Supporting Information

Hydrogen Bonding Reinforcement as a Strategy to Improve Upper Critical Solution Temperature of Poly(*N*-Acryloylglycinamide-*co*-Methacrylic acid)

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Experimental

Materials

Acrylic acid (AA, 99%), Methacrylic acid (MAA, 99%), N,N′-Methylenebis(acrylamide) (BIS, 99%) and 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (V-50, 97%) were purchased from Sigma-Aldrich. N-isopropylacrylamide (NIPAM, 99%) were purchased from J&K Scientific. N-acryloylglycinamide (NAGA) and the chain transfer agent (CTA) 2-ethylsulfanylthiocarbonylsulfanyl-propionic acid methyl ester were synthesized according to our previous reports, respectively.[1-2] The liquid monomers were passed through a column of Al_2O_3 to remove the inhibitor prior to use.

Characterization

Turbidimetry measurements (1 wt%) were carried out at 500 nm on a Lamda 35 UVvis spectrometer with deionized water as reference at a heating/cooling rate of 1 °C min^{-1}. Hydrodynamic diameters (D_h) of copolymers (10 mg mL^{-1}) and nanogels (5 mg mL^{-1}) were measured on a Malvern ZS90 at a heating/cooling rate of 1 °C min^{-1} after being allowed to equilibrate at the setting temperatures for 2 min, respectively. Molecular weight and dispersity were determined using S2 Waters size exclusion chromatography (SEC) equipped with Styragel HR4 and HR5 columns and a Waters 2410 refractive index detector. The measurements were made using DMSO with LiBr (2 mg mL^{-1}) as eluent at 70 °C column temperature. Prior to the measurements, the samples were allowed to dissolve at 70 °C for 2h. Samples soluble in N,N-Dimethylformamide (DMF) were also run using DMF (LiBr, 2 mg mL^{-1}), Styragel HR4, HR5, and HR6 columns, column temperature 30 °C) as an eluent. The flow rate was 1.0 mL min^{-1} in all cases. Calibration was made with pullulan standards (Shodex Standard) in DMSO or PMMA standards in DMF. ^{1}H NMR spectra of the copolymers and nanogels were recorded on a Varian Mercury plus (500 MHz) spectrometer using D_2O as the solvent. Varible-temperture ^{1}H NMR spectra of copolymer solutions were recorded on a Varian Mercury plus (500 MHz) spectrometer using D_2O as the solvent (concentration = 10 wt%) at a decrement of 1 ^{o}C. FTIR spectra were recorded on a Nicolet Nexus 6700 FTIR spectrometer equipped with a DTGS detector at 25 ^{o}C. High resolution transmission electron microscope (HRTEM) images were taken on a Tecnai G2 F20 S-Twin with an accelerating voltage of 200 kV. Diluted polymer dispersions were placed onto carbon-coated copper grids and dried at room temperature.

Synthesis of Poly(N-Acryloyl Glycinamide) (PNAGA) Homopolymer

CTA 2-ethylsulfanylthiocarbonylsulfanyl-propionic acid methyl ester (3.6 mg, 0.016 mmol), NAGA (410 mg, 3.20 mmol) was dissolved in 8.3 mL of water. The solution was degassed with nitrogen at 0 °C for 40 min before immersion into a preheated oil bath at 70 °C. When the temperature was stabilized, a degassed solution of V-50 (0.43 mg, 0.0016 mmol) in water was injected *via* a microsyringe. After designated time the reaction was stopped by exposing the solution to air and cooling. The polymer PNAGA was purified by extensive dialysis against distilled water and lyophilized.

Synthesis of Poly(methacrylic acid) (PMAA) Homopolymer

CTA 2-ethylsulfanylthiocarbonylsulfanyl-propionic acid methyl ester (3.6 mg, 0.016 mmol) and MAA (275mg, 3.20 mmol) was dissolved in 2.8 mL of water. The solution was degassed with nitrogen at 0 °C for 40 min before immersion into a preheated oil bath at 70 °C. When the temperature was stabilized, a degassed solution of V-50 (0.43 mg, 0.0016 mmol) in water was injected *via* a microsyringe. After designated time the reaction was stopped by exposing the solution to air and cooling. The polymer PMAA was purified by extensive dialysis against distilled water and lyophilized.

