



Supporting Information

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Materials and Methods

Materials - All chemicals were reagent grade or higher and purchased from Sigma-Aldrich (Sydney, Australia) unless otherwise stated. Mesitylene (1,3,5-trimethylbenzene) was redistilled from sodium and subsequently stored over molecular sieves until use. Succinimidyl 10-undecenoate was prepared as described previously [1]. Milli-Q water (~18 M Ω cm) was used for preparing solutions and cleaning silicon surfaces. Si(100) wafers (B-doped, resistivity 0.07 Ω cm) were purchased from the Institute of Electronics Materials Technology (ITME, Warsaw, Poland). C-terminus amidated peptide GRGDS was purchased from GenScript Corporation (NJ, USA). Donkey serum and Alexa Fluor 594 anti-mouse IgG antibodies were purchased from Jackson ImmunoResearch and mouse monoclonal anti-paxillin antibodies were purchased from BD Transduction Laboratories.

Rugate Filter Preparation – Rugate filters were formed as described previously [2]. Briefly, p⁺-type Si(100) wafers (~1 cm²) with a resistivity of 0.07 Ω cm were cleaned in acetone and ethanol with sonication for 5 minutes each. The wafer was dried under a stream of nitrogen and placed on a rubber O-ring (inner diameter 0.6 cm) in an electrochemical cell. The wafer was back-contacted with a polished steel electrode (anode) and the cell filled with 25% HF in ethanol (equal parts 50% HF in water and absolute ethanol). A circular platinum electrode (cathode) was immersed in the ethanolic HF solution above the wafer. A computer controlled sinusoidal current density profile was applied to the wafer using a custom LabVIEW 6.1 interface to produce a porosity varying between 54.5 and 57.5 %. After etching, the HF solution was removed and the cell was dismantled. The wafer was rinsed thoroughly with ethanol and pentane, dried under a stream of nitrogen and the optical characteristics measured.

Modification of PSi Rugate Filters with Monolayers – A 0.2 M solution of succinimidyl 10-undecenoate in mesitylene containing 20 mM triethylsilyl chloride as an in situ drying agent [3,4] was placed into a flame-dried Schlenk flask kept under argon, degassed by five freeze-pump-thaw cycles (liquid N₂, high vacuum, T_{room}) and held at room temperature under an argon atmosphere. The degassed solution was refluxed at 170° C for 15 minutes in an oil-bath to react residual water remaining in the flask with Et₃SiCl, brought to 150° C and the freshly etched silicon rugate filter added. After hydrosilylation at 150° C for 8-18 hours, the PSi samples were withdrawn from the solution, rinsed with solvents and blown dry under a gentle stream of argon.

Spectroscopic Characterization of Modified PSi Photonic Crystals - The modified PSi photonic crystals were analyzed using FTIR spectroscopy to monitor the different steps in the modification process via the formation of the appropriate bonds and to assess the extent of adventitious surface oxidation. The wetting behavior of the modified PSi was evaluated by comparing the reflectance spectrum of the photonic crystal measured in air to the reflectance spectrum of the sample immersed in water. A sufficiently hydrophobic monolayer prevented the ingress of water in the pores. Under these circumstances the optical reflectance spectrum measured in water remained unchanged relative to that measured in air. In contrast, a hydrophilic surface coating allowed water to enter the pores resulting in a shift of the features in the reflectance spectrum (reflectance peaks or plateaus) to higher wavelengths. This shift to higher wavelengths is observed because water has a higher refractive index than the air it replaces inside the photonic structure.

Coupling of (Bio-)Molecules Selectively to the Top (External) Surface of the PSi Photonic Crystals - Samples that prevented the infiltration of water (via hydrophobicity) were exposed to aqueous solutions (1 mM) of either hexa(ethylene glycol) amine, ethanolamine or the peptide GRGDS for 1 hour to selectively derivatize the exterior of the mesoporous rugate filters.

Modification of the Internal Surfaces of the PSi Photonic Crystal - Subsequently the interior of the structure was modified by reaction with either 1 mM octylamine or 20 mM hexa(ethylene glycol) amine in anhydrous acetonitrile. Interior modification was also achieved using the same molecules in water by pre-wetting the structure with ethanol prior to adding an *aqueous* solution of the molecules to be immobilized on the internal surfaces.

