Noncovalent structural locking of thermoresponsive polyion complex micelles, nanowires, and vesicles via polymerization-induced electrostatic self-assembly using arginine-like monomer

Qingqing Zhao, Qizhou Liu, Chao Li, Lei Cao, Lei Ma, Xiyu Wang and Yuanli Cai*

Electronic Supplementary Information

Materials. Synthesis of 2-hydroxypropyl methacrylamide[1] (HPMA), 2-aminoethyl methacrylamide hydrochloride[2] (AEMA), 4-cyano-4-ethylsulfanylthiocarbonylsulfanylpentanoic acid[3] (CEP), and sodium phenyl-2,4,6-trimethylbenzoylphosphinate[4] (SPTP) are described elsewhere. 2-Acrylamido-2-methylpropanesulfonic acid (AMPS) monomer was purchased from Sigma-Aldrich; N,N'-di-BOC-1H-pyrazole-1-carboxamidine (PCA) from 3A Chemicals; deuterium oxide (D_2O, 99.8% D), DCl (20% in D_2O, 99.5% D), dimethyl sulfoxide-d_6 (DMSO-d_6, 99.9%D) and chloroform-d (99.8%D) from J&K; other reagents from Aladdin; these reagents were used as received. Deionized water was obtained from a Direct-Q 5 UV Millipore system.

Synthesis of 2-guanadinoethyl methacrylamide hydrochloride (GEMA) monomer. GEMA monomer was synthesized using literature procedures.[5] AEMA precursor (8.0 g, 49 mmol), triethylamine (22.0 mL, 158 mmol) and water (16.0 mL) were added in a 250 mL flask. PCA (10 g in 144 mL acetonitrile, 32 mmol) was added dropwise into the flask under stirring for 30 min and stirred at room temperature for 1 day. The solvents were removed by rotary evaporation. The mixture was poured into water (800 mL) and filtered. The solids were washed with water, freeze-dried to obtain BOC-protected GEMA (9.66 g, yield: 81%). GEMA was obtained by hydrolysis of BOC-protected GEMA in 4.0 M HCl in dioxane (7.0 molar to BOC) at 25 °C overnight, in which monomer was precipitated. After filtration, the solids were washed with dioxane and ethyl ether, dried in a vacuum oven to afford GEMA monomer (yield: 4.5 g, 83%). ^{1}H NMR (400 MHz, in DMSO-d_6, δ/ppm): 1.87 (3H, C**H$_3$**C=CH$_2$), 3.24 (4H, CONHC**H$_2$**C**H$_2$**), 5.73/5.36 (2H, CH$_3$C=C**H$_2$**), and 8.2-6.7 (CON**H**CH$_2$, guanidinium protons).

Synthesis of poly(2-hydroxypropylmethacrylamide) (PHPMA). This macro-CTA was synthesized according to our previous procedures.[6] HPMA monomer (10.26 g, 71.75 mmol), CEP chain transfer agent (0.19 g, 0.72 mmol) were dissolved in 2-butoxyethanol/water (30:70 w/w, 10.51 g) and adjusted to pH 2.5 in a 50 mL flask. SPTP initiator (55.6 mg, 0.18 mmol) was added into the flask. The flask was sealed and immersed in a water bath at 25 °C. The solution was bubbled with argon gas in the dark for 1 h, and irradiated with visible light for 1.5 h. The reaction was quenched by exposure to air. ^{1}H NMR: 65% conv. The polymer was precipitated into acetone, washed using this solvent, and dried in a vacuum oven. Yield: 6.36 g, 95%. ^{1}H NMR: DP = 63, PHPMA$_{63}$; SEC: M_n = 8.7 kDa, $Đ$ = 1.17.

Synthesis of poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS). This polyanion was synthesized using our previous procedures.[7] First, AMPS monomer (42.5% in water at pH 2.5, 10.64 g; 19.7 mmol) and CEP chain transfer agent (52.6 mg in 2.62 g methanol; 0.20 mmol) were added in a 50 mL flask. The solution was adjusted to pH 2.5. SPTP initiator (15.3 mg, 49.37 μmol) was added into the flask in the dark. The flask was sealed, immersed in a water bath at 25 °C. The solution was bubbled with argon gas for 1 h, and irradiated with visible light for 3.5 h. The reaction was quenched by exposure to air. ^{1}H NMR: >99% conv. RAFT end-group (trithiocarbonate, TTC) was removed by H_2O_2 oxidation reaction at H_2O_2/TTC = 30 under stirring at 80 °C for 1 day.[8] The polymer was dialyzed against water and freeze-dried. Yield: 4.02 g, 89%. ^{1}H NMR: DP = 100; SEC: M_n = 22.7 kDa, $Đ$ = 1.12; UV-vis: without TTC and thiol impurities.

