Supporting Information

Compartmentalization and Unidirectional Cross-Domain Molecule Shuttling of Organometallic Single-Chain Nanoparticles

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Characterization. *UV-Vis Spectroscopy* was performed using a Shimadzu UV-3600 spectrometer. Intermediate thiol protection was confirmed using Ellman method.[1] Ellman reagent was prepared by dissolving 5, 5'-dithiobis-2-nitrobenzoic acid in 0.1 M phosphate buffer (0.4 mg/mL, pH 8.0). Polymer (**P-2**, Figure S1; 4.0 mg) was dissolved in phosphate buffer (2.0 mL). Solution was bubbled with argon gas for 1 h. Ellman reagent (50 μL) was added. The solution was stirred for 10 min before UV-vis spectroscopy. *^{1}H NMR Spectroscopy* was conducted on a Unity INOVA 400NB NMR spectrometer. *Size Exclusion Chromatography (SEC)* was conducted on a PL-GPC220 integrated 3 system with RI detector and a set of columns (2×PLGel MIXED-B+1×PLGel MIXED-D). PMMA standards (Agilent, 1.95–1048 kDa) were used for calibration. The calibration and analysis proceeded using DMF eluent that contained 10.0 mM LiBr at a 1.0 mL/min flow rate at 80°C. NH_3^+ and imidazolium-motifs were converted into nonionic derivatives by reaction with di-*tert*-butyldicarbonate in the presence of triethylamine in methanol at room temperature for 3 days. The solution was filtered using a 200 nm filter prior to SEC measurement. *Dynamic Light Scattering (DLS)* was performed on a Brookhaven BI-200SM setup equipped with 35-mW He-Ne laser, BI-200SM goniometer and BI-TurboCorr digital correlator. Temperature was fixed at 25°C using a BI-TCD controller. Dusts were removed using a 200-nm syringe filter. Sample was measured at an angle of 90°. Number-average hydrodynamic diameter ($D_{h,\,DLS}$) was determined by cumulants analysis in CONTIN routine. Data were averaged over 5 runs. *NMR Diffusion-Ordered Spectroscopy (DOSY)* was conducted on an Agilent direct-Drive II 600MHz NMR spectrometer using a 5 mm OnNMR probe and a gradient amplifier, fulfilled in a Dbppste-cc pulse sequence with *z*-direction gradient strength to 60 G/cm at 25°C. Proton pulse length was 8.9 μs. Gradient length was set to 2 ms and gradient strength was incremented over 1.13–28.26 G/cm in 15 steps, scanning for 128 times. Gradient recovery delay time was set to 1 s at acquisition time of 1.7 s. The diffusion delay (Δ) was set to 400 ms. Diffusion coefficient (D) was analyzed using a Vnmrj3.2 software, using the signal C**H**(OH)CH_3 of PHPMA as calibration standard. The mean hydrodynamic diameter ($D_{h,\,DOSY}$) was calculated using Stokes-Einstein Equation, $D_{h,\,DOSY} = kT/3\pi\eta D$, in which k is Boltzmann constant, T is solution temperature, and η is solvent viscosity (D_2O: 1.100×10^{-3} Pa s).[2] *Aqueous Electrophoresis* was performed on a Malvern Zetasizer Nano-ZS90 instrument. *Transmission Electron Microscopy (TEM)* was performed on a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 120 kV. Sample was prepared by diluting solution to 0.5 mg/mL. 10 μL aliquot was dripped onto the copper grid, deeply frozen using liquid nitrogen, and dried under reduced pressure. Number-weighed diameter (D_n) was determined by statistical analysis from >500 particles, using a Nano Measurer 1.2 software. *Fourier Transform Infrared (FTIR)* spectra of **SCNP-1** and **SCNP-2** were recorded on a Nicolet 6700 spectrometer. Samples were prepared by freeze-drying in a Labconco Freezone2.5L freeze-drier prior to FTIR measurements.

