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Glycopolymer probes of signal transduction†

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Abstract

Glycans are key participants in biological processes ranging from reproduction to cellular communication to infection. Revealing glycan roles and the underlying molecular mechanisms by which glycans manifest their function requires access to glycan derivatives that vary systematically. To this end, glycopolymers (polymers bearing pendant carbohydrates) have emerged as valuable glycan analogs. Because glycopolymers can readily be synthesized, their overall shape can be varied, and they can be altered systematically to dissect the structural features that underpin their activities. This review provides examples in which glycopolymers have been used to effect carbohydrate-mediated signal transduction. Our objective is to illustrate how these powerful tools can reveal the molecular mechanisms that underlie carbohydrate-mediated signal transduction.

1. Introduction

All cells, from prokaryotes to eukaryotes, are cloaked in a glycan coat termed the glycocalyx.[1, 2] This coat is composed of a variety of glycosylated proteins and lipids that report on cell type, environment, and the metabolic state of the cell. It was originally thought that the primary role of the glycocalyx was to act as a physical barrier against the cell's environment; however, the presence of carbohydrate-binding proteins on the cell surface augurs the vital role of cell surface glycans in cell–cell recognition. The interaction of cell surface glycans with cell surface carbohydrate receptors is not only important for cell adhesion—it also can trigger signal transduction. This mode of information transfer is fundamental for many biological processes, including fertilization and implantation[3–6], pathogen invasion[7], immune system activation[8, 9] or attenuation[10–14], and cell proliferation.[15] Recognition of the wide-ranging contributions of glycans to signaling is mounting.[16, 17]

This growing appreciation of glycan function is providing impetus to develop ligands to probe and perturb protein-carbohydrate interactions. While methods to isolate and characterize glycans from biological sources are advancing, it can be challenging to elucidate the molecular features involved in glycan recognition and function. Chemical synthesis is a powerful ally in addressing this challenge. It offers access to glycans whose structures can be varied to dissect glycan function.[18] One especially valuable class of synthetic ligands for illuminating carbohydrate recognition is multivalent displays.

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A hallmark of many protein–carbohydrate interactions is multivalency. Most carbohydrate-binding proteins, whether on the cell surface or secreted, are oligomers. They can exist as dimers, trimers, tetramers, or even higher order clusters.[19–21] These oligomeric proteins can bind either to multiple carbohydrate residues within a glycan or to multiple glycans on the surface of cells (Figure 1).[22–25] The advantages of multivalency have been revealed through chemical biology studies. While it is well-appreciated that multivalent binding can enhance the functional affinity (observed affinity, also termed avidity) of cell surface protein–carbohydrate interactions,[22, 26–29] it is often overlooked that multivalent binding can also improve specificity.[28, 30] If individual interactions at the cell surface were to occur with high functional affinity, it could be problematic. During the encounter of two cells (one with carbohydrate ligands and the other with a protein-binding partner), for example, the summation of multiple high affinity interactions would render binding irreversible. In contrast, low affinity multivalent interactions are kinetically labile[31]; therefore, they provide the mean to capture a cell of interest while still allowing for reversibility if the wrong cell type binds initially.[19]

The properties of multivalent carbohydrate derivatives (i.e., their ability to exhibit high functional affinity and increased specificity) have stimulated the development of methods to synthesize defined multivalent carbohydrate derivatives, including polymers bearing pendant carbohydrates (glycopolymers). These agents can be employed as potent inhibitors, but their ability to cluster carbohydrate receptors provides them with an additional property —they can activate signaling.[32] Accordingly, glycopolymers have been used to mimic either polysaccharides (i.e., glycosaminoglycans), glycoproteins, mucins, or even larger entities such as viral particles or clustered glycans in cell surface microdomains.[29, 33] Because they are synthetically tractable, these surrogates can be altered to optimize a desired activity or to probe a specific biological process.[34]

Over the last two decades, many scaffolds have been developed for displaying carbohydrates. These range from dendrimers,[35, 36] to oligomeric bioconjugates[37], to polymers[38–40], to quantum dots[41–43] and nanoparticles.[44–46] While each scaffold has unique benefits, it is the polymers that can exhibit the greatest variation in valency, individual binding group spacing, and overall architecture. Access to this structural diversity has been made possible by advances in polymer synthesis, which have provided the means not only to synthesize polymers of different structures, but also to control the properties of the polymers that result. By varying the structure of the monomer or the polymerization conditions, a multitude of complex topologies can be accessed (Figure 2).[47] Thus, polymers can be generated to carry out systematic investigations into the effect of glycan structure on biological function.

Despite the benefits of employing glycopolymers to study signal transduction, to date this strategy has been surprisingly underutilized. Given the many new roles for glycans that are being revealed, we hope that this review will stimulate research into carbohydrate-mediated signal transduction. Because signal transduction necessitates robust glycopolymer recognition by protein receptors, we provide an overview of the structural features of glycopolymers that can influence their mechanism of protein recognition. We use these studies to provide some general parameters that might influence the abilities of glycopolymers to affect cell signaling. We also discuss examples of how glycopolymers have been employed to address specific problems in signal transduction. While there are many exciting examples where glycopolymers have been used to elicit an immune response[48–54], we highlight those that focus on a specific signal transduction pathway.

2. Maximizing protein recognition of glycopolymers

In the 1970s, Lee[55] and Horejsí[56] described methods for the synthesis of polyacrylamide-based glycopolymers. Their demonstration that these synthetic conjugates could bind lectins spurred investigations using glycopolymers as functional glycan mimics. This led many researchers to explore glycopolymers as potent inhibitors of carbohydrate-binding proteins.[57, 58] Accordingly, many polymerization strategies have now been applied to the generation of glycopolymers (Figure 3). The kind of radical polymerization reactions carried out initially (free radical polymerization) have now been complemented by controlled polymerization reactions, such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain-transfer polymerization (RAFT). Non-radical polymerization strategies also have emerged, including the ring-opening metathesis polymerization (ROMP), which has been used extensively in signaling studies. The synthesis of glycopolymers is beyond the scope of this review, but there many excellent resources on the topic.[39, 40, 48, 59, 60]

For glycopolymers to affect signal transduction, they must bind effectively to at least one but often multiple copies of their protein receptor. Using controlled polymerization methods, many aspects of the glycopolymer structure can be altered systematically to optimize its activity. Some key parameters include the length of the polymer, the density of the carbohydrate ligands, the flexibility of the polymer backbone, and the overall structure of the glycopolymer. Thus, glycopolymers can be tailored to inhibit endogenous glycan interactions with a cell surface receptor or to cluster receptors for signaling.[39, 61] Previously, we reported general principles for designing multivalent ligands[62]; subsequent studies have provided additional insight into how glycopolymer features influence activity. Some examples follow that document the interplay between glycopolymer structure and function.

2.1 Glycopolymer length and functional affinity

The advent of living polymerization reactions provided a means to alter glycopolymer length. Living polymerizations, in which the rate of elongation is more rapid than termination, are ideal processes for assembling glycopolymers of defined lengths. The separation between binding sites can vary for an oligomeric carbohydrate binding protein, and ligands that can occupy multiple binding sites will have increased functional affinity.[63] Glycopolymers of defined lengths can serve as “measuring sticks”; their length (and valency) can be optimized to span multiple binding sites within a carbohydrate-binding protein or between carbohydrate-binding proteins on a cell (Figure 4). For living polymerization reactions with rapid initiation rates, the length of the polymer (degree of polymerization, DP) can be easily predicted and controlled by varying the ratio of initiator to monomer.[64–67] In this scenario, an initiator to monomer ratio of 1:100 would afford a 100mer. This strategy of varying polymer length to bridge different lectin binding sites was shown using glycopolymers generated by the ring-opening metathesis polymerization. Specifically, glyopolymer length was optimized to facilitate binding to the tetrameric lectin concanavalin A (Con A).[66] The most active glycopolymers were those that could bridge at least two binding sites within the Con A tetramer. Glycosylated dendrimers are another popular scaffold for targeting oligomeric lectins[35, 68–72], as different generations or different linkers can be tested to find those that span carbohydrate-binding sites within an oligomeric lectin. To this end, Cloninger and coworkers have employed spin-labels and electron paramagnetic resonance spectroscopy to characterize the spatial distribution of carbohydrates on dendrimers.[73, 74]

2.2 Density of the carbohydrate ligands

Another method to influence glycopolymer functional affinity involves altering the carbohydrate epitope density on a polymer backbone. Studies employing glycopolymer ligands can complement those using natural glycans, whose variation in density can influence their activity.[75] With controlled polymerization reactions, the density of the carbohydrate ligand can be manipulated in two ways—copolymerization of a monomer bearing the carbohydrate epitope with a biologically inert monomer, or post-polymerization functionalization of a polymer with the carbohydrate ligand and a biologically inert ligand (Figure 2).[76] Using either approach, the overall length of the polymer can be kept constant, while the level of carbohydrate substitution can be varied.

Cairo et al. found that increases in binding epitope density enhanced the ability of multivalent ligands to bind avidly to the lectin Con A.[77] The authors used ROMP to generate glycopolymers of various mannose densities by polymerization of different ratios of mannose-substituted monomers and biologically inert galactose-bearing monomers (Con A does not recognize galactose). Their study revealed that as mannose composition increased, both the avidity and the ability of the glycopolymer to cluster Con A increased.

An examination of the role of ligand density at the cellular level was conducted by Rubinstein and coworkers.[78] They sought to prepare glycopolymers that could exploit the observed upregulation of the galactose-binding lectin galectin-3 in metastatic tumor cells. They therefore prepared *N*-(2-hydroxypropyl)methacrylamide (HPMA) conjugates displaying different levels (10%, 20%, or 30%) of a galactose, galactosamine, lactose, or trivalent galactose derivative. They tested the interaction of these glycopolymers with three different colon cancer cell lines (Colo-205, SW-480, and SW-620).[78] The level of galactose substitution influenced binding to some cell lines, but not others. For example, all three glycopolymers bound the SW-480 cell line, while the glycopolymers with the highest substitution levels were the most effective ligands for the Colo-205 cells and SW-620 cells. While the molecular mechanisms underlying these differences have not been determined fully, the results underscore that carbohydrate residue density can be an important parameter in devising effective cell targeting agents.

These aforementioned examples might lead one to conclude that increasing the density of carbohydrate ligands on a polymer always leads to enhanced activity, but such a conclusion would be erroneous. One counter illustration is from Kiick and coworkers, who examined the ability of a series of glycopolymers to inhibit the pentameric cholera toxin (CT),[79] a member of the medically important class of AB5 toxins that have been targets for inhibitor design.[80–82] Glycopolymers were produced by coupling different mole ratios of an amino galactoside to a poly-glutamic acid backbone. Interestingly, the authors found that the glycopolymer with the lowest level of galactose conjugation was the most effective CT inhibitor. A glycopolymer that consisted entirely of galactose interacted with CT much more weakly. The authors argue that when the density of the galactose moieties decreases, the spacing between the galactose moieties increases, thus approaching the approximate distance between binding sites on CT. Because the activity is reported on a galactose residue basis, galactose residues that do not contribute to protein binding decrease the activity. In a subsequent study, the authors engineered random coiled poly-glutamic acid polymers that were designed to contain galactose moieties spaced apart at specific distances.[83] The glycopolymer that had the largest spacing between galactose moieties bound CT with the highest affinity, despite having the lowest valency. Although these glycoconjugates do not yet match the efficacy of the pentavalent glycan ligands devised by the Fan and Bundle groups,[80–82] the glycopolymers are quite potent, especially when considering they are unable to occupy all five binding sites within the toxin.

Like the glycopolymer strategy used by Kiick and coworkers, other methods have been developed that allow precise placement of saccharide residues on a polymer backbone, including polypeptides composed of repeating folded domains[84], poly(amidoamines)[85], and modified nucleic acids.[86–91] Hartman and coworkers have devised an iterative approach for assembling polymers with controlled carbohydrate ligand spacing.[85] Their strategy entailed the sequential coupling of either an inert ethylenedioxy monomer or an alkynyl monomer that could be functionalized with an azide-bearing mannoside to generate mono-, di-, and tri-mannoside glycopolymers. The spacing between the mannose ligands of the polymers was estimated to be 10 nm for di-mannoside and 7 nm for tri-mannoside. Interestingly, the glycopolymer containing one mannose moiety bound Con A with an IC_{50} of 8 μM, much lower than that of other monovalent glycan ligands. The authors postulate that the enhanced affinity is due to the highly hydrated ethylenedioxy units on the polymer backbone[92–94], but it also possible that the backbone itself contributes to the higher affinity.[95] In addition, the authors appended the mannosides to the backbone via a triazole linker, which may also have contributed to the enhanced binding. The glycopolymer that contains two mannose residues with an estimated 10 nm spacing exhibited only a moderate enhancement and was less potent than the monovalent ligand on a saccharide residue basis (IC_{50} to 5 μM). The glycopolymer with the 7 nm spacing between saccharide residues was designed to be the most effective since it most closely matched the distance between binding sites in Con A (~6.5 nm). Indeed, it was the best ligand (IC_{50} value of 1 μM) but the modest enhancement observed is not indicative of multivalent binding. Nevertheless, this defined approach could be used to prepare glycopolymers to examine whether carbohydrate spacing affects signal transmission.

