Supporting Information

One-step hydrothermal route to programmable stimuli-responsive hydrogels

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Supporting Information I: Materials and methods

Materials:

N-Isopropylacrylamide (NIPAM), the crosslinker *N*,*N*′-methylenebisacrylamide (MBA), the initiator ammonium persulfate (APS) and the accelerator tetramethylethylenediamine (TEMED) tetramethylrhodamine isothiocyanate-dextran (red dextran, average molecular weight = 65,000-85,000 Da), 1 M HCl and 1 M NaOH were obtained from Sigma-Aldrich (Sigma-Aldrich. Co. Ltd., Singapore) and used without further purification.

Methods:

Scanning electron microscopy (SEM) observation:

SEM (JEOLJSM-6430 F, JEOL, Japan) was used to investigate the interior morphology of un-treated PNIPAM hydrogel (HT 0 h) and hydrothermally treated (5 h, 15 h and 25 h) PNIPAM hydrogels. The fully swollen PNIPAM hydrogels were transferred into liquid nitrogen for 20 min and freeze-dried at -80°C with at a vacuum pressure of 0.28 Pa for 48 h to remove the water. The dried PNIPAM hydrogel samples were carefully adhered to carbon double-side tape, coated with Pt and observed under an accelerating voltage of 5 keV.

Fourier transform infrared (FTIR) spectroscopy:

FTIR spectrophotometer (IRPrestige-21, Shimadzu, Japan) was used to determine the changes in the chemical structures of the PNIPAM hydrogels after hydrothermal treatment. The studies were performed with a total of 32 scans at a resolution of 4 cm^{-1} for each sample. The thin film PNIPAM hydrogel samples were first freeze-dried at

-80°C at a vacuum pressure of 0.28 Pa for 48 h to remove the water. The dried PNIPAM hydrogel thin films were then cut into small pieces and directly subjected to FTIR observation for analysis. For the powder samples, including the NIPAM monomers, APS and MBA, the samples were first mixed with KBr and ground into fine powders. These mixed fine powders were then compressed into transparent thin films with a compressor, which were then subjected to FTIR observation for analysis.

pH responsiveness of the hydrothermally treated PNIPAM hydrogels:

PNIPAM hydrogels, which were hydrothermally treated for different durations (0 h, 5 h, 15 h and 25 h), were cut into rectangles and placed into chamber slides filled with 1 ml of H_2O. The chamber slides with the PNIPAM hydrogels were then observed using bright field microscopy to record the original length (D_o). Next, 1 M HCl solutions were continuously added to the chamber slides until no further shrinkage of the PNIPAM hydrogels samples was observed. The lengths were then recorded as D. Following the addition of the HCl solution, 1 M NaOH was added to adjust the pH back to 7.5. The length of the hydrogel after adding NaOH was recorded again. This process was repeated three times, with 3 samples tested each time to obtain the average values and standard deviations.

Determination of the swelling ratios of the PNIPAM hydrogel samples:

Dried PNIPAM hydrogels that were hydrothermally treated for 0 h, 5 h, 15 h and 25 h were weighed and soaked in DI H_2O at room temperature. The soaked hydrogels were then removed at predetermined time intervals. The surface water on the hydrogel was carefully removed using filter paper, and the hydrogels were weighed until no

further weight changes were observed. For each batch of samples, three samples were used to obtain the mean values and standard deviations. The swelling ratios of the PNIPAM hydrogels were determined using the following equation[1]:

$$Swelling\ ratio\ (\%) = \frac{Ws - Wo}{Wo}\%$$

where W_s is the weight of swollen PNIPAM hydrogel and W_o is the weight of the original dried PNIPAM hydrogel.

Reference 1: Kim, S. J.; Park, S. J; Kim, S. I. *Reactive & Functional Polymer*, **2003**, *55*, 53-59.

Differential scanning calorimetry (DSC):

DSC was performed using a Mettler Toledo analyzer (DSC 1 STAR^e System, Singapore) equipped with a liquid nitrogen cooling system, which was calibrated with an indium standard. Each hydrogel sample, which weighed 2~3 mg, was encapsulated in an aluminum pan and heated from 10 to 60°C at a heating rate of 2°C/min under a nitrogen atmosphere. All hydrogel samples were equilibrated in DI H_2O at 4°C for 24 h prior to the DSC experiments.

