Electronic Supplementary Material (ESI) for ChemComm.

pH Responsive Polymersome Pickering Emulsion for Simple and Efficient Janus Polymersome Fabrication

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Supporting Information

Materials: All the chemicals were purchased from Sigma-Aldrich. *tert*-Butyl acrylate, styrene, 4-vinylbenzyl chloride were distilled under vacuum to remove the inhibitors before polymerization. Unless stated otherwise, other chemicals were used without further purification.

Instrumentation:

Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on an ATI Matson Genesis Series FT-IR spectrometer fitted with an ATR cell. The vibrations (ν) are given in cm^{-1}.

Nuclear magnetic resonance (NMR) spectra were recorded on a *Varian Inova 400* (400 MHz for ^1H) spectrometer. ^1H NMR chemical shifts (δ) are reported in parts per million (ppm) relative to a residual proton peak of the solvent; δ = 7.26 for $CDCl_3$. Multiplicities are reported as s (singlet), d (doublet), t (triplet) and m (multiplet). Broad signals are indicated by the addition of br. Coupling constants are reported as J value in Hertz (Hz).

Molecular weights of the block copolymers were measured on a *Shimadzu size exclusion column* equipped with a guard column, a Polymer Laboratories gel 5 μm mixed D column and differential refractive index (RI) and UV (λ = 254 nm) detection. The system was eluted with tetrahydrofuran (THF), analysis grade, using a flow rate of 1 mL/min at 35 °C. The calibration was performed with polystyrene standards ranging from 580 to 377,400 g/mol.

Confocal laser scanning microscopy was performed on a *Leica TCS SP5* confocal microscope equipped with an *HCX PL APO40×N.A. 1.2* water immersion lens.

Rhodamine B, Nile red, and FITC-dextran were excited using a HeNe 561 nm laser and a 488 argon ion laser. Emission was detected between 580 and 650 nm for Rhodamine B and Nile red, between 495 and 545 nm for FITC-dextran.

Transmission electron microscopy (TEM) was performed on a *JEOL JEM 1010* microscope with an acceleration voltage of 60 kV equipped with a charge-coupled device (CCD) camera. Sample specimens were prepared by placing a drop (10 μL) of a diluted aqueous vesicle solution on an *EM science* carbon-coated copper grid (200 mesh) for 15 min. The grid was purified from salts and other impurities by placing a drop of MilliQ on it, which was immediately removed. The grid was finally air dried for at least 6 hours and analyzed without further treatment.

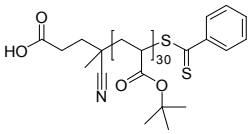
Cryogenic TEM (Cryo-TEM) was performed on a JEOL TEM 2100 microscope with an acceleration voltage of 120 kV. A small droplet of the suspension (5μL) was placed on a holey carbon film supported on a TEM copper grid. Following the preset procedure of the Vitrobot vitrification system (FEI), the specimen was blotted and plunged into a liquid ethane reservoir cooled by liquid nitrogen. The vitrified samples were transferred to a Gatan 914 cryo-holder and cryo-transfer stage cooled by liquid nitrogen. During observation of the vitrified samples, the cryo-holder temperature was maintained below -175 °C to prevent sublimation of vitreous water. The images were recorded digitally by a Gatan 890 ultra scan CCD camera with the software package.

Scanning electron microscopy (SEM) was performed on a *JEOL JSM T300* microscope (30 kV). The samples were prepared by placing 20μL of a solution on a silicon wafer, which was subsequently air dried for at least 12 hours. Samples were next coated in 1.5 nm Pt/Au by using a BALZERS sputter machine. The structures were visualized without further treatment.

Dynamic light scattering (DLS) and zeta potential experiments were performed on a Malvern Zetasizer Nano S equipped with a He-Ne (633 nm, 4 mW) laser and an Avalanche photodiode detector at an angle of 173°. All DLS data were processed using a Dispersion Technology Software (Malvern Instruments).

