Facile One-Pot Approach for Preparing Dually Responsive Core−Shell Nanostructure

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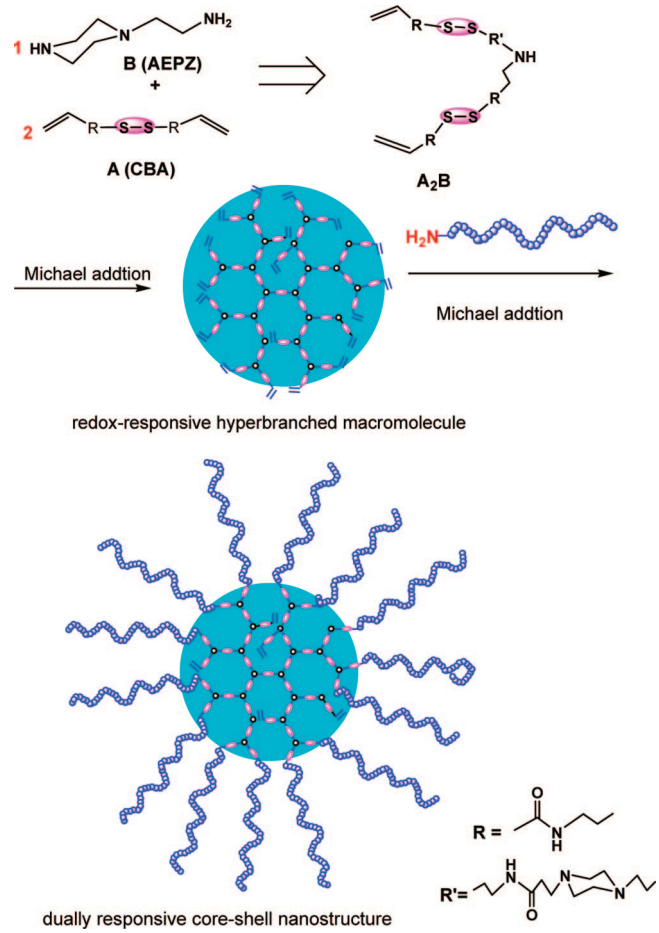
Owing to many potential applications in the controlled drug delivery and release,[1] the preparation of phase transfer materials[2] and nanomedicine materials,[3] core−shell nanostructure with a hyperbranched polymer/dendrimer core has attracted great attention recently. However, the multistep syntheses involved in the preparation limit their general applicabilities. It is desirable to develop a facile synthesis method to access core−shell nanostructure.[1c] In addition, although some core−shell nanostructures with a hyperbranched polymer/dendrimer core have been constructed, the dually responsive core−shell nanostructure, which will have more attractive properties in nanomedicine and nanotechnology, is much less explored yet due to the synthetic difficulties.[4] Recently, Guan et al. reported the one-pot synthesis of water-soluble dendritic nanocarriers.[1c] Here we report a facile one-pot approach for preparing core−shell nanostructure with a redox-sensitive hyperbranched polymer core and a temperature-responsive shell.

Michael-addition polymerization is one of the most used methods for preparing hyperbranched and dendritic polymers.[3d,5,6a] The approach for preparing the dually responsive core−shell nanostructure is shown in Scheme 1: Michael-addition polymerization of triamine (B, 1-(2-aminoethyl)piperazine, AEPZ) with double molar bisacrylamide (A, *N*,*N*′-cystamine bisacrylamide, CBA) forms hyperbranched polymer with redox-sensitive disulfide bonds in the backbone and vinyl terminals, then the amino-ended poly(*N*-isopropylacryamide) (PNIPAM-NH_2) was directly added into the above polymerization mixture, the vinyl terminals further reacted with PNIPAM-NH_2 via Michael-addition reaction, thereby linking temperature-responsive shell to the redox-sensitive hyperbranched core. Consequently the dual stimuli-responsive core−shell nanostructure (the core is responsive to redox agent[6] and the shell is responsive to temperature) was obtained simply via one-pot approach.