Rheological measurements of PNIPAM hydrogels:

Amplitude-sweep test of the PNIPAM hydrogel was performed with a controlled-stress rheometer (Anton Paar, PP25/TG-SN6539, Singapore) to investigate its storage (G′) and loss (G″) module. An oscillating deformation was applied, and G′/G″ was measured as a function of the applied strain between 0.1% and 500% at a constant frequency (f = 1 Hz) and temperature (T = 25°C). The PNIPAM hydrogel samples were cut into flat disks and measured in a plate-plate rheometer geometry with an upper plate diameter of 25 mm. It is important to ensure that adequate contact

between the hydrogel samples and the plates is maintained without significantly compressing the hydrogel.

Stress-strain measurements of crosslinker-free PNIPAM hydrogel:

Tensile mechanical analysis was conducted on the crosslinker-free PNIPAM hydrogel films. The crosslinker-free PNIPAM hydrogel films were cut into rectangular specimens with a width of 4 mm and a thickness of approximately 200 μm. The stress-strain measurements of the hydrogel films were performed using an Instron universal testing machine (Instron Singapore Pte. Ltd., Singapore) at room temperature. The speed of the crosshead was 2 mm/min.

Supporting Information II: Synthesis of fully-crosslinked PNIPAM hydrogels and their hydrothermal treatment

The fully-crosslinked PNIPAM hydrogels were synthesized using conventional redox initiation polymerization methods. Briefly, 100 mg of NIPAM, 10 mg of MBA and 10 mg of APS were dissolved in 1 ml of DI H_2O. The solution was de-gassed under vacuum for 30 min and filled into a shaped mold maintained at 4°C. The polymerization was then initiated by adding 10 μl of a 0.1% TEMED aqueous solution. The polymerization was performed at 4°C for 3 h to form PNIPAM hydrogels. The PNIPAM hydrogels were then peeled out and washed with DI H_2O several times. Next, these PNIPAM hydrogels were placed in 8 L of DI H_2O and stirred at 4°C for 20 days to completely remove the un-reacted NIPAM, APS, MBA and TEMED; the water was regularly changed and replenished with new DI H_2O

during this process. The washed PNIPAM hydrogels were subsequently transferred to a Teflon-lined autoclave containing 20 ml of DI H_2O and heated at 180°C for different times. At predetermined time intervals, the autoclave was cooled to room temperature. The hydrothermally treated PNIPAM hydrogels were then washed with DI H_2O several times and incubated in DI H_2O at 4°C for 24 h before analysis.

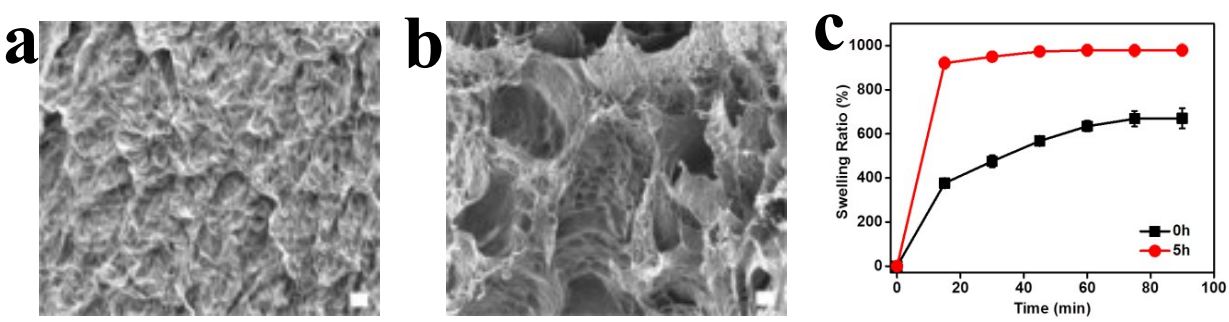


Figure S1. (a-b) SEM Images of the PNIPAM hydrogels in the dried state (a) PNIPAM hydrogel hydrothermally treated for 0 h (b) the PNIPAM hydrogels hydrothermally treated for 25 h; (c) Enlarged view of Figure 1g showing the swelling ratio of the 0 h and 5 h hydrothermally treated PNIPAM hydrogels.

