Supporting Information

Hierarchical self-assembly of protoporphyrin IX-bridged Janus particles into photoresponsive vesicles

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1 Material

Protoprophyrin IX (PPIX), N-Hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), Dicyclohexylcarbodiimide (DCC), Boron trifluoride etherate (48%, $BF_3 \cdot Et_2O$), Methyl viologen hydrate (MV, 98%), 3-Ethyl-3-oxetanemethanol (EHO) and p-toluenesulfonyl chloride (TsCl), (±)-Glycidol (96%), Ethyl 2, 3-dibromo-3-phenylpropionate (DPP, 98%) were purchased from J&K Chemical Ltd (Beijing, China). β-Cyclodextrin (β-CD) was obtained from Beijing solarbio science & Technology Co.,Ltd. Ethylenediamine ($C_2H_8N_2$), Sodium hydroxide (NaOH), Potassium carbonate (K_2CO_3), N, N-Dimethylformamide (DMF), Triethanolamine (TEA, AR) was purchased from Sinopharm Chemical Reagent Co., Ltd. Potassium iodide (AR, KI) was purchased from China National Pharmaceutical Group Corporation. All the other chemical reagents in the study were in analytical grade and utilized without further purification. Deionized water (specific resistance 18.25 MΩ) was used in all experiments.

2 Characterizations

^{1}H NMR and ^{13}C NMR analysis were conducted on a JOEL JNM-ECA600 spectrometer in DMSO-d6 at ambient temperature. FT-IR measurements were conducted on an AVATAR 360 FT-IR spectrometer (Thermo Nicolet). The samples for FT-IR measurement were prepared by dispersing the powder in KBr and compressing the mixtures to form disks. The UV-Vis absorption spectra were recorded with a JASCO V-650 spectrometer in water with 1 cm quartz cell at ambient temperature. SEM was conducted on a JEOL JSM-7500F. The samples for SEM observation were prepared by depositing liquid drops of the solution onto the surfaces of cleaned silicon slices, and air-dried at ambient temperature. The samples were coated with a thin film of platinum before measuring. The visualized images of the assemblies were obtained at ambient temperature using a TECNAI T20 TEM. Samples for TEM observation were prepared by casting one drop of the micellar solution on carbon-coated

copper grid and then dried at room temperature. All samples were observed by tungstophosphoric acid staining. AFM was conducted on an Ntegra Prima microscope (NT-MDT) equipped with a silicon cantilever, AN-NSC01 (radius < 10 nm, resonance frequency = 43-81 kHz, spring constant = 0.6–3.7 N/m), with a noncontact model at room temperature. The samples for AFM observations were prepared by depositing a drop of the solution (0.5 mg/mL) onto the surface of silicon slice and dried naturally.

3 Syntheses

3.1 NH_2-β-CD

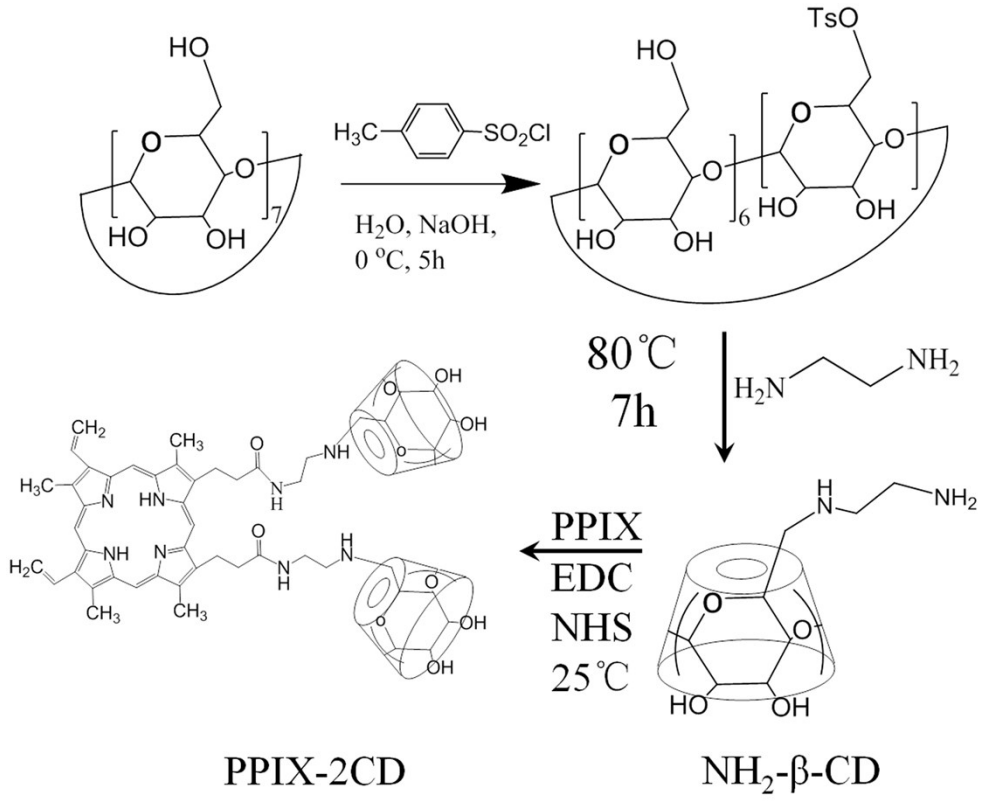
Mono-[6-Ts-6-deoxy]-β-CD was prepared based on the literature[1] (Scheme S1). A three-necked, round-bottomed flask equipped with a large magnetic stirring bar and thermometer was charged with β-CD (25 g, 22 mmol) and a solution of 12.5 g of NaOH in 0.7 L of water. The solution was stirred at 0–5 °C in an ice water bath, and TsCl (10 g, 52.5 mmol) was added to one portion. The reaction mixture was stirred vigorously for 2 h at 0-5 °C. Another portion of TsCl (15 g, 78.5 mmol) was added, and the reaction mixture was stirred at this temperature for another 3 h. The reaction mixture was filtered through Celite in a fritted glass funnel to separate unreacted TsCl. The filtrate was cooled at 0-5 °C, and 10% aqueous hydrochloric acid (HCl, 350 mL) was added. The resulting solution was stored overnight in a refrigerator at 0 °C and then filtered. The product was dried to a constant weight over Drierite in a vacuum desiccator to yield 10.7 g of a white solid. This material was recrystallized (three times) by dissolving it in 100 mL of water at the boiling point and then cooling to room temperature. Storage in a refrigerator overnight provided 5.7 g (yield = 20.3%) of mono-[6-Ts-6-deoxy]-β-CD as a white solid.

