

Electronic supplementary information (ESI) for

**Synthesis and UCST-type thermoresponsive property of polypeptide based
single-chain nanoparticles**

Liang Zhao,^a Xiaodan Wang,^b Liwei Sun,^b Rongtao Zhou,^b Xu Zhang,^b Lin Zhang,^a

Zelai Zheng,^a Ying Ling,^a Shifang Luan,^{b,*} and Haoyu Tang^{a,c,*}

^a *Key Laboratory of Polymeric Materials and Application Technology of Hunan Province, College of Chemistry, Xiangtan University, Xiangtan, Hunan, 411105, China*

^b *State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, China*

^c *Institute of Functional Nano & Soft Materials (FUNSOM), Soochow University, Suzhou 215123, China*

Correspondence to: Haoyu Tang (Email: htang@xtu.edu.cn or hytang@suda.edu.cn) or Shifang Luan (sfluan@ciac.ac.cn),

Materials

Propargyl bromide (99%), trifluoroacetic acid (99%), copper bromide (CuBr, 99%), *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA, 99%), NaI (98%), (3-bromopropyl)triphenylphosphonium bromide (98%), and 1,4-diiodobutane (98%) were purchased from Energy Chemical. *N,N*-dimethylformamide (DMF, 99.9%) was dried over molecular sieves before use. Chloroform-*d* (CDCl₃, D.99.8%) + silver foil was purchased from Cambridge Isotope Laboratories, Inc. Sodium azide (NaN₃, 99.5%) and dimethyl sulfoxide-*d*₆ (DMSO-*d*₆, 99.9 atom % D, contains 0.03% v/v TMS) were purchased from Sigma-Aldrich. Proteinase K (≥ 30 units/mg protein, lyophilized) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Deionized water (DI H₂O) was obtained from Aquapro AR1-100L-P11 water-purification system (Ever Young Enterprises Development Co., Ltd., P. R. China). γ -3-Methylthiopropyl-L-glutamic acid based *N*-carboxyanhydride (MTPLG-NCA) and poly(ethylene glycol) bis(2-aminoethyl ether) (PEG-BA, $M_n = 4000$, $n = 90$) were prepared according to reported procedures.^{1, 2} The L929 murine fibroblasts cell line was obtained from Shanghai ASTRI Cell Resource Center, Chinese Academy of Sciences. Cell Counting Kit-8 (CCK-8) was purchased from Boster Biological Technology Co. Ltd.

Instrumentation

¹H and ¹³C nuclear magnetic resonance (¹H and ¹³C NMR) spectra were recorded on a Bruker ARX400 MHz spectrometer at room temperature. Chemical shifts (δ) were reported in the units of ppm and referenced to the protonic impurities. Gel

permeation chromatography (GPC) measurements were performed on a PL-GPC120 setup equipped with a column set consisting of two PL gel 5 μm MIXED-D columns (7.5 mm \times 300 mm, effective molar mass range of 0.2-400.0 kg $\cdot\text{mol}^{-1}$) and PL-RI differential refractive index (DRI) detector. DMF containing 0.01 M LiBr was used as the eluent at 80 $^{\circ}\text{C}$ at a flow rate of 1.0 mL $\cdot\text{min}^{-1}$. Narrowly distributed polystyrene standards in the molar mass range of 2.95-871 kg $\cdot\text{mol}^{-1}$ (PSS, Mainz, Germany) were utilized for calibration. FTIR spectra were recorded on a Thermo Scientific Nicolet 6700 FTIR spectrometer equipped with an attenuated total reflection (ATR) sample holder. Solid samples were placed on the diamond crystal window and pressed with a metal probe. Spectral measurements were carried out in the transmittance mode (scan range = 4000-600 cm^{-1} , resolution = 2 cm^{-1} , number of scans = 2, 25 $^{\circ}\text{C}$). Elemental analysis was recorded using an Elementar Vario EL instrument. Circular dichroism (CD) measurements were carried out on a JASCO J820 CD spectrometer (Japan Spectroscopic Corp.). The polymer aqueous solutions (0.5 mg $\cdot\text{mL}^{-1}$) were placed in a quartz cell with a path length of 0.2 cm. CD data were collected with the high tension voltage (i.e., the voltage applied to the photomultiplier) less than 600 V. Two scans were conducted and averaged between 185 and 250 nm with a resolution of 0.5 nm. The data were processed by subtracting the solvent (i.e., DI H₂O) background and smoothing with the FFT-Filter method with points of window of 8. The CD spectra were reported in mean residue ellipticity (MRE) (units: deg $\cdot\text{cm}^2\cdot\text{dmol}^{-1}$) which was calculated by the equation $[\theta]_{\lambda} = \text{MRW} \times \theta_{\lambda} / 10 \times d \times c$, where MRW is the mean residue weight (MRW = the molecular weight of polypeptide repeating unit), θ_{λ} is the

