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## ARTICLE TYPE

**Poly(N-isopropylacrylamide) hydrogels fabricated via click chemistry: well-defined  $\alpha,\omega$ -bis propargyl linear poly(N-isopropylacrylamide)s as crosslinkers**Jianquan Wang,<sup>\*,a</sup> Zeyu Kang,<sup>a</sup> Bin Qi,<sup>a</sup> Qiushi Zhou,<sup>a</sup> Shengyuan Xiao,<sup>b</sup> Ziqiang Shao<sup>a</sup>

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A series of poly(N-isopropylacrylamide) (PNIPA) hydrogels were fabricated through click chemistry by using a well-defined azido-PNIPA carrying pendant azido groups, and linear  $\alpha,\omega$ -bis propargyl PNIPAs with different chain lengths. Here linear  $\alpha,\omega$ -bis propargyl PNIPAs were synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization by using a bis propargyl terminal chain transfer agent, whose chain lengths were modulated by changing polymerization conditions. The obtained hydrogels showed increasing *ESR* values in swollen state and rising volume phase transition temperatures (VPTTs) with increasing molecular weights of bis propargyl PNIPAs, ascribed to the lengthening distance between crosslinks and improving hydrophilicity. The incorporation of amine together with crosslinking modified the hydrophilicity of a click hydrogel, resulting in the elevated VPTT and additional pH sensitivity. The present study provided a facile method to regulate swelling properties and/or impart special functions for PNIPA hydrogels, by adjusting the chain length of crosslinkers or by introducing other functional groups.

**Introduction**

Reversible-deactivation radical polymerization (RDRP) techniques, also referred to as controlled/living radical polymerization (CRP), provide powerful tools to design and synthesize well-defined functional macromolecules with complex architectures. In general, nitroxide-mediated polymerization (NMP),<sup>1</sup> atom transfer radical polymerization (ATRP),<sup>2</sup> and reversible addition-fragmentation chain transfer (RAFT) polymerization<sup>3</sup> are the three most commonly used RDRP methods.<sup>4</sup> Using these approaches, narrow polydispersity macromolecules with desired molecular weights and architectures can be readily prepared; as well, special functional moieties can be easily incorporated to specific position on polymer chains via functional initiators, chain transfer agents (CTAs) and modification of terminal groups.<sup>2, 3, 5</sup>

Poly(N-isopropylacrylamide) (PNIPA), a well-known temperature sensitive polymer whose aqueous solution behaving a lower critical solution temperature (LCST) around 32 °C,<sup>6</sup> has been extensively studied in both fundamental and technical aspects, due to their wide potential applications in biomaterials, separation, sensors, actuators, and so on.<sup>7-11</sup> The application of RDRP to NIPA enables the design and synthesis of various well-defined NIPA-based polymers containing special functional groups. Iron oxide/PNIPA core-shell structure was constructed via the “grafting-from” NMP of NIPA in the presence of 1,2-diol moiety ended NMP initiator capable of attaching the surface of Fe<sub>2</sub>O<sub>3</sub> nanoparticles.<sup>12</sup> PNIPAs with different end groups of

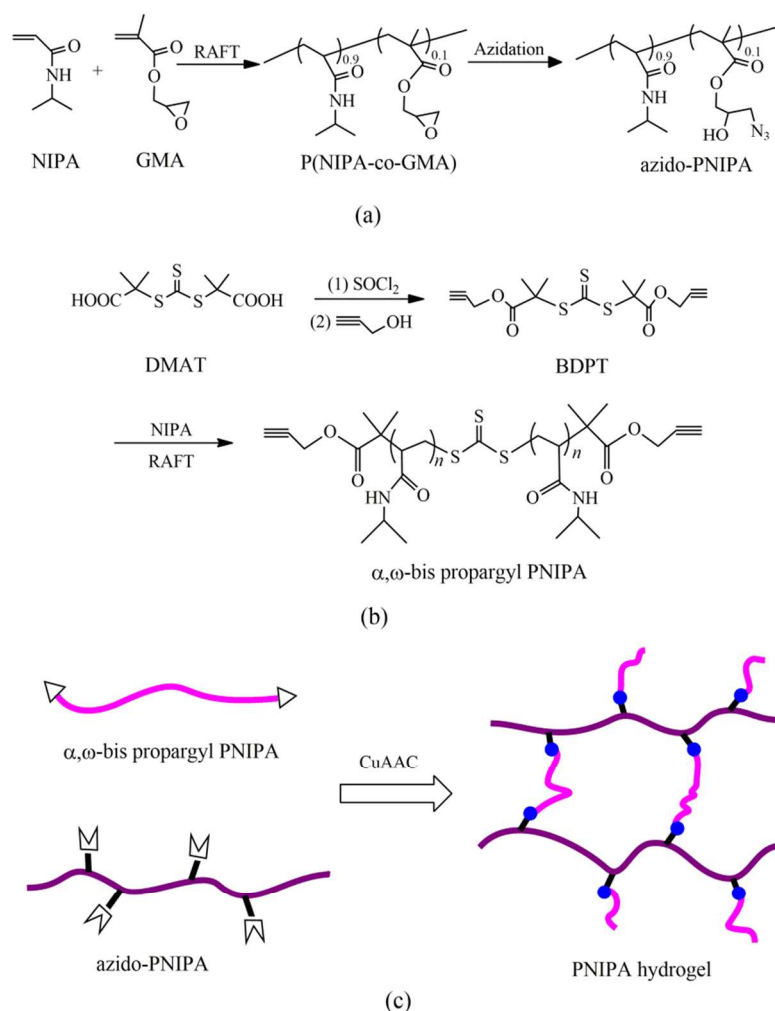
varying polarity were synthesized through ATRP of NIPA initiated by corresponding chloropropionate and chloropropionamide compounds in order to investigate the end group effect on thermal response of PNIPA.<sup>13</sup> Azido- or alkynyl-terminated PNIPA polymers with well-defined structures were easily prepared based on the appropriate ATRP initiators or RAFT agents, and they were utilized to perform further click reaction with partner-group-containing substrates.<sup>14-20</sup> In addition, RAFT agents featured with thiocarbonylthio groups, such as dithioesters and trithiocarbonates, facilitate post-polymerization modification of RAFT polymers for further end-functionalization.<sup>21, 22</sup> Different telechelic PNIPAs were obtained through aminolysis of thiocarbonylthio moieties and subsequent Michael addition of the resulting thiols to  $\alpha, \beta$ -unsaturated carbonyl derivatives in near quantitative yields.<sup>23-25</sup>