Supporting Information III: Effects of hydrothermal treatments on fully-crosslinked PNIPAM hydrogel and poly acrylamide (PAM) hydrogel

Poly acrylamide (PAM) hydrogel, a non-responsive hydrogel, was also synthesized and subjected to hydrothermal treatments. PAM hydrogel was synthesized using similar procedures as that of synthesizing PNIPAM hydrogel. Briefly, 100 mg acrylamide, 8 mg MBA and 8 mg APS were dissolved in 1 ml DI H_2O. The solution were de-gassed under vacuum for 30 min and filled into a shaped mold maintaining at 4 ^oC. The polymerization was then initiated by adding 2 µl of 0.1% TEMED aqueous solution. Polymerization was carried out at 4 ^oC for 4 h to form PAM hydrogels. The PAM hydrogels were then peeled out and washed with DI H_2O for several times. After that, these PAM hydrogels were put into 8 L DI H_2O and kept stirring at 4 ^oC for 20 days to completely remove the un-reacted acrylamide, APS, MBA and TEMED, during which water was regularly changed and replenished with new DI H_2O. The washed PAM hydrogels were subsequently transferred to a Teflon-lined autoclave containing 20 ml DI H_2O and heated at 180 ^oC for 5h. Effects of hydrothermal treatment on PAM and PNIPAM hydrogels were compared.

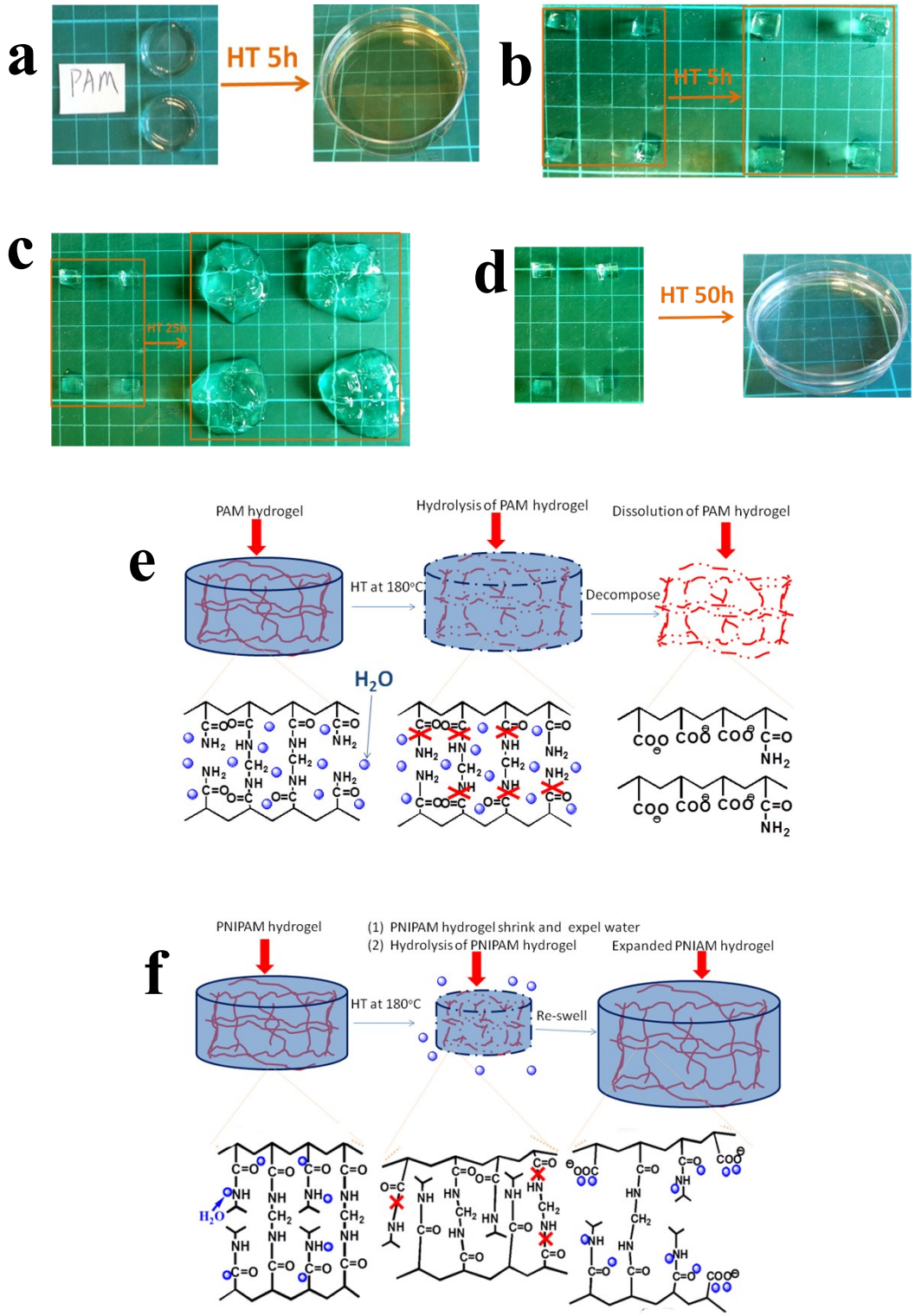


Figure S2. (a) Photographs showing the appearance of PAM hydrogel before and after 5 h hydrothermal treatment, in which PAM hydrogels were dissolved after 5 h hydrothermal reaction; (b) photographs showing the appearance of PNIPAM hydrogel before and after 5 h hydrothermal treatment, in which PNIPAM hydrogels slightly

