Chapter 4

Self-Assembled Monolayers of Carbohydrate Derivatives on Gold Surfaces

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Additional information is available at the end of the chapter

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Abstract

Self-assembled monolayers (SAMs) presenting carbohydrates (glycans) have been widely prepared on gold surfaces to mimic the carbohydrate surfaces that are involved in molecular recognition phenomena in living cells. The binding affinity of carbohydrate immbolized on SAM surfaces to various carbohydrate-binding proteins (such as lectins) can be studied by optical, electrochemical, piezoelectrical and thermal sensing techniques. The lectins present on the surface of pathogens (e.g., bacteria or viruses) can be used as targets for capturing onto carbohydrates immobilized on SAM surfaces. The immobilized carbohydrates can also be used for detecting different types of disease biomarkers present in bodily fluids. Synergistic properties of carbohydrate SAMs and gold nanoparticles can be used for vaccine preparation and drug delivery. By studying different types of glycans, their properties, and the behavior toward recognition of specific pathogens and biomarkers, we can develop not only new therapeutics but also enhance the diagnostic strategies of various diseases. In this chapter, we discuss carbohydrate-terminated SAMs and their common preparation strategies. Next, we focus on roles of different components of SAMs, characterization techniques, and applications.

Keywords: self-assembled monolayers (SAMs), carbohydrates, gold surface, click reaction, biosensing, carbohydrate-lectin interaction, *E-coli* detection

1. Introduction

Carbohydrates are biological molecules, present widely in nature in diverse forms and have varieties of functions [1]. Their role in living organisms is indispensable, whether it is as a structural support (e.g., cellulose and chitin), in energy storage (e.g., glycogen and starch), in the immune system, or for fertilization and development [2, 3]. The glycans are the carbohydrate

parts of glycoconjugates, such as glycoproteins, glycolipids, or proteoglycans, and their importance in human health and disease is an ever expanding field [3]. A major part of the field concerns the study of the organization of carbohydrates at interfaces and their interaction with carbohydrate-binding proteins. Self-assembled monolayers (SAMs) of carbohydrate-terminated alkanethiols and of other carbohydrate derivatives conjugated to species pre-immobilized on gold such as branched polymers and dendrimers have served as model systems in many studies of these interactions and in the development of biosensors based on carbohydrate recognition [4, 5]. These SAMs have been formed both by direct immobilization of carbohydrate-terminated alkanethiols and by conjugation of glycans to pre-formed SAMs with reactive terminal groups [6]. The complexity of the carbohydrates immobilized range from monosaccharides to complex oligosaccharides of varied biological functions. Central to these studies is the goal of understanding the structure and organization of the SAMs, and this has been approached using a range of methods, including surface analysis, surface spectroscopy, scanning probe microscopy, and electrochemical methods. The binding of proteins to these SAMs has been followed using methods, including surface plasmon resonance (SPR), impedance spectroscopy, and quartz crystal microbalance (QCM). In this chapter, we will seek to review the literature concerning SAMs containing terminal carbohydrates, their fabrication by direct or indirect coupling methods, and their structural characterization. The applications of these SAMs in protein-binding studies and biosensor development will also be discussed.

2. Self-assembled monolayers

Organic molecules having functional head-groups (e.g., thiols, disulfides, and amines) and tail groups at the end of hydrophobic chains (e.g., alkanes and polyethylene glycols) can easily self-assemble on noble metal surfaces lowering the free energy at the interface to form densely packed monolayer films, called self-assembled monolayers (SAMs) [7]. Different types of functional groups can be attached to the terminal end of the hydrophobic part depending on the nature of the study, through which further chemistry can be performed linking fields of the material chemistry and organic/biochemistry. SAMs of organosulfur compounds are the most-studied SAMs to date because of strong thiol-gold bond formation [8, 9]. A schematic diagram of an ideal alkanethiol SAM immobilized on gold surfaces having terminal functional groups is shown in **Figure 1,** and the chemisorption reaction between thiol and gold is shown in Eq. (1) [10].

$$\mathrm{R{-}SH} + \mathrm{Au}_n^0 \rightarrow \mathrm{R{-}S^{(-)}{-}Au^{(+)}} \cdot \mathrm{Au}_n^0 + 1/2\mathrm{H}_2 \tag{1}$$

One of the common methods of preparing SAMs of organosulfur derivatives is by immersing a metal substrate into dilute (1–10 mM) ethanolic solution of the desired organosulfur compound for 12–24 h under ambient conditions [11]. When sulfur atoms come in contact with a clean metal surface, they start forming monolayers instantly; however, the molecules reorganize themselves if left in the solution over a longer period, minimizing the density of defects [12]. Alkyl chains of SAMs arrange themselves in trans-conformation with nearly 20–30° tilt from normal to the metal surface [9]. However, studies have shown that overall arrangement

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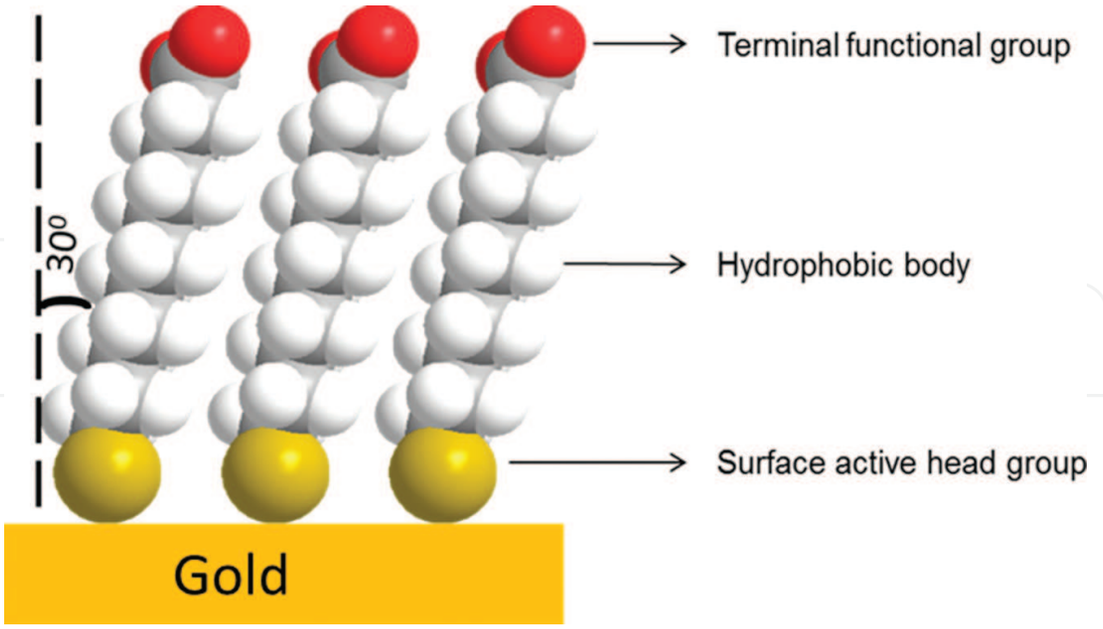

Figure 1. Schematic depiction of an ideal SAM formed on a gold substrate.

and binding of SAMs on gold surfaces depend on numbers of factors, including length of alkyl chains, the nature and distance between terminal functional groups, concentration and purity of adsorbate, immersion time, and substrate morphology [11].

3. Preparation of SAMs having terminal carbohydrates

Preparation of microarrays of carbohydrates to mimic the cell surface for the in vitro study of their interactions with pathogens or other biological molecules is very important. Microarrays present a surface onto which pathogens can undergo multivalent attachment amplifying the relative affinities as on cell surfaces and above that of a single ligand. The pathogens or biological molecules captured on the array can also be easily harvested and further tested. There are different methods to prepare arrays of carbohydrates on solid surfaces; one of the popular methods is through SAMs formation. Here, we will be specifically focusing on the SAM of carbohydrates prepared on a gold substrate through organosulfur molecules. The two common approaches (**Figure 2**) for forming SAMs of carbohydrates on gold surfaces are, (1) direct method: The carbohydrate molecules of interest are modified with the organosulfur molecule first, followed by direct SAMs formation on the gold surface; and (2) indirect method: The SAM of organosulfur molecule having a suitable terminal functional group is prepared on the gold surface first, followed by a reaction to conjugate it to the carbohydrate of interest.

3.1. Indirect methods

The indirect method of forming carbohydrate SAMs does not require preparation of organosulfur molecules already linked to the carbohydrate of interest. The strategy also avoids

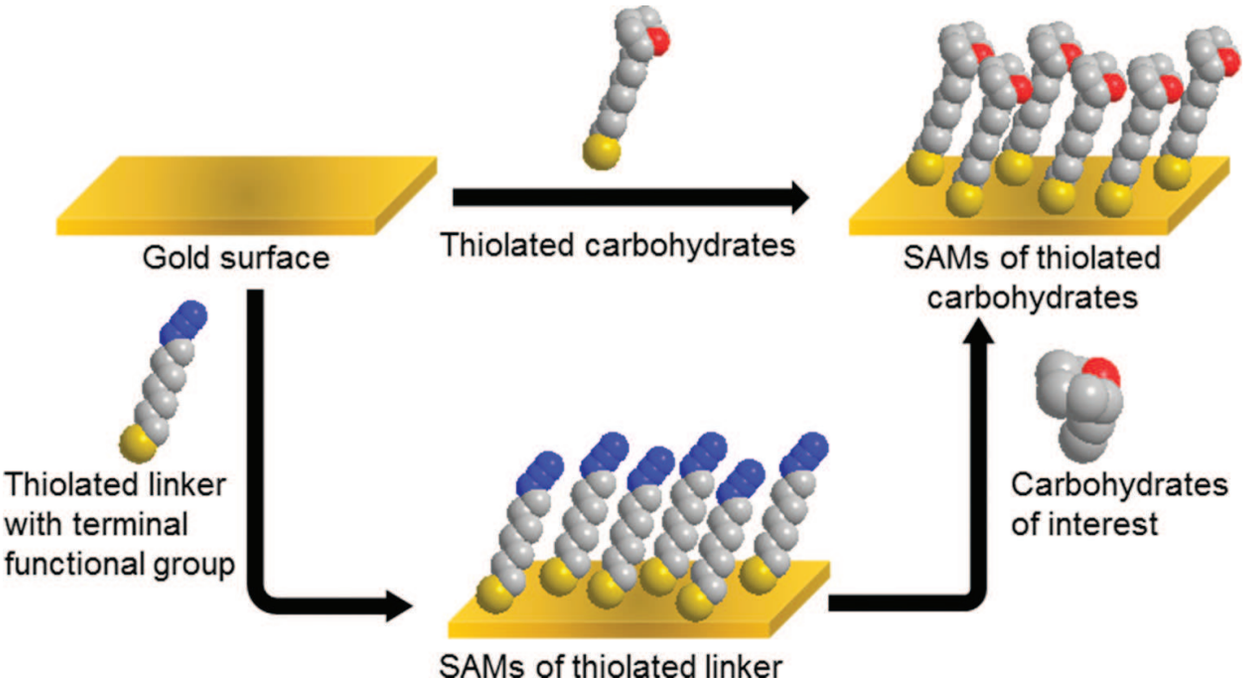

Figure 2. Schematic of two different strategies for forming the SAM of carbohydrates on gold surfaces.

