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A Responsive Hyperbranched Polymer Not Only Can Self-Immolate but Also Can Self-Cross-Link

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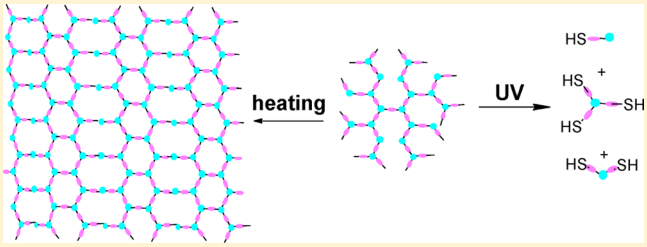
Supporting Information

ABSTRACT: Though many responsive polymers have been prepared, none of them can both self-immolate and self-cross-link via responding to the changes of the environment. Here, we introduce a new responsive hyperbranched polymer, which not only can self-immolate but also can self-cross-link via responding to the external stimuli. Moreover, the obtained polymer can form a bioreducible nanogel in its aqueous solution simply via heating, and the formed nanogel can self-immolate via UV irradiation.



INTRODUCTION

Nature can synthesize tailored macromolecules or biomacromolecules that can both assemble and disassemble via responding to the stimulus changes of the environment to sustain life and maintain biological function.[1] The synthetic macromolecules with responsibility are often prepared for a broad range of applications,[2−7] and now these macromolecules are playing a very important role in drug delivery,[8,9] diagnostics,[10−12] biosensors,[10,13] microelectromechanical systems,[14] coatings,[15] and textiles.[1] Though many responsive polymers have been synthesized, most of them cannot self-degrade. In order to meet the demands in the field of biomaterials, the polymers that can self-degrade have been papered recent years,[16] which have self-immolative linkers in the backbone.[17,18] This new class of linker, which becomes labile via activation, leading to the rapid degradation of the parent polymer, has gained popularity in recent years in controlled release.[18−21] This ability has prompted numerous studies on the design and development of new self-immolative linkers and the kinetics surrounding their disassembly. However, most of the prepared polymers have poor solubility in water, and their preparation procedure is very complex.[17] On the other hand, disulfide-containing hyperbranched polymers have attracted great interest of polymer chemists for constructing bioreducible polymers used in nanomedicine.[22−24]

Based on previous findings, these polymers cannot self-immolate under external stimuli. In the current study, we introduce a novel macromolecule that can self-immolate and self-cross-link via responding to the changes of the environment. This macromolecule bears disulfide bonds in the backbone, *N*,*N*′-dimethylamine side units and 2-((2-nitrobenzyl)thio)ethanol terminals. The *N*,*N*′-dimethylamine unit is pH- and temperature-responsive, and hence the *N*,*N*′-dimethylamine side unit can be used as responsive units to trigger its assembly. Disulfide bonds in the backbone can undergo disulfide exchange via heating the aqueous solution of polymers, which can reversibly cross-link the macromolecules.[25−28] 2-((2-Nitrobenzyl)thio)ethanol terminals can *in situ* release thiols via UV irradiation,[29,30] which can further break the disulfide bonds in the backbone. Therefore, this kind of macromolecules not only can self-cross-link but also can self-immolate via responding to the stimuli-changes of the environment, which will have potential applications in nanomedicine.

EXPERIMENTAL SECTION

The synthesis of *N*-(2-aminoethyl)-4-(((2-hydroxyethyl)thio)methyl)-3-nitrobenzamide is shown in Scheme 1.

Synthesis of 4-(Bromomethyl)benzoic Acid (1). *p*-Toluic acid (10.88 g, 80 mmol), *N*-bromosuccinimide (NBS, 17.10 g, 96 mmol), and benzoyl peroxide (BPO, 200 mg, 0.8 mmol) were added to 100 mL of $CHCl_3$. The reaction was refluxed for 14 h under vigorous stirring. After cooling to room temperature, the precipitate was collected via filtration. The desired 4-(bromomethyl)benzoic acid (**1**) (12.5 g, yield is 72.5%) was then obtained by washing with hot water (3 × 100 mL), drying in vacuum, and recrystallizing from hot methanol. ^{1}H NMR (300 MHz, d_6-DMSO): δ (ppm), 7.92 (d, 2H), 7.56 (d, 2H), 4.75 (s, 2H).

