

## Supporting Information

### Impact of cyclization and dendronization on multi-tunable thermoresponsive behaviors of polyacrylamide copolymers

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## Experimental

### Materials

The chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd unless otherwise stated. *N*-isopropylacrylamide (NIPAM, 97%, Sigma-Aldrich) was recrystallized twice from mixtures of hexane and toluene, and *N,N*-dimethyl acrylamide (DMA, 98%, Sigma-Aldrich) was passed through a basic alumina column to remove the inhibitor. 2,2'-Azobis(isobutyronitrile) (AIBN, 99%) was recrystallized twice from ethanol. *N,N*-Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were dried over CaH<sub>2</sub> and distilled under reduced pressure, and dioxane was distilled over sodium and benzophenone. Acryloyl chloride (99%, Aladdin), D,L-homocysteine thiolactone hydrochloride (98%, Aladdin), *N,N*-dimethylethylenediamine (DMDA, 99%), acrylamide (AM, 99%, TCI), phosphazene base 1-*tert*-butyl-4,4,4-tris(dimethylamino)-2,2-bis[tris(dimethylamino)phosphoranylidenamino]-2Λ5,4Λ5-catenadi(phosphazene) (*t*-BuP<sub>4</sub>, ~0.8 M in THF, Sigma-Aldrich), and other reagents were used as received. The organic solvents were purified using standard procedures. *N*-Acryloylhomocysteine thiolactone (ATL)<sup>1</sup> and 3-(2-formyl-3-methylphenoxy)propyl 4-(benzodithioyl)-4-cyanopentanoate (FBCP)<sup>2</sup> were synthesized according to literature procedures.

### Synthesis of linear and cyclic P(NIPAM-*co*-ATL) copolymers<sup>3</sup>

A telechelic linear P(NIPAM-*co*-ATL) (abbreviated as *l*-PNA) was initially synthesized by RAFT copolymerization. FBCP (45.6 mg, 0.10 mmol), AIBN (1.6 mg, 0.010 mmol), NIPAM (1.13 g, 10.0 mmol) and ATL (0.343 g, 2.0 mmol) were successively added to a Schlenk tube, and then dioxane was added to reach the desired volume of 4.0 mL. The solution in the sealed tube was bubbled with nitrogen for 20 min, followed by RAFT copolymerization at 70 °C for 20 h. The polymer solution was immediately cooled down and repeatedly precipitated into cold diethyl ether. After filtration and vacuum drying, *l*-PNA (1.18 g) was obtained in 77% conversion. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 10.66 (s, CHO), 5.5-8.5 (m, PhH, ArH and CONH), 4.71 (m, CONHCH of ATL unit), 4.30 (m, ArOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 4.14 (m, ArOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.99 (s, CONHCH of NIPAM unit), 3.1-3.6 (m, CH<sub>2</sub>S of ATL unit), 0.5-2.8 (m, other CH, CH<sub>2</sub> and CH<sub>3</sub> originating from CTA and monomer units).

On this basis, *l*-PNA was subjected to intramolecular ring-closure via UV-induced Diels-Alder reaction using a low-pressure mercury lamp (120 W). In a typical run, *l*-PNA (25 mg) and acetonitrile (500 mL) were added to a round-bottom flask, and then 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 isolate cyclic P(NIPAM-*co*-ATL) (abbreviated as *c*-PNA), followed by repetitive ring-closure reaction using recycled acetonitrile and fresh *l*-PNA to increase the amount of cyclic copolymer. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 5.7-8.5 (m, PhH, ArH and CONH), 5.46 (m, ArCHOH), 4.76 (m, CONHCH of ATL unit), 4.33 (m, ArOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 4.12 (m, ArOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.98 (s, CONHCH of NIPAM unit), 3.65 (s, ArCH<sub>2</sub>), 3.1-3.6 (m, CH<sub>2</sub>S of ATL unit), 0.5-2.8 (m, ArOCH<sub>2</sub>CH<sub>2</sub>, CH<sub>3</sub>C(CN)CH<sub>2</sub>CH<sub>2</sub>CO, and other CH, CH<sub>2</sub> and CH<sub>3</sub> originating from monomer units).

### Synthesis of linear and cyclic P(NIPAM-*co*-ATL)-*g*-DMDA/AM copolymers

Starting from *l*-PNA and *c*-PNA, tandem amine-thiol-ene reactions were adopted to synthesize P(NIPAM-*co*-ATL)-*g*-DMDA/AM (abbreviated as PNADA) copolymers. In a typical run, the solution of *l*-PNA (0.250 g, 20 μmol, containing 0.30 mmol of thiolactone group) and AIBN (33 mg, 0.20 mmol) in DMF (2.0 mL) was bubbled with nitrogen for 10 min, followed by heating at 85 °C for 8 h. After cooling down, 0.5 mL of DMF solution comprising DMDA (53 mg, 0.60 mmol) and triethylamine (30 mg, 0.30 mmol) was added to the Schlenk tube under nitrogen, and then the aminolysis was conducted at ambient temperature for 48 h. Finally, acrylamide (85 mg, 1.2 mmol) was added to the Schlenk tube under nitrogen, and the mixture was further stirred at 25 °C for 24 h. The copolymer solution was concentrated and repeatedly precipitated into excess diethyl ether. After filtration and vacuum drying, *l*-PNADA (0.27 g, 90% yield) was isolated as solid powders. According to similar procedures, *c*-PNA was used as a reactive precursor to generate *c*-PNADA via amine-thiol-

ene reactions.

*l*-PNADA:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  10.66 (s, *CHO*), 5.8-8.5 (m, *ArH* and *CONH*), 4.45 (m, *CONHCHCONH*), 4.29 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 4.14 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 3.98 (s, *CONHCH* of NIPAM unit), 3.34 (m, *CONHCH}\_2*), 2.4-3.0 (m, *CH}\_2\text{SCH}\_2\text{CH}\_2\text{CONH}\_2*, *NCH}\_2* and *ArCH}\_3*), 0.4-2.4 (m, other *CH*, *CH}\_2* and *CH}\_3* originating from end groups and monomer units).

