Ship-in-a-bottle entrapment of molecules in porous nanocapsules

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Chemicals

All solvents used were HPLC grade. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipids, Inc. as a dry powder. P-divinylbenzene (DVB), tert-Butylstyrene (tBuSt), p-tolyl aldehyde and pyrrole were purchased from Sigma-Aldrich. Methanol, chloroform and methylene chloride were purchased from Fisher Scientific and used as received. DVB and tBuSt were passed through an alumina column to remove the inhibitor shortly before the synthesis. Pyrrole was distilled prior to the use.

Synthesis of nanocapsules

Polymer hollow nanocapsules were prepared by the following procedure: tert-Butylstyrene (24 μL, 1.33×10^{-5} mol), p-divinylbenzene (19 μL, 1.33×10^{-5} mol), and 2,2-dimethoxy-2-phenylacetophenone, DMPA, (UV initiator; 0.33 mg, 1.3×10^{-6} mol) were added to a solution of DMPC (60 mg, 8.85×10^{-5} mol) in $CHCl_3$. The monomers were purified on a column of neutral alumina prior to the addition. The $CHCl_3$ was evaporated using a stream of purified argon to form a lipid-monomer film on the wall of a culture tube. The film was further dried under vacuum for 30 min to remove traces of $CHCl_3$. The dried film was hydrated with deionized water to give a dispersion of multilamellar vesicles, which was then extruded at 35 °C through a polycarbonate Nucleopore track-etch membrane (Whatman) with 0.2um pore size using a Lipex stainless steel extruder (Northern Lipids). Oxygen was removed by passing purified argon through the solution. The sample was irradiated (λ=254 nm) in a photochemical reactor equipped with a stirrer (10 lamps of 32 W each; 10-cm distance between the lamps and the sample) for 60 min. Methanol (10 mL) was added, and the precipitate was washed 3-5 times with methanol.

Synthesis of H_2TTP

Meso-tetra-p-tolylporphyrin was prepared by Adler method in propionic acid.[7a]

Synthesis of encapsulated H_2TTP

A suspension of nanocapsules in methanol was centrifuged at approx. 2,000 g for 5 minutes. Methanol was decanted, and the culture tube with the compact precipitate was covered with Parafilm™ and kept upside down for several hours to drain excess methanol without drying the capsules. This method was used to allow rapid dispersion of nanocapsules in acetic acid, and it is not required for other solvents. In our experience, nanocapsules freeze-dried from benzene can be readily dispersed in a broad range of solvents. Glacial acetic acid (2 mL), pyrrole (40 μL) and p-tolylaldehyde (68 μL) were added to the precipitate, and the reaction mixture was refluxed for 30-40 min. The reaction mixture was cooled, methanol (10 mL) was added, and the precipitate was washed with methanol 5-6 times. Non-entrapped H_2TTP was separated by washing (3-5 times) with 5:3 methylene chloride – methanol mixture until supernatant became colorless. Aliquots of supernatant were analyzed with UV-vis spectroscopy.

Metallation of H2TTP

Metallation of free and entrapped H_2TTP with $Zn(OAc)_2$ were done by stirring the corresponding porphyrin in large excess of $Zn(OAc)_2$ in methylene chloride at room temperature.[7b] MnTTPCl was prepared by refluxing the freebase porphyrin with excess of $MnCl_2 \cdot 4H_2O$ in a mixture of glacial acetic acid and acetic anhydride.[7d] FeTTP was prepared by refluxing free or entrapped H_2TTP with excess of $Fe(OAc)_2$ in the glacial acetic acid.[7b] Purification of free metallo - porphyrins was done by flash chromatography on silica - gel using methylene chloride as eluent.

Transmission Electron Microscopy (TEM)

TEM images were acquired on a JEOL JEM1200EXII microscope. Samples were negatively stained with phosphotungstic acid (pH 5.9) on a carbon grid.

UV/VIS Spectroscopy

UV/VIS spectra were recorded on Agilent Technologies 8453 UV spectrophotometer in quartz cuvette in the range 200-800 nm.