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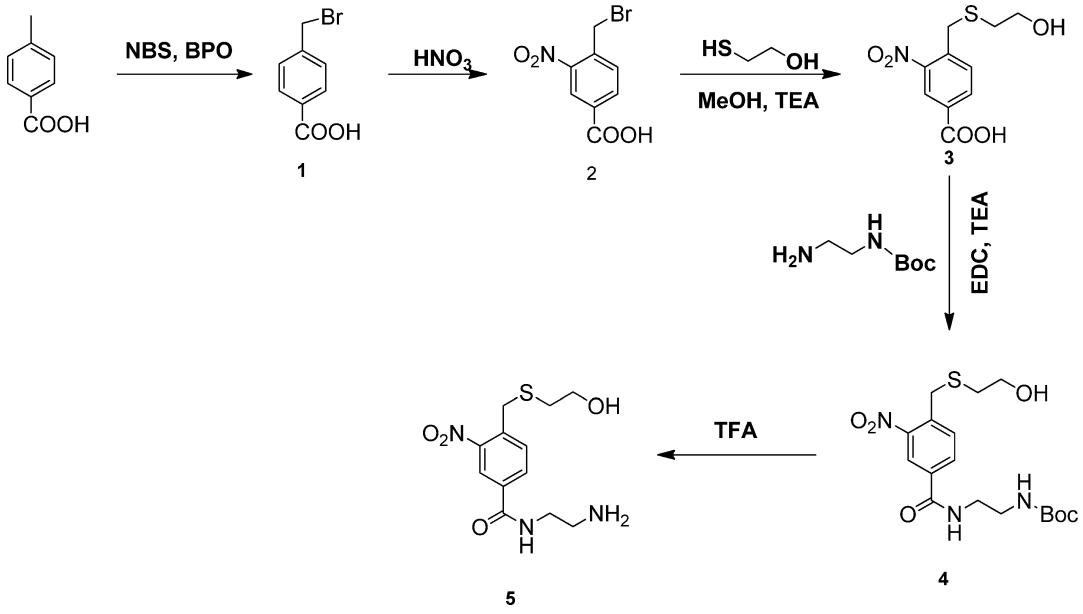
Scheme 1. Synthesis of *N*-(2-Aminoethyl)-4-(((2-hydroxyethyl)thio)methyl)-3-nitrobenzamide



Synthesis of 4-Bromomethyl-3-nitrobenzoic Acid (2). 4-(Bromomethyl)benzoic acid (**1**) (5.0 g, 23.2 mmol) was slowly added to fuming HNO_3 (50 mL) at −10 °C using a NaCl−ice bath. After 2.5 h, the reaction mixture was poured onto crushed ice; the product (4.5 g, 17.3 mmol, yield is 74.6%) was obtained by filtering, washing with cold water, and recrystallizing from dichloromethane−hexane (1/1, v/v). ^{1}H NMR (300 MHz, d_6-DMSO): δ (ppm), 8.48 (s, 1H), 8.24 (d, 1H), 7.90 (d, 1H), 4.99 (s, 2H).

Synthesis of 4-(((2-Hydroxyethyl)thio)methyl)-3-nitrobenzoic Acid (3). 2-Mercaptoethanol (0.94 g, 12 mmol, 1.2 equiv) and triethylamine (TEA, 2.02 g, 20.0 mmol, 2 equiv) were added to a solution of 4-bromomethyl-3-nitrobenzoic acid (2.60 g, 10 mmol, 1 equiv) in 40 mL of methanol under stirring at room temperature; subsequently, the reaction was performed at 50 °C for 5 h. Then, the reaction solution was concentrated and poured into 100 mL of HCl solution (2 M). The product (2.2 g, 8.5 mmol, yield is 85%) was obtained by filtering, washing with water, and drying in a vacuum. ^{1}H NMR (300 MHz, d_6-DMSO): δ (ppm), 8.41 (s, 1H), 8.16 (d, 1H), 7.72 (d, 1H), 4.11 (s, 2H), 3.45 (t, 2H), 2.48 (t, 2H).

Synthesis of *tert*-Butyl (2-(4-(((2-Hydroxyethyl)thio)methyl)-3-nitrobenzamido)ethyl)carbamate (4). 4-(((2-Hydroxyethyl)-thio)methyl)-3-nitrobenzoic acid (2.1 g, 8.2 mmol, 1 equiv) and triethylamine (TEA, 1.7 g, 16.4 mmol, 2 equiv) were dissolved in 50 mL of dichloromethane; subsequently, *tert*-butyl (2-aminoethyl)-carbamate (1.44 g, 9.0 mmol, 1.1 equiv) and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, 2.34 g, 12.3 mmol, 1.5 equiv) were added into the mixture. After stirring at room temperature for 24 h, the reaction solution was concentrated and poured into 100 mL of 5% acetic acid solution. The product (1.15 g, 2.87 mmol, yield is 35%) was obtained by filtering, washing with water, and drying in a vacuum. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm), 8.45 (s, 1H), 8.05 (d, 1H), 7.78 (s, broad, 2H), 7.56 (d, 1H), 5.04 (s, broad, 1H), 4.13 (s, 2H), 3.73 (t, 2H), 3.57 (t, 2H), 3.43 (t, 2H), 2.66 (t, 2H), 1.45 (s, 9H).

