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Reversible Thermo-sensitivity Induced from Varying Hydrogen Bonding between the Side Residues of Rationally Designed Polypeptides

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Experimental Section

Materials

γ-Benzyl-L-glutamate *N*-carboxyanhydrides (BLG-NCA, 98%) was purchased from Nanjing Shuofeng biological medicine technology Co. and preserved at -30 °C under a nitrogen atmosphere prior to use. Hexylamin was purchased from Aladdin reagent and used without further purification. *N*, *N*-Dimethylformamide (DMF) was refluxed with calcium hydride (CaH_2) and distilled in vacuum. Ethyl acetate was purchased from Aladdin, stored over CaH_2 and obtained by filtration. Hydrobromic acid (HBr, 33 wt% in acetic acid), 1-Ethyl-3-(3-dimethyl-aminopropyl)-1-carbodiimide hydrochloride (EDC·HCl) and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich and used as received. Diethyl ether was cooled to -20 °C before use. All other reagents and solvents were purchased from Aladdin and used without further purification.

General characterization.

The ^{1}H Nuclear Magnetic Resonance (NMR) spectra (500 MHz) in deuterated trifluoroacetic acid (CF_3COOD) were recorded at room temperature on a Bruker NMR spectrometer. The temperature-dependent ^{1}H NMR measurements (400 MHz)

in deuteroxide (D_2O) were performed after the sample tube was kept at a predetermined temperature for 15 min. Both the molecular weight and molecular weight distribution were determined by Gel Permeation Chromatography (GPC) on a USA Waters 1515 HPLC using DMF containing 0.01 M LiBr as eluent at a flow rate of 1.0 mL/min. Fourier Transform Infrared Spectroscopy (FTIR) was recorded on a Nicolet 5700 spectrometer at room temperature (25 °C). The samples were milled with KBr crystal (Aldrich) and pressed into disks before measurements. Circular dichroism spectra were recorded on a JASCO/J-810 Circular Dichroism (CD) with a wavelength range from 180 nm to 260 nm with sample concentration of 0.5 mg/mL. The solution was placed into a quartz cell with a pathlength of 0.1 cm. Temperature-dependent ultraviolet spectroscopy were performed on a Perkin Elmer Lambda Bio40 UV spectrophotometer by monitoring the transmittance of a 500 nm light beam through a quartz sample cell with concentration of 2 mg/mL. The solution was heated and cooled at a rate of 0.5 °C/min from 10 °C to 60 °C. The thermal analysis was carried out on a US Dimond Differential Scanning Calorimeter (DSC) working with 5-7 mg samples in open aluminium pans. The samples were first heated from 0 to 150 °C at the heating rate of 20 °C/min and maintained at 150 °C for 5 min to eliminate thermal history before quenching to 0°C. Then the samples were reheated to 150 °C at the heating rate of 10 °C/min. T_g was determined as the inflection point of the spectra in the second heating process. All the Matrix Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF MS) experiments were performed on an Applied Biosystems 4700 Proteomics Analyzer. The matrix material used was *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene] malononitril (DCTB) for PDEGA and α-Cyano-4-hydroxycinnamic acid (CHCA) for PIGA.

Synthesis of poly(L-glutamate).

According to the literature[17,18], poly (γ-benzyl-L-glutamate) (PBLG) was prepared by ring-opening polymerization of BLG-NCA with an amino-terminated initiator. A typical procedure was described as follow. BLG-NCA (10 g, 3.8 mol) were suspended in 10 mL of water-free DMF bubbled with nitrogen flux in a flamed-dried flask. After

the monomer was completely dissolved, a predetermined amount of hexylamine (varied with the targeted DP) was injected to the solution. The reaction mixture was then stirred at room temperature for 72 h. The product PBLG was precipitated in dry diethyl ether and dried under vacuum.