Another means of generating polymer constructs with defined glycan spacing is to use peptide nucleic acid (PNA) backbones.[89, 96] For example, multivalent carbohydrate displays of this type have been used to probe how the dimeric antibody 2G12 interacts with mannosylated gp120 on HIV (Figure 5).[86] Because 2G12 can neutralize HIV, it is thought that compounds that bind tightly to 2G12 might serve as haptens for the development of effective anti-HIV antibodies. The displays were produced by appending mannose-containing oligosaccharides to a single-stranded PNA and hydridizing the mannosylated PNA to single-stranded DNA strands. The interactions of these assemblies with 2G12 revealed the importance of carbohydrate spacing. The authors used this strategy to generate a potent inhibitor of HIV infection.[87] While the synthesis of PNA displays is more labor intensive than assembling glycopolymers by polymerization, the study highlights how the spacing of carbohydrate ligands can be important for generating potent inhibitors.

These examples all serve to illustrate that glycopolymers with the appropriate spacing of carbohydrate ligands exhibit enhanced avidity. The relevance of carbohydrate residue density and spacing in the context of signal transduction are still unexplored. For signaling to occur, multivalent ligands must organize their protein targets into competent signaling complexes. This process typically requires that the multivalent ligand can cluster multiple receptors. The ability to position carbohydrate ligands at defined distances provides the means to test whether different receptor orientations alter signaling. Indeed, data is emerging that suggests it may be important (see, for example, the results of the Kiick Group described in Section 3.1). Still, it seems likely that glycopolymers that activate signaling receptors need only to cluster them. If signaling complex assembly allows for different arrangements of receptors, glycopolymers with a variety of carbohydrate spacings should be capable of activating signaling. For randomly modified glycopolymers, extremely high levels of carbohydrate substitution may sterically block protein binding; consequently, an intermediate density of carbohydrates offers a balance amongst enhancing functional affinity, avoiding unfavorable steric interactions, and presenting carbohydrate ligand arrangement capable of facilitating receptor clustering.[10, 11, 97]

2.3 Glycopolymer length and receptor clustering

The length of a glycopolymer can influence its ability to cluster receptors and how many receptors are in the cluster (Figure 6). Both of these parameters can impact whether a signal is transmitted and how effectively. It has been shown for some glycopolymer scaffolds, that as their length and valency increases, so does the number of receptor copies that they bind.[77] This relationship between glycopolymer length and the number of copies of lectin bound could be observed directly using transmission electron microscopy.[98]

An example in which longer glycopolymers more effectively transmit signals than do shorter polymers has been described. Bacteria must move towards nutrients and away from toxins for survival.[99–101] One attractant for *Escherichia coli* that promotes chemotaxis is galactose. Signals from galactose are transmitted through the transmembrane chemoreceptor Trg. The chemoreceptors cluster at the poles of the bacteria[102], suggesting that they might communicate with each other. Indeed, galactose-substituted polymers of different lengths uncovered the importance of chemoreceptor–chemoreceptor interactions for *E. coli* responses to attractants. The attractant potency of the glycopolymers depends on their ability to alter the intrinsic organization of the chemoreceptors (e.g., cluster them) in the membrane. Specifically, galactose-substituted polymers of sufficient lengths could induce chemoreceptor clustering. These glycopolymers were more potent attractants than monovalent or oligomeric attractants incapable of mediating receptor clustering.[103] Moreover, the galactose-presenting polymers could potentiate responses to other attractants, a result that highlights the ability of the chemoreceptor arrays to act as a kind of sensory organ to detect and integrate signals.[104] These studies reveal that glycopolymers can be used to explore the molecular mechanisms critical for signaling. They also highlight that glycopolymer length can influence signal strength. A similar relationship between glycopolymer length and signal transmission was observed in studies using glycopolymers as glycosaminoglycan analogs (vide infra).[105, 106]

2.4 Flexibility of the polymer backbone

The flexibility of the polymer backbone can also impact a glycopolymer's ability to bind to protein receptors.[62] Initially, one might assume that a rigid polymer with the correct spacing might interact with receptors more tightly, as any multivalent interaction would avoid a conformational entropy penalty. A potential downside of rigid polymers, however, is that they typically are less capable of adapting to protein interfaces; therefore, they will be less apt to adopt a spatial arrangement of glycan residues that complements those of an oligomeric carbohydrate-binding protein (or cluster carbohydrate-binding proteins in a membrane). Indeed, Kobayashi and colleagues noted that rigid glycopolymers often bind weakly to lectins.[107] For glycopolymers that present their glycans in a variety of orientations, the backbone rigidity might have little impact on binding.[66]

Immobilized glycopolymers also have been used to probe the role of polymer rigidity in protein recognition.[108] For example, arrays of glycopolymers of varying flexibility were tested for binding to Con A.[109] When no cross-linker was added to the polymerization, little interaction with Con A was observed. When a low mole fraction (0.5%) cross-linker was added, the lectin readily bound. When a higher level of cross-linker was employed (1.5%), binding to Con A was reduced significantly. Similarly, Miura investigated Con A binding to glycopolymeric hydrogels of different stiffness.[110] They referred to these three states as follows: a flexible swollen state, an intermediate transition state, or a stiff collapsed state. Con A most avidly bound to the transitional hydrogel with intermediate flexibility, the flexible swollen hydrogel was next, with binding to the stiff hydrogel being the weakest.

The flexibility of the linker that connects the carbohydrate to the polymer backbone may also contribute to protein recognition. For example, Stenzel and coworkers generated galactose glycopolymers with either a stiff poly 2-hydroxyethyl methacrylate (pHEMA) linker or a flexible polyethylene glycol (PEG) linker.[111] The authors investigated the polymers' ability to inhibit the plant lectin ricin and found the glycopolymers with the flexibile PEG linker were more effective inhibitors. Given the ability of more flexible backbones and linkers to adopt a conformation/orientation that leads to effective interactions, it seems likely that the most active signaling agents will be glycopolymers that maintain this balance and can rapidly and effectively promote protein clustering.[10, 11, 33, 34, 103, 106]

2.5 Glycopolymer architecture

Advances in controlled polymerization reactions have afforded access to diverse glycopolymer topologies (Figure 2). The role of glycopolymer shape in signal transduction, however, has yet to be explored extensively. A number of studies do suggest that the shape of a multivalent carbohydrate ligand is a critical determinant of its activity. If one considers a carbohydrate-modified surface as "an insoluble glycopolymer", it is apparent that how glycans are displayed influences their functional affinities and protein binding specificities. One study showed that members of the selectin family bind only weakly to surfaces displaying monovalent sulfated galactose derivatives but avidly to surfaces that present multivalent sulfated galactose glycopolymers.[112] The carbohydrate residues were identical, but the multivalent displays had different features. Thus, the manner in which the carbohydrate moiety is displayed can have a marked influence on binding. Differences in glycan presentation have been shown to be important both for implementing glycan array technology and interpreting the data it affords.[113] To this end, different methods to fabricate arrays are being examined, from using glycolipids to immobilizing oligosaccharides via short linkers to presenting glycans on surface-linked protein or glycopolymer scaffolds.[108, 114–118] These data indicate that the architecture of a glycopolymer will undoubtedly influence its functional affinity for its receptor. Still, functional affinity is just one factor to consider in optimizing glycopolymer signals.

As discussed previously, signal strength can depend upon how many receptors a ligand can recruit to the signaling complex, the orientation of clustered receptors,[119] and the rate at which a glycopolymer induces clustering. The link between signal transduction and receptor endocytosis[120–122] offers another means by which glycopolymer topology might alter activity. There is growing evidence that the size of a ligand, its shape, and perhaps even whether it is soluble or insoluble all are factors that influence signaling, endocytosis, and trafficking.[123, 124] For instance, the dendritic cell lectin Dectin-1 is capable of eliciting an immune response to particulate antigen; however, soluble antigen fails to elicit a response.[125]

While the field currently is lacking systematic investigation of how the shape of glycopolymers influences their signaling ability, there has been an assessment of how different multivalent carbohydrate ligands influences their mode of interaction with a lectin.[77] Gestwicki et al. generated a variety of multivalent mannosylated ligands, including bi- and tri-valent small molecules, globular protein conjugates, dendrimers, linear polymers of controlled lengths, and high molecular weight polydisperse polymers, and evaluated these conjugates in a battery of assays that report on different aspects of their ability to interact with Con A. Compounds were evaluated for their ability to block Con A binding to immobilized glycan, but also many activities relevant for signal transduction: induction of lectin clustering (a quantitative precipitation assay[126] that also assessed the stoichiometry of lectin to ligand), the rate of induction of clustering (turbidity assay[127]), and the distance between receptors in a cluster (fluorescence quenching[128]). The oligovalent small molecules

and dedrimers were not potent inhibitors nor were they able to cluster Con A, suggesting these types of structures might be less effective at eliciting signaling. Alternatively, ligands with high molecular weights, such as the carrier protein conjugates and polydisperse polymers, could bind many copies of Con A; however, fluorescence quenching experiments indicated the bound receptors were not in close proximity to one another. These data suggest that the larger ligands may not be as efficient at eliciting signal transduction. Well-defined glycopolymers efficiently clustered the lectin, and proteins within the cluster were proximal. In another study comparing star polymer scaffolds, significant differences in lectin clustering also were observed. Medium-sized star-polymers were more efficient at clustering the lectin than were large star-polymers.[129] Linear glycopolymers, however, were more efficient at interacting with Con A than the star-shaped polymers. Together, the data indicate that ligand architecture can have a profound effect on the mechanism by which a glycopolymer engages its receptor.

Comparisons between linear glycopolymers, which typically exist as individual entities in solution, and diblock copolymers, which have the propensity to form micelles in solution, have revealed differences in their ability to cluster proteins.[130] Contrasting linear glycopolymer **1** (Figure 7) and micelles formed from the assembly of block copolymer **2** was instructive. Con A interacted more strongly with the glycopolymeric micelle than with the linear polymer. Subsequently, the potency of the micelles was augmented by generating block copolymers that display the carbohydrate binding groups in clusters (Figure 8).[131,132] As predicted, the clustered glycopolymer-derived micelle was even more effective at clustering Con A.

The density of glycopolymer presented by the micelle can be altered by doping in an inert polymer during self-assembly. This strategy was employed by Wooley and colleagues, who utilized ATRP to generated a diblock glycopolymer containing a mannoside moiety at one terminus and a diblock polymer with no functionality on its terminus.[133] Mixing various ratios of the two polymers in water resulted in their assembly into micelles with a mannose composition that ranged from 0% to 100%. Increasing the ratio of mannose glycopolymers in the cross-linked micelle increased its ability to block Con A mediated hemaglutinin.

The factors that govern protein recognition by the aforementioned conjugates, including glycopolymeric micelles are complex. The investigations carried out to date on glycopolymeric micelles suggest that they are highly effective at clustering proteins. It seems reasonable, therefore, to postulate that they can be used to deliver powerful signals. Their utility for this purpose, however, has not been investigated. Indeed, many interesting glycopolymer structures have not been tested as signal activators.

3. Mimics of surface glycans

Since all cells present a glycan exterior, it is not surprising that signal transduction can arise from interactions between cell-surface carbohydrate-binding proteins and cell surface-glycans. Glycopolymers have been used to mimic a variety of different types of cell surface glycans, including N-glycoproteins, mucins, and glycosaminoglycans. In this way, glycopolymers can be used to probe how cells communicate with each other or how a pathogen facilitates infection.

3.1 Mimics of mucins

The ability of glycopolymers to mimic mucins[53, 134] has been explored extensively in the context of selectin-mediated inflammation. In the inflammatory response, leukocytes are recruited to a site of injury or infection.[1, 135, 136] The process involves multiple steps: leukocytes roll across the endothelium; adhere tightly to the endothelium wall; and migrate

into the inflamed tissue. In certain disease states, aggressive leukocyte migration is detrimental.[137, 138] A key mediator of leukocyte migration is the cell-surface lectin, L-selectin, which interacts with glycoproteins displayed on the endothelium of blood vessels.[138, 139] Physiological L-selectin ligands are highly glycosylated mucin-like glycoproteins capped with sialyl Lewis x (sLe^x) epitopes.[140] Because its natural ligand is multivalent, it was postulated that clustering of L-selectin could be important for its function.

A number of different types of glycoproteins were investigated as inhibitors of L-selectin.[141–143] Evidence that glycopolymers could cluster this leukocyte surface receptor was obtained using tailored ligands. Using ROMP, a series of different length glycopolymers bearing a 3,6-disulfogalactose ligand and a fluorescent tag were screened for binding to L-selectin-positive cells.[144] Because each polymer bore only one fluorescent tag, it was possible to determine the ratio of L-selectin bound to polymer. As the valency of the polymer increased, so did the ratio of L-selectin to polymer, providing evidence that the ligands cluster L-selectin on the cell surface.

