Supplementary Material (ESI) for Chemical Communications

A Renewable, Chemoselective, and Quantitative Ligand Density Microarray for the Study of Biospecific Interactions

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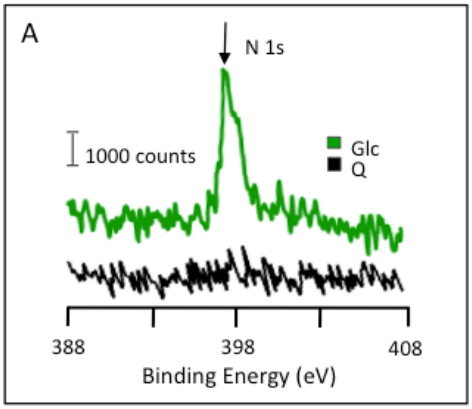
Supplementary Information

X-ray Photoelectron Spectroscopy Characterization



B				
Surface Group	Au			
Contact Angle (°) ± Std	59.31 ± 3.1	64.19 ± 1.2	58.69 ± 1.5	33.32 ± 1.8

Figure S1. X-ray photoelectron spectroscopy (XPS) and contact angle characterization of (A) Q-terminated SAMs before (black) and after reaction with glc-ONH_2 (green). The 1s nitrogen peak found at 398 eV, corresponds to the presence of an oxime linkage after reaction with glc-oxyamine. (B) Contact angle measurements of water taken on bare gold and SAMs presenting quinone-, hydroquinone-, and carbohydrate-terminated groups.

Electrochemical Characterization: SAM & Ligand Density Quantification

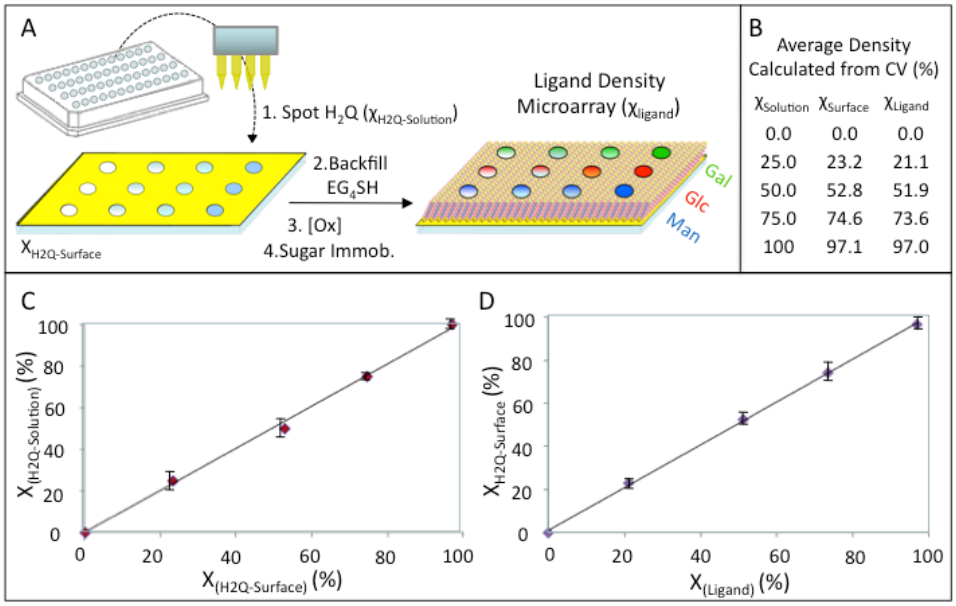
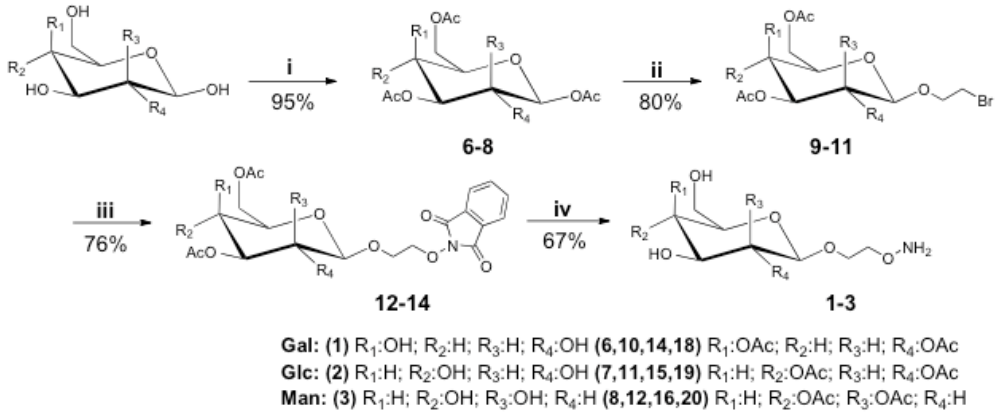


