Electronic Supplementary Information (ESI)

Layer-by-Layer Preparation of Polyelectrolyte Multilayer Nanocapsules via Crystallized Miniemulsions

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1. Experimental Section

1.1. Materials

Poly(acrylic acid) (PAA) in the form of sodium salt (M_W^{GPC} = 5100), poly(allylamine hydrochloride) (PAH; M_W^{GPC} = 17500), *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Tetrahydrofuran (THF) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were obtained from Thermo Fisher Scientific. 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99%) and *n*-docosane (99%) were purchased from Acros Organics. Cetyltrimethylammonium bromide (CTAB) was purchased from MP Biomedicals™. All of these commercial chemicals were used as obtained. Deionized water, obtained from a Thermo Scientific Barnstead Nanopure ultra-pure water system, was used for the preparation of all aqueous solutions or water-based dispersions. Amicon Ultra-4 regenerated cellulose centrifugal filter units with molecular weight cut-off (MWCO) of 30 kDa were used for centrifugal purification cycles.

1.2. Instruments

Dynamic light scattering (DLS) was used to determine hydrodynamic size and size distribution of nanoparticles (NPs) and nanocapsules (NCs). The measurements were performed on Zetasizer Nano ZS90 (Malvern Instruments Ltd.) with a 4 mM 633 nm He-Ne laser as the light source. The temperature was maintained at room temperature, and the measuring angle was 90°. The correlation decay functions were analyzed by cumulants method to obtain size distribution. All measurements were repeated at least five times.

Transmission electron microscopy (TEM) images were obtained with a JEOL 2010 microscope. TEM samples were prepared using 400 mesh carbon-coated copper grids. TEM grids were dip coated with the aqueous solutions of NPs or NCs with DLS count rate of ~250 kcps. Water was then completely removed from surface of grids under vacuum and no staining was applied for TEM samples.

FT-IR spectra were obtained on a Bruker Tensor 27 system using attenuated total reflectance (ATR) sampling accessories. Differential scanning calorimetry (DSC) analysis was conducted under nitrogen atmosphere by using TA Instruments Q200 equipped with a RCS-90 cooling device, with the temperature range of 10-60 °C and a heating or cooling rate of 5 °C/min.

Sonication was performed using a Branson 250 Analog Sonifier. For miniemulsion preparation, a continuous mode of sonication with power level of 2 was used for 30 minutes. For LBL process, a pulse mode of sonication using the minimum power level of 1 with 10% cycle time was employed during the polyeletrolyte deposition step.

1.3. Preparation of crystallized miniemulsion NPs

Monomer surfactant and crosslinker surfactant were synthesized at first according to the method reported by Li et. al.[1] Then, crystallized miniemulsion NPs were prepared. In a representative miniemulsion formulation, monomer surfactant (30 mg), crosslinker surfactant (30 mg), *n*-docosane (70 mg), DMPA (1 mg), and water (3.5 mL) were added to a 10-mL vial. The mixture was heated up to 50 °C in an oil bath. *n*-Docosane melted upon heating and two

immiscible phases were observed. The biphasic mixture was ultrasonicated for 30 min, with a constant oil bath temperature of 50 °C. The resulting transparent miniemulsion was allowed to cool down to room temperature, and subsequently bubbled with nitrogen for 15 min, followed by UV irradiation (λ_{max} = 365 nm) for 60 min to covalently stabilize the surfactant layer. Then the solution was dialyzed against ultra-pure water for 3 days.