expanded without dissolution; (c) photographs showing the appearance of PNIPAM hydrogel before and after 25 h hydrothermal treatment, in which PNIPAM hydrogels still maintain their shape without dissolution; (d) photographs showing the appearance of PNIPAM hydrogel before and after 50 h hydrothermal treatment, in which PNIPAM hydrogel dissolved after 50 h hydrothermal reaction; (e-f) schematic illustration showing the hydrothermal process of (e) PAM hydrogel and (f) PNIPAM hydrogels.

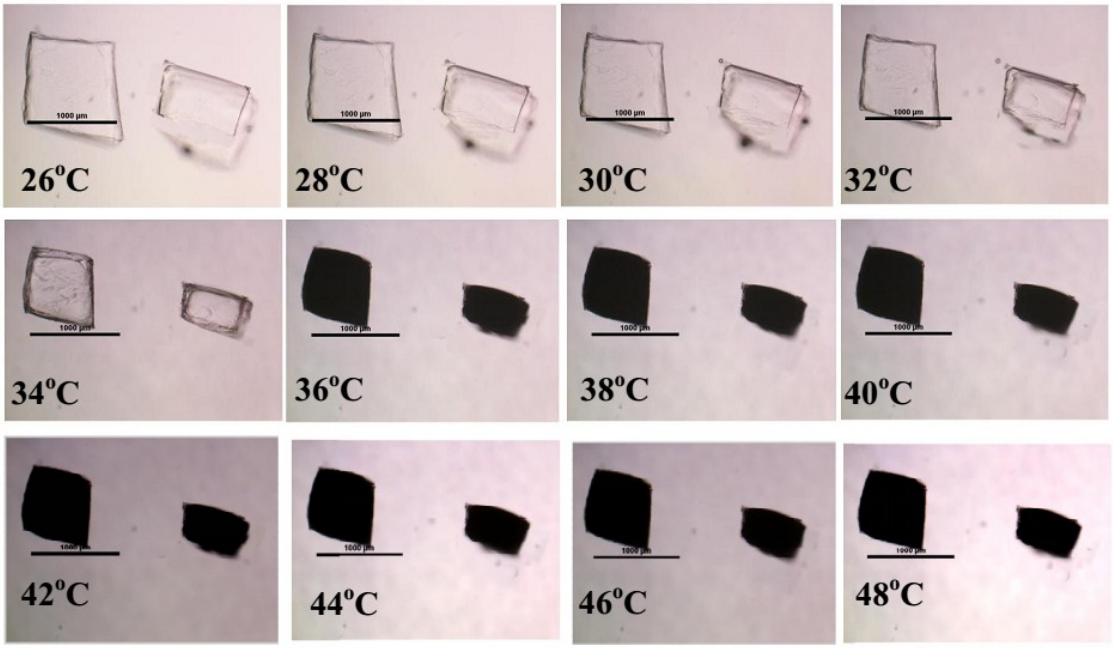
Figure S2 showed the different appearance of PAM and PNIPAM hydrogels after hydrothermal treatments. It was found that PAM hydrogel was completely dissolved while PNIPAM hydrogel maintained its original shapes with slight expansion instead of dissolution after 5h and 25h hydrothermal treatment. The difference between PAM and PNIPAM hydrogel after hydrothermal treatment may be due to their different hydrophobicity/hydrophilicity balance (Figure S2e-S2f). PAM hydrogel is a non-temperature-responsive hydrogel, in which above 90% of its content is free water that can be hydrothermally activated to supercritical water. During the hydrothermal reaction, the supercritical water can freely diffuse in and out of PAM hydrogel, which can cause hydrolysis of amide bonds in PAM hydrogels, leading to the de-crosslinking of PAM hydrogel network. On the other hand, PNIPAM hydrogel is a temperature-responsive hydrogel showing a LCST at around 32 ^{o}C. Consequently, PNIPAM hydrogel would undergo de-swelling and dehydration during the initial hydrothermal stage, in which water molecule was expelled out the hydrogel and hydrophobic interaction force became dominant. Because of the shrunk hydrogel

network and hydrophobic nature of PNIPAM hydrogel after its de-swelling, it is difficult for supercritical water to freely diffuse inside the PNIPAM hydrogel and much slower hydrolysis of amide bonds in PNIPAM hydrogel than in PAM hydrogel is resulted. Only after 50h hydrothermal treatment, complete dissolution of PNIPAM hydrogel was observed (Figure S2d).

