Electronic Supplementary Material (ESI) for Chemical Communications

Bioreducible nanogels/microgels easily prepared via temperature induced self-assembly and self-crosslinking

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1. Materials

Both 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) (95%, Aldrich) and oligo(ethylene glycol) methacrylate (OEGMA, Mn ～ 475, Aldrich) were purified by passing through a column filled with basic alumina to remove the inhibitors. 2, 2'-Azobisisobutyronitrile (AIBN, Sigma) was re-crystallized twice from ethanol. Absolute ethanol, dichloromethane, cystamine dihydrochloride, hydrofluoric acid (40 wt %), and sodium hydroxyl were purchased from Sinopharm Chemical Reagent Co. Ltd. (SCRC, China) and used as received. Acryloyl chloride (SCRC, China) was freshly distilled before use. 1-(2-Aminoethyl)piperazine (AEPZ, 99%, Sigma-Aldrich), *N*,*N*'-methylene bisacrylamide (MBA, 99%, Sigma-Aldrich), methanol (99.8%, Sigma-Aldrich), acetone (99.5%, Aldrich), *N*,*N*-dimethylformamide (DMF, >99.0%, Sigma-Aldrich) were used as received without further purification. Water was deionized to 18MΩ-cm resistivity using the Nanopure system.

2. Characterizations

^{1}H NMR and ^{13}C NMR studies were performed on a Bruker spectrometer (300 MHz). The weight-average molecular weight (M_w), number-average molecular weight (M_n) and polydispersity index of the polymers were determined by size exclusion chromatography (SEC) using a Shimadzu LC-10ADVP liquid chromatography equipped with a Polymer Labs PL gel 5 μm mixed C column. The system was

equipped with a refractometer. *N*,*N*-dimethylformamide (DMF) was used as an eluent at a flow rate of 1.0 mL/min and temperature of 35 °C. FT-IR spectra were recorded on a Bruker spectrometer using KBr window and all the measurements were performed at ambient temperature (ca. 25 ^{o}C). The determination of hydrodynamic diameters was performed by dynamic light scattering using a on a Malvern Zetasizer Nano ZS90. G' and G" were conducted on a AR G2 (TA Co., USA) with a Peltier device for temperature control. The determination of hydrodynamic diameters was performed by dynamic light scattering using a Malvern Zetasizer Nano ZS90 instrument (Malvern Co.) equipped with a 4 mW He-Ne laser (λ= 633 nm) at 90^{o} scattering angle. The reported method was applied to analyze the DLS autocorrelation function. The hydrodynamic diameter d_h was calculated using the Stokes-Einstein equation as $d_h = \kappa_B T/3\pi\eta_o D$, where κ_B is the Boltzmann constant, T is the absolute temperature, η_o is the sample viscosity and D is the diffusion coefficient.

3. **Synthesis of cumyl dithiobenzoate (**CDB**).**

Cumyl dithiobenzoate was prepared as following method: benzyl chloride (6.3 g, 50.0 mmol) was added dropwise into a flask containing elemental sulfur (3.2 g, 100.0 mmol), 25-28% sodium methoxide solution in methanol (40.0 g) and methanol (35 mL). Then the solution was heated and refluxed at 80 ^{o}C for 20 h, consequently, the mixture was filtered to remove the solid. After removing methanol, the resulting solid was dissolved in water and acidified by hydrogen chloride, and washed with diethyl ether (50 mL×3), diethyl ether layer was dried in anhydrous sodium sulfate after it was washed with water (30 mL×3). Dithiobenzoic acid was obtained via removing diethyl ether. This acid was then dissolved in tetrachloromethane and reacted with α-methylstyrene (1:1). Cumyl dithiobenzoate was isolated by column chromatography.

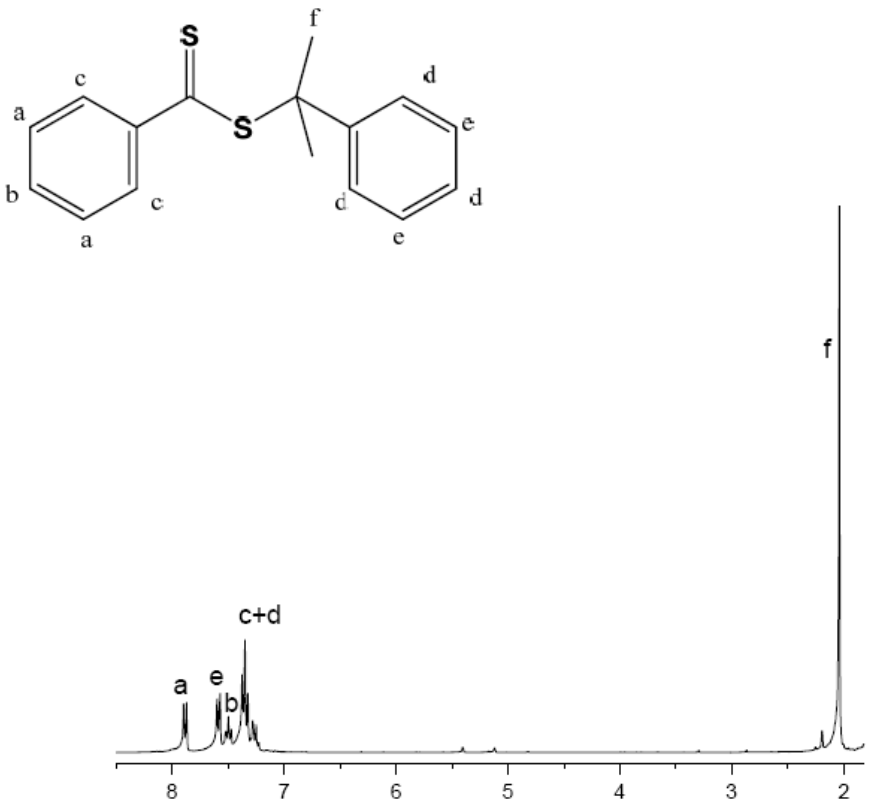


Figure S1. ^{1}H NMR spectrum of cumyl dithiobenzoate

4. Synthesis of *N,N'*-cystaminebisacrylamide (CBA).

Cystamine dihydrochloride (5.8 g, 25 mmol) was dissolved in 50 mL water in a 250 mL flask. Aqueous solution of NaOH (10 mL, 10 M) and solution of acryloyl chloride (4.7 g, 50 mmol) in dichloromethane (5 mL) were added dropwise at the same time, respectively, under stirring at 0 °C. The reaction was performed for 3 h at room temperature after the addition was complete. The product was obtained by filtration and crystallization from ethyl acetate.

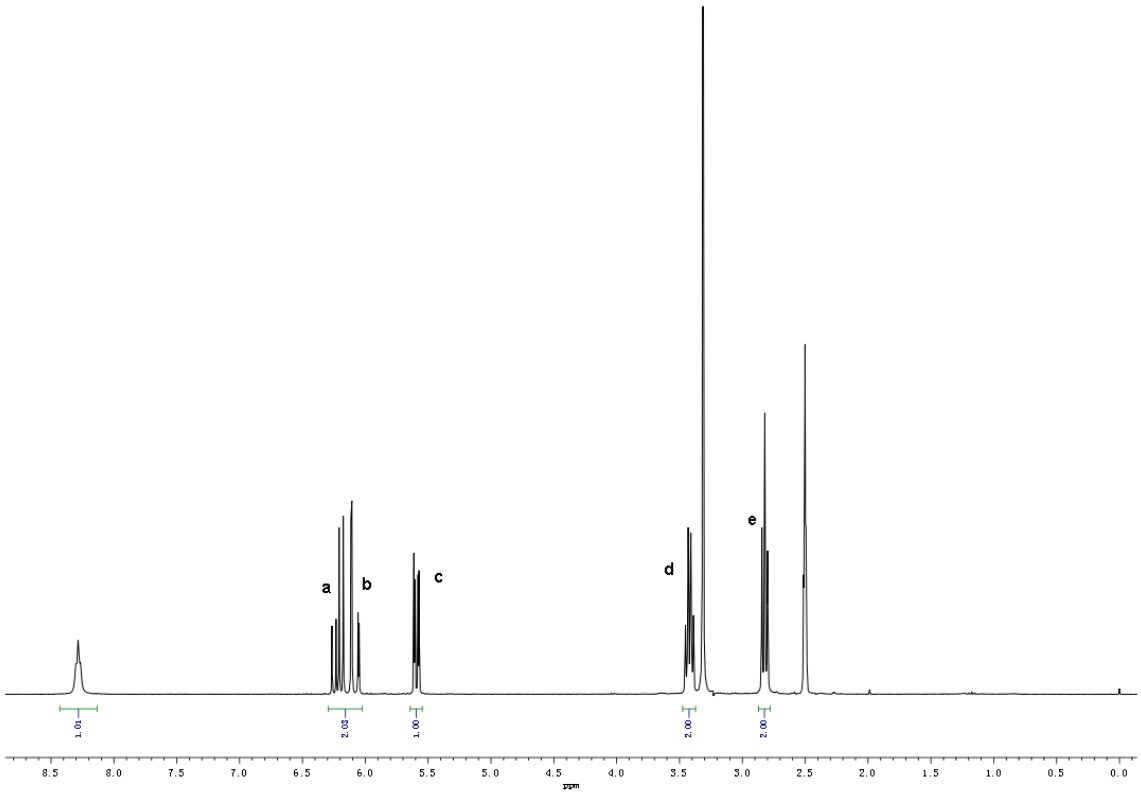


Figure S2. ^{1}H NMR spectrum of CBA in d_6-DMSO.

