

A Dual-Responsive Supra-Amphiphilic Polypseudorotaxane Constructed from a Water-Soluble Pillar[7]arene and an Azobenzene-Containing Random Copolymer

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S Supporting Information

ABSTRACT: Macromolecular supra-amphiphiles refer to a kind of macromolecular amphiphiles whose hydrophilic and hydrophobic parts are connected by noncovalent forces. They have applications in various fields, such as drug delivery, sensor systems, and biomedical materials. Here we report a novel molecular recognition motif between a new thermoresponsive water-soluble pillar[7]arene (WP7) and an azobenzene derivative. Furthermore, we utilized this recognition motif to construct the first pillararene-based supra-amphiphilic polypseudorotaxane which can self-assemble to form vesicles in water. Due to the dual-responsiveness of the molecular recognition motif (the thermoresponsiveness of WP7 and photo-responsiveness of azobenzene), the reversible transformations between solid nanospheres based on the self-assembly of the polymer backbone and vesicles based on the self-assembly of the supra-amphiphilic polypseudorotaxane were achieved by adjusting the solution temperature or UV–visible light irradiation. These dual-responsive aggregation behaviors were further used in the controlled release of water-soluble dye calcein molecules.

Macromolecular amphiphiles have drawn growing interest in chemistry and materials science due to the ability of these chemicals to offer a rich variety of morphologies and transitions as well as their potential applications in many fields, such as drug delivery, sensor systems, and biomedical materials.¹ Macromolecular supra-amphiphiles refer to a kind of macromolecular amphiphiles whose hydrophilic and hydrophobic parts are connected by noncovalent forces.^{2–4} Compared with traditional macromolecular amphiphiles, macromolecular supra-amphiphiles can be easily obtained in a facile and dynamic manner instead of tedious and time-consuming synthesis due to the dynamic and reversible nature of noncovalent interactions.³ Due to the introduction of noncovalent forces, macromolecular supra-amphiphiles show abundant stimuli responsiveness, which could greatly enhance the versatility of these materials in a variety of applications. However, to date, most macrocycle-based macromolecular supra-amphiphiles were prepared by cyclodextrin based host–guest recognition;⁴ macromolecular supra-amphiphiles prepared by other macrocycle-based (such as calixarenes⁵ and cucurbiturils⁶) host–guest recognition motifs have rarely been reported. In view of this, employing new

macrocycle-based molecular recognition motifs to construct macromolecular supra-amphiphiles will push the development of macromolecular amphiphiles. Pillararenes,⁷ a new generation of macrocyclic hosts next to crown ethers, cyclodextrins, calixarenes, and cucurbiturils,^{5,6,8} have been extensively employed to construct functional supramolecular materials, such as supra-molecular polymers^{7c} and transmembrane channels,^{7d} due to their unique structures and high functionality. Recently, pillararene-based supra-amphiphiles have been actively explored due to their potential applications in many areas.⁹ Moreover, various morphologies, such as micelles, vesicles, and nanotubes, have been observed.⁹ However, all these studies focused on small-molecule supra-amphiphiles. Compared with aggregates fabricated from small-molecule supra-amphiphiles,¹⁰ aggregates prepared from macromolecular supra-amphiphiles have exhibited higher thermodynamic stability and durability due to their mechanical and physical properties.¹¹ Consequently, it is essential to develop pillararene-based macromolecular supra-amphiphiles, not only out of academic interest but also because of their potential applications. To the best of our knowledge, pillararene-based macromolecular supra-amphiphiles have not yet been reported. Additionally, though molecular recognition of pillar[5,6]arenes has been widely investigated, host–guest chemistry based on pillar[7]arenes has been rarely explored.^{7f,g}

