Supporting Information for

Adjustable dual temperature-sensitive hydrogel based on self-assembly cross-linking strategy with high stretchable and healable properties

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EXPERIMENTAL SECTION

Materials.

Acrylylchloride (98%, Tokyo Kasei Kogyo Company, Japan), glycinamide hydrochloride (98%, Energy chemical, China), cystamine dihydrochloride (98%, Energy chemical, China), potassium persulfate (KPS, Energy chemical, China), N,N,N',N'-tetramethylethylenediamine (TMEDA, Energy chemical, China). Methanol (Sinopharm Chemical Reagent limited corporation, china), ethyl alcohol (Sinopharm Chemical Reagent limited corporation, china), ether (Sinopharm Chemical Reagent limited corporation, china), sodium hydroxide (Sinopharm Chemical Reagent limited corporation, china), hydrochloric acid (Sinopharm Chemical Reagent limited corporation, china) were used without further purification. All other chemicals and solvent are analytical reagents. Corresponding molds are customized at Guanghui Plexiglass Factory.

Characterization.

Nuclear magnetic resonance (NMR) and IR spectroscopy. All NMR spectra were obtained using either a Bruker HW600 MHz spectrometer (AVANCE AV-600) and recorded in $CDCl_3$ (internal reference 7.26 ppm) or DMSO (internal reference 2.50 ppm). The German Bruker Fourier Infrared Spectrometer (ALPHA) was used for IR spectroscopy.

UV/Vis spectroscopy. The hydrogel sample was directly synthesized in the UV-vis cell prior to the test. The transmittance curves of the PNIPAm/PNAGA double network hydrogels were built by turbidity measurement to collect the transmittance at

700 nm with a scanning rate of 0.1°C min^{-1} from 0 to 65 °C using a Shimadzu UV-2700 spectrophotometer equipped with a PTC-348WI temperature controller (±0.1°C).

Differential Scanning Calorimetry (DSC). The phase transition of hydrogels was investigated by differential scanning calorimetry using a DSC Q200 from TA instrument. Hydrogels at preparation state (ca. 80 mg), equilibrated with a reference filled with the same quantity of pure water, were submitted to temperature cycles between 0 and 110°C under nitrogen atmosphere. The heating and cooling rates were fixed at 2°C min^{-1}.

Dynamic mechanical analysis (DMA). The storage moduli and loss moduli of the hydrogel samples (cylinders, 10 mm in diameter and 4 mm in height) were measured on a NETZSCH Dynamic Mechanical Analyzer with a compression mode at 1 Hz in a temperature range of 0^{o} C-75^{o} C with heating rate of 2^{o} C min^{-1}, and the load was set at 0.6 N. The measurement was performed in a nitrogen atmosphere.

Determination of equilibrium water contents (EWCs). The EWCs of the hydrogels were measured at different temperature using a gravimetric method. The hydrogel samples were fully swollen in the deionized water. Then they were taken out, gently wiped with filter paper, and immediately weighed on a microbalance. Afterwards, the hydrogels were dried in a vacuum oven at 60 ºC until a constant weight was obtained. The EWC is defined as the following Equation 1.

$$EWC = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (1)$$

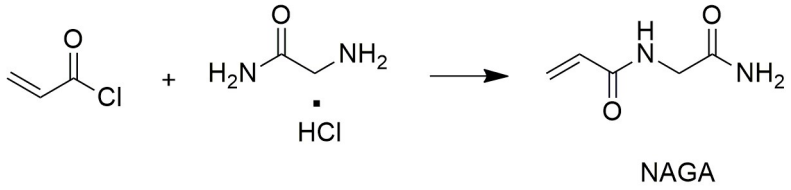
Where m_{wet} is the wet weight at different temperatures and m_{dry} is the dry weight of each sample. The average values and errors were calculated from at least four independent data for each specimen.

Scanning electron microscope (SEM) analysis. The morphology of the hydrogels at different temperature was observed by a FEI inspect F50 scanning electron microscope at accelerated electron energy of 5.0 kV. The hydrogel specimens at different temperature were flash frozen in liquid nitrogen for 5 min and immediately lyophilized for 48 hours to remove water. The cross-sections of the specimens were coated with gold by a sputter coater for 20 seconds before measurement.

Measurement of mechanical properties. All mechanical properties of the hydrogels were tested on MTS Exceed E42 electronic universal testing machine equipped with pneumatic clamps (DQB203B) at room temperature. In this study, all the samples were fully equilibrated in deionized water before test. For tensile test, hydrogels with a thickness of 0.5 mm were cut into rectangle (40 mm in length and 10 mm in width). The rate of extension was fixed at 50 mm min^{-1} for tensile test and loading-unloading test. The cylinder-shaped hydrogels (10 mm in diameter and 10 mm in height) was used for compression test and the crosshead speed was set at 10 mm min^{-1}.

The temperature of the film and the environment. A thermal imager (SMART SENSOR ST9450) was used to record the temperature of the surfaces of hydrogels and environment.

Synthesis of Monomer NAGA.



NAGA

N-(2-amino-2-oxoethyl) acrylamide (NAGA). The synthetic procedures is similar to that of the reference.[1-3] Glycinamide hydrochloride (3.15 g, 28.5 mmol), potassium carbonate (5.9 g, 42.75) 25 mL cold deionized water, and 10 mL cold diethyl ether were added into a 100 mL reaction flask, which was placed in an ice bath. Subsequently, a solution of acryloyl chloride（3.1 g, 34.2 mmol）in 15 mL diethyl ether was added dropwise under stirring at 0 ºC for about one hour. Then the mixture was further stirred for 5 hours at room temperature. After that, 6 mol L^{-1} HCl was added into the solution to adjust pH to 2. Next, the mixture was washed three times with 100 mL of diethyl ether to remove the organic phase and the remaining diethyl ether was evaporated under vacuum. Again, the pH of the solution was adjusted to neutral with the 2 mol L^{-1} NaOH, and the mixture was freezedried. The raw product was washed three times with 100 mL of ethanol/methanol mixture (4/1, V/V). Then the ethanol and methanol were removed by rotary evaporation and the mixture left was recrystallized at 0 ºC to obtain the resultant NAGA which was dried in vacuo as a white solid (2.26 g, Yield: 62%). IR spectrum of NAGA in Figure S1a displays its feature bands: ν = 3389 cm-1 (m, NH), 3314 cm-1 (s, NH), 3191 cm-1 (m, NH), 1662 cm-1 (vs, C=O), 1626 cm-1 (vs, C=O), 1556 cm-1 (vs, NH).