1.4. PAA/PAH coating over crystallized miniemulsion NPs

The polyelectrolytes were deposited using crystallized miniemulsion NPs as the core substrate by LBL technique. The stock aqueous solutions of PAA and PAH were prepared with the concentration of 10 mM with respect to their monomeric units. An aqueous solution of template NPs was added to a glass vial. While the solution in the vial was sonicated mildly at around room temperature, the PAA solution was added to the vial in an excess amount. The sonication was allowed to continue for 30 min with temperature maintained at room temperature to avoid the melting of docosane cores. Unabsorbed PAA was removed by three washing cycles with ultra-pure water using a centrifugal filter unit (MWCO = 30 kDa, 4000 rpm) in order to obtain NPs/PAA. Subsequently, PAH was deposited in the same way and followed by the same washing cycles as the case of PAA, except that NPs/PAA was used as template. Multilayer of PAA/PAH alternating assemblies were obtained by repeating the alternative deposition cycle. The hydrodynamic size, zeta potential, and colloidal stability was monitored at each step through DLS measurements.

1.5. Chemical crosslinking of the deposited PAA/PAH multilayers

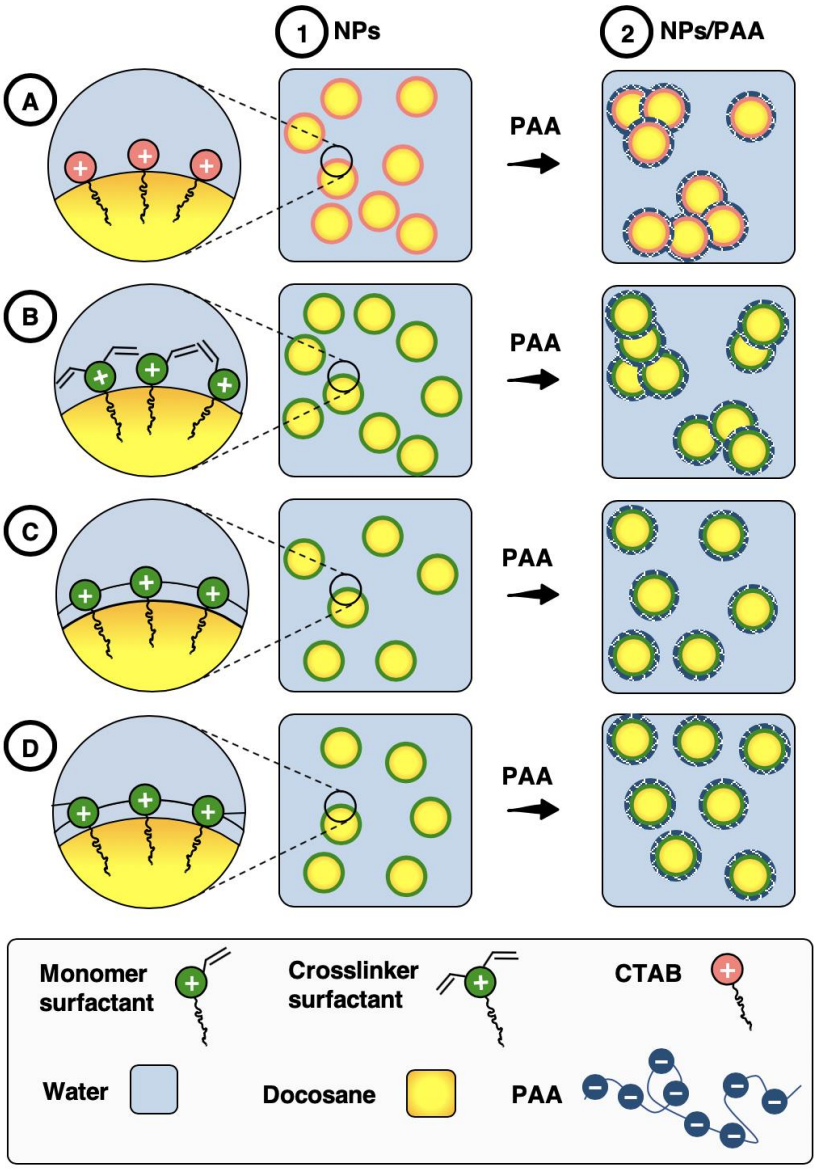
The carboxylic acid group of PAA and amine group of PAH were utilized to conduct chemical crosslinking. Hence, 200 μL solution of NPs/$[PAA/PAH]_3$ was treated for 1 h with EDC (20 μL, 400 mM) and NHS (20 μL, 100 mM) in aqueous solution to trigger crosslinking reaction. Crosslinking process was monitored by measuring zeta potential of the reaction system with DLS and the zeta potential value of the NPs/$[PAA/PAH]_3$ decreased from +54 ± 2 mV to +34 ± 3 mV. The final reaction mixture was washed by three washing cycles with ultra-pure water using a centrifugal filter unit to give a purified solution of shell-crosslinked NPs/$[PAA/PAH]_3$.

