Electronic Supplementary Information

Supramolecular Hydrogels Constructed by Red-Light-Responsive Host-Guest Interactions for Photo-Controlled Protein Release in Deep Tissue

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1. Synthesis

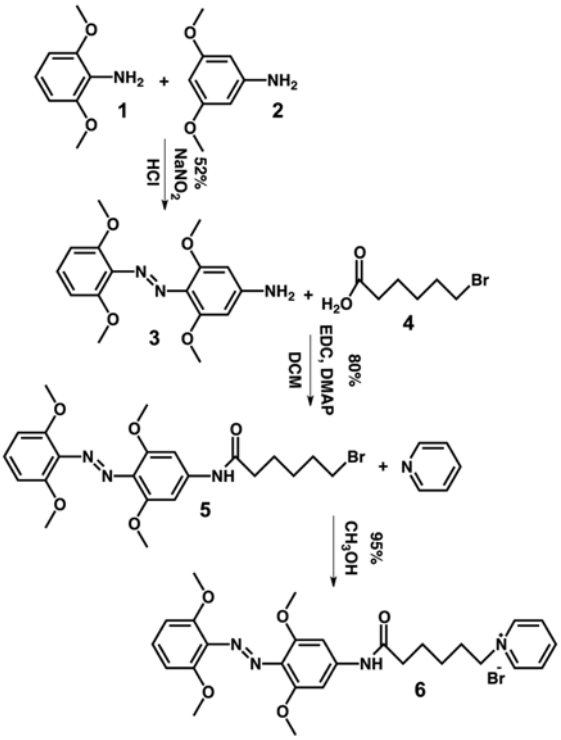

Figure S1. Route for the synthesis of 6 (mAzo-Py).

Synthesis of 3 (mAzo-NH$_2$). 2, 6-Dimethoxy-aniline (1 in Figure S1) (0.459 g, 3.00 mmol) was dissolved in the mixture of 0.56 mL of H_2O and 0.73 mL of HCl (37 *wt. %*). After the

solution was cooled to 0~5 ^{o}C, $NaNO_2$ (0.207 g, 3.00 mmol) in 2 mL H_2O was added slowly. The solution was stirred for 20 min and the temperature of the solution was kept at 0~5 ^{o}C. The diazonium salt was then slowly added to a suspension of 3.5-dimethoxy-aniline (0.459 g, 3.00 mmol) in 20 mL of H_2O at 0~5 ^{o}C. The pH of the mixture was adjusted to 8~9 by adding saturated sodium bicarbonate solution and the mixture was stirred overnight. The red solid was filtered and purified by chromatography using 1/1 methanol/ethyl acetate as eluent to get the product 3. Yield: 52%. ^{1}H-NMR (DMSO-*d6*, 250 MHz): δ=7.15 (t, J=8.3 Hz, 1H; Ar-H), δ=6.71 (d, J=8.4 Hz, 2H; Ar-H), δ=5.99 (s, 2H; Ar-NH_2), δ=5.94 (s, 2H; Ar-H), δ=3.67 (ds, 12H; -OCH_3). ^{13}C-NMR (DMSO-*d6*, 63 MHz): δ=156.1 (Ar-C), δ=153.4 (Ar-C), δ=151.0 (Ar-C), δ=126.6 (Ar-C), δ=123.4 (Ar-C), δ=105.3 (Ar-C), δ=89.9 (Ar-C), δ=55.9 (-OMe), δ=55.4 (-OMe). MS m/z=317.2.

Synthesis of 5. 4 (0.246g, 1.26 mmol), EDC (0.253g, 1.32 mmol) and DMAP (0.020g, 0.16 mmol) were dissolved into 60 mL of dichloromethane (DCM) and the mixture was stirred for 10 min. Then, 3 (0.420 g, 1.32 mmol) was added into the solution. The reaction was kept at room temperature for 24 h. The result solution was purified by chromatography using DCM as eluent to get the product 5. Yield: 80%. ^{1}H-NMR (DMSO-*d6*, 250MHz): δ=10.14 (s, 1H; -CO-NH-), δ=7.24 (t, J=8.3 Hz, 1H; Ar-H), δ=7.15 (s, 2H; Ar-H), δ=6.78 (d, J=8.4 Hz, 2H; Ar-H), δ=3.72 (ds, 12H; -OCH_3), δ=3.55 (t, J=6.7 Hz, 2H; -CH_2-Br), δ=2.37 (t, J=7.2 Hz, 2H; -CH_2-CO-), δ=1.95-1.35 (m, 6H; -CH_2-). ^{13}C-NMR (DMSO-*d6*, 63 MHz): δ=171.6 (-NH-CO-), δ=153.0 (Ar-C), δ=151.2 (Ar-C), δ=141.7 (Ar-C), δ=133.9 (Ar-C), δ=128.6 (Ar-C), δ=105.3 (Ar-C), δ=95.6 (Ar-C), δ=56.1 (-OMe), δ=55.9 (-OMe), δ=36.4 (-CH_2-CONH-), δ=35.0 (-CH_2-Br-), δ=32.0, 27.2, 24.1 (-CH_2-). MS m/z=493.1.

Synthesis of 6 (mAzo-Py). 5 (0.080g, 0.16 mmol) was dissolved in THF (20 mL). Then, pyridine (20 mL) was added. The reaction mixture was stirred overnight at 60 ^{o}C under Ar. The solvents were removed by rotated evaporation. The obtained red solid was dissolved in

methanol. The solution was added stepwise with stirring to plenty of petroleum ether, and the red precipitate was filtered and dried under vacuum to get the 6. Yield: 95%. ^{1}H-NMR (D_2O-*d2*, 250MHz): δ=8.82 (d, J=7.0 Hz, 2H; Py-H), δ=8.51 (t, J=7.8 Hz, 1H; Py-H), δ=8.03 (t, J=7.2 Hz, 2H; Py-H), δ=7.42 (t, J=8.5 Hz, 1H; Ar-H), δ=6.91 (s, 2H; Ar-H), δ=6.87 (d, J=8.5 Hz, 2H; Ar-H), δ=4.61 (t, J=7.2 Hz, 2H; -CH_2-Py), δ=3.85 (ds, 12H; -OCH_3), δ=2.41 (t, J=7.2 Hz, 2H; -CH_2-CO-), δ=2.12-1.30 (m, 6H; -CH_2-).