PIESA Synthesis of $A_{63}B_x/C_{100}$ PICs. Typically, GEMA monomer (84 mg, 0.41 mmol), PHPMA$_{63}$ macro-CTA (62.9 mg, 6.8 μmol) and PAMPS$_{100}$ (93.9 mg, 0.41 mmol units) were dissolved in water (0.91 g) in a 5 mL flask. The solution was adjusted to pH 2.5. SPTP initiator (1.0% w/w in water; 52.7 mg, 1.7 μmol) was added into the flask. The flask was sealed and immersed in a water bath at 25 °C. The solution was bubbled with argon gas in the dark for 1 h, and irradiated with visible light overnight. The reaction was quenched by exposure to air. The sample was studied without purification. ^{1}H NMR: >99% conv. Other PIESA syntheses proceeded under the above conditions, but, changing x (GEMA/PHPMA$_{63}$) for PIESA at 25 °C, or reaction temperature for PIESA synthesis of $A_{63}B_{60}/C_{100}$ spheres, nanowires and vesicles.

Instrumentation. Incident visible light at $I_{420\ nm}$ = 0.20 mW/cm^2 was obtained from a home-made system equipped with 400 W mercury lamp, JB400 filter, UV-A radiometer and ventilator. The polymer sample was dried in a Labconco Freezone 2.5L freeze-drier. The solution pH was probed using an OHAUS ST3100 digital pH-meter.

Characterization. *^{1}H NMR spectroscopy* was performed on INOVA 400 MHz NMR instrument at 25 °C or otherwise mentioned. *Size exclusion chromatography* (SEC) was conducted on a PL-GPC220 integrated system equipped with a refractive index detector and a column set (2 × PLGel MIXED-B + 1 × PLGel MIXED-D). DMF containing LiBr (10 mM) was used

as eluent. PMMA standards (Agilent, 1.95 – 1048.0 kDa) were used for calibration. The calibration and analysis were performed at a flow rate of 1.0 mL min^{-1} at 80 °C. To prevent column adsorption, guanidinium groups were reacted with di-*tert*-butylpyrocarbonate in dioxane/water (1:1, v/v) at pH 12 at 4 °C for 1.5 days and room temperature for 1 day. The solvents were removed by rotary evaporation. Solids were dispersed in dioxane/methanol (1:1, v/v) and passed through silica column to remove salts. After rotary evaporation, the copolymer was dissolved in DMF. The solution was filtered using a Millipore Millex-FG 0.2 µm filter prior to SEC studies. *Aqueous electrophoresis* was conducted on a Malvern Zetasizer Nano-ZS90 instrument. Data were averaged over 5 runs. *Dynamic Light Scattering (DLS)* was performed on a Brookhaven BI-200SM setup equipped with 22-mW He-Ne laser (λ = 633 nm), BI-200SM goniometer and BI-TurboCorr digital correlator. The final dispersion was diluted to 1.0 mg/mL and measured at 90°, at 25 °C or otherwise mentioned. The data were obtained by cumulants analysis in CONTIN routine, averaged over 5 runs. *Transmission Electron Microscopy (TEM)* was conducted on a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 120 kV. The final dispersion was diluted to 1.0 mg/mL. Aliquot (10 µL) was dropped on a carbon-film-coated copper grid, frozen in liquid nitrogen for 0.5 h and freeze-dried under reduced pressure prior to TEM studies. *Atomic Force Microscopy (AFM)* was conducted on a Bruker Multimode 8 microscope in a peak force quantitative nanomechanical mode. Silicon wafer was immersed into piranha solution at 80 °C for 1.5 h, washed with water, ethanol, acetone, ethanol and water under ultrasonic agitation. Final dispersion was diluted to 1.0 mg/mL. Aliquot (10 µL) was dropped onto a clean silica wafer, frozen in liquid nitrogen and freeze-dried under reduced pressure for AFM studies.

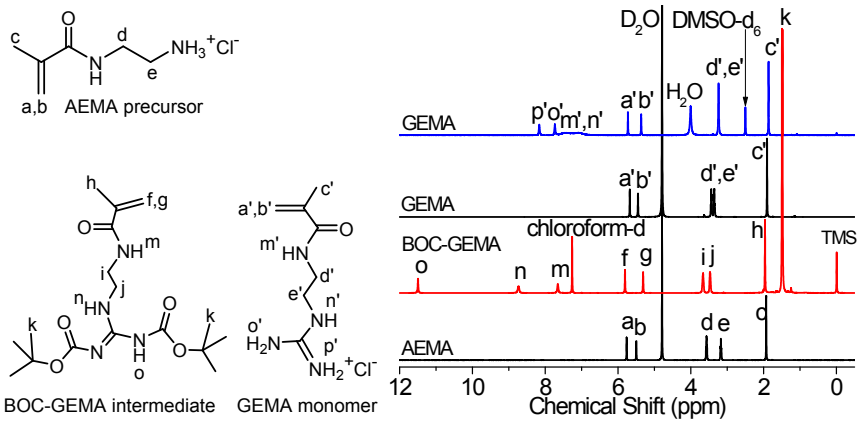

Fig. S1 ^{1}H NMR spectra of AEMA precursor, BOC-GEMA intermediate and GEMA monomer as recorded in (black) D_2O, (red) chloroform-d and (blue) DMSO-d_6.

