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Glycosylated Self-Assembled Monolayers for Arrays and Surface Analysis

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Abstract

Over the past few decades, carbohydrates (glycans) have received growing attention for their many roles in biological systems, including pathogenesis, receptor-ligand interactions, and cell signaling. To unravel the biology of this important category of biomolecules, a host of new tools have been developed for glycomics investigation. At the forefront is the carbohydrate microarray, developed to immobilize functional glycans on a solid substrate to rapidly screen a variety of potential binding partners (carbohydrates, proteins, nucleic acids, cells, and viruses).

The essential role played by surface modification on glycan microarray performance requires new methods to rigorously characterize glycan surface chemistries. Due to their highly reproducible nature and well-studied properties, self-assembled monolayers (SAMs) on gold are powerful models for presenting glycans on a solid substrate, engineering biomimetic microenvironments and exploring the bioactivity of immobilized carbohydrates via surface plasmon resonance (SPR). However, it can be challenging to prepare high quality glycosylated SAMs (glyco-SAMs) that retain their biological function following surface immobilization. Herein, a selection of versatile methods for the preparation of glyco-SAMs using natural and chemically modified glycans is described. This chapter will highlight the following three immobilization techniques: (1) direct self assembly using thiolated glycosides onto gold, (2) tethering aminated glycosides onto amine-reactive SAMs, and (3) conjugating natural glycan onto divinyl sulfone-activated SAMs.

Keywords

Surface plasmon resonance; Self-assembled monolayer; Glyco-SAM; Thiolated glycoside; Divinyl sulfone

1. Introduction

Surface-immobilized carbohydrates can be used to explore glycan-mediated recognition and binding events, e.g. pathogen and toxin detection, (1, 2) cell adhesion (3), and characterizing the carbohydrate-specificity of novel carbohydrate-binding proteins (lectins) (4). However, displaying bioactive mono- and oligosaccharides on a surface often requires significant chemical derivation prior to immobilization. Functional groups, such as thiols (5), amines (6), fluorocarbons (7), or azides (8), must typically be introduced into a chemically modified glycan to be paired with surfaces bearing compatible chemistries (maleimide, amine-reactive activated ester, hydrophobic, or alkyne). These linking strategies represent a highly rational and engineered approach to surface modification, but can also become a significant synthetic burden to the user. Carbohydrate synthesis frequently requires multiple synthetic steps,

complex protecting group manipulations, and deprotection strategies; (9) introducing linkers for surface immobilization only increases the difficulty of said synthesis.

Due to the lack of a universal synthetic strategy for producing linker-modified glycans, the following describes the preparation of glyco-SAMs based upon three complementary immobilization methods: thiolated glycosides on gold, aminated glycans on amine reactive SAMs, and free reducing sugar on vinyl sulfone-activated SAMs (Scheme 1). Whether the glycans are prepared synthetically to include a thiolated linker, functionalized via simple amination chemistries, or isolated from natural sources, these techniques permit the facile preparation of bioactive monolayers presenting glycans for biological assays, biosensing or surface analysis applications.

2. Materials

2.1. Preparation of Au Substrates

Titanium (2 nm) and gold (45 nm) films sequentially deposited onto cleaned silicon wafers (Silicon Valley Microelectronics, San Jose, CA) and SF-10 glass (SCHOTT Glass Technology, Duryea, PA) for surface analysis and SPRI, respectively. Metal films prepared via electron beam evaporation at the Washington Technology Center (Seattle, WA) (see Note 1).

