Reversible, high-affinity surface capturing of proteins directed by supramolecular assembly

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Supporting Information

Chemicals and Materials. Commercially available chemicals and solvents were purchased from Aldrich Chemicals, Sigma Aldrich, Fisher Chemicals and used as received. The heptakis-(6-deoxy-6-thio)-β-cyclodextrin was acquired from Cyclodextrin-Shop (Tilburg, Netherlands) to > 97% purity. The 6-thio-D-glucopyranose was acquired from CarbonSynth, USA (Illinois, USA) to 95% purity. Phosphate buffered saline (PBS) solution was prepared from a 10× concentrated PBS solution (1.37 M sodium chloride, 0.027 M potassium chloride, and 0.119 M phosphate buffer) from Fisher Bio-Reagents. Polycrystalline gold substrates were purchased from George Albert PVD, Germany and consisted either of a 30 nm gold layer deposited onto glass covered with a thin layer (5 nm) of titanium as the adhesion layer (for contact angle and cyclic voltammetry analysis) or 100 nm gold layer on 100-4inch-silicon wafer, precoated with titanium as the adhesion layer (for ellipsometry and XPS analysis). Polycrystalline gold substrates employed in SPR were purchased from Reichert Technologies, USA, consisted of 49 nm gold with 1 nm chromium.

SAM Preparation. The gold substrates were cleaned by immersion in piranha solution (70% H_2SO_4, 30% H_2O_2) at room temperature for 8 minutes, rinsed with ultra-high quality (UHQ) water and dried with an argon flow. (*Caution: Piranha solution reacts violently with all organic compounds and should be handled with care*). For the preparation of the heptakis-(6-deoxy-6-thio)-β-cyclodextrin SAMs, the clean gold substrates were immersed for 12, 24 and 48 h in 0.1 mM or 1 mM DMF solutions of heptakis-(6-deoxy-6-thio)-β-cyclodextrin. Subsequently, the gold substrates were rinsed with DMF and ultra-high quality (UHQ) water and dried under an argon flow. For the preparation of the glucose-terminated SAMs, the clean gold substrates were immersed for 24 h in 1 mM DMF solutions of 6-thio-D-glucopyranose. Subsequently, the gold substrates were rinsed with DMF and UHQ water and dried under an argon flow.

Contact Angle. Contact angles were determined using a contact angle Attension apparatus, equipped with a Navitar camera that was attached to a personal computer for video capture. The dynamic contact angles were recorded as a micro-syringe was used to quasi-statistically add liquid to or remove liquid from the drop. The drop was shown as a live video image on the PC screen. Oneattension software was used for the analysis of the contact angle of a droplet of UHQ water at the three-phase intersection. The contact angle averages and standard errors were determined from three different surfaces of each type of SAM (in triplicate).

Ellipsometry. The thickness of the deposited monolayers was determined by spectroscopic ellipsometry. A Jobin-Yvon UVISEL ellipsometer with a xenon light source was used for the measurements. The angle of incidence was fixed at 70°. A wavelength range of 280–820 nm was used. The Delta-Psi software was employed to determine the thickness values and the calculations were based on a three-phase ambient/SAM/Au model, in which the SAM was assumed to be isotropic and assigned a refractive index of 1.50. The thickness reported is the average of three different surfaces of each type of SAM (in triplicate), with the errors reported as standard deviation.

X-ray Photoelectron Spectroscopy (XPS). XPS spectra were obtained on the K-Alpha (Thermo Scientific, East Grinstead, UK) instrument based at University of Newcastle (NEXUS), UK. XPS experiments were carried out using a microfocused monochromatic AlKα source (Energy/Voltage/Current/Power: 1486.6 eV/12 kV/3 mA/36 W) at an emission angle of zero degrees and a spot size of 0.32 mm^2. Samples were analyzed with charge neutralization. The survey spectra were recorded with pass energy of 150 eV, step size of 0.4 eV and dwell time of 10 ms. The high resolution spectra were collected with pass energy of 40 eV, step size of 0.1 eV and a dwell time of 100 ms. For each surface type (β-CD–(SH)7 SAMs formed using different concentrations (0.1 mM and 1 mM) and times (12 h, 24 h and 48 h)), 3 measurements each from 2 individual chips were taken. XPS fitting was performed using the CASA XPS processing software. Sensitivity factors used in this study were: Au 4f 17.12; S 2p, 1.68.

