Doubly-Responsive Hyperbranched Polymers and Core-Crosslinked Star Polymers with Tunable Reversibility

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EXPERIMENTAL

Materials. Acryloyl chloride (96%), cystamine dihydrochloride (97%), and triethylamine (TEA, 99%) were purchased from Alfa Aesar and used without further purification. *N*-isopropylacrylamide (NIPAM, TCI, 98%) was recrystallized twice from hexane. *N*,*N*-dimethylacrylamide (DMA, 99%) was purchased from Sigma and purified by passing through a basic alumina column prior to polymerization. Anhydrous *N*,*N*-dimethyl formamide (DMF, Sigma, 99.8%), dimethylphenylphosphine (DMPP, Sigma, 99%) tri-*n*-butylphosphine (Bu_3P, Aldrich, 99%), dithiothreitol (DTT, Acros, 99%), potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$, Aldrich, 99%), hexane (Fisher), diethyl ether (Fisher), sodium hydroxide (NaOH, Fisher), hydrochloric acid (HCl, Fisher), and $CDCl_3$ (Cambridge Isotopes, 99.8%D) were used as received. 2,2′-Azobisisobutyronitrile (AIBN, Sigma, 98%) was recrystallized twice from methanol. Dichloromethane (DCM) and tetrahydrofuran (THF) were purified using a solvent purification system. The 4-cyano-4-(dodecylsulfanylthiocarbonyl)-sulfanyl pentanoic acid (CDP) RAFT agent was synthesized according to a previously reported procedure.[1] The divinyl crosslinker *N*,*N*´-

bis(acryloyl)cystamin (BAC) was synthesized according to the procedure[2] described elsewhere (see Figure S1 for the ^{1}H NMR spectrum of BAC).

Instrumentation. Number-average molecular weights (M_n) and molecular weight distributions ($Đ$) of hyperbranched polymers were determined by size-exclusion chromatography (SEC) in DMAc with 0.05 M LiCl at 50 °C and a flow rate of 1.0 mL/min (Agilent isocratic pump, degasser, and autosampler: columns: PLgel 5 µm guard + two ViscoGel I-series G3078 mixed bed columns: molecular weight range 0-20 × 10^3 and 0-100 × 10^4 g/mol). Detection consisted of a Wyatt Optilab T-rEX refractive index detector operating at 658 nm and a Wyatt miniDAWN Treos light scattering detector operating at 659 nm. Absolute molecular weights and dispersities were calculated using the Wyatt ASTRA software. Hydrodynamic diameter values were determined using a dynamic light scattering (DLS) instrument (Malvern Zetasizer Nano–ZS at 173° equipped with a 4 mW, 633 nm He–Ne laser and an Avalanche photodiode detector. Polymer solutions (10 mg/mL) were filtered through a 0.45 µm syringe filter prior to measurement, and each experiment was performed in triplicate to obtain the average value. UV-Vis spectroscopy measurements were obtained using a Varian Cary 500 Scan UV-Vis NIR Spectrophotometer. The ^{1}H NMR spectra were recorded on an Inova 500 MHz spectrometer. Transmission electron microscopy (TEM) images were captured on an H-7000 TEM microscope (Hitachi, Japan) operating at an accelerating voltage of 100 kV. Samples (0.1 mg/mL or 1 mg/mL in DI-water) were prepared using copper grids and 0.5% uranyl acetate stain.

Synthesis of *N,N´*-Bis(acryloyl)cystamin (BAC). Degradable cross-linker BAC was synthesized according to a previous report.[2] Cystamine dihydrochloride (5.63 g, 25 mmol)

was dissolved in a heterogeneous mixture of 40 mL 3.5 M NaOH and 30 mL dichloromethane (DCM) in a 250-mL round-bottomed flask equipped with a magnetic stir bar. The reaction mixture was placed in an oil bath preheated to 50 °C and acryloyl chloride (4.46 mL, 55.0 mmol) in 15 mL of DCM was added dropwise with constant stirring over 20 min. The mixture was allowed to react for 3h at 50 °C. The aqueous phase of reaction mixture was discarded while it was still warm; the organic phase was cooled to room temperature, and the white crystal product precipitated directly from the solution. The crystal product was purified by recrystallization from DCM (Yield 56%). The molecular structure of BAC was confirmed by ^{1}H-NMR spectrum (Fig. S1, $CDCl_3$, δ, ppm): 2.87 (S-C***H***$_2$, 2H, t), 3.65 (NH-C***H***$_2$, 2H, q), 5.67 (CH***H***=CH, 1H, m), 6.16-6.35 (C***H***H=C***H***, 2H, m) and 6.63 (N***H***, 1H, s).

