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Nanoconfinement-Induced Selective Protein Trapping within Hybrid Nanowells

Grazia M.L. Messina[1], Cristiana Passiu[2], Antonella Rossi[2,3] and Giovanni Marletta[1]*

[1] Laboratory of Molecular Surfaces and Nanotechnology (LAMSUN), Department of Chemical Sciences, University of Catania and CSGI, Viale A. Doria 6, 95125, Catania, Italy
[2] Department of Materials, ETH Zurich, Vladimir-Prelog-Weg 5, 8093, Zürich, Switzerland

[3] Dipartimento di Scienze Chimiche e Geologiche, Università degli Studi di Cagliari, Cittadella Universitaria di Monserrato, I-09100 Cagliari, Italy

* Corresponding Author. E-mail: grmessi@unict.it

Preparation of hybrid nanowell arrays

Ordered hybrid organic/inorganic nanowell arrays were prepared by using a modified NP-based lithography, involving a spin-coating step of a monodisperse colloidal suspension of selected size NPs onto a gold substrate.

Figure S1 shows an homogeneous nanowell arrays obtained on large area.

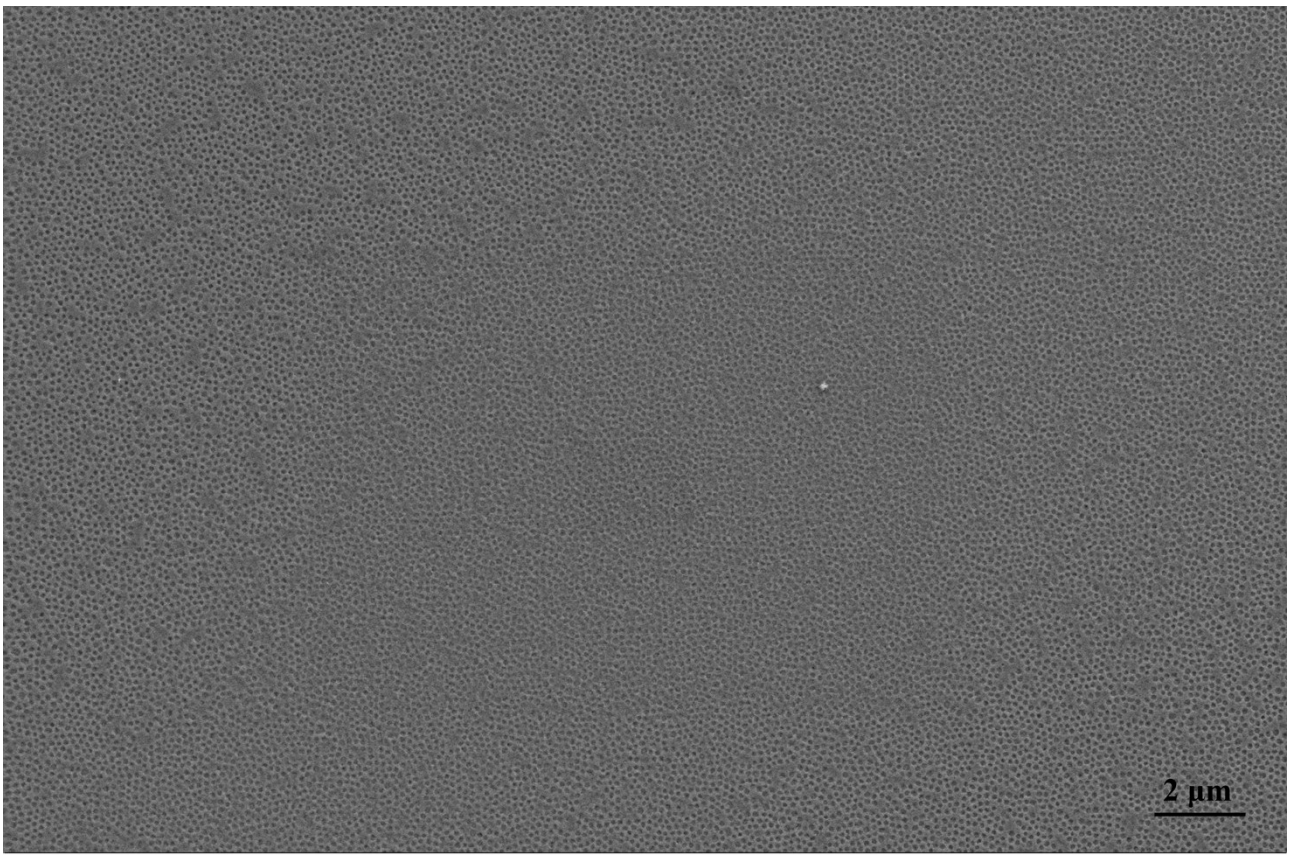

Figure S1. SEM micrograph (SE2 detector) of a nanowell hybrid array on a large sample area of 27x17 µm2, showing the high spatial homogeneity of the structure. EHT=5.00 kV, WD=4.2 mm.

