Synergistic Self-Assembly of Templating Scaffolds with Building Blocks for Directed Synthesis of Organic Nanomaterials

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Chemicals. Hexadecyltrimethylammonium bromide (CTAB), Sodium dodecylbenzenesulfonate (SDBS), tert-butyl methacrylate (t-BMA), butyl methacrylate (BMA), tert-butyl styrene (t-BST), ethylene glycol dimethacrylate (EGDMA), p-divinylbenzene (DVB) were purchased from Sigma-Aldrich. BMA and EGDMA were passed through alumina column to remove the inhibitor shortly before sample preparation. The photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (DPA), manufactured by Sigma-Aldrich, was used without any additional purification. The pH indicator dye Nile Blue A (NB A), also from Sigma-Aldrich, was dissolved in tris buffer (10mM, pH 7.4) and filtered before use. The solvents and other chemicals used in this study were HPLC and ACS reagent grade, respectively and were used as received.

Surfactants mixture. Samples were prepared by first making stock solutions of cationic and anionic surfactant at the desired concentration in distilled deionized water. Stock solutions of each pure surfactant were equilibrated at temperature 40 ºC. Samples were prepared by mixing the stock solutions at the desired ratio, at the room temperature, and compositions reported are on a weight percent basis. After brief vortex mixing, the solutions were not subjected to any type of mechanical agitation.

Concurrent loading of monomers into surfactant vesicles. To prepare stock solutions, SDBS (100 mg) and CTAB (100 mg) were mixed in separate vials with t-BMA (32 μL, 0.193 mmol), BMA (32 μL, 0.199 mmol), EGDMA (32 μL, 0.166 mmol) and initiator 2,2-dimethoxy-2-phenyl-acetophenone (3 mg, 0.01 mmol). Each mixture was hydrated in 10 mL of deionized water. Each stock solution was equilibrated at temperature 40 °C during 30 min and filtered through a 0.2 μm filter prior to sample preparation. Samples were prepared by mixing the stock solutions at 15:85 volume ratios corresponding to 15:85 weight ratio of SDBS to CTAB and were additionally equilibrated at temperature 30 ºC during 1hour. After brief vortexing, the solutions were not subjected to any type of mechanical agitation.

Synthesis of nanocapsules. The sample prepared as described above was irradiated for 1.5 hours with UV light (λ=254 nm) in a photochemical reactor (10 lamps, 32W each; the distance between the lamps and the sample was 10 cm) using quartz tube with path length of light of approximately 3 mm. Following the polymerization, a solution of NaCl (0.02 mL of 3 N) in methanol (10 mL) was added to the reaction mixture to precipitate the nanocapsules. The nanocapsules were separated from the reaction mixture and purified by repeated centrifugation and resuspension steps using methanol (3 drops of NaCl (3 M, 0.02 mL) were added to aid precipitation), water-methanol mixture and water as washing solutions.

Dynamic Light Scattering (DLS). Hydrodynamic diameter and polydispersity index (PDI) measurements were performed on a Malvern Nano-ZS zetasizer (Malvern Instruments Ltd., Worcestershire, U.K.). The Helium-Neon laser, 4mW, operated at 633 nm, with the scatter angle fixed at 173°, and the temperature at 25 °C. 80 µL samples were placed into disposable cuvettes without dilution (70 µL, 8.5 mm center height Brand UV-Cuvette micro). Each data point was an average of 10 scans.

High Performance Liquid Chromatography (HPLC). 50 µL of sample was carefully taken from vial and mixed with 950 µL of methanol to lyze vesicles. 20 µL of the resulting mixture sample was injected into a split-mode injector. Analytical HPLC was performed with a Waters 600 pump and Waters 2487 dual wavelength UV-vis detector. The detection wavelengths used were 220 and 230 nm. The column was a Nova-Pak C18, 3.9 mm diameter × 150 mm length. HPLC grade methanol was used as the mobile phase. The flow rate was 0.5 mL/min and retention times were 5.86, 6.37 and 6.53 min (for EGDMA, BMA and t-BMA respectively). Samples and concentration standards were run at least 5 times for each measurement, and the data were averaged. When necessary, the experimental samples were diluted further so that the signals would fall within a range of concentrations where the detector response was highly reproducible and the relationship between area and concentration could be modeled with a simple polynomial. Standard samples of known concentrations of t-BMA, BMA and EGDMA and their mixtures were prepared by serial dilution of stock solutions in methanol and were analyzed by HPLC as described above. A calibration curve was produced by fitting HPLC data from monomers solutions in methanol at different concentrations via the least-squares fitting method, and was used to interpolate between the points.

