Pseudo-Block Copolymer Based on Star-Shaped Poly(*N*-isopropylacrylamide) with a *β*-Cyclodextrin Core and Guest-Bearing PEG: Controlling Thermoresponsivity through Supramolecular Self-Assembly

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Stimuli-responsive polymers are often referred to as "intelligent" polymer systems because they exhibit reversible property changes in response to changes in environmental factors such as pH or temperature.[1,2] Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most popular thermoresponsive polymers, which shows dramatic and reversible phase transition behavior in water with the lower critical solution temperature (LCST) at 32 °C.[3] This unique property makes PNIPAAm and its copolymers "intelligent" systems for many promising applications, particularly in the areas of biotechnology, biomedicine, and nanotechnology, e.g., for controlled drug delivery, gene delivery, cell and enzyme immobilization, biosensor, and bioaffinity separation.[4−17] The adjustment and control of LCST of these materials are essential for their applications. To this end, copolymerization of NIPAAm with other hydrophilic or other stimuli-responsive monomers has been done, and various random, graft, and block copolymers or copolymer conjugates of PNIPAAm with different functions and applications were prepared.[18−25]

Cyclodextrins (CDs) are a series of natural cyclic oligosaccharides composed of 6, 7, or 8 D-(+)-glucose units linked by α-1,4-linkages, and named α-, *β*-, or *γ*-CD, respectively.[26] The geometry of CDs gives a hydrophobic inner cavity having a depth of ca. 7.0 Å, and an internal diameter of ca. 4.5, 7.0, and 8.5 Å for α-, *β*-, and *γ*-CD, respectively, and various molecules can be fitted into the cavities to form supramolecular inclusion complexes, which have induced a lot of interesting development of supramolecular systems for biomedical and pharmaceutical applications.[27−31] Herein, we present a novel system comprising a *β*-CD-core PNIPAAm star polymer which forms supramolecular self-assembly with poly(ethylene glycol) (PEG) bearing an adamantyl end through host−guest complexation. The block copolymer like supramolecular self-assembly, here we call "pseudoblock copolymer", shows tunable LCST because of the different lengths of the PEG blocks. Through forming the pseudoblock copolymer, this work has demonstrated a novel supramolecular approach controlling the thermoresponsive property of a PNIPPAm copolymer.

A star polymer was synthesized with a *β*-CD core as a molecular recognition moiety and multiple PNIPAAm arms as actuation moieties (Scheme 1a). This work started from the synthesis of a *β*-CD-based macroinitiator (4Br-*β*-CD), from which the *β*-CD-core star polymer (*β*-CD-(PNIPAAm)$_4$) was obtained by the growth of PNIPAAm arms via copper(I)-mediated atom transfer radical polymerization (ATRP) method. The *β*-CD-based macroinitiator with an average degree of substitution (DS) of 4 was synthesized by treating *β*-CD with 4 equivalents of 2-bromo-isobutyric bromide, where DS is defined as the number of grafted initiation group, −OCO−C-(CH$_3$)$_2$Br, per CD molecule. Its purity was ensured by a purification method based on the difference in solubility of the initiator-modified *β*-CDs with different DS in water and acetone. The DS of the purified macroinitiator was determined by means of ^{1}H NMR spectroscopy and X-ray photoelectron spectroscopy (XPS). Both methods indicated that the average DS was very close to 4 (Supporting Information).

During the ATRP of NIPAAm in methanol using 4Br-*β*-CD as initiator in the presence of copper(I) bromide as catalyst and tris[2-(dimethylamino)ethyl]amine (Me$_6$-TREN) as ligand, the monomer conversion was controlled to be less than 50% to limit possible side reactions such as bimolecular termination or thermal initiation. The chemical composition of the final star polymer was determined by ^{1}H NMR, GPC and XPS, and the values of the number-average molecular weight (M_n) based on different measurements were found to correlate well with the predicted molecular weights calculated from the feed initiator concentration and the consumption of monomer, showing that the above-mentioned side reactions were negligible under our polymerization conditions (Table 1). On the basis of the ^{13}C NMR results of the macroinitiator, it was proposed that the initiation groups were mainly grafted onto the smaller rim of the *β*-CD ring in the macroinitiator, thus it was reasonably deduced that PNIPAAm arms in the final star polymer were mainly bonded to the smaller rim of the *β*-CD core (Supporting Information).

