

Electronic Supplementary Information

Synthesis, Thermomorphic Characteristics, and Fluorescent Properties of Poly[2,7-(9,9-dihexylfluorene)]-*block*-poly(*N*-isopropylacrylamide)-*block*-poly(*N*-hydroxyethylacrylamide) Rod-Coil-Coil Triblock Copolymers

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Experimental

Materials

2,7-Dibromo-9,9-dihexylfluorene, trimethylborate, 4-bromobenzyl alcohol, sodium carbonate, tetrakis(triphenylphosphine) palladium(0), 4-dimethylaminopyridine, copper(I) bromide (98%), and *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA) were provided from Aldrich Chemical Co. and used without purification. *N*-hydroxyethylacrylamide was purchased from Kohjin Co., Japan, and used as received. *N*-isopropylacrylamide was provided by Kohjin Co., Japan, and re-crystallized three times from hexane/toluene (7/3, v/v) prior to use. Tris[2-(dimethylamino)ethyl]amine (Me₆TREN) was donated by Mitsubishi Chemical Co., Japan, and distilled over CaH₂ under reduced pressure. Triethylamine was obtained from Wako Pure Chemical Ind., Ltd., and distilled over CaH₂ prior to use. 5-Hexynoic chloride was synthesized in terms of the literature.^{S1} *N*-(2'-azidoethyl)-2-chloropropionamide was prepared according to our previous study.^{S2}

Synthesis of alkynyl-terminated PF (4)

The synthetic scheme for alkynyl-terminated PF is shown in Scheme 1(a). 2-Bromo-9,9-dihexylfluoren-7-boronic acid (**2**) was prepared base on the literature.^{S3} α -(4-Hydroxymethylphenyl)- ω -bromo-poly[2,7-(9,9-dihexylfluorene)] (**3**) was synthesized by Suzuki coupling reaction with 4-bromobenzyl alcohol as the end capper.^{S4} A flame-dried Schlenk flask with a magnetic stir bar was charged with the mixture of **3** (1.26 g, 0.5 mmol), 4-dimethylaminopyridine (61 mg, 0.5 mmol), anhydrous triethylamine (70 μ l, 0.5 mmol), and anhydrous tetrahydrofuran (THF, 4 ml). Then 5-hexynoic chloride (325 μ l, 2.5 mmol) was added dropwise with an argon-purged syringe under magnetic stirring. The mixture was immersed into an oil bath at 45 °C for 48 h. After cooling to ambient temperature, the mixture was diluted with THF and precipitated into methanol. The obtained residue was dried overnight under vacuum, and a brown powder of α -{4-[(5-hexynoate)methyl]phenyl}- ω -bromo-poly[2,7-(9,9-dihexylfluorene)] (**4**) was obtained (967 mg, 73% yield). ¹H NMR in CDCl₃, δ (ppm) 0.47-1.75 (22H, -CH₂C₅H₁₁), 1.78-1.95 (2H, -OC(O)CH₂CH₂-), 1.96-2.21 (4H, -CH₂C₅H₁₁), 2.23-2.33 (2H, -CH₂C $\equiv$ CH), 2.35-2.5 (2H, -OC(O)CH₂-), 2.56-2.68 (1H, -CH₂C $\equiv$ CH), 5.12-5.28 (2H,

-CH₂OC(O)CH₂-), 7.01-8.03 (10 H, fluorene aromatic protons and phenyl group). The molecular weight analysis of (**4**) was conducted by gel permeation chromatographic (GPC) using THF eluent; $M_{n, \text{GPC (THF)}} = 2660 \text{ g mol}^{-1}$, $M_w/M_n = 1.42$.

Synthesis of azide-terminated PNIPAAm-*b*-PHEAA diblock copolymers (**5**)

The synthetic scheme for azide-terminated PNIPAAm-*b*-PHEAA block copolymer is shown in Scheme 1(b). The block copolymers with varying compositions were prepared by adjusting the comonomer/initiator molar ratio, as shown in Table 1. The preparation of PNIPAAm₁₂₀-*b*-PHEAA₃₀ (**5a**) is exemplified below: a mixture of CuCl (8 mg, 8.3×10^{-2} mmol) and Me₆TREN (23 μ l, 8.3×10^{-2} mmol) in 1:1 ethanol/water (0.5 ml, v/v) was placed on one side of a H-shaped glass ampoule and stirred at room temperature. NIPAAm (1.132 g, 10 mmol) and *N*-(2'-azidoethyl)-2-chloropropionamide (15 mg, 8.3×10^{-2} mmol) in 1:1 ethanol/water (4.5 ml, v/v) were added to the other side of the ampoule. Argon was bubbled through both mixtures for 10 min to remove any oxygen. After sealing, both mixtures were mixed and kept at 20 °C for 4 h. Samples of the reaction medium were taken under argon and prepared for the ¹H NMR and GPC analyses. The conversion of PNIPAAm was calculated by the ratio of integrated peak areas between the methylene signal adjacent to the azido group (signal 4 and 5, at 3.27 ppm) and the methylene signals of PNIPAAm (signal 2, at 1.44 ppm) in the ¹H NMR spectrum (Fig. S1(b)). The M_n of PNIPAAm was then estimated as 13500 g/mol. The molecular weight analysis of PNIPAAm was also measured by GPC with dimethylformamide (DMF) as the eluent; $M_{n, \text{GPC (DMF)}} = 13800 \text{ g mol}^{-1}$, $M_w/M_n = 1.28$. The degassed HEAA solution in 1:1 ethanol/water (2.0 M, 1.25 ml) was then added to the reaction system, and the mixture was further stirred for 20 h for the second polymerization. The conversion of HEAA was also determined by the ¹H NMR spectrum of the reaction mixtures in D₂O. The polymerization was quenched by exposure to air, and then the mixture was diluted with ethanol. Subsequently, the copper complex was removed through a silica column. In order to remove unreacted monomer, the resultant solution was dialyzed against water with a Spectra/Por 6 dialysis membrane (molecular weight cutoff of 1000 g mol⁻¹) for 48 h, with replacement of the water at regular time intervals. The obtained aqueous solution was freeze-dried overnight to form PNIPAAm₁₂₀-