The obtained PBLG (1g) above was dissolved in dichloroacetic acid (10 mL) at 25 °C in a 25 mL Schlenk flask and a solution of HBr in acetic acid (33%, 6 mL) was added with stirring. The reaction mixture was dropwisely precipitated in 250 mL dry diethyl ether after kept stirring at room temperature for 2 h, followed by filtration. The poly (L-glutamate) (PGA) was achieved by further washing twice with diethyl ether and vacuum dry (yield: 80%).

Synthesis of homopolymers poly(*N*-isopropyl-L-glutamine) and poly(*N*-diethylin-L-glutamine)

First, PGA (0.2 g) was added to a 25 mL Schlenk flask followed by addition of 10 mL of dry DMF. After the solid was dissolved in DMF completely, EDC·HCl (2.2 g) and NHS (1.3 g) were introduced and stirred at room temperature under a nitrogen atmosphere for 2 h. Next, isopropylamine or diethylamine (3 times amount of carboxyl group in PGA) was added dropwisely and the solution was continuously kept stirring for 24 h. Subsequently, the reaction mixture was transferred to the dialysis tubing (MWCo = 1000 Da). The samples were dialyzed against deionized water for 2 days with water changed per 8 h. Dialyzed polymers were lyophilized to isolate poly(*N*-isopropyl-L-glutamine) (PIGA) or poly(*N*-diethylin-L-glutamine) (PDEGA) as white solids (yield: 65%). Based on NMR results, the conversion of carboxyl group in PGA was nearly 100%.

Synthesis of random copolymer poly(*N*-isopropyl-L-glutamine)-*co*-poly (*N*-diethylin-L-glutamine)

The synthetic procedure of random copolymers was similar to that of PIGA and PDEGA above. After the EDC·HCl (2.2 g) and NHS (1.3 g) was added to the solution of PGA (0.2 g, DP=20) in DMF (10 mL) for 2 h, a predetermined amount of

diethylamine (according to targeted ratio of IGA and DEGA segments, the ratio of diethylamine and carboxyl group was 0.5, 1, 1.5, 2, 2.5, 3. 3.5 respectively) was injected into the flask using a springe and the mixture solution was stirred at room temperature for 30 h. Subsequently, isopropylamine in excess (2 times amount of carboxyl group) was added into the solution and kept stirring for another 30 h. Then random polymer poly(*N*-isopropyl-L-glutamine)-*co*-poly(*N*-diethylin-L-glutamine) (PIGA-*co*-PDEGA) was obtained by dialysis with deionized water (yield: 60%). According to NMR results, the carboxyl group of PGA was completely substituted.

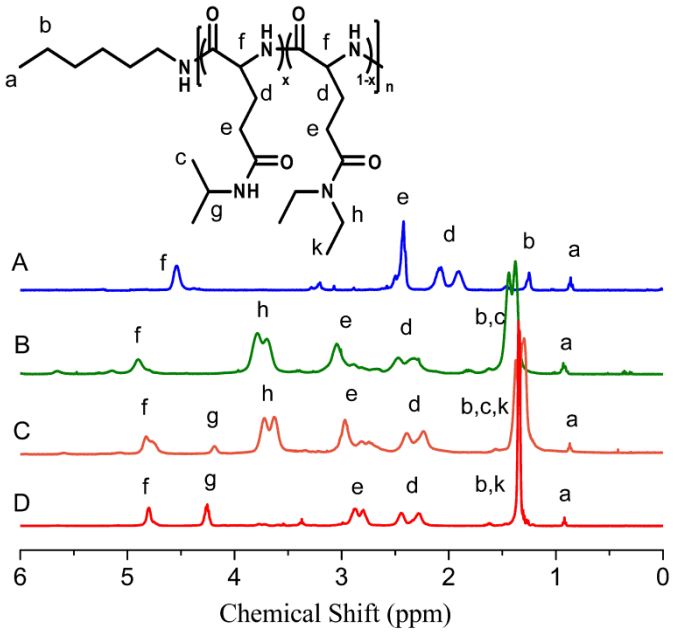

Fig. S1 ^{1}H NMR spectra of (A) poly (L-glutamate) (PGA), (B) poly(*N*-isopropyl-L-glutamine) (PIGA), (C) poly(*N*-isopropyl-L-glutamine)-*co*-poly (*N*-diethylin-L-glutamine) (PIGA-*co*-PDEGA) and (D) poly(*N*-diethylin-L-glutamine) (PDEGA) in CF_3COOD

