Patterned Chemisorption of Proteins by Thin Polymer Film Dewetting

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Electronic Supplementary Information

1. Experimental Section

Surface preparation

All sample preparation was performed in a class-100 laminar flow cabinet to reduce particle contamination. Glassware and all tools used were sonicated in re-distilled ethanol and acetone for at least 1 minute and blown dry in a stream of pure nitrogen before use. The substrates used were silicon wafers with a native oxide layer of thickness 1.92 nm (MMRC Pty Ltd, Malvern VIC Australia). The substrates were sonicated in re-distilled ethanol and acetone and blown dry in a pure nitrogen stream to remove particulate contaminants. Substrates were then cleaned with a CO_2 snow jet (Applied Surface Technologies, NT, USA) to remove residual particles and treated in a plasma cleaner (Harrick Plasma, Itacha NY, model PDC-002) to remove trace organic contaminants.

Hydroxylated silicon surfaces were obtained by immersion in piranha solution (7:3) for at least 30 minutes. The wafers were then rinsed thoroughly and sonicated for 5 minutes in Milli-Q® water to remove excess piranha solution. After sonication, the films were blown dry with a pure nitrogen stream and treated with an air plasma cleaner for a further 3 minutes. This ensures that the entirety of the silicon substrate has been hydroxylated. Functionalisation of the silicon substrates with (3-aminopropyltrimethoxy) silane (APTMS) was performed according to a published protocol:[1,2] the wafers were immersed in a 5.7 mM solution of APTMS (97%, Sigma Aldrich) in anhydrous toluene for 4 hours. The wafers were then sonicated in anhydrous toluene for 15 minutes to remove excess unbound APTMS and annealed in a vacuum oven for 20 hours at 150 °C to improve the layer order and orientation.[2] Thermal treatment of APTMS induces breakup of the hydrogen bonds between the amines and silanol groups. This re-arrangement within the APTMS layer exposes amine groups to air, leading to a greater surface coverage of amines. The annealing also results in the re-arrangement and cross-linking of the silane film, characterised by the decrease in thickness of 2 – 3 nm. The substrates were stored in a desiccator if not used immediately. Aldehyde functionality was introduced by immersing the substrates in 0.1 M glutaraldehyde (50%) in Milli-Q® water for 1 hour and sonicated in Milli-Q® for 1 minute to remove unbound glutaraldehyde.

