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Supporting Information

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Functionalization of Imprinted Nanopores in Sub-Nanometer Thin Organic Materials

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Supporting Information

Materials

1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), 20 mg/ml chloroform solution was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Other materials were purchased from Sigma-Aldrich.

HPLC measurements of 1 in liposomes

Liposomes were prepared as described in the main text using the following amounts of materials: t-butylstyrene: 17.6 µl (9.63×10^{-2} mmol), p-divinylbenzene: 13.7 µl (9.62×10^{-2} mmol), 2,2-dimethoxy-2-phenyl-acetophenone (3 mg, 1.2×10^{-3} mmol) DMPC: 20 mg (5.9×10^{-2} mmol), **1**: varying amounts between 1.45×10^{-3} mmol and 8.70×10^{-3} mmol. After extrusion, a 200 µl aliquot was carefully taken from the bottom of the sample vial, mixed with 200 µl of methanol to lyze liposomes, and the solution was analyzed with HPLC (Waters 600 pump and Waters 2487 dual wavelength UV-vis detector equipped with a semi-preparative flow cell) using 50% hexane/50% ethyl acetate as eluent. The detection wavelengths used were 220 and 255 nm. The column was a Nova-Pak Silica HRC18, 6 µm, 60Å, 19×300 mm. At a flow rate of 10 mL/min the retention time of **1** was 4.50 min. Three independent analyses were performed for each data point. Solutions of **1** in methanol were used for the calibration curve (Figure S1).

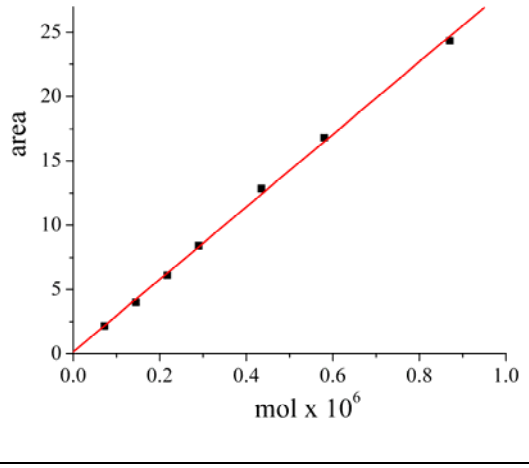

Figure S1. Calibration curve for HPLC measurements of **1**.

FTIR measurements of 1 in nanocapsules

Liposomes containing t-butylstyrene, p-divinylbenzene, **1** and 2,2-dimethoxy-2-phenyl-acetophenone were prepared as described above. Prior to UV-polymerization, a stream of purified nitrogen or argon was passed through the solution to remove oxygen. The sample was irradiated with UV light (λ=254 nm) in a photochemical reactor equipped with a stirrer (10 lamps, 32W each; the distance between the lamps and the sample was 10 cm) for 60 min. After the polymerization, 10 ml methanol were added to precipitate nanocapsules. The precipitate was washed with methanol (3-5 times with 5 ml) to remove lipid and unreacted monomers. Methanol was decanted and the precipitate was resuspended in 2 ml of benzene to give a translucent solution. Samples were freeze-dried on an ice bath under high vacuum to give white powder.

Samples for FTIR analysis were prepared by a thorough mixing of 10 mg of material with 10 drops (140 mg) of light mineral oil. Measurements were performed on a Thermo Nicolet

380 FTIR spectrometer using a Thermo Spectra Tech cell. The oil layer was sandwiched between two 2 mm thick NaCl windows separated by a 0.1 mm Teflon spacer to allow for quantitative measurements. Representative spectra of **1** and glucose pentaacetate (a structural analog of **1** but without a polymerizable moiety) are shown on Figure S2.