Synthesis of P(NAGA-*co*-AA) Copolymers

The total monomer concentration was 10% and the molar ratio of [CTA]/[Mono]/[V-50] was controlled at 1/200/0.1. The target degree of polymerization (DP) was 200, and the actual DP of all thermosensitive copolymers was around 200. The molar ratio of NAGA/AA was varied from 90:10 to 30:70. An exemplary synthesis of the copolymers is given for P(NAGA$_{100}$-*co*-AA$_{96}$). CTA 2-ethylsulfanylthiocarbonylsulfanyl-propionic acid methyl ester (3.6 mg, 0.016 mmol), NAGA (205 mg, 1.60 mmol) and AA (116 mg, 1.60 mmol) were dissolved in 3.2 mL of water. The solution was degassed with nitrogen at 0 °C for 40 min before immersion into a preheated oil bath at 70 °C. When the temperature was stabilized, a degassed solution of V-50 (0.43 mg, 0.0016 mmol) in water was injected *via* a microsyringe. After designated time the reaction was stopped by exposing the solution to air and cooling. The copolymers were purified by extensive dialysis against distilled water and isolated *via* lyophilization. The resulting copolymers were named as PNA-X (X is the AA in molar percentage).

Synthesis of P(NAGA-*co*-MAA) Copolymers

The total monomer concentration was 10% and the molar ratio of [CTA]/[Mono]/[V-50] was controlled at 1/200/0.1. The target degree of polymerization (DP) was 200, and the actual DP of all thermosensitive copolymers was around 200. The molar ratio of NAGA/MAA was varied from 99:1 to 20:80. An exemplary synthesis of the copolymers is given for P(NAGA$_{94}$-*co*-MAA$_{94}$). CTA 2-ethylsulfanylthiocarbonylsulfanyl-propionic acid methyl ester (3.6 mg, 0.016 mmol), NAGA (205 mg, 1.60 mmol) and MAA (138 mg, 1.60 mmol) were dissolved in 3.4 mL of water. The solution was degassed with nitrogen at 0 °C for 40 min before immersion into a preheated oil bath at 70 °C. When the temperature was stabilized, a degassed solution of V-50 (0.43 mg, 0.0016 mmol) in water was injected *via* a microsyringe. After designated time the reaction was stopped by exposing the solution to air and cooling. Aliquots were sampled at predetermined time intervals for polymerization kinetics study. The copolymers were purified by extensive dialysis against distilled water and isolated *via* lyophilization. The resulting copolymers were named as PNM-X (X is the MAA in molar percentage).

Synthesis of P(NAGA-*co*-MAA)-*b*-P(NIPAM-*co*-BIS) diblock Nanogels by RAFT Dispersion Polymerization

P(NAGA-*co*-MAA)-*b*-P(NIPAM-*co*-BIS) core-shell diblock nanogels were synthesized by RAFT dispersion polymerization in a water at 70 °C, at a total solids content of 10% (w/v). In a representative procedure for the synthesis of P($NAGA_{131}$-*co*-MAA_{59})-*b*-P($NIPAM_{198}$-*co*-BIS_5), P($NAGA_{131}$-*co*-MAA_{59}) copolymer Macro-CTA (0.16 g, 0.0073 mmol), NIPAM (0.16 g, 1.45 mmol), and BIS (0.0056 g, 0.036 mmol) were dissolved in 3.3 mL of water. The solution was degassed with nitrogen in an ice bath for 40 min before immersion into a preheated oil bath at 70 °C. After the temperature was stabilized, a degassed solution of V-50 (0.24 mg, 0.00086 mmol) in water was injected *via* a microsyringe. The monomer conversion was found to be near-quantitative (>99%) after 6 h of polymerization as determined by ^{1}H NMR. The nanogels were purified *via* dialysis against water in a dialysis tube (MWCO 14 kg mol^{-1}) at 25 °C. The nanogels are abbreviated according to the DP of shell and core. For example, N190-198 represents a nanogel sample with DPs of P(NAGA-*co*-MAA) shell and PNIPAM core being 190 and 198, respectively.