*c*-PNADA:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  5.8-8.5 (m, *PhH*, *ArH* and *CONH*), 5.50 (m, *ArCHOH*), 4.44 (m, *CONHCHCONH*), 4.28 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 4.14 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 3.98 (s, *CONHCH* of NIPAM unit), 3.65 (s, *ArCH}\_2*), 3.34 (m, *CONHCH}\_2*), 2.37-3.10 (m, *CH}\_2\text{SCH}\_2\text{CH}\_2\text{CONH}\_2* and *NCH}\_2*), 0.40-2.37 (m, other *CH*, *CH}\_2* and *CH}\_3* originating from linking group and monomer units).

### Synthesis of linear and cyclic P(NIPAM-*co*-ATL)-*g*-DMDA/DDMA copolymers

Amide-based Michael addition reaction<sup>4</sup> was adopted to convert the primary amide to triple tertiary amide group. In a typical run, *l*-PNADA (0.120 g, 8.0  $\mu\text{mol}$ , containing 0.12 mmol of *-CONH}\_2*), DMA (0.119 g, 1.2 mmol) and DMSO (1.05 mL) were added to a Schlenk tube under nitrogen. After bubbling with nitrogen for 10 min, *t*-BuP<sub>4</sub> solution (0.15 mL, 0.12 mmol) was added to the tube under nitrogen, followed by stirring at 25 °C for 24 h. Most of solvent was removed under reduced pressure, and *l*-PNADD was isolated by diluting with THF and precipitating into diethyl ether. After vacuum drying, *l*-PNADD (0.132 g, 92% yield) was isolated as solid powders. According to similar procedures, *c*-PNADA was used to generate *c*-PNADD via Michael addition reaction.

*l*-PNADD:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  6.0-8.5 (m, *ArH* and *CONH*), 4.68 (m, *CONHCHCONH*), 4.36 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 4.14 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 3.96 (s, *CONHCH* of NIPAM unit), 3.30 (m, *CONHCH}\_2* and *CONCH}\_2*), 2.92 (m, *CON(CH}\_3)\_2*), 2.3-2.84 (m, *CH}\_2\text{SCH}\_2*, *CH}\_2\text{CON}*, *CH}\_2\text{N(CH}\_3)\_2*, and *ArCH}\_3*), 0.4-2.3 (m, other *CH*, *CH}\_2* and *CH}\_3* originating from end groups and monomer units).

*c*-PNADD:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  6.0-8.5 (m, *ArH* and *CONH*), 5.50 (m, *CHOH*), 4.75 (m, *CONHCHCONH*), 4.36 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 4.14 (m, *ArOCH}\_2\text{CH}\_2\text{CH}\_2\text{O}*), 3.97 (s, *CONHCH* of NIPAM unit), 3.64 (s, *ArCH}\_2*), 3.29 (m, *CONHCH}\_2* and *CONCH}\_2*), 2.93 (m, *CON(CH}\_3)\_2*), 2.26-2.84 (m, *CH}\_2\text{SCH}\_2*, *CH}\_2\text{CON}* and *CH}\_2\text{N(CH}\_3)\_2*), 0.4-2.26 (m, other *CH*, *CH}\_2* and *CH}\_3* originating from linking group and monomer units).

### Characterization

$^1\text{H}$  NMR spectra (400 or 600 MHz) of various copolymers were recorded in  $\text{CDCl}_3$  on a Varian Unity Inova 400 MHz spectrometer or an Agilent ProPulse 600 MHz spectrometer at 25 °C. Temperature-variable  $^1\text{H}$  NMR spectra of copolymer solutions were recorded in  $\text{D}_2\text{O}$  on an Agilent ProPulse 600 MHz spectrometer at different temperatures. Apparent molar mass ( $M_{n,\text{GPC}}$ ) and dispersity ( $D = M_w/M_n$ ) of various polymers were measured on a TOSOH HLC-8320 gel permeation chromatography

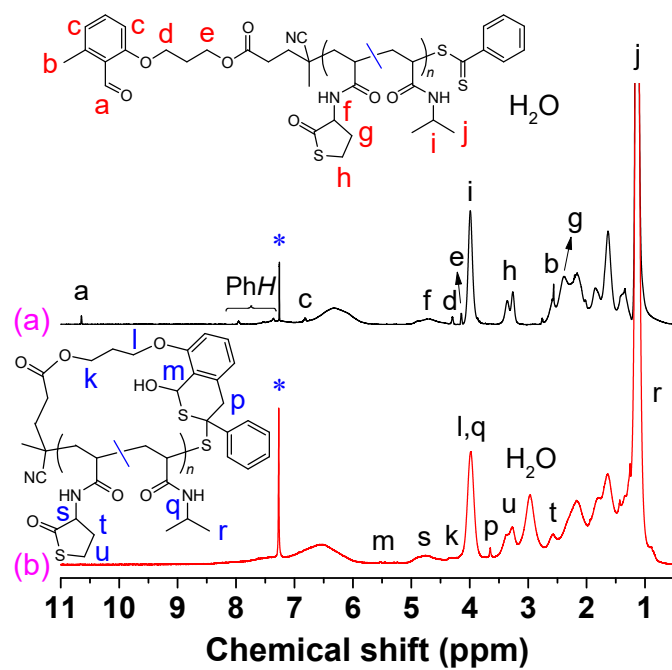
(GPC) using three TSKgel SuperMultipore HZ-M columns at 40 °C. DMF was used as an eluent at a flow rate of 0.35 mL min<sup>-1</sup>, and the samples were calibrated with PMMA standard samples. To determine phase transitions of copolymer solutions, turbidity analysis was performed at 500 nm on a Shimadzu UV-3150 UV-vis spectrophotometer equipped with a thermoregulator, and the cloud point (LCST-type  $T_{c,l}$  or UCST-type  $T_{c,u}$ ) was calculated as the temperature at the half of the maximal and minimal transmittances. Fluorescence spectra of copolymer solutions were recorded at different temperatures using a Shimadzu RF-5301 fluorescence spectrometer with an excitation wavelength of 330 nm. To determine apparent hydrodynamic diameter ( $D_h$ ), particle size distribution (PD) and Zeta potential of copolymer assemblies formed in aqueous solution, dynamic light scattering (DLS) analysis was performed using Zetasizer Nano-ZS from Malvern Instruments equipped with a 633 nm He-Ne laser using back-scattering detection. Transmission electron microscopy (TEM) images were measured using a Hitachi HT7700 electron microscope with an acceleration voltage of 120 kV to determine the morphology of nano-objects, in which freeze-drying technique was used to stabilize the shape of copolymer assemblies.