Synthesis of *N*-(2-Aminoethyl)-4-(((2-hydroxyethyl)thio)-methyl)-3-nitrobenzamide (5). *tert*-Butyl (2-(4-(((2-hydroxyethyl)-thio)methyl)-3-nitrobenzamido)ethyl)carbamate (1.14 g) was dissolved in 40 mL of dichloromethane; subsequently, trifluoroacefic acid (TFA, 10 mL) was added under stirring at room temperature. After 12 h, the product (0.68 g, yield is 80%) was obtained by concentrating the reaction solution, washing with diethyl ether twice, and drying in a vacuum. ^{1}H NMR (300 MHz, D_2O): δ (ppm), 8.41 (s, 1H), 8.98 (d, 1H), 7.61 (d, 1H), 4.11 (s, 2H), 3.68 (t, 2H), 2.60 (t, 2H), 3.22 (t, 2H), 2.61 (t, 2H).

Synthesis of *N,N′*-Cystaminebis(acrylamide) (CBA). After the mixture of cysteamine hydrochloride (11.6 g, 50 mmol) in water (50 mL) was cooled to 0 °C, acryloyl chloride (9.30 g, 100 mmol) in CH_2Cl_2 (10.0 mL) and aqueous NaOH solution (1.0 M, 100 mL) were added simultaneously via separated dropping funnels in 1 h at 0 °C. The reaction mixture was stirred for more 2 h at room temperature. Then, the reaction mixture was washed three times with deionized water, and the white powders were collected by filtration, recrystallized twice from ethyl acetate. Yield is 51%. ^{1}H NMR (300 MHz, d_6-DMSO): δ (ppm), 8.32 (s, 2H), 6.21 (q, 2H), 6.09 (d, 2H), 5.59 (d, 2H), 3.41 (t, 4H), 2.80 (t, 4H).

Synthesis of Hyperbranched Poly(amidoamine)s with 2-((2-Nitrobenzyl)thio)ethanol Terminals.[31] *N,N′*-Cystaminebis-(acrylamide) (CBA, 260 mg, 1.0 mmol) and *N,N′*-dimethyldipropylenetriamine (DMDPTA, 79.6 mg, 0.5 mmol) were added into a vial containing 5.0 mL of methanol/water mixture (7/3, v/v). Subsequently, the polymerization was carried out at 50 °C in the vial under an argon atmosphere. After the polymerization has been carried out for 5 days, *N*-(2-aminoethyl)-4-(((2-hydroxyethyl)thio)methyl)-3-nitrobenzamide (AHTMN, 600 mg) was added into the polymerization mixture to change the vinyl terminals into 2-((2-nitrobenzyl)-thio)ethanol terminals. M_n is 8.7 kDa and PDI is 2.09.

Preparation of Bioreducible Nanogels. The prepared hyperbranched polymer was dissolved in NaH_2PO_4 aqueous solution; the concentration was 5.0 mg/mL. pH value of the solution was tuned to 9.0 via adding NaOH solution; subsequently, the solution was heated and remained at 50 °C for 30 min, and bioreducible nanogels formed.

RESULTS AND DISCUSSION

2-Mercaptoethanol has good solubility in water, affording it with wide applications in biosystems. 2-Mercaptoethanol can act as a reducing agent to reduce the disulfide bond, like GSH.[32,33] When the disulfide-containing poly(amidoamine) is treated with 2-mercaptoethanol, the disulfide bonds will be broken (Figure 1A); for example, poly(amidoamine) with molecular weight of 7.8 kDa and PDI of 1.9 (see Supporting Information) can be reduced into small molecules with molecular weight of ∼300 Da after being treated with 2-mercaptoethanol for 30 min (Figure 1B). On the other hand, 2-((2-nitrobenzyl)thio)ethanol is protected unit for thiol, which is able to *in situ* release 2-mercaptoethanol via UV irradiation.[29,30] Therefore, we can envisage that if we prepare

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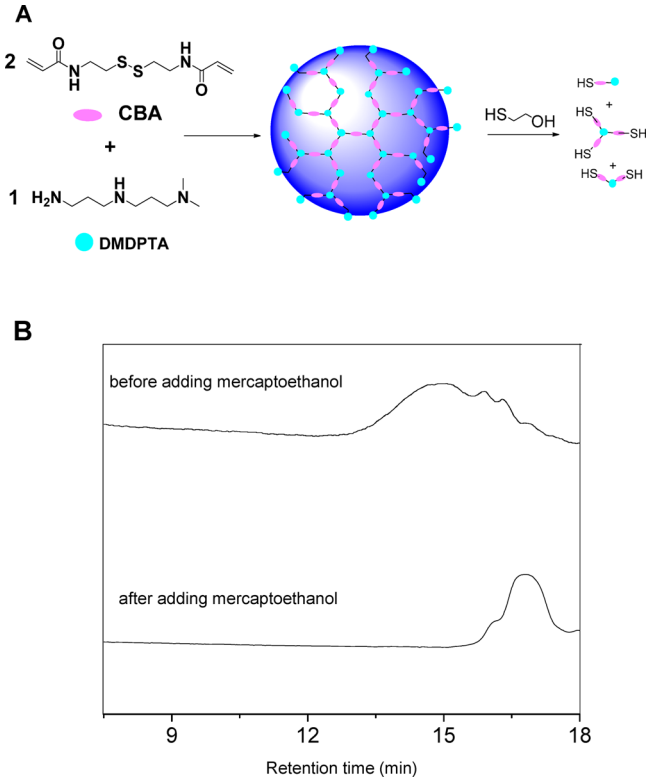


Figure 1. (A) Scheme of 2-mercaptoethanol reducing the disulfide-containing poly(amidoamine). (B) GPC traces of disulfide-containing poly(amidoamine) before and after being treated with 2-mercaptoethanol.

