

## Supporting Information

### Topology-directed multi-tunable self-assembly of linear and tadpole-shaped amorphous-responsive-crystalline terpolymers

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## Experimental

### Materials

The chemicals were purchased from TCI unless otherwise stated. Styrene (St, 99%) was purified by successive washing with 5% NaOH aqueous solution and water, drying over Na<sub>2</sub>SO<sub>4</sub> and distilled under reduced pressure. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 97%) was passed through a basic alumina column, and  $\epsilon$ -caprolactone (CL, 99%, Sigma-Aldrich) was distilled from CaH<sub>2</sub> under reduced pressure. 2,2'-Azobis(isobutyronitrile) (AIBN, 99%) was recrystallized twice from ethanol. *N,N'*-dicyclohexylcarbodiimide (DCC, 95%, Macklin), 4-dimethylamino pyridine (DMAP, 97%, Macklin), stannous octoate (Sn(Oct)<sub>2</sub>, 97%, Sigma-Aldrich), and other reagents with analytical grade were used directly. The organic solvents were dried and distilled according to standard procedures. 4-Cyanopentanoic acid dithiobenzoate (4-CPDB),<sup>1</sup> 2,2,5-trimethyl-(1,3)-dioxane-5-carboxylic acid (TDCA),<sup>2</sup> 2-(3-hydroxypropoxy)-6-methyl-benzaldehyde (HMBA),<sup>3</sup> and 3-(2-formyl-3-methyl phenoxy)propyl 2-((4-cyano-4-phenylthiocarbonylsulfanyl)pentanoyloxymethyl)-2-hydroxymethyl propionate (FCHP)<sup>4</sup> were synthesized and purified according to reference methods.

### Synthesis of PDMAEMA (P1)

FCHP (57 mg, 0.10 mmol), AIBN (1.6 mg, 0.010 mmol), DMAEMA (1.10 g, 7.0 mmol), and dioxane were successively added to a Schlenk tube, and the total volume was 2.3 mL. The solution was degassed by bubbling N<sub>2</sub> for 20 min. RAFT polymerization was conducted at 70 °C for 6 h. The polymer solution was concentrated and repeatedly precipitated from THF solution into hexane. After vacuum drying 40 °C, 0.72 g (60% conversion) of PDMAEMA was obtained as red powders. GPC and <sup>1</sup>H NMR analyses:  $M_{n, \text{GPC}} = 6.90$  kDa,  $D_M = 1.12$ , and  $M_{n, \text{NMR}} = 6.86$  kDa ( $DP_{\text{PDMAEMA}} = 40$ ). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  10.66 (s, CHO), 6.7-8.0 (m, terminal PhH and ArH), 3.8-4.5 (m, ArOCH<sub>2</sub>, CH<sub>2</sub>OCO, and CH<sub>2</sub>O of PDMAEMA), 3.68 (m, CH<sub>2</sub>OH), 0.7-2.8 (m, other CH, CH<sub>2</sub> and CH<sub>3</sub> originating from end group and PDMAEMA).

### Synthesis of linear PDMAEMA-*b*-PSt (P2)

To a Schlenk tube were added P1 (0.343 g, 0.050 mmol), St (1.04 g, 10 mmol) and dioxane solution comprising 0.8 mg (0.005 mmol) of AIBN, and then dioxane was added until the total volume was 5.0 mL. After degassing via bubbling N<sub>2</sub> for 20 min, the chain extension polymerization was conducted at 70 °C for 18 h. The polymer solution was concentrated, and the diblock copolymer was isolated by dissolving in THF and precipitating into hexane. After vacuum drying at 40 °C, 0.51 g (16% conversion) of PDMAEMA-*b*-PSt was obtained as red powders. GPC and <sup>1</sup>H NMR analyses:  $M_{n, \text{GPC}} = 9.87$  kDa,  $D_M = 1.10$ , and  $M_{n, \text{NMR}} = 10.2$  kDa ( $DP_{\text{PSt}} = 32$ ). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  10.66 (s, CHO), 6.2-8.0 (m, terminal PhH and ArH, and PhH of PSt), 3.8-4.5 (m, ArOCH<sub>2</sub>, CH<sub>2</sub>OCO, and CH<sub>2</sub>O of PDMAEMA), 3.68 (m, CH<sub>2</sub>OH), 0.7-2.8 (m, other CH, CH<sub>2</sub> and CH<sub>3</sub> originating from end group, PDMAEMA and PSt).

