CellPress

Cell
Leading Edge

Voices

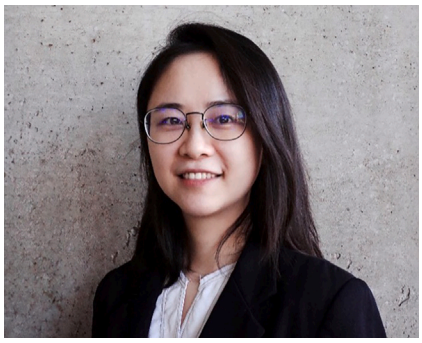
What comes next in glycobiology

Glycans, with their variable compositions and highly dynamic conformations, vastly expand the heterogeneity of whatever factor or cell they are attached to. These properties make them crucial contributors to biological function and organismal health and also very difficult to study. That may be changing as we look to the future of glycobiology.

Peter H. Seeberger
Max-Planck Institute for Colloids and Interfaces, Germany

The advent of molecular glycobiology

Carbohydrates, the predominant biopolymer on earth by weight, are essential components of all living organisms and important nutrients. Glycans, often as conjugates with proteins and lipids, are involved in essential functions from cell-cell recognition to intracellular sorting processes. Polysaccharide and glycoconjugate biosynthesis is not under direct genetic control but involves a plethora of enzymes that help to synthesize, modify, and degrade glycans. The detailed molecular understanding of polynucleotides and polypeptides, thanks to quick and reliable sequencing and synthesis technologies, is not yet a reality for polysaccharides. Moving in that direction, rapid access to production of defined glycans by automated glycan assembly gives rise to the tools required to tackle fundamental aspects of glycobiology. Establishing structure-function relationships for glycans helps to address immunological and medical questions as the basis for the development of diagnostics, vaccines, and medications. Finally, 50 years after the advent of molecular biology, we are beginning to understand at the molecular level. Many questions await. How are antibodies against glycans on the cell surface of bacteria in the gut involved in autoimmune disease? How does carbon storage in polysaccharides in the ocean work, and what does it mean for climate models? Will completely defined glycoprotein therapeutics revolutionize the pharmaceutical industry? The coming years will see a revolution in glycobiology driven by sequencing and synthesis technologies.



Yun Ge
Shenzhen Bay Laboratory, China

Glycan decoder wanted

Deciphering glycan structure-property relationships is pivotal for advancing glycobiology. However, diverse compositions and intricate linkages increase the heterogeneity of glycans, posing challenges to structural analysis. Current methods to determine complex glycan structures mostly rely on breaking down glycan complexes into fragments for mass spectrum analysis. While recent advances such as low-temperature scanning tunneling microscopy enable direct glycoconjugate imaging, there's a need for multidisciplinary approaches for *in situ* high-throughput glycan decoding with spatiotemporal resolution and low cost.

One potential solution is translating glycan information into amplifiable, sequenceable DNA code. Glycan binder-DNA conjugates could theoretically decode glycan-encoded information *in situ*, enabling further joint profiling with DNA, RNA, and proteins for a panoramic view of structure-function relationships. However, unlike proteins recognized by specific antibodies, glycans lack high specificity and affinity recognition agents, hindering accurate and high-throughput decoding. Looking to the future, there are opportunities to design glycan binders with specificity and affinity. Innovative approaches like *de novo* protein design or targeted evolution of numerous glycosyltransferases and glycosidases into new binders show promise in providing tools that offer greater specificity and affinity than lectins. An expanded glycan recognition toolkit will lay the foundation for selective glycan editing in complex biological contexts, advancing our understanding and application of glycobiology.

