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Synthesis and thermally-triggered self-assembly behaviors of a dumbbell-shaped polymer carrying β-cyclodextrin at branch points

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ABSTRACT

We report the design and synthesis of a thermally-responsive dumbbell-shaped polymer based on one β-cyclodextrin (β-CD)-terminated polyethylene glycol (PEG) segment and several poly (N-isopropylacrylamide) (PNIPAM) segments through combining click reaction with atom transfer radical polymerization. Thermally-triggered self-assembly and molecular inclusion behaviors of the polymer were investigated by UV–vis spectroscopy, dynamic light scattering (DLS), ^{1}H NMR in D_2O, fluorescence spectroscopy and transmission electron microscopy. These results indicated that the polymer presents both thermal sensitivity and molecular inclusion properties due to the existence of PNIPAM segments and β-CD units. Above the lower critical solution temperature (LCST) of PNIPAM segment, the polymer can self-assemble into nano-sized assemblies, where PNIPAM segments serve as the core and PEG segments are organized as the corona. By using 1-adamantane acid sodium as guest molecule, the polymer shows size-adjusted aggregation behaviors based on the inclusion interactions between β-CD and guest molecule.

1. Introduction

During the past decade, studies have focused on the synthesis of branched polymers with different topological architectures [1–6]. The alteration of polymer segments in branched architectures can produce various novel structures with interesting chemical and physical properties, such as special morphologies either in bulk or in solvent compared with the corresponding linear analogs [7–27]. Branched polymers are important in the study of morphological diversity and may also have potential applications in drug delivery, biomedical engineering, and nanocomposite materials [16,17]. Till now, branched polymers can be classified into several main types, including the H-shape [7–12], Π-shape [13–15], star-shape [16–19], dendrimers [20,21], hyperbranched [22–27], and so on.

Compared to the above-mentioned branched polymers, there are only a few reports on the synthesis, self-assembly behaviors or other functional properties of dumbbell-shaped polymers [28–38]. Theoretically, dumbbell-shaped polymers can be defined as an A_nBA_n type star-linear-star polymer, where n represents the arm number in polymer terminals for constructing a dumbbell-shape polymer. The differences in polymer chain specie and the chain packing density between star and linear blocks endow this kind of polymer with unique chemical and physical properties compared with other branched polymers [30–36]. Generally, the dumbbell-shaped polymer could be synthesized by a traditional reaction of difunctional living anionic polymers with multifunctionalized chlorosilanes [30]. However, the related complicated synthesis approaches have limited the development of this polymer to some degree. Additionally, to obtain more information on the relationships between dumbbell-shaped topological structure and polymer solution properties, a deeper investigation on the self-assembly behaviors is also required. In recent years, Li et al. [39] constructed a dumbbell-shaped polymer based on the supramolecular self-assembly of β-cyclodextrin (β-CD), which can form inclusion complexes with various small molecules through hydrophobic interaction [40–42]. However, the cavity of β-CD was occupied by adamantane to obtain the final dumbbell-shaped polymer. Thus, the specific inclusion and molecular recognition functions of β-CD toward guest molecules can not be expected. To overcome this limitation, we aim to construct a dumbbell-shaped polymer containing two β-CD units at branching points (Scheme 1). In this polymer structure, the space-free from β-CD hydrophobic cavity

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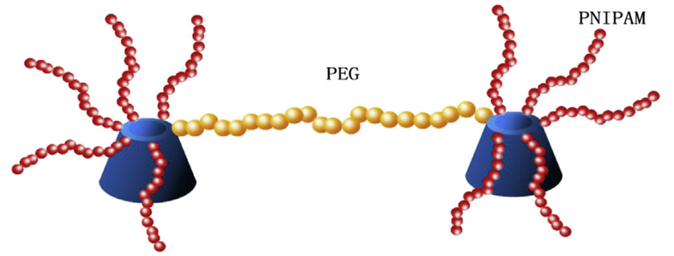


Scheme 1. Schematic representation for 3D topological structure of the designed dumbbell-shaped polymer.

can be utilized to adjust the self-assembly behavior of polymer and the size of formed assemblies through inclusion interaction with guest molecules.

Herein, we synthesized a dumbbell-shaped polymer with functions both from the thermal sensitivity of poly(N-isopropylacrylamide) (PNIPAM) segments [24,25] and from the molecular inclusion property of β-CD. To integrate several polymeric chains into one macromolecule, β-CD can act as an ideal bridge to obtain the dumbbell-shaped polymers. According to our previous work and related literature [18,25–27,51–53], rich activated hydroxyl groups in β-CD can be easily chemically modified, and endow β-CD an excellent bridge compound for the synthesis of miktoarm polymers. Thus, the dumbbell-shaped polymer PEG-2[CD-$(\text{PNIPAM}_r)_x$] was first synthesized through click reaction and atom transfer radical polymerization (ATRP) based on the modified β-CD monomer. After a well-defined characterization for the dumbbell-shaped polymer structure via Fourier transform infrared (FT-IR), ^{1}H NMR, and size exclusion chromatography/multiangle (SEC/MALLS), the thermally-triggered self-assembly behaviors and the effect of guest molecule inclusion on the size of formed nano-assemblies were further investigated using UV–vis spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM) and fluorescence spectrophotometer.

2. Experimental

2.1. Materials

Mono-6-deoxy-6-azido-β-cyclodextrin (β-CD-N_3) was prepared according to literature [43]. 2-Chloropropionic acid (97%, Alfa Aesar), propiolic acid (98%, Alfa Aesar), tris[2-(dimethylamino) ethyl]amine (Me_6TREN, 99%, Alfa Aesar), pyrene (Alfa Aesar, 99%), N-isopropylacrylamide (NIPAM, Acros, 99%), $Cu(Ph_3)_3Br$ (Acros, 99%) were all purchased from the companies attached and used without further purification. PEG ($M_n = 4000$Da), *N,N′*-Dicyclohexylcarbodiimide (DCC, 95%) and 4-Dimethylaminopyridine (DMAP, 95%) were ordered from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. CuCl was stirred with acetic acid overnight, then washed with ethanol and dried under vacuum at 25 °C. *N,N*-dimethyl formamide (DMF), *N,N*-dimethylacetamide (DMAc) were dried with 3Å grade molecular sieve before use. Alk-wang resin was prepared according to the literature [44].