b-PHEAA₃₀ (**5a**) as a white solid (1.248 g, 87% yield). ¹H NMR in D₂O, δ (ppm) 0.89-1.08 (6H, -CH(CH₃)₂), 1.20-1.72 (2H, -CH₂CH-), 1.74-2.16 (1H, -CH₂CH-), 3.02-3.38 (2H, -CONHCH₂-), 3.40-3.67 (2H, -CH₂CH₂OH), 3.68-3.87 (1H, -CH(CH₃)₂). For the second polymerization, the signal of the methylene protons adjacent to the azido group was not observed in ¹H NMR spectrum (Fig. S2(a)), which was due to the overlap with the methylene group of PHEAA. However, the block ratio of PNIPAAm-*b*-PHEAA was determined by comparing the integrated peak areas of methylene protons of PHEAA (signal 5, at 3.51 ppm) and methine proton of PNIPAAm (signal 6, at 3.74 ppm) in Fig. S2(a). The M_n of the PNIPAAm-*b*-PHEAA could be calculated from peak integration as 16800 g mol⁻¹. The molecular weight analysis of PNIPAAm-*b*-PHEAA was also measured by GPC with DMF eluent; $M_{n, \text{GPC (DMF)}} = 17300 \text{ g mol}^{-1}$, $M_w/M_n = 1.35$.

Synthesis of PF-*b*-PNIPAAm-*b*-PHEAA rod-coil-coil triblock copolymers (**6**)

Click reactions between alkynyl-terminated PF and azide-terminated PNIPAAm-*b*-PHEAA were performed according Scheme 1(c). Typical procedures for the synthesis of triblock copolymer are described below: **4** (80 mg, 0.03 mmol), **5a** (520 mg, 0.03 mmol), and CuBr (17 mg, 0.12 mmol) were added to a dried Schlenk flask. The reaction mixture was evacuated for 30 min and backfilled with argon. A solution of degassed PMDETA (25 μ l, 0.12 mmol) in 4:1 THF/ethanol (5 ml, v/v) was added, and the flask was immersed in a preheated oil bath at 40 °C for 48 h. The reaction was quenched by exposure to air. After dilution with THF, the copper complex was removed through a silica column. The reaction mixture was evaporated at room temperature, and re-dissolved in water. In order to remove un-reacted reactants completely, the solution was extracted with diethyl ether and dialyzed against THF and water with a KOCH HFM-181 dialysis membrane (molecular weight cutoff of 18000 g mol⁻¹) for 4 days, respectively, with replacements of the solvent at regular time intervals. The light yellow powder of PF₇-*b*-PNIPAAm₁₂₀-*b*-PHEAA₃₀ (**6a**) (372 mg, 62 %) was obtained after lyophilization of the aqueous solution present inside the dialysis membrane. The GPC analyses using DMF eluent (Fig. S4) showed the single peak without appreciable shoulders and tailings at the both sides and confirm that the un-reacted reactants have disappeared. ¹H NMR in CDCl₃, δ (ppm) 0.92-1.43 (6H, -CH(CH₃)₂), 1.49-

2.00 (2H, $-CH_2CH-$), 2.01-2.58 (1H, $-CH_2CH-$), 3.11-3.62 (2H, $-CH_2CH_2OH$), 3.71-3.94 (2H, $-CH_2CH_2OH$), 3.93-4.16 (1H, $-CH(CH_3)_2$), 4.90-5.11 (1H, $-CH_2CH_2OH$), 6.11-7.12 (1H, $-CONH-$), 7.23-7.82 (10H, fluorene aromatic protons and phenyl group). $M_{n, GPC (DMF)} = 20900 \text{ g mol}^{-1}$, $M_w/M_n = 1.28$. Typical PF₇-*b*-PNIPAAm₁₂₀-*b*-PHEAA₃₀ was analyzed by elemental analysis, and results are shown as follows where all values are given as percentages. Anal. Calcd: C, 64.78; H, 9.41; N, 10.95. Found: C, 64.45; H, 9.39; N, 10.86.

Preparation of micelles of block copolymer in solution

Preparation of block copolymer micelles for the thermoresponsive study, the copolymer in water (0.1 wt%) was raised to the specific temperature of 25 to 80 °C, respectively, and held for 72 h to reach thermodynamic equilibration.

Characterization

The ¹H spectra were recorded using a JEOL JNM-A400II instrument. For the determination of the conversion, the ¹H NMR spectrum of the polymer mixture was measured in D₂O or CDCl₃ at room temperature. The IR spectra were recorded using a PerkinElmer Paragon 1000 FT-IR instrument. GPC analysis was performed on a Lab Alliance RI2000 instrument (two column, MIXED-C and D from Polymer Laboratories) connected with one refractive index detector from Schambeck SFD GmbH. All GPC analyses were performed on THF or DMF eluent at the flow rate of 1 ml/min at 40 °C and calibrated with polystyrene. Elemental analyses for C, H, and N were performed by Heraeus Vario EL-III.

The phase transition of the prepared polymer solution was recorded by monitoring the transmittance of a 550 nm light beam on a Mini 1240 UV-Visible spectrophotometer (Shimadzu). The polymer concentration in water was 0.1 wt%, and the temperature was raised from 25 to 80 °C in increments of 2 °C every 100 min. TEM was performed on a JEOL 1230 operating at an acceleration voltage of 100 kV, and the high resolution TEM (HRTEM) imaging was performed on an FEI Tecnai G2 20 TEM at 200 kV. To preserve the original micelle morphologies as in aqueous solution, the polymer solutions were quickly deposited onto a 200-mesh carbon-coated copper grid at -80 °C before being freeze-dried for 48