1.6. Preparation of nanocapsules (NCs)

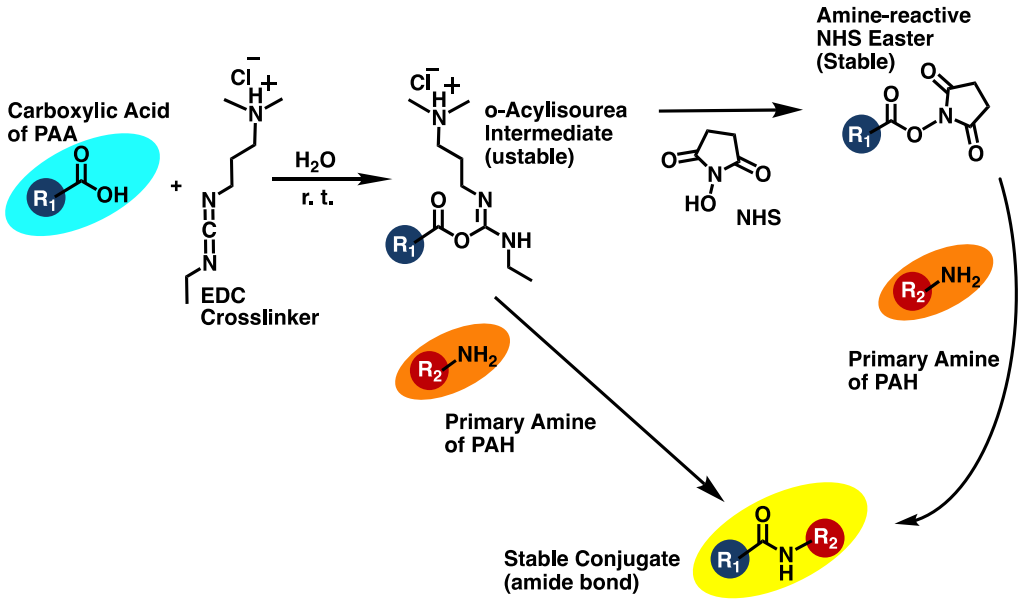
For the preparation of NCs with crosslinked PEM shells, the *n*-docosane cores of shell-crosslinked PAA/PAH-coated NPs (obtained from ESI Sec. 1.5) were dissolved by dialysis using dialysis membrane (MWCO = 30 kDa) against THF for 3 days at room temperature. The resulting NCs were dispersed in water by further dialysis against ultra-pure water for 3 days at room temperature.

For the preparation of NCs with non-crosslinked PEM shells, the aforementioned procedure was also used, but PAA/PAH-coated NPs with non-crosslinked PEM shells (obtained from ESI Sec. 1.4) were used as the precursor NPs.

2. Supplementary Schemes and Figures



Scheme S1. Colloidal stability of crystallized miniemulsion NPs for surface deposition of PAA. These NPs are stabilized by a) CTAB, b) monomer surfactant, c) polymerized monomer surfactant, and d) crosslinked monomer/crosslinker surfactants.