observed ellipticity (mdeg) at the wavelength λ (i.e., 222 nm), d is the path length (mm), and c is the concentration ($\text{mg}\cdot\text{mL}^{-1}$). Dynamic light scattering (DLS) was conducted on a Zetasizer Nano ZS90 (Malvern Instruments Ltd, UK) with a He Ne laser ($\lambda = 633 \text{ nm}$) at a scattering angle of 90° (25°C). In a heating/cooling cycle, the aggregation size was recorded at every 2°C augment after a 5 min thermal equilibration at each measurement. The heating or cooling rate for variable temperature DLS measurements were around $2^\circ\text{C}\cdot\text{min}^{-1}$. Ultraviolet–visible (UV–vis) spectra were measured using an Agilent Cary 100 spectrometer. The polymer solutions placed in a quartz cell with a path length of 1.0 cm. The transmittance at 500 nm was recorded at every 2°C augment after a 5 min thermal equilibration at each measurement. The heating or cooling rate for variable temperature UV-vis measurements were around $2^\circ\text{C}\cdot\text{min}^{-1}$.

References

1. M. Mutter, *Tetrahedron Lett.*, 1978, **19**, 2839-2842.
2. C. Ge, L. Zhao, Y. Ling and H. Tang, *Polym. Chem.*, 2017, **8**, 1895-1905.