h to achieve dryness. The micelles of diblock copolymers were immediately quenched under such a low temperature and vitrified the polymer chains into glassy state without changing the morphologies. The similar quench method to prepare the TEM samples was also used in other studies.^{S5-S7} Subsequently, specimens were stained by exposure to the vapor of OsO₄ prior to TEM observation. The micellar aggregates were also characterized by the tapping mode of the MultiMode AFM with a Nanoscope 3D controller (Digital Instruments). For the AFM surface structure at elevated temperature, the MultiMode heater/cooler was added to provide the thermal-control of sample temperature. The cantilever used was fabricated from phosphorus-doped Si with a spring constant of 3 N m⁻¹ and a resonance frequency of 76 kHz. To observe micelle morphologies under AFM retains the original morphologies as in the solution, the sample for the AFM measurement was prepared by drop-coating the copolymer solution onto a mica substrate at -80 °C before being freeze-dried for 48 h to achieve dryness and obtain the surface structure. The hydrodynamic diameters of micellar aggregates in water at different temperature were determined by DLS using a Brookhaven Instruments Zeta Plus apparatus fitted with a 15 mW laser ($\lambda = 678$ nm) and with the detector set at 90°. Each reported value is the average of 10 measurements. PL spectra of the copolymer solutions were recorded by Fluorolog-3 spectrofluorometer (Jobin Yvon). The PL quantum efficiency of copolymer solutions were measured using a Fluorolog-3 with 380 nm excitation, as described in our previous study.^{S8,S9,S10}

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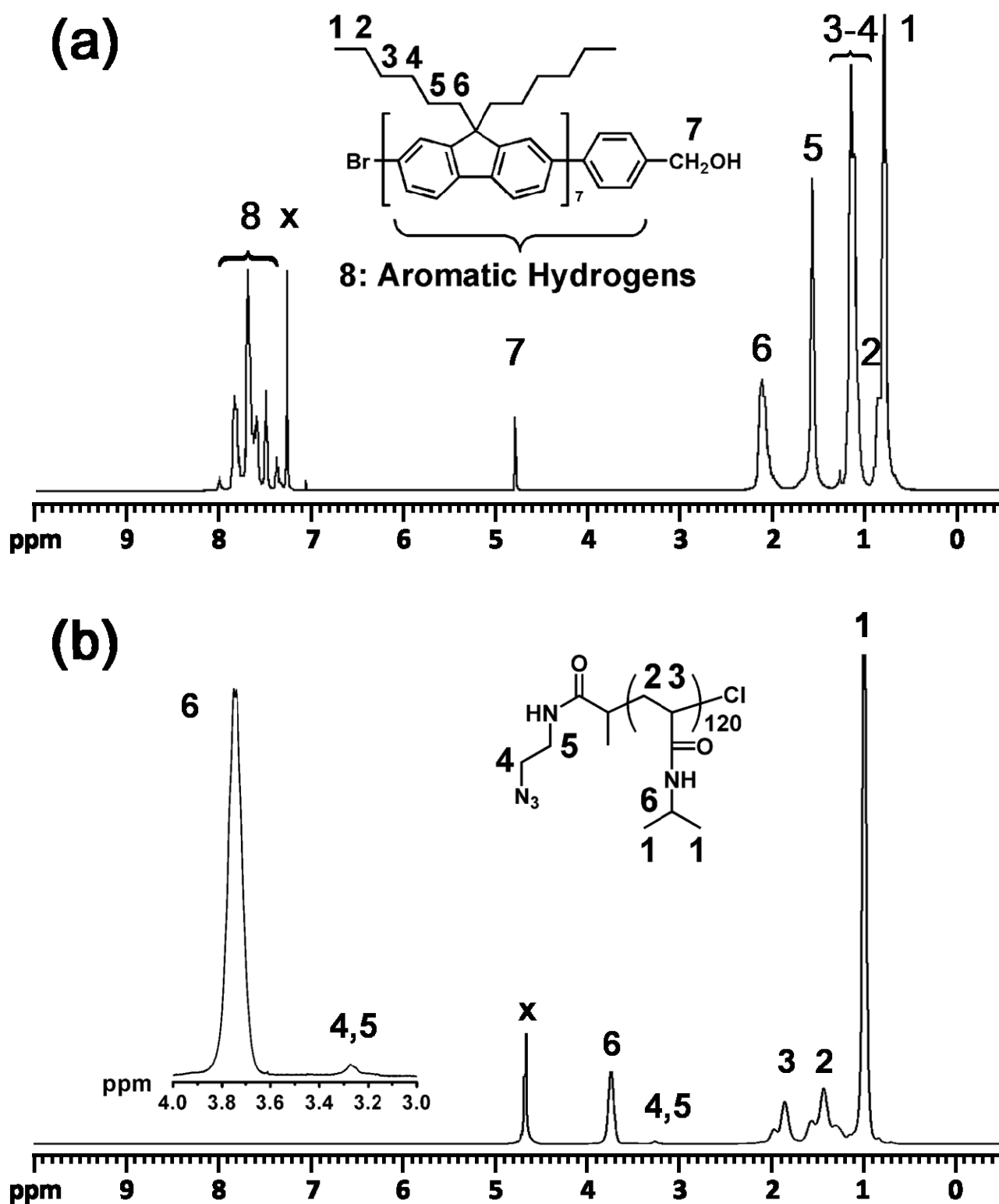


Fig. S1. ^1H NMR spectra of (a) hydroxyl-terminated PF (**3**) in CDCl_3 and (b) the reaction medium of PNIPAAm in D_2O .