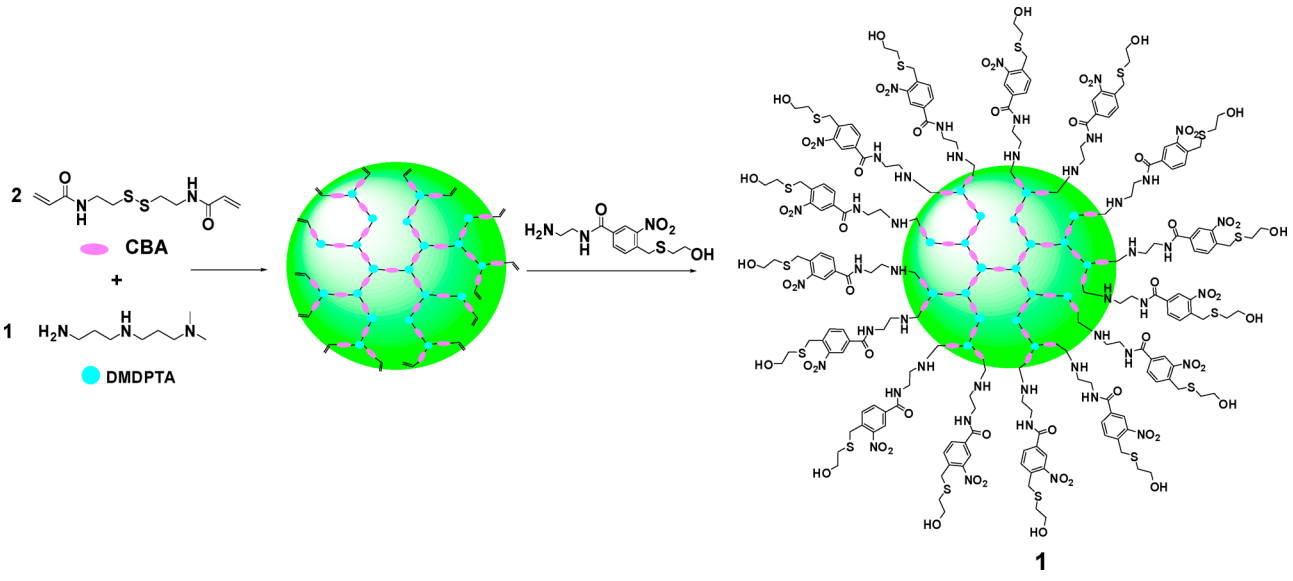
a hyperbranched polymer with both disulfide bonds and 2-((2-nitrobenzyl)thio)ethanol units, 2-((2-nitrobenzyl)thio)ethanol will release 2-mercaptoethanol via UV irradiation, and the released 2-mercaptoethanol can break the disulfide bonds in the polymer backbone, leading to the self-immolation of the hyperbranched polymer.

Michael addition polymerization of *N,N′*-cystaminebis(acrylamide) (CBA) and *N,N′*-dimethyldipropylenetriamine (DMDPTA) at the molar ratio of 2:1 produces hyperbranched poly(amidoamine) with vinyl terminals (HPAA-vinyl)[31] as shown in Scheme 2. Subsequently, *N*-(2-aminoethyl)-4-(((2-hydroxyethyl)thio)methyl)-3-nitrobenzamide was added into the polymerization mixture; the hyperbranched polymer with 2-((2-nitrobenzyl)thio)ethanol terminals was obtained with M_n of 8.7 kDa and PDI of 2.09. In its ^{1}H NMR spectrum, it is clear that the peaks corresponding to vinyl units are absent while the peaks corresponding to 2-((2-nitrobenzyl)thio)ethanol appear (Figure S7), indicating that 2-((2-nitrobenzyl)thio)ethanol terminals have been linked onto the hyperbranched polymer.