Electrochemistry. The cyclic voltammetry (CV) experiments were performed with a Gamry Instrument Reference 600 potentiostat. The reference electrode (Silver/Silver Chloride Reference Electrode) and the cable (Reference 3000 Main Cell Cable Kit 60 cm) were both purchased from Gamry Instruments. The CV data were acquired using the Gamry Instruments Framework software and analysed with Gamry Echem Analyst software. After SAM formation, the modified gold surfaces were immersed in a 0.1 M DMF solution of ferrocene carboxylic acid((FCA) (purity 97%) for 4 hours. Subsequently, the immersed samples were rinsed in UHQ water. Cyclic voltammetry was performed on the gold modified surfaces using 0.2 M sodium sulfate as supporting electrolyte. The potential was scanned between 0 V and −0.8 V at a scan rate of 0.3 V/s. The CVs were performed in triplicate, with each replicate being a new gold modified surface as a working electrode. The geometric area was controlled by the use of a 1 cm diame-

ter O-ring. In order to demonstrate that the reaction was only occurring at the surface and not in the electrolyte solution, the CV experiments were performed at different scan rates on the 1 mM β-CD–(SH)7 SAMs, showing a linearity in the progression between scan rate and anodic peak current (for ferrocene oxidation), Figure S1. The linearity observed indicates that the ferrocene associated with the obtained faradaic response are confined to the β-CD–(SH)7 SAM gold surface.

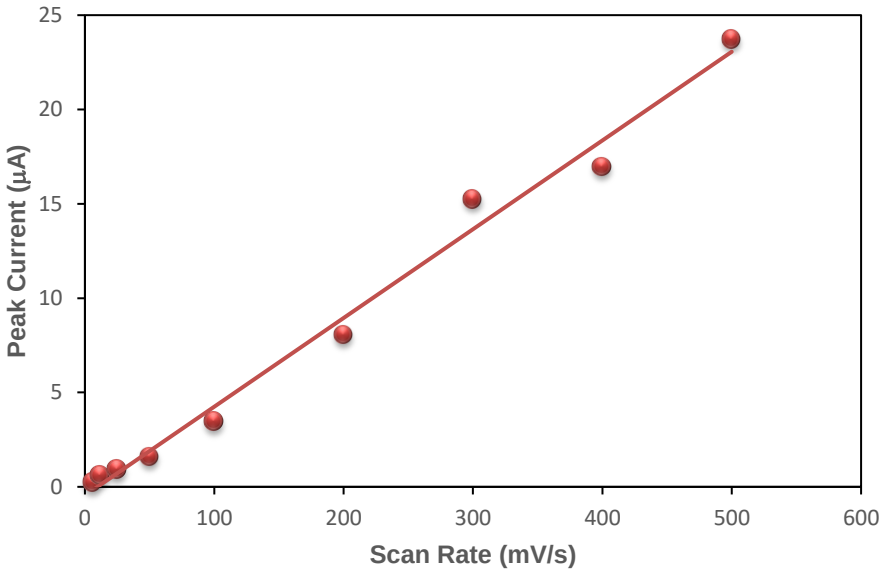

Figure S1. Plot of anodic peak currents at different scan rates at the β-CD–(SH)7 SAM gold surface.

Surface Plasmon Resonance. SPR experiments were performed with a Reichert SR7000DC Dual Channel Spectrometer (Buffalo, NY, USA) at 25˚C. For the insulin experiments, PBS was used as a buffer, while 2-ethanesulfonic acid (MES) buffer was employed for the α-chymotrypsin, RNAse A and cytochrome C studies. The MES buffer was prepared by dissolving 1 g of MES in 250 ml UHQ water and adjusting the pH to 6 with 10 M sodium hydroxide solution. An aqueous 10 mM sodium dodecylsulfonate (SDS) solution was employed as the regeneration solution. Prior to the binding studies between the gold-modified surfaces and the different proteins, the sensor chips were washed by exposure to three SDS injections for 30 seconds. The proteins were then injected at different concentrations over the sensor chip for 8 min, followed by injection of buffer for 8 min in order to allow any dissociation of the protein from the surface. The surfaces were re-used multiple times by conducting a regeneration step involving three SDS injections for 30 seconds. The flow rate was kept constant at 15 µl/min throughout all the SPR experiments.

