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REVIEW

Carbohydrate–protein interactions and their biosensing applications

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Abstract Carbohydrate recognition is clearly present throughout nature, playing a major role in the initial attachment of one biological entity to another. The important question is whether these prevalent interactions could provide a real suitable alternative to the use of antibodies or nucleic acid for detection and identification. Currently, examples of carbohydrates being employed in biological detection systems are limited. The challenges of using carbohydrate recognition for detection mainly come from the weak affinity of carbohydrate–protein interactions, the lack of versatile carbohydrate scaffolds with well-defined structures, and the less developed high-information-content, real-time, and label-free assay technology. In this review, we focus on discussing the characteristics of carbohydrate–protein interactions in nature and the methods for carbohydrate immobilization based on surface coupling chemistry in terms of their general applicability for developing carbohydrate- and lectin-based label-free sensors. Furthermore, examples of innovative design of multivalent carbohydrate–protein interactions for sensor applications are given. We limit our review to show the feasibility of carbohydrate and lectin as recognition elements for label-free sensor development in several representative cases to formulate a flexible platform for their use as recognition elements for real-world biosensor applications.

Keywords Carbohydrate–protein interactions · Biosensors · Carbohydrate immobilization · Lectins

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Carbohydrate recognition in biology

Naturally occurring carbohydrates

Carbohydrates are composed of polyhydroxy units known as monosaccharides, of which some of the more common monosaccharides are glucose, galactose, mannose, fucose, sialic acid, *N*-acetylgalactosamine, and *N*-acetylglucosamine (Fig. 1). These units are joined together via a glycosidic bond between the 'anomeric' hydroxyl group of one monosaccharide and any of the hydroxyl functions of the second monosaccharide, with loss of a molecule of water. The number of different possible disaccharides and higher oligosaccharides is quite phenomenal: more than 10 million tetrasaccharides can be assembled from just nine monosaccharides. Carbohydrates in nature represent a very widely distributed class of biopolymers found in almost all animals and plants, which occur as monosaccharides, oligosaccharides, polysaccharides, or glycoconjugates [1].

In contrast to proteins in which their sequences are unique, oligosaccharides and polysaccharides are typically composed of the same monosaccharide unit repeated over and over such as in cellulose and starch. Carbohydrates were considered less information-rich molecules and were once called "molecules in search of a function" [2]. In the last 20–30 years, the role of carbohydrates in cellular recognition

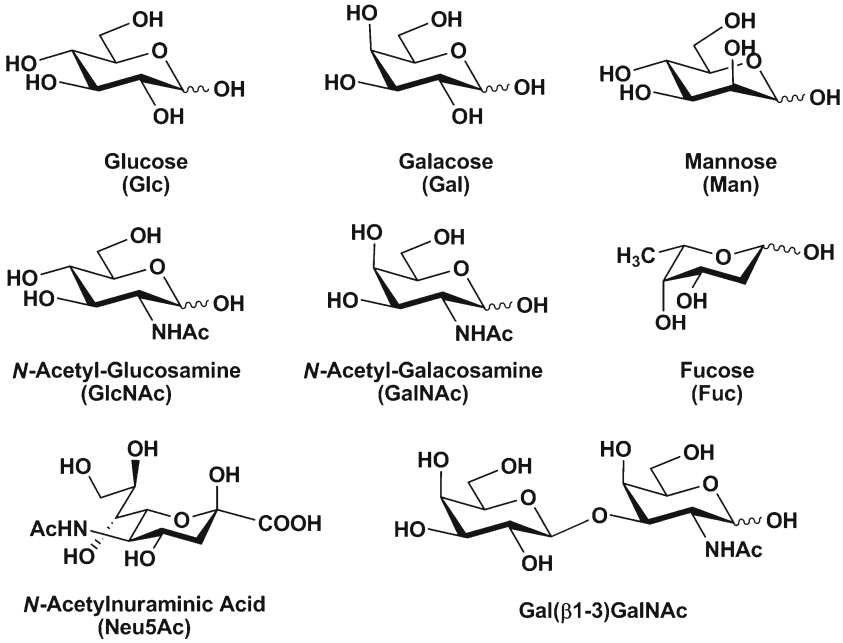

Fig. 1 Examples of carbohydrate structures recognized by lectins (see Table 1 for further information)

and function has started to be thoroughly recognized and understood [3–7]. Carbohydrates play key roles in mediating interactions among cells and between cells and other elements in the cellular environment, and can operate at cell surfaces as a dense coating named glycocalyx [8]. In the glycocalyx, carbohydrates appear mainly conjugated to proteins and lipids resulting in complex carbohydrates such as glycoproteins [9], glycolipids [10], or proteoglycans [11]. Glycosylation is considered one of the most common post-translational modifications of proteins which is responsible for modulating protein functions both on the cellular surfaces and within cells [12]. Alterations in glycosylation patterns have been observed for all types of malignant cells and cells of many types of diseased tissues when compared with the normal cells or tissues [13]. In addition, glycosyltransferases and glycosidases with altered activity levels may promote alterations in the expression of glycoconjugates in cancer cells [14]. These alterations can affect interactions between tumor cell-surface glycans and endogenous lectins, which may determine the metastatic potential of the tumor cell [15] and an increased expression of unusual terminal sequences [16]. These alterations can also result in increased exposure of terminal galactose residues, such as those found in the cancer-associated T antigen (Galβ1–3GalNAc) and the Lewis trisaccharide (Galβ1–4(Fucα1–3)GlcNAc) [15]. Mucins appear to be the major carriers of altered glycosylation in carcinomas (cancers of epithelial origin) [17]. The increased size of tumor cell-derived glycopeptides has been explained by an increase in β1–6 branching of N-glycans, resulting from increased expression of UDP-GlcNAc:*N*-glycan GlcNAc transferase V [17]. In addition, the change in expression of UDP-GlcNAc:*N*-glycan GlcNAc transferase V seems to result from increased transcription of its gene (also called *MGAT5*) and can be induced by various mechanisms, including viral and chemical carcinogenesis [17]. Genetic alterations allow malignant cells to overexpress growth signals and become indifferent to the inhibitory effects of tumor suppressor gene products such as Rb and p53 [13]. In addition, complex carbohydrates are considered to act as receptors for complementary binding proteins, typically lectins. They are a class of proteins of non-immune origin that agglutinate cells, precipitate complex carbohydrates or polysaccharides, and their interaction with polysaccharides resembles the antibody–antigen and enzyme–substrate reactions. The complementary binding between carbohydrates on neighboring cells has been considered either non-specific or non-canonical [18]. Carbohydrate–protein interactions have been implicated in cell-to-cell interaction, ligand–receptor recognition, blood group typing, and transport of biological macromolecules, and to some extent in the immune recognition processes [19] (Fig. 2). Oligosaccharides present on cell surface contribute to critical biological processes such as leukocyte–endothelial cell adhesion [20–22], fatal diseases (congenital disorders of glycosylation [23–25]) caused by a deficiency in the production of glycosidases, bacterial and viral infection, and biosynthesis of glycoproteins. Biological researches are active in the design of synthetic glycoconjugates as tools for the fundamental study of glycobiology and as candidates for future generations of therapeutic and pharmaceutical reagents. Carbohydrate recognition has become the forefront of scientific research.

Characteristics of carbohydrate–protein interactions

Carbohydrate–protein interactions occur through glycoproteins, glycolipids, or polysaccharides displayed on cell

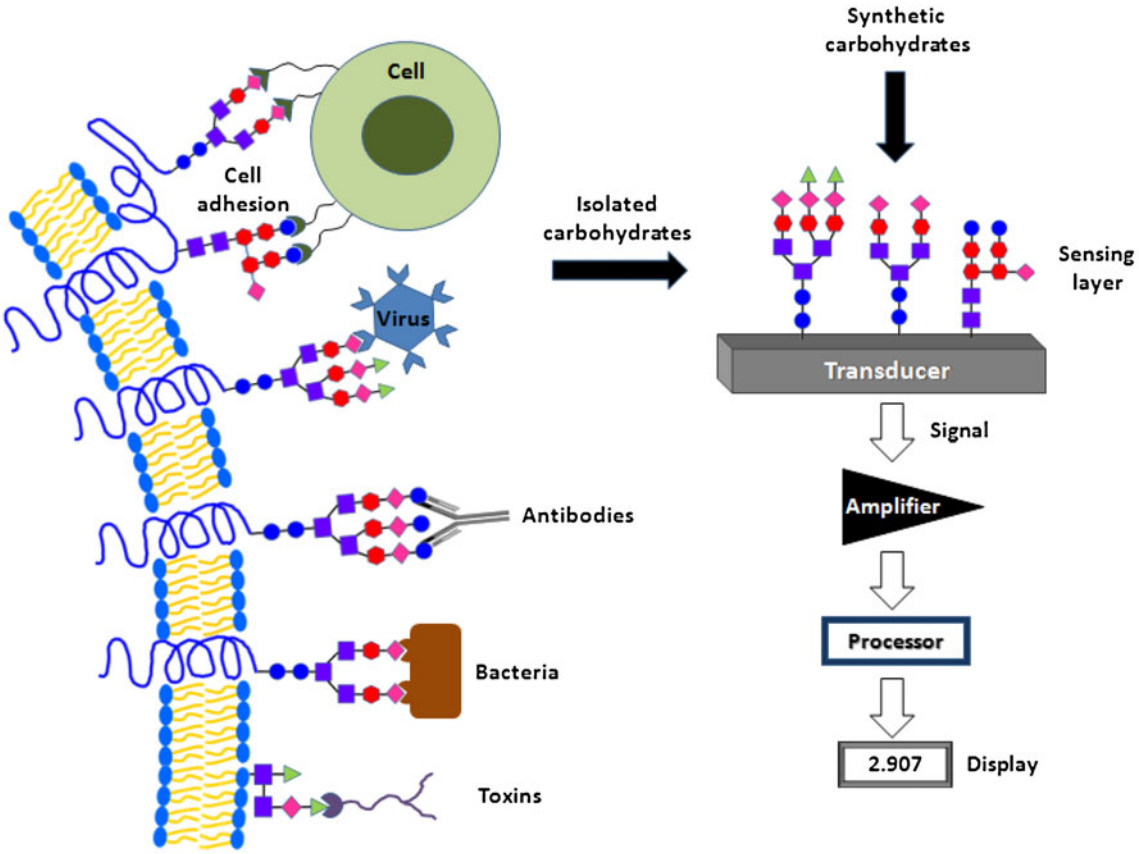

Fig. 2 Multivalent protein–carbohydrate interactions at the cell surface (*left*) and development of a biosensor using carbohydrates (*right*). A biosensor consists of a bioreceptor for specific detection of the respective analyte in spatial contact with a transducer for converting the signal into an electrically manageable format and a signal processing unit