5. **Synthesis of disulfide containing hyperbranched poly(amido amine).**

The synthesis of disulfide contained hyperbranched poly(amido amine) via Michael addition copolymerization (as shown in **Scheme S1**) is similar to early papers.[1-3] In a typical experiment, AEPZ (129.0 mg, 1.0 mmole), CBA (521.0 mg, 2.0 mmole) (the molar ratio of AEPZ to CBA is 1:2) were added into a vial and dissolved in methanol/water mixture (6.0 mL, 8/2 v/v). The reaction was allowed to proceed at 50 ^{o}C for 38 h, yielding hyperbranched polymer with vinyl terminals. Then AEPZ (200 mg) was added to terminate the vinyl units. The yield is 71%, MW is 25K and PDI is 2.2.

Scheme S1. The synthesis of disulfide containing hyperbranched poly(amido amine).

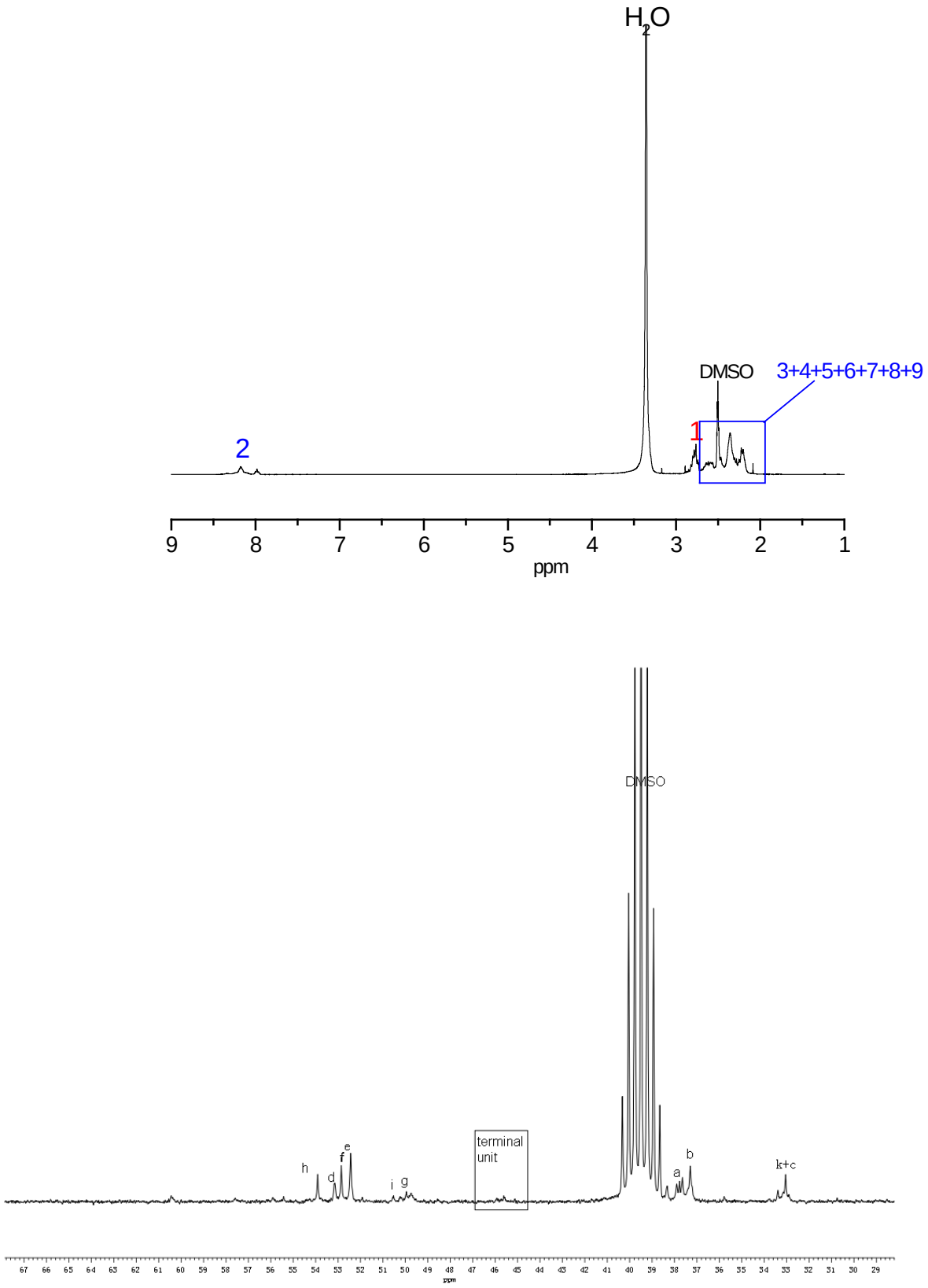


Figure S3. ^{1}H NMR and ^{13}C NMR spectra of disulfide containing hyperbranched poly(amido amine) in d_6-DMSO.