Supporting Information IV: The swelling/shrinking kinetic of original PNIPAM hydrogel and its hydrothermally treated samples

The swelling/shrinking kinetic of original PNIPAM hydrogel and its hydrothermally treated samples is studied by recording their size changes at different time and different temperature (Figure S3). Briefly, the original PNIPAM hydrogel and its hydrothermally treated samples are put into a chamber slide with temperature controller which is attached to the bright field microscope (TP-2.0, Tokai Hit, Japan). The temperature is steadily increased from 26^{o}C to 48^{o}C. At each temperature interval, the hydrogels samples are maintained for 5 minutes to ensure no further size changes are observed. It was found that the un-treated PNIPAM hydrogel shrink from 100% to 50% of its original size at 34oC, while the 5h, 15h and 25h hydrothermally treated PNIPAM hydrogel shrink from 100% to 50% of its original size at 36^{o}C, 42^{o}C and 46^{o}C, respectively (Figure S3).

a


26°C
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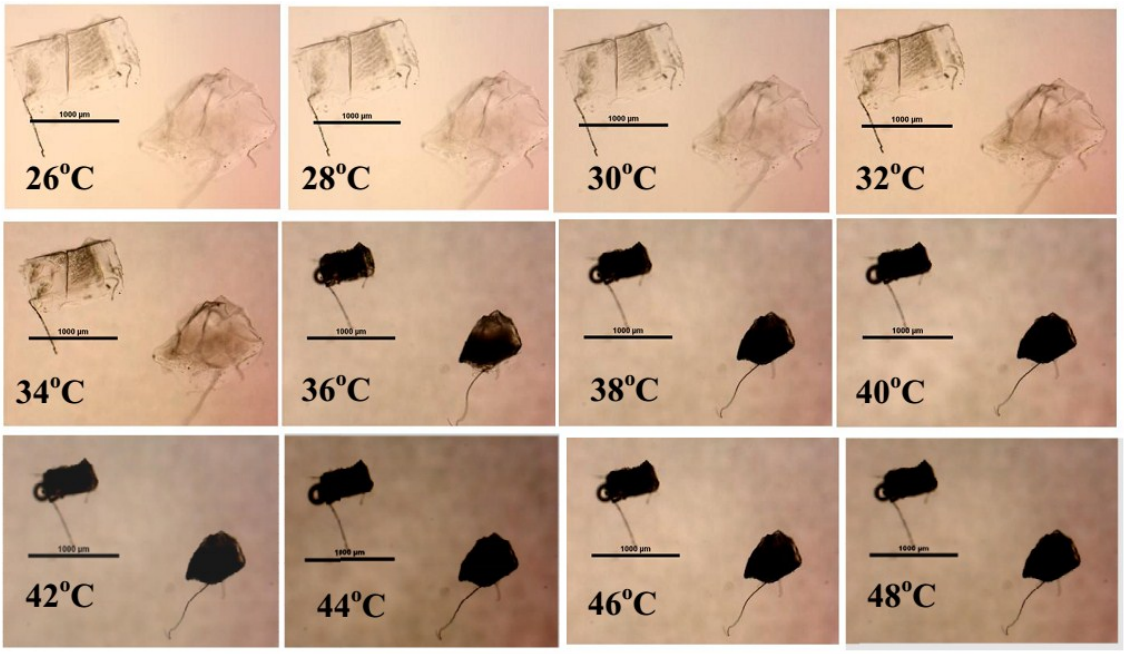
30°C
32°C
34°C
36°C
38°C
40°C
42°C
44°C
46°C
48°C