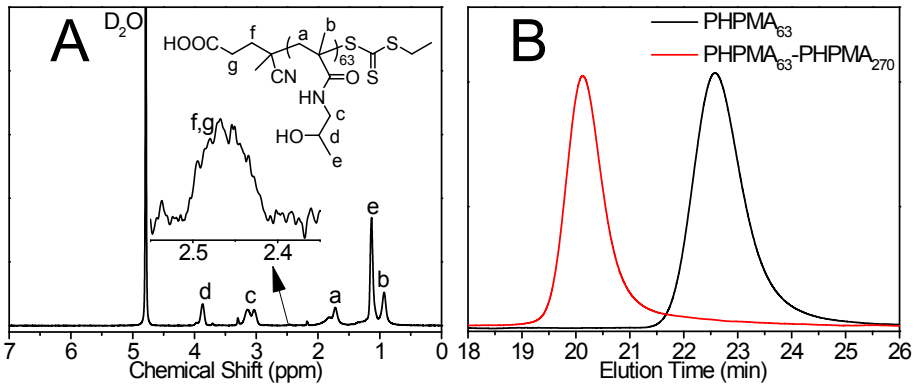

Fig. S2 (A) ^{1}H NMR spectra of $PHPMA_{63}$ macro-CTA, in which DP was calculated according to DP = 4 × I_d / I_{f+g} = 63. (B) SEC traces of $PHPMA_{63}$ macro-CTA and chain-extended $PHPMA_{63}$-$PHPMA_{270}$, in which the clear shift of SEC trace suggests high fidelity of RAFT end-groups (trithiobarbonate, TTC).

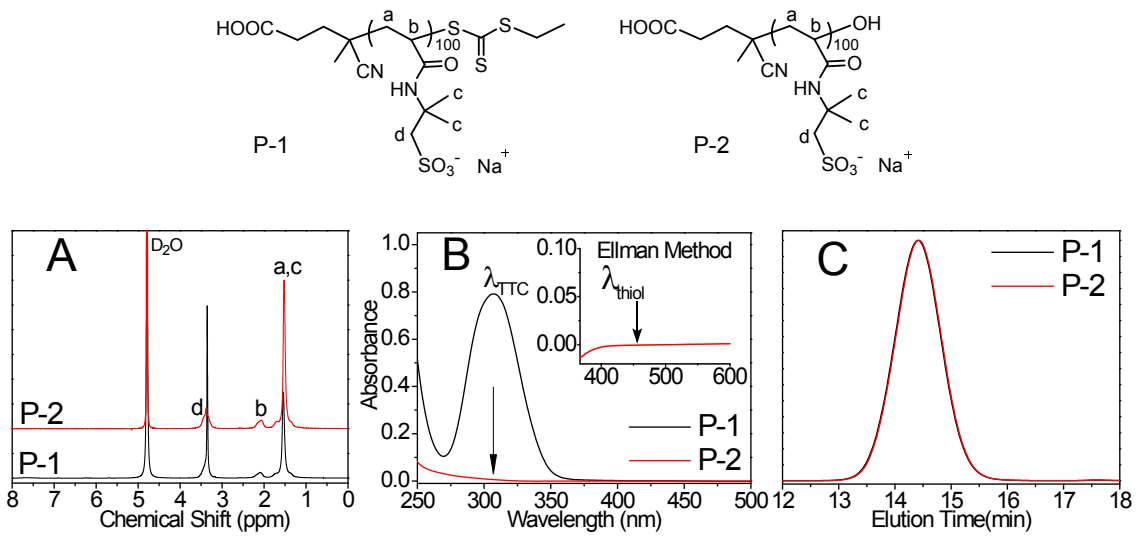

Fig. S3 (A) ^{1}H NMR and (B) UV-vis spectra, and (C) SEC traces of $PAMPS_{100}$ (**P-1**, black) and that after H_2O_2 oxidation removal of TTC end-groups (**P-2**, red).

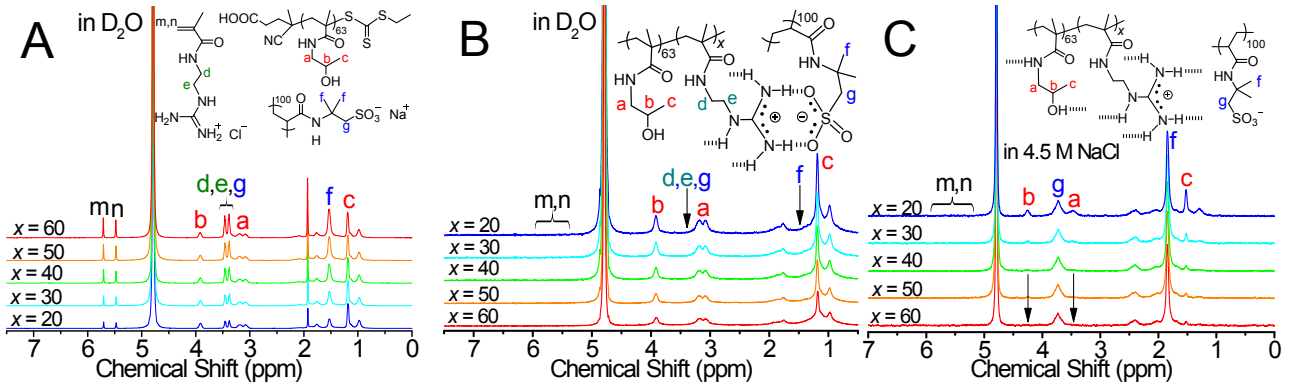

Fig. S4 ^{1}H NMR spectra of reaction mixtures (A) before and (B) after polymerization at GEMA/PHPMA/SPTP = x : 1 : 0.25, n^{+}/n^{-} = 1, at 20% w/w solids in water at pH 2.5 under visible light irradiation at 25 °C overnight (C: the final solutions after salted at 4.5 M NaCl).

