

Review

Carbohydrate amphiphiles for supramolecular biomaterials: Design, self-assembly, and applications

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SUMMARY

Carbohydrate-containing biopolymers such as glycoproteins, proteoglycans, and glycolipids are an incredibly diverse and complex set of natural building blocks used by biological systems to endorse a wide range of molecular functions that are critical to life processes. Simplified synthetic analogs of these polymeric glycoconjugates can capture some of these functions and are increasingly exploited toward the development of supramolecular biomaterials. These carbohydrate amphiphiles can mimic structural aspects through cooperative molecular self-assembly or target distinct signaling pathways through engineered (multivalent) interactions with biological systems. Herein, we discuss the supramolecular principles that regulate the glyceme function(s) and the translation of these in the design of supramolecular biomaterials in which carbohydrates are used as information-rich structural elements with huge potential as therapeutic supramolecular biomaterials.

INTRODUCTION

Carbohydrates are involved in numerous essential processes within the life cycle of cells and organisms—they are a source of energy and important metabolites; they sustain the swelling pressure and mechanical properties of different tissues; and are also responsible for activating cascades of cellular events, ranging from adhesion to differentiation and apoptosis. Their functions are exerted mainly through combinatorial presentation of multiple non-covalent interactions. Because of their structural diversity (Figure 1), carbohydrates can display a vast number of ligand structures with induced fit and cooperativity that render their multivalent interactions specific and selective. The incorporation of carbohydrates in biomedical systems increases the level of molecular complexity, thus giving rise to an exclusive class of bioinformation coding materials that are not accessible using more conventional materials based on peptides, proteins, and other polymers.^{1,2}

To capitalize on multivalency and the structural richness of carbohydrates, supramolecular approaches are better suited than covalent synthesis for several reasons: they introduce a dynamic aspect to the system, offering the possibility of more complex communication with its environment through reversible formation, responsiveness, and adaptability; they can generate larger functional and organized structures that would extend the multivalent interactions over longer distances; and their morphology can be tuned to match the targeted biointerface.^{3,4} However, intermolecular carbohydrate-carbohydrate interactions (CCIs) in simple sugars are not strong enough to generate structured supramolecular scaffolds amenable to

The bigger picture

New concepts that develop supramolecular assemblies from intricate biofunctional carbohydrates are yet to come. Currently, the design of most carbohydrate amphiphiles for supramolecular biomaterials is limited to simple (mono- and di-) saccharides as biofunctional units while the cells are surrounded by complex polysaccharides in their native environment. Moreover, assembly strategies and principles used so far are typically similar to the ones applied for peptide amphiphiles and do not consider specific carbohydrate features such as carbohydrate-carbohydrate interactions. Carbohydrates' structural diversity represented by the chiral and topological abundance, as well as multivalency, is also largely unexplored. Exploitation of this diversity is expected to bring breakthroughs in the field of supramolecular biomaterials, potentially resulting in biomaterials that more closely resemble the key features of the extracellular matrix.

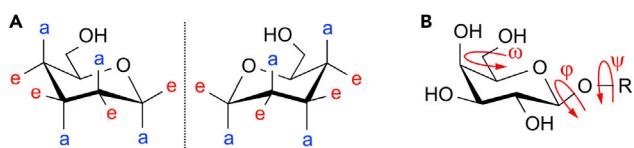


Figure 1. Structural diversity of carbohydrates

(A and B) The structural diversity of carbohydrates is determined by (A) numerous isomers in which each hydroxyl group can be in axial (a) or equatorial (e) position and (B) the induced fit set by the three dihedral angles (ω , ϕ , ψ).

biomedical applications. Thus, diverse approaches have been used to display carbohydrates onto functional biomaterials. In this review, we will discuss different types of carbohydrate amphiphiles and strategies employed in their self-assembly, which can generate a plethora of biomaterials with multivalent presentation of carbohydrates that can be tuned in terms of morphology, function, and bioactivity. Finally, we will highlight some of the recent biological applications of supramolecular carbohydrate systems.

THE ROLE OF MULTIVALENCY IN CARBOHYDRATE BIORECOGNITION

Non-covalent interactions can be up to 100 times weaker than covalent bonds. To compensate for the weakness of these interactions while preserving their reversibility, nature has adopted the concept of multivalency—the ability of a substrate to form multiple individual bonds with a ligand to enhance binding affinity and ensure selectivity and specificity of recognition.⁵ Simultaneously occurring multiple binding events increase the binding contact surface resulting in an interaction that is much stronger and more selective than the individual bonds.

Molecular basis of carbohydrate-binding interactions

Carbohydrates interact with their environment through well-defined signatures determined by their sequence and preferred (induced) three-dimensional conformations. Decades of crystallographic and NMR studies have identified the molecular interactions that govern carbohydrate binding and recognition in biological systems.⁶ Each carbohydrate, ranging from simple monosaccharide to proteoglycans, has a unique imprint that can be recognized by other biomolecules.⁷ The position and arrangement of the functional groups determine the formation of hydrophilic and hydrophobic regions through which polar and non-polar interactions can take place, respectively. Despite the structural diversity of the carbohydrate interactome, the nature of interactions at the molecular level are a conserved set: hydrogen bonding is the main driving force, whereas hydrophobic interactions and metal bridges balance and stabilize the complexes (Figure 2).

Hydrogen bonding

Two types of hydrogen bonding have been identified in carbohydrate interactions: (1) cooperative hydrogen bonds in which the carbohydrate's hydroxyl groups act as both acceptor via the oxygen and donor through the hydrogen (red arrows in Figure 2A1) and (2) bidentate hydrogen bonds (Figure 2A2) in which two adjacent hydroxyl groups of the carbohydrate bind to two different atoms of a planar polar amino-acid side chain (typically aspartate, glutamate, asparagine, glutamine, or arginine).^{8,9} Cooperative hydrogen bonding occurs intramolecularly and/or in CCI, while bidentate hydrogen bonds are mainly involved in carbohydrate-protein interactions (CPIs) in which amide groups of asparagine act as donors and the acidic side chains of aspartate and glutamate as acceptors, thus providing a specific arrangement, selective for adjacent equatorial/equatorial or equatorial/axial

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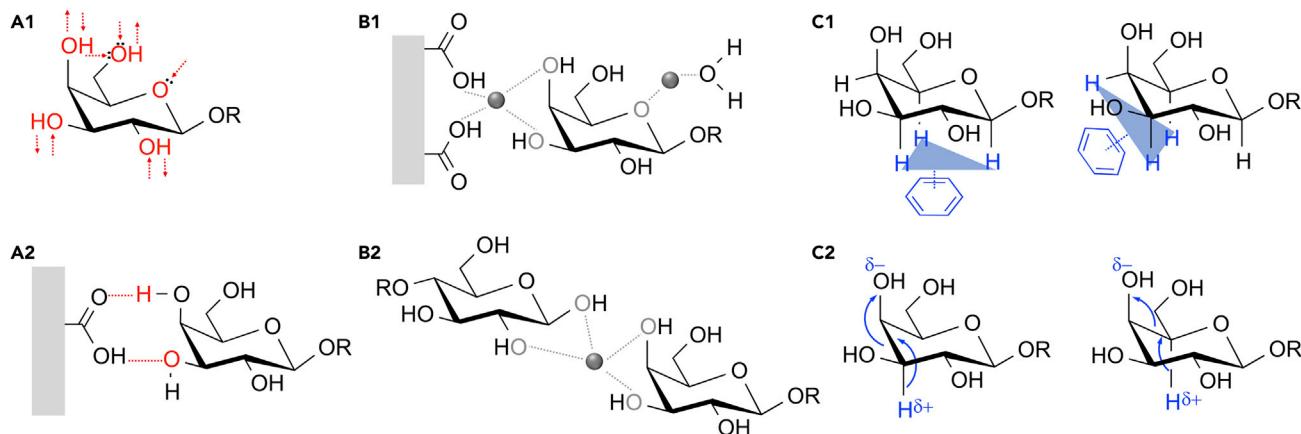


Figure 2. Schematic presentation of non-covalent interactions in which carbohydrates can be engaged

(A) Hydrogen bonding can be either (A1) cooperative or (A2) bidentate.

(B) Complexation with cations, in which (B1) proteins' amino acids, (B2) carbohydrates, and/or (B1) water can also occupy coordination sites.

(C) Hydrophobic CH- π stacking that results from the London dispersion forces and stereoelectronic effects (electron-deficient CH bonds are formed via hyperconjugations, as shown in C2).

hydroxyl groups. (In the biological milieu, water plays an important role in hydrogen bonds networks: the loss of bound water in protein binding pockets at the expense of carbohydrates comes with an entropic cost. For more details on this issue, the reader is referred to Lemieux's work on the role of water in carbohydrate biorecognition.¹⁰)

Interactions with cations

The X-ray crystal structures of some lectins reveal the presence of divalent cations near the carbohydrate-binding site. The metal ions either fix the positions of certain amino acids that interact with the carbohydrate or form bridges between the carbohydrate ligand and the binding pocket (Figure 2B1). Among different metals, calcium ions (Ca^{2+}) are the most common coordination centers with the carbohydrates' hydroxyl groups or the ring oxygen as ligands, and the other coordination sites can be occupied either by amino-acid residues or water molecules (Figure 2B1).¹¹ In CCIs, the coordination sites are occupied by hydroxyl groups or water molecules (Figure 2B2). In complex carbohydrates (e.g., glycoconjugates, glycosaminoglycans), such coordination can result in formation of bridges between different carbohydrate chains—an indispensable feature in cell-cell adhesion, which increases the adhesion force. Cations can also compensate the negative charge of carbohydrates that contain acidic groups. Thus, the concentration of the cations plays a crucial role not only in balancing the adhesive forces versus the charge repulsion but also in modulating the cell surface charge.