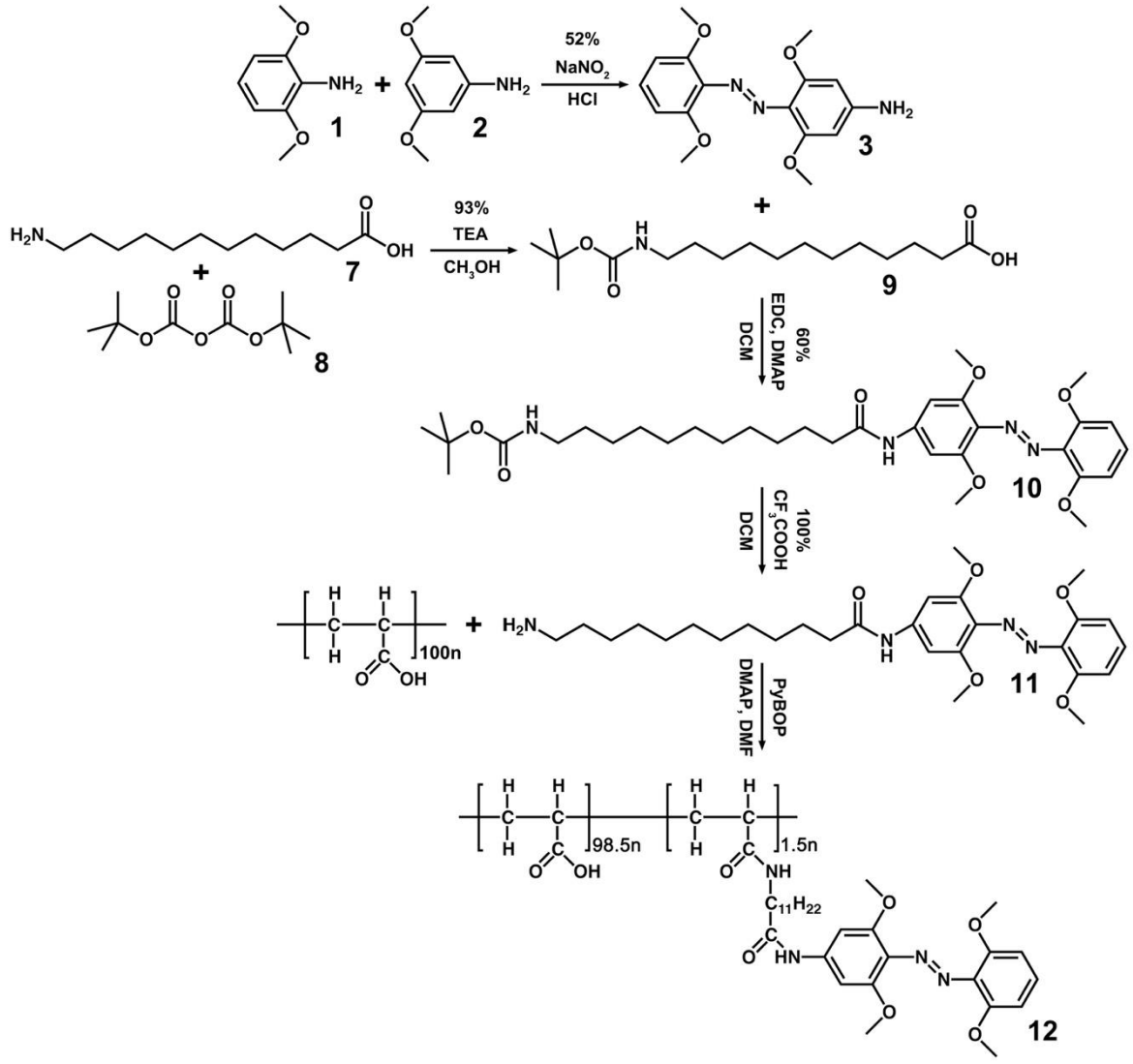

Figure S2. Route for the synthesis of 12 (PAA-mAzo).

Synthesis of 9. 12-Aminododecanoic acid (7 in Figure S2) (1.000 g, 4.65 mmol), di-tert-butyldicarbonate (8 in Figure S2) (1.010 g, 4.65 mmol) and triethylamine (TEA) (0.775 mL, 5.58 mmol) were dissolved into 15 mL of methanol. The reaction was kept 60 ^{o}C overnight. After removing the solvent using a rotary evaporator, the obtained solid was dissolved in ethyl acetate, and washed with HCl solution (0.25 M) twice. The white solid was then dried in a vacuum oven to obtain the product (9). Yield: 93%. ^{1}H-NMR: δ=11.98 (s, 1H; -COOH), δ=6.76 (t, J=5.6 Hz, 1H; -CO-NH-), δ=2.89 (q, J=6.5 Hz, 2H; -CH_2-), δ=2.19 (t, J=7.3 Hz, 2H; -CH_2-), δ=1.49 (t, J=7.1 Hz, 4H; -CH_2-), δ=1.38 (s, 9H; -CH_3), δ=1.24 (s, 14H; -CH_2-). ^{13}C-NMR: δ=174.4 (-COOH), δ=155.5 (-CO-NH-), δ=77.2 (Me_3-C-), δ=33.6, 29.4, 29.0, 28.9, 28.7, 28.5, 28.2, 26.2 (-CH_2-), δ=24.5 (-CH_3). MS m/z=315.2.

Synthesis of 10. Compound 9 (0.157 g, 0.50 mmol), EDC (0.096 g, 0.50 mmol) and DMAP (0.020 g, 0.16 mmol) were dissolved in 10 mL of DCM at room temperature. The mixture was stirred for 20 min. Then, 3 (0.159 g, 0.50 mmol) was added into the solution. The reaction was kept at room temperature for 24 h. Then, the result solution was purified by chromatography using 1/2 acetone/ethyl acetate as eluent to get the product 10. Yield: 60%. ^{1}H-NMR: δ=7.29 (t, J=8.3 Hz, 1H; Ar-H), δ=7.18 (s, 3H; Ar-H and O=C-NH-Ar), δ=6.90-6.70 (m, 3H; Ar-H and O=C-NH-C), δ=3.75 (d, J=2.0 Hz, 12H; -OCH_3), δ=2.92 (q, J=6.5 Hz, 2H; -CH_2-), δ=2.32 (t, J=7.3 Hz, 2H; -CH_2-), δ=1.66 (t, J=7.1 Hz, 4H; -CH_2-), δ=1.41 (s, 9H; -CH_3), δ=1.30 (s, 14H; -CH_2-). ^{13}C-NMR: δ=179.8 (-CO-NH-Ar), δ=156.6 (Ar-C), δ=156.1 (-CO-NH-), δ=151.1 (Ar-C), δ=149.4 (Ar-C), δ=128.4 (Ar-C), δ=125.5 (Ar-C), δ=104.1 (Ar-C), δ=91.5 (Ar-C), δ=71.0 (Me_3-C-), δ=55.1 (-OMe), δ=54.8 (-OMe), δ=33.3, 29.4, 29.0, 28.9, 28.8, 28.7, 28.2 25.3 (-CH_2-), δ=24.4 (-CH_3). MS m/z=614.7.

Synthesis of 11. Compound 10 (0.179 mg, 0.30 mmol) and 3 mL of CF_3COOH were dissolved in 10 mL of DCM. The mixture was stirred at room temperature for 1 h. The resulted solution was washed using saturated Na_2CO_3 solution. Then, the residual DCM was

removed using a rotary evaporator. The dark red solid was further washed by water for several times to obtain the Compound 11. Yield: >99%. ^{1}H-NMR: δ=7.29 (t, J=8.3 Hz, 1H; Ar-H), δ=7.18 (s, 2H; Ar-H), δ=6.80 (d, J=8.4 Hz, 2H; Ar-H), δ=6.17 (s, 2H; -NH_2), δ=3.74 (d, J=2.1 Hz, 12 H; -OCH_3), δ=2.92 (q, J=6.5 Hz, 2H; -CH_2-), δ=2.37 (t, J=7.3 Hz, 2H; -CH_2-), δ=1.66 (t, J=7.1 Hz, 4H; -CH_2-), δ=1.88-1.48 (m, 4H; -CH_2-), δ=1.30 (s, 14H; -CH_2-). ^{13}C-NMR: δ=179.6 (-CO-NH-Ar), δ=152.9 (Ar-C), δ=151.1 (Ar-C), δ=145.1 (Ar-C), δ=127.2 (Ar-C), δ=122.4 (Ar-C), δ=105.3 (Ar-C), δ=95.6 (Ar-C), δ=56.0 (-OCH_3), δ=55.8 (-OCH_3), δ=33.3, 29.7, 29.0, 28.9, 28.8, 28.7, 28.2, 26.1 (-CH_2-). MS m/z=515.6.

Synthesis of 12 (PAA-mAzo). To prepare PAA-mAzo (12 in Figure S2), PAA (0.288 g, 4.00 mmol of the repeat unit), PyBOP (0.062 g, 0.12 mmol), DMAP (0.015 g, 0.12 mmol) and 11 (0.062 g, 0.12 mmol) were dissolved in 20 mL of DMF at room temperature. The reaction was kept at room temperature for 24 h. The product was purified by dialysis using a dialysis tube (cut-off molecular weight: 7000). The grafting density determined by ^{1}H-NMR is 1.5%.

