PREFACE

Awareness of the biological significance of glycans and glycoconjugates continues to grow by leaps and bounds (Varki, 2017). However, the chemical complexity of these biomolecules presents major obstacles to deciphering the overarching rules and cryptic codes that are programmed into their macromolecular structures. Unlike nucleic acids and proteins, glycans are linear and branched biopolymers, and there is a major expansion of structural diversity due to the far greater variation in the regio- and stereochemistry of the linkages that establish the glycopolymer architecture. The functional group reactivity of carbohydrate monomers is also considerably more difficult to differentiate, making the development of methods for building glycopolymers through chemical synthesis and for selectively labeling them for analysis a daunting task. Another fundamental difference is that in nature many glycans are assembled using a "one enzyme–one linkage" strategy, and thus, assembly using enzyme-based strategies can require a considerable foundation of knowledge and reagents. Finally, glycopolymers isolated from nature are commonly highly heterogeneous as their biosynthesis is not template driven and lacks mechanisms to ensure production of unique products. This characteristic adds a major hindrance to structure–function analyses. Nevertheless, the widespread implications of glycans in fundamental biology and in human health and disease make glycobiology research into the structures, actions, and interactions of complex carbohydrates, in areas far beyond the venue of primary metabolism, a demanding, fascinating, and rewarding adventure!

I am delighted to present two volumes of *Methods in Enzymology* in the theme of Chemical Glycobiology. Taking its cue from chemical biology in general, the strategies and methods presented bring approaches from diverse fields of biochemistry, biophysics, bioinformatics, organic chemistry, and engineering to bear on glycans and their myriad functions. As you will see, gaining traction in glycobiology-related research demands synergistic approaches and the volumes expertly illustrate the enormous range of methods and the types of knowledge that can be derived. The chapters showcase these approaches in the context of focused biological studies and I am confident that the delineation of these methods will inspire researchers to apply the presented strategies to their own unique challenges in glycobiology.

Volumes 597 and 598 encompass two interrelated areas: "Chemical Glycobiology Part A: Synthesis, Manipulation, and Applications of Glycans" and "Chemical Glycobiology Part B: Monitoring Glycans and Their Interactions."

The central theme of Volume 597 concerns enzymes that assemble and process glycans and glycoconjugates. Genes encoding these enzymes comprise approximately 5% of an organism's genome (Henrissat, Suriola, & Stanley, 2009), so navigating through the multitudes of glycosidases, glycosyltransferase, and glycan-modifying enzymes that ultimately control the structures of naturally occurring glycans is not an easy task. In the first chapter, Withers describes the function-driven discovery of novel glycosidases from environmental DNA samples representing uncultivatable microorganisms. To complement this, next Kwan presents the application of structure-driven directed evolution to tailor the activity of known glycosidases toward important technological applications, such as processing cell-based blood group antigens. Glycosylation impacts protein structure, function, and interactions in many ways, and the next chapters highlight the application of different approaches for glycosylating peptides and proteins. DeLisa enlists powerful engineering techniques to exploit the advantageous properties of bacterial single-subunit oligosaccharyl transferases for glycoconjugate formation. In contrast, Krauss applies a combination of mRNA display and bioorthogonal labeling with high-mannose glycans to evolve neoglycoconjugate peptides that bind to the HIV broadly neutralizing antibody 2G12. These types of semisynthetic glycopeptides are powerful probes, capturing the phenomenon of multivalency common in glycan epitopes and their potential as candidates in vaccine development.

It is only with molecularly well-defined substrates in hand that progress on understanding biological activity can really be made. As such, synthesis, through both chemical and enzymatic approaches, is critical in glycobiology. The technical challenges here start at the most basic level; even simple carbohydrate building blocks may not be readily available. This limitation is particularly acute in prokaryotes where the diversity of carbohydrate monomers is quite astounding. In Chapters 5 and 6, Imperiali and the team of Gilbert, Schoenhofen, and Young describe the chemoenzymatic synthesis and utilization of prokaryote-specific nucleotide-activated carbohydrates, including derivatives of bacillosamine and legionaminic acid. In related efforts, Field next shows how sugar nucleotides can be accurately profiled, providing further insight into the cellular utilization of activated carbohydrate monomers. Together, these efforts pave a path forward toward understanding the significance of highly modified carbohydrates in bacterial pathogenesis. Complex glycoconjugates, such as gangliosides, can also be prepared and fluorescently labeled via total chemical synthesis, described by Ando and Kiso. Various modified gangliosides prepared by synthesis are employed in imaging studies on lipid rafts described in Volume 598. In the final chapter of this section, Wang demonstrates approaches for remodeling glycoproteins chemoenzymatically to install unique glycans, thereby circumventing the undesirable heterogeneity of naturally-produced therapeutic glycoprotein biologics.