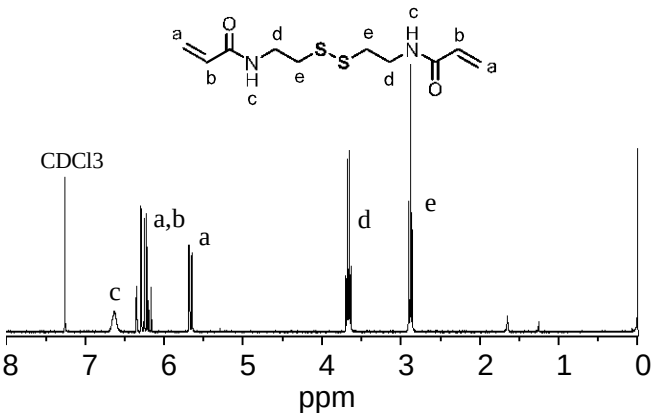

Fig. S1 ^{1}H NMR spectrum of BAC in $CDCl_3$.

Synthesis of Hyperbranched Poly(*N*-isopropylacrylamide-*co*-*N,N´*-bis(acryloyl)cystamine) [P(NIPAM-*co*-BAC)] Copolymers by RAFT Polymerization.

Typically, a mixture of NIPAM (1.00 g, 8.83 mmol), BAC (92.0 mg, 0.353 mmol), CDP (35.6 g, 0.0883 mmol), and AIBN (2.90 mg, 0.0176 mmol) was dissolved in DMF (5 g) and

placed in a 20 mL septum-sealed glass vial equipped with a magnetic stir bar. The reaction vial was purged with N_2 for 25 min and placed in a preheated heating block at 70 °C. Aliquots were removed from the reaction vial periodically using a N_2-purged syringe. Monomer conversion was determined by ^{1}H NMR spectroscopy, and the M_n and $Đ$ were obtained by SEC. The polymerization was quenched by cooling in an ice-water bath and exposure to air. The polymer was precipitated from diethyl ether (×5) to give a light yellow powder that was dried under high vacuum for 24 h.

Synthesis of Star Polymers Poly(*N*–isopropylacrylamide-*co*-*N,N´*-bis(acryloyl)cystamin)-*star*-poly(*N,N*-dimethylacrylamide) [**P(NIPAM-*co*-BAC)-*star*-PDMA] with Hyperbranched Cores *via* RAFT Polymerization.** A typical RAFT chain extension polymerization procedure is as follows: DMA (0.400 g, 4.03 mmol), P(NIPAM-*co*-BAC)-macro-CTA (0.389g, 0.0403 mmol), and AIBN (1.32 mg, 0.00807 mmol) were dissolved in DMF (4 g) and placed in a 20 mL septum-sealed glass vial equipped with a magnetic stir bar. The number of CTA units in P(NIPAM-*co*-BAC) hyperbranched polymers was calculated from the initial feed ratio of NIPAM, CDP, and BAC, and considering monomer conversion. The reaction vial was purged with N_2 for 25 min and placed in a preheated heating block at 70 °C. Aliquots were drawn from the reaction vial periodically using a N_2-purged syringe to measure monomer conversion by ^{1}H NMR spectroscopy, and the M_n and $Đ$ were obtained by SEC. The polymerization was quenched by cooling in an ice-water bath and exposure to air after 75 min. The polymer was precipitated from diethyl ether (×5) to give a light yellow powder that was dried under high vacuum for 24 h, Yield- 87%.

