pH Triggered Self-Assembly of Core Cross-Linked Star Polymers Possessing Thermoresponsive Cores

Supporting Information

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Materials:

All chemicals were purchased from Sigma-Aldrich or Alfa Aesar and were used as received without further purification. *N,N'*-dimethylacrylamide was purified by vacuum distillation at 60 ˚C. *N*-isopropylacrylamide was recrystallized from hexane prior to use. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer at 300 and 75 MHz, respectively, with the residual solvent signal as an internal standard. FTIR spectroscopy was performed on a Varian 800 FTIR instrument (Varian Inc.). High-resolution mass spectrometry was performed on a Waters LCT premier mass spectrometer (Waters Inc.). Fluorescence emission spectra were recorded on a Digilab F-2500 Fluorescence Spectrophotometer (Hitachi) (λ_{ex} = 550 nm). pH Measurements were recorded with a Hanna HI 98103 instrument that was calibrated daily with commercial buffer solutions (Sigma-Aldrich). The pH adjustments of cross-linking reactions were carried out using small aliquots (5 µL) of 1M $NaOH_{(aq)}$ and 1M $HCl_{(aq)}$ solutions.

Gel Permeation Chromatography (GPC):

Gel permeation chromatography (GPC) was conducted on a Varian ProStar instrument (Varian Inc.) equipped with a Varian 325 UV-vis dual wavelength detector (254 nm), a Dawn Heleos II multi-angle laser light scattering detector (Wyatt Technology Corp.), a Viscotek 3580 differential RI detector, and a pair of PL gel 5 µm Mixed D 300 × 7.5 mm columns with guard column (Polymer Laboratories Inc.) in series. Near monodisperse methyl methacrylate standards (Agilent Technologies) were used for calibration. Data collection was performed with Galaxie software (Varian Inc.) and chromatograms analyzed with the Cirrus software (Varian Inc.) and Astra software (Wyatt Technology Corp.).

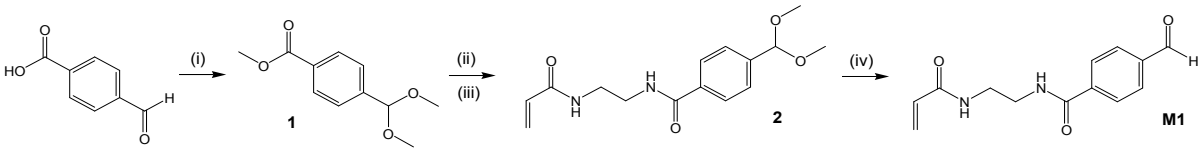
Dynamic Light Scattering (DLS) Analysis:

Hydrodynamic radii (R_h) of polymers and core cross-linked star (CCS) polymers in aqueous solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a MALVERN Instruments HPPS-ET 5002 operating at 20 ˚C with a 633-nm laser module. Measurements were made at a detection angle of 173˚ (back scattering), and Malvern DTS 4.20 software was utilized to analyze the data. All determinations were made in duplicate.

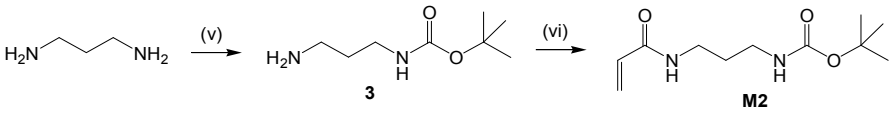
Lower Critical Solution Temperature (LCST) Measurements:

The lower critical solution temperatures (LCST) of polymers **P1** and **P2b** (5 mg/ mL) were measured on a Cary 100 Bio UV-Vis spectrophotometer (Varian Inc.) fitted with a peltier block. Transmittance of polymer solutions was monitored at 550 nm as a function of temperature (cell path length 10 mm).

Experimental:



S1. Synthesis of *N*-ethylacrylamide-2-(4-formylbenzamide) (**M1**): (i) $CH(OCH_3)_3$, MeOH, H_2SO_4, 80 °C, 48 h. (ii) 1,2-diaminoethane, 130 °C, 24 h. (iii) Acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 16 h. (iv) 1M $HCl_{(aq)}$, 2 h.



S2. Synthesis of *N*-(*tert*-butoxycarbonyl)-propylaminoacrylamide (**M2**): (v) Boc_2O, $CHCl_3$, 0 °C, 16 h. (vi) Acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 16 h.

Methyl 4-(dimethoxymethyl)benzoate[1] (1):

A solution of 4-carboxybenzaldehyde (15.4 g, 102.6 mmol), trimethylorthoformate (32.7 g, 307.8 mmol) and H_2SO_4 (8 drops) in MeOH (100 mL) was heated under reflux conditions for 48 h. The reaction mixture was transferred to a separating funnel with saturated $NaHCO_{3(aq)}$ (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL). The organic extracts were combined and dried over Na_2SO_4, filtered and evaporated to dryness to afford a crude liquid which was purified by vacuum distillation to afford the title product as a clear liquid (19.8 g, 92%). ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 6H, $CH(OC\mathit{H}_3)_2$), 3.89 (s, 3H, $OC\mathit{H}_3$), 5.42 (s, 1H, $C\mathit{H}(OCH_3)_2$), 7.51 (d, 2H, Ar, J = 8.1 Hz), 8.02 (d, 2H, Ar, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 52.2, 53.0, 103.0, 127.1, 129.8, 130.8, 143.8, 167.1.

N-Ethylacrylamide-2-(4-(dimethoxymethyl)benzamide) (2):

