

Electronic Supplementary Material (ESI) for RSC Advances

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## Supporting information

### **A dumbbell-like supramolecular triblock copolymer and its self-assembly of light-responsive vesicles**

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## 1. Materials and methods

4-phenylazophenol (Alfa, 98%), 2-bromoisobutyryl bromide (Alfa, 97%), propargyl bromide (J&K Chemical, 80 wt% solution in toluene), potassium carbonate (Sinopharm Chemical Reagent Co., Ltd, AR), sodium azide ( $\text{NaN}_3$ , Aldrich, 99.5%), ruthenium tetroxide ( $\text{RuO}_4$ ) (Acros, 0.5 wt% aqueous solution) were used as received. Styrene (Sinopharm Chemical Reagent Co., Ltd, AR) was washed with an aqueous solution of sodium hydroxide (5 wt% aqueous solution) three times and water until neutralization in order, then dried with anhydrous magnesium sulfate for 24 h and distilled at reduced pressure. Copper bromide (J&K Chemical, 98%) was purified by stirring in acetic acid and washed with ethanol three times. Pentamethyldiethylenetriamine (PMDETA, J&K Chemical, 98%) was stirred overnight over  $\text{CaH}_2$  and distilled under reduced pressure prior to use. N,N-dimethylformamide (DMF), trimethylamine, toluene and tetrahydrofuran (THF) (Sinopharm Chemical Reagent Co., Ltd, AR) were disposed with calcium hydride and distilled under vacuum prior to use. All the other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd and used as received.

## 2. Instruments and Measurements

### Nuclear Magnetic Resonance (NMR)

$^1\text{H}$  NMR spectra and  $^{13}\text{C}$  NMR with deuterium oxide ( $\text{D}_2\text{O}$ ), dimethylsulfoxide- $\text{d}_6$  ( $\text{DMSO-d}_6$ ), deuterated chloroform ( $\text{CDCl}_3$ ) or N,N-dimethylformamide- $\text{d}_7$  ( $\text{DMF-d}_7$ ) as solvents were performed on a Varian Mercury Plus 400-MHz spectrometer at 293K. TMS was used as the internal reference. The chemical shifts were referenced to residual peaks of deuterated solvents:  $\text{D}_2\text{O}$  (4.70 ppm);  $\text{DMSO-d}_6$  (2.48 ppm);  $\text{CDCl}_3$  (7.26 ppm);  $\text{DMF-d}_7$  (8.00 ppm).

### Gel Permeation Chromatography (GPC)

The molecular weights of the samples were measured by GPC on a HLC-8320GPC (TOSOH, EcoSEC GPC System) system at 40 °C with N,N-dimethylformamide

(DMF) as mobile phase at a flow rate of 0.6 mL/min.

### **Mass spectra**

Mass spectra were performed on an ultra high performance liquid chromatography-quadrupole time-of-flight (UPLC & Q-TOF-MS) Waters-ACQUITY™ mass spectrometer. The solvent is methanol and the concentrations of samples are 100~1000 µg/mL.

### **UV-vis absorption spectroscopy (UV-vis)**

UV-vis absorption spectra were performed on a Perkin Elmer Lambda 20 UV-Vis spectrometer. Samples of self-assemblies solutions with certain concentrations were added to a 1cm quartz cuvette (wavelength range: 200-800nm, wavelength accuracy: ±0.5 nm) for the measurements.

### **Dynamic Light Scattering (DLS)**

DLS measurements were performed with aqueous solutions on a Malvern Zetasizer Nano S (MalvernInstruments, Ltd.) equipped with a 4 mW He-Ne laser light operating at = 633 nm. All samples were measured at room temperature with a scattering angle of 173°.

### **Scanning Electron Microscope (SEM)**

SEM measurements were performed on Nova NanoSEM 450 (FEI), with an accelerating voltage 5 kV. The samples for SEM observations were prepared by depositing several drops of the self-assemblies solution (1 mg/mL) onto the surface of clean silicon chips, and the samples were air-dried at room temperature for 24 hours. The samples were coated with a thin film of gold before measuring.

### **Transmission Electron Microscope (TEM)**

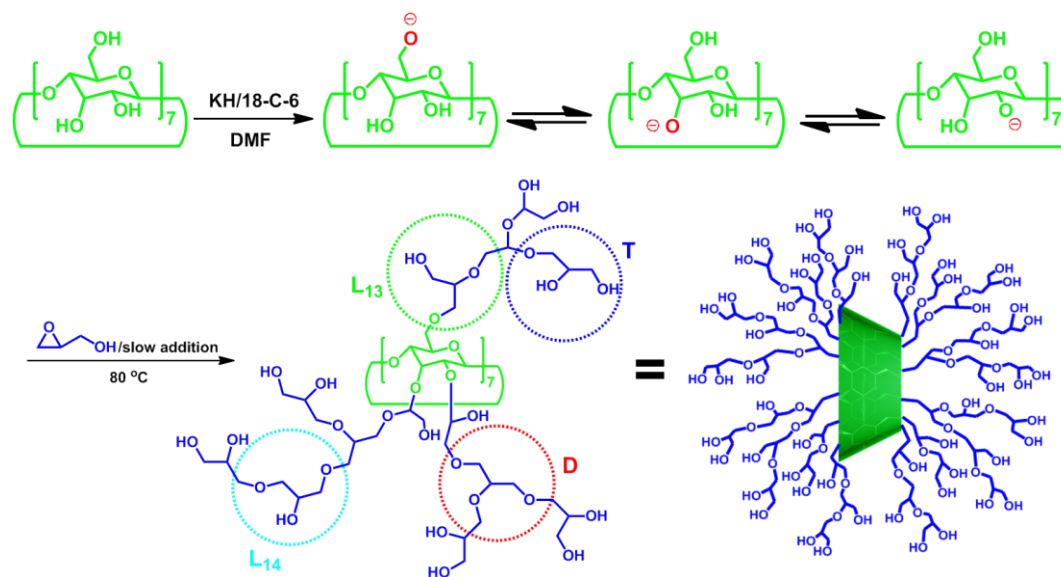
TEM measurements were performed with a JEOL JEM-2100 instrument at a voltage of 200 kV. The samples were prepared by dropping self-assemblies solutions

(0.1 mg/mL) onto carbon-coated copper grids placed on a clean watch glass, and then stained by adding 2 drops of 0.5wt% RuO<sub>4</sub> aqueous solution onto the watch glass and keeping the system sealed for 2 h. Then the grids were air-dried at room temperature for 24 hours.

### 3. Synthesis and characterizations of CD-g-HPG

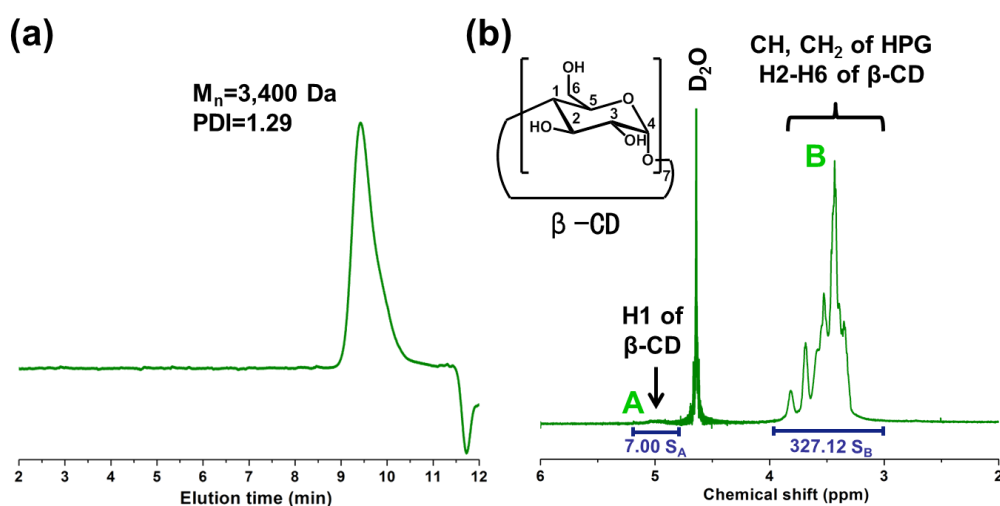
The preparation of CD-g-HPG was according to our previous work.<sup>1,2</sup> As shown in Figure S1, it was synthesized through the anionic ring-opening multibranching polymerization (ROMBP) method with  $\beta$ -CD as a multi-hydroxyl initiator and adding the glycidol monomer very slowly. Here, the slow addition of monomer was to avoid the formation of homopolymers, as previously reported by Frey et al. in the anionic ROMBP process.<sup>3</sup>