## References

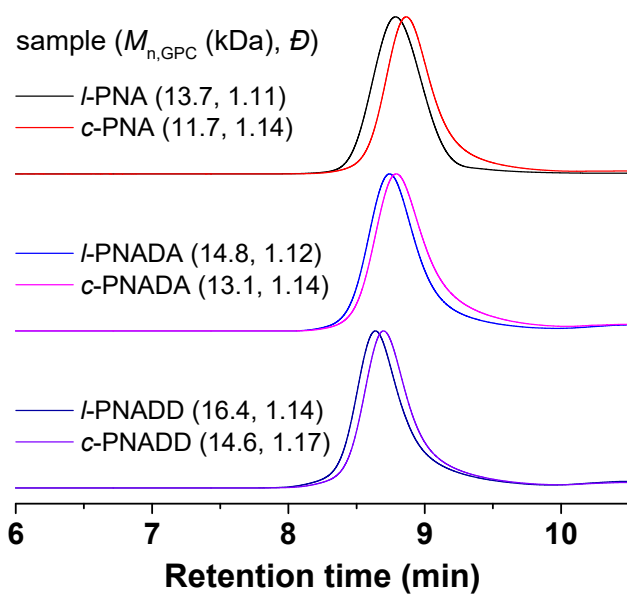
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- 2 Q. Q. Tang, Y. Wu, P. Sun, Y. M. Chen and K. Zhang, *Macromolecules*, 2014, **47**, 3775–3781.
- 3 H. C. Zhang, W. T. Wu, X. Q. Zhao and Y. L. Zhao, *Macromolecules*, 2017, **50**, 3411–3423.
- 4 H. J. Yang, Z. Y. Ren, Y. K. Zuo, Y. Y. Song, L. Jiang, Q. M. Jiang, X. Q. Xue, W. Y. Huang, K. J. Wang and B. B. Jiang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 50870–50878.

**Table S1** Summary of LCST-type cloud point ( $T_{c,l}$ ), critical aggregation temperature with enhanced light scattering intensity ( $T_c(\text{DLS})$ ), and critical aggregation temperature with elevated fluorescence intensity ( $T_c(\text{FL})$ ) of copolymer solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) formed in water or at pH 10

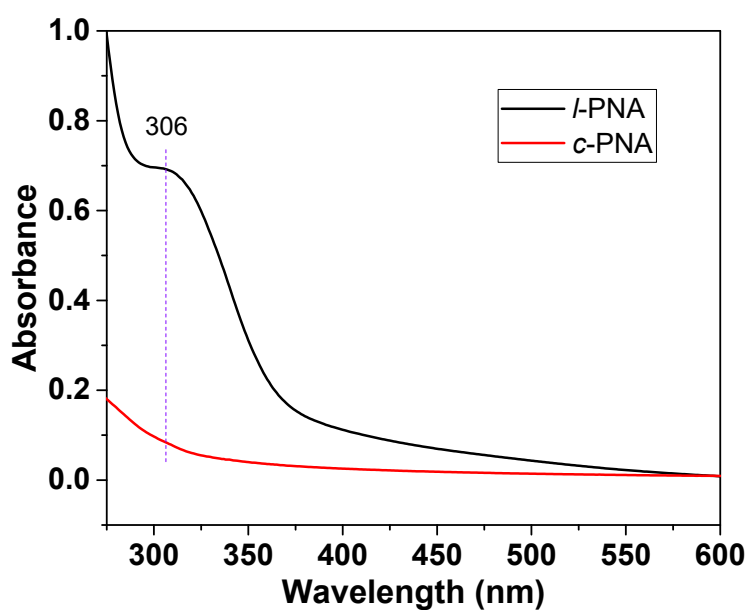
run	polymer	pH	$T_{c,l}$ (°C)	$T_c(\text{DLS})$ (°C)	$T_c(\text{FL})$ (°C)	$T_{c,l} - T_c(\text{DLS})$ (°C)	$T_{c,l} - T_c(\text{FL})$ (°C)
1	<i>l</i> -PNADA	8.1	43.3	41.6	38.5	1.7	4.8
2	<i>l</i> -PNADA	10	47.3	45.3	42.8	2.0	4.5
3	<i>c</i> -PNADA	8.1	47.5	45.4	43.6	2.1	3.9
4	<i>c</i> -PNADA	10	53.7	50.8	47.5	2.9	6.2
5	<i>l</i> -PNADD	7.6	44.0/80.6	39.4/78.5	37.8/74.4	4.6/2.1	6.2/6.2
6	<i>l</i> -PNADD	10	68.7/88.1	86.0	82.0	2.1	6.1
7	<i>c</i> -PNADD	7.5	60.6	58.8	55.1	1.8	5.5
8	<i>c</i> -PNADD	10	70.2	68.6	65.1	1.6	5.1