The macromolecular guests used in this work were synthesized by coupling the molecular recognition moiety, adamantane, with the biocompatible hydrophilic PEGs (Scheme 1b). The mono- or bifunctional PEGs were attached with adamantyl group(s) quantitatively, which was confirmed by ^{1}H NMR spectroscopy and GPC analysis (Supporting Information). It was found that the thermosensitive behavior of the *β*-CD-core star PNIPAAm in aqueous solution was changed dramatically upon the host−guest complexation with the adamantane-modified PEG guests. Thus, the LCST of these self-assembling systems can be easily tuned through changing the ratio of adamantyl moiety to CD core and/or through the length of the PEG block like in a conventional block copolymer system.

Figure 1 showed the detailed information of thermoresponsive behavior for aqueous star polymer *β*-CD-(PNIPAAm)$_4$ in the presence of adamantyl-containing PEGs, which was investigated by cloud point technique. It can be seen from Figure 1a, the LCST of the star polymer was ca. 33.5 °C. The LCST of the star polymer shifted to a higher temperature upon the addition of the macromolecular guest MPEG-2K-Ad (Figure 1a). The LCST significantly changed with a LCST increase (ΔLCST, referenced to pure star polymer) of 2.9 °C when the guest/host

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Scheme 1. Synthetic Procedures for (a) the β-CD-Based Macroinitiator (4Br-β-CD) and β-CD-Core Star Polymer (β-CD-(PNIPAAm)$_4$) via ATRP (Both Have an Average Degree of Substitution of 4) and (b) the Adamantyl-Containing PEGs

