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EPR technique presentation, with some examples of its applications on irradiated sucrose mixtures

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Electron Paramagnetic Resonance (EPR) is a highly selective spectroscopic technique used to study paramagnetic species, which are characterized by the presence of unpaired electrons. Based on the interaction between the electron magnetic moment and an external magnetic field, EPR allows you to get detailed information about the electronic structure, molecular dynamics, and local chemical environment of free radicals, transition metal ions, and defects in materials. It's also the only technique capable of directly detecting free radicals in any sample, solid or liquid, without false positives.

Over the past decade, the evolution of instrumentation, which has made all EPR techniques accessible with almost "press-button" pulsed instruments as well as benchtop EPR equipment, has significantly expanded the field of EPR applications, making it an essential tool in many areas. This contribution aims to give an overview of the basic principles of Continuous Wave EPR, highlighting some of its applications on the degradation of polysaccharides.

EPR for the study of biomacromolecules and their aggregates

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Electron paramagnetic resonance spectroscopy provides extremely site-specific information regarding polarity, viscosity, and the degree of order in complex molecular and supramolecular systems thanks to the use of appropriate radical probes or labels. Spectra analysis can be highly sophisticated and may require mechanical simulation. Nevertheless, a great deal of valuable, unambiguous information can be obtained from the determination of more accessible spectral parameters, such as coupling constants, line widths, and relative signal intensities. Combined with the current availability of highly sophisticated "benchtop" instruments, this broadens the potential user base to include non-experts.

This presentation will briefly outline the experimental methods used to obtain spectra and the various approaches to interpreting them. Several case studies involving biological macromolecules will then be presented, beginning with biological membranes (including examples of bacterial membranes containing lipopolysaccharides) and continuing with peptides and proteins and their interactions with biomembranes. The presentation will then move on to the typical spectra of melanin pigments and phenolic polymers. Lastly, the focus will be on the application of the spin-labeling technique to study the dynamics of polysaccharides in an aqueous solution.

NMR of pharmaceutical polysaccharides

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Solution NMR is a powerful and versatile analytical technique for the characterization of pharmaceutical polysaccharides, offering non-destructive analysis, minimal sample preparation, and atomic-level structural insight. Despite challenges such as spectral overlap and structural heterogeneity, NMR enables reliable qualitative and quantitative analysis across a wide range of systems. In particular, 1D 1H NMR plays a key role in quality control, as exemplified by heparin, where it is used in pharmacopeia methods for identity testing and impurity detection, including contaminants such as OSCS. Complementary approaches such as 13C NMR provide enhanced spectral resolution and allow differentiation of polysaccharide origin, while maintaining robustness within regulatory frameworks. In biopharmaceutical applications, NMR is essential for the characterization of polysaccharide-based vaccines and related materials throughout development and manufacturing. It supports structural elucidation, quantification of critical attributes such as O-acetylation, and monitoring of conjugation processes. Advanced techniques, including diffusion-ordered spectroscopy (DOSY) and multidimensional experiments (e.g., TOCSY, NOESY, HSQC), extend the analytical capabilities to molecular size determination, interaction studies, and detailed structural assignment in complex mixtures. Overall, NMR provides a comprehensive toolkit for ensuring the quality, safety, and functional understanding of pharmaceutical polysaccharides.

Decoding the Glycan Landscape: How Sugar Structure Shapes Function and Recognition

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Deciphering the structural complexity of carbohydrate-based biomolecules remains a difficult challenge due to the intrinsic diversity of sugar world, which still limits automated analyses. Yet, this task is essential for understanding molecular interactions at the atomic level, particularly, but not only, in host–microbe communication. By integrating complementary biophysical approaches, including NMR spectroscopy, computational modeling, native MS, and immunological assays, we can unravel the structures, properties, and functions of glycans and their roles in recognition mechanisms that define the *sugar code*.

This lecture will explore the dual nature of the glycocode: its beneficial role as a mediator of cross-talk, homeostasis, and immune system development, and its harmful role when exploited by pathogens and cancer cells. I will place a special emphasis on the glyco-features of bacterial cell surfaces that fine-tune eukaryotic immune responses.