Figure S2. A quantitative comparison of alkanethiol solution density transferred to gold surfaces and the SAM and ligand density microarrays. (A) The general strategy to generate a ligand density microarray for carbohydrate immobilization. (B) The average density percentages calculated by integrating the redox peak area observed from the CV data and applying Q = nFAΓ. (C) A plot describing the relationship between the ratio of alkanethiol solution ($\chi_{H2Q\text{-}Solution}$) orginally transferred to the surface and average-measured surface density of H_2Q molecules ($\chi_{H2Q\text{-}Solution}$). (D) The correlation between $\chi_{H2Q\text{-}Surface}$ and the density of ligand bound, χ_{Ligand}. The slope of both plots is linear, indicating that the H_2Q and ligand density within the spots on the SAM surface is in accord with the spotting solution H_2Q concentration. The averaged percentages of H_2Q and ligand on the surface, according to solution densities, 0, 25, 50, 75 and 100 %.

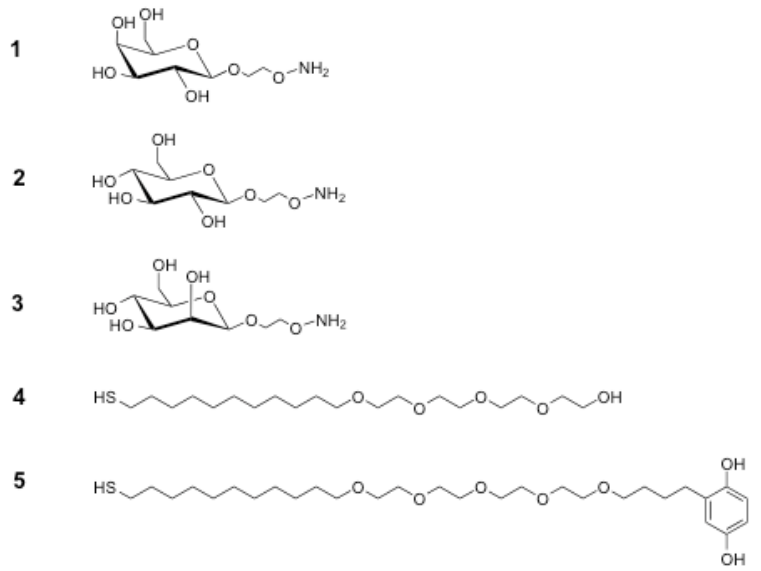
Electrochemistry was also used to quantify the density of H_2Q/Q and immobilized ligands following microarray construction and conjugation, respectively. Alkanethiol solutions containing different ratios of H_2Q/EG_4SH (0:100, 25:75, 50:50, 75:25, and 100:0) were transferred to bare gold surfaces, generating highly reproducible and consistent 24 x 24 microarrays of 100 µm in diameter, SAM spots. To determine whether the corresponding percentage of H_2Q molecules was being printed on the surface, we integrated the CV peak area to calculate the total charge and applied the relationship $Q = nFA\Gamma$ (where Q = total charge, n = number of electrons, 2 for H_2Q, F = Faraday constant, A = surface reaction area [(spot size) x (number of spots)], and surface density of H_2Q (Γ_{H2Q} (molecules/µm^2)). The surface density can then be directly correlated to the spotting solution concentration of H_2Q by plotting $\chi_{H2Q\text{-}Surface}$ versus $\chi_{H2Q\text{-}Solution}$ (Fig. S2C) ($\chi_{H2Q\text{-}surface}$ represents the ratio of H_2Q molecule on a mixed SAM surface, and $\chi_{H2Q\text{-}Solution}$ represents the ratio of H_2Q in the microwell solution.) Following transfer, all surfaces were electrochemically oxidized, reacted with gal-, glc-, and man-ONH_2 (90 mM in MeOH, 4 h), and the oxime linkages were analyzed by CV (in triplicate for each sugar at each density, Figure S2B). Likewise, $\chi_{H2Q\text{-}Surface}$ can be correlated to the density of ligand bound, χ_{Ligand} (Fig. S2D). The slope of both plots is linear, indicating that H_2Q and ligand density is in accord with the spotting solution H_2Q ratio.