### Synthesis of cyclic PDMAEMA-*b*-PSt (P3)

P3 was prepared by UV-induced Diels-Alder ring-closure reaction. A low-pressure mercury lamp (120 W, CEL-LPH120-254, Beijing China Education Au-light Co., Ltd.) served as the UV light source. In a typical run, P2 (20 mg) and acetonitrile (500 mL) were added to a round-bottom flask, and the solution was subjected to UV irradiation (with a dose of around 0.10 mW cm<sup>-2</sup>) at 25 °C for 15 h. Evaporation of the solvent allowed to recycle the solvent, and P3 was isolated as yellow solid powders. Repetitive ring-closure reaction using recycled acetonitrile and fresh P2 was performed to enhance the amount of cyclic copolymer. GPC analysis:  $M_{n, \text{GPC}} = 8.42$  kDa,  $D_M = 1.12$ . <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.2-7.6 (m, PhH and ArH of linking group and PhH of PSt), 5.23 (m, ArCHOH of linking group), 3.8-4.5 (m, ArOCH<sub>2</sub>, CH<sub>2</sub>OCO, and CH<sub>2</sub>O of PDMAEMA), 3.64 (m, CH<sub>2</sub>OH), 3.48 (m, ArCH<sub>2</sub> of linking group), 0.5-2.9 (m, other CH, CH<sub>2</sub> and CH<sub>3</sub> originating from linking group, PDMAEMA and PSt).

### Synthesis of P4 and P5

CL polymerization using P2 as a macroinitiator gave linear triblock terpolymer PCL-*b*-PDMAEMA-*b*-PSt (P4), and ROP of CL initiated with P3 afforded tadpole-shaped terpolymer PCL-*b*-[*c*-(PDMAEMA-*b*-PSt)] (P5). In a typical run, the THF solution comprising P2 (0.204 g, 0.020 mmol) and five-fold triethylborane (Et<sub>3</sub>B) was stirred in a Schlenk tube for 1 h, followed by reduced pressure to remove excess Et<sub>3</sub>B and solvent. Afterwards, CL (91 mg, 0.80 mmol), Sn(Oct)<sub>2</sub> (4.1 mg, 0.010 mmol) and dry toluene were added to the Schlenk tube under nitrogen, and the total volume was 1.6 mL. The mixed solution was subjected to three freeze-vacuum-thaw cycles, followed by polymerization at 100 °C for 20 h. The product was purified by precipitation into hexane, and 0.286 g (93% conversion) of P4 was isolated after vacuum drying. Similarly, ROP of CL initiated with P3 macroinitiator gave P5 in 90% conversion.

P4: GPC and <sup>1</sup>H NMR analyses:  $M_{n, \text{GPC}} = 17.0$  kDa,  $D_M = 1.10$ , and  $M_{n, \text{NMR}} = 14.3$  kDa ( $DP_{\text{PCL}} = 36$ ). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  10.66 (s, CHO), 6.2-7.5 (m, ArH and PhH of PSt), 3.8-4.5 (m, ArOCH<sub>2</sub>, CH<sub>2</sub>OCO, and CH<sub>2</sub>O of PDMAEMA and PCL), 3.65 (m, terminal CH<sub>2</sub>OH), 0.5-2.9 (m, other CH, CH<sub>2</sub> and CH<sub>3</sub> originating from linking group, PDMAEMA, PSt and PCL).

P5: GPC and <sup>1</sup>H NMR analyses:  $M_{n, \text{GPC}} = 15.3$  kDa,  $D_M = 1.10$ , and  $M_{n, \text{NMR}} = 14.2$  kDa ( $DP_{\text{PCL}} = 35$ ). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.2-7.6 (m, PhH and ArH of linking group, and PhH of PSt), 5.23 (m, ArCHOH of linking group), 3.8-4.5 (m, ArOCH<sub>2</sub>, CH<sub>2</sub>OCO, and CH<sub>2</sub>O of PDMAEMA and PCL), 3.65 (m, terminal CH<sub>2</sub>OH), 3.48 (m, ArCH<sub>2</sub> of linking group), 0.5-2.9 (m, other CH, CH<sub>2</sub> and CH<sub>3</sub> originating from linking group, PDMAEMA, PSt and PCL).