Determination of Cloud Point (CP). The turbidity of aqueous hyperbranched polymer solutions at various temperatures was measured with a UV-Vis spectrophotometer by monitoring the change in %-transmittance (%T) at λ = 500 nm. The polymer solutions (10.0 mg/mL) were passed through a 0.45 μm membrane filter prior to analysis. The samples were placed in the UV-Vis spectrometer, and the temperature of the solutions was increased at 1 °C intervals with 2 min equilibration periods at each temperature before measurement. The cloud point was defined as the temperature corresponding to 10% reduction in %T value.

DLS was used to deterimine the CP of the hyperbranched polymers, which was defined as the temperature at which the size of the particles increased by 50%. The concentration of the polymer solutions was 10.0 mg/mL for the cloud point study.

Degradation of Disulfide Bonds into Thiols in the Hyperbranched Polymers. The cleavage of disulfide bonds in the hyperbranched polymer was carried out using Bu_3P and DMPP at ambient temperature. The degradation reactions of **HBP5** (20.0 mg) were conducted in a 20 mL septum-sealed vial in DMAc (2 mL) using Bu_3P (24.7 μmol) or DMPP (36.1 μmol) as reducing agent. The reaction mixture was purged with N_2 for 5 min and it kept under stirring at 25 °C for 45 min. Aliquots were withdrawn for SEC analysis to monitor degradation of the hyperbranched polymer.

To study DTT-induced degradation kinetics, **HBP10** (80.0 mg, 3.53×10^{-5} mol S-S bond) was dissolved in DMAc (8 mL) in a 20 mL septum-sealed vial equipped with a small magnetic stir bar, and DTT (61.7 mg, 4.00×10^{-4} mol) was added. The reaction mixture was purged with N_2 for 5 min to remove atmospheric oxygen and then kept under stirring. Aliquots were withdrawn periodically from the stock solution by a syringe purged with N_2

and passed through 0.45µm Millipore PTFE filters and were subsequently analysed using SEC in DMAc and DLS to monitor the degradation of disulfide bonds.

Degradation of disulfide bonds was also carried out in deionized (DI) water using DTT as reducing agent in a 20 mL septum-sealed vial equipped with a small magnetic stir bar. In a typical example, **HBP10** (50.0 mg, 2.21×10^{-5} mol S-S bond) was dissolved in DI water (10 mL) and DTT (77.1 mg, 4.90×10^{-4} mol) was added. The solution was separated equally into two different vials and purged with N_2 for 5 min to remove dissolved oxygen. One vial was kept under stirring at 25 °C (bellow the CP), and another was kept under stirring at 45 °C (above the CP) for 5 h. Aliquots were passed through 0.45µm Millipore PTFE filters and analysed by DLS at 25 °C and 45 °C to obtain the size of the degradation product.

Synthesis of Thiol Functionalized Linear Poly(*N*-isopropylacrylamide-*co*-*N*-(2-mercaptoethyl)acrylamide) (P(NIPAM-*co*-MEA)) and Poly(*N*-isopropylacrylamide-*co*-*N*-(2-mercaptoethyl)acrylamide)-*b*-poly(*N*,*N*-dimethylacrylamide) (P(NIPAM-*co*-MEA)-*b*-PDMA). Thiol-functionalized linear P(NIPAM-*co*-MEA) was synthesized by disulfide bond reduction using Bu_3P in THF at RT.[3] Typically, P(NIPAM-*co*-BAC) (**HBP9**, 500 mg, 2.21×10^{-4} mol S-S bond) was dissolved in THF (5 mL) in a 20 mL screw cap vial equipped with a magnetic stir bar using an excess of Bu_3P (89.4 mg, 4.42×10^{-4} mol) for 24 h under N_2. The degradation of disulfide bonds in **HBP9** was confirmed by SEC (data not shown here). After complete reduction, the solution was transferred to a 3 kDa molecular weight cut off (MWCO) dialysis bag and dialyzed against acidic water (pH ≈ 3.0) for two days, followed by dialysis in DI water under N_2 for one day. The thiol containing linear P(NIPAM-*co*-MEA) was isolated by lyophilization. Similarly, we obtained thiol-functionalized P(NIPAM-*co*-