Materials. Block copolymers, $PHisAM_{58}$-*b*-$PHPMA_{57}$-*b*-$PAEMA_{58}$ (^{1}H NMR: M_n = 29.6 kDa; SEC: M_n = 24.9 kDa, $Đ$ = 1.09), $PHPMA_{55}$-*b*-$PAEMA_{57}$ (^{1}H NMR: M_n = 17.5 kDa; SEC: M_n = 19.3 kDa, $Đ$ = 1.07) and $PHPMA_{60}$-*b*-$PHisAM_{56}$ (^{1}H NMR: M_n = 20.1 kDa; SEC: M_n = 16.9 kDa, $Đ$ = 1.09) were synthesized *via* visible light mediated RAFT polymerization reported elsewhere.[3] 2-Hydroxyethyl acrylate (HEA), dimethylphenylphosphine (Me_2PPh) were purchased from Sigma-Aldrich; deuterium oxide (D_2O, 99.8% D), sodium deuteroxide (99.5% D, 40% in D_2O) and deuterchloric acid (99.5% D, 20% in D_2O) from J&K; ascorbic acid (AA), $CuCl_2$ and other reagents from Aladdin. These reagents were used as received. Deionized water (>18.2 MΩ/cm) was obtained from a Direct-Q 5 UV Millipore system, and used for the synthesis and characterization.

End-Group Protection. $PHisAM_{58}$-*b*-$PHPMA_{57}$-*b*-$PAEMA_{58}$ (**P-1**; 0.85 g, 28.7 μmol trithiocarbonate, TTC) was dissolved in methanol/water mixture (2:8, w/w; 4.0 g). The solution was adjusted to pH 10.0, bubbled with argon for 40 min, and stirred at 25°C overnight. Sample (0.5 mL) was taken, dialyzed against water at pH 2.5, and freeze-dried as the intermediate polymer, **P-2**. The solution was acidified to pH 3.5. HEA (0.17 g, 1.44 mmol) and Me_2PPH (7.9 mg, 57.4 μmol) were added. Solution was stirred at 25°C for 24 h, dialyzed against water at pH 2.5, and freeze-dried to afford the final product, **P-3**. Yield: 0.72 g, 85%. Figure S1a shows that the bands at $\lambda_{max,\,TTC}$ = 304 nm and $\lambda_{max,\,SH}$ = 408 nm (*inset*) disappeared. End-group protection was confirmed by ^{1}H NMR spectroscopy (Figure S1b), and >99% conv. was determined using Eq. S1. Quantification using Eqs. S2–S4 confirmed that the polymer kept unchanged. Figure S1c shows that SEC traces are identi-

cal at M_n = 24.8 kDa and $Đ$ = 1.12. These confirmed successful preparation of well-defined and end-group protected $A_{58}B_{57}C_{58}$ triblock copolymer.

$$\text{Conversion} = \frac{I_{u+v}}{I_{x+y}} \times 100\% \quad \text{(S1)}$$

$$\text{DP}_{PHisAM} = 2 \times \frac{I_{m+p+q} - I_{x+y}}{I_{x+y}} \quad \text{(S2)}$$

$$\text{DP}_{PHPMA} = 4 \times \frac{I_h}{I_{x+y}} \quad \text{(S3)}$$

$$\text{DP}_{PAEMA} = \frac{I_{c+l} - I_{m+p+q} + I_{x+y}}{I_{x+y}} \quad \text{(S4)}$$