Christine M. Szymanski
University of Georgia, USA

Unraveling the microbial glycan coat

Trillions of bacteria surround us, yet few have been identified, and even fewer have been cultured. Consequently, we know comparatively little about the dense barrier of diverse polysaccharides enveloping each microbe. Structural elucidation of these complex carbohydrates presents challenges, including the need to separate homogeneous glycans in quantities adequate for characterization by mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy. MS does well in detecting known glycans, but many microbial carbohydrates are unique, precluding the routine use of bioinformatics algorithms and artificial intelligence for structure prediction. Although complementary omics approaches play supportive roles, NMR is often the only technique of choice to confidently identify individual atoms and how they are linked, creating a bottleneck owing to the requirement for orders of magnitude more material than is often attainable.

Fortunately, low-field benchtop NMRs are available to assist even inexperienced scientists in following carbohydrate purifications, and techniques such as high-resolution magic-angle spinning NMR enable visualization of abundant and flexible polysaccharides on intact bacterial cells. NMR sensitivity and spectral resolution have improved significantly through the use of cryogenic probes, metabolic labeling, dynamic nuclear polarization, and magnetic field increases, but further advancements are necessary to not only elucidate the polysaccharide structures individual microbes express when grown under nutrient-rich laboratory conditions but also to understand how these protective barriers change in their naturally unfavorable growth environments and in the context of complex microbial communities.

Daniel Kolarich, Morten Thaysen-Andersen, and Nicolle H. Packer
Institute for Glycomics, Griffith University, Australia; Institute for Glyco-core Research, Nagoya University, Japan; School of Natural Sciences, Macquarie University, Australia

The rise of glycomics

All cell surfaces and the extracellular matrix are comprised of glycosylated molecules such as glycoproteins, glycolipids, and proteoglycans. Together, these glycoconjugates form the *glycome*. The glycome is critical for life across all species. It is structurally diverse and dynamic and can only be understood by studying the actual glycosylated products in a phenotypic context. While the genotype is organism-specific, the individual monosaccharide building blocks and their linkages and arrangements give rise to different glycomes within a single organism that differ substantially depending on function and location.

Today's glycomics technologies shed light on the huge heterogeneity of the glycome and its regulation across complex biological systems. Glycomics knowledge is relevant across all species and environmental conditions. Beyond finding new contexts for the numerous glycoforms of known glycoproteins, comprehensive mapping of the glycome now allows discovery of new glycosylation events not seen before.

Our decadal vision is for the integration of glycomics data and glyco-informatics workflows with knowledge derived from molecular, cell, and structural biology, other multi-omics analyses, and generative AI to give a systems-wide understanding of biology. Such convergent knowledge advances promise to deliver new insights into how glycans control a myriad of functions in biological systems as well as innovative solutions to priority medical, agricultural, and sustainability challenges.

Elisa Fadda
School of Biological Sciences, University of Southampton, UK

Computing solutions in glycoscience

Glycosylation is a phenomenally clever evolutionary strategy whereby the rigidity of a template-driven protein structure is complemented by an ever-changing capacity for functionalization through glycans. Yet, this very talent makes glycobiology an extremely challenging field of research, where these processes are encoded in a rich but still largely mysterious biological language known as the GlycoCode**.** Glycans are not only heterogeneous but also have a highly flexible architecture and thus are invisible to most methods in structural biology. Within this framework, I believe that computing has now reached the technological maturity and sophistication to greatly contribute to advancing our understanding of glycobiology. Through computing, we can rebuild the missing glycans on 3D protein structures and reveal the role of glycans in protein folding, structural stability, and function. Computing will also enable new discoveries through the analysis of large datasets available in open-access glyco-bioinformatics repositories. Ultimately, the power of computing, complemented by ever more advanced experimental techniques and ingenuity, will enable us to decipher the GlycoCode, sparking a step-change in the design and development of high-precision, personalized interventions for human health and innovative glyco-bioengineering strategies to support food security and sustainability in the years to come.

Benjamin Davis
University of Oxford and The Rosalind Franklin Institute, UK

Glucose, mannose … otiose?