Table S1. Synthetic Conditions and Results of P(NAGA-*co*-AA) Copolymers.[a]

polymer code	Composition	Conv. (%)[b]	$M_{n,th}$[c] (g/mol)	M_n (GPC)[d] (g/mol)	$Đ$ (GPC)[d]	UCST (°C)[f] (pH=3.5)
PNA10	P(NAGA$_{175}$-*co*-AA$_{19}$)	97	23800	27700 [e]	1.22 [e]	4
PNA29	P(NAGA$_{137}$-*co*-AA$_{55}$)	96	21500	25600	1.21	9.5
PNA40	P(NAGA$_{116}$-*co*-AA$_{77}$)	97	20400	23200	1.23	13.5
PNA49	P(NAGA$_{100}$-*co*-AA$_{96}$)	98	19700	22800	1.24	15
PNA58	P(NAGA$_{82}$-*co*-AA$_{114}$)	98	18700	21500	1.24	15.5
PNA69	P(NAGA$_{61}$-*co*-AA$_{135}$)	98	17500	20200	1.25	16.5

[a] Target DP = ~200. [b] Monomer conversion determined by ^{1}H NMR. [c] Theoretical molecular weight of copolymers = (target DP_{NAGA} × monomer conversion) × M_{NAGA} + (target DP_{AA} × monomer conversion) × M_{AA} + M_{CTA}. [d] Molecular weight determined by GPC (DMF, PMMA). [e] Molecular weight determined by GPC (DMSO, pullulan). [f] The temperature at 50% transmittance of the thermal transition was taken as the UCST upon cooling (1 wt%).

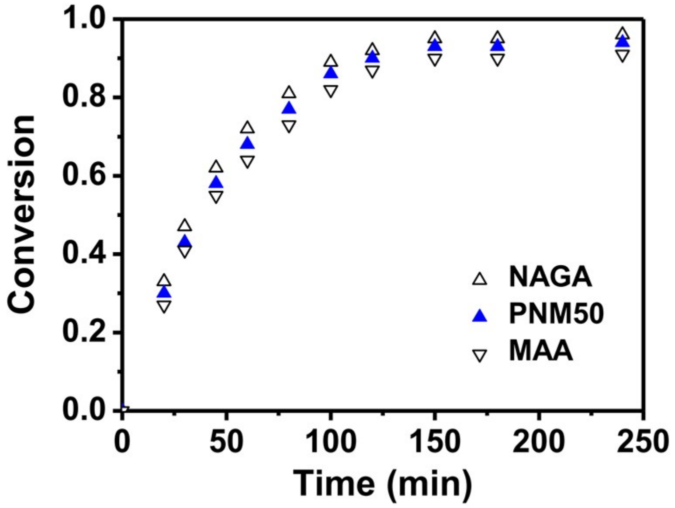

Figure S1. Polymerization kinetics of copolymerization of PNM50, [Monomer]/[CTA]/[V-50] = 200:1:0.1, concentration = 10%.

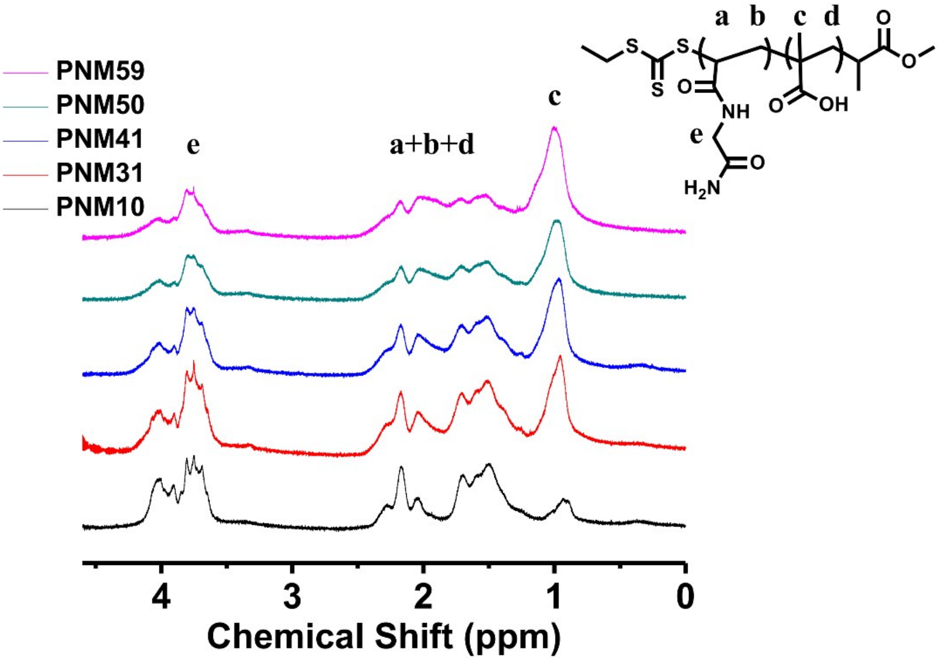

Figure S2. ^{1}H NMR spectra of copolymers in D_2O.

