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Porphyrin-crosslinked Block Copolymer Assemblies as Photophysically-active Nanoscopic Devices

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Measurements. The infrared measurements were obtained with a Shimadzu IR Prestige (Shimadzu Scientific Instruments, Columbia, MD), FTIR spectrophotometer equipped with an ATR accessory. UV–vis spectra were acquired on a Shimadzu UV-3600 system using PMMA cuvettes (or quartz for the acetic acid solution and the 25% Phenol in Toluene). Fluorescence spectroscopy data were acquired on a Shimadzu RF-5301 spectrofluorophotometer, each sample was excited at the maximum absorbance wavelength, and the fluorescence emission spectra in the range of 430–800 nm were recorded. The ^{1}H NMR (300 and 500 MHz) and ^{13}C NMR (75 and 125 MHz) spectra were obtained on either a Varian Mercury 300 MHz or Inova 500 MHz spectrometer, respectively, using the solvent as internal reference. Glass transition (T_g), melting (T_m), and crystallization (T_c) temperatures were measured by differential scanning calorimetry on a Mettler Toledo DSC822^{e} (Mettler Toledo Inc., Columbus, OH), with a heating rate of 10 ºC/min and analyzed using Mettler Toledo Star SW 7.01 software. The T_g was taken as the midpoint of the inflection tangent, upon the third heating scan. Thermogravimetric analysis was performed under N_2 atmosphere using a Mettler Toledo model TGA/SDTA851^{e}, with a heating rate of 10 ºC/min and analyzed using Mettler Toledo Star SW 7.01 software. Gel permeation chromatography was conducted on a system equipped with a Waters Chromatography, Inc. (Milford, MA) model 1515 isocratic pump, a model 2414 differential refractometer, and a three-column set of Polymer Laboratories, Inc. (Amherst, MA) Styragel columns (PL_{gel} 5μm Mixed C, 500 Å and 10^4 Å, 300 x 7.5 mm columns). The system was equilibrated at 35 °C in tetrahydrofuran (THF), which served as the polymer solvent and eluent (flow rate set to 1.00 mL/min). Polymer solutions were prepared at a known concentration (*ca*. 3 mg/mL) and an injection volume of 200 μL was used. Data collection was performed with Precision Detectors, Inc. Precision Acquire software. Data analysis was performed with Precision Detectors, Inc. Discovery 32 software. The differential refractometer was calibrated with standard polystyrene material (SRM 706 NIST) of known refractive index increment dn/dc (0.184 mL/g). The dn/dc values of the analyzed polymers were determined using refractive index detector data. The AFM instrumentation consisted of a MFP-3D system (Asylum Research, Santa Barbara, CA) and standard silicon tips (Silicon probe, Al reflex coated, 160mm; normal spring constant, 42 $N \cdot m^{-1}$; resonance frequency, 300 kHz, manufactured by Olympus). The sample solutions were drop (4 µL) deposited onto freshly cleaved mica and allowed to settle freely for 30 s, after which the excess solution was removed by filter paper and the

mica surface was allowed to dry in air. Samples for transmission electron microscopy (TEM) measurements were diluted with 1 wt% of phosphotungstic acid (PTA) stain solution (v/v, 1:1). Carbon coated copper grids were plasma treated following receipt from Electron Microscopy Science. Micrographs were collected at 100000× magnification and calibrated using a 41 nm polyacrylamide bead from NIST. The number average particle diameters (D_{avg}) measured for the histograms were generated from the analysis of a minimum of 150 particles from at least three different micrographs. Hydrodynamic diameters (D_h) and size distributions for the nanoparticles in aqueous solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a Delsa™Nano C Zeta Potential and Submicron Particle Size Analyzer. Solutions were filtered through a 0.22 μm Millex-GV PVDF membrane filter (Millipore Corp., Medford, MA) and then centrifuged in a model 5414 microfuge (Brinkman Instruments, Inc., Westbury, NY) for 2 min to remove dust particles. The calculations of the particle size distributions and distribution averages were performed with the Delsa Nano software package (Beckman Coulter, MA), which employed single-exponential fitting, cumulants analysis, non-negatively constrained least-squares (NNLS), and CONTIN particle size distribution analysis routines.