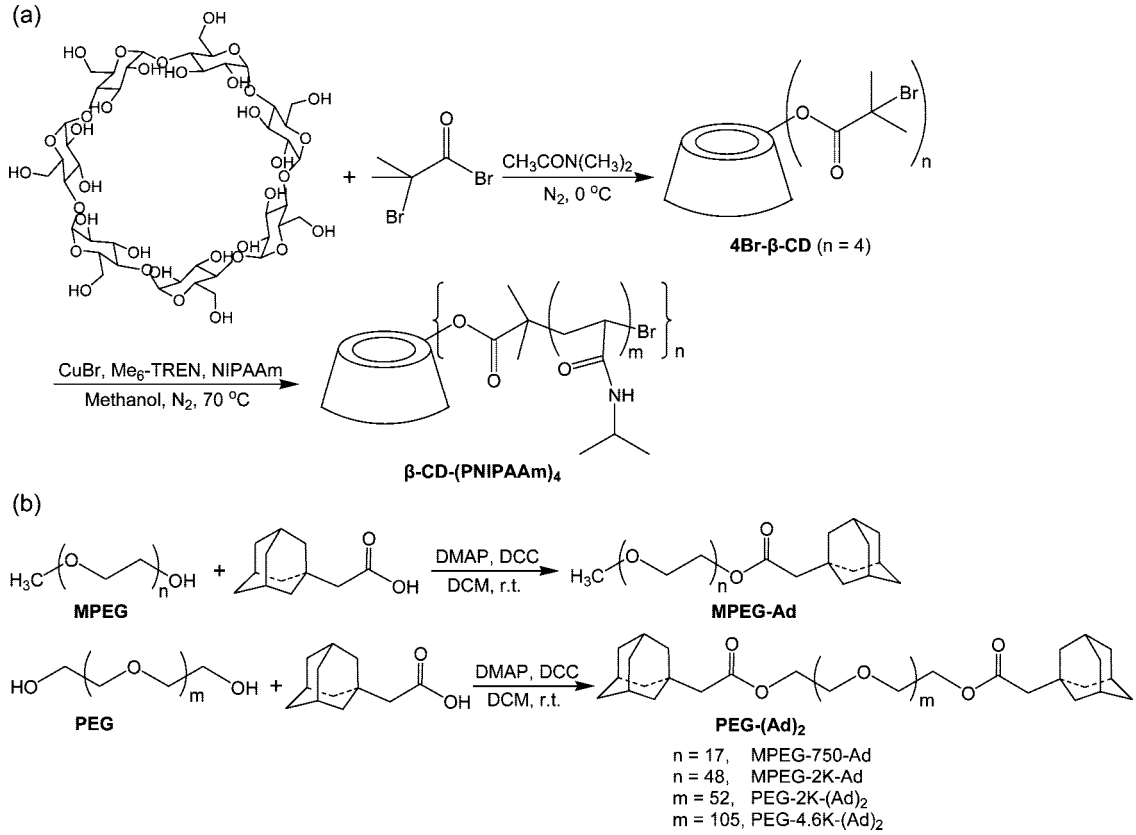


Table 1. Polymerization Conditions and Characterization Results for the β-CD-Core PNIPAAm Star Polymer[a]

	molecular weight, M_n				
conversion [%][b]	$M_{n,predicted}$[c]	$M_{n,NMR}$[d]	$M_{n,XPS}$[e]	$M_{n,GPC}$[f]	PDI[f]
46.8	7851	8166	9169	4101	1.24

[a] ATRP of NIPAAm in methanol at 70 °C for 4 h with $[M]_0/[I]_0/[Cu]_0/[L]_0 = 30/1/1/1.2$, which represented the ratio of initial concentrations of monomer, total initiation sites of macroinitiator, CuBr and the ligand Me_6-TREN, respectively. [b] Calculated based on the method described in Supporting Information. [c] The predicted M_n was calculated based on the monomer conversion and the feed initiator concentration. [d] Calculated based on the ^{1}H NMR (D_2O) result of the final star polymer by comparing the integration of the signals for the methyl protons of isopropyl group (PNIPAAm arm) at around 1.04 ppm to those at the region of 3.40−4.00 ppm for the methine proton of isopropyl group (PNIPAAm arm) and the protons due to the β-CD core. [e] Calculated from XPS-derived [N]/[C] ratio. [f] Estimated by GPC calibrated with monodispersed PEG standards, PDI = M_w/M_n.

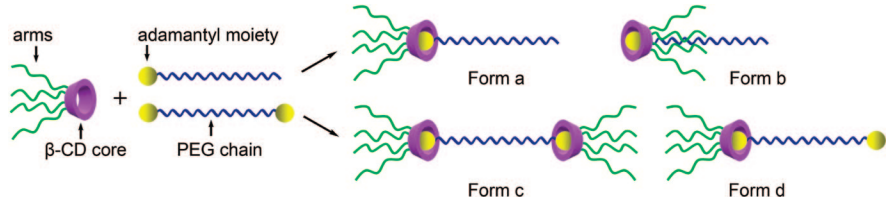
ratio, i.e., adamantyl moiety/CD core molar ratio was 0.5. The ΔLCST further increased another 2.2 °C, resulting in a LCST of ca. 38.6 °C when the guest/host ratio reached to 1.0. However, the LCST became almost saturated upon further increase in the guest/host ratio beyond 1.0, indicating that the self-assembling system involves a 1:1 complexation between the adamantyl moiety and the β-CD core. The adamantyl-containing MPEG could be incorporated to the β-CD core of the star polymer via inclusion complexation to form a supramolecular block copolymer. The PEG block increased the solubility of the assembling system, resulting in a higher LCST of the star polymer.