Click chemistry, usually classified into azide-alkyne cycloaddition, thiol-ene addition, and Diels-Alder cycloaddition, has been widely used as a forceful tool to fabricate well-defined and complex structures or to impart special functions. As the premier example of these three typical click reactions, azide-alkyne cycloaddition has been applied in many fields including organic synthesis, bioconjugation, polymer preparation and modification, network formation, etc.<sup>26-28</sup> In 2006, Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) was pioneeringly utilized to prepare PVA- and PEG-based hydrogels, opening a novel effective crosslinking method for hydrogel fabrication.<sup>29, 30</sup> Especially for the click PEG hydrogels, the high efficiency of CuAAC click chemistry endowed the resulting hydrogels with more ideal structures and consequent improved mechanical

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**Scheme 1** Synthetic route of  $\alpha,\omega$ -bis functional PNIPAs and hydrogels. (a) azido-PNIPA was prepared through RAFT polymerization of NIPA and GMA and following azidation of pendant epoxys; (b)  $\alpha,\omega$ -bis propargyl PNIPAs were synthesized via RAFT polymerization using bis propargyl terminal BDPT as CTA; (c)  $\alpha,\omega$ -bis propargyl PNIPAs were applied as crosslinkers to react with azido-PNIPA containing pendant azido-groups through click chemistry.

properties.<sup>30</sup> Certainly this promising crosslinking strategy has also been introduced to the construction of PNIPA hydrogels. Zhang's group synthesized a series of PNIPA hydrogels through CuAAC, using poly(N-isopropylacrylamide-co-hydroxyethyl methacrylate) [P(NIPA-co-HEMA)],  $\beta$ -cyclodextrin ( $\beta$ -CD), and cellulose carrying alkyne or azido groups as materials.<sup>31-33</sup> Ooi et al prepared telechelic azido-ended PNIPAs via RAFT polymerization, followed by click reaction with a tetra-acetylene crosslinker, obtaining highly regular PNIPA networks.<sup>16</sup> Pasale and coworkers fabricated multi-responsive hyaluronan-PNIPA hybrid hydrogels using azido-grafted hyaluronic acid and  $\alpha,\omega$ -bis propargyl end-capped PNIPA.<sup>20</sup> We constructed PNIPA hydrogels utilizing a well-defined linear PNIPA with pendant azido groups crosslinked by bis- and tetra-alkynyl terminated compounds.<sup>34</sup>

As described above, both RDRP and click chemistry provide valid strategy for preparation of well-defined polymer structures.

Therefore it is necessary to prepare well-defined PNIPA hydrogels via the two versatile methods so as to study the structure-property relationship more accurately. In the present work, a series of well-defined  $\alpha,\omega$ -bis propargyl telechelic linear PNIPAs with different molecular weights were synthesized through RAFT polymerization by using an  $\alpha,\omega$ -bis propargyl terminal CTA. Then these obtained bis-functional PNIPAs acted as macromolecular crosslinkers to react with a linear PNIPA carrying pendant azido groups (azido-PNIPA), to produce hydrogels via CuAAC. The effect of bis-functional PNIPAs chain length on hydrogel properties was studied, and the possibility to incorporate other functional groups such as amino, carboxyl, or luminous units together with crosslinking was explored as well. The synthetic route of click PNIPA hydrogels is shown in

Scheme 1.

## Experimental

### Materials

N-isopropylacrylamide (NIPA), glycidyl methacrylate (GMA), tetrabutylammonium hydrogen sulphate and polystyrene standards were purchased from TCI (Shanghai) Development Co., Ltd. Thionyl chloride and 4,4'-azobis(4-cyanovaleric acid) (V501) were acquired from Alfa Aesar China (Tianjin) Co., Ltd. 2,2'-Azobis(isobutyronitrile) (AIBN), propargyl alcohol and propargyl amine were obtained from Tianjin Heowns Biochemical Technology Co., Ltd. Other chemicals (AR grade) were bought from Beijing Chemicals Co., China. Prior to use, NIPA was recrystallized from hexane, AIBN was recrystallized from ethanol, GMA was passed through basic alumina, and carbon disulfide (CS<sub>2</sub>), 1,4-dioxane and tetrahydrofuran (THF) were distilled. All other reagents were used as received.

### Synthesis of RAFT CTAs

S,S'-bis( $\alpha,\alpha'$ -dimethyl- $\alpha$ ''-acetic acid) trithiocarbonate (DMAT), a bis-carboxyl terminated CTA for RAFT polymerization was prepared according to literature.<sup>35</sup> In detail, CS<sub>2</sub> (13.7 g, 180 mmol), chloroform (53.8 g, 450 mmol), acetone (26.1 g, 450 mmol), tetrabutylammonium hydrogen sulfate (1.2 g, 3.5 mmol), and mineral oil (60 mL) were added into a 500 mL three-neck round-bottom flask in ice bath, and the mixture was purged with N<sub>2</sub> under stirring. After 10 min, 50 wt% sodium hydroxide aqueous solution (100 g, 1.25 mol) was dropped into the above stirring mixture under N<sub>2</sub> protection for over 90 min, and the reaction was kept for 12 h at room temperature. At the end of the reaction, the mixture was strongly bubbled with N<sub>2</sub> for 20 min in order to remove the residual chloroform and acetone. Then the resultant mixture was added to 500 mL of water under severely stirring to dissolve the solid, and liquid separation was performed to remove oil phase. Slowly dropping 60 mL of concentrated HCl into the separated aqueous phase in water bath, a great deal of yellow precipitate was generated, then it was filtered and washed with water. The collected solid was recrystallized from ethanol to get yellow crystal (21.3 g, yield 42%). m.p. 172-175 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) ppm:  $\delta$  1.67 (s, 12H, >C(CH<sub>3</sub>)<sub>2</sub>), 12.77 (s, 2H, -COOH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>) ppm:  $\delta$  25.1 (s, -CH<sub>3</sub>), 55.9 (s, >C(CH<sub>3</sub>)<sub>2</sub>), 173.8 (s, -COOH), 219.8 (s, -C(=S)S<sub>2</sub>). HR-MS: *m/z* 304.99527 [M+Na]<sup>+</sup> (C<sub>9</sub>H<sub>14</sub>NaO<sub>4</sub>S<sub>3</sub>, cal. 304.99519, err. 0.262 ppm). Elemental analysis: C 37.89(cal. 38.30), H 5.09(cal. 4.96), S 33.86(cal. 34.04).