^{1}H NMR (600 MHz, DMSO) δ 8.30 (s, 1H), 7.38 (s, 1H), 7.06 (s, 1H), 6.31 (m, J = 17.1, 10.2 Hz, 1H), 6.09 (m, J = 17.1, 2.1 Hz, 1H), 5.60 (m, J = 10.2, 2.1 Hz, 1H), 3.72 (d, J = 5.8 Hz, 2H).

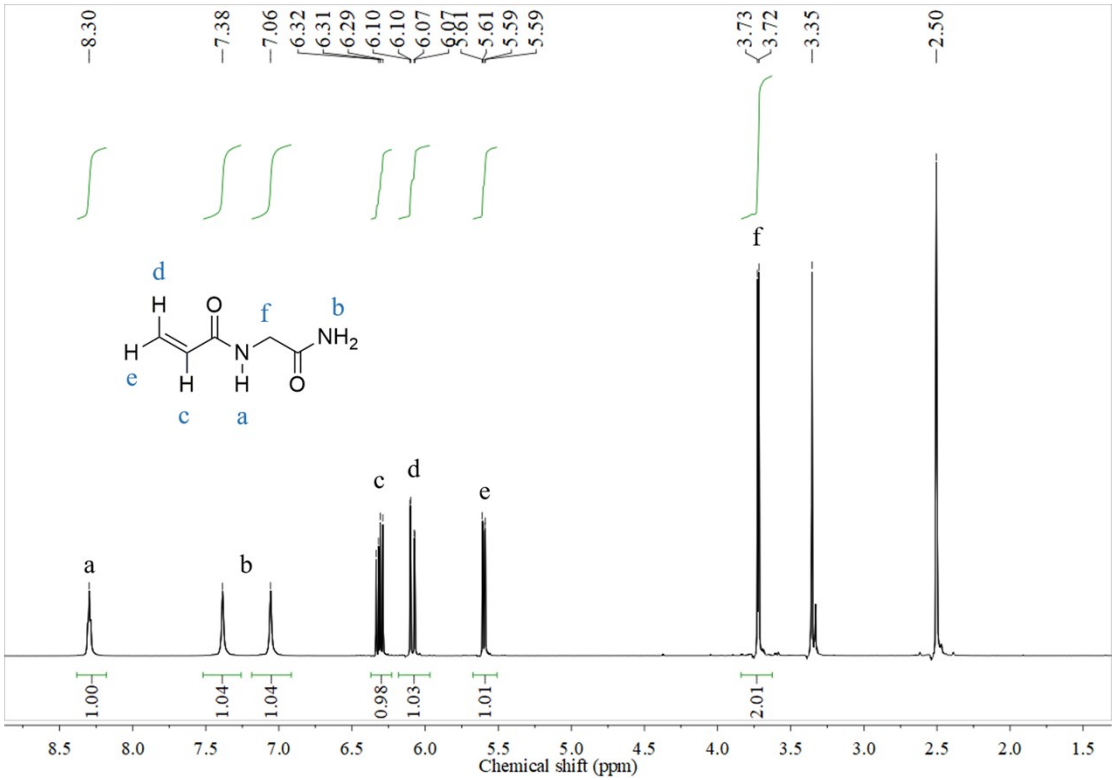
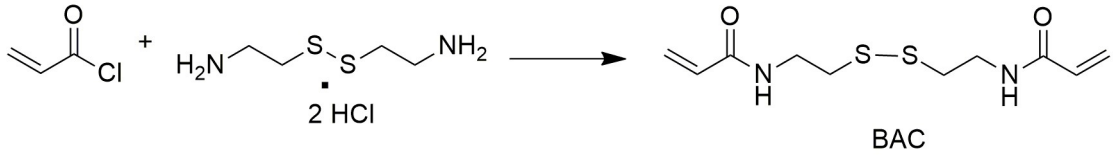


Figure. S1. ^{1}H NMR spectrum of **NAGA**.

Synthesis of crosslinker BAC.



N,N'-bis(acryloyl)cystamine (BAC). The synthetic procedures is similar to that of the reference.[4-6] Cystamine dihydrochloride (0.588g, 2.61mmol) was added into distilled water (10 mL) in a flask in an ice bath. Then, acryloyl chloride (0.498g, 5.5mmol) in 5ml dichloromethane and aqueous NaOH solution (0.22g, 11mmol，1.1ml H_2O) were added dropwise to the flask. Subsequently, the mixture was stirred at room temperature for 6 h. BAC was extracted from dichloromethane and washed

three times with distilled water. The product was obtained after rotary evaporation with a yield of 82%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.71 (s, 2H), 6.33 (d, J = 17.0 Hz, 2H), 6.22 (m, J = 17.0, 10.2 Hz, 2H), 5.68 (m, J = 10.2, 1.5 Hz, 2H), 3.67 (q, J = 6.3 Hz, 4H), 2.88 (t, J = 6.5 Hz, 4H).