2.2. Preparation of Solutions

1. Thiolated mannoside: 4–10 mM thiolated mannoside (5) in ultrapure water. Store in single-use aliquots at -20°C and dilute at 0.1–1 mM prior to daily use (see Note 2).
2. 11-Mercaptoundecanol: 10 mM 11-Mercaptoundecanol powder (Sigma-Aldrich, St. Louis, MO) in ethanol (200 proof, USP) (Decon Labs, King of Prussia, PA). Store in single-use aliquots at -20°C and dilute at 1 mM prior to daily use (see Note 3).
3. HEPES buffer (10 $\times$): 0.1 M HEPES, 1.5 M NaCl, 10 mM Ca^{2+} and Mn^{2+} , pH 7.4. Store at room temperature.
4. PBS buffer (10 $\times$): 27 mM KCl, 1.37 M NaCl, 0.1 M phosphate, pH 7.4. Store at room temperature.
5. Carbonate buffer: 0.5 M Na_2CO_3 , pH 10 or pH 11. Store at room temperature.
6. Surface plasmon resonance imaging (SPRI) running buffers: Filter and degass via lab vacuum (4 torr) for at least 30 min prior to daily use (see Note 4).

¹High quality surfaces require clean gold substrates. To ensure the purity of the gold surface, storage conditions must be carefully controlled – in particular, fresh gold substrates must be stored in a polydimethylsiloxane (PDMS)-free environment. Gold can be easily contaminated by PDMS, sulfur, and iodine, even following only brief exposure to the environment in a chemical hood or contaminated lab bench. Gold substrates should be packed/unpacked in a contaminant-free laminar flow hood and stored under an inert atmosphere in the dark.

²Unless stated otherwise, all aqueous solutions need to be prepared in ultrapure water (18.2 M $\Omega\cdot\text{cm}$). In the event where disulfide formation needs to be avoided, 0.5 stoichiometric equivalents of tris(2-carboxyethyl)phosphine (TCEP) can be added to the aqueous thiol solution. This amount of TCEP has not been observed to foul SAMs, according to XPS analysis.

³Unless otherwise stated, all ethanolic solutions are prepared using 200 proof ethanol (USP test) established to be free from copper contamination. The existence of copper in ethanol affects SAM formation and packing density. To quickly ascertain the purity of the ethanol in question, elemental composition of a hydrophilic SAM prepared from the ethanol can be performed by XPS to identify the presence of copper; for this application SAMs bearing oligo(ethylene) glycol on Au can be used.

⁴Glass vials with occasional magnetic stirring are recommended for degassing. Glassware should be checked for scratches and cracks to mitigate the implosion hazard.

7. BSA-T buffer (10×): 10 mg/mL Bovine serum albumin powder (Sigma-Aldrich, St. Louis, MO), 0.05% Tween-20 (Sigma-Aldrich, St. Louis, MO), pH 7.4. Filter and store at -20°C and dilute in 1× PBS or 1× HEPES for daily use.
8. Con A-HRP (10×): 2,500 ng/mL Con A horseradish peroxidase conjugate (EY Laboratories, San Mateo, CA), 10× BSA-T HEPES, pH 7.4. Store at -20°C , and dilute in 1× HEPES for daily use.
9. Con A (10×): 10 mg/mL Concanavalin A (MP Biomedicals, Solon, OH), 10× HEPES, pH 7.4. Store at -20°C , and dilute in 1× HEPES for daily use.
10. RCA120-HRP (10×): 2,500 ng/mL Ricin horseradish peroxidase conjugate (EY Laboratories, San Mateo, CA), 1× BSA-T PBS, pH 7.4. Store at -20°C , dialyze overnight against 1× PBS, and dilute in 1× PBS for daily use.
11. RNase A: 10 mg RNase A (New England Biolabs, Ipswich, MA), 10× PBS, pH 7.4. Store at -20°C .
12. RNase B: 10 mg RNase B (New England Biolabs, Ipswich, MA), 10× PBS, pH 7.4. Store at -20°C .
13. Divinyl sulfone (DVS): 10% v/v divinyl sulfone (Sigma-Aldrich, St. Louis, MO), 0.5 M sodium carbonate buffer, pH 11. Mix vigorously prior to daily use.
14. Urea: 8 M urea (Sigma-Aldrich, St. Louis, MO). Sonicate and store at room temperature.
15. Glycine: 10 mM glycine (Sigma-Aldrich, St. Louis, MO), pH 2. Filter and store at room temperature.