Sugar residues are rarely assembled into single polymeric sequences. Dazzling molecular permutations can therefore create the risk of not seeing the wood for the (glycan) trees. I wonder if it is perhaps the heterogeneity of that "wood" that may be the very point? I personally no longer see a direct (glyco)code in these oligo/polysaccharide moieties, and the oft-invoked "non-templated" and metabolic aspects of glycobiology invoke in me a response of "And … ?" along with two linked ideas.

Idea 1: Embrace mixtures. Rather than focusing on dilution of putative sequential information transmitted from gene-to-glycosylation patterns, where mixtures limit current analyses, might we instead posit roles for component patterns in stochastic function? Here, single-molecule methods might simultaneously unpick both composition and its change upon stimulus to enable structure-function hypotheses. Alternatively, the study of nucleic acids as molecular biology finds power from informational amplification; corresponding glyco-amplification—a "sugar PCR"—might vitally correlate structure to function at greater scale and also give confidence in low-level glycoconjugates, both their existence and function, that could surprise us.

Idea 2: Consider tuning. Might (sub)populations of glycosylation patterns create a (tuneable) physicochemical fuzzy logic with distributed fitness? Even at the same attachment site (e.g., Ser/Thr-phosphorylation vs. O-GlcNAcylation), simple analogies in function fail. Glycans instead seemingly provide graduated responses where rigid, arrayed residues perhaps act in a modular, thresholded fashion to provide functions that range from zoonosis to immune response. If true, the enzymologist in me would rejoice at broad control exerted by relatively few kinetic events.

Shoko Nishihara
GaLSIC, Soka University, Japan

Understanding regulation and variation

Recent advances, including exploration of glycan functions in stem cells and the increasing variety of glycan types and expression, suggest two key issues for the future: regulated glycan expression and glycan variations.

Various glycans regulate stem cell pluripotency and differentiation by modulating extracellular signaling as ligand co-receptors/stabilizers and by controlling pluripotent transcription factors. Intriguingly, in the early pluripotent state transition, PRC2, a chromatin-remodeling complex, simultaneously regulates the expression of >40% of genes needed to synthesize these glycans. What else contributes? How is the expression of every functional glycan coordinately regulated during early embryogenesis?

The evolutionary diversity of glycosyltransferases has given rise to numerous species-specific glycans, while individual-specific glycans derived from SNPs in glycosyltransferases provide diversity for pathological processes. Moreover, the amount of cell-surface glycan shows significant variance around the median predicted from gene expression owing to the complexity of glycan synthesis and degradation. Moving forward, glycobiologists will seek to clarify the biological meaning of the vast variation of glycans in evolution and disease.

In exploring these issues, the acquisition and processing of comprehensive arrays of data, such as genomic, transcriptomic, glycomic, and glycoproteomic data, will require bioinformatics, mathematical statistics, and even AI. Therefore, the fusion between wet and *in silico* research will be essential to explore the glycobiology world.

Gabriel A. Rabinovich
Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Biología y Medicina Experimental, and Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

A sweet future for immunotherapy

While the development of immunotherapy has revolutionized cancer treatment, its efficacy remains limited for many patients, underscoring the need for other innovative therapeutic approaches. The growing recognition of glycans' regulatory functions, their altered expression in tumor microenvironments, and their multivalent interactions with glycan-binding proteins have led to the design of novel therapies intended to enhance or reprogram antitumor immune responses. Strategies targeting glycan-mediated pathways aim to activate anti-cancer immune mechanisms by antagonizing the inhibitory effects of Siglecs and galectins, enhancing cancer vaccine immunogenicity, and reprogramming adoptive cell therapies, such as chimeric antigen receptor (CAR)-T cells. However, despite promising results from preclinical studies and clinical trials, several hurdles must be overcome to expedite the translation of these findings to patients, including a lack of specific biomarkers and clinical correlates of therapeutic responses. Integrating glycomics and other omics methods with spatial technologies, high-resolution imaging, and flow cytometry approaches may facilitate the identification of specific lectin-glycan signatures associated with individual tumor ecosystems. Although numerous challenges lie ahead, current evidence suggests a sweet future for cancer immunotherapy. Hopefully, many of these findings will also translate into therapeutic advances for other chronic inflammatory conditions, including autoimmune, neurodegenerative, and cardiovascular diseases.