### Self-assembly in DMF-*d*<sub>7</sub> and mixed solvent

Copolymer (P4 or P5, 6.0 mg) and cosolvent (DMF-*d*<sub>7</sub>, 3.0 mL) were added to a vial, and stirring overnight allowed to form the pre-assembled solution. Afterwards, 2.0 mL of copolymer solution was subdivided into five vials (with 0.2-0.6 mL of solution per vial), followed by slow addition of D<sub>2</sub>O via metering pump (with addition rate of 1.0 mL h<sup>-1</sup>) under stirring. After addition, the mixture was further stirred overnight to reach an equilibrium, and copolymer assemblies formed in the mixed solvent were obtained. The volume ratio of D<sub>2</sub>O to DMF-*d*<sub>7</sub> in each vial was 1, 2, 3, 5 and 10, respectively. Finally, various solutions formed in the single or mixed solvent were subjected to <sup>1</sup>H NMR and DLS analyses.

### Thermo/pH-induced morphological transformations

The solvent switch method was used to prepare P4 and P5 assemblies formed in aqueous solution. Copolymer (6.0 mg) was initially stirred in DMF (3.0 mL) for 8 h to form the pre-assembled solution, followed by slow addition of deionized water (8 mL) in 8 h under vigorous stirring. After stirring for another 5 h, the resultant solution was transferred into a dialysis membrane tubing (MWCO 3000) and subjected to dialysis against deionized water for 36 h. Afterwards, deionized water was carefully added

to reach a total volume of 12 mL, and copolymer assemblies with a  $c_p$  of  $0.5 \text{ mg mL}^{-1}$  was obtained. The solution pH of initial copolymer solutions was 7.5 (for P4 assemblies) and 7.4 (for P5 assemblies), respectively. Dilute HCl and NaOH aqueous solution were carefully added to copolymer solutions to reach pH 3.0 or 10, followed by stirring overnight in closed vials. To investigate thermo-induced self-assembly, copolymer solutions were kept at the predetermined temperature for a period of time (typically for 30 min), followed by sample preparation using freeze-drying technique. The processes for heating and thermostatic placement were continuously conducted until the highest temperature was reached. On this basis, DLS and TEM measurements were conducted to reveal thermo/pH-induced self-assembly.

### **Determination of critical aggregation concentration**

Critical aggregation concentration (CAC) of P4 and P5 was obtained by fluorescence approach. The aforementioned self-assembly using DMF/water (volume ratio of 1 : 3) mixture and dialysis were used to prepare copolymer aqueous solution. After diluting with deionized water and stirring, copolymer solutions with predetermined concentrations were obtained. A predetermined amount of pyrene solution in acetone was added into volumetric flasks. As the solvent was evaporated, copolymer aqueous solution with different concentrations were added to each flask. The mixture was stirred overnight to reach an equilibrium, and the concentration of pyrene in each flask was fixed at about  $6.0 \mu\text{mol L}^{-1}$ . The excitation spectra were recorded with an excitation wavelength of 335 nm to monitor the emission fluorescence at 372 and 383 nm. Concentration-dependent plots of intensity ratios at the bands of 383 and 372 nm ( $I_3/I_1$ ) were drawn. The CAC was deduced as the intersection concentration by extrapolating the intensity ratio in different concentration regions.

### **Characterization**

$^1\text{H}$  NMR spectra were recorded on a Varian Unity Inova 400 MHz spectrometer at  $25 \text{ }^\circ\text{C}$ , in which  $\text{CDCl}_3$ ,  $\text{DMF-}d_7$  or  $\text{DMF-}d_7/\text{D}_2\text{O}$  mixture served as the solvent. Apparent molar mass ( $M_{n,\text{GPC}}$ ) and dispersity ( $D_M$ ) of linear or nonlinear polymers were measured on a TOSOH HLC-8320 gel permeation chromatography (GPC) using three TSKgel SuperMultipore HZ-M columns at  $40 \text{ }^\circ\text{C}$ . DMF was used as an eluent at a flow rate of  $0.35 \text{ mL min}^{-1}$ , and the samples were calibrated with PMMA standard samples. Differential scanning calorimetry (DSC) was measured on Q200 DSC from TA Instruments using a heating/cooling rate of  $10 \text{ K min}^{-1}$ . To determine the cloud point ( $T_c$ ) of copolymer assemblies formed in aqueous solution, turbidity analysis was performed at 600 nm on a Shimadzu UV-3150 UV-Vis spectrophotometer equipped with a thermoregulator. Fluorescence spectra of copolymer solutions were recorded at  $25 \text{ }^\circ\text{C}$  using a FLS920 fluorescence spectrometer with an excitation wavelength of 350 nm. To determine apparent hydrodynamic diameter ( $D_h$ ), peak hydrodynamic diameter ( $D_{\text{peak}}$ ),