Glycoconjugates feature prominently on cell surfaces and have emerged as intriguing candidates for vaccine development. Recently, there has been important progress in experimental approaches for defining and developing suitable scaffolds for presentation of glycan epitopes as antigens. Importantly, scaffolds should take into account multivalency effects, which are central to the avidity of glycan interactions. As an example of these technological advances, Feldman describes how outer membrane vesicles from nonpathogenic *E. coli* expressing virulence-associated glycoconjugates from pathogens can be employed in animal models of infection and Seeberger designs and develops synthetic glycan microarrays that are exploited to great advantage in the identification of minimal B cell epitopes to be used as antigens. Synthetic glycans also feature in the chapter from Davis wherein the glycans reflect epitopes that are part of the conserved core structures of cell-surface glycoconjugates. The agents show promising properties, and sera raised against "internal glycans" show cross-reactivity to unrelated cell-wall glycans from other pathogens. In addition to vaccines aimed at mitigating infectious disease, a growing area of investigation concerns tumor-associated carbohydrate antigens for anticancer vaccine development. In the final chapter of this volume, Huang implements synthetic virus-like particles densely modified with the Tn antigen, to elicit high titers of antiglycan IgG antibodies.

In Volume 598, the focus is on the application of biophysical approaches for monitoring glycans and interrogating their physiologic interactions. In the first chapter, Bewley employs a plethora of biophysical approaches at various levels—ranging from individual oligosaccharides to entire virions—to investigate interactions between high-mannose glycans that decorate the HIV and an algal lectin known as Griffithsin. The approaches described by Bewley are complemented by very different strategies in the following two chapters. Solis shows how microarrays and the quartz crystal microbalance-based technology can be exploited for exploring host receptor recognition of bacterial surface glycans in their native environment. Next, Guo and Turnbull apply fluorescence resonance energy transfer with quantum dot-based assemblies, which present multivalent mannose-type ligands, for investigating multivalent interactions with fluorophore-labeled DC-SIGN C-type lectins. In a departure from investigating cell-surface glycans, Hsieh-Wilson turns our attention to profiling dynamic O-GlcNAc glycosylation of serine and threonine in cytosolic proteins using a bioorthogonal tagging strategy. The O-GlcNAc modification of eukaryotic proteins is an important regulator of many physiological and pathological processes and a relative newcomer in our understanding of the intricate processes of cellular signaling.

Glycan array technology is a cornerstone of glycobiology research. Liu and Feizi, a pioneer in glycan array technology and the developer of neoglycolipids (NGLs) as lipid-linked oligosaccharide probes, reveal how NGL arrays provide valuable insight into glucan polysaccharide recognition, employing minimal amounts of isolated glycan samples and highly accessible technology. In an accompanying chapter, Zeng employs glycomic profiling with a powerful microfluidic lectin barcode system, which enhances the capacity of lectin arrays for focused glycomic profiling. This is illustrated in a chapter reporting highly sensitive assessment of tissue-specific changes in a cancer biomarker.

The final section of Volume 598 exemplifies chemical biology strategies that enable selective imaging of glycans and the enzymes that act upon them. In the context of diseases resulting from defects in glycoconjugate processing, approaches for quantitative fluorescence-based live-cell imaging of glucocerebrosidase activity, which has a causal relationship Gaucher's disease, are described. The Vocadlo and Aerts groups present complementary approaches targeting the cerebrosidases based on fluorogenic substrates and activity-based probes that result in covalent modification. Expanding activity-based probes into the realm of glycosyltransferases, Wagner shows that covalent probes for these targets can also be developed when suitable active-site-targeted ligands are available. These probes label enzymes in cell lysates and will be valuable for profiling disease-related changes in glycosyltransferase expression and activity.

Illustrating the power of collaborative initiatives, fluorescently-labeled gangliosides, presented in Volume 597, are deployed by Suzuki and Kusumi in Volume 598 to reveal domain organization in the plasma membrane by single-molecule imaging. Metabolic labeling of glycoconjugates, through exogenous delivery of carbohydrate precursors, has proven time and again to be extremely valuable in glycobiology, and the final three chapters present recent developments that exploit and enhance this powerful approach.

Wittmann and Zumbusch present an exciting approach that enables simultaneous detection of the location and glycosylation state of proteins in live cells. Chen reveals opportunities for greatly expanding metabolic labeling approaches through the development of cell- and tissue-selective liposome-mediated delivery of glycan precursors. Finally, Bertozzi illustrates the application of fluorogenic trehalose-based probes for the detection of the mycobacterial trehalose glycolipids, which will provide important insight into trehalose mycolate biosynthesis and mycomembrane dynamics in diverse experimental and clinical samples.

It is clear that glycans play a wide range of fundamental roles in all aspects of life in mono- and multicellular organisms, and the essentiality and diversity of carbohydrates in biology are no longer well-kept secrets! The development of these volumes on the theme of chemical glycobiology has provided me with an outstanding opportunity to spotlight the significance and impact of chemical biology approaches on the advancement of glycobiology. Each of the chapters highlights aspects of an exciting frontier where major new insight will certainly be derived from collaborative and synergistic approaches.

In conclusion, I would like to take this opportunity to thank the authors for their valuable contributions and for detailing the creative and powerful approaches that are presented in these two volumes. I am also grateful to the editorial and production team of *Methods in Enzymology* for their valuable assistance.

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