
Article:
Richter, RP orcid.org/0000-0003-3071-2837 and Lortat-Jacob, H (2018) Editorial overview: Carbohydrates: Ménage à trois with glycosaminoglycans-a serious rendezvous, not a gag! Current Opinion in Structural Biology, 50. iv-vi. ISSN 0959-440X

https://doi.org/10.1016/j.sbi.2018.07.004

Editorial overview: Carbohydrates.
Ménage à trois with glycosaminoglycans - A serious rendezvous, not a gag!

Ralf P. Richter and Hugues Lortat-Jacob

Hugues Lortat-Jacob
Institut de Biologie Structurale, Centre National de la Recherche Scientifique, CEA, Université de Grenoble-Alpes, France
E-mail: Hugues.Lortat-Jacob@ibs.fr

Hugues Lortat-Jacob trained as a biochemist at the University of Paris and completed a PhD at the Pasteur Institute in Lyon (1991). He obtained a research position at the CNRS and is now Research Director at the Institute for Structural Biology in Grenoble (France), where he set up and leads the "SAGAG" (Structure & Activity of GlycosAminoGlycans) team. Using an interdisciplinary combination of structural, analytical and biological approaches, his lab aims at characterizing the binding mechanisms and the structures of complexes involving glycosaminoglycans and cytokines, chemokines or pathogens, which biological functions are investigated, with respect to their implications in inflammatory or infectious disorders. His work led to the engineering of carbohydrate-based molecules targeting the cytokine interferon-gamma and the HIV envelope glycoprotein gp120. His group also contributes to significant methodological developments, including microarray screening of GAG-derived libraries, kinetic analysis, GAG sequencing approaches and the generation of isotopically labelled GAGs for NMR based structural analysis. His recent interest focuses on the heparin sulfate biosynthetic machinery.

Ralf P Richter
Associate Professor in Physical Chemistry of Biological Systems,
School of Biomedical Sciences, Faculty of Biological Sciences,
School of Physics and Astronomy, Faculty of Mathematics and Physical Sciences, and
Astbury Centre for Structural Molecular Biology,
University of Leeds,
Leeds LS2 9JT, UK
E-mail: r.richter@leeds.ac.uk

Ralf Richter trained as a physicist at the Universities of Marburg (Germany) and Gothenburg (Sweden) before completing his PhD in chemistry at the University of Bordeaux (France) in 2004. He worked as postdoctoral fellow at the University of Heidelberg (Germany), as ERC funded research group leader at CIC biomaGUNE (Spain), and also as Chair of Excellence in Grenoble. In 2016, Ralf moved to Leeds (UK) where he is now associate professor. His lab exploits physics and chemistry tools to resolve the principles that underpin the self-organization, structure and function of glycan-rich extracellular matrices and other soft biological structures.

Glycosaminoglycans (GAGs) are evolutionarily ancient and essential components of the cell surface and extracellular matrices. This family of long, unbranched and complex polysaccharides appeared concomitant with the metazoan phyla and is (perhaps not surprisingly considering its origin date and tissue location) vital for inter-cellular communication. It includes glycans such as heparan sulfate (HS) and chondroitin sulfate (CS) that are bound to core proteins to form proteoglycans along with hyaluronan (HA) which lacks a core protein. In recognition of their importance in a steadily increasing number of key biological mechanisms, this Carbohydrate issue of *Current Opinion in Structural Biology* focuses on GAGs.

The first member of the GAG family, heparin, was discovered almost exactly 100 years ago. This molecule, whose glycan nature was only recognized much later, exerts its main biological activity (anticoagulation) by promoting the interaction of proteins (thrombin and antithrombin), a mode of action that, as we know now, characterizes virtually all GAGs.

What do GAGs do? In a broad sense, they can be considered the 'matchmakers' of proteins. They provide the space in which extracellular signaling proteins such as cytokines, growth factors and morphogens diffuse and reside. They promote the encounter between proteins, hundreds of which have been identified, and thereby control the formation of complexes between signaling proteins and receptors in both time and space. GAGs become an intimate part of the resulting complex which carries the function, and thus effectively engage in a 'ménage à trois'. Such interactions, which control protein availability, stability, structure and reactivity, and can lead to specific processing, are at the heart of the regulation of most major biological systems. Their intrinsic structural disorder and high degree of conformational flexibility along with their tremendous sequences variability are critical to enable dynamic and new modes of interaction, permitting robust responses in plastic environments.

The connection between GAGs and structural biology is thus not quite as obvious as it is with other classes of biomolecules. Their interactions with a plethora of structurally unrelated proteins are also characterized by a certain fuzziness. In this issue, we have therefore considered the field in a broader sense than usual, and included concepts and methods that are unconventional in structural biology.

The main questions that have guided the becoming of this issue reflect views on two distinct scales. On the molecular level: What is glycan structure and how is it 'encoded' – by the sequence, by the dynamics, or by the adoption of a more defined structure upon binding to its protein ligands? On the supramolecular level: How do glycans organize proteins, and *vice versa*, and how does this lead to the formation and dynamic remodeling of extracellular matrix as an essential player in cell-cell and cell-matrix communication?