2.3. XPS Analysis

Sample X-ray photoelectron spectroscopy (XPS) composition data was acquired on a Kratos AXIS Ultra DLD instrument equipped with a monochromatic Al-K X-ray source ($h\nu = 1,486.6\text{ eV}$). All the samples were grounded and data were obtained at 0° take-off angle in the hybrid mode. The take-off angle is defined as the angle between the sample surface normal and the axis of the XPS analyzer lens. Compositional survey and detailed scans (N 1s, O 1s, and S 2p) were acquired using a pass energy of 80 eV. High-resolution scans (C 1s) were acquired using a pass energy of 20 eV. Data analysis was performed with the CasaXPS software (Casa Software Ltd.).

2.4. ToF-SIMS Analysis

Sample time-of-flight secondary ion mass spectrometry (ToF-SIMS) spectra was acquired using an ION-TOF TOF.SIMS 5–100 system (ION-TOF GmbH, Münster, Germany). Positive spectra were recorded by rastering a pulsed 25 keV Bi_3^+ primary ion source over a $(100 \times 100)\text{ }\mu\text{m}^2$ area, while maintaining the total ion dose below the static limit (i.e. 10^{12} ions/cm^2). The mass resolution (m/m) for the positive ion spectra was typically 6,000 at $m/z = 27$ for all spectra, which were mass calibrated using the CH_3^+ , C_2H_3^+ , C_3H_7^+ , and AuC_2H_4^+ peaks.

2.5. SPRi

Sample carbohydrate-mediated protein binding was performed on a surface plasmon resonance imaging system (SPRi), SPRImagerII (GWC Technologies). The SPRImagerII was operated at room temperature using a standard flow cell and a peristaltic pump (BioRad-EconoPump) at $100\text{ }\mu\text{L/min}$. All surfaces were passivated with BSA-T for 30 min and equilibrated in an appropriate running buffer prior to protein binding. Data acquisition consisted of the averaging of 30 images over a short duration to create an average image.

The SPR signal is measured via the average pixel intensity, which can be converted to normalized percentages change in reflectivity according to GWC protocol. Urea (8 M) or glycine (10 mM, pH 2.0) was used to strip bound protein and regenerate the array surfaces. For sensorgram acquisition, a 500 $\mu\text{m} \times 500 \mu\text{m}$ region-of-interest (ROI) was selected. For visual clarity, the contrast and brightness of SPR difference images were adjusted by ImageJ software (U.S. National Institutes of Health, Bethesda, MD).

3. Methods

Preparing high quality self-assembled monolayers (SAMs) bearing carbohydrates is a prerequisite for exploring the bioactivity and surface chemistry of surface resident glycans. Ensuring the quality of glyco-SAMs is complicated by the cleanness of the substrate, purity of reagents, intrinsic instability of SAMs, and adventitious contamination originating from self-assembly and storage. Therefore, to produce reliable and reproducible glyco-SAMs for analysis, it is necessary to verify surface quality, track surface contaminants and their origins, establish SAM stability, and examine the compositional change following surface treatment. XPS (10) and ToF-SIMS (11) are routinely utilized to obtain surface information at a molecular level, e.g. atomic composition, the chemical environment of elements at the interface, and surface species relevant to the immobilized biomolecules (Figs. 1 and 2) (12, 13).

Ultimately, it is necessary to determine whether glycan presented on the surface retain their bioactivity. This is best accomplished via a biological assay using a natural binding partner, such as a lectin. Exploiting the sensitivity of the enzyme-linked lectin assay, ELLA (14) is a common and low cost method that derives its origins from the enzyme-linked immunosorbent assay (ELISA). As a label-free method, SPR is routinely employed to directly probe carbohydrate–protein interactions at the interface, providing accurate structure/function information on binding specificity and kinetics (Fig. 3) (15).