Small-Angle Neutron Scattering (SANS). SANS measurements were performed with the CG-3 Bio-SANS instrument at the High Flux Isotope Reactor (HFIR) facility of Oak Ridge National Laboratory.[1] Hellma cylindrical quartz cells (Model# 120-*QS*) of 1 mm thickness were used to hold the liquid samples. Three different instrument configurations were employed to collect data over the range of scattering vectors, 0.0015 $Å^{-1}$ < Q < 0.357 $Å^{-1}$, employing sample-to-detector distances of 2.529 m, 6.829 m at a fixed neutron wavelength (λ) of 6 Å and 15.329 m

at a fixed wavelength of 12 Å. $Q=(4\pi/\lambda)\sin\vartheta$ and 2ϑ is the scattering angle. In each case the center of the area detector (1 m x 1 m GE-Reuter Stokes Tube Detector)[2] was offset by 350 mm from the beam. The instrument resolution was defined using circular aperture diameters of 40 mm for source and 14 mm for sample separated by distances: 7309 mm for 0.02 $Å^{-1} < Q < 0.35$ $Å^{-1}$, 9332 mm for 0.007 $Å^{-1} < Q < 0.1$ $Å^{-1}$ and 17430 mm for 0.0015 $Å^{-1} < Q < 0.03$ $Å^{-1}$. The relative wavelength spread $\Delta\lambda/\lambda$ was set to 0.15. The scattering intensity profiles $I(Q)$ versus Q, were obtained by azimuthally averaging the processed 2D images, which were normalized to incident beam monitor counts, and corrected for detector dark current, pixel sensitivity, solid angle and background from the quartz cell.

Small-Angle X-Ray Scattering (SAXS). SAXS measurements were performed at Sector 5 (DND-CAT) of the Advanced Photon Source at Argonne National Laboratory. The energy was set at 17.0 keV. Simultaneous SAXS and WAXS (wide-angle) data were collected using two area detectors, with a Q range from 0.01 $Å^{-1}$ to 3.0 $Å^{-1}$. 1.5 mm diameter quartz tubes were used in a "vacuum-flow" configuration. The samples were exposed to x-rays only while flowing at 4 µl/s in order to reduce x-ray damage. Typically, 8 frames were taken, each with 6 seconds of integration time, and then combined. Data reduction was performed by the beamline software package.

Correlograms from DLS measurements.

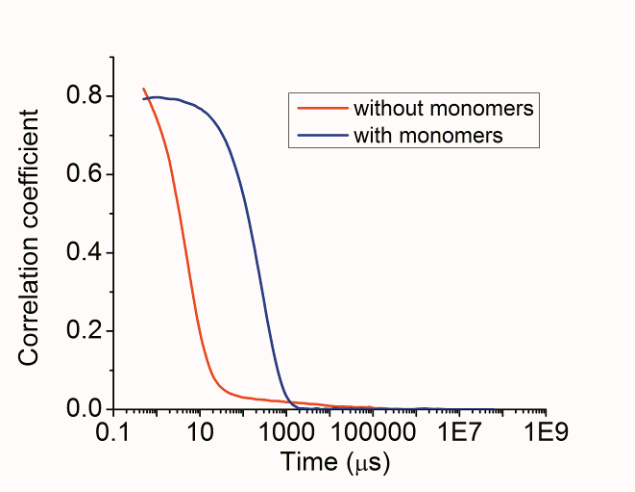


Figure S1. DLS Correlogram from the sample prepared in the presence of monomers is characteristic of uniform monomodal distribution (blue curve). In the absence of monomers, the correlogram corresponds to a non-uniform multimodal distribution.

DLS data before and after the polymerization

DLS analysis was performed on vesicle/monomer assemblies before and after the polymerization. Following the polymerization, the sample was treated with an aqueous solution of Triton X-100 (TX100) to remove the outer layer of CTAB and SDBS while keeping nanocapsules dispersed in water. Aggregate data are shown on Figure S2 for comparison. Average size and size distribution remained unchanged throughout this process, indicating no change in morphology induced by the polymerization of monomers in the hydrophobic interior of the surfactant bilayer. After the treatment with TX100, the size and size distribution of particles corresponding to the size of surfactant/monomer vesicles remained the same, suggesting that nanocapsules preserve their shape and size. This observation is in agreement with the permeability studies described below and with TEM and SEM images of dried nanocapsules shown on Figure 1 of main manuscript. The relative proportion of smaller particles increased, consistent with higher concentration of surfactants in the mixture as a result of addition of TX100.