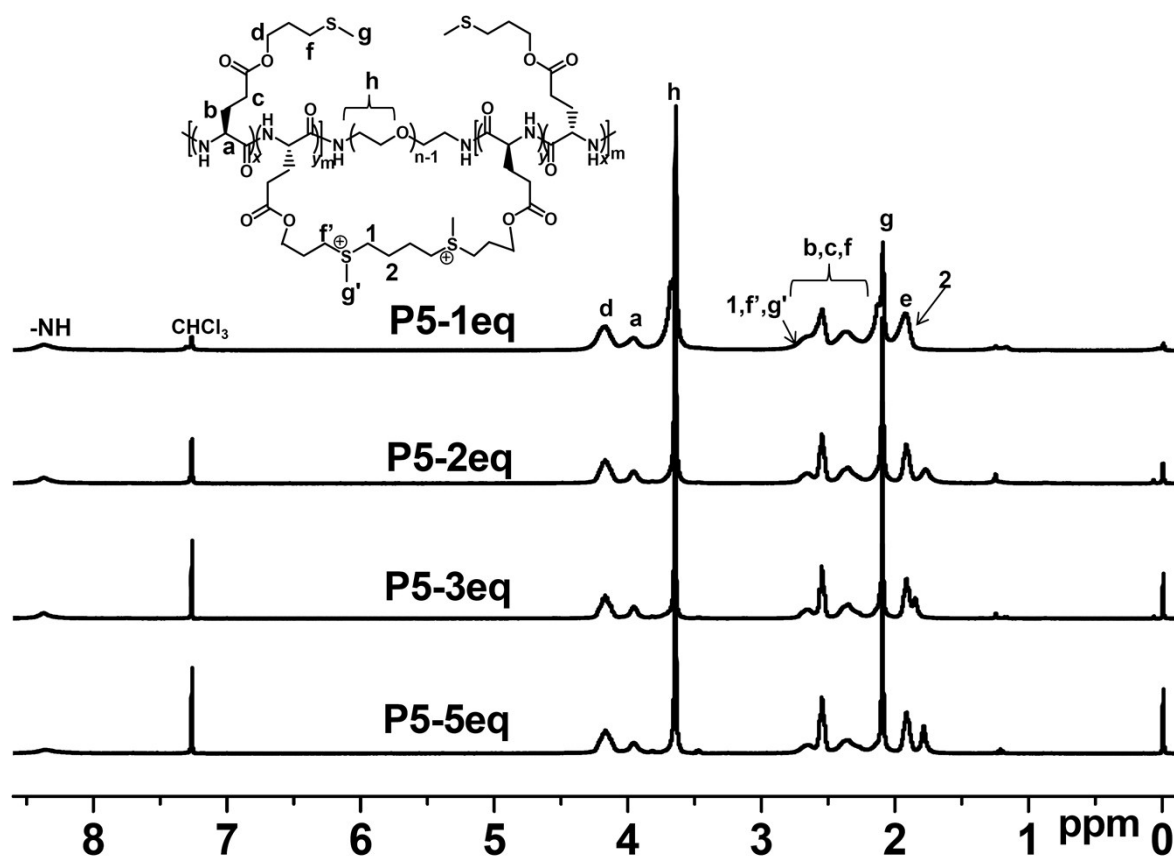


Fig. S1 ^1H NMR spectra of P5 with various crosslinking densities in CDCl_3 .