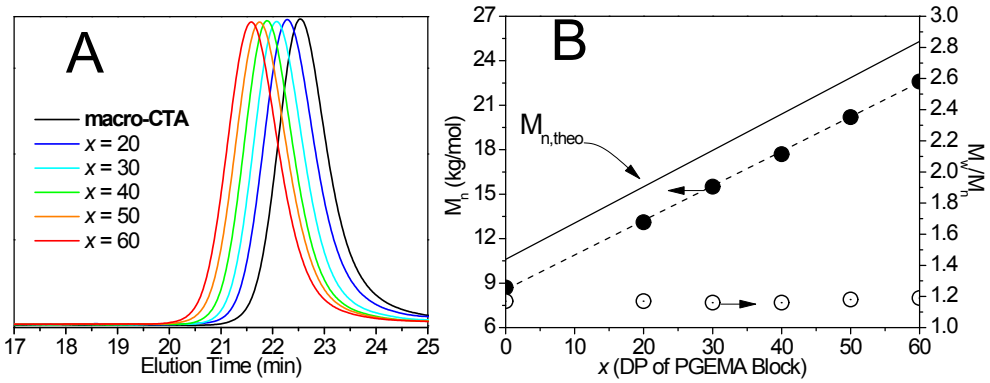

Fig. S5 (A) SEC traces of $PHPMA_{63}$-$PGEMA_x$ ($A_{63}B_x$) block copolymers at labelled x values. (B) Number-average molecular weight (M_n), dispersity ($Đ$, M_w/M_n) vs x (DP_{PGEMA}).

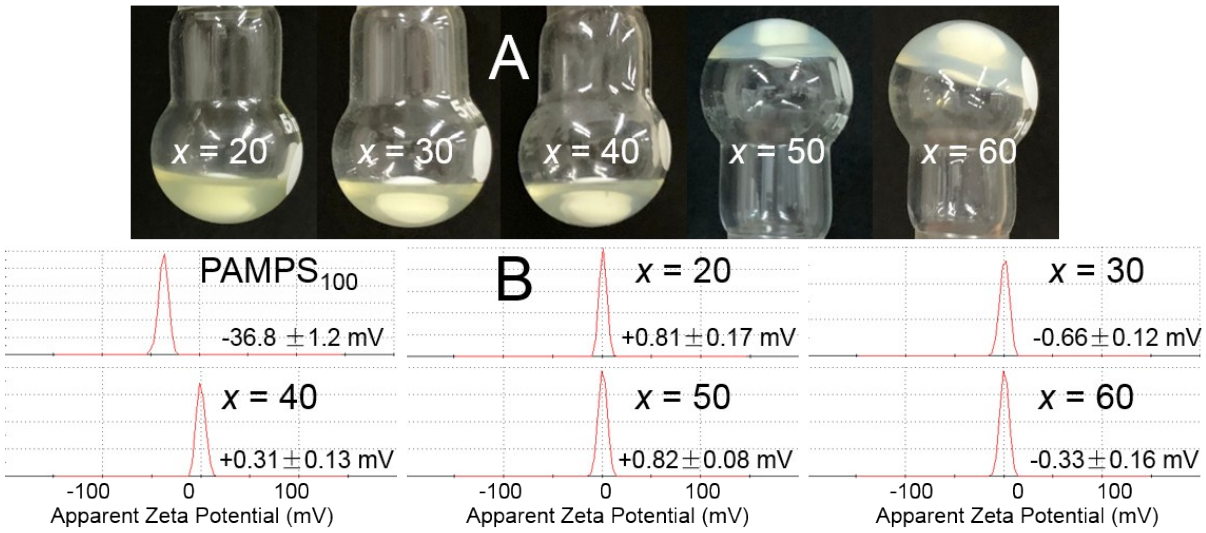

Fig. S6 (A) Digital photographs of final $A_{63}B_x/C_{100}$ dispersions at labelled *x* values. (B) The ζ-potential results of these PIC particles (1.0 mg/mL in water at pH 2.5).

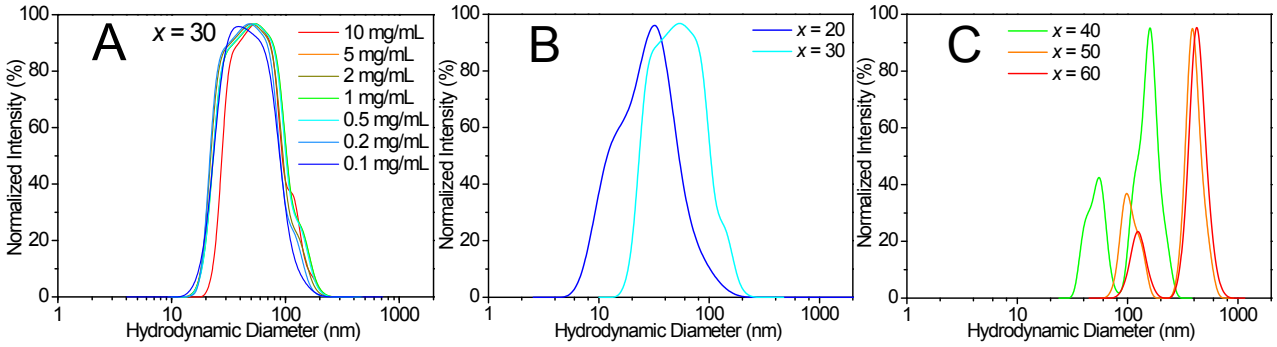

Fig. S7 Particle size distributions of $A_{63}B_x/C_{100}$ PICs: (**A**) at *x* = 30 at labelled concentrations; (**B, C**) at labelled *x* values at 1.0 mg/mL in water at pH 2.5.