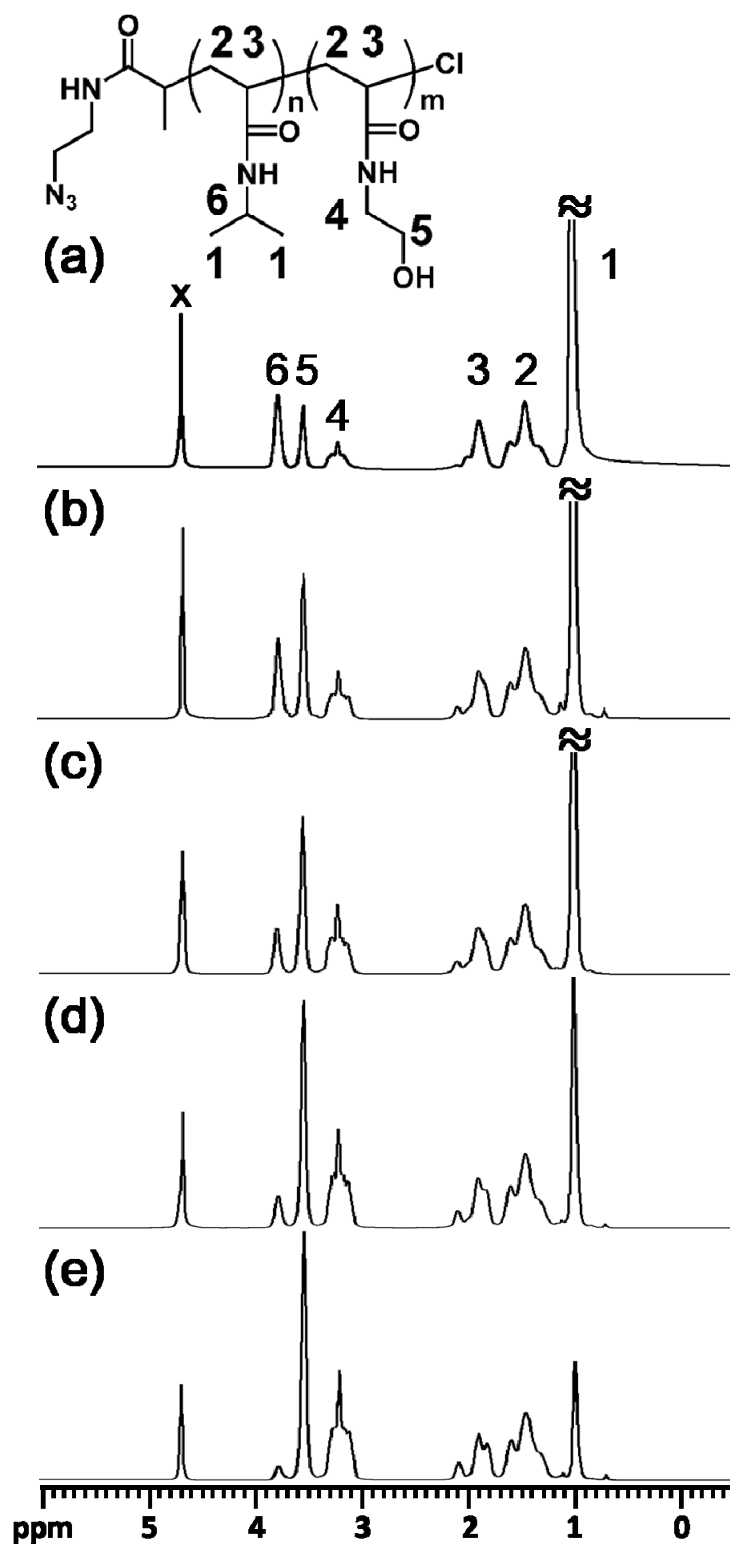


Fig. S2. ^1H NMR spectra of (a) PNIPAAm₁₂₀-b-PHEAA₃₀ (**5a**), (b) PNIPAAm₁₀₀-b-PHEAA₅₀ (**5b**), (c) PNIPAAm₇₅-b-PHEAA₇₅ (**5c**), (d) PNIPAAm₅₀-b-PHEAA₁₀₀ (**5d**), and (e) PNIPAAm₃₀-b-PHEAA₁₂₀ (**5e**) in D₂O.

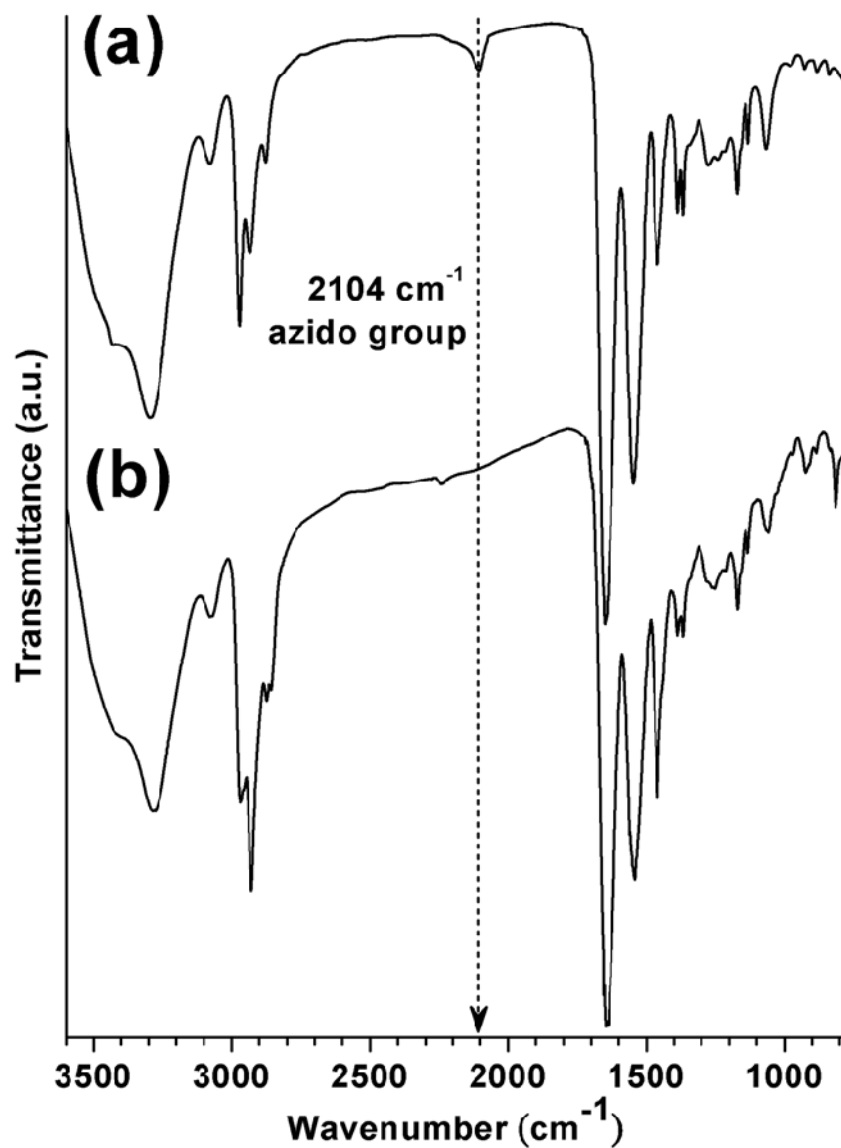


Fig. S3. IR spectra of (a) PNIPAAm₁₂₀-*b*-PHEAA₃₀ (**5a**) and (b) PF₇-*b*-PNIPAAm₁₂₀-*b*-PHEAA₃₀ (**6a**).

