Supporting Information

Precise generation of selective surface-confined glycoprotein recognition sites

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1. Chemical Materials

All chemicals and solvents were purchased from Merck Companies (Aldrich Chemicals, Sigma Aldrich and Fischer Chemical) with all used as received. The 10x phosphate buffered saline (PBS) solution was purchased from Fisher Bio-Reagents. RNase B from bovine pancreas, RNase A from bovine pancreas, α1-acid glycoprotein from bovine plasma and HRP from horse radish root were all purchased from Sigma Aldrich. Polycrystalline gold substrates for the contact angle, ellipsometry, AFM and XPS studies consisting of 100 nm Au layer on silicon wafer were purchased from George Albert PVD (Germany). Polycrystalline gold substrates employed in SPR were purchased from Reichert Technologies, USA, consisted of 49 nm gold with 1 nm chromium.

2. Characterization methods

2.1. NMR Spectroscopy

All ^{1}H NMR spectra were undertaken on a Bruker AV300 FT/NMR Spectrometer (300 MHz) at room temperature, with shifts measured in ppm. All spectra were measured relative to $CDCl_3$ and data processed using MestReNova Version 6.1 (Mestrelab Research). J coupling constants are reported in Hz, and the multiplicity of the signals is expressed as: s= singlet, d=doublet, t=triplet q=quartet, m=multiplet.

2.2. Circular dichroism

Circular dichroism (CD) spectra were measured using a Jasco J1500 CD Spectrometer (ASCO UK Ltd., Great Dunmow, Essex). All experiments were undertaken using a 400 μL quartz crystal cuvette with a 1 mm path-length and at 25 °C. The measured wavelength range was between 270-190 nm, with a data pitch of 0.1 nm and scanning speed of 100 nm/min. 5 repeat accumulations were used to generate each measurement. Each final spectrum was subtracted from the equivalent buffer blank.

2.3. Dynamic Contact Angle

All contact angle measurements were taken using an Attension Theta Contact Angle Meter (Biolin Scientific). An automated microsyringe was used to inject and retract the water droplet onto and from the surfaces from which the advancing and receding angles were then measured by recording the change in the pinning angle by video (acquisition rate 35 frames per second). OneAttension software was then used to analyse the advancing and receding angles. For each surface type (i.e. SAM or polymer), at least 3 measurements each from 3 individual chips were taken (n=9) from which the average and standard deviation values were calculated.

2.4. Ellipsometry

The thicknesses of the SAM and polymer surfaces were measured with spectroscopic ellipsometry using a Jobin-Yvon UVISEL ellipsometer with a xenon light source. The incident angle of the light was fixed at 70° for all measurements and the wavelength range was 220-800 nm. The calculation of the film thicknesses were based on a three-phase ambient atmosphere/SAM/Au model. The SAM was assigned a refractive index of 1.49 and assumed to be isotropic. The thicknesses were reported using averages of at least 3 independent surfaces with 3 measurements obtained from each surface (n=9) and the standard deviation was calculated from these values.

2.5. X-ray Photoelectron Spectroscopy (XPS)

XPS spectra were acquired using an Escalab 250, Thermo Scientific K-Alpha. The system used a monochromatic Al kα source with a photon energy of 1486.68 eV and for each measurement a spot size of 0.2 mm^2 was used with a take-off angle of 90° to the surface of the plane. All measurements were undertaken at a pressure of ~7.5 x 10^{-9} Torr. The SAM samples were taped onto a stainless steel plate using Shintron tape and clipped with stainless steel clips. Each SAM

measurement was undertaken with the charge neutraliser on to prevent charging issues. However, for polymer samples (poly(MEBA) or poly(MEBA)-APBA)), the charge neutraliser was turned off as this prevented charging of the surfaces. The high resolution spectra were obtained using a pass energy of 40 eV and 0.1 increments. Survey scans were obtained using a pass energy of 200 eV and 0.4 increments. Sensitivity factors for all samples were: S (2p), 1.67; Au (4f 7/2), 9.58; Au (4f 5/2), 7.54. N (1s), 1.8; B (1s), 0.486; C (1s), 1.0; Br (3d 5/2), 1.68; Br (3d 3/2), 1.16; O (1s), 2.93.

