

## Supporting Information For

# On the Two-step Phase Transition Behavior of the Poly(*N*-isopropylacryamide) (PNIPAM) Brush: Different Zones with Different Orders

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### Materials

Tris[2-(dimethylamino)ethyl]amine (Me<sub>6</sub>TREN) was prepared as described in the literature. Oligo (ethylene glycol) methacrylate with an average  $M_n$  of 500 g/mol (OEGMA-OH) was purchased from Sigma-Aldrich and filtered through basic alumina to remove stabilizers. *N*-Isopropylacrylamide (NIPAM) was recrystallized from cyclohexane before use. Cuprous bromide (CuBr) and cuprous chloride (CuCl) were purified by washing several times by methanol. Methanol, dichloromethane, isopropanol (IPA), and diethyl ether were purified by distillation. Unless otherwise specified, all other chemicals were used as received.

### POEMGA-*g*-PNIPAM Preparation

Synthesis of Poly [oligo (ethylene glycol) methacrylate] (POEGMA-OH). 1.5 g (3 mmol) of OEGMA-OH, 19.5 mg (0.1 mmol) of ethyl  $\alpha$ -bromoisobutyrate, 9.9 mg (0.1 mmol) of CuCl, 31.2 mg (0.2 mmol) of 2,2'-bipyridine and 2.25 g of ethanol were charged into a reaction tube. After three freeze-pump-thaw cycles, the mixture was sealed under vacuum and the reaction was allowed to continue at 60°C for 5h. The

solution was diluted by THF, passed through alumina column to remove the catalyst, and then precipitated in diethyl ether for three times. The precipitate was collected by centrifugation and dried at 35°C under vacuum for 24 h before further characterization (0.8 g, yield 53%). <sup>1</sup>H NMR analysis (in CDCl<sub>3</sub>, δ): 4.07 (bs, 2 H, -C(=O)OCH<sub>2</sub>-), 3.84–3.40 (m, ~38 H, OEG), 1.95–0.65 (m, 5H, backbone).

Synthesis of Polyinitiator POEGMA-Br. P(OEGMA-OH) (0.5 g, 1 mmol) dissolved in 20 mL of dry dichloromethane and triethylamine (0.404 g, 4 mmol) were added in a 100 mL three-necked round-bottomed flask under ice bath. Then, 0.46 g (2 mmol) of 2-bromoisobutyryl bromide dissolved in 10 mL of dry dichloromethane was added dropwise at 0 °C in 1h. The mixture was stirred for another 1h at 0 °C, followed by stirring at room temperature for 24 h. The insoluble salt was removed by filtration, and the POEGMA-Br was then precipitated by pouring the solution into diethyl ether. After centrifugation and washing, the polymer was dried in a vacuum oven at room temperature for 24 h (0.519 g, yield 80%). <sup>1</sup>H NMR analysis (in CDCl<sub>3</sub>, δ): 4.07 (bs, 2 H, -C(=O)OCH<sub>2</sub>-), 3.84–3.40 (m, ~38 H, OEG), 1.99 (s, 6H, Br-C(CH<sub>3</sub>)<sub>2</sub>), 1.95–0.65 (m, 5H, backbone).

Synthesis of POEMGA-g-PNIPAM by ATRP. To prepare the copolymer, an example ATRP reaction procedure was described as follows: a reaction tube was charged with NIPAM (1.13 g, 100 mmol), POEGMA-Br (0.649 g, 1 mmol), CuBr (14.4 g, 1 mmol), Me<sub>6</sub>TREN (0.304 g, 2 mmol) and IPA/H<sub>2</sub>O (4/1mL). After three freeze-pump-thaw cycles, the mixture was sealed under vacuum and the reaction was allowed to continue at 0 °C. After the reaction finished, the reaction solution was diluted by THF, passed through a short alumina column to remove the catalyst, and then precipitated in diethyl ether for twice. The precipitate was collected by filtration and dried at 35 °C under vacuum for 24 h before further characterization (1.01 g, yield 90%). <sup>1</sup>H NMR analysis (in CDCl<sub>3</sub>, δ): . 3.99 (bs, -NHCH(CH<sub>3</sub>)<sub>2</sub>), 3.67–3.60 (m, OEG), 2.50–0.50 (m, backbone), 1.14 (bs, -NHCH(CH<sub>3</sub>)<sub>2</sub>).