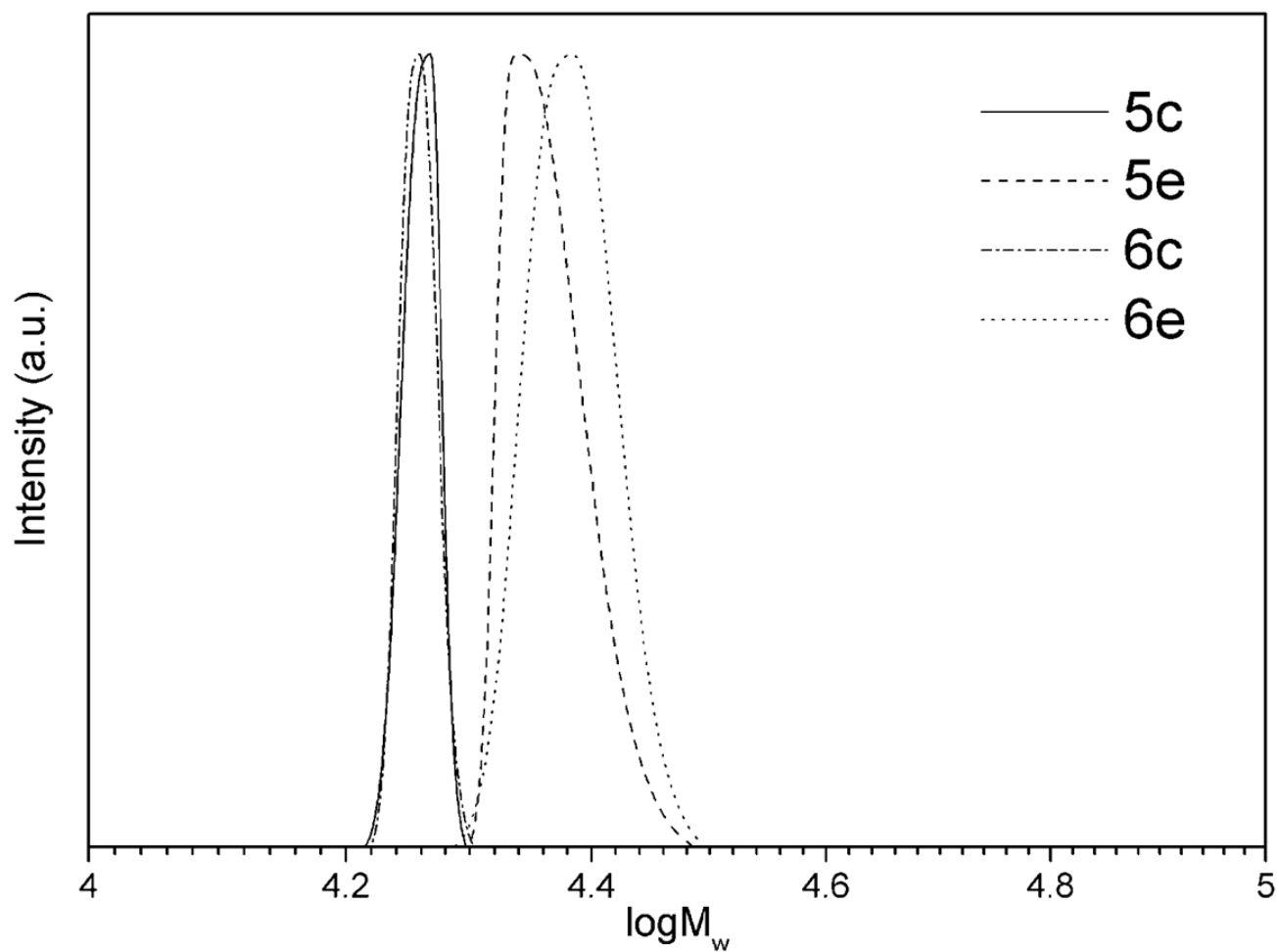


Fig. S4. GPC traces of PNIPAAm-*b*-PHEAA and PF-*b*-PNIPAAm-*b*-PHEAA block copolymers with DMF as the eluent.