The synthesis of NH_2-β-CD was as follows:[2, 3] mono-[6-Ts-6-deoxy]-β-CD (5.0 g) was added to a three-necked flask equipped with a thermometer, followed by the addition of 5 mL of ethylenediamine to dissolve fully under the protection of nitrogen (N_2). The mixture was heated to 70 °C and stirred for 7 h. The remaining nonreactive ethylenediamine was removed

using the rotary evaporation apparatus. As the resultant cooled, methanol was added to the white precipitate to withstand repeated washing with acetone. The solid products were dried under vacuum at ambient temperature.

3.3 PPIX-2CD

PPIX (113 mg, 0.2 mmol), EDC (154 mg, 0.8 mmol), and NHS (94 mg, 0.8 mmol) were dissolved in dry DMF (3 mL) and stirred for 1 h at ambient temperature. The mixture was added to a NH_2-β-CD (643 mg, 0.54 mmol) solution dissolved in DMF (3 mL) in advance. The reaction mixture was stirred for 72 h at ambient temperature and protected from light. Subsequently, the solvent was evaporated under vacuum and the residue was washed exhaustively with methanol to remove unreacted PPIX. Output was dialyzed against deionized water for 2 d, and PPIX-CD was filtered out (MWCO of the dialysis bag = 2 kDa; Solarbio). The dissolved product was freeze-dried to obtain a dark red solid (yield= 31%).