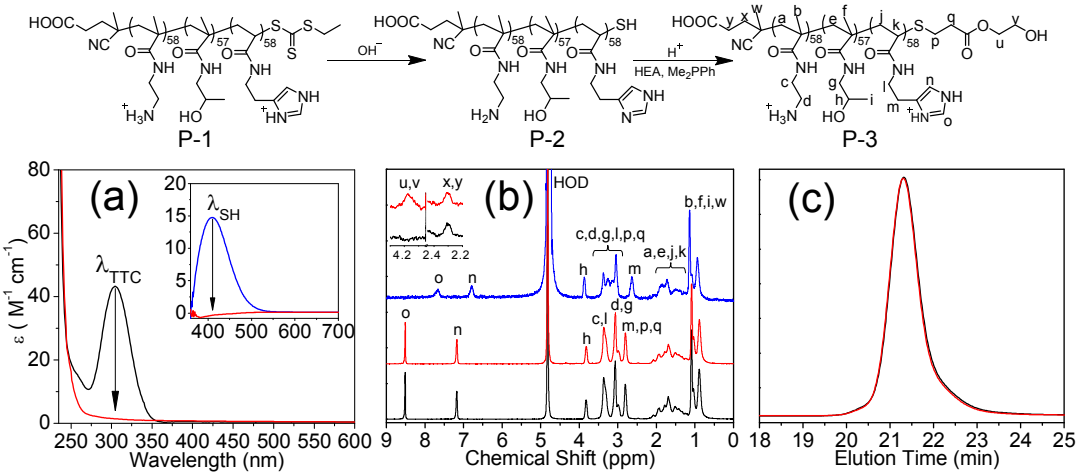

Figure S1. Schematic illustration of end-group protection. (a) UV-vis spectra of **P-1** (*black*), **P-3** (*red*); *inset*: Ellman solutions of **P-2** (*blue*), **P-3** (*red*). (b) ^{1}H NMR spectra of **P-1** (*black*), **P-3** at pH 2.5 (*red*), 6.4 (*blue*). (c) SEC traces of **P-1** (*black*), **P-3** (*red*).

Preparation of Dumbbell-Shape SCNP-1. Briefly, **P-3** (0.300 g, 1.17 mmol HisAM + AEMA) and $CuCl_2$ (50 mg, 0.29 mmol) were dissolved in 150 mL water. The solution was acidified at pH 2.5, and neutralized stepwise to pH 6.4 using 0.5 M NaOH. The solution was stirred at room temperature overnight. Sample was taken and analyzed using UV-vis spectroscopy, FTIR, ^{1}H NMR, DOSY, DLS, and TEM.

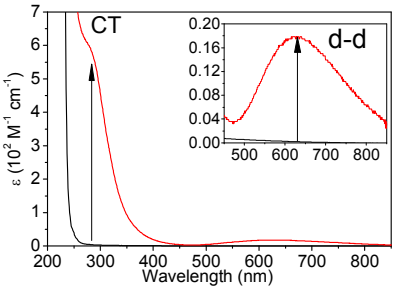

Figure S2. UV-vis spectra of the **P-3** solutions at [Cu(II)]/[ligands] =1/4, pH 2.5 (*black*) and 6.4 (*red*).

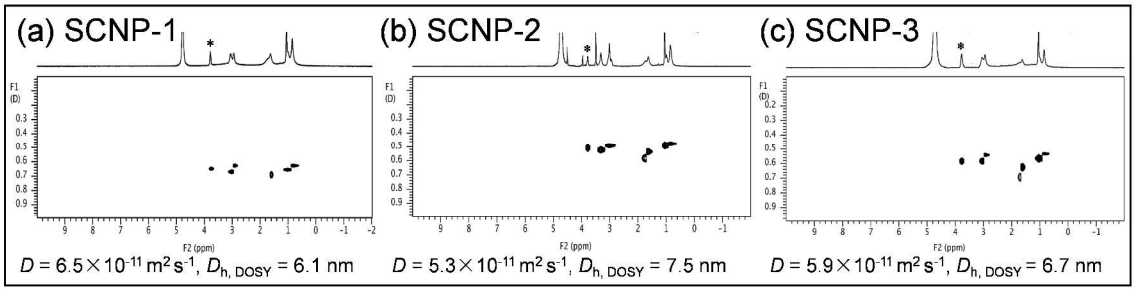

Figure S3. DOSY spectra of (a) **SCNP-1**, (b) **SCNP-2** prepared by the reduction of SCNP-1 at f =2.0, and (c) **SCNP-3** prepared by air oxidation of SCNP-2. Herein, D and $D_{h,\,DOSY}$ are diffusion coefficient and mean hydrodynamic diameter, respectively.