2.2. Synthesis of alkynyl-terminated PEG, PEG-Alkynyl

PEG-Alkynyl was prepared according to the literature [45]. PEG (4.0 g, 1 mmol) was first dried under vacuum at 80 °C for 6 h, and dissolved with 40 mL of fresh distilled toluene. Then *p*-TsOH (34.44 mg, 0.2 mmol) was added. After stirring about 30 min, Propiolic acid (840 mg, 12 mmol) was added to the system. The mixture was allowed to react at 80 °C for 48 h under reflux, and then precipitated into an excess of cold diethyl ether. The collected precipitate was redissolved with CH_2Cl_2 (100 mL) and washed three times with $NaHCO_3$ aqueous solution (50 mL). The organic phase was dried over anhydrous Na_2SO_4. After filtration, the solution was concentrated by rotary evaporation and precipitated into cold diethyl ether. The product was washed three times with diethyl ether and dried under vacuum at 25 °C for 2 days. Yield: 85.3%. FT-IR (KBr): 3226 cm^{-1} (ν, $\equiv$C–H); 2098 cm^{-1} (ν, C$\equiv$C); 1725 cm^{-1} (ν, C=O); 1110 cm^{-1} (ν, C–O–C). ^{1}H NMR (DMSO-d_6, TMS): $\delta = 4.57$ (2H, *H*C$\equiv$C–$COOCH_2$–); $\delta = 4.3$–4.15 (4H, HC$\equiv$C–COOC*H*$_2$–); 3.7–3.3 (348H, –OC*H*$_2$C*H*$_2$O–).

2.3. Synthesis of β-CD-terminated PEG, PEG-2CD

The synthesis of PEG-2CD was accomplished by click reaction between PEG-Alkynyl and β-CD-N_3. At first, a mixture of β-CD-N_3 (1160 mg, 1 mmol), PEG-Alkynyl (1710 mg, 0.4329 mmol) and DMF (20 mL) was bubbled with nitrogen gas for 15 min. $Cu(PPh_3)_3Br$ (186 mg, 0.2 mmol) was then added into the above reaction system. After being bubbled with nitrogen gas for 30 min, the reaction was conducted at 60 °C for 18 h, and then, Alk-wang resin (500 mg) was added and allowed this system to react for another 8 h under nitrogen atmosphere. The mixture was exposed to air and diluted with THF, followed by passing it through a neutral Al_2O_3 column, and then, subjected to rotary evaporation. The residues were precipitated into cold diethyl ether and washed with diethyl ether three times. Finally, the product was dried under vacuum at 25 °C for 1 day. Yield: 72%. FT-IR (KBr): 3370 cm^{-1} (ν, O–H); 2870 cm^{-1} (ν, C–H); 1720 cm^{-1} (ν, C=O); 1105 cm^{-1} (ν, C–O–C in PEG); 1033 cm^{-1} (ν, C–O–C in β-CD). ^{1}H NMR (DMSO-d_6, TMS): $\delta = 7.82$ (2H, methine proton in 1,2,3-triazole); 5.8–5.6 (28H, 2,3–OH); 4.8–4.7 (14H, 1-H); 4.6–4.3 (12H, 6–OH overlaps with HC$\equiv$C–COOC*H*$_2$–); 3.85–3.3 (348H, –OC*H*$_2$C*H*$_2$O–, overlaps with 2,3,4,5,6-H in β-CD).

2.4. Synthesis of macroinitiator, PEG-2(CD-Cl_x)

PEG-2CD was first dried under vacuum at 50 °C for 6 h, and then, PEG-2CD (1500 mg, 0.237 mmol), DCC (586.7 mg, 2.85 mmol), DMAP (69.49 mg, 0.569 mmol) were added to a 50 mL round-bottomed flask and dissolved with 10 mL of anhydrous DMAc. Under the conditions of ice-water bath and magnetic stirring, 2-CPA (309.1 mg, 2.85 mmol, dissolved in 5 mL DMAc) was dropwise added to the flask. The mixture was stirred at room temperature for 48 h, filtered and precipitated into an excess of cold diethyl ether. The precipitate was dissolved in anhydrous methanol, and subjected to a dissolution–filtration–evaporation cycle for five times. The final product (PEG-2(CD-Cl_x)) was precipitated into an excess of cold diethyl ether, and dried in a vacuum oven overnight at 30 °C. FT-IR (KBr): 2870 cm^{-1} (ν, C–H); 1725 cm^{-1} (ν, C=O); 1106 cm^{-1} (ν, C–O–C in PEG); 1035 cm^{-1} (ν, C–O–C in β-CD). ^{1}H NMR (DMSO-d_6, TMS): $\delta = 7.85$ (2H, methine proton in 1,2,3-triazole); 5.95–5.65 (28H, 2,3–OH); 4.95–4.8 (14H, 1-H); 4.8–4.65 (5H, –C*H*$_2$OOC–C–CH(CH_3)Cl); 4.55–4.4 (5H, –C*H*$_2$OOC–C–C*H*(CH_3)Cl), 4.4–4.25 (4H, –C=COOC*H*$_2$–); 4.25–4.1 (10H, –C*H*(CH_3)Cl); 3.9–3.3 (348H, –OCH_2CH_2O–, overlaps with 2,3,4,5-H in β-CD); 1.7–1.45 (29H,–CH(C*H*$_3$)Cl).