SPR Sensorgrams for the Interaction Between Glucose-Terminated SAMs and Different Proteins

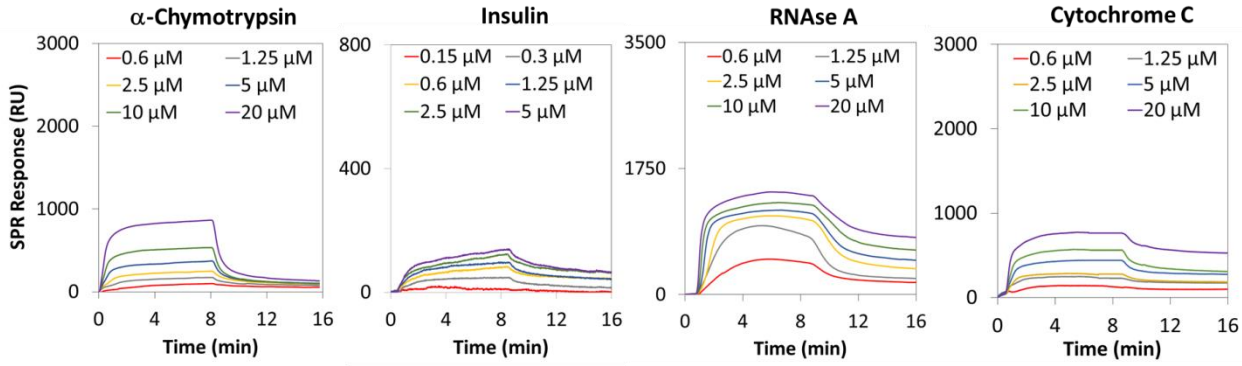

Figure S2. SPR sensorgram traces performed with 1 mM 24 h glucose-terminated SAMs and different concentrations of α-chymotrypsin, insulin, RNAse A and cytochrome C.

Time of Flight Secondary-Ion Mass Spectrometry (TOF-SIMS). The 1 mM 24 h β-CD–(SH)7 SAMs and 1 mM 24 h glucose-terminated SAMs were immersed in a 1 mM Cytochrome C in PBS solution for 2 h. Following protein immobilization, the samples were washed with PBS buffer followed by submersion in UHQ water for 1 min. The samples were then dried under argon. ToF-SIMS spectra were acquired using ToF-SIMS IV (ION-TOF GmbH, Munster, Germany). 25 keV Bi_3^+ primary ions were used for analysis. Static conditions were maintained in order to analyze only outermost 2 nm of the surface. 3 samples of each type were analyzed with 4 areas of 250x250 μm measured per sample. Three control samples, glucose-terminated SAMs, β-CD–(SH)7 SAMs without protein and bare gold have been analyzed to exclude possibility of advantageous contaminants (Figure S3).