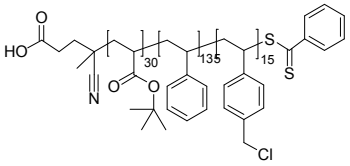
Synthesis:

Poly(tert-butyl acrylate)$_{30}$-chain transfer agent (PtBA$_{30}$-CTA)



A flame-dried Schlenk tube was charged with *tert*-butyl acrylate (2.88 g, 22.5mmol, 50equiv), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (126 mg, 0.45mmol, 1.0 equiv), AIBN (11.2 mg, 0.135mmol, 0.3equiv), 1.5mL dry DMF and a stirring bar. The mixture was degassed by three freeze-pump-thaw cycles. The Schlenk tube was placed in a preheated oil bath of 70 ˚C and the polymerization was monitored by ^{1}H-NMR spectroscopy. When a conversion of 60 % was reached, the polymerization was terminated by removal of the heat source. After cooling down to room temperature, the reaction mixture was diluted with THF and transferred into an Erlenmeyer flask. Precipitation was induced upon addition of diethyl ether (200 mL) and the resulting pink solid was filtered and dried under vacuum. This process was repeated to obtain a pure product (2.3 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0-7.5 (w, arom. H), 2.4-1.6 (br. s, acrylate backbone), 1.5 (s, *tert*-butylCH_3). Mn (GPC): 4.0kDa, PDI =1.12, Mn (^{1}H NMR): 4.2kDa.

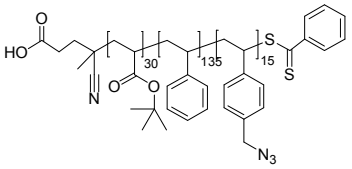
Poly(tert-butyl acrylate)$_{30}$-b-Poly(styrene$_{135}$-co-4-vinylbenzyl chloride$_{15}$) PtBA$_{30}$-b-P(S$_{135}$-co-4VBC$_{15}$)



A flame-dried Schlenk tube was charged with styrene (1.5 g, 14.4mmol, 288equiv), 4-vinylbenzyl chloride (0.244 g, 1.6mmol, 32 equiv), PtBA$_{30}$-CTA (206 mg, 0.05mmol, 1.0 equiv), AIBN (1.24 mg, 0.015mmol, 0.3equiv), 1.5mL dry DMF and a stirring bar. The mixture was degassed by three freeze-pump-thaw cycles. The Schlenk tube was placed in a preheated oil bath of 75 ˚C and the polymerization was monitored by ^{1}H-NMR spectroscopy. When a conversion of 50 % was reached, the polymerization was

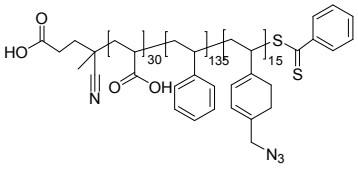
terminated by removal of the heat source. After cooling down to room temperature, the reaction mixture was diluted with THF and transferred into an Erlenmeyer flask. Precipitation was induced upon addition of methanol (200 mL) and the resulting pink solid was filtered and dried under vacuum. This process was repeated to obtain a pure product (488 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25-6.10 (br. s, arom. H), 4.55 (s, CH_2 in benzyl), 2.4-1.25 (br. s, acrylate and styrene backbone), 1.5 (s, CH_3 in *tert*-Butyl). Mn (GPC): 19.6kDa, PDI =1.19, Mn (^{1}H NMR): 20.6kDa.

Poly(tert-butyl acrylate)$_{30}$-b-Poly(styrene$_{135}$-co-4-vinylbenzyl azide$_{15}$) PtBA$_{30}$-b-P(S$_{135}$-co-4VBA$_{15}$)



A flame-dried flask was charged with *PtBA$_{30}$-b-P(S$_{135}$-co-4VBC$_{15}$)* (409 mg, 0.02mmol, 1.0 equiv),sodium azide (39 mg, 0.6mmol, 30equiv), 5mL dry DMF and a stirring bar. The pink solution gradually faded and turned into a pale turbid solution. After 3 hours, precipitation was induced upon addition of methanol (200 mL) and the resulting white solid was filtered and dried under vacuum. This process was repeated to obtain a pure product (406 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25-6.10 (br. s, arom. H), 4.25 (s, CH_2 in benzyl), 2.4-1.25 (br. s, acrylate and styrene backbone), 1.5 (s, CH_3 in *tert*-Butyl). Mn (GPC): 19.9kDa, PDI =1.22, Mn (^{1}H NMR): 21.0kDa.