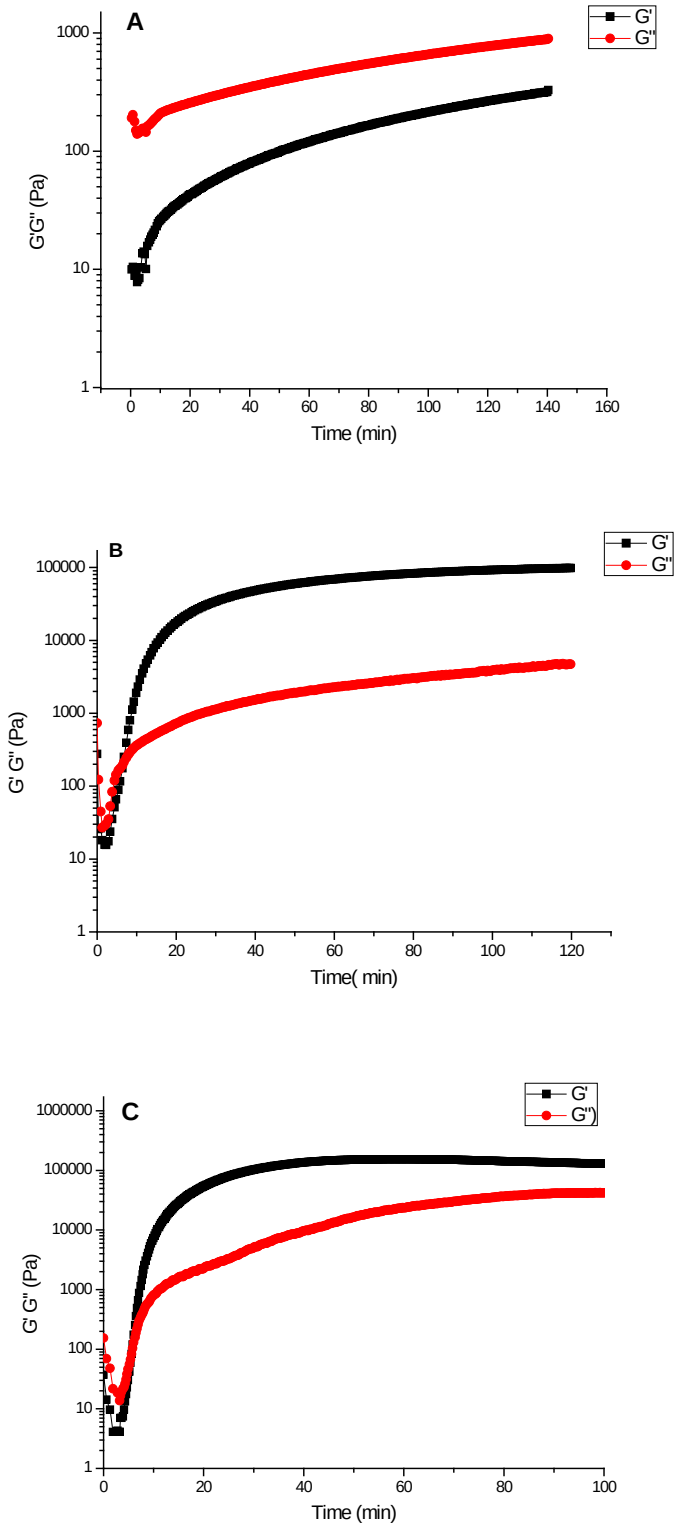
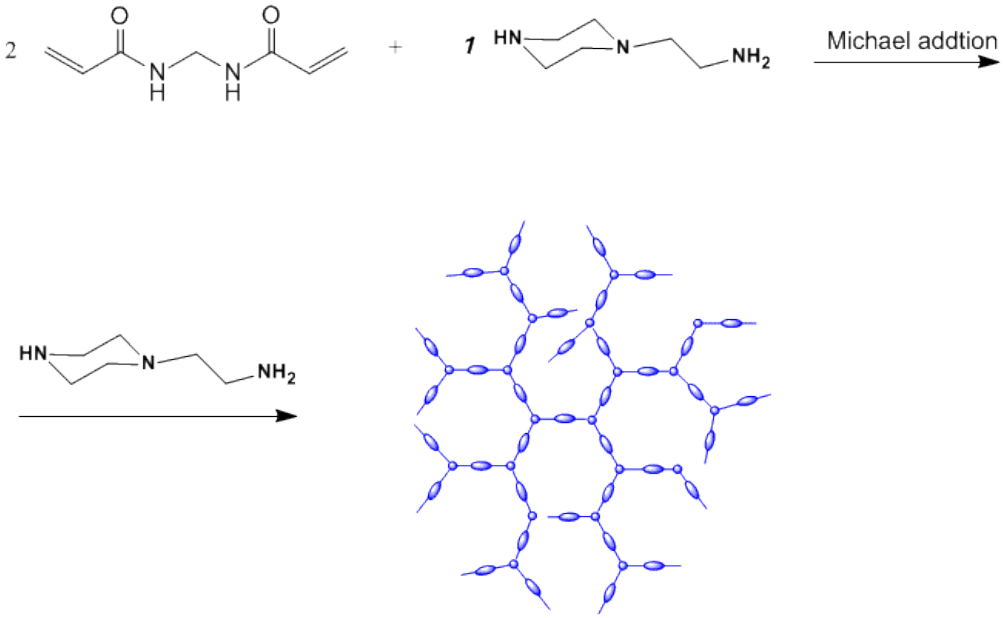


Figure S4. The variation of G' and G" with time at 25°C (A), 45 °C and 60 °C (C).

6. Synthesis of hyperbranched poly(amido amine) without disulfide bond.

The synthesis of hyperbranched poly(amido amine) without disulfide bond by

Michael addition copolymerization (as shown in **Scheme S2**) is similar to early papers.[1-3] In a typical experiment, AEPZ (129.0 mg, 1.0 mmol), and MBA (258.6 mg, 2.0 mmol) (the molar ratio of AEPZ to MBA is 1:2) were added into a vial and dissolved in methanol/water mixture (6.0 mL, 8/2 v/v). The reaction was allowed to proceed at 50 ^{o}C for 53 h, yielding hyperbranched polymer with vinyl terminals. Then AEPZ (200 mg) was added to terminate the vinyl units. The yield is 62%, *M*W is 28K and PDI is 1.7.



Scheme S1. The synthesis of hyperbranched poly(amido amine) without disulfide linkages.

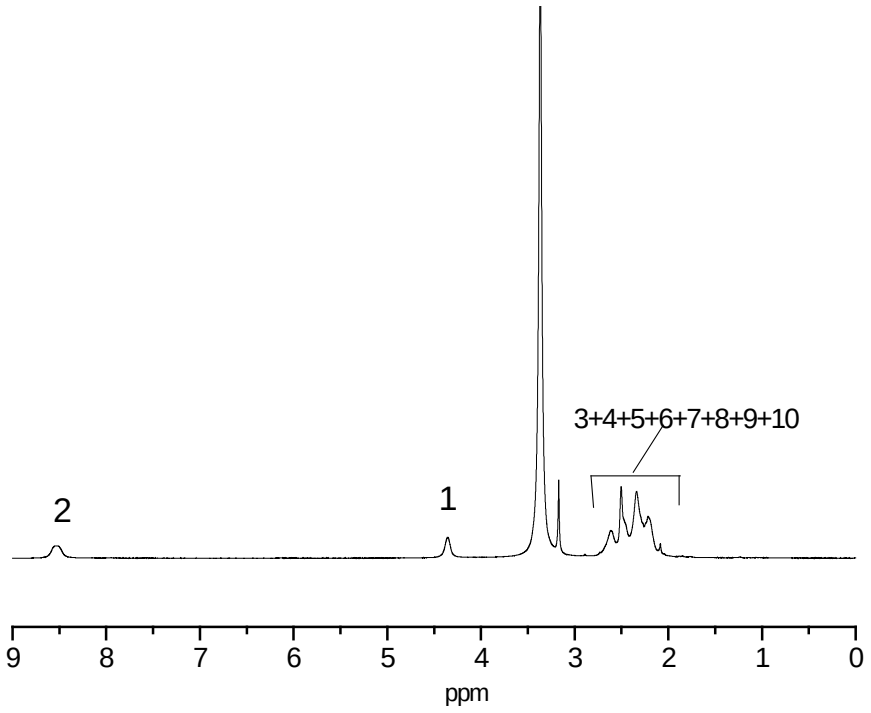
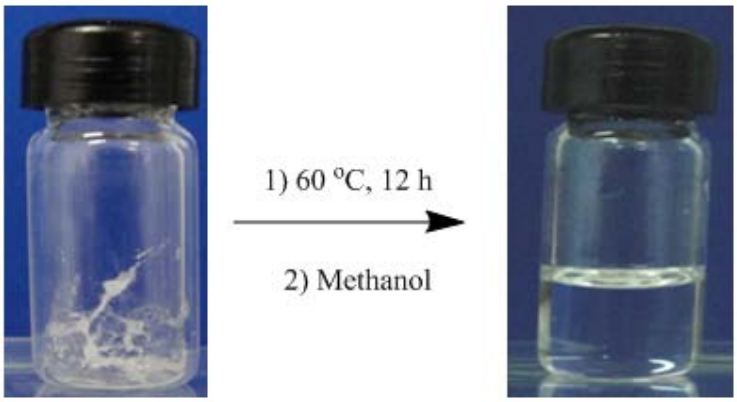


Figure S5. ^{1}H NMR spectrum of hyperbranched poly(amido amine) without disulfide bond in d_6-DMSO