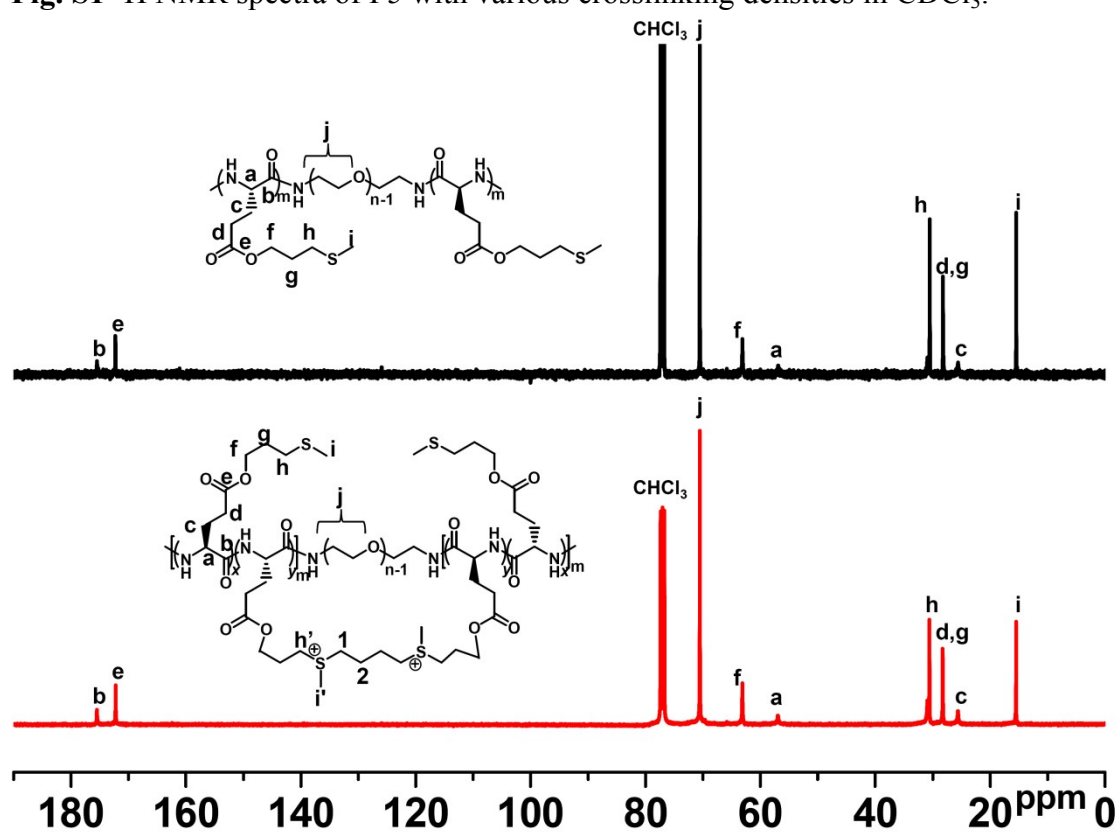


Fig. S2 ^{13}C NMR spectra of P1 and P5-5eq in CDCl_3 .

Table S1. Elemental analysis results of P1 and P5-5eq.

Samples	N [%]	C [%]	H [%]	S [%]
P1	4.62	50.25	7.478	10.706
P5-5eq	4.67	50.15	7.554	10.734

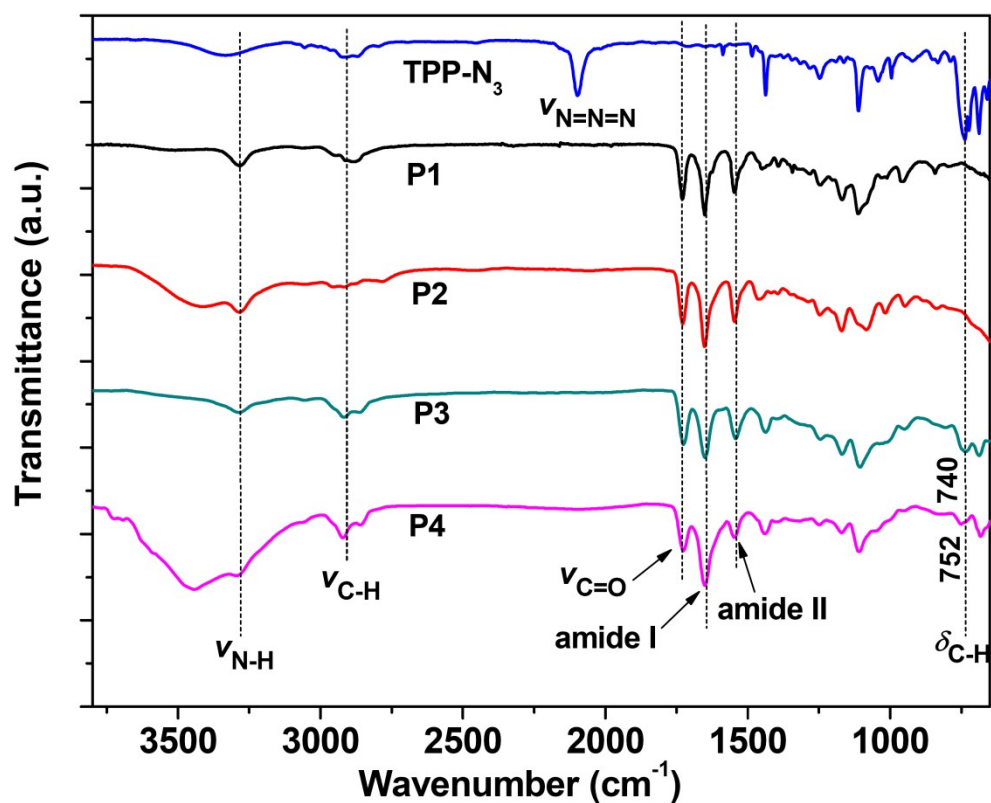


Fig. S3 FTIR spectra of TPP-N₃, P1-P4 in the solid-state.