A solution of methyl 4-(dimethoxymethyl)benzoate (**1**, 6.0 g, 28.5 mmol) in 1,2-diaminoethane (100 mL) was heated under reflux for 24 h then evaporated to dryness. The viscous yellow oil obtained was dissolved in CH_2Cl_2 (100 mL) and Et_3N (5.7 g, 56.3 mmol) added. The solution was cooled to 0 ˚C in an ice bath. Acryloyl chloride (2.6 g, 28.5 mmol) in CH_2Cl_2 (50 mL) was added dropwise over 30 min. The reaction was stirred overnight at room temperature then transferred to a separating funnel with saturated $NaHCO_{3(aq)}$ (150 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL). The organic extracts were combined and dried over Na_2SO_4, filtered and evaporated to dryness to afford a crude solid which was purified by column chromatography [SiO_2, EtOAc-Et_3N (95:5)] to afford the title product as a white solid (3.3 g, 40 %). ^{1}H NMR ($CDCl_3$): δ 3.28 (s, 6H, $CH(OC\mathit{H}_3)_2$), 3.52 (m, 4H, $(C\mathit{H}_2)_2$), 5.37 (s, 1H, $C\mathit{H}(OCH_3)_2$), 5.58 (dd, 1H, J = 9.6 Hz), 6.14 (dd, 1H, J = 17.1 Hz), 6.23 (dd, 1H, J = 17.1 Hz), 7.37 (s, 1H, N*H*), 7.45 (d, 2H, Ar, J = 8.1 Hz), 7.79 (d, 2H, Ar, J = 8.1 Hz), 7.84 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$): δ 41.3, 53.1, 103.1, 127.3, 128.2, 130.0, 131.3, 134.6, 142.1, 167.5, 168.6. FT-IR (wavenumber, cm^{-1}): 3290 (N–H), 3096 (C–H, alkene), 2947 (C–H, alkyl), 1634 (C=O), 1593 (C=O), 1448 (C=C, aromatic), 1413 (C=C, aromatic). $HRMS^+$ $C_{15}H_{21}N_2O_4$: Theoretical: 293.1501. Actual: 293.1503.

N-Ethylacrylamide-2-(4-formylbenzamide) (M1):

A solution of *N*-ethylacrylamide-2-(4-(dimethoxymethyl)benzamide) (**2**, 1.4 g, 4.8 mmol) in 1M $HCl_{(aq)}$ (20 mL) was stirred at room temperature for 2 h then neutralized with saturated

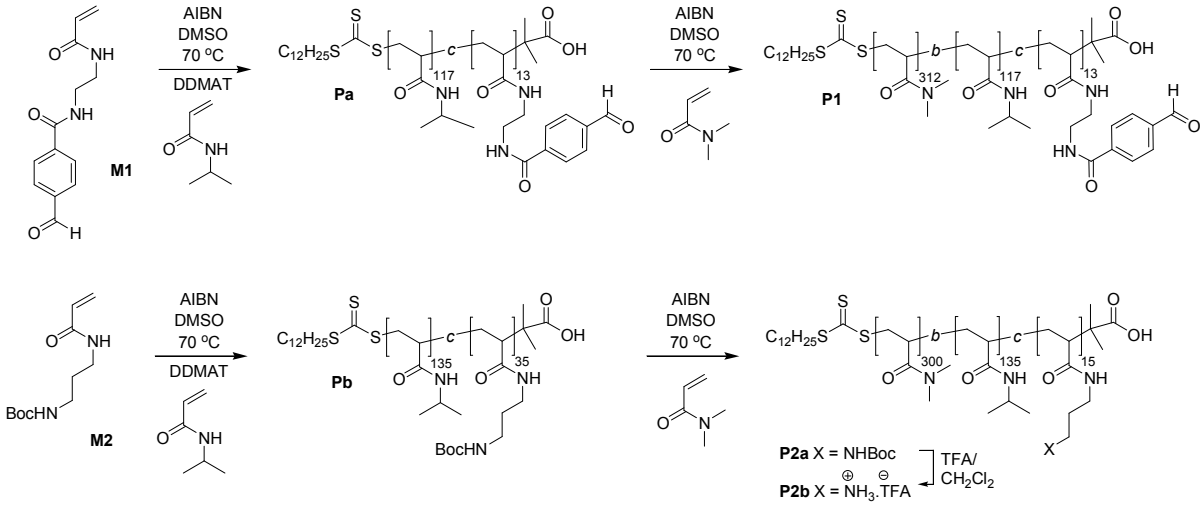
$NaHCO_{3(aq)}$ (100 mL). The aqueous layer was extracted with EtOAc (3 × 150 mL). The organic extracts were combined and dried over $MgSO_4$, filtered and evaporated to dryness to afford the title product as a white solid (0.99g, 84 %). ^{1}H NMR (DMSO-d_6): δ 3.72 (m, 4H, (CH_2)$_2$), 5.59 (dd, 1H, J = 9.6 Hz), 6.09 (dd, 1H, J = 17.1 Hz), 6.23 (dd, 1H, J = 17.1 Hz), 7.99 (d, 2H, Ar, J = 8.4 Hz), 8.03 (d, 2H, Ar, J = 8.4 Hz), 8.23 (s, 1H, N*H*), 8.79 (s, 1H, N*H*), 10.07 (s, 1H, C*H*O). ^{13}C NMR (DMSO-d_6): δ 38.7, 125.2, 128.3, 129.6, 132.3, 138.2, 140.1, 165.5, 166.1, 193.0. FT-IR (wavenumber, cm^{-1}): 3264 (N–H), 3091 (C–H, alkene), 2943 (C–H, alkyl), 1699 (C=O, aldehyde), 1627 (C=O, amide), 1549 (C=O, amide), 1447 (C=C, aromatic), 1414 (C=C, aromatic). HRMS$^+$ $C_{13}H_{15}N_2O_3$: Theoretical: 247.1083. Actual: 247.1085.

_N_-(_Tert_-butoxycarbonyl)-l,3-diaminopropane[2] (3):

A solution of 1,3-diaminopropane (25.0 g, 337 mmol) in $CHCl_3$ (200 mL) was cooled to 0 ˚C in an ice bath. A solution of di-*tert*-butyl dicarbonate (10.5 g, 48 mmol) in $CHCl_3$ (100 mL) was added dropwise over 30 min. The reaction mixture was stirred overnight at room temperature then filtered and the filtrate evaporated to dryness. Saturated $NaCl_{(aq)}$ (150 mL) was added to the clear oil obtained. The solution was filtered to remove the bis-protected product then transferred into a separating funnel with diethyl ether (150 mL), the aqueous layer was extracted with diethyl ether (2 × 150 mL). The organic extracts were combined and dried over Na_2SO_4, filtered and evaporated to dryness to afford the title product as a clear liquid (6.1 g, 73 %). ^{1}H NMR ($CDCl_3$): δ 1.20 (s, 2H, NH_2), 1.38 (s, 9H, C(CH_3)$_3$), 1.54 (p, 2H, CH_2-CH_2-CH_2, J = 6.6 Hz), 2.68 (q, 2H, CH_2N, J = 6.6 Hz), 3.14 (q, 2H, CH_2N, J = 6.6 Hz), 5.03 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$): δ 28.8, 31.5, 33.9, 40.0, 79.4, 156.5. FT-IR (wavenumber, cm^{-1}): 3230 (N–H), 3066 (C–H, alkene), 2941 (C–H, alkyl), 1686 (C=O), 1652 (C=O). HRMS$^+$ $C_8H_{19}N_2O_2$: Theoretical: 175.1447. Actual: 175.1449.