2.6. Atomic Force Microscopy

All AFM images were acquired using an Asylum Research MFP-3D AFM (Oxford Instruments, UK) operating in Intermittent Contact Mode at a temperature of 18 ^{o}C and a relative humidity of <40 %. Images were composed of 512 x 512 pixels and the scanning velocity was 10 μm/s. Rectangular pyramidal-tipped Si cantilevers (PPP-NCL, Windsor Scientific, UK) were employed; their nominal length, width, and tip diameter were 225 μm, 38 μm and <10 nm, respectively. Images were analysed using Scanning Probe Image Processor software (Image Metrology, Denmark). Images were then presented using Gwyddion software (Version 2.51). The Ra roughness of the modified gold surfaces were calculated using the 'Statistical Quantities' tool of Gwyddion software (Version 2.51). Here, random points across the surfaces were selected and the Ra values recorded. The average Ra roughness was calculated from 3 points each from 3 individual chips (n=9).

2.7. Surface Plasmon Resonance (SPR)

All protein SPR experiments were run on a Reichert SR7000DC Dual Channel Spectrometer (NY, USA). All SPR chips were purchased from Reichert (Depew, NY, USA). The running buffer used for all experiments was degassed 1 x PBS containing 96 mM glycine, 10 mM HEPES, and 0.01 % sodium dodecyl sulfate adjusted to pH 8.6 using potassium hydroxide. Before each

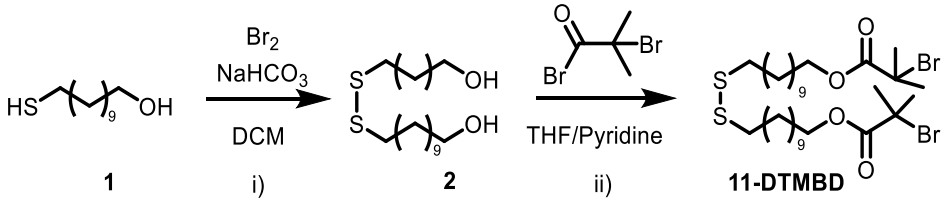
measurement the acidic regeneration buffer (consisting of equal parts of 75 mM malonic, phosphoric, oxalic and formic acid) was run across the surface to remove any contaminates during set up. The protein solutions were injected across the surfaces at 40 μL/min for 10 mins, following which the running buffer was used for the 15 minutes dissociation phase. The surfaces were then regenerated using the acidic regeneration buffer for 10 minutes. Data sets were analysed using Scrubber 2 (BioLogic Software, Campbell, Australia). All SPR responses at R_{eq} were plotted against the concentration of the injected proteins (C_p) and fitted to a 1:1 steady-state model using Scrubber 2. The model uses a non-linear least-squares regression method to fit data to the Langmuir adsorption isotherm (Equation S1), with K_D being the dissociation constant and R_{max} the maximum analyte binding capacity of the surface.

$$R_{eq} = \left(\frac{C_p}{C_p + K_D}\right) R_{max} \qquad \text{Equation S1}$$

3. Methods

3.1. Synthesis of 11,11'-dithiobis[1-(2-bromo-2-methylpropionyloxy)undecane] (11-DTMBD)

Following previous synthetic protocols,[1,2] 11,11'-dithiobis[1-(2-bromo-2-methylpropionyloxy)undecane] (11-DTMBD) was synthesised using a two-step reaction process as shown in Scheme S1 to obtain a highly pure stock of initiator.



Scheme S1 – Synthetic strategy for the synthesis of 11-DTMBD.

Step i) 11-mercaptoundecan-1-ol **1** (1.97 mmol, 0.404 g) was dissolved in 15 mL dichloromethane (DCM) in a round bottomed flask, to which was added 10 % sodium hydrogen carbonate (2 mL). A solution of bromine (1.00 mmol, 0.160 g) was added dropwise to the well stirred mixture. The colour of the bromine quickly disappeared upon addition to the flask. The solution was then left to react overnight. DCM (20 mL) was then added to the mixture, the organic phase separated and the aqueous phase extracted with DCM (40 mL). The organic phases were combined and dried with magnesium sulfate. The solution was filtered and the solvent was then evaporated to give disulphide **2** (0.4 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H ppm: 1.30 (overlapped, 28 H, -CH_2), 1.58 (overlapped, 8 H, -CH_2), 2.70 (t, J = 7.3 Hz, 4H, -SCH_2), 3.66 (dd, J = 11.9 Hz, 4H, -OCH_2).