2.5. Synthesis of dumbbell-shaped polymers: PEG-2[CD-$(\text{PNIPAM}_r)_x$]

PEG-2[CD-$(\text{PNIPAM}_r)_x$] was synthesized by ATRP using PEG-2(CD-Cl_x) as the macroinitiator and NIPAM as the monomer. The typical procedure was as following. A mixture of PEG-2(CD-Cl_x) (410 mg, 0.05568 mmol), NIPAM (2264.5 mg, 20 mmol) and Me_6TREN (128.4 mg, 0.668 mmol) in 4 mL of DMF was bubbled with nitrogen gas for 15 min, and then, CuCl (55.4 mg, 0.557 mmol) was added. The solution was bubbled with nitrogen gas again for 30 min and sealed under nitrogen atmosphere. After polymerization at 40 °C for 4 h, the reaction was terminated by exposing it into air, and diluted with THF. The product was passed though a column of neutral alumina for removal of copper catalyst, and then was evaporated out of the solvent. The residues were precipitated into an excess of cold diethyl ether several times, and dried in a vacuum at 30 °C oven overnight. The final product of PEG-2[CD-(PNIPAM$_r$)$_x$] was obtained. Yield: 62%. SEC/MALLS: $M_{n,SEC}$ = 30,600 g/mol, M_w/M_n = 1.29.

2.6. Polymer characterization

Fourier Transform Infrared (FT-IR) spectra were recorded on a NICOLET iS10 IR spectrometer. The ^{1}H NMR spectra were obtained from a Bruker Avance 300 spectrometer (Bruker BioSpin, Switzerland) operating at 300 MHz (^{1}H) in DMSO-d$_6$ or D_2O. The molecular structure parameters of the polymer synthesized were determined on a DAWN EOS size exclusion chromatography/multiangle laser light scattering (SEC/MALLS). HPLC grade DMF containing LiCl (0.01 mol/L) (at 40 °C) was used as eluant at a flow rate of 0.5 mL/min. Waters 515 pump and differential refractometer (Optilab rEX) were employed.

2.7. Investigation of thermally-triggered self-assembly behaviors of the dumbbell-shaped polymer

The LCST of the dumbbell-shaped polymer was measured by UV–vis spectroscopy (Shimadzu UV-2550 model). The transmittance of the polymeric aqueous solution (0.5 mg/mL) was recorded from 25 °C to 50 °C. Temperature corresponding to the onset of the decrease in transmittance was defined as LCST.

The self-assembly process of the dumbbell-shaped polymer was investigated by DLS and transmission electron microscopy (TEM). DLS measurements were carried out by a Malvern Zetasizer Nano ZS instrument. The DLS data of the polymeric solution (0.2 mg/mL for PEG-2[CD-(PNIPAM$_r$)$_x$] were recorded at temperatures of 25 °C, 35 °C, 40 °C and 50 °C, respectively. Each sample was kept at a constant temperature for 5 min before data collection. TEM observations were conducted on a Hitachi H-600 electron microscope at an acceleration voltage of 75 kV. The samples were prepared by placing 10 μL of polymer aqueous solution on copper grids in a biochemical incubator thermostatted at 50 °C.

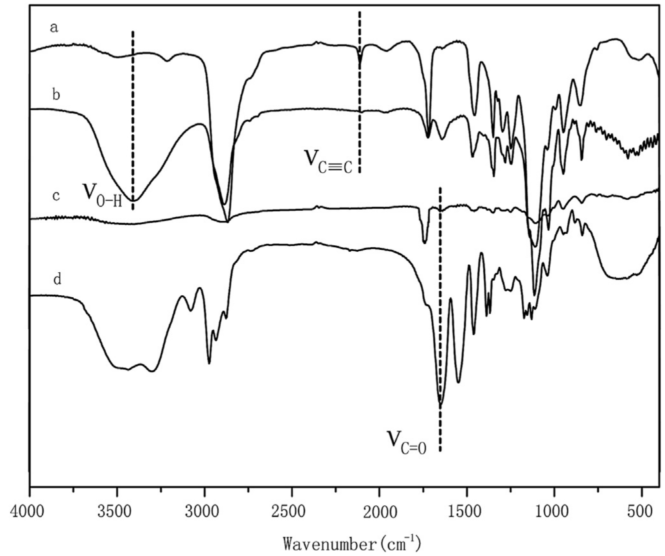
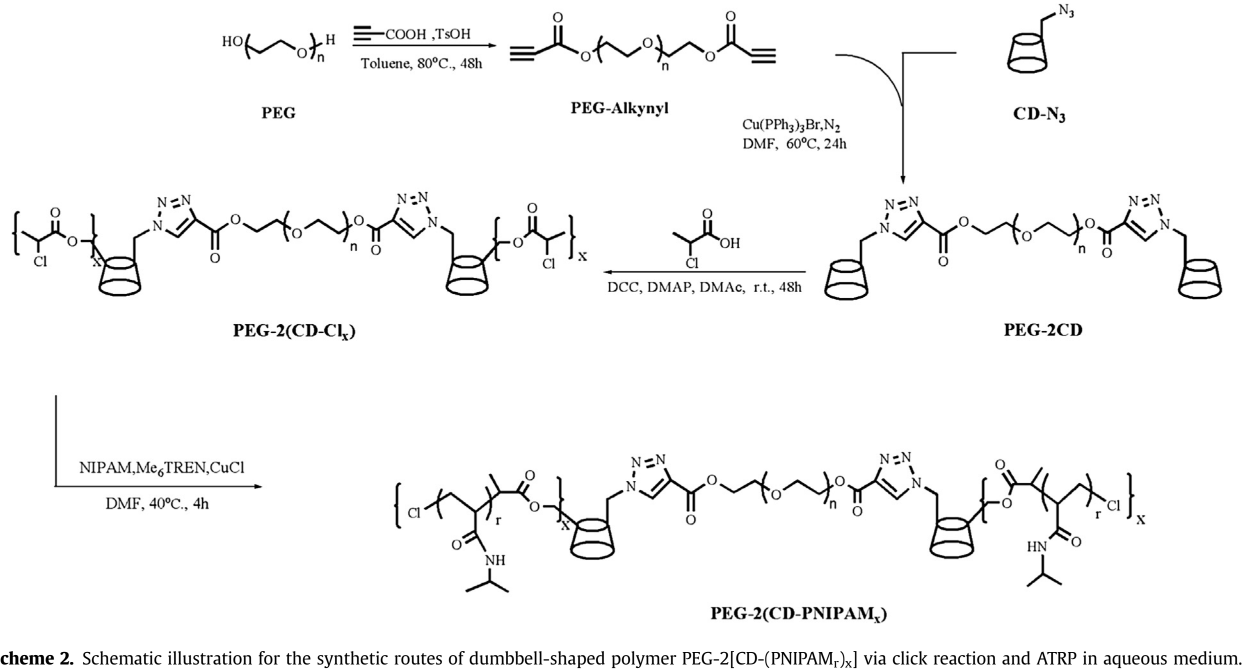