3.1. Preparing Glyco-SAMs

3.1.1. Direct Self-Assembly Using Thiolated Glycosides—Molecular self-assembly of thiols onto gold is arguably one of the most well studied methods for surface modification (16). Thiolated biomolecules, including carbohydrates, can be used to produce highly uniform and reproducible SAMs and are ideal as model surfaces for surface analysis and bioassays. In addition to the ease with which thiol-based SAMs can be assembled onto gold surfaces, their composition can be controlled through mixed self-assembly (10, 15). While no single chemistry has been described for the generalized synthesis of thiolated glycosides for surface modification, a wide variety of published methods describe the synthesis of thiol-bearing linkers for the assembly of modified carbohydrates onto gold surfaces or maleimide-activated substrates (5, 15, 17, 18).

1. Self-assembly of thiolated glycosides to form a uniform glyco-SAM is achieved by immersing fresh gold substrate in a 0.1–1 mM solution of the thiolated glycan for 2–12 h at ambient temperature (incubations longer than 2 h do not significantly affect the quality of the glyco-SAM). Assembly may be performed from thiol solutions in water, ethanol or an ethanol-water mix, in accordance with the solubility of the thiolated glycoside. During self-assembly, the sample should be stored in the dark, and kept under an inert atmosphere, if possible (see Notes 2 and 3).
2. Following self-assembly, the modified gold substrate must be rinsed thoroughly. This can be achieved by dipping the sample into a vigorously stirring bath of fresh ultrapure water (50–100 ml) for 1 min (see Note 5).

3. The rinsed glyco-SAMs should be gently dried by a stream of argon and stored in the dark under an inert atmosphere (see Note 6).

3.1.2. Tethering Aminated Glycans onto Sams Bearing Activated Esters—

While thiol-modified glycosides are ideal for direct self-assembly onto gold, the synthetic complexity of their preparation often precludes their use by all but the most highly specialized synthetic laboratories. As a less synthetically burdensome alternative, aminated glycans can easily be prepared by a variety of simple methods from natural isolated reducing sugars (4, 19–21). Glycans bearing a nucleophilic amine are ideal partners for amine reactive chemistries. Utilizing modified surfaces bearing activated ester functionality, it is possible to covalently couple aminated glycans directly to a surface via an amide bond. This provides a facile route for the synthesis of stable glycosylated surfaces, while minimizing the synthetic complexity of the precursors.

1. Amine-reactive surfaces are prepared by assembling SAMs bearing activated esters, e.g. hydroxysuccinimide or tetrafluorophenyl (11, 22). To prepare the activated surface, fresh Au substrates should be immersed in 1 mM ethanolic solution of the amine reactive disulfide for 2 h at room temperature to form the precursor SAMs for bioconjugation.
2. After SAM formation, rinse the surfaces in a stirring ethanol bath (50 ml) for 1 min (see Note 5).
3. Gently dry the cleaned surfaces by a stream of argon and store in the dark under an inert atmosphere (see Note 6).
4. The amine-reactive surfaces can be modified by printing or covering the surface with 0.1–1 mM aminated glycan (23) (pH 7.4) for hydroxysuccinimide ester or pH 10 carbonate for tetrafluorophenyl ester for 24 h at room temperature.
5. To quench unreacted active ester functionality, immerse the glycosylated substrate in BSA-T for 30 min, thoroughly rinse in a stirring water bath for 1 min (see Note 5), and dry by a stream of argon prior to storage in the dark under an inert atmosphere.

3.1.3. Conjugating Isolated Glycans onto Hydroxyl-Terminated Sams via Divinyl Sulfone Chemistries—

One of the ultimate goals for carbohydrate surface modification is a universal method for the covalent conjugation of glycans that does not require chemical synthesis of the precursor sugar species. In certain cases, the difficulty of carbohydrate synthesis, or lack of structural identification, prohibits synthetic access to the prerequisite glycans. In other instances, it is desirable to avoid repeated synthesis of glycosylated targets that have been prepared previously for alternative array surface chemistries (e.g. thiolated vs. aminated glycans). As an alternative, divinyl sulfone (DVS) represents a unique method for the covalent conjugation of thiolated-, aminated-, and unmodified sugars and proteins through the modification of a hydroxyl-terminated SAM or polymer. Upon DVS activation, hydroxyl-bearing surfaces display the highly electrophilic vinyl sulfone group, which will react with glycans bearing amines, thiols and hydroxyls under slightly basic conditions via a Michael addition (1,4-addition) (24–26).