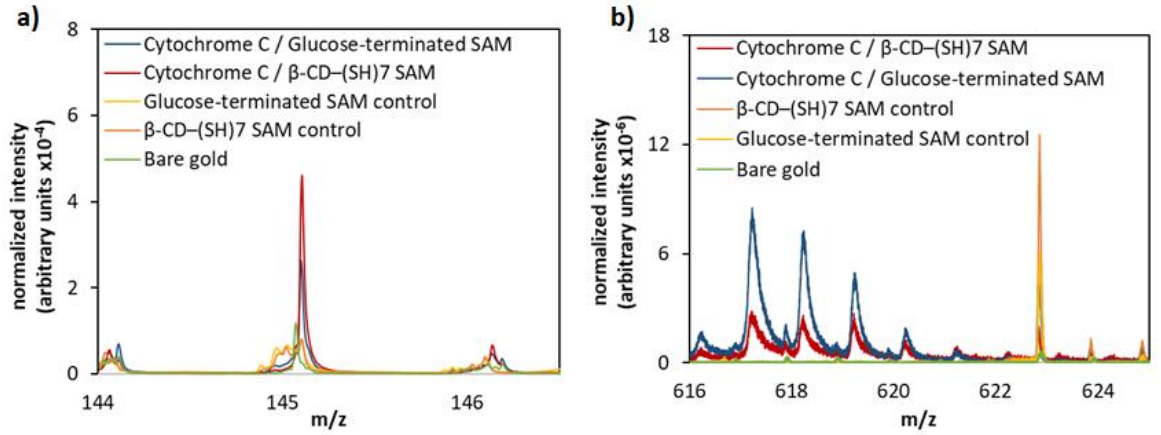

Figure S3. Overlay of spectra showing glucose-terminated SAMs and β-CD–(SH)7 SAMs with and without protein and bare gold samples. Regions of spectra focused on a) $C_5H_9N_2O_3^+$ and b) $C_{34}H_{33}N_4O_4Fe^+$ peaks show no advantageous contaminations from SAM layer.

Statistical Analysis of TOF-SIMS Results. The mass scales of the positive ion ToF-SIMS spectra were calibrated to the CH_3^+,$C_2H_3^+$,$C_3H_5^+$, and $C_7H_7^+$ peaks. Principal component analysis (PCA) was performed to compare peak intensity patterns in the cytochrome C glucose-terminated SAMs and cytochrome C β-CD–(SH)7 SAMs. For statistical analysis, a peak search was done in SurfaceLab and peaks of intensity above 100 ion counts and signal to noise ratio (S/N)>3 were added to peak list. Ion peaks known to be unrelated to protein, such as 23 m/z (sodium), and 39 m/z (potassium) were removed from the list. Peak areas normalized to total ion count were extracted and loaded into Matlab R2017b. PCA was run using a Matlab GUI, simsMVA. Results of principal component analysis are shown in Figure S4. First principal component (PC1) explains 86.56% of the variance in the whole dataset. Loadings and scores are co-localized, therefore peaks on the positive side of loadings correspond to glucose-terminated SAM and peaks on the negative side of loadings correspond to β-CD–(SH)7 SAMs (Figure S4). Spectral intensity patterns of two types of samples are significantly different from each other, with positive loadings representing peaks more prevalent in protein on glucose-terminated SAMs (blue) and negative loadings representing peaks more prevalent in protein on β-CD–(SH)7 SAMs (red).

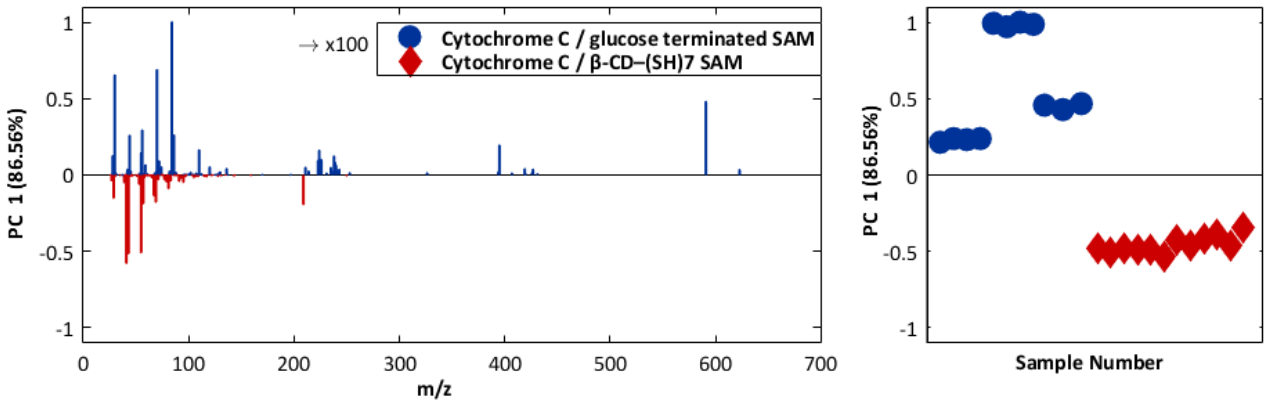

Figure S4. a) Loadings of first principal component (PC 1) represent variables (peaks) responsible for variation between sample groups. b) Positive scores represent Cytochrome C on glucose-terminated SAMs (blue), negative scores represent protein on β-CD–(SH)7 SAMs (red). Protein on β-CD–(SH)7 SAMs were found to exhibit less variation within sample group.