Fig. 1. FT-IR spectra of alkynyl-terminated PEG, PEG-Alkynyl (a), β-CD-terminated PEG, PEG-2CD (b), macroinitiator PEG-2(CD-Cl$_x$) (c) and dumbbell-shaped polymer PEG-2 [CD-(PNIPAM$_r$)$_x$] (d).

The self-assembly architecture of the dumbbell-shaped polymer was characterized by ^{1}H NMR. The polymer concentration in D_2O was set as 0.2 mg/mL for PEG-2[CD-(PNIPAM$_r$)$_x$], which was the same as the concentration used in DLS measurement and TEM observation.

The influence of addition of guest molecules on aggregation behavior of the dumbbell-shaped polymer in aqueous solution was investigated by DLS. The concentration of guest molecules 1-adamantane acid sodium was 0.2 mM (Ada-COONa). The polymeric



Scheme 2. Schematic illustration for the synthetic routes of dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$] via click reaction and ATRP in aqueous medium.

solution was 0.2 mg/mL and the Ada-COONa was prepared by the reaction of 1-adamantane acid with sodium hydroxide. The DLS data of the polymeric solution were recorded at temperatures of 50 °C. Each sample was kept at a constant temperature for 5 min before data collection.

The emission spectra of pyrene aqueous solution (6×10^{-6} mol/L) in the presence of dumbbell-shaped polymer at various temperatures were recorded by fluorescence spectroscopy from 370 to 650 nm with an excitation wavelength of 350 nm.

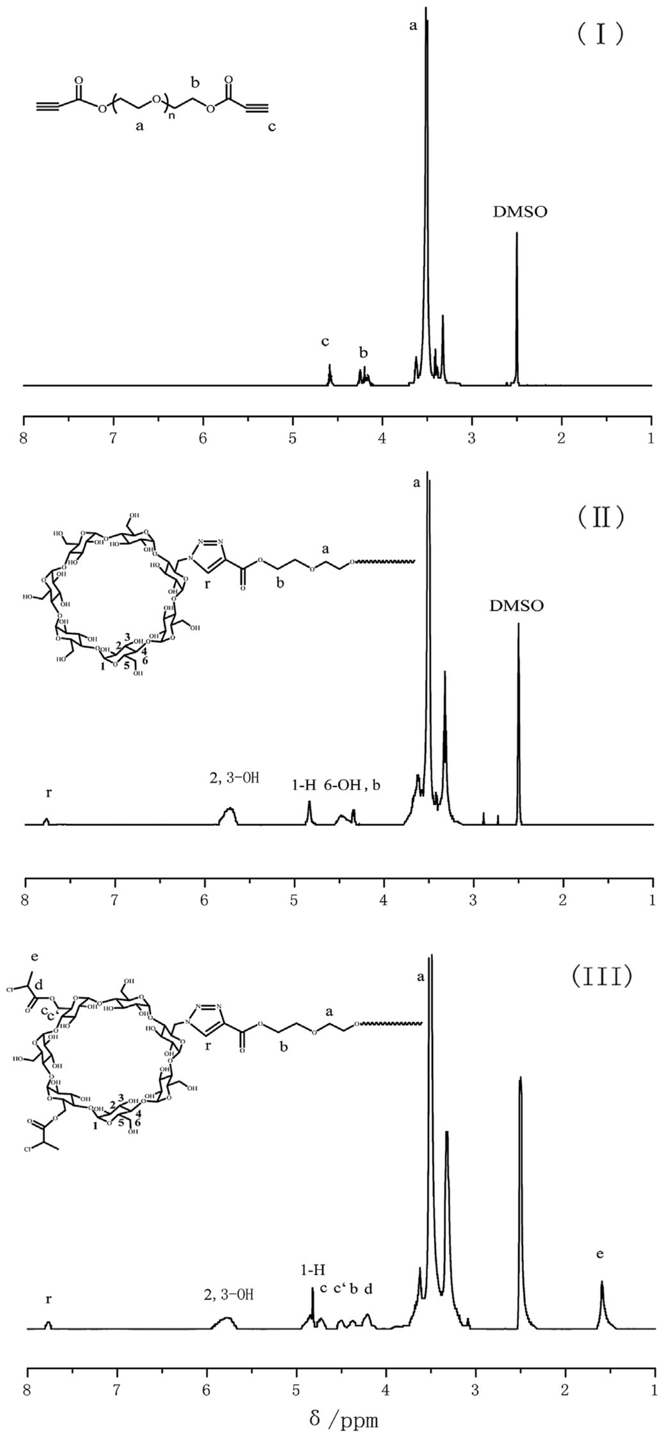


Fig. 2. ^{1}H NMR spectra of the alkynyl-terminated PEG, PEG-Alkynyl (I), β-CD-terminated PEG, PEG-2CD(II) and macroinitiator PEG-2(CD-Cl_x)(III).