Materials. All reagents and solvents were obtained from commercial sources (Sigma-Aldrich, Acros, Fluka, Rapp Polymer, and Ace Synthesis) and used without further purification except in the case of *tert*-butyl acrylate and 4-acetoxystyrene which were filtered through neutral alumina prior to polymerization.

Preparation of $PtBA_{86}$. To a flame dried 25 mL schlenk flask equipped with a stir bar was added *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (0.524 g; 1.61 mmol), *tert*-butyl acrylate (31.0 g; 242 mmol), and 2,2,5-Trimethyl-4-phenyl-3-aza-3-nitroxide (18 mg; 82 µmol). The flask was sealed with a rubber septum and placed under vacuum. The reaction was degassed with three cycles of freeze-pump-thaw, after which the flask was allowed to warm to room temperature under nitrogen. The reaction was accomplished by immersion in a 125 °C oil bath for the duration of the polymerization. The reaction progress was monitored by removing aliquots of sample and obtaining ^{1}H NMR spectra. At the desired monomer conversion, the reaction was quenched by immersing the flask into liquid nitrogen and subsequent exposure to air. The reaction mixture was combined with 40 mL of THF and precipitated (x3) into a 4 liter solution of 1:1 mixture of ice and methanol. The reaction achieved 57% conversion and the product was isolated in 74% yield (13.6 g). $(M_n)_{NMR}$ = 11,300 Da, $(M_n)_{GPC}$ = 12,800 Da, $(M_w)_{GPC}$ = 11,300 Da, PDI: 1.1. T_g = 42 °C $T_{decomposition}$: [(222 °C - 248 °C) 44% mass loss; (248 °C – 310 °C) 11% mass loss; (310 °C – 463 °C) 34% mass loss; 11% mass remaining]. ^{1}H NMR [500 MHz, CD_2Cl_2]: δ 7.40-7.08 (br, aromatic protons of chain initiator), 2.39-1.99 (br, –C***H***CH_2– of the polymer backbone), 1.93-1.71 (br, C***H***$_3$C-), 1.69-1.14 (br, –CHC***H***$_2$– of the polymer backbone, alkyl protons of the chain of initiator) 1.06 (chain of initiator *tert*-butyl minor diastereomer), 0.93 (chain end of initiator *tert*-butyl major diastereomer), 0.39 (chain end C***H***$_3$). ^{13}C NMR [75 MHz, $CDCl_3$]: δ 174, 80, 42, 37, 36, 28. IR (cm^{-1}): 3017-2810, 1721, 1441, 1366, 1264, 1140.

Preparation of $PtBA_{86}$-*b*-$PAcS_{55}$. To a flame dried 50 mL Schlenk flask equipped with a stir bar was added $PtBA_{86}$ (4.1 g; 0.36 mmol), 4-acetoxystyrene (8.8 g; 54 mmol), 10.0 g of DMF, and 2,2,5-trimethyl-4-phenyl-3-aza-3-nitroxide (7 mg, 30 µmol). The flask was sealed with a rubber septum and placed under vacuum. The reaction was degassed with three cycles of freeze-pump-thaw, after which the flask was allowed to warm to room temperature. The reaction was accomplished by immersion in a 125 °C oil bath for the duration of the polymerization. The reaction progress was monitored by removing aliquots of sample and obtaining ^{1}H NMR spectra. At the desired monomer conversion, the reaction was quenched by immersing the flask into liquid nitrogen and subsequent exposure to air. The reaction mixture was combined with 30 mL of THF and precipitated (x3) into a 80:20 mixture of methanol and water. The reaction achieved 37% conversion and the product was isolated in 94% yield (6.8 g). $(M_n)_{NMR}$ = 20200 Da., $(M_n)_{GPC}$ = 23,400 Da, $(M_w)_{GPC}$ = 22,700 Da, PDI: 1.1. T_g = 42 °C, 110 °C $T_{decomposition}$: [(40 °C - 82 °C) 32% mass loss; (238 °C- 250 °C) 12% mass loss; (250 °C- 382 °C) 13% mass loss; (382 °C- 445 °C) 24% mass loss; 19 % mass remaining]. ^{1}H NMR [500 MHz, CD_2Cl_2]: δ 7.33-7.11 (br, aromatic protons of chain initiator), 6.98-6.28 (br, aromatic of 4-acetoxystyrene unit), 2.38-2.11 (br, methyl of acetate groups), 2.11-1.11 (br, –C***H***C***H***$_2$– of the polymer backbone, alkyl protons of the chain of initiator), 0.93 (chain end of initiator tertbutyl major diastereomer), 0.60 (chain end C***H***$_3$). ^{13}C NMR [75 MHz, $CDCl_3$]: δ 174, 169,

149, 142, 128, 121, 80, 42, 40, 37, 36, 28, 21. IR (cm^{-1}): 3011-2812, 1755, 1722, 1503, 1366, 1188, 1142, 1011.