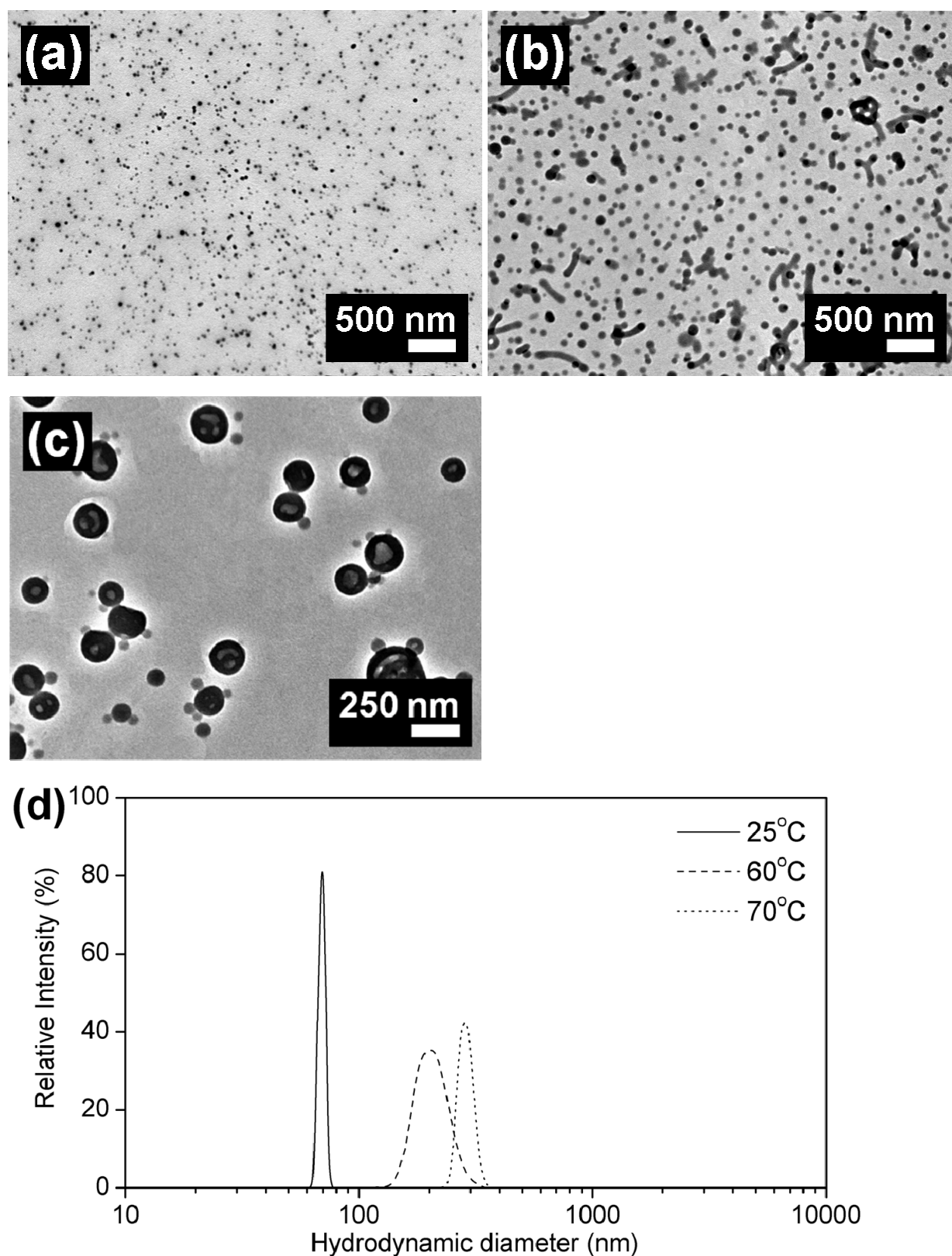


Fig. S5. TEM micrographs of PNIPAAm₁₀₀-*b*-PHEAA₅₀ (**5b**) aggregates in water by increasing temperature from (a) 25, to (b) 60 and (c) 70 °C, respectively. (d) Hydrodynamic diameter of PNIPAAm₁₀₀-*b*-PHEAA₅₀ (**5b**) aggregates in water with changing temperature.