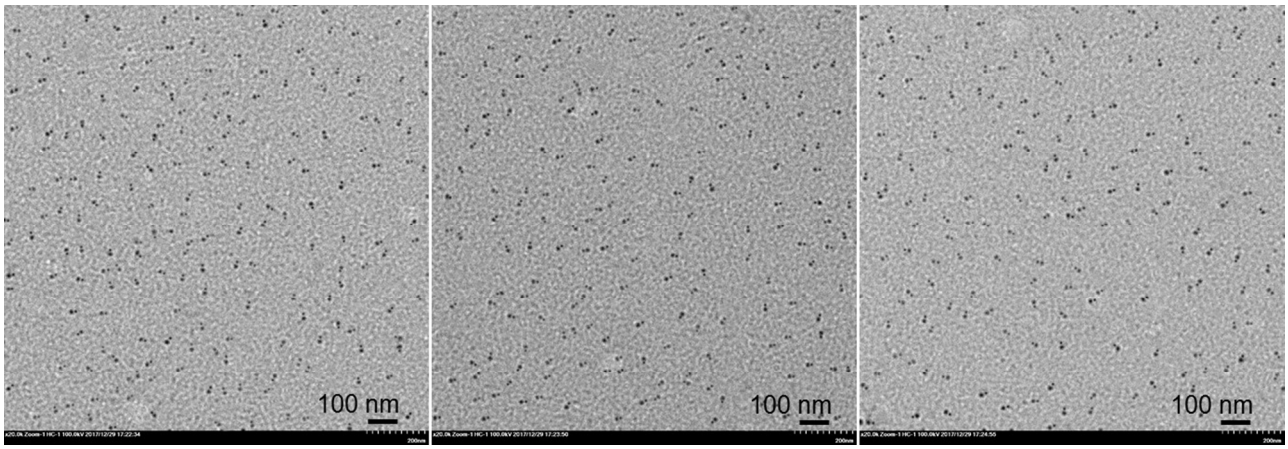

Figure S4. TEM images of **SCNP-1**, which clearly display the discrete double heads. Statistical analysis from >500 particles, using a Nano Measurer 1.2 software, indicated the D_n values of 13.6 and 9.4 nm of these discrete double heads.

Ascorbic Acid Reduction of SCNP-1. Typically, the argon-saturated AA solution (3.0 w/w%, 0, 12.9, 25.7, 38.6, 51.5 mg; 0, 2.0, 4.0, 5.8, 7.8 μmol AA) was added into the argon-saturated **SCNP-1** solution (2.0 mg/mL, 2.0 mL; 3.9 μmol copper ions). The solution was adjusted to pH 6.4, bubbled with argon for 45 min, and stirred at 25°C overnight. Sample was analyzed with UV-vis spectroscopy, FTIR, ^{1}H NMR, DOSY, DLS, and TEM.