Preparation of PAA_{86}-*b*-P*p*HS_{55}. To a flame dried 500 mL round bottom flask, 12.9 g (54.8 mmol of acetate) of $PtBA_{86}$-*b*-P*p*AcS_{55} was dissolved in 200 mL of methanol. A catalytic amount of sodium methoxide (114 mg, 2.11 mmol) was added to the solution by mass using a 25% (by weight) solution of sodium methoxide in methanol. The solution was refluxed for 12 hours and the solvent was then removed. To the dry product 50 mL of trifluoroacetic acid (TFA) was added and stirred overnight. The TFA was removed *via* rotary evaporation, dissolved in THF, and loaded into dialysis tubes (MWCO 6,000-8,000). After 3 days of dialysis, with no less than 2 times per day of changing the water, the product was dried *via* lyophilization and obtained in quantitative yield (9.3 g). T_g = 100 °C $T_{decomposition}$: [(40 °C-144 °C) 4% mass loss; (144 °C -227 °C) 6% mass loss; (227 °C –323 °C) 12% mass loss; (323 °C – 460 °C) 52% mass loss; 26% mass remaining]. ^{1}H NMR [500 MHz, d_6-DMSO]: δ 12.23 (acid proton of acetic acid unit), 8.94 (hydroxyl proton from aromatic hydroxyl of 4-hydroxystyrene unit), 7.32-7.09 (br, aromatic protons of chain initiator), 6.36-5.99 (aromatic protons from 4-hydroxystyrene unit), 2.37-0.93 (br, –C***H***C***H***$_2$– of the polymer backbone), 0.83 (chain end of initiator *tert*-butyl major diastereomer). ^{13}C NMR [75 MHz, d_6-DMSO]: δ 177, 156, 129, 116, 42, 38, 36, 31. IR (cm^{-1}):3615-2660, 1707, 1512, 1431-1408, 1223.

Preparation of PEO_5-*g*-PAA_{81}-*b*-P*p*HS_{55}. In dry DMF, PAA_{86}-*b*-P*p*HS_{55} (1.0 g, 78 μmol) was combined with HOBT (73 mg, 540 μmol) and EDCI (160 mg, 83 μmol) under nitrogen atmosphere. Following stirring for 1 hour, a mono-amine terminated PEO-2000 (1.1 g, 540 μmol) dissolved in 20 mL of dry DMF was added *via* canulation. After stirring for 24 hours, the reaction mixture was transferred into dialysis tubing (MWCO 6,000-8,000) and dialyzed for 1 week (water changed at least twice a day). The product was isolated *via* lyophilization in 77% yield (1.4 g) and contained an average of 5 PEO grafts per polymer chain (determined by NMR). T_g = 30 °C $T_{decomposition}$: [(215 °C – 325 °C) 6% mass loss; (325 °C -475 °C) 73% mass loss; 21% mass remaining]. ^{1}H NMR [500 MHz, CD_2Cl_2]: δ 12.34 (br, COOH of acetic acid unit), 8.98 (HO- from aromatic hydroxyl of 4-hydroxystyrene unit), 7.33-7.14 (br, aromatic protons of chain initiator), 7.00-6.08 (br, aromatic protons from 4-hydroxystyrene unit), 3.75 (terminal methoxy of PEO), 3.51 (CH_2 of PEO), 3.36 (br, –CHCH2– of the polymer backbone), 2.34-2.05 (br, –CHCH2– of the polymer backbone), 1.91-1.71 (br, CH_3C), 1.69-0.99 (br, –CHCH2– of the polymer backbone, alkyl protons of the chain of initiator). ^{13}C NMR [75 MHz, d_6-DMSO]: δ 176, 155, 128, 115, 70, 41, 34, 29. IR (cm^{-1}): 3636-2351, 1709, 1514, 1445, 1348, 1219, 1084.

