

## Supporting Information

### Orthogonal functionalization of nanoporous substrates: controlling 3D surface functionality

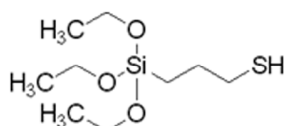
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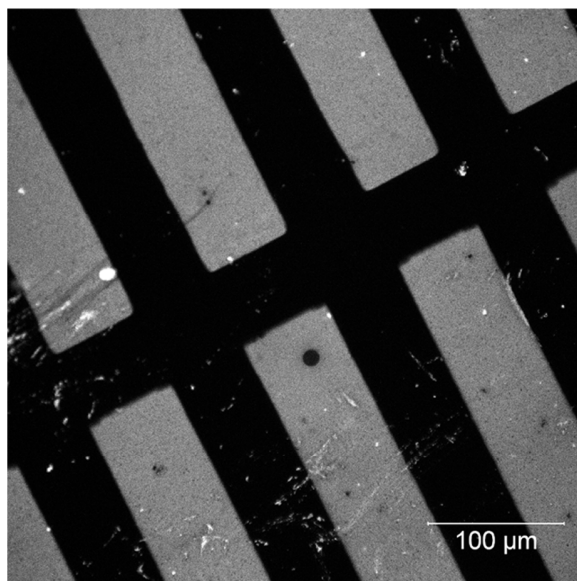
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- Au evaporation through TEM-grid
- Oxygen plasma cleaning, 45 s
- Au-removal with Iodine solution

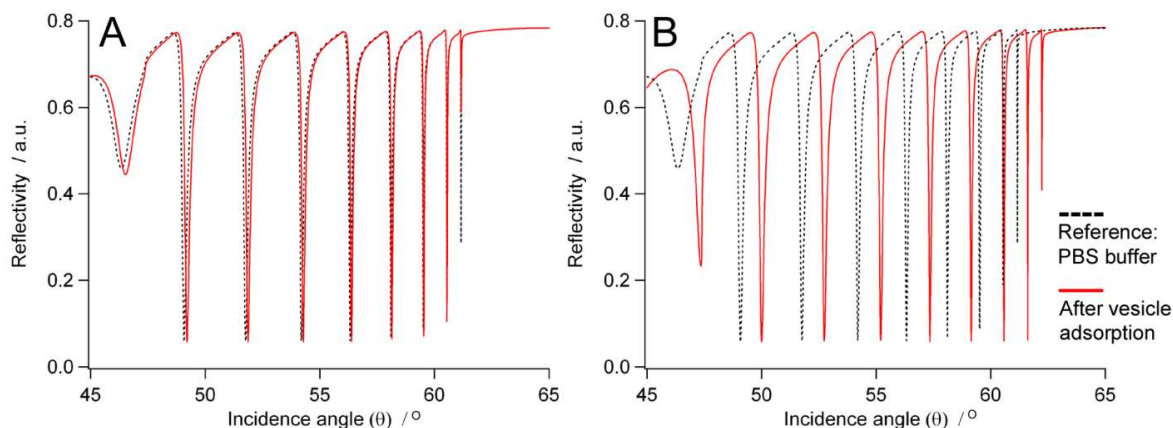
**Au preserves the  
sensitive resonance  
structure of the dye**



