the *N,N'*-diacetylchitobiose disaccharide ($GlcNAc_2$), core residue of *N*-glycans. Given the acyclic nature of the receptor and the unprecedent affinity for $GlcNAc_2$, this molecule has the potential to tune the disaccharide inserted in a complex glycan. In the last years we have developed a second-generation receptor and studied, for the first time in this research field, the recognition of a disaccharide in a complex glycan.[3] The results of this study will be discussed in this communication.

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Glycomic analysis of Cohen syndrome suggests a sweet treatment

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Cohen Syndrome (CS) is a genetic, multisystemic, neurodevelopment disorder caused by mutations in the *VPS13B* gene localized on the chromosome 8q22.2. Symptoms of CS vary significantly among affected individuals with a common core symptomology consisting of facial dysmorphism, intellectual disability, hypotonia, progressive retinopathy, neutropenia, global development, and speech delays.

VPS13B encodes a transmembrane protein involved in endo-lysosomal transport, Golgi apparatus integrity and functionality, leading to an impaired protein glycosylation[1][2].

To detect changes in secreted protein glycosylation, we have investigated by mass spectrometry IgG and total serum N-glycome in a group of CS patients and control subjects, revealing a significant increase of hypo-sialo and hypo-galactosylated N-glycans. Based on this observation, we have set up a pilot therapeutic study, consisting of oral D-galactose administration, for one major affected patient. Interestingly, our galactose-based treatment showed an improvement of clinical parameters such as IGF1 levels, transferrin IEF pattern, coagulopathy normalization, reduction of behavioral difficulties and communicative impairment. Besides, CS patient's glycophenotype considerably ameliorated, with a progressive "normalization" of N-glycan profiles along galactose treatment assessed by MALDI MS, an effective approach to track patient's clinical follow-up.

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Glycosignature in Human Tissue Model Libraries Aided by Artificial Intelligence

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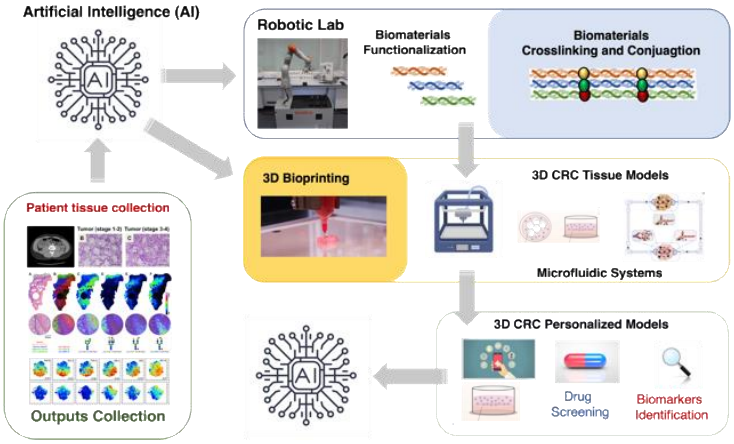
The glycosignature of different human tissues has gained great interest, being organ specific, somehow patient specific, and associated to healthy and pathological states. To spread light to this complexity, associated to the further structural variability of the extracellular matrix (ECM) that composes the tissue models [1], a high throughput approach is required. With the advent of automated manufacturing systems like 3D printing, it is now possible to control the formulation of biomaterials and bioinks, limiting the artisanal chemical approach that is typically used to generate 3D in vitro tissue and organ models [2]. In this study, differently glycosylated patient-specific 3D gastrointestinal tissue models were generated using also Artificial Intelligence algorithms and Machine Learning methodologies, to reduce synthetic effort and predict the most efficient synthetic conditions to generate hybrid multifunctional biopolymers with selected properties and features. The methodology was developed considering polymers of different nature, such as hyaluronic acid and gelatin, functionalized with N_3-PEG-spacer arms and crosslinked with cyclooctine linkers, to generate a library of differently glycosylated bioinks by cell compatible SPAAC click chemistry [3].



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Greener and Streamlined Approaches for the Synthetic Transformations of Complex Organic Compounds: Focus on Glycochemistry

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The large natural abundance, bio-relevance and structural peculiarity of carbohydrates make these compounds crucial synthetic targets in biomedical research as well as convenient precursors of valuable products for disparate applications.[1] This spurs the search for improved synthetic methods in this field and a large effort is currently aimed at both greening and simplifying the most ubiquitous operations such as the differentiation of the saccharide sites by regioselective/orthogonal protection and the stereoselective synthesis of glycoside bonds.[2] Indeed, strategical complexity of these transformations is combined with major experimental issues, due to the moisture sensitivity of reactions, the requirement of lengthy multi-step procedures and the use of noxious and high-boiling solvents.

We have recently demonstrated that most steps in glyco-synthesis can be profitably performed through original solvent-free, under air approaches featuring environmental, experimental and economic advantages with respect to the current state of the art.[3] Herein is presented an overview of their applications, starting from various examples of protection (e.g. with alkyl/silyl ether, acetal, acyl groups) and re-functionalization of saccharide hydroxyls, most of which also addressed to regioselective and one-pot transformations; contextually, it is shown their generalized application in organic reactions, including alcohol elaborations through iodide intermediates, and the recent synthesis of esters and amides (including dipeptides) relying on a solventless revisiting of the carbodiimide chemistry.[4]

The viable application of solventless conditions also to stereoselective glycosidations with readily accessible glycosyl chloride or 1-*O*-acyl donors is finally discussed.

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Regioselective sulfation of polysaccharides from sustainable sources

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Sulfated glycosaminoglycans (GAGs) are highly complex, anionic, linear polysaccharides extracted from extracellular matrix of animals cells. Some of them are exploited in already approved therapeutic treatments, and a significant number of novel drugs are currently under development.[1] Nonetheless, naturally occurring GAGs exhibit variable chemical compositions and biological activities, which could cause unpredictable results during applications (*e.g.* heparin crisis in 2007). However, sulfated polysaccharides can also be obtained in a semi-synthetic way: the introduction of sulfate groups into the backbones of natural unsulfated polysaccharides allows to endow them with bioactivities similar to sulfated GAGs but without risks derived from their typical animal sources.[2] In this frame, a special interest is focused on the sulfation of polysaccharides from eco-sustainable natural and/or biotech sources (algae, fungi, bacteria) already used in the biomedical and/or food fields, in order to improve their properties or to introduce new ones. Regioselective sulfation reactions can be conducted through multi-step strategies consisting in protection-sulfation-deprotection sequences.[3] In particular, the polysaccharides selected to this aim are M-rich alginic acid extracted from brown algae,[4] curdlan from *Agrobacterium* strains,[5] and finally an exopolysaccharide (EPS)[6] from *Vibrio diabolicus* HE800 with a GAG-like structure composed only of aminosugars and uronic acids. Their regioselective sulfation has been performed to obtain new derivatives acting as GAG mimics.