A bis propargyl ended CTA, S,S'-bis( $\alpha,\alpha'$ -dimethyl- $\alpha$ ''-propargyl acetate) trithiocarbonate (BDPT), was prepared by using DMAT as a raw material. DMAT (5.64 g, 20 mmol) was added into a 100 mL three-neck round-bottom flask. Next thionyl chloride (60 mL, 800 mmol) was dropped with stirring under nitrogen protection. Then the mixture was refluxed at 60 °C for 3 h, getting a dark-red solution. Unreacted thionyl chloride was removed under reduced pressure to yield acyl chloride (tan solid). Subsequently, the resultant was dissolved in 15 mL of dried dichloromethane (DCM), and added dropwise to the stirring solution including propargyl alcohol (4.48 g, 80 mmol), dried pyridine (4.74 g, 60 mmol) and dried DCM (15 mL) in ice bath under nitrogen atmosphere. The reactant mixture was left to stir at room temperature for 12 h. Afterwards, the resulting mixture was washed with 0.1 N hydrochloric acid aqueous solution (100

mL  $\times$  3) and water (50 mL  $\times$  3) and dried over Na<sub>2</sub>SO<sub>4</sub>. Then the solvent was removed via rotary evaporation and the resultant was washed with hexane and dried in vacuum to obtain brown viscous liquid (4.68 g, yield 65%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) ppm:  $\delta$  1.53 (s, 12H, >C(CH<sub>3</sub>)<sub>2</sub>), 2.46 (s, 2H,  $\equiv$ CH), 4.67 (s, 4H, -CH<sub>2</sub>-). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) ppm:  $\delta$  24.6 (s, -CH<sub>3</sub>), 55.5 (s, >C(CH<sub>3</sub>)<sub>2</sub>), 172.1 (s, -COO-), 217.6 (s, -C(=S)S<sub>2</sub>), 53.3 (-CH<sub>2</sub>O-), 76.8 (C $\equiv$ CH), 75.3 (C $\equiv$ CH). HR-MS: *m/z* 381.02648 [M+Na]<sup>+</sup> (C<sub>15</sub>H<sub>18</sub>NaO<sub>4</sub>S<sub>3</sub>, cal. 381.02649, err. -0.026 ppm). Elemental analysis: C 50.35(cal. 50.28), H 5.31(cal. 5.03), S 26.35(cal. 26.82).

### Preparation of PNIPA polymers

Azido-PNIPA was synthesized through azidation of RAFT copolymer of NIPA and GMA, with polydispersity index (PDI) as low as 1.06. The obtained azido-PNIPA carries 10 mol% azido side groups relative to the total repeated units in the polymer chain. P(NIPA-co-GMA) was prepared with 9:1 (molar ratio) of NIPA to GMA, using DMAT as CTA and V501 as initiator, in 1,4-dioxane solution at 75 °C. Afterwards, epoxyl units of GMA in the polymer was azidized quantitatively. The detailed procedure and characterization have been reported in our previous paper.<sup>34</sup>

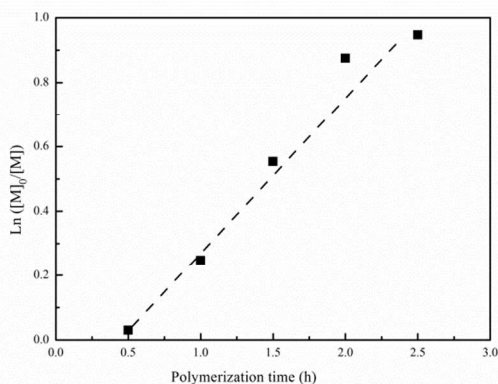
$\alpha,\omega$ -bis propargyl PNIPAs were prepared based on RAFT polymerization of NIPA in the presence of BDPT containing bis propargyl ends. Typically, NIPA (2.26 g, 20 mmol), BDPT (36 mg, 0.1 mmol) and AIBN (1.6 mg, 0.01 mmol) were dissolved in 6.8 g of distilled THF in a 30 mL Schlenk tube. The mixture was subject to three freeze-pump-thaw cycles, and was stirred under nitrogen atmosphere in 65 °C water bath. The polymerization progress was monitored by extracting 0.5 mL of aliquots from the polymerization solution at the desired time interval. The extracted solutions were immediately quenched by opening in air and cooling in liquid nitrogen. The solvent was removed under a gentle airstream and subsequent vacuum drying. The resulting solid was used for <sup>1</sup>H-NMR measurement. The monomer conversion was calculated from the area of signal at 5.6 ppm assigned to the vinyl proton of NIPA and the areas sum of the isopropyl methine proton signals at 4.0 ppm and 4.1 ppm, corresponding to PNIPA and the remaining NIPA monomer, respectively.