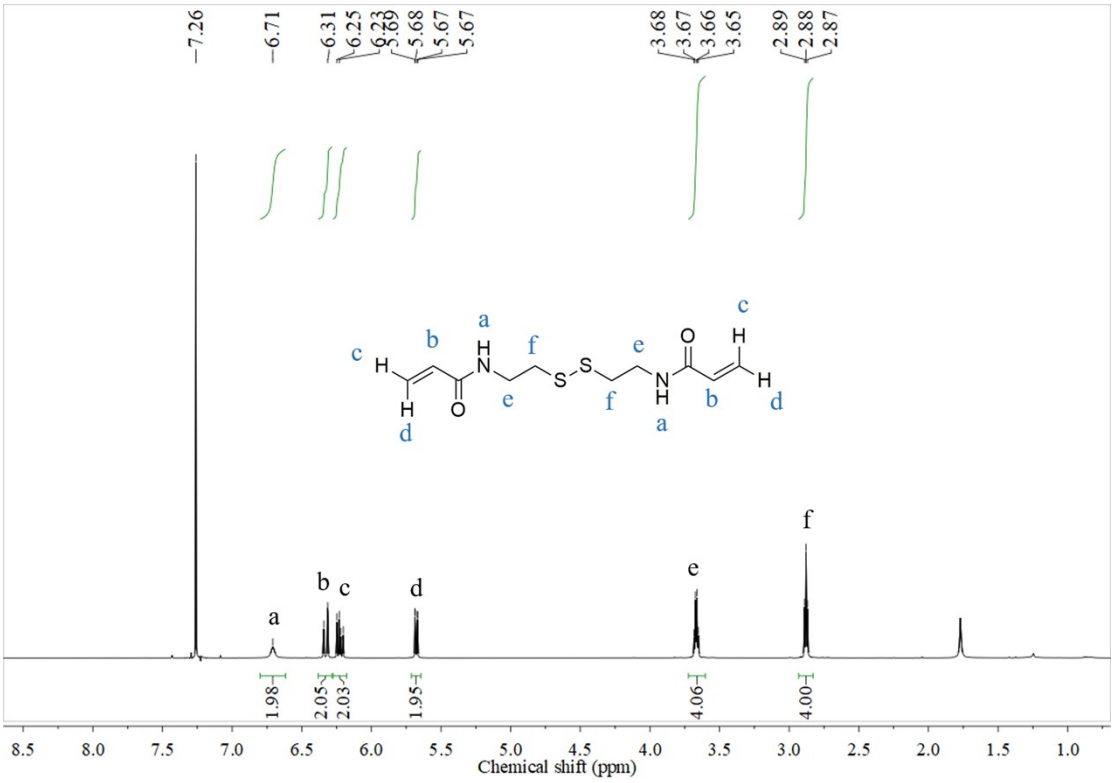


Figure. S2. ^{1}H NMR spectrum of **BAC**.

Preparation of the PNIPAm/PNAGA hydrogel

NIPAm (0.8g, 7.07mmol), BAC (0.6mg, 0.0023mmol) was dissolved in H_2O (10 ml), which was sonicated for 30 minutes to make it completely dissolved. The mixture was deoxygenated under N_2 atmosphere for 2h, then 10mg APS and 10uL TMEDA was added, and the reaction was closed for 24h. The prepared PNIPAm hydrogel was purified by dialysis against pure water (membrane cut-off=3.5 kDa) for one week and the water was changed every 12h to remove unreacted NIPAm monomer, which was freeze-dried to obtain PNIPAm solid (0.52, Yield: 65%).

PNIPAm (11mg), NAGA (239mg, 1.98mmol) and BAC (0.6mg, 0.0023mmol) were self-assembled in H_2O (750uL) and the 5mg KPS and 5uL TMEDA were added to polymerize under thermal initiation to obtain PNIPAm/PNAGA-4 double network hydrogel.

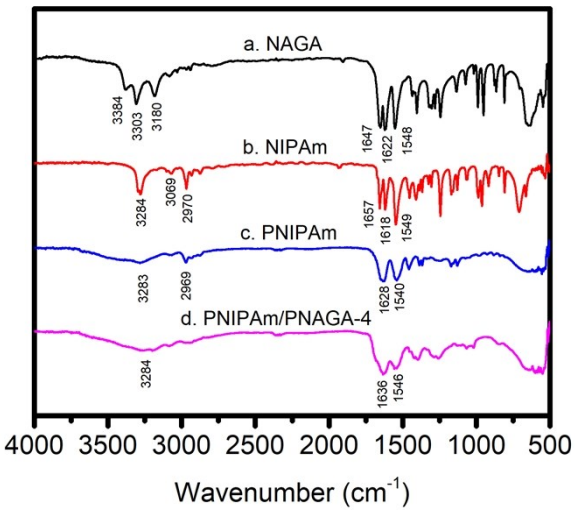


Figure. S3. IR spectra of (a) NAGA (b) NIPAm (c) PNIPAm and (d) PNIPAm/PNAGA-4.

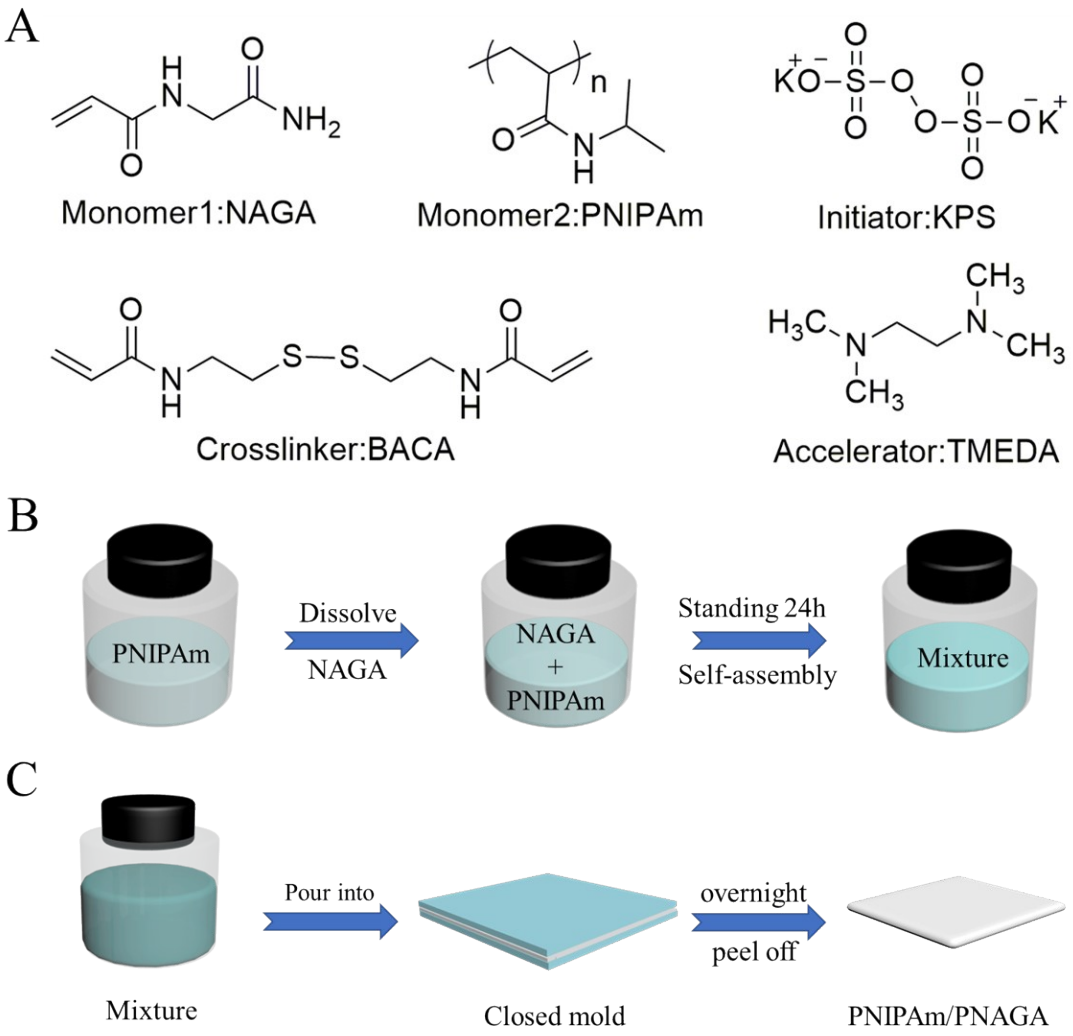


Figure. S4. Preparation process of PNIPAm/PNAGA double network hydrogel. A) The chemical compositions of PNIPAm/PNAGA hydrogel. Self-assembly B) and polymerization B) process for preparing hydrogels.