3. Results and discussion

3.1. Synthesis of macroinitiator PEG-2(CD-Cl_x)

The synthesis of dumbbell-shaped polymer started from the esterification reaction of PEG with propiolic acid in the presence of *p*-TsOH in refluxing toluene [45]. Then, to enhance the functionality of the dumbbell-shaped polymer, two β-CD units as the linkage points were introduced into the ends of PEG by click chemistry (Scheme 2). Fig. 1(a) shows the FT-IR spectrum of PEG-alkynyl, where the appearance of the characteristic absorption peak of alkynyl at 3226 cm^{-1}, 2098 cm^{-1}, and the ester group at 1725 cm^{-1}, together with the disappearance of the stretching vibration peak of hydroxyl group at 3440 cm^{-1}, suggests that the hydroxyl groups of PEG have completely reacted with propiolic acid. Moreover, in the ^{1}H NMR spectrum of PEG-alkynyl (Fig. 2(I)), the appearance of proton peaks **b** and **c** confirms that the hydroxyl groups in PEG terminals were also successfully replaced by ester groups. The calculated peak integral ratio of proton **a** to **b** is about 87, demonstrating that the degree of esterification can be reached up to 96% (Table 1). The result shows that the molecular weight of PEG-alkynyl determined by ^{1}H NMR was in accordance with the date determined by SEC/MALLS. For the click reaction between PEG-Alkynyl and CD-N_3, the organosoluble $Cu(Ph_3)_3Br$ was a suitable catalyst [46,47]. The excess of CD-N_3 can be removed by further click reaction with Alk-wang resin [44]. Fig. 2(II) presents the ^{1}H NMR spectrum of PEG-2CD. Evidently, characteristic signals for β-CD at $\delta = 4.45$, 4.8, 5.7 and protons for 1,2,3-triazole ring at $\delta = 7.82$ appeared, indicating the occurrence of 1,3-dipolar cycloaddition reaction. The signals of 3,5,6-H (protons located at C-3, C-5 and C-6 positions of β-CD) and 2,4-H may overlap with the signals of methylene protons from PEG segments in the region of $\delta = 3.3$–3.8. After the click reaction, the signal of proton b shifts from $\delta = 4.15$–4.3 to $\delta = 4.3$–4.6, and the low-field shift can be ascribed to the change of adjacent group from the alkynyl-ester to the ester-(triazole ring). As shown in Table 1, compared with PEG-alkynyl, the molecular weight of PEG-2CD increased to 6710, which was in accordance with the expected. Based on the FT-IR spectrum of PEG-CD (Fig. 1(b)), the disappearance of absorption peaks of azido groups at 2096 cm^{-1} and alkynyl at 2098 cm^{-1} and 3226 cm^{-1} can further confirm the complete reaction of CD-N_3 with PEG-alkynyl. In addition, the stretching vibration peak of hydroxyl groups at 3370 cm^{-1} implies the successful attachment of β-CD onto the PEG terminals.

To further introduce PNIPAM chains into β-CD moieties, selective esterification was conducted between PEG-2CD and 2-chloropropionic acid under the catalysis of DCC and DMAP. Thus, the macroinitiator PEG-2(CD-Cl_x) carrying several alkyl chlorine groups was obtained. The ^{1}H NMR spectrum for PEG-2(CD-Cl_x) is showed in Fig. 2(III). Signals associated with 2-chloropropionate residue in PEG-2(CD-Cl_x) are discernible at $\delta = 1.60$ (peak e) and $\delta = 4.25$ (peak d). This indicates that alkyl chlorine groups were

Table 1
Molecular structure parameters for PEG-Alkyne, PEG-2CD and PEG-2[CD-$(PNIPAM_r)_x$].

Sample	$M_{n,\ SEC}$[a] (g/mol)	$M_{n,\ NMR}$[b] (g/mol)	M_w/M_n	dn/dc[a] (mL/g)
PEG-alkyne	4210	3950	1.18	0.058
PEG-2CD	6710	6330	1.22	0.061
PEG-2[CD-$(PNIPAM_r)_x$]	30,600	33,740	1.29	0.072

[a] Measured by SEC/MALLS.

[b] Calculated based on the signals at $\delta = 3.52$ ppm to the signals at $\delta = 5.7$ ppm or $\delta = 3.85$ ppm.

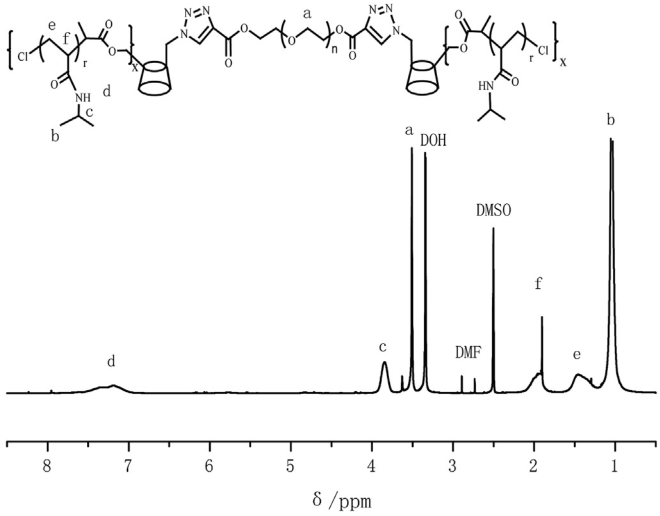


Fig. 3. ^{1}H NMR spectrum of dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$].

successfully introduced into PEG-2CD. Normally, all the hydroxyl groups adjacent to C-2,-3,-6 of β-CD (2,3,6-OH) may react with the 2-chloropropionic acid during the esterification process, whereas selective modification can be carried out via careful controlling the reaction conditions based on the different reactivities from hydroxyl groups at C-2,-3,-6 positions in β-CD. 12 equiv 2-chloropropionic acid was used to react with PEG-2CD to guarantee the occurrence of esterification taking place at the C-6 position of β-CD. By integrating the proton peaks in Fig. 2(III), the molar ratio of 2,3-OH to 1-H (protons at C-1 position) was calculated as to 1.94, indicating that the 2,3-OHs may not involve in the esterification reaction with 2-chloropropionic acid. Besides, the molar ratio of 2-chloropropionate residues to β-CD molecules was calculated to be 9.57, suggesting that each β-CD molecule bears 4–5 chlorines in average. Thus, the degree of substitution, that is the value of x in PEG-2(CD-Cl$_x$), is about 5.