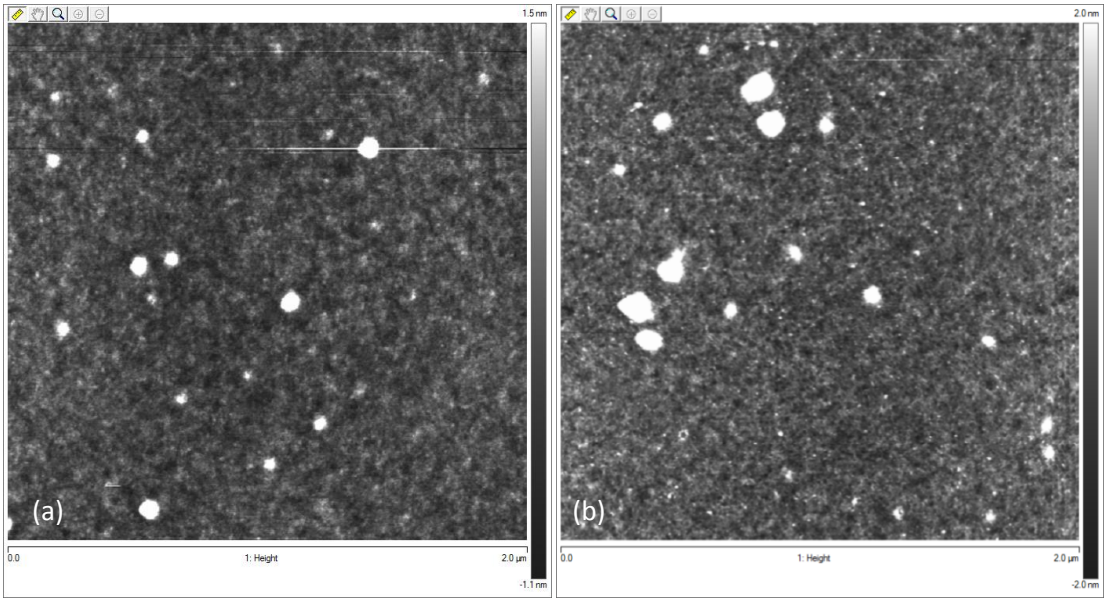
PS films were prepared by spin coating a 20 mg mL^{-1} filtered anhydrous toluene solution of PS (96000 g mol^{-1}, *PDI* 1.04, PSS Germany) at 3000 rpm. Dewetting of the PS films was achieved via solvent annealing. Briefly, the substrate was placed above a reservoir of toluene in an enclosed Petri dish. The dewetting progress was tracked *in situ* using a reflection optical microscope (Nikon Instruments Inc, Melville NY) until the desired hole size was obtained. The thickness of the silane, glutaraldehyde and PS layer was determined by spectroscopic ellipsometry (J.A. Woollam Co.). The wetting properties of the post-annealed silane surface and glutaraldehyde layers were investigated using advancing and receding contact angles of 15 µL sessile droplets of Milli-Q® water with a KSV CAM200 Contact Angle System (KSV Instruments Ltd., Helsinki, Finland). The values reported were an average of 4 measurements per sample with error given by the standard deviation. A Bruker Vertex 80v FT-IR spectrophotometer with a Hyperion 3000 FPA microscope system was employed to identify amine groups on the surface. Samples were characterised using atomic force microscopy (Veeco MultiMode 8) in Tapping Mode.