_N_-(_Tert_-butoxycarbonyl)-propylaminoacrylamide (M2):

A solution of *N*-(*tert*-butoxycarbonyl)-l,3-diaminopropane (**3**, 3.1 g, 17.8 mmol) and Et_3N (2.2 g, 21.4 mmol) in CH_2Cl_2 (75 mL) was cooled to 0 ˚C in an ice bath. Acryloyl chloride (1.6 g, 17.8 mmol) in CH_2Cl_2 (50 mL) was added dropwise over 30 min. The reaction was stirred overnight at room temperature then transferred to a separating funnel with saturated $NaHCO_{3(aq)}$ (150 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL). The organic extracts were combined and dried over Na_2SO_4, filtered and evaporated to dryness to afford a crude solid which was purified by column chromatography [SiO_2, Hexane-EtOAc (1:1)] to afford the title product as a white solid (3.2 g, 79 %). ^{1}H NMR ($CDCl_3$): δ 1.37 (s, 9H, C(CH_3)$_3$), 1.59 (p, 2H, CH_2-CH_2-CH_2, J = 6 Hz), 3.10 (q, 2H, CH_2N, J = 6 Hz), 3.30 (q, 2H, CH_2N, J = 6 Hz), 5.27 (s, 1H, N*H*), 5.56 (dd, 1H, J = 9.6 Hz), 6.10 (dd, 1H, J = 17.1 Hz), 6.20 (dd, 1H, J = 17.1 Hz), 7.02 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$): δ 27.8, 30.5, 36.6, 37.9, 79.6, 125.9, 131.7, 157.0, 166.4. HRMS$^+$ $C_{11}H_{20}N_2O_3Na$: Theoretical: 251.1372. Actual: 251.1374.



S3. Synthesis of aldehyde/ amine functional diblock copolymers **P1** and **P2**.

Aldehyde-Functionalized Copolymer (Pa):

S-1-Dodecyl-*S'*-(α,α-dimethyl-α''-acetic acid)trithiocarbonate[3] (DDMAT) (1 eq, 21.5 mg, 0.059 mmol) and AIBN (0.2 eq, 1.93 mg, 12 μmol) were added to a small schlenk tube. *N*-Isopropylacrylamide (180 eq, 1.20 g, 10.62 mmol) and *N*-ethylacrylamide-2-(4-formylbenzamide) (**M1**, 20 eq, 0.29 g, 1.18 mmol) were then added followed by DMSO (2.5 mL). The reaction mixture was degassed five times, and then the vessel was backfilled with N_2, purged with N_2, and allowed to warm to room temperature. The reaction mixture was then placed in an oil bath at 70 ˚C, and the polymerization was quenched after 1 h. The reaction mixture was dissolved in a minimal amount of THF and added dropwise to a large excess of ice-cold diethyl ether. The polymer precipitate was then isolated by filtration and the precipitation was repeated before drying under high vacuum. Polymer **Pa** was obtained as a pale yellow solid (0.93 g). ^{1}H NMR ($CDCl_3$): 1.09 (br, $CH(CH_3)_2$), 1.61 (br, $CHCH_2$, polymer backbone), 2.12 (br, $CHCH_2$, polymer backbone), 3.49 (br, $(CH_2)_2$), 3.96 (br, $CH(CH_3)_2$), 6.50 (br, NH), 7.89 (br, Ar), 8.04 (br, Ar), 8.45 (br, NH), 10.04 (br, CHO). The composition of **Pa** can be determined by comparing the integration of the aldehyde protons of **M1** with the integration of the $CH(CH_3)_2$ protons of NIPAm. The monomer composition was determined to be 9:1 NIPAm:**M1** (identical to the feed ratio).

Aldehyde-Functionalized Diblock Copolymer (P1):

Pa (1 eq, 0.74 g, 43.8 μmol) and AIBN (0.2 eq, 1.44 mg, 8.8 μmol) were added to a small schlenk tube. *N*,*N*'-Dimethylacrylamide (400 eq, 1.74 g, 17.5 mmol) was then added followed by DMSO (4 mL) and the reaction mixture was degassed five times. The vessel was backfilled with N_2, purged with N_2, and allowed to warm to room temperature. The reaction mixture was then placed in an oil bath at 70 ˚C, and the polymerization was quenched after 1 h. The reaction mixture was dissolved in a minimal amount of THF and added dropwise to a large excess of ice-cold diethyl ether. The polymer precipitate was then isolated by filtration and the precipitation was repeated before drying under high vacuum. Polymer **P1** was obtained as a pale yellow solid (2.09 g). ^{1}H NMR (D_2O): δ 1.10 (br, $CH(CH_3)_2$), 1.36 (br, $CHCH_2$, polymer backbone), 1.62 (br, $CHCH_2$, polymer backbone), 2.90 (br m, $N(CH_3)_2$), 3.86 (br, $CH(CH_3)_2$), 7.89 (br, Ar), 9.97 (br, CHO).

Boc-Amine-Functionalized Copolymer (Pb):

S-1-Dodecyl-*S'*-(α,α-dimethyl-α''-acetic acid)trithiocarbonate[3] (DDMAT) (1 eq, 26.1 mg, 0.072 mmol) and AIBN (0.2 eq, 2.35 mg, 14 μmol) were added to a small schlenk tube. *N*-Isopropylacrylamide (180 eq, 1.46 g, 12.9 mmol), *N*-(*tert*-butoxycarbonyl)-propylaminoacrylamide (**M2**, 20 eq, 0.33 g, 1.43 mmol) were then added followed by DMSO (2.5 mL). The reaction mixture was degassed five times, and then the vessel was backfilled with N_2, purged with N_2, and allowed to warm to room temperature. The reaction mixture was then placed in an oil bath at 70 ˚C, and the polymerization was quenched after 1 h. The reaction mixture was dissolved in a minimal amount of THF and added dropwise to a large excess of ice-cold diethyl ether. The polymer precipitate was then isolated by filtration and the precipitation was repeated before drying under high vacuum. Polymer **Pb** was obtained as a pale yellow solid (1.33 g). ^{1}H NMR ($CDCl_3$): 1.11 (br, $CH(CH_3)_2$), 1.39 (br, $C(CH_3)_3$), 1.62 (br, $CHCH_2$, polymer backbone), 2.11 (br, $CHCH_2$, polymer backbone), 3.09 (br, $NHCH_2CH_2$), 3.58 (br, $NHCH_2CH_2$), 3.97 (br, $CH(CH_3)_2$), 5.50 (br, NH), 6.50 (br, NH). The composition of **Pb** can be determined by comparing the integration of the *Boc* protons of **M2** with the integration of the $CH(CH_3)_2$ protons of NIPAm. The monomer composition was determined to be 9:1 NIPAm:**M1** (identical to the feed ratio).