PNIPAs with different molecular weights were prepared through changing the dosage of compounds and polymerization time, the detail was listed in Table 1. At the end of polymerization, the resultant was quickly quenched by opening in air and cooling in liquid nitrogen. 0.5 mL of the resultant was taken out for the determination of monomer conversion, and the left was rotary evaporated to concentrate, followed by precipitation in diethyl ether. The obtained yellow solid was purified further by two consecutive reprecipitations from THF into diethyl ether.

### Estimation of $\alpha,\omega$ -bis propargyl PNIPA molecular weights

The molecular weights (*M<sub>n</sub>*) of samples were obtained by three methods. Following Equation (1), the theory one was calculated from monomer conversion that was estimated according to <sup>1</sup>H-NMR spectra of unseparated polymerizing resultant including polymer and residual monomer. For the purified polymers, *M<sub>n</sub>* was evaluated via Equation (2) using the peak areas at 4.7 ppm and 4.0 ppm, which correspond to propynyl methylene and





**Fig. 1** Pseudo-first-order kinetic plot of PNIPA RAFT polymerization.

The polymerization condition is as follows: [NIPA]/[BDPT]/[AIBN]=200/1/0.1, the monomer concentration in THF is 25 wt%, polymerization temperature is 65 °C.

isopropyl methine protons, respectively. Furthermore, UV-vis can also be utilized to measure  $M_n$  of a RAFT polymer due to the existence of a characteristic absorbance at about 310 nm for trithiocarbonate group from CTA. Accurately weighed  $\alpha,\omega$ -bis propargyl PNIPA sample (~10 mg) was dissolved in ethanol (10 mL), obtaining ethanol solution with a known polymer concentration. The UV absorbance of the solution, diluted if necessary, at 310 nm was determined. And the concentration of trithiocarbonate group was calculated based on the linear relationship of absorbance (at 310 nm) and BDPT concentration in ethanol, which was derived as Equation (3). Further,  $M_n$  was reckoned applying Equation (4).

$$M_n(\text{theory}) = \text{conversion} \times ([\text{NIPA}]/[\text{BDPT}]) \times M_{\text{NIPA}} + M_{\text{BDPT}} \quad (1)$$

$$M_n(\text{NMR}) = 4 \times (I_{4.0\text{ppm}}/I_{4.7\text{ppm}}) \times M_{\text{NIPA}} + M_{\text{BDPT}} \quad (2)$$

$$C_{\text{BDPT}} = 0.0113 A \quad (3)$$

$$M_n(\text{UV-vis}) = \rho / C_{\text{BDPT}} \quad (4)$$

where  $M_{\text{NIPA}}$  and  $M_{\text{BDPT}}$  mean individual molecular weights of NIPA and BDPT;  $I_{4.0\text{ppm}}$  and  $I_{4.7\text{ppm}}$  mean peak areas at 4.0 ppm and 4.7 ppm for obtained PNIPA  $^1\text{H-NMR}$  spectra, respectively;  $C_{\text{BDPT}}$  means BDPT concentration in ethanol (mol/L),  $A$  is absorbance at 310 nm recorded by UV-vis, and  $\rho$  represents polymer concentration in ethanol (g/L).

### Fabrication of PNIPA hydrogels

In a flat-bottom screw-capped glass vial ( $\phi$ 1 cm), the predetermined amount of azido-PNIPA and bis propargyl PNIPA, total weight of which was 200 mg, were dissolved in 750 mg of DMF. After uniform mixing, 25  $\mu\text{L}$  of 0.5 N  $\text{CuSO}_4$  aqueous solution and 25  $\mu\text{L}$  of 1.0 N ascorbic acid in DMF were added in turn. Then the vial was sealed and shaken, and the gel formed within 15 min. It was left to react for 24 h before the gel was taken out to immerse in 0.2 N ethylene diamine tetraacetic acid disodium salt ( $\text{EDTA} \cdot 2\text{Na}$ ) aqueous solution and then in ethanol to remove copper ions and unreacted precursors. Afterwards, the gel was dried and weighed. The mass of xerogel was divided by 200 to get gel fraction. Through changing the species of bis propargyl PNIPAs, several hydrogels were prepared. In addition, amino groups were simultaneously introduced into a hydrogel in the case that propargyl amine was added into azido- and

propargyl- PNIPAs mixture.

### Swelling properties of hydrogels

Prewritegels were immersed in deionized water at 13 °C, and some time later they were taken out and weighed after removal of excess surface water with wet filter paper. The equilibrium was reached until weight of a swollen gel kept constant. The equilibrium swelling ratio (*ESR*) value was calculated with Equation (5).

$$ESR = (m_e - m_0)/m_0 \quad (5)$$

where  $m_0$  and  $m_e$  are the weights of xerogel and equilibrated swollen hydrogel, respectively.

Afterwards, the temperature was increased gradually to 40 °C by changing the bath temperature. It is worth noting that the gels were kept at a certain temperature for 48 h to ensure swelling equilibrium.

For a hydrogel introduced propargyl amine, the pH sensitivity was investigated in phosphate buffer solutions (PBS) at 25 °C.  $\text{Na}_3\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{NaH}_2\text{PO}_4$ , HCl, and NaCl were used to prepare buffer solutions of different pH values with a constant ionic strength of 0.1 N.

In all of the swelling ratio measurements, three hydrogel samples of the same formulation were used, and the average value was taken.

### Characterization

$^1\text{H-NMR}$  spectra were obtained using a Bruker DRX-500 with  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  as solvent. High-resolution mass spectra (HR-MS) were obtained using a Bruker Apex IV FTMS, equipped with an electro spray ion source (ESI), in positive mode. Both compounds were dissolved in acetonitrile (approx. 1 mmol/L) and infused into the ion source with a syringe pump (5  $\mu\text{L}/\text{min}$ ). The flow of nebulizer gas was 1.0 L/min; the flow of dry gas was 5.0 L/s; the temperature of the dry gas was 250 °C; the scan range was 260-400 Da. Elemental analysis of C, H and S was performed on an Elementar vario EL III. GPC measurements were carried out at room temperature on a Waters instrument attached to a 2414 refractive index detector, using DMF as eluent at a flow rate of 1.0 mL/min, with polystyrenes as standards. Ultraviolet-visible (UV-vis) absorption spectroscopy was performed on a PerkinElmer UV/VIS spectrometer (Lambda 35). The morphology of hydrogels was characterized by scanning electron microscopy (SEM, JSM-6700F). The swollen hydrogels at 20 °C were lyophilized, and sputtered with gold before SEM observation.