For attachment to the glutaraldehyde and carboxylic surface a solution of bovine serum albumin (BSA, Sigma Aldrich) 1 mg mL^{-1} was dissolved in PBS and 2-(N-morpholino)ethanesulfonic (MES) buffer, respectively. PBS was prepared by mixing NaH_2PO_4. $7H_2O$ (10 mM), KCl (3 mM) and NaCl (140 mM) in Milli-Q water and adjusted to pH 7.4 using 1 M NaOH solution. MES was prepared by dissolving 2-(N-morpholino)ethanesulfonic acid (0.05 M, Sigma Aldrich) in Milli-Q water and the pH adjusted to pH 6.5 using 0.1 M MES sodium salt.

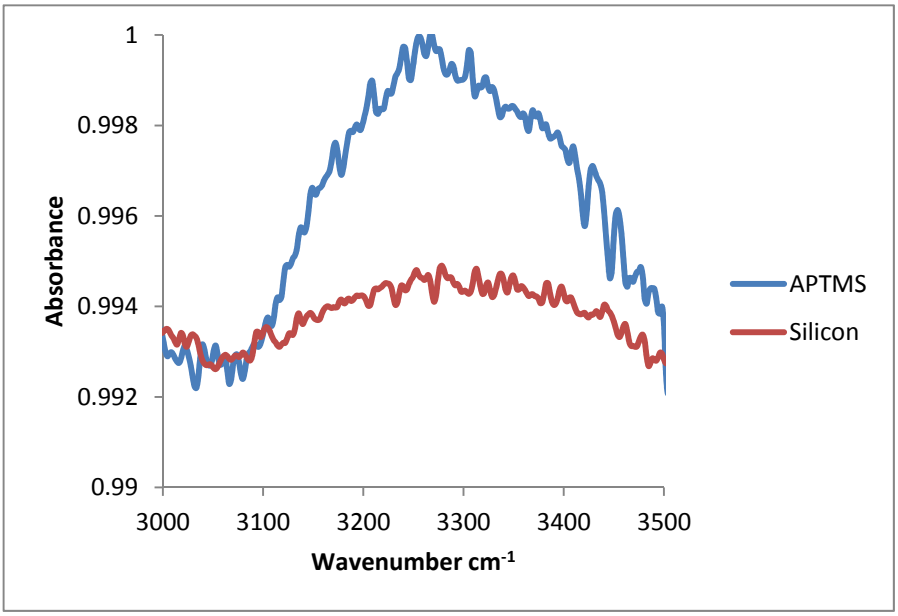
Fluorescein isothiocyanate (FITC, Sigma Aldrich) was used to tag BSA as per the procedure recommended by Invitrogen Molecular Probes™. About 200 µL of a 1-2 mg mL^{-1} solution of FITC-BSA in PBS was deposited on the sample surface and left to incubate for 2 hours. The surfaces were rinsed in 3 mL of PBS and then 5 ml of Milli-Q® water to remove excess salts and non-adsorbed proteins. Fluorescence imaging was performed with an Olympus BX61 optical microscope. The substrates were then immersed in sodium dodecyl sulphate (SDS) surfactant (10 %) at 70°C for 1 hour to remove physisorbed proteins.