MEA)-*b*-PDMA block copolymers from P(NIPAM-*co*-BAC)-*star*-PDMA using excess Bu_3P (5 equiv) as reducing agent. Typically, **HBP7-*star*-PDMA** (500 mg, 5.63×10^{-5} mol S-S bond) was dissolved in THF (5 mL) in a glass vial equipped with a magnetic stir bar, and Bu_3P (56.7 mg, 2.81×10^{-4} mol) was added. The reaction solution was stirred at RT under N_2 for 24 h. The solution was dialyzed against acidic water (pH ≈ 3.0) for two days under N_2 and then in deionized water under N_2 for one day. The polymer was isolated by lyophilization.

Thiol Coupling Reaction of P(NIPAM-*co*-MEA) to Form P(NIPAM-*co*-BAC). The coupling reaction of the thiol groups in P(NIPAM-*co*-MEA) was conducted at different temperatures. A typical procedure for the investigation of disulfide bond formation at various temperatures is as follows. P(NIPAM-*co*-MEA) (50.0 mg) was dissolved in DI water (5 mL) in a 20 mL glass vial, and the resulting solution was divided equally into two different 4 mL glass vials equipped with magnetic stir bars. The coupling reaction of thiol groups was induced by addition of an oxidizing agent, $K_3[Fe(CN)_6]$ (5.00 mg, 1.52×10^{-5} mol), and the reaction solutions were kept at 25 °C or 45 °C, respectively, with stirring. The coupling reaction of thiol groups at 25 °C was investigated by monitoring the SEC and DLS of reaction solution with time, while the reaction solution at 45 °C formed a microgel, which precipitated from the solution.

Thiol Coupling Reaction of P(NIPAM-*co*-MEA)-*b*-PDMA Block Copolymers. Formation of stars from degraded linear P(NIPAM-*co*-MEA)-*b*-PDMA block copolymers was investigated by a coupling reaction between the thiol groups present in the linear block copolymers in the presence of $K_3[Fe(CN)_6]$ as an oxidizing agent at 45 °C. Typically, the thiol-containing P(NIPAM-*co*-MEA)-*b*-PDMA (50 mg) polymer was placed in a 20 mL glass

vial with a magnetic stir bar in DI water (5 mL), and $K_3[Fe(CN)_6]$ (5.00 mg, 1.52×10^{-5} mol) was added. The coupling reaction was performed at 45 °C for 14 h, and the reformation of stars was confirmed by the size and $M_{n,SEC}$ of the polymers using DLS and SEC respectively. Star polymer was isolated by lyophilization, and the size was confirmed by TEM analysis. Similarly, the thiol coupling reaction with P(NIPAM-*co*-MEA)-*b*-PDMA was performed at 25 °C for 24 h.

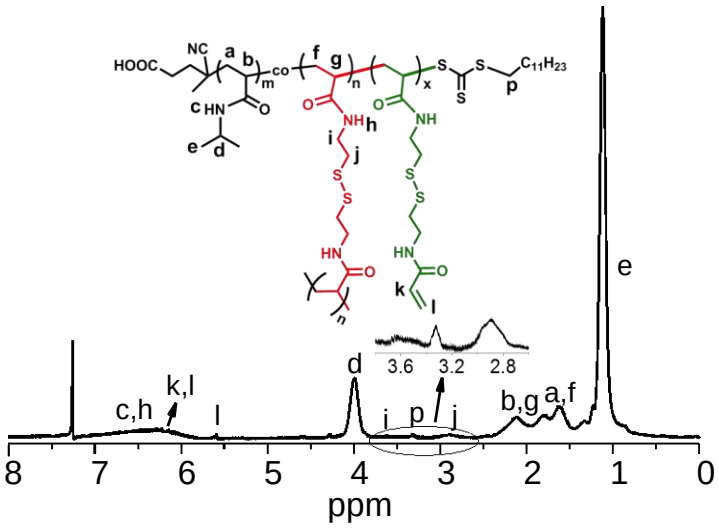

Fig. S2 ^{1}H NMR spectrum of P(NIPAM-*co*-BAC) (**HBP6**) hyperbranched copolymer in $CDCl_3$.