the possibility that the prepared molecules may decompose or oxidize by the time they are used and might not form stable SAMs. An advantage of the indirect SAM formation method is that each step can be tracked in situ [13], which can be imagined as building a tower by stacking bricks on top of one another with cement between the bricks, whereas the direct method is like transferring a whole tower in one piece. Use of indirect methods for the preparation of carbohydrate SAMs date back to the 1990s. In 1995, Lofas used a step-by-step method to form SAMs containing dextran, a hydrophilic linear polymer based on 1,6-linked glucose units, on a gold surface [14]. As a first step, a SAM of 16-mercaptohexadecan-1-ol was formed on a gold surface, followed by the reaction of the exposed hydroxyl groups with epichlorohydrin to prepare terminal epoxy groups. The epoxy group was found to capture the dextran randomly under basic conditions. The immobilized dextran was subsequently reacted with bromoacetic acid to introduce terminal functional carboxylic groups, which were in turn activated using a mixture of N-ethyl-N'-dimethylaminopropyl-carbodiimide and N-hydroxysuccinimide to capture monoclonal antibodies (MAbs). Three different types of MAbs were then tested against their antigen, HIV protein p24. The as-prepared sensor was sensitive enough to distinguish differences in the affinity of the three MAbs toward p24 [14]. During the two decades since, different types of strategies have been discovered for preparing SAMs of carbohydrates using indirect methods, applicable for immobilizing both simple and complex carbohydrate molecules.

Click chemistry-based reactions are popular indirect methods for formation of carbohydrate SAMs. Click reactions skip the tremendous synthetic efforts required for the preparation of thiolated carbohydrates to be used for direct SAM formation. In addition, this reaction can

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tolerate a wide variety of functional groups and can be performed over a broad range of temperature and pH with minimal by-product formation [15, 16].

One of the early uses of click reaction for the carbohydrate SAM formation on a gold surface was performed by Houseman and Mrksich in 2002. They used Diels-Alder reaction to connect benzoquinone-terminated SAM surfaces to different common monosaccharides derivatized with cyclopentadiene and prepared carbochips [17]. The carbochips were then utilized for profiling lectin-binding specificity to their corresponding monosaccharides using SPR and confocal fluorescence microscopy as shown in **Figure 3**. The same group soon reported another click reaction strategy based on maleimide-thiol chemistry for preparing SAMs of carbohydrates [18]. They synthesized four different monosaccharides (mannose, galactose, glucose, and N-acetylglucosamine) having thiol groups at the anomeric centers, which reacted selectively with the terminal maleimide groups of pre-immobilized SAMs on gold surfaces to present carbohydrate-terminated SAMs. They then prepared a carbohydrate array to study the specificity of these carbohydrates to their corresponding lectins using confocal fluorescence microscopy, as before. This strategy of maleimide-thiol reaction can be used for preparing carbohydrate array SAMs having terminal mannoses and interrogate GFP-transfected *E coli* bacteria creating a highly biorepulsive linker [19].

The most commonly used click reaction for preparing carbohydrates SAMs, however, is the Cu (I)-catalyzed Huisgen 1, 3-dipolar cycloaddition or the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction [15]. The first step in this method is to form an organosulfur SAM having either alkyne or azide terminal groups [20, 21]. If the alkyne group is presented at the SAM's surface, it reacts with azide groups available on anomeric positions of carbohydrate molecules of interest and vice versa [22]. This click reaction results in formation of a 1,2,3-triazole ring with the SAM presenting the terminal carbohydrate [23]. Zhang and coworkers used this chemistry to immobilize azido sugars—mannose, lactose, and α-Gal trisaccharides—and studied their interactions with their corresponding specific lectins [24]. Using QCM, the apparent affinity constant (K_a) for the interaction of mannose–Con A, lactose–*Erythrina cristagalli* lectin, and α-Gal trisaccharides–polyclonal anti-Gal antibody were found to be (8.7 ± 2.8) × 10^5, (4.6 ± 2.4) × 10^6 and (6.7 ± 3.3) × 10^6 M^{-1}, respectively. The data were further supported with SPR, AFM, and electrochemical experiments. The prepared sensor was found to be very selective having negligible nonspecific adsorption. Later, the Tamiya laboratory used this strategy to electrochemically detect Alzheimer's related peptide amyloid-β by immobilizing sialic acid derivatives on gold nanoparticles deposited on a carbon electrode [21]. On capturing the peptides Aβ(1-40) and Aβ(1-42), a characteristic oxidation peak was observed at 0.6 V (vs. Ag/AgCl) through differential pulse voltammetry, which was further confirmed by AFM images showing increase in roughness on the surface after capturing of peptides by the sialic acid. In another study, using circular dichroism spectroscopy and fluorescence microscopy, SAM of sialic acid and 6-sulfo-GlcNAc was found to have tendency to change the conformations of Aβ(1–42) into β-sheet while also aggregating Aβ into fibrils having a larger diameter compare to that created by SAM of β-Glc [25]. In the same work, binding affinities of different common monosaccharide SAMs to their corresponding lectins were also studied and were found to be the range of 10^{-7}–10^{-8} M.

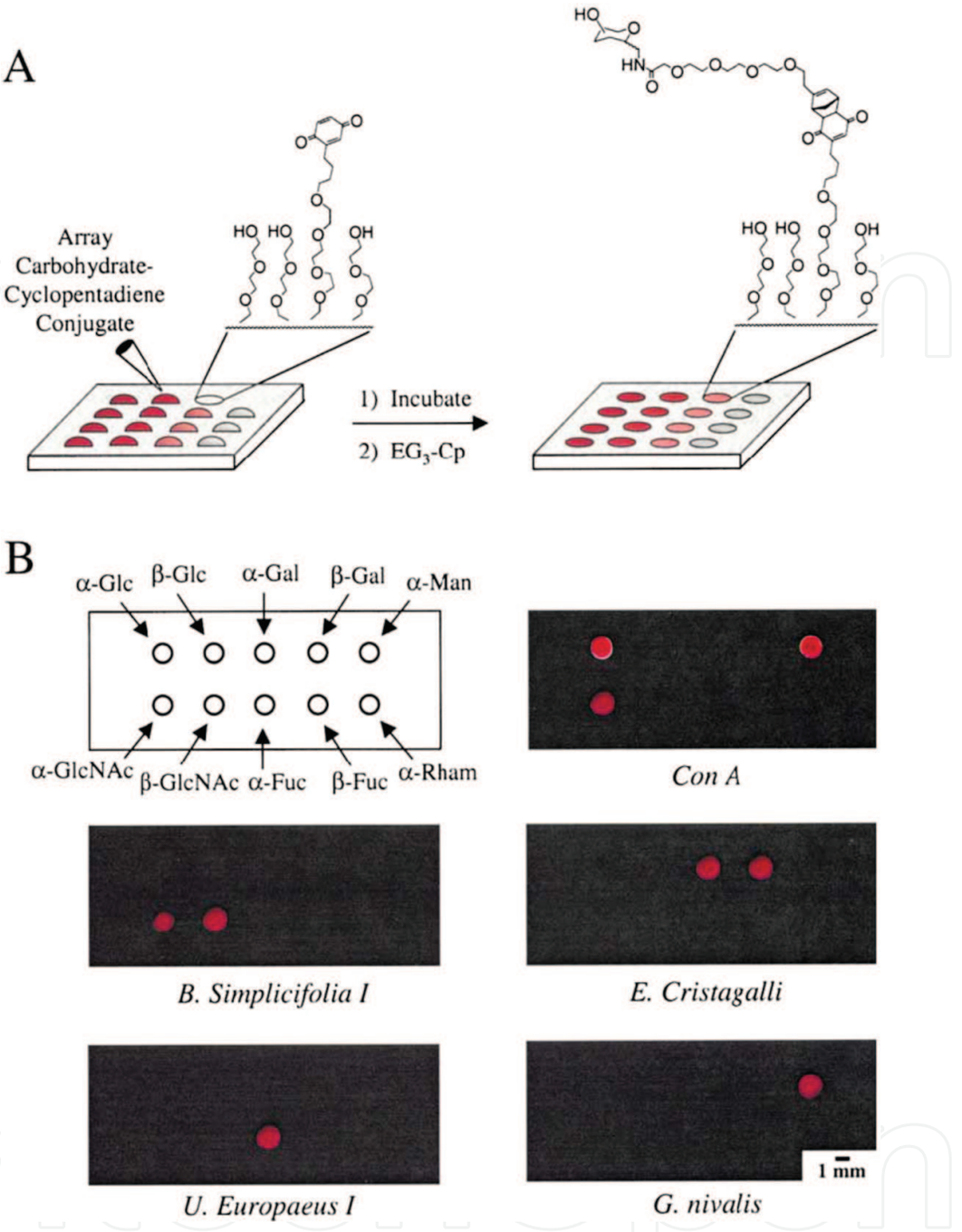

Figure 3. Profiling lectin binding specificities with a carbohydrate array (A). Carbohydrate arrays are prepared by spotting solutions of carbohydrate diene conjugates onto discrete regions of a monolayer presenting benzoquinone groups. After the reaction is complete, benzoquinone groups in the remaining regions of the monolayer can be inactivated by treatment with tri(ethylene glycol)-cyclopentadiene conjugate (EG3-Cp). (B) Identical carbohydrate chips were separately incubated with each of five rhodaminelabeled lectins (2 μM in DPBS) for 30 min, gently rinsed, and evaluated by confocal fluorescence microscopy. Fluorescent images of the resulting arrays are shown for each lectin. These images reveal that the proteins associate specifically with their known carbohydrate ligands on the array. Reprinted with permission from Ref. [17], Copyright 2002, Elsevier.