Quartz crystal microbalance with dissipation (QCM-D) (Q-Sense E4) was used to monitor the adsorption of BSA. The QCM sensors were cleaned by sonication in ethanol and acetone for 5 minutes. After sonication, the sensors were blown dry with a pure nitrogen stream and treated with an air plasma cleaner for 3 minutes. The sensors were then immediately placed in the QCM-D instrument and equilibrated in the required solutions. All the QCM data presented are extracted from the 7th overtone (~35 MHz). The Sauerbrey model was used to calculate the mass of the various adsorbed layers.[3]

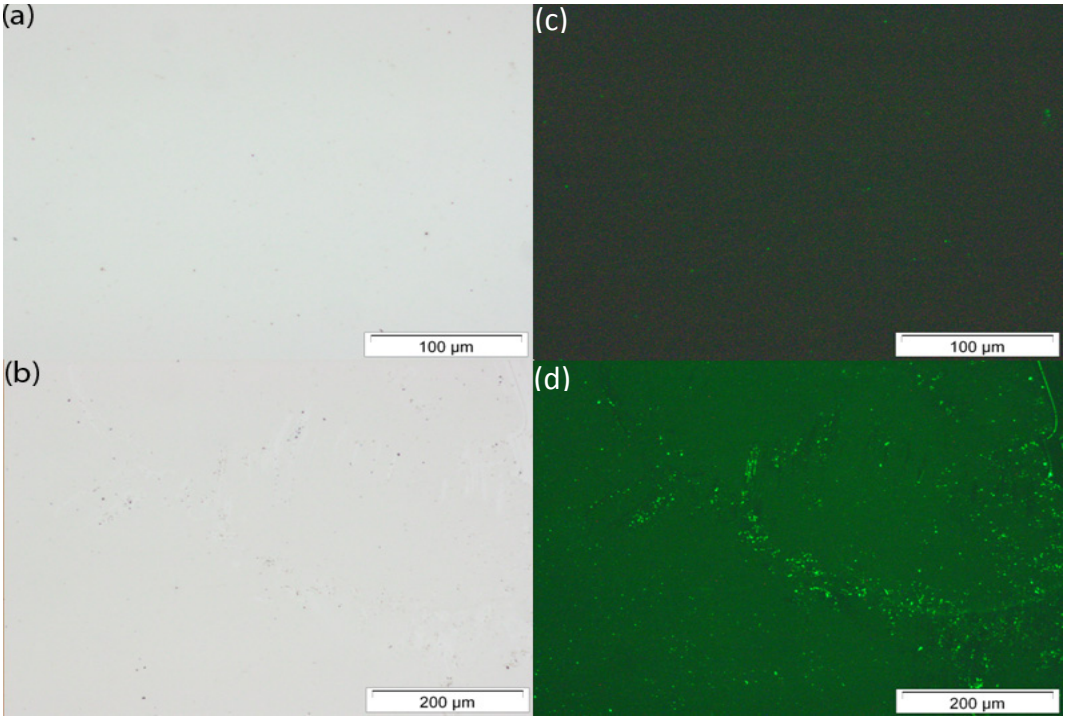
Figures



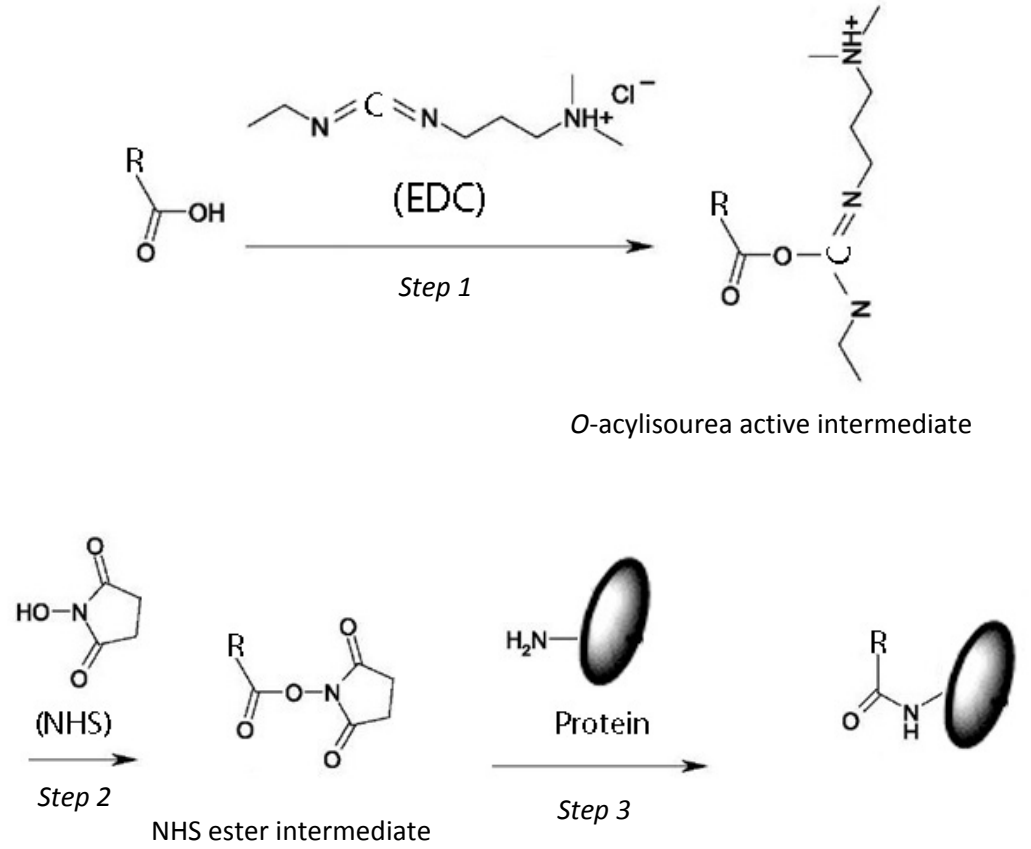
S1: Tapping Mode AFM topography image of (a) an APTMS layer on silicon wafer after thermal annealing and (b) after functionalisation with glutaraldehyde. The approximately circular structures seen in the images are due to the aggregation of APTMS and are on average 10 - 20 nm in height and 100 nm in lateral size. The RMS roughness of the film is 0.9 nm. The roughness of the underlying silicon substrate is typically 0.2 nm.