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Multiscale structure in heterogeneous biomass – to resolve it with chemical resolution by NMR spectroscopy

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Modifications of cellulose reducing end groups investigated by solution state NMR: Imidazole structures formed through a Maillard reaction cascade

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A recently reported facile one-step isolation of cationic cellulose nanocrystals (CNCs) was achieved by high temperature treatment (120 - 160 °C) of different cellulosic feedstocks in reactive eutectic media (REM) consisting of ammonium formate and high boiling organic acids.[1] The performed characterization suggested the introduction of charged nitrogen-containing moieties selectively to the isolated CNCs' reducing end groups (REGs). Given the operative reaction conditions, a reductive amination according to a Leuckart Wallach reaction was proposed. However, selectivity and underlying reaction mechanism could not be confirmed by any direct analytical technique. This left room for scrutiny which motivated the current follow-up effort. The application of solution NMR measurements using a direct-dissolution electrolyte allowed further insights into the occurring reactions.[2] A modification predominantly restricted to the REGs was confirmed and no significant changes of the cellulose backbone shifts were apparent. In contrast to the initial proposal, no resonances corresponding to amine functionalities were present. Instead, peaks in the range of aromatic *N*-heterocycles were seen. Their constitution was established using a CNC prepared from a low molecular-weight cellulose model compound and the application of different 2D NMR experiments. Unexpectedly, the major product was identified as a furanosyl imidazole structure. This was further confirmed by high-resolution mass spectrometry. The proposed furanosyl imidazoles, in monomeric form, are known in literature. They were prepared from glucose in a high temperate reaction with formamidine acetate.[3] Under the conditions prevailing during CNC preparation, the same structures are likely to be formed over a multistep Maillard reaction cascade – involving the imidazole ring closure over a Weidenhagen or Bredereck type reaction. To the best of our knowledge, this represents the first account of a neat Maillard reaction product formation directly on the REGs of cellulose.

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Controlled Synthesis of Crosslinkable Cellulose Acrylate Derivatives with Tunable Solubility

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This work investigates the controlled synthesis of cellulose acrylate derivatives through systematic variation of reaction temperature and the amounts of base and acrylate anhydride. By adjusting these synthetic parameters, it was possible to modulate the degree of substitution and obtain cellulose esters with tunable solubility in different solvents. The study addresses the challenge of achieving predictable and reproducible cellulose modification, since homogeneous acylation often leads to variable substitution patterns and material properties. [1, 2] Structural characterization of the products was carried out by a comprehensive set of NMR techniques, allowing detailed analysis of the modified cellulose derivatives. The results show that reaction conditions play a decisive role in determining both the extent of functionalization and the final solubility behaviour of the products. This approach provides a straightforward and versatile route to cellulose-based materials with tailored properties, supporting their potential use in advanced formulations and functional applications.

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NMR spectroscopy of different bacterial α-glucans in $P_{4444}OAc$ / DMSO-d_6

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Bacterial α-glucans show a high structural diversity, as they have different backbone linkage-types as well as side chains at different positions and of different lengths. Furthermore, the glucan structure and water-solubility are influenced by the synthesis conditions. However, detailed knowledge of the structure of bacterial α-glucans is necessary to establish structure-function relationships and to enable their targeted pharmaceutical or food applications. By using NMR spectroscopy, different structural features can be analyzed, however, severe signal overlap or insolubility in the most common solvent, D_2O, limits the information obtained on fine structures. In recent years, tetrabutylphosphonium acetate ($P_{4444}OAc$) in DMSO-d_6 was developed as a solvent for NMR spectroscopic analysis of cellulose.[1] Therefore, the aim of this study was to establish a $P_{4444}OAc$/DMSO solvent system for α-glucans and to find marker signals by using a sample set of bacterial α-glucans with all common structural elements. By using 5 % $P_{4444}OAc$ in DMSO-d_6 at 40 °C, all α-glucans could be dissolved, and well resolved ^1H NMR spectra were obtained for all samples. Several 2D experiments were used to assign the anomeric signals to the different structural elements. Characteristic signals for all side chain types, elongated side chains as well as highly branched regions and insoluble glucans were identified and demonstrated the potential of the approach. Overall, the established conditions enable a simple, comprehensive NMR spectroscopic analysis of the fine structures of water-soluble and water-insoluble bacterial α-glucans.