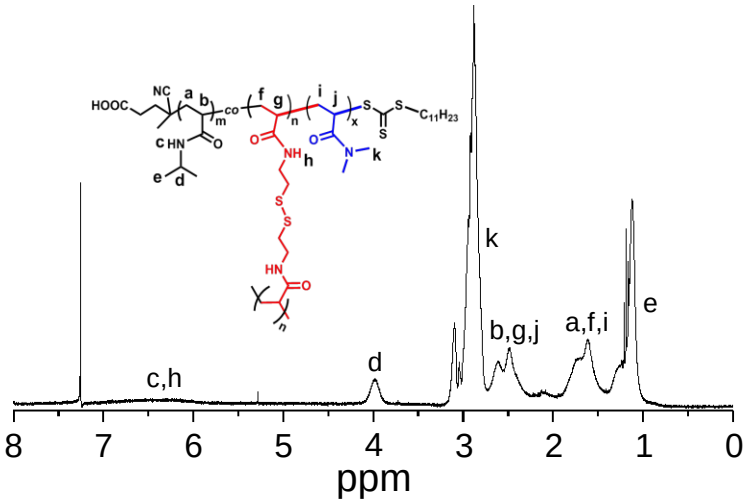

Fig. S3 ^{1}H NMR spectrum of P(NIPAM-*co*-BAC)-*star*-PDMA (**HBP7-*star*-PDMA**) copolymer in $CDCl_3$.

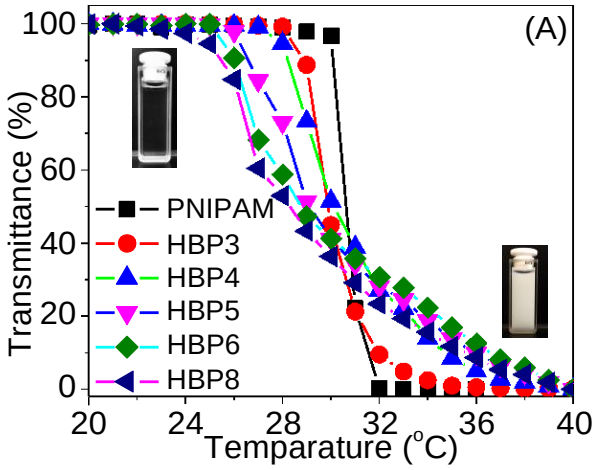

Fig. S4 Plot of % transmittance of PNIPAM and P(NIPAM-*co*-BAC) as a function of temperature with inset photos of representative solutions above and below the CP.

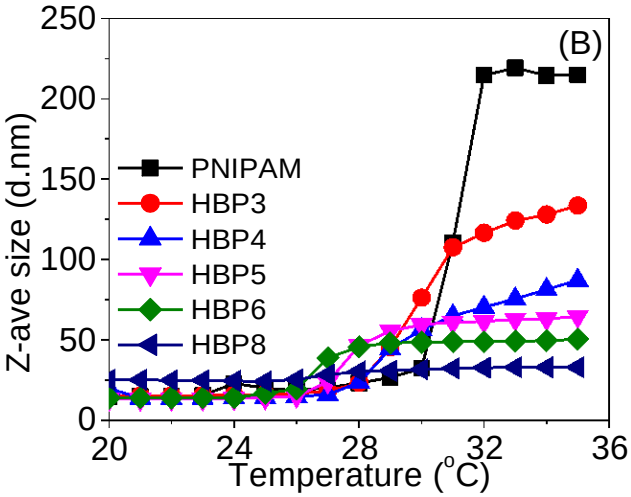

Fig. S5 Plot of changes in Z-average size as a function of temperature, as determined by DLS of PNIPAM and P(NIPAM-*co*-BAC) in aqueous solution (10 mg/mL).