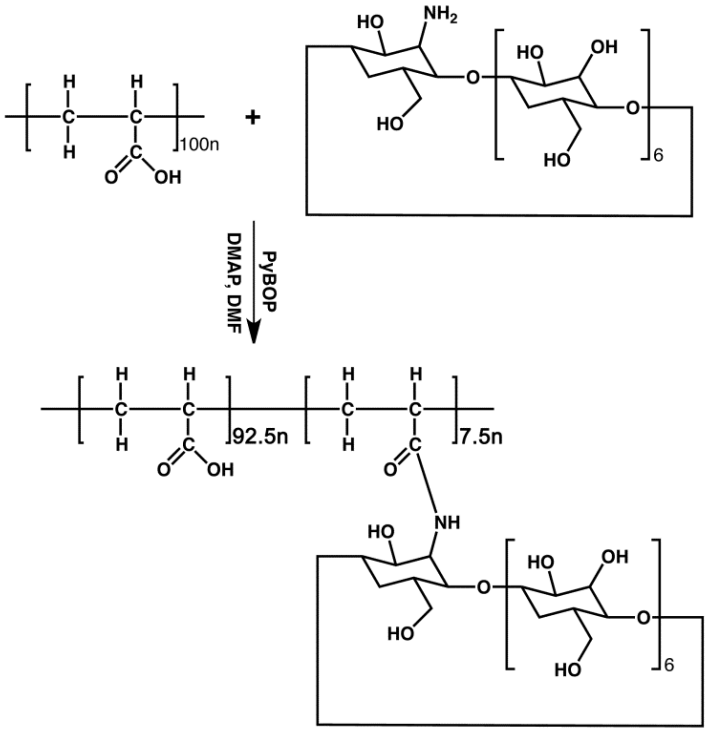

Figure S3. Route for the synthesis of PAA-β-CD.

Synthesis of PAA-β-CD. PAA (0.288 g, 4.00 mmol of the repeat unit), PyBOP (0.208 g, 0.40 mmol), DMAP (0.015 g, 0.12 mmol) and NH_2-β-CD (0.453 g, 0.40 mmol) were dissolved into 20 mL of DMF. The reaction mixture was kept at room temperature for 24 h. The product was purified by dialysis using a dialysis tube (cut-off molecular weight: 7000). The grafting density determined by ^{1}H-NMR is 7.5%.

2. Photoisomerization of mAzo and host-guest interactions between mAzo and β-CD

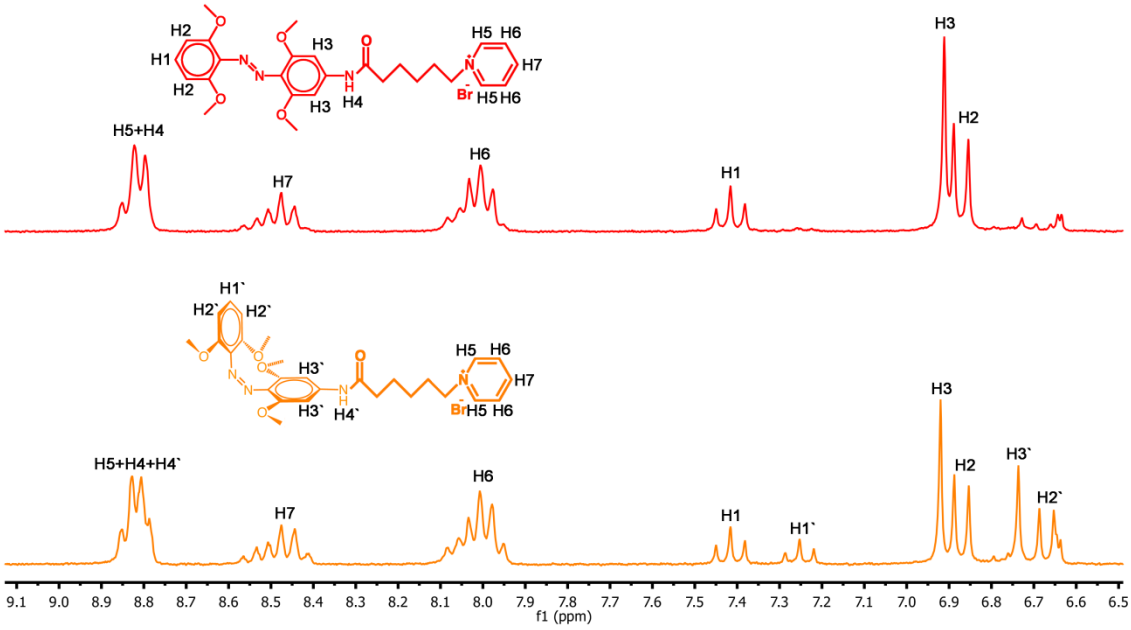

Figure S4. ^{1}H-NMR (250 MHz, D_2O, 298K) spectra of mAzo-Py before (red) and after (orange) 625-nm light irradiation. Photoisomerization of mAzo-Py can be induced by red light.

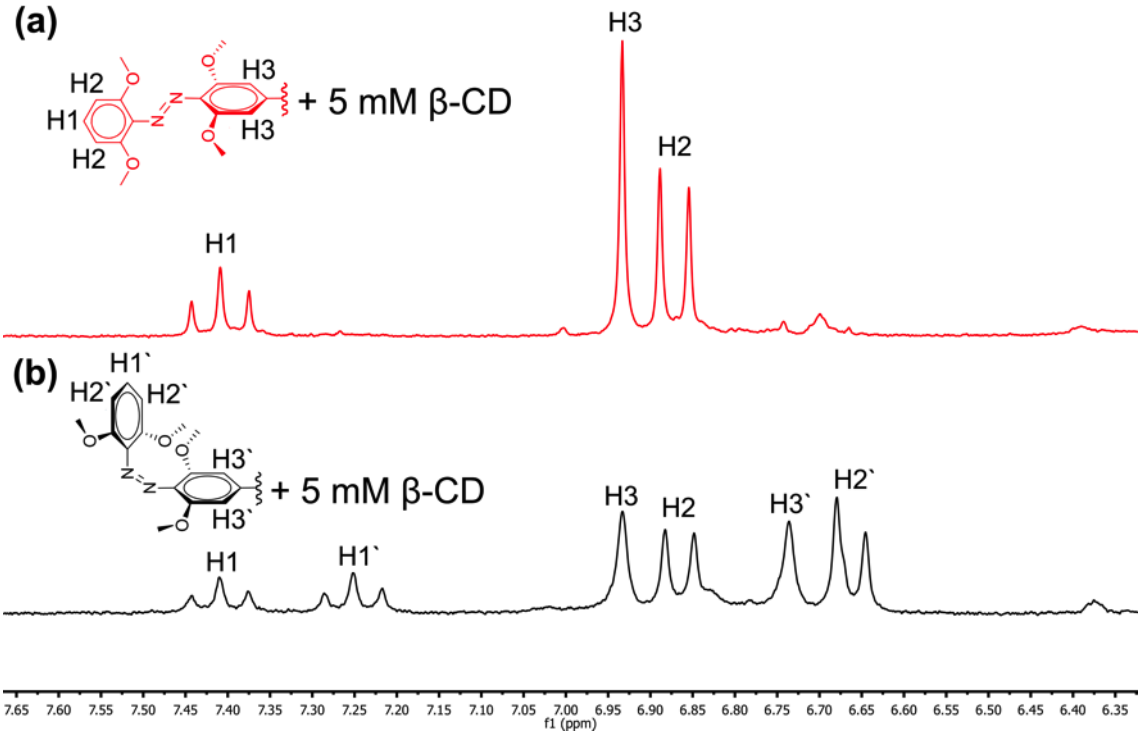

Figure S5. ^{1}H-NMR (250 MHz, D_2O, 298K) spectra of mAzo-Py/β-CD (1:5) before (a) and after (b) 625-nm light irradiation. Red light can induce the photoisomerization of mAzo-Py in the presence of β-CD. The concentration of mAzo-Py is 1 mM.