To create a well-defined multivalency, SAMs of glycodendrimers of various generations were prepared using CuAAC reactions on a gold surface (**Figure 4**) and characterized using

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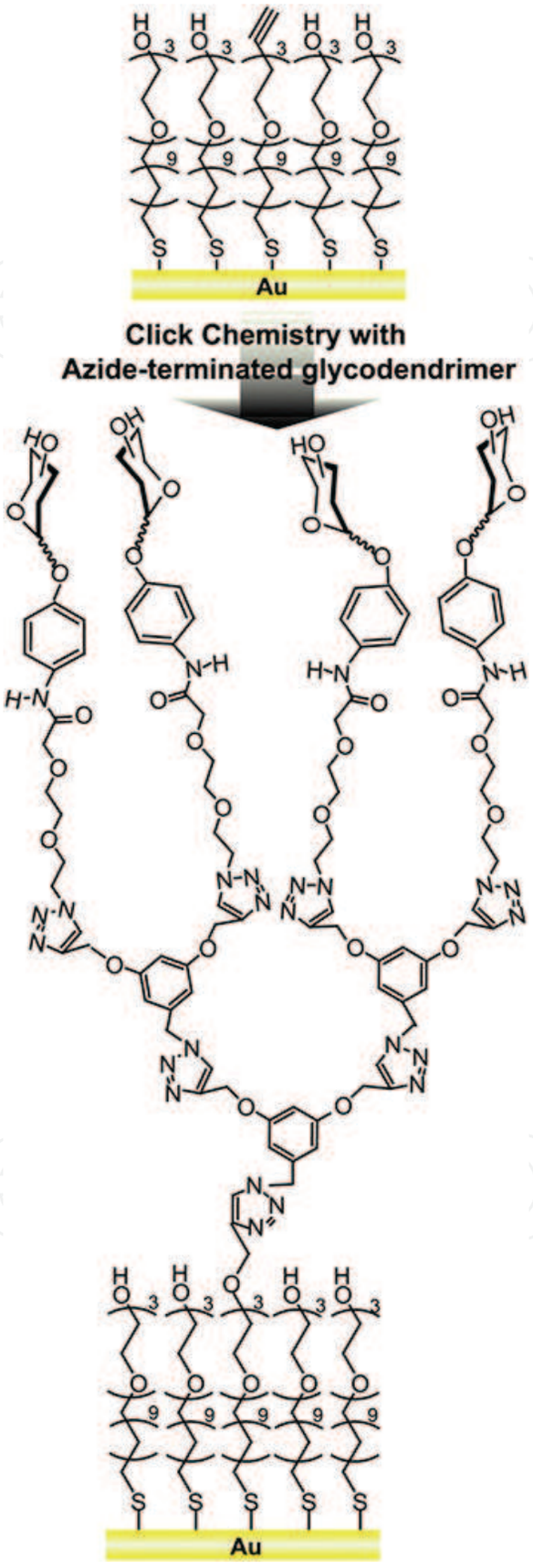


Figure 4. Schematic illustration for the process of glycodendrimer immobilization. Reprinted with permission from Ref. [4], Copyright 2009, Elsevier.

XPS, ellipsometry, MALDI-ToF mass spectrometry, cyclic voltammetry, ^{1}H and ^{13}C NMR, contact angle goniometry and FTIR [4, 26]. The equilibrium association constants (K_a) and kinetic association rate constants (k_{on}) and dissociation rate constants (k_{off}) for the interactions of terminal monosaccharides (α-Man, β-GlcNAc, and β-Gal) to their corresponding lectins were determined using SPR [4]. Similarly, dendritic mannosylated surfaces were used to enhanced recognition of *E. coli* bacteria with generation one dendrimer detecting 3570

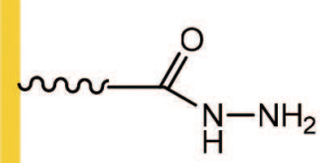
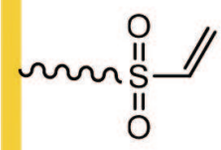
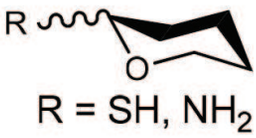
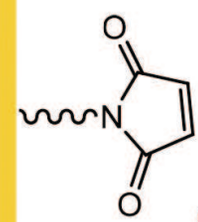
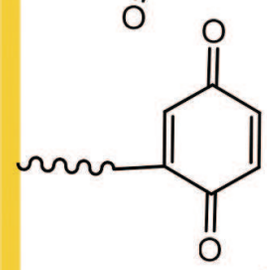
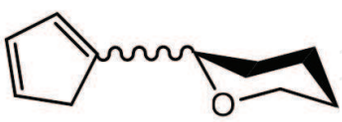
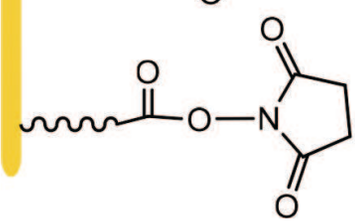
Pre-formed SAM	Functionalized carbohydrates	Reactive functional groups
		Alkyne/Azide [4, 13, 21, 24, 25, 27]
		Azide/Alkyne [20]
		Amine/Isothiocyanate [5, 27]
		Hydrazide/unmodified carbohydrates [28]
		Vinyl sulfone/amine or thiol [29]
		Maleimide/thiol [18, 19,30]
		Benzoquinone/cyclopentadiene [17]
		N-Hydroxysuccinimide-ester/amine [31]

Table 1. Different strategies to prepare SAMs of carbohydrate using indirect method.

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cells/pmol of end groups, whereas generation three dendrimer detected 4170 cells/pmol of end groups [26].

Recently, the CuAAC along with amine/isothiocyanate click reactions were used by Grabosch and coworkers to prepare mannosylated SAMs on gold surfaces [27]. The surface was then used for selective recognition of GFP-transformed *E. coli* bacterial with a minimum of nonspecific interactions because of the prepared biorepulsive linker. There are many other pairs of functional groups [17–19, 28–31], which can react together for the successful preparation of carbohydrates SAM using indirect method. Some commonly used functional groups are listed in **Table 1**.

3.2. Direct method

The indirect method of forming SAM of carbohydrates has some advantages, but the direct method has also been widely used within last two decades. In this method, the carbohydrate (or glycan) of interest is directly functionalized with an organosulfur molecule, and the

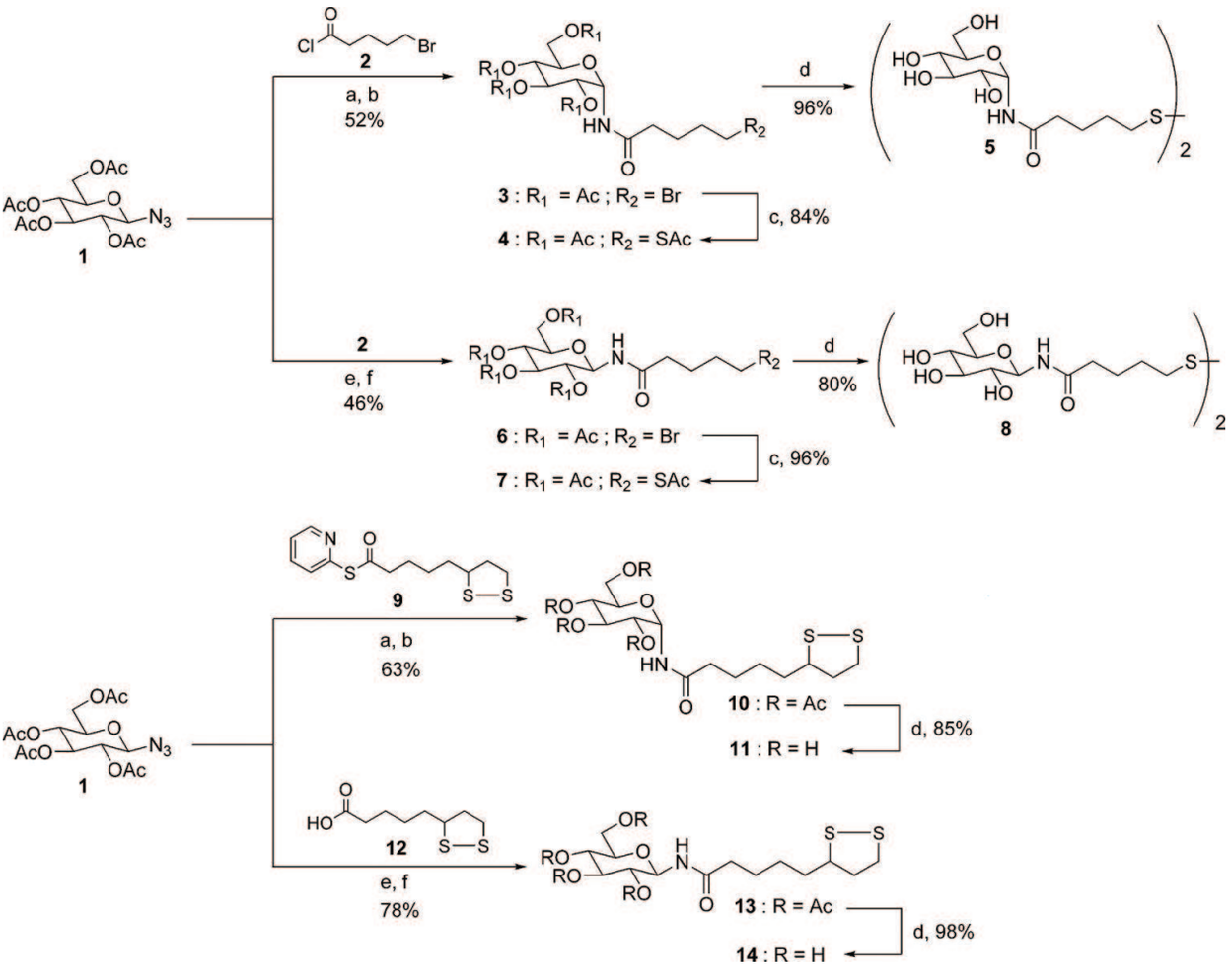
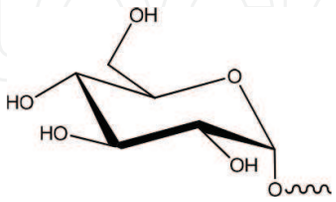
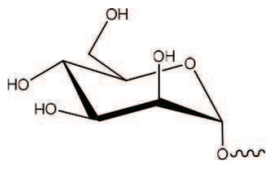


Figure 5. Scheme for synthesis of glucopyranosylamide conjugates. (a) PPh_3, 1,2-dichloroethane, 95°C, 16 h. (b) coupling partner, 24 h. (c) KSAc, DMF, RT, 20 h. (d) NaOMe, MeOH, RT. (e) PMe_3, DIEA, 1,2-dichloroethane, RT, 30 min. (f) coupling partner, RT, 24 h. Reprinted with permission from Ref. [34], Copyright 2007, American Chemical Society.

synthetic product is characterized and confirmed before forming SAMs. In the next step, freshly cleaned gold surface is incubated on ethanolic solution of the functionalized molecule for 1–24 h at ambient conditions to prepare the SAM. This method avoids multistep surface reactions. In 1996, Fritz and coworkers reported SAMs of a hexasaccharide molecule functionalized with alkanethiols on gold surfaces [32]. The conditions for high-density SAM formation were explored by performing experiments with or without protecting the hydroxyl groups with acetyl groups and determining whether unprotecting it after or before the immobilization gave the optimal condition. The optimal condition was found to be adsorption of the unprotected molecules from solution. The Russell laboratory later synthesized mannose-terminated alkanethiols to prepare SAMs and selectively capture Con A, and the expected selective capture of Con A was determined using reflection absorption infrared spectroscopy (RAIRS) and surface plasmon resonance (SPR); L-fucose-specific lectin *Tetragonolobus purpureas* was not captured [33]. The detailed synthetic strategies for the preparation of carbohydrate functionalized organosulfur SAM is outside the scope of this chapter. A representative scheme for preparation of the SAM of glucopyranosylamide is shown in **Figure 5**, which shows strategies for introducing different disulfide linkers to the acetyl protected glucose units having azide groups at the anomeric centers [34].