The final purified product after a long dialysis process was carefully characterized by GPC, <sup>1</sup>H NMR and <sup>13</sup>C NMR. The GPC curve (Figure S2a) shows a unimodal distribution with a number-average molecular weight ( $M_{n,PMMA}$ ) of 3,400 Da and a narrow polydispersity of 1.29. Figure S2b shows the <sup>1</sup>H NMR spectrum of pure CD-g-HPG as well as the assignments. We could see that all the signals attributed to protons of CD-g-HPG, indicating the obtaining of purified product. The degree



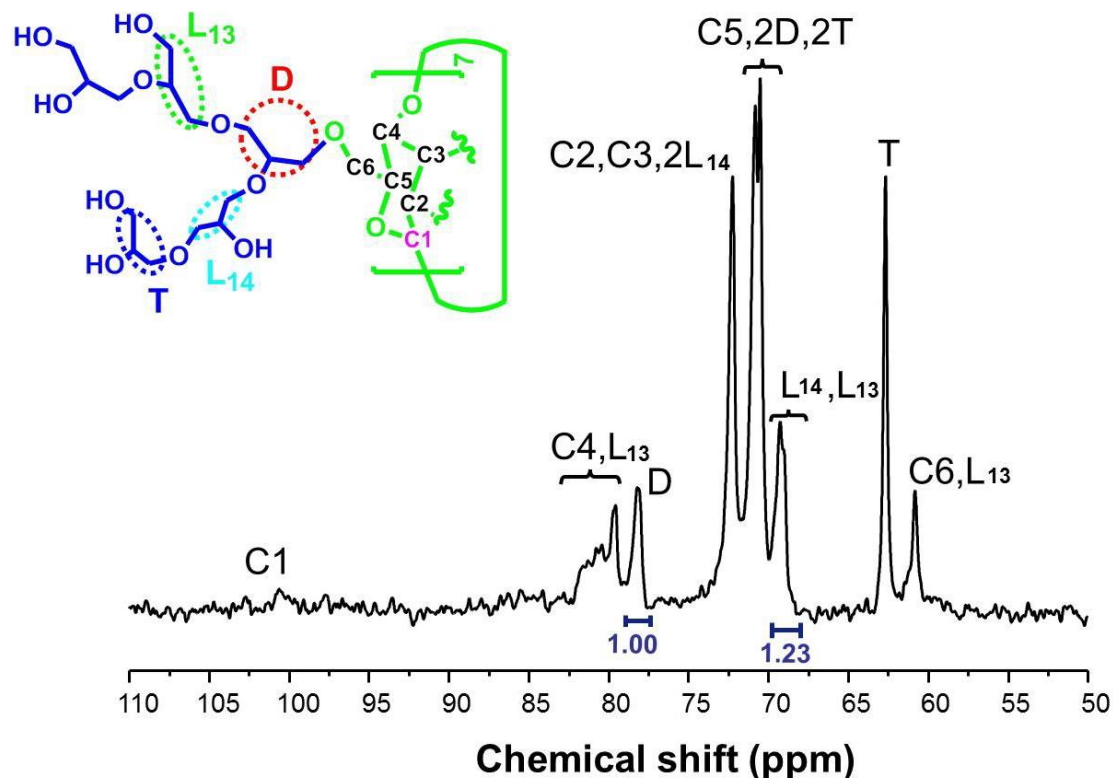
**Figure S1.** The synthetic scheme of CD-g-HPG.<sup>1</sup>

of polymerization (DP) of HPG in the CD-g-HPG could be calculated by comparing the integral of peak A (H1) and B (CH, CH<sub>2</sub> of HPG and H2-H6 of CD). The calculation formula is  $DP = [(S_B - 6S_A)/5]/(S_A/7)$  and the result is 57. According to the molecular weight of glycidol monomer (74 g/mol) and  $\beta$ -CD (1135 g/mol), the molecular weight of CD-g-HPG calculated by <sup>1</sup>H NMR is 5,300 Da, which is higher than that measured by GPC. The difference is owing to that the hydrodynamic volumes of hyperbranched polymers are smaller than those of linear polymers used for calibration in the GPC measurements and hyperbranched polymers often exhibit adsorption by GPC columns because of their large number of end groups. Therefore, we chose the  $M_n$  calculated by <sup>1</sup>H NMR as the accurate molecular weight for the following experiments.



**Figure S2.** (a) GPC curve of CD-g-HPG with DMF as eluent; (b) <sup>1</sup>H NMR spectrum of CD-g-HPG.

The quantitative <sup>13</sup>C NMR spectrum of CD-g-HPG (Figure S3) also presents all the signals from CD and HPG groups. The peak located at 101 ppm is attributed to C1 of CD groups, which is a typical peak to verify that the polymer contains CD groups. In addition, all the dendritic units, linear units and terminal units of the grafted HPG can be clearly discerned. Then the degree of branching (DB) of the grafted HPG could be calculated by comparing the integral of peaks D with that of peaks L<sub>13</sub> and L<sub>14</sub>. The calculation formula is  $DB = 2S_D/(2S_D + S_{L13} + S_{L14})$  as reported by Frey,<sup>3</sup> and the result is 0.62.