Hydrophobic interactions

Despite being highly polar and solvated molecules, carbohydrates can engage in hydrophobic interactions through non-polar regions that are created by the localization of several axial protons on the same face of the carbohydrate ring. Hydrogen bonds provide a structural framework that enables molecules approaching to close proximities where van der Waals forces contribute to the stability of carbohydrate-protein complexes. The additional contribution of CH- π interactions was demonstrated experimentally and theoretically relatively recently.¹²⁻¹⁴ These studies showed that aromatic amino-acid residues can interact with different faces of the carbohydrate ring giving rise to a parallel stacking geometry (Figure 2C1). While

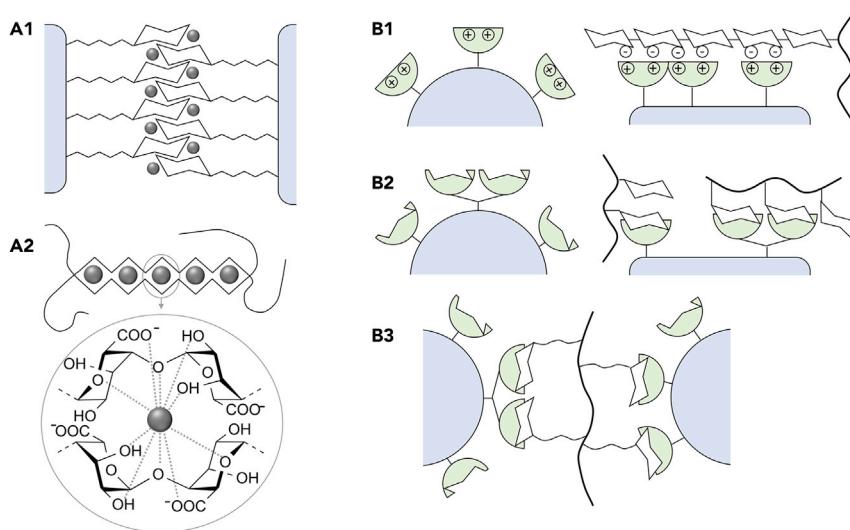


Figure 3. Examples of multivalency

(A and B) (A) Carbohydrate-carbohydrate interactions that take place during (A1) cell-to-cell interactions or (A2) alginate chains in the presence of metal ions (gray) and (B) protein-carbohydrate interactions between: (B1) glycan-binding proteins and glycosaminoglycans; (B2) lectins and glycoconjugates with multivalent carbohydrate presentation can promote lectin clustering; and (B3) multiple lectins and multivalent carbohydrate forming cross-linked nets. Cells are presented in blue, receptors in light green, and the metal ions in dark gray.

London dispersion forces are the main attractive forces of CH- π interactions, the weaker electrostatic interaction between the electron-rich aromatic ring and electron-deficient C-H bonds of the carbohydrate (Figure 2C2) controls the directionality of the bond. A comparison between different aromatic amino acids shows that all four of them, namely, tryptophan, tyrosine, phenylalanine, and histidine, engage in CH- π interactions with carbohydrates but there is a strong preference for tryptophan followed by tyrosine, phenylalanine, and histidine.¹³ This order reflects the electrostatic surface potential and electron-richness of their respective π -systems.

Multivalent carbohydrate-binding modes

A key feature of carbohydrate interactions is the combinatorial impact of the above interactions that must be multiplied and organized in a cooperative fashion to enhance and modulate the strength of binding and render it biologically functional (Figure 3). Of note, multiple simultaneous interactions have unique collective properties that are different than the simple arithmetic sum of the respective monovalent interactions. Multivalent ligands usually bind to their receptors in a sequential manner so that the entropic cost is paid in the initial binding. The entropic barrier of subsequent interactions is therefore lower, resulting in favorable binding.⁵

Multivalent CCIs

In biological systems, CCIs draw attention owing to their involvement in cell-cell adhesion and recognition processes.^{15,16} The weak CCI are advantageous during the first step of cell surface screening and together with their induced fit, as compared with protein-protein interactions (e.g., integrins, cadherins), allow fine-tuning for primary connections between the cells' glycocalyx through the formation of low-affinity and reversible bonds (Figure 3A1).^{16,17} These are then reinforced, if the fit is right, through multiple, simultaneous, and specific interactions, before less reversible processes can take place to form dynamic but stable complexes. In

most biological systems, bivalent ions, particularly Ca^{2+} , promote CCIs by locking the carbohydrate chains in their complementary arrangement to provide an optimal fit between cells and/or by crosslinking the carbohydrate chains from interacting cells. Such cross-links might only be involved as a driving force in the initial cell-to-cell approach or provide additional adhesion forces per binding site, leading to the formation of the so-called carbohydrate zippers (Figure 3A2). The zippers are usually formed by the systematic repetition of membrane proteoglycans at the cell surface and their adhesion strengths are within the piconewton range. While this binding mechanism is considered to be general for CCI, other binding modes may also occur, illustrating the tremendous value of multivalency in creating specificity and modulating binding forces.

Multivalent CPIs

Most of the specific biological roles of carbohydrates are mediated by CPIs. If we exclude the glycan-specific antibodies, the proteins that interact with carbohydrates can be categorized into two major groups: lectins and glycosaminoglycan-binding proteins (GBPs). Their monovalent interactions, i.e., protein binding to a monosaccharide at a single site, are generally weak (K_d in the micromolar range) but the binding affinities can be enhanced dramatically by adopting protein conformations that facilitate multivalent binding. The spatial arrangement of the binding sites can modulate the affinity of the interaction and thus allows selectivity. The carbohydrate density is also important because of the avidity (collective affinity) effect, but too high density can cause steric hindrance and compromise the selectivity.

Various binding modes exist and depend on the protein structure, the nature and complexity of carbohydrate, the environment, and the particular function (Figure 3B). GBPs interact with sulfated glycosaminoglycans via clusters of positively charged amino acids, e.g., Cardin-Weintraub sequences (Figure 3B1). On the other hand, lectins recognize specific carbohydrate termini and bind them into structurally defined pockets (Figures 3B2 and 3B3). Multivalent carbohydrates can either bind to subsites of single or multiple lectins. In the case of single lectins, the subsites are generally clustered to face the carbohydrate, facilitating face-to-face binding through multiple identical interactions. The clustering of receptor subsites increases the valency on the protein side, thereby strengthening the interaction. This phenomenon is known as the “cluster glycoside effect” and the overall interaction can be stronger than the sum of the individual interactions in some cases. Lectins that are involved in signal transduction processes use different binding modes with cross-linked nets between multiple lectins and one or more multivalent glycoconjugates (Figure 3B3). These cross-links create networks of non-covalent interactions that can be either linear, two dimensional, or three dimensional. One-dimensional cross-links result from interactions between bivalent carbohydrates and bivalent lectins, where each carbohydrate site is bound to a different lectin. More complex two- and three-dimensional networks are formed with carbohydrate ligands and proteins with higher valencies, often causing aggregation and precipitation.

DESIGN OF CARBOHYDRATE SELF-ASSEMBLING BLOCKS

The interactions of carbohydrates showcase how cooperative supramolecular multivalent interactions can (1) build specificity through spatial complementarity and (2) increase binding affinity through the multiplication of interaction sites. A fundamental understanding of these molecular features provides opportunities for exploiting multivalency, which illustrates the potential of designed carbohydrate systems for biomedical applications.

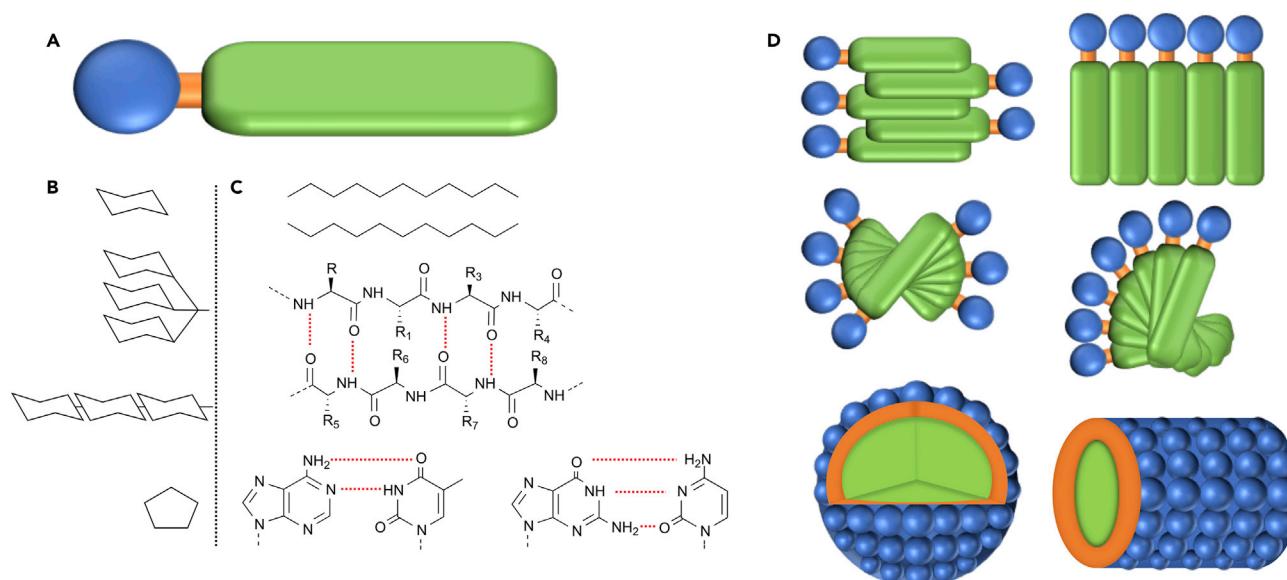


Figure 4. Types of carbohydrate amphiphiles and their self-assembly modes

(A) General structure of carbohydrate amphiphile (carbohydrate moiety, blue; linker, orange; hydrophobic portion, green).
(B and C) The main structural components of natural carbohydrate amphiphiles: (B) the carbohydrate part can be simple six- (e.g., glycosphingolipids) or five- (e.g., nucleotides) carbon monosaccharide, as well as linear (e.g., proteoglycans, mucines) or branched (e.g., glycosphingolipids, glycoproteins) oligosaccharide, and (C) the hydrophobic portion can be made of lipids, proteins, or nucleobases.
(D) Some of the modes by which these amphiphiles assemble.