It was found that the amino units in a trifunctional amine have different reactivity in Michael-addition polymerization of trivalent amine with bisacrylamide/bisacrylate, hence the topology of the produced polymers can be tuned simply via varying reaction conditions such as temperature or the molar ratio of trivalent amine to bisacrylamide/bisacrylate.[5,6a] Generally, in Michael-addition polymerization of AEPZ with equal molar CBA, AB-type intermediate formed at the starting stage and 2° amine (produced during the polymerization) did not take part in reaction due to high steric hindrance, which resulted in the formation of linear poly(amido amine) (no peak at ∼50 ppm was observed in ^{13}C NMR spectrum, indicating that 2° amine (produced during the polymerization) did not take part in polymerization,[5] thereby no dendritic unit formed, seeing supporting materials). However, in Michael-addition polymerization of AEPZ with double molar CBA, A_2B-type intermediate produced at starting stage, 2° amine

Scheme 1. Outline of One-Pot Approach for Preparing a Dually Responsive Core−Shell Nanostructure



(produced during the polymerization) participated in the polymerization in late stage,[5] which leads to the formation of hyperbranched poly(amido amine) with M_n of 11800. The appearance of peaks at ∼50 ppm (see Figure 1) and absence of peaks at ∼46.5 ppm compared with ^{13}C NMR spectrum of linear poly(amido amine) indicate that most of the 2° amine (produced during the polymerization) units participated in the polymerization of AEPZ and double molar CBA, leading to the formation of hyperbranched structure as shown in Scheme 1. However, there was no obvious difference between dendritic units and linear units in the produced hyperbranched poly(amido amine) in ^{13}C NMR and ^{1}H NMR, which is similar to previous finding,[5] thus degree of branching based on the definition by Hawker and Fréchet[7] could not be obtained from the NMR spectra of hyperbranched poly(amido amine). On the basic of that R_g/R_h for the produced poly(amido amine) is ∼0.98 (R_g/R_h is 1.50∼2.0 for linear flexible polymers in good solvent, 0.8∼1.5 for hyperbranched /dendritic macromolecules, and 0.78 for uniform spheres)[8] combined with that most of the 2° amine (produced during the polymerization) have participated in the polymerization, we can conclude the formation of hyperbranched structure (see Supporting Information).

The as-synthesized hyperbranched polymers have disulfide linkages in the backbone and vinyl terminals, which can be demonstrated by the results of ^{1}H NMR and ^{13}C NMR. The disulfide linkages in the backbone are sensitive to reducing agent

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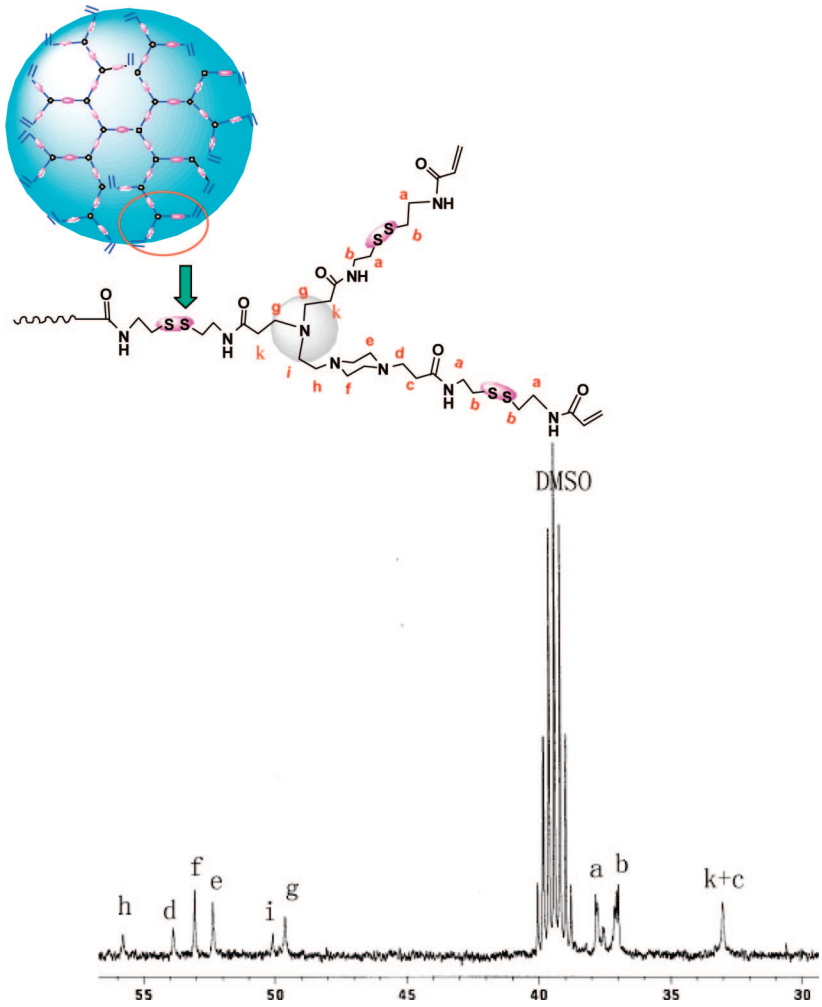
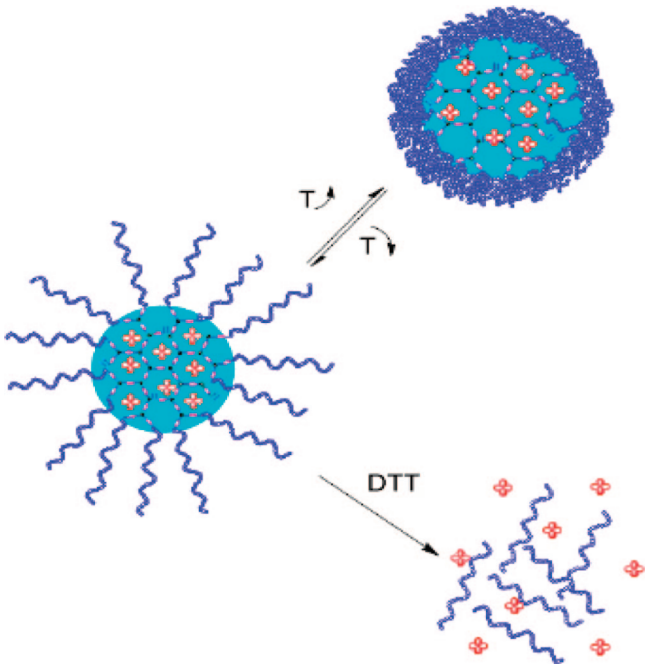


