Supplementary information

A Shish-Kebab-like Supramolecular Polymer and its Light-Responsive self-assembly into nanofibers

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1. Materials

4-phenylazophenol, (TCL, 95%), 6-Bromo-1-Hexanol (Adamas, 98%+), N,N-dimethylformamide, Potassium Carbonate, Sodium Hydroxide，p-Toluenesulfonyl Chloride , Potassium Hydroxide (Sinopharm Chemical Reagent Co., Ltd, AR), β-Cyclodextrin (Adamas, 99%) was re-crystallized from deionized water 3 times, Glycidol (Acros, 96%) was purified by vacuum distillation. Potassium hydride (Acros, 30wt%, dispersion in mineral oil) was purified by washing with dry tetrahydrofuran for 5 times and then dried in vacuum. 18-Crown-6 ether (Alfa Aesar, 99%), 3-Bromopropyne (Adamas, 99%), Sodium azide, Copper(I) Bromide (Aldrich, 98%), N, N, N, N', N'-Pentamethyldiethylenetriamine (Alfa Aesar, 98%).

2. Instruments and measurements

2.1 Nuclear Magnetic Resonance (NMR)

^{1}H NMR, ^{13}C NMR spectra with deuterated chloroform ($CDCl_3$), N, N-dimethylformamide-d_7 (DMF-d_7), dimethylsulfoxide-d_6 (DMSO-d_6) or deuterium oxide (D_2O) as solvents at 298 K. Residual protic solvent of deuterated solutions and tetramethylsilane (TMS) were used as the internal reference in the ^{1}H and ^{13}C NMR spectra.

2D-NOSEY ^{1}H NMR spectrum of β-CD-*g*-HPG-AZO (in DMF-d_7 and D_2O, 90/10, v/v) was recorded using Bruker AVANCEIII 400 spectrometer at 298 K.

2.2 Ultra Performance Liquid Chromatography and Mass Spectrometry

The mass spectrometry (MS) spectrum of AZO-*g*-OH and AZO-Alk was obtained on a ACQUITYTM UPLC & Q-TOF MS Premier (USA).

2.3 Gel Permeation Chromatography (GPC)

The molecular weights of the products were measured by GPC on an HLC-8320 GPC (TOSOH, EcoSEC GPC System) system at 40 ºC with N, N-dimethylformamide as mobile phase at a flow rate of 0.6 mL min^{-1}.

2.4 Dynamic Light Scattering (DLS)

DLS measurements were performed in H_2O/DMF solutions with a Malvern Zetasizer Nano S (MalvernInstruments, Ltd.) equipped with a 4 mW He-Ne laser light operating at λ= 633 nm. All samples were kept at about 1 mg mL^{-1} and measured at 25 ºC with a scattering angle of 173 º.

2.5 Scanning Electron Microscopy (SEM)

SEM measurements were performed on a NOVA NanoSEM 450 (FEI). The samples for SEM observations were prepared by depositing several drops of the solution (1 mg mL^{-1}) onto the

surface of silicon wafer, and the samples were air-dried at room temperature. The samples were coated with a thin film of gold before measuring.

2.6 Atomic Force Microscopy (AFM)

Atomic Force Microscopic (AFM) images of nanofibers were taken on a BioScope Resolve scanning probe microscopy (SPM) system (Bruker, USA) by PeakForce Tapping mode, and Scanasyst Air tips (Bruker, USA) with a force constant of ~0.4 N m^{-1} and resonance vibration frequency of ~70 kHz were used. The samples for AFM observations were prepared by depositing several drops of the solution (0.1 mg mL^{-1}) onto the surface of fresh cleaved mica and dried at room tempertaure for 24 h.

2.7 Transmission Electron Microscopy (TEM)

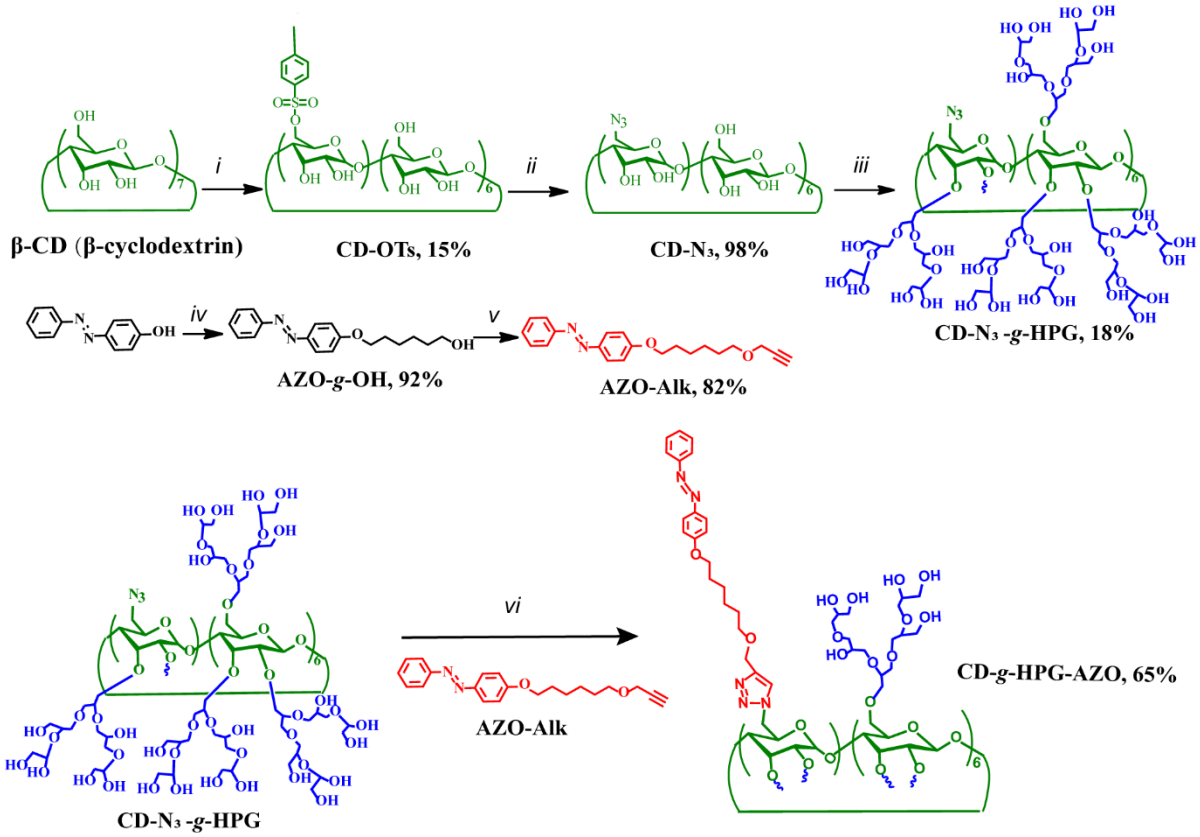
TEM measurements were performed with a Tecnai G2 SpiritBiotwin (FEI) instrument at a voltage of 120 kV. The samples were prepared by dropping solutions (0.1 mg ml^{-1}) onto carbon-coated copper grids, and the grids were dried at room temperature for 24 h.

2.8 UV-vis absorption spectroscopy (UV-vis)

UV-vis absorption spectra were measured by UV-2600 (SHIMADAZU(CHINA) CO., LTD). Samples of self-assembly with certain concentration were added to a 1 cm quartz cuvette (Wavelength range: 200-800 nm, Wavelength Accuracy: ±0.5 nm) for the measurements.

3. Synthesis of CD-*g*-HPG-AZO

The whole synthesis route of CD-g-HPG-AZO was shown in Scheme S1.



Scheme S1 Synthesis of CD-*g*-HPG-AZO.

3.1 Synthesis and characterization of 6-hydroxyoxo-4-azobenzene (AZO-*g*-OH)

A typical synthesis process was as follows: 6-Bromo-1-Hexanol (2.17 g, 12 mmol) and 4-phenylazophenol (1.98 g, 10 mmol) were dissolved in dried DMF (30mL). Potassium carbonate (2.07 g, 15 mmol) was added to the solution, and then the solution was immersed in an oil bath at 75 ℃ for 24 h. After cooled to room temperature, the solution was poured into cold water (50 mL) and extracted with chloroform (50 mL). The organic phase was washed with 1 M HCl and saturated

NaCl for three times, and then dried with anhydrous $NaSO_4$. After filtered, the solvent was evaporated under reduced pressure to afford the crude product as a yellow solid. The crude product was chromatographed on a silica gel using ethyl acetate (EA) and dichloromethane (DCM) (EA: DCM=1:15, v/v) as eluent, and the purified product is a pale yellow solid (2.74 g, yield: 92%).

The final product of 6-hydroxyoxo-4-azobenzene (AZO-*g*-OH) was characterized by ^{1}H NMR, ^{13}C NMR and Mass Spectrometry.

δ H (400 MHz, $CDCl_3$) 8.04–7.81 (4 H, m), 7.62–7.38 (3 H, m), 7.05–6.96 (2 H, m), 4.05 (2 H, t, J 6.5), 3.74–3.57 (2 H, m), 1.84 (2 H, dq, J 13.0, 6.5), 1.61 (2 H, tt, J 8.3, 4.0), 1.57–1.40 (6 H, m).

δ C (101 MHz, $CDCl_3$) 161.86 (s), 152.96 (s), 147.05 (s), 130.55 (s), 129.25 (s), 124.99 (s), 122.75 (s), 114.90 (s), 68.41 (s), 63.08 (s), 32.88 (s), 29.39 (s), 26.10 (s), 25.78 (s).