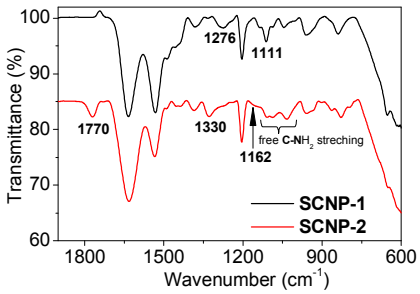

Figure S5. FTIR spectra of dried **SCNP-1** (*black*) and **SCNP-2** (*red*). Cu(II)-imidazole's C=N ring stretching vibration[4] at 1276 cm^{-1} (**SCNP-1**) upshifted to 1330 cm^{-1} [Cu(I)-imidazole's C=N ring stretching vibration[5] in **SCNP-2**], suggesting the conversion of Cu(II)-imidazole into Cu(I)-imidazole. Moreover, Cu(II)-NH_2 complex C-N stretching[6] at 1111 cm^{-1} (**SCNP-1**) disappeared and Cu(I)-NH_2 complex's C-N stretching[7] at 1162 cm^{-1} is unobservable in **SCNP-2**, and the C-N stretching[6] of free alkyl-NH_2 in **SCNP-2** is observed. These results suggest that C-block's NH_2-ligands in **SCNP-2** have been dissociated. In addition, ascorbic acid C=O stretching[8] at 1770 cm^{-1} was observed in **SCNP-2**. These results confirmed that **SCNP-1**'s Cu(II)-ligands converted into solely Cu(I)-imidazole complex in **SCNP-2**. That is, C-block disassembled and A-block reassembled upon the metal reduction at f = 2.0.

The fraction of unfolded C-block (PAEMA%) was determined by ^{1}H NMR studies using Eq. S5, where $I_{3.4}$, $I_{3.9}$ are integral signals CONHC**H**$_2$CH$_2$NH$_3^+$ of AEMA unit at λ=3.4 ppm, C**H**(OH)CH$_3$ of HPMA unit at 3.9 ppm (Figure 2B), respectively.

$$\text{PAEMA\%} = \frac{I_{3.4}}{2 \times I_{3.9}} \times \frac{DP_{PHPMA}}{DP_{PAEMA}} \times 100\% \qquad \text{(S5)}$$

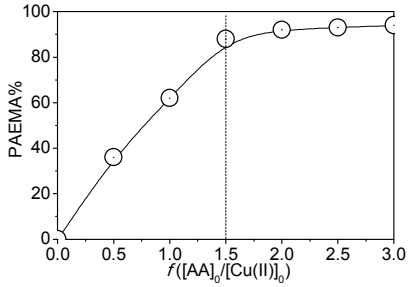

Figure S6. The variation of unfolded C-segmental fraction (PAEMA%) upon reduction with AA at different f values.

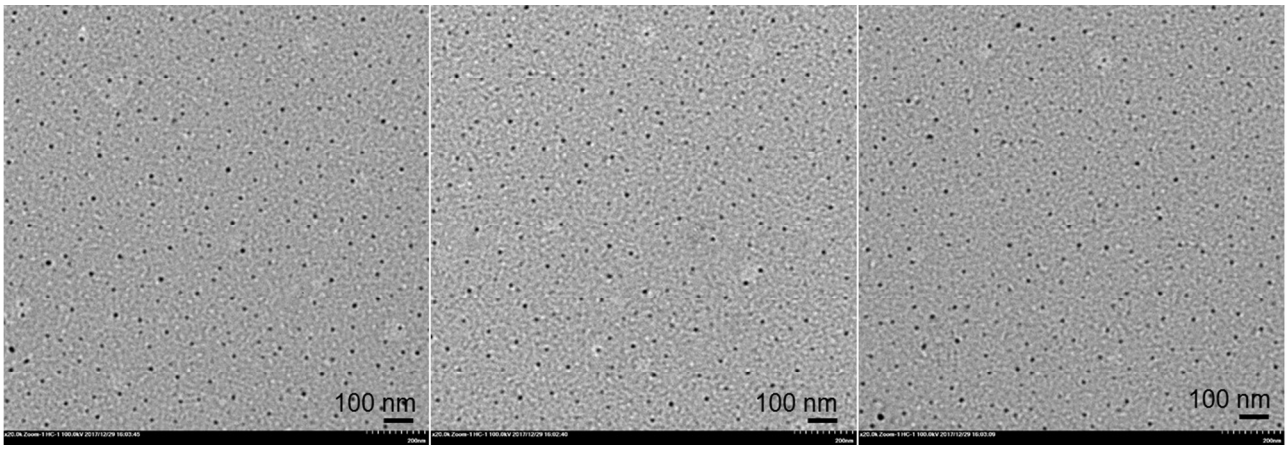

Figure S7. TEM images of **SCNP-2** prepared by reduction of SCNP-1 at f = 2.0, which confirmed the spherical shape of as-formed SCNPs. Statistical analysis from >500 particles using a Nano Measurer 1.2 software indicated a D_n value of 15.6 nm.