Boc-Amine-Functionalized Diblock Copolymer (P2a):

Pb (1 eq, 1.04 g, 54.8 μmol) and AIBN (0.2 eq, 1.8 mg, 10.9 μmol) were added to a small schlenk tube. *N*,*N*'-Dimethylacrylamide (400 eq, 2.17 g, 21.9 mmol) was then added followed by DMSO (4 mL) and the reaction mixture was degassed five times. The vessel was backfilled with N_2, purged with N_2, and allowed to warm to room temperature. The reaction mixture was then placed in an oil bath at 70 ˚C, and the polymerization was quenched after 1 h. The reaction mixture was dissolved in a minimal amount of THF and added dropwise to a large excess of ice-cold diethyl ether. The polymer precipitate was then isolated by filtration and the precipitation was repeated before drying under high vacuum. Polymer **P2a** was obtained as a pale yellow solid (2.37 g). ^{1}H NMR ($CDCl_3$): 1.09 (br, $CH(CH_3)_2$), 1.38 (br, $C(CH_3)_3$), 1.58 (br, $CHCH_2$, polymer backbone), 2.07 (br, $CHCH_2$, polymer backbone), 2.86 (br, $N(CH_3)_2$), 3.95 (br, $CH(CH_3)_2$), 5.50 (br, NH), 6.50 (br, NH).

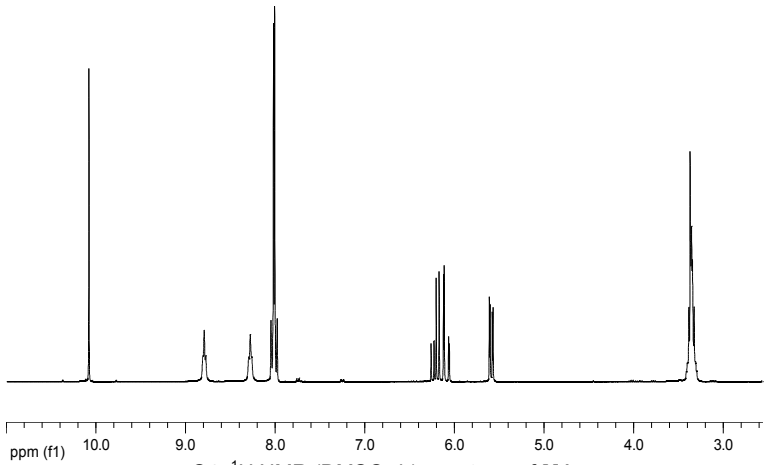
Amine-Functionalized Diblock Copolymer (P2b):

P2a (2.08 g) was dissolved in CH_2Cl_2 (5 mL) and TFA (3 mL) was added, the reaction mixture was left to stir for 2 h. After this time the solvent and excess TFA were removed on the rotary evaporator and the oil obtained was dissolved in a minimal amount of THF and added dropwise to a large excess of diethyl ether. The polymer precipitate was then isolated by filtration and dried under high vacuum. Polymer **P2b** was obtained as a pale yellow solid (1.82 g). ^{1}H NMR (D_2O): δ 1.09 (br, $CH(CH_3)_2$), 1.34 (br, $CHCH_2$, polymer backbone), 1.59 (br, $CHCH_2$, polymer backbone), 2.87 (br, $N(CH_3)_2$), 3.84 (br, $CH(CH_3)_2$).

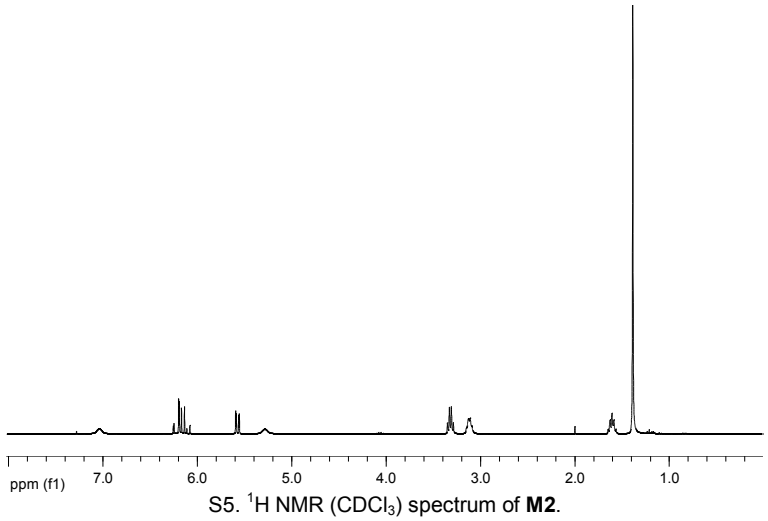
General Procedure for the Formation of CCS Polymers and Encapsulation of Nile Red:

P1 (47.4 mg, 1 μmol, 1 eq) was dissolved in H_2O (4.73 mL) and **P2b** (48.2 mg, 1 μmol, 1 eq) was dissolved in H_2O (4.73 mL), these solution were then combined under rapid stirring resulting in a solution with a total diblock copolymer concentration of 0.211 mM (1 wt %). The pH was adjusted to 11.0 with small aliquots (5 μL) of 1M $NaOH_{(aq)}$ and left to equilibrate overnight at room temperature to allow for the formation of CCS polymers. After this time

Nile Red (9.56 mg, 30 μmol, 30 eq per polymer chain) was added resulting in a solution with a Nile Red concentration of 3.17 mM. This solution was stirred at 45 ˚C for 16 h. After this time the excess Nile Red was removed by filtration. The encapsulation was then confirmed by fluorescence spectroscopy (λ_{ex} = 550 nm). To trigger the disassembly of CCS polymers and the subsequent release of Nile Red the pH was adjusted to 5.5 with small aliquots (5 μL) of 1M $HCl_{(aq)}$ and left to equilibrate overnight at room temperature. Fluorescence spectroscopy (λ_{ex} = 550 nm) confirmed the release of Nile Red.