General principles in the design of self-assembling building blocks

Self-assembly is a bottom-up approach that uses non-covalent (reversible) interactions to generate dynamic multivalent systems.^{18,19} It is ubiquitously used by nature to create complex structures whose biofunctionality is tailored by the generated (nanoscale) morphology and the incorporation and presentation of bioactive moieties. In biological self-assembled systems, carbohydrates are often conjugated to lipids, proteins, or aromatic nitrogenous bases (Figures 4A–4C). Such conjugation imparts the hydrophilic carbohydrates with amphiphilic properties and thus enhances their self-assembling propensity. The diversity of the generated morphologies is striking (Figure 4D) and suggests that synthetic amphiphilic carbohydrate building blocks can be tuned to drive a structure-targeted assembly. Indeed, assembly of diverse synthetic analogs of natural glycoconjugates has shown that the size and structure of the linker^{20–23} and the hydrophobic portion^{22–26} affect the assembly, morphology, and properties of the generated supramolecular systems. The main driving force of the assembly process depends on the structure of the hydrophobic component: CH-π interactions and aromatic stacking prompt the assembly of aromatic amphiphiles,^{24,27,28} hydrogen bonding patterns (including β-stacking) usually underpins the assembly of glycopeptides,^{29,30} and hydrophobic interactions are critical to the assembly of glycolipids.²⁶ Some linkers might enhance the aggregates' stability by participating in supramolecular interactions as well.^{22,24} Finally, the carbohydrate component can also influence the assembly process usually by enhancing the solubility of the amphiphile in water and/or by influencing the morphology and hierarchical order of the self-assembled structures.^{31–33} Additional functionalization of the carbohydrate with polar and charged groups, such as sulfates and phosphates, can be required for some amphiphiles to enhance their solubility.²⁷ Such functionalization can also introduce/change the repulsive Coulombic forces and thus affect the assembly.^{27,34} In supramolecular biomaterials, however,

the carbohydrate is primarily designed in function of a targeted bio-recognition.^{29,30,34–37}

Types of carbohydrate building blocks for self-assembly

Glycopeptides

In biological systems, most proteins are glycosylated, i.e., they are covalently bound to carbohydrates, and form two main classes of bioactive molecules—glycoproteins and proteoglycans. Synthetic glycopeptides emerged as functional mimics of these molecules—they are simpler in composition but recapitulate some of the bioactivity of the natural components upon assembly. The design is usually based on amino-acid sequences with known bioactivity and self-assembly propensity that is functionalized with a monosaccharide, such as mannose,³⁸ glucosamine,^{30,37,39,40} or N-acetylglucosamine,²⁸ to impart biorecognition and/or to change the properties (e.g., self-assembly propensity, interfacial activity) of the peptide assemblies. The carbohydrate can be incorporated via S^{31,41}, N^{37,39,40,42,43}, or O^{29,30,33,38} glycosylation and can also include a linker^{29,38,41,42} between the carbohydrate and the peptide. In the case of very short peptides, e.g., di- and tri-peptides, the balance between solvation and supramolecular interactions might not be sufficient to prompt assembly of stable structures. However, additional modification can be used to create powerful self-assembling motifs. The most used strategies in these cases are to insert additional aromatic segment(s) either as a part of the linker^{29,42} or at the peptide end of the amphiphile.^{37,39,43,44} Such modification introduces additional π-π stacking, enhances the self-assembly propensity of the amphiphile, and can also be used for direct visualization by fluorescence microscopy. The resulting nanoscale assembly of glycopeptides is usually unidirectional, i.e., leading to formation of nanofibers with peptide core and multivalent carbohydrate presentation on the shell—supramolecular structures as minimalistic mimics of native glycoproteins. The carbohydrate density can be tuned by stoichiometry and co-assembly of glycosylated and non-glycosylated peptides to optimize the biointeractions and minimize the steric hindrance. As an alternative to these supramolecular amphiphilic glycopeptides, covalent glycopolymers have also been explored. In particular, polymersomes that mimic cell surface and extracellular vesicles can be generated by assembly of glycopeptide block copolymers.^{45–47} In these supramolecular systems, longer (about 6–8 kDa) carbohydrate chains are exposed on the surface for bio-interactions, thus mimicking proteoglycans. To maintain the amphiphilic character of the copolymer, the peptide portion must also be extended allowing incorporation of longer (17–20 kDa) biofunction-coding sequences.^{46,47} Besides the obvious advantage of mimicking closer the proteoglycans, the design of such long polymers is challenging as it must consider and balance different properties, namely high self-assembling propensity, formation of a targeted morphology, and preservation of the bioactivity of the incorporated elements. The main difficulty is associated with the interconnection between these properties and the inability to change/tailor any of them without affecting the others.

Nucleosides and nucleotides

The formation of double helix by oligomeric nucleic acids represents perhaps the most famous example of supramolecular organization in biological systems. The process is driven by hydrogen bonding between specific Watson-Crick or Hoogsteen pairs of nucleobases. From the molecular design point of view, nucleosides and nucleotides can be considered as short amphiphiles in which a monosaccharide is conjugated to an aromatic nitrogenous base also called as nucleobase (Figure 5A). The nucleobases are N-linked with ribose in RNA or 2-deoxyribose in DNA—a small structural difference that is crucial for the biorecognition of these molecules (e.g., by

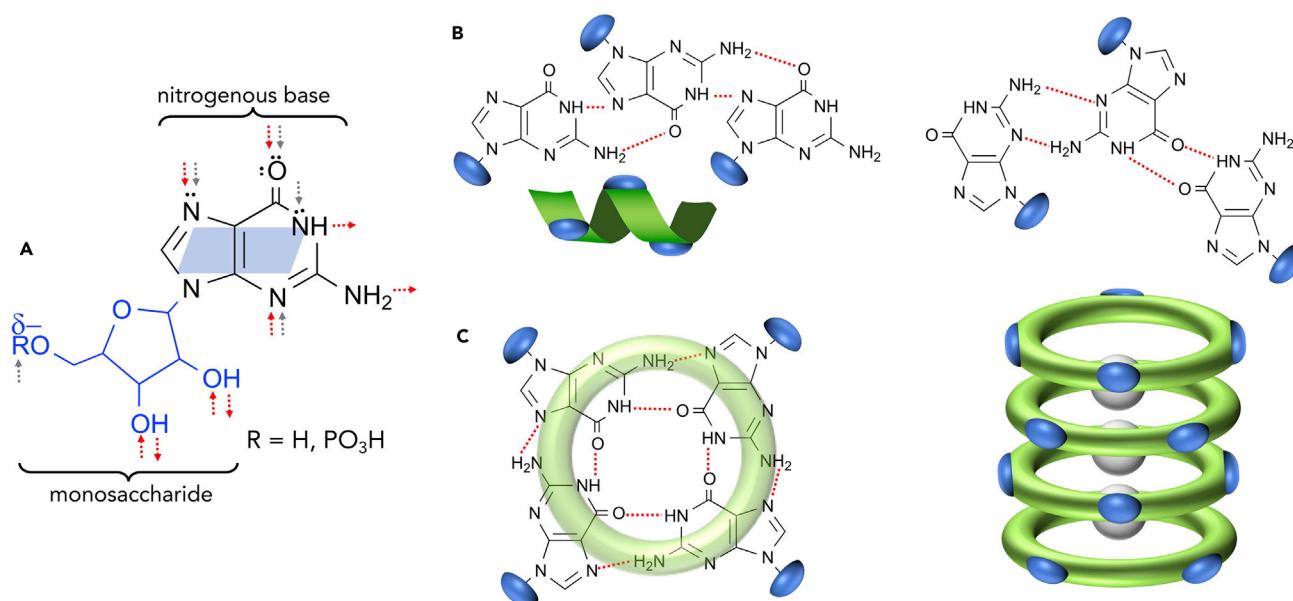


Figure 5. Self-assembly of nucleosides and nucleotides

(A) Supramolecular interactions in which guanosine and its 5' monophosphate nucleotide can participate: hydrogen bonding (red), complexation with metals (gray), π -stacking (light blue), and electrostatic interaction via the phosphate. (B and C) These interactions result in formation of (B) supramolecular ribbons or (C) tetramers (G-quartet) in the presence of metal ions that can further stack in G-quadruplex. In the schematic presentations (B and C) the carbohydrate portion is represented in blue, the nucleobases are represented in green, and the metal ions are represented in gray.

enzymes). Nucleotides can also participate in complexation with metals, π -stacking, and electrostatic interaction via the phosphate group(s) (Figure 5A), which makes them versatile components for synthetic supramolecular biosystems.⁴⁸ Among the five natural nucleosides, guanosine (G) has attracted the attention of the carbohydrate community owing to the recent demonstration that Y RNA can be N-glycosylated at guanosine.⁴⁹ This nucleoside is also of special interest for supramolecular chemists because of its self-complementarity and ability to form ribbons and ordered tetramers (G-quartets) that can further organize into hierarchical structures of higher orders in the presence of metal ions (Figures 5B and 5C). Different metal ions can be used as a trigger of the self-assembly process to generate supramolecular structures with different morphology and gelation properties.^{50–52} Nucleotides can be modified at the pentose or at the nucleic acid to generate a wide variety of amphiphiles.^{53–57} The carbohydrate moieties in these amphiphiles contribute to the assembly process (mainly by hydrogen bonding) but surprisingly, there are no data or reports on their involvement in any specific biorecognition.