Further investigations of L-selectin targeted glycopolymers suggested that they could promote L-selectin-mediated signaling. Specifically, the polymers not only bind to L-selectin but also facilitate its downregulation (Figure 9).[145, 146] Treatment of lymphocytes with glycopolymer **3** resulted in a dramatic decrease in cell-surface L-selectin levels. The data indicated that ligand binding triggered the proteolytic release (or shedding) of L-selectin. Consistent with this mechanism, after cells were exposed to the glycopolymers, soluble L-selectin was detected. Monovalent ligands were unable to mediate shedding of L-selectin. The accumulated data suggest that clustering of L-selectin leads to a signal transduction cascade that results in L-selectin shedding.[147] Thus, glycopolymers are highly potent inhibitors of L-selectin function through multiple mechanisms: they block interactions of L-selectin with endogenous ligands, promote L-selectin loss from the cell surface, and generate a soluble form of the protein that can inhibit cell surface interactions.

In a subsequent study, Kiick and coworkers investigated the role of carbohydrate-spacing in activating L-selectin shedding.[148] Using a polypeptide backbone, sialylated ligands were appended to give a polymer with ligands separated by distances estimated to range from 17 – 35 Å and a polymer with carbohydrate moieties separated by 35 – 50 Å. The polymer with shorter carbohydrate-spacing was more effective at eliciting L-selectin shedding than the polymer with the longer spacing. The diameter of L-selectin is thought to be about 22 Å, which falls in the range of the polymer with shorter carbohydrate-spacing. Still, both polymers should be capable of clustering L-selectin, so the origin of the differences is not obvious. The authors raise the possibility that optimal ligand spacing may be important in eliciting signal transduction.

3.2 Mimics of pathogen glycans

The carbohydrates on the surfaces of pathogens can engage host receptors and activate signaling. Many pathogen recognition receptors (PRRs) of the innate immune system have evolved to recognized conserved carbohydrate epitopes on the pathogen surface. One key family of PRRs includes the C-type lectin receptors (CLRs), whose members are named for their dependence on calcium ions to facilitate carbohydrate binding.[149] Several CLRs are found on dendritic cells (DCs).[150] DCs are the major antigen-presenting cells of the immune system, and DC lectins function as antigen receptors, as regulators of DC migration, and as facilitators that mediate binding to other immune cell types.[151, 152] The multiple functions of DC lectins contribute to an appropriate immune response.

One CLR of particular interest, DC-specific ICAM-3-grabbing non-integrin (DC-SIGN), is a lectin implicated in numerous functions.[153, 154] Through interactions with high mannose glycans or fucose-containing Lewis-type antigens on self-glycoproteins ICAM-3 and ICAM-2, DC-SIGN can mediate T cell interactions and trans-endothelial migration.[155–157] In addition, DC-SIGN is thought to play a role in pathogen recognition and processing, as anti-DC-SIGN antibodies are internalized, processed and presented to T cells.[158] Despite its putative role in healthy immune function, DC-SIGN is exploited by a variety of pathogens, which deploy pathogen-specific mechanisms.[159, 160] DC-SIGN binds to the mannosylated envelope glycoprotein gp120 on HIV-1 to mediate infection of T cells *in trans*.[7, 156, 161] Alternatively, binding of DC-SIGN to mannosylated glycoproteins on the surface of *Mycobacterium tuberculosis* activates signaling pathways that lead to immunosuppression.[162] How each pathogen exploits DC-SIGN to take advantage of distinct escape routes remains unclear. Glycopolymers may prove critical tools in understanding the role of antigen structure on DC-SIGN function.

Initial studies of DC-SIGN primarily focused on inhibitors (Figure 10), as inhibitors could block its ability to disseminate viruses, such as HIV.[163–166] Haddleton and coworkers, for instance, synthesized mannose-substituted glycopolymers using ATRP.[167] They controlled the ratio of a mannoside (DC-SIGN ligand) and galactoside (non-binding) moiety incorporated into the polymers. This strategy of using a non-binding carbohydrate group as a spacer ligand is identical to that employed by Cairo et al. (Section 2.2). As its mannose density increased, so did the ability of the glycopolymers to disrupt the gp120-DC-SIGN interaction. Polymer scaffolds also served as vehicles to present non-carbohydrate glycomimetics (such as **4**)[168] to DC-SIGN.[97] The functional affinity of the glycomimetic was found to be enhanced significantly over that of the monovalent glycomimetic.

Increasing evidence suggests that the propensity of pathogens to appropriate DC-SIGN arises from the lectin's ability to participate in signal transduction complexes.[169, 170] DC-SIGN also is involved in antigen uptake. It can internalize a variety of synthetic multivalent ligands, including mannose-functionalized gold nanoparticles[171], glycoprotein surrogates[16], mannosylated dendrimers[172], and glycopolymeric nanoparticles[173] Whether these synthetic multivalent mannose derivatives promote signaling in dendritic cells is largely unknown.

Data are emerging that implicate DC-SIGN in transducing signaling. Narasimhan and coworkers generated mannosylated polyanhydride nanoparticles that were capable of eliciting DC maturation, a phenomenon that occurs upon interaction with antigen; however, the outcome was not entirely DC-SIGN-dependent.[173] Additionally, a study demonstrated that glycopeptidic dendrimers can be internalized by DC-SIGN, activate DC-SIGN signal transduction, and even deliver a peptide antigen for presentation to T cells.[70] In general, DC-SIGN signals in collaboration with another class of PRRs, the Toll-like receptors (TLRs).[174] TLRs bind to a wide variety of pathogenic epitopes and ultimately results in cytokine production. DC-SIGN stimulation in the presence of TLRs leads to an amplification of TLR-induced cytokine production. One potential means of regulating cytokine production is by controlling the structural features of the DC-SIGN ligand, such as the type of carbohydrate ligand, its valency, or its ability to present other epitopes that promote cross-talk between receptors.[170] Glycopolymers and related displays might therefore not only inhibit DC-SIGN but also elicit tailored signals.

There are hints that glycopolymer-promoted DC-SIGN signaling may lead to new insight into DC-SIGN function in immunity and pathogen evasion of the immune system. A recent study by Prost et al. suggests that glycopolymers that bind to DC-SIGN do elicit signaling.[16] Specifically, glycoprotein surrogates displaying glycomimetic **4** promote signal transduction. In contrast, Ribeiro-Viana et al. produced mannosylated second-generation

glycodendrimers that were internalized by DC-SIGN, but no signaling was detected.[172] While neither compound has been assessed for its ability to cluster DC-SIGN, the larger glycoprotein surrogates are likely more capable of mediating DC-SIGN clustering. Thus, the extent of DC-SIGN clustering may determine the level of signal transmission. Forthcoming systematic studies using glycopolymers will undoubtedly aid in understanding how DC-SIGN mediates differential responses to distinct pathogens.

4. Mimics of soluble polysaccharides

Glycopolymers share with glycoproteins (including mucins) a general arrangement in which the glycans emanate from a backbone (either synthetic or proteinaceous) (Figure 11). In contrast, glycosaminoglycans and other polysaccharides are linear carbohydrate chains. Proteins can assemble on these linear polysaccharide chains (e.g., heparan sulfate) to mediate signaling.[175, 176] Hsieh-Wilson and coworkers demonstrated that glycopolymers could serve as glycosaminoglycan mimics. Their investigation was prompted by their interest in the role of glycosaminoglycan interactions in axon regrowth. A major barrier in functional recovery after injury to the central nervous system is the inhibitory environment encountered by regenerating axons.[177] After recruitment of astrocytes to the injury site, these cells release chondroitin sulfate proteoglycans (CSPGs).[178–180] The highly sulfated polysaccharides are the principal inhibitory components of axon regeneration, but their mechanism of action was poorly understood. The consensus was that CSPGs primarily acted as an additional physical barrier to axon regrowth. The function of CSPGs in axon regeneration was elusive because of the complexities associated with GSPG structure. Though studies had suggested distinct roles for specific sulfation patterns in GSPGs, these conclusions rested on experiments conducted using heterogeneous polysaccharides.[181–183]

Hsieh-Wilson and colleagues used ROMP to synthesize glycopolymers that served as CSPG mimics.[105] They assembled polymers bearing disaccharide and tetrasaccharide sequences derived from the biologically active chondroitin sulfate-E (CS-E) epitope. The ability of the glycopolymers to promote outgrowth of hippocampal neurons was compared to that of a natural CS-E polysaccharide. As expected, the natural CS-E polysaccharide inhibited 100% of neurite outgrowth. Intriguingly, the glycopolymer bearing the tetrasaccharide also inhibited 100% of neurite outgrowth. The activity of the disaccharide-substituted glycopolymer depended on its length—as glycopolymer length increased so neurite outgrowth decreased.

Access to CSPG surrogates allowed for definitive studies on the role of specific CSPG sulfation patterns in axon regeneration. Homoglycopolymers **5** – **7** containing key disaccharides from chondroitin sulfate polysaccharides CS-A, CS-C, or CS-E were synthesis (Figure 12).[106] The CSPG mimics were analyzed for inhibition of neurite outgrowth. For comparison, the natural polysaccharides enriched in the three sulfation patterns were tested. Interestingly, only CS-E glycopolymer **7** and the CS-E enriched preparations blocked neurite outgrowth. These data suggest that sulfation at both the 4- and 6-position of *N*-acetylgalactosamine in CS polysaccharides is required for inhibition.

The authors found that CS-E glycopolymer **7** could promote signaling. CSPGs and myelin inhibitors activate Rho/Rho-kinase (ROCK) and epidermal growth factor receptor (EGFR) pathways to impede axon regeneration.[184–186] Pharmacological inhibition of ROCK and EGFR with synthetic inhibitors reversed the inhibitory effects of CS-E glycopolymer **7** on the cells. Analogous effects were observed on cells treated with natural polysaccharides enriched with CS-E. The ability of CS-E surrogates to elicit downstream signaling events suggests that they interact directly with a cell-surface receptor. CSPGs can engage protein tyrosine phosphatase PTPs, a glycosaminoglycan binding cell-surface receptor[187, 188], and

this interaction may be involved in axon regeneration. PTPs was screened in a carbohydrate array and found to interact only with the sulfation pattern present in CS-E polysaccharides. In addition, deletion of PTPs attenuated the inhibitory effects of glycopolymer **7**. Therefore, it appears CS-E enriched CSPGs may signal through PTPs to inhibit axon regeneration.

These investigations highlight the power of glycopolymers to act as functional mimics of chondroitin sulfate proteoglycans. They also underscore the remarkable ability of glycopolymers to mimic a wide range of glycans. Further highlighting that point, antibodies raised against CS-E glycopolymer were specific for the CS-E epitope and had no cross-reactivity with other sulfated glycans.[106] These studies highlight the utility of glycopolymers for activating and probing signaling pathways that involve proteoglycans or other polysaccharides.

5. Assembling multireceptor complexes

CD22 is a member of the Siglec (sialic acid binding immunoglobulin-like lectins) family. Siglecs are prevalent on the surfaces of immune cells, where they play key roles in innate and adaptive immunity.[189, 190] Glycopolymers have been used to probe how one member of the Siglec class, CD22, leads to attenuation of B cell responses.

Initiation of an immune response and the prevention of autoimmunity is influenced by the ability of the B cell antigen receptor (BCR) to transmit signals that both positively and negatively regulate B lymphocyte survival, proliferation, and differentiation.[191] To aid in discriminating between self- and non-self, co-receptors that modulate BCR signaling help ensure these distinctions are made. CD22 is an inhibitory coreceptor that can attenuate BCR signaling.[190] Studies in CD22-deficient mice suggest that CD22 acts to increase the threshold for B cell activation.[192–194] CD22 recognizes a-(2,6)-linked sialylated glycans[195], which are present on some antigens but also abundant on the surface of B cells.[195] C*is* interactions between CD22 and proximal surface glycans can mask the coreceptor to exogenous (*trans*) ligands. No binding of even multivalent trisaccharide CD22 ligands was observed, until the cells were treated to remove or disable cell surface sialic acid residues.[14, 196–199] Paulson and coworkers, however, generated modified oligosaccharide derivatives with higher affinity for CD22, and multivalent displays of these ligands could out-compete the cis interactions to bind CD22 in *trans*.[14] Data indicate that the functional role of the *cis* interactions between CD22 and surface glycoproteins is to sequester CD22 from the BCR prior to antigen stimulation.[13, 200]

In addition to the importance of *cis* interactions, evidence had been mounting that *trans* interactions are important.[12201] A molecular mechanism by which *trans* interactions alter signaling can be formulated. CD22 possesses cytoplasmic motifs that can recruit a phosphatase to the BCR signaling complex to counteract kinases that lead to B cell activation. If *trans* interactions are important, antigens that can co-cluster CD22 and the BCR should give rise to attenuated immune activation. Mixed glycopolymers provided an ideal vehicle with which to test this model, both at the level of signal transduction in cells[11] and immune suppression in vivo.[202]

Glycopolymers were used to elucidate a role for *trans* interactions with CD22 in modulating B cell signaling (Figure 12).[198] Glycopolymers were synthesized that display glycan ligands for CD22 (Figure 12, R^2 = H) and the 2,4 dinitrophenyl (DNP, R^1 = $-NO_2$) hapten that could engage the BCR. DNP-displaying polymers had been shown to elicit activation in a B cell line with a DNP-binding BCR[10], and this cell line was used to test whether *trans* interactions could influence signaling through CD22 (Figure 13).[11] Specifically, if only *cis* CD22 interactions are relevant, CD22 would be masked, and the polymers would interact solely

with the BCR to promote B cell activation. In contrast, if the polymers engage both the BCR and CD22 through *trans* interactions, B-cell activation would be dampened.