In order to confirm that the shift of LCST was caused by the complexation between the adamantyl moiety of guest polymer and the β-CD core of the star polymer, a series of control experiments were carried out following the same procedure as described above. First, pure MPEG-2K (containing no adamantyl group) was used to replace MPEG-2K-Ad. As expected, no detectable changes of LCST were observed no matter how much of MPEG-2K was added to the aqueous solution of the star polymer. On the other hand, instead of MPEG-2K-Ad, pure adamantane acetic acid (containing adamantyl moiety but no hydrophilic PEG chain) was used as guest. A slight down-shift in LCST (ΔLCST is ca. 0.5 °C) of the aqueous star polymer solution was observed with a guest/host ratio at 1.0. The change is minimal as compared to that caused by MPEG-2K-Ad (ΔLCST is ca. 5.1 °C), indicating that the effect of PEG chain is significant.

Subsequently, we investigated the effect of the PEG chain length and structure of the adamantyl-containing PEGs on the LCST behaviors of the star polymer (Figure 1b, all guest/host ratio was fixed to be 2.0). When monoadamantyl-terminated PEGs were used as macromolecular guests, the LCST of the self-assembling systems were ca. 37.8 and 39.5 °C for MPEG-750-Ad and MPEG-2K-Ad, respectively, indicating that the LCST increased with the molecular weight of MPEG. The same trend was also found in the case of bis-adamantyl-terminated PEGs, and the LCST of the self-assembling systems were ca. 38.5 and 39.3 °C for PEG-2K-$(Ad)_2$ and PEG-4.6K-$(Ad)_2$, respectively. Therefore, the LCST of the self-assembling systems in our work can be easily tuned and controlled by the chain length of PEG as well as the shape and structure of the adamantyl-containing macromolecular guests.

We propose the possible modes of complexation between the adamantyl-containing PEGs and the β-CD-core star polymer (Scheme 2). In the case of MPEG-Ad (with one adamantyl end group), there are two possible forms of complexes. In form a, the adamantyl end approaches the wider side of the β-CD core, forming the self-assembling system with the PEG chain located

Scheme 2. Schematic Representation of the Inclusion Complexes Formed via Host−Guest Interactions between β-CD-Core Star Polymer and Adamantyl-Containing PEGs



on the wider side of the β-CD core. In form b, the methoxy end of MPEG-Ad approached the wider side of the β-CD core, and the PEG chain threading through the β-CD cavity, forming the self-assembling system with the PEG chain located on the narrow side of the β-CD core. In the case of PEG-$(Ad)_2$ (with two adamantyl end groups), the adamantyl end can only form complex from the wider side of the β-CD core. However, there will be two possible self-assembling structures: the ABA triblock (form c) and the AB diblock (form d) architectures.

To further confirm the host−guest complexation of the β-CD core star polymer with the adamantyl-containing PEGs, 2D-NOESY NMR measurements were carried (see Supporting Information, Figure S6). It was found that the three signals of the methylene (*H*a, *H*c) and methine (*H*b) protons of the adamantyl moiety were all correlating well with the inner protons C(3)*H* and C(5)*H* of β-CD core, indicating that there