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Stereospecific One-Pot Reiterative Synthesis of Linear 1,4-Oligosaccharides

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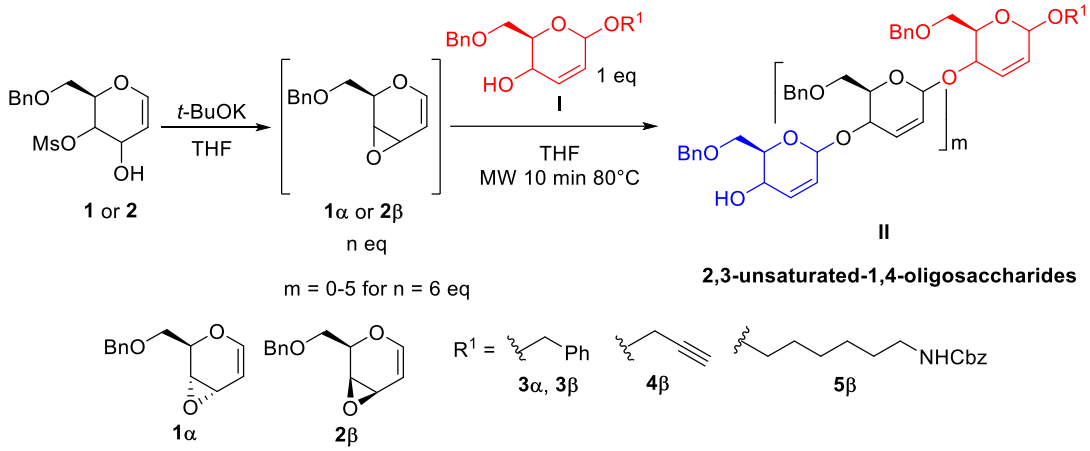
Glycans organic synthesis, even of linear typologies, suffers of a strong complexity of chemical actions.[1] The possibility of realizing one-pot process, where the reactive specie takes charge of both the regio- and stereoselective aspects of the transformation in a reiterative fashion is remarkably attractive.[2]

In the last years we have reported glycal derived chiral epoxides **1α** and **2β** as stereoselective innovative glycosil donors for the preparation of D-Manno and D-Gulo-based glycosides (of the type **I**), thanks to an efficient substrate-dependent stereocontrolled 1,4-conjugated nucleophilic addition process.[3]

Herein we describe a smart reiterative one-pot version of this process for the synthesis of a series of 2,3-unsaturated-1,4-oligosaccharides (**II**): it consisted in a fast (10 min) microwave assisted reaction of an allylic alcohol initiator of the type **I** with either the in situ formed epoxide **1α** or **2β**. The molar ratio of initiator and reactive epoxide controls the products distribution around different sizes of oligomers. The reiterative process has been proved to be completely 1,4-regioselective, and by 2D-NMR analysis to be totally stereospecific: the oligomerization of **1α** resulted in α-manno-1,4-oligosaccharides, while vinyl epoxide **2β** gave rise to β-gulo-1,4-oligosaccharides.

Differently functionalized initiators allowed the construction of oligosaccharides containing a high value first monosaccharide unit. In addition, the double bond has been dihydroxylated by means of OsO_4 protocols, in a total diastereoselective manner.



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Towards Covalent Ligands for the *N*-Terminal Domain of the Bacterial Lectin BC2L-C

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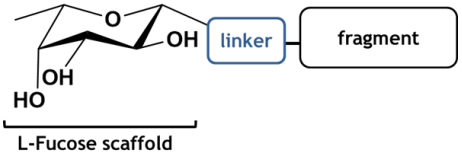
Burkholderia cenocepacia is an opportunistic human pathogen responsible for deadly lung infections in immunocompromised individuals, and it is highly resistant to most clinically useful antibiotics.[1] This bacterium employs lectins as virulence factors to target host tissues through recognition of and adhesion to the glycoconjugates on the host cells' surface. Among these lectins, the *superlectin* BC2L-C has been proposed as a major player in the adhesion process and in the formation of bacterial biofilm.[2] In particular, its *N*-terminal fucose-binding domain (BC2L-C-nt) represents an interesting target for design of new antimicrobials that may prevent lectin-mediated bacterial adhesion to the host cells. Based on fragment- and structure-based design studies, we have synthesized and characterized by ITC and X-ray crystallography a group of glycomimetics targeting BC2L-C-nt and containing a fucose residue connected to fragments able to engage a secondary site in the lectin, near the fucose-binding region.[3,4]



This resulted in the first BC2L-C-nt synthetic ligands showing up to a 10-fold affinity gain over the parent monosaccharide .[4,5]

With the aim of improving the binding affinity, the next generation of ligands was designed to covalently target specific nucleophilic amino acid residues, (Cys72 and Lys108) near the binding site. These glycomimetics were selected in silico through non-covalent and covalent docking protocols, and the most interesting ones were synthetized and subjected to the first biophysical studies showing promising results.

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Synthesis and physio-chemical properties of sulphated tamarind (*Tamarindus indica L.*) seed polysaccharide

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Tamarind seed polysaccharide (TSP) is a galactoxyloglucan isolated from seed kernel of Tamarindus indica. It is applied in the industrial fields, due to its physical, chemical, and biological properties (1). It is a neutral and water-soluble polysaccharide with a high viscosity which makes solubilization difficult. The absence of charges on the chain made electrostatic interactions not possible with charged biological or chemical molecules. For this reason, a chemical modification of this biopolymer is carried out adding sulphated groups on the TSP chain. The presence of negatively charged groups may allow a better solubilization of the polysaccharide and also specific binding to proteins or receptors, giving new biological properties to TSP (2). The sulfation reaction of TSP was performed in one-step process, using dimethylformamide as a solvent, and sulfur trioxide pyridine complex as reagent.

Characterization of the chemical-physical properties of the sulfated products are carried out through different analytical approaches to verify the successful synthesis. Studies of viscosity, morphology, chemical structure, and molecular weight distribution are conducted to obtain the complete characterization of the synthesized products. By potentiometric titration, the substitution degree is obtained and the distribution of sulphated groups on the sugars chain is stuedied by NMR spectroscopy and mass spectrometry characterizing the hydrolyzed sulfated TSP sample obtained by enzymatic degradation with cellulase and xyloglucanase.

Sulphates TSP products have a molecular weight in the range of 400 kDa 1000 kDa, a substitution degree on the repetitive unit of TSP of 5-50% of the hydroxyl groups which can be sulphated, exhibit lower viscosity than TSP and they show higher solubility than TSP.