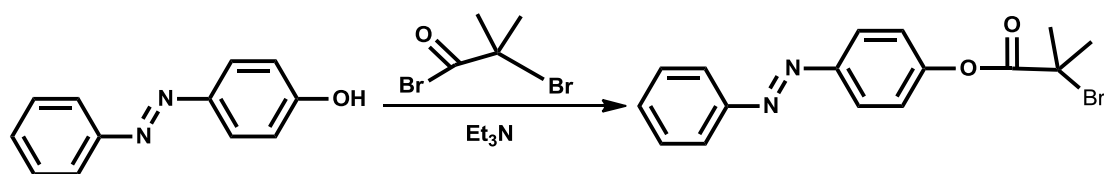


**Figure S3.** The quantitative  $^{13}\text{C}$  NMR spectrum of CD-g-HPG in  $\text{D}_2\text{O}$  at 293K.

#### 4. Synthesis and characterizations of AZO-Br (initiator of atom transfer radical polymerization)

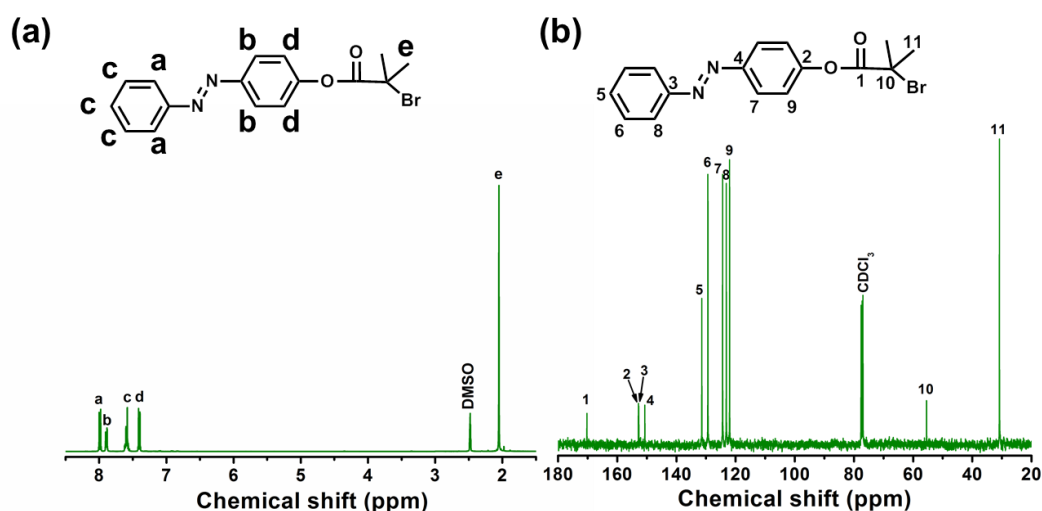
2-bromo-2-methylpropionic acid-4-phenylazophenol ester (AZO-Br) was synthesized via an esterification reaction between 4-phenylazophenol and 2-bromo-2-methylpropionyl bromide (Figure S4). A typical synthetic process was as follows: 4-phenylazophenol (1.98 g, 10 mmol), dried triethylamine ( $(\text{Et})_3\text{N}$ , 2.8 mL, 20 mmol) and 80 mL dried  $\text{CHCl}_3$  were added into a 250 mL single-neck round-bottom flask. Stirring to the complete dissolution of the sample, 2-bromo-2-methylpropionyl bromide (4.6 g, 20 mmol) dissolved in 30 mL  $\text{CHCl}_3$  was added into the previous solution dropwise at  $0\text{ }^\circ\text{C}$ . Then the mixture was stirred at room temperature for 24 hours. After stopping the reaction, the solution was filtered, washed with deionized water three times and dried with anhydrous magnesium sulfate overnight. The solvent was then removed by rotary evaporation. The obtained crude product was purified by recrystallization three times from ethanol, and a pale yellow

solid named as AZO-Br was obtained (3.16 g, yield: 74%).

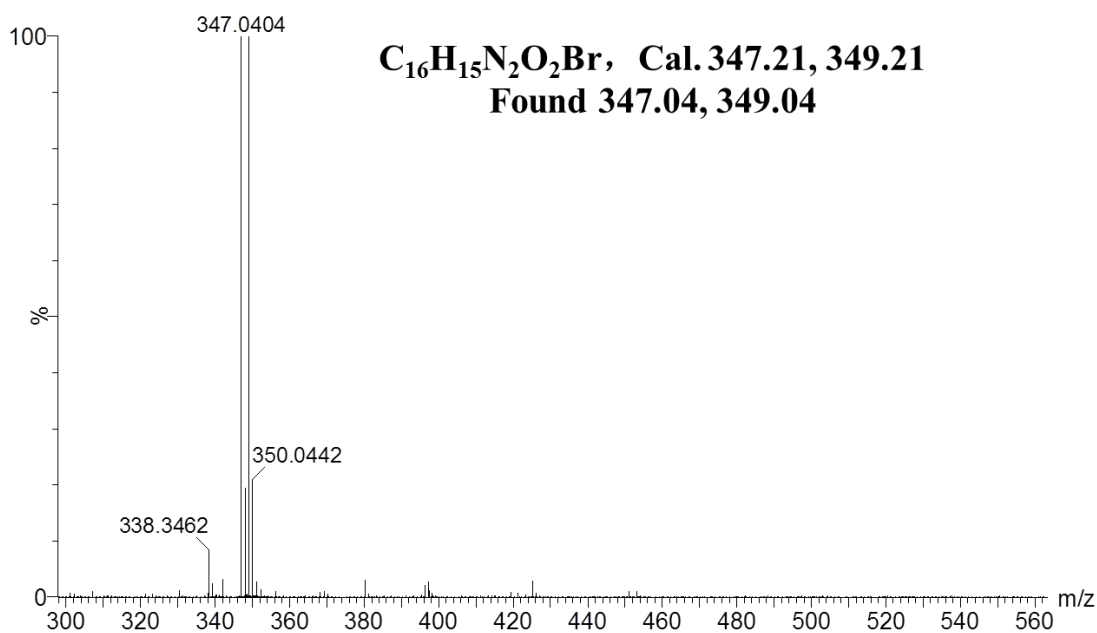


**Figure S4.** The synthetic scheme of AZO-Br.

<sup>1</sup>H NMR, <sup>13</sup>C NMR and Quadrupole-Time-of-Flight Mass Spectrometer (Q-TOFMS) were used to characterize the final product AZO-Br (Figure S5, S6). From the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra, we could clearly see that all the signals attributed to protons and carbon atoms of AZO-Br, indicating the successful obtaining of purified AZO-Br. In addition, the mass spectrum of AZO-Br showed two main molecular ion peaks (m/z, 347.04 and 349.04) with same abundance. Since bromine atoms have two isotopes (<sup>79</sup>Br and <sup>81</sup>Br) with almost the same abundance in nature, the two molecular ion peaks in the mass spectrum are ascribed to the quasi-molecular ion peaks ([M]<sup>+</sup>) of AZO-<sup>79</sup>Br and AZO-<sup>81</sup>Br. The characterization results were consistent with the theoretical molecular weight of AZO-Br (C<sub>16</sub>H<sub>15</sub>O<sub>2</sub>N<sub>2</sub>Br, 347.21 (50%), 349.21 (50%)), further confirming that we had successfully synthesized AZO-Br.



**Figure S5.** The <sup>1</sup>H NMR (a) and <sup>13</sup>C NMR (b) spectra of AZO-Br.

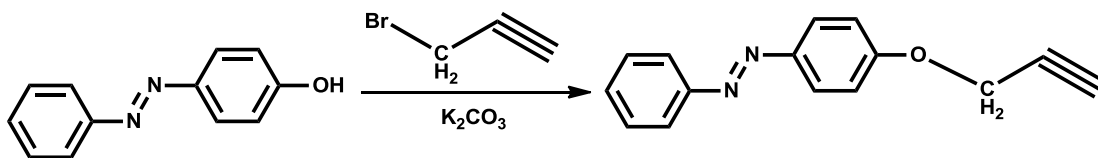


**Figure S6.** Mass spectra of AZO-Br.

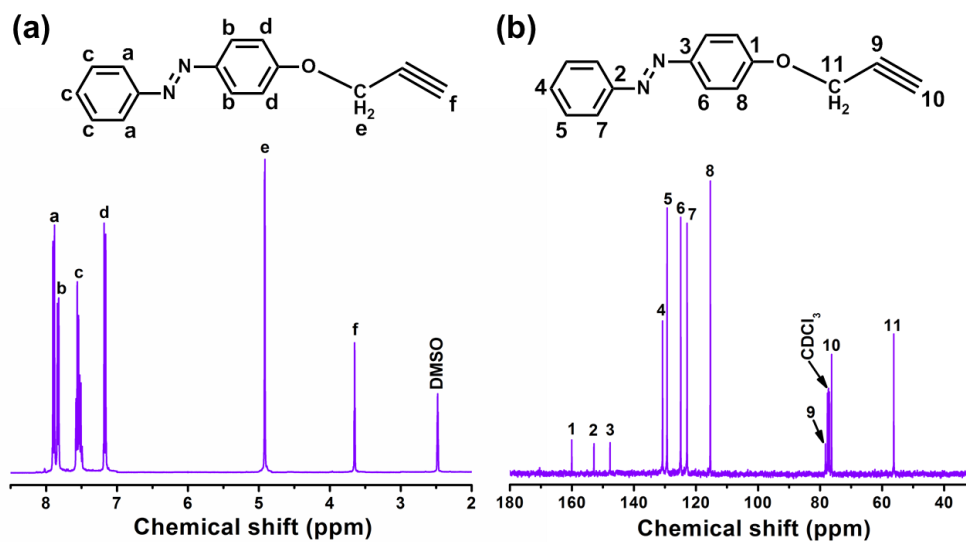
## 5. Synthesis and characterizations of AZO-PP

4-propyneoxy-azobenzene (AZO-PP) was synthesized via a substitution reaction between 4-phenylazophenol and propargyl bromide (Figure S7). A typical synthetic process was as follows: 4-phenylazophenol (1.98 g, 10 mmol), propargyl bromide (1.49 g, 10 mmol), potassium carbonate (2.07 g, 15 mmol) and 30 mL dried toluene were added into a 150 mL single-neck round-bottom flask. Stirring to the complete dissolution of the sample, the flask was immersed in an oil bath at 75 °C for 24 hours. After cooled to room temperature, 50 mL deionized water was poured into the mixture and the organic phase was extracted after stirring intensively. The organic phase was washed with 1M HCl and saturated NaCl three times respectively, and then dried with anhydrous MgSO<sub>4</sub>. After filtered, the solvent was evaporated by rotary evaporation to afford the crude product as a brown solid. The crude product was chromatographed on a silica gel using petroleum ether and ethyl acetate with a volume ratio of 4:1 as eluent, and the purified product is a orange-yellow solid (1.26 g, yield: 40%).