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NMR-Based Structural Investigation of the O-Antigen from a Pan-Drug Resistant *Pseudomonas aeruginosa* Isolate

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen responsible for severe biofilm-associated infections, particularly in cystic fibrosis (CF) patients. CF is a hereditary multisystem disorder caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), leading to impaired ion transport and accumulation of thick mucus in the lungs, which favors chronic bacterial colonization and persistent infections. Among CF-associated pathogens, *P. aeruginosa* is particularly relevant due to its strong biofilm-forming ability and high antibiotic resistance.[1] In this work, we report the structural characterization of the lipopolysaccharide (LPS) from a pan-drug resistant (PDR) *P. aeruginosa* isolate recovered from a cystic fibrosis patient. Particular attention was devoted to the polysaccharide moieties of the LPS. The O-antigen was purified by gel filtration chromatography and investigated through chemical analyses and advanced NMR spectroscopy techniques, allowing the elucidation of its structural features. In parallel, lipid A was obtained by mild acid hydrolysis and analyzed by high-resolution mass spectrometry to evaluate structural heterogeneity associated with bacterial adaptation and antimicrobial resistance. Overall, this study highlights the importance of combining analytical and spectroscopic approaches, particularly NMR spectroscopy, for the detailed characterization of complex bacterial polysaccharides involved in pathogenicity and chronic infections.

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UNRAVELING BACTERIAL GLYCANS: FROM LPS AND CPS STRUCTURES TO PROTEIN INTERACTIONS

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Glycans play a crucial role in the health and infectivity of Gram-negative bacteria by shaping their outer structures and interactions with the host. These bacteria possess lipopolysaccharides (LPS) in the outer membrane and sometimes capsular polysaccharides (CPS). LPS consists of a polysaccharide linked to lipid A, the endotoxin component, while variations in lipid A and the O-antigen affect virulence and immune responses. CPS further increases antigenicity and provides protective external layers around the bacterial cell. [1,2] Many bacteria interacting with humans are still chemically poorly understood, limiting therapeutic development. This study aims to structurally characterize their surface glycans.[3] Here, I report the structural characterization of LPS and CPS from Phocaeicola vulgatus (P. vulgatus), a beneficial commensal bacterium widely distributed in the gut microbiota.[4] Interestingly, P. vulgatus releases BSAP-3, an antibacterial toxin targeting the LPS core of competing strains via its MACPF domain, driving microbiota diversity and affecting host biology. Therefore, the interaction between P. vulgatus LPS and BSAP-3 has been evaluated using NMR and biophysical approaches. [5,6] I will explore further bacterial glycans, including: the structure and properties of CPS from Neisseria subflava, a poorly characterized bacterium associated with the life cycle of better-known Neisseria pathogens [7] and Akkermansia muciniphila, a mucin-degrading bacterium, in its interaction with host immune receptors, such as Galectin-4, a lectin recognizing β-galactosides, highlighting potential host–microbe glycan interactions. [8,9]

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Role of Glucansucrases from Lactic Acid Bacteria (LAB) to produce novel α-glucans

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Lactic Acid Bacteria (LAB) encodes carbohydrate active enzymes (CAZymes) responsible for the production of homopolymeric and heteropolymeric exopolysaccharides (EPS) structures. Glucansucrases are the main enzymes for the production of homopolymeric α-glucans utilising sucrose as the substrate converting these cheap substrate to glucan structures containing α-(1→6), α-(1→4), α-(1→3) and α-(1→2) bonds. Recent developments in the sequencing technology have increased the number of whole genome available LAB strains that resulted in the identification of potential new subgroups of glucansucrases that could be responsible for the production of new polysaccharide structures. Branching sucrases (BS) are good examples as a subgroup of glucansucrases acting on sucrose for the hydrolysis but cannot perform the polymerisation reaction to synthesize the α-glucans. Also glucansucrases can be subjected to genetic manipulations targeting the alteration of their activity that could also result in the formation of novel α-glucan structures. In this study, the role of LAB originated glucansucrases for the production of novel α-glucans was discussed.