particle size distribution (PD) and light scattering intensity of copolymer assemblies formed in aqueous solution, dynamic light scattering (DLS) analysis was performed using Zetasizer Nano-ZS from Malvern Instruments ( $T = 25\text{ }^{\circ}\text{C}$ ) or BI200-SM from Brookhaven Instruments (for temperature-variable DLS measurement) equipped with a 633 nm He-Ne laser using back-scattering detection. To check the morphology of nano-objects, transmission electron microscopy (TEM) images were measured using a Hitachi HT7700 electron microscope with an acceleration voltage of 120 kV. A drop of copolymer solution or suspension was casted on a carbon-coated copper grid at a fixed temperature, immediately quenched with liquid nitrogen, followed by freeze-drying to prepare the samples for TEM measurement.

### References

- 1 Y. Mitsukami, M. S. Donovan, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2001, **34**, 2248–2256.
- 2 Q. Li, F. Li, L. Jia, Y. Li, Y. Liu, J. Yu, Q. Fang and A. Cao, *Biomacromolecules*, 2006, **7**, 2377–2387.
- 3 Q. Tang, Y. Wu, P. Sun, Y. Chen and K. Zhang, *Macromolecules*, 2014, **47**, 3775–3781.
- 4 C. Miao, X. Zhu, J. Zhang and Y. Zhao, *Polym. Chem.*, 2020, **11**, 6259–6272.

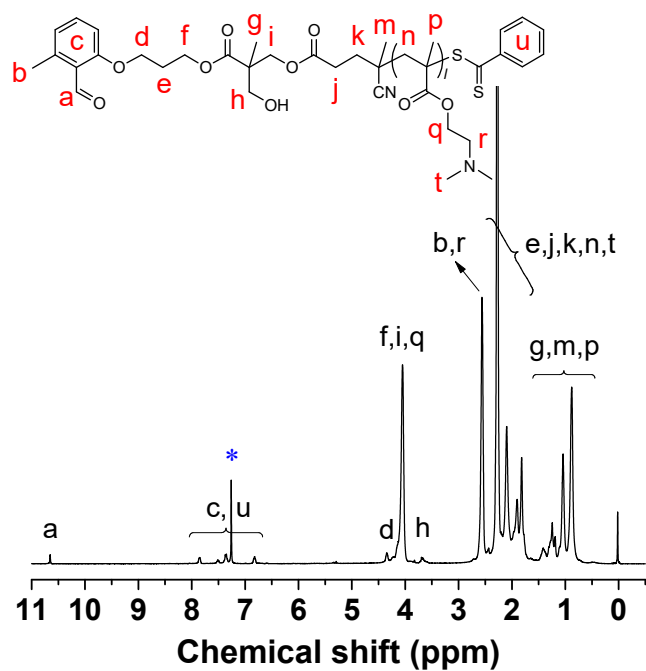


Fig. S1 <sup>1</sup>H NMR spectrum of P1 recorded in CDCl<sub>3</sub> (\*).