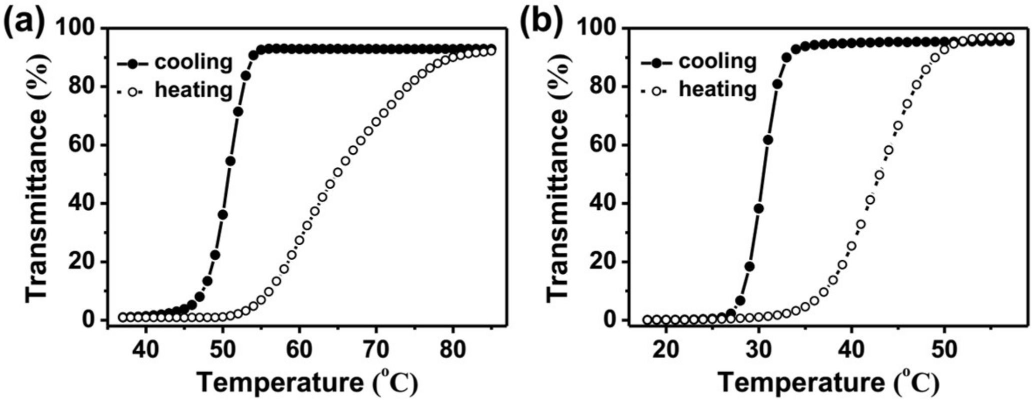

Figure S3. Thermal transitions of PNM50 (1 wt%) measured by turbidimetry at pH 1 (a) and pH 4 (b) during cooling/ heating cycle, respectively.

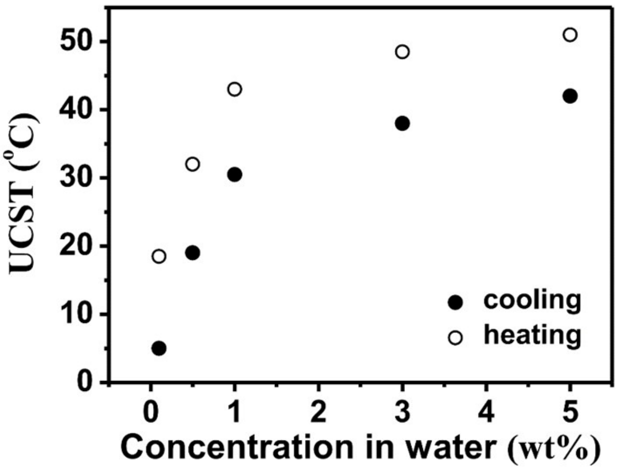

Figure S4. Dependence of UCST of PNM50 on the polymer concentration in pH 4 buffer solution.