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Bacterial Extracellular Vesicles as Trojan Horse Antibiotic Carriers against Multidrug-Resistant Biofilms

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The spread of multidrug-resistant bacteria is largely driven by the overuse and misuse of antibiotics,[1] with the aggravating factor of biofilm formation.[2] Because the extracellular biofilm matrix acts as a protective barrier, treating these infections is highly challenging, highlighting the need for alternative therapeutic strategies. Lipid-based delivery systems are among the most widely used strategies to overcome multidrug-resistant and biofilm-related infections because of their safety, biodegradability, and favorable physicochemical properties.[3] in this context, bacterial extracellular vesicles may represent a promising alternative therapeutic platform. Here, we have characterized the extracellular membrane vesicles produced by the *Shewanella vesiculosa* bacterium as a possible platform for delivering antibiotics and obtaining information about the vesicles' dimensions and molecular weight. In addition, we have reconstructed the outer membrane of this Gram(-) bacteria, considering the different components, that is, the lipid part, the lipopolysaccharides (LPS), and the capsular polysaccharides (CPS). The information obtained from the chemical-physical characterization of the membrane that mimics the bacterium has allowed us to optimize the preparation of an innovative delivery system, capable of acting as a Trojan horse.

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Quantitative Analysis of Cellulose I and Cellulose II Phases in Industrial Pulps by Solid-State NMR

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When cellulose is dissolved and regenerated, or sufficiently swollen under alkaline conditions, its crystalline structure changes from cellulose I into cellulose II. The proportions of these two crystalline structures influence the physical properties of pulp. The aim of this study was to quantify both crystalline cellulosic phases combined in pulp samples, using solid-state NMR spectroscopy and wide-angle X-ray scattering (WAXS). Both methodological approaches require reference materials: natural cellulose I from cotton linters, regenerated cellulose II from highly oriented lyocell fibers (NMR) or pulp originated from an alkali cellulose (WAXS), and fully amorphous cellulose were used to acquire their solid-state ^{13}C-CP/MAS-NMR spectral profiles. After calibrating the contact time of cross polarization, the ^{13}C-NMR spectra were recorded for five solid industrial pulp samples. The complex pulp spectra had to be referenced, intensity normed, and were fitted as a linear combination of the reference material spectra. This fitting procedure allowed for the quantification of the relative proportions of cellulose I and cellulose II. In parallel, WAXS diffractograms were measured from the same pulp samples. The WAXS data were analyzed to estimate the content of cellulose I and II based on their characteristic diffraction peaks, providing an independent method. The NMR analysis results in a cellulose II content from 30 to 62 % across the different pulp samples. The relative deviation between the WAXS and solid-state NMR analysis are between 1 to 8 %. WAXS analysis evaluates crystalline long-range differences in interplanar spacing while NMR studies focus on differences in the near electronic environment of nuclei. Solid-state NMR spectroscopy and WAXS provide detailed insights into the structure of materials, which influence pulp properties and are valuable analytical tools for quality control and the optimization of pulp and paper manufacturing processes.

^{13}C solid-state NMR application to characterization reversible swelling of starch granules

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Starch reversible swelling refers to the process by which starch granules swell by absorbing water at low temperature (under the gelatinization temperature around 60°C). This work aims at assessing the relevance of solid-state NMR, and particularly the VCT-CPMAS technique, in probing the fine structure of hydrogen bonds and their dynamics in starch granules under varying hydration levels at low temperatures (30°C). Using various starches of different origins (wheat, waxy wheat, rice, and tapioca) hydrated at around 12 or 40% (wet basis), we could identify multiple populations of relaxation and proton spin diffusion times that were attributed to starch domains with different degrees of structural organization (amorphous and semicrystalline) and hydration levels [1].

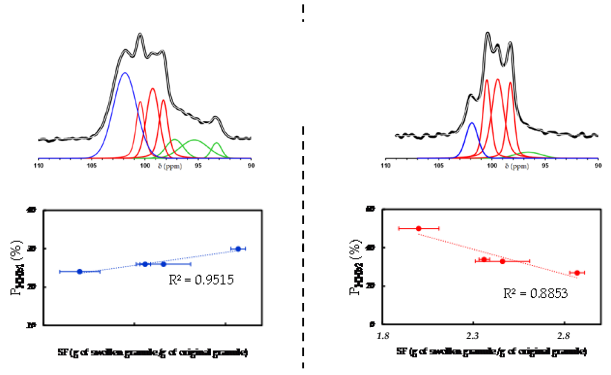

Figure 1. Effect of water content on starch organization and correlation between swelling factor and spin diffusion proportions.