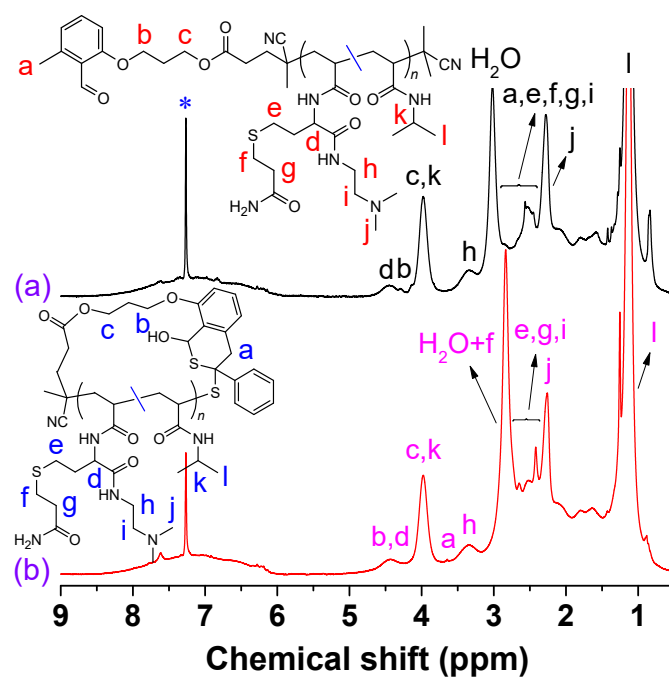
**Fig. S1**  $^1\text{H}$  NMR spectra of linear (a) and cyclic (b) P(NIPAM-co-ATL) copolymers recorded in  $\text{CDCl}_3$  (\*) at 25 °C.



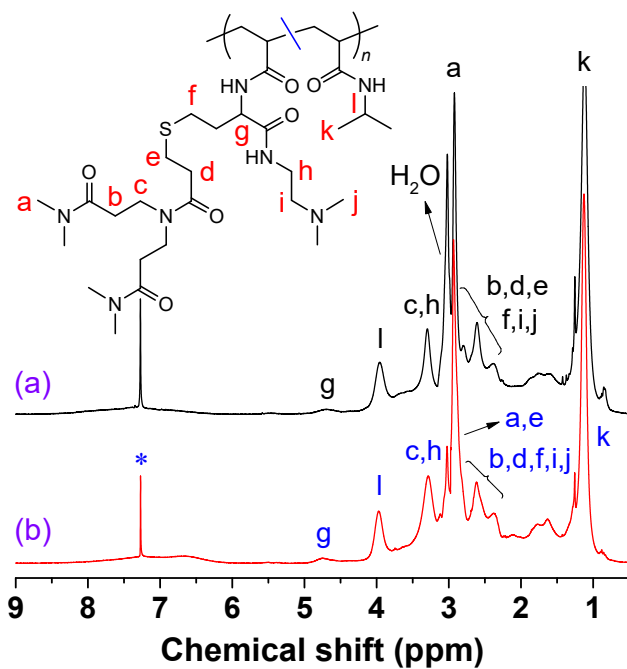
**Fig. S2** GPC traces of various copolymers.



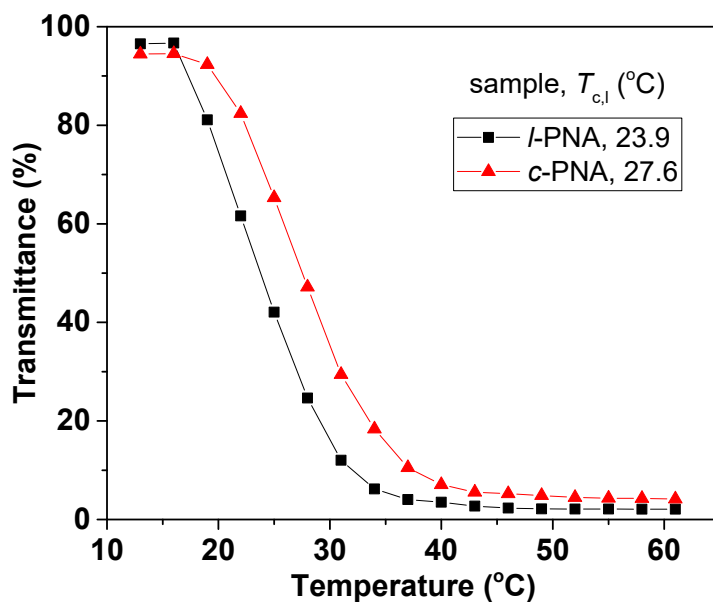
**Fig. S3** UV-vis spectra of copolymer solutions ( $c_p \approx 0.3 \text{ mg mL}^{-1}$ ) formed from *l*-PNA and *c*-PNA in water at 25 °C.



**Fig. S4**  $^1\text{H}$  NMR spectra of linear (a) and cyclic (b) P(NIPAM-*co*-ATL)-*g*-DMDA/AM copolymers recorded in  $\text{CDCl}_3$  (\*) at 25 °C.