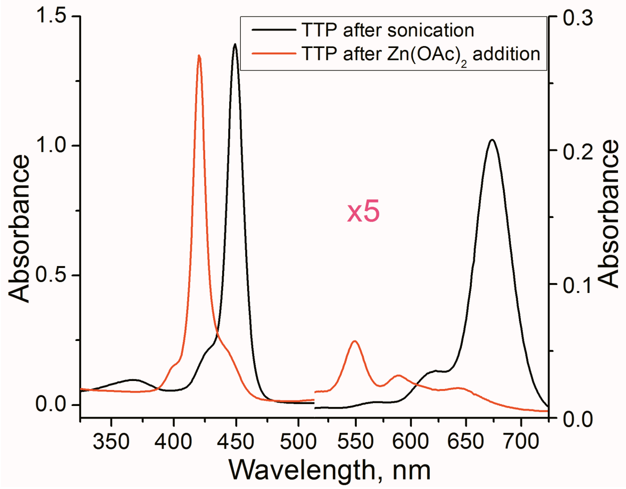

Fig. S1 Change of porphyrin UV spectra upon sonication. [9]

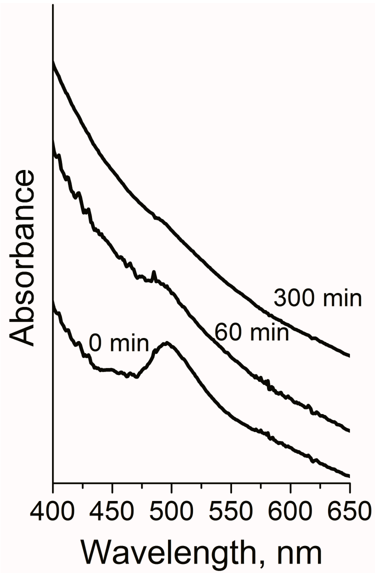

Fig. S2 Evolution of UV spectra of the entrapped porphyrin upon reaction with $MnCl_2 \cdot 4H_2O$; Spectra were recorded in methylene chloride after repeated washing with 5:3 methylene chloride – methanol mixture.

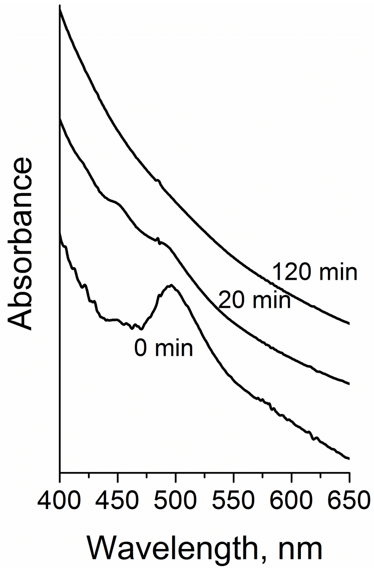

Fig S3 Evolution of UV spectra of the entrapped porphyrin upon reaction with $Fe(OAc)_2$; Spectra were recorded in methylene chloride after repeated washing with 5:3 methylene chloride – methanol mixture.

FTIR Spectroscopy

IR-spectra of freeze-dried samples in KBr pellets were recorded on Thermo Nicolet 380 FTIR spectrometer.