surfaces. The carbohydrate-binding proteins are also called lectins. In contrast to antibodies, lectins are not the products of the immune system, their structures are diverse, and their specificity is restricted to binding to carbohydrates. There are typically three classical lectin families—legume lectins, C-type lectins, and galectins—as well as other plant lectins, viral proteins (e.g., influenza hemagglutinin), toxins (e.g., cholera toxin), anti-carbohydrate antibodies, and pentraxins. The binding between lectin and monovalent carbohydrate is typically weak since the lectins generally possess shallow binding pockets that are exposed to solvents [26–28]. Carbohydrates interact with lectins through hydrogen bonds, metal coordination, van der Waals and hydrophobic interactions [29, 30]. Hydrogen bonds are common in saccharide structures due to the presence of hydroxyl, amine, and carboxyl groups. The availability of large numbers of hydroxyl groups on carbohydrates enables the formation of the complex networks of hydrogen bonds in which the hydroxyl group serves both as a donor and an acceptor to form cooperative hydrogen bonds between the corresponding polysaccharide amphiphilic surfaces and the protein surfaces [31]. The lectin and carbohydrate hydrogen bonds are both direct and water mediated. Divalent cations, such as Ca^{2+} and Mn^{2+}, are involved in carbohydrate recognition either indirectly, by shaping the binding site, or through direct binding of the carbohydrate to Ca^{2+} as in the C-type lectins. For example, concanavalin A (Con A), the lectin of the jack bean (*Canavalia ensiformis*), is one of the best characterized lectins. Each Con A monomer (M_r= 26,500) consists of 237 amino acids. In solution, above pH 6.9 Con A is a tetramer; below pH 5.9 it is a dimer. The lectin possesses a transition metal ion binding site S1 (typically Mn^{2+}), a Ca^{2+} binding site S2, and a saccharide binding site [32]. Both metal ions stabilize the molecular conformation and therefore they are necessary for carbohydrate binding [33]. Specific interactions of Con A with α-D-mannose and α-D-glucose are favored. Besides the hydrophilic character of carbohydrates, hydrophobic interactions play a major role in carbohydrate recognition by lectins. For example, the interaction between aromatic residues and galactose (Gal) in the combining sites of Gal-specific lectins was attributed to an interaction between an extended patch of partially charged aliphatic protons on the B factor of the hexose ring and the partial negative charge on the π electrons of the aromatic system.

The studies of protein–carbohydrate or lectin–carbohydrate interactions have been challenged by the complexity and heterogeneity of cell surfaces, the inherent structure complexity of carbohydrates, and the typical weak affinities of the binding. Carbohydrate–protein interactions are much weaker than protein–protein interactions, by about a factor of 10^2–10^3 from typical antibody equilibrium dissociation constant (K_D). The K_D value of lectin binding with a simple monosaccharide is approximately 1 mM [34]. However, in biological context, this limitation has been overcome by multivalent interactions, i.e., simultaneous contacts between the clustered carbohydrates on cell surfaces and protein receptors that contain multiple carbohydrate recognition domains [35]. The interaction between lectin and carbohydrate is not a simple monomeric binding event, but an oligo- or polymeric binding mechanism [36, 37]. Lectins often have more than one carbohydrate binding site, and it has been shown that the occurrence of two simultaneous binding events can increase the avidity of interaction by more than 100-fold [36, 38, 39], and even as high as 10,000-fold [40]. For example, interaction of the ganglioside G_{M1} with cholera toxin has been investigated thoroughly, and has been reported to have a K_D from approximately 1 nM [41] to as low as 1 pM [42], depending on the experimental setup. This level of affinity is very similar to, if not higher than, that of a typical antibody interaction. The true affinity is probably about 1 nM, a value derived from an experiment using a liposome antibody capture method [41]. The lower K_D value, however, was derived from an experiment utilizing an immobilized lipid bilayer, in which hydrophobic interactions most likely stabilized complex formation [43]. The affinity of the self-interaction of a synthetic disaccharide, based on the aggregation factor from the marine sponge *Microciona prolifera*, is another carbohydrate event to have been significantly enhanced by creating a multivalent binding system [44]. The overall avidity of self-recognition of a neoglycoconjugate of the disaccharide had a $K_D \sim 10$ μM, as opposed to the monomeric interaction which was too weak to be measured. The interaction between charged a mono- or oligosaccharide and lectin is inclined to have a slightly higher affinity: the affinity of sialic acid for the lectin from *Sambucus nigra* has a K_D of approximately 1 μM [45]. The charged polysaccharides heparin and heparan sulfate interact with a number of proteins, with high affinities [46–48]. Lipoprotein lipase binds to heparan sulfate with a $K_D \sim 100$ pM [46], human acidic fibroblast growth factor binds to heparin with a $K_D \sim 50$ nM [47], and protease nexin-1 binds to heparin with a $K_D \sim$ 20 nM [48]. The multivalency of lectin–carbohydrate binding has been shown, as predicted, to significantly increase the observed K_D values towards 1 nM [38, 40]. Hence, lectin–carbohydrate interactions can be on a par with those of antibodies. Overall it can be concluded that with the incorporation of high densities of carbohydrate on the cell surface, the multivalent contact points (hydrogen bonds, van der Waals' interactions, and hydrophobic stacking) between the carbohydrates and the proteins can result in the so-called glycoside cluster effect, which led to almost a logarithmic enhancement of the binding potencies [49] (Fig. 3).

Sensors using carbohydrates

Antibodies and DNA are the most used recognition elements in biosensor developments. But antibodies may be unstable and prone to denaturation so they cannot be used in extreme environments such as acidic, basic, in organic solvent, or at high temperature or pressure. Similar problems exist in the nucleic acid assays which are often lengthy and require several purification steps. One possible alternative principle is to explore carbohydrate–protein recognition as an alternative detection and identification principle. As opposed to antibody/nucleic acid detection, carbohydrate–protein recognition could enable identification of unexpected or even novel agents, by using very few carefully chosen carbohydrate epitopes, since carbohydrates are recognized as possessing

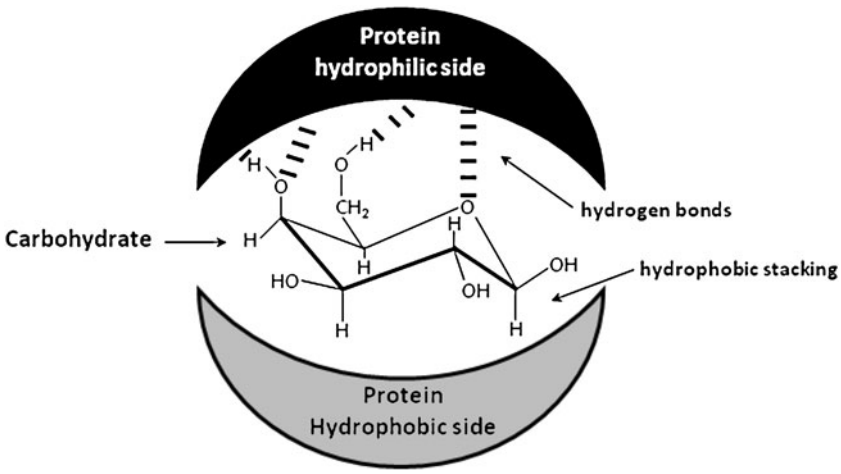

Fig. 3 Multivalent contact points between carbohydrate and protein

broad interaction specificity. The desired fingerprinting of a high number of agents with relatively few carbohydrate epitopes could therefore be a real possibility. The ability to coat an assortment of carbohydrates onto a sensor surface could lead to the development of microarray carbohydrate-recognition-based biosensors. In addition, carbohydrates are more stable and smaller than an antibody/nucleic acid, and they can neither be denatured easily nor lose their activity; moreover, small oligosaccharides can achieve higher ligand densities and consequently higher sensitivity of carbohydrate biosensors. Numerous techniques have been applied to investigate carbohydrate–protein interactions, such as isothermal titration calorimetry (ITC) [50, 51], surface plasmon resonance (SPR) [52], capillary electrophoresis (CE) [53], nuclear magnetic resonance (NMR) [54], quartz crystal microbalance (QCM) [55], fluorescence spectroscopy [56], and atomic force microscopy (AFM) [57, 58]. Since carbohydrates do not absorb light very well, fluorescence labeling of the carbohydrates or proteins is often used to evaluate carbohydrate–protein interaction via optical methods [18]. However, fluorescence labeling of the carbohydrates or proteins requires additional steps. The presence of the labels themselves may introduce additional interferences to the "true" binding process. This motivates the development of biosensors, especially label-free biosensors such as SPR and QCM, for studying carbohydrate–protein interactions.

Table 1 summarizes some examples of biosensors using carbohydrate recognition elements. Most carbohydrate biosensors, especially label-free biosensors, require the immobilization of the carbohydrate recognition elements on solid surfaces such as polymer [59], glass [60], gold [61], nanoparticles (NPs), or quantum dots [62]. The immobilization of the carbohydrate receptors at the surfaces of the biosensor has been achieved through three main approaches: (i) physisorption, (ii) covalent immobilization, (iii) bioaffinity-based interaction. Physisorption [63] of carbohydrate receptors is a convenient technique and it relies on the weak interactions between carbohydrate and the surface. Nitrocellular and polystyrene are mainly used as the substrates. In order to increase the interaction, monosaccharides or oligosaccharides are modified with an anchoring tail such as a lipid [64] or polymer [59]. The covalent immobilization of the carbohydrates has been achieved by reacting carbohydrates with unfunctionalized or functionalized surfaces, in which either unmodified carbohydrates or functionalized carbohydrates are used. Reductive amination is the top choice for immobilization of underivatized carbohydrates, in which the reaction of the reducing end of the carbohydrate with a hydrazine [65] or oxime [66] on the surface is followed by the reduction of the Schiff base formed by reductive reagents such as hydrides. The immobilization of unmodified saccharides is advantageous for complex carbohydrates from nature sources. However, the main drawback is that it leads to the opening of the reducing-end sugar ring that might be crucial for reorganization as a receptor or to maintain the sugar structure.