HRMS (MALDI^{+}) m/z: calcd. for $C_{18}H_{22}N_2O_2$ $(M)^{+}$: 299.1715; found: 299.1765.

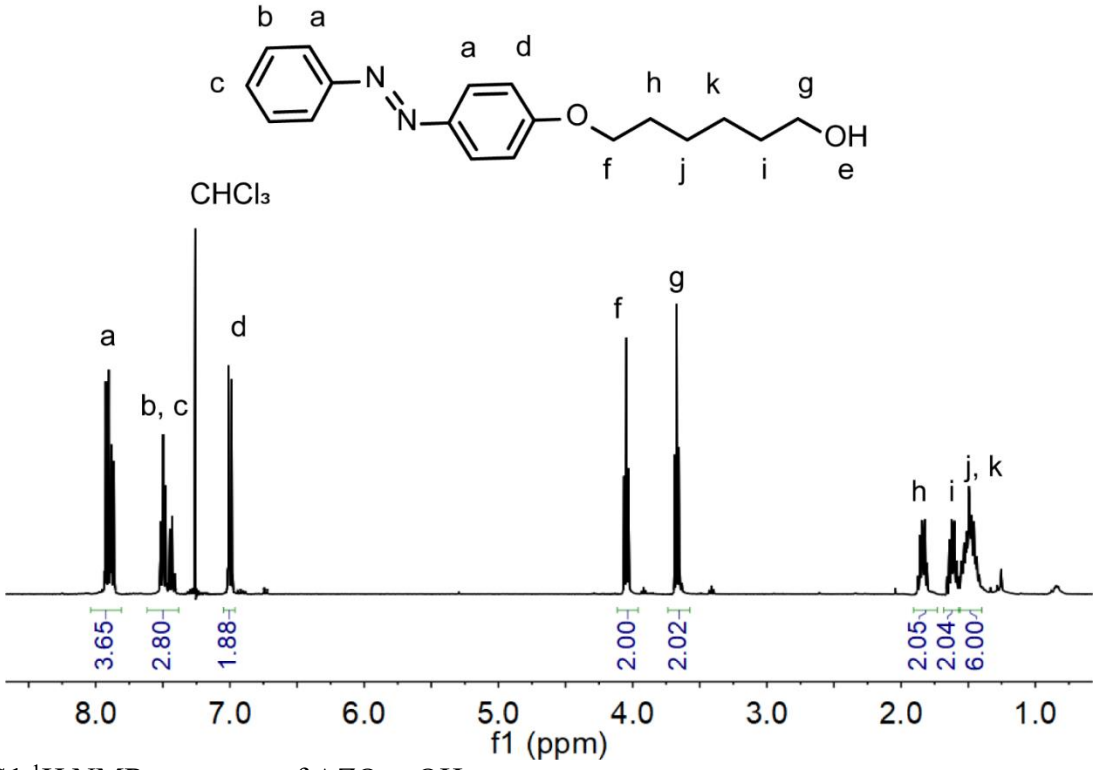


Fig. S1 ^{1}H NMR spectrum of AZO-*g*-OH.

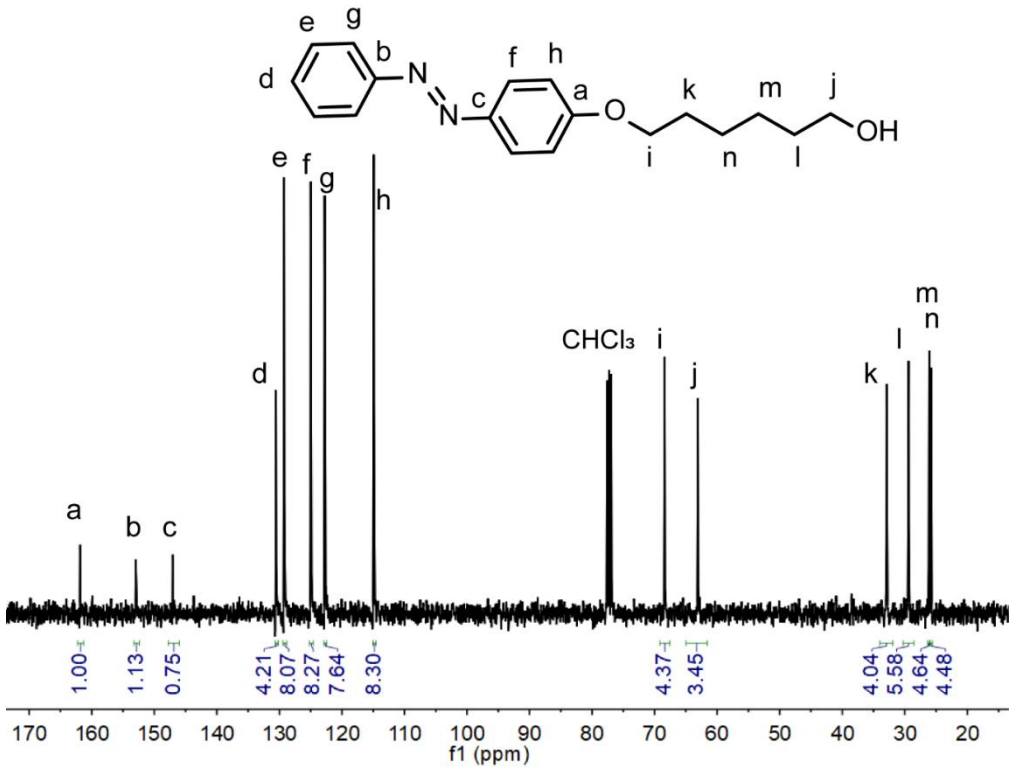


Fig. S2 ^{13}C NMR spectrum of AZO-*g*-OH.

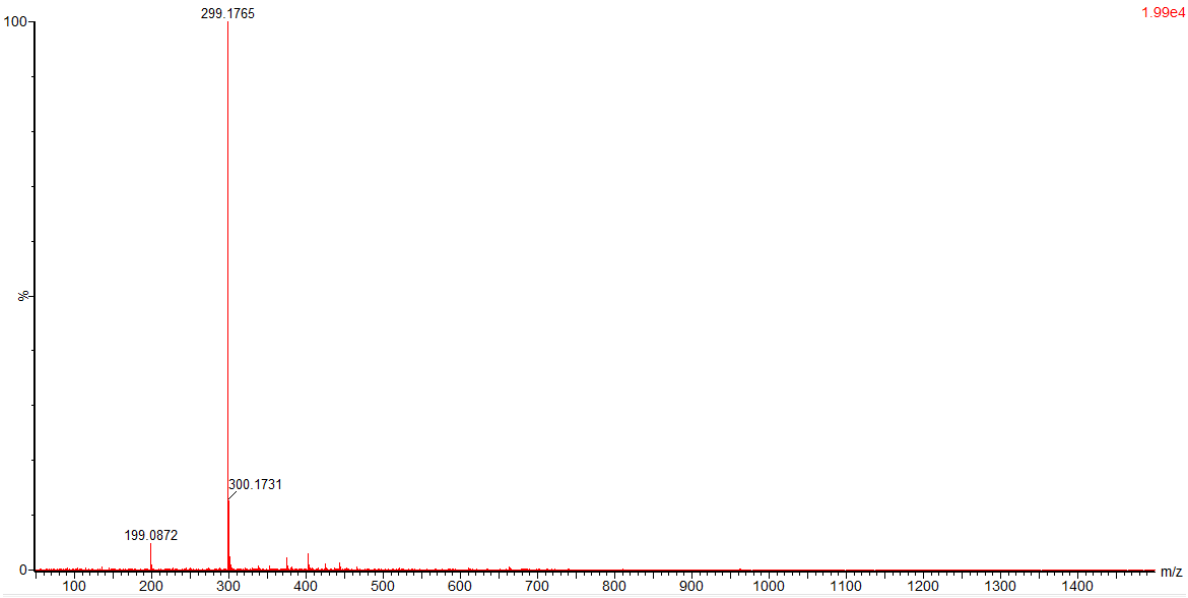


Fig. S3 MS spectrum of AZO-*g*-OH.

3.2 Synthesis and characterization of 6-(propynyloxy) hexyloxy-4-azobenzene (AZO-Alk)

6-hydroxyoxo-4-azobenzene (AZO-*g*-OH) (600 mg, 1.98 mmol) and 18-Crown-6 ether (15 mg, 0.06 mmol) were dissolved in Toluene (25 mL). Potassium hydroxide (222 mg, 3.96 mmol) was added to the solution, and then the solution was immersed in an oil bath at 25 ℃. Then 3-Bromopropyne (588.8 mg, 4.95 mmol) was added dropwise. After reaction for 48 h, the color of the solution change to yellow, then stop the reaction. The solution was washed with 1 M HCl and saturated NaCl for three times, and then dried with anhydrous $NaSO_4$. After filtered, the solvent was evaporated under reduced pressure to afford the crude product as a yellow solid. The crude product was chromatographed on a silica gel using ethyl acetate (EA) and petroleum ether (PE) (EA: PE=1:5, v/v) as eluent, and the purified product is a pale yellow solid (553.6 mg, yield:82%).

The final product of AZO-Alk was characterized by 1H NMR, 13C NMR and Mass Spectrometry.

δ H (400 MHz, $CDCl_3$) 7.85 (2 H, dd, J 19.0, 8.4), 7.40 (2 H, dt, J 25.4, 7.2), 6.93 (1 H, d, J 8.8), 4.07 (1 H, d, J 1.8), 3.97 (1 H, t, J 6.5), 3.46 (1 H, t, J 6.5), 2.35 (1 H, d, J 1.6), 1.83–1.69 (1 H, m), 1.65–1.52 (1 H, m), 1.51–1.30 (2 H, m).