To determine the outcome, a DNP homopolymer, CD22L homopolymer, and CD22L/DNP copolymer were employed. A hallmark of B cell signaling involves the influx of intracellular Ca^{2+}. If BCR signaling is activated, as with DNP homopolymer[10], an influx of Ca^{2+} should be observed. The CD22L homopolymer did not elicit any change in intracellular Ca^{2+}, consistent with its inability to bind the BCR. If the copolymer could recruit CD22 to the BCR complex, B cell activation should be suppressed and Ca^{2+} influx should be attenuated. It is that outcome that was observed. These data indicate that co-clustering of CD22 and the BCR results in signal attenuation. These glycopolymers were used further to identify which proteins involved in BCR signaling were activated and which were deactivated upon co-clustering of CD22 and the BCR.

Paulson and coworkers carried out in vivo studies that highlight the role of *trans* interactions with CD22 for attenuating B cell activation.[202] Using a polyacrylamide polymer functionalized with a nitrophenyl hapten (R^1 = H) and a high affinity CD22 ligand (R^2 = biphenyl), mice were treated with the glycopolymers and their B cell response was measured. The glycopolymers were non-immunogenic, and they promoted long-lived tolerance, preventing B cell responses when the mice were challenged with an immunogen. When mice deficient in CD22 were treated with these polymers, no tolerogenic response was observed. Together, the cell-based and in vivo investigations lead to the importance of *trans* CD22 interactions. They support a role for antigen glycosylation as an innate form of self-recognition.[201]

These experiments also demonstrate the utility of using glycopolymers to address mechanistic questions in signaling. Polymer scaffolds can present multiple ligands for multiple receptors. Thus, they are ideal tools to dissect cross-talk between cell surface receptors. Additionally, the glycopolymers described in this section can serve as therapeutic leads for the design of agents that inhibit or suppress autoimmune responses.

6. Conclusions and Future Outlook

Cell surface protein receptors are tasked with the essential role of sensing the cell's environment and initiating a rapid response to ensure survival. These receptors rarely act as individual entities, but instead work in concert to form highly sensitive, macromolecular signaling assemblies. Glycopolymers that can promote and stabilize these complexes are powerful agents for understanding and exploiting mechanisms of signal transduction. The advent of new polymerization methods that can yield glycopolymers of diverse architecture provides the means to optimize glycopolymers to elicit or inhibit signal transduction. Thus, they can elucidate critical aspects of signal transduction that elude traditional approaches. How glycopolymer topology influences signaling is largely unexplored, and this arena remains an exciting frontier.[125, 203–205]

Glycopolymers may also prove useful in dissecting the role of oligosaccharides in the assembly of multiprotein supramolecular complexes in signal transduction. In addition to their utility in understanding signal transduction, glycopolymers have possible therapeutic uses.[206] Their ability to modulate immune responses may lead to new therapeutic strategies.[48–54, 207] For example, the Bundle and Paulson groups described heterobifunctional glycopolymers that template IgM onto the surface of lymphoma cells to elicit humoral cytotoxicity.[199] Glycopolymers are also being explored in vaccine development[208] and drug delivery.[209–212] We hope that this overview will spur new applications of synthetic glycopolymers to explore and exploit diverse signal transduction processes.

References and Notes

1. Ito S. Philos Trans R Soc Lond B Biol Sci. 1974; 268:55–66. [PubMed: 4155091]
2. Costerton JW, Irvin RT, Cheng KJ. Annu Rev Microbiol. 1981; 35:299–324. [PubMed: 7027902]
3. Schröter S, Osterhoff C, McArdle W, Ivell R. Hum Reprod Update. 1999; 5:302–313. [PubMed: 10465522]
4. Florman HM, Wassarman PM. Cell. 1985; 41:313–324. [PubMed: 2986849]
5. Pang PC, Chiu PCN, Lee CL, Chang LY, Panico M, Morris HR, Haslam SM, Khoo KH, Clark GF, Yeung WSB, Dell A. Science. 2011; 333:1761–1764. [PubMed: 21852454]
6. Genbacev OD, Prakobphol A, Foulk RA, Krtolica AR, Ilic D, Singer MS, Yang ZQ, Kiessling LL, Rosen SD, Fisher SJ. Science. 2003; 299:405–408. [PubMed: 12532021]
7. Kwon DS, Gregorio G, Bitton N, Hendrickson WA, Littman DR. Immunity. 2002; 16:135–144. [PubMed: 11825572]
8. Kooyk, Yv; Rabinovich, GA. Nat Immunol. 2008; 9:593–601. [PubMed: 18490910]
9. Rabinovich GA, van Kooyk Y, Cobb BA. Ann N Y Acad Sci. 2012; 1253:1–15. [PubMed: 22524422]
10. Puffer EB, Pontrello JK, Hollenbeck JJ, Kink JA, Kiessling LL. ACS Chem Biol. 2007; 2:252–262. [PubMed: 17432821]
11. Courtney AH, Puffer EB, Pontrello JK, Yang ZQ, Kiessling LL. Proc Natl Acad Sci U S A. 2009; 106:2500–2505. [PubMed: 19202057]
12. Collins BE, Blixt O, DeSieno AR, Bovin N, Marth JD, Paulson JC. Proc Natl Acad Sci U S A. 2004; 101:6104–6109. [PubMed: 15079087]
13. Collins BE, Smith BA, Bengtson P, Paulson JC. Nat Immunol. 2006; 7:199–206. [PubMed: 16369536]
14. Collins BE, Blixt O, Han S, Duong B, Li H, Nathan JK, Bovin N, Paulson JC. J Immunol. 2006; 177:2994–3003. [PubMed: 16920935]
15. Hernandez JD, Baum LG. Glycobiology. 2002; 12:127R–136R.
16. Prost LR, Grim JC, Tonelli M, Kiessling LL. ACS Chem Biol. 2012; 7:1603–1608. [PubMed: 22747463]
17. Varki A. Glycobiology. 1993; 3:97–130. [PubMed: 8490246]
18. Kiessling LL, Splain RA. Annu Rev Biochem. 2010; 79:619–653. [PubMed: 20380561]
19. Weis WI, Drickamer K. Annu Rev Biochem. 1996; 65:441–473. [PubMed: 8811186]
20. Rini JM. Annu Rev Biophys Biomol Struct. 1995; 24:551–577. [PubMed: 7663127]
21. Gabius HJ, André S, Jiménez-Barbero J, Romero A, Solís D. Trends Biochem Sci. 2011; 36:298–313. [PubMed: 21458998]
22. Lundquist JJ, Toone EJ. Chem Rev. 2002; 102:555–578. [PubMed: 11841254]
23. Sacchettini JC, Baum LG, Brewer CF. Biochemistry (Mosc). 2001; 40:3009–3015.
24. Mann DA, Kanai M, Maly DJ, Kiessling LL. J Am Chem Soc. 1998; 120:10575–10582.
25. Dam TK, Roy R, Pagé D, Brewer CF. Biochemistry (Mosc). 2002; 41:1359–1363.
26. Page MI, Jencks WP. Proc Natl Acad Sci U S A. 1971; 68:1678–1683. [PubMed: 5288752]
27. Kiessling LL, Pohl NL. Chem Biol. 1996; 3:71–77. [PubMed: 8807830]
28. Lee RT, Lee YC. Glycoconj J. 2000; 17:543–551. [PubMed: 11421347]
29. Mammen M, Choi SK, Whitesides GM. Angew Chem Int Ed. 1998; 37:2755–2794.
30. Mortell KH, Weatherman RV, Kiessling LL. J Am Chem Soc. 1996; 118:2297–2298.
31. Rao J, Lahiri J, Isaacs L, Weis RM, Whitesides GM. Science. 1998; 280:708–711. [PubMed: 9563940]
32. Perelson AS. Math Biosci. 1981; 53:1–39.
33. Kiessling LL, Gestwicki JE, Strong LE. Angew Chem Int Ed. 2006; 45:2348–2368.
34. Kiessling LL, Gestwicki JE, Strong LE. Curr Opin Chem Biol. 2000; 4:696–703. [PubMed: 11102876]
35. Cloninger MJ. Curr Opin Chem Biol. 2002; 6:742–748. [PubMed: 12470726]

36. Röglin L, Lempens EHM, Meijer EW. Angew Chem Int Ed. 2010; 50:102–112.
37. Manimala JC, Roach TA, Li Z, Gildersleeve JC. Glycobiology. 2007; 17:17C–23C.
38. Becer CR. Macromol Rapid Commun. 2012; 33:742–752. [PubMed: 22508520]
39. Ting SRS, Chen G, Stenzel MH. Polym Chem. 2010; 1:1392–1412.
40. Miura Y. J Polym Sci, Part A: Polym Chem. 2007; 45:5031–5036.
41. Hild WA, Breunig M, Göpferich A. Eur J Pharm Biopharm. 2008; 68:153–168. [PubMed: 17869074]
42. Gao X, Wang T, Wu B, Chen J, Chen J, Yue Y, Dai N, Chen H, Jiang X. Biochem Biophys Res Commun. 2008; 377:35–40. [PubMed: 18823949]
43. Kikkeri R, Lepenies B, Adibekian A, Laurino P, Seeberger PH. J Am Chem Soc. 2009; 131:2110–2112. [PubMed: 19199612]
44. Chien YY, Jan MD, Adak AK, Tzeng HC, Lin YP, Chen YJ, Wang KT, Chen CT, Chen CC, Lin CC. Chem Bio Chem. 2008; 9:1100–1109.
45. Song EH, Osanya AO, Petersen CA, Pohl NLB. J Am Chem Soc. 2010; 132:11428–11430. [PubMed: 20669964]
46. Reynolds M, Marradi M, Imberty A, Penadés S, Pérez S. Chem Eur J. 2012; 18:4264–4273. [PubMed: 22362615]
47. Matyjaszewski K. Science. 2011; 333:1104–1105. [PubMed: 21868664]
48. Spain SG, Cameron NR. Polym Chem. 2010; 2:60–68.
49. Roy R, Baek MG, Rittenhouse-Olson K. J Am Chem Soc. 2001; 123:1809–1816. [PubMed: 11456798]
50. Baek MG, Roy R. Biorg Med Chem. 2002; 10:11–17.
51. Hulikova K, Benson V, Svoboda J, Sima P, Fiserova A. Int Immunopharmacol. 2009; 9:792–799. [PubMed: 19303462]
52. Hulikova K, Svoboda J, Benson V, Grobarova V, Fiserova A. Int Immunopharmacol. 2011; 11:955–961. [PubMed: 21349367]
53. Rele SM, Cui W, Wang L, Hou S, Barr-Zarse G, Tatton D, Gnanou Y, Esko JD, Chaikof EL. J Am Chem Soc. 2005; 127:10132–10133. [PubMed: 16028900]
54. Geng J, Mantovani G, Tao L, Nicolas J, Chen G, Wallis R, Mitchell DA, Johnson BRG, Evans SD, Haddleton DM. J Am Chem Soc. 2007; 129:15156–15163. [PubMed: 18020332]
55. Schnaar RL, Lee YC. Biochemistry (Mosc). 1975; 14:1535–1541.
56. Horejsí V, Smolek P, Kocourek J. Biochim Biophys Acta. 1978; 538:293–298. [PubMed: 620071]
57. Spaltenstein A, Whitesides GM. J Am Chem Soc. 1991; 113:686–687.
58. Matrosovich MN, Mochalova LV, Marinina VP, Byramova NE, Bovin NV. FEBS Lett. 1990; 272:209–212. [PubMed: 1699804]
59. Ladmiral V, Melia E, Haddleton DM. Eur Polym J. 2004; 40:431–449.
60. Vázquez-Dorbatt V, Lee J, Lin EW, Maynard HD. Chem Bio Chem. 2012; 13:2478–2487.
61. Deniaud D, Julienne K, Gouin SG. Org Biomol Chem. 2011; 9:966–979. [PubMed: 21173976]
62. Kiessling LL, Strong LE, Gestwicki JE. Annu Rep Med Chem. 2000; 35:321–330.
63. Braun P, Nägele B, Wittmann V, Drescher M. Angew Chem Int Ed. 2011; 50:8428–8431.
64. Lynn DM, Mohr B, Grubbs RH. J Am Chem Soc. 1998; 120:1627–1628.
65. Fraser C, Grubbs RH. Macromolecules. 1995; 28:7248–7255.
66. Kanai M, Mortell KH, Kiessling LL. J Am Chem Soc. 1997; 119:9931–9932.
67. Lynn DM, Kanaoka S, Grubbs RH. J Am Chem Soc. 1996; 118:784–790.
68. Roy R. Trends in Glycoscience and Glycotechnology. 2003; 15:291–310.
69. Lindhorst TK, Kieburg C. Angew Chem Int Ed. 1996; 35:1953–1956.
70. García-Vallejo JJ, Ambrosini M, Overbeek A, van Riel WE, Bloem K, Unger WWJ, Chiodo F, Bolscher JG, Nazmi K, Kalay H, van Kooyk Y. Mol Immunol. 2013; 53:387–397. [PubMed: 23103377]
71. Branderhorst HM, Ruijtenbeek R, Liskamp RMJ, Pieters RJ. Chem Bio Chem. 2008; 9:1836–1844.