4. Types of terminal carbohydrates

It is possible to immobilize or form microarrays of a wide variety of carbohydrates on gold surfaces using SAM techniques. Different types of monosaccharides [17, 21, 27, 35–46], disaccharides [42, 43, 47–50], oligosaccharides [45, 46, 49–54], polysaccharides [14], and dendrimers [4, 5, 26] can be immobilized as a terminal functional group of the SAM depending on the nature of the study. **Table 2** lists some of the carbohydrates used as terminal functional groups of the SAM and their applications. The detailed applications of the carbohydrates will be discussed later.

Sacc.	Terminal carbohydrates	Applications
Monosaccharides	Glucose (α-D-Glc) 	-to study the interaction with lectins [17, 55]
	Mannose (α-D-Man) 	-to specifically detect *E. coli* bacteria [27, 56]-to load anti-HIV prodrug candidates on gold nanoparticles using "thiol-for-thiol" ligand place exchange reactions [57]-to study the interactions with lectin Con A to understand more complex carbohydrate-proteins [58, 59]

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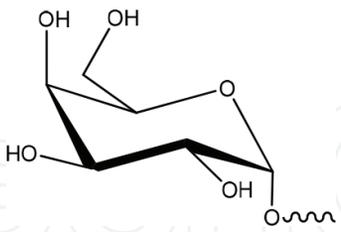
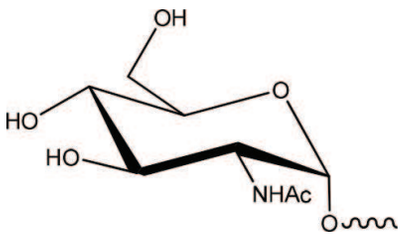
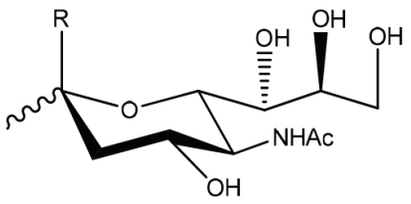
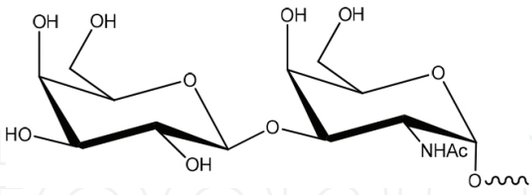
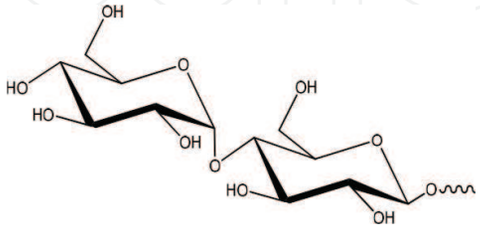
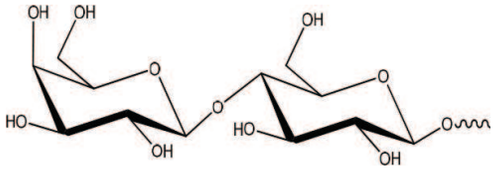
(cont.)

Sacc.	Terminal carbohydrates	Applications
	Galactose (α-D-Gal) 	-to study the specific interactions with different types of lectins, for example, lectin from *E. cristagalli* [17], Jacalin [58], peanut agglutinin- to use as a proteins resist surface [60]-sensitive and facile detection of deadly plant protein, ricin [47]-to use as an anti-fouling surface for marine fouling organisms [35, 48]
	N-acetylglucosamine (GlcNAc) 	-wheat germ agglutinin binding study [36]- to study the enzymatic modification of the immobilized carbohydrates[37]
	N-acetylneuraminic acid (sialic acid)  R = COO^- or OH	-to detect Alzheimer's disease linked protein amyloid-beta (Aβ) at low concentration [21, 38]- to optically detect virus based on plasmonic properties of the sialic acid-linked gold nanoparticles assembled on the surface of viruses [39]
Disaccharides	Thomsen–Friedenreich antigen (TF_{ag})β-D-Gal-(1–4)-D-GalNAc 	-to study binding properties and immune response of this tumor-associated carbohydrate antigen [40] and in antitumor therapeutics [41]
	Maltose 	-to detect nanomolar concentration of Con A with high signal-to-noise ratio [42, 43]-to prepare antifouling surface against proteins and common marine fouling species [48]
	Lactose 	-to study the interaction with lectin *Erythrinacristagalli*-sensitive and facile detection of deadly plant proteinous toxin, ricin [47]-nM-level detection of galectins, a biomarker for cancer and other serious diseases [44, 45]

(cont.)

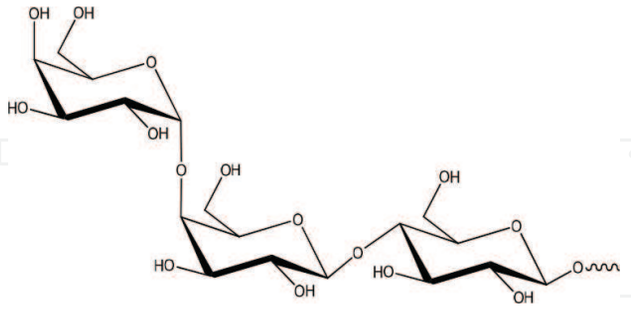
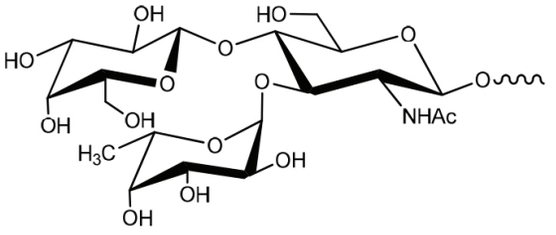
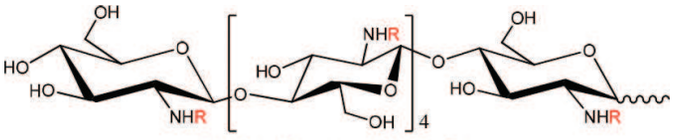
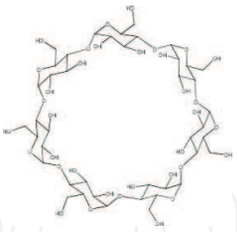
Sacc.	Terminal carbohydrates	Applications
Oligosaccharides	Globotriose (α-D-Gal-(1-4)-β-D-Gal-(1-4)-β-D-Glc) 	- to probe Shiga-like toxins[46]- as potential therapeutics for Shiga toxins when used with gold nanoparticles [50]-to discover the peptides that can inhibit Shiga toxin [49]
	Lewis x (le^x) 	-to study the carbohydrate-carbohydrate interactions on gold nanoparticleSelf-recognition in aqueous solution was demonstrated by mimicking glycosphing olipid clusters [51, 52]
	Chitohexaose  GlcNAc6: R = Ac, Glc6: R= H	-for immobilization of cells and use the surface for cell culture applications [53, 54]
	β-Cyclodextrin 	-to enhance the loading capacity of anticancer drug methotrexate on gold nanoparticles[45]
Dendrimers	Generation 2 dendrimer with terminal carbohydrates (**Figure 4**) Generation 3 dendrimer with terminal mannose group	-to determine association constants and kinetic rate constants with corresponding specific lectins [4, 5]-to enhance bacteria sensor [26]

Table 2. Commonly used carbohydrate molecules to prepare SAM on gold surface and their applications.

5. Role of linkers and mixed SAMs

The spacer connecting the sulfur atom to the terminal carbohydrates has an important role in the arrangement and application of SAMs. The most commonly used linkers are repeating

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units of a methylene group (-CH_2), ethylene glycol (-OCH_2CH_2) and the combination of both [29]. However, linkers can also be made from aromatic compounds [55], dendrimers [4, 5], and peptides [56]. Connecting the different types of linkers previously present on the thiols or carbohydrates may introduce complex structures to the linker; for example, triazoles from alkyne/azides reaction, thiourea-bridge from isothiocyanide/amine reaction and amides from N-hydroxyester/amine reaction. The main goals of the linkers are to provide strong support to the terminal group with a proper orientation, keep the terminal group far from the substrate, and resist non-specific interactions with proteins.

It has been reported that due to kinetic and thermodynamic reasons, longer chain linkers are relatively ordered and robust [11]. The length of the linker is also important for the arrangement of the SAM, which can significantly change the orientation of SAM as shown by Yatawara and coworkers [30]. Alkanethiol linkers of terminal glucose having 11 and 16 carbons chain have similar orientations, but are totally different compared to a cysteine containing linker. A study comparing the effect of thioctic acid amide and alkanethiol linkers on the interactions of terminal mannoses with specifically binding lectin Con A, non-specific lectins and with the highly adsorbent "sticky" proteins, fibrinogen and cytochrome c was performed [57]. The results show that the thioctic acid amide-based linker was better at resisting the nonspecific interactions while specifically binding terminal mannose to its corresponding lectin. It was claimed that this linker can resist adsorption of fibrinogen and cytochrome c better than ethylene oxide-based SAMs.

Even though the longer chain linkers are preferred due to various advantages, monolayers of oligosaccharides (e.g., hyaluronan, chitohexaose, and chitosan hexamer) have been successfully immobilized on gold by modifying the reducing ends of the carbohydrates with thiosemicarbazide (TSC) (**Figure 6**) [53, 58, 59]. The immobilized oligosaccharides SAMs were then used for specifically capturing different types of cell to be used in cell culture applications [53, 59, 60].

Mixed SAMs of carbohydrates are generally formed by two different constituents of thiolated molecules, one having terminal carbohydrates and other lacking carbohydrate molecules. The

Figure 6. Schematic illustration of thiosemicarbazide (TSC)-derivatization and self-assembly immobilization of GlcNAc6 and GlcN6. The photograph is an optical image of carbohydrate-SAMs. Reprinted with permission from Ref. [53], Copyright 2011, The Royal Society of Chemistry.

most common way of making mixed SAMs of carbohydrates on gold surfaces is by co-adsorption of the two components from solution [61]. Other methods to prepare mixed SAMs are by adsorption of asymmetric disulfides on gold surfaces [18] and by ligand exchange reaction [62]. In the ligand exchange reaction, new thiolated molecules are introduced on the surface of the already formed SAMs by a thiol-for-thiol mechanism [62]. However, newly introduced molecules may not yield homogeneously mixed SAMs. The relative ratio of the component of the mixed SAM on the surface depends on the mole ratio of the components in the solution. However, increasing mole ratio of one component in solution does not necessarily increase its ratio on surface in a directly proportional manner [11].