⁵It is essential to thoroughly rinse the surface of unbound molecular species, while minimizing exposure to heat, light, and oxygen. A stirring water bath, rather than sonication, should be used to remove free thiol/disulfide from the surface. The water bath avoids unnecessary heating of the sample and potential damage to the glyco-SAM.

⁶Unless stated otherwise, all glyco-SAMs must be stored at room temperature in a dark and oxygen-free environment. Petri dishes in glass jars backfilled with argon and sealed with parafilm can reduce airborne contamination and oxidation of the thiol-based SAM. Alternatively, samples can be stored in a vacuum desiccator. However, care must be exercised to avoid the introduction of contaminating species when venting the desiccator.

1. Hydroxyl-terminated SAMs can be prepared by immersing fresh Au substrates in 1 mM 11-mercaptoundecanol for 2 h at room temperature to form a uniform SAM.
2. Dip surfaces in a stirring ethanol bath (50 ml) for 1 min and dry under a stream of argon for 1 min (see Note 5).
3. Immerse the cleaned hydroxyl-bearing surface in a solution of DVS (10% v/v, 0.5 M carbonate buffer, pH 11) with vigorous shaking at room temperature for 1 h.
4. The DVS-treated surface should be thoroughly rinsed with a stream of water, dried under a stream of argon, and stored in the dark at 4°C.
5. Functionalize the DVS activated surface by printing or covering with a solution of free reducing sugar (0.5–20% w/v, 0.5 M carbonate buffer, pH 10) at ambient temperature for 16–24 h. Thiolated and aminated glycosides should be conjugated at 0.1–1 mM using pH 7.4 PBS.
6. Quench the printed/functionalized surface for 30 min with BSA-T, thoroughly rinse with water, dry under a stream of argon for 1 min and store in the dark at 4°C.

3.2. Carbohydrate-Mediated Protein Binding

3.2.1. Enzyme-Linked Lectin Assay—ELLA is a popular qualitative probe for interrogating carbohydrate-modified surfaces based on a modified enzyme-linked immunosorbent assay (ELISA). Utilizing commercial peroxidase or alkaline phosphatase-conjugated lectins, ELLAs provide users with an accurate method to compare the bioactivity of carbohydrates on various glycosylated substrates. This method is particularly attractive for general use, as it requires little to no specialized equipment for array fabrication or analysis. The following ELLA-based assay for the bioavailability of glyco-SAMs is based upon the quantification of HRP-modified lectins via a commercial fluorogenic peroxidase substrate.

1. The QuantaBlu fluorogenic peroxidase substrate kit (Thermo Fisher Scientific, Rockford, IL) must be equilibrated to room temperature for 30 min and the QuantaBlu working solution prepared in accordance with the manufacturer's specifications.
2. Prior to ELLA, glyco-SAMs must be immersed in the appropriate working buffer (PBS or HEPES, depending on the lectin) and incubated for 30 min at room temperature to rehydrate the glycosylated surface. Small gold-coated chips can be placed at an angle in a 24-well plate and fully immersed by 1.5 ml buffer. Following incubation, the buffer can be removed by aspiration (see Note 7).
3. To passivate the glyco-SAM substrates against nonspecific protein interactions, gently add BSA-T PBS or HEPES (1.5 ml) to each well and incubate for 1 h at room temperature. Remove the BSA-T buffer and rinse both sides of the glyco-SAM sample three times with BSA-T (see Note 7).
4. Carefully remove the glycosylated substrate from the 24-well plate using forceps and clean by flushing three times with PBS or HEPES working buffer on both sides of the chip. Place cleaned chips in a fresh 24-well plate, resting flat on the surface.