Glycolipids

The role of glycolipids in cell membrane structuring and cell-cell interactions has inspired the development of diverse amphiphiles that contain fatty chain(s) as a lipophilic component complementing the hydrophilic carbohydrate(s) (usually mono- or di-saccharide) (Figure 6A). The driving force of their assembly is a microphase separation that results in the formation of discrete lipophilic and hydrophilic regions. While these amphiphiles seem structurally simple, they can form aggregates with various morphologies, such as micelles, vesicles, twisted fibers, helical coils, and nanotubes, among others. To assemble such varieties of adaptive and functional systems, living organisms use fatty acids of different sizes (14 to 24 carbons) and saturations (0 to 6 unsaturated bonds).⁵⁸ Studies with synthetic glycolipids show that

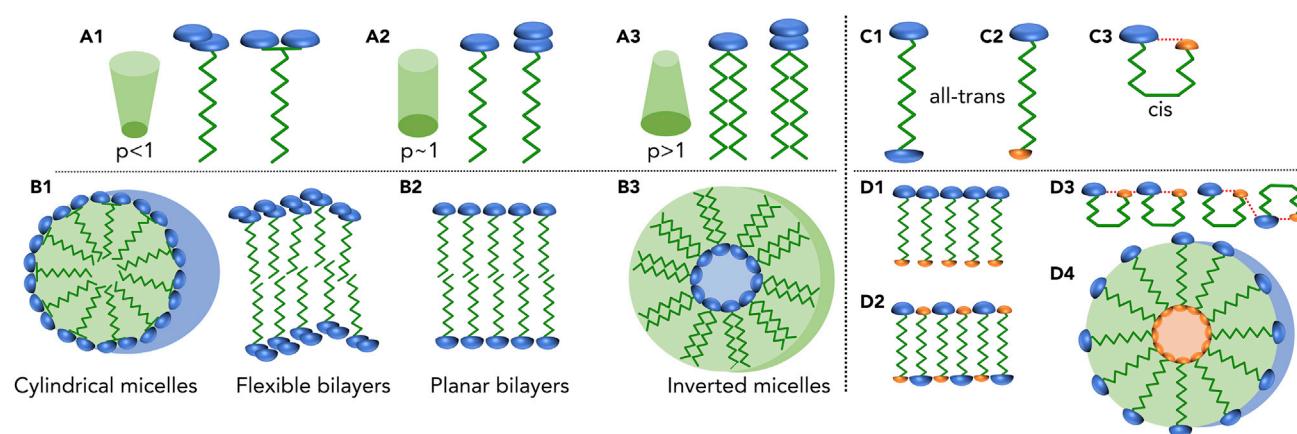


Figure 6. Structure and assembly of glycolipids

(A and B) General structure of (A) glycolipids⁶⁰ and (B) their possible assemblies that can be predicted from the critical packing parameter (p). (C and D) (C) Bolaamphiphiles can be (C1) symmetric and (C2 and C3) asymmetric. The assembly of asymmetric ones is pH sensitive due to $-COOH$ or $-NH_2$ groups (orange) that can participate in (C3) intramolecular and (D) intermolecular hydrogen bonding.

the amphiphilic properties can be tuned by the length of the fatty acids, while their number (one or two) and/or saturation can change the packing parameter and thus affect the morphology of the generated aggregate (Figure 6).^{59–62} The fatty acids saturation is also important for permeability of the generated systems—a crucial parameter in the design of delivery systems. Unsaturated chains assemble in bilayers with enhanced water permeability and this effect is proportional to the number of the unsaturated bonds.^{58,60} When disaccharides are used instead of monosaccharides as a polar component, the structure is stabilized by additional hydrogen bonding between the hydroxyl groups and thus the resulting membrane is less flexible/permeable.⁶¹ The bond between the carbohydrate and the fatty acid (α - or β -) as well as between the monosaccharides (1,4- or 1,6-) also influence the order of the system—a more stable smectic A phase is formed by β -linked glycolipids and 1,4-linked disaccharides, for which the packing parameter is about 1 (Figure 6).⁶¹

Bolaamphiphiles can be considered as a subclass of glycolipids in which both ends of the lipid chain are functionalized with saccharide groups. Bolaamphiphiles can be symmetrical, i.e., bearing the same carbohydrate units at both termini^{53,63} or asymmetrical having a carbohydrate moiety at one end and another polar group (e.g., $-COOH$) at the other.^{64–67} The role of the lipid length and saturation is similar as described above but the presence of a second polar unit (and charged for asymmetrical amphiphiles) extends the assembly configurations, e.g., asymmetric bolaamphiphiles can form monolayers by parallel (Figure 6D1) or antiparallel (Figure 6D2) stacking that can further interact in head-to-head or head-to-tail mode to form bilayers and more complex structures.^{64,65} The asymmetric bolaamphiphiles are of interest for biomaterials development (particularly as delivery systems) because of their pH sensitivity due to the second polar group (usually $-COOH$ or $-NH_2$), which can participate in intra- and intermolecular hydrogen bonding with the carbohydrate (Figures 6C and 6D).^{65,66}

Other carbohydrate amphiphiles

Aside from amphiphiles inspired by natural biomolecules, carbohydrates can be functionalized with synthetic hydrophobic portions structurally tailored to promote an assembly of a targeted morphology. The design of these amphiphiles can include different number of aromatic rings that promote assembly by π - π and/or CH- π

stacking.^{28,36,68,69} Diblock copolymers obtained by functionalization of the carbohydrate reducing end with different hydrophobic polymers is another configuration. The main advantage of these amphiphiles is the possibility for custom design of the hydrophobic portion to include stimuli-responsive (e.g., light, pH) or functional (e.g., conducting, fluorescent) units.^{34,70–73}

SUPRAMOLECULAR APPROACHES TO ASSEMBLE CARBOHYDRATE AMPHIPHILES

Triggers of self-assembly in aqueous media

Self-assembly processes are disorder-to-order transitions that are driven by the balance between repulsive and attractive forces of the involved entities. From a thermodynamic perspective, it can be described by the Gibbs free energy (ΔG , Equation 1):

$$\Delta G = \Delta H - T\Delta S \quad (\text{Equation 1})$$

and occurs only if ΔG is negative, i.e., only if the energy gain associated with entropy (ΔS) decrease is compensated by an enthalpy (ΔH) decrease and below a critical temperature. In aqueous media, ΔG decrease depends on the supramolecular interactions between the amphiphiles but is also controlled by the solvation dynamics and local water organization.^{74,75} Physical (e.g., temperature, light) and chemical (e.g., pH, ionic strength) factors that affect the balance between these interactions can result in different thermodynamic minima corresponding to different supramolecular organizations and biofunctionality. Comprehensive understanding of the role of these factors on the dynamics and timescale of the assembly process is progressing and will allow development of new materials in a predictable/targeted manner.

Temperature

Temperature is the most commonly used physical trigger of laboratory-based self-assembly. In aqueous solutions and below a critical temperature, water molecules structured around the hydrophobic portions prevent the supramolecular interactions between the amphiphile molecules. Heating above the critical temperature induces molecular mobility: the structural water molecules are lost, allowing approximation and induction of supramolecular ordering of the amphiphiles.⁷⁵ Balancing the enhanced disorder in water and induced order in the amphiphiles is important for maintaining the overall positive entropy. The critical temperature depends on the structure of the amphiphile, especially on the ratio between the hydrophobic and hydrophilic portions as demonstrated in the case of glycolipids.^{76,77} Any change in the system that affects this ratio alters the critical temperature. As an example, co-assembly process, in which a second component is added to the system, can be used as a strategy to reduce the critical temperature and also to add functional motifs, i.e., as a multipurpose approach targeting generation of functional diversity under mild conditions.⁷⁸ Application of slow heating-cooling cycles (thermal annealing) can also be applied when non-homogeneous, kinetically trapped arrested phases are formed and can interrupt the assembly of the system toward equilibrium. In this case, the temperature is increased to disassemble the arrested phase and the formation of a new phase is controlled by the cooling rate. The transition between different minima is another process that can be manipulated by controlled temperature changes. An example is the glycolipid n-octyl- β -D-glucoside, which, at 23°C, can assemble either in hexagonal or cubic phase as a function of its concentration (Figure 7), i.e., 65% and 80%, respectively.⁷⁹ A temperature-induced transition is observed for either of these phases, although the needed input is different—hexagonal to micelle transition is observed at 40°C, while cubic to lamellar transition