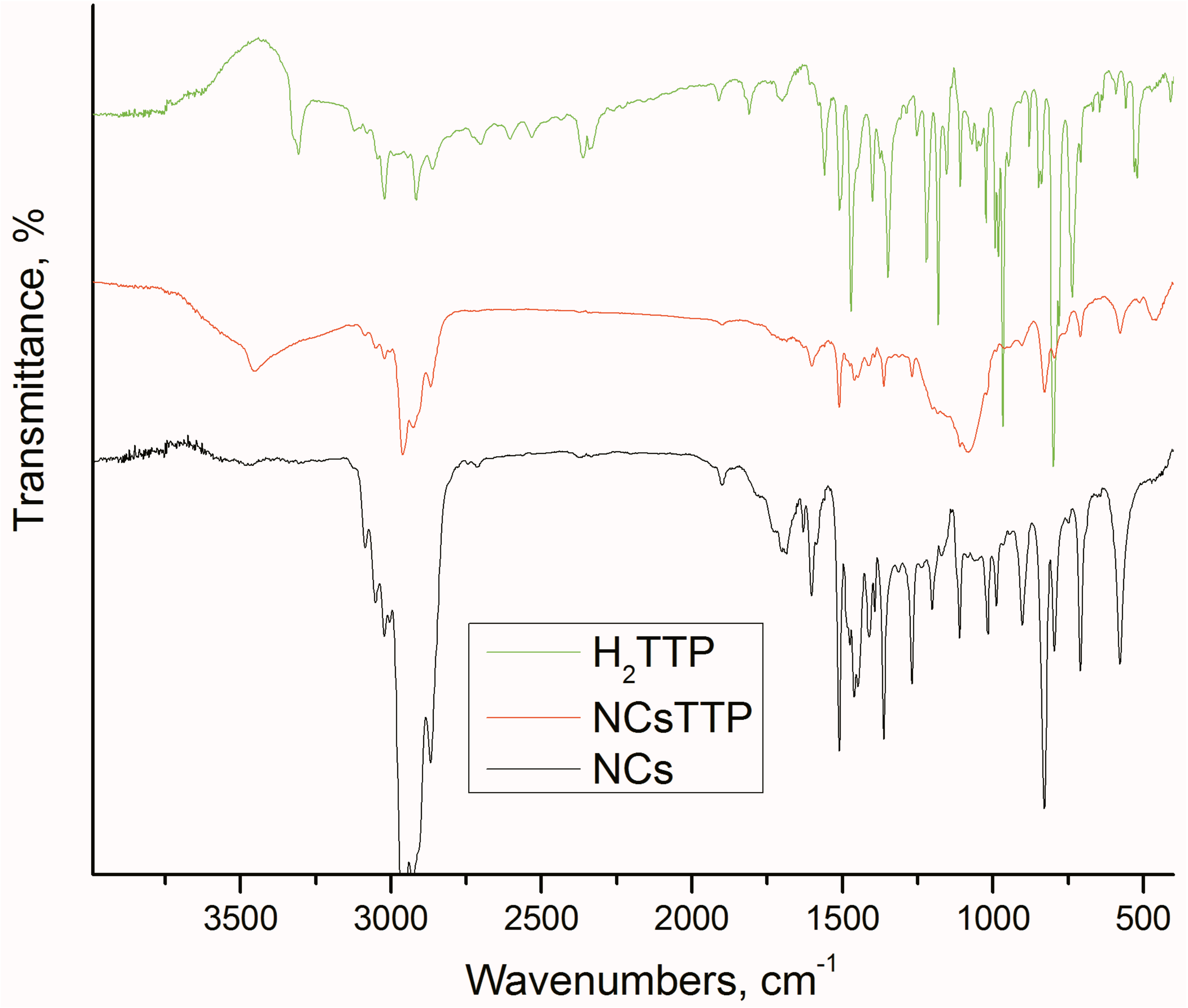

Fig S4. IR-spectra of NCs, NCsTTP and H_2TTP

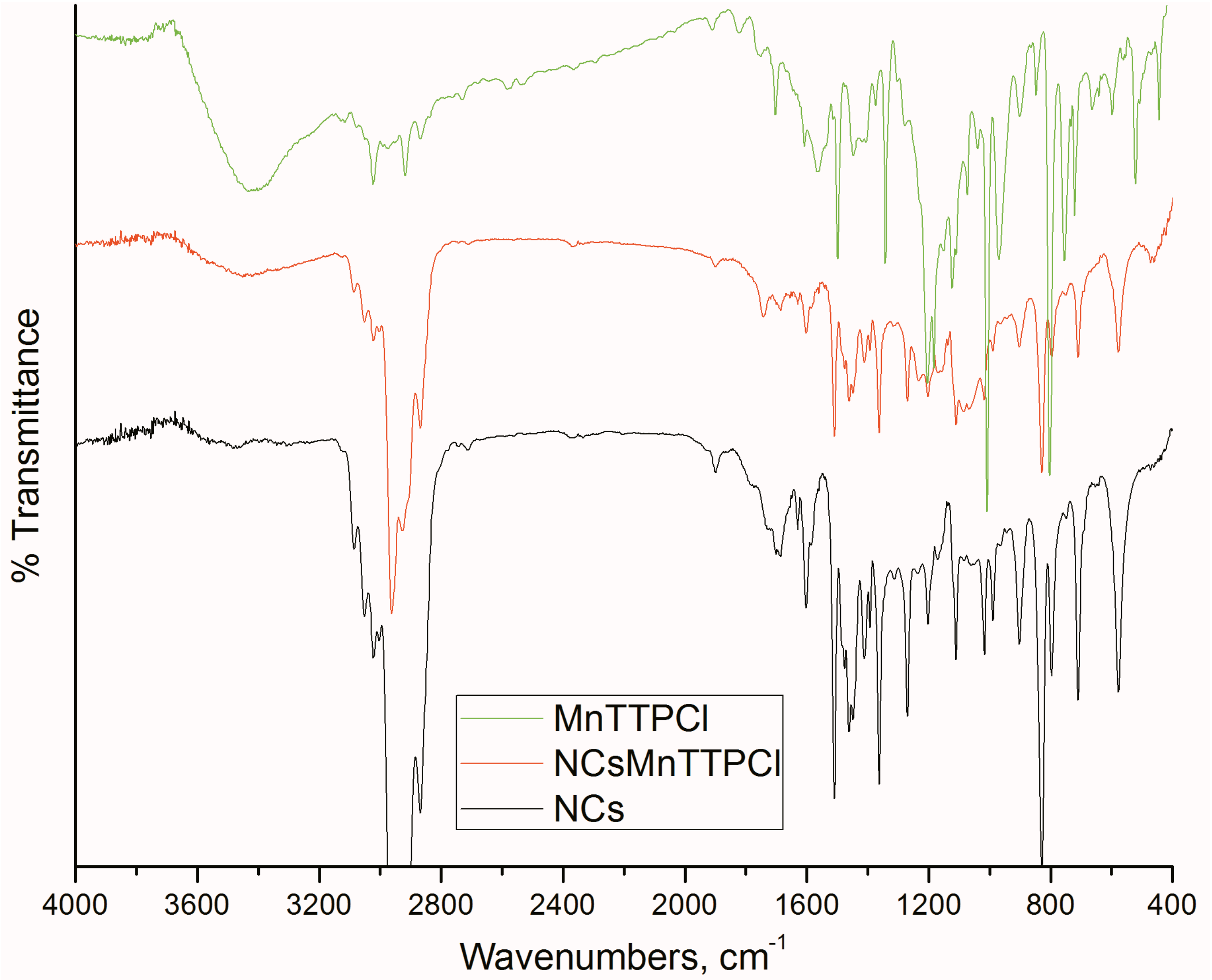

Fig S5. IR-spectra of NCs, NCsMnTTPCl and MnTTPCl

Fluorescence measurements

Fluorescence measurements were performed in quartz cuvette with SLM 8000 spectrometer, which was upgraded and interfaced to computer by OLIS, Inc. Emission spectra were recorded in the 500 to 800 nm region with λ_{ex} = 419 nm.

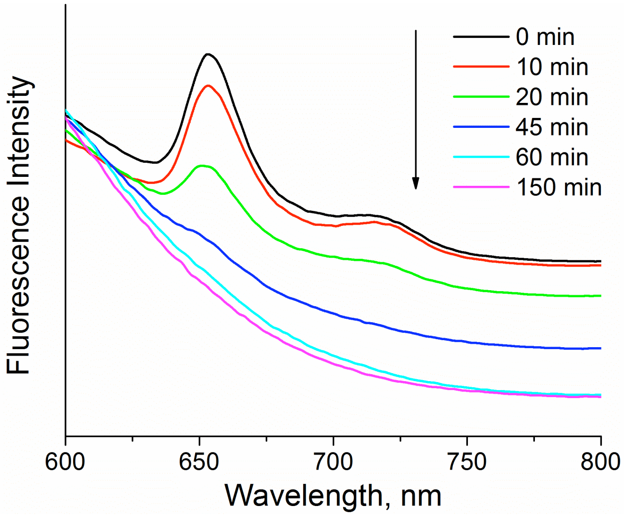

Fig S6 Evolution of fluorescence spectra of the entrapped porphyrin upon reaction with $MnCl_2 \cdot 4H_2O$; Spectra were recorded in