Nanocapsules with "invisible" walls

Electronic Supplementary Information

Sergey Dergunov, Beata Miksa, Bill Ganus, Ernö Lindner, and Eugene Pinkhassik

General: (Materials: 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), 20 mg/ml chloroform solution was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). 4-tert-butyl styrene (t-BSt), the cross-linker p-divinylbenzene (DVB), the photoinitiator 2,2-dimethoxy-2-phenylacetophenone, procion red MX-5B (PR), 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (pyranine), bromophenol blue (BPB), triton X-100 (TX-100) were obtained from Sigma-Aldrich. The monomers t-BSt and DVB were purified through Al_2O_3 columns to remove the inhibitors. The UV spectra of absorbance were done by using Agilent Technology UV-Vis Spectrophotometer model 8453 with polystyrene cuvettes.

Synthesis and FTIR measurements of nanocapsules

t-Butylstyrene (17.64 μL, 9.63×10^{-5} mol), p-divinylbenzene (13.70 μL, 9.62×10^{-5} mol), and initiator– 2,2-Dimethoxy-2-phenyl-acetophenone (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. 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 nanocapsules as white powder.

Dye retention. Nanocapsules were prepared as described above from t-butylstyrene (17.64 μL, 9.63×10^{-5} mol) and divinylbenzene (13.70 μL, 9.62×10^{-5} mol) in DMPC liposomes (40 mg) extruded in the presence of Procion Red (10 mM) with a syringe mini-extruder from Avanti Polar Lipids with 0.1 μm polycarbonate filters. Non-entrapped dye was removed by using two different techniques: precipitation in methanol and the size exclusion chromatography. In the first method, capsules were precipitated with excess methanol immediately after polymerization, the sample was centrifuged (3500 rpm, 10min) and the supernatant was carefully decanted. The washing step was repeated 3-5 times until the supernatant showed no coloration. Absence of dye in the supernatant was confirmed with UV. The capsules were suspended in benzene, and the suspension was freeze-dried to yield colored powder. In the size exclusion method, the sample (0.5 ml) after polymerization was mixed with 0.5 ml of 0.2% Triton X-100 solution, and the sample was passed through a Sephadex G-25 column (10 ml) twice to ensure complete removal of free dye. The faster-eluting nanocapsule fraction was collected.

Nanocapsule characterization. The average size of the capsules was estimated by the transmission electron microscopy (JEOL Transmission Electron Microscope model: JEM-1200EX II) and DLS (Dyna Pro NanoStar, Wyatt Technology). For TEM the samples were negatively stained with phosphotungstic acid (2 %, pH = 5.9) on a carbon support film on specimen grid (Electron Microscopy Sciences, 200 mesh copper).

Stopped-flow measurements. Pyranine or pyranine-loaded nanocapsules (100 µl) were rapidly mixed with NaOH solution (20 µl) using a stopped-flow apparatus (Aviv Model 410, CD spectrometer) at 25^0 C. The solution was irradiated at 450 nm using a 150 W suprasil xenon lamp. Emission was measured using a fluorescence photomultiplier tube equipped with a 475 nm cut-off filter and oriented at a 90^0 angle with regard to the excitation axis. Similarly, BPB or BPB-loaded nanocapsules (60 µl) were rapidly mixed with HCl solution (20 µl, 1 mM or 5 mM). UV absorbance was recorded at 598 nm. In these experiments, nanocapsules were suspended in 0.2% aqueous solution of Triton X-100. Data were collected at 100-µsec intervals for 1 sec using Aviv software. In each experiment, five to ten raw tracings were averaged and analyzed subsequently.

Size-exclusion separation of nanocapsules from non-entrapped dye.

Size-exclusion chromatography was performed using Sepharose-4B as a separation medium and an aqueous solution of Triton X-100 (0.2%) as an eluent. The surfactant was used to solubilize polymer nanocapsules in water. Figure S1 shows the progression of a mixture through the column and excellent separation of capsules from free dye. Separation of nanocapsules from non-entrapped Procion Red (MW 615) is shown for simplicity of visual observation. Pyranine (MW 524) and bromophenol blue (MW 670) exhibit similar separation characteristics. To ensure removal of traces of non-entrapped dye, nanocapsules fraction was run through the column again.

Additional separations of nanocapsules on a Sepharose-4B column showed no free dye. To investigate long-term retention of encapsulated molecules, samples of purified nanocapsules were run through the column after standing in ambient temperature for two weeks. No free dye was observed.