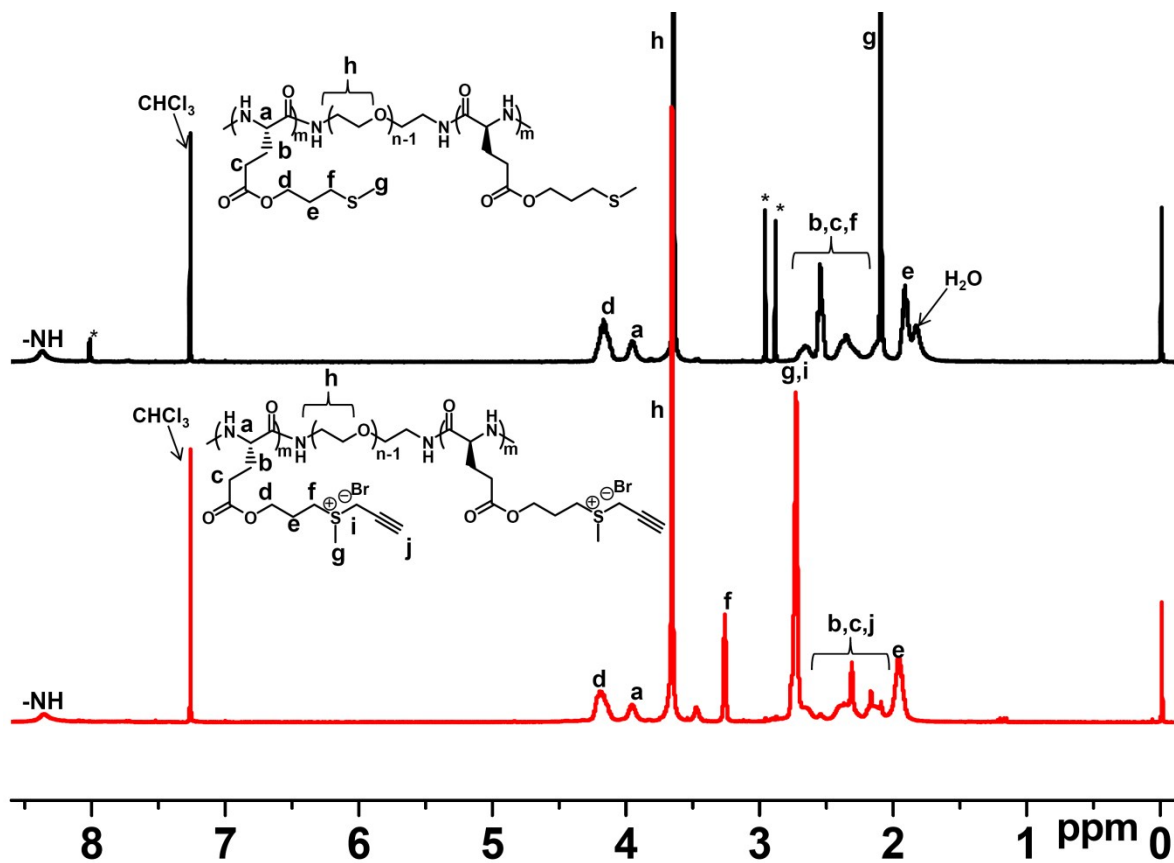


Fig. S4 ¹H NMR spectra of P1 (top curve) and P2 (bottom curve) in CDCl₃.