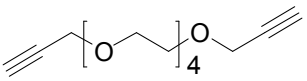
Poly(acrylic acid)$_{30}$-b-Poly(styrene$_{135}$-co- 4-vinylbenzyl azide$_{15}$) PAA$_{30}$-b-P(S$_{135}$-co-4VBA$_{15}$)



A flask was charged with *PtBA$_{30}$-b-P(S$_{135}$-co-4VBA$_{15}$)* (411 mg, 0.02mmol, 1.0 equiv), trifluoroacetic acid (274 mg, 2.4mmol, 120equiv), 5mL dichloromethane, 1mL H_2O and a stirring bar. After 24 hours, precipitation was induced upon addition of

methanol (200 mL) and the resulting white solid was filtered and dried under vacuum. This process was repeated to obtain a pure product (320 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25-6.10 (br. s, arom. H), 4.25 (s, CH_2 in benzyl), 2.4-1.25 (br. s, acrylate and styrene backbone). Mn (GPC): 20.1kDa, PDI =1.25, Mn (^{1}H NMR): 20.8kDa.

4,7,10,13,16-pentaoxanonadeca-1,18-diyne



To a dispersion of NaH (433 mg of a 60% dispersion in oil, 11.3 mmol, 2.2 equiv) in toluene (40 mL) at 0 °C was added dropwise a solution of tetraethylene glycol (1.00 g, 5.15 mmol, 1.0 equiv) in toluene (10 mL). The reaction mixture was stirred for 1h at 0 °C after which propargyl bromide (1.68 g of an 80% solution in toluene, 11.3 mmol, 2.2 equiv) was added. After stirring for an additional 2 h at 0 °C the reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL). The product was extracted with CH_2Cl_2 (3 × 50 mL), dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (EtOAc/heptane, 1:2) afforded the product as a colorless oil (957 mg, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.21 (d, *J*=2.4 Hz, 4H), 3.73–3.63 (m, 16H), 2.44 (t, *J*=2.4 Hz, 2H).

Preparation of PVP capped Pt-NP

The procedure was followed as reported earlier.[1] In brief, 20 mg of PVP (Mn~10000) was added to 2 ml of a 20 mM solution of $K_2PtCl_4$and was left dissolving for at least 24 hrs. 35 mg of ascorbic acid dissolved in 1ml of MilliQ water was added at once to the platinum salt solution and the vial was sonicated for 40 min at room temperature to generate PVP-capped PtNP of size ~30 nm. Three centrifugation/washing cycles were performed to remove the excess of PVP before further use of the particles for the entrapment experiments. After the final wash, freshly prepared PtNP were redispersed in MilliQ water.

Polymersome preparation procedures

Preparation of crosslinked polymersomes

Block copolymer *PAA_{30}-b-P(S_{135}-co-$4VBA_{15}$)* (10 mg) was placed in a vial and 2 mL of THF:1,4- dioxane (1:3 v/v) was added to the vial to dissolve the block copolymer. To this solution, 2 mL of ultrapure water (MilliQ, 18.2 MΩ) was gently dripped into within 2 hours with magnetic stirring. The polymersomes were allowed to self-assemble for 30 min. Then, 6 μL of 4,7,10,13,16-pentaoxanonadeca-1,18-diyne, followed by copper sulfate (1.0 mg), sodium ascorbate (2.0 mg) and bathophenanthrolinedisulfonic acid disodium salt hydrate (1.2 mg) were added into the suspension for polymersome crosslinking. After 24 hours, the obtained suspension was dialyzed against ultrapure water (MilliQ, 18.2 MΩ) for 48 hours to remove organic solvent and excess of crosslinker. The final solution of the block copolymer was adjusted to be 50 mg/mL by concentrating the polymersome solution.

Preparation of pH-responsive PPE

For polymersome Pickering emulsion formation 25 μL of polymersome solution was first diluted by HCl (pH 3) to 20 mg/mL and then mixed with 200 μL of ethyl acetate. The PPE was formed by a vibration homogenizer at room temperature for 2 minutes. The PPE disassembled to single polymersomes when the pH value of the water phase was adjusted to pH7 by concentrated NaOH solution. The above procedures were repeated by several rounds.