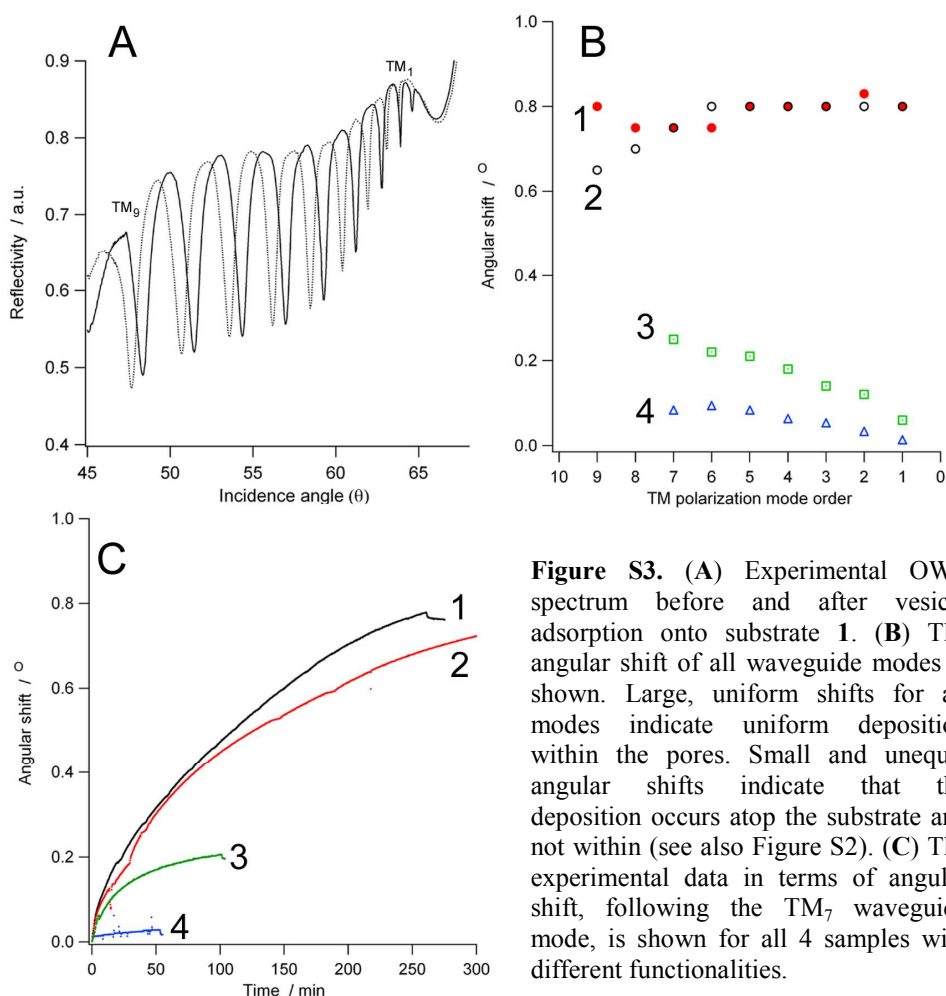
Mercaptopropyl-triethoxysilane  
SH-TES



**Figure S1.** A glass slide was silanized with mercaptopropyl-triethoxysilane (SH-TES), followed by reacting the thiol groups with cyanine-3-maleimide dye (maleimide-Cy3). The substrate was then patterned with a TEM-grid (see also Figure 4) during Au evaporation (15 nm). The Au layer protects the sensitive chemical structure of the Cy3 dye during the 45 s O<sub>2</sub> plasma treatment. Shown on the right is a confocal fluorescence image of the substrate after Au removal with iodine solution (I<sub>2</sub>/KI).