3.2. Synthesis of dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$]

Using PEG-2(CD-Cl$_x$) as the macroinitiator and NIPAM as the monomer, the A$_n$BA$_n$ type dumbbell-shaped polymer (A and B represent PNIPAM segment and PEG segment, respectively), PEG-2[CD-(PNIPAM$_r$)$_x$], was synthesized by ATRP. The reaction was conducted at 40 °C for 4 h, and then, the removal of NIPAM was achieved by the precipitation step. Fig. 1(d) shows the FT-IR spectrum of PEG-2[CD-(PNIPAM$_r$)$_x$], the appearance of absorption peak of amido at 1640 cm^{-1} proved the successful polymerization. Fig. 3 shows the ^{1}H NMR spectrum of PEG-2[CD-(PNIPAM$_r$)$_x$]. As can be seen, the several characteristic peaks of PNIPAM at $\delta = 7.24$, 3.85, 1.12, and one peak of PEG at $\delta = 3.52$ are clearly observed. However, the proton resonance peaks from β-CD can not be observed clearly because the increased molecular weight. The total degree of polymerization (DP) of PNIPAM was determined as 235 by calculating the integral ratio of peak c (methine protons of PNIPAM side chain) versus peak a (methylene protons of PEG main chain), leading to each arm with about 24 NIPAM units. Thus, the molecular weight of PEG-2[CD-(PNIPAM$_r$)$_x$] was calculated to be 32,800 from NMR analysis. The result is in accordance with the result of SEC/MALLS (M_n = 30,600 g/mol) as showed in Table 1. Fig. 4 displays the SEC traces of PEG-2(CD-Cl$_x$) and PEG-2[CD-(PNIPAM$_r$)$_x$], and the observed monodispersed peak further indicates the narrow molecular weight distribution.

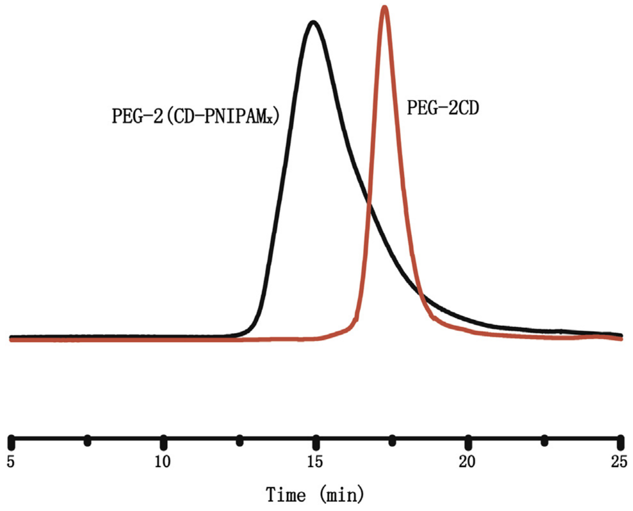


Fig. 4. DRI traces of SEC/MALLS chromatograms of PEG-2[CD-(PNIPAM$_r$)$_x$] and PEG-2CD.

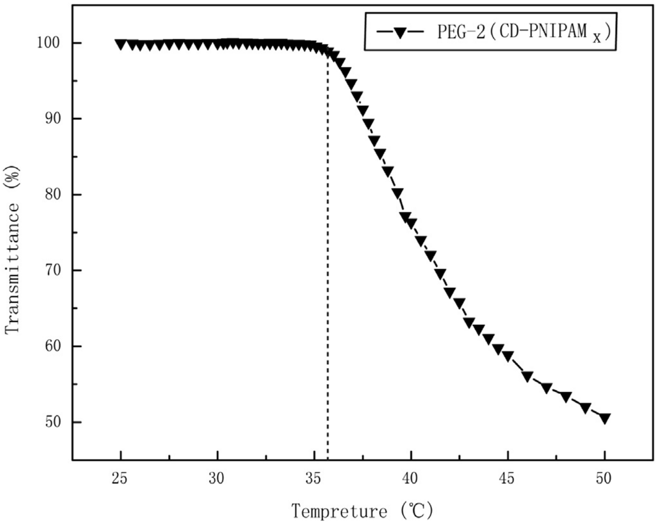


Fig. 5. Determination of LCST of dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$] (0.5 mg/mL).

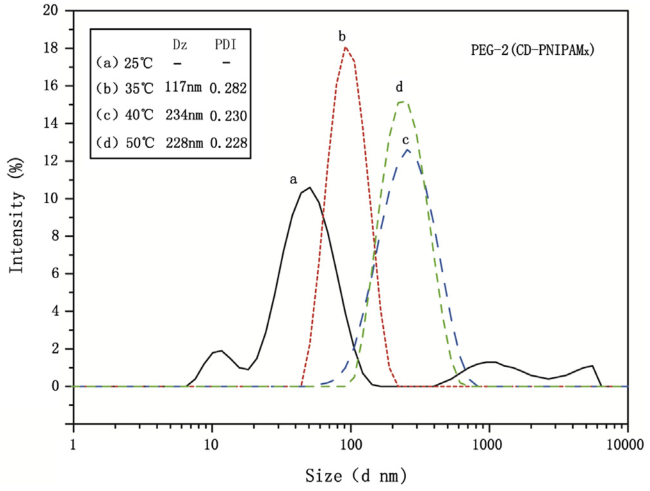


Fig. 6. Size distributions of the assemblies formed by dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$] (0.2 mg/mL) in aqueous solution.