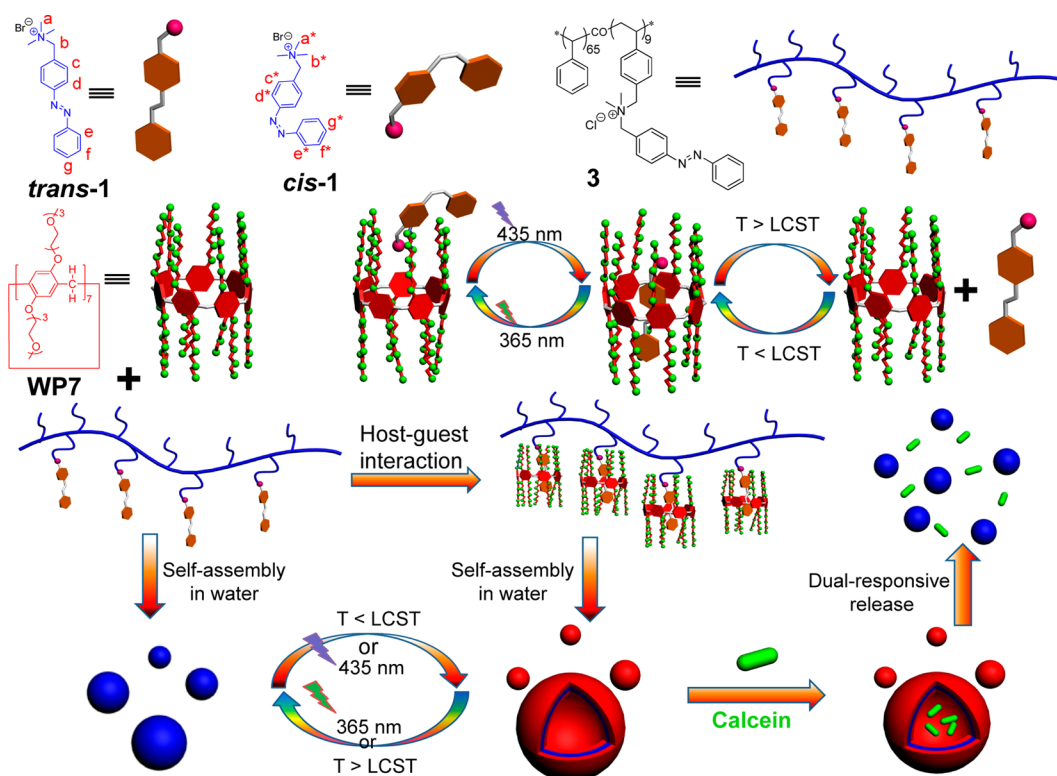
Herein, we report a novel molecular recognition motif between a new thermoresponsive water-soluble pillar[7]arene (WP7) and an azobenzene derivative (Scheme 1). This new recognition motif in water has dual thermo- and photo-responsiveness. Furthermore, we utilize this recognition motif to construct the first pillararene-based supra-amphiphilic polypseudorotaxane, which can self-assemble to form vesicles in water. Due to the thermoresponsiveness of WP7 and photoresponsiveness of the azobenzene unit, the reversible transformations between solid nanospheres based on the self-assembly of the polymer backbone and vesicles based on the self-assembly of the supra-amphiphilic polypseudorotaxane are achieved by adjusting the solution temperature or UV–visible light irradiation. These reversible conversions are further used in the controlled release of water-soluble dye calcein molecules.

The synthetic methods for azobenzene containing random copolymer **3** and WP7 are shown in Schemes S1 and S2. WP7 was prepared by etherification of *per*-hydroxylated pillar[7]arene,

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Scheme 1. Chemical Structures of WP7, 1, and Polymer 3, and Illustration of the Dual-Responsive Controlled Assembly and Disassembly of WP7/3 Supramolecular Vesicles and the Process of Dual-Responsive Release of Calcein Molecules



which was synthesized according to a previously reported procedure.^{7f} Like pillar[5,6,10]arenes with tri(ethylene oxide) groups,¹² WP7 also showed LCST behavior in water. As shown in Figure S8, a clear aqueous solution of WP7 (2.00 mM) turned to be turbid upon heating to 50 °C. It became clear again as the temperature fell down. Moreover, the LCST behavior of WP7 has a concentration dependence feature (Figure S9). As the concentration of WP7 increased, T_{cloud} of WP7 decreased, which showed the same phenomena with analogous pillar[5,6,10]-arenes.¹²