Scheme S1. The synthetic route of PPIX-2CD

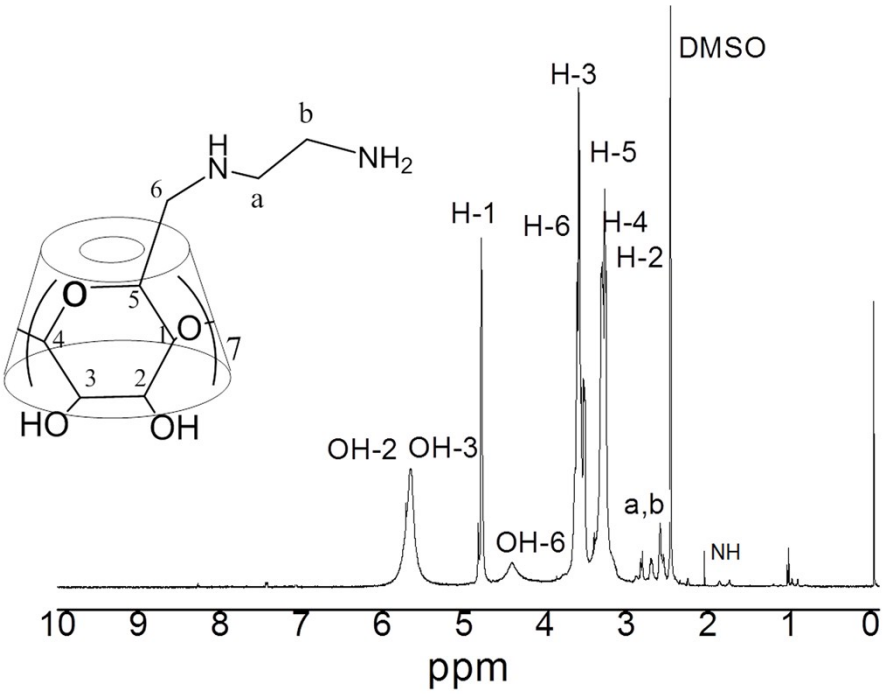
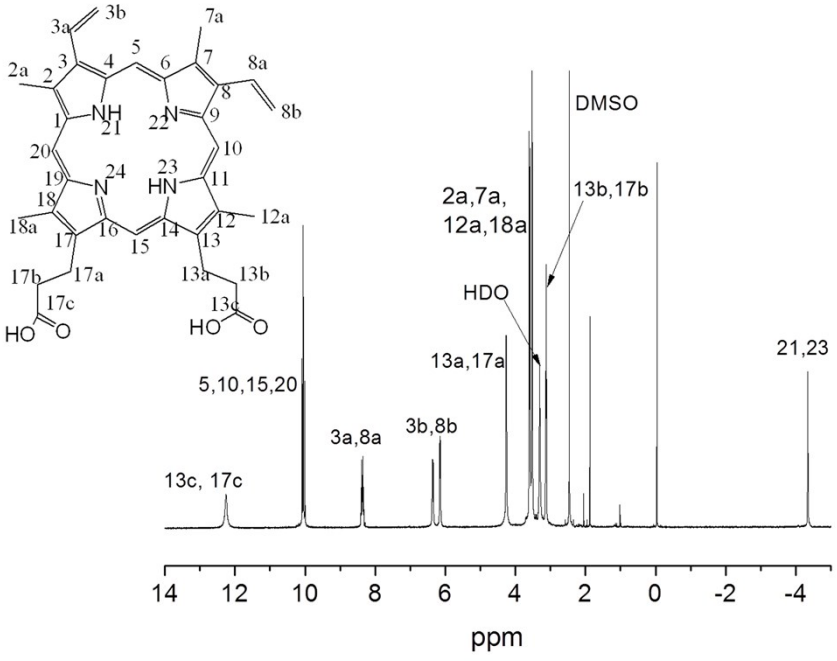


Figure S1 ^{1}H NMR spectrum of NH_2-β-CD



FigureS2 ^{1}H NMR spectrum of PPIX

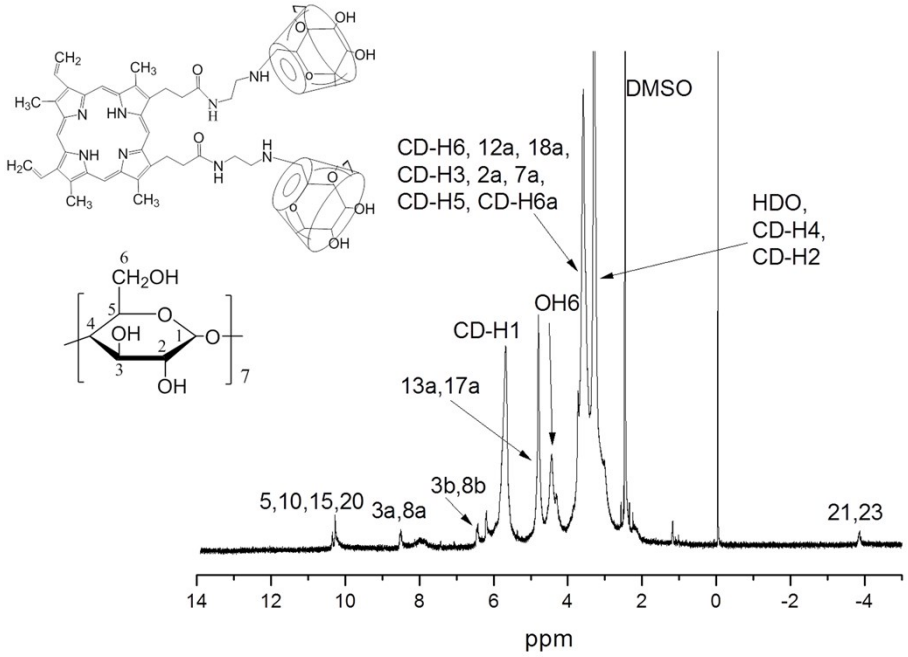


Figure S3 ^{1}H NMR spectrum of PPIX-2CD

Figure S3 shows that the ^{1}H NMR spectrum of DMSO-d6 allowed the satisfactory characterization of the product. The carboxyl group protons of parent PPIX were absent, indicating that a reaction occurred at the carboxyl site. Parts of the PPIX signals caused by the exocyclic double bonds (δ 6.5 and δ 8.6 ppm) were observed, along with those of the meso-positions (δ 10.3 ppm) and pyrrole endocyclic NH (δ 3.8 ppm). By contrast, the anomeric proton signals of CD (δ 5.0 ppm) were reasonably separated from those of the CD primary (δ 4.5 ppm) and secondary (δ 5.8 ppm) hydroxyl groups.