In their paper, Richter, Baranova, Day and Kwok apply soft-matter physics concepts to investigate how GAGs self-organize within extracellular matrices and better understand how their interactions with proteins contribute to matrix assembly and structuration. They provide evidence that immobilized GAGs can form mechanically soft and swollen brushes that can be cross-linked by binding proteins. Cross-linking induces microphase separation and local compaction of the matrix resulting in unique matrix architectures. Using this approach, they give

a rational explanation on how perineuronal nets may form: specialized matrix structures that enwrap neurons and feature discontinuity, providing holes for synapse formation and stabilization, and signal transmission.

Mourão, Vilanova and Soares review how GAG structure can be investigated, at the atomic level, through NMR spectroscopy, a methodology that is becoming increasingly important in the structural analysis of sulfated glycans. Using fucoidan, a highly heterogeneous GAG-like polymer of sulfated fucose from marine invertebrates, the authors described how NMR-based analyses can reveal the chemical structure (identity and linkages of the buiding blocks, sulfation pattern), the conformation and the dynamics of these molecules. This is illustrated with polysaccharides composed of regular repetitive fucose units with sulfation patterns differing in a species-specific manner, which play a key role in the acrosome reaction of sea urchins and with fucosylated chondroitin sulfates composed of a vertebrate-like chondroitin sulfate decorated with species-specific fucose branches such as in the cell-wall of sea-cucumbers.

Compagnon Schindler, Renois-Predelus and Daniel, review selected aspects of mass spectrometry based approaches, applied to GAG structural analysis and sequencing. They describe, in particular, alternative fragmentation techniques to the well-established collision induced or electron-based methods such as laser induced ion photo-fragmentation in the UV and IR domains. They also introduce a different approach, integrating ion mobility spectrometry or ion spectroscopy, whereby molecular structures are directly analyzed, with mass spectrometry and detail how this can be exploited to elucidate GAG oligosaccharide sulfation patterns.

Almond examines the current theoretical methods used to investigate glycosaminoglycan structure, dynamics and interactions, from the monosaccharide to the macromolecular scale. He highlights how computational modeling techniques based on quantum mechanics, molecular mechanics, molecular dynamics, coarse graining and docking provide an understanding of GAG structure and dynamics across multiple scales.

In their opinion article, Kjellen and Lindahl examine how proteins recognize these characteristics and bind GAGs. They highlight that binding affinity and specificity are determined by the charge distribution but also by the dynamics and conformation of GAG chains. Interactions may be nonspecific, essentially reflecting charge density, or highly specific, dependent on rare GAG structural features. Yet other GAG epitopes bind protein ligands with intermediate specificity and variable affinity. Studies of heparan sulfate biosynthesis point to stochastic but strictly regulated, cell-specific polymer modification. Together, these features allow for graded modulation of protein functional response where the functional optimum is not maximal binding but the tissue-specific modulation of protein function.

Sankaranarayanan, Nagarajan and Desai expand on this question and review the computational methods available to address how and to what extent the GAG sequence defines the specificity of protein binding. With a focus to help non-computational researchers undertake computational studies, they explore how one can analyze the degree of specificity – from highly specific to essentially non-specific – in a given GAG–protein system, and how emerging tools can

address structural biology of higher order GAG–protein complexes and the design of GAG-based drugs.

Experimentally, the lack of structurally defined GAG derived oligosaccharides has long hampered the reliable investigation of how GAG sequences contribute to protein binding specificity. Xu, Arnold and Liu detail novel chemoenzymatic approaches to produce homogeneous and defined HS materials whose progressively expanded structural coverage, in conjunction with micro-array analyses, enable to highlight the relationship between structure and biological activity.

Similarly, Zulueta, Chyan and Hung show that chemical synthesis also provides libraries of structurally pure HS oligosaccharides, including sequences that are considered to be inaccessible by natural enzymatic means. Using fibroblast growth factors and heparin-binding hemagglutinin from *Mycobacterium tuberculosis* as examples, they detail how such libraries, in conjunction with binding, X-ray and NMR based studies help to identify the structural determinants involved in protein-GAG recognition as well as the relative contributions of hydrogen bonding, electrostatic and hydrophobic interactions.

Finally, to close this GAG rendezvous, Townley and Bülow provide examples of how these interactions are functionally key to biological systems. Their review focuses on genetic approaches to characterize GAG motifs and their function in defined signalling pathways during development. They highlight that specific GAG sequences can function in defined cellular contexts by modulating protein-protein interactions, where a given protein can interact with different GAG motifs in different contexts. They also discuss a new coding approach for GAGs that would enable computational analyses of GAG sequences such as alignments and position weight matrices to describe GAG motifs.

Multicellular life would not be possible without GAGs. We have already come some way to understand how GAGs make proteins meet and cells communicate. More often than not, communication requires subtleness, and on the molecular scale this is certainly where GAGs excel. We hope that new tools and approaches, such as those reviewed here, along with a fresh look on these old molecules, will be of use to further resolve and exploit how they can do it.