The close-packed colloidal structure is then embedded in a thin PMMA film by spin-coating a polymer solution of suitable concentration. It is possible to tune the polymer concentration having different nanowell diameter and depth.

Here we report a table to summerize the results obtained by using different PMMA concentration.

PMMA Concentration	Nanowell depth (nm)	Nanowell diameter
1 mg/ml	14.8 (1)	69.8 (5)
2mg/ml	38.6 (2)	99.3 (2)
5mg/ml	9 (2)	67.2 (6)
10mg/ml	Nanoparticle completely covered by PMMA	

In particular it is evident that when a concentration of 5 mg/ml is used the nanowell depth is complicated to estimate because of the tip radius and its convolution. In this case the PMMA covers about three quarters of the nanoparticles so it's a little bit more difficult to remove the spheres by etching and the diameter is reduced as you can note.

We used a PMMA concentration of 2 mg/ml because is this the best condition for our experiments.

By increasing the size/depth of the nanowell we aspect an increasing of protein adsorption inside the nanopores in the case of HSA as well as of Lyz outside the pores, we have this experimental evidence with a similar system see ref. 14

This method is really versatile because it is possible to use nanoparticles of different diameter and deposit them by spin coating on surfaces just by changing the spin coating parameters.

Opening of the Au nanowell bottom.

In order to confirm the effective opening of the nanowells' Au bottom, we have exposed the samples to a solution of thiols, comparing the thiol adsorption for nanowell arrays with the one of bare PMMA films.

11-mercaptoundecanoic-acid was purchased as lyophilized powder (Sigma) and solubilized in ethanol, to reach a concentration of 2×10^{-3} M. Each QCM-D experiment started with the sensor running in ethanol (out-gassed by 30 min sonication under vacuum), then the addition of the thiol solution and, after 15 minutes the exchange of the thiol solution in the measurement with ethanol, to check both desorption and stability of the adsorbed layer. We reported the first 20 min of adsorption that is significant to see the kinetic effect on the two different surfaces.

Fig. S2 shows that while a very low adsorption (2-3 Hz, roughly corresponding to mere physisorption of loose molecules, i.e., 35 – 52 ng cm-^{2}) occurs for the bare PMMA films, a markedly higher uptake of irreversibly adsorbed thiol (12-13 Hz of adsorbed molecules, i.e., 175-192 ng cm^{-2}) is measured for the nanowell arrays. It is to note that, the thiol-chemisorption is expected to be relatively low since the open nanowell bottom is not so big, so the distinctive sign of thiol chemisorption being the abrupt shape of the mass uptake for nanowell arrays

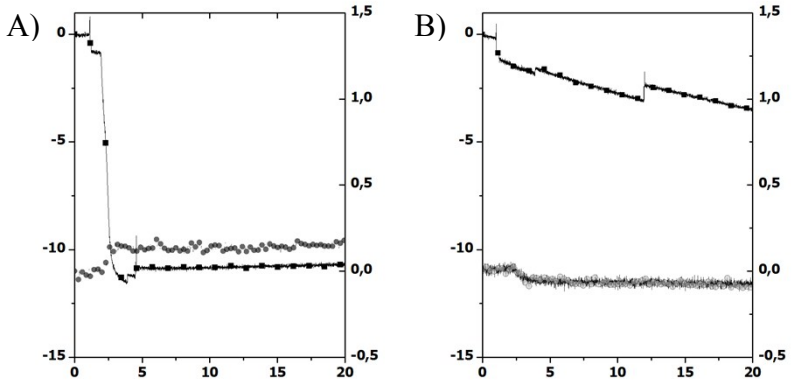

Fig. S2. Frequency shifts (Full squares, left hand side axis) and dissipation shifts (full circles, right hand side axis) from QCM-D measurements for adsorption of Thiol on A) Nanowell arrays and B) PMMA surface. The traces have been simplified removing the noise and full square and full circle are used to draw the eye.

Selective protein adsorption within nanowells

Protein and antibodies adsorption experiments were performed to study the capability of this system to induce the confinements of proteins.

We analysed the adsorption of two globular proteins, respectively HSA (1.44×10-5 M in PBS, at 25 °C) and Lyz (1.44×10-5 M in PBS, at 25 °C) in two different experiments and later we studied

the response of their own antibodies, as the experiments are not sequential we are interested to know the only response of the protein to its antibody. We tried to test the interactions of Ab-HSA and Ab-Lyz on bare nanowell surface but from section analysis none significant change in depth is visible even if we expect an aspecific adsorption on all sample.

As it is known by literature data yje surface free energy influences the protein adsorption, so we measured the wettability of our sample to understand the behavior of protein confinements.

Here we report a table containing the value acquired on homogeneous surface before and after the etching by HF.

Substrate	Water Contact Angle (θ°)
Au untreated	72.1 (0)
Au after HF treatment	67.0 (1)
PMMA untreated	65.5 (1)
PMMA after HF treatment	66.3 (0)

It is evident that the WCA is not dramatically different so it is not necessary to evaluate the protein adsorption before and after the treatment.