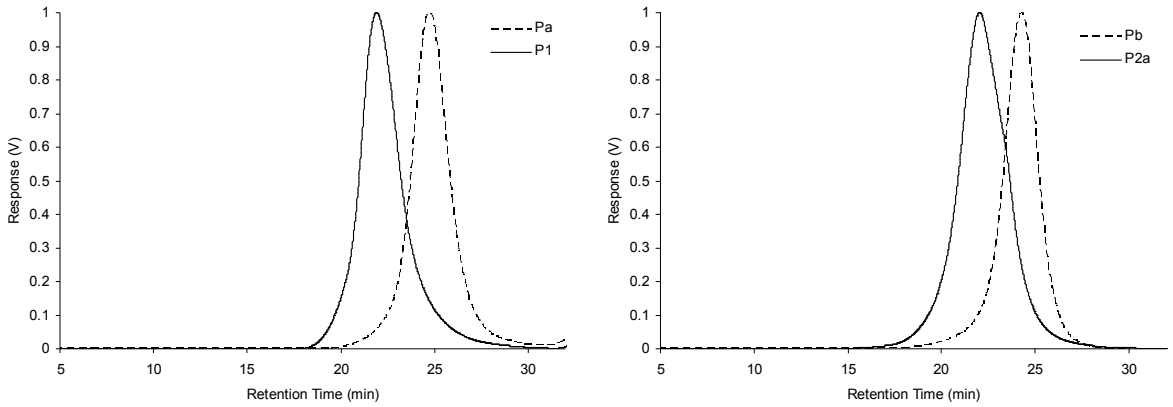
S4. ^{1}H NMR (DMSO-d_6) spectrum of **M1**.



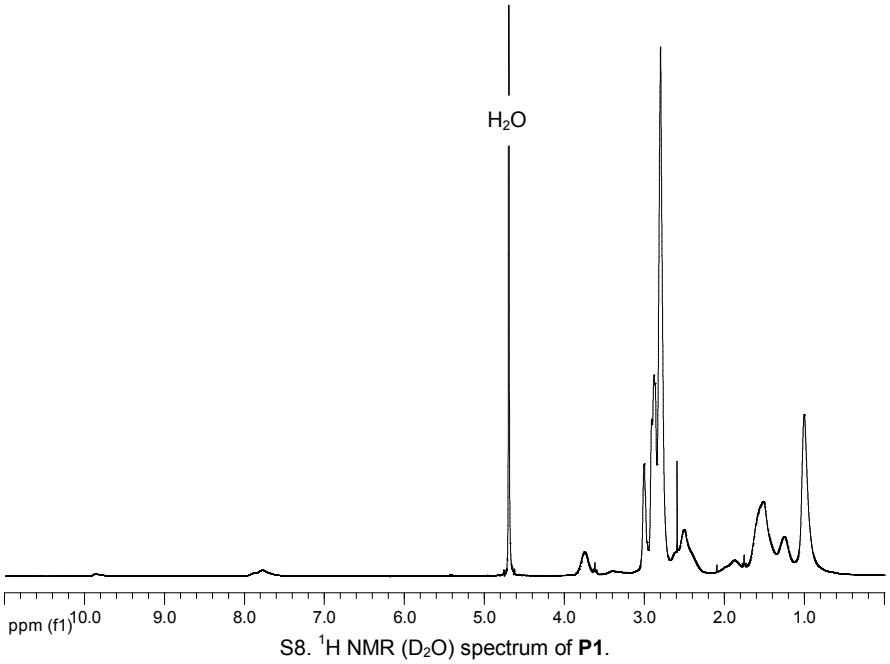
S5. ^{1}H NMR ($CDCl_3$) spectrum of **M2**.

polymer	(macro)chain transfer agent	monomer	co-monomer	initiator	solvent	temp (˚C)	time (h)	M_n[a] (Da)	M_w[a] (Da)	PDI[a] (M_w/M_n)
Pa	DDMAT (1 eq)	NIPAm (180 eq)	**M1** (20 eq)	AIBN (0.2 eq)	DMSO	70	1	16,100	21,200	1.32
P1	**Pa** (1 eq)	DMA (400 eq)	-	AIBN (0.2 eq)	DMSO	70	1	47,400	65,600	1.38
Pb	DDMAT (1 eq)	NIPAm (180 eq)	**M2** (20 eq)	AIBN (0.2 eq)	DMSO	70	1	22,200	27,200	1.23
P2a	**Pb** (1 eq)	DMA (400 eq)	-	AIBN (0.2 eq)	DMSO	70	1	48,200	68,900	1.43

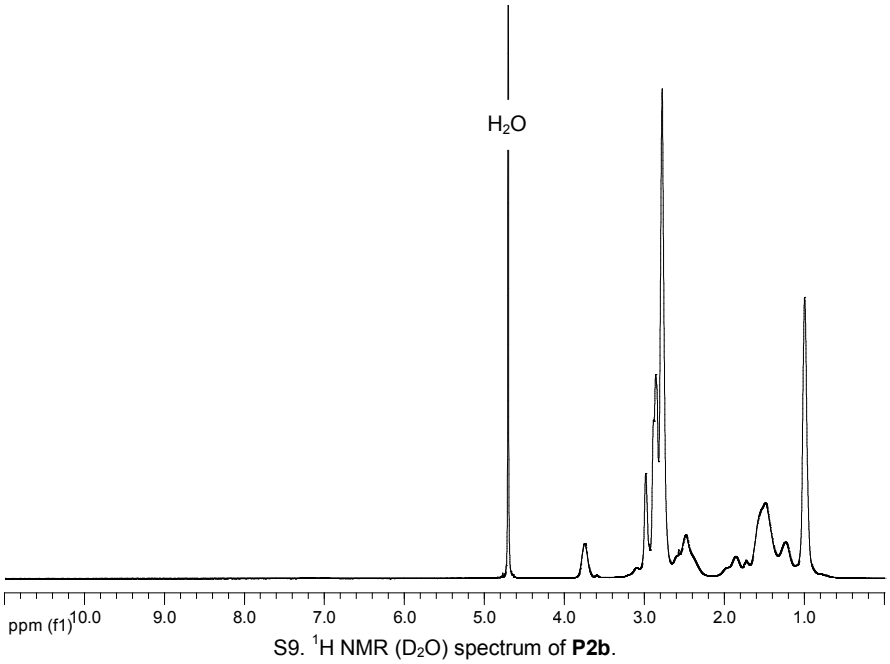
S6. Characterization of diblock copolymer building blocks. [a] As determined by gel permeation chromatography in DMF (0.6 mL/min) calibrated against near monodisperse methyl methacrylate standards. AIBN: azobis(isobutyronitrile), DMSO: dimethyl sulfoxide, NIPAm: *N*-isopropylacrylamide, DMA: *N,N'*-dimethylacrylamide, DDMAT: *S*-1-dodecyl-*S'*-(α,α-dimethyl-α''-acetic acid)trithiocarbonate.