Endothelial Cell Capture - Pig aortic endothelial cells (PAEC, Cell Application) were cultured in M199 medium containing 20 % (v/v) fetal bovine serum (FBS) and 0.1 mg/mL heparin at 37 °C in 5 % CO₂. Confluent cells were serum-starved (0.2 % FBS) overnight followed by re-plating on PSi surfaces for 4 h in 10 – 20 % FBS. PSi rugate filters were washed twice in warm phosphate buffered saline and fixed in 4% paraformaldehyde at RT for 20 min. For immunofluorescence, fixed cells were blocked with 5% normal donkey serum in 0.1% saponin and immunolabelled for 1 h each with mouse anti-paxillin and Alexa Fluor 594-conjugated anti-mouse IgG antibodies, washed after each incubation period and mounted with mounting media containing anti-fading agents (ProSciTech). Endothelial cell images were obtained with a DM IRE2 Microscope (Leica, Australia) equipped with photon-multiplier tubes and Leica acquisition software. Alexa Fluor 594 fluorescence was excited at 800 nm with a Verdi/Mira 900 multi-photon laser system.

Spectroscopy - The reflectivity spectra of porous silicon samples were monitored throughout all stages of chemical and biological modification. Monochromatic light from a 250 W projector lamp was coupled through a J/Y SPEX 1681 spectrometer and modulated with an optical chopper. Entrance and exit slit widths were adjusted in conjunction with focusing optics to minimise the spot size on the sample (~1 mm diameter). Reflectivity spectra were measured by illuminating the sample at normal incidence and the reflected light collected using a beam splitter for collection at a silicon detector. Fourier transform infrared (FTIR) spectra of rugate filters were measured in transmission mode on a ThermoNicolet AVATAR 370-FTIR spectrometer and data collected using OMNIC Professional software (Thermo Fisher Scientific, Inc., USA).

Supplementary Results

Influence of Monolayer Quality and Oxide Formation on the Wetting Behaviour of the Pores – The internal surfaces (pore walls) of the PSi have to be sufficiently hydrophobic to prevent the ingress of water into the pores. This hydrophobicity can be achieved when the monolayer is of sufficient quality and free of oxide [5]. Figure S1 shows the FTIR spectra of two different PSi rugate filters after reaction with succinimidyl 10-undecenoate. Both spectra reveal the presence of the NHS ester terminated monolayer (see Table S1 for assignment of the peaks corresponding to the organic monolayer). There is no appreciable level of silicon dioxide present in the structure shown in Figure S1a and the spectrum is characteristic of a structure with sufficient hydrophobicity to prevent any measurable penetration of water. In contrast, when oxidation of the PSi structure was apparent from the FTIR spectrum (Figure S1b), water was able to penetrate into the mesopores and thus, selective exterior derivatization was impossible.

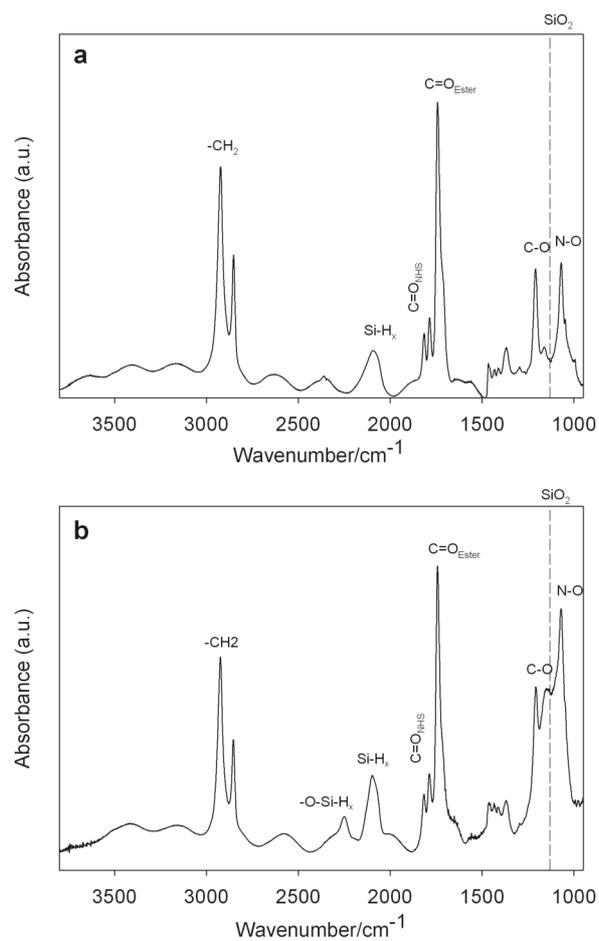


Figure S1. Transmission FTIR spectra of two different PSi rugate filters after reaction with succinimidyl 10-undecenoate associated with (a) insignificant and (b) with considerable levels of silicon dioxide.