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Using solid-state NMR to elucidate the changes in cellulose structure and xylan conformation of nanocellulose

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Drying and redispersing nanocellulose has been an ongoing challenge in the scientific community. Successful attempts at solvent-exchanging water for isopropyl alcohol (IPA) resulted in fully redispersed cellulose nanofibers (CNF)[1]. Still, many questions remain pertaining to the changes in the cellulose structure and the mechanisms that make it possible. We used ^{1}H and ^{13}C NMR 1D solid-state spectroscopy[2] to unveil the structure of cellulose and xylan conformation and elucidate the changes in the molecular structure of CNF that make drying and redispersion viable. Three types of CNF (unmodified, TEMPO-oxidized, and oxalic-acid oxidized) were analysed. C4 and C6 peaks showed that cellulose 1β structures remain mostly intact in all samples. ^{13}C spectra showed that, for CNF samples, the main differences observed were the removal of a portion of the hemicellulose in IPA-solvent exchanged samples as seen at 63.5 ppm and 102.5 ppm. Carbon spectra showed that IPA-dried TEMPO-CNF had more static disorder on the surface in comparison with air dried and freeze dried samples. The proton spectra of IPA-dried and redispersed samples showed smaller pools of water compared to air-dried and freeze-dried samples. The differences found herein highlight the importance of understanding cellulose conformation and its water interactions. Next steps include DP measurements as well as experiments to differentiate free and bound water.

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Decoding Ligand-Protein Interactions in STING and Siglec-7

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The STING and Siglec-7 proteins represent pivotal checkpoints in the immune response to cancers and pathogens. STING is responsible for the activation of a pathway leading to the production of type I interferons after binding to 2'3'-cyclic GMP-AMP (2'3'-cGAMP), synthetized upon detection of cytosolic dsDNA[1][2]; while Siglec-7 acts as an inhibitory receptor for natural killer cells, recognizing α(2,6)- and α(2,8)-linked sialic acids exposed on cells' surfaces[3]. Tumours and pathogens frequently subvert or exploit these pathways to evade immune surveillance[4][5].
Therefore, STING and Siglec-7 emerge as potential targets for new medical therapies: thus, this work will present the dissection of their interaction with different ligands. While for STING the focus was centered on cyclic dinucleotidic compounds, for Siglec-7 the analysis involved a suite of glycans including α(2,8)-linked sialylated oligosaccharides, core 2 glycans, and the cancer-associated DSGb5 with its monosialylated derivatives. Protein and ligand-based NMR experiments were the main techniques used to determine the interacting amino acids and the ligand binding epitopes and conformations; whereas ITC and fluorescence titrations were employed to determine binding affinity and thermodynamic parameters. Finally, complementing the acquired data with docking and molecular dynamics provided insight into 3D complexes formed. The obtained results allowed for a through characterization of the ligand-protein interactions and could conceivably be used for future drug design.

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Phosphatidylserine lipids affect the location of chondroitin sulfate at the membrane surface

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Chondroitin sulfate (CS) is a negatively charged polysaccharide and a key component of the extracellular matrix [1], found in close proximity to membrane phospholipids. Its overexpression at the surface of cancer cells suggests a potential role as a biomarker [2,3], yet CS–lipid interactions remain poorly characterised [4,5]. In this work, QCM-D, neutron reflectometry, FTIR spectroscopy, and MD simulations were combined to investigate CS interactions with phosphatidylcholine (POPC) bilayers and mixed PC/PS bilayers mimicking the lipid composition of cancer or inflamed cells, where phosphatidylserine (PS) is exposed at the outer leaflet [6].CS adsorption was confirmed on POPC bilayers, consistent with prior studies on DPPC monolayers [5], with MD simulations identifying electrostatic contacts between CS sulfate groups and the choline moiety as the primary stabilising interactions, further supported by FTIR data. In contrast, no CS adsorption was detected on POPC/POPS bilayers, attributed to electrostatic repulsion from the anionic PS headgroups. Comparison with literature data on DPPG monolayers [5] suggests that headgroup identity, rather than net charge alone, governs CS–membrane association.To our knowledge, this is the first study probing CS–bilayer interactions in a physiologically relevant PC/PS mixture, offering mechanistic insight into how membrane lipid composition modulates extracellular matrix organisation at the cell surface.

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Integrating Compositional Analysis and Machine Learning for Dietary Fibre Estimation in Plant Products

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Fruits and vegetables are major sources of dietary fibre. To support sustainable nutrition, pectin, cellulose, and total dietary fibre were quantified in fifty fruit and vegetable species from market, and the dataset was integrated into the FibreApp mobile application for image-based estimation.