Surface functionalizations with desired functional group such as carboxylic acid, thiol, or maleimides for covalent immobilization of carbohydrates with functional aglycons have been widely used. Among them, amine reacting with either carboxylic acid [59], aldehyde [73], or epoxy groups [65], and thiol reactivity towards the maleimide group [74] via Michael addition reaction have been demonstrated. In addition, cycloaddition reaction such as Diels–Alder addition [75], Cu(I)-catalyzed 1,3-dipolar cycloaddition known as click chemistry [76], and Staudinger ligation [77] have been developed for carbohydrate receptor immobilization. All these methods have advantages such as that they can be conducted with chemical selectivity and in aqueous mild conditions, which is very important for complex carbohydrates immobilization. Chevolot's group [78] has derived carbohydrates with a photoactivatable aryl-diazirine, which can be converted to a reactive carbine upon photoirradiation at 350 nm and reacts with the substrate. The advantage of the photochemistry method is the versatility of the reaction with respect to the substrate and the possibility of using photolithography for obtaining specific patterns of carbohydrate receptors on the surface. Finally, bioaffinity-based immobilizations have also been employed for carbohydrate immobilization. Among them, streptavidin–biotin interaction [79] has been mostly used, in which biotinylated

Table 1 Representative biosensors based on carbohydrates

Types of carbohydrates as recognition element	Analytes	Transducer	Sensing mechanisms	Refs.
Mannose	*Escherichia coli* W1485	Piezoelectric	Carbohydrate–protein interaction	[67]
Dextran sulfate and fucoidan	Hepatocyte growth factor/scatter factor (HGF/SF)	Mass-sensitive	Specific binding of HGF to sulfated polysaccharides	[68]
Sialic acids	Alzheimer's amyloid-beta (Aβ)	Electrochemical	Saccharide–protein interactions	[69]
Alginate	Mucosal pathogenic bacteria	Optical	Carbohydrates as receptors for pathogenic bacteria	[70]
Mannose	*Escherichia coli* ORN178	Piezoelectric	Carbohydrates as receptors for pathogenic bacteria	[71]
Gangliosides (GD3, GM1, and GM2)	Antibodies, antibody fragments, or binding partners	Optical	Interaction between gangliosides and antibodies and antibody fragments or other binding partners	[72]

glycosides were immobilized onto streptavidin-coated surface. In addition, DNA hybridization for surface immobilization of carbohydrate was also reported [80].

Sugar self-assembled monolayers for studying protein–carbohydrate interactions

The high sensitivity and selectivity of a carbohydrate biosensor often depend on the immobilized carbohydrate structure. The density and orientation of carbohydrates on the surface can often determine the physicochemical properties of the carbohydrate recognition elements. The immobilization of carbohydrates derivatized with functional aglycons has been the major approach in carbohydrate-based biosensor fabrication, especially the carbohydrate self-assembled monolayer (SAM) (Fig. 4). A SAM is formed by the spontaneous association of molecules under equilibrium conditions that yields a stable, structurally well-defined two-dimensional aggregate. SAM technology reduces the variation of the ligand immobilization, a critical factor for array development. SAMs offer extensive control over carbohydrate presenting pattern, density, and orientation, which is beneficial to elicit a clear structure–activity relationship of multivalent interactions [81, 82]. SAMs are also inherently manufacturable. More importantly, SAMs are very useful in surface-based, real-time, label-free analysis methods such as SPR and QCM.

Two main strategies have been developed in our laboratory for carbohydrate SAM formation. One uses an aglycon bearing a function that can react directly with the surface (gold in particular), such as thiolated or disulfide aglycons (Fig. 5a). The thiol functions have strong affinity for noble metals such as gold, silver, copper, etc. As shown in Fig. 5a, an α-Gal trisaccharide was tailored with a thiol linker, which facilitates the formation of SAMs on the gold surface. The binding between α-Gal trisaccharide and anti-Gal antibody was thoroughly characterized. Many other groups have taken advantages of this direct immobilization of thiol- or disulfide-containing biomolecules. The second strategy uses an aglycon bearing a function that reacts with a chemically functionalized surface (Fig. 5b) such as the preformed functionalized SAMs [83, 84]. Several interphase reactions have been identified for anchoring sugar units, including Diels–Alder reaction [75], thiol addition to activated maleimide [85], and disulfide exchange [86]. We demonstrated that the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction can be used for anchoring azidosugars onto preformed SAM templates incorporated with alkyne terminal groups [87]. Azidosugars are readily accessible and have been used extensively in understanding cellular processes through chemoselective ligation reaction between an azide and a functionalized phosphine, termed the Staudinger ligation by Bertozzi and others [88]. This reaction can take place in aqueous medium and also tolerates a variety of conditions and functionalities. It occurs quantitatively with high fidelity. The mannose, lactose, and α-Gal trisaccharide SAMs were fabricated using the above methods and the so-formed sugar SAMs were characterized for their specific carbohydrate–protein interactions (i.e., mannose–Con A; lectin from *Erythrina cristagelli* (ECL)–lactose; α-Gal–anti-Gal). This strategy needs much less synthetic effort compared with making carbohydrate thiolates and promises to be an alternative method for rapid and flexible construction of arrays of carbohydrate SAMs for elucidating carbohydrate functions in biological systems [89, 90].

Recent studies showed that protein activities are highly dependent on the ligand density on the SAM surface [91, 92]. In general, fine tuning of ligand density could be accomplished by changing the ratio of functionalized thiol and dilute thiol. For chemical transformation on surfaces, the surface reactivity is a key factor that impacts the presentation of the sugar, as the reaction on the surface may not act as well as its solution-phase counterpart [83]. Diminished reactivity has been observed due to partially blocked access of an external reagent to the monolayer-embedded reaction center [84, 93]. Since carbohydrates are generally much smaller than proteins, in most cases, the sugar density as low as 1% suffices optimal binding with protein in the ordered environment. In addition, several reports have described acceleration of reaction on SAMs and enhanced reactivity could be achieved with enforced juxtaposition of the reactive surface functional groups or the favorable

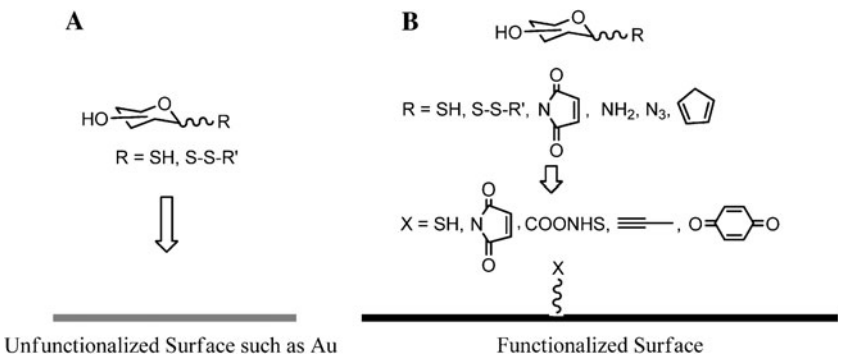

Fig. 4 Covalent immobilization of carbohydrate receptors on biosensor surfaces: reacting carbohydrates with unfunctionalized (**a**) and functionalized (**b**) surface

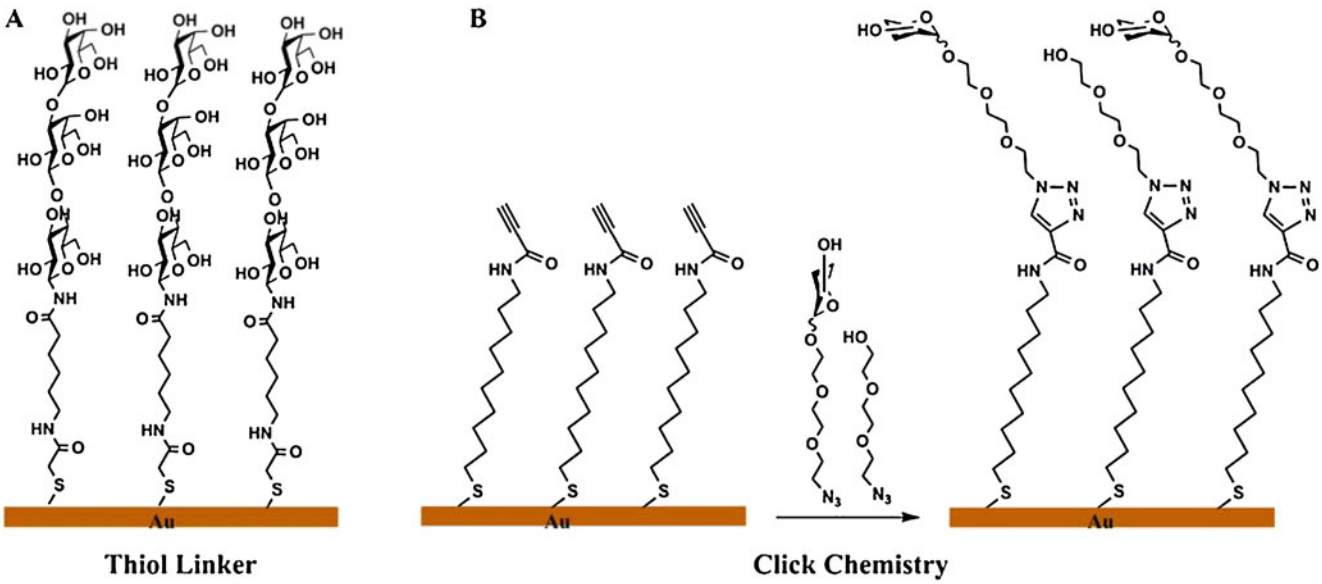

Fig. 5 Carbohydrate SAMs for studying protein–carbohydrate interactions

orientation of the reactive group [94–96]. Surface inertness to non-specific adsorption is another important issue for the study of carbohydrate–protein interaction, as proteins easily adsorb to most man-made materials non-specifically, which will greatly interfere with the specific recognition process. By tethering an oligo ethylene glycol (OEG) unit to the SAM, it could effectively prevent the non-specific adsorption, and these inert SAM surfaces are now the most used approach for the fabrication of carbohydrate-based biosensors [97–99].

Immobilized glycopolymers for studying carbohydrate–protein interactions

Glycopolymers have been shown to enhance the multivalent carbohydrate–protein interactions [100, 101]. Alkanethiol-containing glycopolymers and cross-linked surface-grafted glycopolymer (Fig. 5) have been reported to mimic the biological multivalent interaction of carbohydrate–protein recognition on surface. As shown in Fig. 6a, in the first example, glycopolymers containing mannose and alkanethiol linker were synthesized through substituting preactivated poly[*N*-(acryloyloxy)succinimide] (pNAS) with amine-containing monomer. This thiol functional glycopolymer allows it to be chemically adsorbed onto gold surfaces. The ratio of mannose unit and alkanethiol unit to acrylamide can be optimized to generate a multivalent binding cavity. In the second example, cross-linked glycopolymer was directly synthesized on the gold surface. An α-linked mannose-conjugated acrylamide monomer was synthesized by free radical polymerization with acrylamide, a cross-linker, and a surface linker directly on the gold surface. The surface linker, with an active carbon–carbon double bond, was pre-immobilized on the gold surface by the thiol anchor. Thus, a cross-linked mannose-conjugated polymer thin layer was grafted onto a gold surface (Fig. 6b). The use of cross-linked polymer decreased the flexibility of the polymer backbone between the carbohydrate binding sites. Therefore, the cost of conformational entropy for multivalent binding was minimized. Additional size selectivity could be obtained by controlling the amount of the cross-linking reagent used. The glycopolymer made has shown substantially minimized possible cross-reactivity and significantly enhanced specificity and sensitivity of detection through both the specific multivalent carbohydrate–target interactions and the size and shape of the cross-linked cavity.