The ¹H NMR spectroscopy was first used to study the host-guest complexation between WP7 and the azobenzene unit on copolymer 3 by using 1 as the model compound because of the relatively poor water-solubility of copolymer 3. In comparison with the spectrum of free *trans*-1 (Figure 1a), the resonance peaks corresponding to the protons on *trans*-1 shifted upfield in the presence of WP7 (Figure 1b). Upfield shift changes were observed for the signals related to protons H_d, H_e, H_c, H_f and H_g ($\Delta\delta = -0.15, -0.08, -0.40, -0.07,$ and -0.06 ppm for H_d, H_e, H_c, H_f and H_g, respectively) upon addition of an equivalent amount of WP7 (Figure 1b). Moreover, the peaks of the protons on WP7 also exhibited slight chemical shift changes in the presence of *trans*-1 arising from the interactions between WP7 and *trans*-1 (Figure S15). These phenomena provided evidence for the formation of an inclusion complex between WP7 and *trans*-1 (Figure 1) mainly driven by hydrophobic interaction. The complexation stoichiometry between WP7 and *trans*-1 was determined to be 1:1 in aqueous solution by isothermal titration calorimetry (ITC) and the complexation association constant was $(1.97 \pm 0.24) \times 10^4 \text{ M}^{-1}$ (Figure S10a).

When a solution containing *trans*-1 was irradiated with UV light at 365 nm for 10 min, new peaks appeared (Figure 1d). Yet complete isomerization did not occur. At equilibrium, the molar

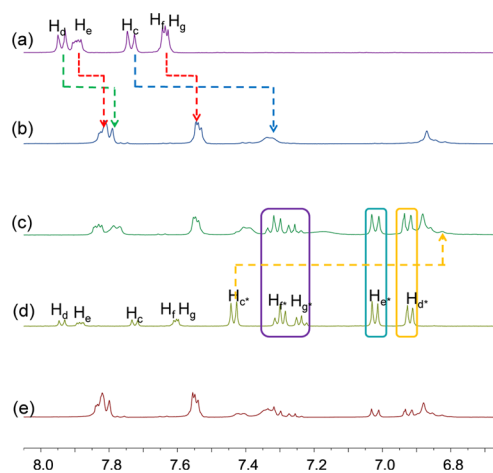


Figure 1. Partial ¹H NMR spectra (400 MHz, D₂O, 298 K): (a) 2.00 mM *trans*-1; (b) 2.00 mM *trans*-1 and 2.00 mM WP7; (c) 2.00 mM *trans*-1 and 2.00 mM WP7 after irradiation at 365 nm for 10 min; (d) 2.00 mM *trans*-1 after irradiation at 365 nm for 10 min; (e) 2.00 mM *trans*-1 and 2.00 mM WP7 after further irradiation at 435 nm for 10 min.

ratio of the *trans* to *cis* form of 1 changed to 30:70 (Figure 1d). Irradiation of the mixture with visible light (435 nm) allowed recovery of *trans*-1, but not complete recovery. Complexation of WP7 with *cis*-1 was examined by ¹H NMR (Figure 1c). When a mixture of WP7 and *trans*-1 was irradiated with UV light at 365 nm for 10 min, the chemical shift of proton H_{c*} on the benzene ring of *cis*-1 shifted upfield from 7.45 to 6.83 ppm. On the contrary, no or very small peak shifts were observed for protons H_{d*}–H_{g*} of *cis*-1 (Figure 1, spectra c and d). This indicated that the trimethylammonium group on *cis*-1 was bound by a rim of WP7, while the rest of *cis*-1 was outside the cavity of WP7. By

contrast, the remaining small proton peaks from *trans*-1 broadened. With the increase of the percentage of *cis*-1, the association constant for the host–guest complexation decreased to $(1.87 \pm 0.13) \times 10^3 \text{ M}^{-1}$ (Figure S10b). Upon irradiation with light at 435 nm for 10 min, almost all *cis*-1 went back to *trans*-1, and the proton signals related to the mixed solution of WP7 and *trans*-1 went back to the original state (Figure 1e), suggesting that the photocontrollable threading–dethreading switch between WP7 and 1 was achieved.

On the basis of the LCST behavior of WP7, the dethreading/rethreading process of the complex WP7 \supset *trans*-1 could also be controlled by the heating/cooling treatment. ^1H NMR provided powerful evidence for the thermo-controlled complexation between WP7 and *trans*-1 (Figures S11 and S12). As the temperature of the aqueous solution increased to 60 °C, the intensity of the aromatic signals of *trans*-1 almost returned to their uncomplexed values (Figure S11b). However, as the temperature of this solution decreased, the complexation between WP7 and *trans*-1 was recovered; most aromatic signals of 1 shifted upfield again (Figure S11f). Therefore, we demonstrated that the assembly and disassembly of the WP7 \supset *trans*-1 complex could be controlled upon cooling or heating.