Scheme S2. The crosslinking reaction between PAA (R_1-COOH) and PAH (R_2-NH_2) to form amide bond.[2]

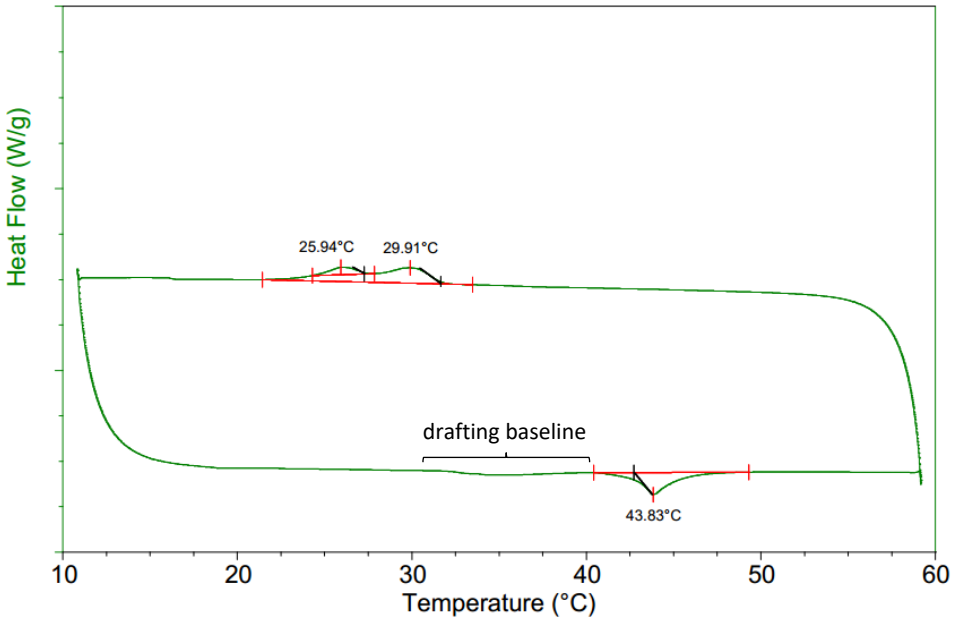


Fig. S1. DSC analysis of the miniemulsion system (prior to covalent stabilization of the surfactant monolayers). The crystallinity of the *n*-docosane-based dispersed phase at room temperature was verified, although the bimodal crystallization peaks in cooling cycle and the drafting baseline in heating cycle suggest the possibility of the presence of different types of crystalline domains.

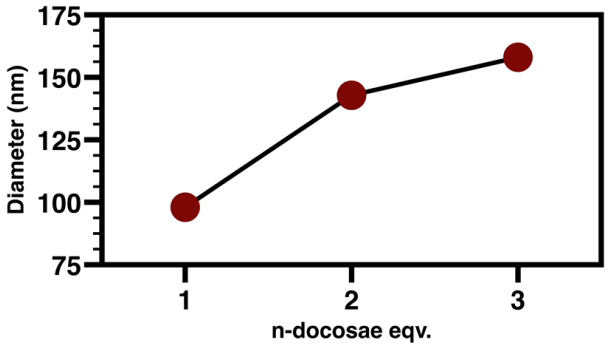


Fig. S2. The variation of volume-average hydrodynamic diameter ($D_{h,V}$) of the crystallized miniemulsion NPs (prior to covalent stabilization of surfactant monolayers, at room temperature) with the increase of *n*-docosane amount in the miniemulsion formulation. The amount of *n*-docosane in the representative formulation described in ESI Sec. 1.3 was defined as one equivalent amount (there was no other formulation difference, except *n*-docosane amount).