δ C (101 MHz, $CDCl_3$) 161.88 (s), 152.97 (s), 147.05 (s), 130.53 (s), 129.25 (s), 124.99 (s), 122.75 (s), 74.37 (s), 70.31 (s), 68.42 (s), 58.23 (d, J 12.2), 29.69 (s), 29.36 (s), 26.11 (d, J 4.7).

HRMS (MALDI$^+$) m/z: calcd. for $C_{21}H_{24}N_2O_2$ $(M)^+$: 337.1817; found: 337.1941.

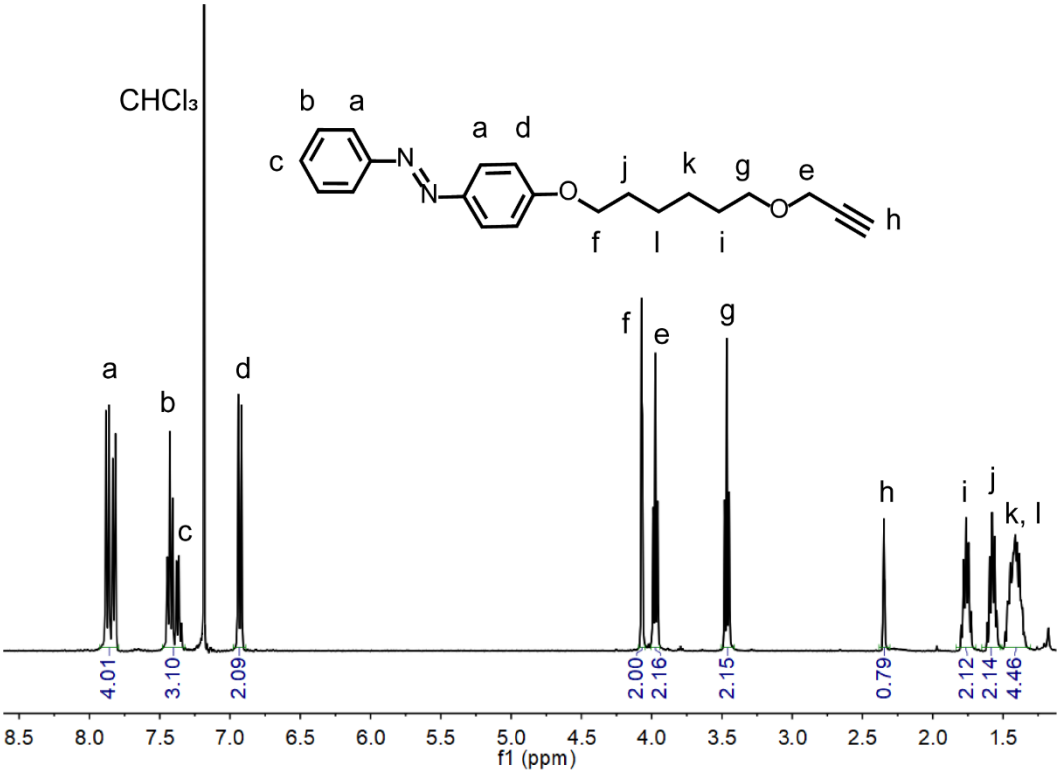


Fig. S4 ^{1}H NMR spectrum of AZO-Alk.

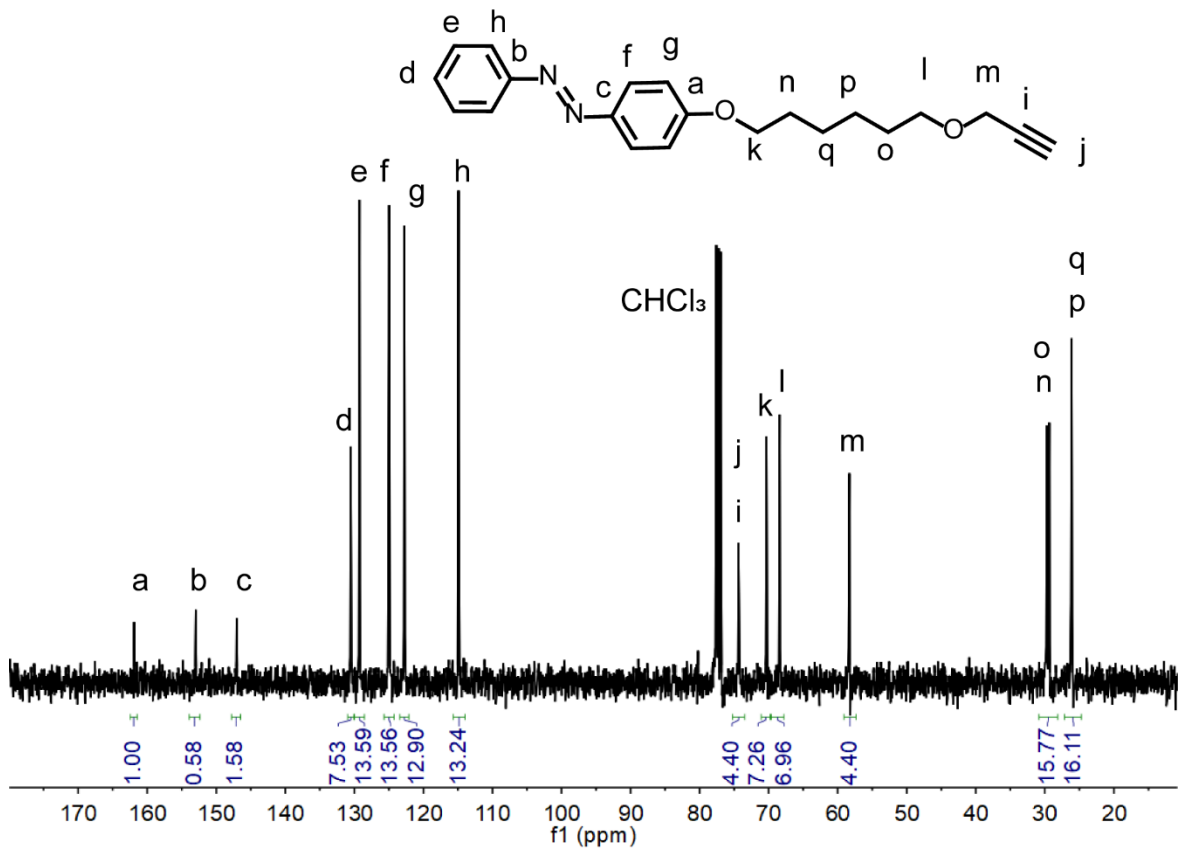


Fig. S5 ^{13}C NMR spectrum of AZO-Alk.

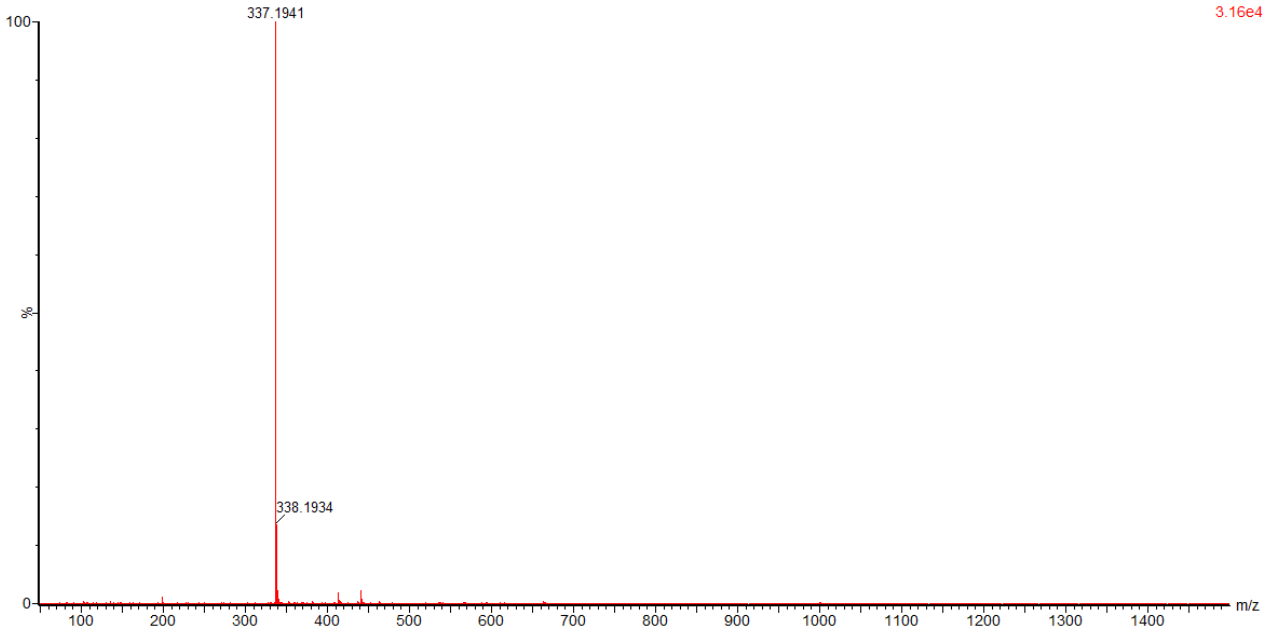


Fig. S6 MS spectrum of AZO-Alk.