A



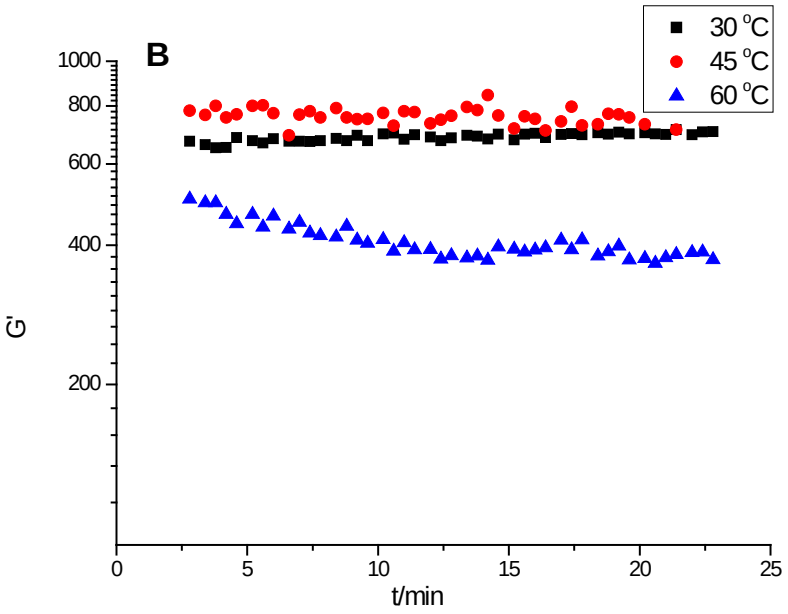
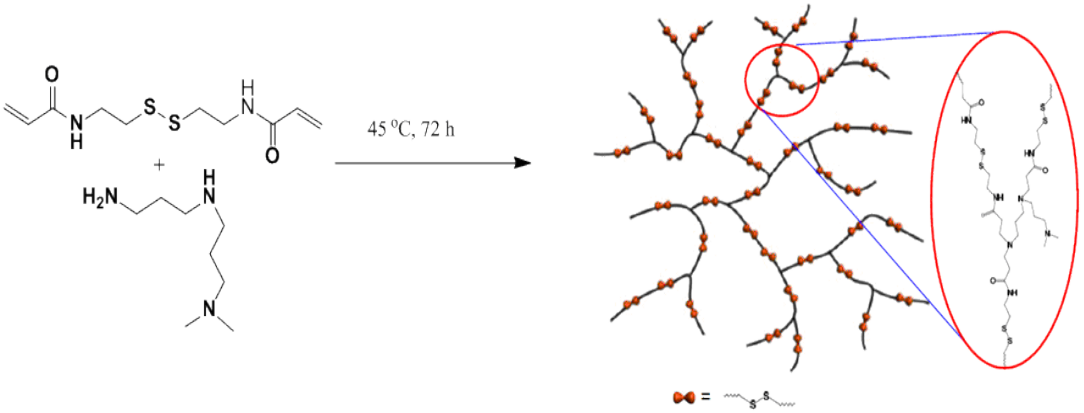


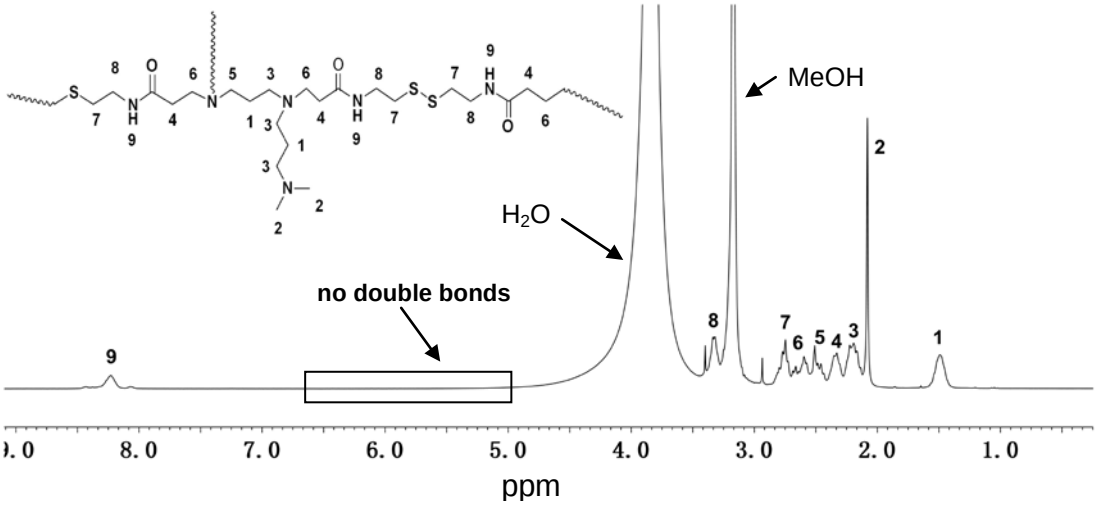
Figure S6. A. Hyperbranched poly(amido amine) (30.0mg) without disulfide is uncrosslinked after it has been kept at 60 ^{o}C for 12 h; B. The variation of G' with time at 30, 45, 60 ^{o}C.

7. **Synthesis of temperature-responsive disulfide containing hyperbranched poly(amido amine).**

The synthesis of disulfide contained hyperbranched poly(amido amine) via Michael addition copolymerization (as shown in **Scheme S3**) is similar to early papers.[1-4] One typical procedure is: N,N-dimethyldipropylenetriamine (DMDPTA, 159.0 mg, 1.0 mmole), CBA (260.2 mg, 1.0 mmole) were added into a vial and dissolved in methanol/water mixture (3.0 mL, 5/5 v/v). The reaction was allowed to proceed at 45 ^{o}C for 72 h, yielding disulfide containing hyperbranched poly(amido amine) with MW of 9.5K and PDI of 1.2, the yield is 79%.



Scheme S3. The synthesis of temperature-responsive disulfide containing hyperbranched poly(amido amine) (S-HPAA).



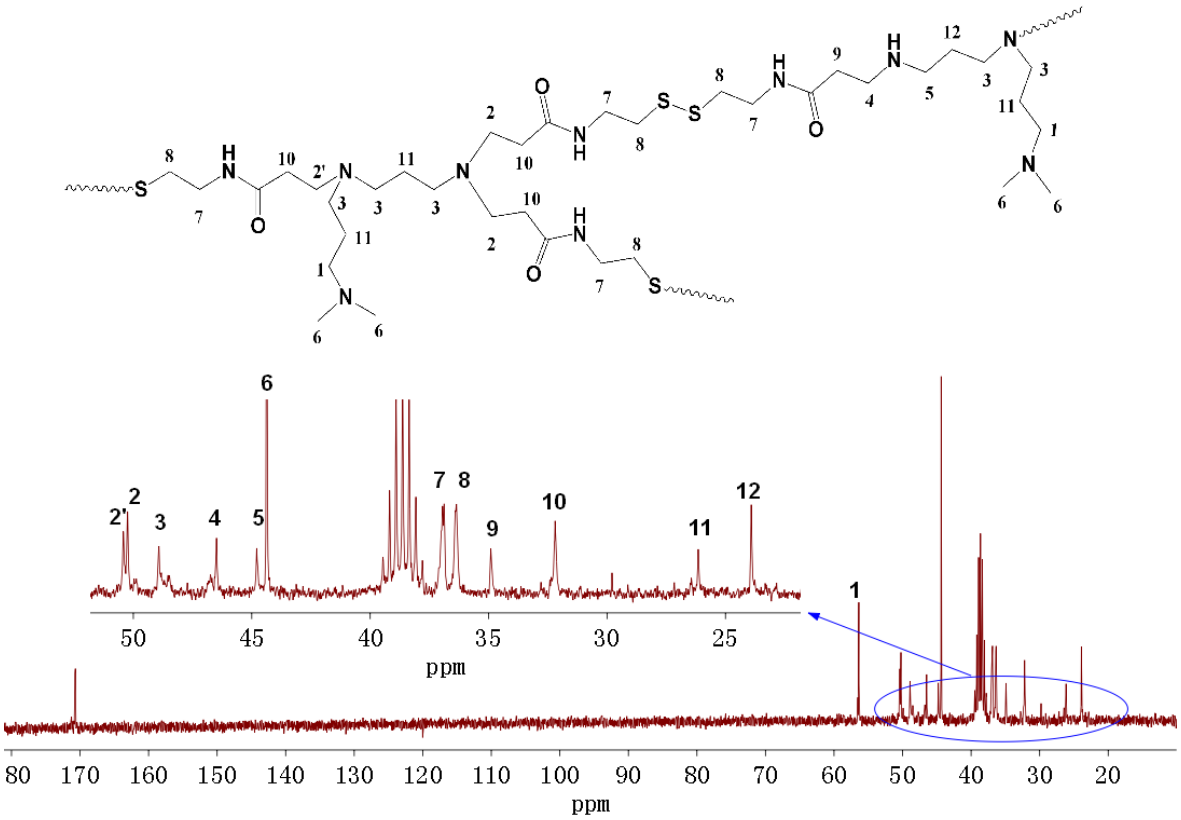


Figure S7 ^{1}H NMR and ^{13}C NMR spectra of temperature-responsive disulfide containing hyperbranched poly(amido amine) (S-HPAA).

1) S-HPAA is uncrosslinked upon heating in solution.