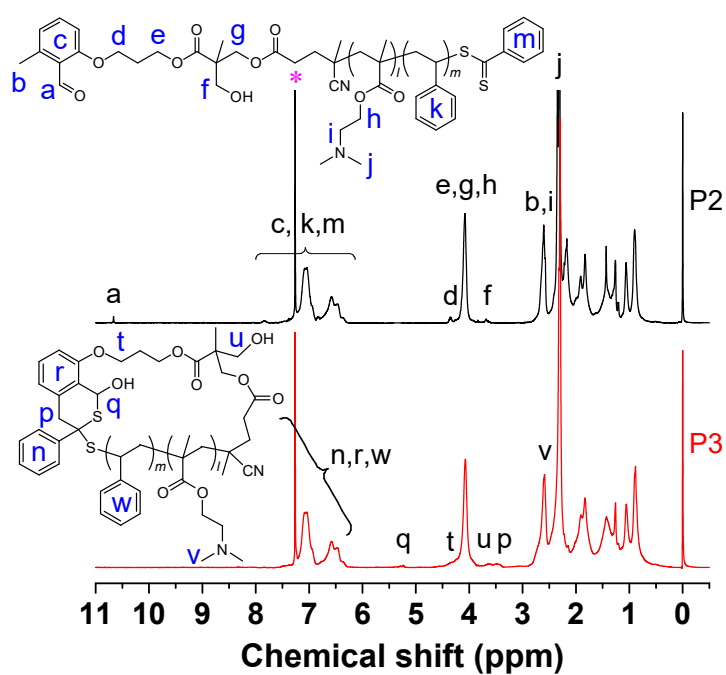
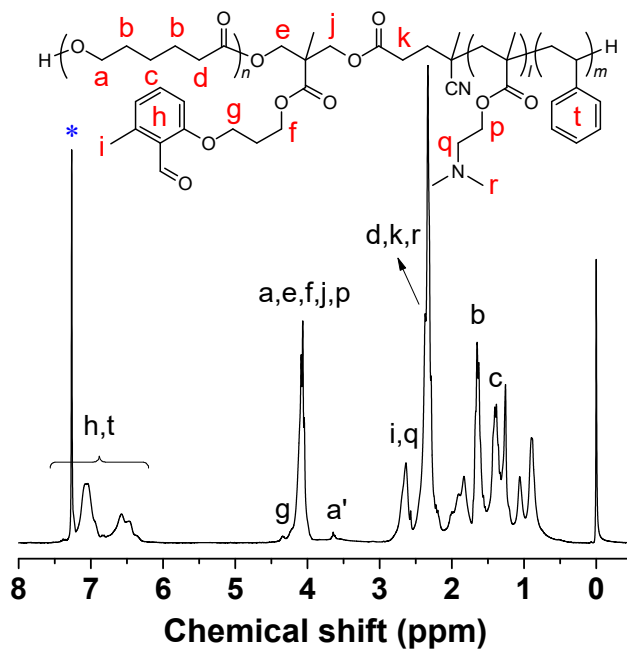
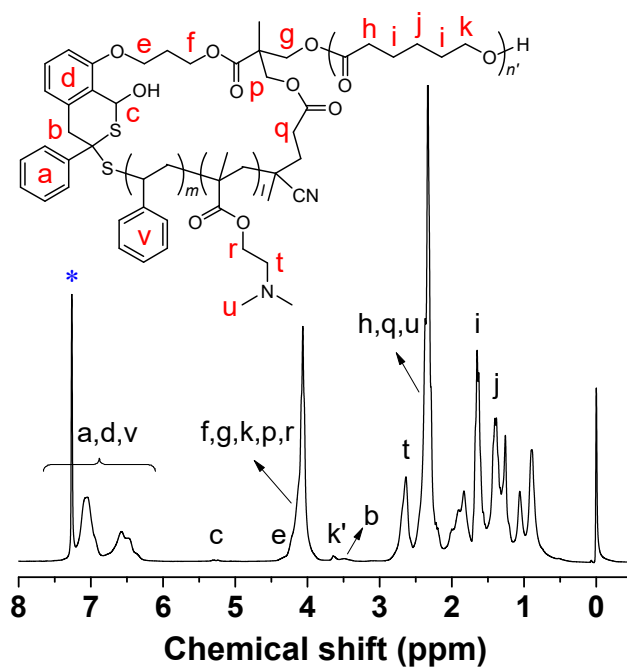