b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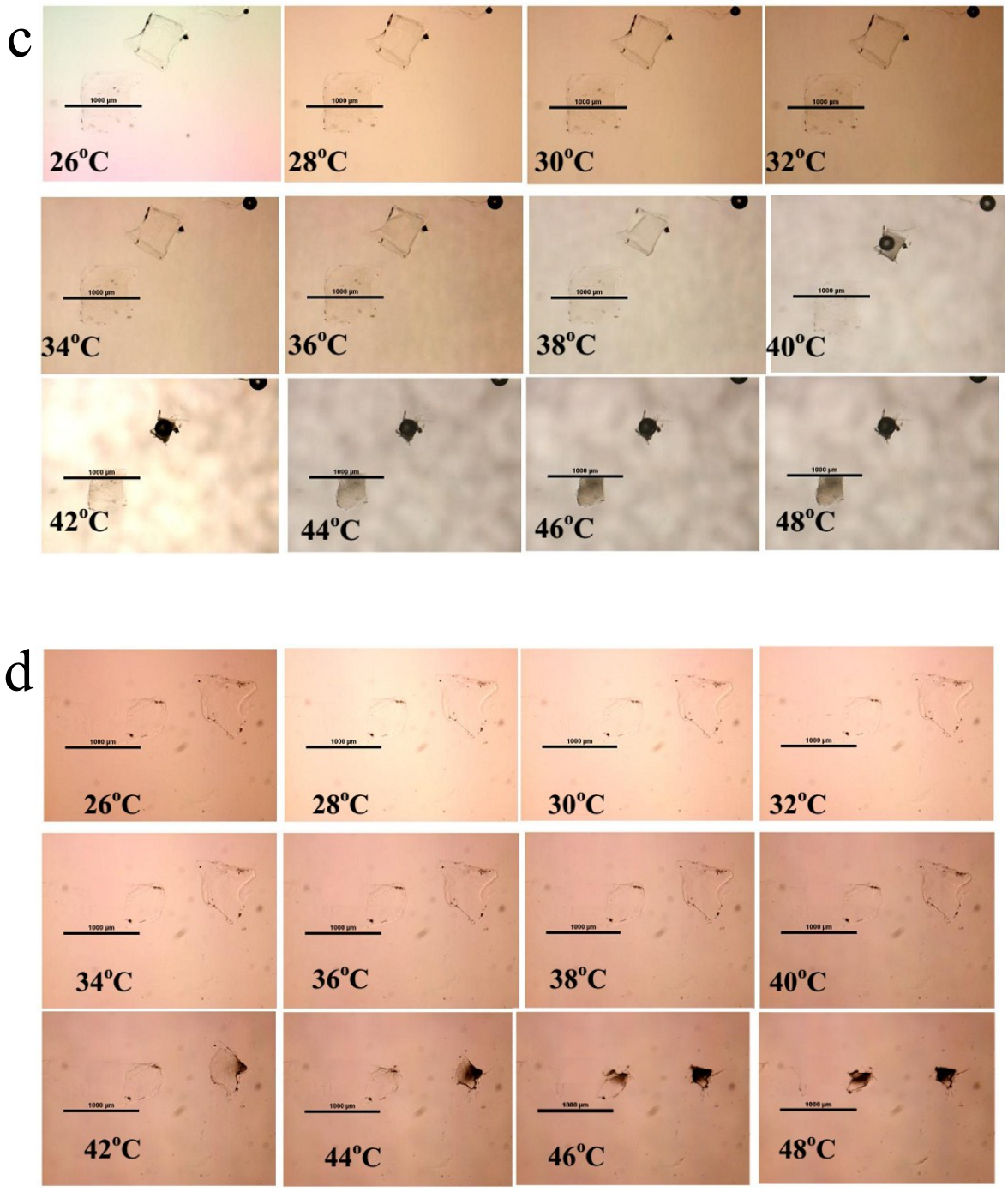

Figure S3 Microscopic observations showing the size change of hydrogel samples to different temperature, (a) original PNIPAM hydrogel (b) 5h hydrothermally treated PNIPAM hydrogel (c) 15h hydrothermally treated PNIPAM hydrogel and (d) 25h hydrothermally treated PNIPAM hydrogel; the scale bar is 1000μm..

Supporting Information V: Effects of treating time and treating temperature on the density and pH changes of PNIPAM hydrogels to investigate the reaction kinetic of the hydrothermal process

(a) Effects of hydrothermal treating time: To characterize effects of treating time, the hydrothermal treating temperature was fixed to be 180^{o}C in this experiment. Many previous researches reported that obvious hydrolysis only started above 180^{o}C.[1-6] After different hydrothermal treating time (0h, 5h, 15h and 25h), the density of the hydrogel and pH of the supernatant were characterized (**Figure S4**). The morphology of lypophilized PNIPAM hydrogels being treated for different time (0h, 5h, 15h and 25h) were shown in **Figure S5**. With increases of hydrothermal treating time, increased volume and decreased density of lypophilized hydrogels were observed (**Table 1**). The results suggested that the amide groups in *N,N*-methylenebisacrylamide (MBA) crosslinkers of the PNIPAM hydrogel were hydrolyzed into carboxyl groups, which lead to the de-crosslinking of the PNIPAM hydrogel networks. Consequently, a part of PNIPAM polymer chains containing carboxyl groups were dissolved into the aqueous solution and cause its pH changes. It was consistent with the finding that pH of the aqueous supernatant decreased steadily with increasing of hydrothermal treating time (**Table 1**).