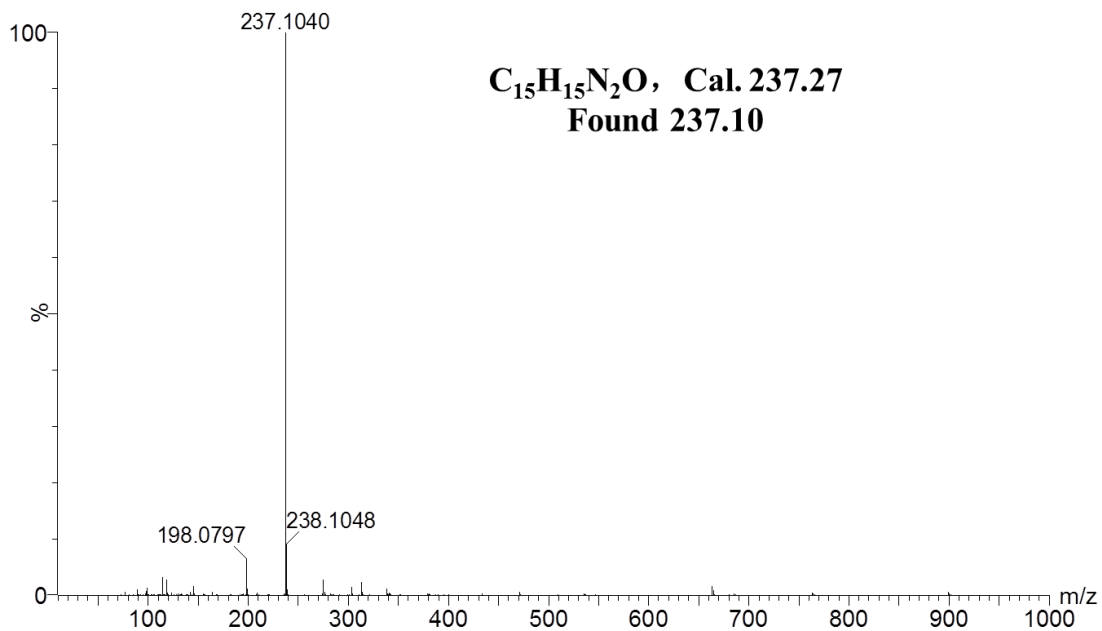




**Figure S7.** The synthetic scheme of AZO-PP.



**Figure S8.** The <sup>1</sup>H NMR (a) and <sup>13</sup>C NMR (b) spectrum of AZO-PP.

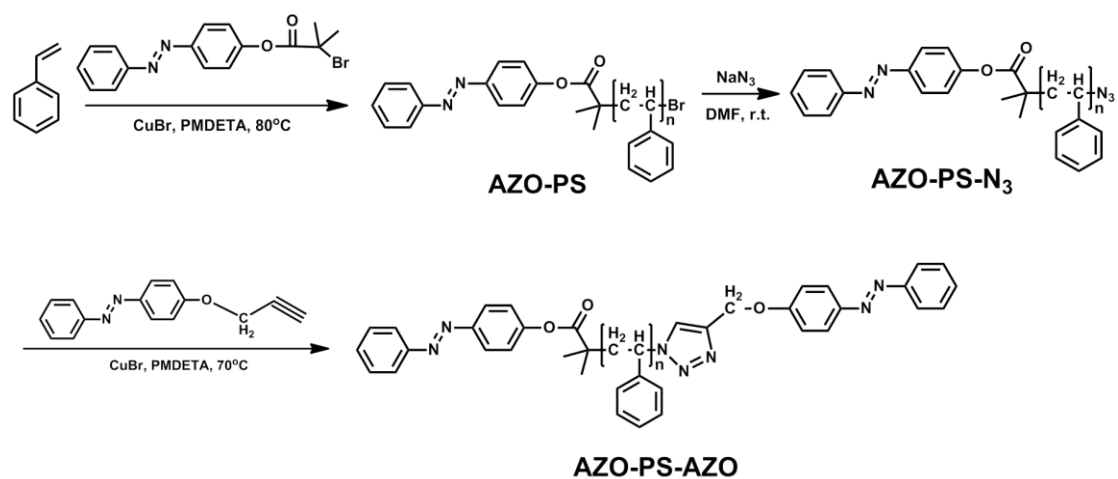


**Figure S9.** Mass spectra of AZO-PP.

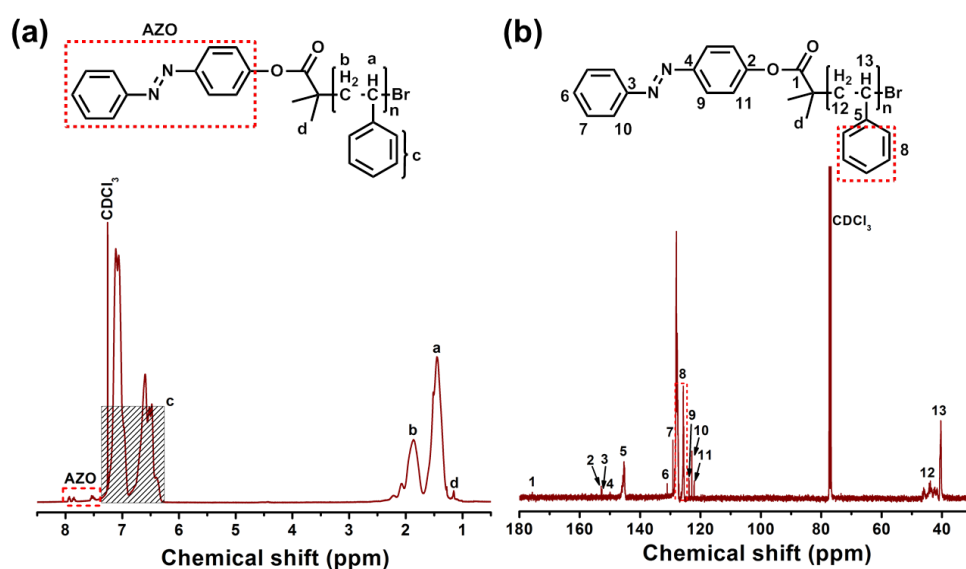
$^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and Q-TOFMS were used to characterize the final product AZO-PPs (Figures S8 and S9). Well-resolved protons and carbon atoms signals assigned to AZO-PPs were clearly observed, indicating the obtaining of purified compounds. Meanwhile, the mass spectrum of AZO-PP showed a main molecular ion peaks ( $m/z$ , 237.10), which was ascribed to the quasi-molecular ion peak ( $[\text{M}+\text{H}]^+$ ) of AZO-PP. The characterization results were consistent with the theoretical molecular weight of AZO-PP ( $\text{C}_{15}\text{H}_{15}\text{N}_2\text{O}$ ) adding one hydrogen ion (237.27), further confirming that we had successfully synthesized AZO-PP.