A main goal of making a mixed SAM is to minimize non-specific interactions and create a biorepulsive background, as the hydrophobic chain of the linker might not be able to resist non-specific interactions. Mixed SAMs are also prepared to control the density of the terminal carbohydrates, as it has been found that crowding of receptors on the substrate surface is not an optimal condition for the binding of proteins or other analytes [63]. The crowded receptors may interact with each other or hinder the binding of the approaching analyte to the nearby receptors.

Mixed SAMs having two different types of terminal carbohydrates can also be created for a dual function. Aykac¸ and coworkers prepared SAMs of two different carbohydrates, lactose and β-cyclodextrin, on gold nanoparticles to selectively detect human galectin-3 through lactose whereas at the same time loading anticancer drug methotrexate on β-cyclodextrin [45]. This synergistic effect of two different terminal carbohydrates was found to be very effective for site-specific delivery of anticancer drug than when they are used individually.

6. Types of head-group

SAMs of carbohydrates on gold surfaces are extensively prepared based on thiol or disulfide head-groups. Thiols have higher solubility and normally form a well-ordered surface compared to disulfides [64]. However, they are susceptible to oxidation, forming sulfonates or disulfides and degrade over the time [11]. Disulfides, being the less soluble component, may precipitate out forming multilayer contamination if not prepared carefully [11]. In spite of this, disulfides are frequently used as head-groups for carbohydrate-terminated SAMs [65]. The carbohydrate-terminated SAMs formed by using dialkyl disulfide groups are found to be indistinguishable from those formed from the corresponding thiol and are believed to be formed by the cleavage of disulfide bond [66, 67]. However, such phenomenon is not very well studied or understood for the disulfides present in the cyclic form such as in the case of lipoic acid-based linkers. To reduce the problem of oxidation of thiols, they can be protected using different strategies and reduced in situ just before SAM formation. This can be done by keeping them as disulfides before and reducing them to corresponding thiols using dithiothreitol [68] or by first protecting the thiols using the S-trityl group followed by detritylation using trifluoroacetic acid and triethylsilane in dichloromethane [19].

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7. Gold substrates for SAMs formation

Gold substrates are so far the most used and studied substrate for the formation of carbohydrate SAMs not only because they are capable of supporting stable SAMs due to Au-S bonding but also due to their conductivity, chemical and physical stability, and biocompatibility. SAMs of carbohydrates can be prepared on gold surfaces having different morphologies, such as planar (e.g., bulk or thin-films) to nanostructured surfaces (e.g., nanoparticles, nanostructured films, and nanoporous structures). Nanostructures of gold are intriguing to scientists because they can strongly scatter and absorb light due to large optical field enhancements [69] and have a high surface area-to-volume ratio [70] while still maintaining their other important bulk properties. Because of these properties, nanostructures of gold have application in diverse fields, including biomedicine (drug delivery), energy (hydrogen storage, solar cell, and battery), optics (sensors), and electronics (computer chips, information storage) [69, 71]. Carbohydrates SAM on nanoparticles can be prepared using direct and indirect methods similar to those explained before. However, they can also be prepared; at the same time, gold nanoparticles are prepared using reduction of gold salts by keeping the desired thiolated carbohydrates in the same solution mixture. The ligand exchange reaction is another way to introduce the SAM of desired carbohydrates to the already formed carbohydrate SAMs on nanoparticles surfaces [62]. SAMs prepared on nanoparticles may not be exactly same as the SAMs prepared on a planar surface due to the high radius of curvature of nanoparticles [11]. Since the carbohydrate immobilized nanoparticles are free to move around, they are used for studying the self-recognition of different carbohydrates [52], as possible inhibitors of lung cancer metastasis [72] and as an antitumor agent [41, 73]. SAMs of carbohydrates are also immobilized on other robust nanostructures of gold such as nanostructured gold film [74] and nanoporous gold (np-Au), which can be used as a biosensor transducer [75]. np-Au is a three-dimensional structure having pores (inter-ligament gaps) and ligaments with widths on the order of a few nanometers to a few hundreds of nanometers [70, 76]. np-Au was also used as a solid support for synthesizing disaccharides and trisaccharides starting from simple monosaccharide-terminated SAMs [77, 78].

8. Characterization techniques

There are wide varieties of methods to characterize the successful formation of SAM having terminated carbohydrates on gold surfaces and to study their interaction with other biomolecules. However, there is no single technique that alone can characterize the carbohydrates SAMs and their interactions completely, so different techniques should be used to support the result obtained from one method. Based on the purpose of the study, some of the most frequently used techniques are now discussed.

8.1. Surface wettability

The wettability of the surface before and after the modification by carbohydrate-terminated SAMs can be determined using contact angle goniometry by measuring the contact angle

between water droplet and the surface [35]. The contact angle can be calculated by first taking images of the droplet of water on the surface and using software to fit different models. If contact angle is greater than 90°, the surface is considered hydrophobic; and if smaller than 90°, the surface is considered hydrophilic [79]. Unprotected carbohydrate-terminated surfaces typically create low contact angles owing to their hydrophilic nature. The static contact angle determined using a sessile droplet is the common method to check the surface wettability. However, due to the deviation from an ideal nature of the surface, there is always a contact angle hysteresis ranging from advancing contact angle to the receding contact angle [80]. The Liedberg group compared the wettability of the surface created with methylated and nonmethylated galactose-terminated SAMs on gold surfaces [81]. It was found that nonmethylated galactose surfaces had contact angle <10° demonstrating its hydrophilic properties, whereas methylated galactose surfaces had contact angle >70° demonstrating relative hydrophobicity. Dietrich and coworkers used contact angle goniometry to measure the contact angle of dimannoside-terminated SAMs on gold surfaces, which was found to be 36° ± 2° [79]. The reported contact angle is high compared to a pure hydroxyl-terminated surface, attributed to the exposed hydrophobic aliphatic linker. Fyrner and coworkers measured the advancing contact angle of oligo(lactose)-based thiol SAMs on gold, and it was found to be <10° [82]. This demonstrates a very hydrophilic surface as expected because of the highly hydroxylated oligosaccharides moieties.

8.2. Thickness and roughness

Ellipsometry is a powerful optical technique used for measuring the thickness and roughness of the carbohydrate-terminated SAM surface. This technique is based on changes in the polarization of incident radiation upon interacting with the surface of interest. The Konradsson group utilized this technique to study the increase in thickness of the oligo(lactose)-based SAM by introducing one, two, and three lactose units [82]. As expected, the thickness of the immobilized SAMs for one lactose unit 15.7 ± 0.2 is increased to 20.9 ± 0.2 for two units and to 29.8 ± 0.2 for three units. Another study using ellipsometry showed that $(CH_2)_{15}$ alkanethiol linker gave a height of 22 Å, which the addition of globotriose increased to 32 Å and on insertion of triethylene glycol between globotriose and the alkanethiol linker increased to 40 Å [68]. The results also showed that the thickness of the mixed monolayer increases nonlinearly with an increase in the ratio of the globotriose in solution, as the composition of globotriose in the incubation solution differs from that immobilized on the gold surface.

Atomic force microscopy (AFM) is another important tool, which can be used to directly image the sample to see the roughness on the surface with the resolution on the order of a fraction of a nanometer. It consists of cantilever having sharp tip made of silicon or silicon nitride that gets deflected upon interaction with the surface. Depending on the nature of study, deflecting force or force needed to keep the cantilever at a constant height can be measured using mainly two different modes, contact mode and non-contact mode (tapping mode). The Penades group prepared SAMs of disaccharide maltose on flat gold (111) surface as a neoglycoconjugate to mimic the glycolipids of cell membranes and characterized them using AFM [83]. They have found that despite the bulkiness of the carbohydrate groups, the SAMs were found to be well-ordered and densely packed comparable to SAMs of alkanethiols. A

similar conclusion was reached when α- or β-linked glucopyranosylamide derivatives SAMs were prepared on gold and studied using the contact mode of AFM [34]. The study has also found that acetate analogues of the same compound form multilayered films instead of forming SAMs under the similar deposition conditions. AFM was also used for monitoring the interactions of mannose-terminated SAMs to Con A by observing changes in roughness on the surface before and after the immobilization of Con A [24]. Increase in roughness and the average *z*-dimension value from ≈2.5 to ≈4.0 nm was observed after Con A immobilization. Relatively smaller height of Con A compared to the one obtained from X-ray crystallography (≈6.3–8.9 nm) was attributed to the tip-induced changes when contact mode was used. **Figure 7** shows work by Chikae and coworkers where AFM was used for monitoring changes in roughness on the surface after each modification step [21]. **Figure 7A** shows the AFM image of clean flat gold substrate, **7B** is the image after immobilization of sialic acid SAM, and **7C** and **7D** represent the images after capturing of Aβ(1–40) and Aβ(1–42), respectively after incubation of 20 μM Aβ peptides at room temperature for 180 min.

In a slightly different direction, AFM was also used for controlling the spacing of already immobilized carbohydrate SAMs by increasing the imaging force above the displacement threshold [84], which was then used to monitor the binding affinity of viral envelope glycoprotein gp120 to SAM of galactosylceramide prepared at controlled edge-to-edge gaps. The protein shows better immobilization when edge-to-edge separation of SAMs falls between 1.3 and 9.4 nm, with a 4.8 nm gap giving the optimal binding.

8.3. Chemical composition

X-ray photoelectron spectroscopy (XPS) is a powerful surface characterization technique, which provides useful information about elemental and chemical composition of carbohydrate SAMs. Cheng and coworkers utilized the XPS technique to determine the carbon, sulfur, and oxygen composition of diluting SAM, linker, and functionalized mannose [85]. In another study, high-resolution XPS analysis of C1s spectrum was performed by Dhayal and Ratner to identify the O-C-O functionality and separate/quantify the relative coverage of carbohydrate molecules in mixed SAMs [86]. They also found that the ratio of thiolated molecules in the solution is directly dependent on types of SAMs formed on the surface. High-resolution XPS was also used to track the nitrogen atom after the alkyne/azide click reaction [87]. The N1s spectra clearly does not show any peak before the click reaction, but after the click reaction, the N1s spectra shows a peak at 402 eV supporting the formation of triazole rings.