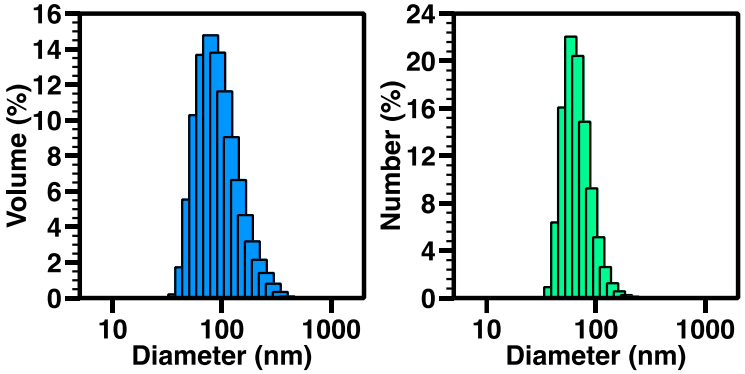


Fig. S3. DLS results of crystallized miniemulsion NPs (after covalent stabilization of surfactant monolayers) obtained from the representative miniemulsion formulation described in ESI Sec. 1.3: a) volume profile, and b) number profile. The zeta potential of these NPs was +63 mV.

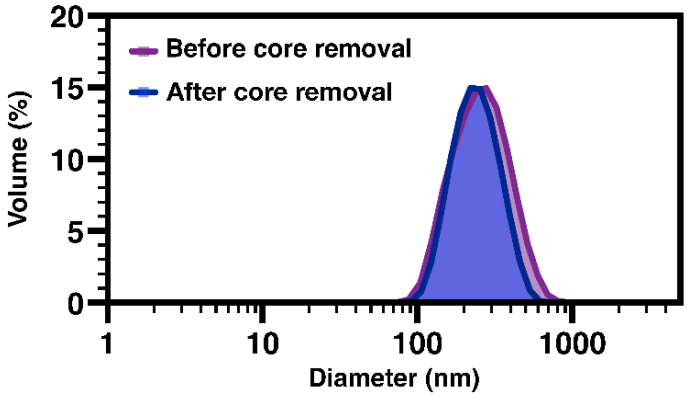


Fig. S4. Volume profiles of hydrodynamic size distribution of shell-crosslinked NPs/$[PAA/PAH]_3$ (before core removal) and the corresponding NCs (after core removal through dialysis) in water.

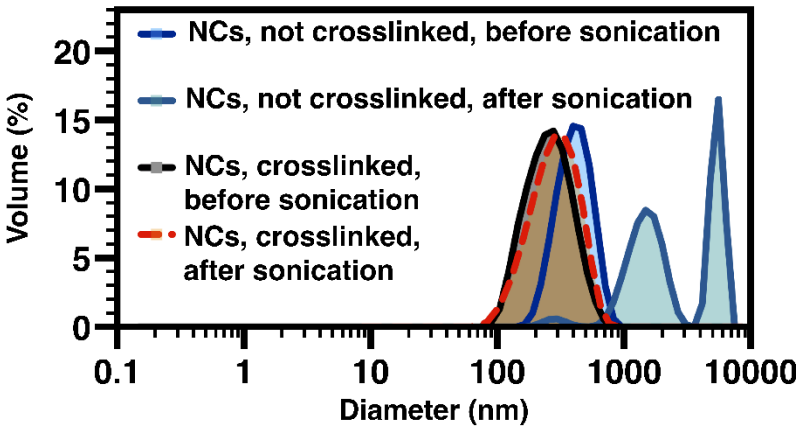


Fig. S5. Volume profiles of hydrodynamic size distribution of NCs with crosslinked and non-crosslinked PEM shells in water before and after sonication treatment for 1 min using a Branson 250 Analog Sonifier at minimum power level of 1.

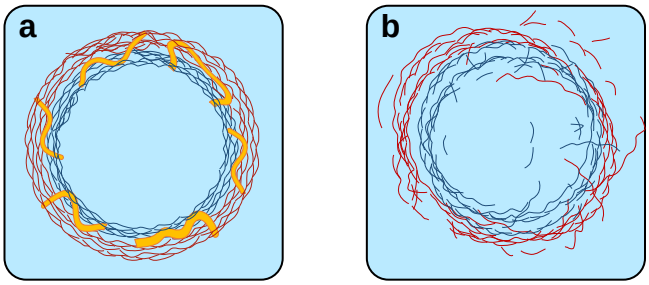


Fig. S6. Conceptual illustration showing the structural stability of (a) crosslinked NC versus (b) non-crosslinked NC upon sonication treatment.