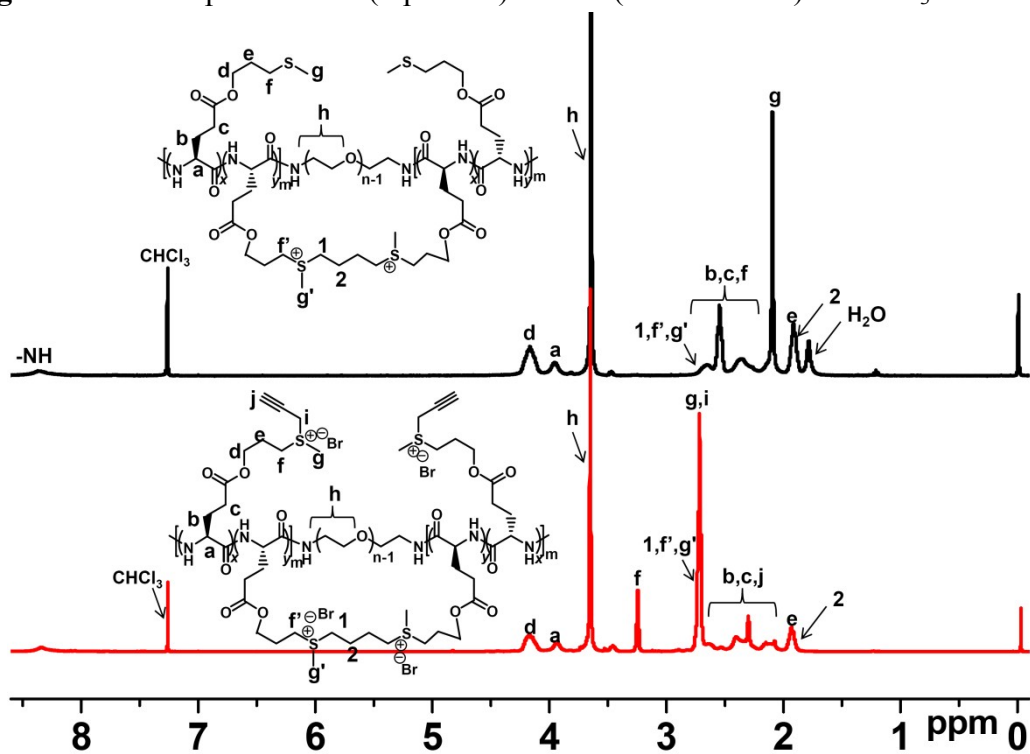


Fig. S5 ¹H NMR spectra of P5 (top curve) and P6 (bottom curve) in CDCl₃.

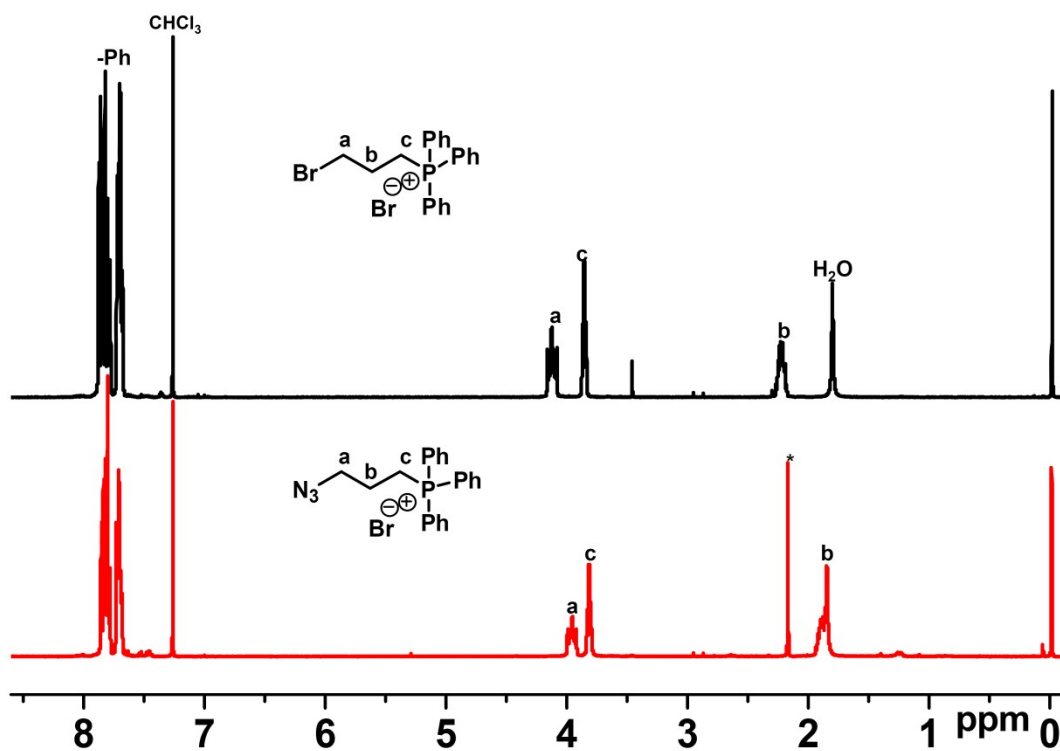


Fig. S6 ¹H NMR spectra of TPP-Br (top curve) and TPP-N₃ (bottom curve) in CDCl₃.