72. Euzen R, Reymond JL. Biorg Med Chem. 2011; 19:2879–2887.
73. Walter ED, Sebby KB, Usselman RJ, Singel DJ, Cloninger MJ. J Phys Chem B. 2005; 109:21532–21538. [PubMed: 16853796]
74. Sebby KB, Walter ED, Usselman RJ, Cloninger MJ, Singel DJ. J Phys Chem B. 2011; 115:4613–4620. [PubMed: 21469686]
75. Dam TK, Brewer CF. Glycobiology. 2010; 20:270–279. [PubMed: 19939826]
76. Burns, JA.; Gibson, MI.; Becer, CR. Functional Polymers by Post-Polymerization Modification. Wiley-VCH Verlag GmbH & Co. KGaA; 2012. p. 237-265.
77. Gestwicki JE, Cairo CW, Strong LE, Oetjen KA, Kiessling LL. J Am Chem Soc. 2002; 124:14922–14933. [PubMed: 12475334]
78. David A, Kopecková P, Kopeček Ji, Rubinstein A. Pharm Res. 2002; 19:1114–1122. [PubMed: 12240936]
79. Polizzotti BD, Kiick KL. Biomacromolecules. 2006; 7:483–490. [PubMed: 16471920]
80. Kitov PI, Sadowska JM, Mulvey G, Armstrong GD, Ling H, Pannu NS, Read RJ, Bundle DR. Nature. 2000; 403:669–672. [PubMed: 10688205]
81. Fan E, Merritt EA, Verlinde CLMJ, Hol WGJ. Curr Opin Struct Biol. 2000; 10:680–686. [PubMed: 11114505]
82. Fan EK, Zhang ZS, Minke WE, Hou Z, Verlinde C, Hol WGJ. J Am Chem Soc. 2000; 122:2663–2664.
83. Polizzotti BD, Maheshwari R, Vinkenborg J, Kiick KL. Macromolecules. 2007; 40:7103–7110. [PubMed: 19169374]
84. Hollenbeck JJ, Danner DJ, Landgren RM, Rainbolt TK, Roberts DS. Biomacromolecules. 2012; 13:1996–2002. [PubMed: 22681396]
85. Ponader D, Wojcik F, Beceren-Braun F, Dernedde J, Hartmann L. Biomacromolecules. 2012; 13:1845–1852. [PubMed: 22483345]
86. Gorska K, Huang KT, Chaloin O, Winssinger N. Angew Chem Int Ed. 2009; 48:7695–7700.
87. Ciobanu M, Huang KT, Daguer JP, Barluenga S, Chaloin O, Schaeffer E, Mueller CG, Mitchell DA, Winssinger N. Chem Commun. 2011; 47:9321.
88. Englund EA, Wang D, Fujigaki H, Sakai H, Micklitsch CM, Ghirlando R, Martin-Manso G, Pendrak ML, Roberts DD, Durell SR, Appella DH. Nat Commun. 2011; 3:614–617. [PubMed: 22233624]
89. Scheibe C, Wedepohl S, Riese SB, Dernedde J, Seitz O. Chem Bio Chem. 2013; 14:236–250.
90. Das I, Désiré J, Manvar D, Baussanne I, Pandey VN, Décout JL. J Med Chem. 2012; 55:6021–6032. [PubMed: 22698070]
91. Spinelli N, Defrancq E, Morvan F. Chem Soc Rev. 2013
92. Jayaraman N. Chem Soc Rev. 2009; 38:3463–3483. [PubMed: 20449063]
93. Clarke C, Woods RJ, Gluska J, Cooper A, Nutley MA, Boons GJ. J Am Chem Soc. 2001; 123:12238–12247. [PubMed: 11734024]
94. Kadirvelraj R, Foley BL, Dyekjær JD, Woods RJ. J Am Chem Soc. 2008; 130:16933–16942. [PubMed: 19053475]
95. Sigal GB, Mammen M, Dahmann G, Whitesides GM. J Am Chem Soc. 1996; 118:3789–3800.
96. Schlegel MK, Hütter J, Eriksson M, Lepenies B, Seeberger PH. Chem Bio Chem. 2011; 12:2791–2800.
97. Garber KCA, Wangkanont K, Carlson EE, Kiessling LL. Chem Commun. 2010; 46:6747–6749.
98. Gestwicki JE, Strong LE, Kiessling LL. Angew Chem Int Ed. 2000; 39:4567–4570.
99. Falke JJ, Bass RB, Butler SL, Chervitz SA, Danielson MA. Annu Rev Cell Dev Biol. 1997; 13:457–512. [PubMed: 9442881]
100. Hazelbauer GL, Falke JJ, Parkinson JS. Trends Biochem Sci. 2008; 33:9–19. [PubMed: 18165013]
101. Adler J. Annu Rev Biochem. 1975; 44:341–356. [PubMed: 1094913]
102. Maddick JR, Shapiro L. Science. 1993; 259:1754–1757. [PubMed: 8456303]
103. Gestwicki JE, Strong LE, Kiessling LL. Chem Biol. 2000; 7:583–591. [PubMed: 11048949]

104. Gestwicki JE, Kiessling LL. Nature. 2002; 415:81–84. [PubMed: 11780121]
105. Rawat M, Gama CI, Matson JB, Hsieh-Wilson LC. J Am Chem Soc. 2008; 130:2959–2961. [PubMed: 18275195]
106. Brown JM, Xia J, Zhuang B, Cho KS, Rogers CJ, Gama CI, Rawat M, Tully SE, Uetani N, Mason DE, Tremblay ML, Peters EC, Habuchi O, Chen DF, Hsieh-Wilson LC. Proc Natl Acad Sci U S A. 2012; 109:4768–4773. [PubMed: 22411830]
107. Hasegawa T, Kondoh S, Matsuura K, Kobayashi K. Macromolecules. 1999; 32:6595–6603.
108. Godula K, Rabuka D, Nam KT, Bertozzi CR. Angew Chem Int Ed. 2009; 48:4973–4976.
109. Yu L, Huang M, Wang PG, Zeng X. Anal Chem. 2007; 79:8979–8986. [PubMed: 17973352]
110. Hoshino Y, Nakamoto M, Miura Y. J Am Chem Soc. 2012; 134:15209–15212. [PubMed: 22946923]
111. Kumar J, McDowall L, Chen G, Stenzel MH. Polym Chem. 2011; 2:1879–1886.
112. Gestwicki JE, Cairo CW, Mann DA, Owen RM, Kiessling LL. Anal Biochem. 2002; 305:149–155. [PubMed: 12054443]
113. Oyelaran O, Gildersleeve JC. Curr Opin Chem Biol. 2009; 13:406–413. [PubMed: 19625207]
114. Rillahan CD, Paulson JC. Annu Rev Biochem. 2011; 80:797–823. [PubMed: 21469953]
115. Blixt O, Head S, Mondala T, Scanlan C, Huflejt ME, Alvarez R, Bryan MC, Fazio F, Calarese D, Stevens J, Razi N, Stevens DJ, Skehel JJ, van Die I, Burton DR, Wilson IA, Cummings R, Bovin N, Wong CH, Paulson JC. Proc Natl Acad Sci U S A. 2004; 101:17033–17038. [PubMed: 15563589]
116. Bochner BS. J Biol Chem. 2004; 280:4307–4312. [PubMed: 15563466]
117. Liang PH, Wu CY, Greenberg WA, Wong CH. Curr Opin Chem Biol. 2008; 12:86–92. [PubMed: 18258211]
118. Adams EW, Ratner DM, Bokesch HR, McMahon JB, O'Keefe BR, Seeberger PH. Chem Biol. 2004; 11:875–881. [PubMed: 15217620]
119. Lemmon MA, Schlessinger J. Cell. 2010; 141:1117–1134. [PubMed: 20602996]
120. Conner SD, Schmid SL. Nature. 2003; 422:37–44. [PubMed: 12621426]
121. Sorkin A, von Zastrow M. Nat Rev Mol Cell Biol. 2009; 10:609–622. [PubMed: 19696798]
122. Doherty GJ, McMahon HT. Annu Rev Biochem. 2009; 78:857–902. [PubMed: 19317650]
123. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Biochem J. 2004; 377:159–169. [PubMed: 14505488]
124. Gratton SEA, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM. Proc Natl Acad Sci U S A. 2008; 105:11613–11618. [PubMed: 18697944]
125. Goodridge HS, Reyes CN, Becker CA, Katsumoto TR, Ma J, Wolf AJ, Bose N, Chan ASH, Magee AS, Danielson ME, Weiss A, Vasilakos JP, Underhill DM. Nature. 2011; 472:471–475. [PubMed: 21525931]
126. Khan MI, Mandal DK, Brewer CF. Carbohydr Res. 1991; 213:69–77. [PubMed: 1933954]
127. Easterbrook-Smith SB. Mol Immunol. 1993; 30:637–640. [PubMed: 8487780]
128. Matko J, Edidin M. Methods Enzymol. 1997; 278:444–462. [PubMed: 9170326]
129. Chen Y, Chen G, Stenzel MH. Macromolecules. 2010; 43:8109–8114.
130. Hetzer M, Chen G, Barner-Kowollik C, Stenzel MH. Macromol Biosci. 2010; 10:119–126. [PubMed: 19731270]
131. Kumar J, Bousquet A, Stenzel MH. Macromol Rapid Commun. 2011; 32:1620–1626. [PubMed: 21858890]
132. Martin AL, Li B, Gillies ER. J Am Chem Soc. 2009; 131:734–741. [PubMed: 19072231]
133. Joralemon MJ, Murthy KS, Remsen EE, Becker ML, Wooley KL. Biomacromolecules. 2004; 5:903–913. [PubMed: 15132680]
134. Thoma G, Patton JT, Magnani JL, Ernst B, Öhrlein R, Duthaler RO. J Am Chem Soc. 1999; 121:5919–5929.
135. Vestweber D, Blanks JE. Physiol Rev. 1999; 79:181–213. [PubMed: 9922371]
136. Worthylake RA, Burridge K. Curr Opin Cell Biol. 2001; 13:569–577. [PubMed: 11544025]