Repeat the above experimental steps. Keeping the NAGA content unchanged in the experiment, changing the pNIPAm content, a series of double-network hydrogels were obtained from PNIPAm/PNAGA-0 to PNIPAm/PNAGA-9, where values such as 0 represent the quality of PNIPAm. This series of hydrogels is used to test the capability of temperature response.

Repeat the above experimental steps. Keeping the PNIPAm content unchanged in the experiment, changing the NAGA content, a series of double-network hydrogels

were obtained PNIPAm/PNAGA-4, PNIPAm/PNAGA-10, PNIPAm/PNAGA-11, PNIPAm/PNAGA-12 and PNIPAm/PNAGA-13,which was used to test the mechanical properties[36].

Table S1. Preparation of PNIPAm/PNAGA hydrogels with different monomer ratios

Sample	NAGA (mg)	PNIPAm (mg)	BAC（mg）	H_2O (μL)	Percentage of PNIPAm (%)
PNIPAm/PNAGA-0	239	0	0.6	750	0
PNIPAm/PNAGA-1	239	2	0.6	750	0.2
PNIPAm/PNAGA-2	239	5	0.6	750	0.5
PNIPAm/PNAGA-3	239	8	0.6	750	0.8
PNIPAm/PNAGA-4	239	11	0.6	750	1.1
PNIPAm/PNAGA-5	239	15	0.6	750	1.5
PNIPAm/PNAGA-6	239	25	0.6	750	2.5
PNIPAm/PNAGA-7	239	35	0.6	750	3.4
PNIPAm/PNAGA-8	239	45	0.6	750	4.3
PNIPAm/PNAGA-9	239	55	0.6	750	5.3
PNIPAm/PNAGA-10	119	11	0.6	750	1.25
PNIPAm/PNAGA-11	155	11	0.6	750	1.2
PNIPAm/PNAGA-12	195	11	0.6	750	1.15
PNIPAm/PNAGA-13	286	11	0.6	750	1.05

Transmittance of hydrogels from PNIPAm/PNAGA-5 to PNIPAm/PNAGA-9 at different temperatures and 700nm wavelength.

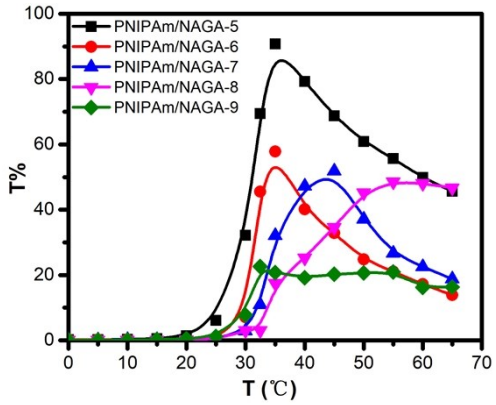


Figure. S5. Transmittance curves of double network hydrogels with different monomer ratios at different temperatures and 700nm wavelength from PNIPAm/PNAGA-5 to PNIPAm/PNAGA-9.

In order to further confirm the influence of the monomer ratio on the temperature response of the hydrogel, we further increased the PNIPAm content and obtained the above-mentioned light transmittance curves of hydrogels with different monomer ratios at different temperatures and 700nm wavelength from PNIPAm/PNAGA-5 to PNIPAm/PNAGA-9.

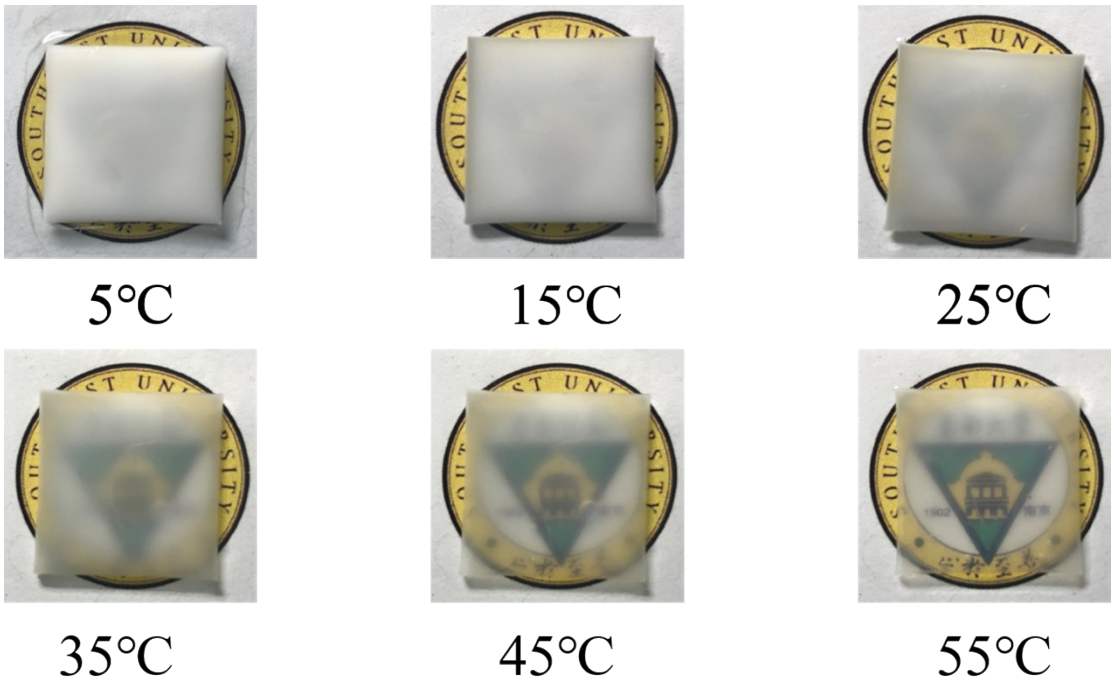


Figure. S6. Photograph of the thermal response of PNIPAm/PNAGA-8 at different temperatures

When the content of PNIPAm increased to 45mg, it was found that the light transmittance showed a UCST behavior on the macroscopic scale, and the LCST behavior disappeared. This is mainly due to the competition between various hydrogen bonds in the hydrogel and the hydrogen bonds between PNAGA and PNIPAm. The photograph (**Figure. S6**) above is the thermal response of PNIPAm/PNAGA-8 at different temperatures.