Infrared (IR) spectroscopy provides the information regarding the chemical composition present on the SAM surface based on frequencies and intensities of the molecular vibration. A simple carbohydrate molecule show characteristic bands in two different regions; the first is a broad band at around 3300–3500 cm^{-1} due to stretching of OH groups and the second is several modes between 1200 and 1000 cm^{-1} due to hemiacetal of carbohydrates (O-C-O) [13]. Increasing broadness of OH band and shifting to lower frequency indicate higher intermolecular and intramolecular hydrogen bonding, creating well-ordered densely packed monolayer formation [33]. Several other special molecules might be introduced through the linker when preparing the carbohydrate SAM that can be easily seen through the change in

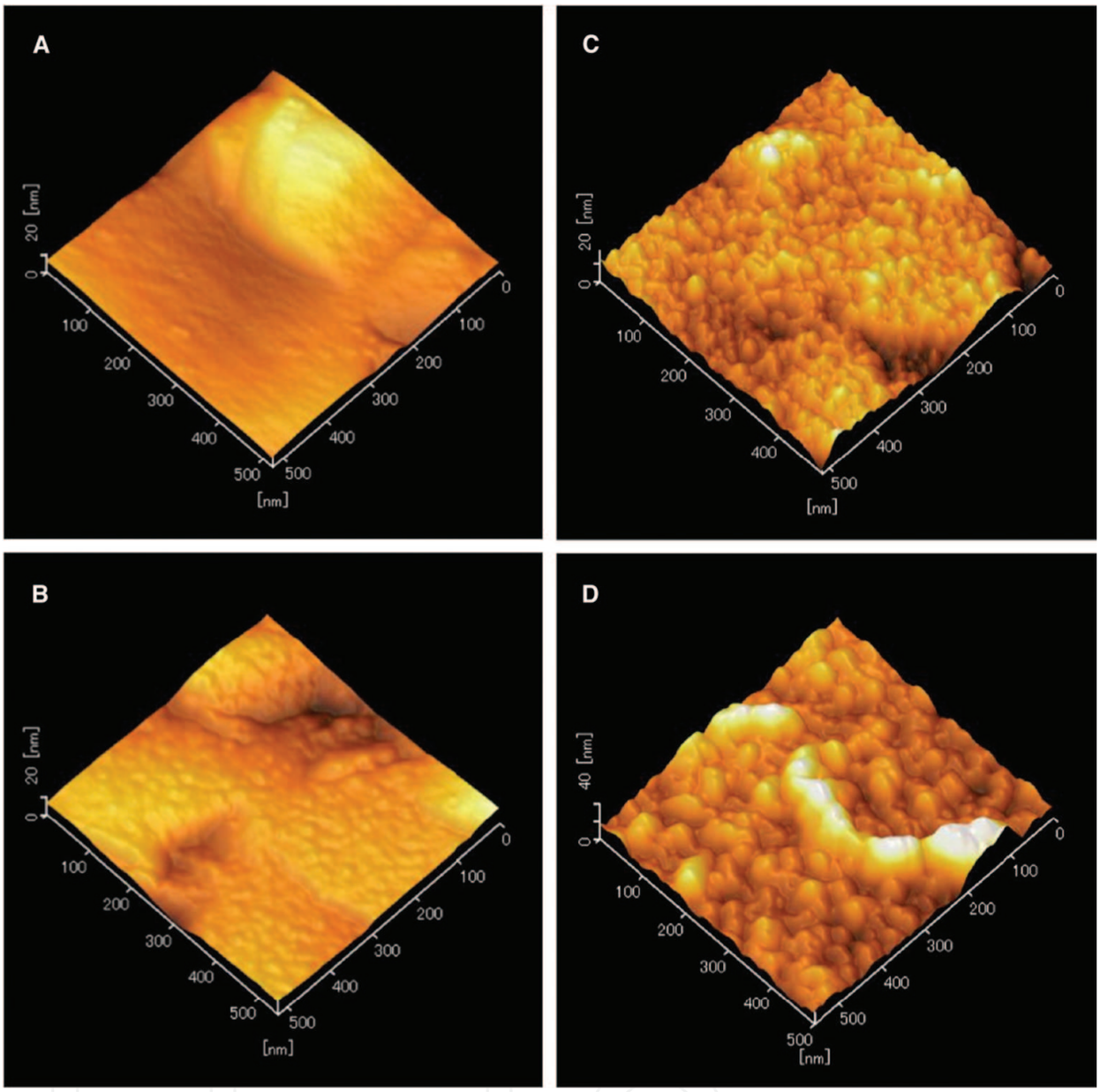

Figure 7. AFM images of the bare gold substrate (A); after cycloaddition of the sialic acid (B); the attachment of 20 μM Aβ peptides after incubation at RT for 180 min; Aβ(1–40) (C) and Aβ(1–42) (D). Reprinted with permission from Ref. [21], Copyright 2008, Elsevier.

position and sudden appearance or disappearance of the bands. Amide group present in the linker of the carbohydrate SAM was found to show bands at ≈1650 and ≈1560 cm^{-1} for amide I and amide II, respectively [34, 88]. Similarly, if the hydroxyl groups are protected with an acetyl group, a strong band will be seen at 1765 cm^{-1} due to ester functionalities.

8.4. Binding affinity

Surface plasmon resonance (SPR) is a popular biosensing tool based on change in the refractive index at the metal-sample interface. It is used for studying the affinity-based interactions

of the biomolecules such as protein-protein interactions, label-free immunoassay, enzyme-substrate interactions, DNA hybridization, and diagnosis of virus-induced diseases in real time [89]. This method has also been widely used for studying the interactions of carbohydrate SAMs with proteins. Schlick and Cloninger studied the inhibition property of glycodendrimer for the binding of Con A to the SAM presenting mannose [90]. The equilibrium dissociation constant (K_d) of Con A to the mannose functionalized surface was initially determined to be 78 nM. The smaller K_d value for this interaction was attributed to multivalent interactions on the surface. A multivalent glycodendrimer framework was used to inhibit the interaction of mannose-functionalized dithiol SAMs on gold surfaces to Con A, whose IC_{50} values were found to be in the range from 260 to 13 nM. In another study, SPR was used for one-step detection of galectins, a β-galactoside-binding lectin well-known as a biomarker for different cancer, by preparing mixed SAMs of thiolated lactoside and triethylene glycol (TEG) [44]. The sensor designed is very sensitive that it can detect ≈ 1 nM galectin-4 and -8, in spite of very weak interactions of galectins and lactoside with $K_d \approx 1.0 \times 10^{-3} - 1.0 \times 10^{-6}$ M because of suppression of nonspecific interactions by TEG.

Localized surface plasmon resonance (LSPR) is a relative new biosensing technique. Scientists are exploring this technique because of its simplicity and possibility to be miniaturized, decreasing the application cost [91]. This technique is very sensitive and supports label-free a real-time biosensing. Similar to SPR, LSPR-based biosensing also depends upon the change in RI with output data commonly represented by measuring wavelength or intensity shift. LSPR-based biosensing, however, also depends on shape, size, and composition of the material used as a transducer [92]. The important step of LSPR-based biosensing is the fabrication of plasmonic sensitive metal nanostructures. Wide varieties of nanostructures have been created using different techniques with the goal of finding simple preparation methods, highly sensitive structures for detecting and studying biomolecules and their interactions, chemically and physically robust structures, and those that can be easily regenerated. Even though nanostructures of many noble metals can generate the LSPR signal, nanostructures of gold and silver are frequently used for LSPR-based biosensing [74]. Silver-based nanostructures show better sensitivity with sharper peaks than gold-based nanostructures [93]. However, silver nanostructures are prone to oxidation causing change in plasmonic properties and also weakening thiol-metal binding [94], making gold nanostructures the metal of choice for LSPR-based biosensing. Bellapadrona and coworkers prepared LSPR sensitive gold island films by evaporation of gold on glass followed by annealing [95]. As-prepared structures were used to form mannose SAM, and interaction with Con A was monitored by change in peak intensity and wavelength. The binding kinetics of mannose to Con A were also determined whose k_{on} and k_{off} are 2.0×10^4 M^{-1} s^{-1} and 2.6×10^3 s^{-1}, respectively. The detection limit of Con A on mannose-terminated SAM was achieved down to <5 nM. Previously, our group has also prepared a robust and sensitive nanostructured gold film (NGF) using a simple electrochemical method [74]. By immobilizing mixed SAMs presenting mannose, we were able to show the real-time interaction of Con A to mannose-terminated SAMs.

LSPR was applied to study monolayers of colloidal Au nanoparticles supported on glass. These were modified by polymer brushes presenting multiple glucose residues, and LSPR was used to determine a binding constant of $5.0 \pm 0.2 \times 10^5$ M^{-1} noted as larger than that for

Con A binding to methyl α-D-glucopyranoside of 2.4±0.1 × 10^3 M^{-1} in solution due to multivalent binding effects [96]. The use of supported gold nanoparticles modified with a polymer brush presenting many mannose units was also applied to follow Con A binding, resulting in an apparent association constant of 7.4 ′ 0.1 × 10^6 M^{-1}, noted as much greater than that for Con A to methyl α-D-mannopyranoside in solution of 7.6±0.2 × 10^3 M^{-1}, with the difference attributed to multivalent binding [97]. Galactose presenting polymer brushes was also used to modify colloidal gold monolayers and their binding of the lectin RCA120 was followed by LSPR, and the binding of HepG2 cells which contain galactose receptors was followed by optical microscopy [98]. The interaction of wheat germ agglutinin (WGA) with a disulfide-modified telomer polymer on a colloidal gold monolayer was also followed by LSPR [99].

Cyclic voltammetry: Cyclic voltammetry (CV) is one of the commonly used electroanalytical techniques for the study of SAMs. In this technique, the potential is linearly scanned back and forth making a triangular waveform in appropriate electrolyte solution. SAMs of carbohydrates and their interactions with biomolecules are normally determined by monitoring a decrease in peak current of a voltammogram. Li and coworkers used mannose-terminated SAMs to capture *E. coli* bacteria and the change in the peak current before and after the immobilization of bacteria was monitored [100]. It was found that the peak current decreased exponentially reaching a plateau within 5 min due to immobilization of *E. coli,* which means that there is a less free space surrounding the electrode for the probe molecules to reach the surface of the electrode. However, cyclic voltammetry may not always be a suitable technique to study the interactions, as the peak current may reach a plateau beforehand as the well-ordered SAM on gold surfaces leaves no further possibility for the peak current to decrease, which also means that if no probe molecules can penetrate the SAM, they also cannot penetrate any other biomolecules immobilized on SAM surface, and no change in current response will be seen [21, 75].