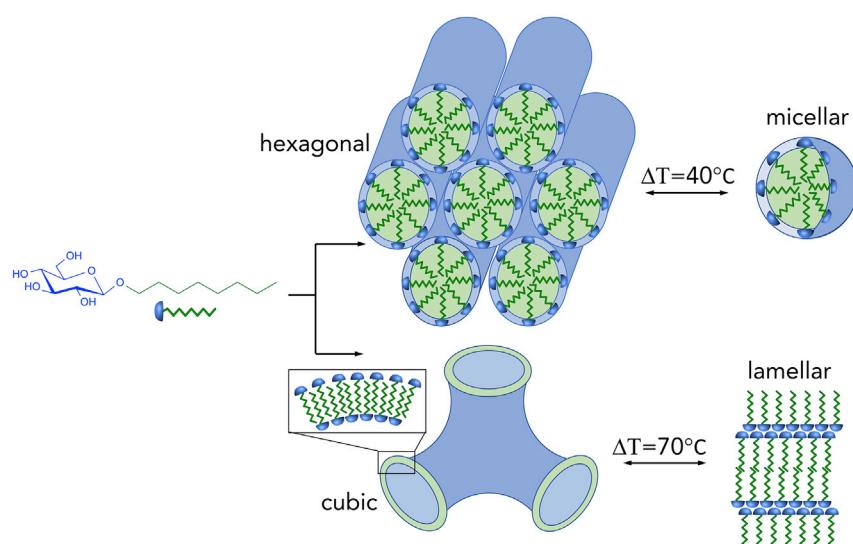


Figure 7. Temperature-induced transition in a glycolipid self-assembly: Hexagonal to micellar and cubic to lamellar phase

Figure is adapted from elsewhere.⁷⁹ Copyright 2012 American Chemical Society.

occurs at 70°C. Because temperature affects both thermodynamics and kinetics of the system, the whole thermal history, i.e., the heating temperature and time, the cooling rate, the final temperature, and the time after cooling, is important for the assembly pathway and critical for the properties of the generated system.⁸⁰

pH

pH-triggered assembly/disassembly associated with morphological transition and differential diffusion kinetics is an attractive tool for the development of customized stimuli-responsive biomaterials.⁸¹ There are various molecular designs that rely on different pH-sensitive processes. The most common approach includes functionalization of the amphiphile with basic/acidic functional groups (Figure 8).^{65,66,81} Protonation/deprotonation of such molecules is associated with the rebalancing of the supramolecular (electrostatic) interactions and may also influence conformations. Asymmetric bolaamphiphiles (Figures 6C2 and 6C3) are an illustrative example for such behavior. Protonation/deprotonation of these amphiphiles triggers different conformation-dependent interactions: the intermolecular hydrogen bonding in *trans*-bolaamphiphiles results in the formation of a well-organized monolayer (Figure 6D2), while in *cis*-isomers both inter- and intramolecular hydrogen bonding occur (Figures 6D3 and 8B), and the balance between these controls the assembly process. Depending on the amphiphile structure, pH change can result in either loosening of the packing within the assemblies, while preserving their overall shape, or the formation of nanostructures with different morphologies (Figure 8A). pH responsiveness of nanotubes formed by asymmetric bolaamphiphiles functionalized with amino groups and glucose at their ends exemplified the former behavior.⁸¹ Upon pH decrease, the amino groups that form the inner surface of the tube are protonated gradually and the electrostatic repulsions between them result in change of the diffusion coefficient across the nanotube wall. However, different morphologies are often generated due to the activation of a new assembly regime, as shown in the example of acidic sophorolipids (Figure 8B).⁶⁶ At low ionization degrees, the assembly is concentration driven and results in the formation of micelles. Upon charge introduction (ionization of COOH to COO⁻), repulsive electrostatic interactions favor the formation of large tubular aggregates.

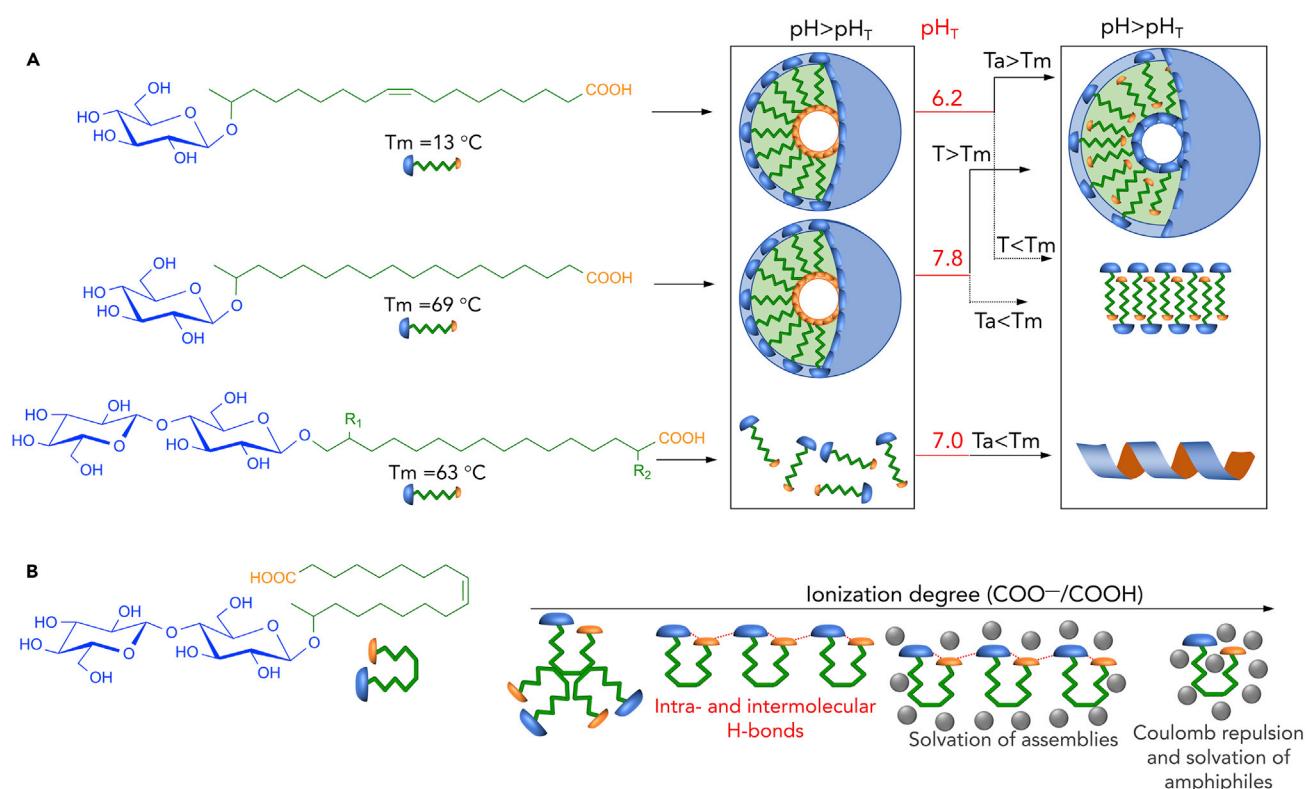


Figure 8. Effect of the environmental conditions on the assembly of bolaamphiphiles

Examples of (A) pH-induced phase transition in bolaamphiphiles self-assembly and (B) different regimes of assembly of bolaamphiphiles as a function of the ionization degree. Abbreviations used: pH_T , transition pH; Ta , ambient temperature; Tm , melting temperature. The figure is based on data published elsewhere.^{66,67} Copyright 2012, 2016 American Chemical Society.

Another approach in using pH to control self-assembly is through covalent activation, which can be based on the introduction of pH-labile linkages such as acetals.^{82,83} In this case, the amphiphiles assemble at neutral pH and degrade with a decrease in pH, which may be useful for design of responsive drug delivery systems. pH change can also occur endogenously when multiple pH-sensitive groups are engaged in the supramolecular interactions that govern the assembly process. Such endogenous change can be applied for sequential assembly of multi-component systems, as demonstrated in the case of aromatic amphiphiles of sorbitol functionalized with either acylhydrazide or carboxylic acid.⁷⁸ In this system, the assembly of the acylhydrazide decreases the pH, which, in turn, triggers the assembly of the carboxyl derivative.

Enzymes

Enzyme-catalyzed reactions can be used as selective triggers of self-assembly and have the advantage of triggering assembly while keeping the overall conditions constant. The process is known as enzyme-instructed or biocatalytic self-assembly (EISA or BSA).^{36,84} BSA is based on the transformation of an enzyme-sensitive precursor into a self-assembling molecule and thus is highly selective and sensitive—the kinetics can be regulated by altering enzyme and precursor concentration. The rate of the enzymatic action controls the kinetics, which, in turn, impacts the morphology of the assembly formed. BSA can be applied *in situ* to trigger assembly in cells. Among different enzymes, phosphatases³⁶ and glycosydases^{85,86} have been selected as triggers owing to their involvement in different pathologies. Some years