## Discussion and Results

### Preparation of $\alpha,\omega$ -bis propargyl PNIPAs

In order to prepare well-defined linear PNIPA chains with a telechelic propargyl moiety on either end, a bis propargyl functional RAFT CTA named as BDPT was firstly synthesized based on the esterification of bis carboxyl DMAT and propargyl alcohol in two steps, with acyl chloride as an intermediate product. The structures of DMAT and BDPT were confirmed by  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , HR-MS and element analysis. The relevant spectra are displayed in Figure S1-S6. RAFT polymerization of NIPA using BDPT and AIBN as CTA and initiator, respectively, was performed in distilled THF at 65 °C. Figure 1 shows the

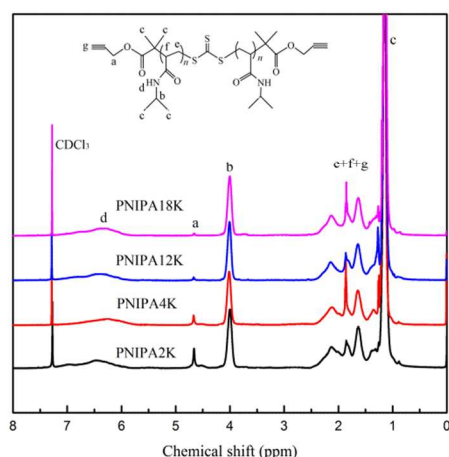
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**Table 1** The detailed information of obtained PNIPAs

| Samples  | [NIPA]/[BDPT]/[AIBN] | Polymerization time (min) | Conversion (%) | $M_n$ (kg/mol)      |                  |                     | PDI (GPC) |
|----------|----------------------|---------------------------|----------------|---------------------|------------------|---------------------|-----------|
|          |                      |                           |                | Theory <sup>a</sup> | NMR <sup>b</sup> | UV-vis <sup>c</sup> |           |
| PNIPA2K  | 30/1/0.1             | 75                        | 64             | 2.5                 | 2.2              | 2.0                 | 1.12      |
| PNIPA4K  | 50/1/0.1             | 180                       | 68             | 4.2                 | 4.5              | 4.1                 | 1.20      |
| PNIPA12K | 200/1/0.1            | 120                       | 58             | 13.5                | 12.1             | 13.1                | 1.18      |
| PNIPA18K | 250/1/0.1            | 180                       | 65             | 18.7                | 18.2             | 20.3                | 1.22      |

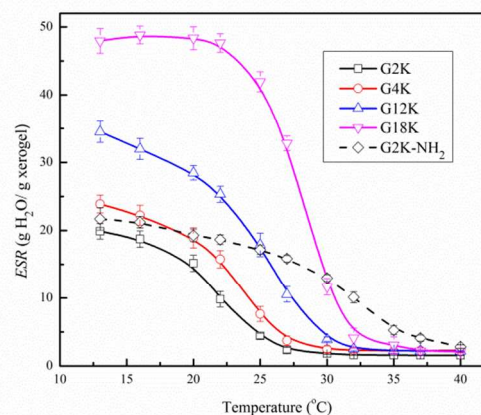
<sup>a</sup> Calculated according to Equation (1); <sup>b</sup> Derived based on Equation (2); <sup>c</sup> Reckoned with Equation (4).**Fig. 2** <sup>1</sup>H-NMR spectra of  $\alpha,\omega$ -bis propargyl PNIPAs with different macromolecular weights. The resonances of related protons are assigned.

polymerization kinetics of a certain formulation, in line with the basic characteristics of living polymerization.

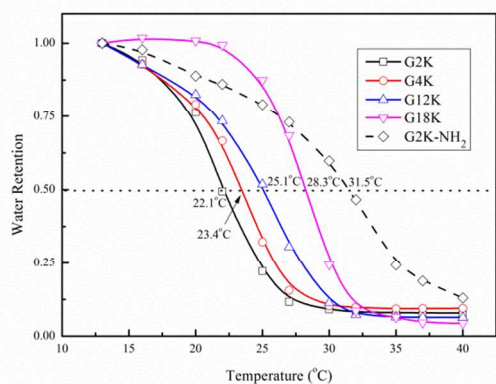
Several  $\alpha,\omega$ -bis propargyl PNIPA samples with different molecular weights were prepared through changing the polymerization formulation and time. The monomer conversion was controlled to a lower level (<70 %) in order to avoid undesirable side reactions and to ensure the retaining of propargyls on both ends of polymers.<sup>3</sup> The detailed polymerization conditions and some characterizations of obtained polymers are listed in Table 1. It can be seen that the molecular weights calculated by various methods show close values, which prove the well defined nature of obtained PNIPAs in combination with the measured PDI values lower than 1.25. In addition, the incorporation of CTA end groups was also confirmed by <sup>1</sup>H-NMR spectra, as revealed in Figure 2. Meanwhile, the assignment of peaks is also illustrated. It is necessary to point out that molecular weights through GPC measurements are relative ones to polystyrene standards, leading to greater deviation from real ones. Thus the GPC  $M_n$  values were not listed in Table 1, while only PDI values were shown.