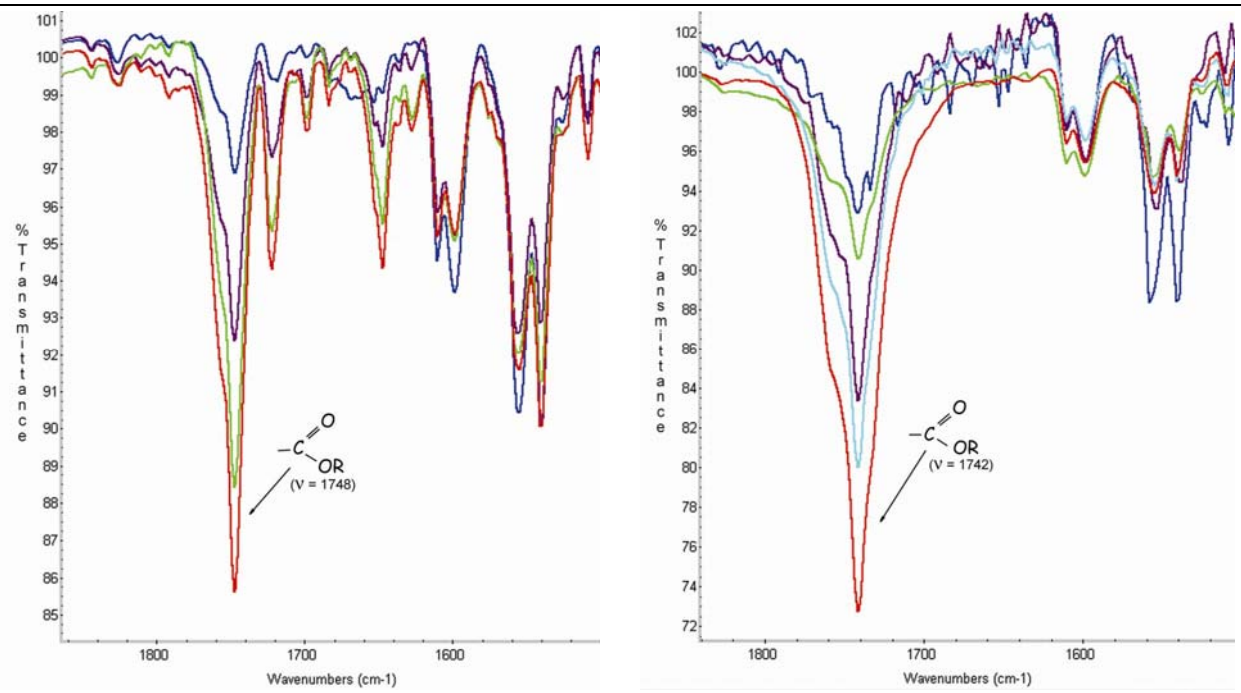

Figure S2. FTIR spectra of **1** (**A**) and glucose pentaacetate (**B**) at different concentrations

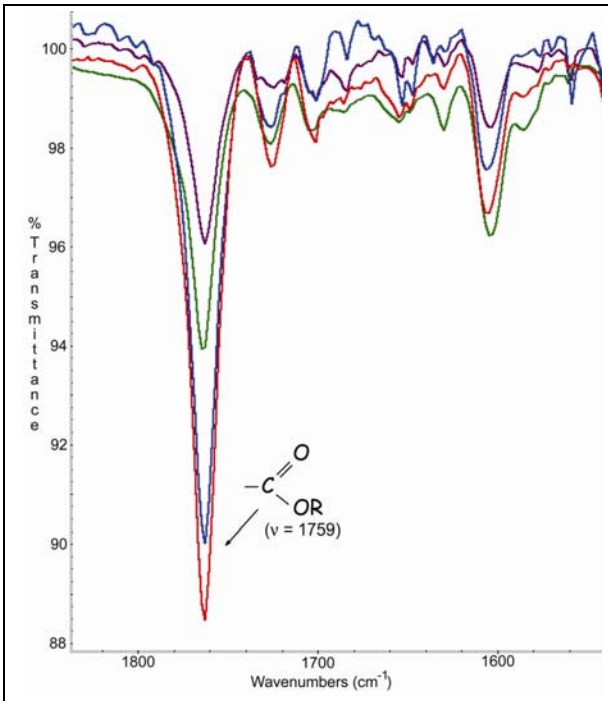

Figure S3. FTIR spectra of nanocapsules with **1**.

