NMR IN MICROBIAL GLYCOBIOLOGY

MINI SYMPOSIUM

BOOK OF ABSTRACTS

24 JANUARY 2023

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NMR IN MICROBIAL GLYCOBIOLOGY

ORGANISERS:

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PROGRAMME

10:00 - 10:30

Paola Cescutti, Università degli Studi di Trieste

NMR spectroscopy applied to the determination of polysaccharides primary structure

10:30 - 11:00

Barbara Bellich, Università degli Studi di Trieste

Solution-state NMR to investige molecular interactions of polysaccharides and Quorum sensing molecules

11:00 - 11:30

Primož Šket, Slovenian NMR Centre

Insights into bio-macro-molecules in solutions: NMR spectroscopy

11:30 - 12:00

Mojca Kranjc, University of Ljubljana, Biotechnical faculty

Isolation and characterization of extracellular polysaccharides in bacterium Bacillus subtilis

INTRODUCTION

Nuclear magnetic resonance spectroscopy (NMR) or magnetic resonance spectroscopy (MRS), is a powerful technique for the structural analysis of biomolecules. It has also proven to be extremely valuable for studies in microbial glycobiology, especially since microbes continue to surprise us with the variability and diversity of their glycan structures. Understanding these structures can help identify their function, which is important for understanding bacterial physiology. Bacteria commonly grow in biofilms in which they are surrounded by an extracellular matrix that not only provides them with structural support but also offers several functional advantages such as interaction with quorum-sensing molecules that enable bacterial communication and biofilm maturation. The extracellular matrix is composed of various macromolecules, including polysaccharides, nucleic acids and proteins.

The mini-symposium on NMR in microbial glycobiology is organized as the final event of the bilateral project BI-IT-18-20-006: »Mechanism of KKP lectin targeting biofilm of pathogenic and probiotic bacteria«, led by Prof. Dr. Paola Cescutti and Dr. Barbara Bellich from the University of Trieste on the Italian side and the group of the Faculty of Biotechnology with Assoc. Prof. Dr. Anja Klančnik, in collaboration with Dr. Jerica Sabotič from the Jožef Stefan Institute on the Slovenian side.

Prof. Dr. Paola Cescutti and her group mainly study the structure and function of bacterial polysaccharides. Many different bacterial species have been studied for their polysaccharides: Among others, intensive studies were conducted on the opportunistic pathogen of cystic fibrosis, Burkholderia cepacia complex, which revealed the ability of this microorganism to synthesize very different polysaccharides. She also investigated the biological functions of polysaccharides as scavengers of reactive oxygen species and inhibitors of antimicrobial peptides. In recent years, she has collaborated with Dr. Barbara Bellich to study the structures and biological functions of polysaccharides produced by bacteria in biofilms.

INTRODUCTION

Together we have used 1D and 2D NMR spectroscopy to elucidate the structure of wall teichoic acid of Listeria innocua (Barbara Bellich, Nika Janež, Meta Sterniša, Anja Klančnik, Neil Ravenscroft, Roberto Rizzo, Jerica Sabotič and, Paola Cescutti. Characterisation of a new cell wall teichoic acid produced by Listeria innocua ŽM39 and analysis of its biosynthesis genes. Carbohydrate Research 511 (2022) 108499). The joint work in support of the bilateral collaboration was funded under Slovenian Research Agency projects J4-9299, J4-1771, J4-3088, J4-455, J4-4548 and program groups P4-0116, P4-0127 and P4-0432.

This mini-symposium focuses on carbohydrate NMR spectroscopy, which provides invaluable information about the structure and conformation of carbohydrates and is obtained by various types of NMR analyses. NMR spectroscopy is also used to obtain information about the structure and dynamics of DNA or RNA and their different forms. NMR spectroscopy of carbohydrates presents many challenges, of which a particularly important and difficult step is sample preparation. In our experience, NMR spectroscopy is indeed a powerful analytical tool for the structural elucidation of macromolecules, of which microbial polysaccharides offer particularly many surprises and are therefore very interesting to study. The mini-symposium will also strengthen the cross-border cooperation between Slovenia and Italy, thus enabling further research collaborations.