### Formation of PNIPA hydrogels

A series of PNIPA hydrogels were fabricated through CuAAC using well-defined  $\alpha,\omega$ -bis propargyl PNIPAs and azido-PNIPA

**Fig. 3** The change of ESR values with temperature for hydrogels. Different ESR values were shown in swollen state for various samples; G2K-NH<sub>2</sub> revealed gentler deswelling with temperature than G2K.

with pendant azido moieties, as shown in Scheme 1. Herein, the azido-PNIPA is the same one as used in our previous paper,<sup>34</sup> i.e., there is 10 mol% of azido-contained monomer and 90 mol% of NIPA distributed in polymer chains, which was synthesized by RAFT polymerization of NIPA and GMA with molar ratio of 90 to 10 followed by azidation of epoxyl in GMA. In hydrogel preparation, the individual amounts of azido- and propargyl-contained PNIPAs were designed based on the principle that 25 mol% of azido-groups was used to react with all of the propargyl moieties. Corresponding to the individual molecular weights of used  $\alpha,\omega$ -bis propargyl PNIPAs, the hydrogel samples were nominated as G2K, G4K, G12K and G18K, respectively. It is noteworthy that besides those for crosslinking, there is still 75 mol% of azido groups theoretically left on polymer chains, which provide effective sites to simultaneously incorporate other useful groups for imparting some special functions. Herein, as an example, another sample named G2K-NH<sub>2</sub> with additional pH sensitivity was prepared based on the sample G2K, for which propargyl amine in stoichiometric amount of 75 mol% of azido groups was fed together with PNIPA2K. Under the catalysis of CuSO<sub>4</sub>/ascorbic acid system, all the samples held the gel fraction higher than 90 %, demonstrating the high crosslinking efficiency of click chemistry in these systems.

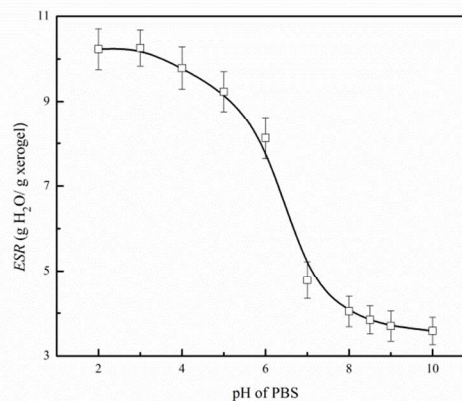


**Fig. 4** Water retention of samples at different temperature relative to *ESR* at 13 °C. VPTT became greater with the increasing molecular weights of bis propargyl PNIPAs; G2K-NH<sub>2</sub> displayed delayed LSCT than G2K due to more hydrophilicity of the former.

### Temperature sensitivity of PNIPA hydrogels

For the prepared PNIPA hydrogels, the change of *ESR* values with temperature was examined, and the results are shown in Figure 3. In general, the *ESR* values in swollen state display a tendency that higher-molecular-weight PNIPA crosslinked hydrogels have greater swelling capacities. For instance, the *ESR* values of G2K, G4K, G12K and G18K are 20, 24, 34 and 48, respectively, at initial temperature (13 °C); and the *ESR* sequence keeps consistence at temperatures below 30 °C. In the case that the feed ratio of azido to alkynyl groups is fixed, for an  $\alpha$ ,  $\omega$ -bis propargyl PNIPA to crosslink azido-PNIPA presented in this work, the higher molecular weight of the former means the longer distance between crosslinks, which provide larger space to accommodate water molecules. This result is in agreement with that of PNIPA hydrogels prepared from azido-PNIPA and multi-alkynyl terminated small molecules researched by us,<sup>34</sup> and that of PEG hydrogels derived from PEG-bisazide and a polyester with pendant alkynyl groups, reported by others.<sup>36</sup> Besides, the influence of hydrophobic groups within polymer chains is also objective. There are two kinds of hydrophobic moieties, such as carboxylic acid ester and trithiocarbonate group (originated from RAFT agent), which are located at both ends and in the middle of bis propargyl-PNIPA chains, respectively. The lower molecular weight of polymer implies the more relative content of above two kinds of hydrophobic groups. It is a potential contribution to cause the discrimination of *ESR* values for different hydrogels in swollen state.

As expected, all the PNIPA hydrogels showed deswelling behavior with increasing temperature, as revealed in Figure 3. It need pointing out that G2K, G4K and G12K samples behaved continuous deswelling process when the temperature was gradually elevated from 13 to 32 °C; while G18K appeared relatively stable *ESR* values till 22 °C and following significant deswelling with further temperature rising to 35 °C. Analogue to LCST of a linear PNIPA, volume phase transition temperature (VPTT) is a parameter to characterize a corresponding hydrogel.<sup>6</sup> Here, VPTT values of samples seem different from one another by preliminarily judging from the trace of *ESR*-temperature curve for each one. For a negative temperature-sensitive hydrogel,



**Fig. 5** Effect of pH on *ESR* values for G2K-NH<sub>2</sub> at 25 °C. The different protonation levels of amino groups decided the *ESR* values at various pH values.

VPTT can be defined as the temperature at which the swelling ratio decreased to a half of its value at initial temperature, or be regarded as the temperature at which the swelling ratio of hydrogel decreased most dramatically.<sup>37</sup> According to the former definition, the curves of *ESR* versus temperature in Figure 3 were normalized based on the data at initial temperature (13 °C), plotted in Figure 4, where water retention was defined as the ratio between *ESR* at a certain temperature and that at 13 °C. From Figure 4, the VPTT values are estimated as follows: G2K: 22.1 °C; G4K: 23.4 °C; G12K: 25.1 °C; G18K: 28.3 °C. It can be inferred that the VPTT shifts to lower values with decreasing molecular weights of  $\alpha$ ,  $\omega$ -bis propargyl PNIPAs, and all of them are lower than 32 °C of a conventional pure PNIPA hydrogel.<sup>38</sup> It is known that either LCST or VPTT is dependent on the hydrophilic/hydrophobic balance of a polymer system, and the incorporation of hydrophobic component to PNIPA can improve the dehydration of isopropyls and accompanied hydrophobic interactions with raising temperature, further leading to a decline in LCST or VPTT.<sup>39-41</sup> Inside the prepared hydrogels, there are hydrophobic trithiocarbonate group and 3-azido-2-hydroxypropyl methacrylate (azidized GMA) within azido-PNIPA chains in addition to those in bis propargyl PNIPA. It is the presence of these hydrophobic moieties that reduced VPTT values of obtained hydrogels compared with that of a conventional one (32 °C). As described above, for  $\alpha$ ,  $\omega$ -bis propargyl PNIPAs, higher molecular weight of polymer corresponds to lower relative content of hydrophobic parts, which in turn makes VPTT deviate from 32 °C to a smaller extent. And the lower is PNIPA molecular weight, the larger this deviation becomes. In this consideration, the changing trend of VPTT with different samples becomes understandable. Furthermore, the distinction in *ESR*-temperature curve shapes of samples, i.e., the continuous alterations for G2K, G4K and G12K samples from 13 to 32 °C versus relative constancy from 13 to 22 °C and subsequent dramatic descent at higher temperatures for G18K, may be associated with their different hydrophilic performances. For less hydrophobic G18K sample, hydrophobic interactions arises only when the temperature reaches a certain degree above 22 °C; while for the other three more hydrophobic samples, hydrophobic interactions has occurred at lower temperatures and