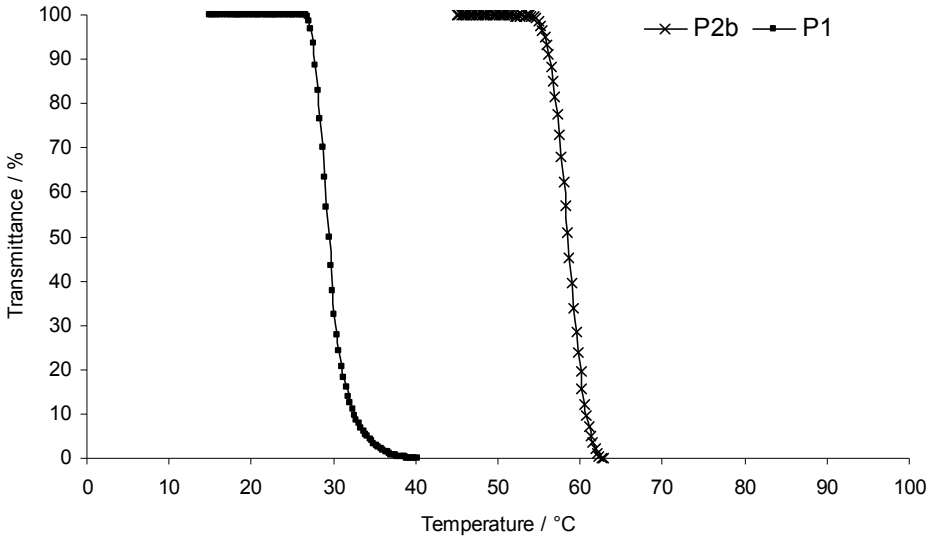
S7. Differential refractive index (dRI) GPC traces of polymers **Pa** – **P1** and **Pb** – **P2a** in DMF (0.6 mL/ min).



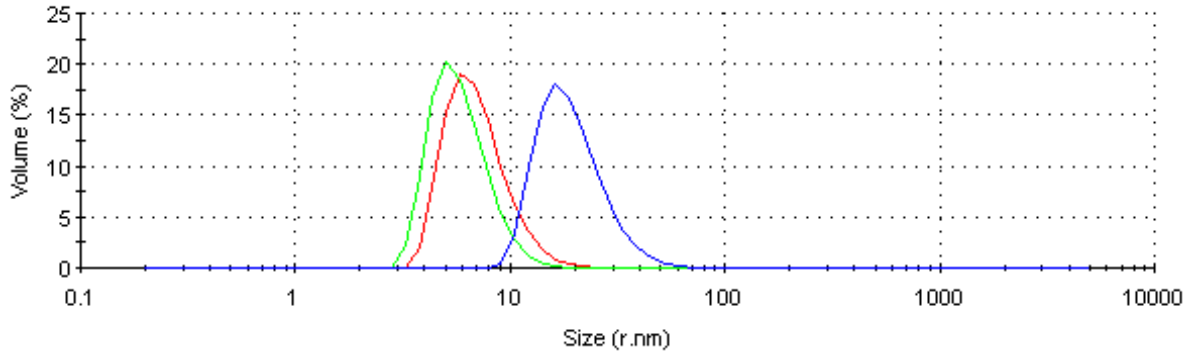
S8. ^{1}H NMR (D_2O) spectrum of **P1**.



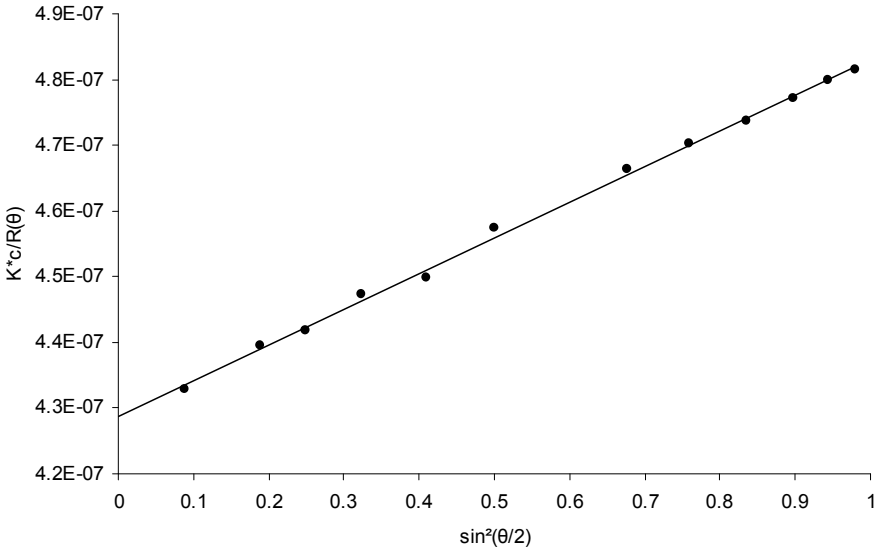
S9. ^{1}H NMR (D_2O) spectrum of **P2b**.