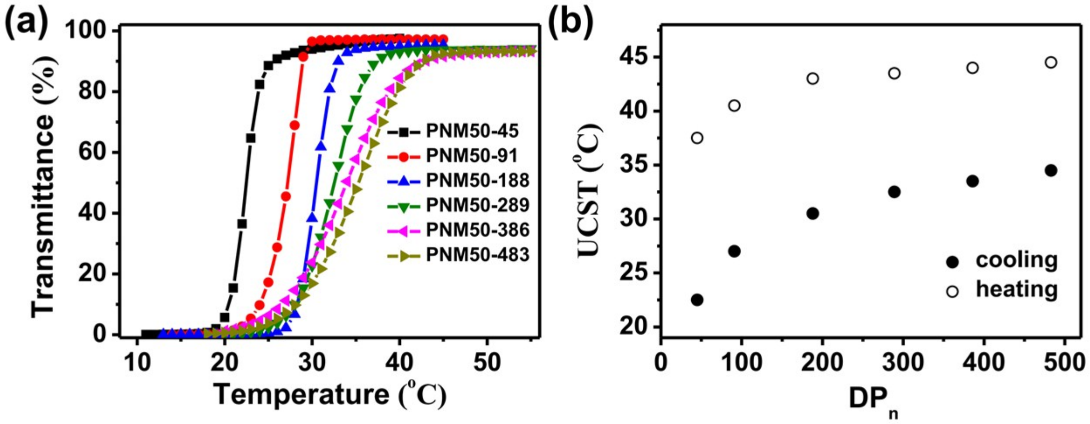

Figure S5. Thermal transitions of the copolymers measured by turbidimetry (1 wt %) in pH=4 buffer solution upon cooling (a) and dependence of UCST of the copolymers on DP (b).

Table S2 Hydrodynamic Diameters and Thermal Transition Temperatures of P(NAGA-*co*-MAA)-*b*-P(NIPAM-*co*-BIS) Diblock Nanogels [a]

nanogel code	D_h (nm) (PDI)[b]	D_n (nm)[c]	UCST/LCST (°C)[d] (pH 4)
N190-99	97 (0.20)	49	8/31
N190-198	122 (0.18)	64	5/31
N190-396	162 (0.15)	85	4/31

[a] BIS : P(NAGA-*co*-MAA) =5:1, 70 °C [b] Hydrodynamic diameter and polydispersity index (PDI) measured by DLS in water at 25 °C. [c] Diameter determined by TEM. [d] Thermal transition temperature measured by variable-temperature DLS upon cooling (5 mg/mL).

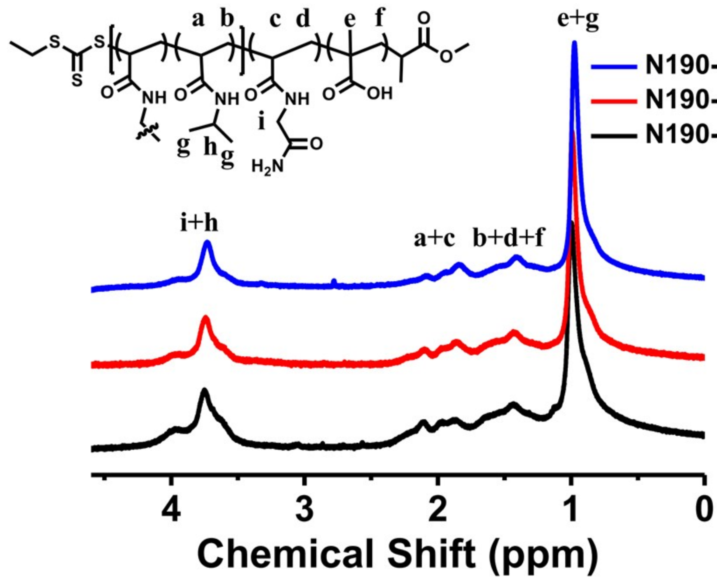

Figure S6. ^1H NMR spectra of nanogels in D_2O.