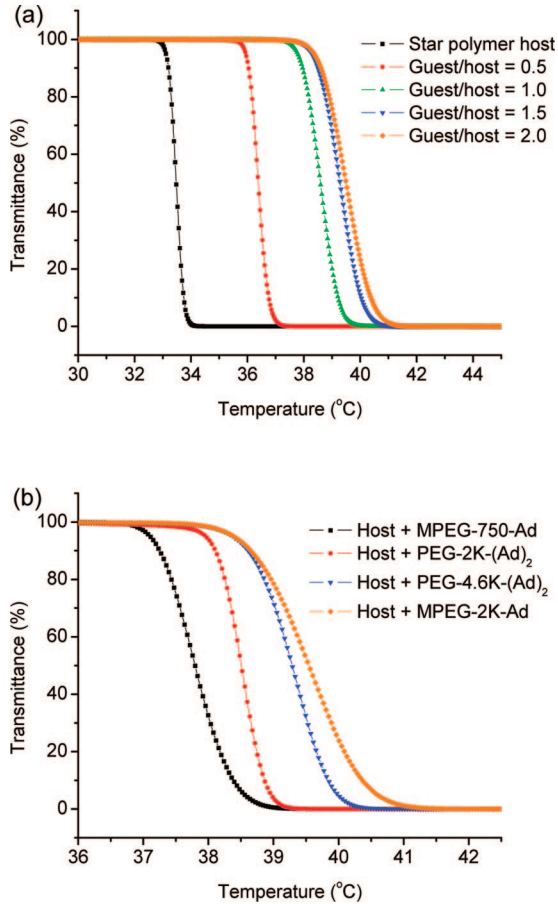


Figure 1. (a) Turbidity variations of aqueous solutions of star polymer β-CD-$(PNIPAAm)_4$ as a function of temperature upon the addition of macromolecular guest MPEG-2K-Ad. $C_{star\ polymer} = 10\ g\ L^{-1}$. (b) Turbidity variations of aqueous mixtures of star polymer and different macromolecular guests as a function of temperature (guest/host = 2.0). $C_{star\ polymer} = 10\ g\ L^{-1}$.

were both Form a and Form b existing in the self-assembling systems when MPEG-Ad was used.

Recently, Ritter and co-workers reported a few copolymers of PNIPAAm bearing hydrophobic guest moieties which can be complexed with methylated CDs or CD dimers.[32,33] The formation of complexes of the hydrophobic guest moieties with a methylated β-CD changed the hydrophobicity of the guest moieties, which subsequently changed the LCST of the PNIPAAm copolymer.[32] In another study, a β-CD dimer formed inclusion complexes with the guest moieties attached to the main chain of PNIPAAm, acting as physical cross-linking and changed the LCST behavior of the PNIPAAm copolymer.[33] In our system, the mechanism is different because the β-CD-core PNIPAAm star polymer forms an block copolymer like supramolecular assembly, whose LCST is altered in a similar way in a PNIPAAm amphiphilic block copolymer, but the LCST can be simply changed with the guest PEGs of different PEG block lengths. This is much more advantageous than a conventional block copolymer system, because the same β-CD-core PNIPAAm star polymer can be easily combined with different guest macromolecules for controlling the LCST of the PNIPAAm star polymer in our system. This is particularly useful in biomedical and pharmaceutical applications, because a conventional vinyl copolymer usually is nonbiodegradable and of high molecular weight to function as thermoresponsive system, but it is known that a water-soluble polymer with high molecular weight (normally above 10 000) is not suitable for filtration through human kidney membrane due to the large hydrodynamic radius.[34] Therefore, a pseudoblock copolymer system based on supramolecular self-assembly between the β-CD-core PNIPAAm star polymer and guest PEGs (molecular weights of both components are less than 10 000) may be much more promising as an intelligent system for biomedical and pharmaceutical applications.

In conclusion, through preparation of a novel multiarm β-CD-core PNIPAAm star polymer, which combined the thermoresponsive properties of its PNIPAAm arms and the molecular recognition abilities of its β-CD core, we have successfully demonstrated a self-assembling system comprising a star-shaped PNIPAAm block and a hydrophilic PEG block. From a practical point of view, the thermoresponsive properties of the β-CD-core star polymer can be easily tuned over a temperature scale around the body temperature by varying the number of guest moieties and molecular weight of the adamantyl-terminated PEGs added to the solution.

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Supporting Information Available: Synthesis and characterization data of macroinitiator, star polymer, and macromolecular guests, figures showing the ^1H and ^{13}C NMR spectra, molecular weight, GPC, and XPS plots and the 2D-NOESY NMR spectra,

and a table of selected characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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