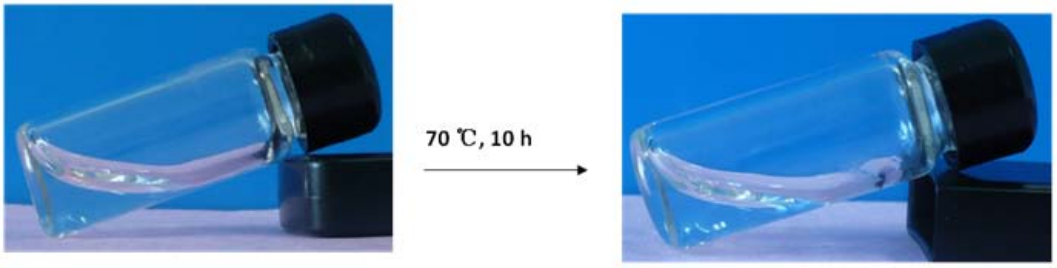


Figure S8 The images of S-HPAA DMSO solution (200 mg/mL) before and after remaining at 70 ^{o}C for 10 h.

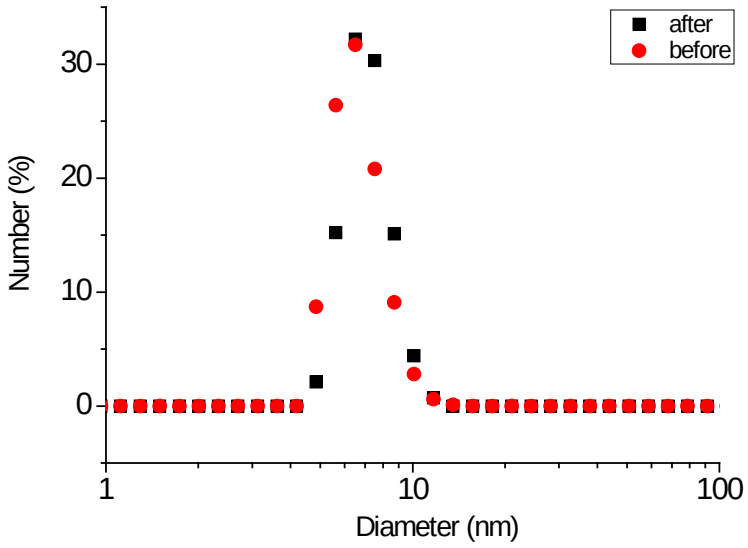


Figure S9. The hydrodynamic diameter of S-HPAA solution (200 mg/mL) before and after remaining at 70 ^{o}C for 10 h.

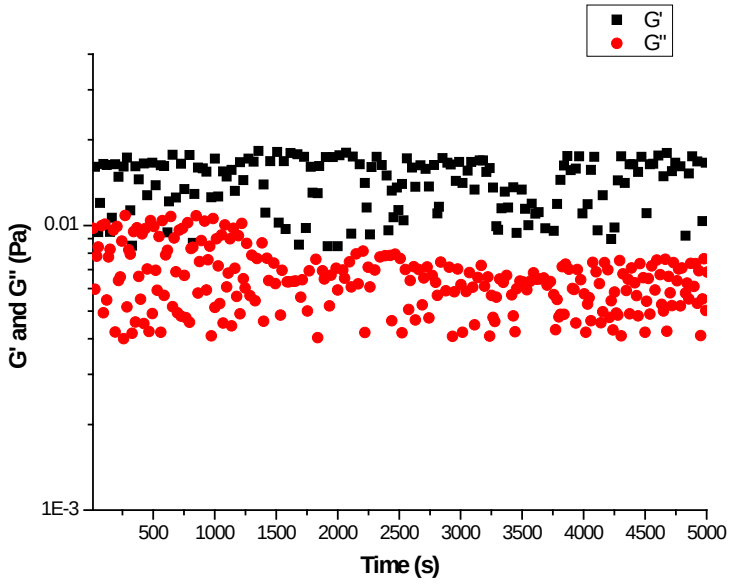


Figure S10. The variation of G' and G" of S-HPAA DMSO solution (200 mg/mL) with time at 70 ^{o}C.

2) S-HPAA is easily crosslinked upon heating in solid state.

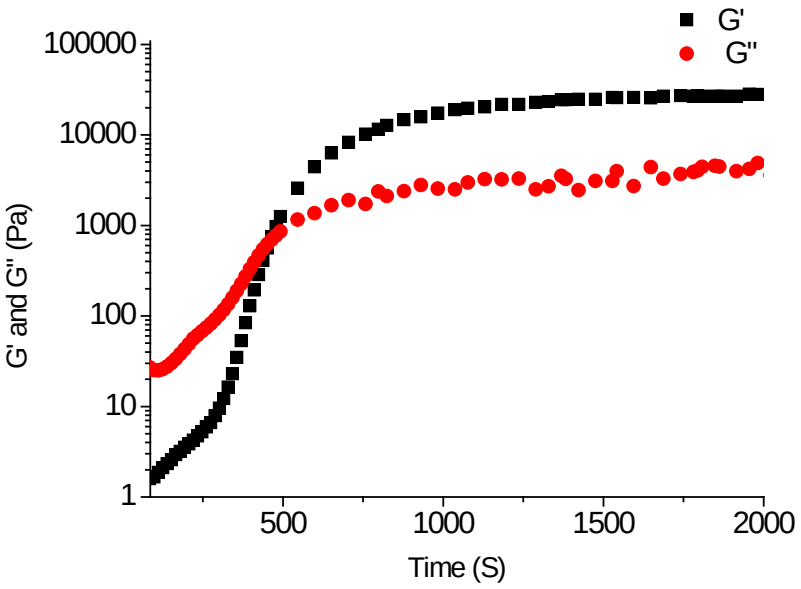


Figure S11. The variation of G' and G" with time at 70 ^{o}C.

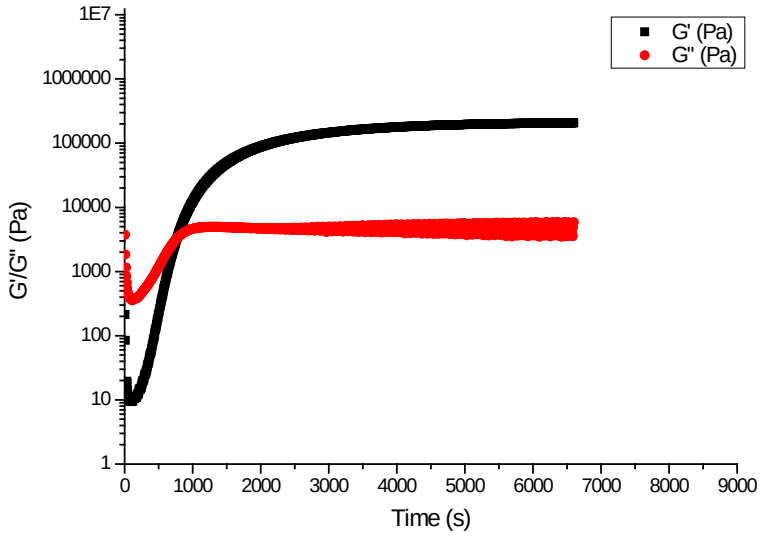
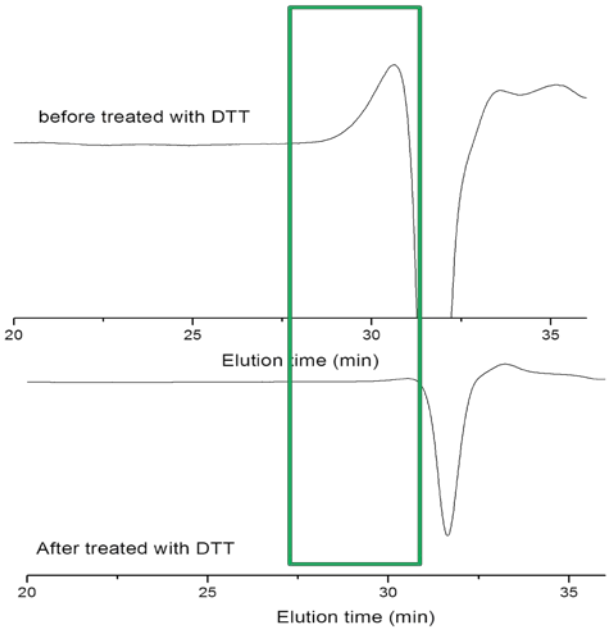


Figure S12. The variation of G' and G" with time at 45 ^{o}C.