The degree of CD (DS_{CD}) grafted onto PPIX was quantified by the ^{1}H NMR spectrum and expressed as Equation S1:

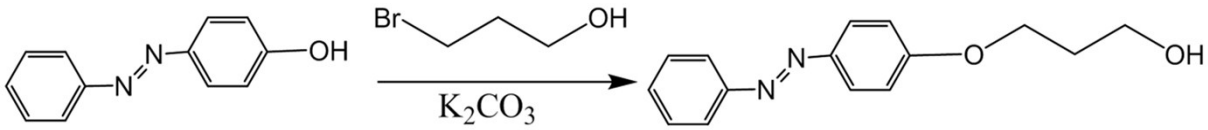
$$DS_{CD} = \frac{A_{\text{CD-H1}}/7}{(A_{\text{PPIX-H5,10,15,20}} + A_{\text{PPIX-H21,23}})/6} \qquad \text{Equation S1}$$

3.4 AZO-HBPO and AZO-HPG

AZO-HBPO and AZO-HPG were synthesized based on previous studies,[4-6] with the following steps. First, 4-(3-hydroxypropyloxy)-azobenzene (AZO-OH) was synthesized using

3-bromo-1-propanol and 4-phenylazophenol as raw materials. Second, monomers underwent cationic ring-opening polymerization using AZO-OH as the initiator, and the monomers were added slowly.

AZO-OH was prepared as follows (Scheme S2):[7] 3-bromo-1-propanol (1.67 g, 12 mmol) and 4-phenylazophenol (1.98 g, 10 mmol) were dissolved in dried DMF (30 mL). K_2CO_3 (2.07 g, 15 mmol) was added to the solution, and the solution was immersed in an oil bath at 75 °C for 6 h. After cooling to room temperature, the solution was mixed with cold water (50 mL) and extracted with chloroform (50 mL). The organic phase was washed with 1 M HCl and saturated NaCl solution and then dried with anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure to obtain a crude product. The crude product was chromatographed on a silica gel using ethyl acetate (EA) and dichloromethane (DCM) (EA/DCM = 1:6, v/v) as eluent. The purified product was also a yellow solid (yield = 65%).



Scheme S2. The synthetic route of AZO-OH

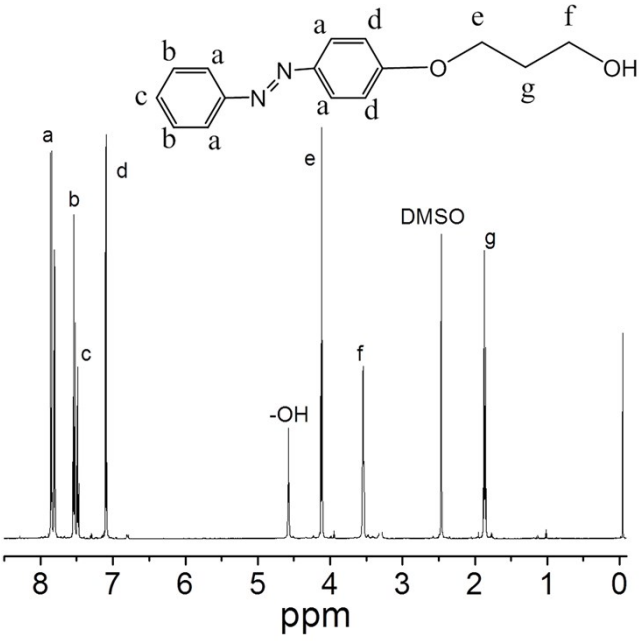
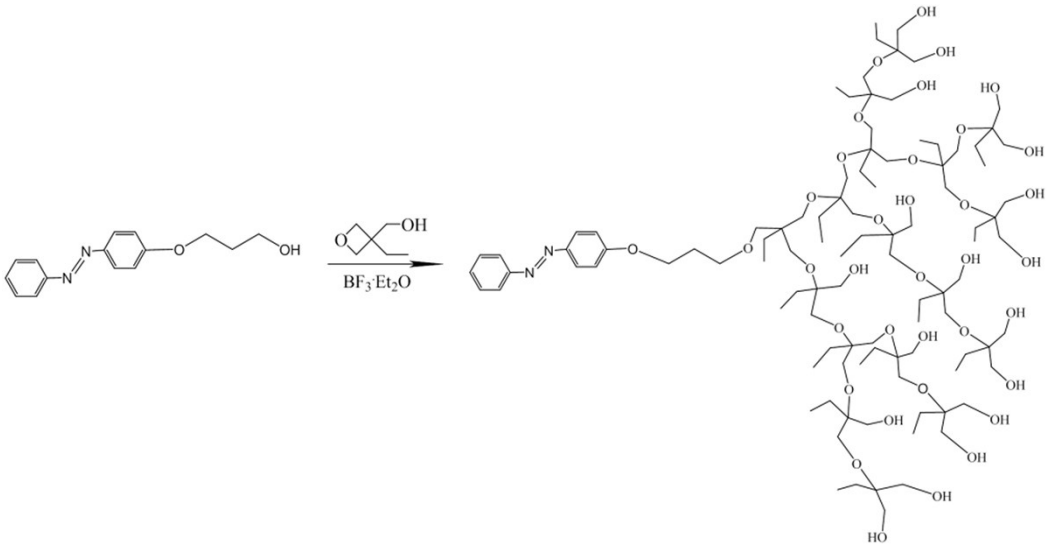