Ascorbic Acid Reduction of Diblock Copolymer Cu(II)-SCNPs. $PHPMA_{60}$-*b*-$PHisAM_{56}$ (0.10 g, 0.28 mmol HisAM), $CuCl_2$ (11.8 mg, 69 μmol) were dissolved in 50 mL water. Solution was acidified to pH 2.5 and then neutralized to pH 6.4 to yield Cu(II)-SCNP. AA (3.0 w/w%, 183 mg; 27.7 μmol) was added into the solution (5.0 mL, 7.0 μmol copper). The solution was adjusted to pH 6.4, bubbled with argon gas for 45 min, and stirred at 25°C overnight to yield Cu(I)-SCNP. AA-reduction of Cu(II)-SCNPs of $PHPMA_{55}$-*b*-$PAEMA_{57}$ proceeded under the same conditions except using $PHPMA_{55}$-*b*-$PAEMA_{57}$ copolymer.

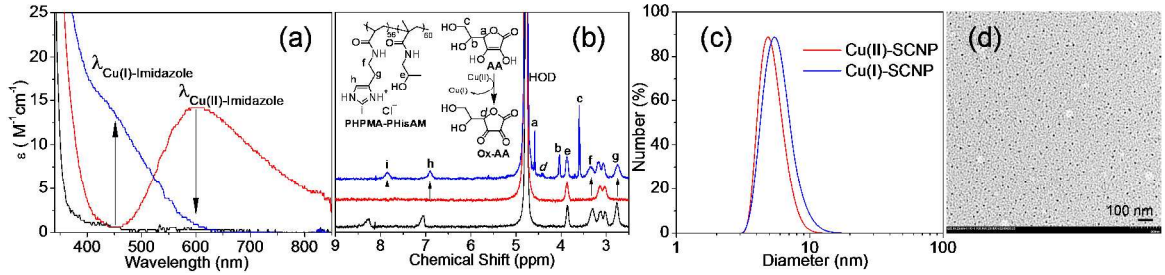

Figure S8. (a) UV-vis and (b) ^{1}H NMR spectra of $PHPMA_{60}$-*b*-$PHisAM_{56}$ (*black*), the Cu(II)-SCNP (*red*) and Cu(I)-SCNP (*blue*). (c) DLS diameter profiles of SCNPs. (d) TEM image of Cu(I)-SCNP. Based on the ^{1}H NMR studies using Eqs. S6-S8, 53% PHisAM segments unfolded, 54% AA (F_{AA}) retained, and only 5% of as-generated Ox-AA ($F_{Ox\text{-}AA}$) are detectable.

$$\text{PHisAM\%} = \frac{I_g}{2\times I_e} \times \frac{DP_{PHPMA}}{DP_{PHisAM}} \times 100\% \qquad \text{(S6)}$$

$$F_{AA} = 2 \times \frac{I_c}{I_e} \times \frac{DP_{PHPMA}}{DP_{PHisAM}} \times \frac{[Cu(II)]_0}{[AA]_0} \times 100\% \qquad \text{(S7)}$$

$$F_{Ox-AA} = 4 \times \frac{I_d}{I_e} \times \frac{DP_{PHPMA}}{DP_{PHisAM}} \times \frac{[Cu(II)]_0}{[AA]_0} \times 100\% \qquad \text{(S8)}$$