3.3 Synthesis and characterization of CD-N_3-*g*-HPG

3.3.1 Synthesis and characterization mono-6-O-(p-toluenesulfonyl)-β-cyclodextrin (CD-OTs) and mono-6-azido-β-cyclodextrin (CD-N_3)

The detailed synthesis process of CD-OTs and CD-N_3 refered to previous literature. [1]The molecular structure of CD-OTs and CD-N_3 was characterized by ^{1}H NMR.

CD-OTs: δ H (400 MHz, DMSO-d_6) 7.73 (2 H, d, J 8.0), 7.43 (2 H, t, J 13.3), 5.91–5.56 (14 H, m), 4.78 (7 H, d, J 27.9), 4.54–4.09 (9 H, m), 3.83–3.05 (46 H, m), 2.40 (2 H, s).

β-CD-N_3: δ H (400 MHz, DMSO-d_6) 5.82–5.56 (1 H, m), 4.82 (1 H, t, J 9.3), 4.55–4.36 (1 H, m), 3.72 (1 H, s), 3.58 (2 H, dd, J 31.6, 8.8), 3.30 (5 H, s).

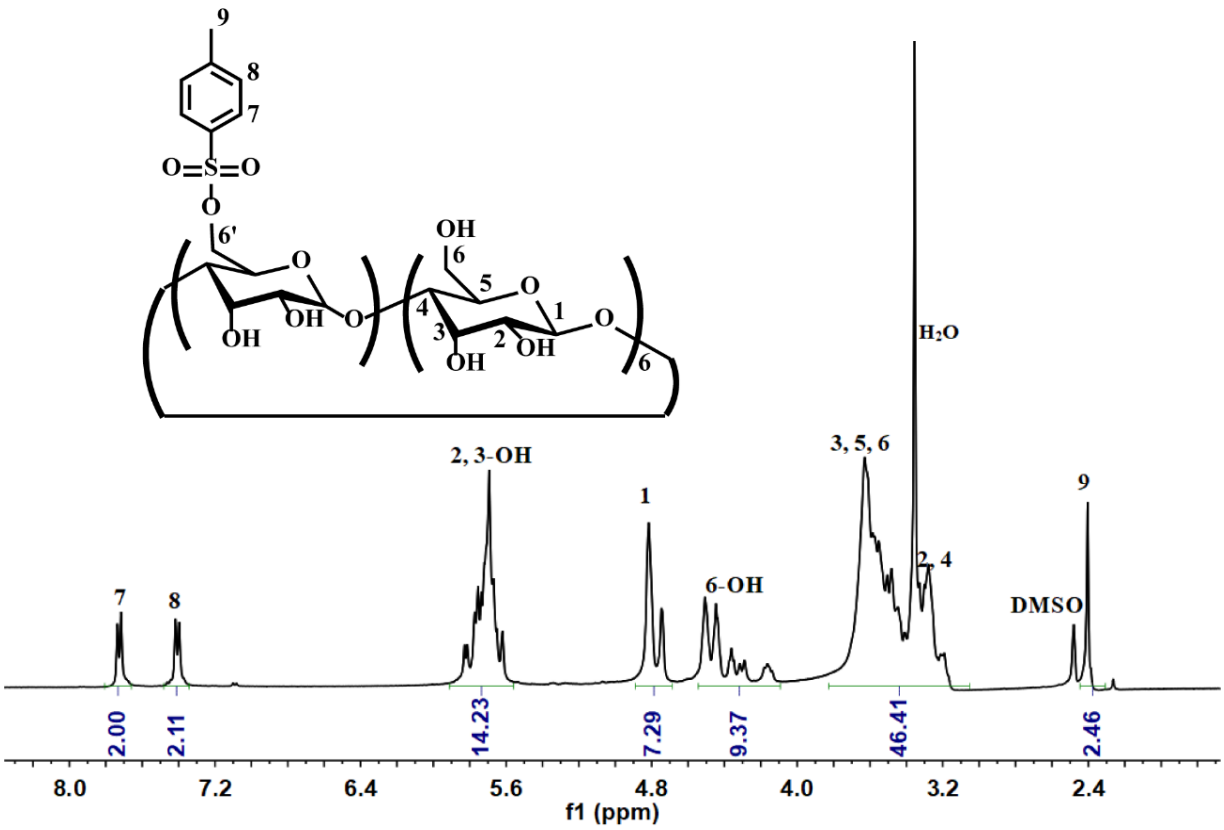


Fig. S7 ^{1}H NMR spectrum of CD-OTs.

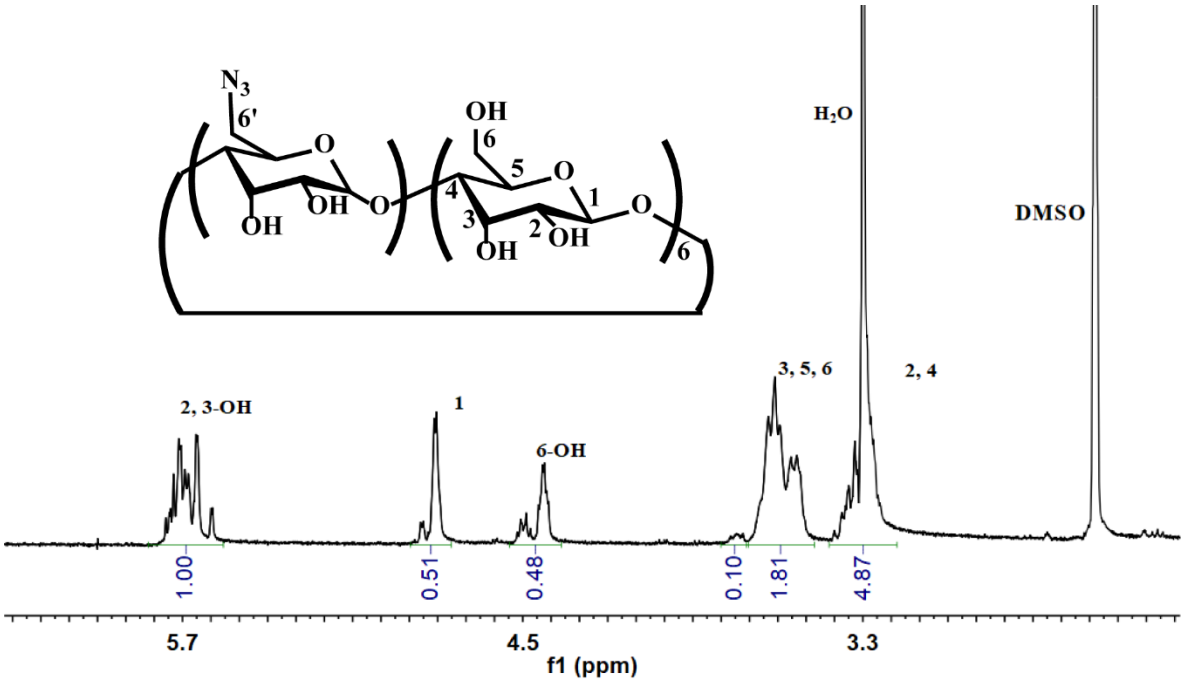


Fig. S8 ^{1}H NMR spectrum of CD-N_3.

3.3.2 Synthesis and characterization of CD-N_3-*g*-HPG

CD-N_3-HPG was synthesis according to the anionic ring-opening multibranching polymerization (ROMBP) method by using CD-N_3 as a multi-hydroxyl initiator, and the glycidol monomer was added very slowly. The whole synthesis route was shown in Figure S9. The detail synthesis process was shown in our previous work.[2, 3] To avoid the formation of the homopolymers, herein, we have used a monomer slow addition technique as previously reported by Frey et al. in the anionic ROMBP process.[4]

The final product of CD-N_3-*g*-HPG was characterized by ^{1}H NMR, GPC.

δ H (400 MHz, D_2O) 5.11 (7 H, s), 4.04–3.20 (335 H, m).

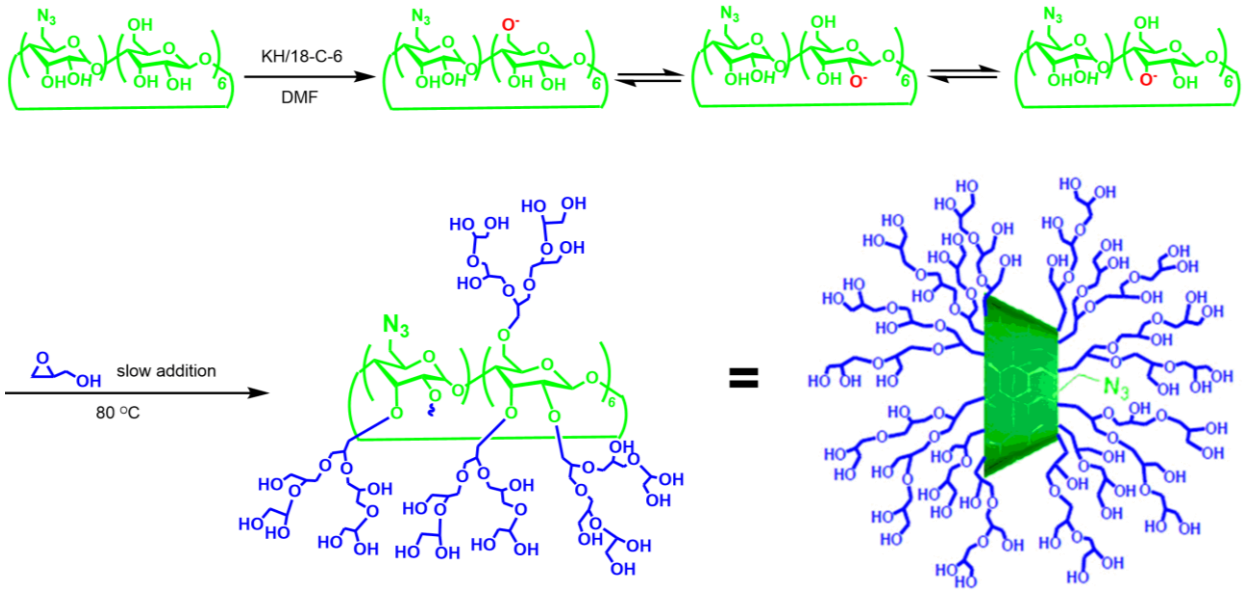


Fig. S9 Synthesis of CD-N_3-*g*-HPG.