Figure S4 ^{1}H NMR spectra of AZO-OH (DMSO-d6, 298K)

The product of AZO-OH was characterized by ^{1}H NMR (Figure S4). Well-resolved proton signals assigned to AZO-OH were clearly observed, which indicated that pure compounds were obtained. ^{1}H NMR (600 MHz, DMSO-d6, 298 K: δ (ppm) = 1.81 (m, 2H, $CH_2CH_2CH_2$), 3.90 (t, 2H, -CH_2CH_2OH), 3.52 (t, 2H, Ar-O-CH_2CH_2), 7.02 (d, 2H, *o*-Ar-H-OCH_2), 7.12 (t, 1H, *p*-Ar-H-N=N), 7.50 (t, 2H, *m*-Ar-H-N=N), 7.90 (q, 4H, *o*-Ar-H-N=N-Ar-H).

AZO-HBPO: The synthesis route is shown in Scheme S3. AZO-OH (0.214 g, 0.83 mmol) was placed in an oven-dried reaction tube and then sealed with a rubber septum. After three cycles of degassing in a vacuum and back-filling with nitrogen gas, dried CH_2Cl_2 (5 mL) and $BF_3{\cdot}OEt_2$ (1.7 mL, 13.5 mmol) were added via a syringe. After the mixture was heated to 35 °C, a solution of 3-ethyl-3-oxetanemethanol (3.14 g, 27 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to the system over 24 h, and the solution was reacted for approximately 24 h. Finally, the reaction was terminated by a small amount of methanol. CH_2Cl_2 was removed by vacuum rotary evaporation, and the crude product was dissolved by THF and dialyzed against ethanol (MWCO = 1000 Da) for 2 d to remove the unreacted AZO-OH and oligomer of EHO. After removal of the solvents, the final product of AZO-HBPO was a viscous yellow solid (yield = 66%).



Scheme S3 The synthetic route of AZO-HBPO

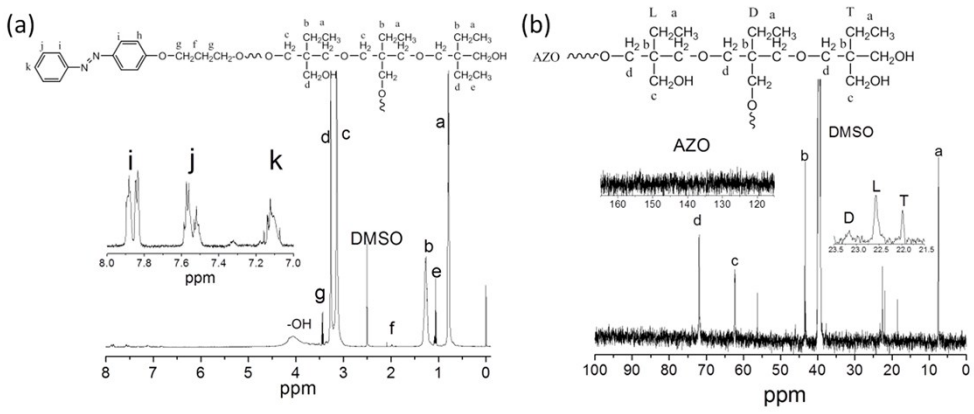


Figure S5 (a) ^{1}H NMR spectra of AZO-HBPO; (b) ^{13}C NMR spectra of AZO-HBPO; (DMSO-d6, 298K)