Preparation of metal nanoparticle-modified JPs

PPEs were prepared as mentioned above. Subsequently, 3 μL concentrated AuNP or PtNP solutions were added to the water phase and the mixture was homogenized again for 2 minutes. The concentration of AuNP with different sizes and PtNP were ~$6.0\cdot10^{14}$particles/mL (10 nm), ~$3.5\cdot10^{12}$particles/mL (50 nm), ~$7.8\cdot10^{11}$particles/mL (80 nm) and ~$8.0\cdot10^{14}$particles/mL, respectively. The concentration of AuNPs was provided by the commercial product specification of Sigma-Aldrich. The concentration of PtNPs was determined according to previous ref.[1] To disassemble the

PPE, the pH value of the water phase was adjusted to pH 7 by concentrated NaOH solution. After homogenizing, the water and ethyl acetate phase separated automatically. Then the ethyl acetate was removed to obtain the dispersed JP aqueous solution. Since the concentrations of AuNPs and PtNPs were relatively low, all the particles were attached to the polymersome surface. No free particles were found in the aqueous solution.

Reference:

1. D. A. Wilson, R. J. M. Nolte, J. C. M. van Hest, *Nature Chemistry* 2012, **4**, 268.

Figures:

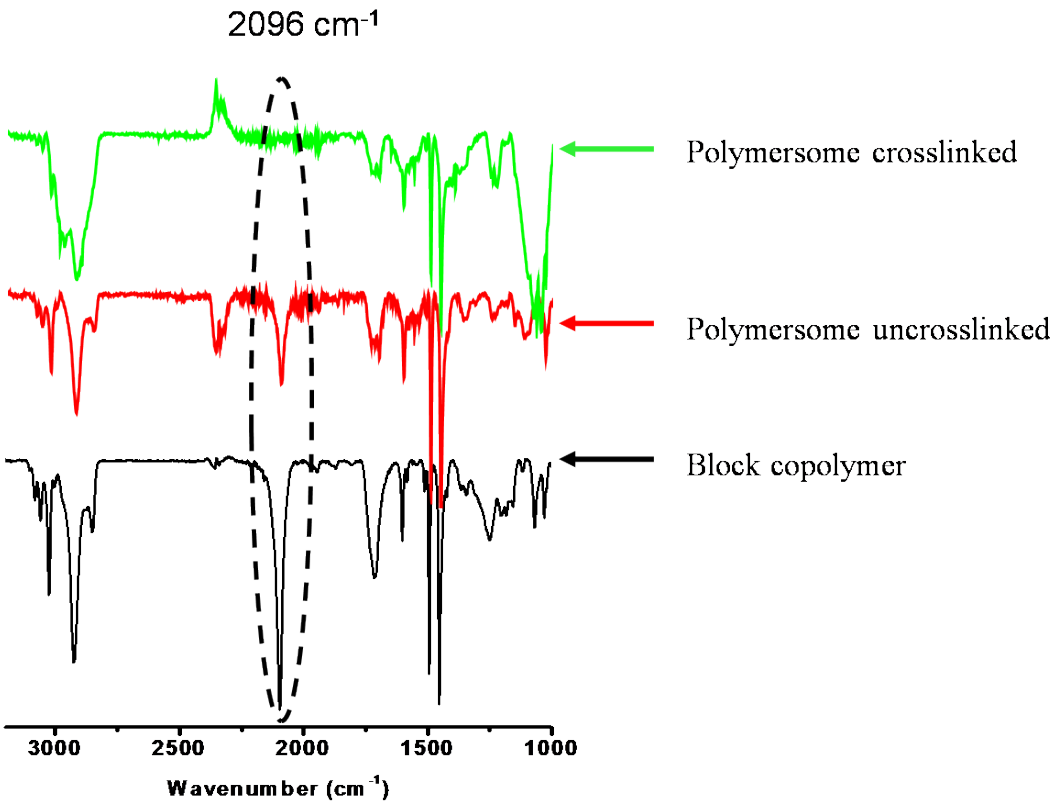


Figure S1. FTIR spectra of block copolymer (black), uncrosslinked polymersome (red), and crosslinked polymersome (green).