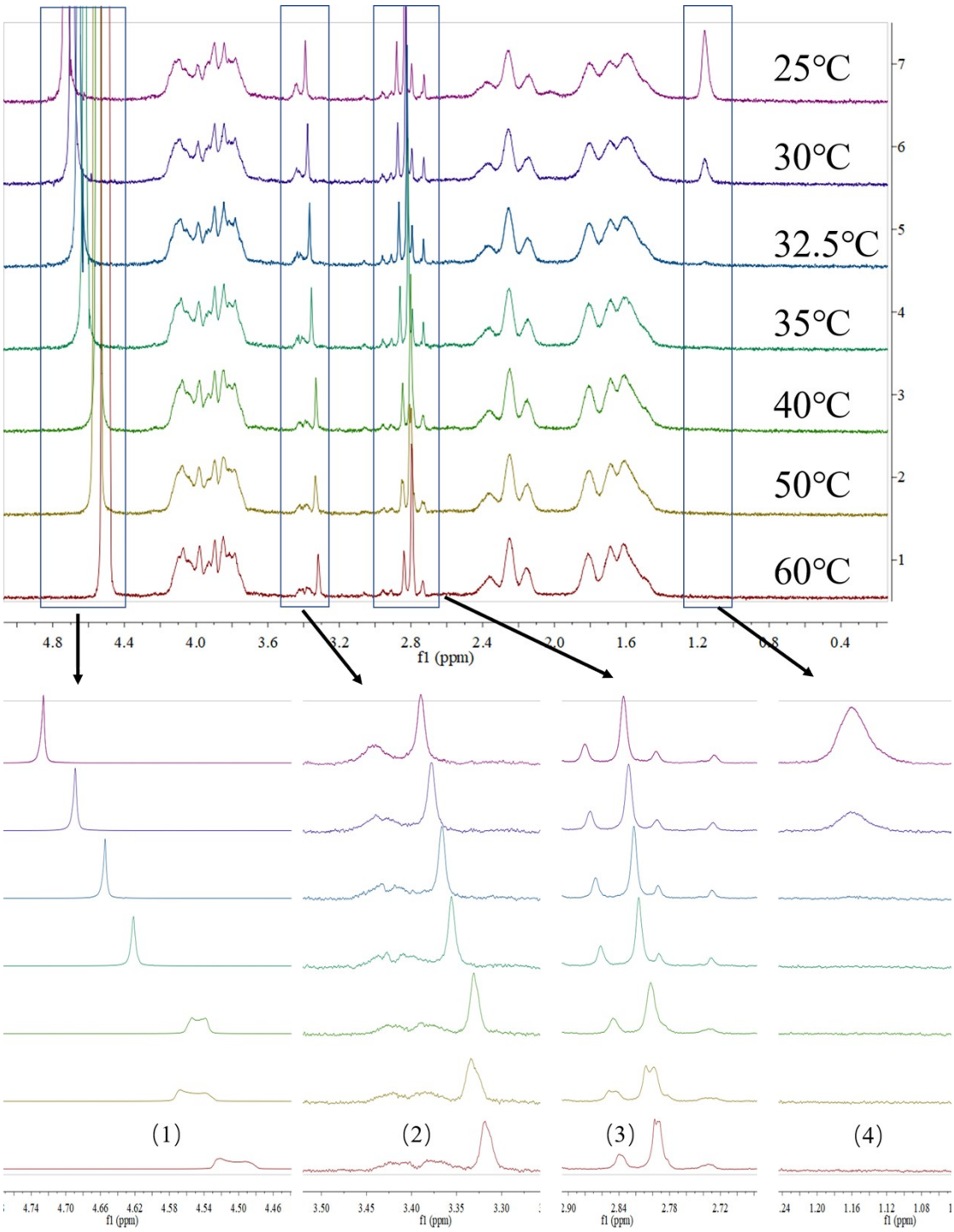


Figure. S7. Temperature variation of 1H NMR spectra of PNIPAm/PNAGA-4 hydrogel in D_2O and zooms corresponding to the NMR signals of D_2O(1), the methine signal of PNIPAm (2), the characteristic peaks of PNAGA (2, 3) and the methyl group of PNIPAm (4), respectively.

At first, we tried to study the structural changes of PNIPAm/PNAGA-4 gel at different temperatures by using temperature variation of 1H NMR, but the freeze-dried PNIPAm/PNAGA-4 cannot be dissolved in D_2O, so it is impossible to study the

structural changes in the original state. Therefore, we reduced the degree of polymerization of PNIPAm/PNAGA-4 to obtain the oligomer of PNIPAm/PNAGA-4, which can be dissolved in D_2O and carried out temperature variation of 1H NMR test.

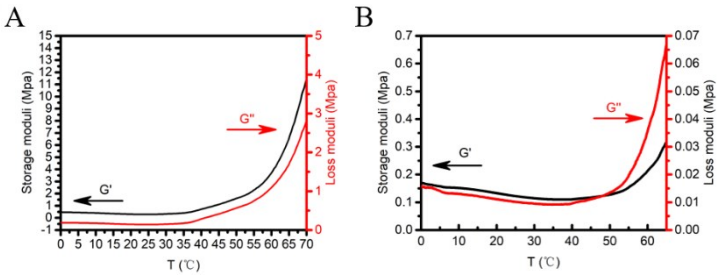


Figure. S8. Variation in storage moduli G' and loss moduli G" of PNIPAm/PNAGA-3 (A), PNIPAm/PNAGA-5 (B) as a function f temperature.

With the increase of PNIPAm content, G' and G" gradually decrease from PNIPAm/PNAGA-3 to PNIPAm/PNAGA-5. G' is always greater than G" throughout the process, indicating that the PNIPAm/PNAGA-3 and PNIPAm/PNAGA-5 hydrogel has always been in a gel state.