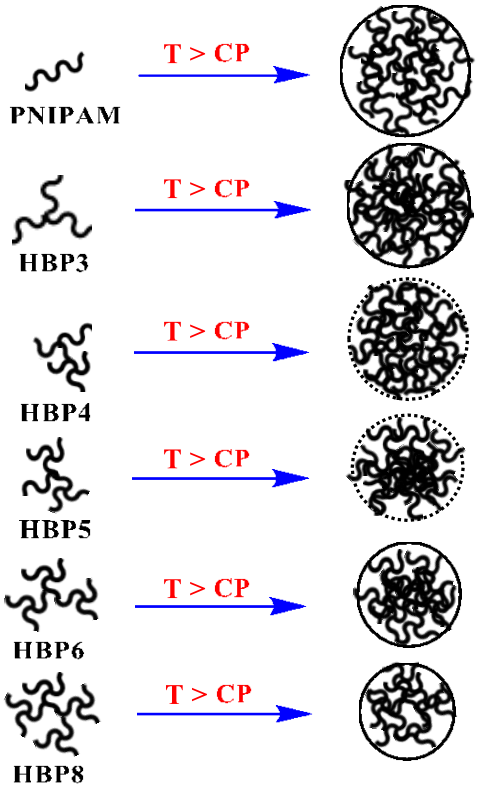

Fig. S6 Schematic illustration of aggregation behavior for PNIPAM and P(NIPAM-*co*-BAC) at a temperature (T) above their CP. Aggregate size above the CP decreases with increasing branching density.

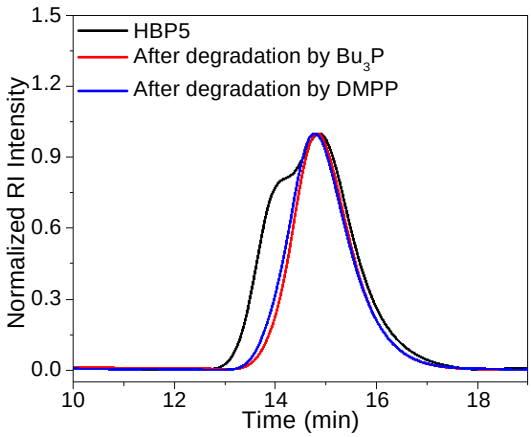

Fig. S7 SEC-refractive index traces of **HBP5** before and after degradation by Bu_3P and DMPP.

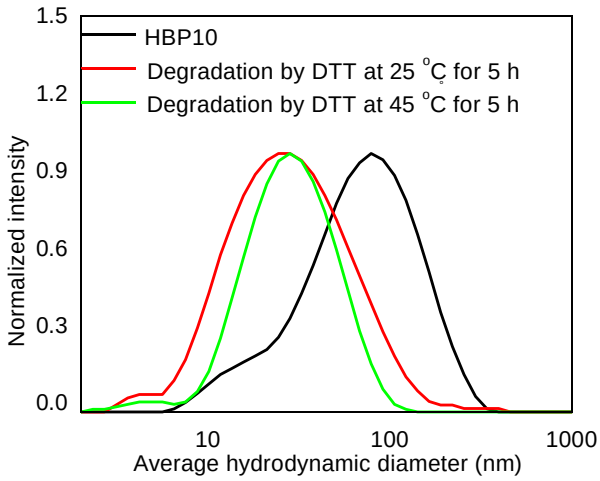

Fig. S8 The size distribution of **HBP10** after degradation by DTT in water at 25 °C and 45 °C for 5 h.

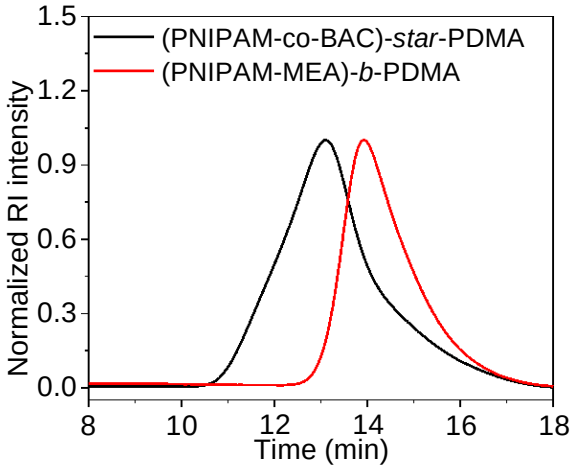

Fig. S9 SEC-refractive index traces of the parent **HBP7-*star*-PDMA** star polymer and the isolated P(NIPAM-*co*-MEA)-*b*-PDMA linear copolymer that resulted after exposure to Bu_3P for 24 h at RT.

