

Priming the Cellular Glycocalyx for Neural Development

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ABSTRACT: Glycans are important contributors to the development and function of the nervous system with enormous potential as therapeutic targets. However, a general lack of tools for tailoring the presentation of specific glycan structures on the surfaces of cells has left them largely unexplored in the biomedical context. In this Viewpoint, we briefly summarize the distinct challenges and complexities of the Glycome. We also highlight an emerging concept of cell surface engineering using synthetic nanoscale mimetics of native glycoconjugates to harness some of the unique biology of glycans, with an eye toward advancing stem cell-based neuroregenerative therapies.

An intricate canopy of biomolecules rich in carbohydrates, called the glycocalyx, envelopes the surfaces of all cells. There, the carbohydrates, or glycans, are anchored to membrane lipid structures or displayed on protein scaffolds. Glycans are composed of monosaccharides linked together into linear or branched oligo- and polysaccharides, giving rise to a vast diversity of information-dense structures. Glycosylation is a highly dynamic posttranslational modification resulting in distinct glycan patterns generated across different tissues, and even developmental or disease stages. The importance of glycans in human biology cannot be overstated; yet, they are largely understudied. Too frequently, the complexity of the Glycome and the lack of tools to study glycans leave them simply ignored, rendered invisible.

The nervous system offers an exquisite trove of unique glycans, matched by the variety of functions they perform (Figure 1).¹ The roles of cell surface neural glycans can be purely physical. For example, glycans can mask sites on proteins

that are susceptible to cleavage by extracellular matrix (ECM) proteases (Figure 1A). They can also prevent the association of binding partners, as in the case of the neural cell adhesion molecule (NCAM), which can be modified with long chains of polysialic acid (PSA) to hinder homophilic interactions with NCAM molecules on opposing cells (Figure 1B). Alternatively, the glycocalyx also harbors ligands for adhesion proteins that control cellular association, migration, and organization of the nervous system (Figure 1C, D). As well, cells present glycans as signals for receptors on neighboring cells and in the ECM that are involved in the regulation of neural network formation and maintenance, synaptogenesis, or learning (Figure 1E, F).

The inability to trace individual glycan structures to the genetic code makes their manipulation in living organisms challenging. This void has provided exciting new opportunities for the development of chemical strategies that enable the elucidation of mechanisms underlying glycan functions.² Synthetic carbohydrate probes have been indispensable in mapping the binding specificities of key glycan receptors of the nervous system. The metabolic engineering of cell surface glycans either through the elimination of specific structural features using small molecule inhibitors of glycan biosynthesis, or through the incorporation of chemically altered unnatural monosaccharide building blocks into the glycocalyx of cells has fueled the discovery of novel biological roles for glycans. Together, advances in chemical glycobiology, molecular biology, genetics, and modern glycan analysis and sequencing technologies have begun to remove the invisibility cloak surrounding the glycocalyx and its contributions to the formation and function of the nervous system.

An increasing understanding of glycan biology has also exposed additional layers of complexity within the Glycome. It is now well accepted that the three-dimensional presentation of glycans throughout the glycocalyx is just as important as the molecular detail of their structures.³ The typically weak interactions between monovalent glycans and their protein receptors ($K_d \sim 10^{-3}$ – 10^{-6} M) are insufficient to generate a specific biological response and have to be enhanced by their presentation in multivalent displays. This provides an

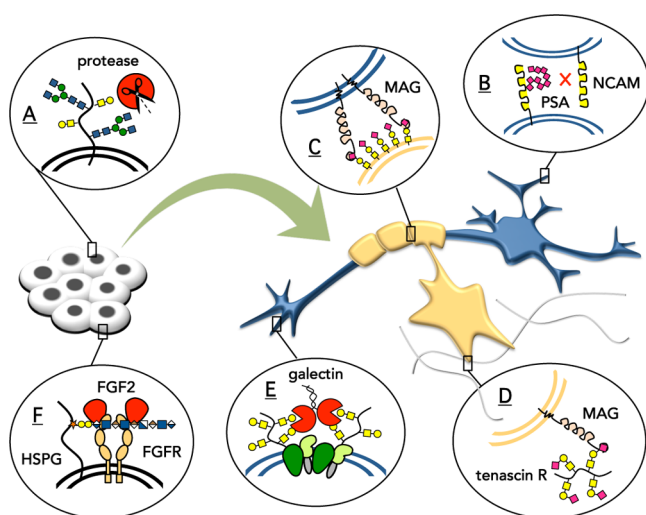


Figure 1. Glycans play key roles in neural development and function. They can have purely protective roles (A) or serve as modulators of cell adhesion and neural network formation (B–D). Glycans also orchestrate the organization of receptor–ligand complexes involved in signaling events (E, F).