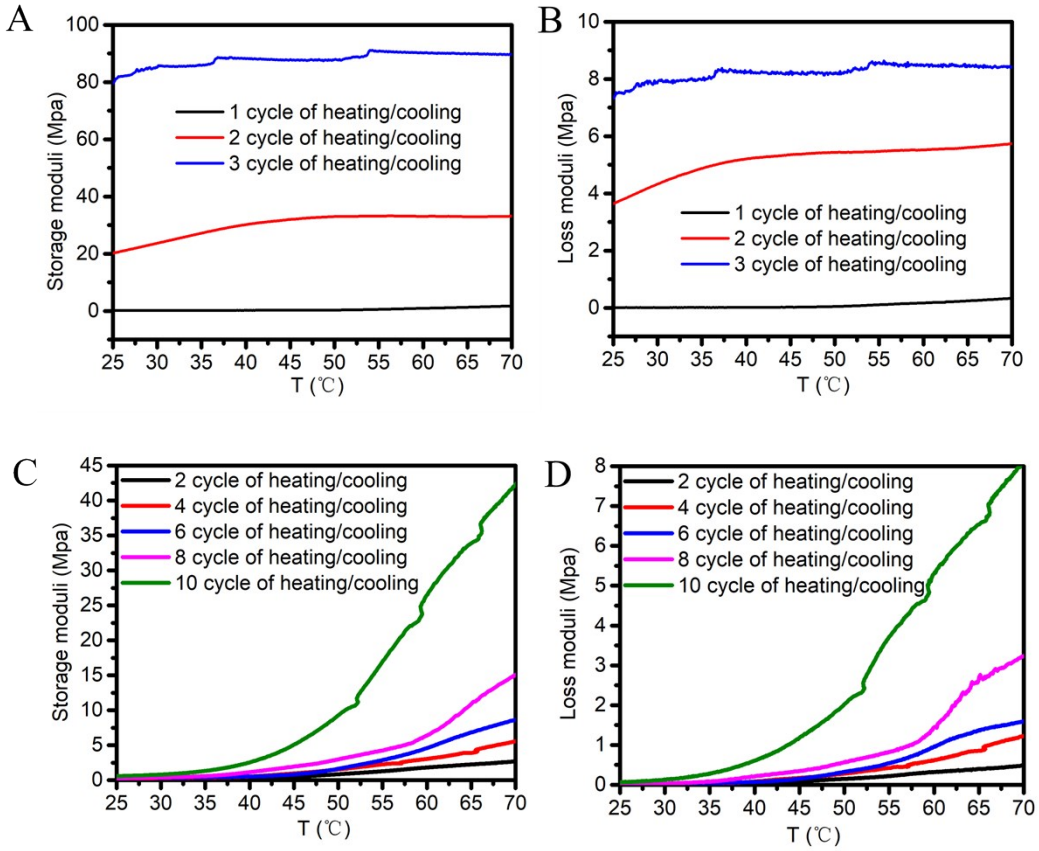


Figure. S9. Variation in storage moduli G' (A) and loss moduli G" (B) of the PNIPAm/PNAGA-4 hydrogel as a function of temperature under continuous heating and cooling cycle conditions; Variation in storage moduli G" (C) and loss moduli G" (D) of the PNIPAm/PNAGA-4 hydrogel after heating and cooling cycles in closed environment as a function of temperature.

We tried to test the DMA of the hydrogel under the conditions of continuous heating and cooling cycles. However, the hydrogel would break after the number of cycles exceeded 3 times, which caused by the continuous loss of water from the hydrogel during the heating process. In order to reduce the water loss during the heating process, we put the gel in a closed environment for heating and cooling cycles. The PNIPAm/PNAGA-4 hydrogel was first heated to 70℃, then cooled to 5℃ in closed environment. Repeat the above cooling and heating process several times and perform DMA test. Figure. S9 A and B show variation in storage moduli G' and variation loss moduli G" of PNIPAm/PNAGA-4 as a function of temperature under continuous heating and cooling cycle conditions. Figure. S9 C and D show variation in storage moduli G' and variation loss moduli G" of PNIPAm/PNAGA-4 after heating and cooling cycles in closed environment as a function of temperature.

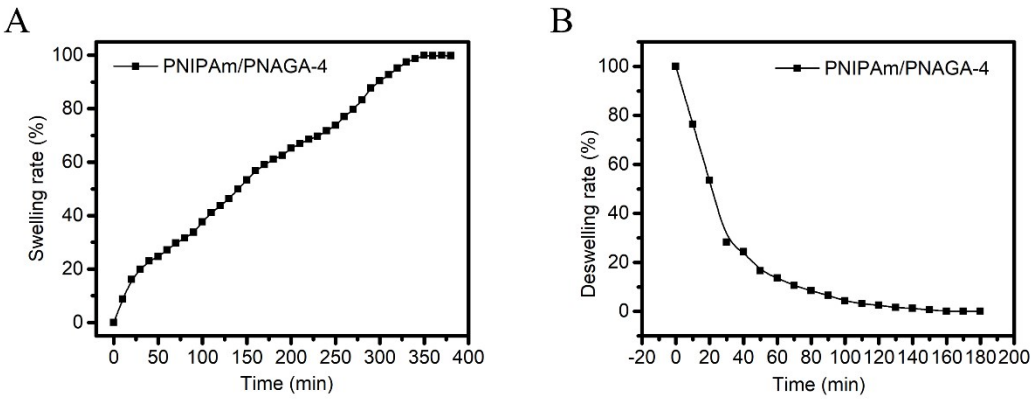


Figure. S10. The swelling (A) and deswelling (B) kinetics of PNIPAm/PNAGA-4 at room temperature.

The swelling and deswelling kinetics of PNIPAm/PNAGA-4 showed that the PNIPAm/PNAGA-4 hydrogel reached equilibrium of swelling and deswelling after 350 min and 180 min at room temperature.