Step ii) 2-bromo-2-methylpropionyl bromide (267 μL, 2.16 mmol) was added to 10 mL anhydrous tetrahydrofuran (THF) in a pressure equalised funnel under an argon atmosphere. The 11,11'-disulfanediylbis(undecan-1-ol) **2** (0.4 g, 0.98 mmol) was added to a separate, stirred solution of 30 mL THF and dry pyridine (175 μL, 2.16 mmol) and kept at 0-5 °C also under an argon atmosphere. The 10 mL solution containing the acid bromide was then slowly added dropwise to the 30 mL disulfide solution. The reaction was stirred for 2 hrs at 0-5 °C and then for a further 16 hrs at room temperature. Afterwards the reaction was diluted with DCM (60 mL) and extracted with cold 1 N HCl (2 × 100 mL), saturated $NaHCO_3$ (1 × 100 mL) and saturated NaCl (1 × 100 mL). The organic phase was retained and all aqueous phases were back extracted with DCM (1 × 100 mL). The solvent was removed under vacuum and the crude product was purified by chromatography on silica gel (hexane/DCM, 1:1). The purified **11-DTMBD** product (0.2 g, 28.9 %) was a clear, colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H ppm: 1.30 (overlapped, 28 H, CH_2), 1.69 (m, 8 H, CH_2CH_2O+CH_2CH_2S), 1.96 (s, 12 H, CH_3), 2.70 (t, J = 7.5 , 4 H, SCH_2), 4.19 (t, J = 6.6, 4 H, OCH_2).

3.2. CD of RNase B with increasing percentages (v/v) of MeOH

To begin with, a 50 μM RNase B solution was prepared using 0.1 M potassium phosphate buffer pre-adjusted to pH 8.6 using tertiary amine tetraethyl ethylenediamine (TEEN). For each variation in the % of MeOH (v/v), 1 mL of the 50 μM RNase B solution was mixed with 1 mL of buffer containing the proportion of MeOH at twice the required final MeOH % (v/v) to give 2 mL of 25 μM RNase B in the buffer/MeOH mixed solution. Once made, each sample was wrapped in parafilm to prevent MeOH evaporation, incubated for 12 hrs to equilibrate and then measured with CD. For all experiments the cuvette was sealed with parafilm to prevent evaporation of the MeOH.

3.3. Formation of the 11-DTMBD SAMs

The gold substrates were cleaned by immersion in piranha solution (70% H_2SO_4, 30% H_2O_2) at room temperature for 10 minutes, rinsed with ultra-high quality (UHQ) water and HPLC grade EtOH. (*Caution: Piranha solution reacts violently with all organic compounds and should be handled with care*). Subsequently, the clean gold substrates were immersed for 24 hrs in 1 mM ethanolic solution of 11-DTMBD. The gold substrates were then rinsed with HPLC grade EtOH and dried using a stream of Argon.

3.4. Polymerisation from 11-DTMBD SAMs

The gold substrates functionalized with 11-DTMBD SAMs were added to individual polymerisation solutions containing *N,N'*-methylenebis(acrylamide) (MEBA, 0.5 mg), 2,2'-bipyridyl (2-bpb, 2.2 mg), Cu(I)Br (1 mg) and Cu(II)Br (2.3 mg) dissolved in 35 mL of deoxygenated (using argon) ultra-high pure H_2O. The MEBA was dissolved first, the solution was degassed to remove oxygen for 1 hr, following which the 2-bpb was added and finally the 1:1.5 Cu(I)Br:Cu(II)Br. The functionalized gold substrates were then added to this 35 mL solution and

subsequently 1.02 µL of ethyl-2-bromoisobutyrate (E-2-BB) was injected. The substrates were polymerised for 30 minutes, during which the solution was continually degassed using a slow stream of argon, after which they were then were removed from the polymerisation solution, rinsed extensively for 3 minutes with ultra-high pure H_2O, then with HPLC grade EtOH and finally dried under a stream of argon.

3.6. Formation of surface molecularly imprinted polymers (MIP) and non-imprinted polymers (NIP) from 11-DTMBD SAMs

The RNase B:APBA complexes for the MIP surfaces were formed using a 75 % (v/v) 10x PBS and 25 % (v/v) MeOH solution at pH 8.6, which was adjusted using TEEN. To 1 mL of this solution, 6.8 mg of RNase B and either 0.88 mg, 1.32 mg or 1.76 mg of APBA were added to form 1:10, 1:15 and 1:20 molar ratios of RNase B:APBA complexes, respectively. The complexation solution was left for 2 hrs to allow the RNase B and APBA to complex. The solutions for the NIP surfaces were prepared in the same way but without RNase B as the template.