Dr. Jerica Sabotič and dr. Anja Klančnik
Organizers

NMR SPECTROSCOPY APPLIED TO THE DETERMINATION OF POLYSACCHARIDES PRIMARY STRUCTURE

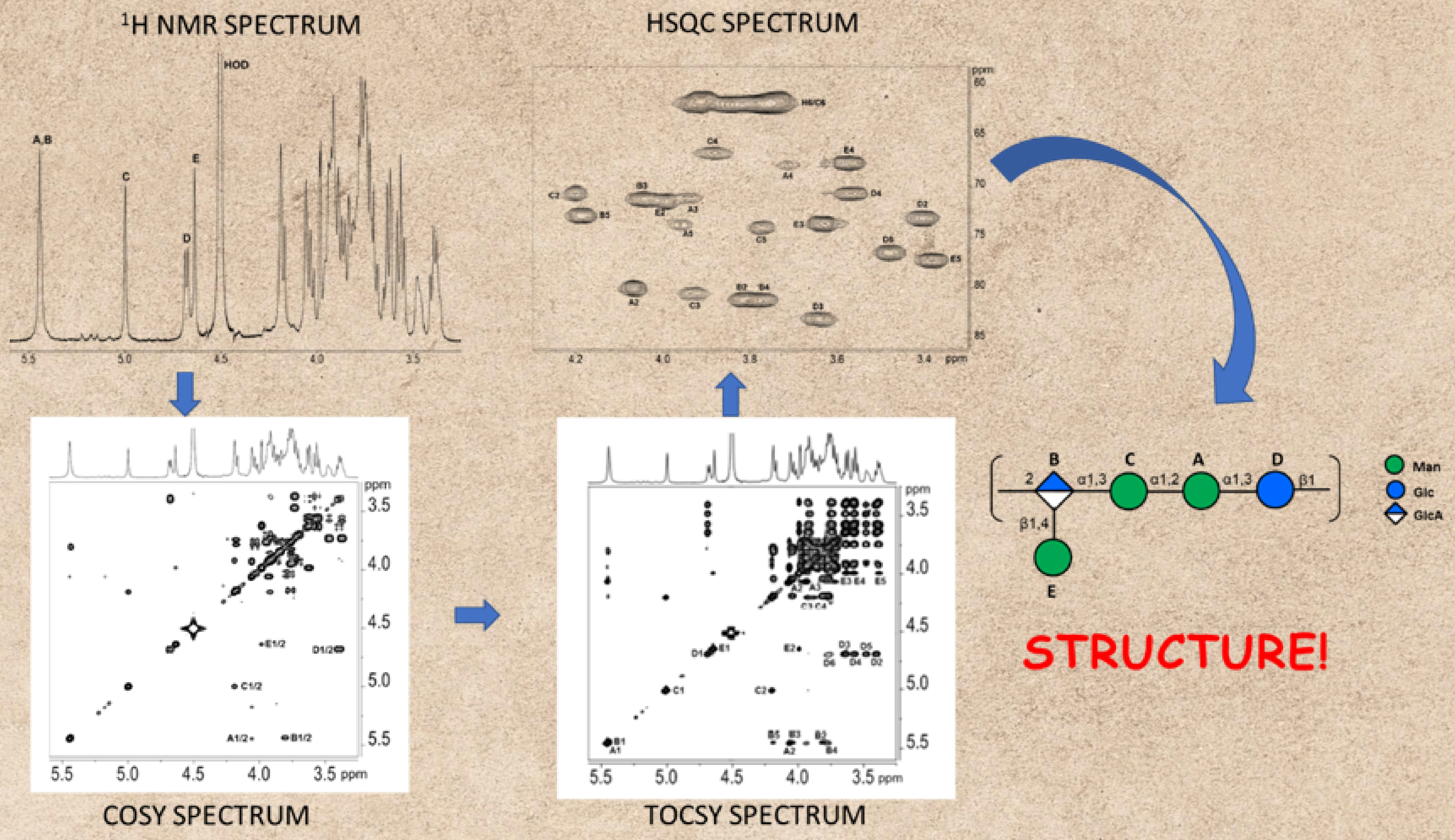
Paola Cescutti

University of Trieste, Department of Life Sciences, Via L Giorgieri 1, Ed. C11, Trieste, Italy

Carbohydrates are widely distributed in nature and have different biological functions: for example, glucose is a major cellular fuel, ribose derivatives constitute the backbone of nucleic acids, cellulose is the most abundant organic substance on this planet, oligosaccharides are key cell recognition antigens in the immune system, and polysaccharides have key roles in the pathogenicity of microorganisms implicated in many human diseases.

Polysaccharides have a high structural diversity deriving from the high variability in monosaccharide composition, anomeric configuration, monosaccharides ring size, linkage position, and sequence. This structural diversity gives rise to the great variance of chemical and biological properties they possess. In nature polysaccharides are found as plant building material, as energy storage or as a protective coating for bacteria, just to name a few examples. In industry, polysaccharides are used in various areas such as food, textile, paper, pharmaceutical, adhesives, and biofuel.