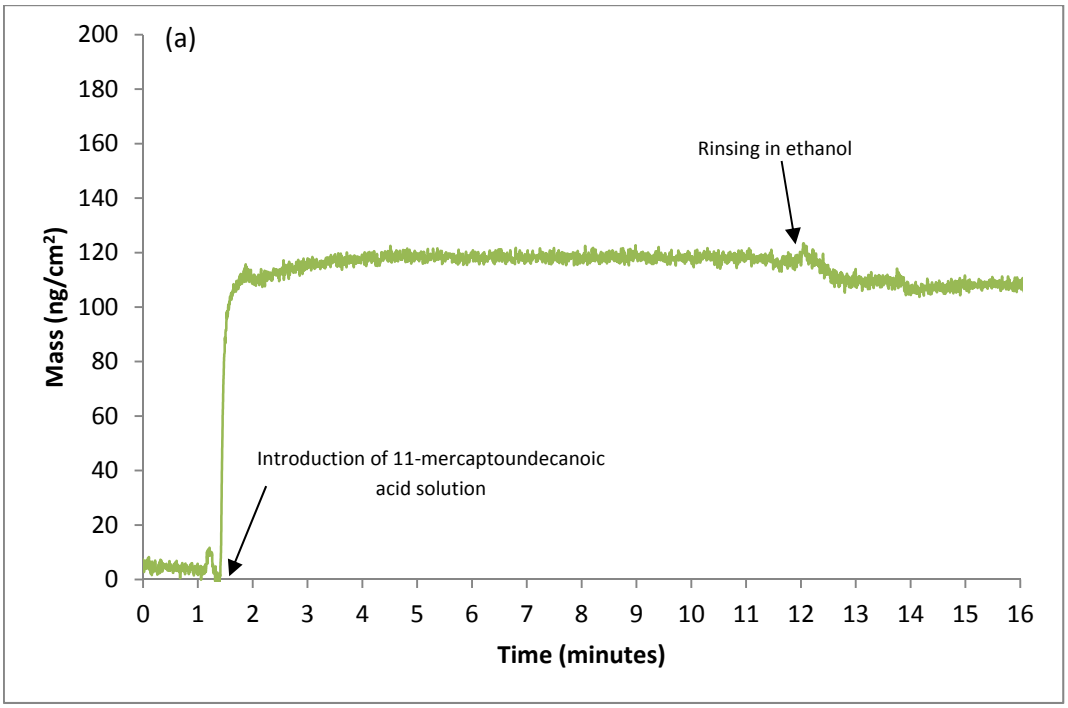
S2: Diagnostic region of an ATR-FTIR spectra for primary amines for the bare silicon (red) and the APTMS layer on silicon wafer after thermal annealing (blue). The spectra are normalised with respect to the largest absorbance value in the primary amine assignment range (3500 – 3300 cm^{-1}) of the silane layer. The APTMS curve shows a higher absorbance compared to the silicon, indicating a higher primary amine content.