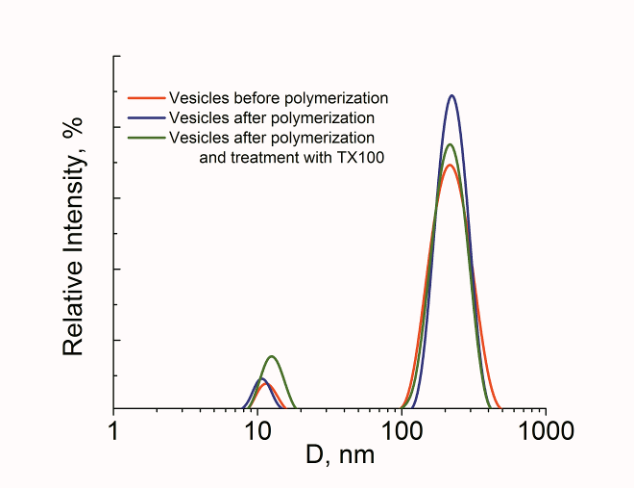


Figure S2. DLS data on vesicle/monomer assemblies before and after the polymerization.

Permeability test

Solution of NBA (1.5×10^{-4} M) was used for hydration of surfactant/monomer mixture instead of water. All other steps were performed as described above. After the polymerization, the sample was treated with methanol to remove surfactant scaffold, and the gel-like precipitate of nanocapsules was washed several times with methanol to remove unentrapped and release dyes. To perform the washing steps, we precipitated nanocapsules with the help of a brief centrifugation (~2000g; 1-2 min). The supernatant was decanted, and the precipitate was

redispersed in fresh methanol using mild vortexing. The washing steps were repeated 5-10 times. Dyes that are larger than the pore size in the polymer shell of nanocapsules remained entrapped during this washing procedure (Figure S3A) and resulted in colored precipitated nanocapsules (Figure 1E in the main manuscript). Dyes that are smaller than the pores in the shells of nanocapsules escape during the washing procedure (Figure S3B) and yield colorless nanocapsules. The permeability test offers an upper limit on the pore size – the smallest cross-section of molecules that remain entrapped in nanocapsules.

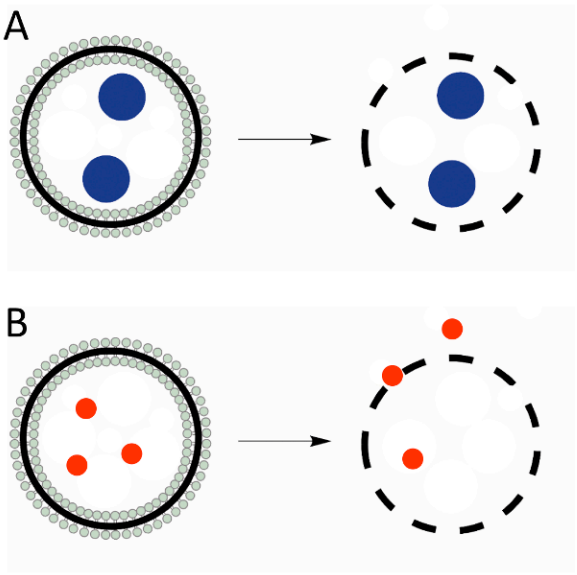


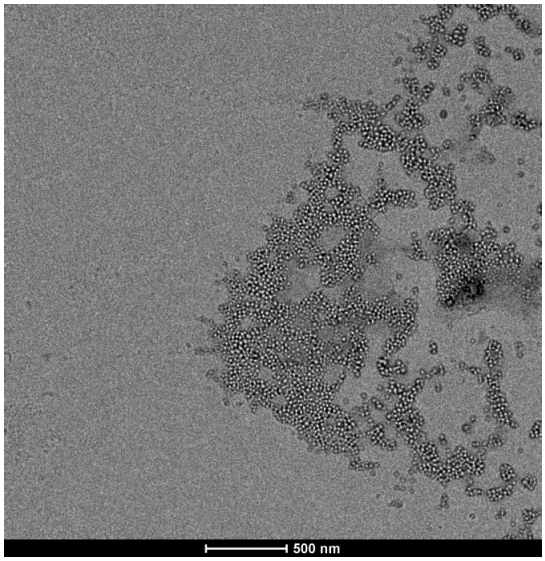
Figure S3. Overview of the permeability test. Vesicles with monomers in the bilayer are prepared using an aqueous solution of a dye that serves as a size probe. Water-soluble dyes are entrapped in the aqueous core of the vesicles. After the polymerization, samples were washed with methanol to remove the surfactant scaffold. During this step, dyes that are smaller than the pores in the polymer shell escape from the nanocapsules (B). Dyes larger than the pores remain entrapped (A).