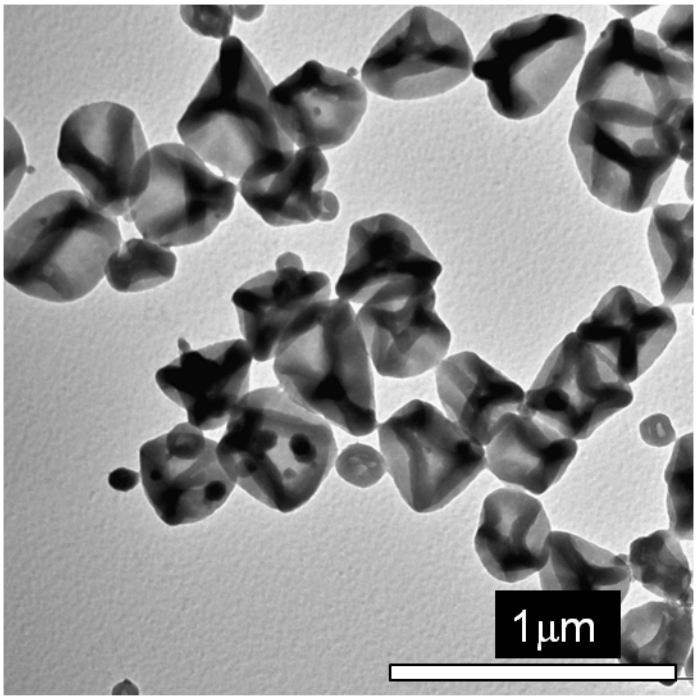


Figure S2. TEM image of polymersomes re-dispersed in water after disassembly from PPE.