## 6. Synthesis and characterizations of AZO-PS-AZO

The synthetic process included three steps, as shown in Figure S10. In the first step, AZO-Br (34.7 mg, 0.1 mmol), CuBr (14 mg, 0.1 mmol), PMDETA (20  $\mu\text{L}$ , 0.1 mmol) and styrene (4.0 g, 38 mmol) were added into a 50 mL round-bottom flask, followed by three freeze-vacuum-thaw cycles. The flask was immersed into oil bath at 80  $^\circ\text{C}$  with stirring. After reaction for 4 hours, the flask was cooled to room temperature and opened to the air. The mixture was dissolved in 25 mL THF and precipitated in methanol for three times. After dried in vacuum, a pale yellow powder solid named as AZO-PS was obtained (1.35 g, conversion: 34%). The obtained AZO-PSs were carefully characterized and proved by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and GPC (Figures S11 and S12). We had prepared two AZO-PS samples with different molecular weights by controlling the ATRP time. As shown in Figure S12 and Table S1, the molecular weights of AZO-PS increased with polymerization time and all polydispersity indexes (PDI) were very small.



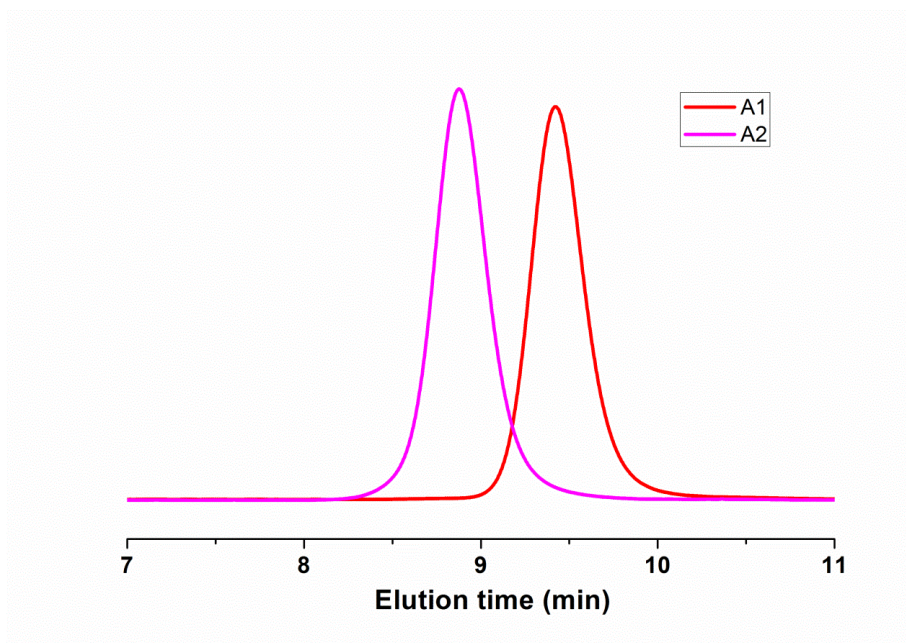
**Figure S10.** The synthetic scheme of AZO-PS-AZO.



**Figure S11.** The <sup>1</sup>H NMR (a) and <sup>13</sup>C NMR (b) spectrum of AZO-PS ( $M_n=5,800$  Da).

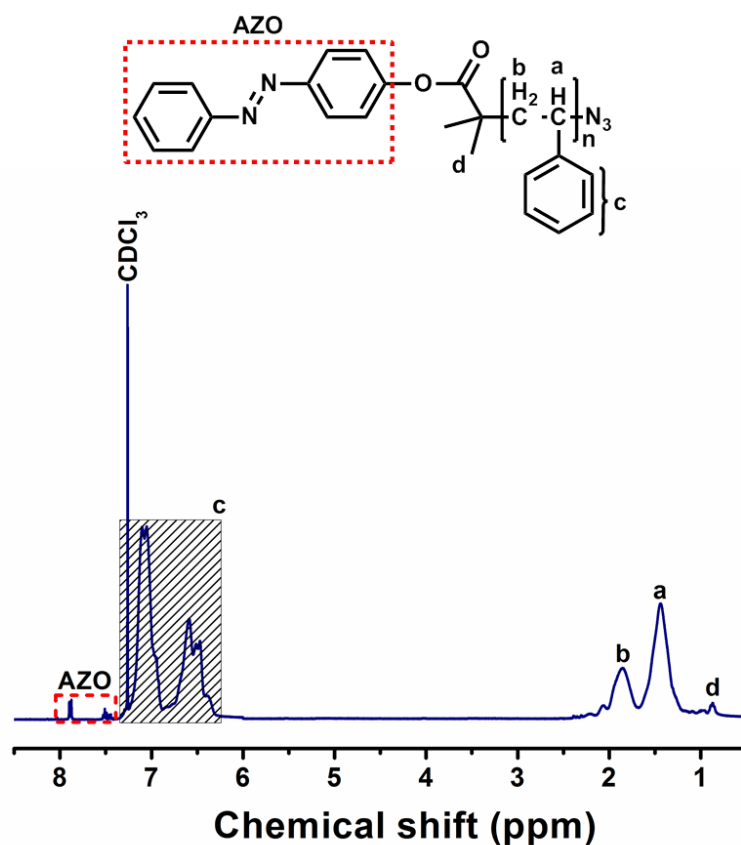
**Table S1.** Molecular weights and PDIs of two AZO-PS samples (A1, A2) with different polymerization time.

Samples	Polymerization time/h	$M_n$	PDI
A1	1.5	5,800	1.20
A2	6	23,000	1.20



**Figure S12.** GPC curves of AZO-PS samples (A1, A2).

In the second step, AZO-PS (0.4 g, 0.05 mmol Br),  $\text{NaN}_3$  (19.5 mg, 0.3 mmol) and 30 mL dried DMF were added into a 150 mL round-bottom flask. Stirring to the complete dissolution of the sample, the mixture was heated to 80 °C and reacted for 24 hours. After cooling to the room temperature, the reaction mixture was precipitated in deionized water for three times. The final precipitation was dried in vacuum and a pale yellow powder solid named as AZO-PS- $\text{N}_3$  was obtained (0.33 g, yield: 78%). The obtained AZO-PS- $\text{N}_3$ s were characterized and proved by  $^1\text{H}$  NMR (Figure S13). It should be noted that the content of azide groups is too low to be detected.

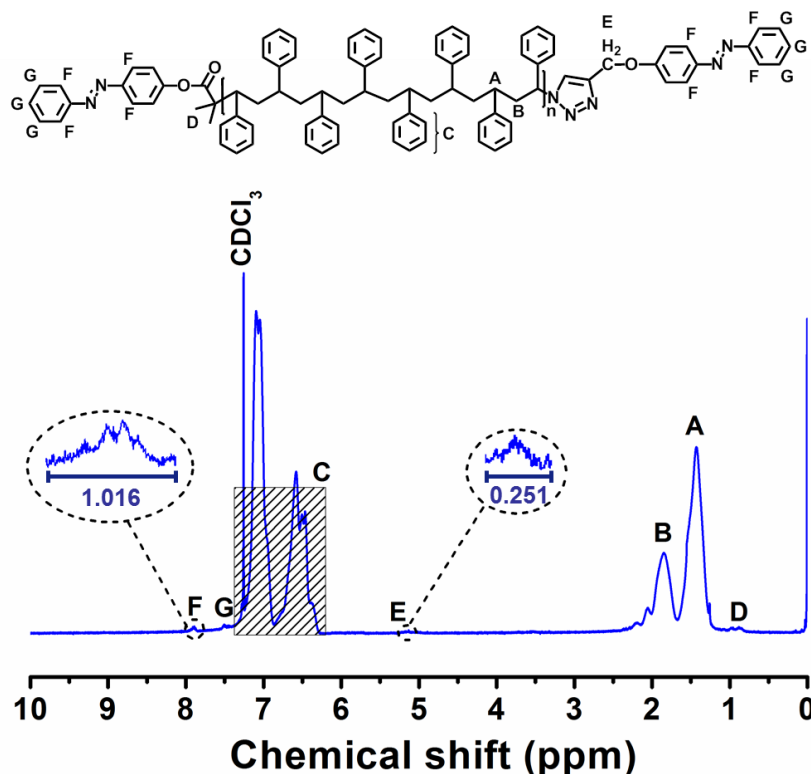


**Figure S13.** The  $^1\text{H}$  NMR spectrum of AZO-PS- $\text{N}_3$  ( $M_{n,\text{PS}}=5,800$  Da). All the signals of PS and AZO segments were attributed. However, the content of azide groups is too low to be detected.