⁷It is essential that the entire gold-coated chip be exposed to the incubation and blocking buffers. By positioning the chips at an angle, propped against the side-wall of the 24-well plate, it is possible to ensure that both the coated and uncoated side are blocked by BSA-T to reduce nonspecific adsorption lectin HRP conjugates to the substrate (bottom side of the chip) and the gold-coated top. Blocking both sides of the chip has been observed to significantly reduce background lectin-binding to the substrate.

5. Add the lectin-HRP solution (250 ng/mL, 1 ml) to each well, and incubate on a shaking platform at room temperature for 1 h (see Note 8).
6. Following incubation, remove the lectin-HRP solution by aspiration and carefully rinse the chip three times with BSA-T on both the gold-coated and unfunctionalized side to remove any unbound lectin-HRP.
7. Carefully remove the glyco-SAM substrates from the 24-well plate using clean forceps and place flat on the surface of a fresh 24-well plate.
8. Add the QuantaBlu working solution (1.0 mL) to each well and incubate on shaking platform at room temperature, in the dark, for 30 min.
9. Add the QuantaBlu stop solution (100 μ L) to each well and mix briefly.
10. Transfer the fully developed QuantBlu solution (50 μ L) to a 96-well plate and measure the Relative Fluorescence Units (RFU) (325 nm ex, and 420 nm em), in accordance with the manufacturer's specifications.

3.2.2. SPRI—Surface plasmon resonance imaging (SPRI) is a powerful method for interrogating complex arrays of immobilized glycans in a high-throughput and label-free fashion. The versatility of SPRI makes it ideal for validating glyco-SAM bioactivity and optimizing surface modification based on the response to protein binding. Glycan arrays for SPRI can be easily prepared using any of the aforementioned glyco-SAM chemistries, including direct assembly of thiolated glycans on a gold surface, coupling of aminated glycans to an activated ester SAM or conjugation of glycan (regardless of source) to a DVS-activated substrate on gold. Arrays can be printed using microcontact printing, (27) pin-based printing, (28) inkjet printing, (4) or prepared by hand (10). The following protocol is based upon a silicone gasket cut to form individual wells and does not require specialized array printing instrumentation.

1. A 1 cm $\times$ 1 cm sheet of culture-well silicone (Grace Bio-Labs, Bend, OR) can be punched using a flat needle (2 mm) to create an array of wells that can be overlaid on a gold surface or activated SAM substrate by gently pressing the pre-punched sheet onto the SPRI chip.
2. Spot $\sim$ 2 μ L of modified or unmodified glycan solution (in accordance with the specific immobilization chemistries) into the silicone well on the SPRI chip and incubate in a 100% relative humidity chamber overnight (see Note 9).
3. Following overnight incubation, remove the SPR chip from the humidity chamber and flush the array spots with water in a bottom-to-top sequence and gently remove the silicone mask. Following removal of the mask, the surface should be briefly rinsed with water (see Note 10).
4. Dry the SPR chip in a stream of argon and immerse the chip in BSA-T on a shaking platform at room temperature for 1 h to passivate unfunctionalized areas on the array surface. The BSA blocking step will make it easier to identify glycan-

⁸Lectin-HRP stored at -20°C gradually loses its peroxidase activity, particularly after a number of freeze-thaw cycles. To verify the HRP activity, a small amount of lectin-HRP should be added to the QuantaBlu working solution and mixed briefly. In the event that the fluorescent product is not observed, the lectin-HRP stock should be discarded in favor of a fresh solution.

⁹The specific concentration of glycans used for surface modification (array fabrication) is a function of the immobilization chemistry. On DVS-activated surfaces, Unmodified reducing sugars are employed as 0.5–20% w/v solutions, pH 10, and aminated and thiolated glycosides are used at 0.1–1 mM, pH 10 and 0.1–1 mM, pH 7.4, respectively. On bare Au and amine-reactive surfaces thiolated glycosides and aminated glycosides are used at 0.1–1 mM.