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Glycoconjugated Europium Complexes as Cancer Diagnostic Probes

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Cancer cells have several metabolic dysregulations. In particular, in recent years the Warburg effect, which is the glucose avidity and the high rate of aerobic glycolysis of cancerous tissues, is recognized as one of the most important hallmarks of cancer.

Therefore, one promising strategy in cancer terapy, for targeting dysregulated tumor-cell metabolism is glycoconjugation, the linking of anticancer agents to glucose or another sugar. The power of glycoconjugation approch is connected with the evidence that glucose transporter GLUT, are widely overexpressed in tumor cells, with respect to normal tissues, and represents a powerful tool to impart selectivity to therapeutic and molecular diagnostic agents [1]. Moreover, recently, lanthanide-based probes gathered attention for their unique photophysical properties: long-lived photoluminescence, unlike common organic fluorophores, large Stokes shift, chemical and photochemical stability in biological medium, high brightness. Thanks to the long luminescence lifetime, time-resolved setup allows an efficient detection, an important advantage for bioassays and luminescence microscopy.

Within this context, our work focuses on the synthesis of glycoconjugated Europium complexes of ligands based on triazacyclononane azamacrocycle bearing charge-transfer antennae with strongly adsorbing p-substituted aryl-alkynyl group [2]. This coordination assures good stability and excellent sensitization of the metal ion. The glycoconjugation with different monosaccharides, has been realized either with classic glycosyl donors (trichloroacetimidate) or through click chemistry of propargyl glycosides directly on an Europium complex bearing azide functionalities (**Figure 1**). The photophysical characterization in water of these new complexes has been realized confirming their remarkable brightness, together with cells imaging experiments and relative uptake studies.

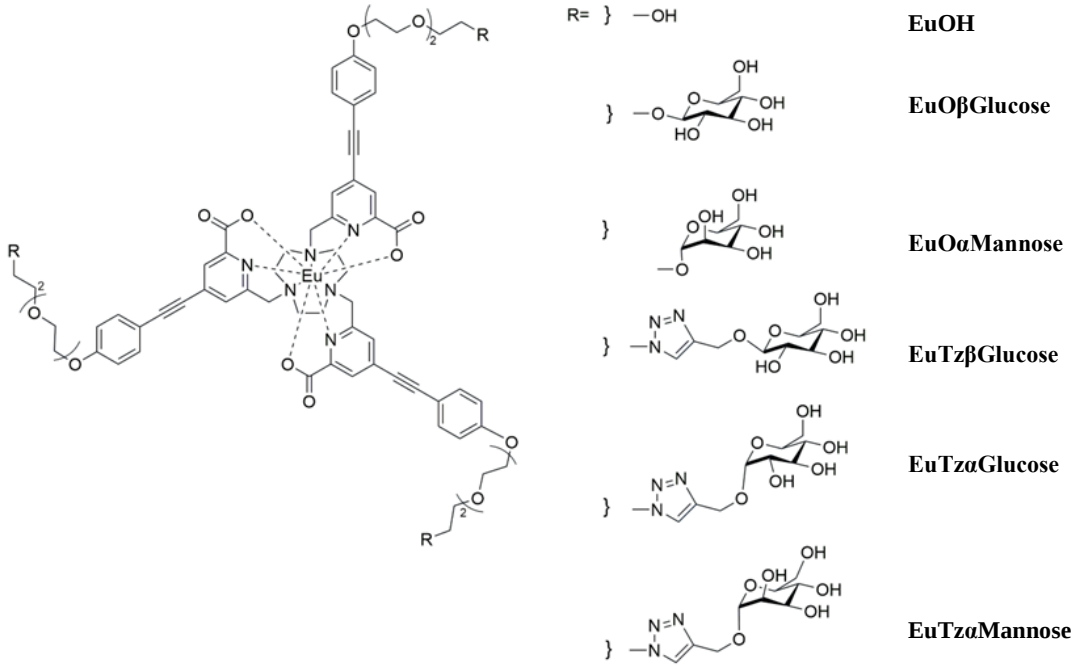

Figure 1: Glycoconjugated Europium (III) complexes synthesized.

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Biochemical Characterization of the First Pyruvyl Transferase Encoded by a Giant Virus.

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Pyruvylation consists in the transfer of a pyruvate moiety to the monosaccharide target in enol or ketal form, where the ketal-pyruvylation, the most widespread in nature, is found in bacteria, yeasts, and algae [1]. Interestingly, the chemical characterization of the mimivirus glycans [2] has extended this type of sugar modification to the viral glycans. Indeed, in one of the two polysaccharides covering mimivirus fibrils, the repeating unit is an N-acetyl-glucosamine modified with a pyruvic acid linked as a ketal to the hydroxy function 4 and 6. Since the sugar pyruvylation is absent in amoeba, the host of mimivirus, it is likely that mimivirus encodes its own pyruvyl transferase enzyme. Bioinformatic studies have identified L143 as a good candidate [3] and the object of this work was to assess the function of L143 enzyme by chemical and spectroscopic studies. There are currently no examples of virally encoded pyruvyl transferase, therefore its characterization is a major challenge. Biochemical assays identified the phosphoenolpyruvate as a donor of the pyruvic acid, for which the enzyme presents a high affinity (Km < 1). Therefore, we demonstrated that the substrate of this reaction is the N-acetyl-glucosamine monosaccharide (Figure 1), the enzyme being unable to work at the level of the nucleotide sugar (UDP-N-acetyl-glucosamine). These results suggested that *in vivo* the reaction substrate could be the full polysaccharide or the disaccharide precursor of the repeating unit, prior the assembly of the polysaccharide, in analogy with the mechanism of action of the yeast pyruvyl transferase (PDB code:5AX7).

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

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Role of EPS in Mitigation of Plant Abiotic Stress: The Case of *Methylobacterium Extorquens PA1*

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Methylobacterium extorquens is a facultative methylotrophic Gram-negative bacterium, often associated with plants[1], that exhibits a unique ability to grow in the presence of high methanol concentrations, which serves as a single carbon energy source[2].

We found that *M. extorquens strain PA1* secretes a mixture of different exopolysaccharides (EPSs) when grown in reference medium or in presence of methanol, that induces the secretion of a peculiar and heterogenous mixture of EPSs, with different structure, composition, repeating units, bulk and a variable degree of methylation[3]. These factors influenced 3D structure and supramolecular assets, diffusion properties and hydrodynamic radius, and likely contribute to increase methanol tolerance and cell stability.

No direct methanol involvement in the EPSs solvation shell was detected, indicating that the polymer exposure to methanol is water mediated. The presence of methanol induces no changes in size and shape of the polymer chains, highlighting how water-methanol mixtures are a good solvent for refEPS and metEPS.