3.3. Thermally-triggered self-assembly behaviors

In the polymer structure of PEG-2[CD-(PNIPAM$_r$)$_x$], PEG is well-known for its highly hydrophilic property and biocompatibility, while PNIPAM is a thermosensitive polymer due to its good coil-to-globule phase transition reversibility, and a well-defined lower critical solution temperature (LCST) around 32 °C [48]. Thus, the synthesized dumbbell-shaped polymer combining the properties both of two polymers can be expected to conduct self-assembly to nano-assemblies by adjusting the temperature of aqueous solution. UV–vis was first used to determine the LCST of dumbbell-shaped polymer. As can be seen from Fig. 5, a sharp decline of transmittance of the solution can be observed with elevating temperature, indicating the thermally-triggered property of dumbbell-shaped polymer. Its LCST can be determined as 35.7 °C, which is higher than that of the LCST of linear PNIPAM reported [48].

By DLS measurement (Fig. 6) and TEM observation (Fig. 7), it was found that PEG-2[CD-(PNIPAM$_r$)$_x$] can self-assemble into nano-sized assemblies above its LCST in aqueous solution. In Fig. 6-a, the size distribution of PEG-2[CD-(PNIPAM$_r$)$_x$] aqueous solution presents a wide polydispersity below LCST. However, when the temperature was raised above the LCST, big aggregates with a Z-average diameter (D_z) ranging from 200 nm to 250 nm were formed between 40 and 50 °C. In a typical TEM image (Fig. 7-A), the nano-assemblies around 200 nm in diameter were observed at 50 °C, which was in agreement with the result of DLS at the same temperature. Furthermore, the nano-assemblies constructed by PEG-2[CD-(PNIPAM$_r$)$_x$] present a dark dense core surrounded by relatively light color corona, reflecting a special micellar feature with nano-sized structure. Both of the DLS and TEM results confirm that the nano-assemblies were indeed formed based on the thermally-induced self-assembly of dumbbell-shaped polymer.

The structure of nano-assemblies of dumbbell-shaped polymer was further confirmed by ^{1}H NMR in D_2O as shown in Fig. 8. At 25 °C, the polymer can be completely dissolved in D_2O, all proton signals for PEG-2[CD-(PNIPAM$_r$)$_x$] are visible under this condition (Fig. 8-A), However, the disappearance of the proton resonance peaks from β-Cyclodextrin may influenced by the following two aspects, firstly, all the hydroxyl groups will be substituted by the deuterium in D_2O, secondly, the increased molecular weight may lead to this result too. When the temperature was raised to 50 °C, almost all signals from PNIPAM segments disappeared, whereas the signal from PEG segment was still remained with only a slight downshift. This fact strongly confirms the formation of nano-assemblies with a special core–shell structure, in which PNIPAM segments consist of the hydrophobic core while PEG segment serves as the hydrophilic shell. Based on the above analysis, a schematic illustration for the formation of PNIPAM-core based assemblies from PEG-2[CD-(PNIPAM$_r$)$_x$] is shown in Scheme 3. In Scheme 3, the central PEG block may form several loops around the core layer of PNIPAM, so the nano-assemblies present a "flowerlike" shape, which can be also confirmed by TEM image (Fig. 7-A).

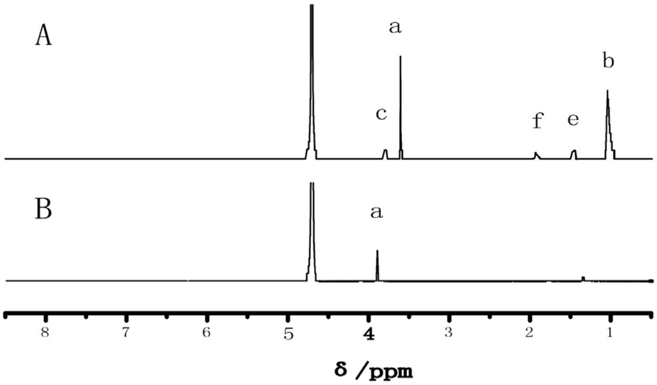


Fig. 8. ^{1}H NMR spectra of dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$] in D_2O at 25 °C (A) and 50 °C (B).

To further investigate the thermally-triggered self-assembly behaviors and corresponding mechanism of PEG-2[CD-(PNIPAM$_r$)$_x$], the fluorescence spectroscopy was employed to monitor its phase transition process using pyrene as a probe. In general, the fluorescence emission for pyrene can be used to discern a hydrophobic region where pyrene is existing [49,50]. The ratio of emission intensity for the first vibronic band to the third for pyrene is usually defined as I_1/I_3, and this parameter is widely used to monitor the formation of hydrophobic micro-domains. We recorded the I_1/I_3 values of pyrene in aqueous solution in the presence of PEG-2[CD-(PNIPAM$_r$)$_x$] against the temperature as showed in Fig. 9. The I_1/I_3 value for pyrene was 1.48 without polymer at 25 °C, whereas the I_1/I_3 value was lowered to 1.19 after the addition of polymer in a temperature range from 25 to 35 °C. This result implies that the inclusion between β-CD and pyrene took place. The molecular interaction between β-CD and pyrene effectively decreases the polarity of pyrene, leading to the lower I_1/I_3 value. Furthermore, before attaining to the LCST of PEG-2[CD-(PNIPAM$_r$)$_x$], the change of the I_1/I_3 value is basically steady with the increase of the temperature. However, the I_1/I_3 value exhibits an