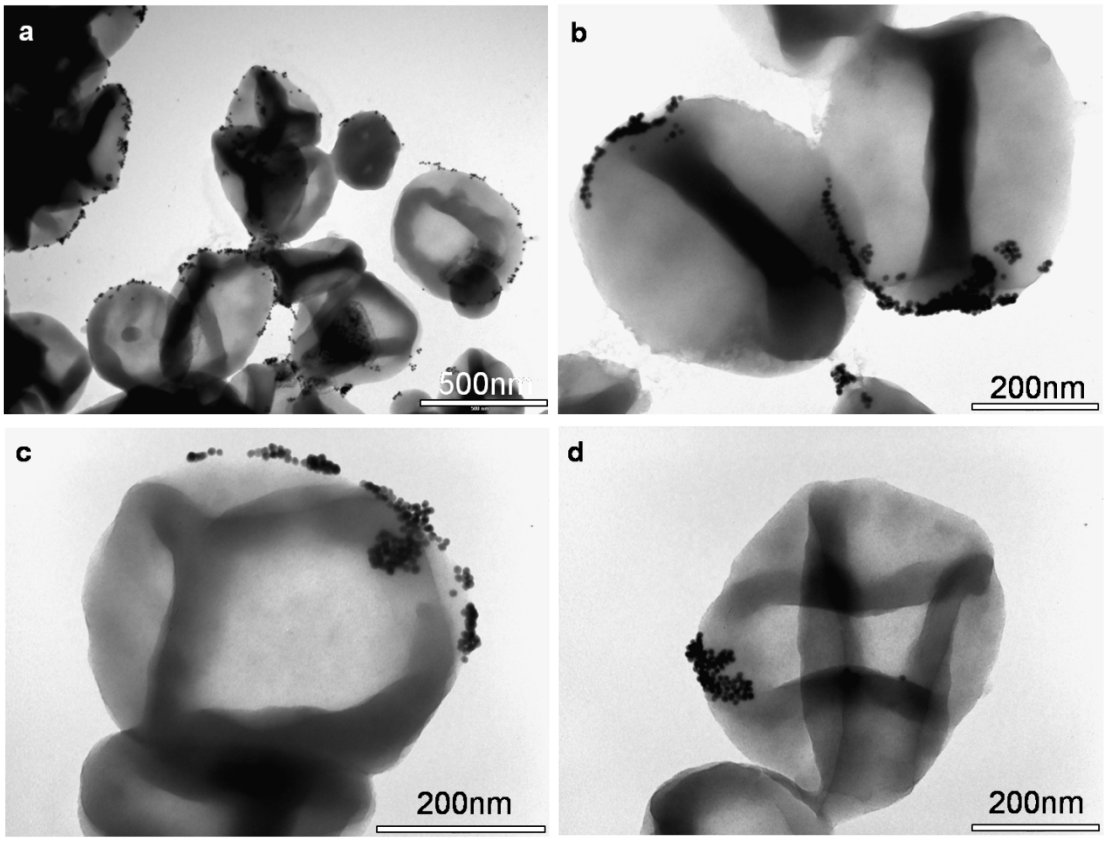


Figure S3. TEM images of AuNP (10 nm) modified JP.

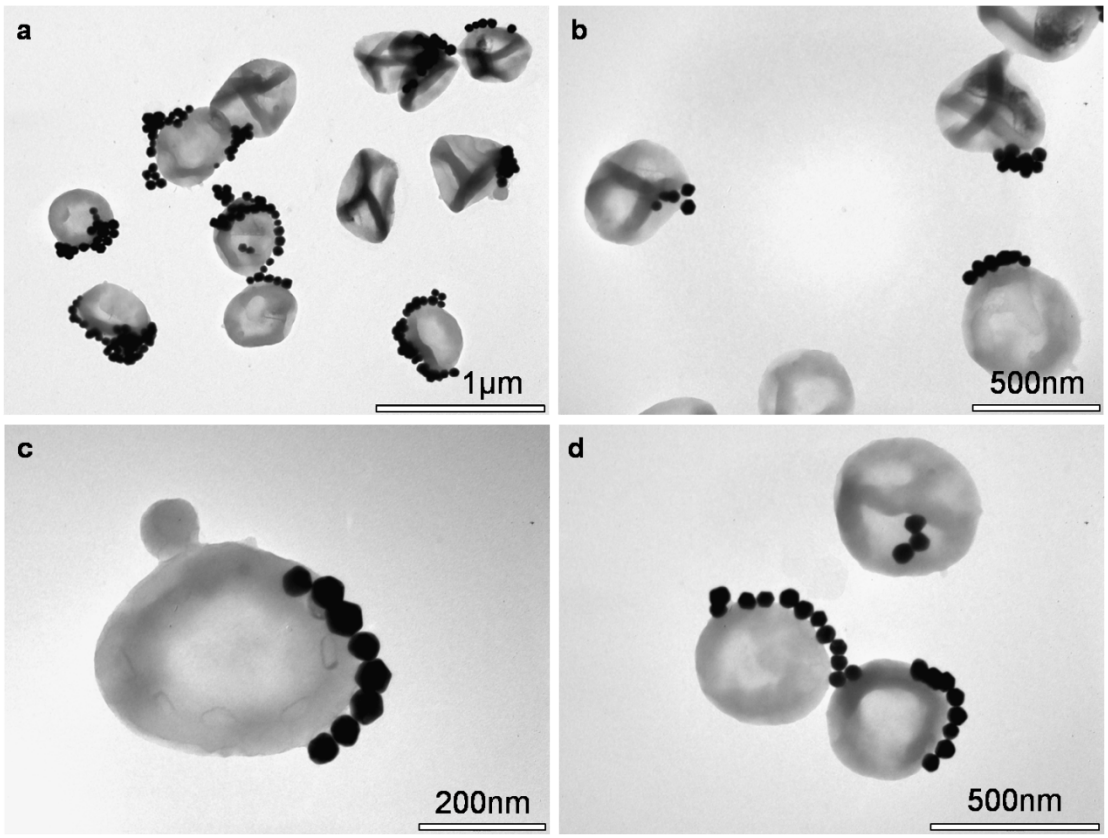


Figure S4. TEM images of AuNP (50 nm) modified JP.