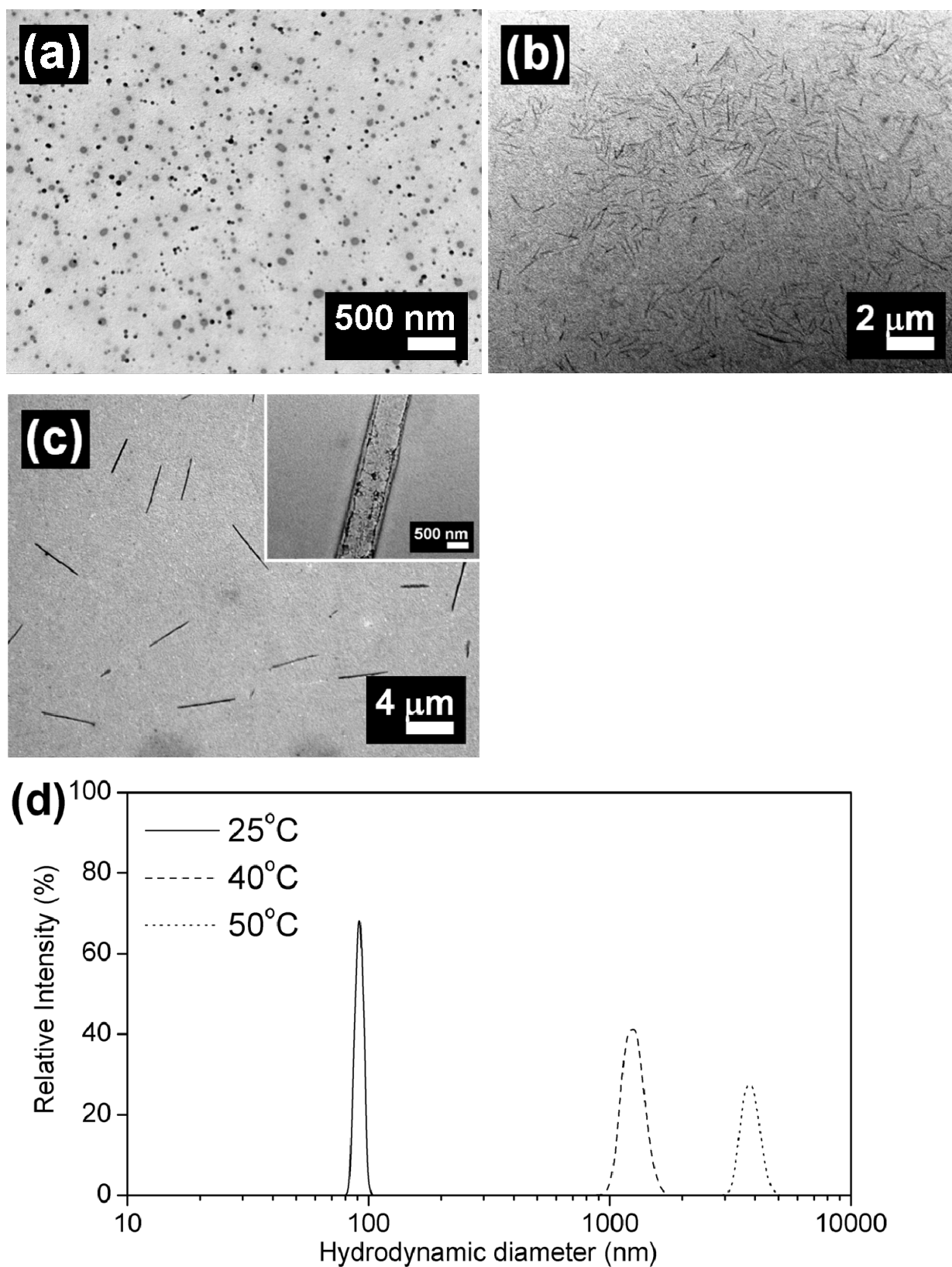
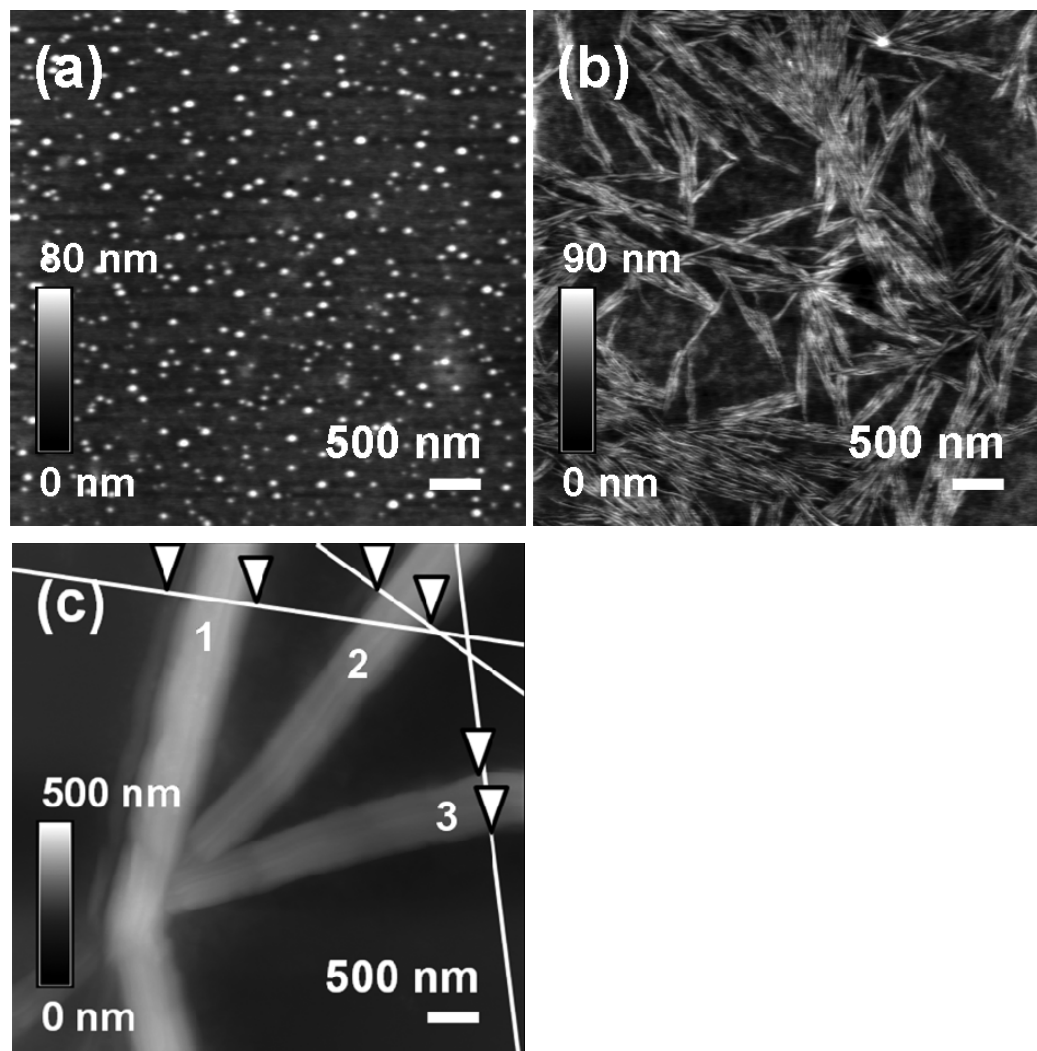


Fig. S6. TEM micrographs of PF₇-*b*-PNIPAAm₁₀₀-*b*-PHEAA₅₀ (**6b**) aggregates in water by increasing temperature from (a) 25, to (b) 40 and (c) 50 °C, respectively. (d) Hydrodynamic diameter of PF₇-*b*-PNIPAAm₁₀₀-*b*-PHEAA₅₀ (**6b**) aggregates in water with changing temperature.



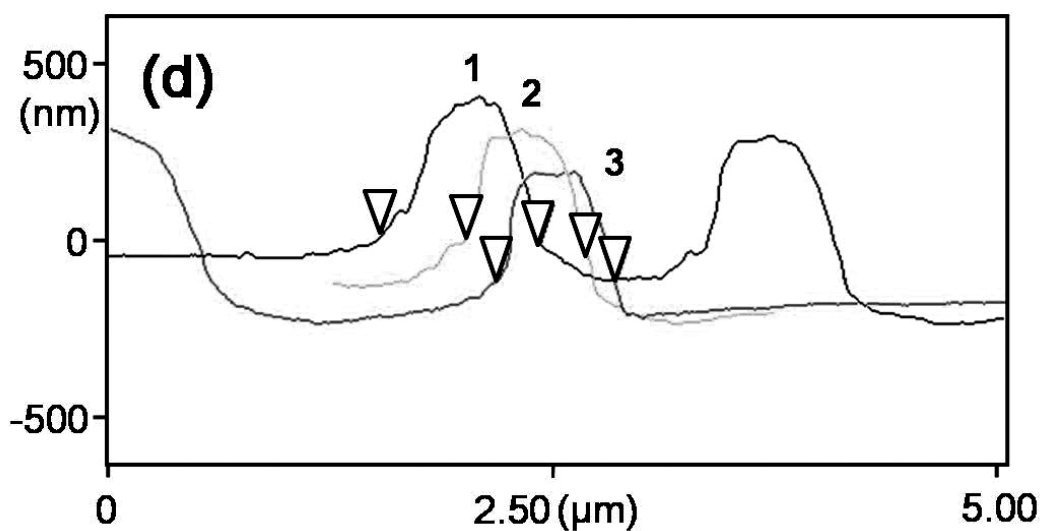
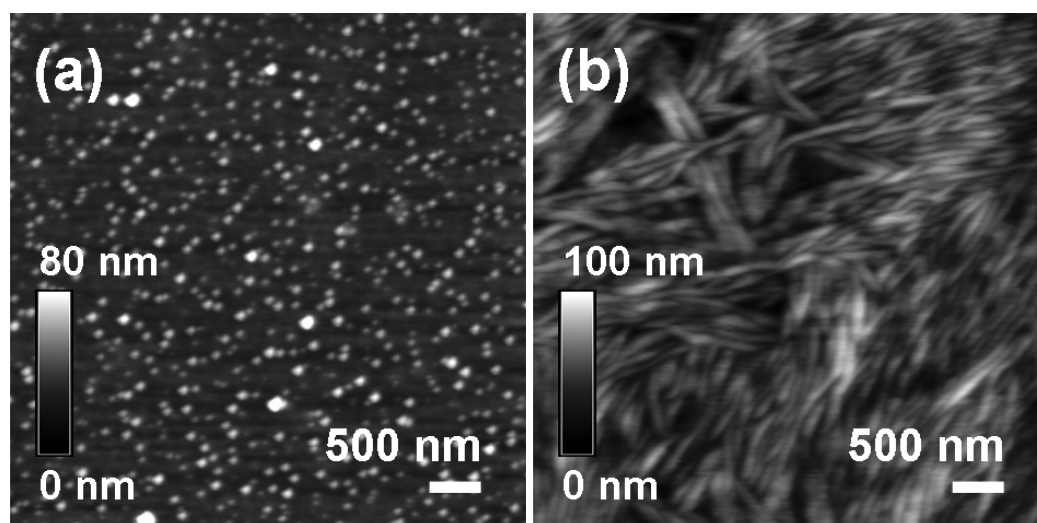