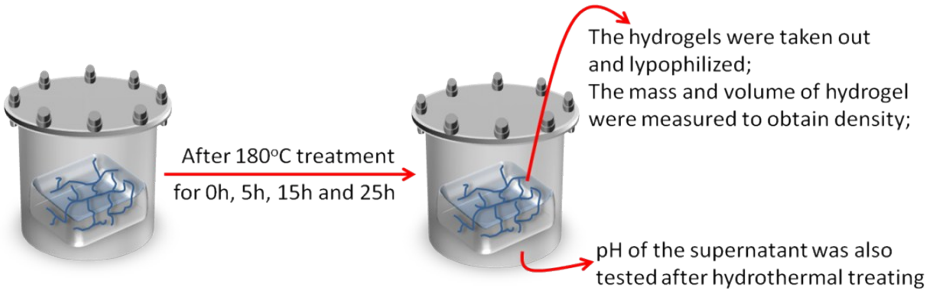

Figure S4. The hydrogels are treated at 180°C for different times and the density and pH of the hydrogels are measured to study reaction kinetic of hydrothermal process

Figure S5. Photographs showing the lypophilized hydrogels being hydrothermally treated at 180°C for different time

Time (h)	Density (g/cm^3)	pH
0	0.18	6.75
5	0.16	6.62
15	0.04	6.26
25	0.01	5.98

Table 1. Effects of hydrothermal treating time on the density of the PNIPAM hydrogels and pH of the aqueous supernatant

(b) Effects of hydrothermal treating temperature: In this experiment, the hydrothermal treating time was fixed to be 25h. The effects of varied treating temperatures at 27°C, 32°C, 37°C, 100°C, 120°C and 150°C were studied (**Figure S6**) by measuring hydrogel's density and pH changes of the supernatant (**Table 2**). When the hydrothermal treating temperature increased from 27°C to 37°C, density of the PNIPAM hydrogel and pH of the aqueous supernatant remained the same, indicating that the reaction kinetics of the hydrothermal process was unaffected. When hydrothermal treating temperature increased from 100°C to 150°C, density of the hydrogel and pH of the supernatant reduced slightly, indicating de-crosslinking of PNIPAM hydrogel network.

In conclusion, both treating temperature and treating time have important effects on the reaction kinetics of hydrothermal process. The reaction kinetics could be accelerated by either increasing the treating temperature or increasing treating time. Our results in this study indicated that hydrolysis of amide groups in PNIPAM hydrogels occurred obviously when treating temperature is above 180°C. At this condition, tuning the treating time could effectively modulate the reaction kinetics to change properties of resulting hydrogels.

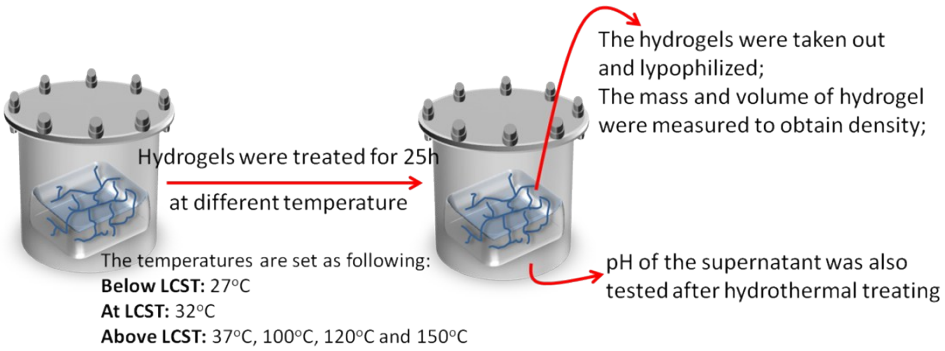

Figure S6. The hydrothermal processes of PNIPAM hydrogels were performed at 27°C, 32°C, 37°C, 100°C, 120°C and 150°C for 25 hours to characterize the effects of hydrothermal treating temperature on hydrogel density and solution pH to study the reaction kinetic.