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful analytical tools for the structure elucidation of polysaccharides. The structural characterization is usually achieved through a series of 1H and 13C one-dimensional (1D) as well as two-dimensional (2D) NMR experiments. Sometimes, due to the complexity of the polysaccharide under investigation together with the limited chemical shift dispersion, supporting chemical analysis to establish the composition and glycosidic linkages is needed. Examples of application of NMR spectroscopy for determining polysaccharides structure will be given.



W. A. Bubb, NMR spectroscopy applied to the determination of polysaccharides structure. Concepts in Magnetic Resonance Vol 19A, Issue 1, Pages 1-19, 2003

https://www.youtube.com/watch?v=BU6GJFA1LUk

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SOLUTION-STATE NMR TO INVESTIGATE MOLECULAR INTERACTIONS OF POLYSACCHARIDES AND QUORUM SENSING MOLECULES

<u>Barbara Bellich</u> (a), Roberto Rizzo (a), Ining A. Jou (b), John W. Brady (b), Paola Cescutti (a)

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Bacteria growing in biofilms are surrounded by an extracellular matrix where exopolysaccharides (Epols) are important factors for the structural stability of the biofilm matrix. The biofilm formation represents a virulence trait common to many bacterial species and it has been associated with the persistence of the infections and the increased tolerance to antibiotics.