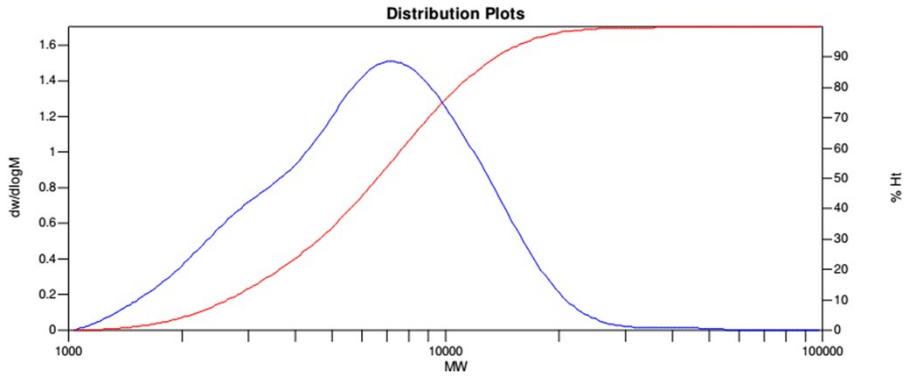


Figure S6 GPC curve of AZO-HBPO with THF as eluent.

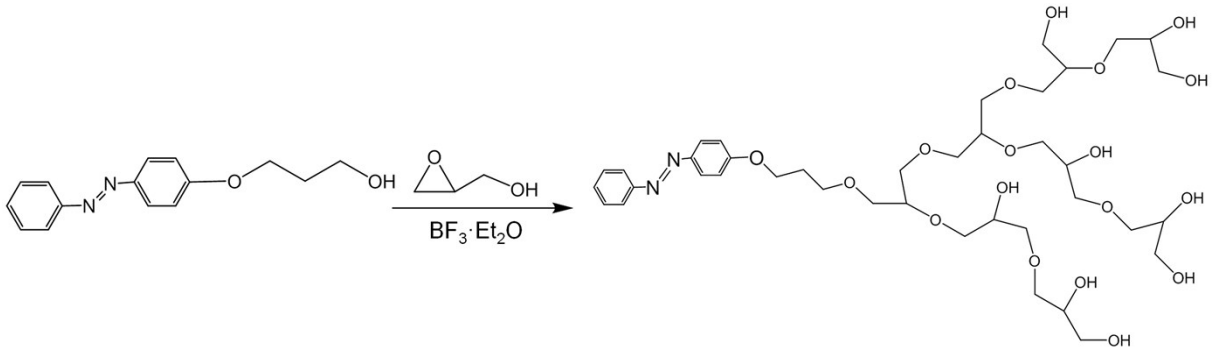
Figure S5a shows the ^{1}H NMR spectrum of AZO-g-HBPO and the peak assignments. Peaks h–k was assigned to the proton signals of the AZO focal point groups. The other strong proton signals were assigned to the HBPO chains. We could directly calculate the degree of polymerization (DP) of HBPO, which was equal to 29, in the so-formed AZO-g-HBPO based on the integrated areas of peaks f–j (AZO focal point) and peaks a–d (HBPO). Thus, the molecular weight of AZO-g-HBPO calculated by ^{1}H NMR was 3332 Da.

Figure S5b shows the quantitative ^{13}C NMR spectrum of AZO-g-HBPO, and the signals of AZO could be significantly observed at δ = 120–160 ppm. The degree of branching (DB) of AZO-g-HBPO was calculated using the integrated areas of three signals at δ = 22.5 ppm, and the result was 46%. GPC measurement with the RI detector (Figure S7) showed a unimodal

distribution with a number average molecular weight ($M_{n,GPC}$) of 4957 Da and a polydispersity of 1.47, indicating that no AZO-OH or homopolymers existed in the system (Figure S7). Thus, we calculated the DB of the grafted HPG using Equation S2 as reported by Frey, [4, 5] the result was 0.2 (where T is the terminal unit, L is the linear unit, and D is the dendritic unit):

$$\mathrm{DB} = \frac{2D}{2D + L} \qquad \text{Equation S2}$$

AZO-HPG: AZO-HPG was synthesized using a method similar to that of AZO-HBPO. The synthesis route is shown in Scheme S4.