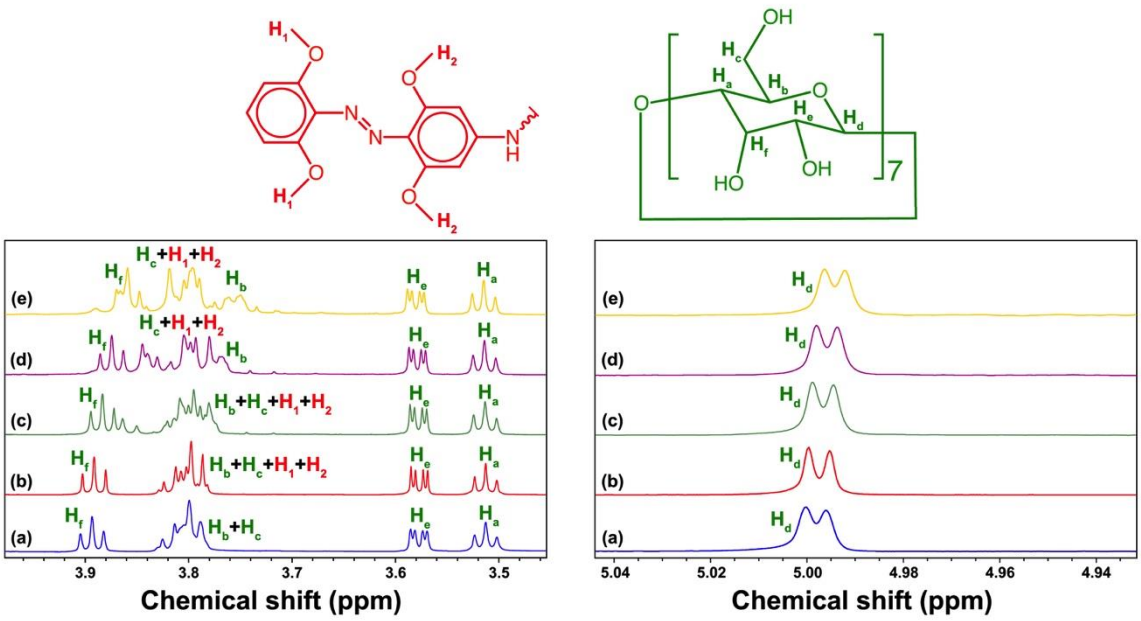

Figure S6. ^{1}H-NMR (700MHz, 298K) spectra of *trans* mAzo-Py and its mixtures with β-CD in D_2O: *trans* mAzo-Py:β-CD=0:1 (a), *trans* mAzo-Py:β-CD=0.5:1 (b), *trans* mAzo-Py:β-CD=1:1 (c), *trans* mAzo-Py:β-CD=2:1 (d) and *trans* mAzo-Py:β-CD=4:1 (e). The concentration of β-CD is 1.67 mg/mL.

H_b, H_d and H_f are protons inside the cavity of β-CD. As the ratio of *trans* mAzo-Py in mAzo-Py/β-CD increases, the signals of H_b, H_d and H_f shift, indicating *trans* mAzo-Py enters the cavity of β-CD. The other protons are outside or at the edge of the cavity of β-CD. As the ratio of *trans* mAzo-Py in mAzo-Py/β-CD increases, the signals of the outer protons of β-CD have no or only slight shift. The result suggests that *trans* mAzo has stronger interactions with inner protons of β-CD. Thus, this experiment confirms the host-guest interaction between *trans* mAzo and β-CD.

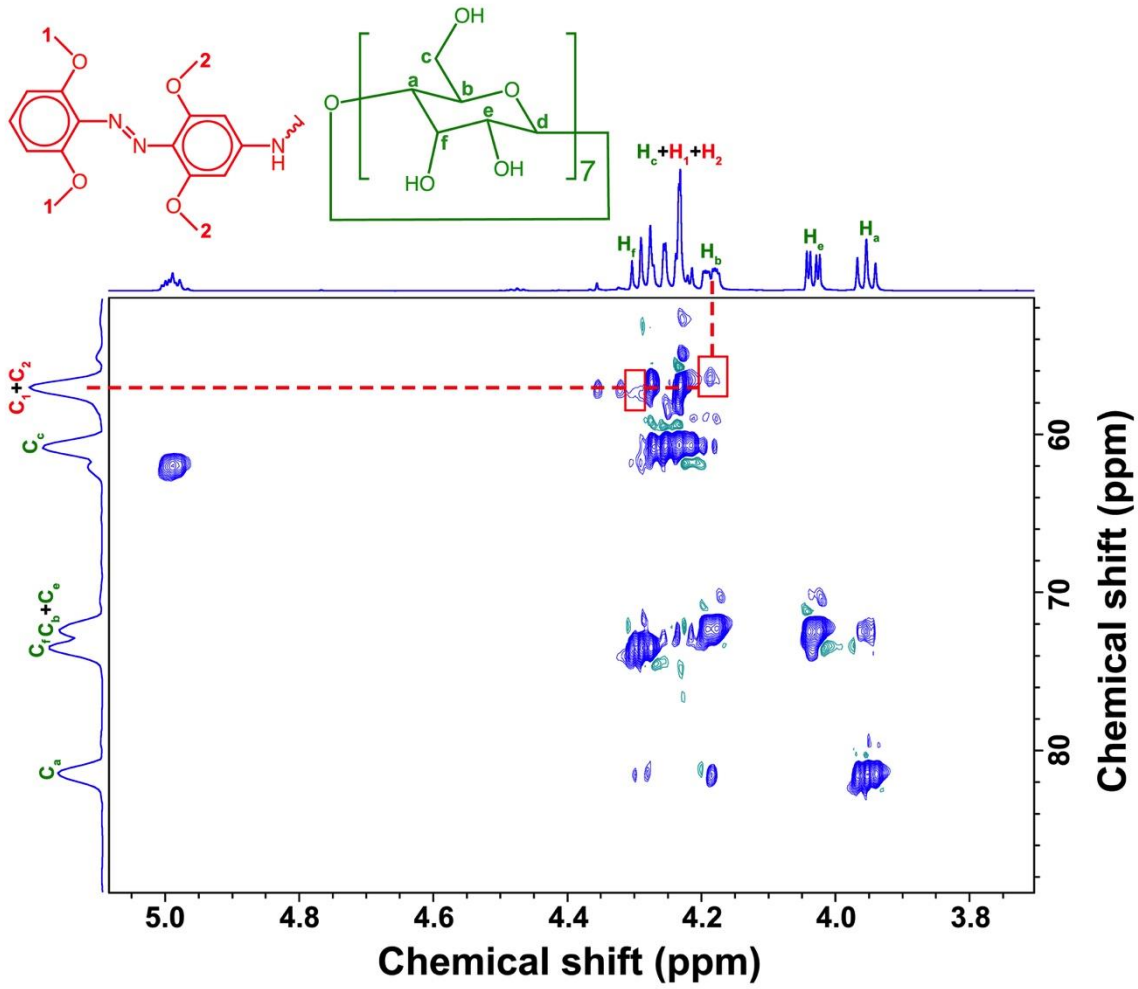

Figure S7. 2D HSQC-NOESY spectrum (700MHz, 298K) of *trans* mAzo-Py/β-CD (2/1 in molar ratio) in D_2O. The concentration of β-CD is 1.67 mg/mL.