3) The bio-reducibility of the prepared S-HPAA and nanogels/microgels



GPC curves of S-HPAA before and after treated with 10 mM DTT

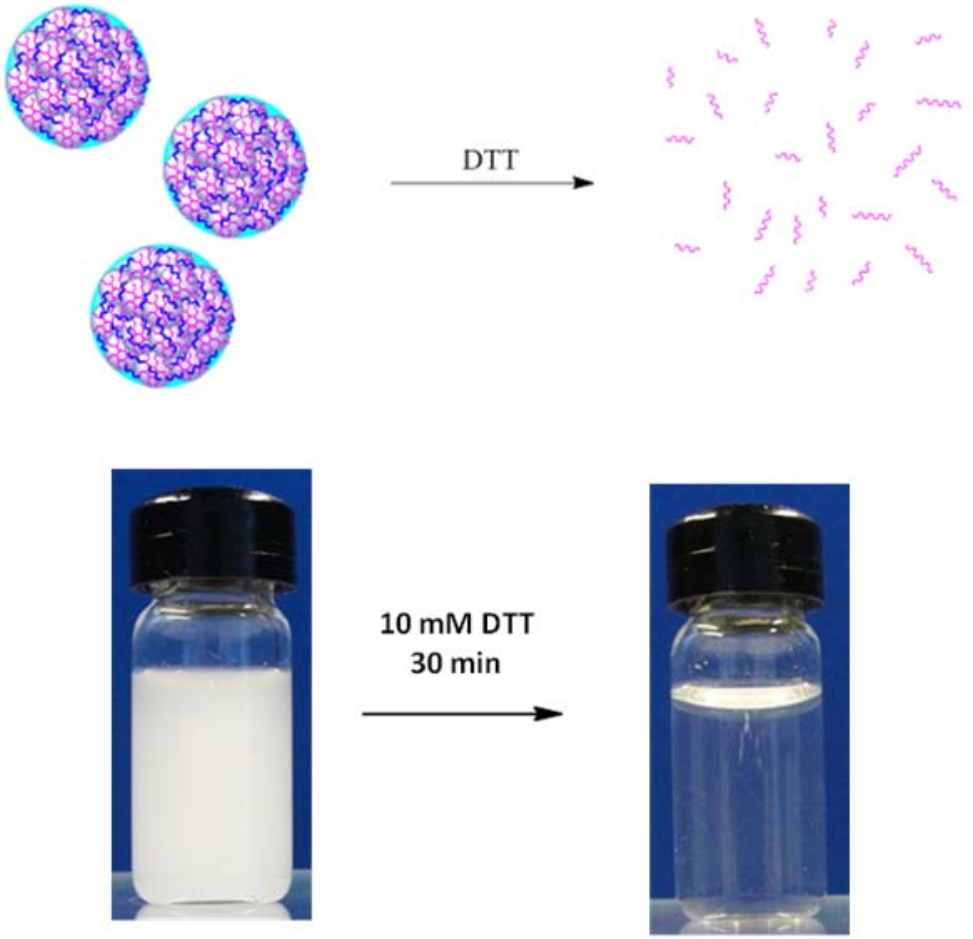
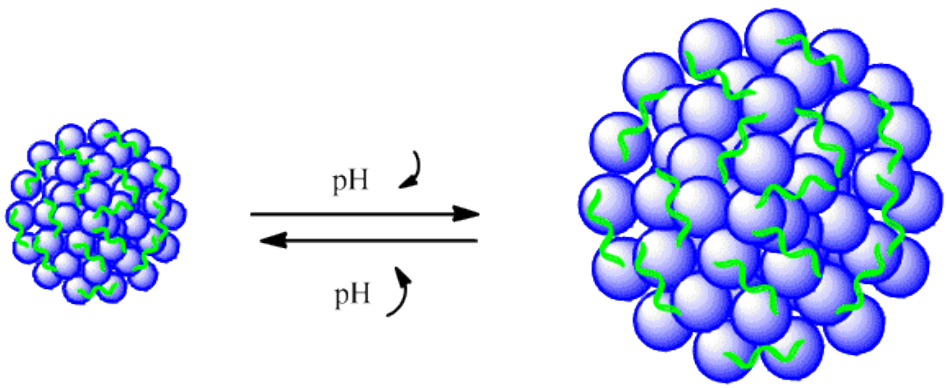


Figure S13 The images of the formed nanogels/microgels before and after being treated with 10 mM DTT.

4) pH-sensitivity of the prepared nanogels/microgels.



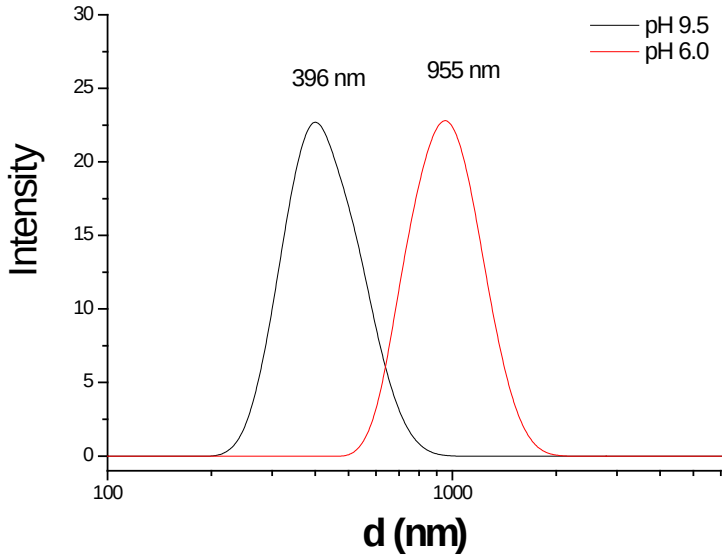


Figure S14. The size of nanogel/microgel responds to pH.

5). Biocompatibility of the prepared nanogels/microgels.

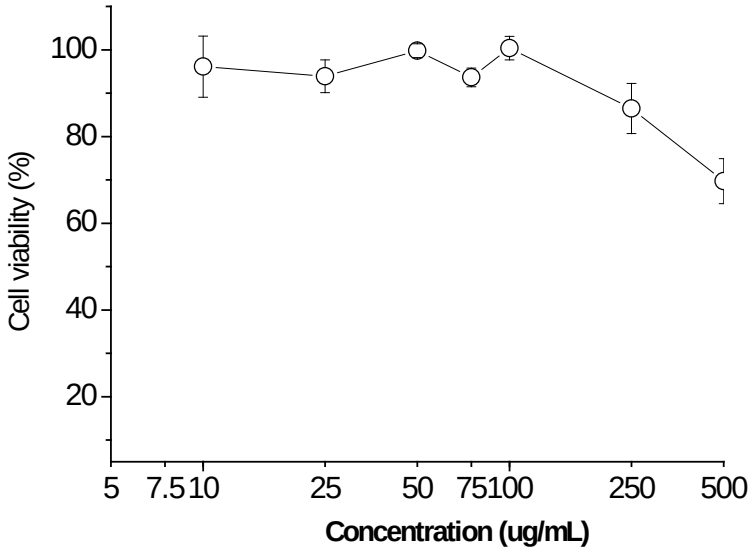


Figure S15 Cytotoxicity of nanogel was determined in HepG2 cells by MTT assay

8. **Control experiment (the polymer without disulfide):**

1) **Synthesis of temperature-responsive hyperbranched poly(amino ester) without disulfide linkage**.

One typical procedure is: N,N-dimethyldipropylenetriamine (DMDPTA, 159.0 mg,

1.0 mmole), bisacrylate (226 mg, 1.0 mmole) were added into a vial and dissolved in methanol/chloroform mixture (3.0 mL, 3/7 v/v). The reaction was allowed to proceed at 50 ^{o}C for 72 h, yielding hyperbranched poly(amino ester) with MW of 10K and PDI of 1.1, the yield is 83%..

Scheme S4. Synthesis of temperature-responsive hyperbranched poly(amino ester) without disulfide linkage.

2) Hyperbranched poly(amido ester) (without disulfide bond) solution becomes opaque after heating the solution to 50 ^{o}C, and the solution becomes clear after cooling the solution to 16 ^{o}C. The process is completely reversible.