Peter D. Kwong
Aaron Diamond AIDS Research Center, Columbia University, and Vaccine Research Center, NIH, USA

Overcoming the viral glycan shield

Enveloped viruses hide beneath lipid membranes, leaving only a few antigens exposed, such as the fusion machines they need for entry. These fusion machines are often targeted by neutralizing antibodies, and viruses use *N*-linked glycan to mask them from humoral recognition. Some viral fusion machines, such as those from HIV-1 or Lassa virus, have taken this to an extreme: *N*-linked glycan comprises half their mass, and structures reveal almost complete surface coverage by glycan. Analyses of HIV-1 coevolution, furthermore, indicate an evolving "glycan shield," whereby changes in glycan provide an efficient means to evade neutralizing antibodies.

Glycan shielding, however, isn't all bad. The ability of glycan to immunologically mask proximal surfaces from antibody recognition has spurred immunogen design strategies, whereby the site-specific introduction of glycan is exploited to reduce off-target responses.

Additionally, holes in the glycan shield, corresponding to site-specific removal of glycan, can be exploited to induce hole-localized immune responses. While development of these responses to achieve neutralization of viruses with filled holes has been observed infrequently thus far, excitement in the vaccine field is building around insights into how germinal centers capture and mature responses to highly glycosylated antigens. Notably, specialized vaccination regimens have been discovered that increase immune responses to highly glycosylated antigens, potentially providing a means to overcome glycan shielding and facilitate effective vaccines against HIV-1 and Lassa virus.

Richard Strasser
University of Natural Resources and Life Sciences, Austria

Glycans in plant biology

Protein-linked glycans are often neglected in the functional characterization of proteins, and the biological role of many glycans that are very structurally conserved across plant species remains unknown. Our understanding of protein glycosylation is largely based on studies conducted with the model plant *Arabidopsis thaliana*. However, there are huge differences between plants. For instance, rice lacking complex *N*-glycans exhibits severe developmental defects that are absent in *A. thaliana*. In numerous plant species, there is mounting evidence that particular glycans are crucial for stress responses, such as adaptation to varying temperatures or pathogen defense. While established roles of glycosylation in protein folding, stability, intracellular trafficking, and signaling may contribute, it is probable that we are only scratching the surface of potential functions. More broadly, to gain new insights into the structure-function relationship of glycans in plants, it is crucial to investigate the underlying mechanisms and identify the significant glycans present at each glycosylation site within individual proteins. Performing systematic high-throughput approaches such as glycoproteome analysis in plants under different physiological or stress conditions and developing plant glycan labeling methods to monitor glycosylation changes in cells in real time are essential to address these issues. These methodological advances, in combination with the characterization of the poorly understood plant lectins, will provide novel insights at the organismal and cellular level.

DECLARATION OF INTERESTS

B.G.D. is a shareholder of SugaROx and holds various patents that seek to exploit the function of glycosylation and that would afford him royalties upon exploitation. G.A.R. is co-founder of Galtec SAS and co-inventor on a family of US and European patents associated with therapeutic and diagnostic applications of galectins in cancer and autoimmune and inflammatory conditions. P.H.S. has a significant financial interest in GlycoUniverse GmbH&CoKG (commercializing the automated synthesis technology and services related to it) and Tacalyx GmbH (developing anti-glycan antibodies to treat cancer patients) as well as advisory roles with both companies. He holds a host of patents in the area of synthetic carbohydrates and their use as vaccines and for the generation of therapeutic antibodies.