The correlation peaks between the carbons of *trans* mAzo-Py (C_1 and C_2) and the inner protons of β-CD (H_b and H_f) shows the host-guest interaction between *trans* mAzo-Py and β-CD. Additionally, there is no correlation peak between the carbons of *trans* mAzo-Py (C_1 and C_2) and the outer protons of β-CD (H_a and H_e). Thus, *trans* mAzo strongly interact with the inner protons of β-CD. These results indicate that *trans* mAzo-Py enters the cavity of β-CD.

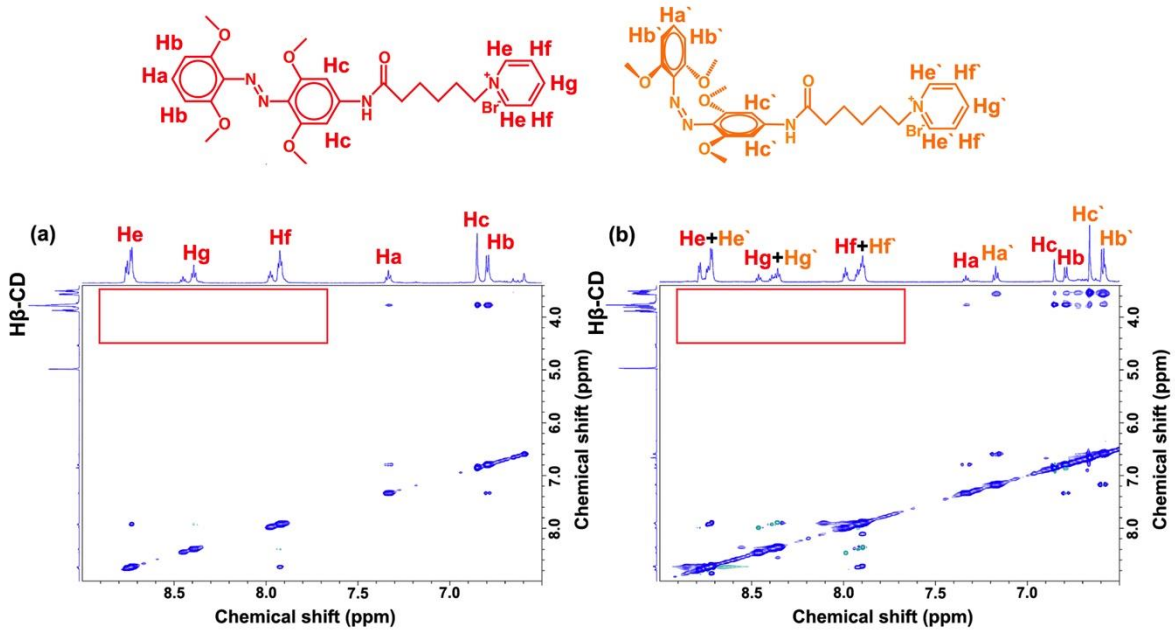

Figure S8. 2D NOESY spectra (700 MHz in D_2O at 298 K) of *trans* mAzo-Py/β-CD (a) and *cis* mAzo-Py/β-CD (b). No corresponding proton was found between pyridine and β-CD (red rectangles) in both of the spectra. The concentration of β-CD is 1.67 mg/mL. The molar ratio of mAzo-Py/β-CD is 1/1.

3. Association constants between mAzo-Py and CDs

^{1}HNMR was used to measure the association constants (K_a) between mAzo-Py and α-CD or β-CD. mAzo-Py and CDs were dissolved in D_2O. The concentration of mAzo-Py was kept as 1.0 mM. The concentrations of CDs were 5, 10, 15 and 20 mM, respectively. ^{1}HNMR spectra of mAzo-Py/CD mixtures in D_2O were measured. Due to the host-guest interaction between mAzo and CDs, the signals of protons will shift. A modified Benesi-Hildebrand equation[2] was used for the calculation of the association constants between mAzo-Py and CDs.

$$\frac{1}{\Delta\delta_{obs}} = \frac{1}{\Delta\delta} \cdot \frac{1}{K_a} \cdot \frac{1}{C_{CD}} + \frac{1}{\Delta\delta} \qquad \text{(S1)}$$

where $\Delta\delta_{obs}$ is the observed shifts of the peaks; K_a is the association constant; $\Delta\delta$ is a constant correlated to the concentration of the mAzo-Py; C_{CD} is the concentration of the CD.

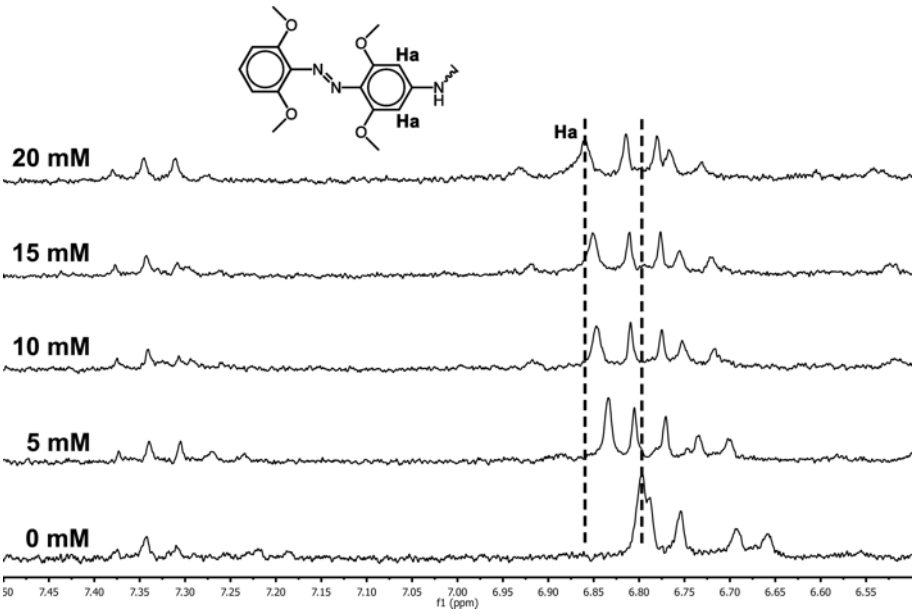

Figure S9. ^{1}H-NMR (250 MHz, 298K) spectra of *trans* mAzo-Py with different concentrations of α-CD (0, 5, 10, 15 and 20 mM) in D_2O. The concentration of mAzo-Py is 1 mM.

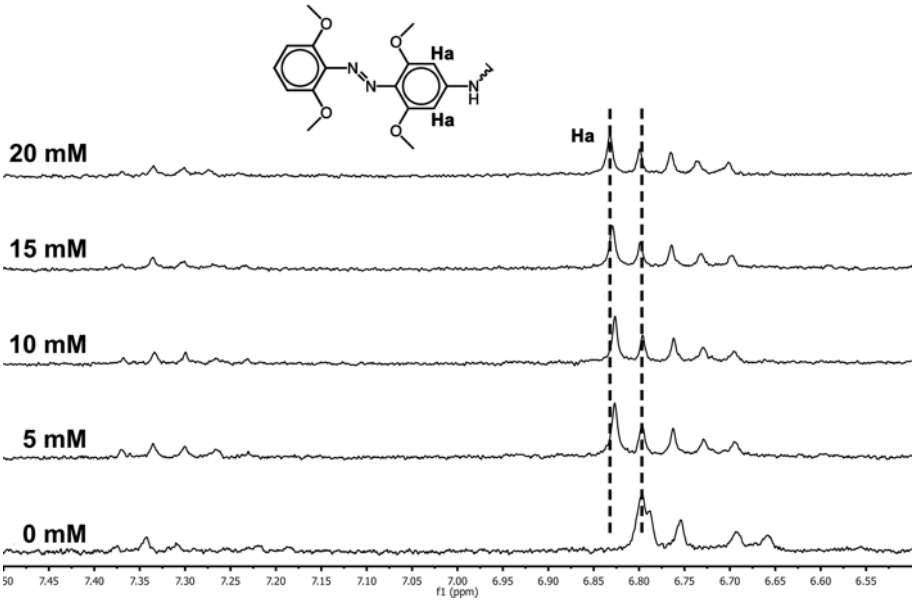

Figure S10. ^{1}H-NMR (250 MHz, 298K) spectra of *trans* mAzo-Py with different concentrations of β-CD (0, 5, 10, 15 and 20 mM) in D_2O. The concentration of mAzo-Py is 1 mM.