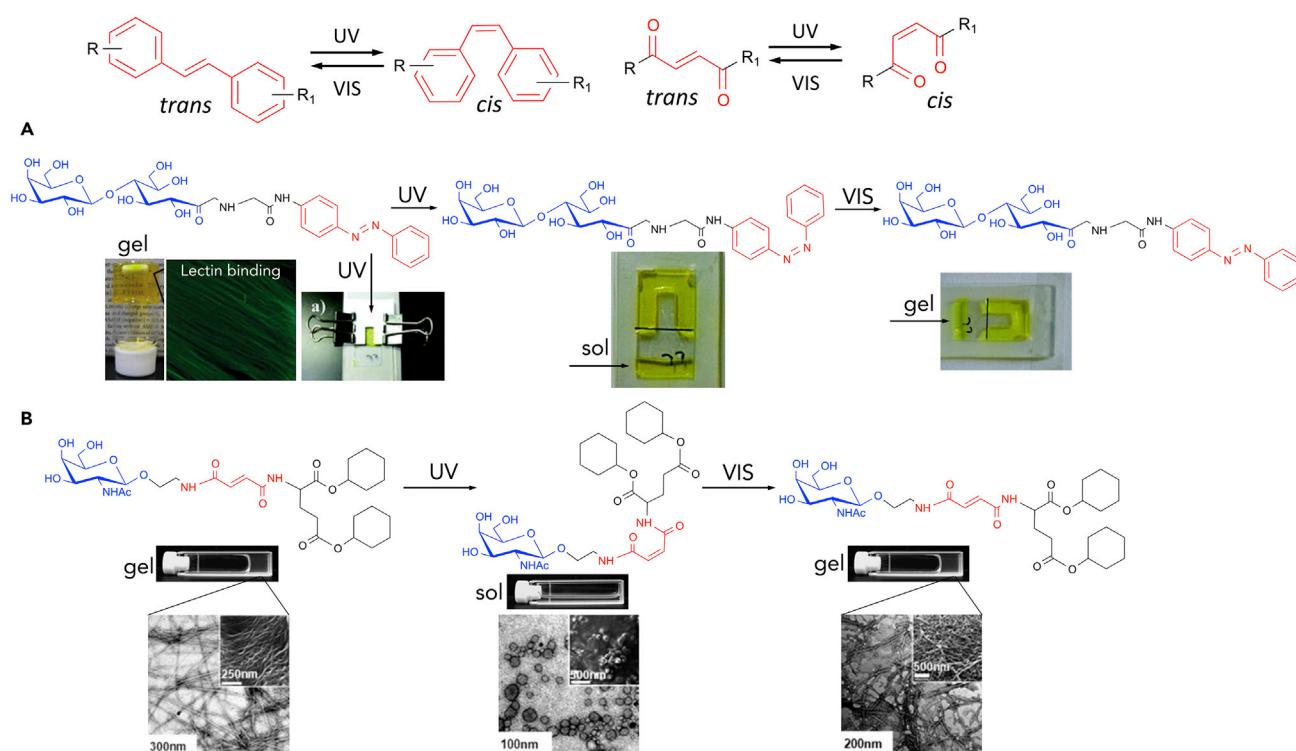


Figure 9. Photo-responsive carbohydrate amphiphiles: Light-sensitive groups are shown in red and the carbohydrate moiety in blue. Figure is adapted with permission from.^{87,89} Copyright 2012 American Chemical Society.

ago, we introduced a simple aromatic amphiphile of glucosamine-6-phosphate as a BSA precursor.³⁶ The enzymatic removal of the phosphate group reduces its solubility and triggers the formation of fibrillar structures that further organize into a supramolecular gel. Enzymatic reactions can also be used to regulate the disassembly of supramolecular glycosylated systems. For example, glycosidase-triggered drug release from amygdalin-fatty acid hydrogels can be manipulated by changing the enzyme concentration.⁸⁶ These approaches can conceptually be extended to other enzymes such as glycokinases and glycosyltransferases.

Light

Light is an attractive trigger for bioapplications, as it is non-invasive and can be tuned by several means, namely, wavelength, duration, and intensity of exposure. The amphiphiles include mono- (e.g., glucose, galactose, N-acetylglucosamine)^{87,88} or disaccharides (e.g., lactose, maltose)⁸⁹ that are conjugated to light-sensitive moieties, such as azobenzene (Figure 9A)^{89,90} or 2-butenedial (Figure 9B)^{87,88} as light-sensitive groups.

In the case of azobenzene derivatives, the assembled structure is sustained by π - π interactions, which are fostered at the planar *trans*-configuration. The structure is additionally stabilized by multiple hydrogen bonds between the carbohydrate units, as demonstrated using different stereoisomers that resulted in systems with different stabilities.⁸⁹ The light irradiation triggers *trans* (visible light)/*cis* (UV light) isomerization, and this conformational change induces a rebalancing of the supramolecular forces. Usually, assembly (and consequent sol-gel transition) is promoted when wavelength within the visible region is used while UV irradiation causes the reverse process. These transitions are reversible upon removal of the stimulus, which

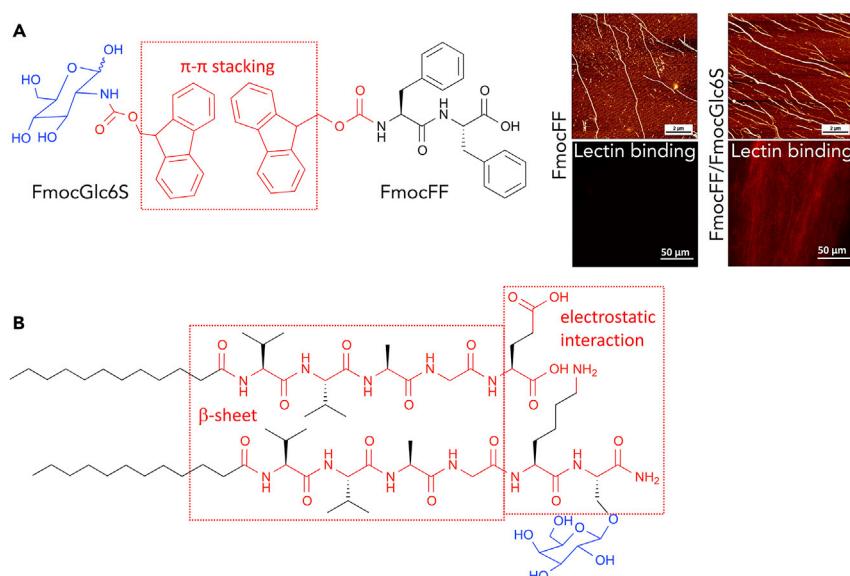


Figure 10. Examples of multi-component systems with carbohydrate amphiphiles

(A and B) Amphiphiles can (A and B) share the same functional units or/and (B) complementary charge that drives the co-assembly.

motivates the use of these systems as nanoprobes for biosensing and *in situ* cell imaging. When 2-butenedial carbohydrate derivatives are used, additional functionalization with lipids must be performed to augment the assembly propensity via hydrophobic interactions (Figure 9B). Like the azobenzene amphiphiles, the *trans*-2-butenedial amphiphiles form gels via tight packing, which is distorted upon UV irradiation and results in gel-to-sol transition.^{87,88}

Multi-component systems

The self-assembly of two (or more) different building blocks provides a powerful approach to generate functional diversity. The co-assembly approaches applied to carbohydrates can be classified in two groups based on the building blocks that are used: (1) the components are able to self-assemble individually, but when they are mixed they influence each other, either by co-assembly (components interact) or by the assembly formed by discrete components^{69,78,91,92} and (2) one of the components self-assembles and a non-assembling component is added to change the final assemblies.^{27,93–95} As an example of the first approach, co-assembly can be promoted by using identical functional units that promote the self-assembly in the design of the amphiphiles, e.g., the components co-assemble by $\pi\text{-}\pi$ stacking or β -sheet formation (Figure 10A)^{27,33,91} and/or by charge complementarity between the co-assembled amphiphiles, i.e., by electrostatic interaction (Figure 10B).⁹² In this case, the carbohydrate moiety is not the main driving force of the self-assembly, but it can alter the structure and function of the supramolecular structures. Recently, we have demonstrated that such an approach is feasible for the development of core-shell systems in which the peptide amphiphile assembles into a structural core, while the carbohydrate amphiphile forms a functional shell of the nanofibers.²⁷ The co-assembled fibers are branched and thicker in comparison with the single component fibers (Figure 10A). They also have different bioactivities due to the introduction of carbohydrate moiety, as demonstrated by the lectin binding assay. The main advantage of this assembly mode is its modularity—using independent peptide and

carbohydrate blocks allows to generate structural diversity, e.g., different functional carbohydrate, different density by simple mixing of two solutions.

In the second approach, bioactive carbohydrates, namely, glycosaminoglycans (GAG, e.g., hyaluronan and heparin), are used as a non-assembling component and the assembly process is induced by the addition of a self-assembling peptide.^{93–97} In most cases, an interfacial polyelectrolyte complexation between the negatively charged glycosaminoglycan and the positively charged peptide is the main driving force of the co-assembly process, as demonstrated by the formation of a nanolayer at the interface between the solutions of the components.^{93,96,98} Both sides of this layer have different properties and they depend on the concentration/viscosity of the used GAG as well as on the order of addition (dropping peptide solution in GAG one or vice versa). In another approach, selective amplification of charged Fmoc-dipeptides from dynamic combinatorial libraries can be achieved by electrostatic interactions with an oppositely charged carbohydrate: cationic chitosan amplifies the peptide containing cysteic acid and they co-assemble into nanosheets, while the negatively charged heparin interacts selectively with the lysine-containing peptide and together form nanotubes.⁹⁶

BIOAPPLICATIONS OF CARBOHYDRATE SUPRAMOLECULAR SYSTEMS

Mimics of extracellular matrix

The extracellular matrix (ECM) is a three-dimensional, highly hydrated, and dynamic assembly of macromolecules that provides structural support for cell adhesion and growth as well as biochemical information that control the cell fate.⁹⁹ Because glycoproteins are the main components of the ECM, supramolecular carbohydrate interactions are central to ECM-cell communication. Mimicking the functions of glycoproteins is limited by the complex and challenging nature of synthetic glycochemistry. However, self-assembling carbohydrate amphiphiles circumvent this issue by providing well-organized 3D matrices with surface-exposed carbohydrate clusters emulating the multivalency and high density of ECM glycoproteins. This approach has been embraced by many in the field, with innovative variations in design elements. The first to explore this approach was Xu, who proposed *de novo* glycoconjugate design with three components that are relevant to ECM mimicry—a self-assembling nucleobase (adenine), an integrin binding sequence (FRGD), and a glucosamine unit (Figure 11A).³⁷ *De novo* glycoconjugates have demonstrated their versatility in influencing cellular pathways through various mechanisms.^{7,13,16} In their rudimentary form, they offer better cell-adhesion properties through CCI than the non-carbohydrate-containing ECM mimics, and are known to enhance stem cell proliferation and differentiation.^{16,37} They can compete with native ECM proteins, offering a better anchoring point for cell adhesion. The morphology of the self-assembled structure has a direct effect on cell-cell interactions and organization. Glycosylated nanoparticles, for instance, facilitate the formation of cellular spheroids from cell monolayers.¹⁰⁰ The morphogenesis is accompanied by imposed physical constraints that alter the accessibility of surface proteins and molecular diffusion from the environment to the inside of the cellular cluster increasing the resistance to cytotoxic drugs.