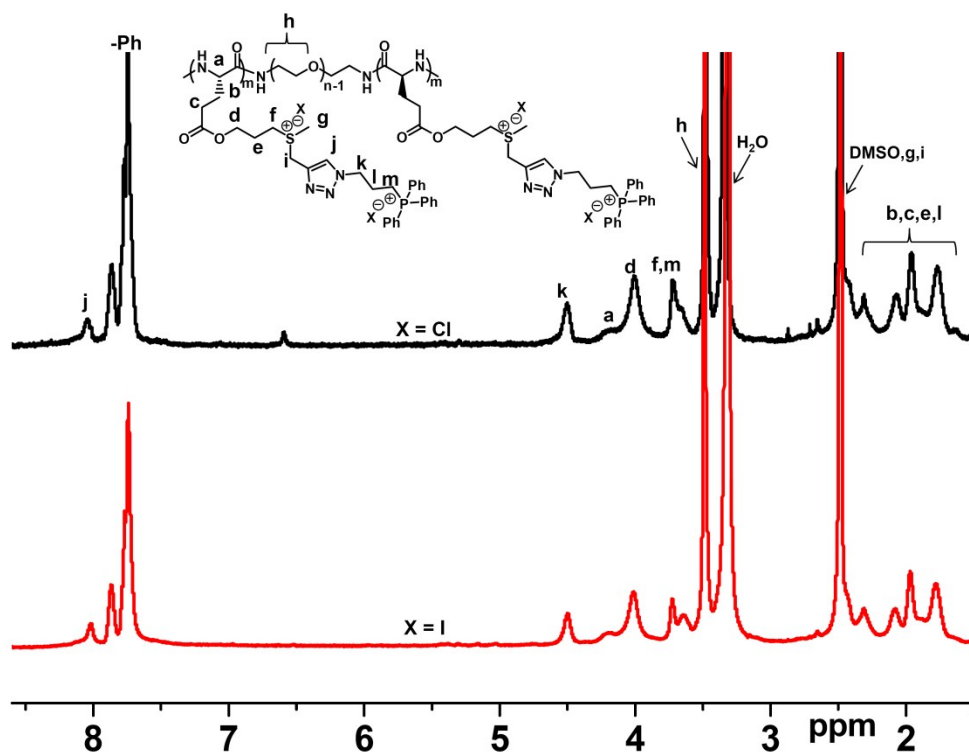


Fig. S7 ¹H NMR spectra of P3 (top curve) and P4 (bottom curve) in DMSO-d₆.