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opportunity to fine-tune the biological responses by setting activity thresholds through valency and spatial organization. For instance, the adhesion molecule, myelin associated glycoprotein (MAG), recognizes sialic acids presented on O-linked glycans. These epitopes are frequently found on glycolipids clustered in axonal plasma membranes (Figure 1C). As well, multivalent arrays of sialylated glycans have been found decorating tenascin R, a potential MAG ligand involved in the process of myelination (Figure 1D). Due to the low affinity of the singular glycan-binding domain of MAG for sialic acids, multivalency is required to trigger a recognition event. The resulting interaction reflects the spatial arrangements of the glycans, which can span distances of up to hundreds of nanometers, effectively amounting to nanoscale encoding of information.

The multivalency of glycan display is often mirrored in their binding partners, which can organize into multimeric complexes with more than one glycan-binding site. This offers many modes through which glycan receptors can engage their ligands, giving rise to higher-order associations that provide additional level of sophistication and control over biological processes. For example, glycoproteins can be cross-linked by multimeric lectins, called galectins, into ordered lattices, which either promote or inhibit intracellular signaling events (Figure 1E). Galectin-mediated interactions may be important in axon-axon and axon-glia associations during sensory innervation of the olfactory system.

The ability to organize receptor complexes is not limited to multivalent glycoproteins. Linear glycosaminoglycan polysaccharides (GAGs) bound to proteoglycans (PGs) recruit growth factors to the cell surface and present them to their receptors. For instance, PGs with heparan sulfate (HS) GAGs composed of alternating units of variously sulfated glucosamine and uronic acids orchestrate the formation of complexes between fibroblast growth factors (FGFs) and their receptors (FGFRs, Figure 1F). HSPGs are critical for early neurogenesis in mouse models, where FGF2 signaling is required for the formation of the neuroectoderm, and continue to play a key role in later stages of development by balancing the opposing effects of FGF2 and pleiotrophin on cell proliferation. The sulfated domains in HS span sufficient distances to reach across both FGF2 and FGFR and stabilize their association in a signaling complex. The sulfation patterns of GAGs are believed to encode selectivity for a number of growth factors and neural differentiation is accompanied by changes in HS sulfation.

An emerging strategy to probe the nanoscale encoding of information by glycans is via glycocalyx remodeling (Figure 2). This strategy involves the insertion of synthetic nanoscale

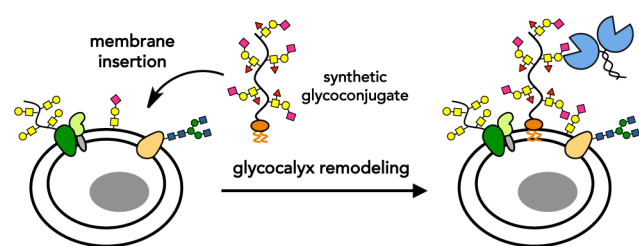


Figure 2. Glycan epitopes of interest in predefined spatial arrangements can be introduced into the plasma membranes of living cells via the insertion of synthetic nanoscale glycoconjugates equipped with a phospholipid anchor. The glycomaterials can be tailored to engage various glycan-binding proteins of interest.

glycomaterials that match the dimensions and approximate the sophistication of native glycoconjugates into the membranes of living cells. This tool has proved useful for scrutinizing and manipulating higher-order glycan interactions that are difficult to analyze via small molecules. An important distinction of this method from existing glycan engineering strategies is that it allows for the addition of specific glycan epitopes in predefined spatial arrangements to the cell surface with minimal perturbations to the existing glycocalyx or other membrane structures. This is difficult to achieve by manipulating glycan biosynthetic pathways, which provides little control over glycan presentation and leads to global alterations to glycosylation patterns through the entire glycocalyx.

Recently, we proposed glycocalyx remodeling as a potential strategy for harnessing cell-surface glycan interactions to influence the outcome of neural differentiation (Figure 3).⁴

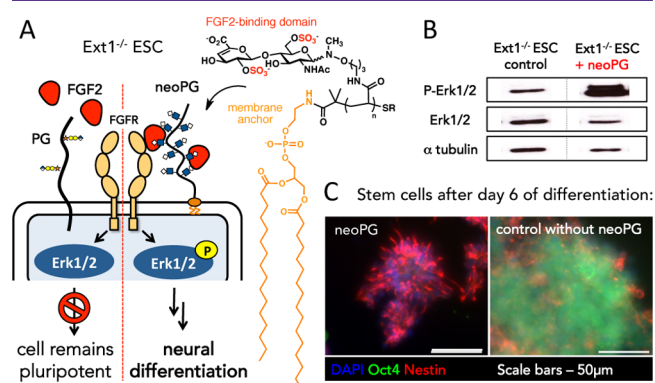


Figure 3. Glycocalyx remodeling strategy for guiding stem cell specification. Neural differentiation in mouse embryonic stem cells deficient in heparan sulfate (HS) biosynthesis (*Ext1*^{-/-} ESCs) can be rescued by the insertion of synthetic HS proteoglycan mimetics with affinity for FGF2 into the cell membrane (A). At the cell surface, the neoproteoglycans (neoPGs) rescued FGF2-mediated Erk1/2 kinase signaling (B) and promoted differentiation into neural rosettes (C).