137. Hemmerich S. Drug Discov Today. 2001; 6:27–35. [PubMed: 11165170]
138. Rosen SD. Am J Pathol. 1999; 155:1013–1020. [PubMed: 10514381]
139. Rosen SD, Bertozzi CR. Curr Opin Cell Biol. 1994; 6:663–673. [PubMed: 7530461]
140. Rosen SD. Annu Rev Immunol. 2004; 22:129–156. [PubMed: 15032576]
141. Spertini O, Cordey AS, Monai N, Giuffrè L, Schapira M. J Cell Biol. 1996; 135:523–531. [PubMed: 8896607]
142. Hicks AER, Nolan SL, Ridger VC, Hellewell PG, Normal KE. Blood. 2002; 101:3249–3256. [PubMed: 12480716]
143. Guyer DA, Moore KL, Lynam EB, Schammel CM, Rogelj S, McEver RP, Sklar LA. Blood. 1996; 88:2415–2421. [PubMed: 8839831]
144. Owen RM, Gestwicki JE, Young T, Kiessling LL. Org Lett. 2002; 4:2293–2296. [PubMed: 12098230]
145. Gordon EJ, Strong LE, Kiessling LL. Biorg Med Chem. 1998; 6:1293–1299.
146. Mowery P, Yang ZQ, Gordon EJ, Dwir O, Spencer AG, Alon R, Kiessling LL. Chem Biol. 2004; 11:725–732. [PubMed: 15157883]
147. Crockett-Torabi E. J Leukoc Biol. 1998; 63:1–14. [PubMed: 9469467]
148. Liu S, Kiick K. Polym Chem. 2011; 2:1513. [PubMed: 23926449]
149. Weis WI, Crichlow GV, Murthy HM, Hendrickson WA, Drickamer K. J Biol Chem. 1991; 266:20678–20686. [PubMed: 1939118]
150. Steinman RM. Eur J Immunol. 2007; 37:S53–S60. [PubMed: 17972346]
151. Figdor CG, van Kooyk Y, Adema GJ. Nat Rev Immunol. 2002; 2:77–84. [PubMed: 11910898]
152. Robinson MJ, Sancho D, Slack EC, LeibundGut-Landmann S, Sousa CRe. Nat Immunol. 2006; 7:1258–1265. [PubMed: 17110942]
153. Geijtenbeek TBH, Engering A, van Kooyk Y. J Leukoc Biol. 2002; 71:921–931. [PubMed: 12050176]
154. Švajger U, Anderluh M, Jeras M, Obermajer N. Cell Signal. 2010; 22:1397–1405. [PubMed: 20363321]
155. Geijtenbeek TBH, Krooshoop D, Bleijs DA, van Vliet SJ, van Duijnhoven GCF, Grabovsky V, Alon R, Figdor CG, van Kooyk Y. Nat Immunol. 2000; 1:353–357. [PubMed: 11017109]
156. Geijtenbeek TBH, Torensma R, van Vliet SJ, van Duijnhoven GCF, Adema GJ, van Kooyk Y, Figdor CG. Cell. 2000; 100:575–585. [PubMed: 10721994]
157. van Liempt E, Bank CMC, Mehta P, Garciá-Vallejo JJ, Kawar ZS, Geyer R, Alvarez RA, Cummings RD, Kooyk Yv, van Die I. FEBS Lett. 2006; 580:6123–6131. [PubMed: 17055489]
158. Engering A, Geijtenbeek TBH, van Vliet SJ, Wijers M, van Liempt E, Demaurex N, Lanzavecchia A, Fransen J, Figdor CG, Piguet V, van Kooyk Y. J Immunol. 2002; 168:2118–2126. [PubMed: 11859097]
159. Kooyk Y, Appelmelk B, Geijtenbeek TBH. Trends Mol Med. 2003; 9:153–159. [PubMed: 12727141]
160. van Kooyk Y, Geijtenbeek TBH. Nature Reviews Immunology. 2003; 3:697–709.
161. de Witte L, Nabatov A, Geijtenbeek TBH. Trends Mol Med. 2008; 14:12–19. [PubMed: 18055263]
162. Geijtenbeek TBH, van Vliet SJ, Koppel EA, Sanchez-Hernandez M, Vandenbroucke-Grauls CMJE, Appelmelk B, van Kooyk Y. J Exp Med. 2002; 197:7–17. [PubMed: 12515809]
163. Mangold SL, Prost LR, Kiessling LL. Chem Sci. 2012; 3:772–777. [PubMed: 22639721]
164. Sattin S, Daghetti A, Thépaut M, Berzi A, Sanchez-Navarro M, Tabarani G, Rojo J, Fieschi F, Clerici M, Bernardi A. ACS Chem Biol. 2010; 5:301–312. [PubMed: 20085340]
165. Lasala F, Arce E, Otero JR, Rojo J, Delgado R. Antimicrob Agents Chemother. 2003; 47:3970–3972. [PubMed: 14638512]
166. Timpano G, Tabarani G, Anderluh M, Invernizzi D, Vasile F, Potenza D, Nieto PM, Rojo J, Fieschi F, Bernardi A. Chem Bio Chem. 2008; 9:1921–1930.
167. Becer CR, Gibson MI, Geng J, Ilyas R, Wallis R, Mitchell DA, Haddleton DM. J Am Chem Soc. 2010; 132:15130–15132. [PubMed: 20932025]

168. Schuster MC, Mann DA, Buchholz TJ, Johnson KM, Thomas WD, Kiessling LL. Org Lett. 2003; 5:1407–1410. [PubMed: 12713285]
169. Gringhuis SI, Van Der Vlist M, Van Den Berg LM, den Dunnen J, Litjens M, Geijtenbeek TBH. Nat Immunol. 2010; 11:419–426. [PubMed: 20364151]
170. Gringhuis SI, den Dunnen J, Litjens M, Van Der Vlist M, Geijtenbeek TBH. Nat Immunol. 2009; 10:1081–1088. [PubMed: 19718030]
171. Arnáiz B, Martínez-Ávila O, Falcon-Perez JM, Penadés S. Bioconjug Chem. 2012; 23:814–825. [PubMed: 22433013]
172. Ribeiro-Viana R, García-Vallejo JJ, Collado D, Pérez-Inestrosa E, Bloem K, van Kooyk Y, Rojo J. Biomacromolecules. 2012; 13:3209–3219. [PubMed: 22920925]
173. Carrillo-Conde B, Song EH, Chavez-Santoscoy A, Phanse Y, Ramer-Tait AE, Pohl NLB, Wannemuehler MJ, Bellaire BH, Narasimhan B. Mol Pharm. 2011; 8:1877–1886. [PubMed: 21882825]
174. Gringhuis SI, Geijtenbeek TBH. Methods in Enzymology: Functional Glycomics, Elsevier Inc. 2010; 480:151–164.
175. Gandhi NS, Mancera RL. Chem Biol Drug Des. 2008; 72:455–482. [PubMed: 19090915]
176. Bishop JR, Schuksz M, Esko JD. Nature. 2007; 446:1030–1037. [PubMed: 17460664]
177. Yiu G, He Z. Nat Rev Neurosci. 2006; 7:617–627. [PubMed: 16858390]
178. McKeon RJ, Schreiber RC, Rudge JS, Silver J. J Neurosci. 1991; 11:3398–3411. [PubMed: 1719160]
179. Busch SA, Silver J. Curr Opin Neurobiol. 2007; 17:120–127. [PubMed: 17223033]
180. Laabs T, Carulli D, Geller HM, Fawcett JW. Curr Opin Neurobiol. 2005; 15:116–120. [PubMed: 15721753]
181. Gilbert RJ, McKeon RJ, Darr A, Calabro A, Hascall VC, Bellamkonda RV. Mol Cell Neurosci. 2005; 29:545–558. [PubMed: 15936953]
182. Properzi F, Carulli D, Asher RA, Muir E, Camargo LM, van Kuppevelt TH, Bten Dam G, Furukawa Y, Mikami T, Sugahara K, Toida T, Geller HM, Fawcett JW. Eur J Neurosci. 2005; 21:378–390. [PubMed: 15673437]
183. Lin R, Rosahl TW, Whiting PJ, Fawcett JW, Kwok JCF. PLoS ONE. 2011; 6:e21499. [PubMed: 21747937]
184. Wang H, Katagiri Y, McCann TE, Unsworth E, Goldsmith P, Yu ZX, Tan F, Santiago L, Mills EM, Wang Y, Symes AJ, Geller HM. J Cell Sci. 2008; 121:3083–3091. [PubMed: 18768934]
185. Sivasankaran R, Pei J, Wang KC, Zhang YP, Shields CB, Xu XM, He Z. Nat Neurosci. 2004; 7:261–268. [PubMed: 14770187]
186. Kaneko M, Kubo T, Hata K, Yamaguchi A, Yamashita T. Neurosci Lett. 2007; 423:62–67. [PubMed: 17662527]
187. Aricescu AR, McKinnell IW, Halfter W, Stoker AW. Mol Cell Biol. 2002; 22:1881–1892. [PubMed: 11865065]
188. Shen Y, Tenney AP, Busch SA, Horn KP, Cuascut FX, Liu K, He Z, Silver J, Flanagan JG. Science. 2009; 326:592–596. [PubMed: 19833921]
189. Crocker PR, Paulson JC, Varki A. Nat Rev Immunol. 2007; 7:255–266. [PubMed: 17380156]
190. Pillai S, Netravali IA, Cariappa A, Mattoo H. Annu Rev Immunol. 2012; 30:357–392. [PubMed: 22224769]
191. Goodnow CC. Proc Natl Acad Sci U S A. 1996; 93:2264. [PubMed: 8637861]
192. Nitschke L, Carsetti R, Ocker B, Köhler G, Lamers MC. Curr Biol. 1997; 7:133–143. [PubMed: 9016707]
193. O'Keefe TL, WillIams GT, Davies DL, Neuberger MS. Science. 1996; 274:798–801. [PubMed: 8864124]
194. Collins BE, Smith BA, Bengtson P, Paulson JC. Nat Immunol. 2005; 7:199–206. [PubMed: 16369536]
195. Stamenkovic I, Sgroi D, Aruffo A. Cell. 1992; 68:1003. [PubMed: 1372195]
196. Razi N, Varki A. Proc Natl Acad Sci U S A. 1998; 95:7469–7474. [PubMed: 9636173]

197. Razi N, Varki A. Glycobiology. 1999; 9:1225–1234. [PubMed: 10536038]
198. Yang ZQ, Puffer EB, Pontrello JK, Kiessling LL. Carbohydr Res. 2002; 337:1605–1613. [PubMed: 12423961]
199. O'Reilly MK, Collins BE, Han S, Liao L, Rillahan C, Kitov PI, Bundle DR, Paulson JC. J Am Chem Soc. 2008; 130:7736–7745. [PubMed: 18505252]
200. Hennet T, Chui D, Paulson JC, Marth JD. Proc Natl Acad Sci U S A. 1998; 95:4504–4509. [PubMed: 9539767]
201. Lanoue A, Batista FD, Stewart M, Neuberger MS. Eur J Immunol. 2002; 32:348–355. [PubMed: 11807774]
202. Duong BH, Tian H, Ota T, Completo G, Han S, Vela JL, Ota M, Kubitz M, Bovin N, Paulson JC, Nemazee D. J Exp Med. 2010; 207:173–187. [PubMed: 20038598]
203. Rombout J, Berg AA, Berg C, Witte P, Egberts E. Journal of Fish Biology. 1989; 35:179–186.
204. Kapsenberg ML, Teunissen M, Stiekema FEM, Keizer HG. Eur J Immunol. 1986; 16:345–350. [PubMed: 3486127]
205. Link A, Zabel F, Schnetzler Y, Titz A, Brombacher F, Bachmann MF. J Immunol. 2012; 188:3724–3733. [PubMed: 22427639]
206. Bernardi A, Jiménez-Barbero J, Casnati A, De Castro C, Darbre T, Fieschi F, Finne J, Funken H, Jaeger K-E, Lahmann M, Lindhorst TK, Marradi M, Messner P, Molinaro A, Murphy PV, Nativi C, Oscarson S, Penadés S, Peri F, Pieters RJ, Renaudet O, Reymond J-L, Richichi B, Rojo J, Sansone F, Schäffer C, Turnbull WB, Velasco-Torrijos T, Vidal S, Vincent S, Wennekes T, Zuilhof H, Imberty A. Chem Soc Rev. 201310.1039/C1032CS35408J
207. Ortega-Muoz M, Morales-Sanfrutos J, Perez-Balderas F, Hernandez-Mateo F, Giron-Gonzalez MD, Sevillano-Tripero N, Salto-Gonzalez R, Santoyo-Gonzalez F. Org Biomol Chem. 2007; 5:2291. [PubMed: 17609761]
208. Lipinski T, Kitov PI, Szpacenko A, Paszkiewicz E, Bundle DR. Bioconjug Chem. 2011; 22:274–281. [PubMed: 21186827]
209. Suriano F, Pratt R, Tan JPK, Wiradharma N, Nelson A, Yang YY, Dubois P, Hedrick JL. Biomaterials. 2010; 31:2637–2645. [PubMed: 20074794]
210. Kim B, Peppas NA. J Biomater Sci, Polym Ed. 2002; 13:1271–1281. [PubMed: 12518804]
211. Ratner E-HSMJMY-HCBGAJCPSSLMSDM, Manganiello MJ, Chow Y-H, Ghosn B, Convertine AJ, Stayton PS, Schnapp LM, Ratner DM. Biomaterials. 2012; 33:6889–6897. [PubMed: 22770567]
212. Pearson S, Scarano W, Stenzel MH. Chem Commun. 2012; 48:4695–4697.

Biographies

Laura L. Kiessling holds the Steenbock Chair in Chemistry and is the Laurens Anderson Professor of Biochemistry. She received her B.S. in Chemistry from MIT and her Ph.D. in Chemistry from Yale University with Professor Stuart Schreiber. She was an ACS postdoctoral fellow in Chemical Biology at the California Institute of Technology with Peter Dervan. In 1991, she joined the University of Wisconsin-Madison. She is the founding and current Editor-In-Chief of ACS Chemical Biology. She is a Fellow of the American Academy of Arts and Sciences, the American Society for Microbiology, and a Member of the National Academy of Sciences. The Kiessling group is focused on elucidating and exploiting the mechanisms of cell surface recognition processes, especially those involving

protein–carbohydrate interactions. Her group interests include understanding how glycans are assembled, elucidating their biological roles, and using this information to co-opt or inhibit glycan interactions.

Joseph Grim was born in Chicago, Illinois. He received his B.S. in Chemistry from the University of Wisconsin—Green Bay in 2008. He then joined the research group of Professor Laura Kiessling at the University of Wisconsin—Madison where he is currently a doctoral candidate. He is designing and synthesizing multivalent glycoconjugates to study the function of C-type lectins in immunity.

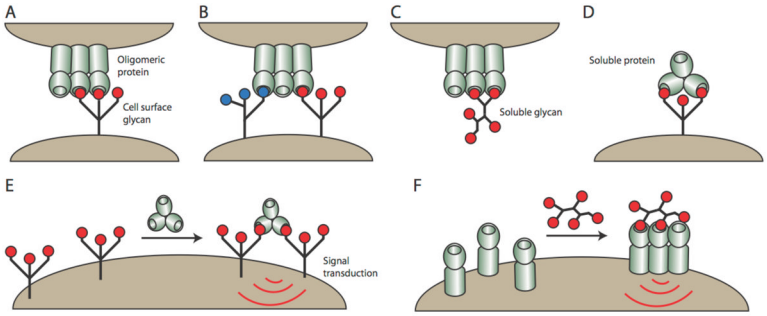

Figure 1.