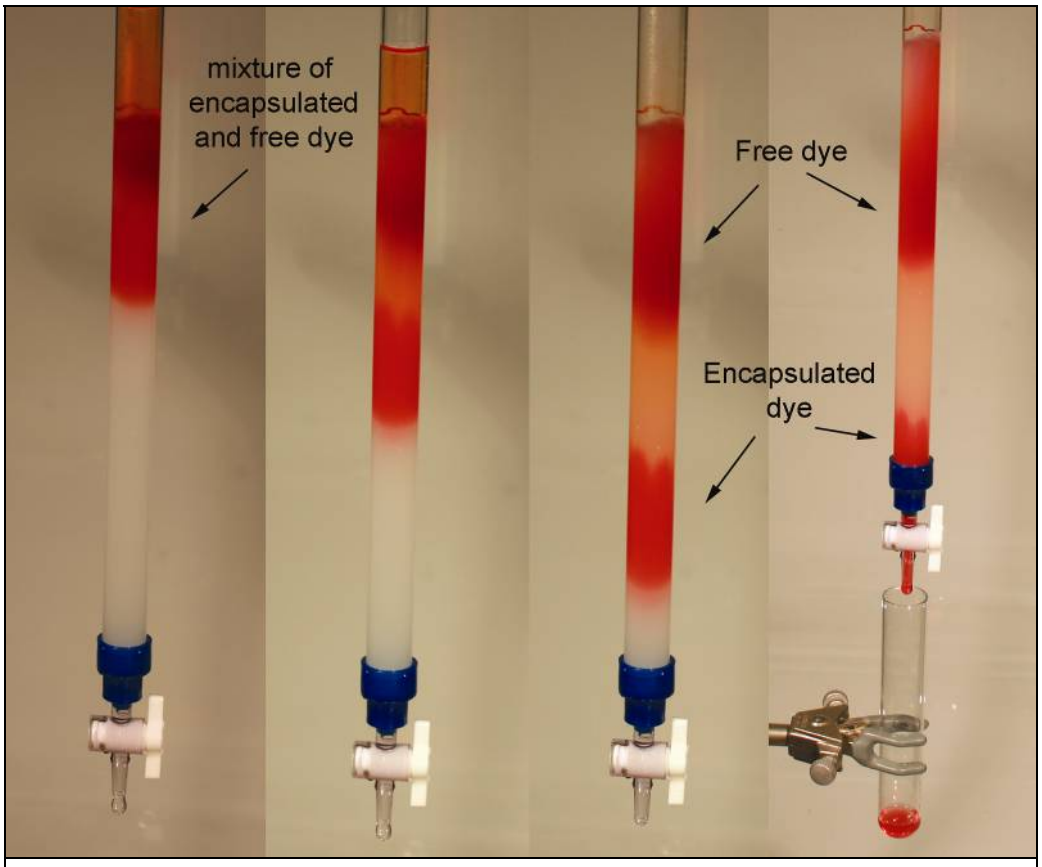

Figure S1. Size-exclusion separation of aqueous suspension of nanocapsules from non-entrapped Procion Red dye.

Spectral properties of free pyranine and bromophenol blue (BPB).

Figure S2 shows excitation (Fig. S2A) and emission (Fig. S2B) spectra of free pyranine in water mixed with NaOH solutions of different concentrations. Response of free BPB to increasing concentrations of acid is shown on Figure S3. Absorption maximum at 430 nm is clearly visible in free BPB and is masked in encapsulated BPB due to light scattering from nanocapsules.

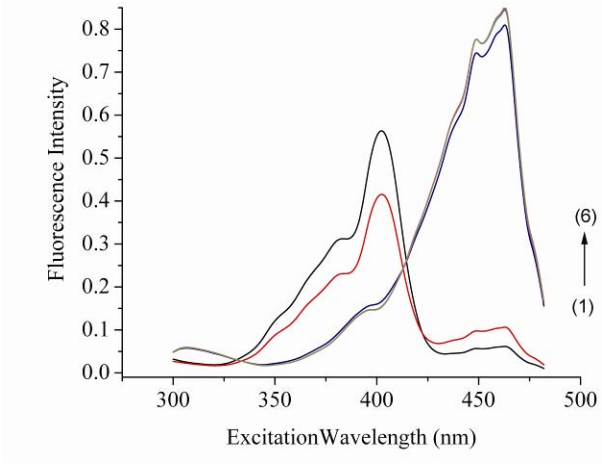

Figure S2A. Excitation (λ_{em}=510 nm) spectra of aqueous solution of pyranine (1) in the presence of (2-6) 0, 0.001, 0.01, 0.1 and 1 M NaOH

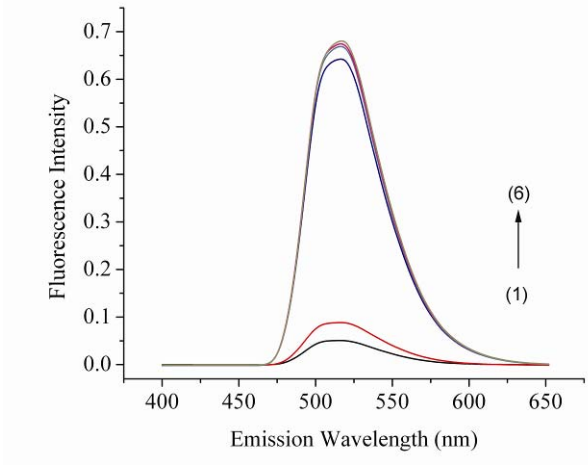

Figure S2B. Emission (λ_{ex}=450 nm) spectra of aqueous solution of pyranine (1) in the presence of (2-6) 0, 0.001, 0.01, 0.1 and 1 M NaOH

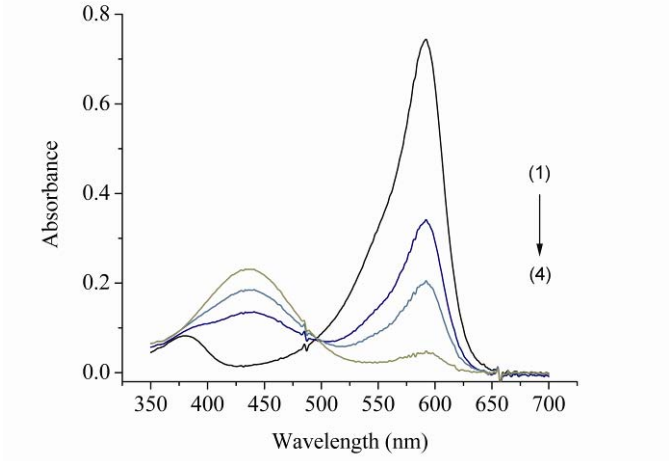

Figure S3. Absorption spectra of aqueous solution of bromophenol blue (1) in the presence of (2-4) 0.4, 1 and 2 mM HCl