Scheme S4 The synthetic route of AZO-HPG

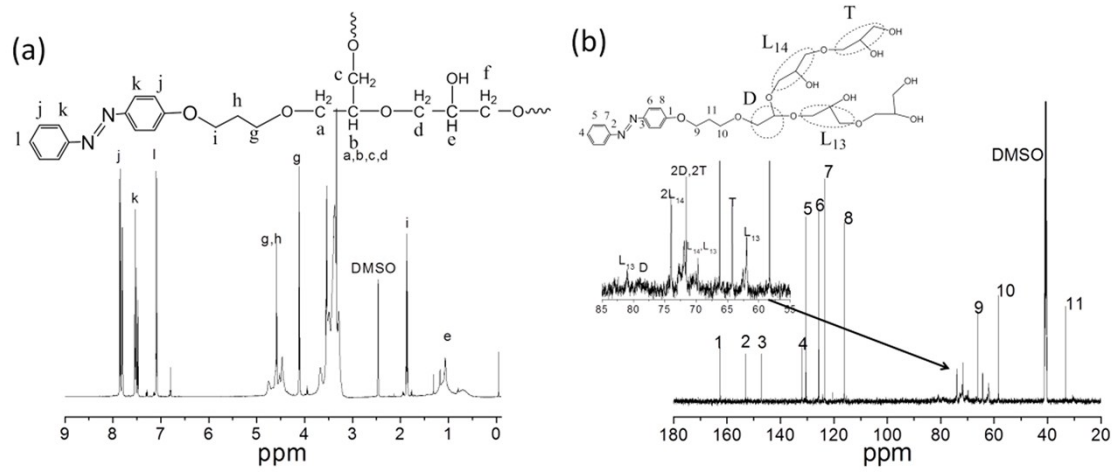


Figure S7 (a) ^{1}H NMR spectra of AZO-HPG; (b) ^{13}C NMR spectra of AZO-HPG; (DMSO-d6, 298K)

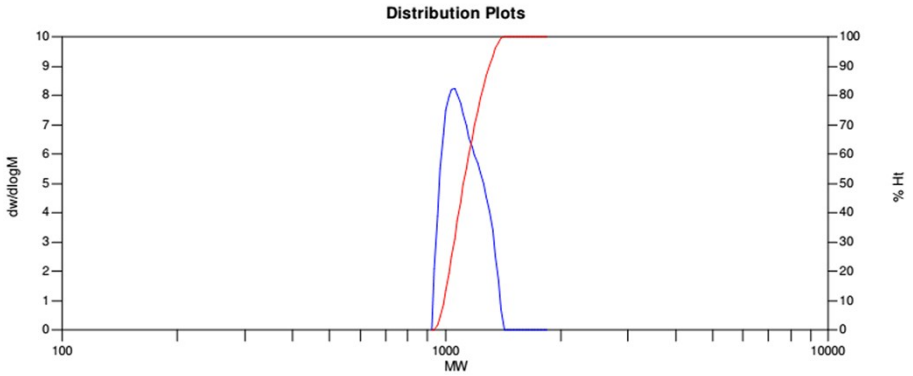


Figure S8 GPC curve of AZO-HPG with THF as eluent.

The synthesis of AZO-HPG was similar to AZO-HBPO. AZO-HPG was carefully characterized by ^{1}H NMR, ^{13}C NMR, and GPC. Figure S7a shows the ^{1}H NMR spectrum of AZO-g-HPG and the peak assignments. Peaks h–k was assigned to the proton signals of the AZO focal point groups. The other strong proton signals were assigned to the HPG chains. We could directly calculate the DP of HPG, which was equal to 10, in the so-formed AZO-g-HPG based on the integrated areas of peaks j–l (AZO focal point) and peaks a–e (HPG). Thus, the average molecular weight of AZO-g-HBPO calculated by ^{1}H NMR was 1000 Da.

Figure S7b shows the quantitative ^{13}C NMR spectrum of AZO-g-HPG, and the signals of AZO were observed at δ = 120–160 ppm. All the dendritic units, linear units, and terminal units of the grafted HPG were clearly discerned. The DB of AZO-g-HPG was calculated using the integrated areas of three signals at δ = 60–90 ppm, and the result was 0.53. GPC measurement with the RI detector (Figure S8) showed a unimodal distribution with a number average molecular weight ($M_{n,GPC}$) of 1111 Da and polydispersity of 1.1, indicating that no AZO-OH or homopolymers existed in the system. Thus, we calculated the DB of the grafted HPG using Equation S3 as reported by Frey,[4] and the result was 0.53:

$$\mathrm{DB} = \frac{2D}{2D + L_{13} + L_{14}} \qquad \text{Equation S3}$$

The synthesis products were also determined by FT-IR (Figure S9). In the spectra of AZO-OH, the absorption bands at 1246 and 1057 cm^{-1} were attributed to the ether band, indicating

that 3-bromo-1-propanol was successfully coupled with 4-phenylazophenol. The absorption bands at 2935 and 2872 cm^{-1} were attributed to the symmetric and asymmetric stretching vibrations of -CH_2-. The bands at 1582 and 1600 cm^{-1} were attributed to the aromatic ring with AZO groups in the π–π conjugate. In the spectra of AZO-HPG and AZO-HBPO, the new absorption band at 1115 cm^{-1} was attributed to the branched chain of methylene. Compared with AZO-OH, the relative intensity of the absorption band at 3400 cm^{-1} was attributed to the terminal hydroxyl group.