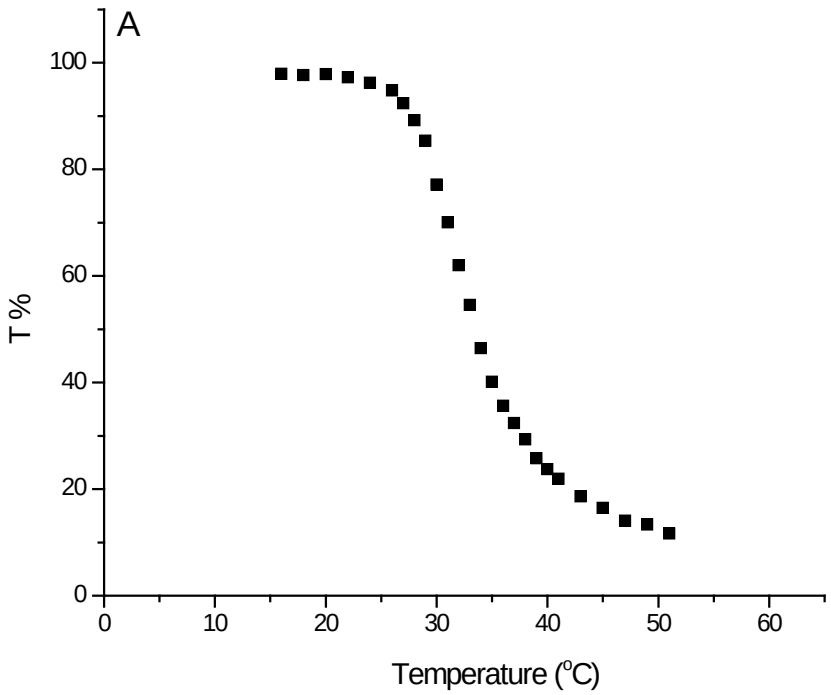


Figure S16. The variation of transmittance of hyperbranched poly(amino ester) (without disulfide bond in the backbone) solution with temperature.

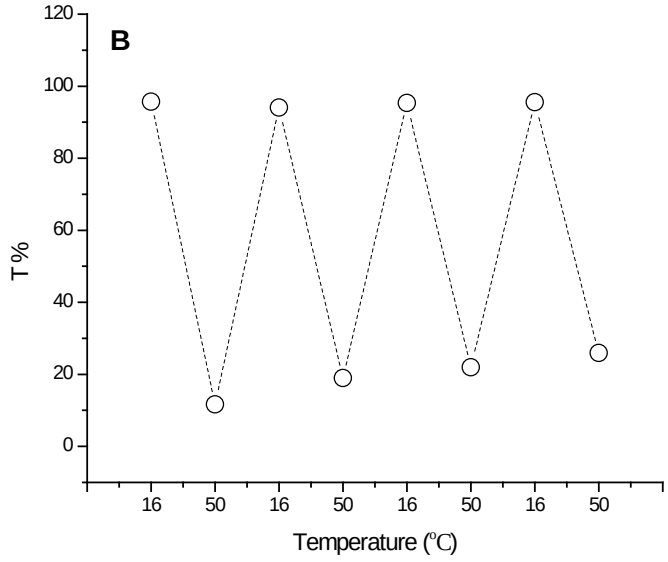


Figure S17. The variation of transmittance of hyperbranched poly(amino ester) (10.0 mg) without disulfide bond in the backbone) solution with the recycle of heating to 50 ^{o}C and cooling to 16 ^{o}C.

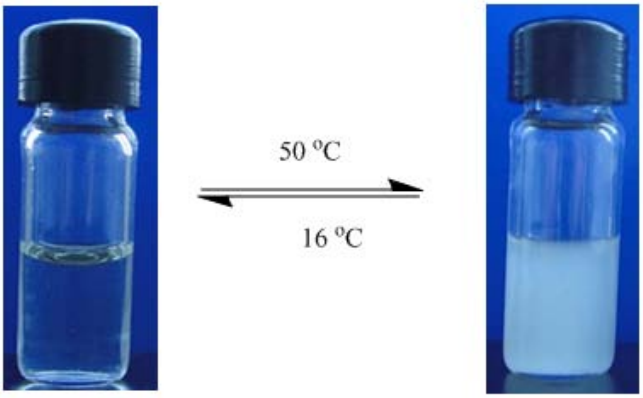


Figure S18 The images of hyperbranched poly(amino ester) solution in H_2O/MeOH (10.0 mg/mL, 9/1).

9. PEG based hyperbranched polymer with disulfide linkages and nanogels/microgels prepared from it.

1) PEG based hyperbranched polymer

In a typical procedure as shown in Scheme S1, MEO_2MA (564 mg, 3.90 mmol),

OEGMA (950 mg, 2.10 mmol), CDB (20.4 mg, 0.075mmol), AIBN (2.5 mg, 0.015 mmol), and CBA (260 mg, 1 mmol) were dissolved with THF (5 mL) in a polymerization tube. The mixture was subjected to several cycles of freeze-pump-thaw, and then the polymerization tube was vacuum sealed. The sealed tube was placed in a preheated oil bath at 60 °C. After 24 h, the polymerization was terminated by rapid cooling in liquid nitrogen and exposure to air. The resulting polymer was isolated and purified by repeated precipitation in cold hexane and dried under vacuum, the polymer has MW of 27K and PDI of 1.53.

Scheme S5. The synthesis of PEG based hyperbranched polymer with disulfide linkages.

2) **Preparation of PEG based bioreducible nanogels/microgels**

PEG based hyperbranched polymer was dissolved in sodium dihydrogen phosphate aqueous solution (pH of 8.0), the concentration is 2.0 mg/mL and 4.0 mg/mL. Subsequently, the solutions were heating and remained at 45 for 25 min, and bioreducible nanogels/microgels formed.

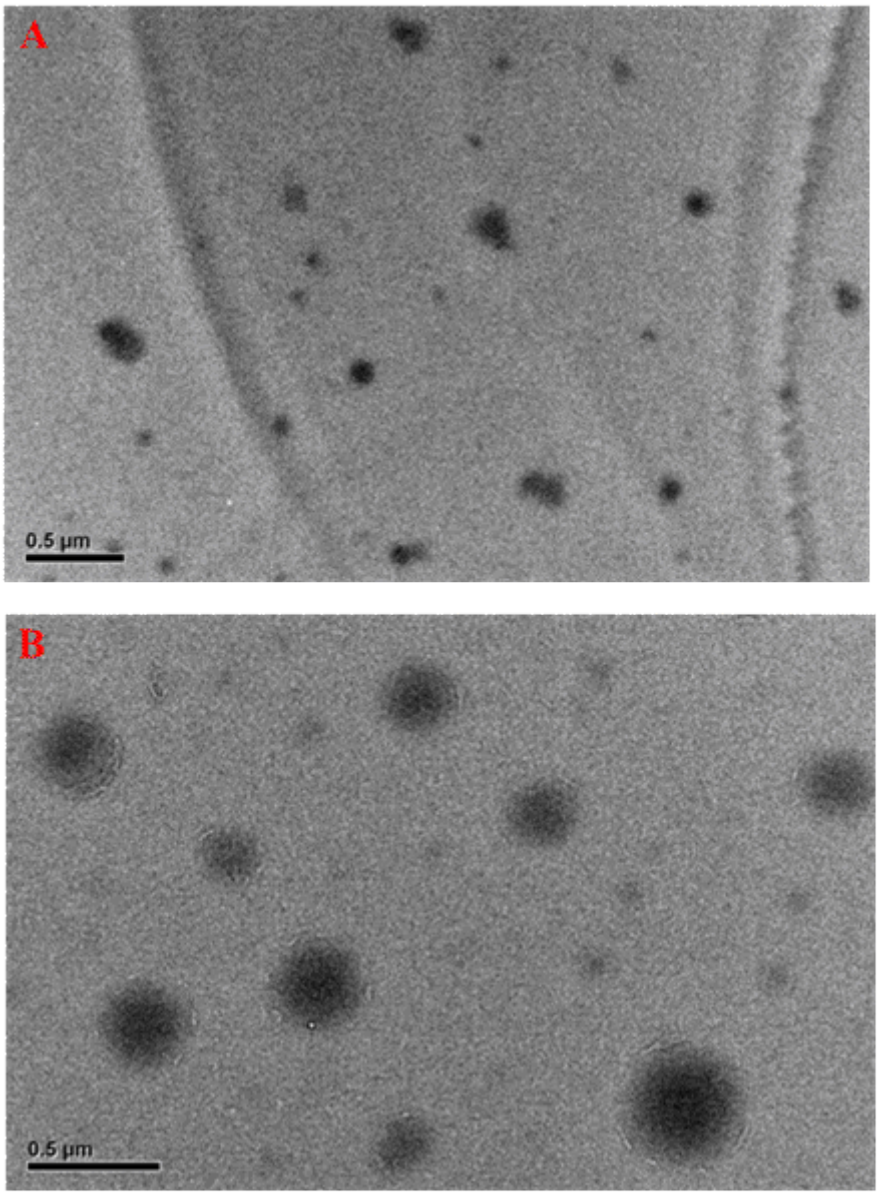


Figure S19. TEM images of nanogel prepared via TISASC method at concentration of 2.0 mg/mL (A) and 4.0 mg/mL (B).