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Towards the Semi-Synthesis of Phosphorylated Glycosaminoglycans

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A very important family of animal-sourced polysaccharides is constituted by sulfated glycosaminoglycans (GAGs). They are highly negatively charged, linear biomacromolecules, consisting of disaccharide repeating units composed by alternating aminosugar and uronic acid (or neutral hexose) monomers, and are extensively decorated with sulfate groups. GAG sulfation is a dynamic, complex post-translational modification process that seems to be a result of evolution in order to let sulfated GAGs play key roles in many physiological and pathological processes typical of higher animals.

GAG mimics displaying phosphate rather than sulfate anionic groups have been poorly investigated, although phosphate *vs.* sulfate differences in size, polarity, acid-base and chelation properties could lend interesting, unreported activities to phosphorylated GAGs (pGAGs). Indeed, a recent *in silico* study comparing the structural flexibility and intra- and intermolecular interaction patterns of native GAGs with their phosphorylated counterparts suggested that pGAGs could bind proteins generally with a stronger affinity than their sulfated counterparts and the differences in the binding modes might be highly protein target-dependent.[1] Therefore, pGAGs could stand as new, promising tools to specifically control biochemical processes where the mediating role of sulfated GAGs is crucial. Nonetheless, the introduction of phosphate groups on polysaccharide backbones presents some concerns, in particular the rather harsh conditions required by the commonly employed methods, and the very low degrees of derivatization and regioselectivity that are generally achieved.[2] With the aim to find a robust access to pGAGs, in this communication we present the results of a screening of several standard and recently proposed phosphorylation methods on an unsulfated chondroitin polysaccharide deriving from the fed-batch fermentation of *Escherichia coli* O5:K4:H4[3] and three partially protected derivatives thereof.

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Development of a platform method for the analysis of glycosylation in recombinant proteins by Mass Spectrometry

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Biopharmaceuticals currently represent the fastest growing sector of the pharmaceutical industry, mainly driven by a rapid expansion in the manufacture of recombinant protein-based drugs. Among them vaccines represent an important class of biomolecules. Glycosylation is the most prominent post-translational modification occurring on these proteins depending on the host cells used for their production, such as mammalian or insect cells and yeast [1]. To develop a versatile analytical platform for the rapid analysis of protein glycosylation we set up a three steps workflow that allowed the precise description and relative quantification of the two major types of glycosylation, such as *N*- and *O*-glycosylation. The first two steps rely on a bottom-up proteomic approach in which the glycosites and the correspondent glycan heterogeneity are investigated and then a glycan shaving followed by fluorescence-liquid chromatography and mass spectrometry allowed the characterization and quantification of all the glycans that decorate the surface of the vaccine protein. The third step is the precise identification of the O-glycosylation by filter-aided sample preparation coupled to LC-MS/MS detection that allowed the isolation of small peptides. In this work we report the glycan profiling of the recombinant protein gB from human cytomegalovirus produced in CHO cell line [2]. We describe the entire developed analytical workflow and the complete glycan characterization, that allowed a deeper product understanding. Glycosylation represents a potential critical quality attributes that requires thorough analysis for a better vaccine design and product consistency [3].

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

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

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Fucosylated calix[4]arene-based ligands for microbial lectins

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Lectins are multimeric proteins able to recognize and bind clusters of specific carbohydrates.[1] Due to this characteristic, lectins modulate various cellular recognition processes, ranging from physiological functions to pathological conditions. Numerous microorganisms exploit lectins affinity for glycosylated biomolecules to adhere to host cells and trigger their infection processes. Fucose is a naturally widespread saccharide, which can be found also in human tissues. For this reason, multiple human-targeting pathogens are equipped with fucose-selective lectins.[2] Many fucose-based compounds have been developed during the years as tools to hinder the adhesive properties of pathogenic microorganisms, in order to hamper their infectivity.[3] In this project, we are investigating the possibility of exploiting multivalent calix[4]arene-based compounds as ligands for fucose-selective lectins. In particular, three fucosylated calix[4]arenes (compounds **1**-**3**) were synthetized; these compounds were all functionalized with four units of α-fucose, but as a result of their geometries they expose these saccharides in different spatial directions. Isothermal Titration Calorimetry (ITC) allowed us to evaluate the binding affinity of the synthetized compounds towards three fucose-selective lectins expressed by pathogenic microorganisms: BambL from *Burkholderia ambifaria*, AFL from *Aspergillus fumigatus* and LecB from *Pseudomonas aeruginosa*.

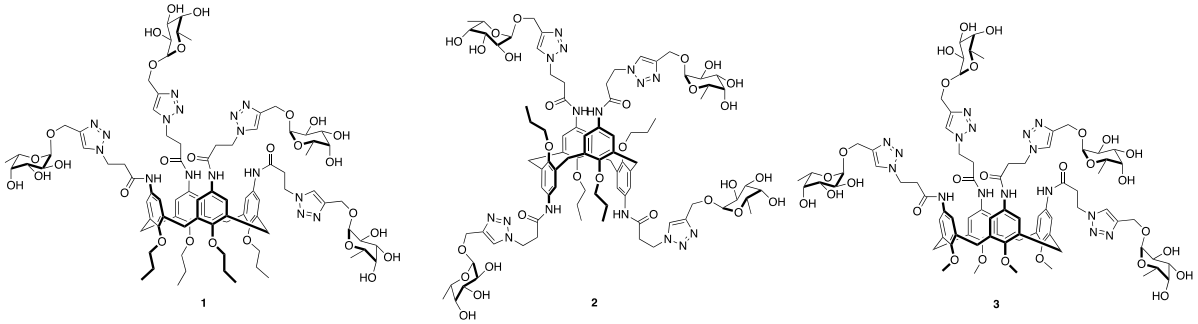

***Figure 1**: The three synthetized calix[4]arene ligands*

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Exploiting bacteriophage enzymes against *K. pneumoniae* infections

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Klebsiella pneumoniae is a Gram-negative opportunistic pathogen considered as one of the most critical bacterial species by several international health organizations. Indeed, infections by multi-drug resistant *K. pneumoniae* (MDR-Kp) represent a major clinical challenge, given their massive spread in hospital settings and the notable complexity of their treatment due to the lack of effective drugs. The bacterial capsule is an important virulence factor; moreover, the capsular polysaccharide (CPS) is also a major component of bacterial biofilms, thus representing an interesting target for the development of alternative antimicrobial strategies.

This study aims to characterize the activity of a bacteriophage endoglycosidase towards the CPS of *K. pneumoniae* strain KpB-1, whose structure has been previously defined in our laboratory (Fig. 1). This strain is of particular interest, since it belongs to the Clonal Group (CG) 258, one of the main responsible for the worldwide pandemic of MDR-Kp.