Preparation of 5-Mesityldipyrromethane. 5-Mesityldipyrromethane was prepared according to the method of Lindsey (J. K. Laha, S. Dhanalekshmi, M. Taniguchi, A. Ambriose, J.S. Lindsey *Organic Process Research and Development* **2003**, *7*, 799).

Preparation of 5,15-Dimesityl-10,20-bis(4-aminomethylphenyl)porphyrin. 5-Mesityldipyrromethane (500 mg, 1.89 mmol, 1 equiv.) and *tert*-butyl 4-formyl-benzylcarbamate (445 mg, 1.89 mmol, 1 equiv.) were placed in a 250 mL round bottom flask and dissolved in anhydrous CH_2Cl_2 (100 mL) under a positive pressure of dry argon. The mixture was stirred for 10 minutes and treated with TFA (250 mL, 3.36 mmol, 2 equiv.). The resulting mixture was protected from the light and stirred at room temperature for 35 minutes. DDQ (750 mg, 3.30 mmol, 2 equiv.) was added and the dark mixture was stirred for another 2 hours at room temperature. At this time triethylamine (2.00 mL) was added and the reaction mixture was filtered through a 1 inch plug of neutral alumina (with the aid of CH_2Cl_2). The filtrate was concentrated and the residue was purified by flash chromatography (SiO_2, Merck 230-400 mesh, 3% methanol/ CH_2Cl_2) to afford 330 mg of a purple solid. This material was immediately dissolved in CH_2Cl_2 (25.0 mL) and treated with TFA (5.00 mL). This mixture was stirred for 1 hour, treated with 10% Na_2CO_3 (adjusting pH to ~ 9.0) and the layers were separated. The aqueous was extracted with CH_2Cl_2 (3 x 50.0 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was crystallized from acetonitrile to afford 127 mg (18.0% yield for two steps) of the desired porphyrin. ^{1}H NMR (DMSO-d_6) δ 8.81 (d, J = 4.0 Hz, 4 H), 8.65 (d, J = 4.0 Hz, 4 H), 8.26 (d, J = 8.0 Hz, 4 H), 8.25-7.95 (bs, 4 H), 7.85 (d, J = 8.0 Hz, 4 H), 7.32 (s, 4 H), 4.35 (s, 4 H), 2.55 (s, 6 H), 1.72 (s, 12 H), -2.80 (bs, 2 H); λabs 419, 515, 550, 592, 648 nm; LCMS (50-95% acetonitrile in water, 0.05% TFA over 10 min., Agilent Eclipse XD8-C18 column, 5 μm, 4.6 x 150 mm; ESI positive ion mode) $[M + H]^+$ = 757.5.

Porphyrin Nanostructures Preparation Procedure. To a 100 mL round bottom flask equipped with a magnetic stir bar was added (PEO_5-*g*-PAA_{81}-*b*-P*p*HS_{55}) (23 mg, 1.0 μmol) and 22 mL of nanopure water. The pH value was raised to 12 through the addition of 1.0 M NaOH solution. The transparent solution was stirred overnight at RT and the micellization was then initiated after decreasing the pH value to 7 by adding 1.0 M HCl dropwise. After further stirring for 12 h at RT, nanopure water was added to the micelle solution for a final volume of 46 mL (micelle concentration 0.5 mg/mL). The shell crosslinked nanoparticles containing porphyrin were prepared through the combination of the 0.5 mg/mL micelle solution (10 mL; 17.5 μmol of carboxylic acid residues) with 0.5 mg (0.66 μmol) of porphyrin (3.8 mol% relative to the acrylic acid residues) for 8% crosslinking extent, or 1.0 mg (1.3 μmol) of porphyrin (7.4 mol% relative to the acrylic acid residues) for 15% crosslinking extent). The porphyrin was added to the micelle solution *via* a 1.0 mg/mL solution of the compound in DMF (0.5 mL of porphyrin in DMF with an additional 0.5 mL of DMF solvent for 8% crosslinking extent or 1.0 mL porphyrin in DMF for 15% crosslinking extent). The solution was stirred for 2 h at RT followed by the addition of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (EDCI, 0.5 mg, 1.7 μmol for 8% crosslinking extent; or 1.1mg, 3.8 μmol for 15% crosslinking extent) as a 6 mg/mL solution in nanopure H_2O. The reaction was stirred overnight at RT and then transferred into pre-soaked dialysis tubing (MWCO ca. 6,000-8,000 Da) and dialyzed against nanopure water for three days to remove the small-molecule reaction by-products and DMF. The micelles containing physically entrapped porphyrin (noncovalently bound) were prepared in an identical fashion except no EDCI was added. The micelle solutions were purified by dialysis (in the same procedure stated above) to remove the DMF present.