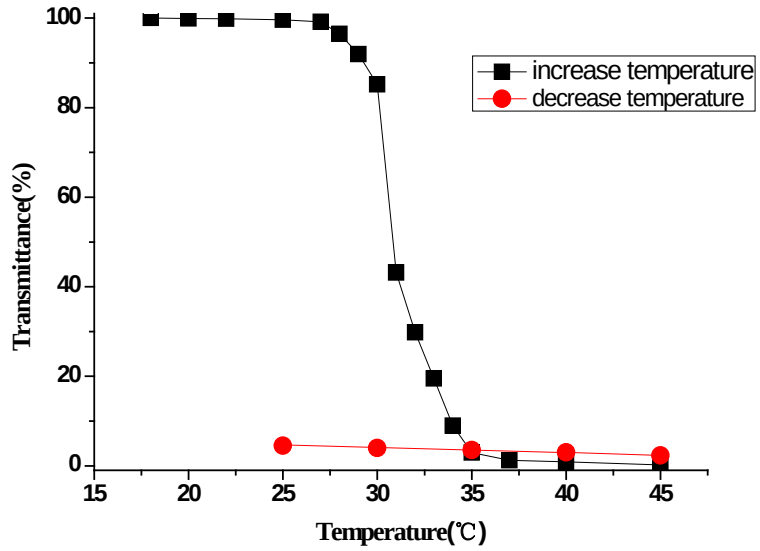


Figure S20. The variation of transmittance of PEG based hyperbranched polymer (with disulfide linkages) solution (4.0 mg/mL) with temperature.

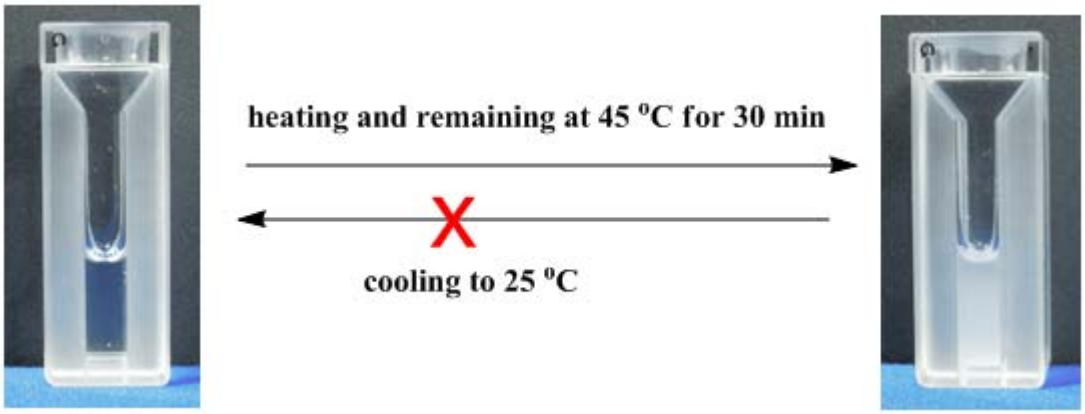


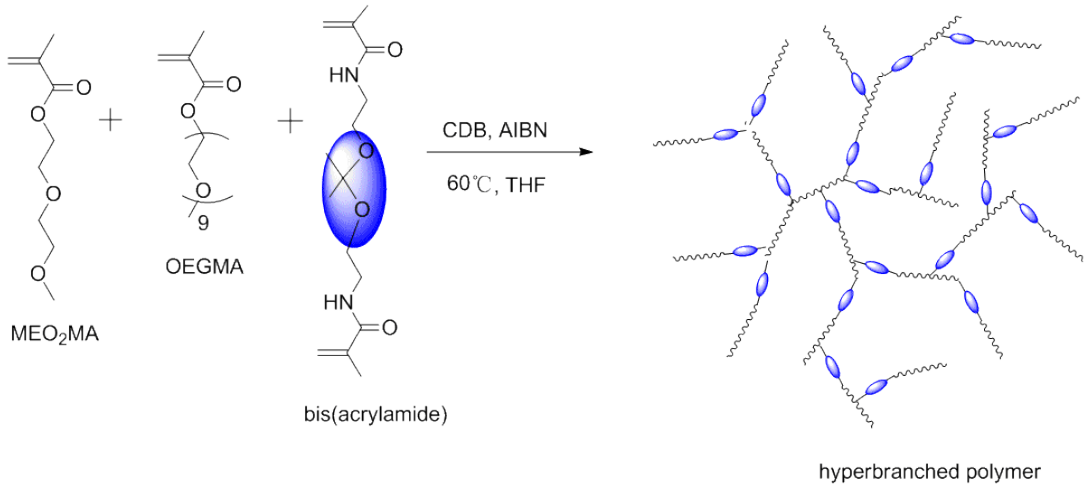
Figure S21. The images of heating and cooling PEG based hyperbranched polymer (with disulfide linkages) solution (4.0 mg/mL).

Figure S22. The hydrodynamic diameter of PEG based hyperbranched polymer solution (4 mg/mL) at 10 ^{o}C and 40 ^{o}C.

2). Control experiment (the polymer without disulfide):

a). PEG based hyperbranched polymer without disulfide linkage.

In a typical procedure as shown in Scheme S2, MEO_2MA (564 mg, 3.90 mmol), OEGMA (950 mg, 2.10 mmol), CDB (20.4 mg, 0.075 mmol), AIBN (2.5 mg, 0.015 mmol), and N,N'-((propane-2,2-diylbis(oxy))bis(ethane-2,1-diyl)) bis(2-methylacrylamide) (299 mg, 1 mmol) were dissolved with THF (5 mL) in a polymerization tube. The mixture was subjected to several cycles of freeze-pump-thaw, and then the polymerization tube was vacuum sealed. The sealed tube was placed in a preheated oil bath at 60 °C. After 24 h, the polymerization was terminated by rapid cooling in liquid nitrogen and exposure to air. The resulting polymer was isolated and purified by repeated precipitation in cold hexane and dried under vacuum, the polymer has MW of 59K and PDI of 1.88.



Scheme S6. The synthesis of PEG based hyperbranched polymer without disulfide linkages.

b). PEG based hyperbranched polymer (without disulfide bond) solution becomes opaque after heating the solution to 45 ^{o}C, and the solution becomes clear after cooling the solution to 25 ^{o}C. The process is completely reversible.

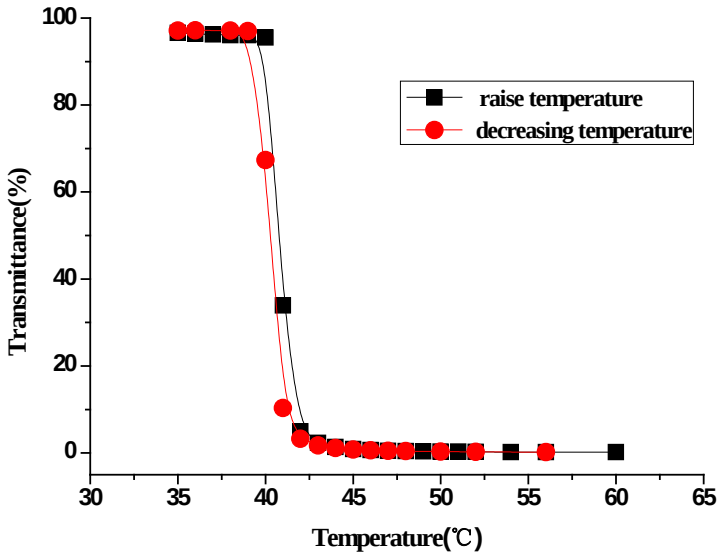


Figure S23. The variation of transmittance of PEG based hyperbranched polymer (without disulfide bond in the backbone) solution (4.0 mg/mL) with temperature.

Figure S24. The images of heating and cooling PEG based hyperbranched polymer (without disulfide linkages) solution (4.0 mg/mL).

References

1. Y. Z. You, Z. Q. Yu, M. M. Cui and C. Y. Hong, *Angew. Chem. Int. Ed.* 2010, **49**, 1099.
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