It has been found that disulfide bonds can undergo intermolecular exchange via heating.[25,26,28,34−36] Thereby, the prepared poly(amidoamine) with 2-((2-nitrobenzyl)thio)-ethanol terminals and disulfide bonds in the backbone can self-cross-link via intermolecular disulfide exchange via heating (Figure 2A,B). The storage modulus G' and loss modulus G'' curves as a function of time or frequency (ω) are a good experimental estimate of the gel transition (cross-linking). The values for G' and G'' of the prepared hyperbranched polymer slightly increase with time at 25 °C, and the G' and G'' curves do not intersect, indicating that the prepared hyperbranched polymer will not cross-link at 25 °C (Figure 2B). However, at 60 °C the values for G' and G'' of the prepared hyperbranched polymer also increase with time and the G' and G'' do intersect, indicating that the prepared hyperbranched poly(amidoamine) can self-cross-link upon heating (Figure 2B).[25,26,37]

Recently, it has been reported that 2-((2-nitrobenzyl)thio)-ethanol can *in situ* release thiol via UV activation.[29,30] Hyperbranched polymer with 2-((2-nitrobenzyl)thio)ethanol terminals can also release 2-mercaptoethanol under UV irradiation. In order to trace the release of 2-mercaptoethanol, we record the absorption spectra of disulfide-containing poly(amidoamine) via UV irradiation in the presence of Ellman's agent (5,5′-dithiobis(2-nitrobenzoic acid)) as shown in Figure 2C. It is clear that the absorption increases with the increase of irradiation time, indicating that 2-mercaptoethanol has been released via UV irradiation and the amount of the released 2-mercaptoethanol increases with the increase of irradiation time. Based on the absorption value, the

Scheme 2. Outline for the Synthesis of Self-Immolative and Self-Cross-Linking Hyperbranched Poly(amidoamine)



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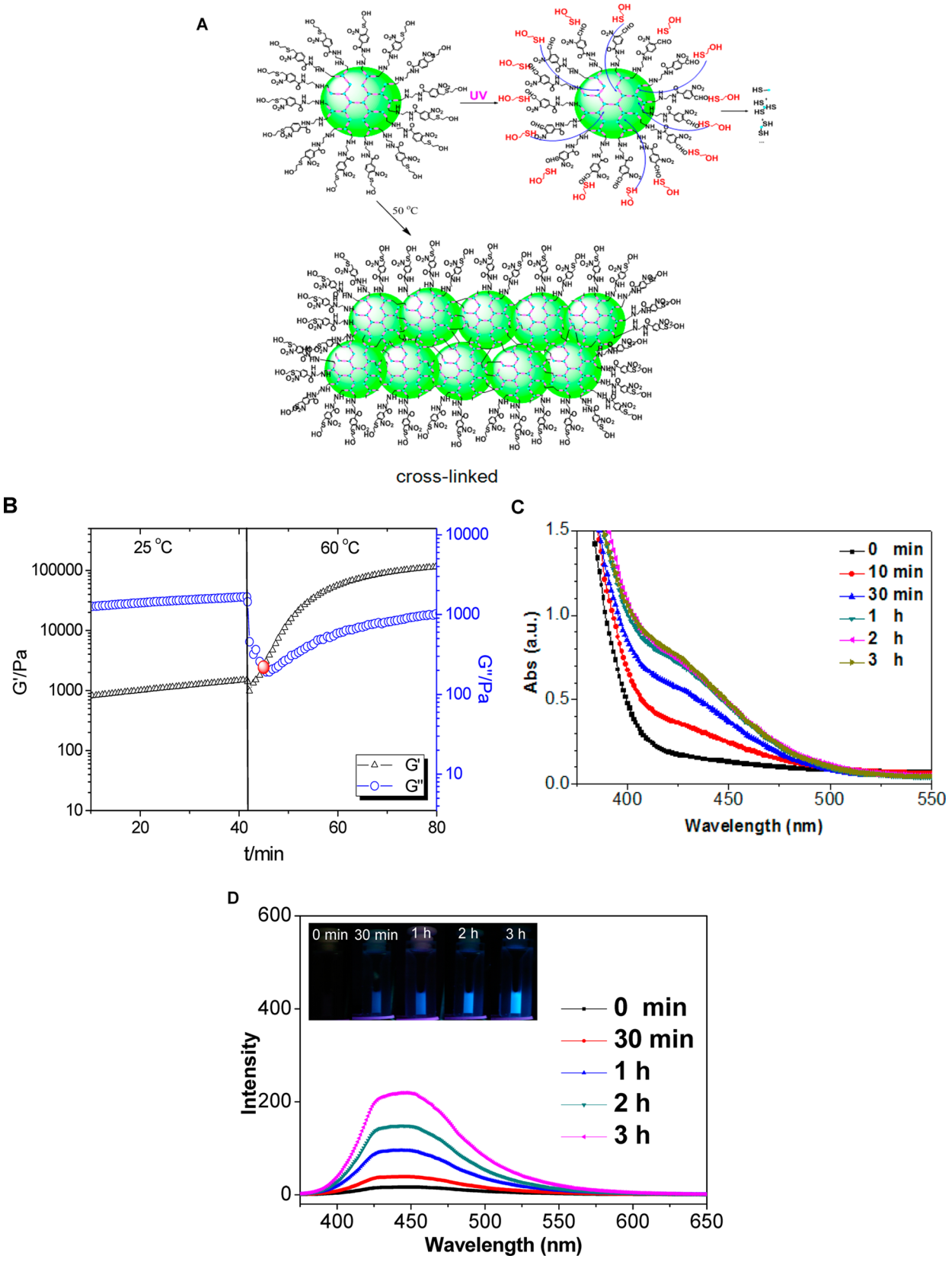