Synthesis of Oxyamine-Containing Carbohydrates



Scheme S1. General synthetic route for oxyamine-functionalization of carbohydrates. Reagents and conditions. (i) NaOAc (3 eq), $ZnCl_2$ (cat), Ac_2O, 90°C, 4 h, 95%; (ii) 2-bromethanol (1.2 eq), boron trifluoro diethyletherate (1.3 eq), $ZnCl_2$ (cat), DCM 0-25°C, 6 h, 80%; (iii) N-hydroxyphthalimide (1.5 eq), $NaHCO_3$ (1.5 eq), DMF, 65°C, 8 h, 76%; and (iv) hydrazine (6 eq), EtOH, 25°C, 48 h, 67%.

List of Surface Molecules



Scheme S2. SAM molecules and carbohydrates used in the study. (1) Galactose-oxyamine (Gal-ONH_2); (2) Glucose-oxyamine (Glc-ONH_2); (3) Mannose-oxyamine (Man-ONH_2); (4) Tetra(ethylene glycol)-terminated alkanethiol (EG_4SH); and (5) Hydroquinone-terminated alkanethiol (H_2Q).

Experimental Section

Fluorescent lectins were obtained from Invitrogen; all other chemicals were obtained from Sigma Aldrich.

Synthesis. Tetra(ethylene) glycol- (EG_4SH) and hydroquinone- (H_2Q) terminated alkanethiols were synthesized as previously reported.[1-2] The synthesis of oxyamine-containing monosaccharides is described below.[3]

1,2,3,4,6-Penta-O-acetyl-β-D-(sugar)pyranose (6-8): Typical procedure; to a solution of acetic anhydride (50mL) was added sodium acetate (5.40 g, 66.6 mmol, 3 eq.). The mixture was refluxed at 90°C for 20 minutes to which D-sugar (4.00 g, 22.2 mmol) was added and stirred for 4 hours. The mixture was then concentrated, dissolved in methanol, and recrystallized with cold water. A white solid was then filtered and dried to afford **(6)** (6.734 g, 77%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.01, 2.09, 2.13, 2.15, 2.18 (s, 15H; CH_3), 4.17-4.13 (2xm, 3H, J=16; CH, CH_2), 5.11-5.09 (2xm, 1H, J=7; CH), 5.34-5.33 (t, 1H, J=4; CH), 5.93-5.92 (m, 1H, J=4; CH), 5.72-5.70 (d, 2H, J=8; CH_2); **(7)** (7.44 g, 85%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.01, 2.03, 2.07, 2.09, 2.13 (s, 15H; CH_3), 4.17-4.14 (m, 2H, J=12; CH_2), 4.40-4.38 (2xm, 1H, J=7; CH), 4.71-4.69 (d, 1H, J=8; CH), 5.32-5.31 (t, 1H, J=4; CH), 5.37-5.36 (t, 1H, J=4; CH), 5.41-4.40 (d, 1H, J=4; CH); **(8)** (8.32 g, 95%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 1.99, 2.02, 2.09, 2.13, 2.16 (s, 15H, CH_3), 4.24-4.22 (m, 2H, J=8; CH_2), 4.43-41 (m, 1H, J=7; CH), 5.02 (s, 1H; CH), 5.34-5.33 (t, 1H, J=4; CH), 5.58-5.57 (m, 1H, J=4; CH), 5.66-5.65 (d, 1H, J=4; CH).