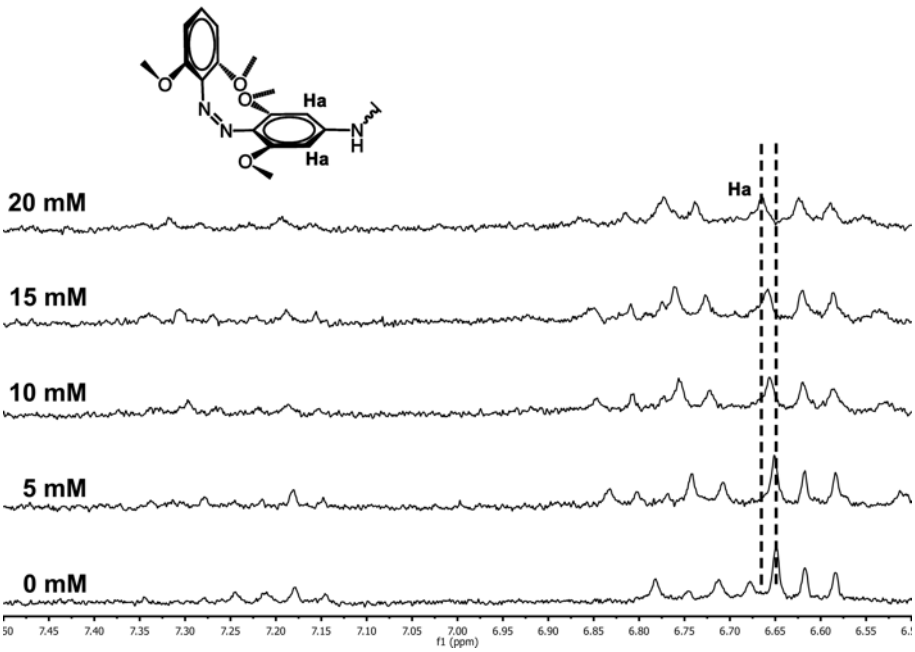

Figure S11. ^{1}H-NMR (250 MHz, 298K) spectra of *cis* mAzo-Py with different concentrations of α-CD (0, 5, 10, 15 and 20 mM) in D_2O. The concentration of mAzo-Py is 1 mM.

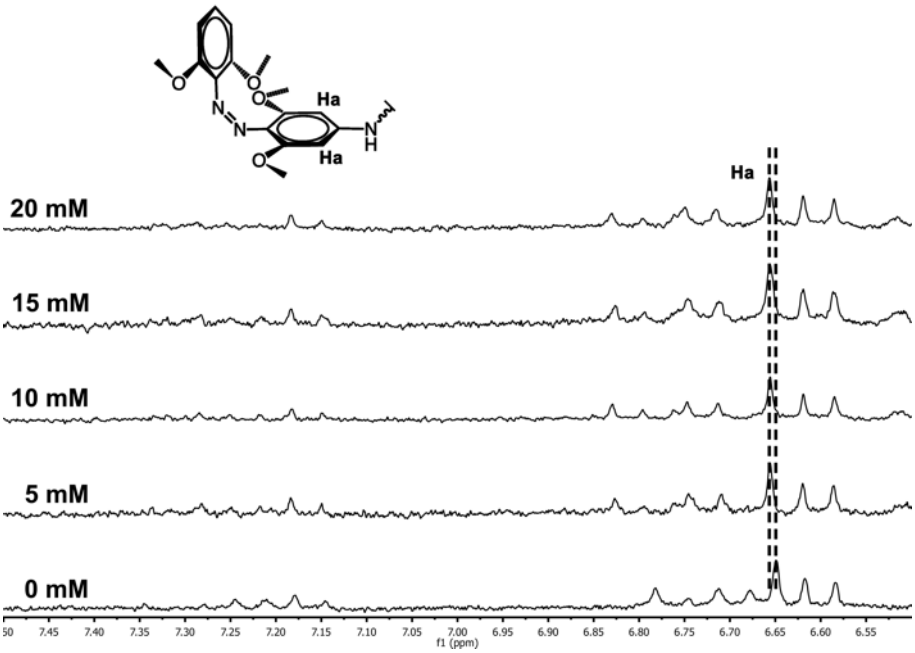

Figure S12. ^{1}H-NMR (250 MHz, 298K) spectra of *cis* mAzo-Py with different concentrations of β-CD (0, 5, 10, 15 and 20 mM) in D_2O. The concentration of mAzo-Py is 1 mM.

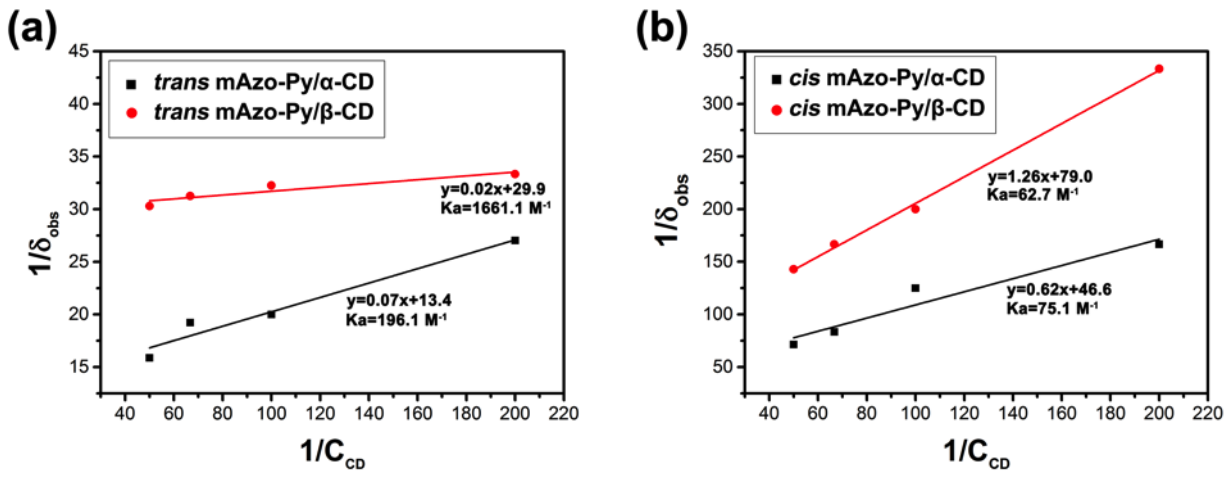

Figure S13. (a) Association constants (K_a) between *trans* mAzo-Py and CDs. The data for calculating the association constants were obtained from Figure S9 and S10. (b) Association constants (K_a) between *cis* mAzo-Py and CDs. The data for calculating the association constants were obtained from Figure S11 and S12. Equation S1 was used to calculate the association constants.