The MIP and NIP polymerization was subsequently carried out following similar protocol as described in section 3.3, in which the 35 mL polymerization solution was adjusted to pH 8.6 using TEEN. Furthermore, due to the addition of the APBA monomer, the amount of MEBA was adjusted to 0.225 mg, 0.213 mg and 0.200 mg for 1:10, 1:15 and 1:20 RNase B:APBA complexes,to account for the change in monomer stoichiometry. Following degassing of the complexation solution for 1 hr with argon, 1 mL of the complexation solution was then added to 1 mL of the degassed polymerisation solution within a 10 mL round-bottomed flask sealed already containing the SAM gold substrates using an argon purged syringe. For the MIPs, the final concentration of RNase B within each 2 mL of this solution was 0.23 mM. The SAM gold substrates were then left to equilibrate within these 2 mL MIP or NIP polymerisation solutions for 5 minutes before 1.02 µL of E-2-BB were then

injected and left to polymerise for either 10, 20 or 60 minutes. Following the polymerisations, each chip was immediately removed from the solution, rinsed extensively with ultra-high pure H_2O, then the SPR acidic regeneration solution (to remove any RNase B bound to the surfaces) and again with ultra-high pure H_2O. Each modified gold substrate was subsequently rinsed with HPLC grade EtOH and finally dried under a stream of argon.

4. Supporting results

4.1. Circular dichroism spectra of RNase B in MeoH

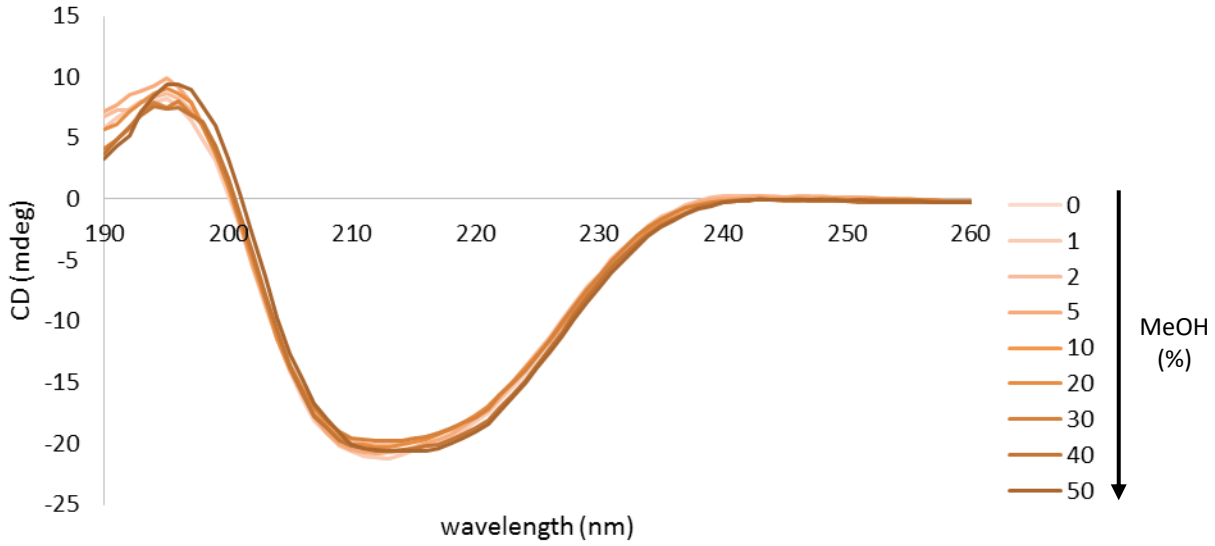


Figure S1 - Far-UV CD spectra of 25 μM RNase B observed with increasing percentages (v/v) of MeOH.