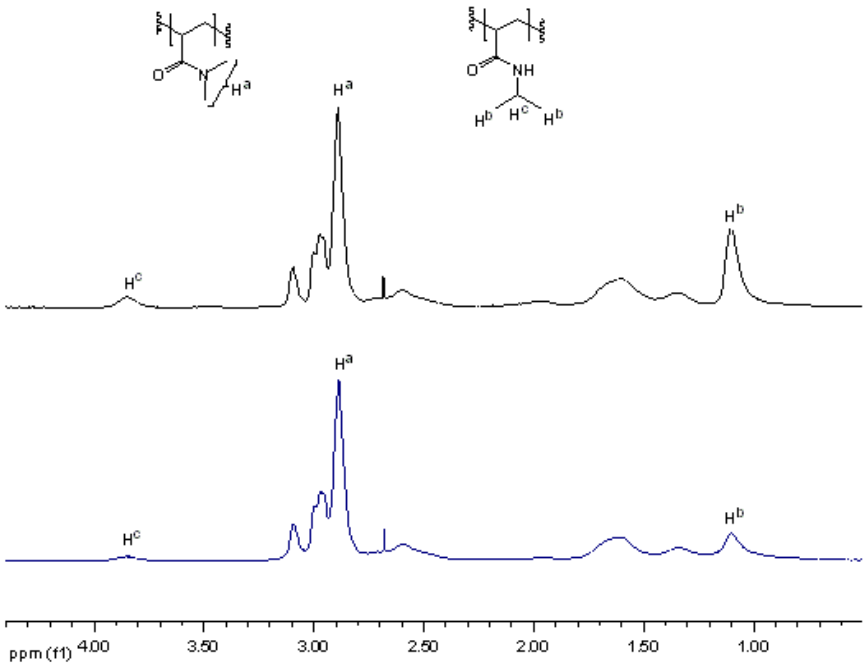
S10. Temperature-turbidity curves for diblock copolymers **P1** and **P2b** at pH 11.0 (5 mg/ mL).



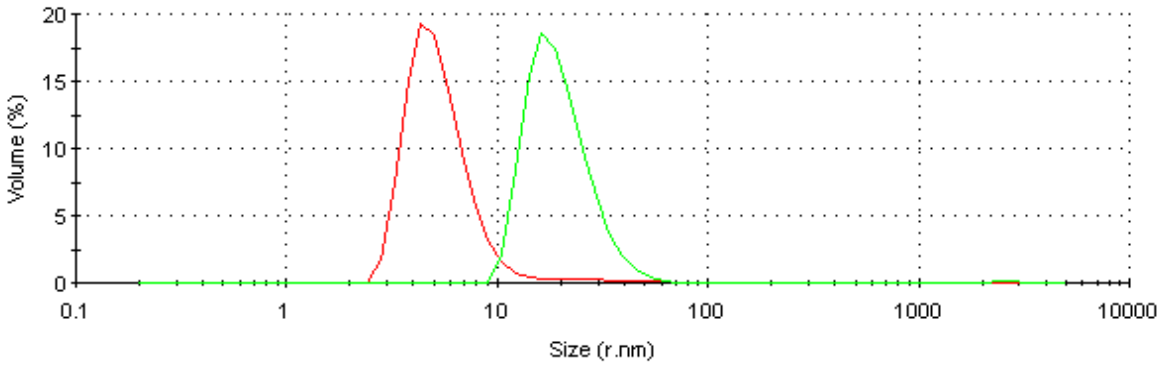
S11. Dynamic light scattering analysis (H_2O, 1 wt %, 20 ˚C) of diblock copolymer building block **P1** (red line) and **P2b** (green line), and CCS (blue line) polymer formed after cross-linking at pH 11.0.



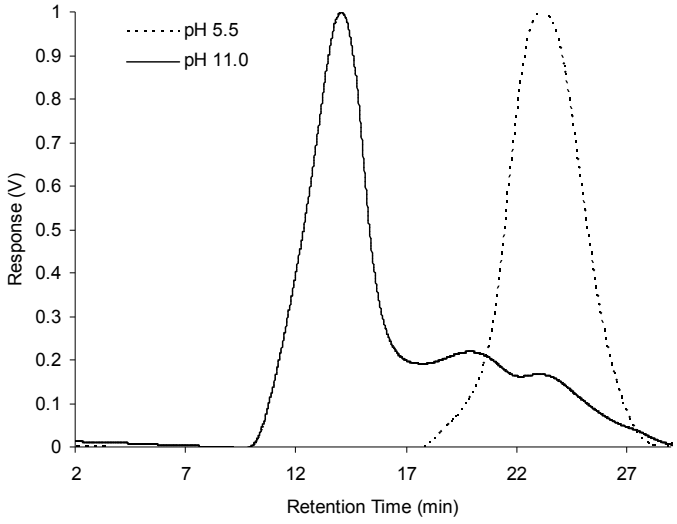
S12. Debye plot for CCS polymer at (2.5 mg/ mL). Batch analysis carried out in H_2O (1 mL/ min) over a range of concentrations (0.3 – 5 mg/ mL), using experimentally determined *dn*/*dc* value of 0.166 mL/ g. M_w was estimated to be 2,332 kDa (49 polymer chains per assembly). R_g = 24.3 nm, R_h = 19.3 nm and structure sensitive ρ parameter (ρ = R_g/ R_h) was found to be ~ 1.26.



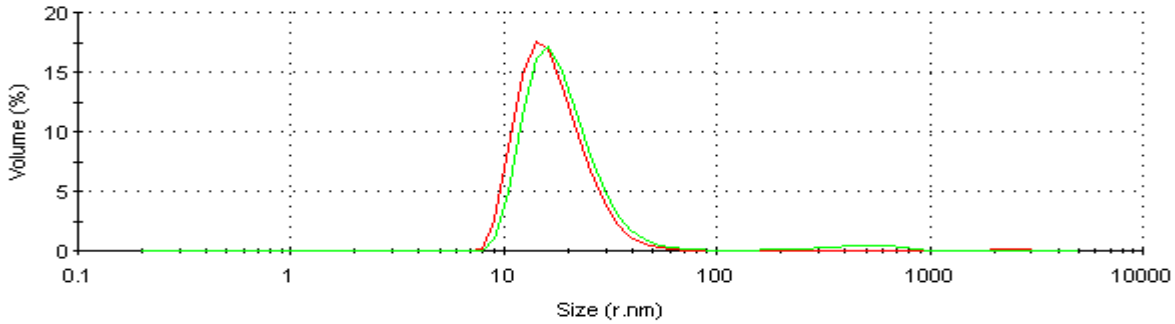
S13. ^{1}H NMR spectra before (top) and after (lower) CCS polymer formation. pH 5.5 (top) and pH 11.0 (lower).