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## ARTICLE TYPE

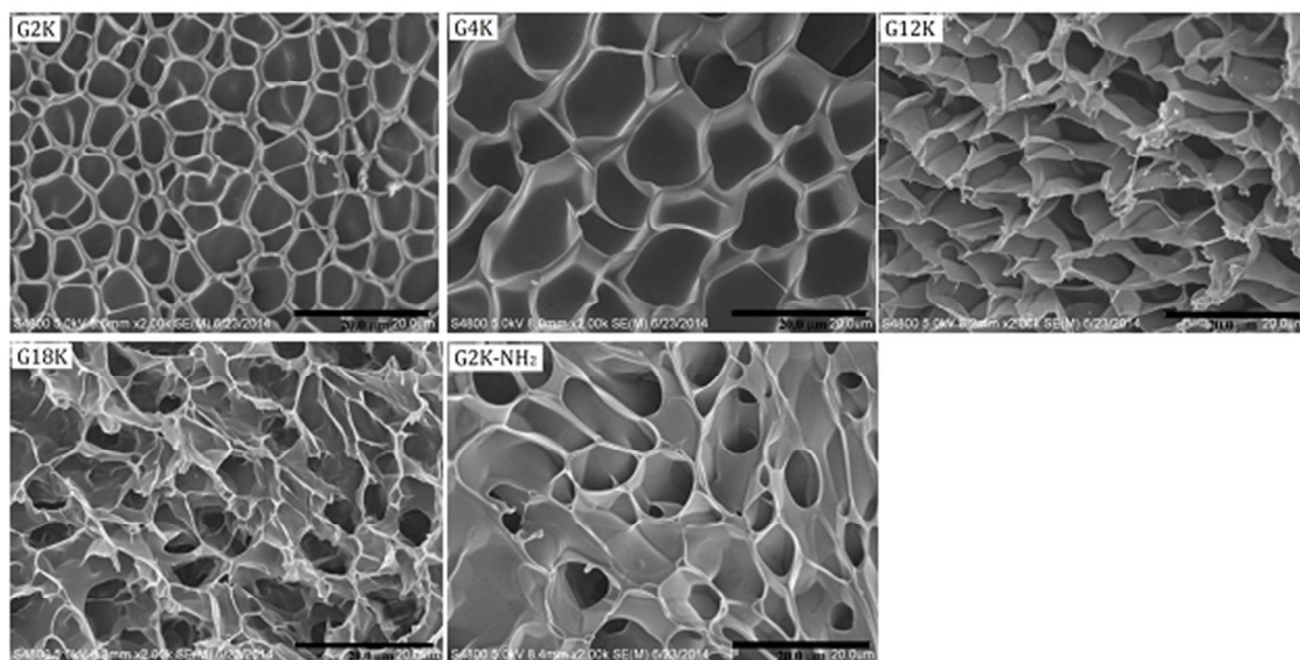


Fig. 6 SEM photographs of freeze-dried hydrogel networks

it becomes stronger with temperature increasing.

On the basis of the existing differences in temperature sensitivity for various samples described above, it is worthy to mention that some parameters such as VPTT and swelling capacity change (usually corresponding to volume alternation) with temperature variation can be facily modulated by adjusting the chain length of bis propargyl PNIPA crosslinkers, in order to meet various demands.

#### Incorporation of amine to G2K

In order to examine the possibility of simultaneously introducing functional groups to a network during click crosslinking, stoichiometric propargyl amine of left azido groups (0.75 equivalents) was fed together with prequantitative PNIPA2K-propargyls of azido groups (0.25 equivalents). The swelling property dependence on temperature was studied along with other hydrogel samples, and the results are shown in Figure 3 and Figure 4. In comparison with G2K, G2K-NH<sub>2</sub> sample decorated with amino groups reveals increased *ESR* values and higher VPTT value. Comparing both samples, the increment of G2K-NH<sub>2</sub> *ESR* relative to that of G2K is small at temperatures below 16 °C, and such a increment becomes especially significant in the range from 20 °C to 35 °C, as demonstrated in Figure 3. For a hydrogel, the *ESR* value, an indicative of water sorption capacity, relies on the water affinity of hydrogel components and network structure. Here the so called network structure may mean the spaces to hold water molecules in networks, which is related with crosslinking density. In this research, both G2K and G2K-NH<sub>2</sub> samples should have similar crosslinking density due to the same

dosage of PNIPA2K, i.e., the mesh size in their fully swollen hydrogels should be approximate. In this regards, water sorption capacity of G2K-NH<sub>2</sub> is restrained to some extent by stretched chains between crosslinks although hydrophilic amines is incorporated, because it has been highly swollen at such low temperatures, like the case of G2K. Therefore, the *ESR* difference between G2K and G2K-NH<sub>2</sub> at temperatures below 16 °C is not so notable. Afterwards, the hydrophobic interaction in hydrogels becomes stronger and stronger with increasing temperature due to the dehydration of isopropyls. However, this interaction is suppressed to a larger degree due to the existence of hydrophilic amino groups, leading to the slowing down of G2K-NH<sub>2</sub> *ESR* changing with temperature increasing. In addition, the hydrophilic/hydrophobic balance is also affected by amino groups, so VPTT of G2K-NH<sub>2</sub> is delayed by near 10 °C compared with that of G2K, as demonstrated in Figure 4.