Table S1. Assignment of the absorbances in the FTIR spectra in Fig S1.

Assignment	Wavenumber/cm ⁻¹
Organic monolayer	
C-H stretches (asymm., symm.)	2920, 2850
Succinimide group	1820, 1790
C=O stretch, ester	1740
N-O stretch	1210
C-O stretch	1070
Silicon substrate	
Residual Si-H _x stretches (x = 1-3)	~2100
Oxide (O-Si-O)	~1100

Monitoring internal modification by Reflectance and FTIR spectroscopy – Reflectance spectroscopy can be used to monitor the deposition of organic layers onto the pores walls. The position of the reflectivity peak depends on the average refractive index of the PSi. Introduction of organic layers (with a refractive index greater than that of air) into the porous scaffold results in a red-shift of the reflectivity peak. The reflectance spectra after the initial steps of the modification protocol are shown in Figure S2 for the same sample as that monitored by FTIR spectroscopy in Figure S3. FTIR spectroscopy in transmission mode is also only sensitive to reactions occurring inside the porous matrix because the external surface area on top of the PSi photonic crystal is negligible compared to the internal surface area (pore walls) of the PSi photonic crystal [6]. Figure S3 shows FTIR spectra of a PSi rugate filter after modification with a NHS ester terminated monolayer (step 1) followed by successive treatment with aqueous (step 2) and organic (step 3) solutions of amines.

Step 1: Monolayer formation. The FTIR spectrum of the as prepared PSi rugate filter showed the characteristic stretches of the silicon hydride species covering the freshly etched PSi surface (Figure S3a). After formation of the monolayer via hydrosilylation with succinimidyl 10-undecenoate the Si-H_x stretches were reduced and the absorbances from the organic monolayer appeared in the FTIR spectrum (Figure S3b, see Table S1 for assignments). The position of the reflectivity peak showed a red-shift by 93 nm after the hydrosilylation reaction relative to the peak position of the as prepared structure (Figure S2), consistent with the formation of a densely packed organic monolayer.

Step 2: External modification. After reacting the hydrophobic NHS ester terminated sample for 1 hour with an aqueous solution of GRGDS peptides there was no change in the FTIR spectra, in particular the carbonyl stretches of the NHS ester were still present (Figure S3c). The position of the reflectance peak the same before (Figure S2b) and after external modification (Figure S2c). Both of these observations were consistent with the *interior* matrix remaining underivatized

Step 3: Internal modification. Next, the interior of the hydrophobic porous scaffold derivatized by incubation of the sample in a solution of amines in acetonitrile. In contrast to the aqueous solution used in step 1, the organic solution could infiltrate the pores [7]. The sample shown in Figure S3 was cleaved into two halves and each half was reacted with a different amine (hexa(ethylene glycol) amine, Figure S3d or octylamine, Figure S3e). The FTIR spectra of the samples displayed loss of NHS ester peaks and appearance of amide bond signatures at 1650 cm⁻¹ and 1560 cm⁻¹. The –NH stretch of the amide at 3330 cm⁻¹ and the –OH stretch of terminal hydroxyl group of the hexa(ethylene glycol) moiety at 3440 cm⁻¹ were also apparent in the FTIR spectra.

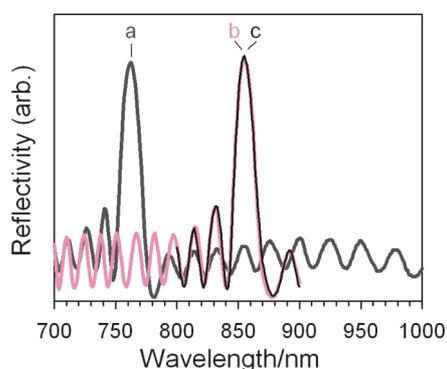


Figure S2. Optical reflectance spectra of a PSi rugate filter after successive stages of the modification process (see Fig. S3 for structures of the internal surface groups): (a) as prepared, (b) after reaction with succinimidyl 10-undecenoate and (c) after reacting the top of the rugate filter with the GRGDS peptide in aqueous solution.