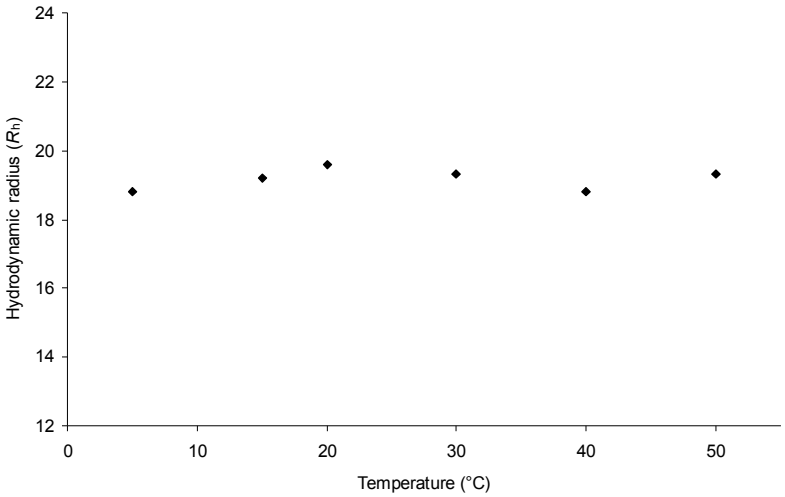
S14. Dynamic light scattering analysis (H_2O, 1 wt %, 20 ˚C) of CCS polymer formation (green line) at pH 11.0 and CCS polymer disassembly (red line) at pH 5.5.



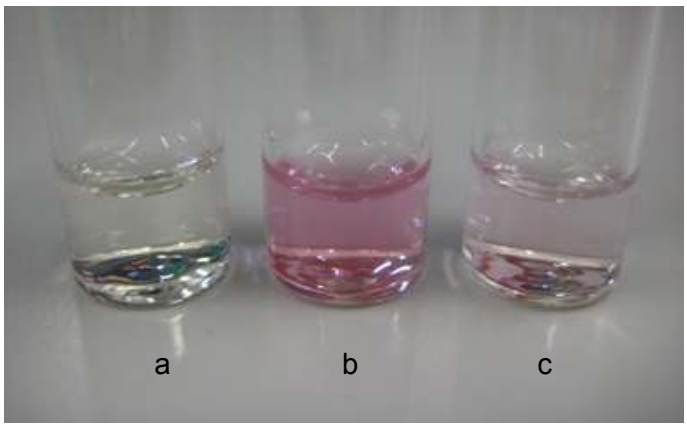
S15. GPC Analysis displaying pH-triggered disassembly of CCS polymer in DMF (0.6 mL/ min). To prevent diblock copolymer building blocks reacting together in column, acetone (1 drop) was added to GPC sample to cap amine functionalities.



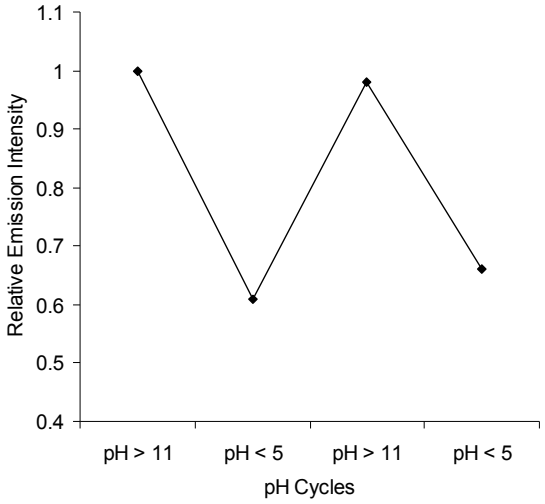
S16. CCS polymer (green line) at pH 11.0 and reduced CCS polymer species (red line) at pH 5.5 after addition of $NaCNBH_3$.



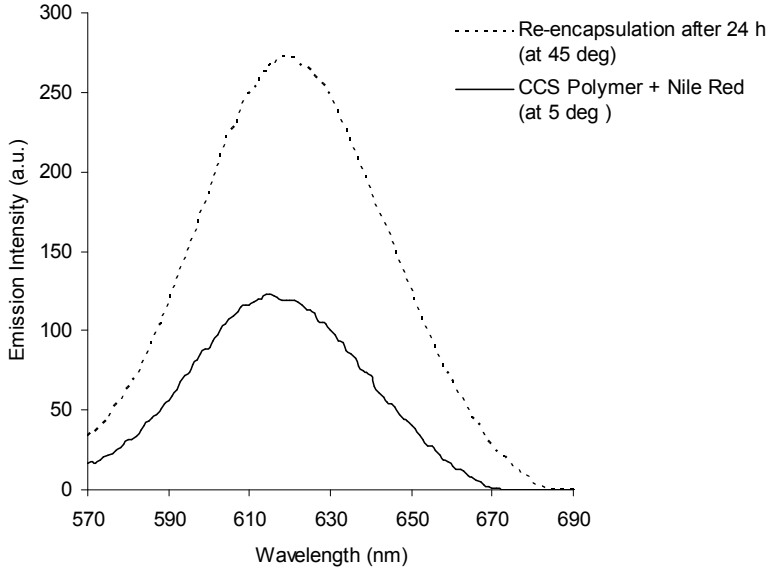
S17. Hydrodynamic radii of CCS polymers (pH 11.0, 1 wt %) as a function of temperature. DLS analysis for data point at 5 ˚C was carried out at 15 ˚C after sample was stored overnight at 5 ˚C.



S18. a: Nile Red in H_2O, b: CCS polymer + Nile Red at pH 11.0, c: CCS polymer + Nile Red at pH 5.5 after CCS polymer disassembly. All solutions have been filtered to remove excess or precipitated Nile Red.



S19. Reversible Nile Red encapsulation cycling from pH 11.0 (cycles 0 and 2) to pH 5.5 (cycles 1 and 3).



S20. Temperature triggered re-encapsulation of Nile Red followed by fluorescence spectroscopy. After cooling to 5 ˚C for 72 h at pH 11.0 (solid line), the solution was heated to 45 ˚C for 24 h at pH 11.0 (dotted line). The increase in fluorescence intensity suggests the encapsulation of Nile Red with the CCS polymers.

References:

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[3] J. T. Lai, D. Filla and R. Shea, *Macromolecules,* 2002, **35**, 6754.