To test the phage endoglycosidase activity on the KpB-1 CPS, phage particles were incubated with pure CPS, the products were separated by size exclusion chromatography and analysed by NMR spectroscopy and ESI-mass spectrometry. The endoglycosidase cleavage site was determined to be the linkage α-L-Rha*p*-(1→3)-β-D-Gal*p*. Furthermore, treatment of preformed KpB-1 biofilm with phage particles resulted in biofilm disruption, suggesting that this endoglycosidase may be used to specifically attack biofilms formed by MDR-Kp of CG258. Therefore, the endoglycosidase was cloned and expressed in *E. coli*, purified, and its activity against pure CPS and biofilms will be evaluated.

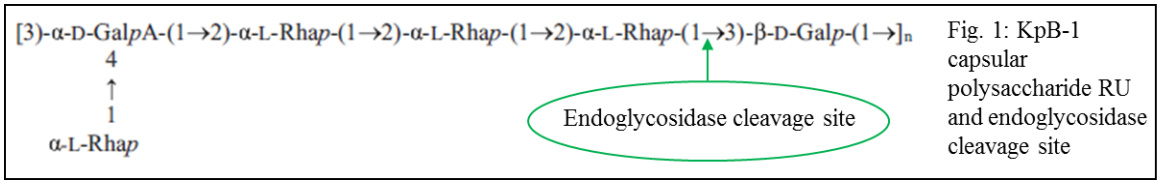

Fig. 1: KpB-1 capsular polysaccharide RU and endoglycosidase cleavage site

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Structural Elucidation of Polysaccharides from *Lactobacillus plantarum*

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In recent times, nutrition has become one of the main health patterns due to disorders related to sedentary lifestyles and therefore consumers consider functional foods as an attractive solution.[1] Among the main ingredients of functional foods are probiotics, food supplements based on live microbes that have beneficial effects on the host organism.[2] Probiotic microorganisms include the lactic acid bacteria (LAB) group, which have several scientifically proven effects on human health, such as antimicrobial activity, immune enhancement and anti-cancer activity. Their probiotic activity derives from the molecules they produce, including polysaccharides, which are used not only as ingredients but also especially as food additives. The wide spectrum of applications of microbial polysaccharides is in fact due both to their properties as thickeners or filmogens and, above all, to their immunomodulatory properties, i.e. anti-cancer, anti-inflammatory or antimicrobial.[3]

Based on the above, the present work focuses on the structural elucidation of polysaccharides produced by *Lactobacillus plantarum*, a gram-positive, mesophilic bacterium belonging to the LAB group, which colonises the human and animal gastrointestinal tract. *L. plantarum* possesses probiotic activity and therefore this bacterium is associated with several beneficial effects due to its ability to: produce antimicrobial molecules, modulate the immune system and strengthen the intestinal microflora.[4] However, the probiotic activity of this bacterium is not fully associated with molecules whose structure and more general chemical nature is known. This is why the present work was based on the extraction, purification and structural characterisation of a polysaccharide expressed by this bacterium, with the aim of being able to associate a possible probiotic function with it.

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Innovative Hybrid Nano-Architectures based on Cyclodextrin-Hyaluronic Acid Bioconjugate and Metal Nanoparticles.

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Polysaccharides such as hyaluronic acid and β-cyclodextrins thanks to their biocompatibility, accessibility, and biological and technological properties are widely employed in several biomedical/pharmaceutical applications. Moreover, their ability to act as stabilizers towards metal nanoparticles has been recently investigated [1-3]. Here, we describe a new synthetic strategy for the preparation of hyaluronic acid β-cyclodextrin (HA-CD) conjugate and its ability to interact with iron nanoparticles (SPIONS) and gold nanoparticles (Au NPs) producing new hybrid glyco-materials for theranostic applications. The chemical-physical characterization of new hybrid nano-Architectures (HACD@Au and HACD@Fe_3O_4 NPs) have been investigated by UV-vis, NMR, Dynamic Light Scattering (DLS), ζ-potential analyses and AC magnetization measurements.

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Hyaluronan-cholesterol nanogels for the enhancement of the ocular delivery of therapeutics

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The anatomy and physiology of the eye have always been a limit to the local delivery of therapeutics. Thus, the use of nanocarriers able to efficiently encapsulate therapeutics appears as an attractive strategy to facilitate the permeation and enhance ocular drug delivery [1-2]. In this light, polysaccharide-based nanogels (NHs) offer several advantages, such as biocompatibility, biodegradability and mucoadhesive property [3-4], and hyaluronic acid (HA) represents a good candidate for NHs preparation since it is found in the eyes [5].

On this basis, an HA's amphiphilic derivative, obtained by grafting the polymeric backbone with cholesterol moieties (HA-CH), was used to obtain self-assembled NHs able to load both hydrophobic (dexamethasone and piroxicam) and hydrophilic (tobramycin and diclofenac) drugs. *Ex vivo* studies by fluorescence microscopy and in-tube analyses with mucin showed that HA-CH NHs can interact with corneal components, without penetrate the stroma. Furthermore, *ex vivo* transcorneal permeation experiments were performed to assess their capability to behave as permeation enhancers, showing that NHs formulations can improve the ocular bioavailability of the instilled drugs by increasing their preocular retention time (hydrophobic drugs) or facilitating their permeation (hydrophilic drugs).

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Investigating the Role of Glycans in Antibiotic Resistance of Biofilm-Associated Infections

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Biofilms are ecosystems in which microbial cells are embedded in a matrix of extracellular polymeric substances (EPS) such as polysaccharides, proteins, lipids, and extracellular DNA. The composition, properties and dynamics of the biofilm matrix influence bacterial growth and antibiotic resistance.[1] The National Institute of Health of the United States declares that over 80% of microbial infections in the body are due to biofilms and are associated with nosocomial infections. Among biofilm-producing bacteria, *P. aeruginosa* is responsible for both acute and chronic infections in individuals with pneumonia or cystic fibrosis (CF). *P. aeruginosa* produces a huge amount of different extracellular polysaccharides and modifies the structure of lipopolysaccharide (LPS) when isolated from patients.[2] It has been demonstrated that the phenotypic alteration and biofilm production are correlated with nutritional limitation within the respiratory tract of CF patients rather than unique characteristics of these bacterial strains and may be the cause of the lack of therapeutic effects.[3] Clinical isolates including *P. aereuginosa* wild type (WT) strains obtained from recently infected CF patients and multidrug-resistant (MDR) and pan-drug-resistant (PDR) will be considered in this presentation. The study of the glycans from these microorganisms will be done by using Mass Spectrometry and NMR spectroscopy. The results obtained will be compared with the reference strain PAO14 to highlight structural differences.