Carbohydrate-binding proteins exist as oligomers and can interact with glycans through a variety of mechanisms. An oligomeric protein can interact with an individual cell-surface glycan (A) or with multiple different cell-surface glycans simultaneously (B). Oligomeric proteins can also interact with soluble glycans or soluble oligomeric lectins can engage cell surface glycans (C & D). Soluble proteins can cluster cell-surface glycoproteins to mediate signal transduction (E). Likewise, soluble glycans can cluster cell-surface receptors to mediate signal transduction (F).

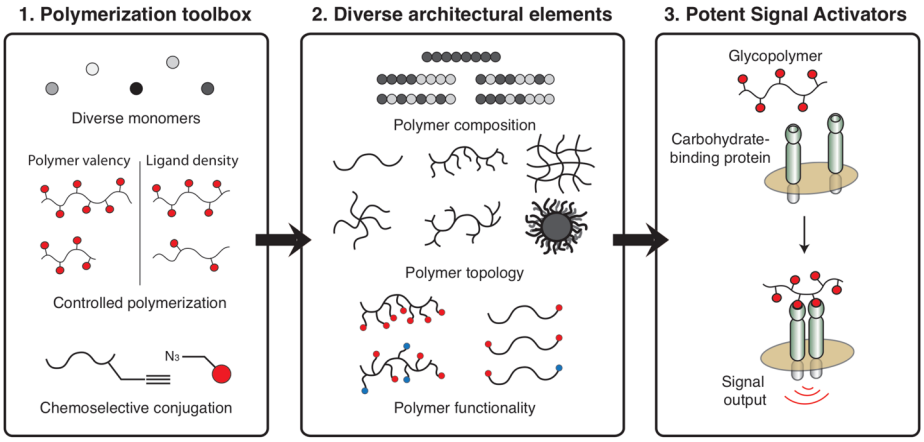

Figure 2.

(Left) Polymers can be assembled from a wide variety of monomers in a controlled manner to generate polymers of a defined length and valency. (Center) The polymers can be generated with many different topologies. In addition, it is possible to generate polymers bearing multiple functionalities, such as biological ligands or fluorophores. (Right) Finally, a key step in eliciting signal transduction is clustering of cell-surface receptors. Polymers bearing carbohydrates can cluster cell-surface proteins to elicit a signaling output. Figure adapted from reference 47.

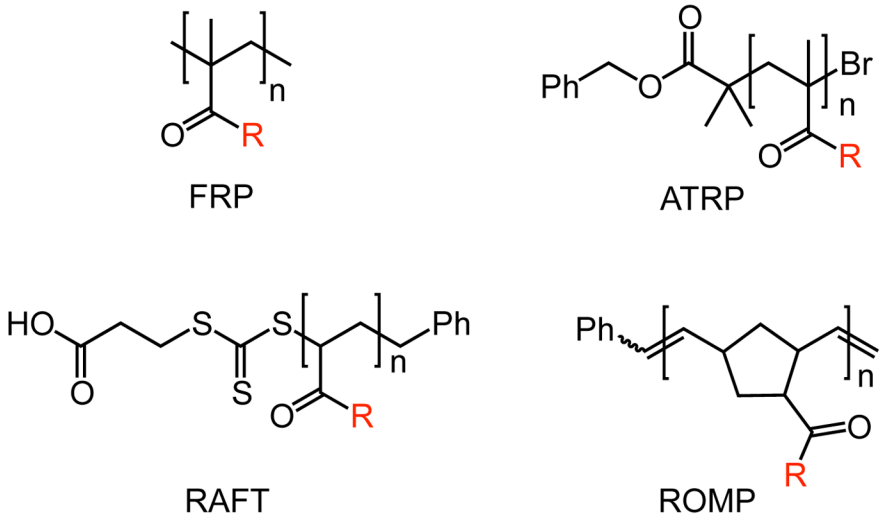

Figure 3.
Example polymer backbones generated from common polymerization strategies used in synthesizing glycopolymers. The R substituent represents a linker bearing a carbohydrate ligand, but for many glycopolymers not every monomer unit bears a carbohydrate ligand.

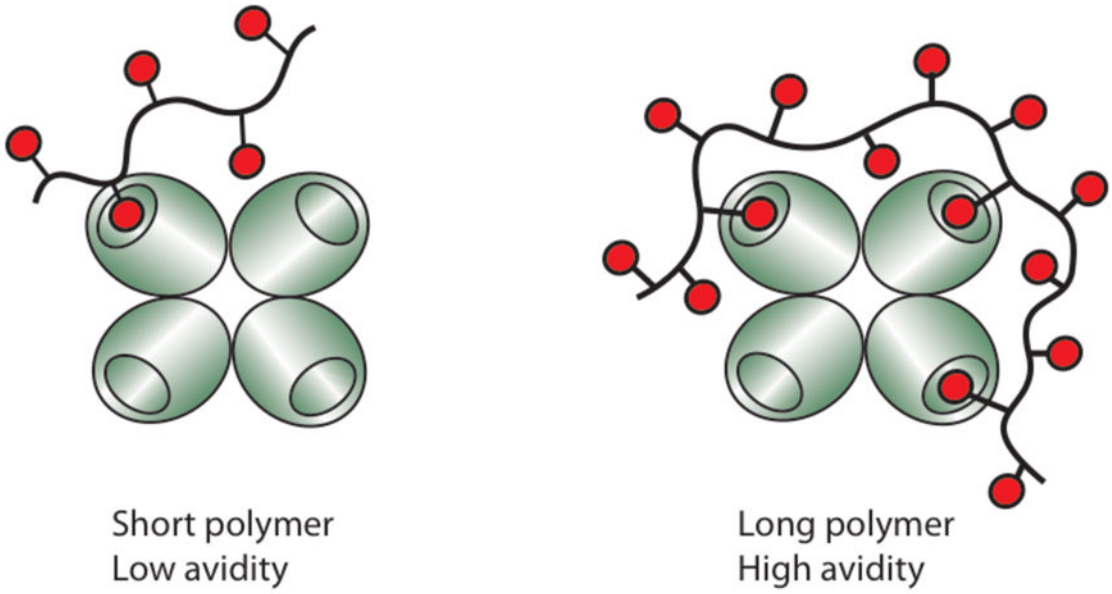

Figure 4.
Increasing polymer length (and valency) allows polymers to span multiple binding sites in oligomeric proteins, thereby increasing their functional affinity (avidity).

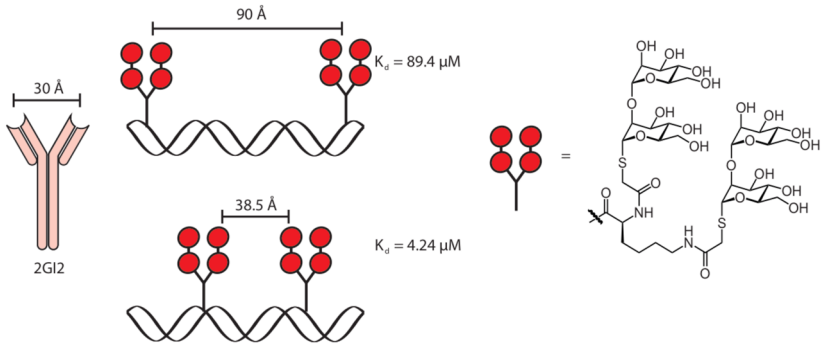

Figure 5.

Peptide nucleic acids (PNAs) were generated to control the spacing of carbohydrates. The library of PNAs was screened against the dimeric antibody 2GI2, which binds to HIV gp120. As the distance between carbohydrates approached the distance between carbohydrate binding sites on 2GI2, the functional affinity increased.

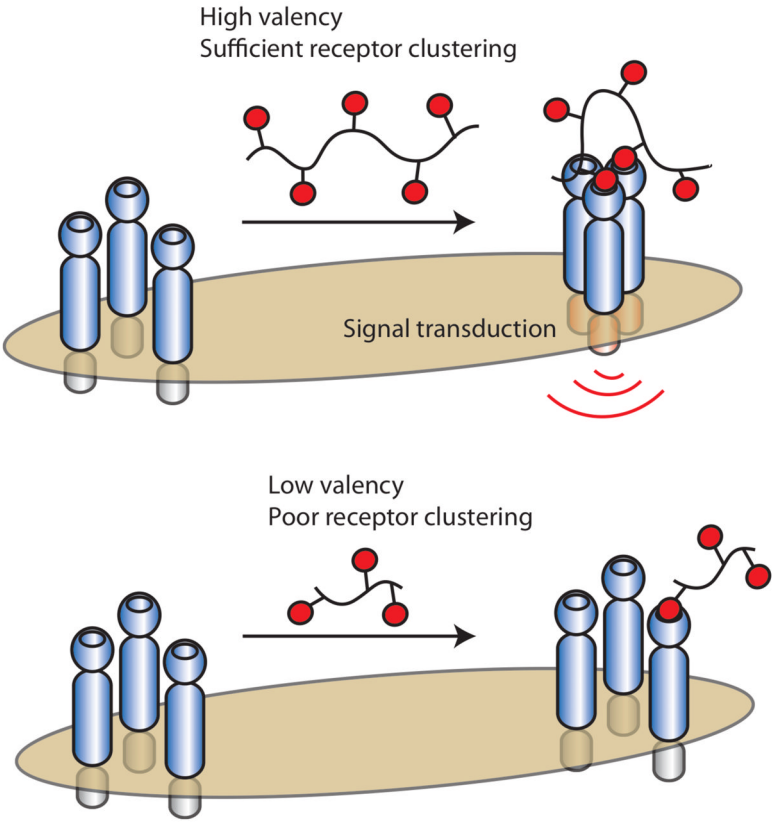

Figure 6.
Polymers of sufficient length are capable of bridging multiple surface receptors, clustering them, and initiating signal transduction.

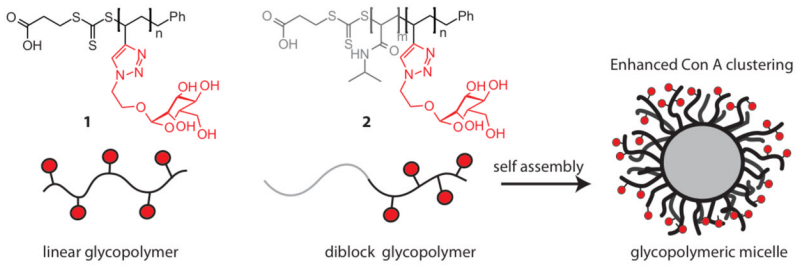

Figure 7.

Stenzel and coworkers synthesized linear glycopolymers and diblock glycopolymers, which self assembled to form glycopolymeric micelles. The glycopolymeric micelle was more efficient at clustering Con A than the linear glycopolymer.