O-(2,3,4,6-tetra-O-acetyl)-β-D-(sugar)pyranosyl-bromoethyloxy (9-11): Typical procedure; to a solution of **6-8** (1.00 g, 2.56 mmol, 1 eq.) and $ZnCl_2$ (catalytic) in anhydrous dichloromethane (15 mL) was added 2-bromoethanol (0.24 mL, 3.33 mmol, 1.3 eq.), followed by the addition of boron trifluoride diethyl etherate (0.41 mL, 3.33 mmol, 1.3 eq.) dropwise at 0°C. The mixture was stirred under inert atmosphere (N_2) for 6h at room temperature. Upon completion, the mixture was then washed with water (2x50mL), sodium bicarbonate (1M) (2x50mL), concentrated, and recrystallized in hexanes to afford a white solid **9** (0.721 g, 59%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.01, 2.12, 2.15, 2.18 (s, 12H; CH_3), 4.10-4.06 (2xm, 2H, J=8, J=16; CH_2), 4.17-4,14 (m, 2H, J=12; CH_2), 4.41-4.39 (m, 2H, J=7; CH_2), 4.76-4.74 (d, 1H, J=8; CH), 5.24-5.23 (t, 1H, J=4; CH), 5.42-5.41 (d, 1H, J=4; CH), (ESI) (*m/z*) [M + H^+]: 454.05; **(10)** (0.93 g, 80%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.01, 2.03, 2.07, 2.09 (s, 12H; CH_3), 3.50-3.49, 3.53-3.51 (2xm, 2H, J=8; CH_2), 3.51-3.49 (m, 2H, J=8; CH_2), 4.11-4.08 (m, 2H, J=12; CH_2), 4.30-4.28 (2xd, 2H, J=8; CH_2), 4.58-4.57 (d, 1H, J=4; CH), 4.99-4.98 (t, 1H, J=4; CH), 5.13-5.12 (t, 1H, J=4; CH), 5.21-5.20 (t, 1H, J=4; CH), (ESI) (*m/z*) [M + H^+]: 454.05; **(11)** (0.81 g, 69%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.01, 2.07, 2.12, 2.18 (s, 12H; CH_3), 3.55-3.52 (t, 2H, J=8; CH_2), 3.91-3.89, 3.87-3.85 (2xm, 2H, J=8; CH_2), 4.17-4.14 (m, 2H, J=12; CH_2), 4.31-4.29 (m, 1H, J=8; CH), 4.89 (s, 1H; CH), 5.29-5.28 (m, 1H, J=4; CH), 5.31-5.30 (t, 1H, J=4; CH), 5.34-5.33 (t, 1H, J=4; CH), (ESI) (*m/z*) [M + H^+]: 454.05.

O-(2,3,4,6-tetra-O-acetyl)-β-D-(sugar)pyranosyl-ethyloxy-N-oxyphthalimide (12-14): *Typical procedure;* to a solution of N-hydroxyphthalimide (1.07 g, 6.59 mmol, 1.5 eq.) in DMF (20 mL) at 65°C was added sodium bicarbonate (0.55 g, 6.59 mmol, 1.5 eq.). The mixture was then stirred for 30 min until fully deprotonated (brown in color) to which **9-11** (2.00 g, 4.39 mmol, 1 eq.) was added. The solution was stirred under inert atmosphere (N_2) for 8 hours. After completion, the mixture was diluted with dichloromethane and washed with water (8x100mL) and 1 M $NaHCO_3$ (3x25mL) or until the excess N-hydroxyphthalimide was completely taken up into the aqueous layer. The organic layer was concentrated and purified by flash chromatography (Hex/EtOAc, 3.5:6.5) to afford a pale yellow oil **12** (1.80 g, 76%). ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.01, 2.07, 2.12, 2.16 (s, 12H; CH_3), 4.13-4.09 (2xm, 2H, J=8, J=16; CH_2), 4.19-4,16 (m, 2H, J=12; CH_2), 4.47-4.45 (m, 2H, J=7; CH_2), 4.80-4.78 (d, 1H, J=8; CH), 5.28-5.27 (t, 1H, J=4; CH), 5.42-5.41 (d, 1H, J=4; CH), 7.85-7.83, 7.80-7.78 (2xm, 4H, J=8; CH), (ESI) (*m/z*) [M + H^+]: 537.15; **(13)** (1.49 g, 63%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.09-2.00 (4xs, 12H; CH_3), 3.78-3.76 (m, 2H, J=8; CH_2), 4.04-4.02, 4.4.39-4.37 (2xm, 2H, J=8; CH_2), 4.09-4.06 (m, 2H, J=12; CH_2), 3.50-3.48 (2xd,