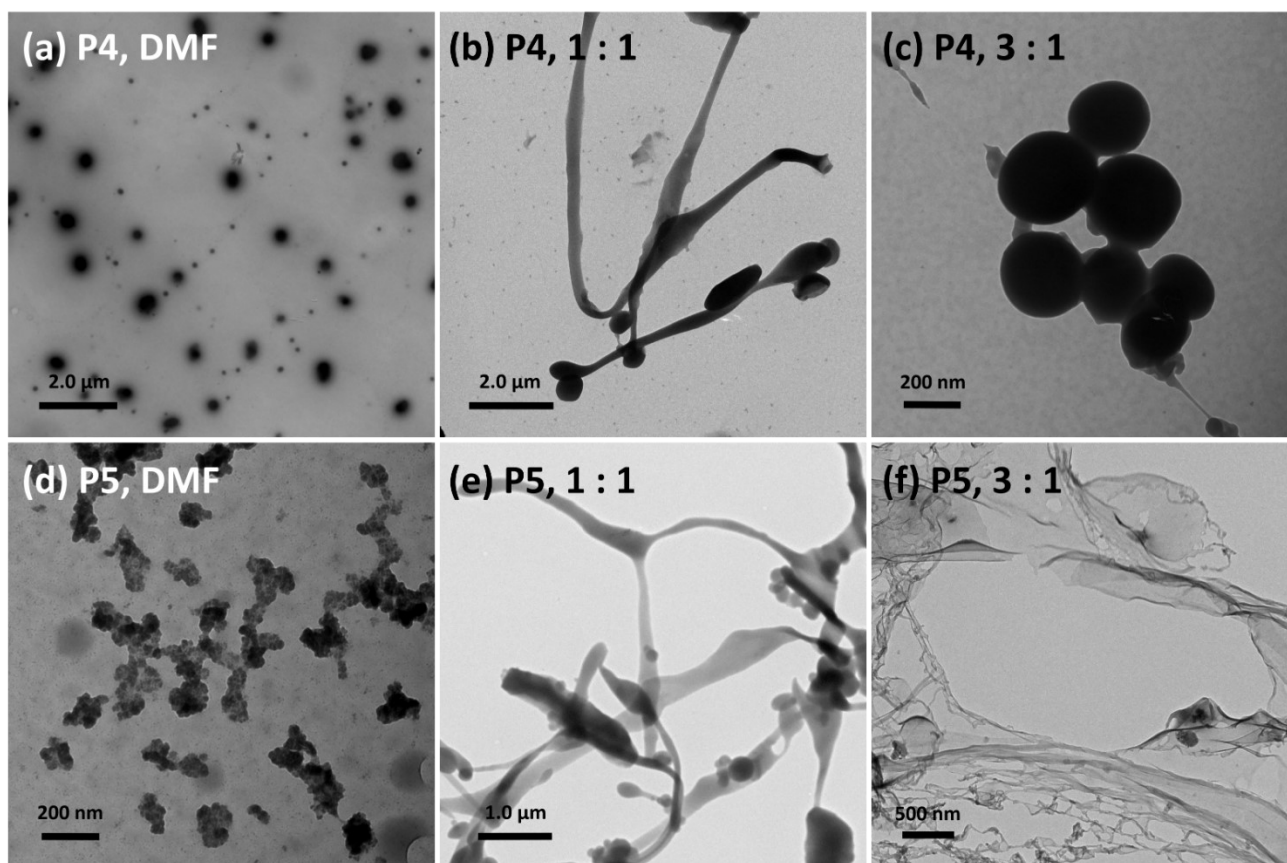
Fig. S2 <sup>1</sup>H NMR spectra of P2 and P3 recorded in CDCl<sub>3</sub> (\*).



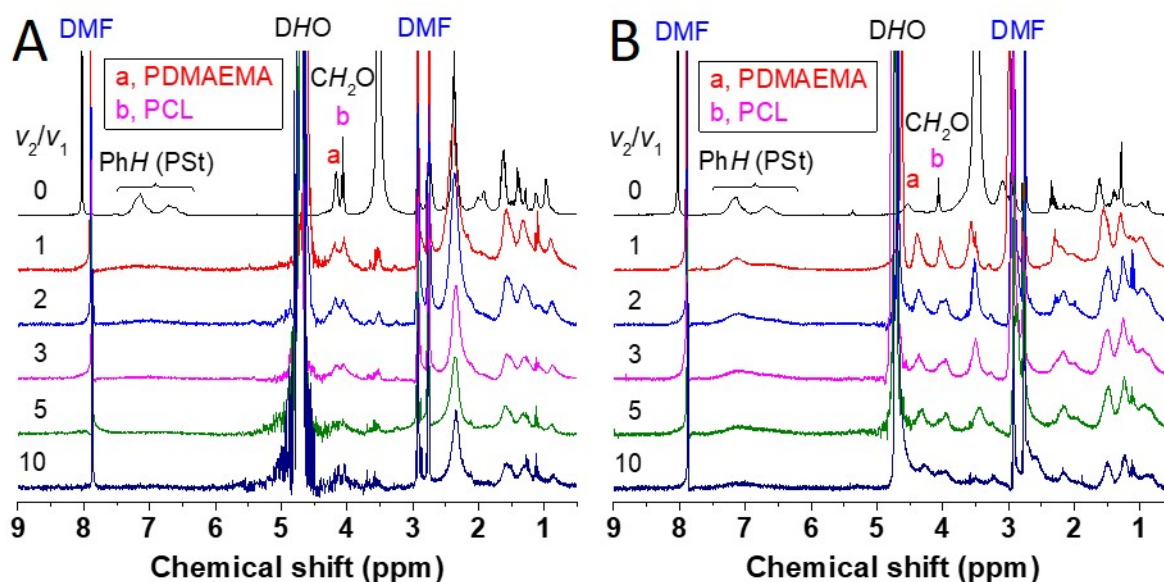
**Fig. S3** <sup>1</sup>H NMR spectrum of P4 recorded in CDCl<sub>3</sub> (\*), in which a' denotes the signal of terminal CH<sub>2</sub>OH of PCL segment.