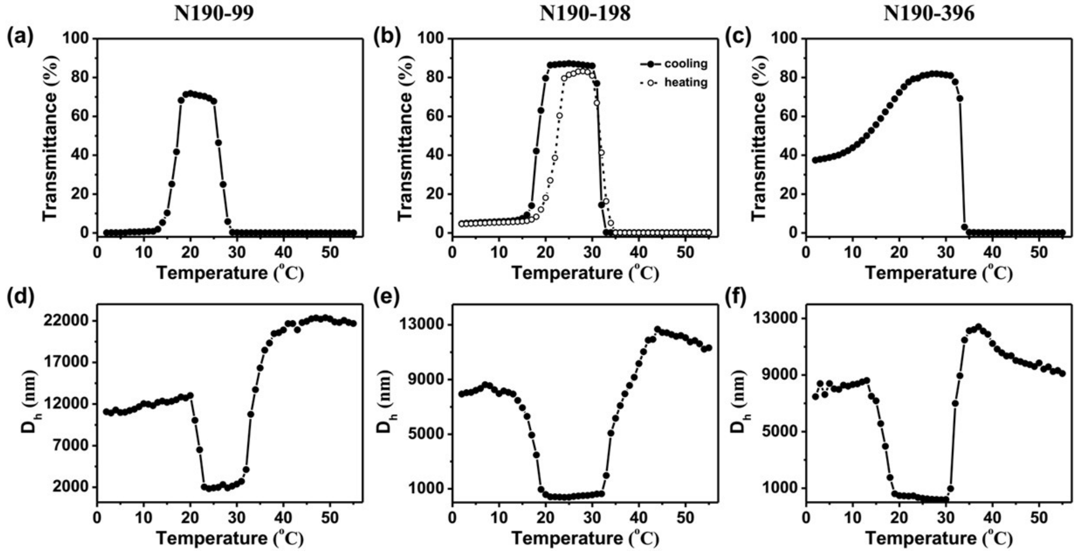

Figure S7. Turbidimetry and variable-temperature DLS measurements (cooling process) of N190-99 (a) and (d), N190-198 (b) and (e), and N190-396 (c) and (f) nanogels respectively in pH =1 buffer solutions at a concentration of 5 mg/mL.

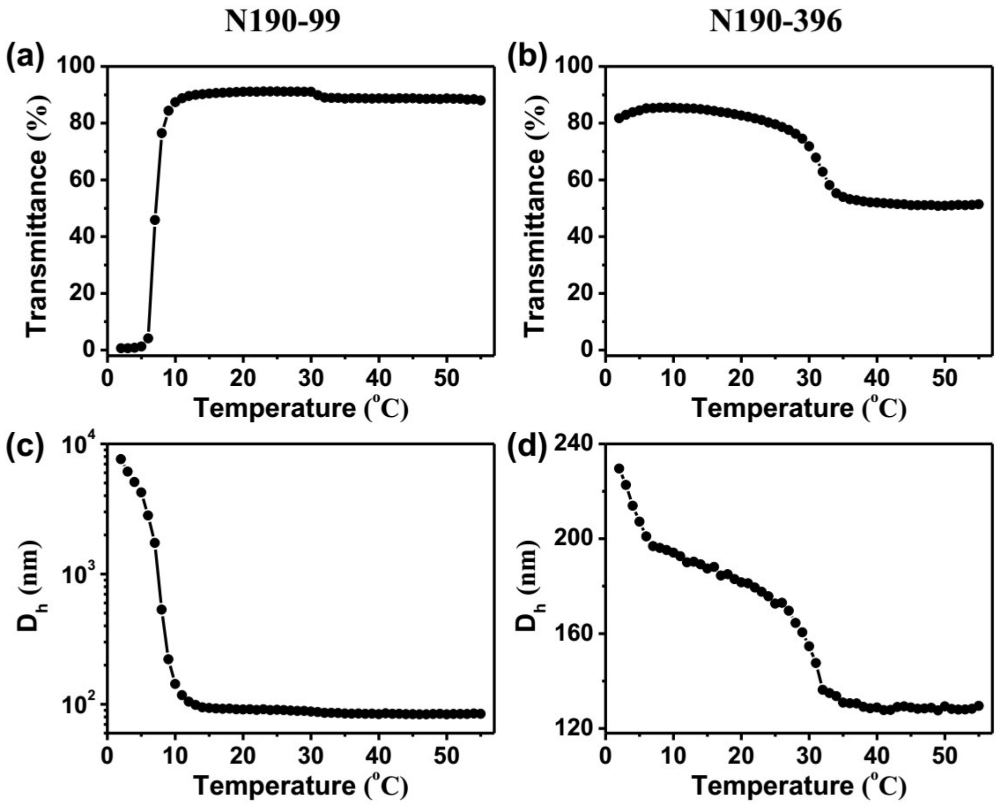

Figure S8. Turbidimetry and variable-temperature DLS measurements (cooling process) of N190-99 (a) and (c), and N190-396 (b) and (d) nanogels respectively in pH =4 buffer solutions at a concentration of 5 mg/mL.