Lectin-based biosensors for detecting carbohydrates

It is well known that carbohydrates are poorly immunogenic and elicit the production of weakly binding antibodies. As a consequence, anti-carbohydrate polyclonal or monoclonal antibodies typically have poor affinity even though monoclonal antibody was used to build an immunosensor for the detection of panose (Glcα1–6Glcα1–4Glc), and maltose (Glcα1–4Glc) with a sensitivity in the range of 1–5 μM [102]. Lectins bind to cell surfaces through specific carbohydrate-containing receptor sites [103]. Given the high specificity of lectins, they have been explored as new bioreceptors for biosensor applications in the analysis of their cognate carbohydrates and glycoproteins. Common lectin-immobilization techniques range from non-covalent attachments to covalent immobilization onto a vast palette of

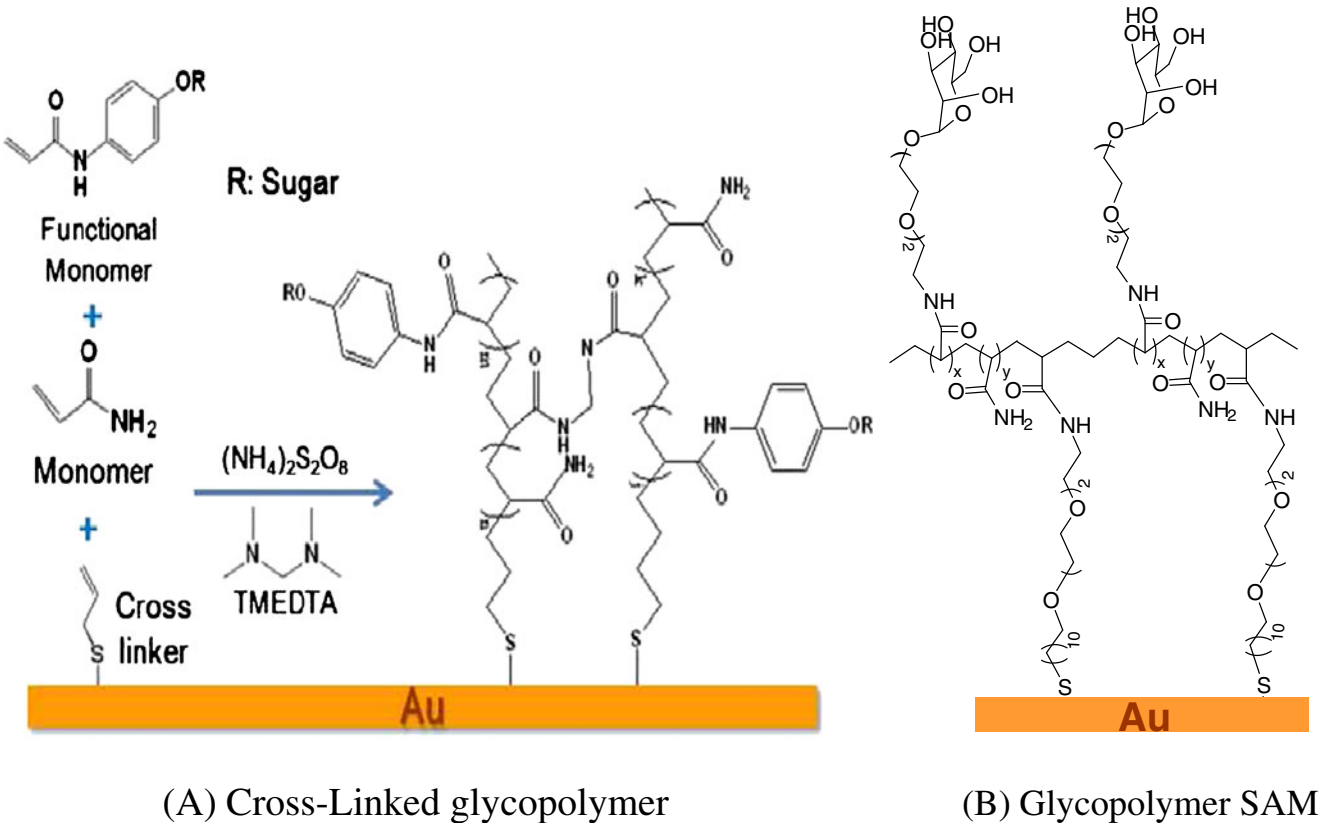

Fig. 6 Glycopolymer formation on gold surface: **a** cross-linked glycopolymer reaction and **b** thiolated glycopolymer

substrates. An example of a non-covalent immobilization was achieved by reversible attachment of Con A to polymyxin B surface via hydrophobic interactions [104]. Specifically, polymyxin B was first covalently immobilized by its primary amine groups to an SPR sensor plate, which allowed Con A to be adsorbed by strong hydrophobic forces. In addition, the immobilization of lectins, such as Con A, on gold NPs is generally ascribed to electrostatic interactions. In order to optimize the immobilization process, Oliveira et al. successfully used polyvinyl butyral (PVB) and gold NPs in the development of an immunosensor [105]. According to the authors, PVB entraps the protein–NP aggregate on the electrode surface. Similarly, this group reported a biosensor based on Fe_3O_4 NPs and CramoLL lectin as an electrochemical dengue serotypes biosensor [106]. In addition, Xi et al. reported an impedimetric biosensor fabricated by simply and reproducibly self-assembling a lectin monolayer on the polyelectrolyte modified electrode through electrostatic interactions [107].

An example of covalent immobilization of a lectin was achieved by flow-injection assay of the pathogenic enterobacteria using a lectin-based QCM biosensor [108]. The biosensing part of the analytical device contained the lectins—Con A, lectins from *Ulex europeus*, *Maackia amurensis*, *Lens culinaris*, wheat germ agglutinin—immobilized on the gold surface of a quartz crystal electrode. The immobilization of lectins was carried out using amine coupling on the surface of the Au crystal modified with 11-mercaptoundecanoic acid (Fig. 7).

The carbohydrate binding lectins in many bacteria are usually in the form of fimbriae (or pili). For example, *E. coli* carries type 1 fimbriae, which is specific for mannose binding. The pili typically have a diameter of 3–7 nm and can extend 100–200 nm in length [109]. The bulk of the fimbrial filament is made up of polymers of the major subunit. Only one of the subunits, usually a minor component of the fimbriae, possesses a carbohydrate combining site and is responsible for the binding activity and sugar specificity of the fimbriae. For example, in type 1 fimbriae, which are made up of hundreds of subunits of four different kinds, the subunit (MW 29–31 kDa) is present in small numbers at intervals along the fimbrial filament and at the distal tip. However, only these subunits appear to be able to mediate mannose-sensitive adhesive interactions, whereas the

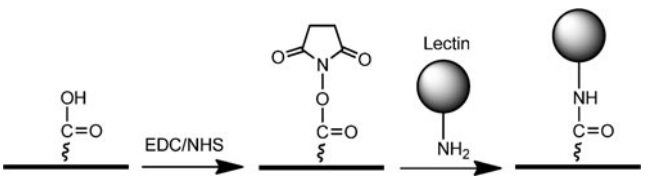

Fig. 7 Amine coupling procedure for immobilization of lectins onto a carboxylic acid surface (*EDC* 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, *NHS N*-hydroxysuccinimide)

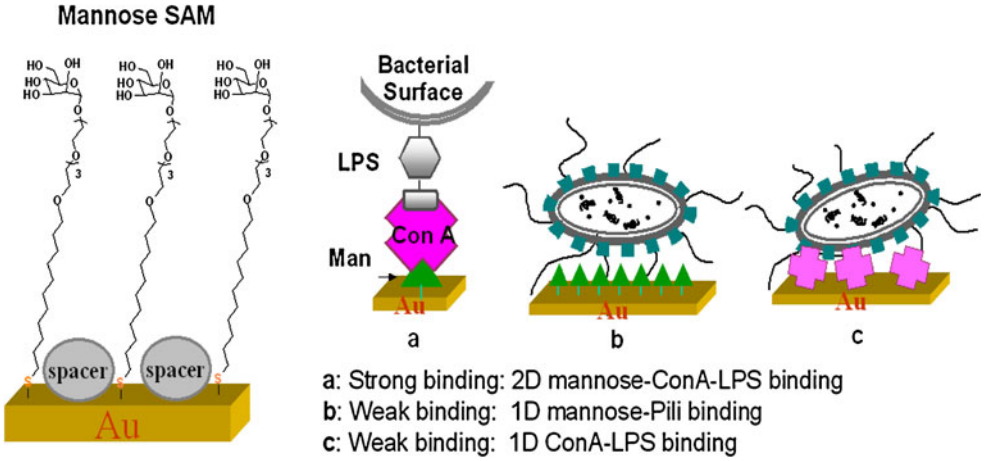

Fig. 8 Schematic of three biointerface binding events: **a** carbohydate-lectin-LPS, **b** carbohydate-protein (in the pili), **c** lectin-LPS binding

subunits at the other positions are inaccessible to the ligand [109]. Consequently, binding is generally of low affinity and not rigid. But in the cell–cell interaction, the adhesions and the receptors often cluster in the plane of the membrane, and the resulting strength of the interaction can be quite strong.