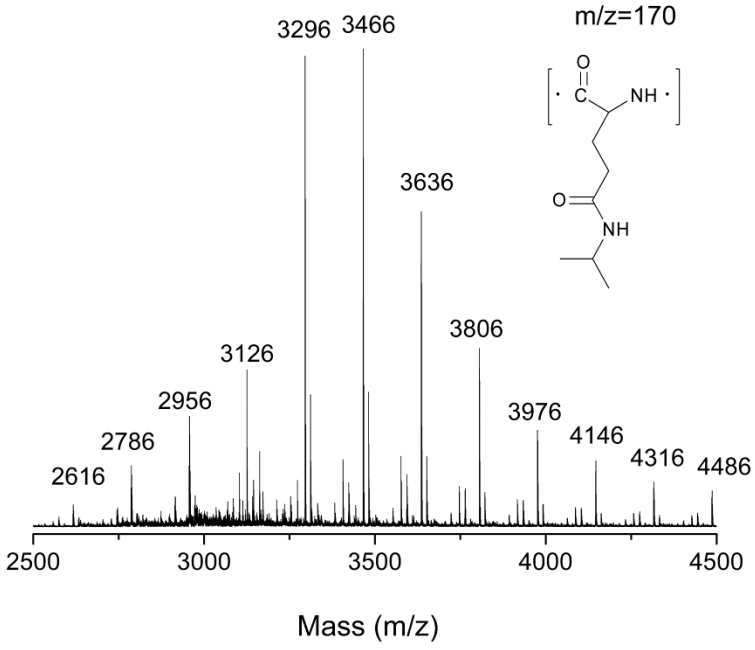

Fig. S2 MALDI-TOF MS spectra of PIGA

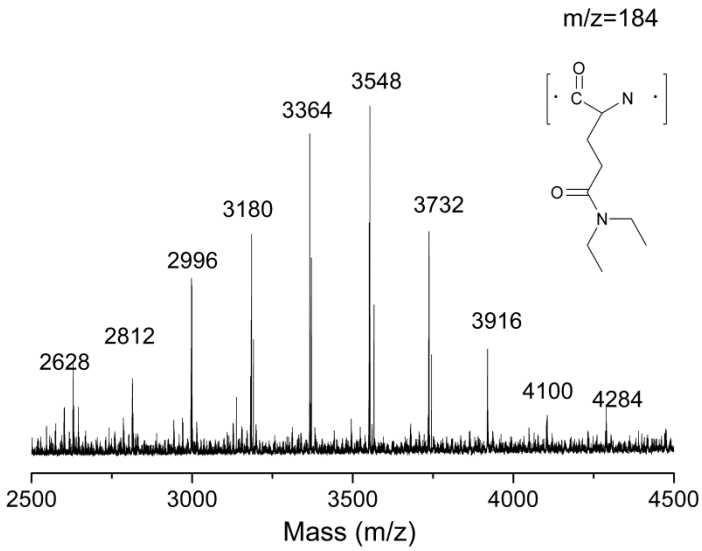

Fig. S3 MALDI-TOF MS spectra of PDEGA

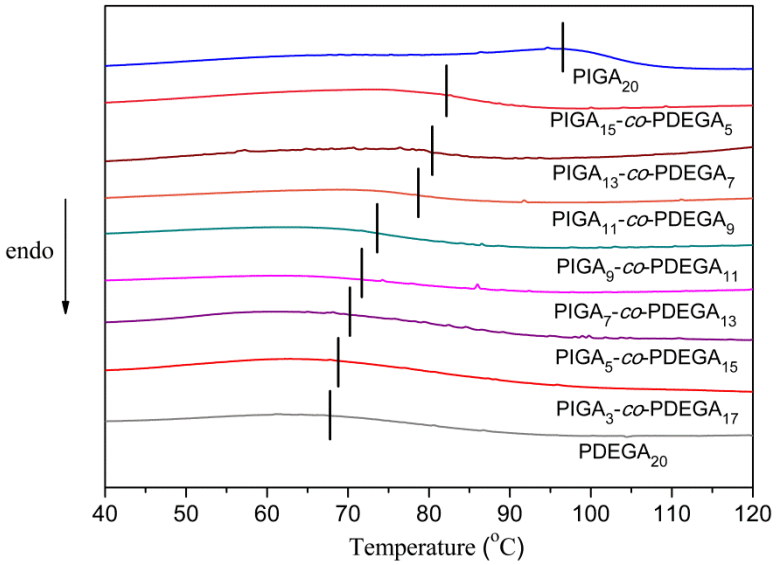

Fig. S4 DSC curves of PIGA, PIGA-*co*-PDEGA and PDEGA in solid state

Table S5 Molecular weight and molecular weight distribution of all polymers

Polymers	Mn[a]	Mn[b]	PDI[b]
$PIGA_{20}$	3700	6100	1.2
$PIGA_{15}$-*co*-$PDEGA_5$	3700	6200	1.2
$PIGA_{13}$-*co*-$PDEGA_7$	3800	6400	1.1
$PIGA_{11}$-*co*-$PDEGA_9$	3800	6600	1.2
$PIGA_9$-*co*-$PDEGA_{11}$	3800	7000	1.1
$PIGA_7$-*co*-$PDEGA_{13}$	3900	7300	1.1
$PIGA_5$-*co*-$PDEGA_{15}$	3900	7700	1.2
$PIGA_3$-*co*-$PDEGA_{17}$	4000	7900	1.2
$PDEGA_{20}$	4000	8200	1.1

[a] Determined by ^{1}H NMR.

[b] Determined by GPC.