In the third step, AZO-PS- $\text{N}_3$  (0.2 g, 0.026 mmol  $\text{N}_3$ ), AZO-PP (12.2 mg, 0.052 mmol), CuBr (7.5 mg, 0.052 mmol), PMDETA (11  $\mu\text{L}$ , 0.052 mmol) and 20 mL dried DMF were added into a 150 mL round-bottom flask followed by three freeze-vacuum-thaw cycles. The flask was immersed into oil bath at 70  $^\circ\text{C}$  with stirring. After reaction for 24 hours, the reaction mixture was dialyzed against (MWCO: 3500 Da) methanol for three days. The solution inside the dialysis bag was collected and evaporated under vacuum to produce some yellow product named as AZO-PS-AZO (0.19 g, yield: 92%).

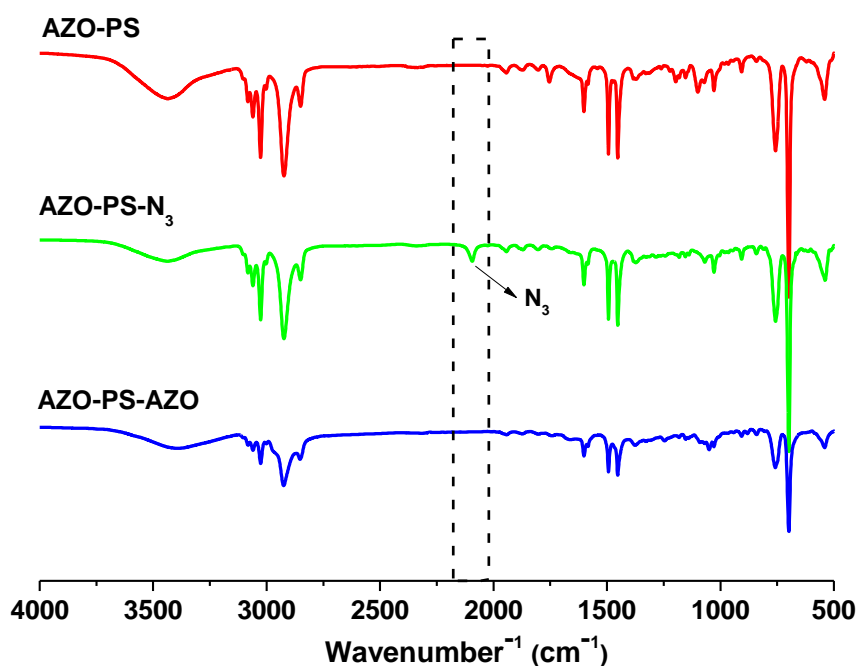
The final product AZO-PS-AZO was characterized by  $^1\text{H}$  NMR (Figure S14). The  $^1\text{H}$  NMR spectrum showed that all the signals were attributed to protons of AZO-PS-AZO, indicating that we had prepared AZO-PS-AZO successfully. Meanwhile, the molar ratio between AZO groups (peak F) and  $\text{CH}_2$  groups adjacent to

the triazole rings (peak E) was calculated by integrating the corresponding proton peak E and F in Figure S14 ( $S_E = 0.251$ ,  $S_F = 1.016$ ). The result is 2.02 : 1 ( $S_F/4 : S_E/2$ ), which is very close to 2 : 1. Although the signals of peak E and peak F are rather weak (Figure S14), the calculated molar ratio still indicates that the target products of AZO-PS-AZO with two AZO groups at its chain ends have been synthesized successfully.



**Figure S14.** The <sup>1</sup>H NMR spectrum of AZO-PS-AZO ( $M_{n,PS}=5,800$  Da).

In addition, FT-IR spectra were used to characterize the intermediate product AZO-PS-N<sub>3</sub> and final product AZO-PS-AZO (Figure S15). Analyzing the FT-IR spectra, we could clearly see that the absorption bands at 2109 cm<sup>-1</sup> ascribed to the characteristic absorption peak of N<sub>3</sub> groups disappeared completely from AZO-PS-N<sub>3</sub> to AZO-PS-AZO, indicating the quantitative conversion of the azide-alkyne click reaction to form AZO-PS-AZOs.



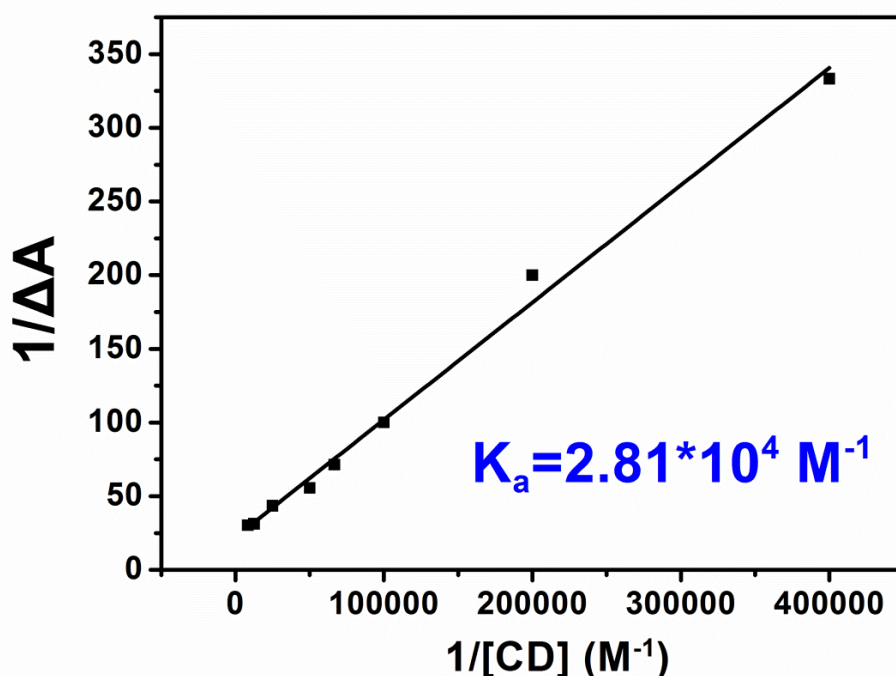
**Figure S15.** FT-IR spectra of AZO-PS ( $M_n=5,800$  Da), AZO-PS-N<sub>3</sub> and AZO-PS-AZO.

## 7. Complexation and self-assembly of DSTCs characterized by DLS measurements

Purified CD-g-HPG (10.6 mg, 0.002 mmol CD groups) and AZO-PS-AZO (6.2 mg, 0.002 mmol AZO groups) were firstly dissolved in the co-solvent of DMF. Then the concentrated DMF solution was divided into many aliquots, followed by adding different volume of water (water/DMF: 0-7 v%) in each aliquot while keeping the same polymer concentration of 0.5 mg/mL. After keeping the aliquots for 24 hours to reach the equilibrium, dynamic light scattering (DLS) measurements were performed to characterize the sizes of aggregates in each aliquot.