We envisioned that such a strategy might help overcome some of the challenges that have prevented stem cell replacement therapies for neural repair from becoming a reality. Successful regenerative procedures will require robust methods for the production of large quantities of specialized neural cell types for transplantation. Currently, cells for transplantation are derived, with variable success, by sequential treatment of pluripotent stem cells with cocktails of various growth factors and other supplements to mimic the conditions that occur during embryogenesis. Most growth factors involved in neurogenesis require HS for their function; yet, these glycans are rarely taken into consideration. This is not surprising in the absence of methods for tailoring specific cell-surface GAG structures to optimize growth factor association and signaling.

To demonstrate the viability of this concept, we generated synthetic mimetics of PGs (neoPGs) with affinity for FGF2 (Figure 3A). For the recognition element in our neoPGs, we selected a sulfated disaccharide motif representing the basic building block of HS. We generated a library of HS disaccharides (diGAGs) with various sulfation patterns by depolymerization of HS by bacterial heparinases, which were subsequently conjugated to a polymer scaffold via reactive side chains. This allowed for a rapid generation of a library of neoPG structures, which were screened in a microarray format for binding of FGF2. The highest affinity neoPG was then

equipped with a lipid tail and introduced into the plasma membranes of mouse embryonic stem cells deficient in HS biosynthesis (Ext1^{-/-} ESCs, Figure 3A). The lack of HS leaves the cells unable to signal through FGF2 and, as a consequence, arrested in an undifferentiated state. At the cell surface, the neoPGs assumed the function of native HSPGs, rescued FGF2-mediated signaling (Figure 3B), and induced neural specification (Figure 3C).

The ability of these simple materials to partake in and steer the complex set of events ultimately resulting in neural differentiation opens numerous new opportunities for controlling this process. The ease with which these materials are generated, coupled with the power of the microarray assay to screen them for affinity toward various growth factors of interest, provides a platform for rapidly identifying custom neoPGs that may eventually be able to prime cells for differentiation into specific cell lineages with higher selectivity and efficiency.

Glycocalyx remodeling may also provide a viable strategy for enhancing the repair of neural tissues after injury. In an alternative approach, Hsieh-Wilson et al. showed that chondroitin sulfate polysaccharides conjugated to liposomes could be delivered to the extracellular leaflet of cortical neuron membranes, where they enhanced nerve growth factor-mediated signaling in a sulfate-dependent manner and promoted neural outgrowth.⁵

Other types of surface glycoproteins can be emulated in synthetic glycopolymers. For instance, Bertozzi and co-workers showed that the crowding of glycans along the backbones of synthetic polymers forces their chains into an extended conformation, giving a nanoscale architecture characteristic of mucins, which are densely glycosylated proteins populating the surfaces of epithelial cells and overexpressed in many adenocarcinomas. Glycocalyx remodeling with these mucin mimetics helped uncover a fundamentally new role for mucins in enabling cancer cell survival in soft tissues.⁶ By exerting mechanical forces against the surrounding matrix, the mucin mimetics drove the formation of focal adhesions and facilitated integrin-mediated signaling required for proliferation. It is interesting to note the potential of glycocalyx remodeling to elucidate the functions of mucins in the nervous system. The glycoprotein, α -dystroglycan, expressed by glia and neurons, and implicated in synapse stabilization, carries a central mucin domain glycosylated with branched O-linked glycans. Dysregulation of glycosylation in dystroglycan results in severe CNS malformation and muscular dystrophy, yet the exact roles of the mucin domain and its glycans in this process are still unclear.

Despite the recent progress in uncovering the functions of neural glycans, much is still unknown about their roles in the development, maintenance, and disease of the nervous system. The concept of surface remodeling with nanoscale mimetics of native glycoconjugates to reveal, and harness, the various functions of the glycocalyx is still in its infancy. However, the control over glycan presentation that synthetic nanoscale glycomaterials offer, and the simple and noninvasive delivery of these materials to the cell surface constitutes a powerful new tool for probing glycan biology in a physiologically relevant context. We anticipate that glycocalyx remodeling will develop into a general strategy for exploiting glycan functions to advance cell-based therapeutics for reversing neurodegeneration and open new opportunities for more effective repair the nervous system.

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Notes

The authors declare no competing financial interest.

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