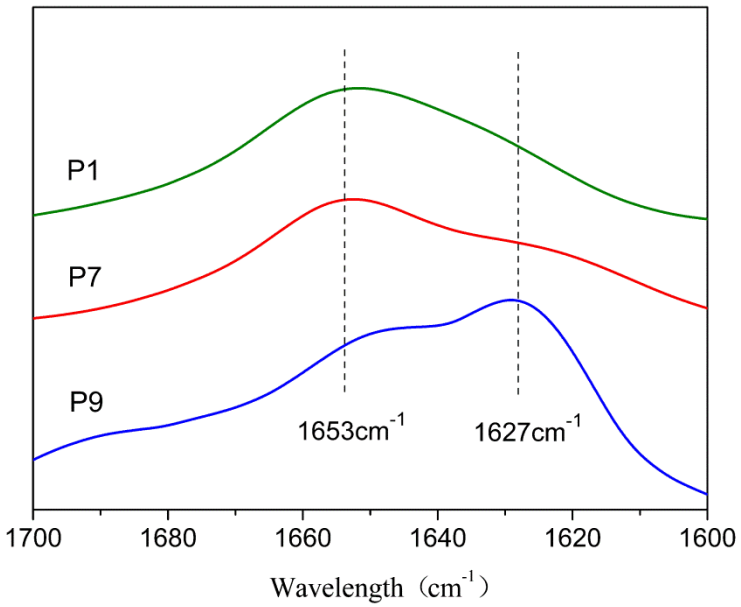

Fig. S6 FTIR spectra of entries P1, P7 and P9 in solid state

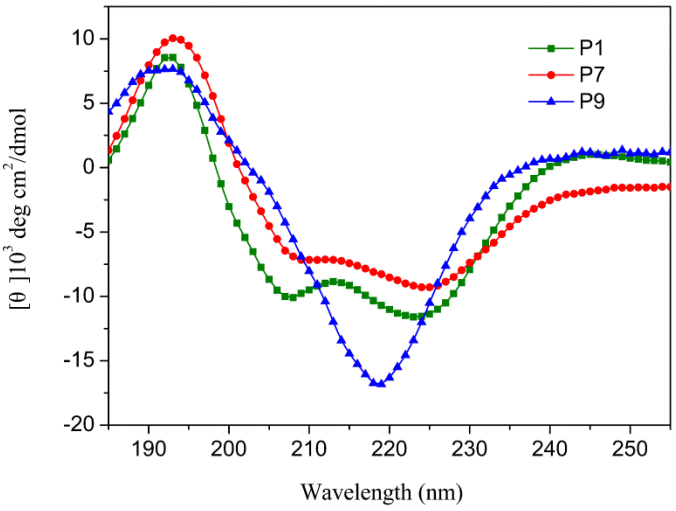

Fig. S7 Circular dichroism spectra of entries P1, P7 and P9 (0.5 mg mL) in the aqueous solution

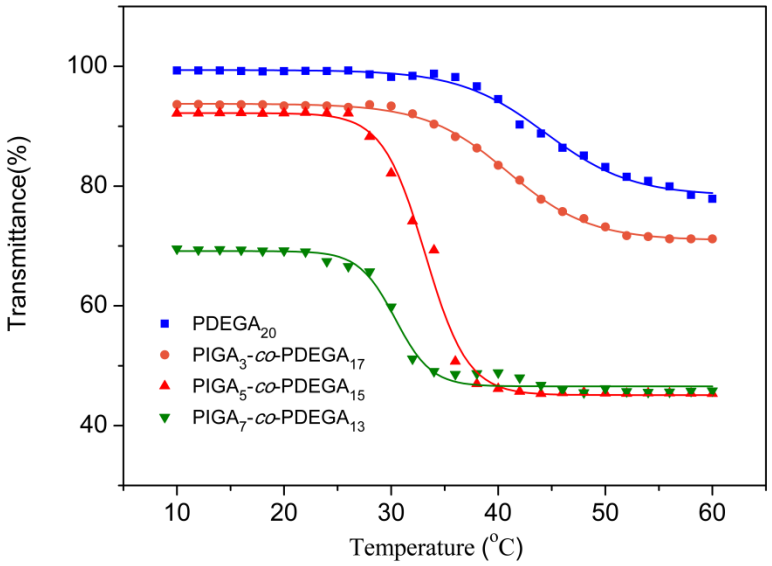