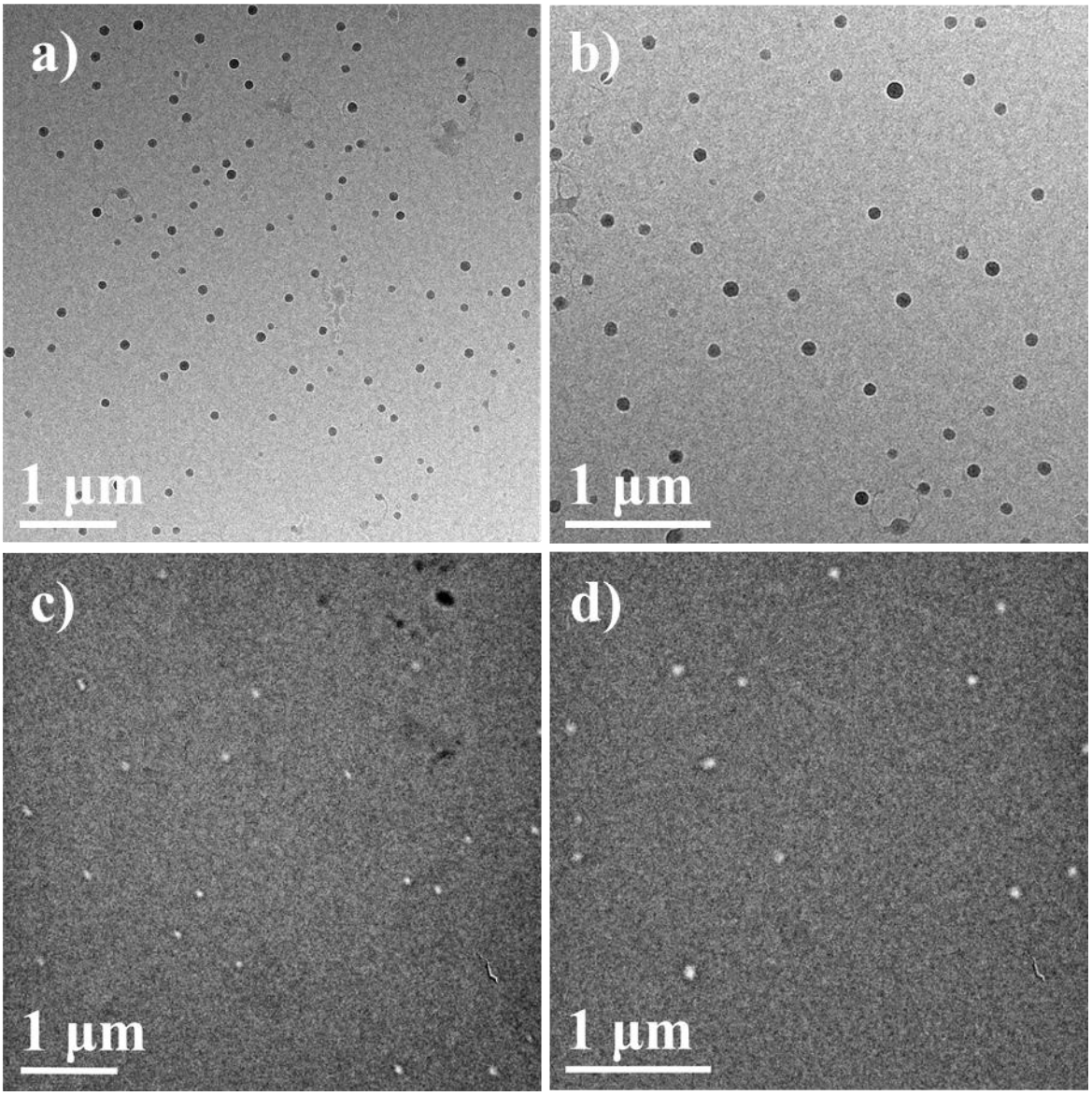


Fig. S7 a-b) TEM images of shell-crosslinked NPs/$[PAA/PAH]_3$; c-d) TEM images of the corresponding crosslinked $[PAA/PAH]_3$ NCs.

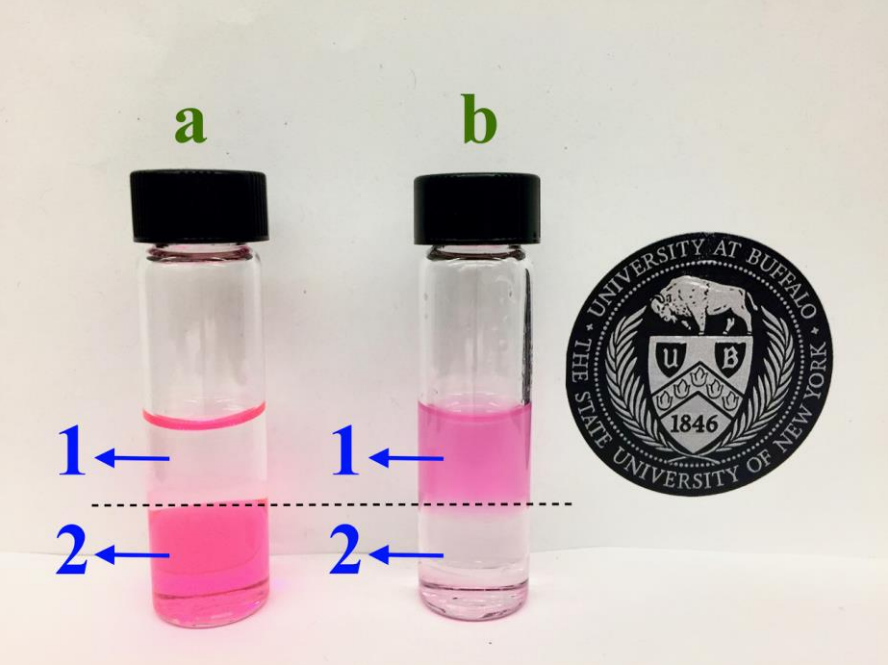


Fig. S8. Photo images of solutions: (a) the aqueous suspension of nile red (top a1 layer) after extraction with DCM (bottom a2 layer), and (b) the aqueous solution of nile red-loaded shell-crosslinked NCs (top b1 layer) after extraction with DCM (bottom b2 layer).

Note: Following our previously established protocol,[3] nile red was encapsulated by shell-crosslinked NCs by mixing an aqueous solution of NCs with an acetone solution of nile red (v/v = 1/4 for acetone/water), followed by acetone evaporation and extraction using DCM. Although nile red is hydrophobic and has poor solubility in water, it can be effectively loaded into the crosslinked NCs because the inner PEM shell surface was adsorbed with covalent-stabilized surfactant monolayers carrying hydrophobic tails. The resulting nile red-loaded NCs can also be readily dispersed in water.

References:

1. Y. Li, E. Themistou, B. P. Das, L. Christian-Tabak, J. Zou, M. Tsianou and C. Cheng, *Chem. Commun.*, 2011, **47**, 11697-11699.
2. Carbodiimide Crosslinker Chemistry, https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/carbodiimide-crosslinker-chemistry.html.
3. Y. Yu, C.-K. Chen, W.-C. Law, J. Mok, J. Zou, P. N. Prasad, and C. Cheng, *Mol. Pharmaceutics*, 2012, 10, 867-874.