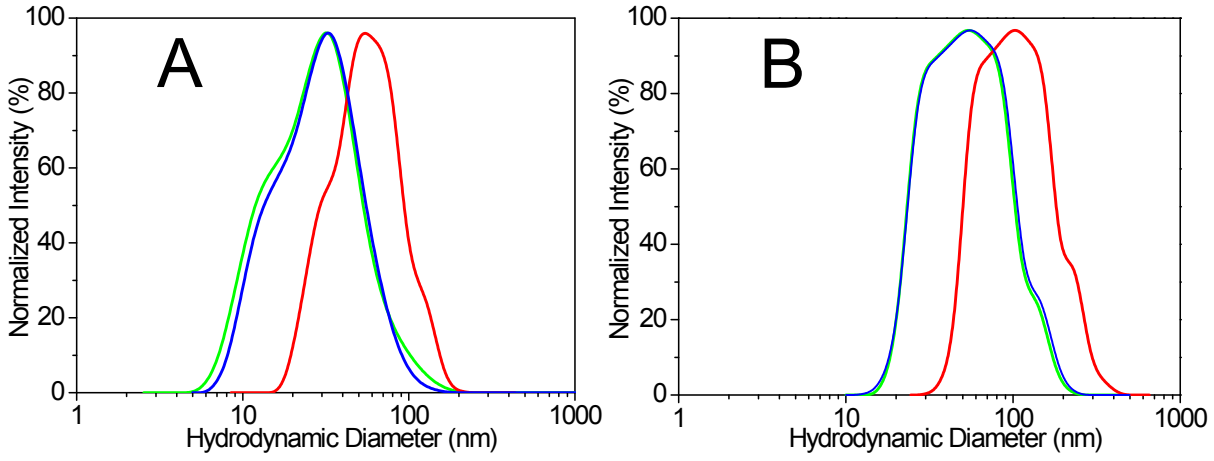

Fig. S8 Thermo-responsive evolution of particle size distributions (initially at 25 °C: green) upon heating to 70 °C (red) and cooling to 25 °C (blue). (A) $A_{63}B_{20}/C_{100}$, (B) $A_{63}B_{30}/C_{100}$.

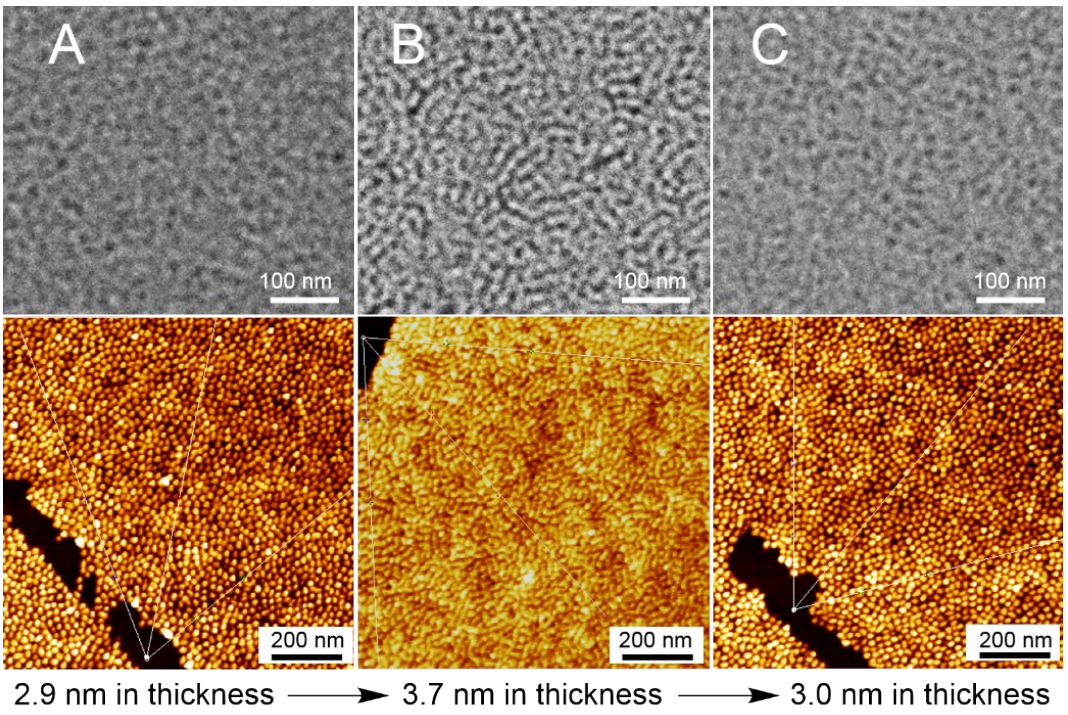

Fig. S9 TEM micrographs (top) and AFM images (bottom) of $A_{63}B_{20}/C_{100}$ monolayer colloidal nanosheets before (A) and after heating to 70 °C (B) and cooling to 25 °C (C).