## 8. Calculation of complexation constant between AZO and CD groups

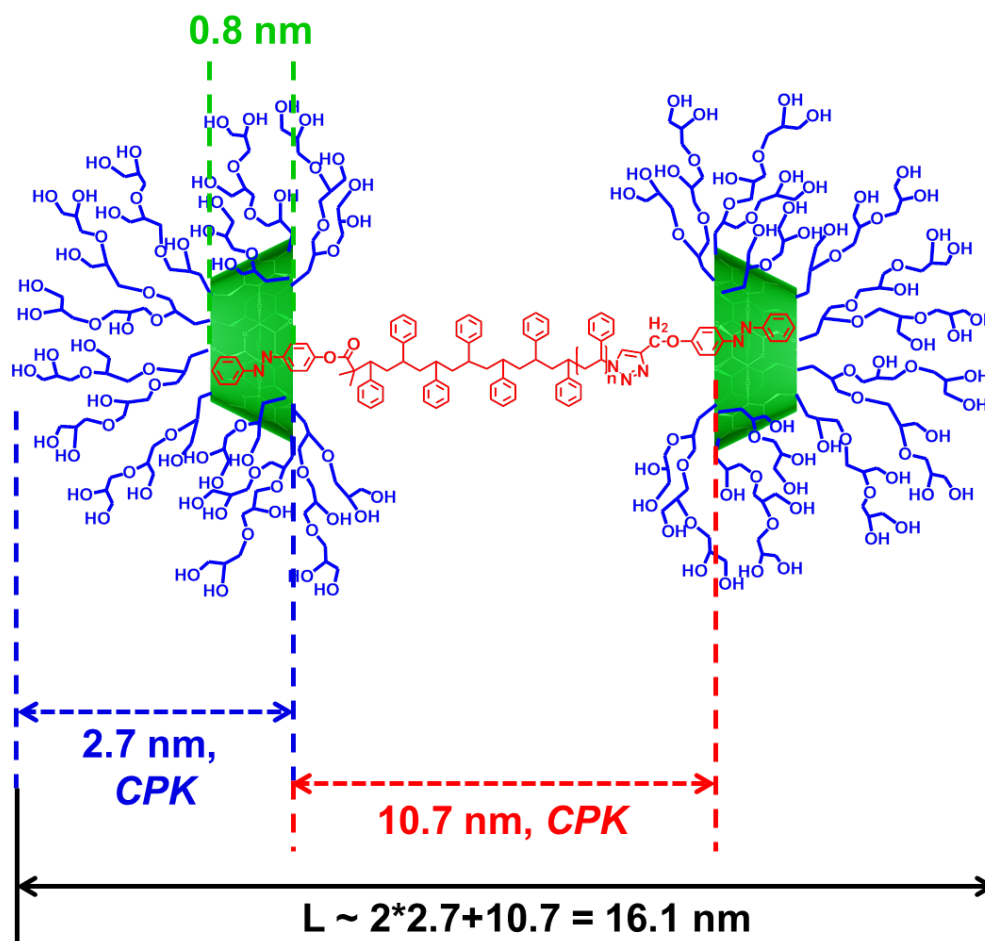
According to the UV-titration experiment (Fig. 1a), we obtained Benesi-Hildebrand plot as shown in Figure S16 by quantitatively calculating the absorbance change of AZO groups ( $\lambda = 350$  nm) versus the concentration of CD-g-HPGs added. The equation of the plot is  $y = 7.9569 \times 10^{-4} + 22.3831$  ( $R^2 = 0.993$ ). Combining with the Benesi-Hildebrand equation, that is,  $1/\Delta A = [1/(K_a \Delta \epsilon [G_0])] * [1/[CD]] + 1/(\Delta \epsilon [G_0])$  ( $\Delta A$  is the absorbance difference of AZO-PS-AZO with the addition of CD-g-HPG;  $K_a$  is the complexation constant between AZO and CD groups;  $\Delta \epsilon$  is the difference of the molar extinction coefficient between AZO and AZO-CD complex;  $[G_0]$  is the concentration of AZO groups, which remains unchanged in the titration;  $[CD]$  is the concentration of CD groups, which is the same with that of CD-g-HPG), we calculated the complexation constant according to the slope and intercept of the fitted curve and the result was  $K_a = 2.81 \times 10^4 \text{ M}^{-1}$ .



**Figure S16.** Benesi-Hildebrand plot at the maximum absorption wavelength ( $\lambda = 350$  nm) of AZO groups.



## 9. Calculation of the molecular length of one DSTC

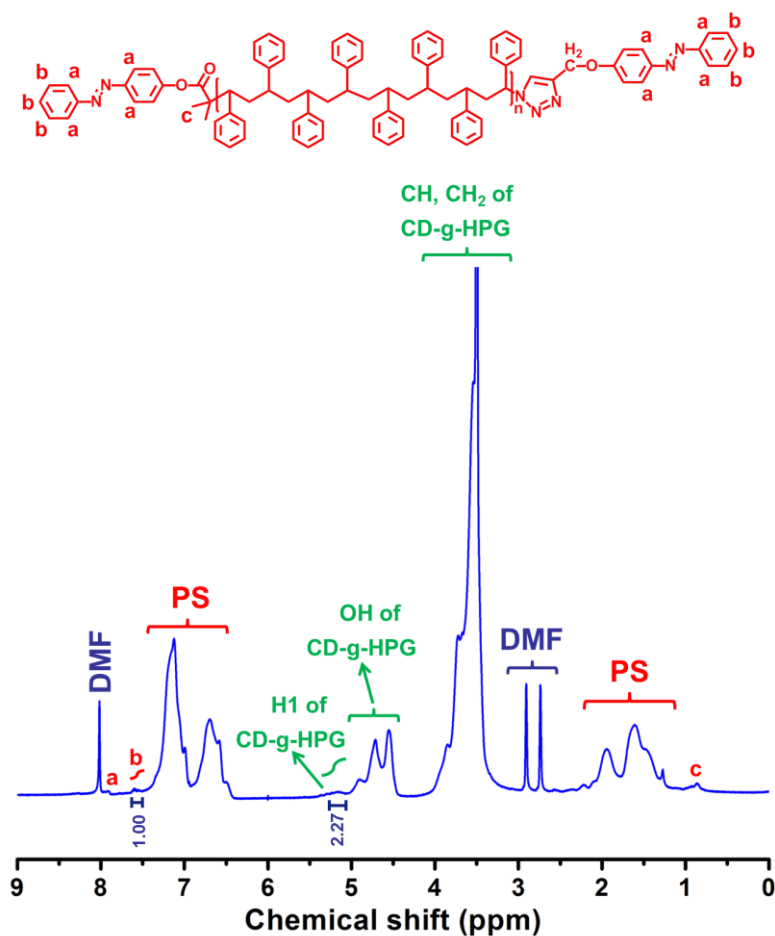


**Figure S17.** Estimating the length of an extended DSTC molecule ( $M_n$  of PS = 5,800 Da) according to the CPK model. The height of  $\beta$ -CD is about 0.8 nm.<sup>4</sup>

## 10. $^1\text{H}$ NMR characterization of collected vesicles

The purified self-assemblies were collected by dialyzing the solution of self-assemblies against (MWCO: 10,000) water to remove free CD-g-HPGs and AZO-PS-AZOs which did not form self-assemblies, followed by collecting the solution inside the dialysis bag and freeze-drying in vacuum.