Moreover, G2K-NH<sub>2</sub> sample is imparted with pH sensitivity because of the incorporation of amine, and *ESR* values at different pH is plotted in Figure 5. Herein, the swelling media are PBSs with an ionic strength of 0.1 N, and the measuring temperature is 25 °C. It can be seen that G2K-NH<sub>2</sub> has a typical pH responsibility in the range between pH 2 and pH 10: the hydrogel swells highly at pH values below 3; deswelling appears and becomes more seriously with gradually increasing pH values from 4 to 10. The change of *ESR* values with pH rising is easy to understand because there are different protonation levels of amine at lower and higher pH values. Namely, the higher protonation degree of amine at lower pH stands for stronger hydrophilicity, and vice versa. Through the curve in Figure 5, it can be induced that



pKa of amino groups in G2K-NH<sub>2</sub> is between 6 and 7, lower than that of propargyl amine (pKa = 8.15),<sup>42</sup> probably as a result of the better electron withdrawing ability of triazole than that of alkyne. In this work, the successful introduction of pH sensitive amino groups accompanied by crosslinking via click chemistry, provides a good reference for endowing of other functions such as electrochemical, light sensitivities, luminescence, and so on.

### SEM photographs of freeze-dried hydrogel networks

In order to characterize internal network structures of hydrogels, SEM measurement was performed for freeze-dried samples, which had been swollen at 20 °C, and the SEM photographs are shown in Figure 6. It can be observed that G4K has bigger pore size than G2K because the former one has longer distance between crosslinks and corresponding higher water content. While for G12K and G18K with much higher ESR values, the pore size seems not higher than that of G4K, and porous structure of G18K clearly becomes non-uniform. In fact, higher water content corresponds to lower polymer fraction in hydrogel, resulting in thinner pore walls. In this case, the supporting force of crosslinked networks becomes weaker, and the partial shrinking and/or collapse is unavoidable in the course of freeze-drying.<sup>43</sup> Meanwhile interconnected porous structure is observed for G18K sample, which should arise from combined action of high water content and freeze-drying process. Comparing the cases of G4K and G2K-NH<sub>2</sub>, both have similar water content at 20 °C before freeze-drying (Figure 3), but the porous structure of the former is relatively regular and that of the latter is interconnected. Actually, G2K-NH<sub>2</sub> sample has more crosslinkages than G4K, and the introduced amine helps the former one to absorb more water than its analogue G2K. Thus the interactions between polymer and water molecules as well as water structures and properties inside G2K-NH<sub>2</sub> and G4K samples may be somewhat different, causing the difference of their network structures.

### Conclusions

Several well-defined linear  $\alpha,\omega$ -bis propargyl PNIPAs with different molecular weights were prepared through RAFT polymerization by using a bis propargyl terminal CTA. They were applied to crosslink with an azido-PNIPA via click chemistry, fabricating a series of PNIPA hydrogels in high efficiency. As molecular weights of bis propargyl PNIPAs increased, the obtained hydrogels showed increasing ESR values in swollen state and rising VPTTs due to the lengthening distance between crosslinks and the weakening hydrophobicity. The presence of residual azido groups besides those for crosslinking supplied a chance to simultaneously incorporate other functions. In this study, amine was introduced as an example, imparted the hydrogel with better water affinity, higher VPTT and additional pH sensitivity. Furthermore, the microscopic structures of freeze-dried hydrogels are found to be different with various samples according to SEM observation. In summary, this work provides an effective approach to adjust temperature sensitivity or to modify some special properties, by controlling the chain length of crosslinkers or by introducing other functional groups, in order to satisfy various application demands. For instance, when they were used as temperature responsive sensors or actuators, their

sensitivities or motion amplitudes can be easily modulated by designing hydrogel formulations.

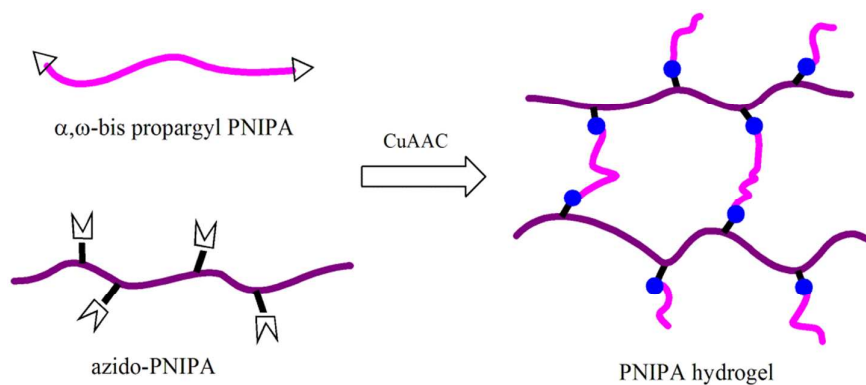
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### Notes and references

- <sup>a</sup> School of Materials Science and Engineering, Beijing Institute of Technology, Beijing, 100081, China. Tel: +86-10-68940942; E-mail: jqwang@bit.edu.cn
- <sup>b</sup> School of Life Science, Beijing Institute of Technology, Beijing, 100081, China.
- † Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/
- ‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.
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Poly(N-isopropylacrylamide) (PNIPA) hydrogels were fabricated through click chemistry by using azido-PNIPA and linear  $\alpha,\omega$ -bis propargyl PNIPAs with different chain lengths. This work provided a facile method to regulate swelling properties and/or impart special functions for PNIPA hydrogels, by adjusting the chain length of crosslinkers or by introducing other functional groups.