Fig. S7. AFM micrographs of PF₇-*b*-PNIPAAm₁₂₀-*b*-PHEAA₃₀ (**6a**) aggregates in water by increasing temperature from (a) 25, to (b) 40 and (c) 50 °C, respectively. (d) The corresponding cross-sectional surface images for the micelle aggregate at 50 °C.



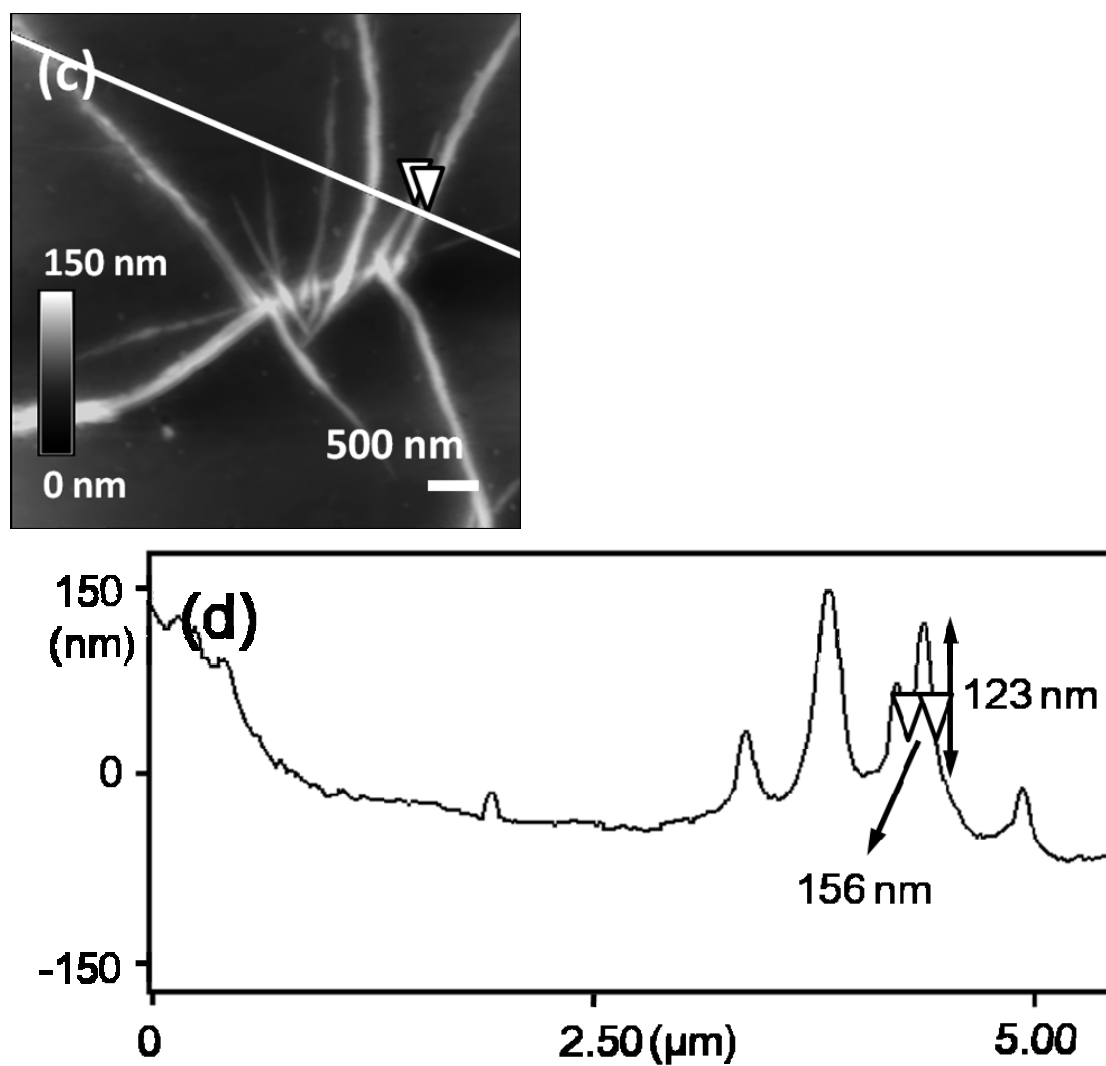


Fig. S8. AFM micrographs of PF₇-*b*-PNIPAAm₇₅-*b*-PHEAA₇₅ (6c) aggregates in water by increasing temperature from (a) 25, to (b) 50 and (c) 60 °C, respectively. (d) The corresponding cross-sectional surface images for the micelle aggregate at 60 °C.