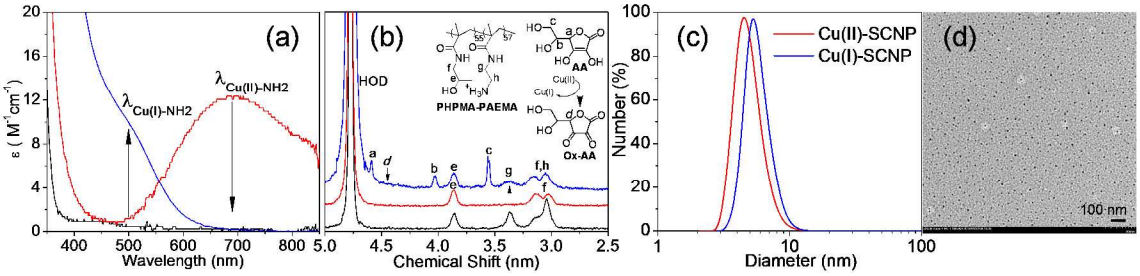

Figure S9. (a) UV-vis and (b) ^{1}H NMR spectra of $PHPMA_{55}$-*b*-$PAEMA_{57}$ (*black*), the Cu(II)-SCNP (*red*) and Cu(I)-SCNP (*blue*). (c) DLS diameter profiles of SCNPs. (d) TEM image of Cu(I)-SCNP. Based on ^{1}H NMR studies using Eqs. S9, S10, 52% PAEMA segments unfolded and 54% AA (F_{AA}) retained. Figure S9b shows that Ox-AA signal *d* disappeared, suggesting full encapsulation of Ox-AA into as-reassembled heads.

$$\text{PAEMA\%} = \frac{I_g}{2\times I_e} \times \frac{DP_{PHPMA}}{DP_{PAEMA}} \times 100\% \qquad \text{(S9)}$$

$$F_{AA} = 2 \times \frac{I_c}{I_e} \times \frac{DP_{PHPMA}}{DP_{PAEMA}} \times \frac{[Cu(II)]_0}{[AA]_0} \times 100\% \qquad \text{(S10)}$$

Air Oxidation of SCNP-2. SCNP-2 solution was bubbled with fresh air at 8-10 bubbles/min at 25°C overnight. Fresh air was obtained by removal of CO_2 and water vapor *via* passing through an anhydrous KOH column. Sample was taken and analyzed with UV-vis spectroscopy, ^{1}H NMR, DOSY, DLS, TEM, and aqueous electrophoresis.