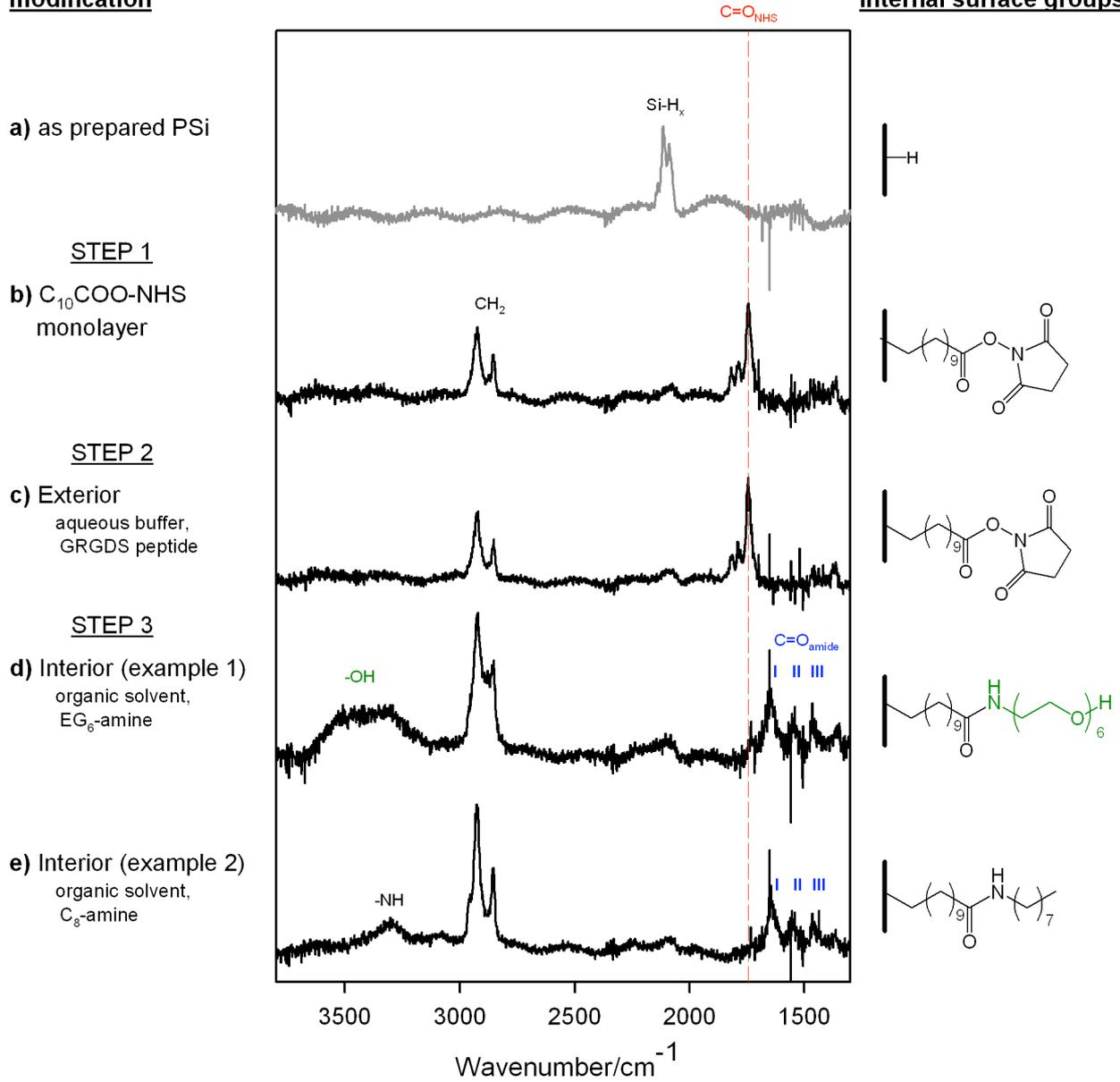
modification**internal surface groups**

Figure S3. Transmission FTIR spectra of a PSi rugate filter after successive stages of the modification process (same sample as in Fig. S2): (a) as prepared, (b) after reaction with succinimidyl 10-undecenoate, (c) after reacting the top of the rugate filter with the GRGDS peptide in aqueous solution and (d,e) after interior modification of the rugate filter (cleaved into two halves) with (d) hexa(ethylene glycol) amine and (e) octylamine using acetonitrile as solvent.