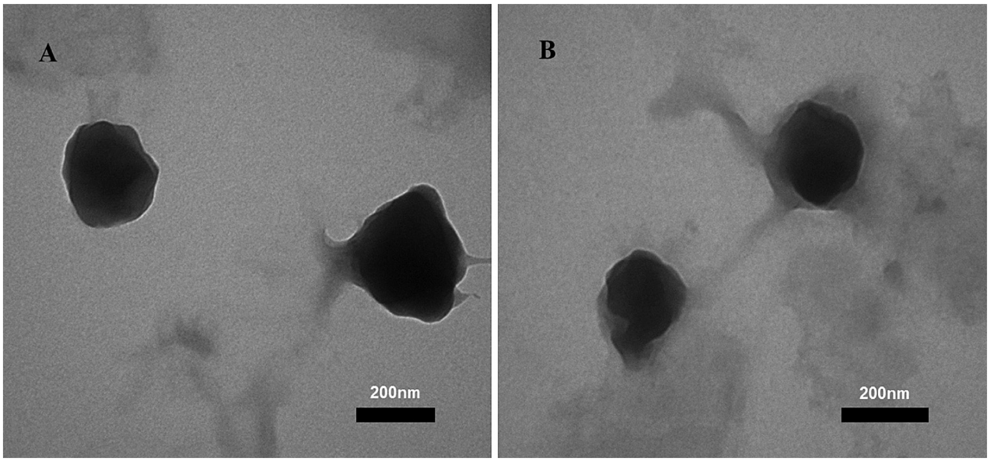
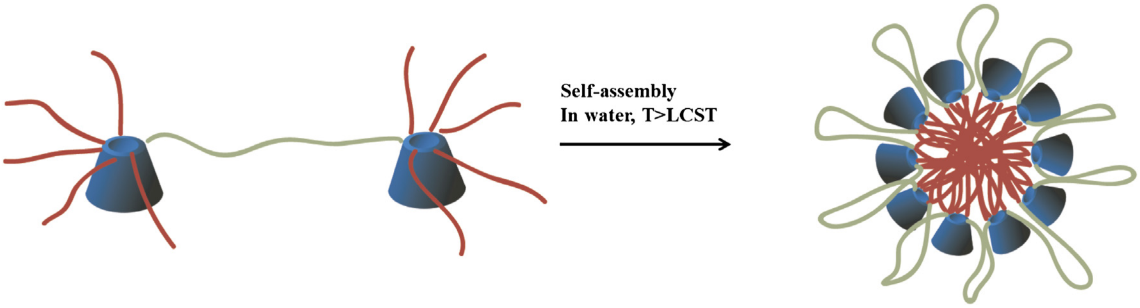


Fig. 7. Typical TEM images of dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$] in aqueous solution at 50 °C (A, 0.2 mg/mL; B, 0.2 mg/mL with 0.2 mM Ada-COONa).



Scheme 3. Schematic illustration for possible self-assembly mechanism of dumbbell-shaped polymer PEG-2[CD-(PNIPAM$_r$)$_x$] in water with the rising of temperature.

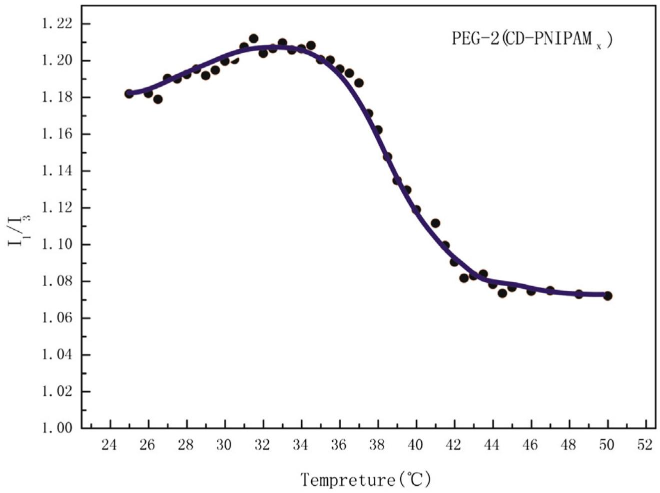


Fig. 9. I_1/I_3 value of the pyrene aqueous solution in the presence of dumbbell-shaped PEG-2[CD-(PNIPAM$_r$)$_x$] (0.5 mg/mL) with the gradual elevation of temperature.

abrupt decrease near LCST, indicating the environment transition for pyrene from a hydrophilic to a hydrophobic state.

According to our previous research [19], Ada can fairly form inclusion complexes with β-CD, and its inclusion constant with β-CD is also high. Thus, Ada-COONa was used as guest molecule to study the influence of molecular inclusion on self-assembly behaviors. Upon the addition of Ada-COO$^-$ (0.2 mM) under the condition of 50 °C, the size of PEG-2[CD-(PNIPAM$_r$)$_x$]/Ada complexes was reduced with an average D_z value of 165 nm (Table 2). The TEM image from the complexes also draws the same conclusion as shown in Fig. 7-B. Additionally, the zeta-potential measured for the complexes is about −17.1 mV (Table 2), which also confirms the same point. Compared with the PEG-2[CD-(PNIPAM$_r$)$_x$] nano-assemblies, the decrease of the size of complexes may be resulted from the molecular inclusion interactions between Ada-COO$^-$ and β-CD. The charges from Ada-COO$^-$ not only can stabilize the complexes, but also give rise to the electrostatic interaction repulsive force within Ada-COO$^-$ groups, leading to the shrink of the size of complexes.

Table 2
Size and zeta-potential of PEG-2[CD-(PNIPAM$_r$)$_x$] without and with Ada-COONa at the same concentration (0.2 mg/mL) at 50 °C.

Sample	D_z (nm)	Zeta-potential (mV)
PEG-2[CD-(PNIPAM$_r$)$_x$]	228	−4.97
PEG-2[CD-(PNIPAM$_r$)$_x$] + Ada	165	−17.1

4. Conclusions

The thermally-responsive dumbbell-shaped polymer was synthesized using a combination of click reaction and ATRP polymerization. The polymer consists of a PEG central segment and two β-CD as bridge units, with which about 4–5 PNIPAM arms are linked. Above the LCST of PNIPAM segment, the polymer can self-assemble into flower-like nano-assemblies, in which PNIPAM segments act as a core part and PEG segments serve as a corona part. The polymer shows the evident molecular inclusion ability using Ada-COONa and pyrene as guest molecules. The addition of Ada-COONa into polymer solution can effectively adjust the size of nano-assemblies. Compared with previously reported dumbbell-shaped polymers, we believe that the dumbbell-shaped polymer in this work can offer more advantages in the fields of molecular inclusion and controlled release for drugs, dyes, and so on.

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