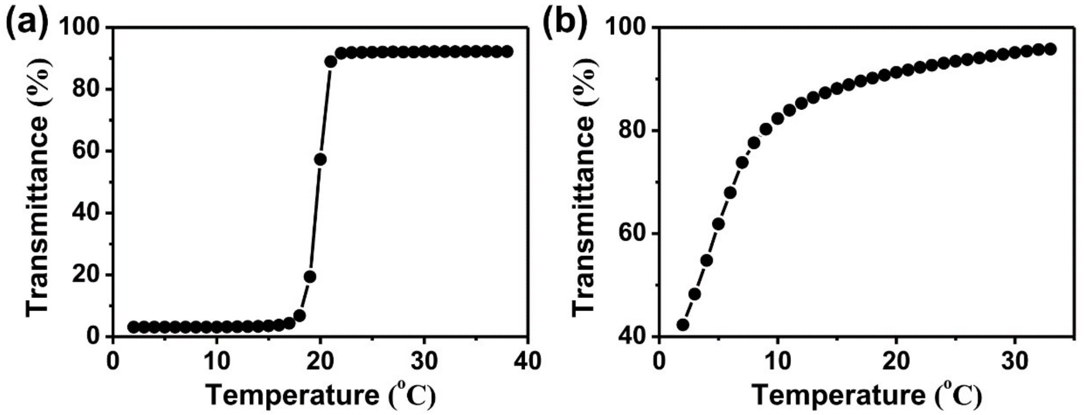

Figure S9. Thermal transitions of PNM31 (2.5 mg/mL) measured by turbidimetry at pH 1 (a) and pH 4 (b) during cooling, respectively.

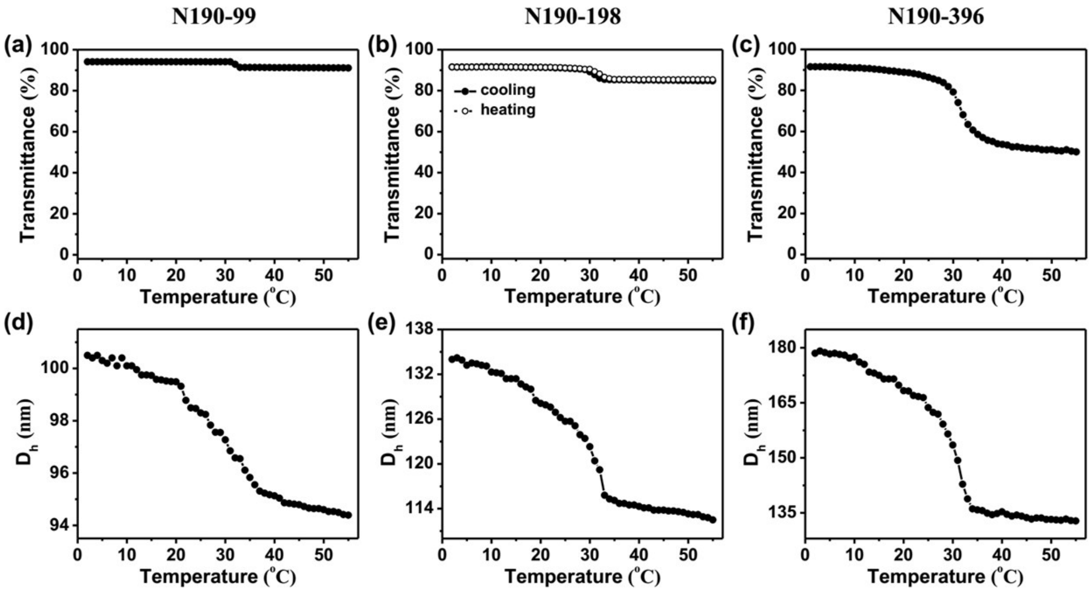

Figure S10. Turbidimetry and variable-temperature DLS measurements (cooling process) of N190-99 (a) and (d), N190-198 (b) and (e), and N190-396 (c) and (f) nanogels respectively in pH =7 buffer solutions at a concentration of 5 mg/mL.

1. Sun, W.; An, Z.; Wu, P., UCST or LCST? Composition -Dependent Thermoresponsive Behavior of Poly(N-acryloylglycinamide-co-diacetone acrylamide). *Macromolecules* **2017,** *50* (5), 2175-2182.

2. Xu, Y.; Li, Y.; Cao, X.; Chen, Q.; An, Z., Versatile RAFT dispersion polymerization in cononsolvents for the synthesis of thermoresponsive nanogels with controlled composition, functionality and architecture. *Polym. Chem.* **2014,** *5* (21), 6244-6255.