Fiber fractions, including cellulose, hemicellulose, lignin, and pectin, were determined using an automatic fiber extractor and a continuous flow analyzer. Supervised machine learning models were trained on >1000 images per species and implemented in a mobile application for Android and iOS.

Significant variability in fibre composition was observed. Citrus fruits were richest in pectin, while lignin was highest in aronia, plums, and grapes. Legumes, mushrooms, and root vegetables were identified as key sources of insoluble fibre. The application demonstrated robust performance under variable conditions, maintaining low misclassification rates and effectively handling multi-class scenarios.

FibreApp integrates experimental data with machine learning to provide a practical, scalable tool for dietary fibre estimation and consumer awareness.

Figure 1. QR codes of FibreApp for iOS and Android.

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Tailoring Cellulose Reactivity: Protection-Driven Regioselective Modification at C2 and C3

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In homogeneous cellulose acylation, the primary C6–OH is usually the most reactive site, which often leads to uneven substitution and limits precise control over material properties. For this reason, regioselective functionalization at C2 and/or C3 remains a key challenge in the design of cellulose-based functional materials.[1]

During the last decade, several strategies have been developed to direct reactivity toward the secondary hydroxyl groups, especially C3, by combining suitable solvent systems with protecting-group chemistry. Common approaches use bulky silyl or trityl-based groups, such as TMS, TBS, or trityl, to temporarily block C6 and one secondary hydroxyl, allowing selective modification of the remaining free position.[2]

This work addresses the development of efficient and controllable methodologies for the chemical modification of cellulose dissolution strategies and the selective introduction of the substituent at the C2 and C3 positions, enabled by temporary protection of the primary hydroxyl group. The complete cellulose derivative characterization was achieved by an array of NMR techniques.

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NMR Insights into Marine Microalgae Polysaccharides for Biomedicine

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In the field of bioregenerative medicine, unicellular marine microalgae are increasingly recognised as sustainable sources of biobased compounds[1]. Among them, coccolithophores such as *Gephyrocapsa huxleyi* produce calcite plates known as coccoliths, characterised by species-specific micro and nanostructures. These mineral elements are embedded within a polysaccharidic matrix that forms an external adhesive coating[2], but the composition is still poorly understood. A major challenge lies in the isolation and chemical characterisation of these coccoliths-associated polysaccharides (CAPs) to elucidate their functional roles and metabolic regulation. CAPs have a predominantly (1→3)-β-D-mannose backbone, different branching patterns, extensive chemical modifications, and a wide variety of monosaccharide components, reflecting remarkable structural heterogeneity[3]. In this context, nuclear magnetic resonance (NMR) spectroscopy represents a key analytical tool, allowing detailed structural elucidation of complex polysaccharides, and offer the possibility of investigating structure-activity relationships by probing CAP–ion interactions and conformational dynamics in solution. Finally, as CAPs are hypothesised to play a key role in guiding crystal growth[4], advanced analytical approaches may support a deeper understanding of their role in biomineralization processes, even considering differences between different species.

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Multi-Scale NMR in Sustainable Polymers Development

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This work presents an integrated NMR approach for the characterization of polymer systems, combining solution-state, solid-state, and Time Domain NMR (TD-NMR). While high-resolution techniques provide detailed chemical structure, TD-NMR offers complementary insights into molecular mobility, hydration, and supramolecular organization, without requiring extensive sample preparation. In the context of sustainable materials development, including bio-based systems such as polysaccharides and cork-derived materials, are particularly interesting. This contribution explores how TD-NMR responses can be correlated with key structural features such as crystallinity, phase heterogeneity, and polymer–water interactions. By integrating information across NMR modalities, this approach aims to provide a coherent, multi-scale understanding of structure–property relationships, highlighting the potential of TD-NMR as a versatile tool for advanced material characterization and future process monitoring applications. *[1,2]*

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Modulating physicochemical and biological properties of polysaccharide bio-based materials by microbial melanin incorporation

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Polysaccharide bio-based materials might show different physicochemical and biological properties depending not only on the carbohydrate structure, but also on the incorporation of bio-active compounds. In this work three different polysaccharides of diverse origins were explored for preparing films, gels and beads in absence or in presence of microbial melanin, biotechnologically produced and purified by *S. nashvillensis*[1].