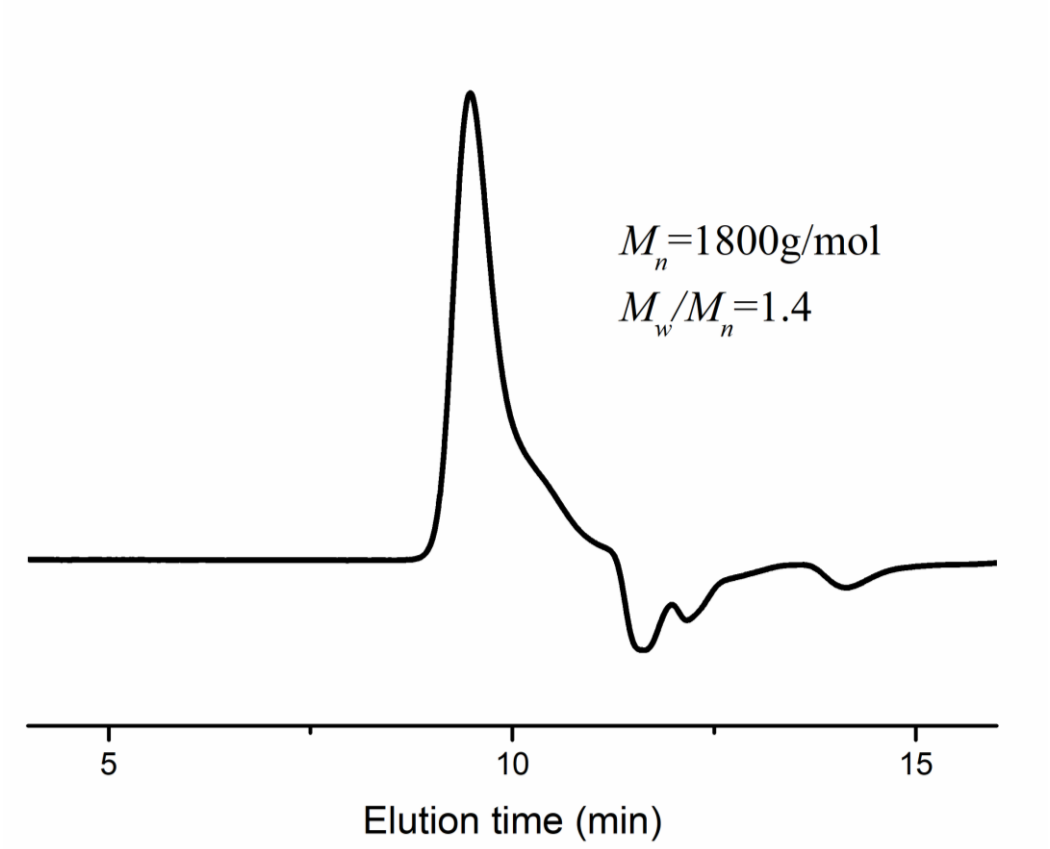


Fig. S10 GPC of CD-N_3-*g*-HPG.

The GPC measurements (Figure S10) showed a unimodal distribution with a number-average molecular weight of 1800 Da and a polydispersity of 1.4. ^{1}H NMR of CD-N_3-*g*-HPG was showed in figure S11.According to our previous work[2, 3], the degree of polymerization (DP) of HPG in CD-N_3-*g*-HPG could be calculated according to the integrated areas of peak A (H1 of CD) and B (CH, CH2 of HPG and H2-H6 of CD), and the calculation formula is shown in Equation 1, which turns out to be 58. Thus, the M_n of CD-N_3-*g*-HPG calculated by 1H NMR is about 5000 Da. Generally, the M_n measured by GPC may be lower than the actual M_n for hyperbranched polymers because the hydrodynamic volumes of hyperbranched polymers are smaller than those of linear polymers used for calibration. Besides, hyperbranched polymers often exhibit adsorption by GPC

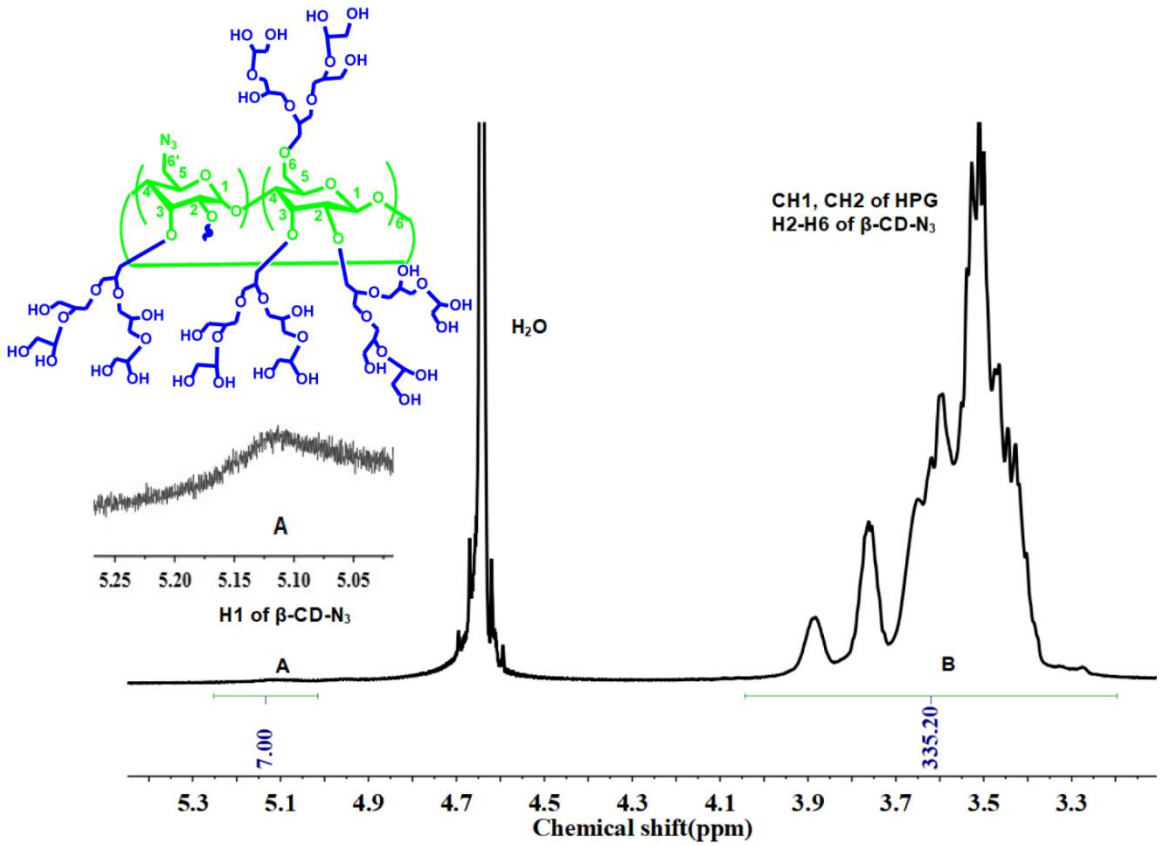


Fig. S11 ^{1}H NMR spectrum of CD-N_3-*g*-HPG.

columns due to their large number of end groups. Therefore, we selected the *Mn* calculated from 1H NMR as accurate molecular weight for following calculation.

$$DP = \frac{(\mathrm{B}-6\mathrm{A})/5}{\mathrm{A}/7} \qquad \text{(equation 1)}$$

3.3 Synthesis and characterization of CD-*g*-HPG-AZO

The synthesis of CD-*g*-HPG-AZO is based on the click chemistry reaction of an alkynyl group with an azide group.[5] Typically, AZO-Alk (100 mg, 0.297 mmol), CD-N_3-*g*-HPG (162.1 mg) and CuBr (29 mg, 0.144 mmol), 20 mL of ultra-dry DMF and 30.46 μL (1.44 mmol) of PMDETA were added into a 100 mL round-bottom flask followed by three freeze-vacuum-thaw cycles. The flask was immersed into oil bath at 70 ℃ with stirring. After reaction for 48 h, the reacted solution was allowed to stand for cooling, and diluted with DMF to form a clear transparent and chromatographed on a neutral aluminum oxide column to remove copper ions and cuprous ions. After evaporating the solvent, a yellow viscous liquid was obtained, which was washed three times with acetone to remove unreacted AZO-Alk. The product was dialyzed against (MWCO: 1000 Da) DMSO for three days. The solution inside the dialysis bag was collected and evaporated under vacuum to produce yellow viscous liquid, which was dried in a vacuum oven at 80 ° C for 48 h to obtain a final product of CD-*g*-HPG-AZO (100 mg, 65% yielded).