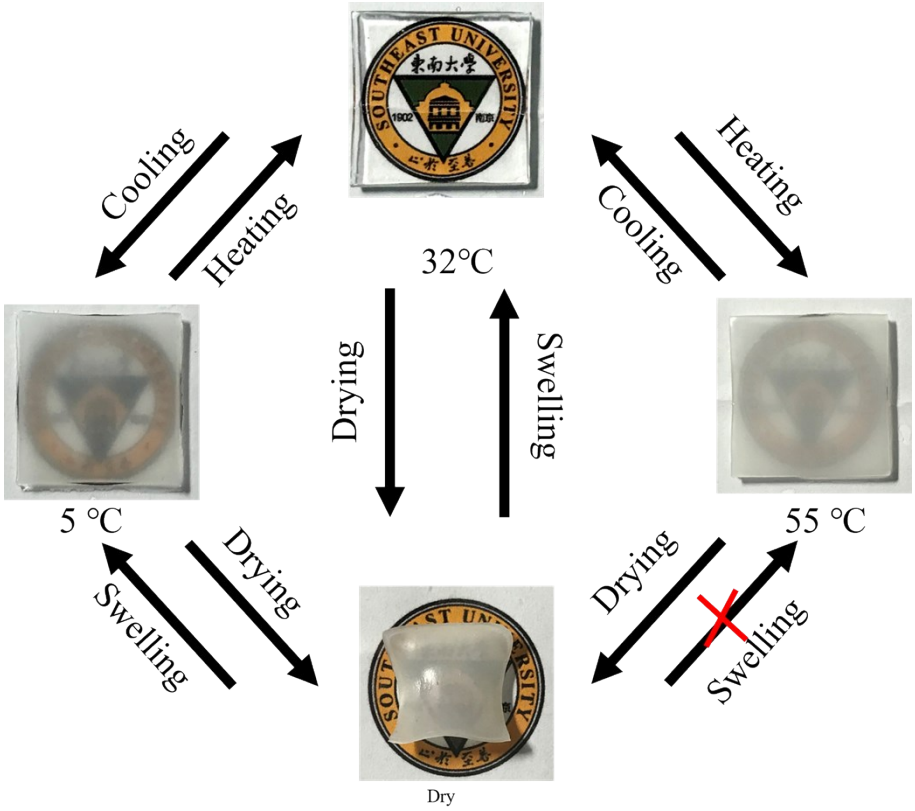


Figure. S11. Correlation photograph of temperature response of the dried hydrogel after swelling at different temperatures.

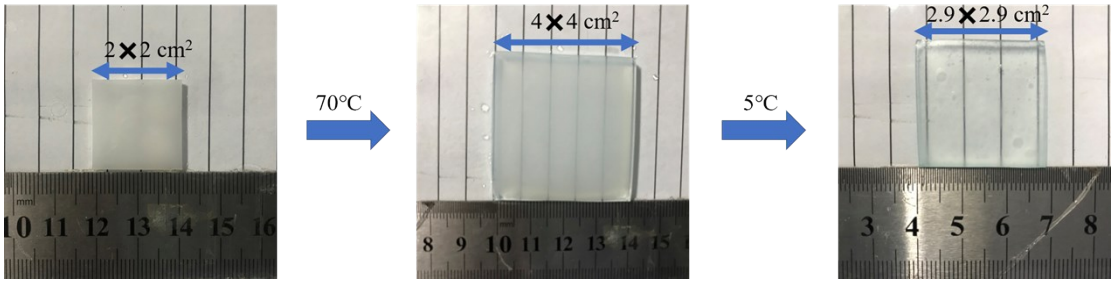


Figure. S12. Photograph of PNIPAm/PNAGA-4 hydrogel swollen at 70 ºC for 24 hours and recovered at 5 ºC for 24 hours

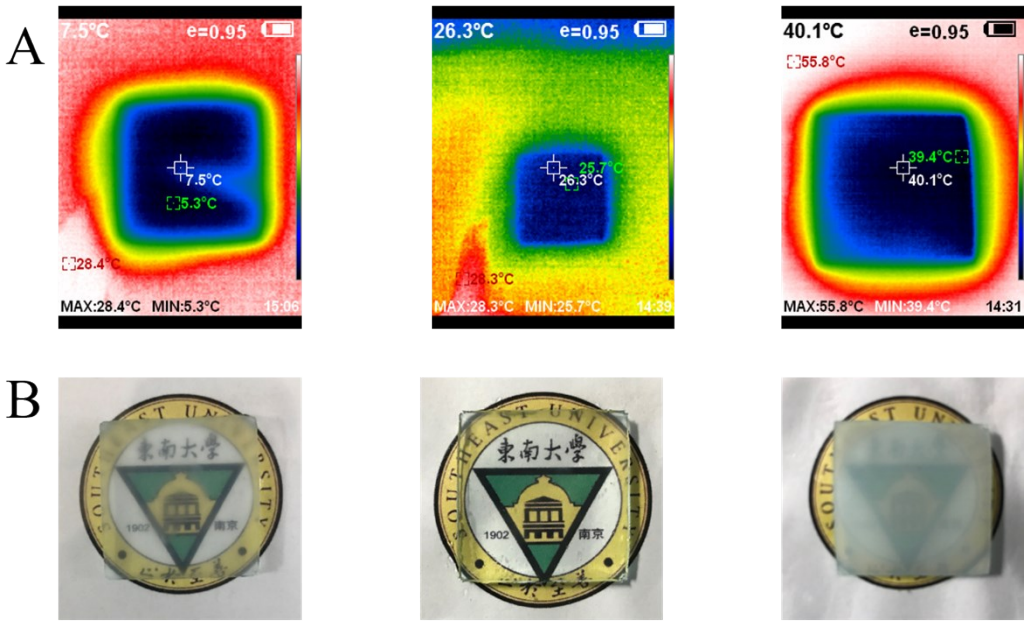


Figure. S13. Photograph of the temperature response of the PNIPAm/PNAGA-4 hydrogel after swelling A) and the corresponding thermographic photograph B）.

In order to further verify the mechanism of temperature response for PNIPAm/PNAGA, we studied the macro-turbidity change of the dried hydrogel after swelling at different temperatures (Figure S11). When the dry PNIPAm/PNAGA-4 was reconstituted at 5℃and 35℃, it can still maintain good reversible UCST and LCST thermoresponsive behavior. However, it was reconstituted at a high temperature at 55℃water, the dual temperature response performance was sharp reduced. In order to clearly observe the macro temperature-sensitive change of the hydrogel after high-temperature reconstitution, the dried hydrogel was put into 70°C

water for 24 h and recovered at 5 ºC for 24 hours (Figure S12). Figure S13 shows photograph of the temperature response of the hydrogel after swelling and the corresponding thermographic photograph. Compared with the original state of PNIPAm/PNAGA-4 hydrogel film, the gel is more transparent at 5°C and the best light transmittance is achieved at 25°C, but the gel still remains opaque at high temperatures. These results confirm that the high temperature swelling greatly breaks the hydrogen bond between PNAGA and PNIPAm and it is difficult to restore the original state of hydrogen bond between the two components before Swelling, which further verify the various dynamic hydrogen bonds mechanism of temperature response for PNIPAm/PNAGA.