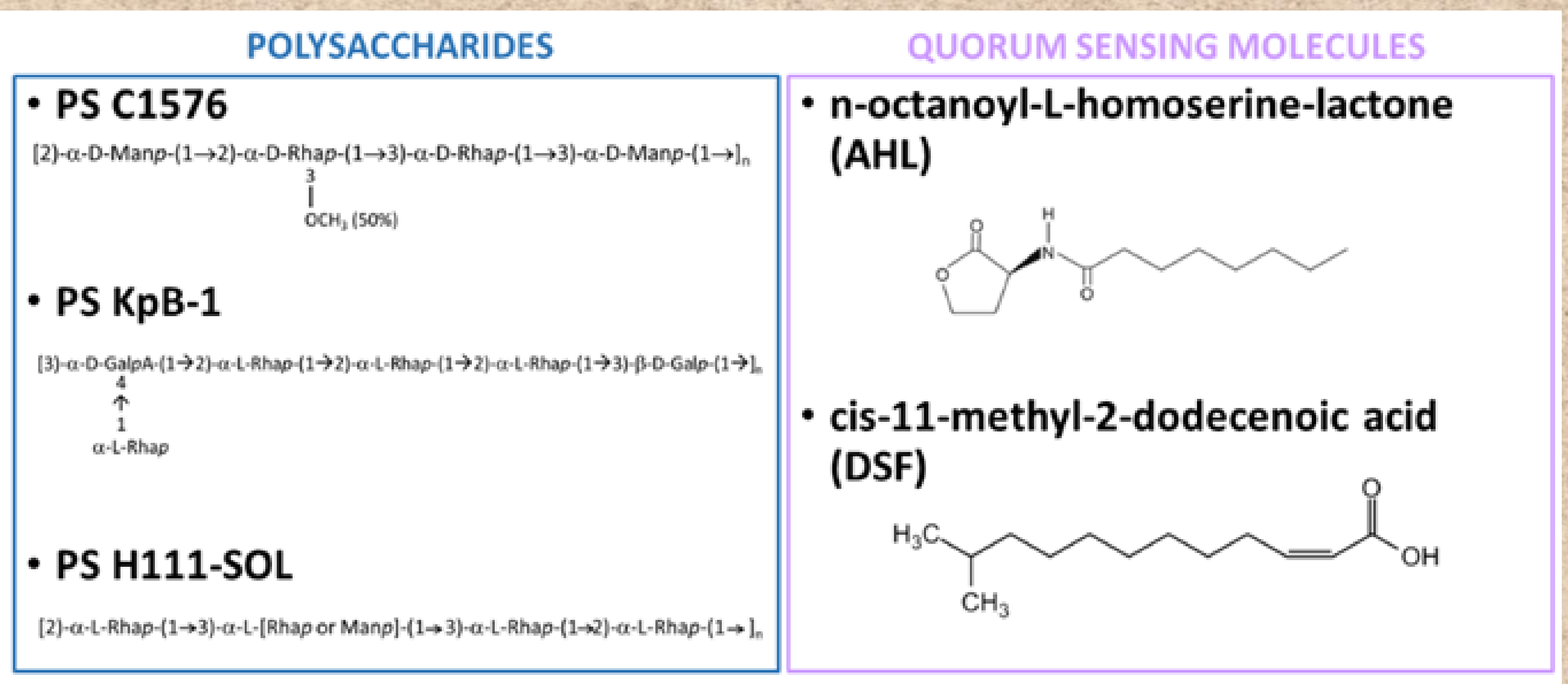
The infections caused by some bacteria of the Burkholderia cepacia complex (Bcc) are difficult or impossible to treat with antibiotics. In particular, Burkholderia cenocepacia and Burkholderia multivorans are the Bcc species most commonly isolated from cystic fibrosis patients.

Besides polysaccharides, small hydrophobic molecules, such as acyl-homoserine lactones (AHLs), are present in the biofilm matrix and they represent a widespread intercellular form of communication, known as Quorum sensing [1]. Such small hydrophobic molecules have a low solubility in aqueous environment, therefore it may be hypothesised that their solubility is somehow enhanced, by interacting with polysaccharides with certain chemical characteristics.

Therefore, we investigated the possibility of interactions at a molecular level between polysaccharides and Quorum sensing molecules (QS mol). Three different polysaccharides extracted from Burkholderia cenocepacia H111, from Burkholderia multivorans C1576 and from Klebsiella pneumoniae KPB-1 were considered. The repeating unit of all polysaccharides is characterized by the presence of rhamnose, which is expected to confer a less polar character to the backbone, if compared to other monosaccharides like glucose or galactose.

The Quorum sensing molecules used for experiments were n-octanoyl-L-homoserine-lactone (AHL) and cis-11-methyl-2-dodecenoic acid, a fatty acid whose activity as signalling molecule is well documented in literature [2].