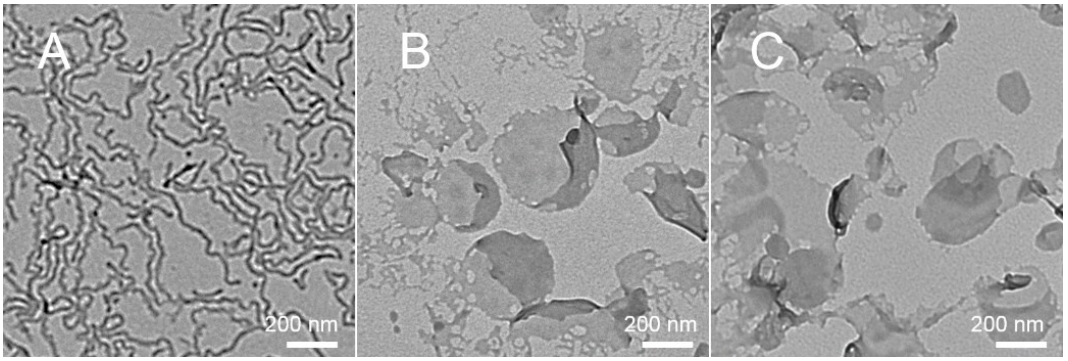

Fig. S10 TEM images of as-synthesized $A_{63}B_{50}/C_{100}$ nanowires (A) and the jellyfishes formed by heating to 70 °C (B) and subsequently cooling to 25 °C (C).

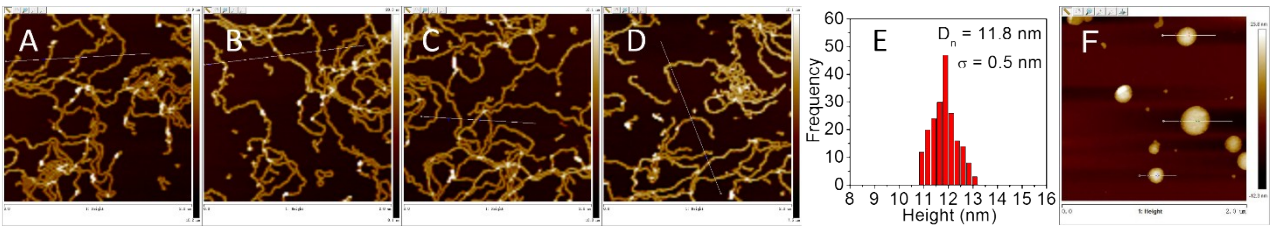

Fig. S11 AFM images of (**A-D**) $A_{63}B_{60}/C_{100}$ nanowires, in which (**E**) the mean height or vertical diameter (D_n) was determined by statistical analysis from 200 points, and (F) the heating-transformed vesicles whose membrane thickness was calculated by: thickness = height/2 = 11.4 nm based on statistical analysis from 200 points.

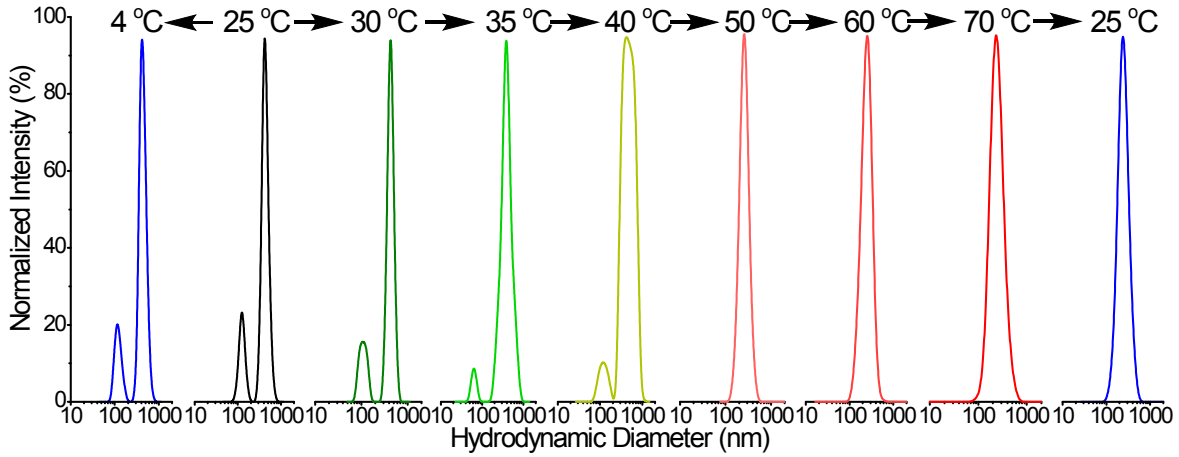

Fig. S12 Evolution of DLS particle size distribution of $A_{63}B_{60}/C_{100}$ PICs in a dispersion (1.0 mg/mL) upon cooling to 4 °C, and stepwise heating to 70 °C finally cooling to 25 °C.