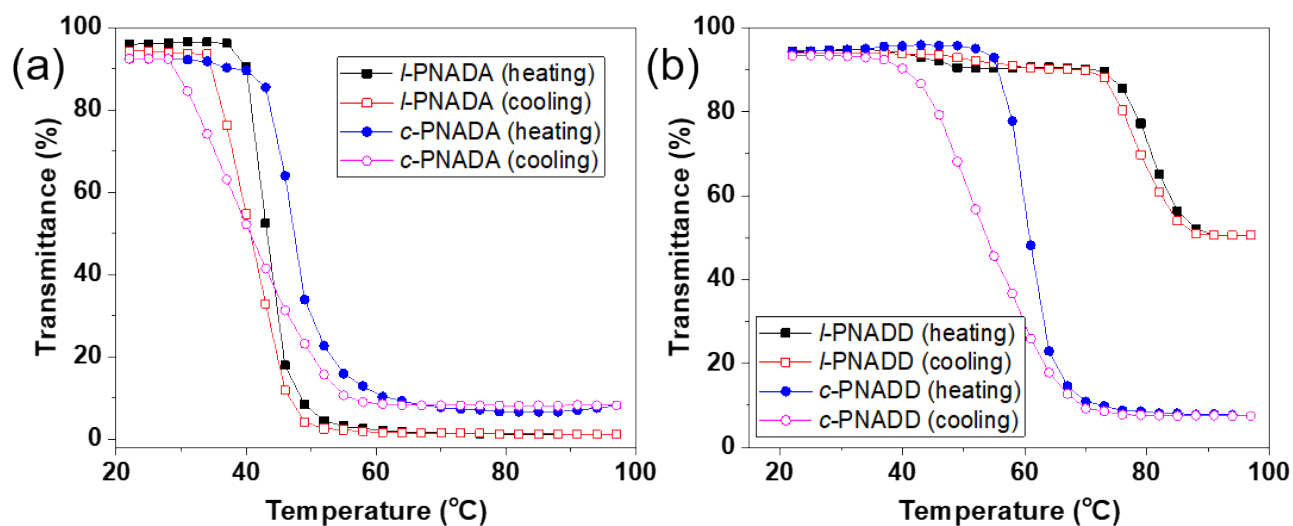


**Fig. S5**  $^1\text{H}$  NMR spectra of linear (a) and cyclic (b) P(NIPAM-*co*-ATL)-*g*-DMDA/DDMA copolymers recorded in  $\text{CDCl}_3$  (\*) at 25  $^\circ\text{C}$ .

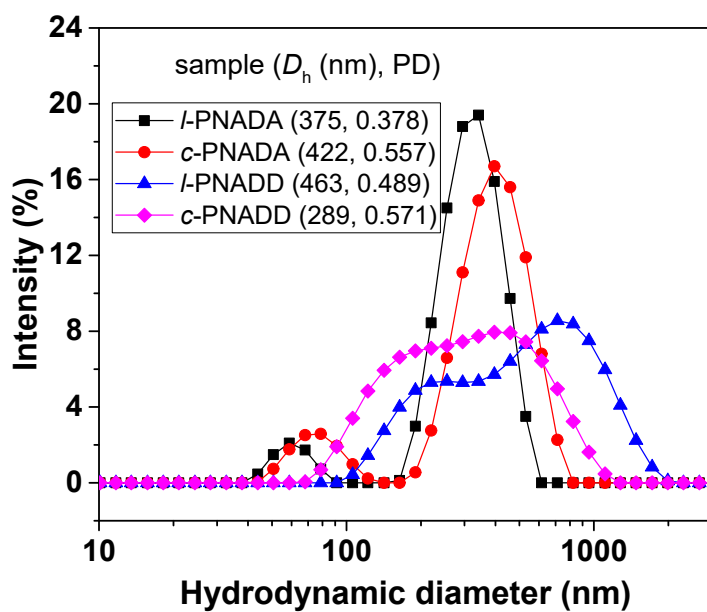


**Fig. S6** Temperature-dependent transmittance of *l*-PNA and *c*-PNA aqueous solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ).