4. Photoresponse of PAA-mAzo

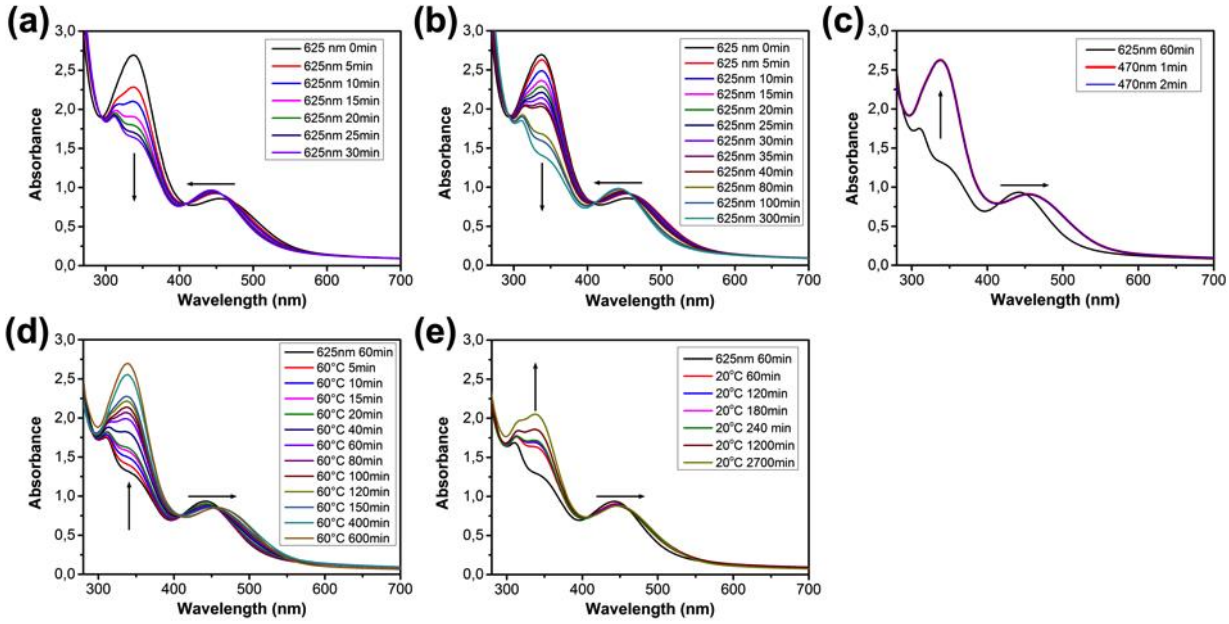

Figure S14. UV/Vis absorption spectra of *trans* PAA-mAzo after 60 mW/cm^2 (a) and 15 mW/cm^2 (b) 625-nm red light irradiation for different time. (c) UV/Vis absorption spectra of *cis* PAA-mAzo after 60 mW/cm^2 470-nm blue light irradiation for different time. The *cis*-to-*trans* isomerization was completed in 1 min. UV/Vis absorption spectra of *cis* PAA-mAzo kept at 60 ℃ (d) and 20 ℃ (e) for different time. Cis-to-trans isomerization could be induced thermally. The half-lives of *cis* isomer at 60 ℃ and 20 ℃ are 60 min and 34 h, respectively.

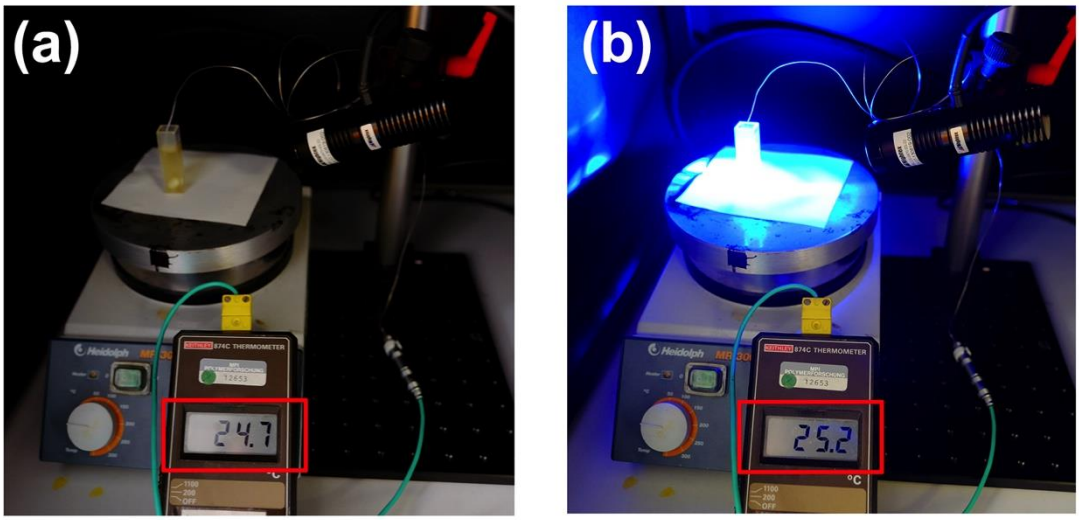

Figure S15. Photothermal effect of 470 nm blue light measured by a thermometer. The temperature of the PAA-mAzo solution (1.43 mg/mL in H_2O) before irradiation is 24.7 ^oC (a), and increased to 25.2 ^oC after blue light irradiation (60 mW/cm^2, 10 min). The measurement demonstrates that the cis-to-trans isomerization under the blue light is a photochemical process.

5. Association constants between PAA-mAzo and CDs

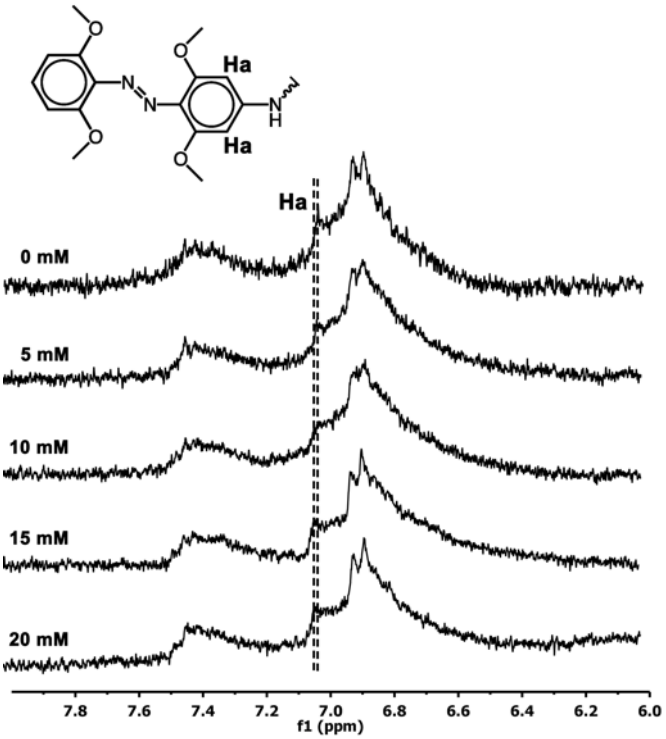

Figure S16. ^{1}H-NMR (250 MHz, 298K) spectra of *trans* PAA-mAzo (1 mM of mAzo groups) with different concentrations (0, 5, 10, 15, 20 mM) of α-CD in D_2O.