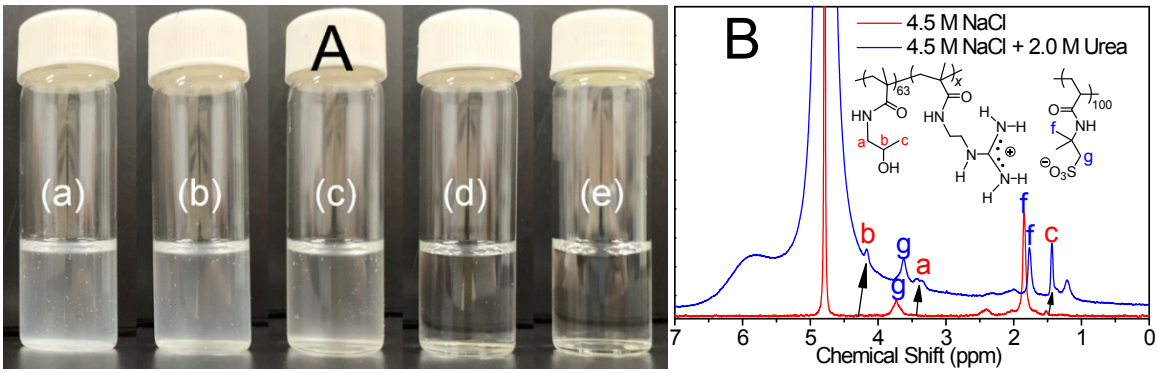

Fig. S13 (A) Digital photographs of $A_{63}B_{60}/C_{100}$ salted solutions (4.5 M NaCl) upon stepwise adding (a) 0 M, (b) 0.5 M, (c) 1.0 M, (d) 1.5 M, and (e) 2.0 M urea. (B) Evolution of ^{1}H NMR spectrum of the salted solution (red, at 4.5 M NaCl) after adding 2.0 M urea (blue), reappearance of both PGEMA and PHPMA signals suggests that PHPMA/PGEMA associative hydrogen bonding interactions occurred in the salted dispersion.

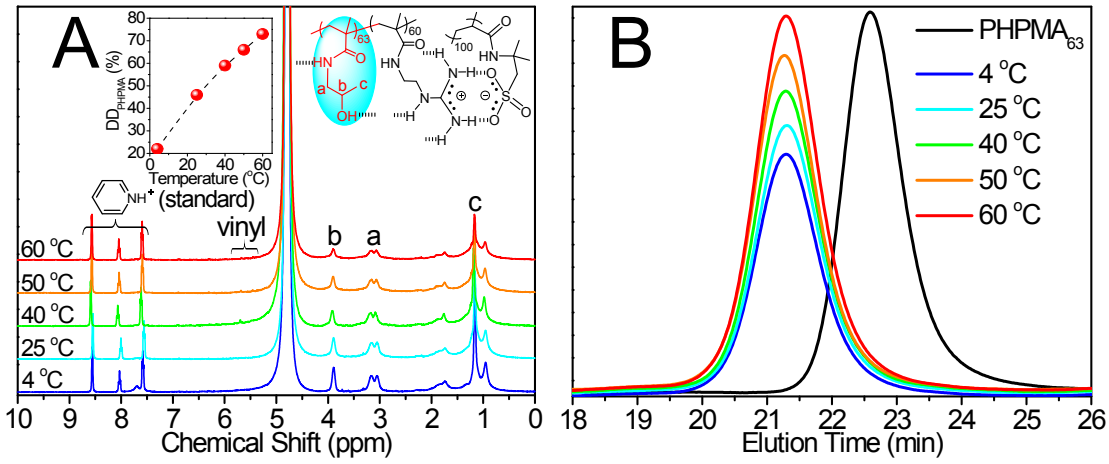

Fig. S14 (A) ^{1}H NMR spectra of final dispersions synthesized via PIESA at GEMA/$PHPMA_{63}$/SPTP = 60: 1:0.25 and n^+/n^-=1:1, 20% w/w solids at labelled reaction temperatures (*inset*: $PHPMA_{63}$ dehydration *vs.* reaction temperature). (b) SEC traces of $PHPMA_{63}$ macro-CTA and the chain-extended block copolymers.

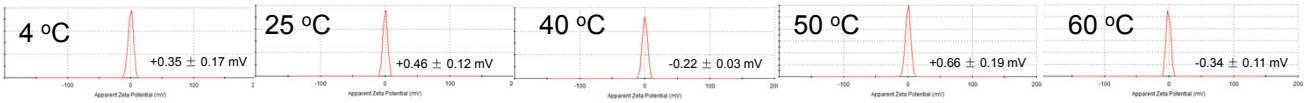

Fig. S15 The ζ-potential results of $A_{63}B_{60}/C_{100}$ PICs synthesized *via* PIESA at labelled reaction temperatures, as determined by the aqueous electrophoresis at pH 2.5.