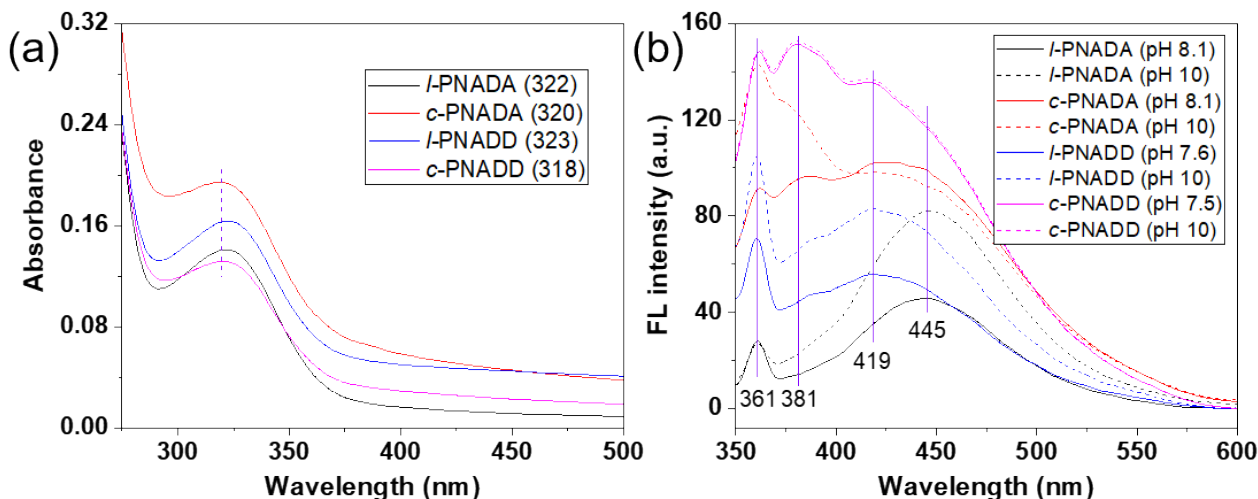




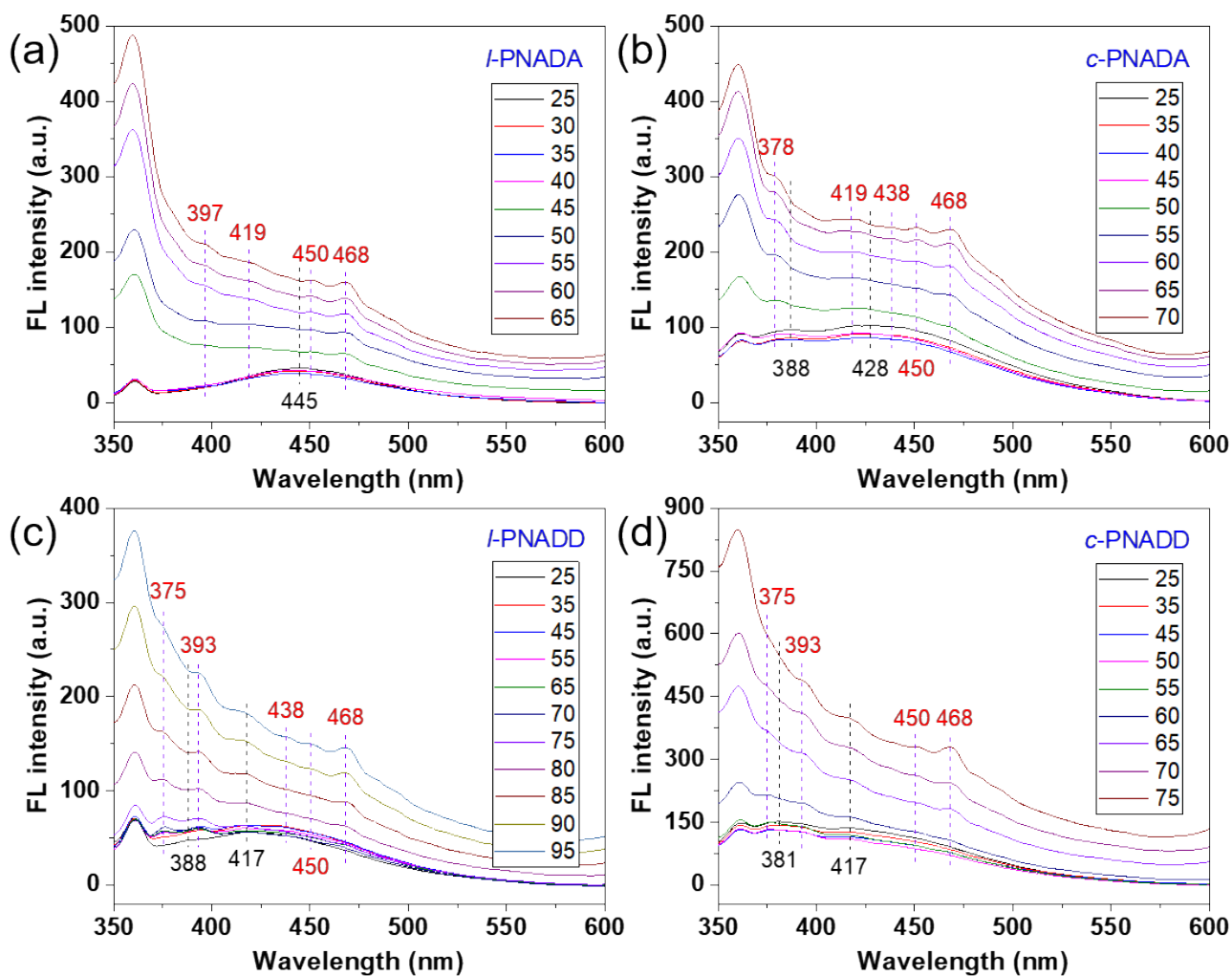
**Fig. S7** Turbidity curves of copolymer solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) formed from *l/c*-PNADA (a) and *l/c*-PNADD (b) in water upon heating and cooling.



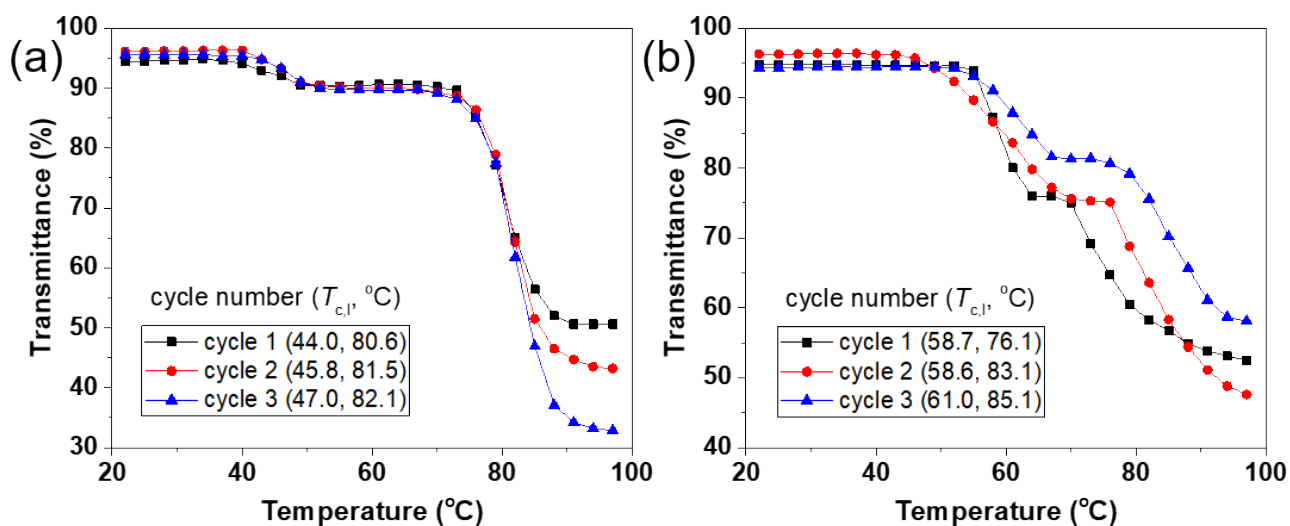
**Fig. S8** DLS plots of copolymer solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) formed in deionized water at 25 °C.



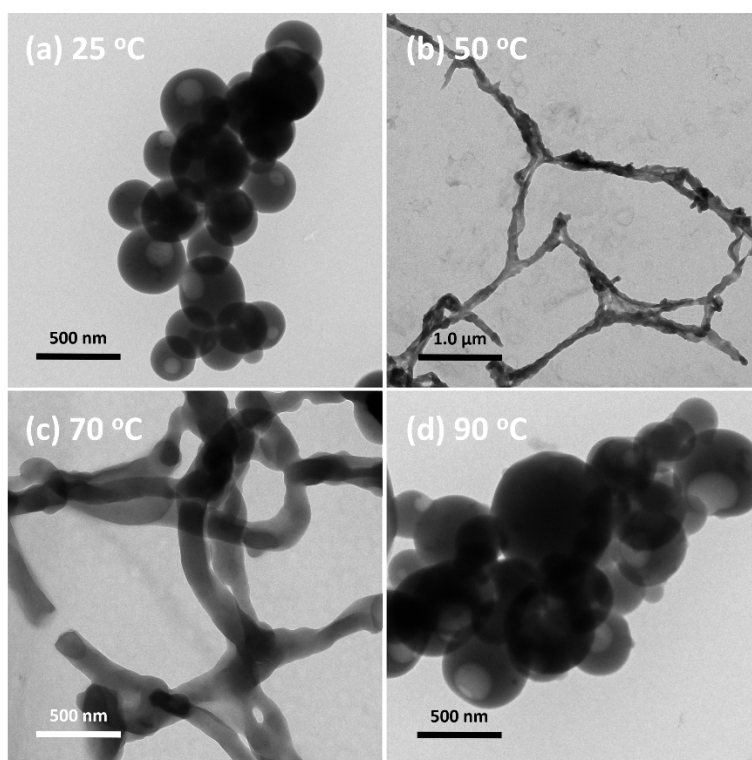
**Fig. S9** UV-vis spectra of copolymer aqueous solutions at 25 °C (a), and fluorescence spectra ( $\lambda_{\text{ex}} = 320$  nm) of copolymer solutions formed in water or at pH 10 at 25 °C (b), in which  $c_p$  is  $1.0 \text{ mg mL}^{-1}$ .