Electrochemical impedance spectroscopy (EIS) is another very sensitive technique used for biosensing [101]. This technique measures the resistive and capacitive properties of materials by applying a small alternating sinusoidal potential, typically 2–10 mV around a fixed potential usually chosen as the formal potential of a redox probe. An impedance spectrum is obtained by varying the frequency over a wide range and is most commonly represented in the form of a Nyquist plot [102]. Interactions of terminal carbohydrates of SAM with other biomolecules are observed by measuring the charge transfer resistance of the electrode before and after the modification of the surface. The Heineman group used EIS to detect the mannose-specific bacteria *E. coli* ORN 178 on mannose-terminated SAM on a gold surface [103]. With the incubation of *E. coli* ORN 178 on the mannose surface, the charge transfer resistance (R_{ct}) increased drastically in the Nyquist plot because of the selective binding, whereas incubating with *E. coli* ORN208 did not show any significant change in the signal supporting non-specificity toward mannose. Monitoring the change in signal with change in concentration of *E. coli* ORN 178, it proved possible to determine the detection capacity of the system, found to be from 10^2 to 10^3 CFU/mL. In another work, EIS was used to study the resistance of different carbohydrate-derived lipoic acid derivatives for nonspecific binding [65]. The interaction of single chain antibody (scFv) with rabbit IgG was studied on a gold surface. It was found that human serum and HeLa cell interfered with the interactions shown by an increase in charge

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transfer resistance. However, when galactose presenting SAM and bovine serum albumin (BSA) was prepared along with the scFv, the resistance decreased compared to scFv alone, but it did not increase upon passing human serum and HeLa cells, showing the effectiveness of the galactose SAM to resist background interactions. When antibody was passed over the SAM surface, it showed an increase in resistance due to selective binding.

Quartz crystal microbalance (QCM) is one of the frequently used techniques for biosensing in which a piezoelectric crystal, most commonly a quartz-crystal coated with a gold electrode, is made to vibrate at a particular frequency [104]. Depending on the increase in mass by the immobilization of the analyte to bioreceptor on the resonator surface, the resonance frequency of quartz crystal decreases, which can be measured electrically and the amount of mass change can be determined [105]. QCM is widely used to study carbohydrate SAMs and their interactions with different biomolecules. Zhang and coworkers used click reaction to immobilize

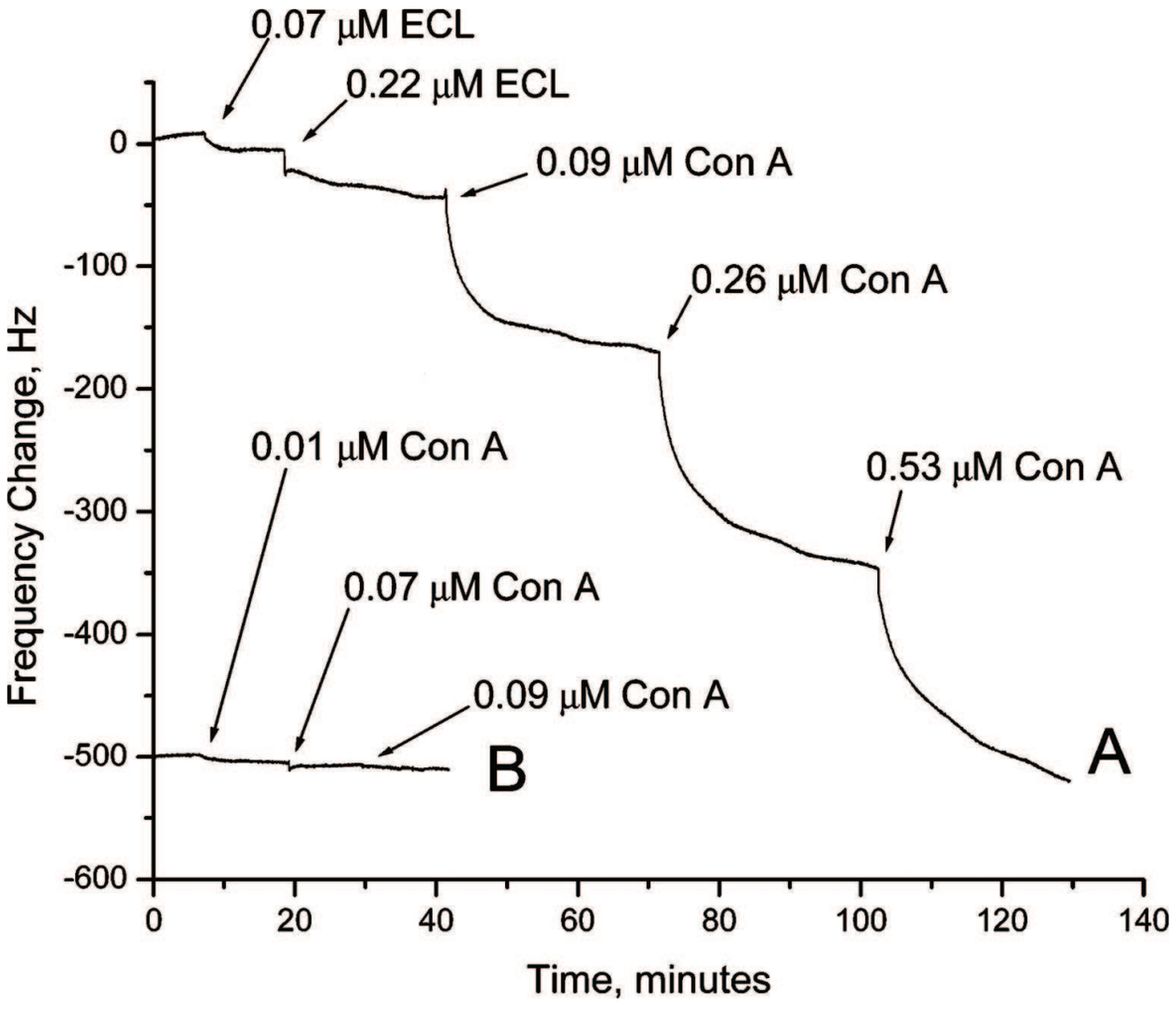

Figure 8. QCM analysis of *Erythrina cristagalli* lectins (ECLs) and Con A binding on (A) mannose SAM and (B) lactose SAM. ECL is specific to lactose but not to mannose, while Con A is specific to mannose but not to lactose. Reprinted with permission from Ref. [24], Copyright 2006, American Chemical Society.

azido sugars (mannose and lactose) on the alkyne-terminated SAM and studied their interactions with con A and *Erythrina cristagalli* lectins (ECLs) using QCM (**Figure 8**) [24]. It can be seen that ECL does not result in a change in frequency on the mannose modified resonator, whereas small concentration of Con A gives larger frequency change due to specific interaction with mannose. Similarly, Con A does not show any interaction with a lactose-functionalized resonator.

9. Applications

The carbohydrates present on the cells surface can act as a receptor for many pathogens to facilitate cell-cell adhesion through which humans can be infected, for example, mannose binds pathogenic bacteria *E. coli* and sialic acid binds influenza virus. Therefore, cell surface can be mimicked by preparing SAMs of carbohydrates to study and understand different types of binding and inhibition studies in vitro. SAMs of carbohydrates are also used for detecting disease biomarkers and carrying drugs.

9.1. Carbohydrate–lectin interactions

The diverse arrangement of carbohydrate in biological molecules makes their study challenging. However, the ubiquitous presence of 10 common monosaccharaides, namely, D-glucose (D-Glc), D-mannose (D-Man), D-galactose (D-Gal), N-acetylglucosamine (D-GlcNAc), N-acetylgalactosamine (D-GalNAc), D-glucuronic acid (D-GlcA), L-fucose (L-Fuc), N-acetylneuraminic acid (Neu5Ac), D-xylose (D-Xyl) and L-iduronic acid (L-IdoA), has made it easier to understand these structures and their functions, mainly by selecting lectins specific to these monosaccharides. Lectins are the proteins having an ability to bind specific types of carbohydrate [106] and hence to variety of glycoproteins, bacteria, and viruses through their carbohydrate-binding moieties [107]. Examples of commonly used lectins include Concanavalin A (Con A)-specific to mannose and glucose [108], peanut agglutinin (PNA) and jacalin-specific to galactosyl (β-1,3) N-acetylgalactosamine sugar sequence [109] and wheat germ agglutinin (WGA)-specific to N-acetylglucosamine.

Carbohydrate-lectin interactions can be studied in solution using techniques like isothermal titration calorimetry [110] or on gold surfaces using SPR, LSPR, EIS, and QCM [75, 104]. It has been found that the interactions between carbohydrate and lectin are stronger when performed on solid surfaces. The reason behind this is the favorable multivalency condition on the solid surface [111]. However, care should be taken when studying the interactions on solid surface as the defects on immobilized film can cause the analyte (protein) to immobilize directly on solid surface and can also precipitate the protein. The main goal of the carbohydrate-protein interactions study is to find the binding constant or to detect protein at low concentration. The change in response before and after the interactions of carbohydrate and lectin mostly in the form of optical, electrochemical, thermal or mass response is recorded. Then the change in response is recorded for wide range of concentrations creating a calibration plot, from which binding kinetics can be determined. The lower the value of K_d, the stronger is

the binding between carbohydrate and lectin. K_d is the important information needed to guide the preparation of different inhibitors.

Since monosaccharides are easier to be derivatized to prepare SAMs, their interactions with their corresponding lectins have been well explored using different techniques. For example, K_d of mannose and Con A interactions are reported in the range of tens of nanomolar to few hundreds of nanomolar [112]. The variation on K_d arises due to the number of factors, including techniques used, type of substrate and SAM, preparation method, and functional activity of lectins. Loaiza and coworkers prepared screen-printed carbon electrodes modified with gold and functionalized with D-glucose and D-mannose SAMs [113]. The substrate was then used for detecting Con A using an electrochemical impedimetric technique finding detection limits of 0.099 and 0.078 pmol for D-glucose and D-mannose SAMs, respectively.

9.2. SAMs of carbohydrates for the detection of *E. coli*

Escherichia coli (E. coli) is a gram negative, rod-shaped bacteria naturally found in the intestines of humans and other animals. Many subset of *E. coli* are harmless; however, they are also the major cause of diarrheal disease among children in low-income countries [114]. The subset of *E. coli* uropathogenic *E. coli* (UPEC) is a leading cause of urinary tract infection (UTI) in humans [115]. According to the National Kidney Foundation, there are nearly 10 million doctor visits each year due to UTI, and one in five women will have it at least once in a lifetime. *E. coli* uses the FimH adhesin present at the tip of the type 1 pili to mediate adherence and invasion to urothelial cells through D-mannose groups present on the cell surface [116]. Invasion of *E. coli* into urothelial cells can not only lead to substantial medical cost for treatment but also damage different parts of the urinary system, including kidney creating potentially life-threatening complication if untreated. The culture and colony counting method is one of the preferred methods for the detection of bacteria, but it normally takes days to obtain the results. Research for developing simple, sensitive, and selective techniques, which can detect, with or without labeling, bacteria cells in shorter periods of time at cheaper price, are ongoing. Mannose microarrays prepared using SAM technique is a promising way to prepare such types of detector. Some of the previously reported work based on *E. coli* detection on mannose SAM surface is discussed below.