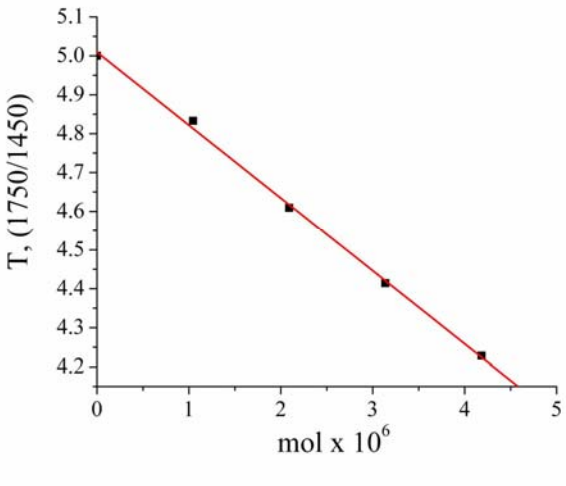

Figure S4. Calibration curve for FTIR measurements of **1**.

The signal of carbonyl group of **1** in nanocapsules (1759 cm^{-1}, Figure S3) is shifted compared with free **1** (1748 cm^{-1}, Figure S2A). The position of the absorption band is not concentration-dependent. Three independently prepared samples were measured for each data point. Samples for the calibration curve (Figure S4) were obtained by mixing varying amounts of **1** with polystyrene (an inert additive) to make up 10 mg of powder, which was then mixed with mineral oil as described above.

Synthesis and FTIR measurements of functionalized nanocapsules

Nanopores with carboxylic acid groups. t-Butylstyrene (17.64 μL, 9.63×10^{-5} mol), p-divinylbenzene (13.70 μL, 9.62×10^{-5} mol), and initiator (3 mg, 0.117×10^{-5} mol) were added to a chloroform solution of DMPC (5.9×10^{-5} mol, 20 mg/ml in chloroform) and **1** (0.87×10^{-5} mol, 2.08 mg/ml in chloroform). Chloroform was evaporated using a stream of purified argon to form a lipid/monomer film on the wall of a culture tube. The lipid film was further dried under vacuum for 30 minutes to remove traces of chloroform. GC and UV-vis analysis confirmed that the concentration of monomers after drying remained the same. The dried film was hydrated with DI water giving a dispersion of multilamellar vesicles. The suspension was extruded at 32°C through a polycarbonate Nucleopore track-etch membrane (Whatman) with 100 nm pore size using a Lipex stainless steel extruder (Northern Lipids). Prior to the polymerization, oxygen was removed by passing purified nitrogen or argon through the solution. The sample was irradiated with UV light (λ=254 nm) in a photochemical reactor equipped with a stirrer (10 lamps, 32W each; the distance between the lamps and the sample was 10 cm) for 60 min. An aliquot of the solution was taken for the measurement of **1** in nanocapsules. Triton X-100 (0.5 ml, 2%) and NaOH (0.5 ml, 1M) were added and the reaction mixture was stirred for 1 hour. Following the alkaline hydrolysis, the reaction mixture was neutralized with HCl. Methanol (10 ml) was added and the precipitate was washed 3-5 times with methanol. Methanol was decanted, and the residue was suspended in benzene. The solution was freeze-dried to give white powder. The presence of carboxylic groups was confirmed by the IR spectroscopy and subsequent transformations to an acid chloride and an amide. The ratio of ester groups to carboxylic acids was found to be 5:1 by IR spectroscopy using the ratio of intensities of the bands corresponding to the carbonyl groups in IR spectra of nanocapsules with **1** before and after hydrolysis