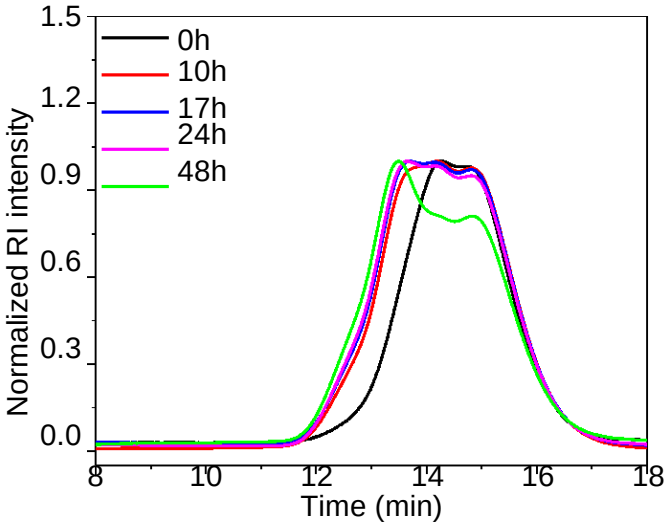

Fig. S10 SEC-refractive index traces for the regeneration of disulfide bonds from P(NIPAM-*co*-MEA) to P(NIPAM-*co*-BAC).

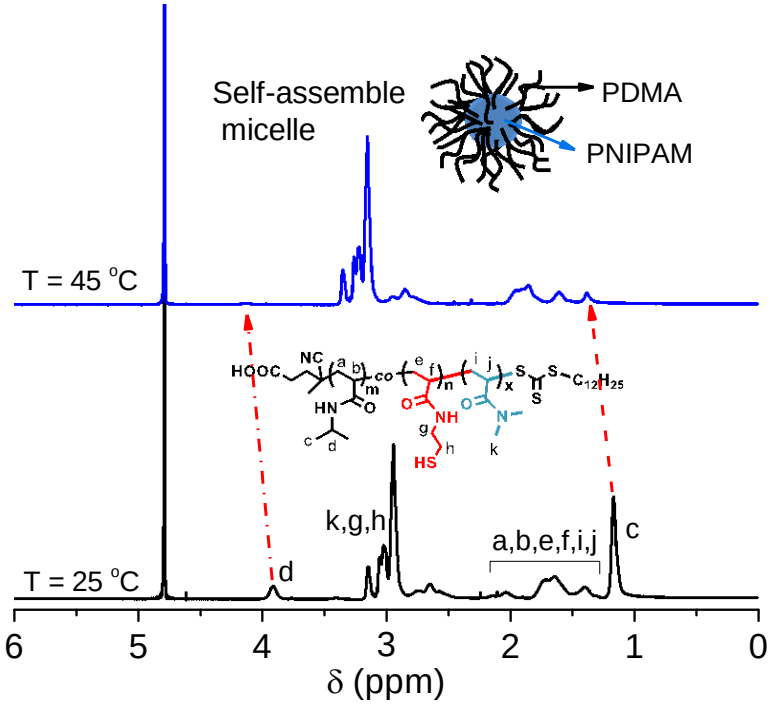

Fig. S11 ^{1}H NMR spectra of P(NIPAM-*co*-MEA)-*b*-PDMA in D_2O at 25 and 45 ºC. The PNIPAM peaks shifted to the lower field with decreasing peak intensity at elevated temperature.[4]

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3. Singh, S.; Topuz, F.; Hahn, K.; Albrecht, K.; Groll, J. *Angew. Chem. Int. Ed.* **2013,** *52*, 3000-3003.

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