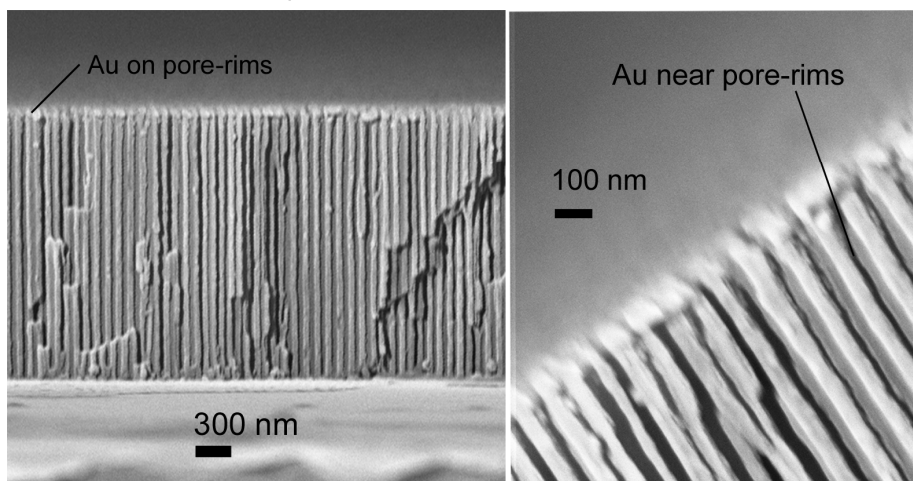
**Figure S2.** Fresnel simulations of the optical waveguide spectra before and after the addition of vesicles on 4  $\mu\text{m}$  thick AAO samples containing pores with diameters of 65 nm for two possible scenarios. The case for a 25 nm film forming only atop is shown in (A). In contrast, the deposition of a 2.5 nm film on the entire surface of the AAO film, atop and within, is shown in (B). When material deposition occurs only atop the film, such as in the case of vesicle adsorption, the waveguide mode angular shifts are smaller and only the higher order modes shift, while the lower order modes remain unchanged, as shown in (A). In contrast, if the entire AAO surface is modified with a thin layer, the optical density of the film increases significantly and a large angular shift is observed for all waveguide modes.



**Figure S3.** (A) Experimental OWS spectrum before and after vesicle adsorption onto substrate 1. (B) The angular shift of all waveguide modes is shown. Large, uniform shifts for all modes indicate uniform deposition within the pores. Small and unequal angular shifts indicate that the deposition occurs atop the substrate and not within (see also Figure S2). (C) The experimental data in terms of angular shift, following the TM<sub>7</sub> waveguide mode, is shown for all 4 samples with different functionalities.