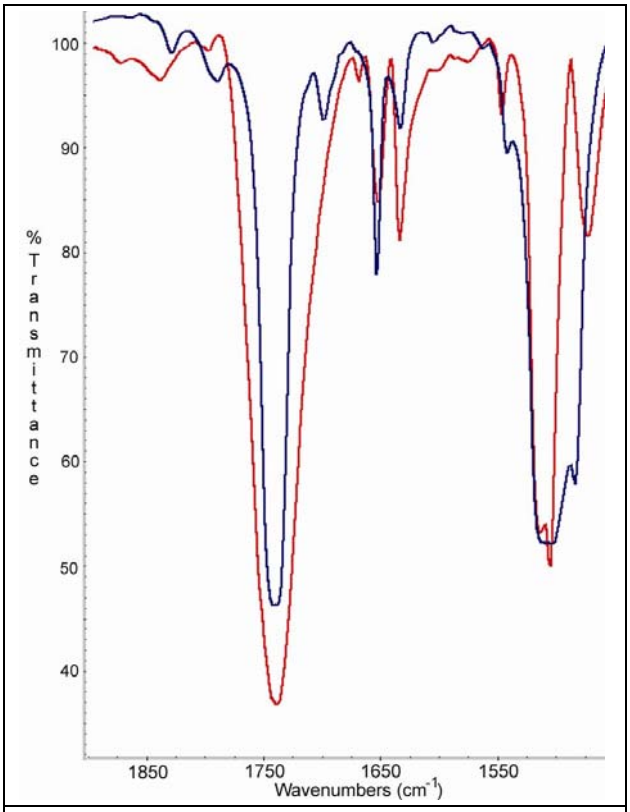

Figure S5. FTIR spectra of benzoic acid (red) and methyl benzoate (blue) at identical concentrations.

(approximately 2.6, Figure 2) and accounting for the difference in carbonyl group intensity between free acid and the corresponding ester (approximately 1.9, Figure S5).