Figure 1. ^{13}C NMR spectrum of the formed hyperbranched core in d_6-DMSO.

Scheme 2. Outline of the Prepared Core−Shell Nanostructure Responsive to Temperature and DTT



such as dithiotreitol (DTT) and glutathione, which can be verified by that the as-synthesized hyperbranched polymer was reduced into small molecules under 20 mM of DTT in 40 min based on SEC results (see Supporting Information). Moreover, the vinyl terminals have high addition reactivity toward amino unit even at room temperature, therefore, they can be used for further Michael addition reaction with amino-contained molecules (such as PNIPAM-NH_2) to link temperature-sensitive shell onto this redox-sensitive hyperbranched core. Poly(*N*-isopropylacrylamide) (PNIPAM) represents one of the most widely investigated temperature-responsive polymers and exhibits a lower critical solution temperature (LCST, the temperature at which the polymer undergoes a change from a hydrated to a dehydrated state). Thus, at temperature below the LCST, the polymer is in the coil (soluble) conformation, while above the LCST, it is in the globule or collapsed (insoluble) state. This property has been exploited in applications ranging widely from rheology modifications to controlled drug delivery.[1d,9a,b] Therefore, we chose PNIPAM as model polymer and linked it on the hyperbranched core. The procedure is: After 120 h' polymerization of AEPZ with double molar CBA (some polymerization mixture was taken out for characterization, M_n = 11800 and PDI = 1.8 for the formed hyperbranched poly(amido amine)), PNIPAM-NH_2 (M_n = 6200, PDI = 1.8) in methanol/water was directly added into this polymerization mixture, and the reaction mixture was stirred for 48 h. The unreacted PNIPAM-NH_2 was removed by viva spin (MW 10000 cutoff). After the 48 h' reaction, the peaks associated with the vinyl units decreased in intensity, ~70% vinyl terminals reacted with PNIPAM based on NMR result, M_n increased to 89000