Figure 2. (A) Scheme for that the hyperbranched polymer can self-cross-link via heating and self-immolate via UV irradiation. (B) Changes of G' and G'' at 25 and 60 °C with time. (C) Absorption for disulfide-containing poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals via UV irradiation in the presence of Ellman's agent at different time. (D) Fluorescent spectra for disulfide-containing poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals via UV irradiation at different time (inset: the fluorescent images of this polymer via UV irradiation at different times).

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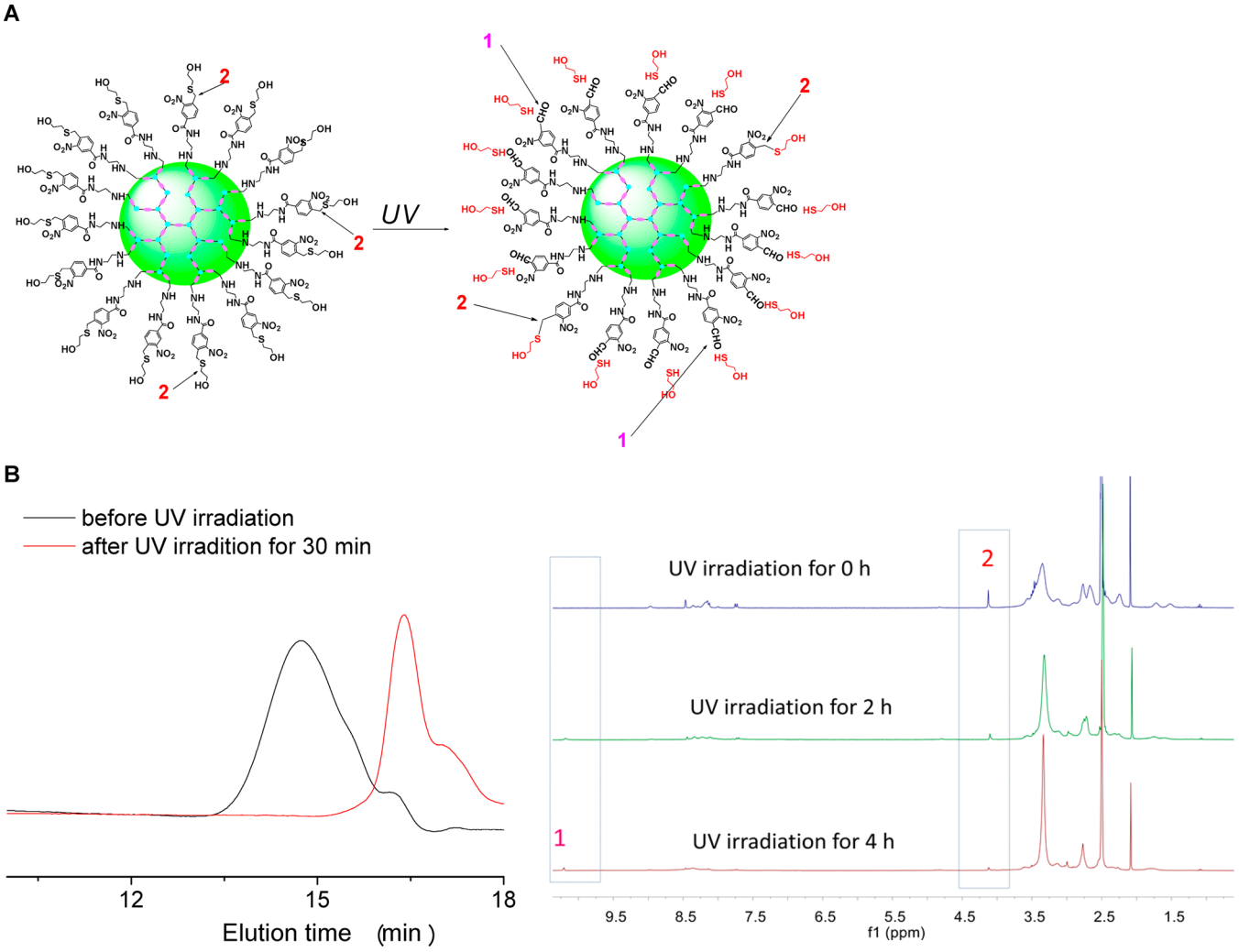

Figure 3. (A) ^{1}H NMR spectra of the disulfide-containing poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals under UV irradiation. (B) GPC traces of disulfide-containing poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals before and after UV irradiation.