Solutions of the polysaccharide incubated with the QS mol were investigated by 1H NMR spectroscopy. The peak intensity of specific signals belonging to anomeric protons of the Epols and to the methyl group of the QS mol was considered. The increased intensity of signals belonging to QS mol means that the PS is able to increase the solubility of the QS mol with respect to dextran (taken as control). From this investigation it turned out that the most interacting polysaccharide is the one extracted from Burkholderia cenocepacia H111, while the less interacting resulted to be that extracted from KPB-1.



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[1] Eberl, L. (2006). Quorum sensing in the genus Burkholderia. International Journal of Medical Microbiology, 296(2-3), 103-110.
[2] Marques, C. N., Davies, D. G., & Sauer, K. (2015). Control of biofilms with the fatty acid signaling molecule cis-2-decenoic acid. Pharmaceuticals, 8(4), 816-835.

ISOLATION AND CHARACTERIZATION OF EXTRACELLULAR POLYSACCHARIDES IN BACTERIUM BACILLUS SUBTILIS

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Bacillus subtilis is one of the most studied model systems for biofilms. A crucial component for robust biofilm formation in B. subtilis is extracellular polysaccharide EpsA-O. The EpsA-O is difficult to isolate in large quantities, which prevents its complete chemical and physical characterization. To improve isolation yield of polysaccharide EpsA-O we tested different liquid and solid growth media.

By using high productive EpsA-O mutant strain (ΔsinR) the highest yield was obtained in MSgg growth medium with an increased concentration of glycerol. Through optimization of sonication, pH, and dialysis steps we greatly increased the yield and purity of the EpsA-O polysaccharide.

At the microscopic, macroscopic and biochemical levels, we observed differences between extracellular material of the productive strain and the negative control (ΔepsA-O). Cells differed in shape and size. The structure of the pellet after centrifugation differed in density, hardness and stickiness.

The extracellular material was different in the content of total sugars, proteins and nucleic acids. Using HPSEC analysis, we observed that EpsA-O polysaccharide formed large polymers (Mw > 3000 kDa). The rheological behaviour of purified EpsA-O isolate solutions was markedly pseudoplastic also at very low concentrations.

Comparable to other industrially important polysaccharides, such as guar gum, EpsA-O is a very viscous polymer. It has large intrinsic viscosity, which agrees with HPSEC results. The critical concentration was determined at 0.2 % (w/v). Oscillation tests revealed that the purified polysaccharides are viscoelastic liquids below 1 % and acquire gel structural properties at higher concentrations.

In contrast to HPSEC and intrinsic viscosity measurements, the purified EpsA-O isolate contained high amount of reducing ends (6 %), which would suggest a 16-unit with one reducing end per chain. The structure of EpsA-O, however, remains to be elucidated by NMR spectroscopy.

Figure 1:
Freeze-dried extracellular polysaccharide EpsA-O isolated from B. subtilis culture.

INSIGHTS INTO BIO-MACRO-MOLECULES IN SOLUTIONS: NMR SPECTROSCOPY

Primož Šket

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Slovenian NMR centre (SLONMR) is a national facility and organizational unit within National Institute of Chemistry (NIC) that offers infrastructure and expertise in the field of NMR spectroscopy to scientists and researchers in academic and industrial institutions. NMR spectroscopy is method which allows the study of the correlations between the structure of materials of biological or other origins, as well as explores their dynamics and molecular recognition. NMR is thus a key to gain insight into the biological functions, chemical structure, and interactions of molecules in both the liquid and solids.

For instance, by the use of NMR spectroscopy in liquid state we are studying interactions of anionic ligands with receptors. We can establish conformational preferences of these molecules before and after interaction with various anions. These structural features are essential for further development of anionic ligands used as anionic transporters. Additionally, we are experts in the determination of nucleic acid structures, especially of guanine rich DNA and RNA oligonucleotides and their interactions with ligands, potential new drug molecules in solution. Some of our research is also focused on characterization of protein structures and their dynamics in solution, for instance of prion proteins.