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Polysaccharides from the Red Microalga *Porphyridium Cruentum*

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Exopolysaccharides (EPS) are high molecular weight carbohydrate polymers secreted by many microorganisms and comprise a substantial part of dissolved carbon in the marine environment. The sulphated extracellular polysaccharides play a crucial role in the development of personal care formulations and represent the future in biomedical applications like drug delivery and wound dressing. Microalgae are proven to be a very promising source of bioactive molecules including polysaccharides. Under stress conditions, such as nutrient starvation and physicochemical environmental conditions, these microalgae synthesize exocellular sugars in form of mucilage to survive. [1] Depending on their ecological niche and physiological needs, marine bacteria produce EPS for various reasons, such as adhesion and colonisation of surfaces and protection of cells from extreme conditions. [2] The chemical structure of sulphated extracellular polysaccharides (sEPS) endows them with peculiar rheological properties and many biological activities, such as antimicrobial, anti-inflammatory and antiviral.[3,4] Different studies report that the red microalga *Porphyridium cruentum* produces a significant amount of sEPS.[5] The biological activities of polysaccharides depend on their structural features such as molecular weight, the types of sugars they are composed of, and the degree of sulphation. Here, we present our data about the characterization of the primary structure of the EPS released by *P. cruentum.*

The polysaccharide has been purified by Gel Filtration Chromatography and High-performance Liquid Chromatography. Glycosyl analysis suggests the presence of mainly Xylose, Galactose, Glucose, and Glucuronic acid, indicating that the polymer is probably a neutral macromolecule.

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Protective Effect of Tamarind-Seed Polysaccharide in a Human 3D *In Vitro* Model of Dry Eye Desease

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Background

Tamarind-seed polysaccharide (TSP) is a branched polysaccharide with a cellulose-like backbone with glycosyl substitution, which (like other polysaccharides) can be potentially used in many pathological states such as dry eye disease (DED). DED is an ophthalmological disorder due to complex etiopathogenetic factors; among these, the impairment of the tear film structure.

Purpose

TSP + HA eye drops were tested on human corneal epithelial 3D tissues (HCE) to evaluate both protective and barrier effect to characterize their safety and efficacy profile.

Materials and methods

Tissues were maintained in low humidity and high temperature (Rh 40%; T 40°C) (HYP-DRY), treated with sorbitol added in basolateral culture medium for 8h, or also on the apical side for 8h. Then, culture medium was replaced with fresh one, and tissues were treated with TSP + HA on the apical side. Tissues were maintained in HYP-DRY conditions for further 16h. Tissue viability and IL-1β were quantified. Furthermore, the barrier effect of TSP + HA was assessed by analyzing caffeine permeability through healthy corneal tissues following the OECD Test guideline 428 for MD. The caffeine permeation was monitored for 2 hours and quantified using HPLC analysis.

Results

In HYP-DRY treated tissues, the inflammatory cytokine IL1-β was significantly higher than control tissues (CT). TSP + HA induced an appreciable reduction in IL-1β release. TSP + HA also decreased caffeine permeability of nearly 35% during the first 15 minutes and 20% after 2 hours.

Conclusion

The analysis demonstrates the barrier effect exerted by TSP + HA, which leads to a protective effect denoted by a decrease in the production of IL-1β.

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Multivalent Iminosugars Inhibit the Levansucrase Enzyme: A New Plant Protection Strategy Against Kiwifruit Canker?

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Bacterial canker disease caused by *Pseudomonas syringae pv. actinidiae* (Psa) biovar 3 devasted the Italian crops of kiwifruit since few years. In this bacterium, the enzyme levansucrase is responsible for the synthesis of levan, a hexopolysaccharide known to be part of the survival strategies of many different bacteria [1]. For these reasons, levansucrase inhibitors may have an important role in the plant protection from this pathogen. Some of us cloned and expressed the two putatively functional levansucrases in *E. coli* and characterized their biochemical properties [2]. The enzyme is deputed both to the hydrolysis of sucrose and to levan polymerization. We have identified inhibitors of both these processes based on a 1,4-deoxy-1,4-imine-D-arabinitol (DAB-1), a pyrrolidine iminosugar ring derived from an inexpensive sugar (D-arabinose) grafted onto a di- or a trivalent scaffold [3]. The best results in terms of inhibition were obtained with compound **1** (**Figure 1**). We report our synthetic efforts to the synthesis of the PEG-ylated analogue **2**, aimed at increasing solubility in order to avoid bioaccumulation and optimize the characteristics of **1**, in view of its application as agrochemical for crop protection.

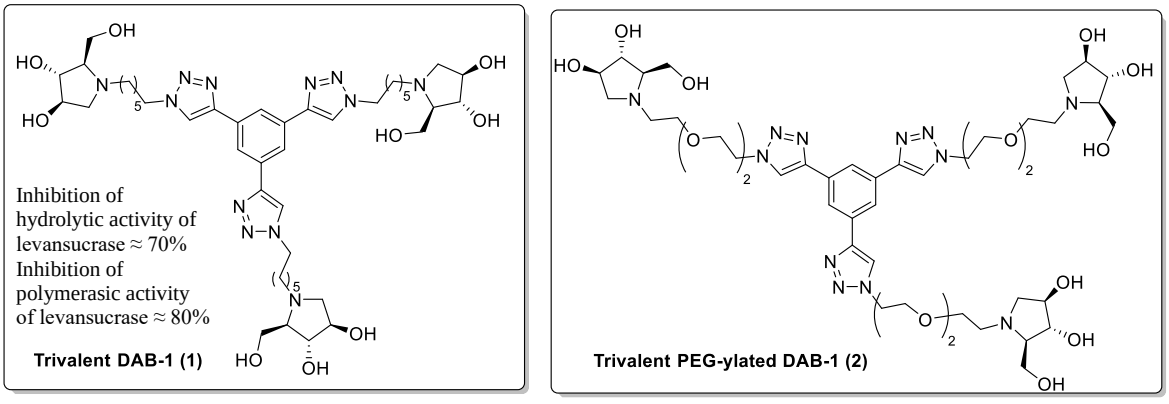

Figure 1: Trivalent DAB-1 (**1**) and its PEG-ylated analogue **2**.

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Theranostic Iminoglyco-NPs targeting the Blood Brain Barrier

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Gaucher's disease (GD) is a rare autosomal recessive disease caused by a deficiency in the activity of the glucocerebrosidase (GCase) enzyme due to mutations in the GBA gene. Studies show that GBA genetic mutations are also a risk factor for Parkinson's disease, suggesting that therapeutic approaches to modulate GCase activity could play an important role in treating both diseases [1]. An innovative therapeutic strategy for the treatment of GD is the use of pharmacological chaperones (PCs) to enhance natural enzymatic activity, although currently no PC has yet reached the market for this pathology. Iminosugars and other substrate analogues acting as competitive inhibitors of GCase are promising candidates as PCs [2]. As the blood brain barrier (BBB) represents an obstacle for the classical enzyme replacement treatment of neuronopathic forms of GD, the synthesis of novel iminosugars functionalized with specific ligands able to enhance the transport through the BBB is being investigated. In addition, since a greater affinity between enzyme and multivalent iminosugars has been demonstrated, the multimerization of the iminosugars onto nanoparticles (NPs) will be carried out to combine the properties of the inhibitors to the multivalency of the NPs, thus yielding smart systems with theranostic properties [3].