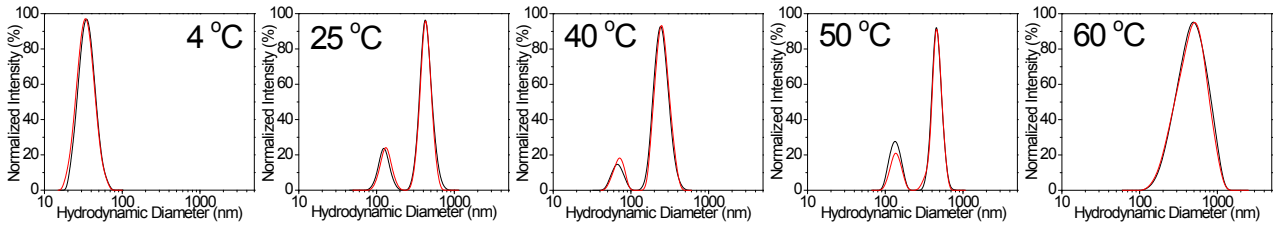

Fig. S16 DLS particle size distributions of (black) as-synthesized $A_{63}B_{60}/C_{100}$ PICs at labelled temperatures, and (red) those after incubation at 1.0 mg/mL at 25 °C for 15 days.

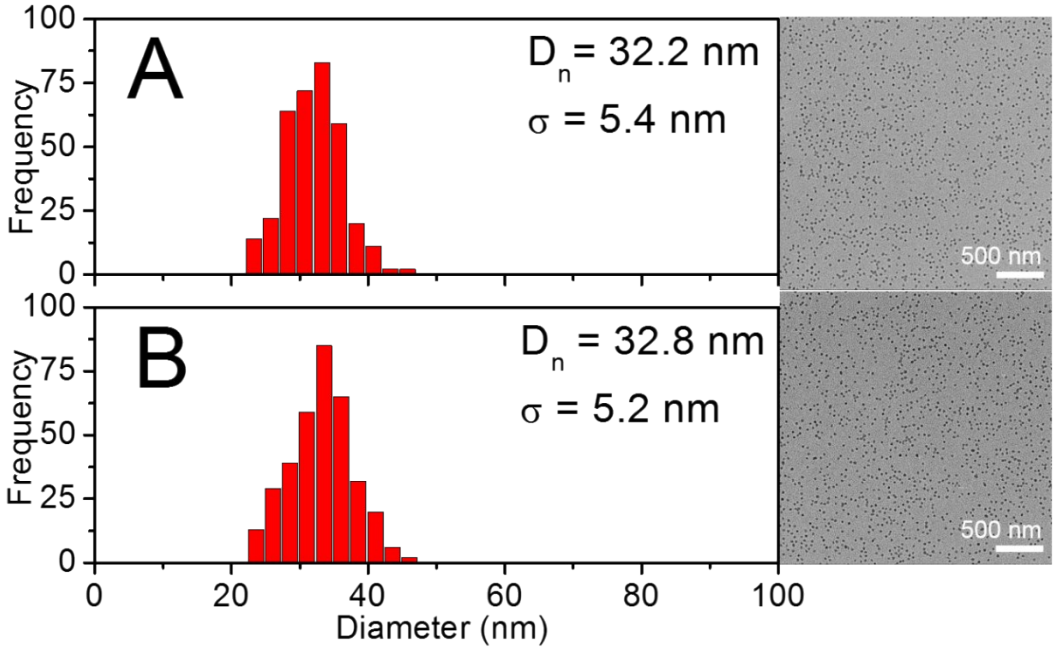

Fig. S17 TEM micrographs and statistical analysis results (D_n = number-average diameter, σ = standard deviation) of (A) $A_{63}B_{60}/C_{100}$ spheres synthesized via PIESA at 4 °C and (B) those after incubation in water at 1.0 mg/mL at 25 °C for 15 days. Herein, D_n and σ were determined by statistical analysis from 350 spheres.

References

1 C. W. Scales, Y. A. Vasilieva, A. J. Convertine, A. B. Lowe and C. L. McCormick, *Biomacromolecules*, 2005, **6**, 1846.
2 G. Liu, H. Shi, Y. Cui, J. Tong, Y. Zhao, D. Wang and Y. Cai, *Polym. Chem.*, 2013, **4**, 1176.
3 X. Xu, A. E. Smith, S. E. Kirkland and C. L. McCormick, *Macromolecules*, 2008, **41**, 8429.
4 J. Y. Tong, Y. Shi, G. H. Liu, T. Huang, N. Xu, Z. G. Zhu and Y. L. Cai, *Macromol. Rapid Commun.*, 2013, **34**, 1827.
5 S. E. Exley, L. C. Paslay, G. S. Sahukhal, B. A. Abel, T. D. Brown, C. L. McCormick, S. Heinhorst, V. Koul, V. Choudhary, M. O. Elasri and S. E. Morgan, *Biomacromolecules*, 2015, **16**, 3845.
6 Q. Yu, Y. Ding, H. Cao, X. Lu and Y. Cai, *ACS Macro Lett.*, 2015, **4**, 1293.
7 Y. Ding, M. Cai, Z. G. Cui, L. L. Huang, L. Wang, X. H. Lu and Y. L. Cai, *Angew. Chem., Int. Ed.*, 2018, **57**, 1053.
8 C. P. Jesson, C. M. Pearce, H. Simon, A. Werner, V. J. Cunningham, J. R. Lovett, M. J. Smallridge, N. J. Warren and S. P. Armes, *Macromolecules*, 2017, **50**, 182.