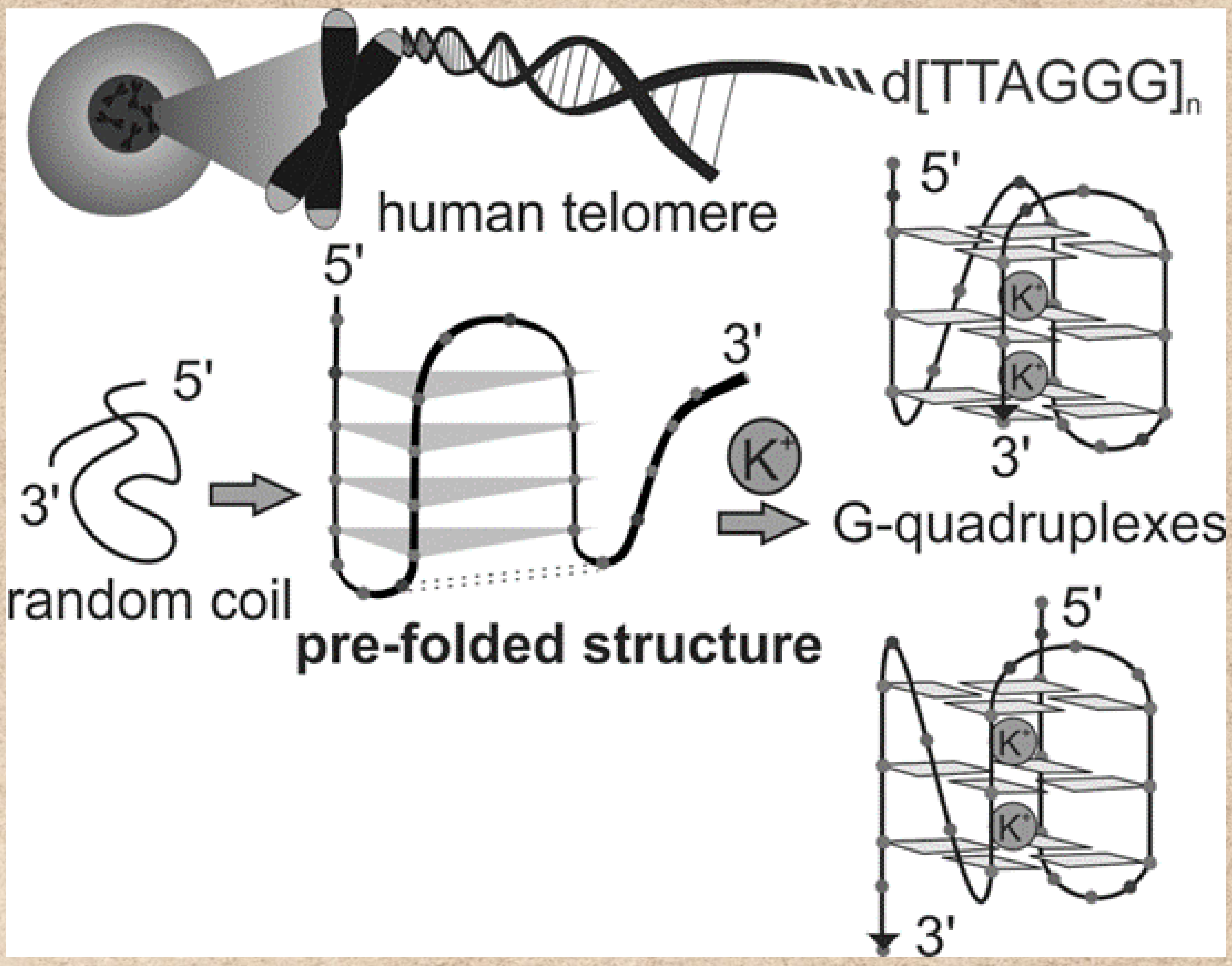

Figure: Studies where we can experimentally characterize structures of pre-folded forms are essential to establish mechanisms of G-quadruplex folding.

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