**Fig. S4** <sup>1</sup>H NMR spectrum of P5 recorded in CDCl<sub>3</sub> (\*), in which k' denotes the signal of terminal CH<sub>2</sub>OH of PCL segment.

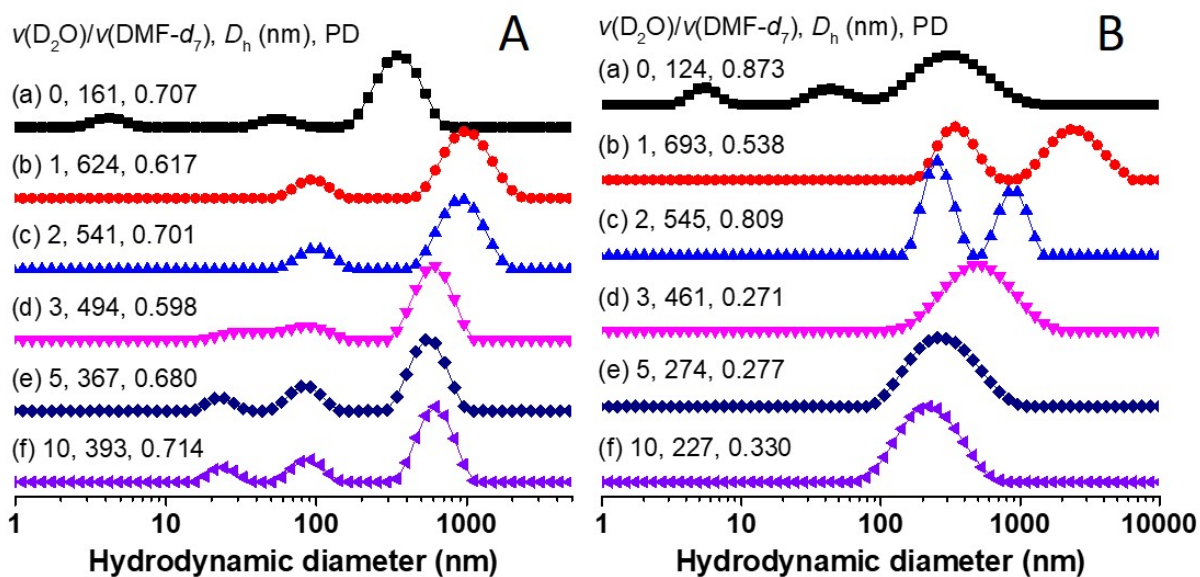


**Fig. S5** TEM images of copolymer assemblies formed from P4 (a-c) and P5 (d-f) in DMF or water/DMF mixture ( $v(\text{H}_2\text{O})/v(\text{DMF}) = 1$  or  $3$ ) at  $25^\circ\text{C}$ , in which  $c_p$  ( $\text{mg mL}^{-1}$ ) was  $2.0$  (a, d),  $1.0$  (b, e) and  $0.5$  (c, f).

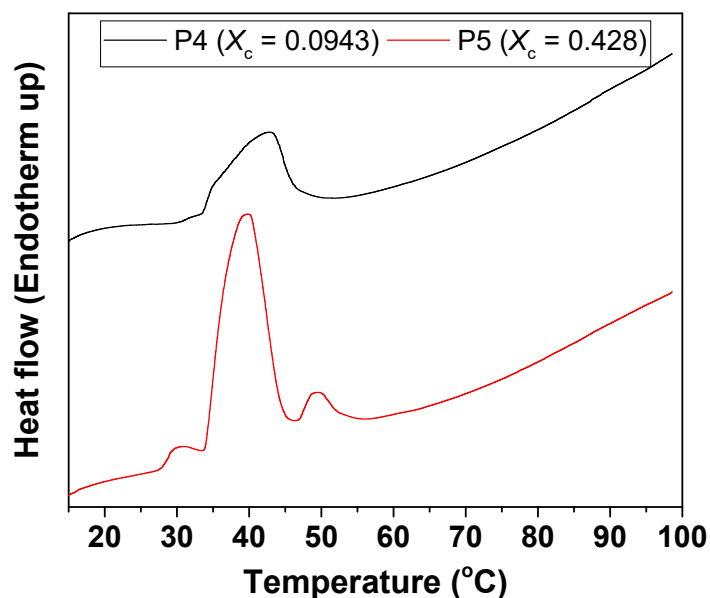


**Fig. S6**  $^1\text{H}$  NMR spectra of P4 (A) and P5 (B) recorded in  $\text{DMF-}d_7$  ( $v_2/v_1 = 0$ ) and  $\text{D}_2\text{O}/\text{DMF-}d_7$  mixture, in which various solutions ( $v_2/v_1 = 1, 2, 3, 5$  and  $10$ ) were obtained by slow addition of  $\text{D}_2\text{O}$  (solvent 2) into copolymer solution ( $c_p = 2.0 \text{ mg mL}^{-1}$ ) in  $\text{DMF-}d_7$  (solvent 1).