¹⁰In the event that glycoprotein or protein spots (e.g. RNase B and RNase A) are used in the SPRI array, particular care must be exercised to avoid contamination of the surface during rinsing. Protein spots should be placed on the edge of the array, and the surface rinsed so that unbound protein flows off the chip, and not across the patterned array.

modified spots on the array by increasing the SPRi contrast between the printed and unprinted regions on the chip. This will subsequently facilitate identification of printed spots during region-of-interest (ROI) selection on the SPR instrument.

5. Prior to installation in the SPRi instrument, the functionalized SPR must be rinsed in a stirring water bath, dried in a stream of argon, and positioned in the instrument according to the manufacturer's recommendations (see Note 11).
6. Running buffer, protein dilutions, and protein stripping solutions (8.0 M urea or 10 mM glycine, pH 2.0) can be applied to the SPR chip to measure SPR response according to a pre-established binding recipe. Care must be taken to avoid caustic solutions or oxidative damage to glyco-SAMs. However, if treated gently, chips can be used in repeated binding and regeneration cycles (see Notes 12 and ¹³).
7. Following an SPR experiment, the regenerated, rinsed and dried glyco-SAMs can be saved and stored at 4°C for additional SPR experiments or surface analysis (see Note 14).

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¹¹This rinsing and drying step is essential to ensure the optical quality of the chip by removing excess BSA-T blocking solution and buffer salts. If the chip is not sufficiently rinsed, remaining protein or salts will interfere with refractive index matching to the SPR prism.

¹²All buffers and solutions should be degassed prior to introduction to the SPR instrument to reduce the appearance of bubbles during binding experiments.

¹³During SPR binding experiments, it is critical to avoid the introduction of air bubbles prior to rinsing the surface with water. Air bubbles have been found empirically to damage glyco-SAMs, as observed by an altered SPRi response. In addition, if bubbles are passed over a surface following the specific capture of proteins, surface-bound proteins can become resistant to stripping by urea or glycine, and the glyco-SAM cannot be completely regenerated.

¹⁴To preserve the quality of the glyco-SAMs, the SPR chip should be thoroughly stripped of bound protein by 8.0 M urea or 10 mM glycine (pH 2) and rinsed with water to remove buffering salts. Following removal from the SPR instrument, the SPR chip (glyco-SAM) must be rinsed with water, dried in a stream of argon, and stored in the dark at 4°C under an inert atmosphere.

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Fig. 1.

XPS characterization of a glyco-SAMs formed using direct self-assembly of thiolated mannosides: (a) XPS survey scan, (b) detail sulfur 2p scan, and (c) high resolution carbon 1s scan, indicating the high quality of SAMs bearing mannose on Au. (Note: sulfur 2p_{3/2} at 162.0 eV and C 1s at 288.0 eV are assigned to bound thiol and mannose acetal species, respectively).

Fig. 2. Representative positive ion ToF-SIMS of a glyco-SAM composed of a thiolated mannoside assembled on to gold. Fragments associated with both the linker chemistries and immobilized glycan can be observed by this method; **(a)** oligo(ethylene) glycol fragment $C_2H_5O^+$ (m/z 45) and **(b)** pyranose (sugar) fragment $C_3H_5O_2^+$ (m/z 73).

Fig. 3. SPRi reflectivity image and the corresponding sensorgrams on a spotted glyco-SAM array composed of thiolated mannose, galactose and the glycoprotein RNase B. The bioactivity of immobilized glycans were verified via lectin binding with Con A (500 nM). The array includes (a) thiolated mannose, (b) the mannosylated glycoprotein RNase B and (c) thiolated galactose.

Scheme 1.

Glycan immobilization on to gold or modified gold substrates. **(a)** Direct self-assembly using thiolated glycosides on to a gold surface. **(b)** Tethering aminated glycans onto amine-reactive surfaces. **(c)** Conjugating natural or modified glycans onto hydroxyl terminated SAMs via divinyl sulfone chemistry.