On the basis of the dual photo- and thermocontrollable threading–dethreading switch between WP7 and 1, we further explored whether it could be used to construct a dual-responsive self-assembly system by introducing the azobenzene-containing random copolymer 3. Due to its poor solubility in water, copolymer 3 was first dissolved in *N,N*-dimethylformamide (DMF, 1.00 mL) with a concentration of 1.00 mM. WP7 was also dissolved in the above solution with a concentration of 7.00 mM. To elucidate the host–guest complexation, 2D Nuclear Overhauser enhancement spectroscopy (NOESY) was employed. As shown in Figure S16, the presence of 2D NOESY cross peaks between protons of H_{AZO} of 3 and protons of H_{ph} of WP7 indicates that the azobenzene species is threaded into the cavity of WP7. Therefore, a 1.00 mM DMF solution of the supra-amphiphilic polypseudorotaxane was obtained. Dripping 0.10 mL of this solution into water (1.90 mL) enabled self-assembly, which was indicated by the formation of a slightly turbid colloidal mixture. DMF was then removed by dialysis.

Then the self-assembly behavior of the supra-amphiphilic polypseudorotaxane in water was investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). First, the average size of the aggregates was determined by DLS to be ~ 250 nm (Figure 2a). The TEM image in Figure 2b shows that these supramolecular assemblies have a typical vesicular structure with an average diameter of ca. 260 nm, as evidenced by the distinct contrast between the periphery and the central part. Few larger particles observed in TEM compared with the DLS result can be attributed to flattening of the soft vesicular particles on the TEM grid while drying and/or fusion of smaller particles induced by a dehydrated hydrophilic corona.¹³ The partially collapsed morphologies also confirmed their vesicular structure (Figure 2c). The enlarged picture (Figure S13b) of the vesicles showed that the wall thickness was about 25 nm. Notably, the vesicles were quite stable and no precipitation was observed after storing for 2 months without any external stimuli.

Surprisingly, upon irradiation with UV light at 365 nm for over 1 h, the vesicles disassembled and the membrane started to disrupt into solid nanospheres based on the self-assembly of the polymer backbone with an average diameter of ~ 50 nm (Figure 3a). The disassembled system could be reassembled by

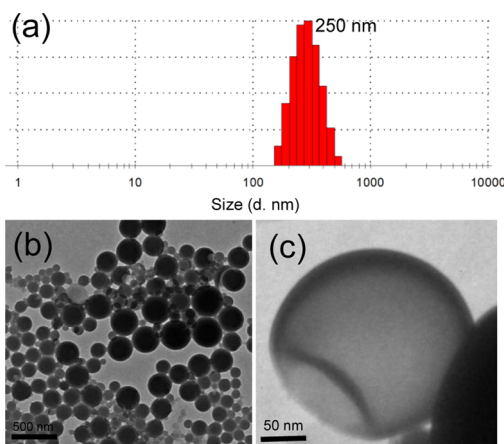


Figure 2. Characterizations of vesicles formed by the supra-amphiphilic polypseudorotaxane WP7 \supset 3: (a) DLS data; (b) TEM image; (c) enlarged TEM image of a partially collapsed vesicle.

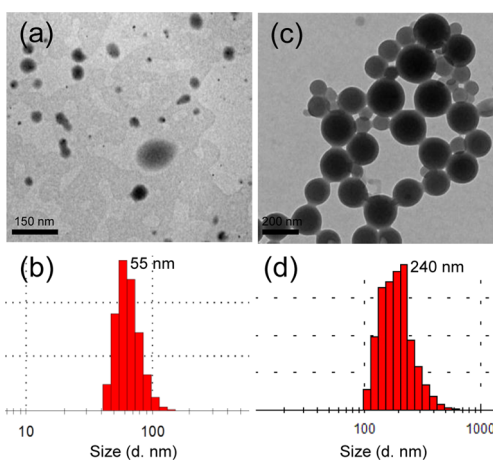


Figure 3. (a) TEM image of an aqueous solution of 7.00 mM WP7 and 1.00 mM 3 after irradiation with UV light at 365 nm; (b) DLS result of (a); (c) TEM image of aqueous solution of WP7 and 3 after further irradiation with visible light at 435 nm; (d) DLS result of (c).

irradiation with visible light at 435 nm, and vesicles with similar shapes and sizes reformed (Figure 3c). DLS was also utilized to monitor the size changes of the self-assemblies formed by the supra-amphiphilic polypseudorotaxane (Figure 3b,d), which were in accord with the TEM results. After irradiation with UV light, the particle size sharply decreased from ~ 260 to ~ 55 nm and then turned back to almost original size (~ 240 nm).