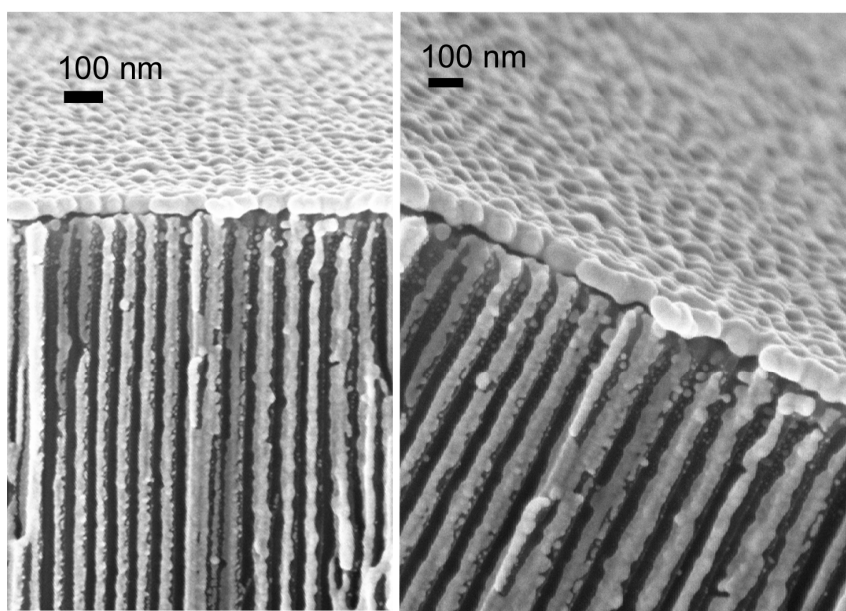
**A 50 nm Au evaporated ATOP**

Au accumulates at pore-rims and forms a thick film

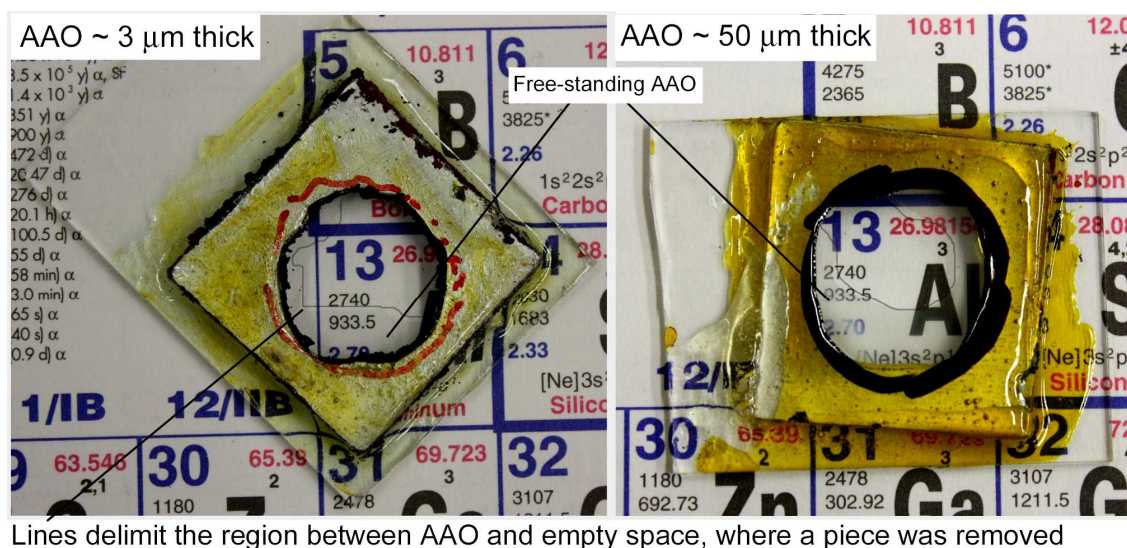


**B 50 nm Au evaporated ATOP + 50 nm Au on CROSS-SECTION**

Pore-interiors remain generally uncoated, Au accumulates on edges



**Figure S4.** (A) 50 nm of Au were evaporated only atop the film before imaging. (B) The AAO substrate was cracked to reveal the cross-section and another 50 nm of Au was evaporated directly on the cross-section: total of 100 nm, 50 nm atop + 50 nm in the cross-section. The resolution is better since the sample is more conductive. These results show that Au does not deposit significantly within the AAO pores (aggregates). Even in the cross-sectional evaporation, Au accumulates at the rims between the linear pore grooves. We do not expect more than about 1-5 % maximum pore interior contamination (for a 4  $\mu\text{m}$  thick AAO), which is approximately the ratio of pore area ( $\pi r^2$ ) to pore-interior area.



**Figure S5.** Photographs showing the transparency of free-standing AAO films (preparation step before mounting the films on glass slides). About half of the AAO free-standing film was broken off in order to show the transparency difference with and without AAO. The AAO on the left-hand side picture is thinner (3  $\mu\text{m}$ ) and much more transparent than a thicker (50  $\mu\text{m}$ ) AAO, shown on the right-hand picture. The thicker AAO is slightly hazy and therefore less transparent.

## Experimentals

Mercaptopropyl-triethoxysilane was purchased from ABCR (Karlsruhe, Germany). Cyanine-3 maleimide was purchased from Toronto Research Chemicals (North York (ON), Canada).

**Silanization of glass substrates (Figure S1).** Regular  $\text{SiO}_2$  microscope glass slides were cleaned in 2 % *vol.* Hellmanex, rinsed, and  $\text{O}_2$  plasma cleaned for 2 min prior to silanization to increase the surface density of OH-groups. The glass slides were then inserted into a glass staining jar and 50  $\mu\text{l}$  of mercaptopropyl-triethoxysilane (SHTES) were added in a glass test tube, inside the chamber. The container was covered with its glass cover and sealed using Scotch vacuum tape from 3M (St-Paul (MN), USA), left in the oven at 130  $^\circ\text{C}$  for 10 min to warm, followed by 3 hr under vacuum. The samples were cooled and 100  $\mu\text{l}$  of cyanine-3-maleimide dye (0.02 mg) dissolved in PBS were added and left to incubate for 2 hr, followed by rinsing with PBS, deionized water and ethanol. They were dried and orthogonally functionalized (see manuscript) using a TEM grid as an evaporation mask to create protected and unprotected areas.