2H, J=8; CH), 4.78-4.77 (d, 1H, J=4; CH), 4.97-4.95 (t, 1H, J=7; CH), 5.09-5.07 (t, 1H, J=7; CH), 5.31-5.29 (t, 1H, J=7; CH), 7.78-7.76 (2xm, 4H, J=8; 4H), (ESI) (*m/z*) [M + H^+]: 537.15; **(14)** (1.53 g, 65%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 1.89 (s, 2H; NH_2), 2.16, 2.12, 2.05, 2.00 (s, 12H; CH_3), 3.77-3.75, 3.74-3.72 (2xm, 2H, J=8, J=16; CH_2), 4.07-4.05 (m, 2H, J=8; CH_2), 2.10-2.08 (m, 2H, J=8; CH_2), 4.31-4.30 (m, 1H, J=4; CH), 4.87 (s, 1H; CH), 5.29-5.27 (m, 1H, J=7; CH), 5.38-5.37 (d, 1H, J=4; CH), 5.40-3.39 (d, 1H, J=4; CH), (ESI) (*m/z*) [M + H^+]: 537.15.

***β-D-(Sugar)pyranosyl-propyloxy-N-oxyamine (1-3)*:** Typical procedure; to a solution of **12-14** (0.912 g, 1.70 mmol) in ethanol (15 mL) was added hydrazine (0.327 mL, 10.2 mmol, 6 eq). The mixture was stirred under inert atmosphere for up 48h. The mixture was then concentrated and purified by flash chromatography (MeOH/DCM, 3:7) to afford a white solid **15** (0.271 g, 67%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.23-2.21 (t, 1H, J=7; CH), 3.50-3.38 (m, 3H; CH, CH_2), 3.64-3.62 (m, 3H, J=7; CH_2, OH), 3.79-3.74 (m, 2H; OH), 3.83-3.79 (m, 4H, J=16; CH_2, CH, OH), 3.96-3.94 (m, 1H, J=8; CH), 4.30-4.28 (d, 1H, J=7; CH); (ESI) (*m/z*) [M+H^+]: 239.10; **16** (0.213 g, 52%), ^{1}H NMR (400 Hz, $CDCl_3$, δ): 3.18-3.16 (t, 1H, J=7; CH), 3.27-3.24 (t, 1H, J=12; CH), 3.36-3.34 (m, 2H, J=8; CH_2), 3.60-3.59, 3.57-3.56 (2xd, 1H, J=4, J=16; CH), 3.75-3.72 (2xm, 2H, J=12; CH_2), 3.78-3.77 (m, 2H, J=8; CH_2), 3.93-3.91 (m, 1H, J=8; CH), 4.36-4.34 (d, 1H, J=8; CH); (ESI) (*m/z*) [M+H^+]: 239.10.

Preparation of gold-coated substrates and monolayers. Glass cover slips (75 mm x 25 mm, Fisher) were immersed into a piranha solution (1:1 volume ratio of H_2SO_4 and 32% H_2O_2) for 4 h, followed by rinsing with deionized water and ethanol. Gold substrates were prepared by electron-beam deposition of titanium (5 nm) and then gold (12 nm for microarray and 50 nm for electrochemical measurements). The gold-coated slides were cut into 1 x 2 cm^2 pieces. In order to form SAMs on gold, the slides were immersed in an ethanolic solution containing alkanethiols (1 mM) for at least 16 hours. Once removed from solution, the surfaces were rinsed with ethanol and dried before use.

Electrochemical immobilization and release of ligands. All electrochemical experiments were performed using a Bioanalytical Systems CV-100W potentiostat. To activate H_2Q-terminated SAMs, electrochemical experiments were performed in a 1 M $HClO_4$ electrolyte solution with an Ag/AgCl electrode (Bioanalytical systems) serving as the reference, the gold monolayer as the working electrode, and a Pt wire as the counter electrode. Surfaces were scanned at a rate of 100 mV/s. Immobilized ligands were confirmed using the same parameters. Ligands were released by applying potential for 15 min (~60 cyclic scans) in PBS buffer (pH = 7) and were characterized in similar conditions.

Sugar-oxyamine immobilization for electrochemical measurements. Surfaces containing mixed SAMs of H_2Q- and EG_4SH-terminated alkanethiols were electrochemically oxidized (1 M $HClO_4$, pH = 0) to generate mixed SAMs of Q- and EG_4SH-terminated groups. Surfaces were then rinsed with ethanol and dried. A 90 mM solution of sugar-oxyamine in ethanol was allowed to react on the quinone surface for 4 hours at 40°C. Once the immobilization was complete, the surfaces were rinsed with ethanol and dried before verification by cyclic voltammetry.

