

Supplementary Material for Protein Nanopatterning and Release from Nanocavity arrays.

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Materials and Methods.

1-nonanethiol (95%), O-(2-mercaptoethyl)-O'-methyl-hexa(ethylene glycol) (PEG-thiol 95%), potassium hydroxide (99.99%),
10 HNaCO₃ (99%) and cysteamine (98%) were purchased from Sigma. Oregon Green fibrinogen (90%) was purchased from
Invitrogen (λ_{max} abs : 496 nm, λ_{max} em : 524 nm). Polystyrene spheres (820 nm diameter) were purchased from Duke Scientific.

Electrochemistry was performed in a standard three electrode cell, using a platinum mesh counter electrode and a Ag/AgCl (sat.
KCl) reference electrode at 23 ± 2 °C. All potentials are quoted with respect to this reference. Raman spectroscopy was carried
15 out on a Horiba Jobin Yvon HR800UV microscope using 633 nm excitation line, focused through a 10x objective lens. The
acquisition time was 30 seconds and three acquisitions were performed to acquire a spectrum. Fluorescence microscopy images
were obtained using a confocal fluorescence microscope (LSM 50, Zeiss) and 64x oil immersion objective (NA = 1.4). The
wavelength of excitation was 488 nm and the fluorescence was collected above 505 nm. Scanning electron microscopy was
performed using a Hitachi S-3000N scanning electron microscope. Atomic force images were obtained using a Nano-scope III
20 (Digital Instruments) and silicon tips (Veeco Probes HMX-10).

Nano-cavity arrays were fabricated in a method similar to that described by Bartlett and co-workers.¹ Gold (400 nm) coated
silicon wafers were cleaned in piranha solution before immersion in a solution of 1 mM cysteamine in ethanol overnight. The
adsorption of cysteamine results in a more hydrophilic surface. The slides were then placed upright in an aqueous solution
25 containing 0.01% W/W 820 nm polystyrene spheres. Evaporation of the solvent at ambient temperature creates a 2-dimensional
array of spheres on the gold surface as the solution meniscus slowly moves down the slide. Electrochemical deposition through
this template was achieved using a commercially available gold salt solution (Technic Inc, TG-25 RTU) at a potential of -0.95 V.
Deposition continued until sufficient charge had been passed to create a 600 nm thick gold film. Monolayers of 1-nonanethiol or
PEG-thiol were formed on the top surface of the arrays by immersion of the slide for 12-14 hours in 1 mM solutions of the desired
30 compound in ethanol.

The polystyrene spheres were removed by sonicating the functionalized arrays in THF for one hour. Consistent with the film
thickness, SEM revealed cavity openings of 700 nm after sphere removal. Adsorption of fibrinogen inside the arrays was
achieved by sonicating the arrays in 50 μ M solutions of Oregon Green Fibrinogen in aqueous 0.1 M HNaCO₃ for 30 minutes.

Characterisation of the Nano-cavity arrays

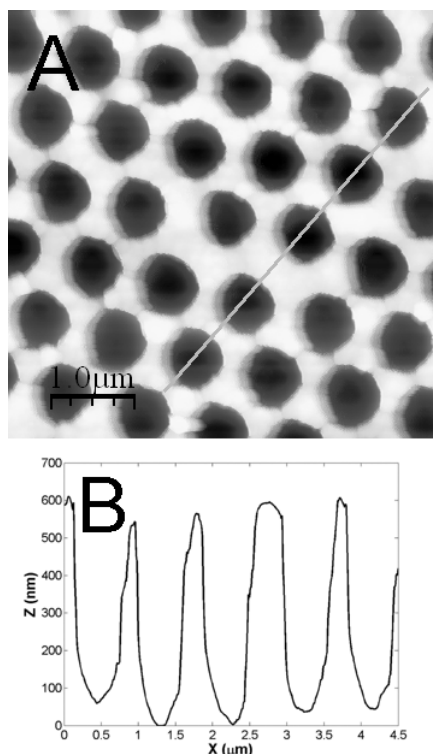


Fig. 1 AFM image of nanocavity arrays formed by electrodeposition through a polystyrene sphere template (A) and height profile corresponding to the grey line across the array (B).

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It has been previously mentioned that the nano-cavity arrays are fabricated by electrodeposition of gold through a sphere template formed on planar gold slides. The amount of charge passed is sufficient for the creation of a gold film of 600 nm thickness. SEM images reveal a cavity opening of approximately 700 nm. Figure 1A shows an AFM image of the array and a cavity opening of 700 nm is also observed. Significantly, AFM can probe the height of the structure and Figure 1B also shows a height profile across several nano-cavities. This profile clearly shows that the depth of the cavities is approximately 600 nm, which is consistent with the charge passed in the electrochemical deposition step. Figure 2 shows a SEM image of the side-on view of the nanocavity array. This image clearly shows that (a) only one layer of cavities is present and (b) the gold is deposited above the half way point of the spheres. Both Figures 1 and 2 strongly suggest that the cavities are partially closed, with a cavity opening of 700 nm and a depth of 600 nm.