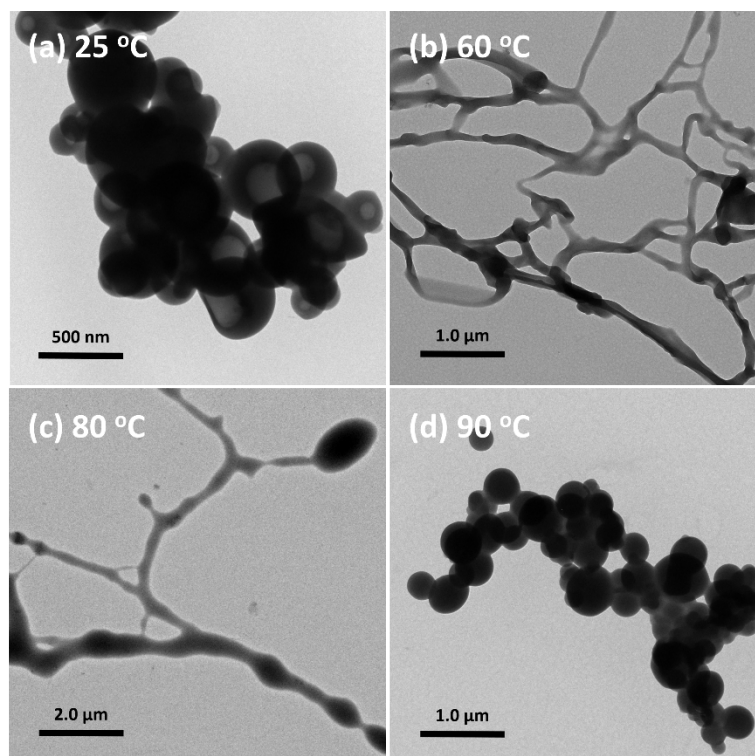
**Fig. S10** Temperature-dependent fluorescence spectra ( $\lambda_{\text{ex}} = 320$  nm) of copolymer solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) formed from *l*-PNADA (a), *c*-PNADA (b), *l*-PNADD (c) and *c*-PNADD (d) in water.



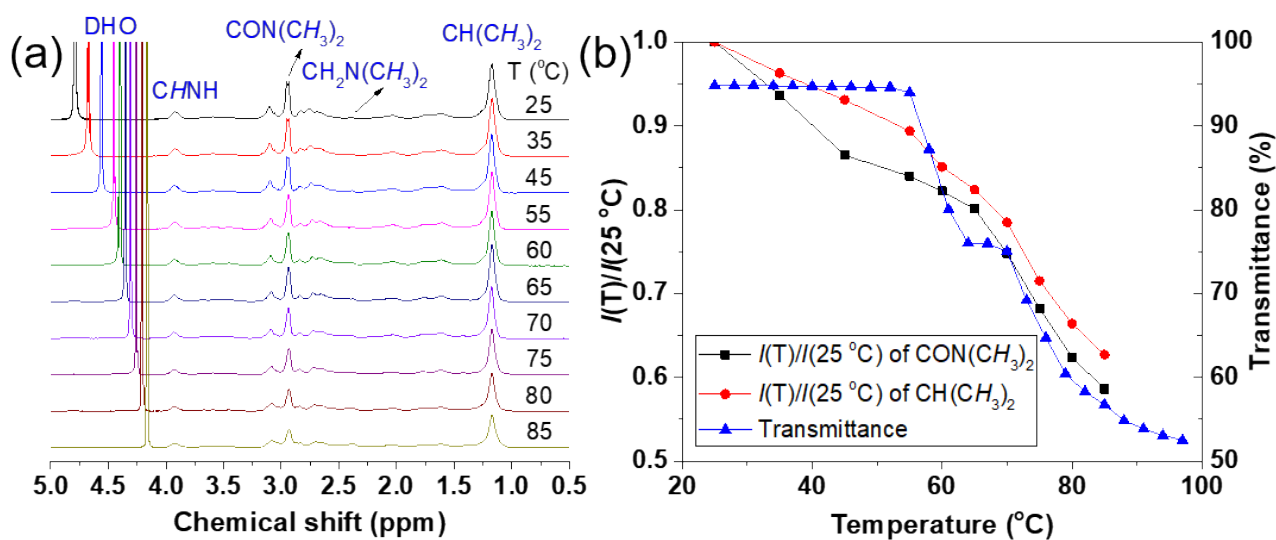
**Fig. S11** Turbidity curves of *l*-PNADD solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) formed in  $\text{H}_2\text{O}$  (a) and  $\text{D}_2\text{O}$  (b) obtained by three heating-cooling cycles, in which only heating curves are listed.