#### Instruments and measurements

<sup>1</sup>H NMR spectra were recorded on Varian Mercury plus 400M spectrometer with CDCl<sub>3</sub> as solvent and TMS as the internal reference. Temperature-variable <sup>1</sup>H NMR spectra of the copolymer were recorded using D<sub>2</sub>O as solvent (concentration =10 wt %) with an increment of 1 °C. The thermal behaviors of the samples were investigated with differential scanning calorimetry (DSC) on a Mettler DSC-1 apparatus. All the experiments were carried out in a nitrogen atmosphere. Each sample weighed about 10 mg and was sealed in an aluminium pan. The cooling and subsequent heating rate was 10 °C min<sup>-1</sup>. All data were based on the second heating process. The AFM image

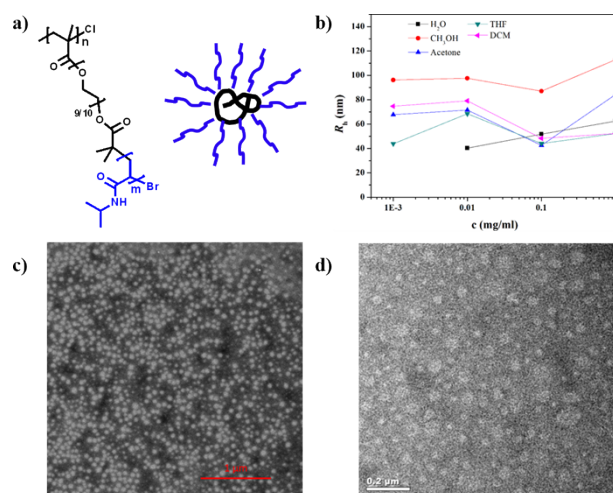
was obtained with a Veeco/Digital Instruments Nano-Scope IV operating in tapping mode with a drive frequency of 270 kHz. The samples were prepared by the spin-coating method after dropping the chloroform dispersion (1 mg/mL) onto a freshly cleaved mica surface. The average hydrodynamic radius of the copolymer in water was estimated on a Zetasizer Nano particle size analyzer (Malvern). Turbidity measurements were carried out at 500 nm on a Lamda 35 UV-vis spectrometer with deionized water as the reference (100% transmittance). Temperatures were regulated manually with a water-jacketed cell holder at the rate of 0.5 °C min<sup>-1</sup> with an increment of 1 °C. SEM image was recorded on a Nova NanoSEM450 scanning electron microscope. TEM image was recorded on a JEM 2010 transmission electron microscope.

The concentration of POEGMA-*g*-PNIPAM in D<sub>2</sub>O was fixed to 10 wt% and placed at 4 °C for a week before FT-IR measurements to ensure complete dissolution. The sample of POEGMA-*g*-PNIPAM solution for FT-IR measurements was prepared by being sealed between two ZnS tablets. All the time-resolved FTIR spectra at variable temperatures were recorded by using a Nicolet Nexus 6700 FTIR spectrometer equipped with a DTGS detector. 32 scans at a resolution of 4 cm<sup>-1</sup> were accumulated to obtain an acceptable signal-to-noise ratio. Temperatures were manually controlled with an electronic cell holder at rate of ca. 0.3 °C/min with an increment of 1 °C (accuracy: 0.1°C). Baseline correction was performed by the software of OMNIC 6.1a.

**Table S1.** Copolymerization results of the resultant copolymers

entry	feed	$N_{\text{NIPAM}}/N_{\text{OEGMA}}$ <i>a</i>	$M_n^b$ (10 <sup>3</sup> )
G0	-	-	7
G25	25:1	30	52
G50	50:1	120	183
G100	100:1	340	514
G200	200:1	780	1152

<sup>a</sup>  $N_{\text{NIPAM}}$ : number of NIPAM segments;  $N_{\text{OEGMA}}$ : number of OEGMA repeat units, determined by <sup>1</sup>H NMR; <sup>b</sup>  $M_n$ : molecular weight determined by <sup>1</sup>H NMR.



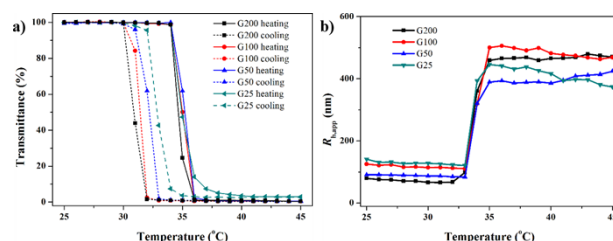
**Fig. S1** (a) Chemical structure and schematic illustration of the resultant copolymers; (b) Hydrodynamic radius of the copolymer at different concentrations in different solution; (c) SEM images of G50 in water; (d) TEM images of G50 water.

Copolymerization results were summarized in Table S1. Hydrodynamic radius distribution of the copolymers in different solution was determined by dynamic light scattering (DLS) as shown in Fig. S1b. Tens to hundreds nanometers even at very low concentration was observed, implying the formation of aggregates in solution. The aqueous solution display light blue at room temperature and the size of copolymer in water below LCST was around 100 nm, as shown in Fig. S1c, d, which is clear evidence for the self-assembly nature and aggregates morphology.