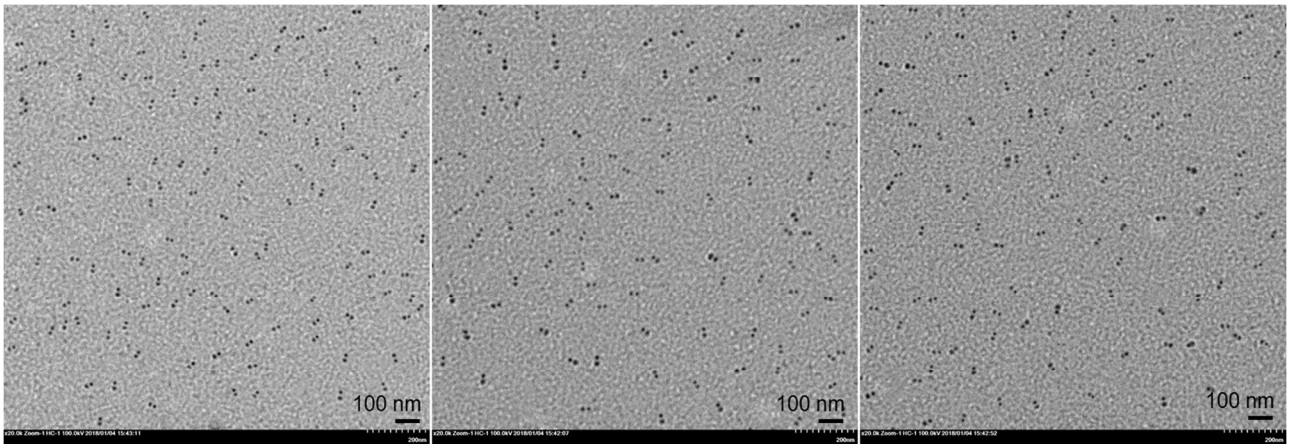

Figure S10. TEM images of **SCNP-3** prepared by air oxidation of SCNP-2 at pH 6.4 overnight, which clearly display their discrete double heads. Statistical analysis from >500 particles indicated the D_n values of 15.5 and 12.6 nm of the discrete double heads.

Salting-Out Effect. This effect was confirmed by comparing **Particle I**, formed by addition of NaCl into SCNP-2 solution, with **Particle II**, formed by addition of AA (equal molar to aforementioned NaCl) into SCNP-1 solution. Briefly, **Particle I** was prepared by adding the argon-saturated NaCl solution (1.76 M, 13 μL; 23.4 μmol) into argon-saturated **SCNP-2** solution (2.0 mL, 3.9 μmol copper ions; [NaCl]/[Cu(I)] = 6.0). **Particle II** was prepared by adding the argon-saturated AA solution (3.0 w/w%, 184 mg; 31.3 μmol AA) into the argon-saturated solution of SCNP-1 solution (2.0 mL; 3.9 μmol copper centers; f = 8.0). These solutions were adjusted to pH 6.4 and stirred at 25°C overnight prior to the measurements.

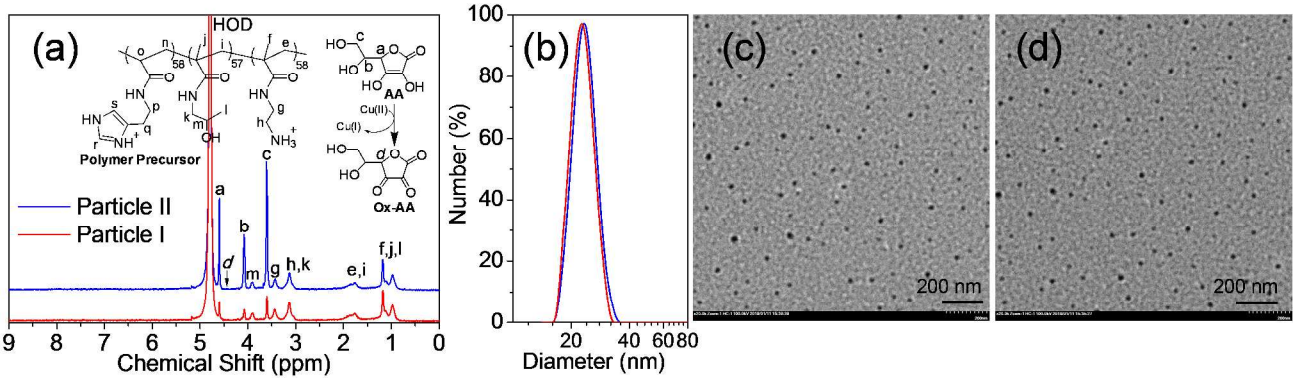

Figure S11. (a) ^{1}H NMR spectra and (b) DLS diameter profiles of **Particle I** (*red*) and **Particle II** (*blue*). TEM images of (c) **Particle I** and (d) **Particle II**.

Figure S11a indicates that, in both cases, A-segments collapsed, C-segments unfolded, and Ox-AA has been encapsulated. DLS and TEM studies confirmed comparable size distributions of these spherical nanoparticles (**Particle I:** $D_{h,\,DLS}$ = 19.1 nm, D_n = 24.1 nm; **Particle II:** $D_{h,\,DLS}$ = 19.4 nm, D_n = 24.6 nm). These results confirmed that the unreacted AA played electrolyte role, which brought about salting-out effect that could induce the undesired intermolecular self-assembly.

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