Lectins are oligomeric with several sugar binding sites and the bacterial cell surface has abundant expression of carbohydrate structures. Thus, we have developed a carbohydrate–lectin sensor based on conjugation of a lectin to both bacterial cell surface lipopolysaccharides and a carbohydrate sensing element immobilized on the Au transducer surface [67] (Fig. 8). In this approach, we combined synergistically a lectin's dual carbohydrate binding activity and explored the unique characteristics of surface-sensitive label-free biophysical methods that allow sensitive measurement of cell surface carbohydrate–protein interaction for bacterial detection (Fig. 8). The major bacterial cell wall component in all gram-negative bacteria families consists of a class of glycoconjugates called lipopolysaccharides (LPS). The LPS fraction comprises about 10–15% of the total molecules in the outer membrane and is estimated to occupy about 75% of bacterial surface area [110]. LPS consists of a hydrophobic domain known as lipid A anchored in the membrane, a polysaccharide (O-antigen) made of repetitive subunits of one to eight sugars and a "core" oligosaccharide at the junction between the two other regions [111]. The O-antigens, being at the outermost cell surface, are at the interface between the bacterium and its environment, and are important virulence factors and antigens for many pathogenic bacteria [112]. Many bacteria are subclassified by the O-antigen on their surface [113]. Therefore, LPS O-antigens are unique to specific bacterial strains (i.e., subspecies) and provide the selective specificity needed for the bacterial detection. As shown in Fig. 9a, the carbohydrate–lectin sensor (i.e., Con A–LPS recognition and Con A–mannose recognition) demonstrated much higher sensitivity for *E. coli* W1485 binding likely due to the significantly enhanced binding rigidity. By using an immobilized carbohydrate that binds with the lectin Con A to facilitate Con A binding with bacterial cell surface LPS, the authors observed a greater

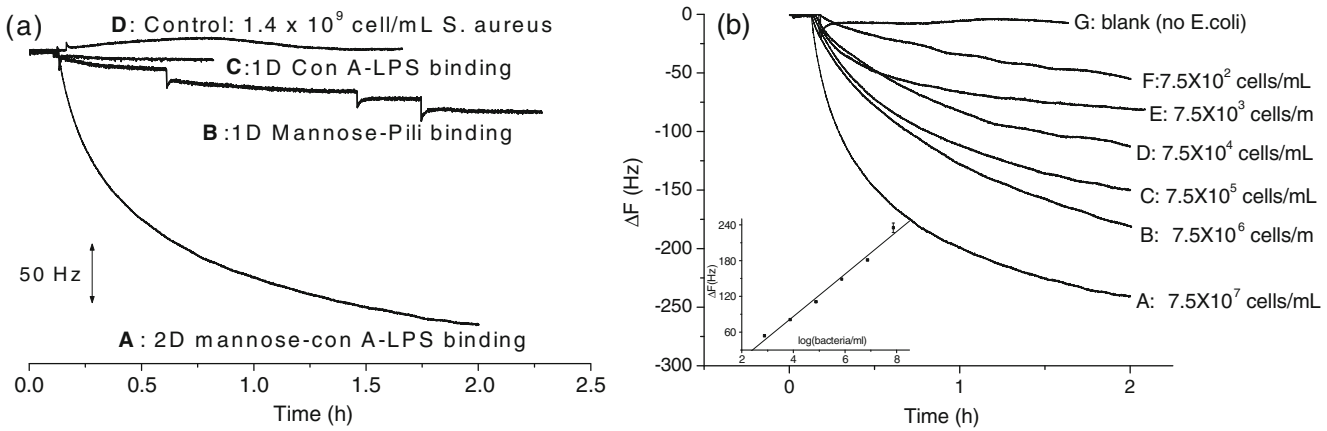

Fig. 9 **a** QCM results for the mannose–Con A, mannose, or Con A sensors for binding with *E. coli* and control experiment in which mannose–Con A sensor was tested with *S. aureus*; **b** real-time QCM results when *E. coli* at various concentrations was added to the mannose–Con A sensors. *Inset* calibration curve (log of *E. coli* concentration vs. QCM frequency signal)

than 10^4-fold increase in binding sensitivity for binding with *E. coli* when compared with either a lectin or a carbohydrate QCM sensor. The linear range is 7.5×10^2 to 7.5×10^7 cells/mL, which is four decades larger than that of the mannose-alone sensor (i.e., 2.9×10^7–2.7×10^8 cells/mL without Con A). The simultaneously determined changes of damping resistances for *E. coli* adhesion experiments were no more than 1.4%. This suggests that the bacterial attachment was rigid, rather than a viscoelastic behavior. Figure 9 also demonstrated that QCM is extremely surface sensitive and can differentiate strong, weak, and non-binding between bacteria and immobilized receptors on the sensor surface (Fig. 8). Little non-specific binding was observed for *Staphylococcus aureus* and other proteins (fetal bovine serum (FBS) and ECL). The significant increase in the sensitivity and specificity of bacterial detection was owing to two factors: the oriented immobilization of Con A enables high affinity binding with LPS O-antigen and the dual carbohydrate–lectin–carbohydrate recognition increase the specificity of the detection.

Table 2 summarizes several examples of the lectin-based biosensors for clinical diagnostic of cancer [107] as well as bacterial and viral infection [114]. Lectin arrays for profiling cell surface carbohydrate expression using Con A, wheat germ agglutinin (WGA), *Ulex europaeus* agglutinin I (UEA), *Ricinus communis* agglutinin (RCA), *Griffonia simplificolia* lectin II, and *Sambucus nigra* agglutinin (SNA) deposited on gold chips [115] were developed and used to analyze cell surface carbohydrate expression via fluorescence. The lectin arrays were used to profile BHK-21, which is a fibroblast cell line derived from baby hamster kidney, and Caco-2, an adenocarcinoma cell line derived from human colon, surface carbohydrate expression. BHK-21 cells and Caco-2 cells showed distinct binding patterns to the lectin arrays. These results showed that the expression levels of fucose and sialic acid on BHK-21 cells are much lower than the levels found on the Caco-2 cells. Unfortunately similar to many protein arrays, lectin arrays suffer some loss of binding activity in the coupling of lectin to the arrays [116–119]. The large sizes of lectins also increase

Table 2 Biosensors based on lectins

Lectins	Biological sensing element	Physical transducer	Sensing mechanisms	Refs.
Con A	Dengue fever and dengue hemorrhagic fever	Electrochemical	Carbohydrate–lectin interaction	[105]
Cratylia mollis lectin	Detection of dengue virus serotypes	Electrochemical	Carbohydrate–lectin interaction	[106]
Con A SNA	Mannose and sialic acid expression on normal and cancer cells derived from human lung, liver, and prostate	Electrochemical	Lectin-based biosensor with the bioconjugates featuring lectin and thionine labels linked to gold NPs for signal amplification	[114]
Con A RCA	Carbohydrates of gram-negative bacteria, gram-positive bacteria, fungus, and human cervical cancer cells	Electrochemical	Carbohydrate–lectin interaction	[107]
Con A UEA	Pathogenic enterobacteria, such as *C. jejuni*, *Helicobacter pylori*	Piezoelectric	Specific cell agglutination	[108]
M. amurensis lectin (MAL)				
L. culinaris agglutinin				
WGA				
Aleuria aurantia agglutinin *Galanthus nivalis* agglutinin	Hyperglycosylated human chorionic gonadotropin produced by malignant gestational trophoblastic neoplasias and male germ cell tumors	Optical	Lectins recognizing carbohydrates	[121]
Phaseolus vulgaris erythroagglutinin				
Pisum sativum agglutinin				
RCA				
MAL				
SNA				
WGA				
WGA MAL	*C. jejuni* strains	Piezoelectric	Lectins recognizing carbohydrates	[120]
L. culinaris agglutinin				
Con A				

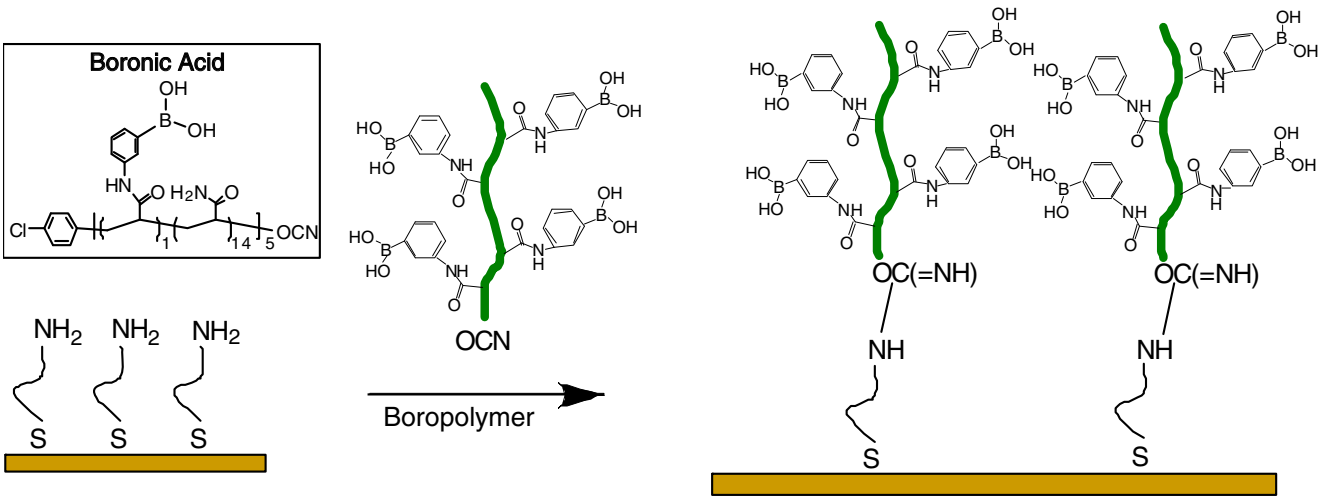

Fig. 10 *O*-Cyanate chain-end functionalized boropolymer and its oriented immobilization onto amine surface via isourea bond formation as carbohydrate receptor

their susceptibility to proteases and encounter non-specific binding. Using the amine coupling chemistry as shown in Fig. 7, Yakovleva et al. reported seven *Campylobacter jejuni* strains typed by a lectin biosensor system [120]. The sensor system was based on a QCM with four commercially available lectins (wheat germ agglutinin, *M. amurensis* lectin, *L. culinaris* agglutinin, and Con A), which were chosen for their differing carbohydrate specificities. The gold surfaces of the quartz crystals were modified with 11-mercaptoundecanoic acid followed by lectin immobilization using a conventional amine coupling technique.

Sensors using synthetic materials to detect carbohydrates or lectins

Since most lectins are isolated from natural sources, which can introduce some variability in their binding affinities dependent on purification. Some lectins can also be cross-specific, binding multiple glycan structures. Furthermore, lectin-based systems lack stability and have short lifetimes, thus requiring replenishment due to their intrinsic protein nature. Alternatively, several molecular architectures have been employed in the development of lectin-like carbohydrate receptors during the past decade or so. Early approaches based on non-covalent interactions were developed through supramolecular chemistry [122]. Sugimoto and co-workers identified peptidic sugar receptors that mimic the non-covalent binding properties of lectins by use of a combinatorial method [123]. The peptide sequence with the greatest affinity displayed a K_a of 3.5×10^5 M^{-1} for D-erythrose binding in aqueous media. Recently, Davis and co-workers reported tricyclic polyamide-based cage-liked receptors that recognize simple sugars [124]. On the other hand, boronic acid-containing compounds have been widely explored as covalent interaction-based carbohydrate receptors [125]. The broad interest in boronic acid-containing receptors results from the relatively low mammalian toxicity shown by boronic acids and their unique interactions with diols: they form cyclic esters with diols in water much more readily than many other acids.