δ H (400 MHz, DMSO-d6) 8.01–7.95 (1 H, m), 7.93–7.71 (4 H, m), 7.55 (3 H, d, J 7.8), 7.12 (2 H, s), 5.30–4.86 (7 H, m), 4.80–4.26 (37 H, m), 3.85–2.89 (219 H, m), 1.79–1.70 (2 H, m), 1.57–1.48 (2 H, m), 1.33 (2 H, s), 1.22 (2 H, s).

As shown in Figure S12, all the protons can be assigned to the 1H NMR spectrum. In addition, the integrated areas of the proton a in nitrogen heterocyclea to the H1 proton peak area on the cyclodextrin after click chemistry reaction is about 1:7. Therefore, it can be proved that only one azobenzene is substituted on each cyclodextrin, which proves that the synthesis of CD-*g*-HPG-AZO is successful.

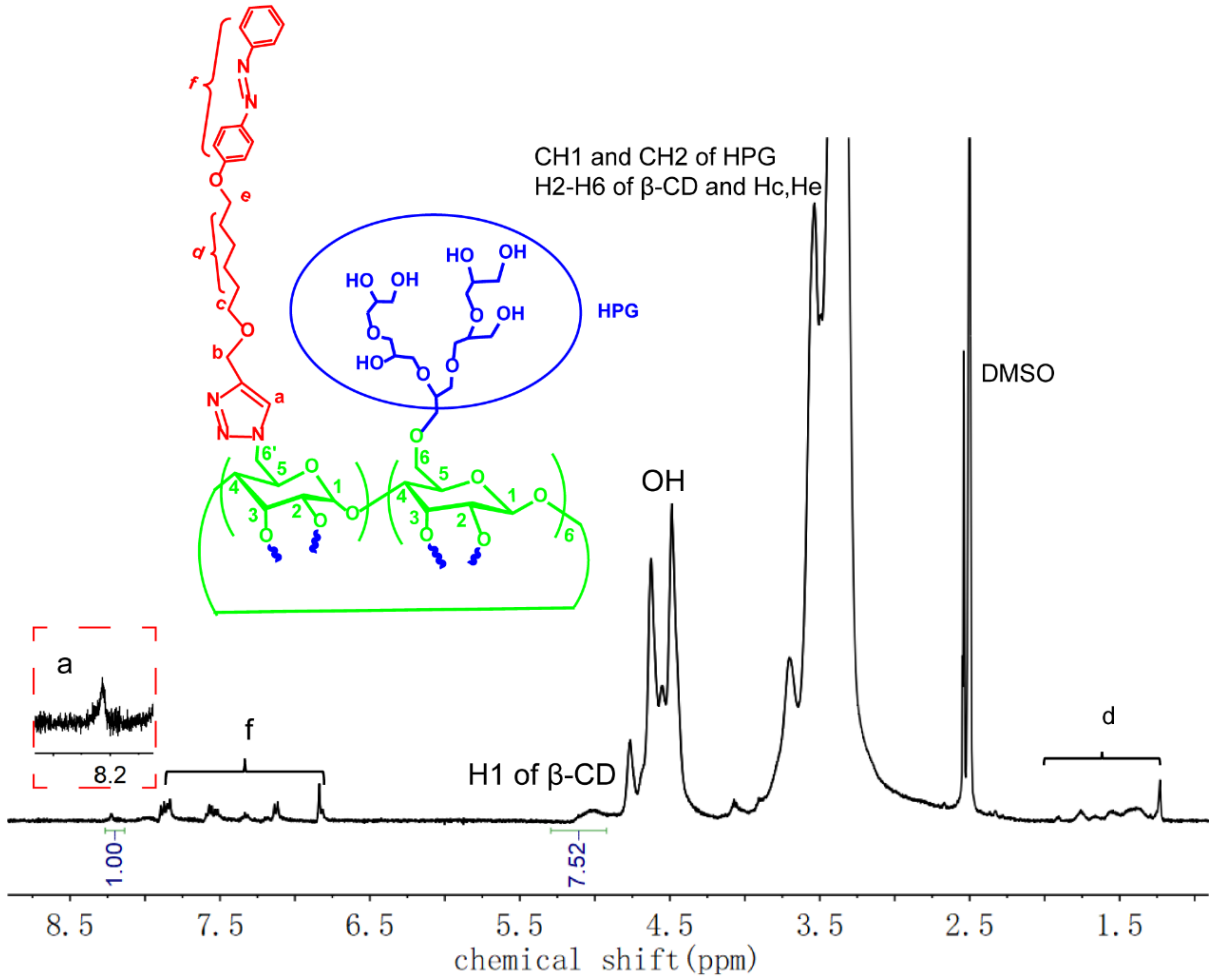


Fig. S12 ^{1}H NMR spectrum of CD-*g*-HPG-AZO.

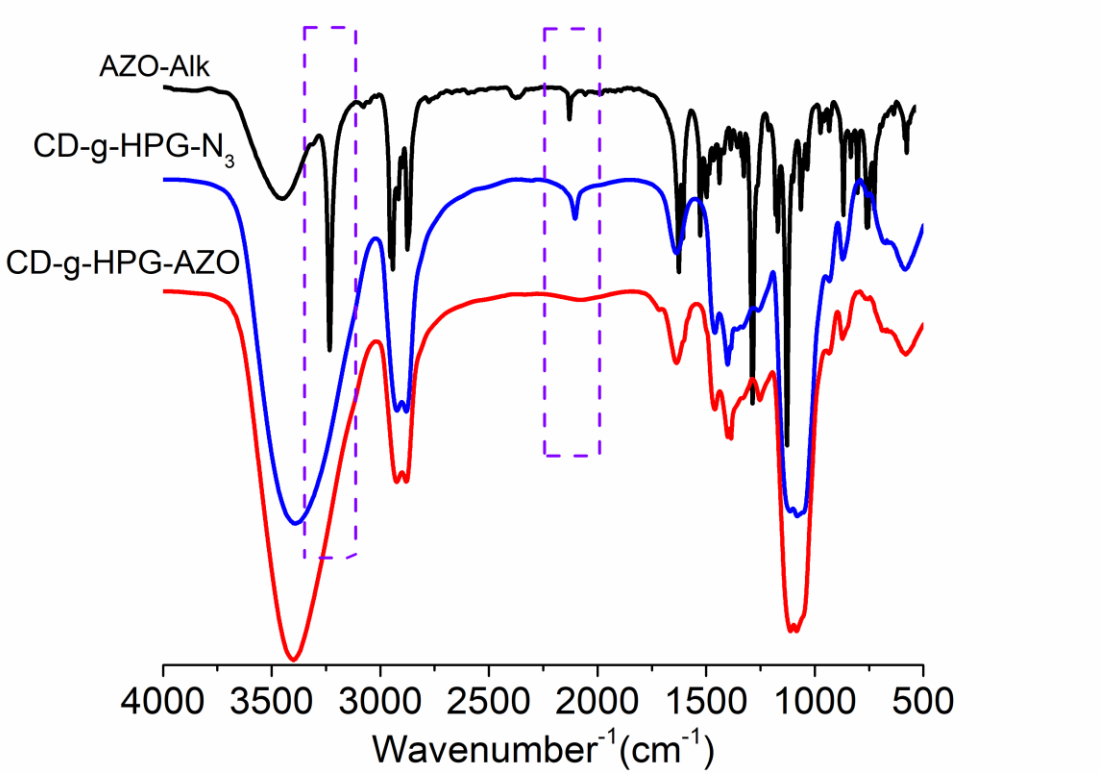


Fig. S13 FTIR spectrum of CD-*g*-HPG-AZO.

As shown in Figure S13, after the click-chemistry reaction occurs, the stretching vibration absorption peak of AZO-Alk at 3225 cm-1 completely disappears in CD-*g*-HPG-AZO. In addition, the CH stretching vibration absorption peak on the alkyne group of AZO-Alk at 2110 cm-1, and the stretching vibration absorption peak of the azide group at 2100 cm-1 in β-CD-N_3-g-HPG, these two peaks disappear completely in CD-*g*-HPG-AZO, indicating that the reaction has proceeded completely.