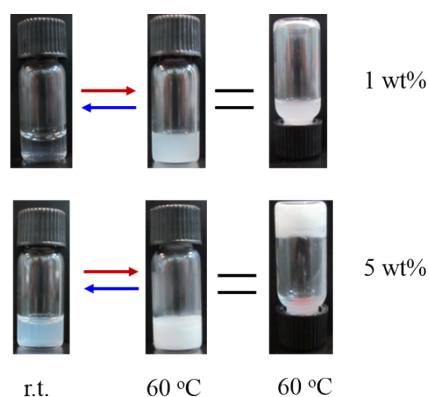
Chemical compositions of the resultant copolymers were estimated from  $^1\text{H}$  NMR spectra with  $\text{CDCl}_3$  as the solvent and the average number of OEG units in OEMGA unit is assumed as 9.5. Partial molar proportions of the two units in the copolymers ( $N_{\text{OEMGA}}$  and  $N_{\text{NIPAM}}$ ) were calculated according to:

$$N_{\text{NIPAM}}: N_{\text{OEMGA}} = 38 I_a : I_e$$

Where,  $I_a$  and  $I_e$  are defined as the relative intensity of isopropyl and OEG groups, respectively.



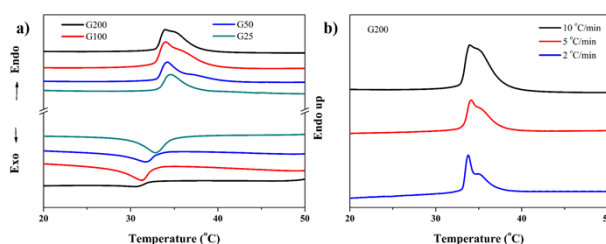
**Fig. S2** (a) Plots of transmittance versus temperature for the copolymer with the concentration of 1 mg/mL; (b) DLS curves of the copolymer in water with the concentration of 1 mg/mL.



**Fig. S3** Illustrations of 1 and 5 wt% the copolymer (G100) aqueous solution were taken at temperatures representative of each regime.

Turbidity and DLS measurements were also performed to determine the  $T_p$  of POEGMA-*g*-PNIPAM at the concentration of 1 mg/mL, as shown in Fig. S2. For each copolymer,  $T_p$  was taken as the middle point of the transmittance versus temperature curves. The phase transition of POEGMA-*g*-PNIPAM is reversible with a small hysteresis, as shown in Fig. S2a. During the heating process, the  $T_p$  was almost the same (*ca.* 35°C), which was slightly higher than the LCST of PNIPAM aqueous solution (*ca.* 32°C). The increase  $T_p$  could be attributed to introduction of POEGMA, which is relatively hydrophilic and possesses no thermo-responsive property in this temperature region. Similar  $T_p$  could also be observed in DLS measurements, as shown in Fig. S2b. However, different  $T_p$  was identified during the cooling process. With increasing side chain length, the  $T_p$  during cooling process decreased from *ca.* 33°C to 31°C. It seems more PNIPAM chain associations tend to occur in an overlapped environment and lead to a lower transition temperature.

As shown in Fig. S3, clear and transparent aqueous solution could be observed when the concentration of POEGMA-*g*-PNIPAM (G100) is 1 wt%, indicating the good solubility of graft copolymer in water. When heated up to 60 °C, the aqueous solution turned white but still with mobility.



**Fig. S4** (a) DSC curves of copolymers with different PNIPAM chain lengths; (b) Normalized DSC curves of G200 with different heating rate.

DSC curves of 10 wt% solution of copolymers during the heating and cooling process was shown in Fig. S4a. Notably, double endothermic peaks located at ~34 °C and ~36 °C were identified in the curves and the shoulder peak at higher temperature region “grow” more obvious with increasing the molecular weight of PNIPAM. However, in the present study, increment of the double phase transition temperature compared with that of the homopolymer was observed due to the existence of POEGMA core which is favorable to the hydrophilic condition.

Thermal behavior of the copolymers with different heating rate was also studied and the normalized curve was shown in Fig. S3b. The higher-temperature shoulder peak became less obvious compared with the lower one with decreasing the heating rate. It has been proved that the response for Au-PNIPAM dispersions is faster than that for PNIPAM. Therefore, the inner and outer zone may collapse cooperatively when the experiment was performed with slow heating rate. However, the response difference may be enlarged with increasing the heating rate.

Table S2 operation details of the sequence order determination during heating process

1602	-	-	+	-	+	-	+	-	-	-
1627	-	+	+	+	+	+	+	-	+	
1646	-	-	+	-	+	-	+	-		
1700	+	+	+	+	+	+	+			
1735	-	-	+	-	-	-				
2873	-	+	+	+	+					
2883	-	-	+	-						
2931	-	-	+							
2942	-	-								
2971	-									
	2987	2971	2942	2931	2883	2873	1735	1700	1646	1627