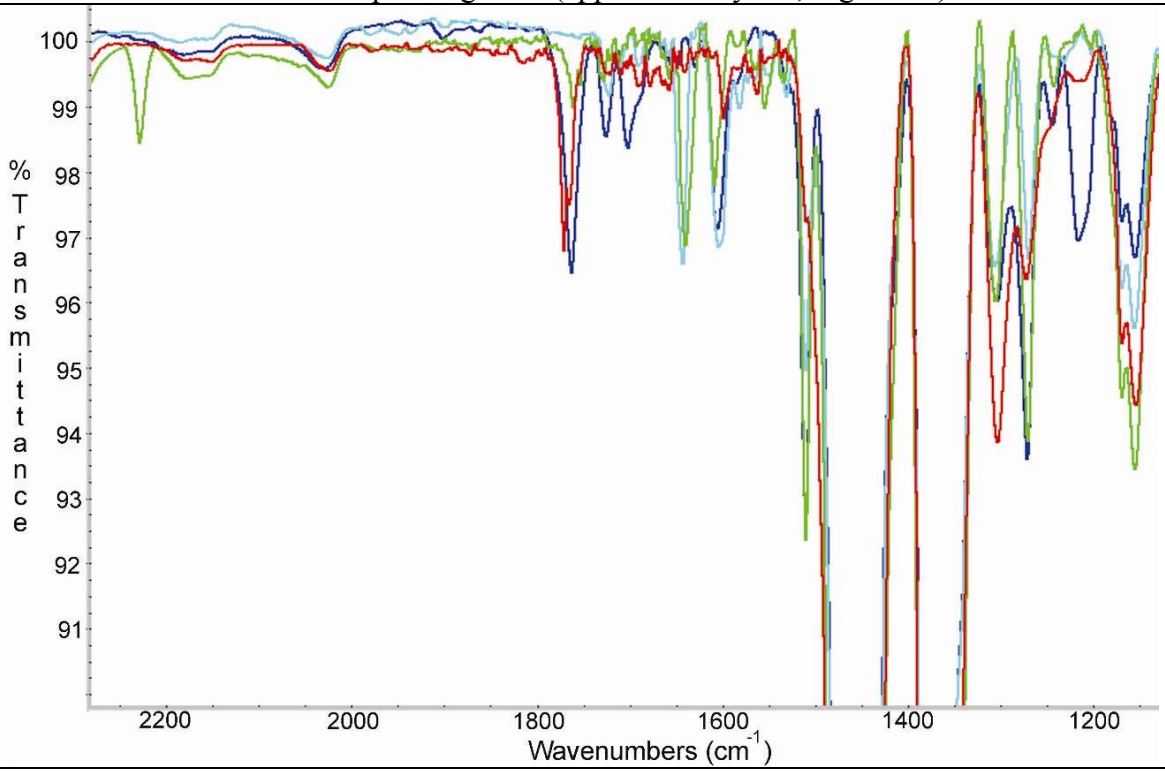

Figure S6. IR spectra of nanocapsules with free carboxylic groups (blue), acid chloride groups (red), amide prepared with benzylamine (light blue) and amide prepared with 4-(aminomethyl)benzonitrile (green).

Nanopores with acid chloride groups. Freeze-dried polymer nanocapsules containing free carboxylic acid groups (10 mg) were suspended in toluene (3 ml) to give a translucent solution. Thionyl chloride (5 ml) was added and the reaction mixture refluxed for 12 h. The reaction mixture was evaporated to dryness, and the residue was washed 3-5 times with toluene. IR spectra revealed a shift in the carbonyl group vibration from 1763 cm^{-1} to 1774 cm^{-1} (Figures 3 and S4) and the disappearance of the band at 1215 cm^{-1} corresponding to the single C-O bond in the carboxylic group (Figure S6).

Nanopores with amide groups. The powder produced in the previous step was resuspended in toluene (3ml), then triethylamine (3 ml) and either 4-(aminomethyl)benzonitrile hydrochloride (0.2 g) or benzylamine (3 ml) were added, and the mixture was refluxed overnight (15 h). Then solution was evaporated to dryness, the residue was dispersed in water, centrifuged and washed 3-5 times in methanol. Methanol was decanted; the

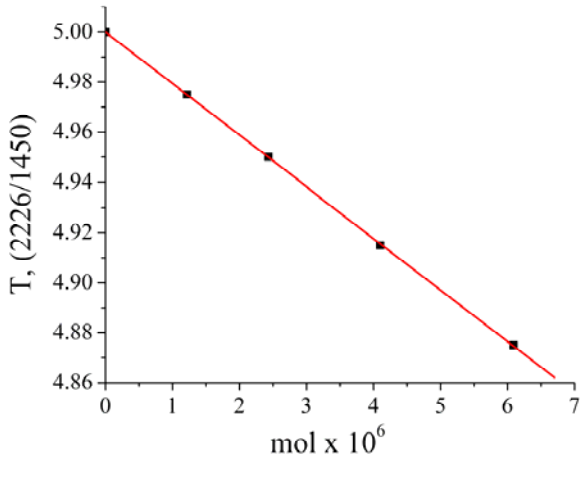

Figure S7. Calibration curve for the FTIR measurement of 4-(aminomethyl)benzonitrile

solid residue was resuspended in benzene (2 ml) and the sample was freeze-dried under high vacuum to give white powder. IR spectra show the signal of the carbonyl group at 1650 cm^{-1}, typical for amides.

FTIR analyses were performed as described above. The amount of amide was determined from the C≡N vibrations. Samples for the calibration curve (Figure S7) were prepared by mixing varying amounts of 4-(aminomethyl)benzonitrile hydrochloride and polystyrene to make up 10 mg of powder, which was mixed with mineral oil as above.

Characterizations

TEM images were acquired on a JEOL JEM1200EX II microscope (100 kV). Samples were negatively stained with phosphotungstic acid (pH=5.9) on a carbon grid. SEM images were obtained with a Philips XL 30 ESEM, coated with 40-60 Å Au-Pd (60:40) layer using EMS 590 X sputter. FTIR spectra were obtained in light mineral oil using 2 mm NaCl discs with Thermo Nicolet 380 spectrometer. Oil layer thickness was 0.1 mm (controlled by a Teflon spacer). For each data point, three independently prepared samples were measured. Spectra were taken with resolution of 4 cm^{-1} and were averaged over 1024 scans. UV measurements were done with an Agilent 8453 UV-vis spectrophotometer using a quartz cuvette with 1 cm optical path length.