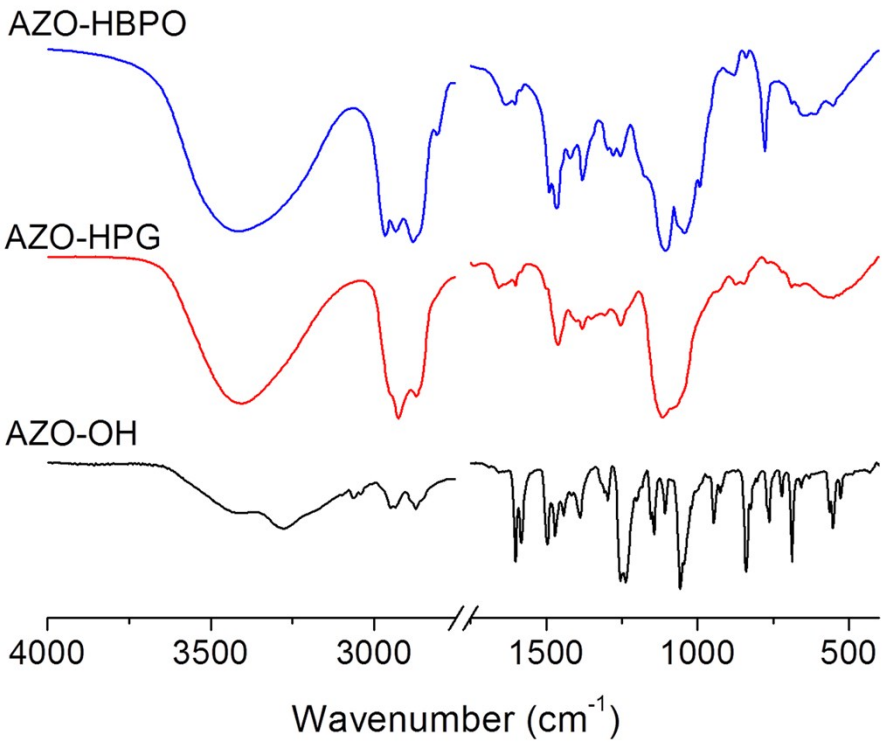


Figure S9 FT-IR spectra of AZO-OH, AZO-HBPO, and AZO-HPG

4 Preparation of PPIX-2CD@AZO nanoparticles

First, mixed PPIX-2CD solution in DMF with AZO-HPG solution in DMF and stirred for 0.5 h. And then AZO-HBPO solution was added with a molar ratio of PPIX-2CD, AZO-HPG, and AZO-HBPO equal to 1:1:1 (0.5 mg/mL PPIX-2CD). After stirring for 3 h at ambient temperature, water was added dropwise to obtain a mixed solution with water/DMF volume ratio of 30% (v/v). The mixed solutions were stirred for 4 h and then dialyzed against water

(MWCO = 3.5 kDa; Solarbio) for 48 h to remove DMF. The final solution had a slight yellow turbidity, indicating the formation of supramolecular self-assemblies. The morphology of the nanoparticles was tested by TEM, SEM, and AFM.

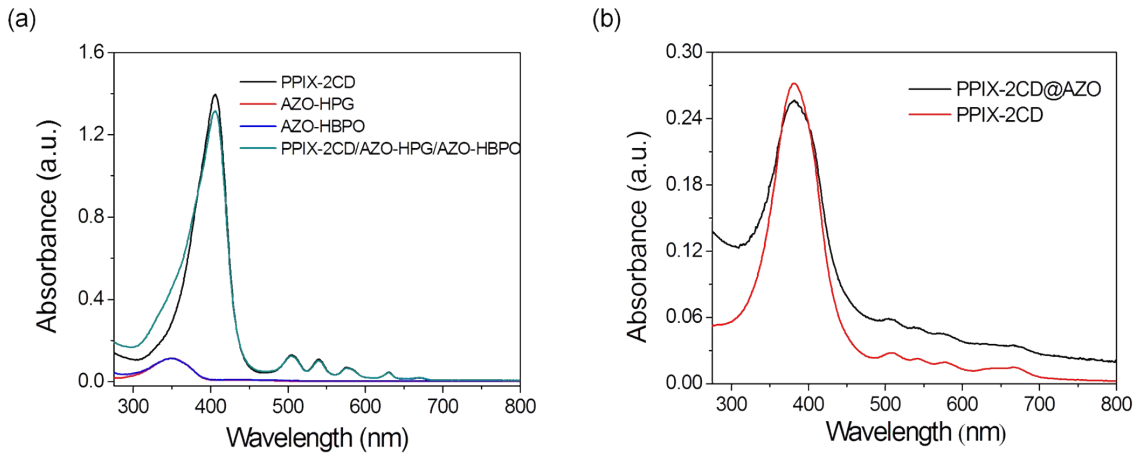


Figure S10 UV-vis spectra (a) PPIX-2CD, AZO-HPG, AZO-HBPO and PPIX-2CD/AZO-HPG/AZO-HBPO in DMF solution; (b) PPIX-2CD and PPIX-2CD@AZO vesicles in aqueous solution.

Reference

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