Observation of Covalent Incorporation. To MWCO 6,000-8,000 dialysis tubing, 5 mL of 15% porphyrin containing micelles were added and dialyzed against DMF for 5 days. The bulk DMF solution became noticeably yellow in color. However, when 5 mL of 15% porphyrin containing SCKs were loaded into MWCO 6,000-8,000 dialysis tubing and dialyzed against DMF for 5 days, the color of the bulk DMF solution did not change.

Photophysical Properties Measurement. For the porphyrin nanostructures, 3 mL of 5 mM PBS (with 5 mM NaCl) at pH 4.5, 6.1, 8.0, 9.5, and 11 were added to PMMA cuvettes. 100 μL of the appropriate nanoporphyrin solution was added to each pH solution and UV-vis and Fluorescence data were obtained. All experiments were completed in triplicate. For the small molecule porphyrin, 3 mL of solvent (50% acetic acid or 75% toluene/25% phenol) were placed in cuvettes. A 0.4 mg/mL solution of porphyrin in 50% acetic acid or 75% toluene/25% phenol was prepared. In each cuvette, 25, 50, 100, 125 or 150 μL of the porphyrin solution was added. Solvent (50% acetic acid or 75% Toluene/25% Phenol) was added to the samples containing 25, 50, 100 or 125 μL of porphyrin so that the total solution volume was 3.15 mL.

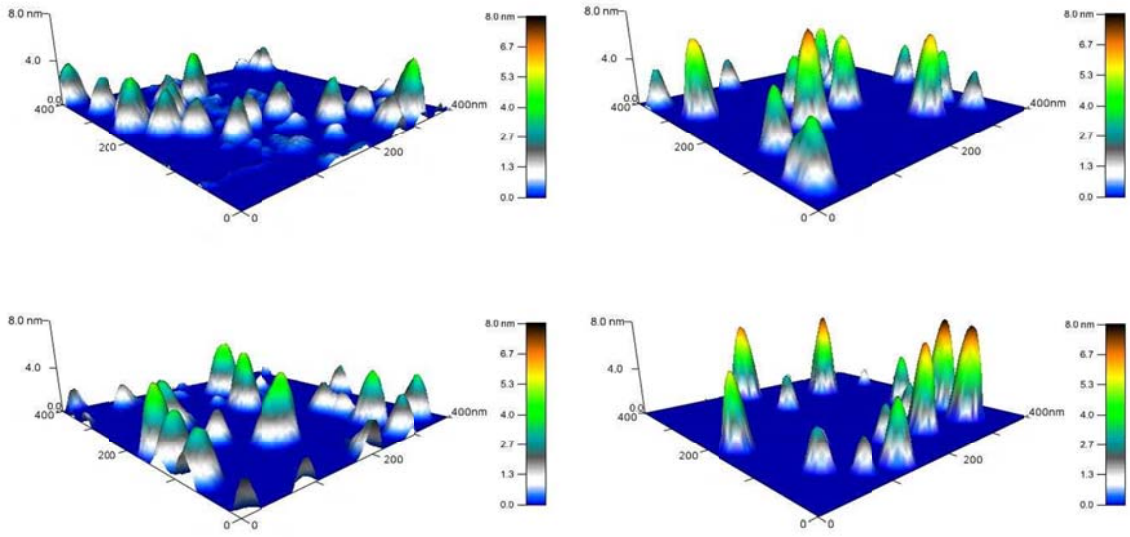

Fig. S1 AFM Images of porphyrin-containing nanoparticles (Top Left: 8% Micelles; Top Right: 8% SCKs; Lower Left: 15% Micelles, Lower Right: 15% SCKs)