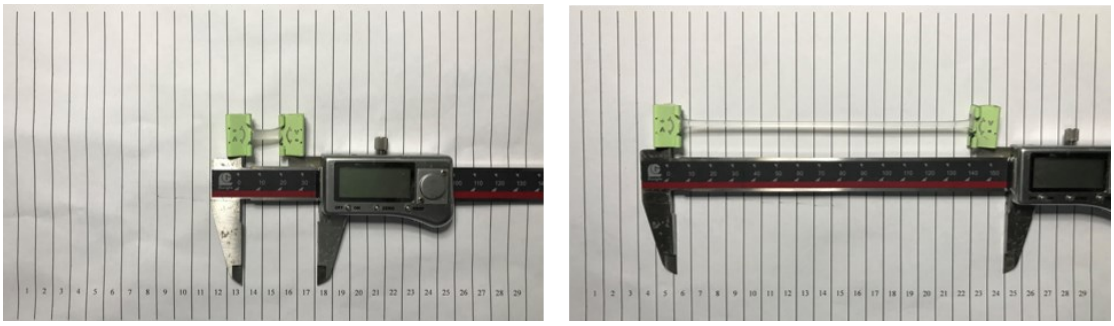


Figure. S14. Photograph of PNIPAm/PNAGA-4 hydrogels showing their ability to withstand stretching.

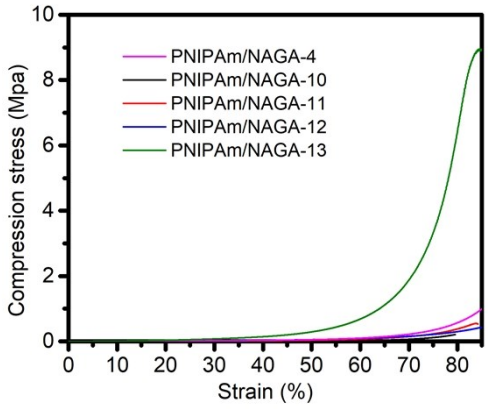


Figure. S15. Compressive stress–strain curves of a series of PNIPAm/PNAGA double-network hydrogels at 85% strain.

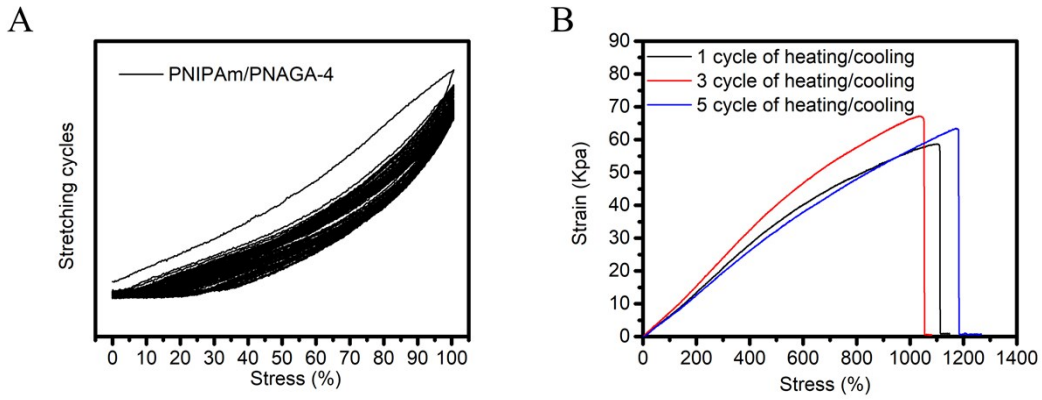


Figure. S16. (A) 50 times stretching-releasing cycles of PNIPAm/PNAGA-4 hydrogel at 100% strain at room temperature and (B) a series of tensile stress-strain curves of PNIPAm/PNAGA-4 hydrogels after heating and cooling cycles in closed environment.

It is difficult for us to carry out the cyclic tensile tests at different temperature because our tensile test instrument has no temperature control device. Therefore, we carry out the following tensile tests: the PNIPAm/PNAGA-4 hydrogel was first heated to 70℃, then cooled to 5℃ in closed environment. Repeat the above cooling and heating process several times and perform tensile fracture test (Figure. S16 B).

Figure. S17. Photograph of PNIPAm/PNAGA-4 healable hydrogel showing the ability to withstand stretching.

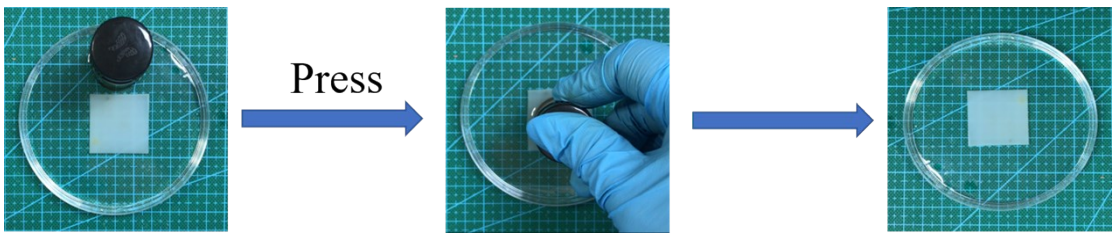


Figure. S18. Hydrogel constant temperature pressing experiment.

In order to eliminate the influence of finger pressure on the gel structure, we conducted a constant temperature finger pressing experiment under constant temperature water bath conditions (Fig. S18 (ESI)). The hydrogel constant temperature pressing test is performed in water to ensure a constant temperature. It was found that under constant temperature, finger pressing had no obvious effect on the gel.

Table S2. Tensile properties of PNIPAm/PNAGA hydrogels prepared with different monomer ratios

Sample	Tensile strength (KPa)	Elongation at break (%)	Young's modulus (KPa)
PNIPAm/PNAGA-4	51.48±0.61	1417±11	5.51±0.16
PNIPAm/PNAGA-10	7.07±0.07	477±2	1.56±0.03
PNIPAm/PNAGA-11	15.73±0.13	790±5	2.29±0.05
PNIPAm/PNAGA-12	27.79±0.36	1036±7	2.93±0.06
PNIPAm/PNAGA-13	47.82±0.48	1140±8	4.53±0.13

References:

1. M. Boustta, P. E. Colombo, S. Lenglet, S. Poujol, M. Vert, *J. Control. Release.,* 2014, **1**.
2. H. Guo, C. Mussault, A. Marcellan, D. Hourdet, N. Sanson, *Macromol. Rapid. Commun.,* 2017, **38**, 17.
3. X. Dai, Y. Zhang, L. Gao, T. Bai, W. Wang, Y. Cui, W. Liu, *Adv. Mater.,* 2015, **27**, 23.
4. H. Zhao, J. Duan, Y. Xiao, G. Tang, C. Wu, Y. Zhang, W. Xue, *Chem. Mater.,* 2018, **30**, 10.
5. W. Yang, C. Pan, X. Liu, J. Wang, *Biomacromolecules,* 2011, **12**, 5.
6. K. Wang, H. Peng, K. J. Thurecht, S. Puttick, A. K. Whittaker, *Polym. Chem.,* 2014, **5**, 5.