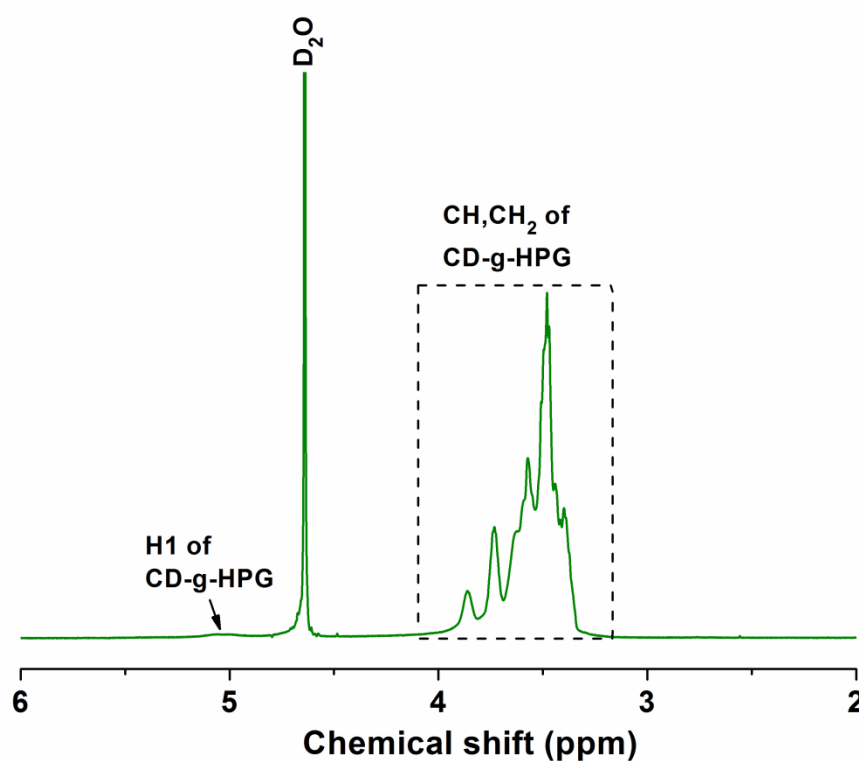
The collected vesicles were characterized by  $^1\text{H}$  NMR measurement in deuterated DMF (Figure S18). All of the proton signals were clearly assigned to CD-g-HPG and AZO-PS-AZO, which certified that the vesicles were composed of CD-g-HPG and AZO-PS-AZO. The integrated area ratio of the H-1 protons in CD units to AZO groups (peak b) in AZO-PS-AZO is approximately 2.27 : 1 ( $S_{\text{H-1}} : S_{\text{a}}$ ), indicating the molar ratio between CD and AZO groups is 0.97 : 1 ( $S_{\text{H-1}}/7 : S_{\text{a}}/3$ ). This value is very close to 1 : 1, which makes us believe that the vesicles are self-assemblies from our DSTCs with the equal stoichiometry of CD and AZO groups.



**Figure S18.** The  $^1\text{H}$  NMR spectrum of the freeze dried vesicles in deuterated DMF.

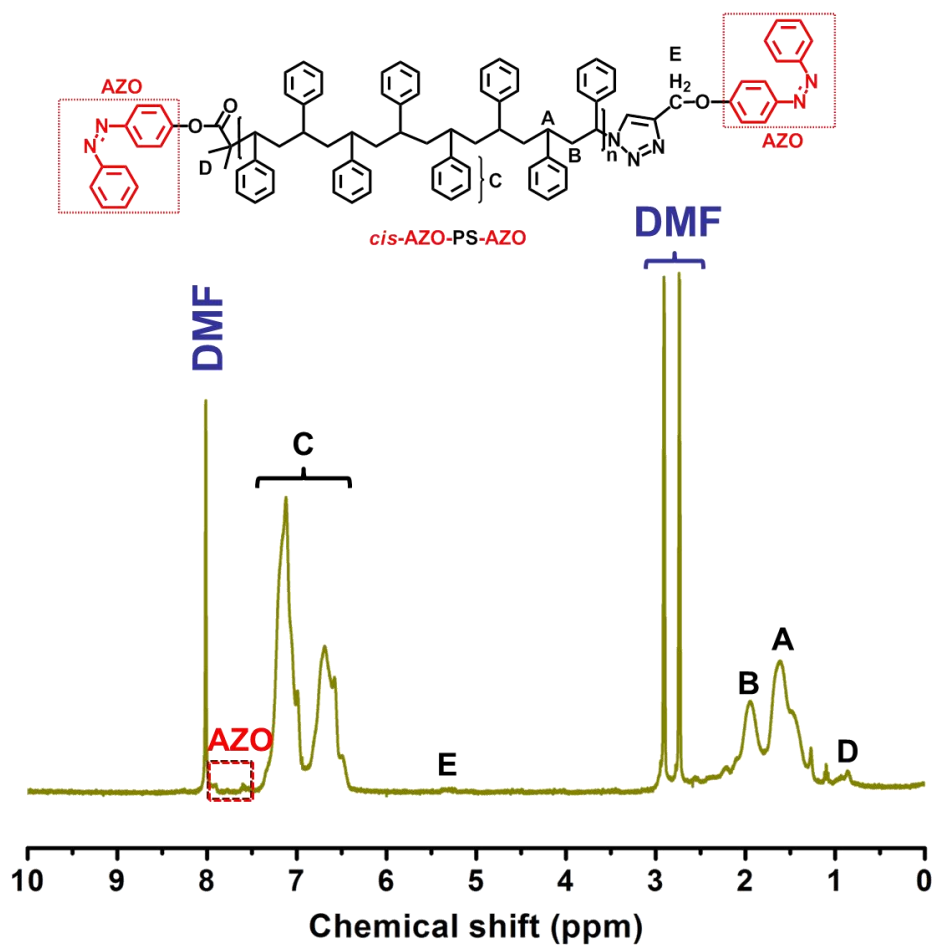
## 11. $^1\text{H}$ NMR characterization of the residual polymers in the solution collected by freeze drying after UV-triggered disassembly

The residual polymers in the solution after disassembly under UV irradiation were collected by freeze-drying, and then re-dissolved in deuterioxide ( $\text{D}_2\text{O}$ ) for  $^1\text{H}$  NMR measurement (Figure S19). The spectrum is almost the same with that of pure CD-g-HPG (Figure S2b), indicating that the residual polymers in the solution after UV-triggered disassembly are CD-g-HPGs.



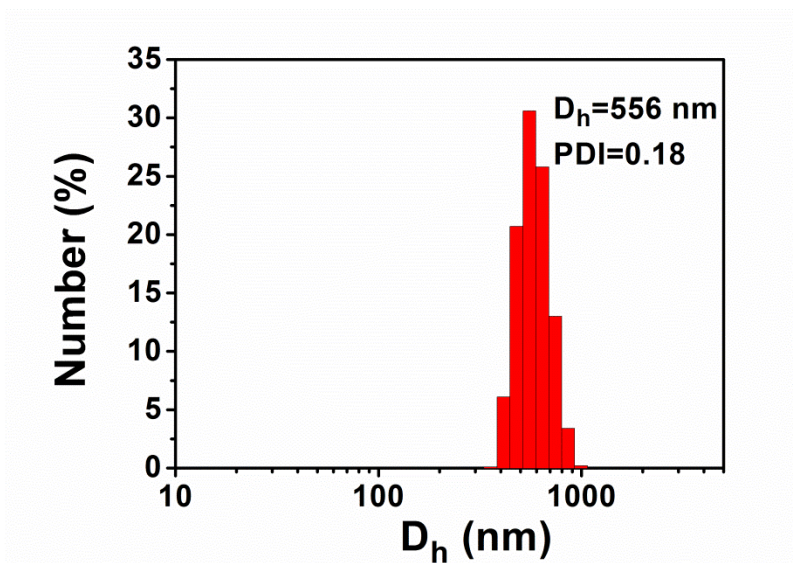
**Figure S19.** The  $^1\text{H}$  NMR spectrum of the residual polymers in the solution collected by freeze drying after disassembly under UV irradiation.

## 12. $^1\text{H}$ NMR characterization of the precipitation after UV-triggered disassembly



**Figure S20.** The  $^1\text{H}$  NMR spectrum of precipitates after disassembly.

### 13. DLS characterization of vesicles self-assembled from DSTCs with larger PS blocks



**Figure S21.** DLS characterization of aqueous vesicle solutions from DSTCs with  $M_n=23,000$  Da in the hydrophobic PS blocks.

#### Notes and references

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