4. Host-Guest complextion of CD-*g*-HPG-AZOs

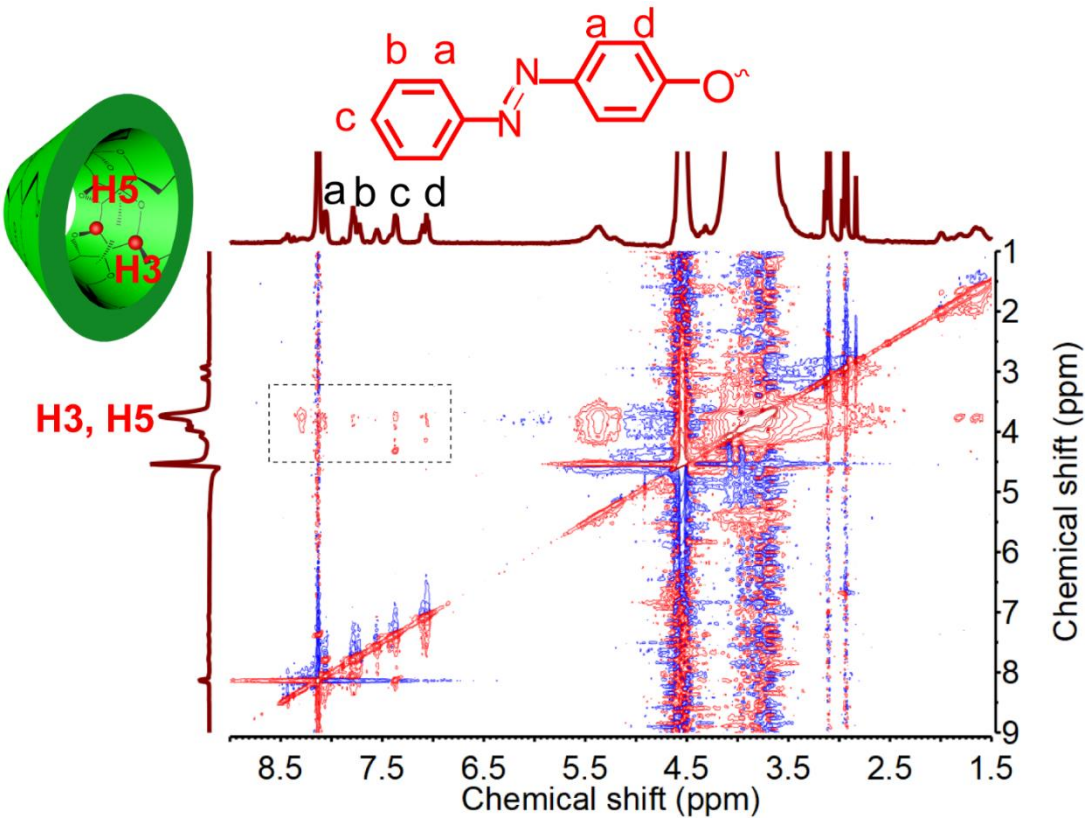


Fig. S14 2D-DOSY ^{1}H NMR spectrum of CD-*g*-HPG-AZOs in D_2O/DMF-d_7 solution (D_2O 10% v). The intermolecular correlations between the H3, H5 protons in the inner cave of CDs and the protons of AZO groups, provides a direct evidence to support the complexation of CD-*g*-HPG and AZO group of CD-*g*-HPG-AZO through the specific AZO/CD host-guest interactions.

5. ^{1}H NMR titration experiments

Typically, the ^{1}H NMR titration experiments was carried out by the sequential addition of CD-*g*-HPG (0, 0.25, 0.625, 1.260, 1.875 and 2.520 mM) into the AZO-ONa/D_2O solution (2.25 mM). All proton signals were calibrated with the D_2O at 4.70 ppm. With the increase of CD-g-HPG concentration, the proton signals of AZO-ONa shifted to low-field, which further supported the

formation of the inclusion complex between the CD-*g*-HPG and AZO-ONa through CD/AZO specific host-guest recognition (Fig. S15a). According to the variation of chemical shift, we obtained Benesi Hildebrand plot as shown in (Fig. S15b). The complexation constant can be calculated by Benesi Hildebrand equation as shown in equation 2. Three Kc values according to the proton peaks of AZO-ONa (peak a, b and c) were obtained and the Kc value in average was 1412 M-1. Generally, the typical Kc between β-CD and AZO group is around ~10^4, thus we speculate the grafted HPG in CD-g-HPG weaken the binding ability of CD/AZO.

$$\frac{1}{\Delta\delta_{ob}} = \frac{1}{\Delta\delta_c K_c}\frac{1}{[CD-g-HPG]} + \frac{1}{\Delta\delta_c} \qquad Equation\ 2$$

Where $\Delta\delta_{ob}$ is the δ difference between AZO-ONa with CD-*g*-HPG and AZO-ONa without CD-*g*-HPG. $\Delta\delta_c$ is δ difference between AZO-ONa fully complexed with CD-*g*-HPG and AZO-ONa without CD-*g*-HPG. K_c is the binding constant between AZO-ONa and CD-*g*-HPG.

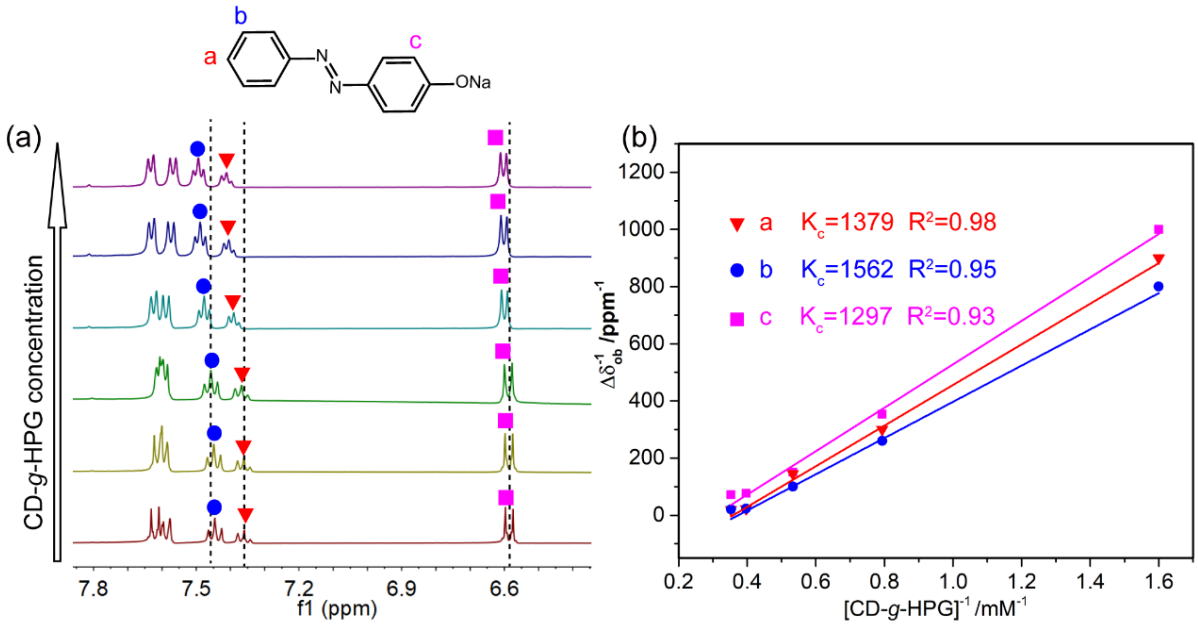


Fig. S15 (a) The shifting of protons of AZO-ONa (2.25 mM) with increasing the concentrations of CD-*g*-HPG (0, 0.25, 0.625, 1.260, 1.875 and 2.520 mM) in D_2O at 293 K. (b) the Benesi Hildebrand plots of three proton signals of AZO groups.

6. DOSY Analysis of the supramolecular polymerization process

Typically, 2.5 mM CD-g-HPG-AZOs in DMF-d_7 and 2.5 mM CD-g-HPG-AZOs in D_2O/DMF-d_7 (10% D_2O, v/v) were recorded, respectively. Bruker Advance III HD 600 (Germany) was used to perform the DOSY NMR experiments at 298 K. The pulse program used in the experiments was Bruker *ledbpgp2s*. Gradient amplitudes varied from 0.936 to 45.734 G/cm in 8 equal steps (8 scans each). Besides, the diffusion gradient length (δ) was 0.004 s and the diffusion time (Δ) was 0.0299 s. According to the fitting results of signals by the Stejskal-Tanner equation (equation 3), the diffusion coefficient could be obtained.

$$I = I_0 e^{-D\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)} \quad equation\ 3$$

Where I is the observed intensity, I_o is the reference intensity, D is the diffusion coefficient, γ is the gyromagnetic ratio of the observed nucleus, g is the gradient strength, δ is the length of the gradient, and Δ is the diffusion time.

As shown in Figure R4a, in DMF-d_7 and D_2O/DMF-d_7 cosolvent, two peaks with different diffusion coefficient values (4.5 and 2.9×10^{-10} $m^2 \cdot s^{-1}$) appeared in the spectrum. The slower diffusion rate of CD-g-HPG-AZOs in D_2O/DMF-d_7 compared to that of CD-g-HPG-AZOs in DMF-d_7 suggested the formation of supramolecular polymer.