One of the roles of ECM proteoglycans is to interact with soluble bioactive molecules secreted by cells (e.g., growth factors, cytokines), and regulate their stability, distribution, and availability to cells. Several designs of carbohydrate amphiphiles have been proposed for the development of supramolecular systems that mimic this ECM feature (Figures 11B–11D).^{27,29,30,101,102} These amphiphiles can be assembled alone (Figures 11C–11E)^{29,33,101,102} or co-assembled with peptide amphiphiles (Figures 11B and

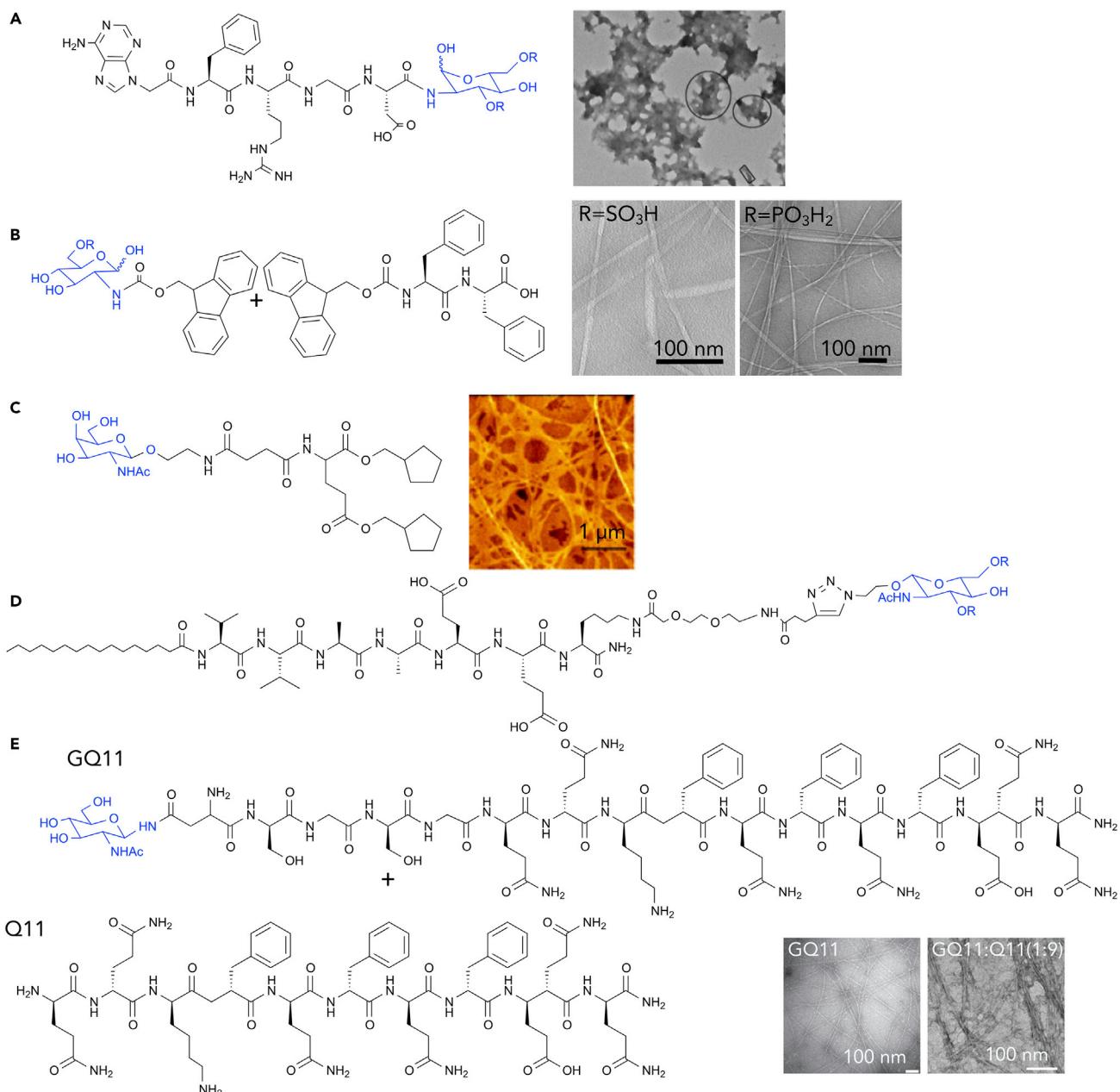


Figure 11. Different carbohydrate amphiphiles used to assemble structural and biofunctional mimics of extracellular matrix

(A–E) R = H, SO₃H. Images reproduced with permissions from elsewhere (A)³⁷ Copyright 2014 American Chemical Society; (C)¹⁰¹ Copyright 2004 American Chemical Society; (E).³² Details on the assemblies (B and D) can be found in Brito et al.²⁷ and Lee et al.,²⁹ respectively.

11E)^{27,91,92,96} to generate nanofibers with surface-exposed carbohydrates. The generated hydrogels have a nanofibrous structure with surface-exposed carbohydrates that protect and enhance the activity of different proteins by CPI.^{27,29,101,102} These systems can be used as biosensors,^{101–104} immuno-suppressive materials,⁴³ or in different tissue repair and engineering strategies.^{27,29,92} Similar to heparin sulfate proteoglycans, sulfation of the carbohydrate unit can enhance the biofunctionality of the system significantly, as shown in the cases of bone morphogenic protein-2 (BMP2)²⁹ and basic fibroblast growth factor (FGF2),²⁷ thus significantly reducing the protein dose needed for an efficient therapy. Co-assembly of glycosylated amphiphiles and their non-glycosylated

analogs can be used to modulate the carbohydrate density on the surface of the assembled system, simply by varying the ratio of each component.⁹¹ As discussed earlier, such a density control is crucial for the bioactivity and selectivity of the system. The exact multivalent arrangement and distancing between the exposed sugar moieties can also be tailored to specific protein binding by post-assembly functionalization of peptide nanostructures.¹⁰⁵

Discrete cellular environments can be co-assembled from hyaluronan and peptide amphiphiles.^{93,94} These two components form hierarchically ordered, remarkably stable macroscopic membranes or sacs that are semipermeable and allow exchange of nutrients and metabolites, but cells are too big to cross them. These systems have an advantage: they can be manipulated during or after the assembly using techniques such as sewing or micropatterning, which makes them versatile tools for tissue engineering and regenerative medicine.

Systems with antimicrobial activity

Bacterial adhesion to the host cell is a crucial step in an infection process that is mediated by CPGs. At this initial stage of the infection, bacteria adhesins/invasins bind to glycoconjugates expressed on the eukaryotic cell surface. Moreover, some bacteria secret toxins (e.g., AB₅-type proteins) that facilitate the invasion process and bind to host cell also via their surface glycans. Multivalency is crucial in these processes, as demonstrated in systematic studies using synthetic branched polymers and dendrimers functionalized with carbohydrates as inhibitors of these interactions.¹⁰⁶ In unidirectional self-assembled systems, the glycosylation of nanofibers can also add to the hierarchical organization of the system by forming lateral carbohydrate-carbohydrate cross-links.³³ The nanofibers are thus able to align rather than intertwine via non-specific hydrophobic interactions, which generally leads to undesired aggregation, producing highly ordered supramolecular architectures. The multivalency and relatively weak nature of the interactions facilitates the sliding and repositioning of nanofibers to reach the most energetically favorable state of alignment. The interaction of glycosylated surfaces with the surrounding water endows them with resistance to non-specific bacteria and proteins while maintaining their selectivity for specific lectins. This is due to the specific multivalent carbohydrate surface display, whose density and arrangement can be modulated for tailored binding by different strategies.