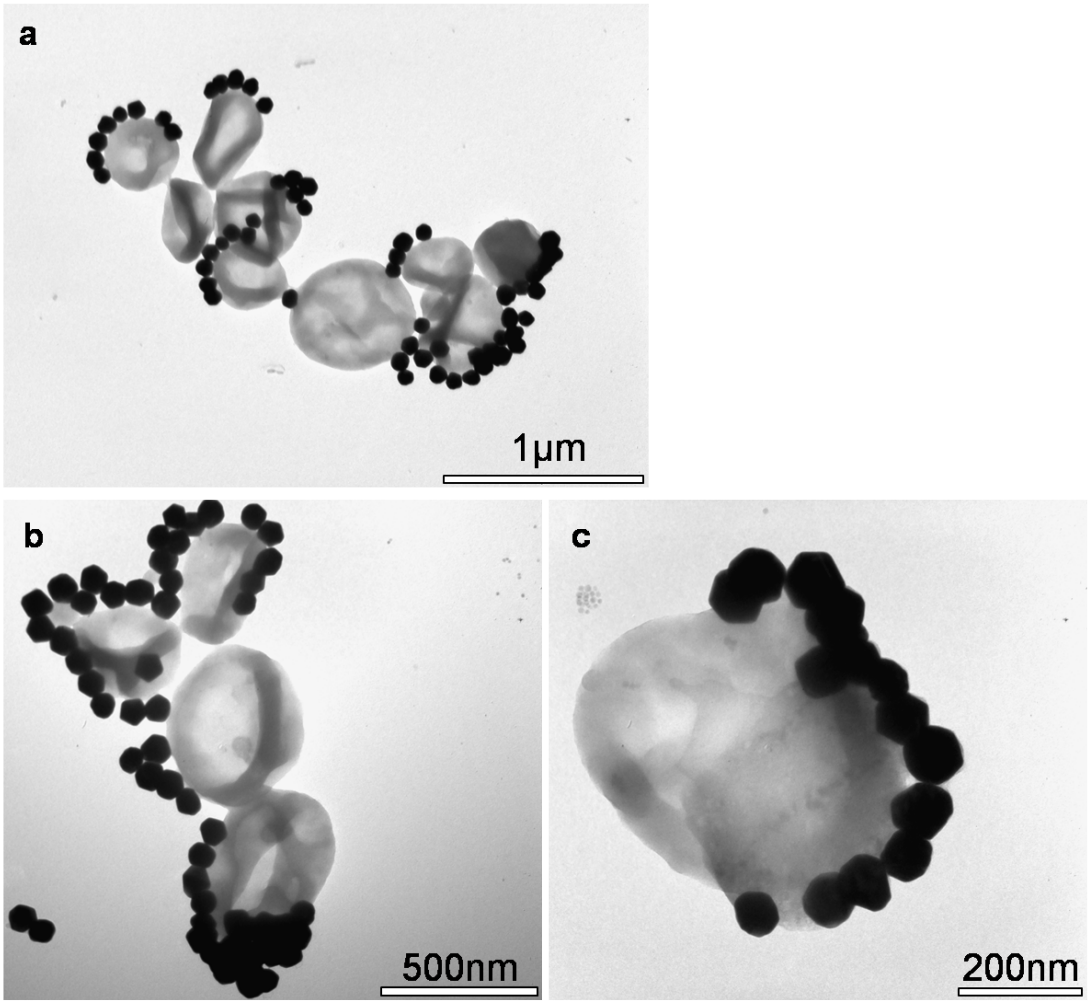


Figure S5. TEM images of AuNP (80 nm) modified JP.