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Design of an effective glycoconjugate vaccine against Group A *Streptococcus*

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No commercial vaccine is yet available against Group A Streptococcus (Strep A), major cause of pharyngitis and impetigo, with a high frequency of serious sequelae in LMICs, and an important driver of antibiotic use.

The highly conserved Group A Carbohydrate (GAC), conjugated to an appropriate carrier protein, has been proposed as an attractive vaccine candidate. GAC consists of a polyrhamnose (polyRha) backbone with alternating *N*-acetylglucosamine (GlcNAc) at the side chain. Both native GAC and the polyRha backbone only have been proposed as vaccine candidates.

Here, GAC and polyRha conjugates have been compared in different animal models, showing higher anti-GAC IgG responses elicited by GAC with stronger binding capacity to Strep A strains. Moreover, we confirmed that GlcNAc is an important component of the GAC epitope motif, and it is recognized by monoclonal antibodies only in the context of the polyRha backbone.

Also, the possibility to use Strep A protein antigens with a dual role of antigen and carrier, to enhance the efficacy of the final vaccine and reduce its complexity, has been explored. All protein antigens resulted good carrier for GAC; however, conjugation to the polysaccharide had a negative impact on the anti-protein functional responses. Therefore, we selected CRM as protein carrier and used a Design of Experiment approach to increase process robustness and conjugation yield.

This work contributes to the development of a vaccine against Strep A and shows how recent advancements in the field of conjugation can lead to improved design of glycoconjugate vaccines.

Investigation of Alternative Technologies for the Development of Polysaccharide-Based Vaccines

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Chemical conjugation to carrier proteins has been traditionally used to improve polysaccharides immunogenicity and to overcome the limitations of T-independent antigens, including lack of immunological memory and efficacy in infants [1]. Bioconjugation is a simplified strategy to obtain glycoconjugate vaccines. Bacteria are genetically mutated to obtain glycosylation of carrier proteins directly *in vivo*. Recently, bacterial protein glycosylation has been exploited to obtain glycosylated proteins in *E. coli* cytoplasm [2]. This system relies on a N-glucosyltransferase (NGT) enzyme which catalyzes the transfer of a single β-linked glucose onto engineered N-x-S/T sequons on recombinant proteins. N-linked glucose can then function as a site-specific primer for the biosynthesis of diverse oligo- and polysaccharides through the addition of different glycosyltransferases, as reported for meningococcus serogroup B and C capsular polysaccharides [3]. In our work, we are investigating this technology for the generation of innovative glycoconjugate vaccines via a fully engineered biosynthetic pathway. For this purpose, *Klebsiella pneumoniae* capsular and subcapsular polysaccharides have been chosen as model polysaccharide antigens for the generation of innovative glycoconjugate using Kp protective protein antigens. A glycosylation pathway was successfully introduced in *E. coli* to modify the carrier proteins with lactose and strategies to extend this unique sugar anchor through addition of further enzymes are currently under study. N-x-S/T sequons have been also inserted in different regions of the protein of interest to understand better the impact of such glycosylations on antigenicity and carrier effect. In parallel, traditional glycoconjugates have been produced using a random approach. Mice studies will be then performed to compare the immune response induced by all these different vaccines constructs. This work will contribute to expand the application of glycoengineering technology for the development of effective glycoconjugates vaccines.

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Combining Solid-State NMR with Structural and Biophysical Techniques to Design Challenging Protein-Drug Conjugates

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Several protein-drug conjugates are currently being used in cancer therapy. These conjugates rely on cytotoxic organic compounds that are covalently attached to the carrier proteins or that interact with them via non-covalent interactions.[1] Human transthyretin (TTR), a physiological protein, has already been identified as a possible carrier protein for the delivery of cytotoxic drugs.[2] Here we show the structure-guided development of a new stable cytotoxic molecule based on a known strong binder of TTR and a well-established anticancer drug. This example is used to demonstrate the importance of the integration of multiple biophysical and structural techniques, encompassing MST, X-ray crystallography and NMR in solution[3], but also showing that solid-state NMR provides an easy access to a more complete structural picture. In particular, we show that solid-state NMR has the ability to reveal effects caused by ligand binding which are more easily relatable to structural and dynamical alterations that impact the stability of macromolecular complexes.

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Antitumor Platinum(II) Hybrid Compounds Based on a Glucosylglycerol Scaffold

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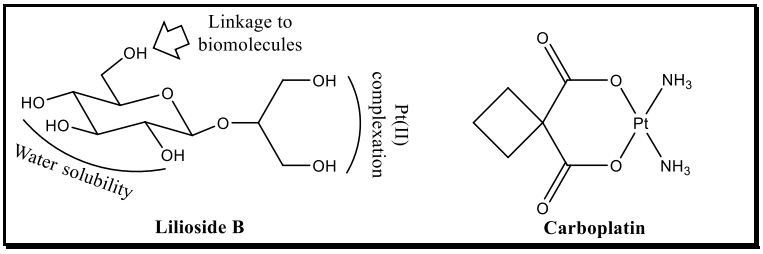
Platinum(II) drugs such as cisplatin, carboplatin and oxaliplatin are antineoplastic drugs clinically available for the treatment of different kinds of cancers, including ovarian carcinoma. However, their use is limited by the occurrence of severe systemic side effects and resistance [1, 2]. For these reasons, the development of new platinum-based compounds endowed with higher selectivity against cancer cells and able to overcome resistance is an active research field. A promising strategy to pursuit these goals is the design of hybrid platinum(II) compounds bearing bioactive ligands able to selectively target cancer cells, improve the platinum-mediated antitumor activity and/or overcome resistance by interacting with selected targets known for their involvement in cancer resistance [3]. In this context, due to its peculiar structure, 2-O-β-D-glucosylglycerol (a natural compound named Lilioside B) [4] could be efficiently used at the same time as the complexing agent of platinum(II) and as the point of attachment of cancer involved bioactive compounds. Thus, the present communication will show some preliminary results on the synthesis and cytotoxicity data, on ovarian cancer cells, of a water soluble platinum(II) hybrid compound in which, similarly to carboplatin, a properly modified 2-O-β-D-glucosylglycerol is able to complex platinum(II).