methylene chloride after repeated washing with 5:3 methylene chloride – methanol mixture.

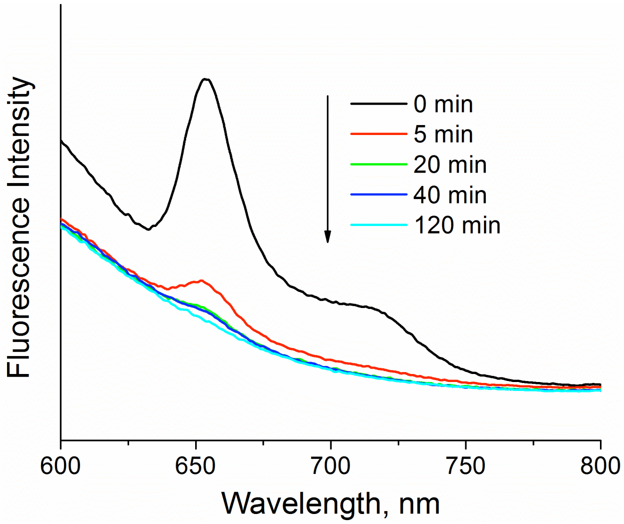

Fig S7 Evolution of fluorescence spectra of the entrapped porphyrin upon reaction with $Fe(OAc)_2$; Spectra were recorded in methylene chloride after repeated washing with 5:3 methylene chloride – methanol mixture.

Atomic absorption spectroscopy (AA)

Atomic Absorption measurements were done on Varian 220 FS G spectrometer using corresponding Varian hollow cathode lamps. Lamps output was monitored to ensure stability and maximized signal following a minimal 30 min warm-up.