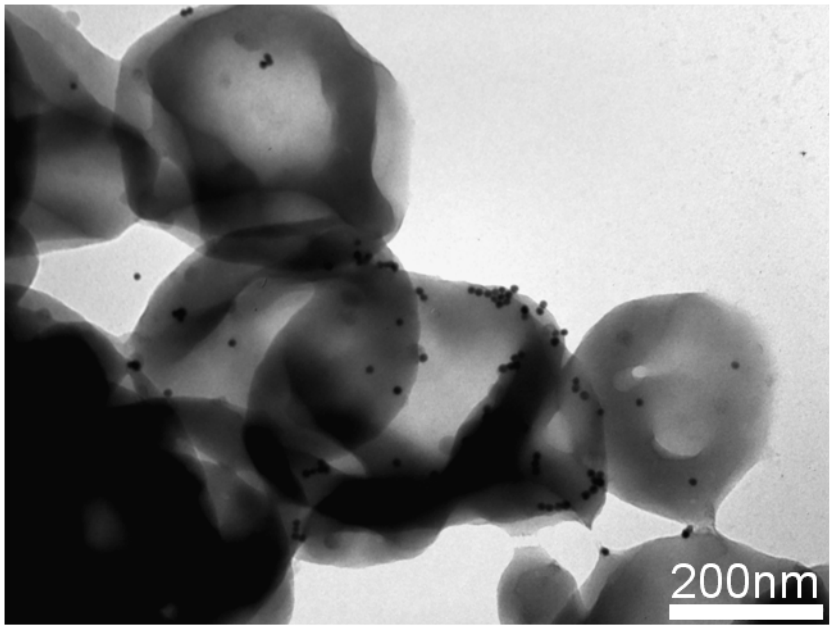


Figure S6. TEM image of polymersomes randomly interacting with AuNP (10 nm) in water.

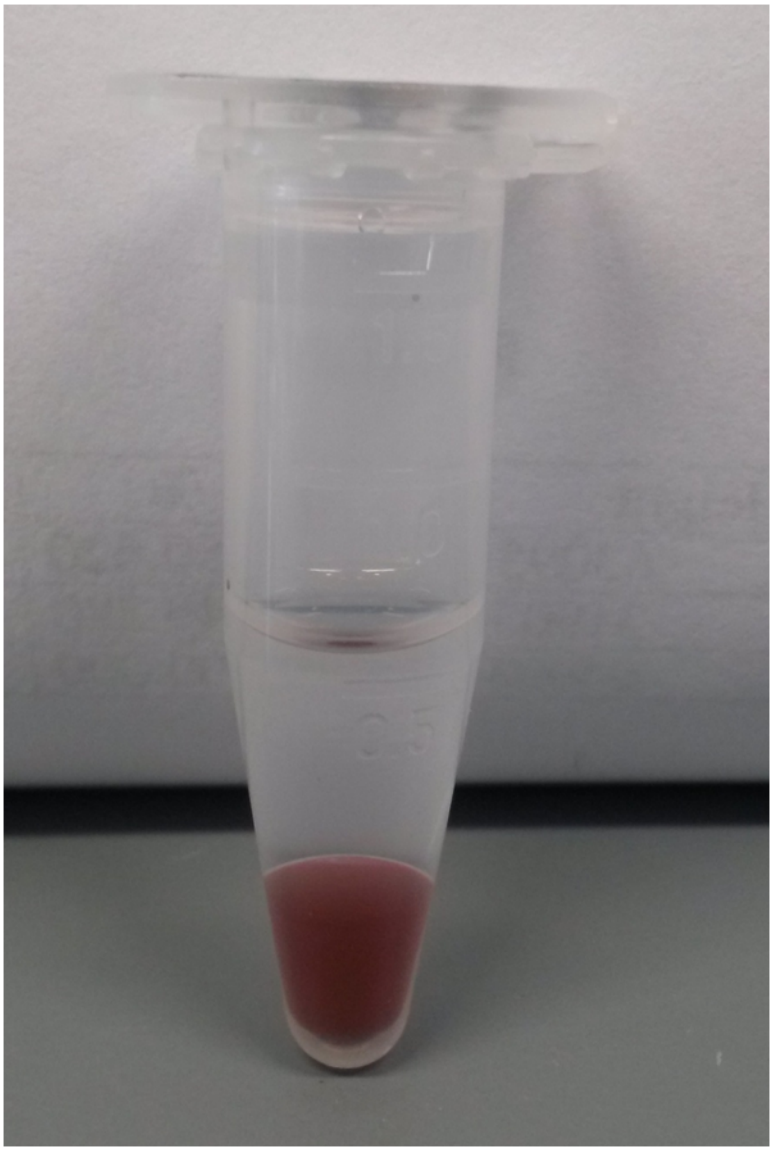


Figure S7. Photo of an aqueous solution of AuNP (50 nm) with ethyl acetate.