Chitosan-based films, containing the microbial pigment, exhibited improved UV-shielding capacity, enhanced antioxidant activity, and modified solubility behavior. A marine microbial exopolysaccharide (HYD721), produced by a *Pseudoalteromonas* sp. strain, was used to prepare melanin-containing hydrogels with enhanced protective antimicrobial properties. In addition, alginate beads loaded with microbial melanin demonstrated improved adsorption performance for bioremediation applications.

In all systems, the interactions between the different polysaccharide matrices and microbial melanin were investigated through FT-IR spectroscopy, scanning electron microscopy (SEM), and thermogravimetric analysis (TGA), highlighting the influence of the pigment on the structural and functional properties of the resulting materials.

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Exopolysaccharides synthesised by beneficial *Lactobacillus* strains autochthonous to brine ecosystems

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A variety of polysaccharides is synthesised by beneficial *Lactobacillus* strains autochthonous to fermentation ecosystems [1,2]. The focus here is on their relative abundance in the typical Croatian fermentation product, sauerkraut. In line with principles of circular bioeconomy, we focused on the fermentation by-products and exploited the capacity of *Lactobacillus* strains isolated from brine to produce exopolysaccharides (EPS). More than 100 bacterial isolates were screened and subjected to *in vitro* analysis to diversify the strains targeting those that express or do not express ropy phenotype associated with secreted EPS. Three distinct genetic clusters were identified across selected 19 strains based on hierarchical clustering [3]. EPS-producing strains were identified by 16S RNA sequencing and after optimization of EPS extraction, their further structural and molecular analysis will be performed.

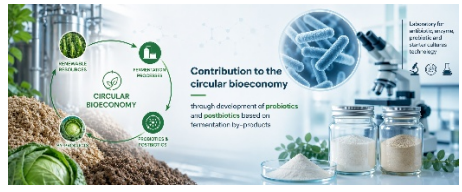

Figure 1. *Exploiting the potential of probiotics and postbiotics from fermentation by-products while respecting the principles of sustainability and circular bioeconomy.*

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Bacterial glycoconjugates as targets for new therapeutics and diagnostics

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Bacterial glycoconjugates play a central role in pathogen–host interactions, environmental adaptation, and bacterial survival. Located on the bacterial cell wall and membranes, these molecules mediate bacterial adhesion and communication with host tissues, contributing to infections, bacterial persistence and adaptation within the host environment.[1–2] In Gram-positive bacteria, their cell wall components are particularly exposed, making them attractive therapeutic targets. Among these, wall teichoic acids (WTAs), anionic glycopolymers covalently linked to the peptidoglycan layer, are involved in cell wall organization, virulence, and host recognition.[1–2] Despite their biological importance, several aspects of WTA-mediated host interactions and biosynthetic pathways remain poorly understood, highlighting their potential as targets for novel antibacterial approaches.[3]

In this project, we focus on the characterization of the molecular mechanisms of bacterial glycoconjugates.[1, 4–5] More specifically, we selected some enzymes involved in WTA biosynthetic pathway in against methicillin-resistant *Staphylococcus aureus*, of particular note for its clinical relevance.[6–7] Starting with TarI, the CTP-transferase responsible for CDP-ribitol synthesis in the WTA pathway,[3] By combining virtual screening of potential inhibitors, molecular dynamics simulations, and NMR studies, we aim to identify innovative strategies to inhibit bacterial growth and contribute to addressing the global challenge of antimicrobial resistance.[8]

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Hydrodynamic characterization via SEC-TDA: a reliable and powerful approach for the characterization of natural, biotechnological, and semi-synthetic polysaccharides