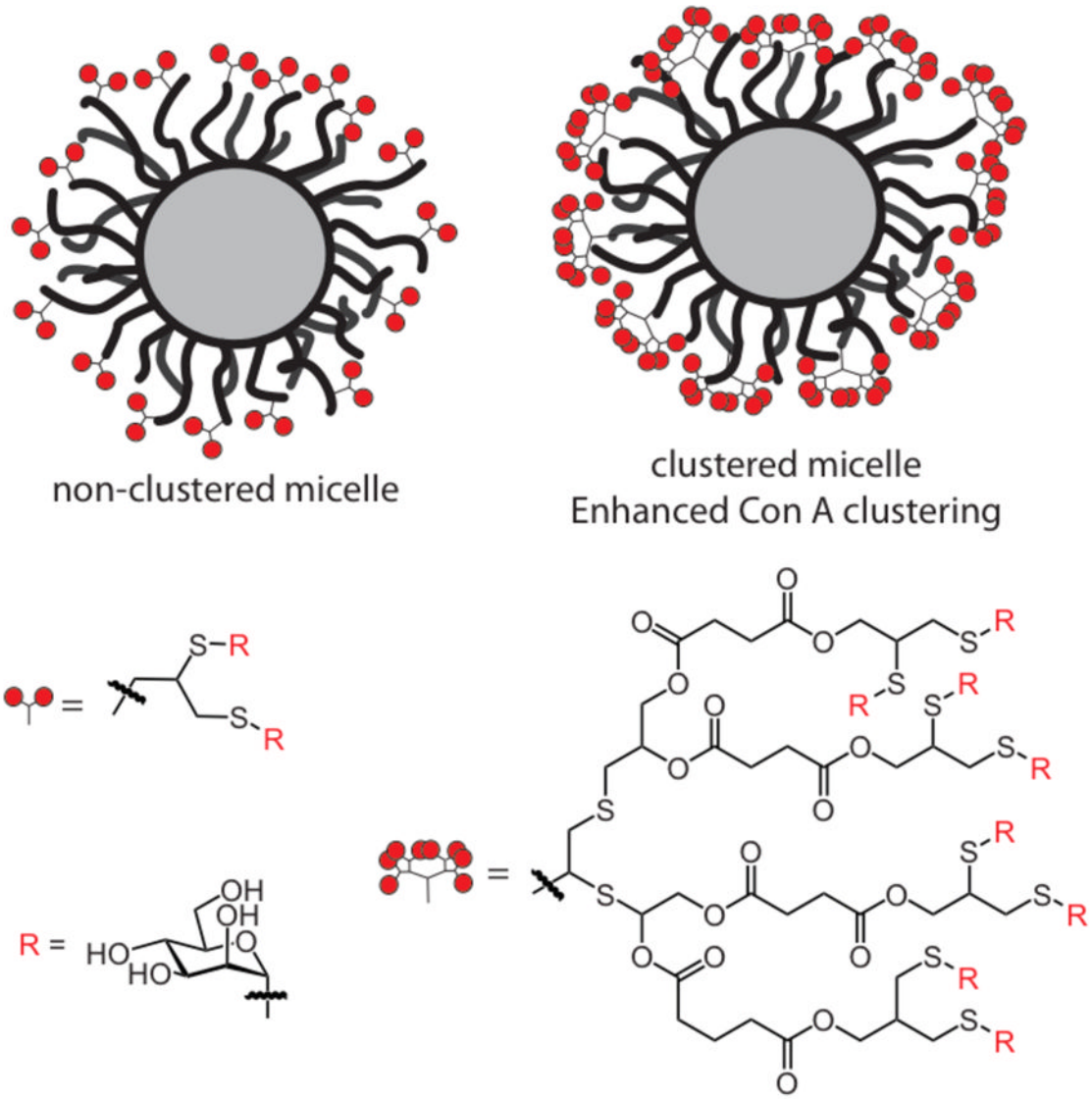

Figure 8.
An investigation of the role of ligand clustering. Glycopolymers displaying carbohydrate clusters were much more effective at clustering the lectin Con A.

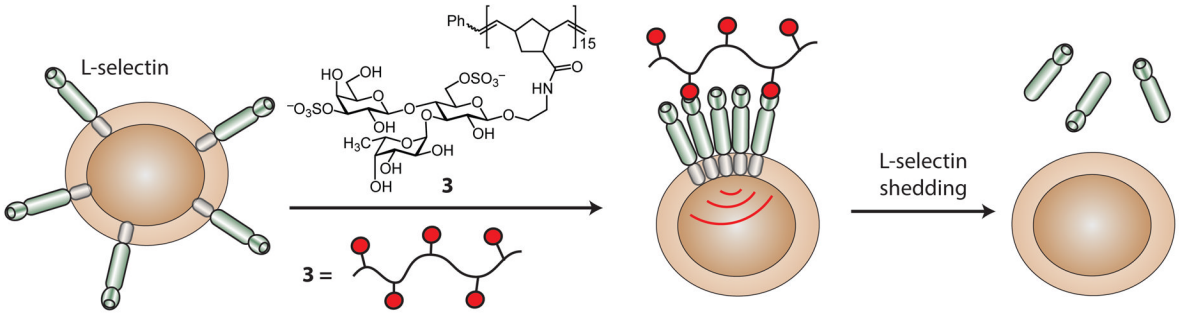

Figure 9.

Glycopolymers were used to promote L-selectin signal transduction. Upon clustering of the surface L-selectin by the glycopolymer, signal transduction occurs that leads to the proteolytic cleavage of L-selectin.

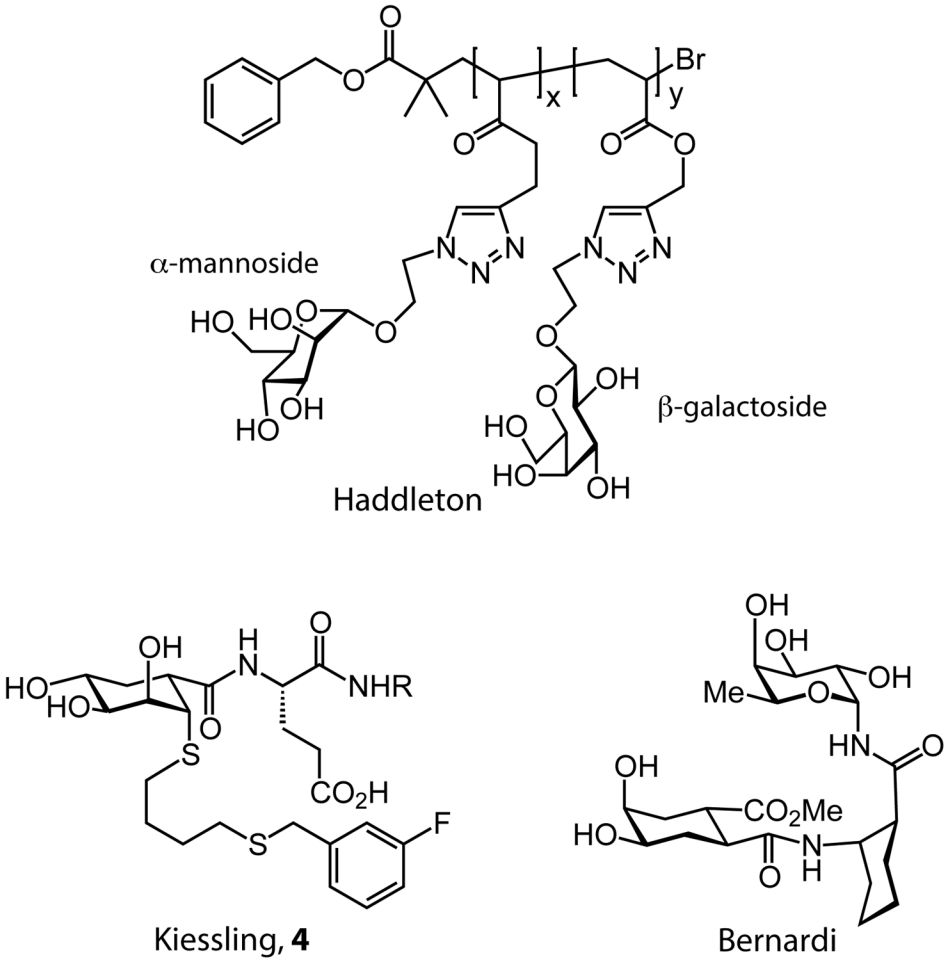

Figure 10.

Various inhibitors of DC-SIGN. For compound **4**, R=H for a monovalent inhibitor; alternatively, R can be a linker appended to synthetic polymer or protein backbone.

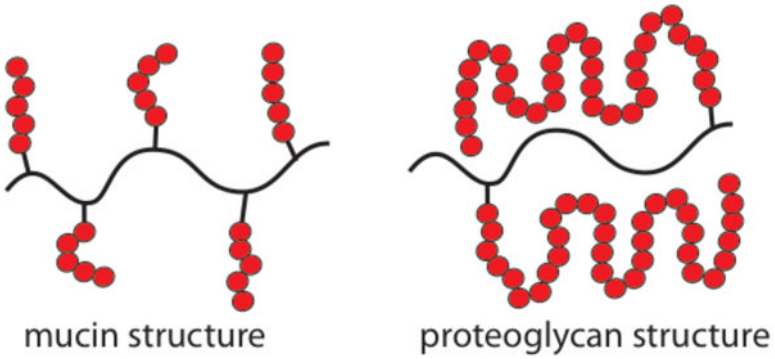

Figure 11.
The structure of mucins closely resembles that of glycopolymers. Proteoglycans, however, have a different binding epitope arrangement than that found in glycopolymers. It is a testament to glycopolymer utility that they can function as glycosaminoglycan mimics.

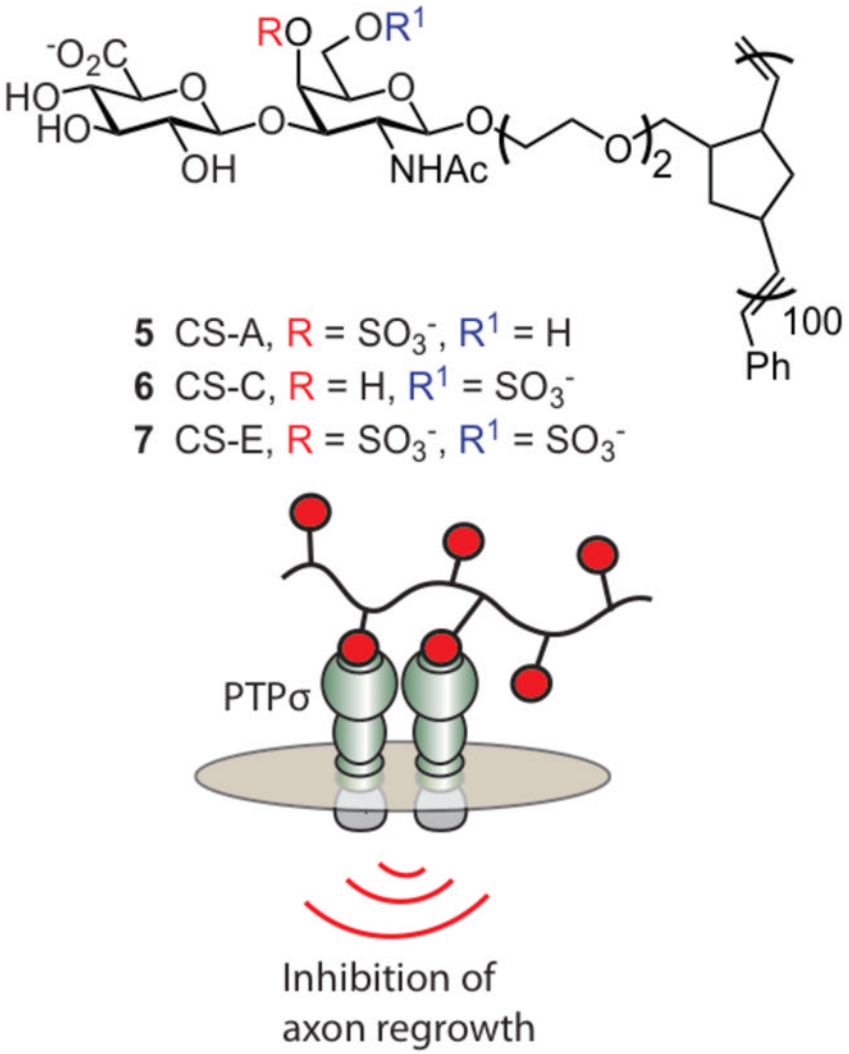

Figure 12.
Glycopolymers were synthesized by Hsieh-Wilson and coworkers bearing carbohydrate epitopes for CS-A, CS-C, and CS-E. The CS-E glycopolymers were found to inhibit axon regrowth through siganling.

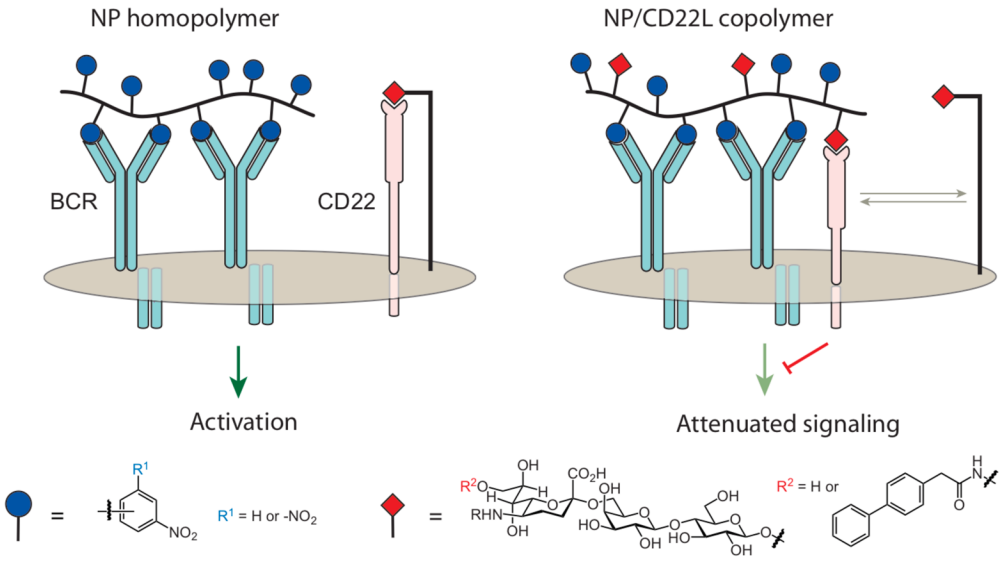

Figure 13.

Polymers were designed to contain a nitrophenyl or dinitrophenyl hapten, a carbohydrate ligand for CD22, or both. The hapent-substituted homopolymer only interacts with the B cell receptor complex (BCR), which activates B cell signaling. A copolymer bearing a hapten and a ligand for the lectin CD22 can interact with both the BCR and CD22, which attenuates B cell activation and suppresses immunity.