During the past decade, boronic acids have been extensively investigated for use in glucose sensors [126], membrane transport agents [127], cell surface recognition and imaging [128], and protective agents and catalysts in synthesis [129]. Multivalent boronic acid-based carbohydrate binders such as boronic acid-containing polymers have been developed as molecular scaffolds with multiple binding sites to access a variety of substrate selectivity and affinity [130]. We have designed an *O*-cyanate chain-end functionalized boronic acid-containing polymer to facilitate the oriented immobilization of the polymer which provides a three-dimensional expression of a carbohydrate receptor (Fig. 10). Briefly, a boronic acid-containing polymer (boropolymer) acting as a multivalent carbohydrate receptor was oriented and immobilized on the cysteamine-coated electrode through isourea bond formation. Carbohydrates were conjugated to AuNPs to generate a multivalent carbohydrate moiety to amplify the response signal. Thus, the binding of the carbohydrate-conjugated AuNPs to the boropolymer surface is multivalent which could simultaneously increase the binding affinity and specificity. We systematically studied the binding between five carbohydrate-conjugated AuNPs and the boropolymer as shown in Fig. 11. The association constant (K_a) of binding of the carbohydrate-conjugated AuNPs and the boropolymer was in the order of fucose < glucose <

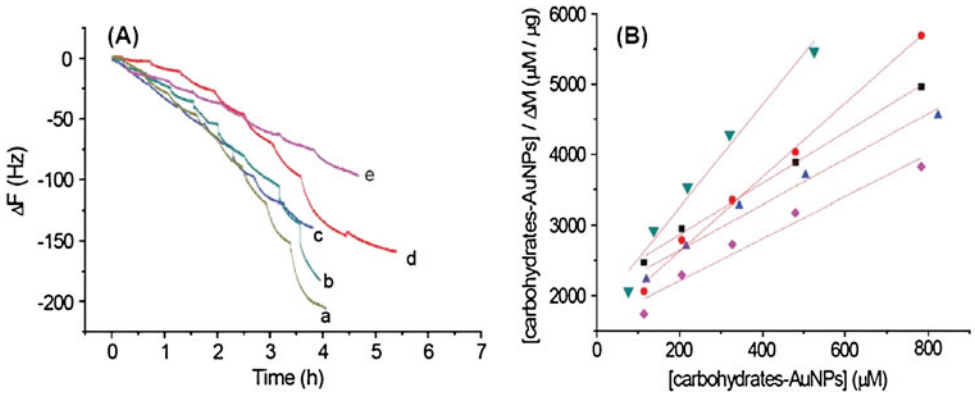

Fig. 11 (**A**) QCM frequency change vs. time curves of boropolymer modified Au quartz electrodes exposed to mannose–AuNPs (**a**), fucose–AuNPs (**b**), galactose–AuNPs (**c**), glucose–AuNPs (**d**), and maltose–AuNPs (**e**) when 2.5, 7.5, 17.5, 37.5, 67.5, 107.5, 157.5, and 257.5 μg/mL carbohydrate–AuNP were added to 1mL buffer in series. AuNPs size, 15 nm. (**B**) [carbohydrates-AuNPs] /Δ*M* vs. [carbohydrates-AuNPs] of carbohydrates–AuNPs binding with boropolymer. Maltose–AuNPs (▼), glucose–AuNPs (■), galactose–AuNPs (●), fucose–AuNPs (▲), mannose–AuNPs (◆)

mannose < galactose < maltose. The linear range of the boronic polymer's detection of mannose-conjugated AuNPs is 23 μM to 3.825 mM with a lower detection limit of 1.5 μM for the carbohydrate analytes. Another benefit of the multivalent binding between carbohydrates and boronic acids is that it is reversible and allows the regeneration of boropolymer surface using 1 M acetic acid, i.e., sequentially capturing and releasing the carbohydrate analytes. Even though the interactions between boronic acids and the carbohydrates are only partially selective, pattern recognition can be used to improve the selectivity for boronic acid recognition element-based carbohydrate biosensors.

Concluding remarks

The modern analytical techniques and synthetic advances in the past decade or so have enabled the understanding of the lectins and glycoconjugates' interactions and their roles in biology (e.g., cell signaling, cell adhesion, fertilization, proliferation, viral/bacterial infection, apoptosis, and the immune response). The carbohydrates' hydroxyl functional groups that are present at almost every carbon atom are ideally suited to form bridges to the side chains of all charged or hydrophilic amino acids. However, carbohydrate–protein interactions are typically weaker than protein–protein interactions since carbohydrates have fairly rigid structure and are mostly only capable of forming hydrogen bonds and hydrophobic interactions. Strong ionic interactions are typically not present in carbohydrates except uronic acids, sialic acids, carbohydrate sulfates or phosphates, ketodeoxynonulosonic acids and 3-deoxyoctulosonic acid in the bacterial endotoxins. Nature uses multivalency to overcome the weak affinity of carbohydrate–protein interaction. Carbohydrate-binding proteins (i.e., lectins) are always multivalent and they bind with multiple oligosaccharide epitopes (the smallest structural element that fills the proteins' binding site). Knowing the nature of carbohydrate–protein interaction, it seems natural to use carbohydrate or lectin structures as receptor elements in biosensor development. However, it is commonly accepted that carbohydrate–protein recognition has low specificity and many other endogenous and exogenous proteins could recognize the carbohydrate ligands and false positive results could be obtained.

In this review, we have shown that carbohydrate SAMs or carbohydrate recognition lectins that combined with an additional binding event, such as a hydrophobic or even a second carbohydrate-mediated interaction, can render affinity similar to or better than that of an antibody interaction. Thus, sensors and sensor arrays using carbohydrate–lectin interactions are able to offer wide flexibility in designing novel carbohydrate-based and carbohydrate-targeted biosensors for detection and analysis of carbohydrate–protein interactions. Characterization of the strength, stoichiometry, and specificity of carbohydrate–protein interactions is fundamental to many practical applications including profiling cell surface carbohydrate and lectin expression, clinical diagnostics of infectious diseases, and environmental as well as food safety monitoring for bacterial outbreak. Carbohydrate-based and carbohydrate-targeted sensors should be developed with minimum sample pretreatment in matrices, such as serum, plasma, urine, or cerebrospinal fluid [131]. In addition, further development and automation will expand the possibilities of carbohydrate and lectin analysis in the clinical sciences [131]. Thus, carbohydrate recognition can be considered a major player in the

biosensor fields for various applications such as bioterrorism defense, environmental pollutant monitoring, forensic analysis, food safety, biological research, and routine clinical tests in laboratory medicine.

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References

1. Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry, 5th edn. Freeman, New York
2. Cook GM (1986) Cell surface carbohydrates: molecules in search of a function? J Cell Sci Suppl 4:45
3. Varkie A (1997) Biological roles of oligosaccharides: all of the theories are correct. Glycobiol 97:97–130
4. Dwek RA (1996) Glycobiology: toward understanding the function of sugars. Chem Rev 96:683–720
5. Drickamer K, Taylor ME (1998) Evolving views of protein glycosylation. Trends Biochem Sci 23:321–324
6. Gagneux P, Varki A (1999) Evolutionary considerations in relating oligosaccharide diversity to biological function. Glycobiol 9:747–755
7. Sears P, Wong CH (2001) Toward automated synthesis of oligosaccharides and glycoproteins. Science 291:2344–2350
8. Frey A, Giannasca KT, Weltzin R, Giannasca PJ, Reggio H, Lencer WI, Neutra MR (1996) Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: implications for microbial attachment and oral vaccine targeting. J Exp Med 184:1045–1059
9. Nakano M, Kakehi K, Tsai MH, Lee YC (2004) Detailed structural features of glycan chains derived from alpha 1-acid glycoproteins of several different animals: the presence of hypersialylated, O-acetylated sialic acids but not disialyl residues. Glycobiol 14:431–441
10. Bush CA, Martin-Pastor M, Imberty A (1999) Structure and conformation of complex carbohydrates of glycoproteins, glycolipids, and bacterial polysaccharides. Ann Rev Biophys Biomol Struct 28:269–275
11. Berninsone PM, Hirschberg CB (2003) The nematode Caenorhabditis elegans as a model to study the roles of proteoglycans. Glycoconj J 19:325–330
12. Dang F, Maeda E, Osafune T, Nakajima K, Kakehi K, Ishikawa M, Baba Y (2009) Carbohydrate-protein interactions investigated on plastic chips statically coated with hydrophobically modified hydroxyethylcellulose. Anal Chem 81:10055–10060
13. Ghazarian H, Idoni B, Oppenheimer SB (2011) A glycobiology review: carbohydrates, lectins and implications in cancer therapeutics. Acta Histochem 113:236–247
14. Powlesland AS, Hitchen PG, Parry S, Graham SA, Barrio MM, Elola MT, Mordoh J, Dell A, Drickamer K, Taylor ME (2009) Targeted glycoproteomic identification of cancer cell glycosylation. Glycobiol 19:899–909
15. Kim YJ, Varki A (1997) Perspectives on the significance of altered glycosylation of glycoproteins in cancer. Glyconj J 14:569–576
16. Oppenheimer SB (2006) Cellular basis of cancer metastasis: a review of fundamentals and new advances. Acta Histochem 108:327–334
17. Varki A, Kannagi R, Toole BP (2009) Glycosylation changes in cancer. In: Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J (eds) Essentials of glycobiology. Cold Spring Harbor Laboratory Press, New York
18. Bucior I, Burger MM (2004) Carbohydrate-carbohydrate interactions in cell recognition. Curr Opin Struct Biol 14:631–637
19. Ahmad S, Khan RH, Ahmad A (1999) Physicochemical characterization of Cajanus cajan lectin: effect of pH and metal ions on lectin carbohydrate interaction. Biochim Biophys Acta 1427:378–384
20. Kaltner H, Stierstorfer B (1998) Animal lectins as cell adhesion molecules. Acta Anatom 161:162–179
21. Yang J, Furie BC, Furie B (1999) The biology of P-selectin glycoprotein ligand-1: its role as a selectin counterreceptor in leukocyte-endothelial and leukocyte-platelet interaction. Thromb Haemostasis 81:1–7
22. Lasky LA (1995) Selectin-carbohydrate interactions and the initiation of the inflammatory response. Annu Rev Biochem 64:113–139
23. Matthijs G (2000) Congenital disorders of glycosylation. Trends Biochem Sci 25:428–432
24. Orlean P (2000) Congenital disorders of glycosylation caused by defects in mannose addition during N-linked oligosaccharide assembly. J Clin Invest 105:131–132
25. Freeze HH, Aebi M (1999) Molecular basis of carbohydrate-deficient glycoprotein syndromes type I with normal phosphomannomutase activity. Biochim Biophys Acta 1455:167–178
26. Kogelberg H, Solis D, Jimenez-Barbero J (2003) New structural insights into carbohydrate-protein interactions from NMR spectroscopy. Curr Opin Struct Biol 13:646–653
27. Gilbert HJ, Bolam DN, Szabo L, Xie H, Williamson MP, Simpson PJ, Jamal S, Boraston AB, Kilburn DG, Warren RAJ (2002) An update on carbohydrate binding modules. Royal Soc Chem 275:89–98
28. Sharon N, Lis H (2001) The structural basis for carbohydrate recognition by lectins. Adv Exp Med Biol 491:1–16
29. Bundle DR, Young NM (1992) Carbohydrate–protein interactions in antibodies and lectins. Curr Opin Struct Biol 2:666–673
30. Spillmann D, Burger MM (1996) Carbohydrate-carbohydrate interactions in adhesion. J Cell Biochem 61:562–568
31. Lemieux RU (1996) How water provides the impetus for molecular recognition in aqueous solution. Acc Chem Res 29:373–380
32. Goldstein IJ, Poretz RD (1986) The lectins. Academic, New York
33. Goldstein IJ, Hollerman CE, Smith EE (1965) Protein-carbohydrate interaction. II. Inhibition studies on the interaction of concanavalin A with polysaccharides. Biochemistry 4:876–883
34. Goldstein IJ, Reichert CM, Misaki A (1974) Interaction of concanavalin A with model substrates. Ann NY Acad Sci 234:283–296
35. Boggs JM, Wang H, Gao W, Arvanitis DN, Gong Y, Min W (2004) A glycosynapse in myelin? Glycoconj J 21:97–110
36. Kitov PI, Sadowska JM, Mulvey G, Armstrong GD, Ling H, Pannu NS, Read RJ, Bundle DR (2000) Shiga-like toxins are neutralized by tailored multivalent carbohydrate ligands. Nature 403:669–672
37. Liang R, Loebach J, Horan N, Ge M, Thompson C, Yan L, Kahne D (1997) Polyvalent binding to carbohydrates immobilized on an insoluble resin. Proc Nat Acad Sci U S A 94:10554–10559
38. Shinohara Y, Kim F, Shimizu M, Goto M, Tosu M, Hasegawa Y (1994) Kinetic measurement of the interaction between an oligosaccharide and lectins by a biosensor based on surface plasmon resonance. Eur J Biochem 223:189–194