Fig. S8 Plots of transmittance as a function of temperature for aqueous solution of PDEGA$_{20}$, PIGA$_3$-*co*-PDEGA$_{17}$, PIGA$_5$-*co*-PDEGA$_{15}$, PIGA$_7$-*co*-PDEGA$_{13}$ (2 mg/mL)

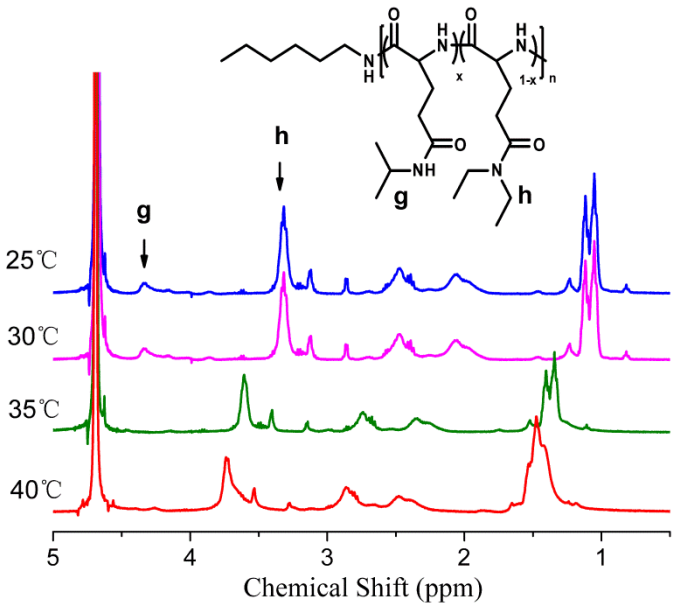

Fig. S9 Temperature dependent ^{1}H NMR for P7 in aqueous solution (30 mg/mL)