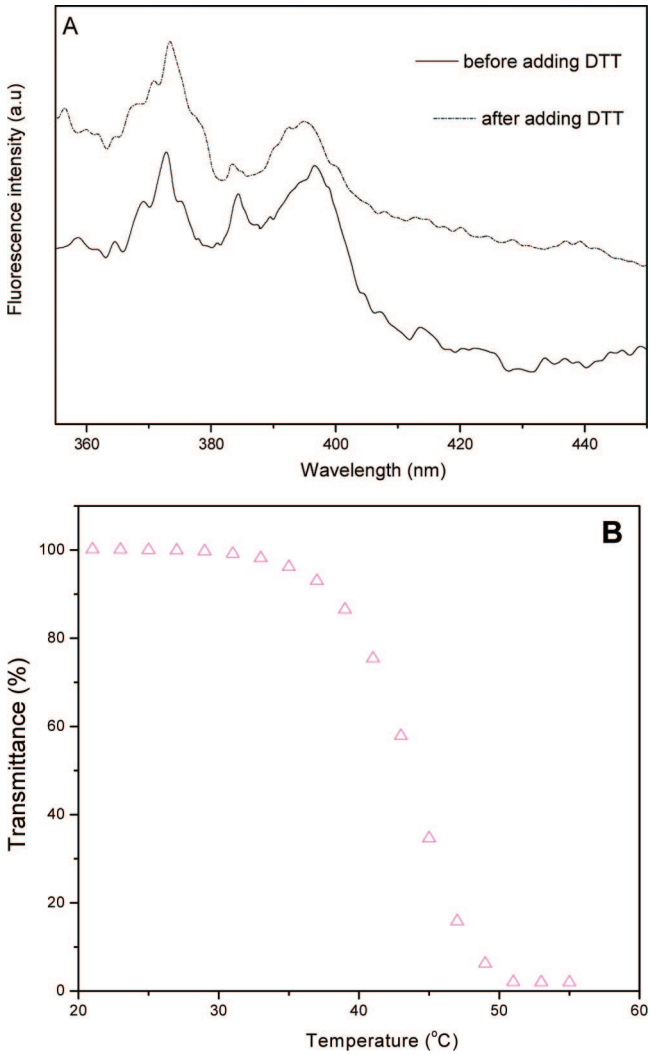


Figure 2. Fluorescence spectra of dually responsive core−shell nanostructure loaded with pyrene before and after adding DTT (A); the transmittance of core−shell nanostructure with temperature (B).

from 11800, all these indicate that temperature-responsive PNIPAM chains were linked onto the redox-responsive hyperbranched poly(amido amine) core.

The as-synthesized core−shell nanostructure is dually sensitive: the core is responsive to redox stimuli and the shell is responsive to temperature (as shown in Scheme 2). When increasing the solution temperature from 25 to 50 °C, the diameter of core−shell nanostructure decreased form 37 to 28 nm, and the size increased to 36 nm upon subsequent cooling to 25 °C. The transmittance decreased with the increase of temperature as shown in Figure 2B, and this procedure is reversible, indicating that PNIPAM shell can be closed and opened induced by temperature as shown in Scheme 2, which was similar to previous findings.[9] Moreover, the hyperbranched core is redox-responsive. We used pyrene to trace the degradability of the redox-sensitive core. The hyperbranched core has poor solubility in water at pH of 7.4, hence pyrene can be loaded into the poly(amido amine) core at room temperature. The reducing agent can cleave the hyperbranched poly(amido amine) core and thereby releasing loaded pyrene. It is well-known that I_1/I_3, the highest energy vibrational band I_1 (372 nm) to the third highest energy vibrational band I_3 (385 nm), is very sensitive to microenvironmental polarity;[10] namely, I_1/I_3 is 1.8 in pure water while I_1/I_3 could be as low as 1.1 in a hydrophobic domain. I_1/I_3 of pyrene in the presence of as-synthesized core−shell nanostructure is ∼1.2 in Figure 2A, indicating that pyrene was loaded into the hyperbranched core. I_1/I_3 increased up to ∼1.6 after adding DTT, which indicates that DTT cleaved the core thereby releasing pyrene and the core is redox responsive.

This hyperbranched poly(amido amine) has very low cytotoxicity (see Supporting Information) due to that it can be reduced into small molecules by glutathione in cell. Also the vinyl terminals of hyperbranched poly(amido amine) have high reactivity to amino units in antibody, peptide, sugar and protein which can provide tissue and cell specific delivery, therefore, this one-pot method can be extended to easily link these targeting biomolecules onto the redox-responsive hyperbranched poly(amido amine), which will have potential applications in nanomedicine.

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Supporting Information Available: Text giving the detailed procedures for syntheses of the resulting polymers and detailed characterizations, including figures showing the ^{1}H NMR and ^{13}C NMR spectra on all the polymers used in experiments, SEC traces, and cytotoxicity of the poly(amido amine), and schemes showing the structures and syntheses. This material is available free of charge via the Internet at http://pubs.acs.org.

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