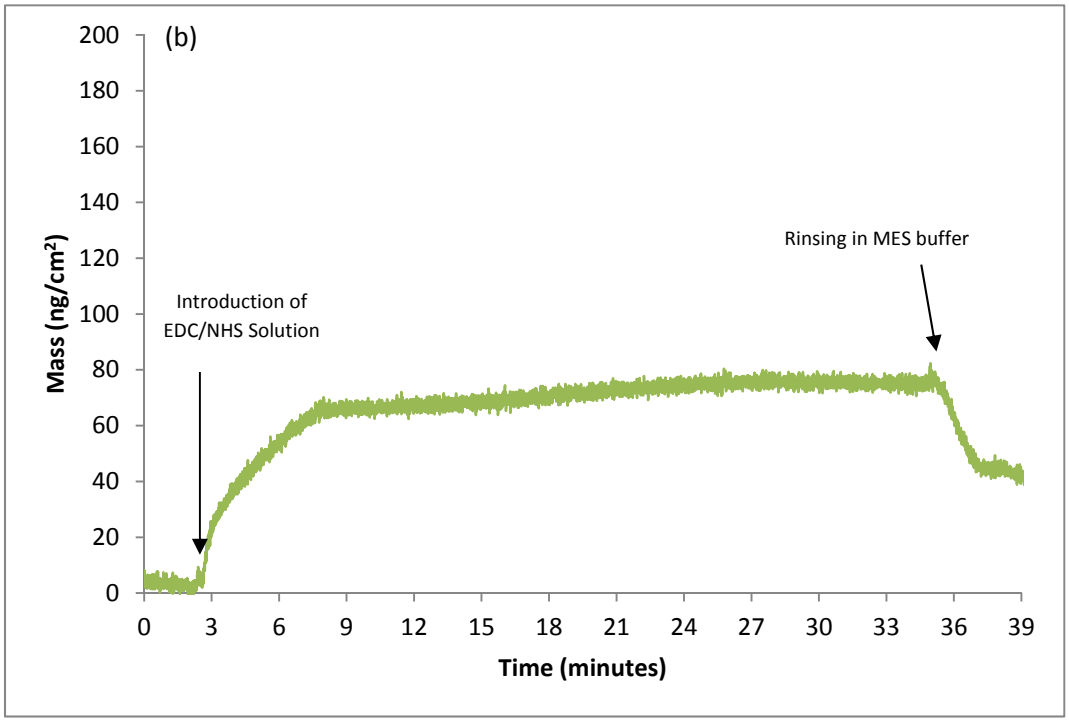


S3: Optical (a,b) and fluorescence micrographs (c,d) of a glutaraldehyde surface before (a,c) and after (b,d) adsorption of FITC-tagged BSA (2 hours adsorption time) and immersion in 10% SDS solution (at 70°C for 1 hour). The fluorescence intensity in part (d) demonstrates that BSA proteins were chemisorbed on the surface and could not be removed by aggressive SDS washing.

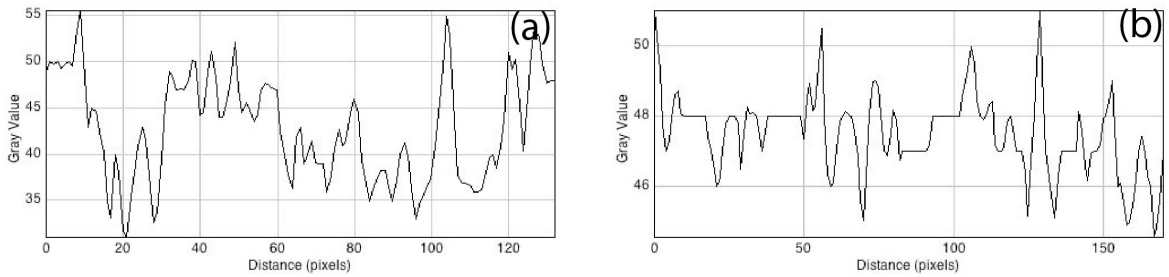


S4: Schematic of the carboxylic group reacting with EDC to produce an *o*-acylisourea intermediate (Step 1). Addition of NHS forms the stable active ester (Step 2) which then reacts with the amine groups on proteins to form a stable amide bond (Step 3).





S5: (a) QCM analysis for the self-assembly of 11-mercaptoundecanoic acid (4 mM in ethanol) onto a gold sensor. The solution was passed over the sensor at a flow rate of 1 mL min^{-1} for approximately 10 minutes. The sensor was then rinsed with ethanol until the signal equilibrated. (b) Activation of the carboxylic acid groups was achieved by flowing a mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDC) and *N*-hydroxysuccinimide (NHS) dissolved in MES buffer (2 mM and 3 mM respectively) at 1 mL min^{-1} for approximately 30 minutes.



S6: Line profile of the greyscale levels in Figures 3a (a) and Figure 3b (b). In part (a) the line is taken across four non-fluorescent patches in the image.

References

1 Salis, A. *et al.* Physical and Chemical Adsorption of Mucor javanicus Lipase on SBA-15 Mesoporous Silica. Synthesis, Structural Characterization, and Activity Performance. *Langmuir* **21**, 5511-5516, (2005).

2 Zhang, F. & Srinivasan, M. P. Self-Assembled Molecular Films of Aminosilanes and Their Immobilization Capacities. *Langmuir* **20**, 2309-2314, (2004).

3 Sauerbrey, G. Verwendung Von Schwingquarzen Zur Wagung Dunner Schichten Und Zur Mikrowagung. *Zeitschrift Fur Physik* **155**, 206-222, (1959).