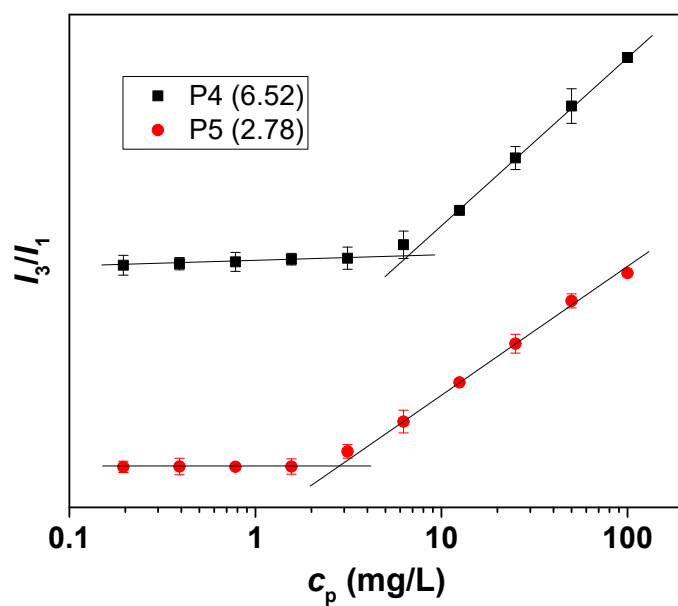




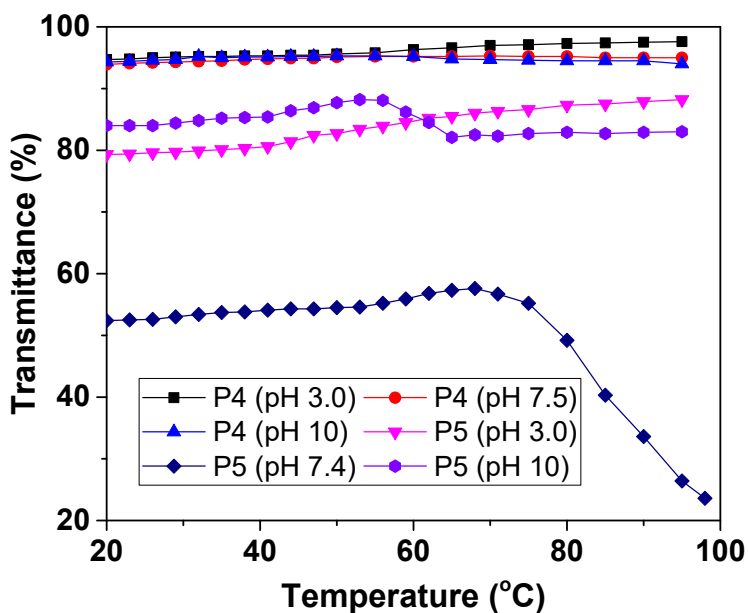
**Fig. S7** DLS plots of P4 (A) and P5 (B) assemblies formed in DMF- $d_7$  and  $\text{D}_2\text{O}/\text{DMF-}d_7$  mixture at 25 °C, in which  $\text{D}_2\text{O}$  was slowly added to copolymer solution in DMF- $d_7$  with initial concentration of 2.0 mg mL<sup>-1</sup>.



**Fig. S8** DSC curves of freeze-dried copolymers originating from copolymer assemblies formed in water ( $c_p = 0.5$  mg mL<sup>-1</sup>).



**Fig. S9** Polymer concentration dependent intensity ratio at the bands of 383 and 372 nm ( $I_3/I_1$ ) obtained from the fluorescence excitation spectra of pyrene.



**Fig. S10** Temperature-dependent transmittances of copolymer assemblies ( $c_p = 0.5 \text{ mg mL}^{-1}$ ) formed from P4 and P5 in aqueous solution at different pHs.