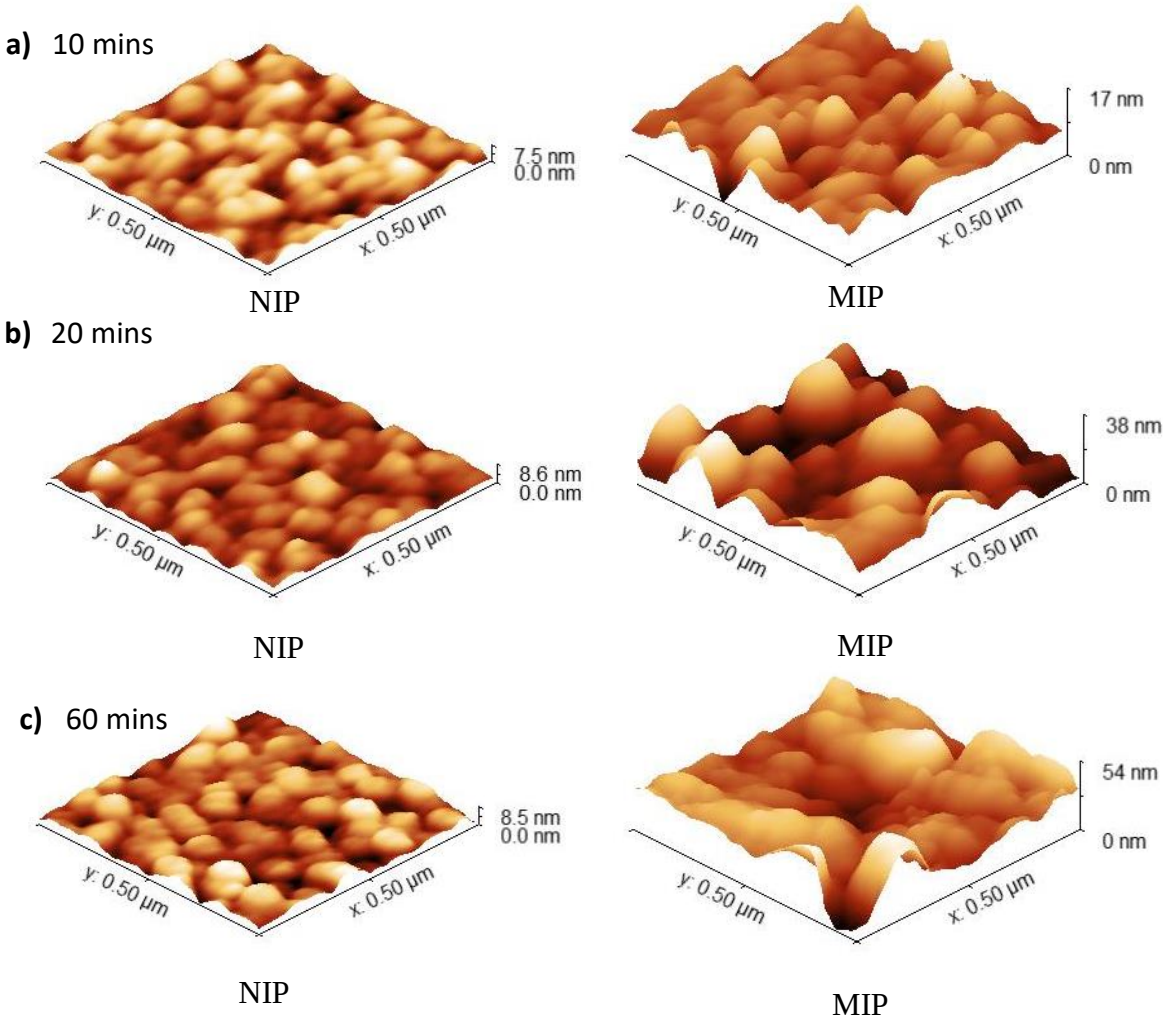


Figure S2. AFM images (0.5 µm^2) that visualise the topography of the poly(MEBA)-APBA NIPs (left) and MIPs (right) incubated in the polymerisation solutions for either (a)10 mins, (b) 20 mins or (c) 60 mins. It should be noted that the *z* heights shown on the 3D projections are only representative of the maximum *z* height of a single pixel of the image and are not thickness values for the surfaces.

Table S1. Characteristics of the proteins investigated by SPR.

Protein	RNase B	RNase A	α1-acid glycoprotein	HRP
Molecular Weight (g/mol)	14,700	13,700	44,000	44,000
Glycosylation (%)	9	0	45	21
Dimensions	3.8 x 2.8 x 2.2	3.8 x 2.8 x 2.2	5.9 x 4.2 x 3.9	4 x 6.7 x 11.7
pI	9.2-9.6	9.2-9.6	2.8- 3.8	9

References

(1) Belegrinou, S.; Malinova, V.; Masciadri, R.; Meier, W. Efficient Two-Step Synthesis of 11,11'-Dithiobis 1-(2-bromo-2-methylpropionyloxy)undecane , a Conventional Initiator for Grafting Polymer Brushes from Gold Surfaces via ATRP. *Synth. Commun.* **2010**, 40, 3000-3007.

(2) Shah, R. R.; Merreceyes, D.; Husemann, M.; Rees, I.; Abbott, N. L.; Hawker, C. J.; Hedrick, J. L. Using Atom Transfer Radical Polymerization to Amplify Monolayers of Initiators Patterned by Microcontact Printing into Polymer Brushes for Pattern Transfer. *Macromolecules* **2000**, 33, 597-605.