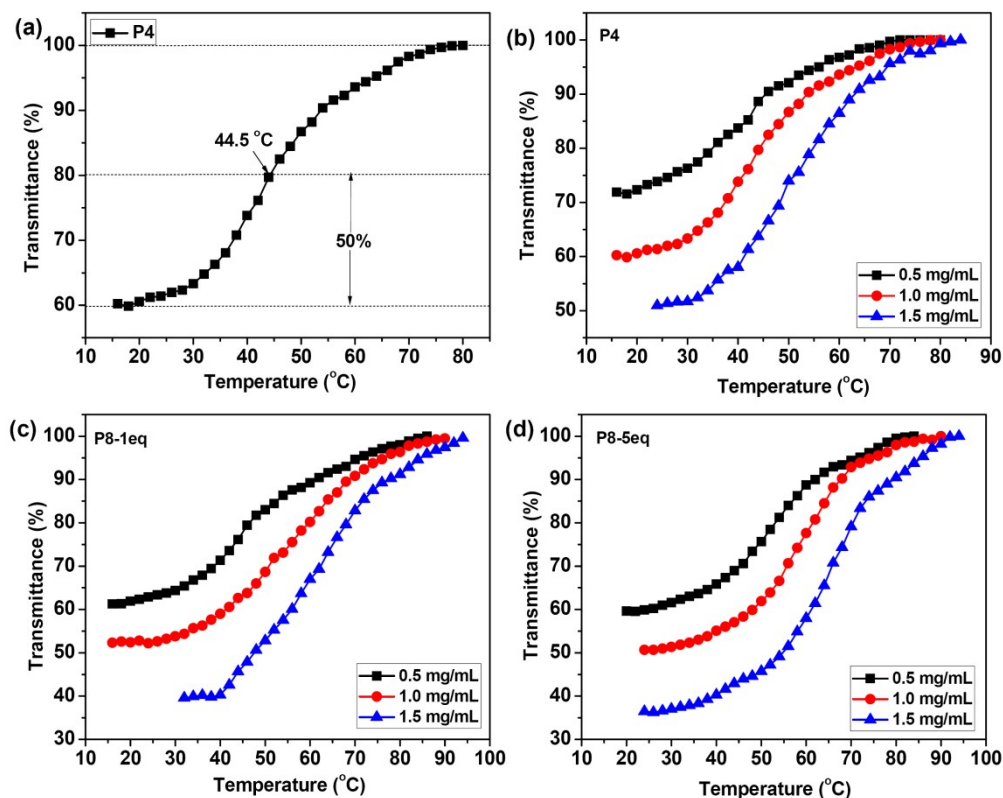


Fig. S8 Plots of transmittance at $\lambda = 500$ nm *versus* temperature for (a) P4 at 0.5 mg·mL⁻¹, (b) P4, (c) P8-1eq, and (d) P8-5eq in DI water at various polymer concentrations.

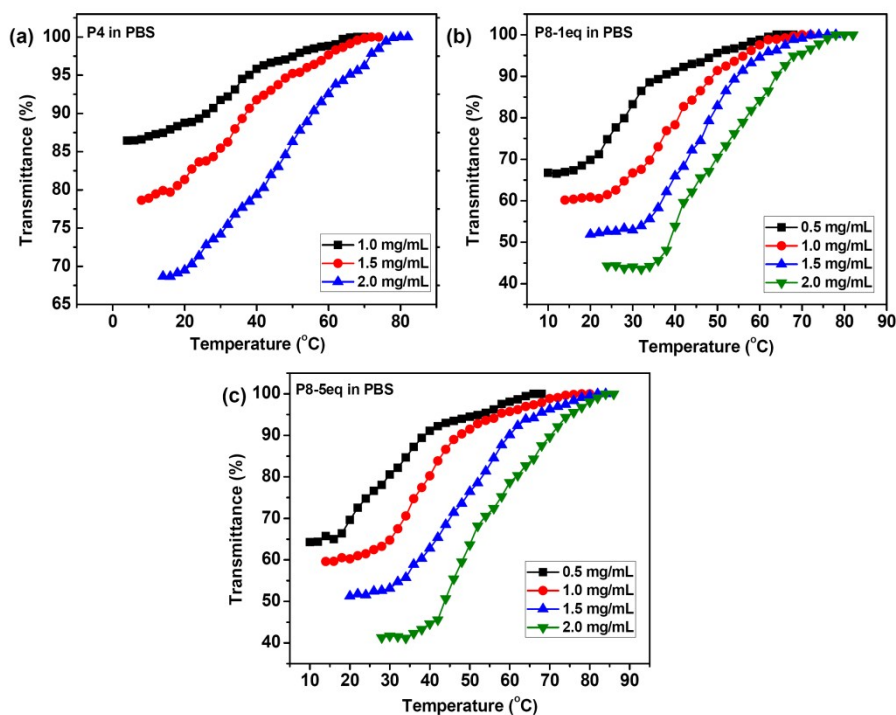


Fig. S9 Plots of transmittance at $\lambda = 500$ nm *versus* temperature for (a) P4, (b) P8-1eq, and (c) P8-5eq in PBS at various polymer concentrations.