Tailoring the wetting behaviour of other types of PSi photonic crystals with organic monolayers – The strategy presented in this study is applicable to other materials with different porosities and pore sizes. As an example we prepared a different type of photonic crystal, PSi Bragg reflectors, by electrochemical etching of highly doped p-type Si(100). The pore sizes in this material were considerably larger and ranged between 30 – 70 nm in diameter. PSi Bragg reflectors exhibit a high reflectivity plateau in their reflectance spectrum. A red-shift is observed in the position of the Bragg plateau when the air inside the structure is replaced by a medium with a higher refractive index (such as water or ethanol). Figure S4a shows the reflectance spectrum of a PSi Bragg reflector modified by reaction with undecenoic acid in air and after immersion of the sample in water. The red-shift of the Bragg plateau verified that water entered the hydrophilic acid-terminated pores. In contrast, when a PSi Bragg reflector was modified with a relatively hydrophobic methyl ester terminated monolayer water could not enter the pores and no shift in the reflectance spectrum was observed relative to the spectrum acquired in air (Figure S4b). Organic solvents with a lower surface tension than that of water readily entered the pores as evident from the pronounced red-shift of the Bragg plateau after immersion of the same sample in ethanol (Figure S5).

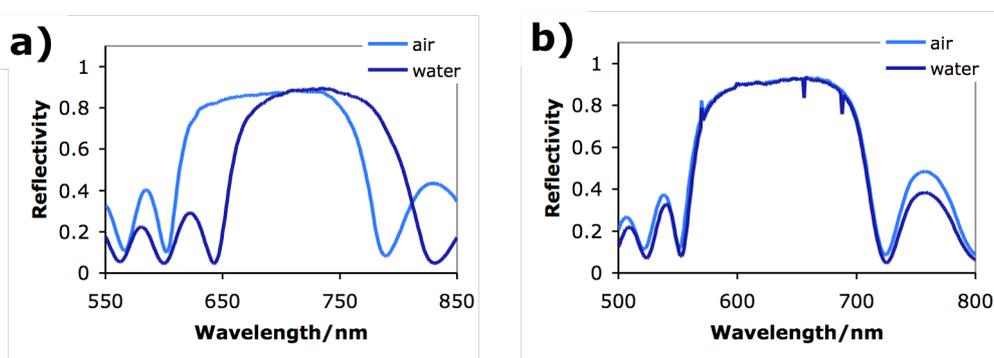


Figure S4. Optical reflectance spectra of PSi Bragg mirrors modified with a monolayer by reaction with (a) undecenoic acid ($C_{10}COOH$) and (b) methyl undecenoate ($C_{10}COOMe$). The reflectivity of each sample was measured in air and in water. Ingress of water into the pores results in a red-shift of the Bragg plateau.

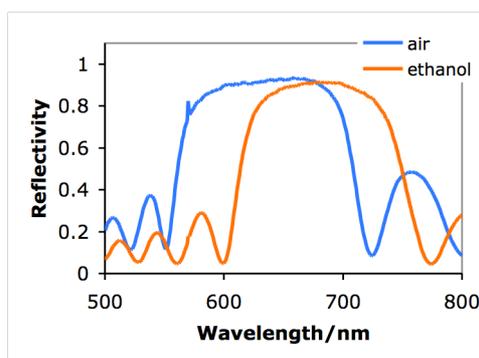


Figure S5. Optical reflectance spectra measured in air and with the sample immersed in ethanol of the same PSi Bragg mirror modified with methyl undecenoate ($C_{10}COOMe$) as shown in Figure S3b.

References and Notes

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5. Silicon oxide can be formed when oxygen and water are not carefully excluded from the reaction.
6. The PSi rugate filters used in this study had the following characteristics: average pore diameter ~12 nm, average porosity 55% and thickness of the PSi layer ~9 μm . On the basis of these parameters the internal surface area is three orders of magnitude larger than the external surface area.
7. Alternatively the porous structure can be wetted using a water miscible organic solvent (e.g. ethanol) followed by immersion in water. In this way the modification of the internal surfaces can also be carried out in aqueous solutions to allow coupling of molecules that are incompatible with organic solvents.