Table 2. Effects of hydrothermal treating temperature on the density of the PNIPAM hydrogels and pH of the aqueous supernatant

Temperature (°C)	Density (g/cm^3)	pH
27	0.18	6.75
32	0.18	6.75
37	0.18	6.75
100	0.16	6.52
120	0.15	6.12
150	0.13	6.08

Supporting Information VI: Synthesis of the partially-crosslinked PNIPAM hydrogels and their hydrothermal treatment

Partially-crosslinked PNIPAM hydrogels were synthesized by interrupting the redox initiation polymerization process. Briefly, 100 mg of NIPAM, 10 mg of MBA and 5 mg of APS were dissolved in 1 ml of DI H_2O. The solutions were de-gassed under vacuum for 30 min and poured into a shaped mold maintaining at 4°C. The polymerization was then initiated by adding 2 μl of 0.1% TEMED aqueous solution. The polymerization was performed at 4°C for 1 h to form PNIPAM hydrogels. The PNIPAM hydrogels were then peeled out and directly transferred to a Teflon-lined autoclave containing 20 ml of DI H_2O and heated at 180°C for different times. The resulting core-shell PNIPAM hydrogels were then removed and washed with DI H_2O three times. Next, the core-shell PNIPAM hydrogels were placed in 8 L of DI H_2O and stirred at 4°C for 5 days; the water was regularly changed and replenished with new DI H_2O.

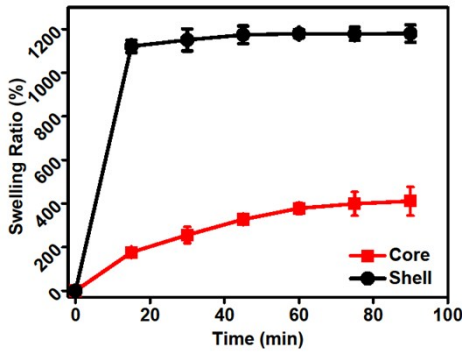

Figure S7 swelling ratios of the hydrogel core and hydrogel shell for a core-shell PNIPAM hydrogels obtained after hydrothermally treating partially-crosslinked PNIPAM hydrogels for 3h.

Supporting Information VII: Comparison of hydrothermal technique with other modifications techniques in programming hydrogel swelling, responsiveness and structure **(Table 3)**

Hydrogel Swelling		
	Hydrothermal technique	**Pore-forming technique [1-6]**
Simplicity/effectiveness	One-pot, one step reaction, tuning reaction time can program the swelling effectively	Need to use pore-forming agents or methods such as solvent casting, particle leaching, gas foaming and electrospinning. Swelling is controlled by pore-forming agents.
Chemicals/solvents	Water is the only reactive medium used	Need organic solvents/chemicals to synthesize or dissolve the porogen
Pore size/pore shape	Have poor control on the pore size/pore shape	Pore size/shape can be precisely controlled by using designated porogen
Hydrogel Responsiveness		
	Hydrothermal technique	**Copolymerizing with AA/MBA[7-10]**
Simplicity/effectiveness	One-pot, one step reaction can transform the hydrogel into hydrogel with both temperature/pH responsiveness; Tuning reaction time can modulate the responsiveness	One-pot, one step reaction can also make hydrogel with both temperature/pH responsiveness; Adjusting amounts of added AA/MBA monomers can modulate the responsiveness of the hydrogel
Chemicals/solvents	Water is the only reactive medium used	Have to use external chemical such as acrylic acid (AA)
Program with LCST and swelling	The responsiveness, LCST and swelling can by simultaneously programmed	It can form hydrogel with both temperature/pH responsiveness and tunable LCST but it has limits in tuning the swelling of hydrogel
Hydrogel Structure		
	Hydrothermal technique	**Microfluidic technique [11-15]**
Simplicity/effectiveness	With partially-crosslinked PNIPAM hydrogel as starting materials, one-pot and one step reaction can form core-shell structural hydrogel; Only need a hydrothermal autoclave as the reactor	Need to use microfluidic devices requiring cleaning room, soft lithography. Multi-step reactions are needed.
Chemical/solvents	Water is the only reactive medium used	Need to use organic oil phase such as mineral oil and surfactants, may have toxic residues
Throughput	Similar to microfluidic technique	Similar to hydrothermal technique
Size of the hydrogel	Can only form hydrogel structure above millimeter scale	Various sizes of hydrogel can be formed; Good at forming hydrogel with micrometer scale
Order of Hierarchical structure	Can only form core-shell hydrogel structure at the current stage	Hydrogels with various order of hierarchical structure can be formed

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