Understanding the microbial force of adhesion to the carbohydrate surface can help develop a new approach for detection and prevention of bacterial infection by blocking or decreasing the adhesion capacity. The forces of adhesion of UPEC to the mannose presenting SAM surface, representing the surface of epithelial cells, have been studied using optical tweezers by Whitesides group [117]. The group was successful to orient the bacteria end-on on mannose surface, from where they can be immediately detached and reattached onto mannose surface and the force required to detach from the surface was measured. In another study, SAMs of octadecanethiol on a gold electrode surface and polydiacetylene derivatives with or without terminal mannose were used to prepare bilayer similar to the biological membrane. Incubation of the prepared electrode in *E. coli* solution having 9×10^8 cells/mL for 5 min changes the initial blue color of the electrode to red, which can simply be observed through naked eyes. The change was further confirmed by resonance Raman spectroscopy,

UV-vis and EIS. Transmission electron microscope (TEM) was used to detect *E. coli* ORN 178 strain and distinguish it from non-mannose binding strain *E coli* ORN 208 on gold nanoparticles (AuNP) encapsulated by SAM of mannose. TEM micrographs of the area of pili clearly show selectively bound mannose encapsulated AuNP on pili of *E. coli* ORN 178 strain, but not on pili of the *E. coli* ORN 208 strain [118]. Recently, detection and comparison study of *E. coli* ORN 178 and *E. coli* ORN 208 was also performed using EIS technique where α-mannoside-terminated SAM was immobilized on a gold disk electrode [103]. The system is sensitive to detect bacteria in the range from 10^2 to 10^3 CFU/mL. To make label free and sensitive sensor for detecting bacteria, the synergistic combination of mannose SAM and Con A was utilized as a molecular recognition unit (**Figure 9**) [104]. Compared to the direct interaction of SAM of mannose to *E coli*, this synergistic combination showed significant improvement in attachment, sensitivity and specificity when monitored through the quartz crystal microbalance (QCM) transducer.

The adhesion of Con A to *E. coli* was through the multiple binding to lipopolysaccharides (O-antigen) exposed on cell wall of *E. coli*. LOD for mannose/Con A sensor was improved down to 7.5×10^2 cells/mL from 3.0×10^7 cells/mL for mannose-alone sensor. Similarly, four decades wider linear range (7.5×10^2 – 7.5×10^7 cells/mL) was found for the mannose/Con A-based sensor compared to mannose-alone QCM sensor [104]. In detecting *E. coli* on mannose-terminated SAMs, it is always preferred to have minimum nonspecific interaction for better selectivity and specificity of the detector. Grabosch et al. introduced a dual click chemistry strategy for creating a biorepulsive background with the exposed mannose terminal SAMs [27]. A polyethylene glycol linker having azide and amine terminal groups was used to support dual click reactions. Azides react with alkynes to form triazole ligation products and amines react with isothiocyanate to form thiourea bridges where the other ends of alkyne and isothiocyanate consist of thiol head group and terminal mannose, respectively. The SAMs of mannose prepared by this dual click strategy was found to be very effective in reducing the nonspecific interactions while specifically and selectively capturing green fluorescent protein (GFP)-tagged *E. coli* strain (pPKL1162) evident by epifluorescence micrographs with and without

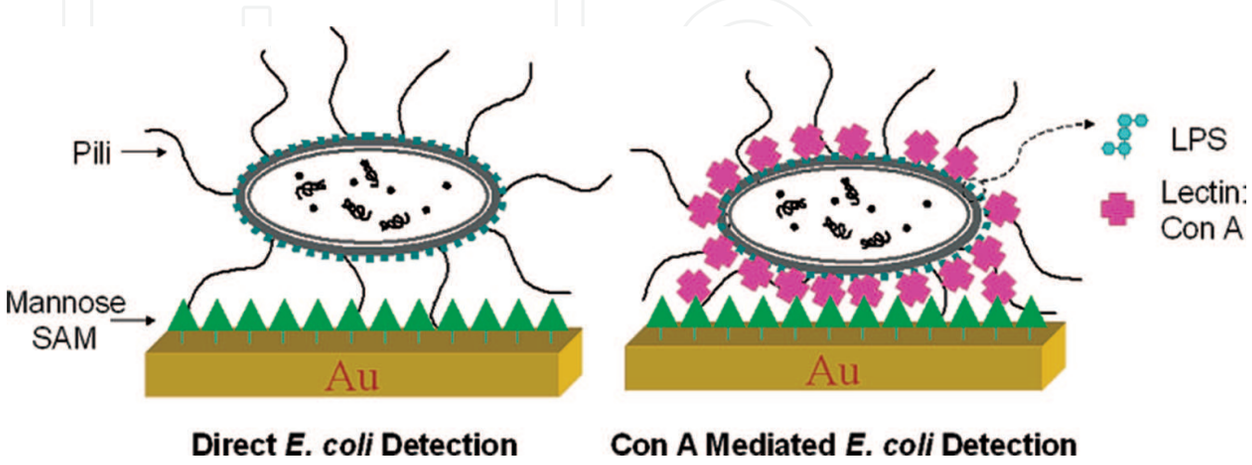

Figure 9. Schematic presentation of direct *E. coli* detection and Con A-mediated *E. coli* detection. Reprinted with permission from Ref. [104], Copyright 2007, American Chemical Society.

http://dx.doi.org/10.5772/66194

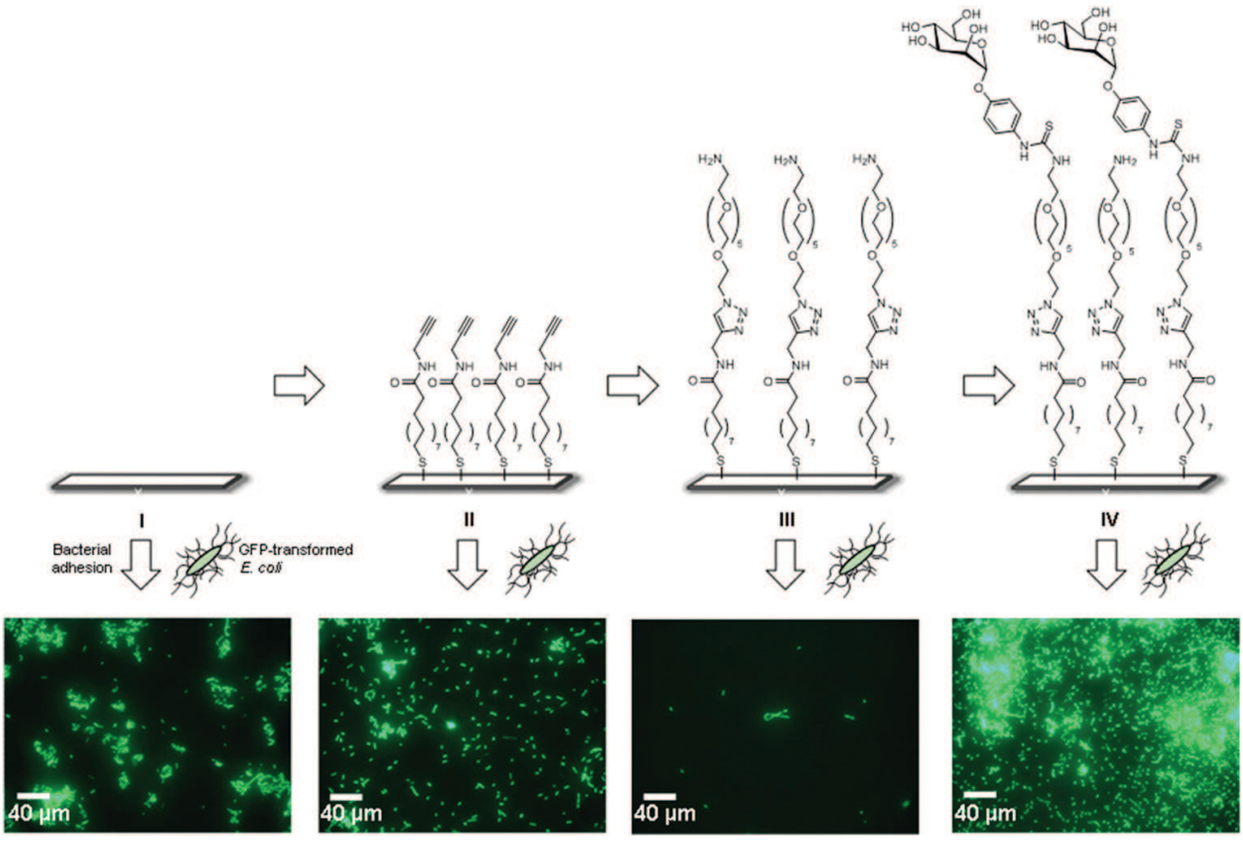

Figure 10. Adhesion of fluorescent bacteria to the different stages of the SAM during the 'dual click' approach. The GFP-transformed *E. coli* bacteria (pPKL1162) enable a fast, direct fluorescence readout to investigate bacterial adhesion on surfaces. The native gold surface (I) was used as reference in each of the other experiments. As can be seen in the epifluorescence micrographs, the (non-specific) adhesivity of the alkyne-terminated SAM II is comparable to the one of the native Au surface. Introduction of the OEG chain reduces the adhesion significantly, while the α-mannosyl-terminated SAM is effectively recognized by the *E. coli* leading to heavy adhesion. Reprinted with permission from Ref. [27], Copyright 2013, The Royal Society of Chemistry.

terminal mannose (**Figure 10**). The same strand of *E. coli* was also successfully detected on self-assembled dendritic monolayer (SADM) having disulfide cores using SPR and resonance-enhanced surface impedance (RESI) [26]. Generation one dendrimer having four terminal mannoses was found to have the binding efficiency of approximately 3600 cells·(pmol of Man)$^{-1}$ whereas generation three dendrimer having 16 terminal mannose have binding efficiency of 4200 cells·(pmol of Man)$^{-1}$. In this work, binding efficiency of generation three dendrimer having terminal mannoses and hydroxides groups to *E. coli* was also compared showing that mannose-terminated surface can improve the attachment of cells by 2.5-fold.

10. Summary

SAMs of carbohydrate can be prepared on gold surfaces to present multivalency and mimic the cell surface to study different physiologically significant interaction in vitro. In this chapter, we have presented the direct and indirect methods for forming SAM of carbohydrates on gold

surfaces. Common strategies of preparing SAMs using indirect method are discussed and presented. We have also tabulated some of the commonly used carbohydrate terminal groups of the SAM, and their applications are presented. Different characterization techniques based on nature of study were also presented. Finally, the application of carbohydrates SAM for lectin and bacteria detection has been discussed.

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