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Synthesis of a Small Library of Compounds Inspired by *Bacteroides fragilis* Lipid A as Potential Vaccine Adjuvants

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Adjuvants are substances that improve the overall efficacy of vaccines, enhancing the response towards antigens and allowing for the use of lower doses of active ingredients[1]. Moreover, adjuvants provide functionally appropriate immune response by modulating the generation of specific immune system cell types[1,2]. Until now, only a few vaccine adjuvants have been approved and their complete mechanism of action must be fully elucidated yet. Therefore, new research efforts are needed with the aim to identify novel chemical entities to be developed as adjuvants. Some low-toxicity Lipid A molecules can positively modulate the human's immune system through their interaction with key components of the innate immune system, such as the Toll-like receptors (TLRs). An illustrative example is "Monophosphoryl Lipid A", a modified Lipid A from *Salmonella minnesota* R595, the first TLR-agonist authorized as vaccine adjuvant[3]. Recently, new Lipid A moieties of *Bacteroides fragilis*, a Gram-negative bacterium belonging to the gastrointestinal microbiota, have been isolated. Their promising immunomodulatory activities[4] and their probable low toxicity make them interesting candidates for the development of new vaccine adjuvants. The glycolipid mixture isolated from *B. fragilis* has been only partially characterized and their exact structures is still not completely established. In this context, our project is focused on the chemical synthesis of a small library of Lipid A structures (Figure 1), based on the available information, with the aim to define their chemical structures and their immunological properties, as well as to investigate their potential application as vaccine adjuvants.

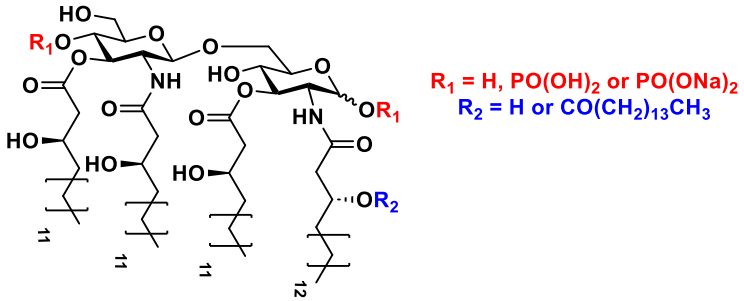

Figure 1. *Schematic representation of the designed lipid A library*

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Multivalent Glycosidic Vectors for the Modulation of the Immune System

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Among the different breast cancer types, the triple negative breast cancer (TNBC) is the most difficult to treat and to recover from.[1] Cancer immunotherapy is nowadays a consolidated strategy and Tumor-Associated Carbohydrate Antigens (TACAs) are used to develop therapeutic cancer vaccines (CVs). In designing a potential TACA-based CV it must be considered that saccharidic antigens suffer from a reduced metabolic stability *in vivo* and a poor T cells-dependent immunogenicity, which compromise a strong immune response, crucial for a promising CV.

To overcome these issues, synthetic organic chemists may offer a solution by designing TACA analogues to mimic the native antigens and which are endowed with a better stability and immunogenicity.[2]

Here, we will discuss about the preparation, characterization, and *in vitro* screening of niosomes displaying multiple copies of the native mucin antigen TnThr or of TnThr mimetic.[3]

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Sustainable Valorization of Polysaccharide Fraction in Food Industry Biomass Wastes

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The transition towards circular economy prompted both academia and industry to investigate new ways of producing chemicals, materials, and fuels from renewable resources such as food industry biomass wastes. Recently, the development of sustainable processes that provide high performance and maximal use of biomass feedstocks into commodities with reduced environmental and economic impact have been highly pursued. In this context, the recovery and reutilization of polysaccharides, one of the most abundant fractions of biomass, play a key role. Hence, in this study, the valorization of the polysaccharide portion found in biomass wastes derived from the production of fruit juice (apple fibers and cherry pomace as feasible underexploited lignocellulosic materials), was investigated through the employment of bio-based ionic liquids (bio-ILs) as green media. The cellulose crystallinity and the presence of the lignin strictly cross-linked to cellulose and hemicellulose fibers make this treatment necessary to increase the accessibility of desired polysaccharides. More in detail, cholinium arginate (ChArg) was successfully employed using mild conditions (90 °C for 1 h) with the two biomasses to obtain cellulose-enriched materials (CRMs). The CRM achieved from apple fibers was then subjected to enzymatic treatment to hydrolyze cellulose into monosaccharides that can subsequently be converted into biofuel. For comparison, also a natural deep eutectic solvent, ChCl:lactic acid and the classical IL, 1-butyl-3-methylimidazolium acetate (BMIM OAc) were tested for the pre-treatment of apple fibers before enzymatic hydrolysis. Conversely, the CRM fraction derived from the treatment of cherry pomace was dissolved in a different bio-IL, cholinium levulinate (ChLevA), in order to prepare an ionogel. All materials have been characterized by FTIR and thermogravimetrical analyses (TGA). The ionogel was also subjected to rheological analyses. Furthermore, both ChArg and ChLev were recovered without significant alterations.

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Mannosylated Liposomes as Advanced Delivery Vehicles for Selective Dendritic Cells Targeting

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Carbohydrates are the major class of biomolecules, usually present as glycoconjugates covalently linked to other macromolecules such as lipids and proteins. These glycoconjugates are involved in a variety of physiological and pathological processes. In particular, mannose-based glycoconjugates are involved in the first step of viruses, bacteria and fungi infections.[1] Mannose Receptor (MR or CD206) is a transmembrane glycoprotein mainly expressed by most tissue macrophages and dendritic cells (DCs). The role of MR is crucial in the recognition of viral and microbial glycans, thus, inducing the consequent internalization of the pathogens (up-take) by macrophages. This is the first event in the immune response against pathogens.[2] Nanoparticles in general and liposomes in particular gained lot of attention in the pharmaceutical field as flexible and multifunctional targeted delivery systems, therefore, becoming the first-choice delivery vehicles for administration of drugs, nutrients and, more recently, mRNA vaccines.[3] The discovery of the role of MR suggested the idea that mannosylation of liposomes surface with mannose glycans, could enhance the recognition promoted by the mannose receptors, thus, representing a suitable strategy to target dendritic cells, in particular macrophages. Here, we report the synthesis of mono, di and tri mannose-based saccharides, endowed each one with an azido group, at the anomeric position to perform the subsequent strain promoted azide-alkyne cycloaddition (SPACC) with multifunctional liposomes showing a cyclooctyne group on their surfaces. The liposomes were decorated with a fluorescent dye, glycosylated on their surface at 5,10 and 20 % and tested *in vitro* to evaluate their uptake ability towards RAW 264.7 cells and, in comparison, to non-glycosylated ones. The 10% monomannosylated liposome showed the highest affinity for macrophages. The achieved results indicate that the obtained nano-system may be used to vehiculate vaccine-inducing antigens, as well as to delivery drugs to dendritic cells (E.G. compounds influencing polarization of macrophages, from M1 to M2 state, or viceversa).

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