Furthermore, when heating the solution, supra-amphiphilic polypseudorotaxane WP7/3 disassembled and the vesicles changed into solid nanospheres with an average diameter of ~ 60 nm (Figure 4a). Moreover, upon cooling the solution, the solid nanospheres turned back to vesicles (Figure 4c). We then carried out variable-temperature DLS measurements (Figure 4b,d) with 0.100 mM aqueous polymer solution to monitor the size changes of the assemblies. With increasing temperature, the particle size sharply decreased from ~ 260 to ~ 58 nm and then turned back to almost original size (~ 255 nm).

The reversible transformations between solid nanoparticles based on the polymer backbone and vesicles formed by the supra-amphiphilic polypseudorotaxane were then utilized for controlled release. Because of the hydrophilic interior of vesicles, they could encapsulate hydrophilic guest molecules. With the use of fluorescent water-soluble calcein as a model, calcein-loaded

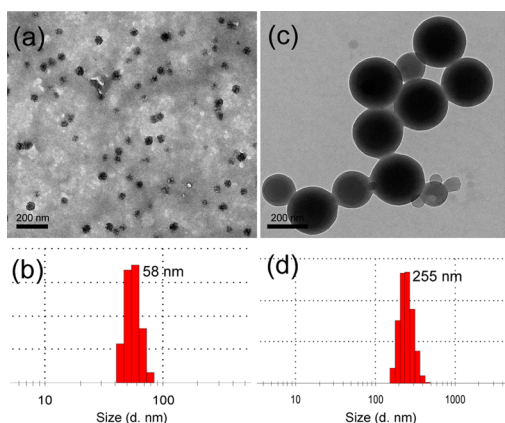


Figure 4. (a) TEM image of an aqueous solution of 7.00 mM WP7 and 1.00 mM **3** upon heating to 60 °C; (b) DLS result of (a); (c) TEM image of aqueous solution of WP7 and **3** after further cooling to 25 °C; (d) DLS result of (c).

WP7/**3** vesicles were prepared to perform controlled release experiments. The solution of calcein-loaded vesicles was dialyzed against the deionized water until the water outside the dialysis tube exhibited negligible calcein fluorescence. By irradiation with UV light at 365 nm or heating the solution to 60 °C, the release of calcein molecules from the inside of vesicles was achieved (Figures S14 and S17). It was interesting that the release behaviors of the encapsulated calcein were quite different under the influences of temperature and photoirradiation. As shown in Figure S17, the thermal stimulus caused a burst calcein release in prior 3 h and about 90% calcein molecules were released after 10 h, while the irradiation with UV light only resulted in 70% release of calcein molecules for 10 h. As we discussed above, because of the disassembly of the complexation between WP7 and **3**, the vesicles disrupted into solid nanoparticles, leading to the release of most encapsulated molecules. However, the irradiation with UV light did not result in a complete disruption of vesicles due to the existence of the equilibrium between *trans*-azo and *cis*-azo. In contrast, in the absence of external stimuli, calcein only showed a low-level free release that is less than 25% within 10 h. Thus, the conclusion can be drawn that WP7/**3** supramolecular polymeric vesicles can serve as nanocapsules that will release functional molecules or drugs by the heating or UV light irradiation treatments.

In conclusion, we have successfully established a novel molecular recognition motif between a water-soluble thermoresponsive pillar[7]arene WP7 and an azobenzene guest **1**. This new recognition motif in water shows dual-responsiveness. Furthermore, we utilized this recognition motif to construct a dual-responsive supra-amphiphilic polypseudorotaxane in water. To the best of our knowledge, this is the first pillararene-based supra-amphiphilic polypseudorotaxane. Due to the thermoresponsiveness of WP7 and photoresponsiveness of the azobenzene unit, the reversible transformations between solid nanospheres based on the self-assembly of the polymer backbone and vesicles based on the self-assembly of the supra-amphiphilic polypseudorotaxane were achieved by adjusting the solution temperature or UV-visible light irradiation. Therefore, the vesicles were further used in the controlled release of water-soluble dye calcein molecules. This work offered a new way to combine polymer science with pillararene supramolecular chemistry to construct functional supramolecular materials.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and additional data. 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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