concentration of the released 2-mercaptoethanol is 1.2 mM after 3 h irradiation when the polymer concentration is 10.0 mg/mL. For the hyperbranched poly(amidoamine) without 2-((2-nitrobenzyl)thio)ethanol terminal, there is almost no absorption in the presence of Ellman's agent as shown in Figure S9. Moreover, after the release of thiol, the system becomes fluorescent (may be due to the produce of 2-nitrobenzaldehyde units). Thereby, we record the fluorescence changes during the self-immolation of poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals. The fluorescence changes are shown in Figure 2D. Similar to absorption, the fluorescence intensity increases with the increase of UV irradiation time while there is almost no fluorescence for the similar hyperbranched poly(amidoamine) without 2-((2-nitrobenzyl)thio)ethanol terminal under UV irradiation (Figure S10). Moreover, based on the self-immolative mechanism (Figure 2A), CH_2 unit between S atom and benzene unit will be absent from the macromolecule after UV irradiation. We record ^{1}H NMR spectra of hyperbranched poly(amidoamine) before and after UV irradiation; it is clear that the signal at about 4.1 ppm for the CH_2 unit between S atom and benzene unit becomes very weak after 4 h UV irradiation (the remaining CH_2 is only ~10%) while there is a new peak at 10.2 ppm for the produced aldehyde unit after UV irradiation (Figure 3A). All these results show that UV can activate the release of 2-mercaptoethanol, which can act a reduction agent to reduce the disulfide bonds. Here, it should be noted that the polymer prepared has no primary amine in the backbone, most of the amine units are tertiary amine, and there are few secondary amines. The reactivity of formed benzaldehyde with the secondary amine in the backbone is very slow; NMR result shows that ~90% of the formed benzaldehyde remained unreacted based on integral value of CHO and benzene protons. We also check the molecular weight change of poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals under UV irradiation for 30 min via GPC; it is clear that molecular weight decreases to 300 Da from 8.7 kDa (Figure 3B). Based on the above result, the prepared hyperbranched poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals can self-immolate via UV irradiation.

Furthermore, this hyperbranched polymer is temperature and pH-responsive (Figure S11).[26] Therefore, this polymer can self-construct a bioreducible nanogel in its solution simply by increasing pH and temperature of the solution; moreover, the formed bioreducible nanogel can self-immolate via UV irradiation as shown in Figure 4A. The hyperbranched polymer can shrink and collapse into nanoparticles via tuning pH of the solution to 9.0, and the size of the nanoparticles is ~380 nm

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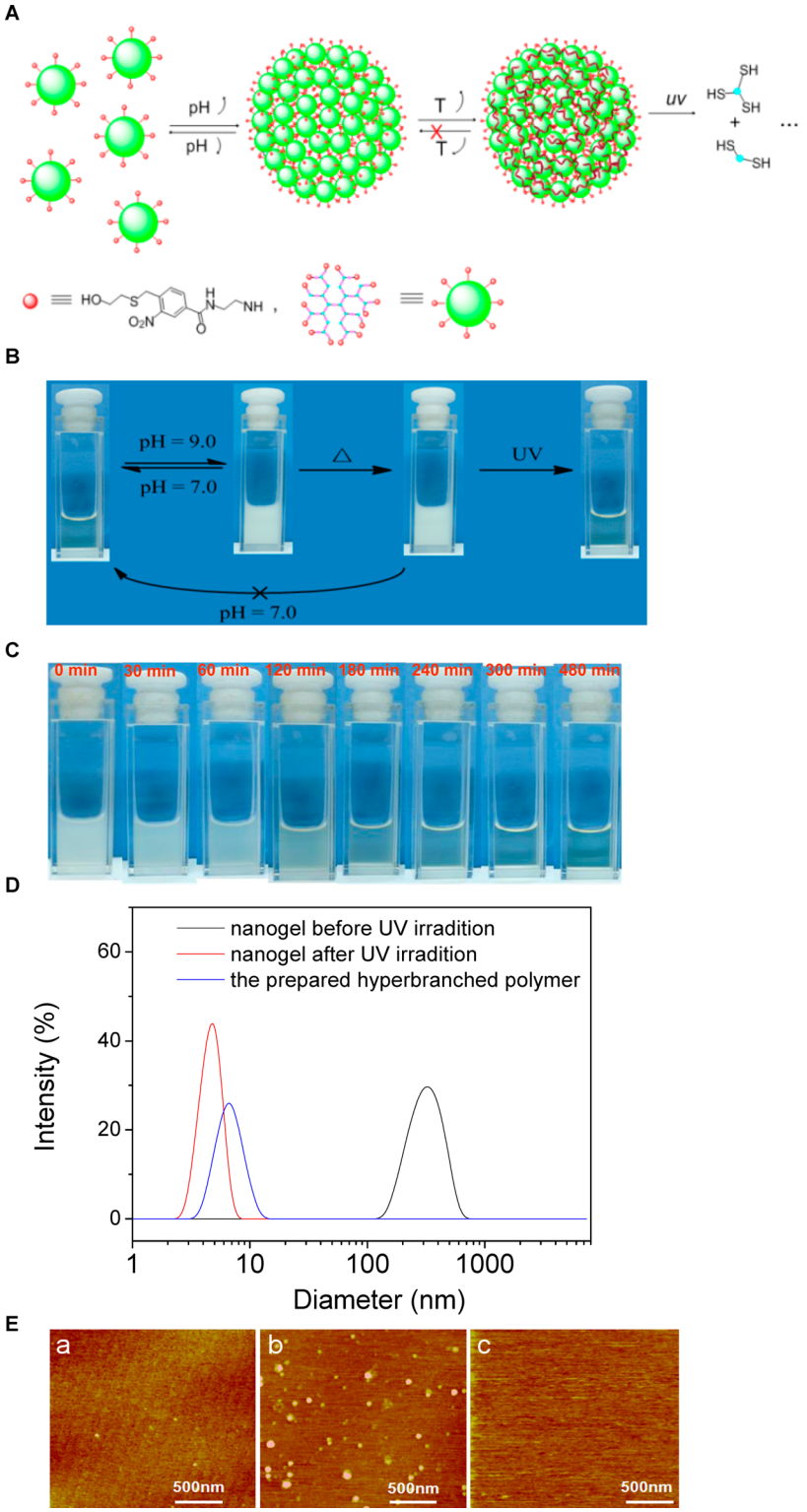