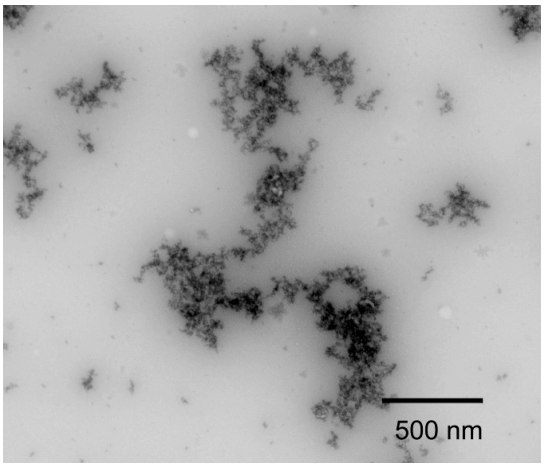
TEM and SEM studies

Electron microscopy images were obtained with a FEI Inspect F50 STEM scanning electron microscope (Hillsboro, OR) at a working voltage of 30 kV. To prepare the sample for TEM analysis, a drop of sample was carefully placed on a 200-mesh carbon grid and excess sample was wiped away with filter paper. Then a drop of 2% Uranyl Acetate was added to the grid to negatively stain the sample. After 2 min, the excess liquid was wiped off. To prepare the sample for SEM analysis, a drop of sample was placed on SEM pin stub specimen mount covered with double coated carbon conductive tabs and dried under vacuum. The studied samples were coated with a 7nm gold-palladium (60:40) layer using EMS 590 X sputter (Hatfield, PA).

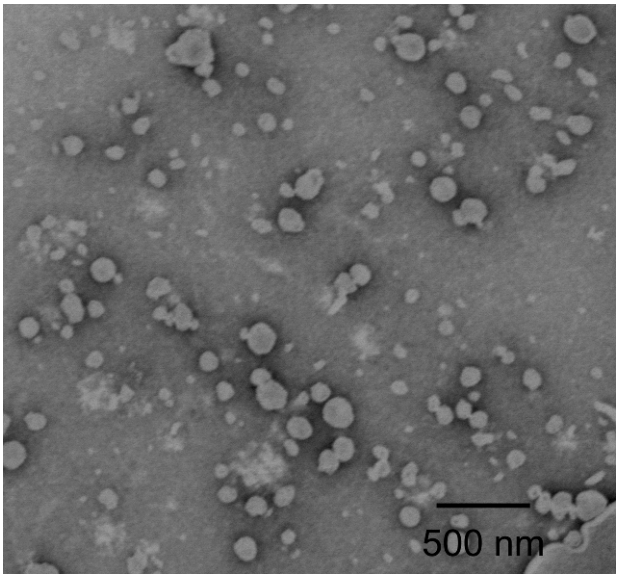
TEM images of surfactant assemblies without monomers (Figure S4A) show predominantly small structures consistent with micelles. In the presence of monomers, TEM images reveal circular structures (Figure S4B) with the same average size as determined by DLS.

A





B



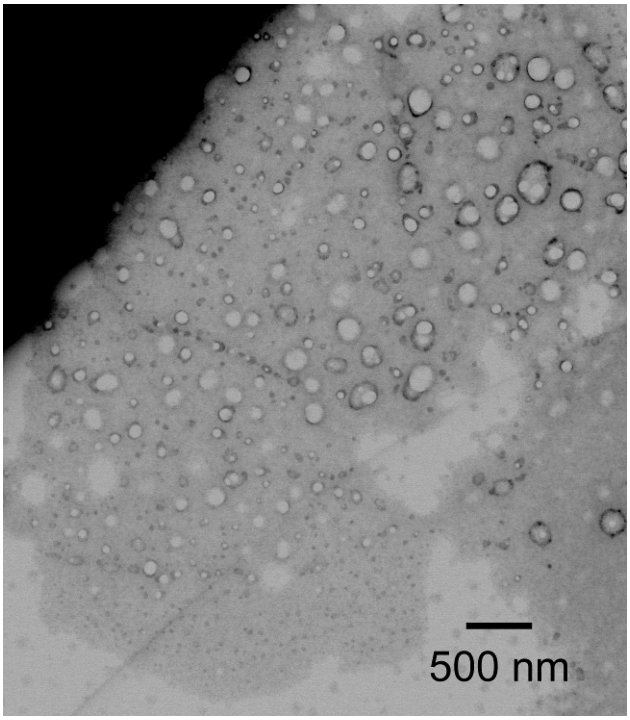


Figure S4. TEM of surfactant assemblies without monomers (A) and with monomers (B).

References

1. G. W. Lynn, W. Heller, V. Urban, G. D. Wignall, K. Weiss and D. A. A. Myles, *Physica B: Condensed Matter,* 2006*,* **385-386**, 880-882**.**

2. G. D. Wignall, K. C. Littrell, W. T. Heller, Y. B. Melnichenko, K. M. Bailey, G. W. Lynn, D. A. Myles, V. S. Urban, M. V. Buchanan, D. L. Selby and P. D. Butler, *J. Appl. Crystallogr.,* 2012*,* 990-998