Preparation of microarrays for density studies. Bare gold substrates (2 x 1 cm^2) were printed using a SpotBot® 2 Complete (TeleChem International, Inc., Sunnyvale, CA). Programming and printing was carried out with SpoCLeGenerator and Spot App. Software. Different densities of mixed SAMs of H_2Q- and EG_4SH-terminated alkanethiols (0, 25, 50, 75 and 100 percent of H_2Q, 1 mM in EtOH) were formed in microarray patterns (12 x 12 arrays, 100 μm diameter, 300 μm spacing, 0.1 s printing time). Substrates were then backfilled with EG_4SH (1 mM in EtOH, 16h), followed by electrochemical oxidation (1 M $HClO_4$, pH = 0) to generate corresponding mixed densities of Q- and EG_4SH-terminated groups. Surfaces were then rinsed with ethanol and dried. A 90 mM solution of sugar-oxyamine in ethanol was allowed to react on the quinone surface for 4 hours at 40°C. Once the immobilization was complete, substrates were rinsed with ethanol and dried before cyclic voltammetry was performed.

Ligand density determination. The amount of sugar-oxyamine immobilized (gal, glc, and man), corresponding to the total charge (Q) on the surface, was quantified by integrating the redox peak area observed from the CV data after reaction with H_2Q-terminated SAM microarrays (different densities of 0, 25, 50, 75, and 100% H_2Q). The total charge was then compared to the theoretical value generated from a 100% converted surface using $Q = nFA\Gamma$ (where Q is the total charge, n is the number of electrons involved in the reaction (in this case, 2), F is Faraday's constant, and Γ is the surface coverage in molecules per surface area). The theoretical value was calculated to be 16.1 $\mu C/cm^2$ for a surface density of 1.66×10^{-10} moles/cm^2.

Preparation of renewable microarrays for lectin adhesion studies. A solution of H_2Q-/EG_4SH-containing alkanethiols (1:9, 1 mM in EtOH) were transferred to a bare gold surface (2 x 1 cm^2) to form microarray features (12 x 12 arrays, 100 μm diameter, 300 μm spacing, 0.1 s printing time). Substrates were then backfilled with EG_4SH (1 mM in EtOH, 16 h), followed by electrochemical oxidation (1 M $HClO_4$, pH = 0) to generate corresponding Q-terminated SAMs. Surfaces were then rinsed with ethanol and dried before the same spotting programmed was employed to randomly print sugar-oxyamines (90 mM in ethanol). Once the immobilization was complete, substrates were rinsed with ethanol and dried before lectin adhesion (1 mg/mL, PBS, 2 h, 25°C). After rinsed and dried with PBS, substrates were visualized by fluorescence microscopy. Ligands were released from the array by applying electrochemical potential for 15 min (~60 cyclic scans) in PBS buffer (pH = 7), and sugar-oxyamines were again transferred via random microspotting. Lectins were added and visualized in a similar.

Fluorescence microscopy. After immobilization of sugars and fluorescently labeled lectins to patterned microarrays, substrates were imaged by fluorescence and brightfield microscopy using a Nikon TE2000E inverted microscope. Image acquisition and processing was carried out with Metamorph software.

X-Ray Photoelectron Spectroscopy (XPS). After immobilization of sugar-oxyamine to quinone surfaces, XPS measurements were preformed on substrates containing the immobilized ligands mentioned, as well as bare gold and quinone SAMs with a Kratos Axis Ultra DLD. A mono Al anode source was used with a specific excitation energy of 1486.6 eV and a 80 eV pass energy was used for the high resolution scans. All binding energies are reference to the C 1s of a saturated hydrocarbon at 284.7 eV.

Contact angle measurements. Gold surfaces with SAMs composed of hydroquinone, quinone, and immobilized sugars were prepared. The static contact angles of these surfaces, as well as bare gold, were measured using 10 μL drops of diionized H_2O using a KSV CAM 200 instrument and software.

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2 W. S. Dillmore, M. N. Yousaf and M. Mrksich, *Langmuir*, 2004, **20**, 7223.

3 S. Cao, F. D. Tropper and R. Roy, *Tetrahedron Lett.* 1995, **51**, 6679; O. Renaudet and P. Dumy, *Tetrahedron Lett.* 2001, **42**, 7575.