Figure 4. (A) Scheme of the *in situ* formation of bioreducible nanogel and its self-immolation under the stimulus changes of the environment. (B) Pictures for the changes of poly(amidoamine) (**1**) solution under pH, temperature, and UV irradiation. (C) Images the formed nanogel under UV irradiation at different times. (D) DSL results for disulfide-containing poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals; the nanogel before and after UV irradiation. (E) AFM images for disulfide-containing poly(amidoamine) with 2-((2-nitrobenzyl)thio)ethanol terminals (a); the formed nanogel before (b) and after UV activation (c).

(DLS) at polymer concentration of 5.0 mg/mL. Without heating the solution, the formed nanoparticles are stabilized by weak intermolecular associations (i.e., van der Waals interactions), and the particles can readily redissolve by decreasing pH to 7.0 (Figure 4B). However, after heating the solution to 50 °C for 30 min, the particles cannot redissolve (Figure 4B); the reason is that the nanoparticles undergo intermolecular disulfide exchange via heating to 50 °C, thereby self-cross-

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linking the nanoparticles into nanogels as shown Figure 4A. The formed nanogels have many 2-((2-nitrobenzyl)thio)-ethanol units, via UV irradiation, 2-((2-nitrobenzyl)thio)-ethanol can *in situ* release 2-mercaptoethanol, and the released 2-mercaptoethanol can break the disulfide bonds in the nanogel; therefore, the nanogel self-immolates via UV irradiation as shown Figure 4. In Figure 4C, it is clear that the solution becomes clear from opaque via UV irradiation for 3 h. However, the nanogel without 2-((2-nitrobenzyl)thio)-ethanol formed from disulfide-containing poly(amidoamine) cannot self-immolate via UV irradiation; the solution keeps opaque via UV irradiation for 3 h (Figure S12). Furthermore, DLS results show that the nanogel with the size of 380 nm self-immolates into small fragments with size of several nanometers via UV irradiation. AFM images also indicate that the nanogel with 2-((2-nitrobenzyl)thio)ethanol can self-immolate via UV activation (Figure 4E).

Polyplexes consisting of gWiz-Luc plasmid DNA and the prepared hyperbranched polymer were prepared in PBS (10 mM) at pH 7.4 with N/P ratios of 20 and 40. The polymer solution was rapidly added to the DNA and mixed by vortex, followed by 20 min incubation at room temperature prior to use. Transfection efficiency was tested in Hela cell lines. After 4 h of incubation, the transfection mixture was removed and the cells were cultured in fresh full DMEM media and under UV irradiation for 0, 10, and 20 min. The result shows that the transfection efficiency increases with the increase of UV irradiation time, indicating that UV irradiation can help the release of DNA in cell (Figure S13).

CONCLUSIONS

A novel multiresponsive macromolecule with disulfide bonds in the backbone, *N,N′*-dimethylamine side units, and 2-((2-nitrobenzyl)thio)ethanol terminals has been prepared via Michael addition reaction. It not only can self-cross-link via intermolecular disulfide exchange via heating but also can self-immolate by the *in situ* released 2-mercaptoethanol from 2-((2-nitrobenzyl)thio)ethanol terminals via UV activation. Furthermore, in aqueous solution, this polymer self-constructs a disulfide-cross-linked nanogel, and this formed nanogel can self-immolate by the *in situ* released 2-mercaptoethanol via UV activation. The self-cross-linking and self-immolative properties of this hyperbranched polymer make it have potential applications in nanomedicine.

ASSOCIATED CONTENT

Supporting Information

Detailed characterization of the small molecules and the corresponding polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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