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Polysaccharides are widely employed in pharmaceutical and biomedical applications owing to their biocompatibility, biodegradability, non-toxicity, and tunable physicochemical properties. In this context, a comprehensive characterization of their molecular and hydrodynamic features is essential for understanding and predicting biomaterial performance. Among available analytical techniques, Size Exclusion Chromatography coupled with Triple Detector Array (SEC-TDA) has emerged as a powerful platform for the advanced characterization of natural and semi-synthetic polysaccharides and for establishing predictive structure–property relationships. The potential of SEC-TDA was investigated through complementary studies on hyaluronan (HA), calcium alginate (Ca-Alg) hydrogels, and phosphorylated chondroitin derivatives. Pharma-grade HAs (60–2500 kDa) and alginates with different viscosimetric grades were characterized in terms of molecular weight (Mw) distribution, intrinsic viscosity, hydrodynamic size, and conformational behaviour. In HA formulations, SEC-TDA enabled the correlation of Mw and concentration with viscosity, shear-thinning behaviour, and depolymerization susceptibility, also identifying dilute, semi-dilute, and concentrated regimes[1]. In Ca-Alg hydrogels, increasing Mw and polymer concentration improved microstructural organization, mechanical stiffness, and degradation resistance[2]. Furthermore, SEC-TDA proved effective in monitoring structural changes in phosphorylated chondroitins, revealing variations associated with regioselective phosphorylation and intermolecular crosslinking phenomena[3]. Overall, SEC-TDA represents a robust analytical tool for rational polysaccharide-based biomaterial design.

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Molecular Recognition of Bacterial Glycoconjugates by Host Immune System

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Bacterial surface glycoconjugates are chemically diverse glycan structures that play a key role in microbe detection by the human immune system. Glycoconjugate recognition by pattern recognition receptors (PRRs)[1] and antibodies[2] depend both on the presence of specific carbohydrate motifs, and on linkage, substitution, charge, and conformational behaviour, and conjugation with lipids or peptides. This project studies these interactions at the molecular level, using bacterial glycoconjugates such as wall teichoic acids[3] and peptidoglycan[7] structures as initial model systems. Here, we present our results on the study of two relevant examples of host lectins, macrophage galactose-type lectin (MGL)[4,5] and regenerating islet-derived lectin (RegIII)[6,7], and how they can interact with microbial surface. Biophysical energetic assays, NMR spectroscopy[8], and computational modelling[9] have been employed to study interactions and evaluate ligand receptor behaviour. Integrating computational analysis adds a layer of conformational understanding of how bacterial glycoconjugates interact with the immune system at the molecular level. Results contribute towards a description of how structural and conformational features of bacterial glycoconjugates play a role in recognition by the host immune system.

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CHEMICAL FUNCTIONALIZATION OF POLYSACCHARIDES WITH SMALL MOLECULES: A ^{1}H-NMR SPECTRUM IS NOT ALWAYS ENOUGH FOR CONFIRMING THE DERIVATIZATION

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Chemical functionalization of polysaccharides is a very important area of development in the field of carbohydrate polymers [1], as it allows to modulate and often to enhance the physicochemical properties and/or the (bio)activities of the native polysaccharide, or even to confer them new ones [2]. In particular, the covalent conjugation of polysaccharides with functional small molecules through suitable chemical reactions is a field of growing and growing interest. Indeed, polysaccharide conjugates with drugs and other small biomolecules generally possess low toxicity and are compatible with living tissues under many circumstances, thus conferring significant advantages in their clinical applications [1].

The structural characterization of complex biomacromolecules such as polysaccharides, and even more polysaccharide conjugates, is very often not trivial at all, but it is mandatory for investigating structure-activitity relationships. Nonetheless, the successfull covalent conjugation of small molecules to polysaccharides is very often claimed by just recognizing the expected signals in ^{1}H-NMR spectra for the functional groups of the conjugated species. More robust data are not seldom provided by measuring additional NMR spectra such as ^{13}C- and, less frequently, 1D- and/or 2D-diffusion-ordered (DOSY) spectra. The latter are particularly significant for the structural investigation of polysaccharide conjugates with small molecules, as they generally allow to distinguish between the signals related to slow diffusing macromolecules and to low molecular weight species, that are not covalently linked to the former and therefore are able to give a fast diffusion in solution [3].

In this communication some examples are presented of conjugation of polysaccharides with small molecules, that could have been claimed to be successfull by not only ^{1}H-NMR but also 1D-DOSY analysis and instead are demonstrated to be not (or poorly) working by a much more careful structural characterization through a set of homo- and heteronuclear 2D-NMR techniques. Although these are specific cases within the frame of the structural modification of the polysaccharides of interest for our research lines – *i.e.* the production of glycosaminoglycan (GAG)-like species from non-animal sourced polysaccharides [4] – they are presented here to fuel the discussion about the definition of suitable guidelines for a robust structural determination of derivatized polysaccharides.

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