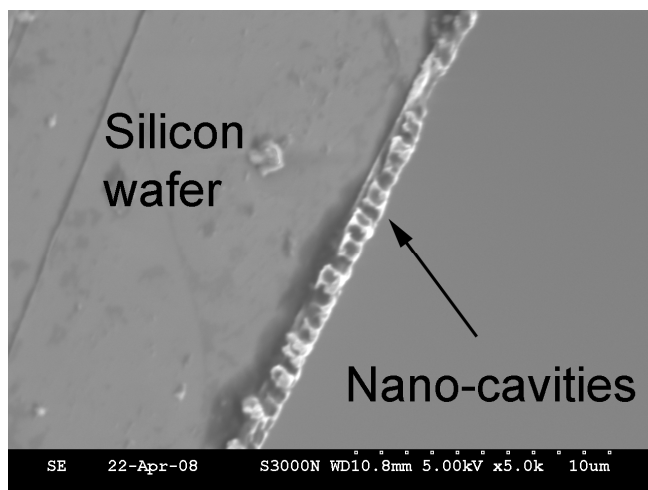


Fig. 2 SEM image showing the side view of the nanocavity array

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Raman Spectroscopy of Thiolated Nano-cavity arrays

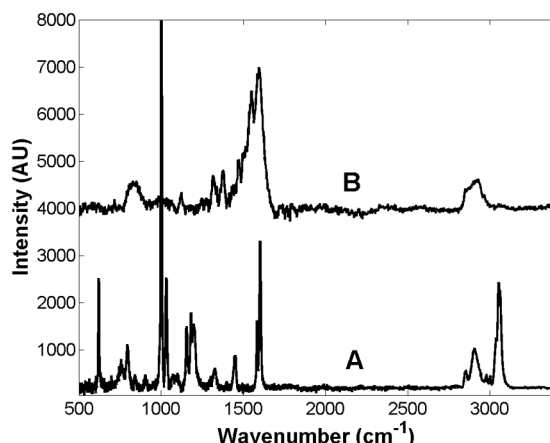


Fig. 3 Raman spectra of nano-cavity arrays which had been exposed to 1-nonanethiol for 14 hours followed by thorough washing with ethanol and Milli-Q water before (A) and after (B) removal of polystyrene spheres.

Raman spectroscopy was used to probe the persistence of the templating spheres during the monolayer formation process. Figure 3 (a) shows the spectrum of the array following overnight deposition of 1-nonanethiol onto the gold/PS sphere array. The Raman spectrum is unchanged from that observed prior to exposure to the thiol deposition solution and is dominated by characteristic modes arising from polystyrene, in particular aromatic ring breathing modes at 1001, 1200 and 1602 cm⁻¹. Saturated and unsaturated CH stretching bands are also evident at 2855-2905 cm⁻¹ and 3050 cm⁻¹ respectively. SEM imaging reveals that 60 minutes sonication in THF successfully removes the templating spheres. Moreover, as shown in Figure 1 (b), the Raman modes associated with polystyrene are absent following sonication indicating that the spheres have been completely dissolved and that there is no polymer residue on the surface. It is important to note that merely soaking the cavities in toluene or THF does not effectively remove the polystyrene, where Raman modes associated with polystyrene remain even after extensive soaking when not accompanied by sonication.

Significantly, Figure 1 (b) shows that following removal of the PS spheres, modes associated with the alkane thiol self-assembled monolayer are observed. The features between 1000-1170 cm⁻¹ and 2800-3000 cm⁻¹ spectral regions are attributed principally to skeletal vibrations of C-C stretching modes and C-H vibration, respectively. Other modes attributable to straight chain alkanes can also be observed, such as CH₂ scissor vibrations observed in the 1450-1500 cm⁻¹ region. The presence of a C-S bend is also suggested by the peak observed at 850 cm⁻¹. The presence of these bands indicates that the 1-nonanethiol layer is not destroyed in the process of removing the spheres from the gold arrays.

Calculation of the top versus interior surface area

The ratio of the top surface area to the interior surface area can be found by analysis of SEM images with common image analysis programs, such as Image J (<http://rsb.info.nih.gov/ij/>). Firstly, the number of cavities in a defined area is determined from an SEM image using this program. The surface area of a single cavity was then calculated by using the equation for the area of a truncated sphere i.e. surface area = $3\pi r^2$, where r is the radius of the sphere. The surface area of a single truncated sphere can then be used to find the internal surface area of the cavities by multiplication by the total number of cavities present in the SEM image area. The top surface area can be found by determining the area of the cavity openings using image analysis software (i.e. finding the area of all the holes in the image) and subtracting this value from the total area of the image. Repeated analysis of several images reveals a ratio of top: interior surface area of approximately 1:5.

Notes and references

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