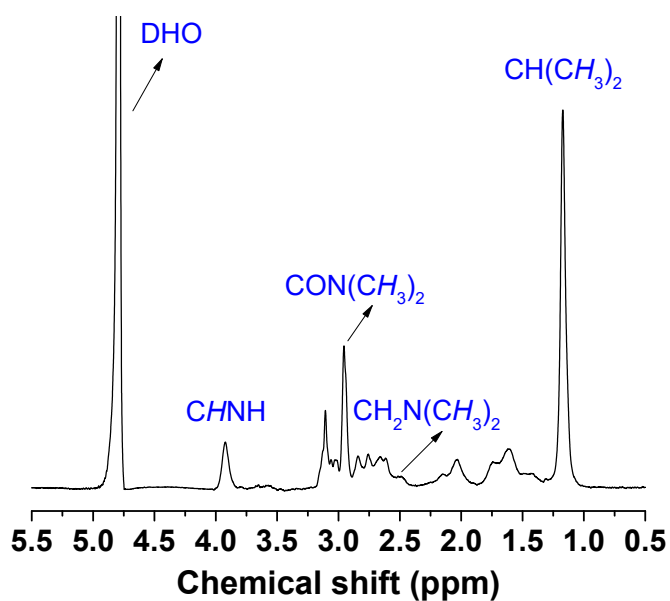
**Fig. S12** Representative TEM images of *c*-PNADA assemblies formed in  $\text{D}_2\text{O}$  ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) at different temperatures.



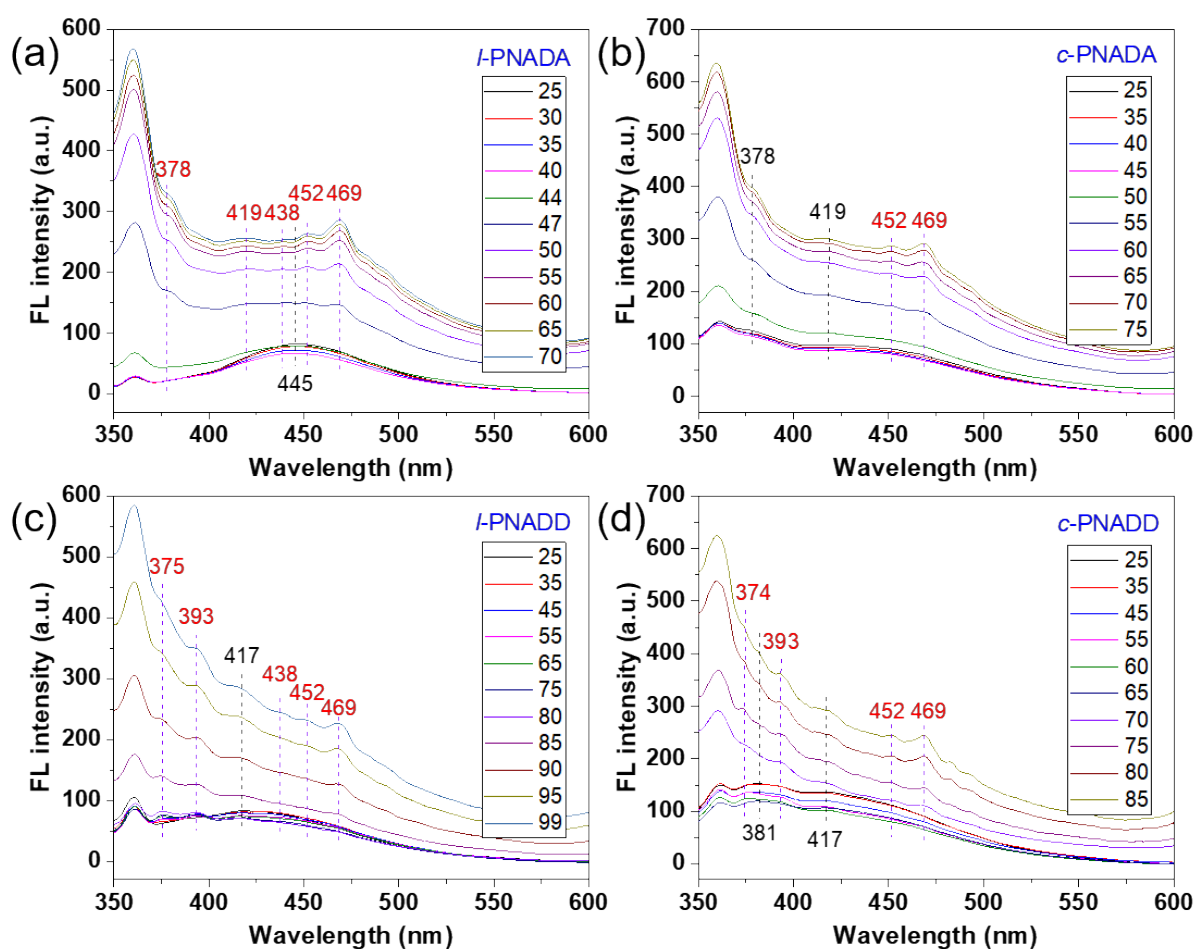
**Fig. S13** Representative TEM images of *l*-PNADD assemblies formed in D<sub>2</sub>O ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) at different temperatures.



**Fig. S14** Temperature-variable <sup>1</sup>H NMR spectra of *l*-PNADD solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) recorded in D<sub>2</sub>O (a), and temperature-dependent integral ratios ( $I(T)/I(25 \text{ }^\circ\text{C})$ ) of CON(CH<sub>3</sub>)<sub>2</sub> and CH(CH<sub>3</sub>)<sub>2</sub> (NIPAM unit) and transmittances of *l*-PNADD solutions in D<sub>2</sub>O (b).



**Fig. S15**  $^1\text{H}$  NMR spectrum of *c*-PNADD solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) recorded in  $\text{D}_2\text{O}$  at  $25 \text{ }^\circ\text{C}$ .



**Fig. S16** Temperature-dependent fluorescence spectra ( $\lambda_{\text{ex}} = 320 \text{ nm}$ ) of copolymer solutions ( $c_p = 1.0 \text{ mg mL}^{-1}$ ) formed from *l*-PNADA (a), *c*-PNADA (b), *l*-PNADD (c) and *c*-PNADD (d) at pH 10.