39. Mammen M, Choi SK, Whitesides GM (1998) Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. Angew Chem Int Ed 37:2755–2794
40. Shinohara Y, Hasegawa Y, Kaku H, Shibuya N (1997) Elucidation of the mechanism enhancing the avidity of lectin with oligosaccharides on the solid phase surface. Glycobiol 7:1201–1208
41. MacKenzie CR, Hirama T, Lee KK, Altmann E, Young NM (1997) Quantitative analysis of bacterial toxin affinity and specificity for glycolipid receptors by surface plasmon resonance. J Biol Chem 272:5533–5538
42. Kuziemko GM, Stroh M, Stevens RC (1996) Cholera toxin binding affinity and specificity for gangliosides determined by surface plasmon resonance. Biochemistry 35:6375–6384
43. Evans SV, MacKenzie CR (1999) Characterization of protein-glycolipid recognition at the membrane bilayer. J Mol Recog 12:155–168
44. Haseley SR, Vermeer HJ, Kamerling JP, Vliegenthart JFG (2001) Carbohydrate self-recognition mediates marine sponge cellular adhesion. Proc Nat Acad Sci U S A 98:9419–9424
45. Haseley SR, Talaga P, Kamerling JP, Vliegenthart JFG (1999) Characterization of the carbohydrate binding specificity and kinetic parameters of lectins by using surface plasmon resonance. Anal Biochem 274:203–210
46. Lookene A, Chevreuil O, Ostergaard P, Olivecrona G (1996) Interaction of lipoprotein lipase with heparin fragments and with heparan sulfate: stoichiometry, stabilization, and kinetics. Biochemistry 35:12155–12163
47. Lee MK, Lander AD (1991) Analysis of affinity and structural selectivity in the binding of proteins to glycosaminoglycans: development of a sensitive electrophoretic approach. Proc Nat Acad Sci U S A 88:2768–2772
48. Herndon ME, Stipp CS, Lander AD (1999) Interactions of neural glycosaminoglycans and proteoglycans with protein ligands: assessment of selectivity, heterogeneity and the participation of core proteins in binding. Glycobiology 9:143–155
49. Lee YC, Lee RT (1995) Carbohydrate–protein interactions: basis of glycobiology. Acc Chem Res 28:321–327
50. Flint J, Nurizzo D, Harding SE, Longman E, Davies GJ, Gilbert HJ, Bolam DN (2003) Ligand-mediated dimerization of a carbohydratebinding module reveals a novel mechanism for protein–carbohydrate recognition. J Mol Biol 337:417–426
51. Dam TK, Brewer CF (2004) Multivalent protein-carbohydrate interactions: isothermal titration microcalorimetry studies. Meth Enzymol 379:107–128
52. Critchley P, Willand MN, Rullay AK, Crout DHG (2003) Carbohydrate–protein interactions at interfaces: synthesis of thiolactosyl glycolipids and design of a working model for surface plasmon resonance. Org Biomol Chem 1:928–938
53. Taga A, Mochizuki M, Itoh H, Suzuki S, Honda S (1999) Capillary electrophoretic determination of the association constant of a protein and a neutral carbohydrate by introducing mercaptoethanesulfonate tags to the carbohydrate. J Chromatogr A 839:157–166
54. Jiménez-Barbero J, Peters T (2003) NMR spectroscopy of glycoconjugates. Wiley-VCH, Weinheim
55. Pedroso MM, Watanabe AM, Roque-Barreira MC, Bueno PR, Faria RC (2008) Quartz crystal microbalance monitoring the real-time binding of lectin with carbohydrate with high and low molecular mass. Microchem J 89:153–158
56. Lee YC (1997) Fluorescence spectrometry in studies of carbohydrate-protein interactions. J Biochem 121:818–825
57. Bucior I, Scheuring S, Engel A, Burger MM (2004) Carbohydrate-carbohydrate interaction provides adhesion force and specificity for cellular recognition. J Cell Biol 165:529–537
58. Guerardel Y, Czeszak X, Sumanovski L, Karamanos Y, Popescu O, Strecker G, Misevic GN (2004) Molecular fingerprinting of carbohydrate structures phenotypes of three porifera proteoglycan-like glyconectins. J Biol Chem 279:15591–15603
59. Fukui S, Feizi T, Galustian C, Lawson AM, Chai W (2002) Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate-protein interactions. Nat Biotechnol 20:1011–1017
60. 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 C-H, Paulson JC (2004) Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc Natl Acad Sci U S A 101:17033–17038
61. Seo JH, Adachi K, Lee BK, Kang DG, Kim YK, Kim KR, Lee HY, Kawai T, Cha HJ (2007) Facile and rapid direct gold surface immobilization with controlled orientation for carbohydrates. Bioconj Chem 18:2197–2201
62. Larsen K, Thygesen MB, Guillaumie F, Willats WGT, Jensen KJ (2006) Solid-phase chemical tools for glycobiology. Carbohydr Res 341:1209–1234
63. Palma AS, Feizi T, Zhang Y, Stoll MS, Lawson AM, Díaz-Rodríguez E, Campanero-Rhodes MA, Costa J, Gordon S, Brown GD, Chai W (2006) Ligands for the beta-glucan receptor, Dectin-1, assigned using "Designer" microarrays of oligosaccharide probes (neoglycolipids) generated from glucan polysaccharides. J Biol Chem 281:5771–5779
64. Huang GL, Zhang HC, Wang PG (2006) Fabrication and application of neoglycolipid arrays in a microtiter plate. Bioorg Med Chem Lett 16:2031–2033
65. Lee MR, Shin I (2005) Fabrication of chemical microarrays by efficient immobilization of hydrazide-linked substances on epoxide-coated glass surfaces. Angew Chem Int Ed Engl 44:2881–2884
66. Zhou X, Zhou J (2006) Oligosaccharide microarrays fabricated on aminooxyacetyl functionalized glass surface for characterization of carbohydrate–protein interaction. Biosens Bioelectron 21:1451–1458
67. Shen ZH, Huang MC, Xiao CD, Zhang Y, Zeng XQ, Wang PG (2007) Nonlabeled quartz crystal microbalance biosensor for bacterial detection using carbohydrate and lectin recognitions. Anal Chem 79:2312–2319
68. Berger M, Welle A, Gottwald E, Rapp M, Länge K (2010) Biosensors coated with sulfated polysaccharides for the detection of hepatocyte growth factor/scatter factor in cell culture medium. Biosens Bioelectron 26:1706–1709
69. Chikae M, Fukuda T, Kerman K, Idegami K, Miura Y, Tamiya E (2008) Amyloid-β detection with saccharide immobilized gold nanoparticle on carbon electrode. Bioelectrochem 74:118–123
70. Chung K-H, Park J-S, Hwang H-S, Kim J-C, Lee K-Y (2007) Detection and kinetics of mucosal pathogenic bacteria binding with polysaccharides. J Microbiol Biotechnol 17:1191–1197
71. Tzeng T-RJ, Cheng YR (2011) Adhesin-specific nanomechanical cantilever biosensors for detection of microorganisms. J Heat Transfer 133:011012–011017
72. Catimel B, Scott AM, Lee FT, Hanai N, Ritter G, Welt S, Old LJ, Burgess AW, Nice EC (1998) Direct immobilization of gangliosides onto gold-carboxymethyldextran sensor surfaces by hydrophobic interaction: applications to antibody characterization. Glycobiol 8:927–938
73. Biskup MB, Müller JU, Weingart R, Schmidt RR (2005) New methods for the generation of carbohydrate arrays on glass slides and their evaluation. ChemBioChem 6:1007–1015