Supramolecular multivalent antibacterials can be assembled by rod-coil amphiphiles (Figure 12) and their efficacy has been demonstrated for *Escherichia coli* (*E. coli*).^{68,107} Because the bacterial adhesin FimH has mannose-binding lectin domain, this carbohydrate has been a primary choice for amphiphile functionalization. The assembled systems exhibit multiple mannoses on their surface that interact and block the lectin. Of note, the supramolecular systems not only affect the protein-carbohydrate interactions but also inhibit the bacterial mobility by triggering agglutination.^{38,69,108} The agglutination depends upon the shape and size of the assembled nanostructure, which can, in turn, be controlled by the amphiphile design as discussed earlier—nanoribbons^{38,108} and long fibers⁶⁹ are more efficient agglutination promoters and the introduction of a rigid segment into the amphiphiles facilitates the assembly of these nanostructures.¹⁰⁹

Cancer management

One of the cancer cells' hallmarks is their accelerated metabolism and higher glucose dependence as compared with non-malignant cells. The metabolic alterations suggest that glucose antagonists can provide efficient tools for cancer

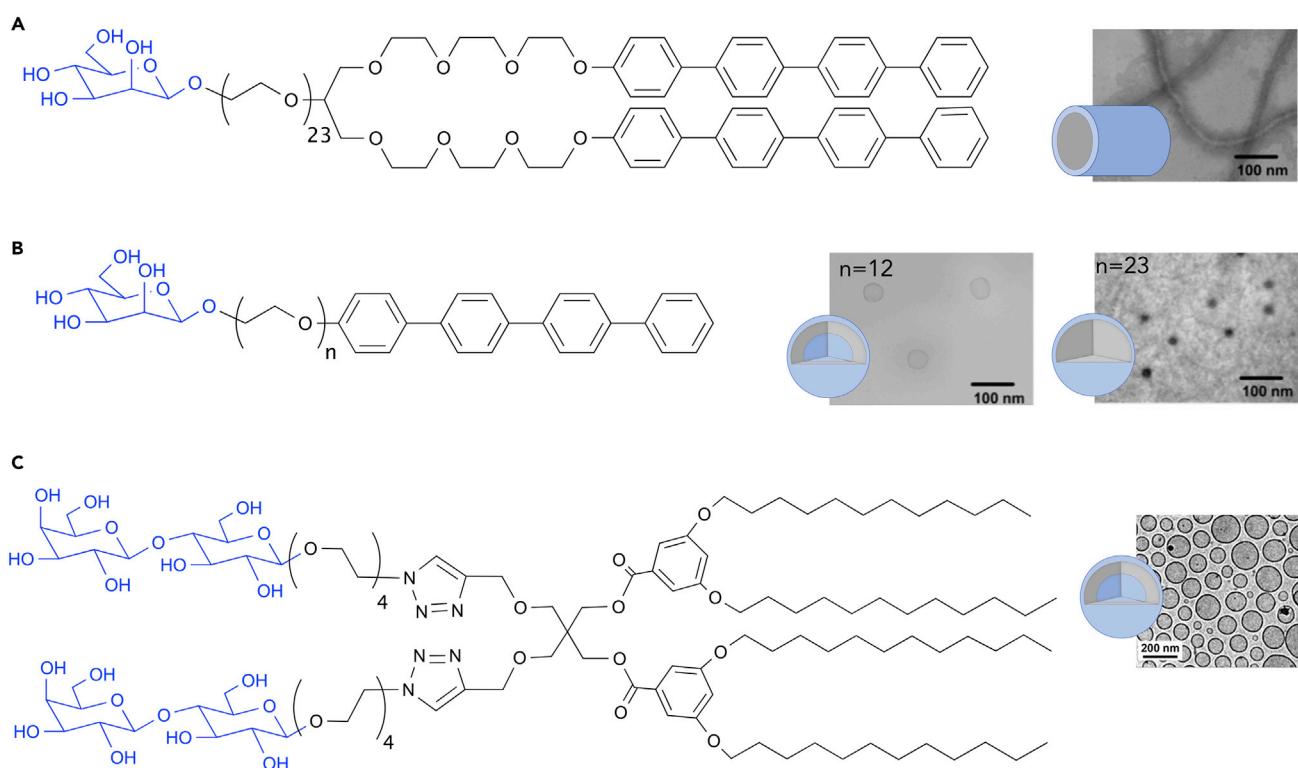


Figure 12. Rod-coil carbohydrate amphiphiles for assembly of multivalent antibacterial systems

Figure is adapted with permission from.^{68,110} Copyright 2005, 2013 American Chemical Society.

management. Few years ago, we described a simple aromatic carbohydrate amphiphile, N-(fluorenylmethoxycarbonyl)-glucosamine-6-phosphate, as a chemotherapeutic agent.³⁶ This amphiphile deprives the glucose metabolism by interacting with glucose transporter 1 (GLUT1) and also by the *in situ* assembly of a supramolecular net around the cancer cells (Figure 13).^{35,36,111}

In aqueous environment, the amphiphile assembles into micelles.²⁷ Alkaline phosphatase, which is overexpressed in different cancers, can cleave the amphiphile's phosphate group, thus triggering micelle-to-fiber transition. Due to the action of membrane-embedded phosphatases, the biocatalytic action is localized at the cell surface, and the result is the formation of a discrete nanocage around cancer cells that acts as a physical barrier between these cells and their environment with the glucose units exposed on the surface of the fibers, similar to an approach using aromatic peptide amphiphiles that was previously introduced by Xu.¹¹² The synergism between physical and biochemical deprivation of cancer metabolism turns this therapeutic strategy selective and efficient especially in 3D environment, e.g., tumor models, where the cancer cells are heavily dependent on glucose and have much higher expression of GLUT1 as compared with the standard 2D cultures.¹¹¹

CONCLUSIONS AND FUTURE TRENDS

While the incorporation of carbohydrates within self-assembling building blocks seemed challenging some years ago, the boom that the fields of systems chemistry and glycochemistry have experienced lately has precipitated an interest in the development of carbohydrate supramolecular systems with biomedical application. Nonetheless, most of the developed systems rely on the same principles used for

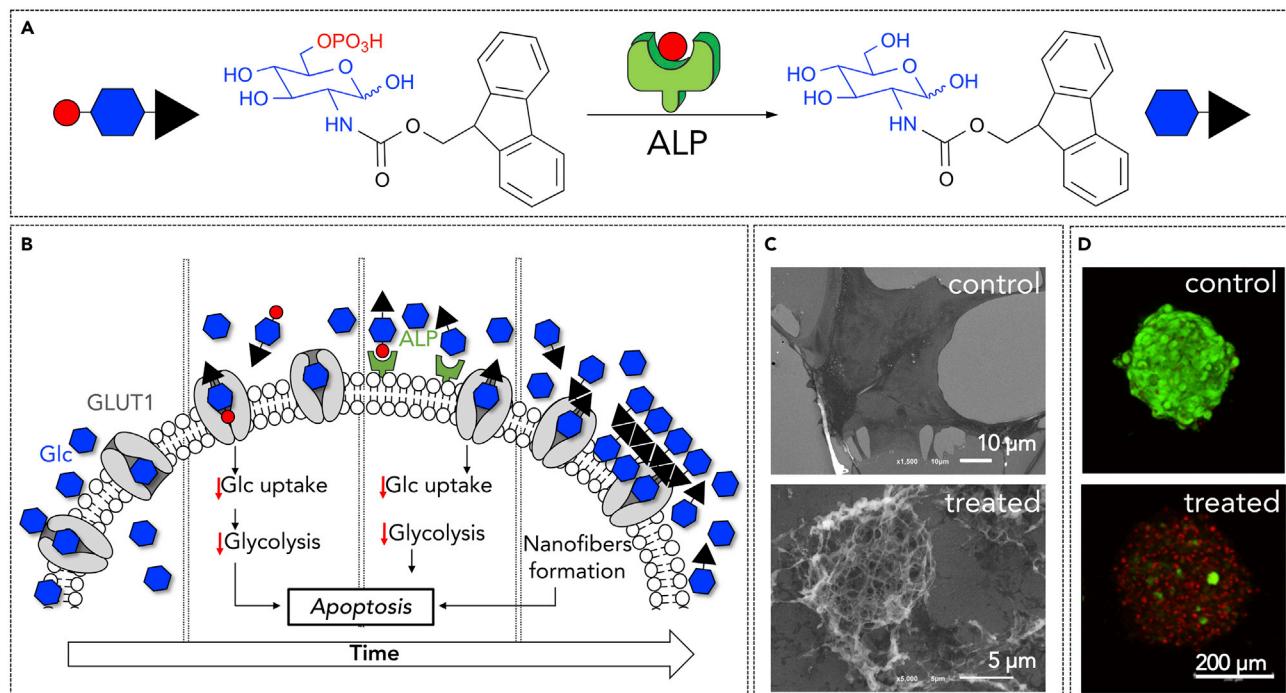


Figure 13. Carbohydrate amphiphiles in cancer management

(A–D) (A) Enzymatic transformation, (B) mechanism of action and effect of N-(fluorenylmethoxycarbonyl)-glucosamine-6-phosphate on (C) osteosarcoma-like cells and (D) spheroids of HS578T breast cancer cells. Adapted from^{35,111} published by the Royal Society of Chemistry and³⁶ published by American Chemical Society.

structurally different building blocks, e.g., polymers and peptides, and do not explore unique carbohydrates features such as CCIs, chiral and topological abundance, and multivalency. Although the cells are surrounded by complex polysaccharides in their native environment, most of the amphiphiles have simple (mono- and di-) saccharides as biofunctional units. Thus, new concepts that develop supramolecular assemblies from intricate biofunctional carbohydrates are yet to come, and these should result in even better performing biomaterials. We opine that the full potential of carbohydrates in self-assembly can be only achieved if we introduce the principles that regulate the glycome functions in the construction of supramolecular biomaterials.

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AUTHOR CONTRIBUTIONS

Conceptualization, I.P., R.A.P., and R.V.U.; writing – original draft, A.B., S.K., and I.P.; review and editing, I.P., R.V.U., and R.A.P.; funding acquisition, I.P., R.V.U., and R.L.R.; supervision, I.P., R.A.P., R.V.U., and R.L.R.

DECLARATION OF INTERESTS

R.V.U. is a member of the Chem advisory board.

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