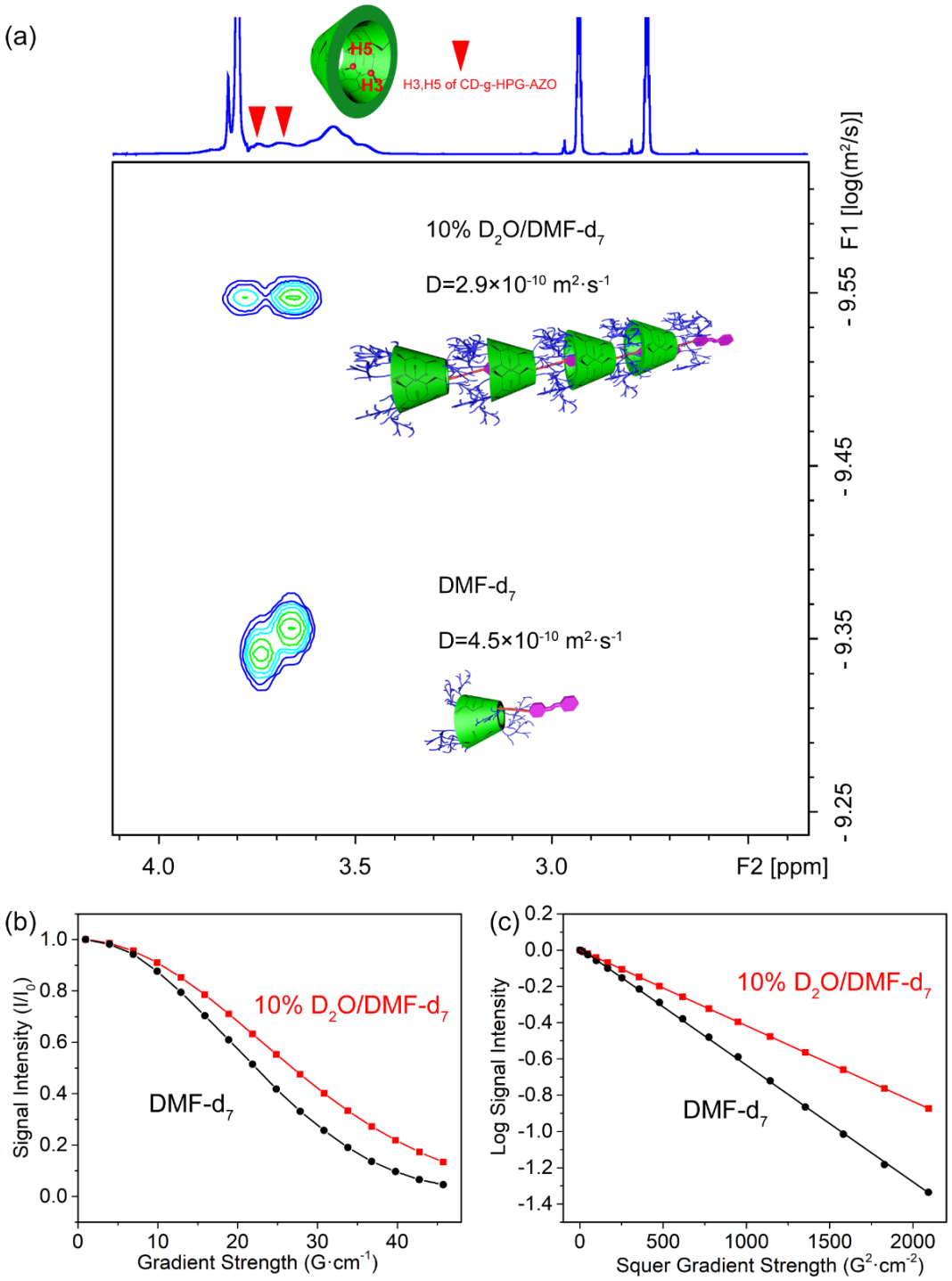


Fig. S16 (a) DOSY NMR spectrum of CD-g-HPG-AZOs in DMF-d_7 and D_2O/DMF-d_7 cosolvent. (b) Relative intensities (I/Io) of the experimental 1H NMR diffusion signals *versus* the NMR gradient strength. (c) Log relative intensities (I/Io) *versus* the square of NMR gradient strength.

According to previous report, the degree of supramolecular polymerization can be roughly estimated according to the Stocks-Einstein equation (equation 4) when assuming that the assemblies are hydrodynamically spherical.[6]

$$D = \frac{k_B T}{6\pi\eta R} \qquad equation\ 4$$

Where T denotes the temperature, k_B is the Boltzmann constant, and η is the dynamic viscosity of the solvent.

As a result, the degree of supramolecular polymerization of SKSPs was calculated by equation 5, which was about 4. This result corresponded well to the DLS results.

$$N \approx \frac{1}{\left(\frac{D}{D_0}\right)^3} \qquad equation\ 5$$

Where D_0 is the diffusion coefficient value in DMF-d_7 and D is the diffusion coefficient value in D_2O/DMF-d_7 mixed solvent.

7. Disassembly of nanofibers by adding competitive guest (AD)

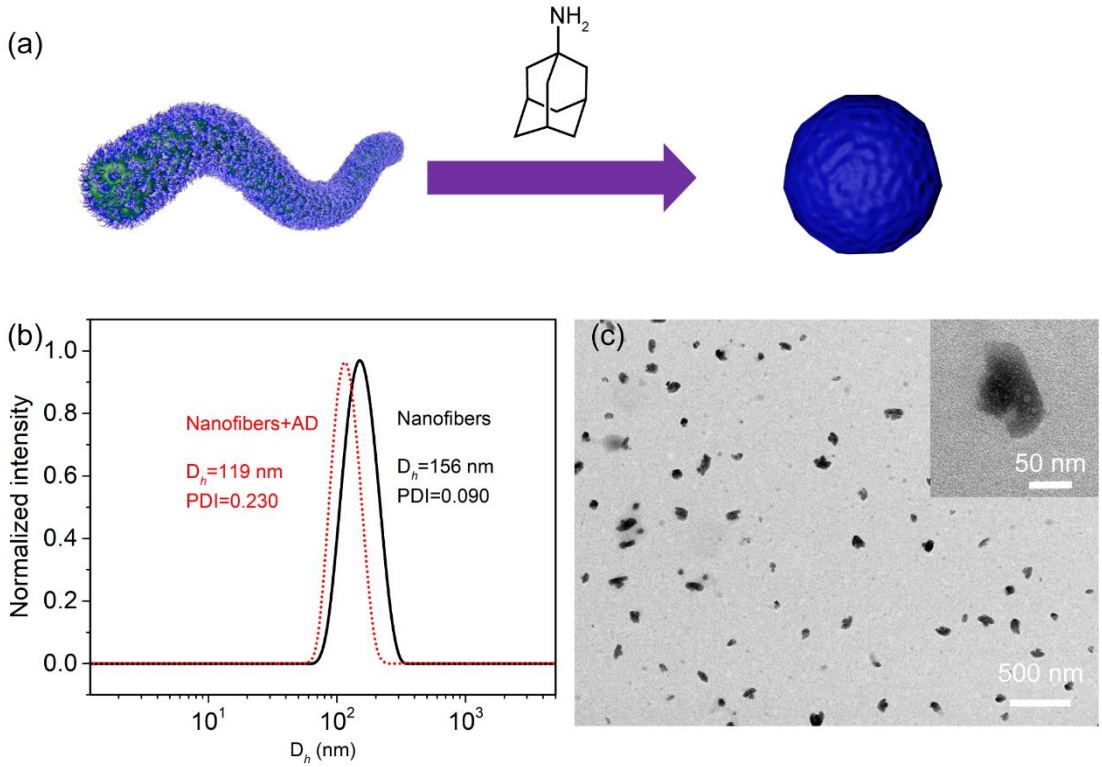


Fig S17 (a) Schematic model of morphology change when AD was added. (b) DLS curve of assemblies before and after adding AD molecules. (c) TEM images of assemblies after adding AD molecules.

8. UV-vis spectrum of CD-g-HPG-AZOs

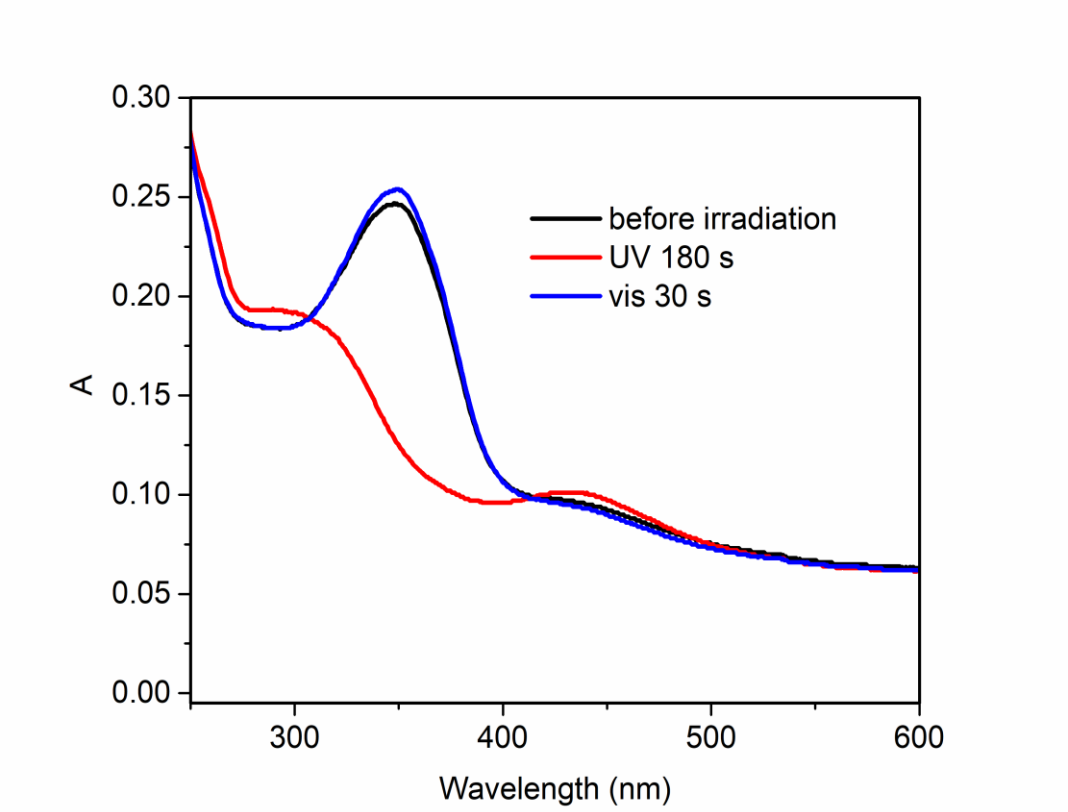


Fig. S18 UV-vis spectrum of assemblies before irradiation (black line), after UV (365 nm, 250 W) irradiation for 180 s (red line) and after vis (450 nm, 200 W) irradiation for 30 s (blue line). The absorbance of the trans-AZO attenuated after UV irradiation for 180 s, while it could be recovered after the further vis irradiation for 30 s.

9. References

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