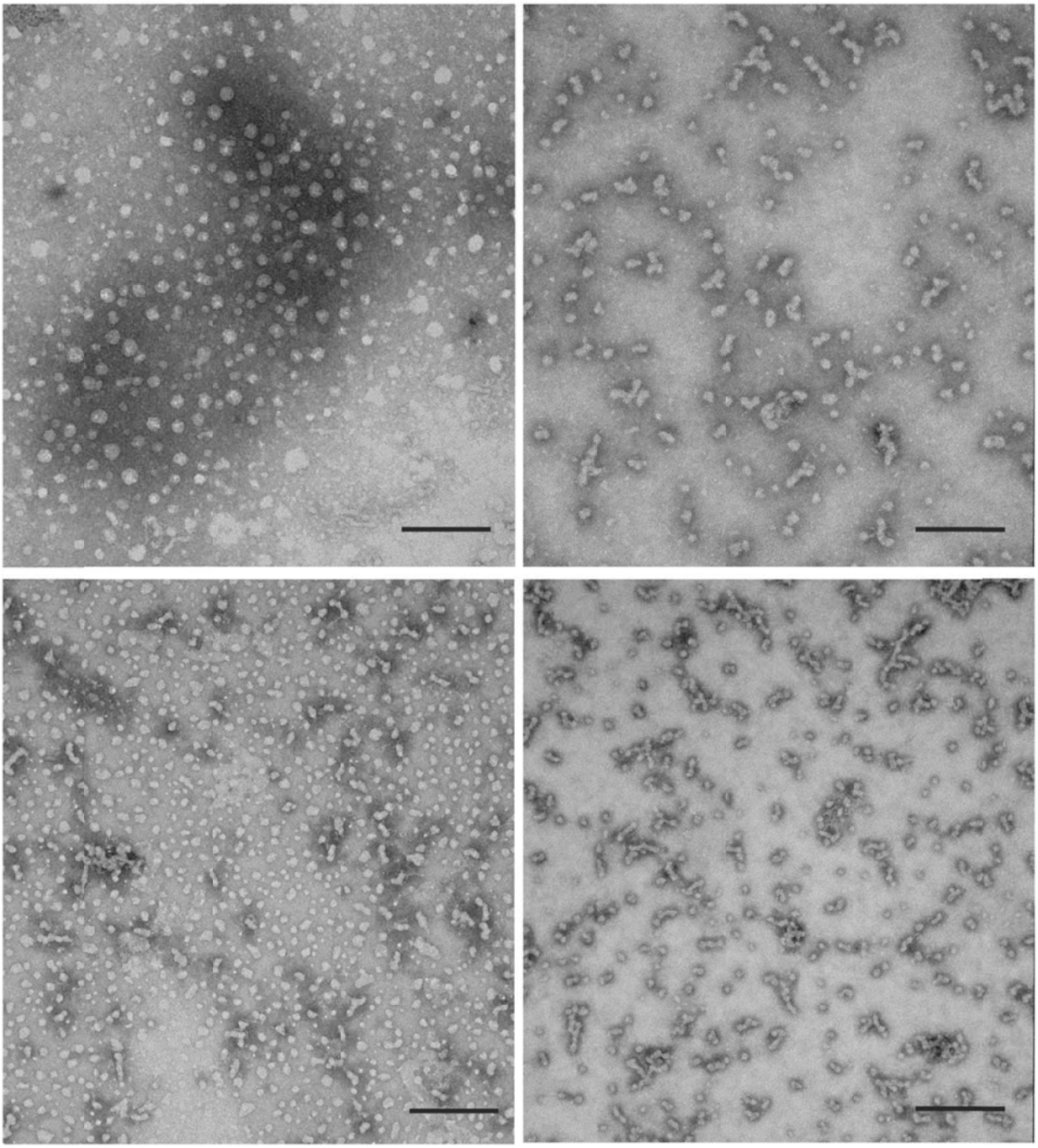

Fig. S2 TEM Images of porphyrin-containing nanoparticles (Top Left: 8% Micelles; Top Right: 8% SCKs; Lower Left: 15% Micelles, Lower Right: 15% SCKs) with 200 nm scale bars