74. Brun MA, Disney MD, Seeberger PH (2006) Miniaturization of microwave-assisted carbohydrate functionalization to create oligosaccharide microarrays. ChemBioChem 7:421–424
75. Houseman BT, Mrksich M (2002) Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. Chem Biol 9:443–454
76. Huang C-Y, Thayer DA, Chang AY, Best MD, Hoffmann J, Head S, Wong C-H (2006) Carbohydrate microarray for profiling the antibodies interacting with Globo H tumor antigen. Proc Natl Acad Sci U S A 103:15–20
77. Köhn M, Wacker R, Peters C, Schröder H, Soulere L, Breinbauer R, Niemeyer CM, Waldmann H (2003) Staudinger ligation: a new immobilization strategy for the preparation of small-molecule arrays. Angew Chem Int Ed Engl 115:5830–5834
78. Chevolot Y, Bucher O, Leonard D, Mathieu HJ, Sigrist H (1999) Synthesis and characterization of a photoactivatable glycoaryldiazirine for surface glycoengineering. Bioconj Chem 10:169–175
79. Bochner BS, Alvarez RA, Mehta P, Bovin NV, Blixt O, White JR, Schnaar RL (2005) Glycan array screening reveals a candidate ligand for siglec-8. J Biol Chem 280:4307–4312
80. Chevolot Y, Bouillon C, Vidal S, Morvan F, Meyer A, Cloarec JP, Jochum A, Praly JP, Vasseur JJ, Souteyrand E (2007) DNA-based carbohydrate biochips: a platform for surface glyco-engineering. Angew Chem Int Ed Engl 46:2398–2402
81. Schreiber F (2004) Self-assembled monolayers. From simple model systems to biofunctionalized interfaces. J Phys: Cond Matt 16:R881–R900
82. Chaki NK, Vijayamohanan K (2002) Self-assembled monolayers as a tunable platform for biosensor applications. Biosens Bioelectron 17:1–12
83. Sullivan TP, Huck WTS (2003) Reactions on monolayers: organic synthesis in two dimensions. Eur J Org Chem 17–29
84. Flink S, van Veggel FCJM, Reinhoudt DN (2000) Sensor functionalities in self-assembled monolayers. Adv Mater 12:1315–1328
85. Houseman BT, Gawalt ES, Mrksich M (2003) Maleimide-functionalized self-assembled monolayers for the preparation of peptide and carbohydrate biochips. Langmuir 19:1522–1531
86. Emily AS, William DT, Laura LK, Robert MC (2003) Surface plasmon resonance imaging studies of protein-carbohydrate interactions. J Am Chem Soc 125:6140–6148
87. Kolb HC, Finn MG, Sharpless KB (2001) Click chemistry: diverse chemical function from a few good reactions. Angew Chem Int Ed 40:2004–2008
88. Saxon E, Bertozzi CR (2000) Cell surface engineering by a modified Staudinger reaction. Science 287:2007–2010
89. Ratner DM, Adams EW, Disney MD, Seeberger PH (2004) Tools for glycomics: mapping interactions of carbohydrates in biological systems. ChemBioChem 5:1375–1383
90. Feizi T, Fazio F, Chai WC, Wong CH (2003) Carbohydrate microarrays - a new set of technologies at the frontiers of glycomics. Curr Opin Struct Biol 13:637–645
91. Horan N, Yan L, Isobe H, Whitesides GM, Kahne D (1999) Nonstatistical binding of a protein to clustered carbohydrates. Proc Nat Acad Sci U S A 96:11782–11786
92. Houseman BT, Mrksich M (1999) The role of ligand density in the enzymic glycosylation of carbohydrates presented on self-assembled monolayers of alkanethiolates on gold. Angew Chem Int Ed 38:782–785
93. Chechik V, Crooks RM, Stirling CJM (2000) Reactions and reactivity in self-assembled monolayers. Adv Mater 12:1161–1171
94. Liu MH, Nakahara H, Shibasaki Y, Fukuda K (1993) Polycondensation of long-chain esters of aromatic amino acids in monolayer at the air/water interface. Chem Lett 967–970
95. Bartz M, Kuther J, Seshadri R, Tremel W (1998) Colloid-bound catalysts for ring-opening metathesis polymerization: a combination of homogeneous and heterogeneous properties. Angew Chem Int Ed 37:2466–2468
96. Tollner K, Popovitz-Biro R, Lahav M, Milstein D (1997) Impact of molecular order in Langmuir-Blodgett films on catalysis. Science 278:2100–2102
97. Pale-Grosdemange C, Simon ES, Prime KL, Whitesides GM (1991) Formation of self-assembled monolayers by chemisorption of derivatives of oligo(ethylene glycol) of structure HS $(CH_2)_{11}OCH_2CH_2)_mOH$ on gold. J Am Chem Soc 113:12–20
98. Seigel RR, Harder P, Dahint R, Grunze M, Josse F, Mrksich M, Whitesides GM (1997) Online detection of nonspecific protein adsorption at artificial surfaces. Anal Chem 69:3321–3328
99. Luk YY, Kato M, Mrksich M (2000) Self-assembled monolayers of alkanethiolates presenting mannitol groups are inert to protein adsorption and cell attachment. Langmuir 16:9604–9608
100. Mrksich M (2000) A surface chemistry approach to studying cell adhesion. Chem Soc Rev 29:267–273
101. Sigal GB, Mammen M, Dahmann G, Whitesides GM (1996) Polyacrylamides bearing pendant α-sialoside groups strongly inhibit agglutination of erythrocytes by influenza virus: the strong inhibition reflects enhanced binding through cooperative polyvalent interactions. J Am Chem Soc 118:3789–3800
102. Engstrom HA, Andersson PO, Ohlson S (2006) A label-free continuous total-internal-reflection-fluorescence-based immunosensor. Anal Biochem 357:159–166
103. Sharon N, Lis H (2003) Lectins, 2nd edn. Kluwer Academic, Dordrecht
104. Masárová J, Deya ES, Carlsson J, Danielsson B (2004) Novel peptide surface for reversible immobilization of concanavalin A. J Biochem Bioph Met 60:163–170
105. Oliveira MDL, Correia MTS, Diniz FB (2009) Concanavalin A and polyvinyl butyral use as a potential dengue electrochemical biosensor. Biosens Bioelectron 25:728–732
106. Oliveira MDL, Nogueira ML, Correia MTS, Coelho LCBB, Andrade CAS (2011) Detection of dengue virus serotypes on the surface of gold electrode based on Cratylia mollis lectin affinity. Sens Actuator B Chem 155:789–795
107. Xi F, Gao J, Wang J, Wang Z (2011) Discrimination and detection of bacteria with a label-free impedimetric biosensor based on self-assembled lectin monolayer. J Electroan Chem 656:252–257
108. Safina G, van Lier M, Danielsson B (2008) Flow-injection assay of the pathogenic bacteria using lectin-based quartz crystal microbalance biosensor. Talanta 77:468–472
109. Lis H, Sharon N (1998) Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem Rev 98:637–674
110. Hsu KL, Pilobello KT, Mahal LK (2006) Analyzing the dynamic bacterial glycome with a lectin microarray approach. Nat Chem Biol 2:153–157
111. Rietschel ET, Kirikae T, Schade FU, Ulmer AJ, Holst O, Brade H, Schmidt G, Mamat U, Grimmecke HD, Kusumoto S, Zahringer U (1993) The chemical-structure of bacterial-endotoxin in relation to bioactivity. Immunobiol 187:169–190
112. Andersson M, Carlin N, Leontein K, Lindquist U, Slettengren K (1989) Structural studies of the O-antigenic polysaccharide of Escherichia coli O86, which possesses blood-group B activity. Carbohydr Res 185:211–223
113. Reeves PR, Hobbs M, Valvano MA, Skurnik M, Whitfield C, Coplin D, Kido N, Klena J, Maskell D, Raetz CRH, Rick PD (1996) Bacterial polysaccharide synthesis and gene nomenclature. Trends Microbiol 4:495–503

114. Zhang X, Teng Y, Fu Y, Xu L, Zhang S, He B, Wang C, Zhang W (2010) Lectin-based biosensor strategy for electrochemical assay of glycan expression on living cancer cells. Anal Chem 82:9455–9460
115. Zheng T, Peelen D, Smith LM (2005) Lectin arrays for profiling cell surface carbohydrate expression. J Am Chem Soc 127:9982–9983
116. Angeloni S, Ridet JL, Kusy N, Gao H, Crevoisier F, Guinchard S, Kochhar S, Sigrist H, Sprenger N (2005) Glycoprofiling with micro-arrays of glycoconjugates and lectins. Glycobiol 15:31–41
117. Pilobello KT, Krishnamoorthy L, Slawek D, Mahal LK (2005) Development of a lectin microarray for the rapid analysis of protein glycopatterns. ChemBioChem 6:985–989
118. Poxton IR (1995) Antibodies to lipopolysaccharide. J Immunol Methods 186:1–15
119. Hsu KL, Pilobello KT, Mahal LK (2006) Analyzing the dynamic bacterial glycome with a lectin microarray approach. Nat Chem Biol 2:153–157
120. Yakovleva ME, Moran AP, Safina GR, Wadström T, Danielsson B (2011) Lectin typing of Campylobacter jejuni using a novel quartz crystal microbalance technique. Anal Chim Acta 694:1–5
121. Kelly LS, Birken S, Puett D (2007) Determination of hyperglycosylated human chorionic gonadotropin produced by malignant gestational trophoblastic neoplasias and male germ cell tumors using a lectin-based immunoassay and surface plasmon resonance. Mol Cell Endocrinol 260–262:33–39
122. Davis AP, Wareham RS (1999) Carbohydrate recognition through noncovalent interactions: a challenge for biomimetic and supramolecular chemistry. Angew Chem Int Ed Engl 38:2978–2996
123. Sugimoto N, Miyoshi D, Zou J (2000) Development of small peptides recognizing a monosaccharide by combinatorial chemistry, Chem Comm 2295–2296
124. Ferrand Y, Crump MP, Davis AP (2007) A synthetic lectin analog for biomimetic disaccharide recognition. Science 318:619–622
125. James TD, Shinkai S (2002) Artificial receptors as chemosensors for carbohydrates. Top Curr Chem 218:159–200
126. Fang H, Kaur G, Wang B (2004) Progress in boronic acid-based fluorescent glucose sensors. J Fluoresc 14:481–489
127. Duggan PJ (2004) Fructose-permeable liquid membranes containing boronic acid carriers. Aust J Chem 57:291–299
128. Yang W, Fan H, Gao X, Gao S, Karnati VVR, Ni W, Hooks WB, Carson J, Weston B, Wang B (2004) The first fluorescent diboronic acid sensor specific for hepatocellular carcinoma cells expressing sialyl Lewis X. Chem Biol 11:439–448
129. Duggan PJ, Tyndall EM (2002) Boron acids as protective agents and catalysts in synthesis. J Chem Soc Perkin Trans 1:1325–1339
130. Patterson S, Smith BD, Taylor RE (1998) Tuning the affinity of a synthetic sialic acid receptor using combinatorial chemistry. Tetrahedron Lett 39:3111–3114
131. Luppa PB, Sokoll LJ, Chan DW (2001) Immunosensors - principles and applications to clinical chemistry. Clin Chim Acta 314:1–26