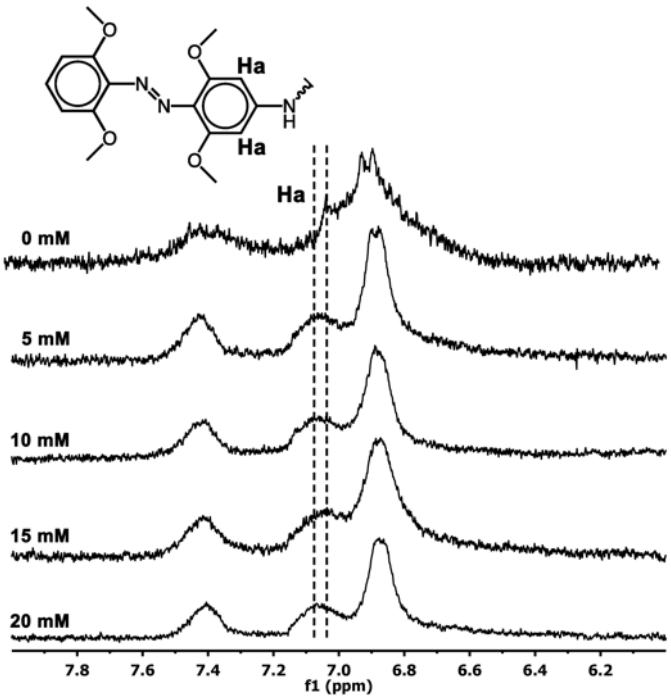

Figure S17. ^{1}H-NMR (250 MHz, 298K) spectra of *trans* PAA-mAzo (1 mM of mAzo group) with different concentrations (0, 5, 10, 15, 20 mM) of β-CD in D_2O.

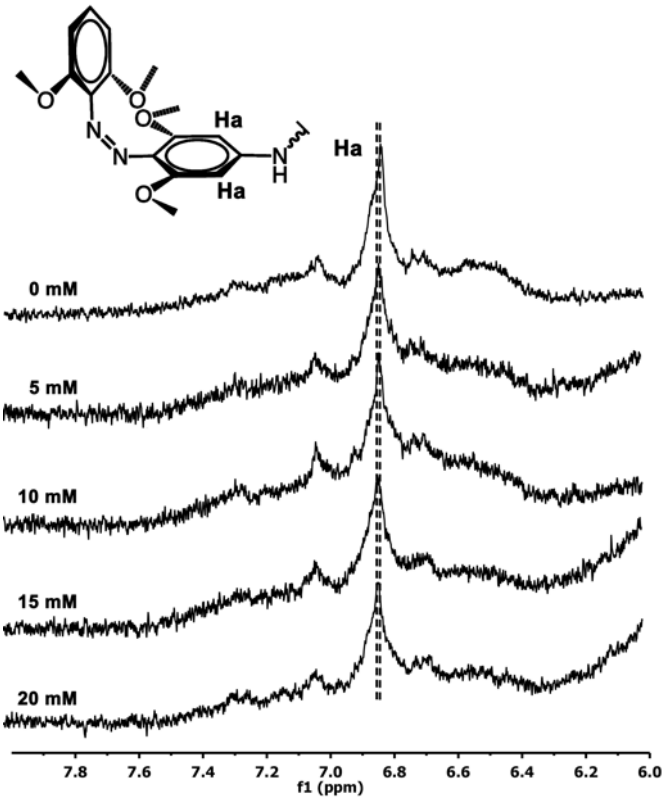

Figure S18. ^{1}H-NMR (250 MHz, 298K) spectra of *cis* PAA-mAzo (1 mM of mAzo group) with different concentrations (0, 5, 10, 15, 20 mM) of α-CD in D_2O.

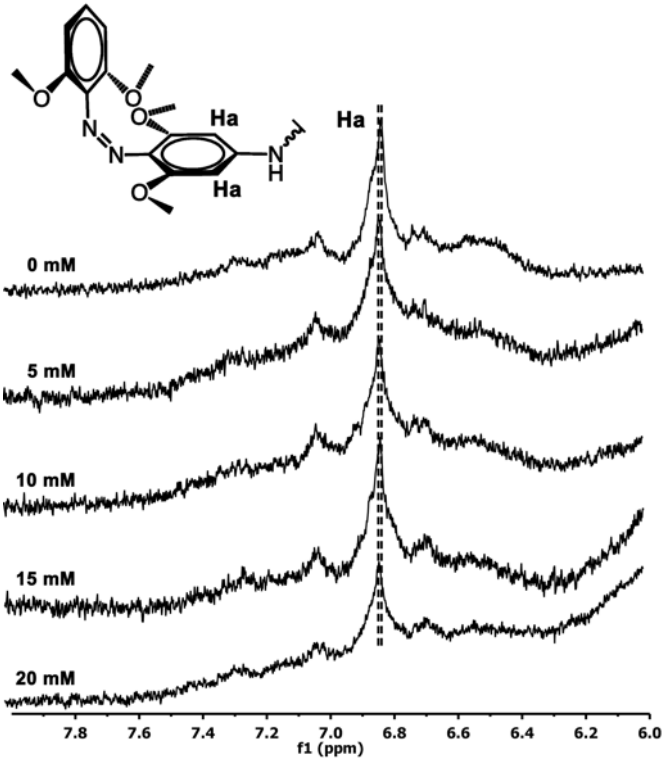

Figure S19. ^{1}H-NMR (250 MHz, 298K) spectra of *cis* PAA-mAzo (1 mM of mAzo group) with different concentrations (0, 5, 10, 15, 20 mM) of β-CD in D_2O.

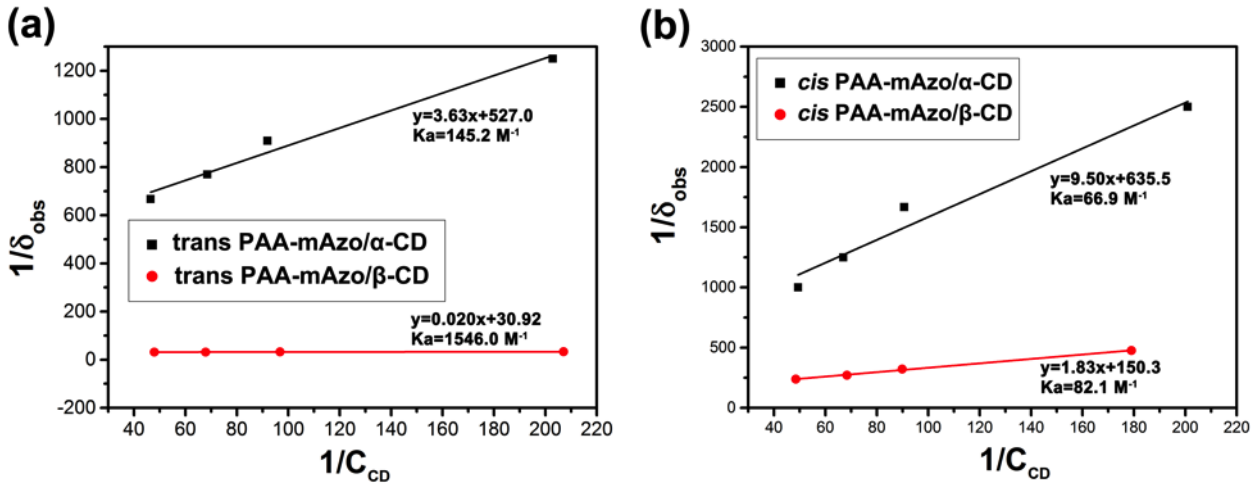

Figure S20. (a) Association constants (K_a) between *trans* PAA-mAzo and CDs. The data for calculating the association constants were taken from Figure S16 and S17. (b) Association constants (K_a) between *cis* PAA-mAzo and CDs. The data for calculating the association constants were taken from Figure S18 and S19. Equation S1 was used to calculate the association constants. The mAzo/CD association constants measured using PAA-mAzo is similar to those measured using mAzo-Py (Figure S13).

6. Association constants between PAA-Azo and CDs

PAA-Azo is used for control experiments (Figure S21). It contains normal azobenzene groups. In our previous work, we synthesized PAA-Azo and studied the host-guest interactions between PAA-Azo and CDs.[3]

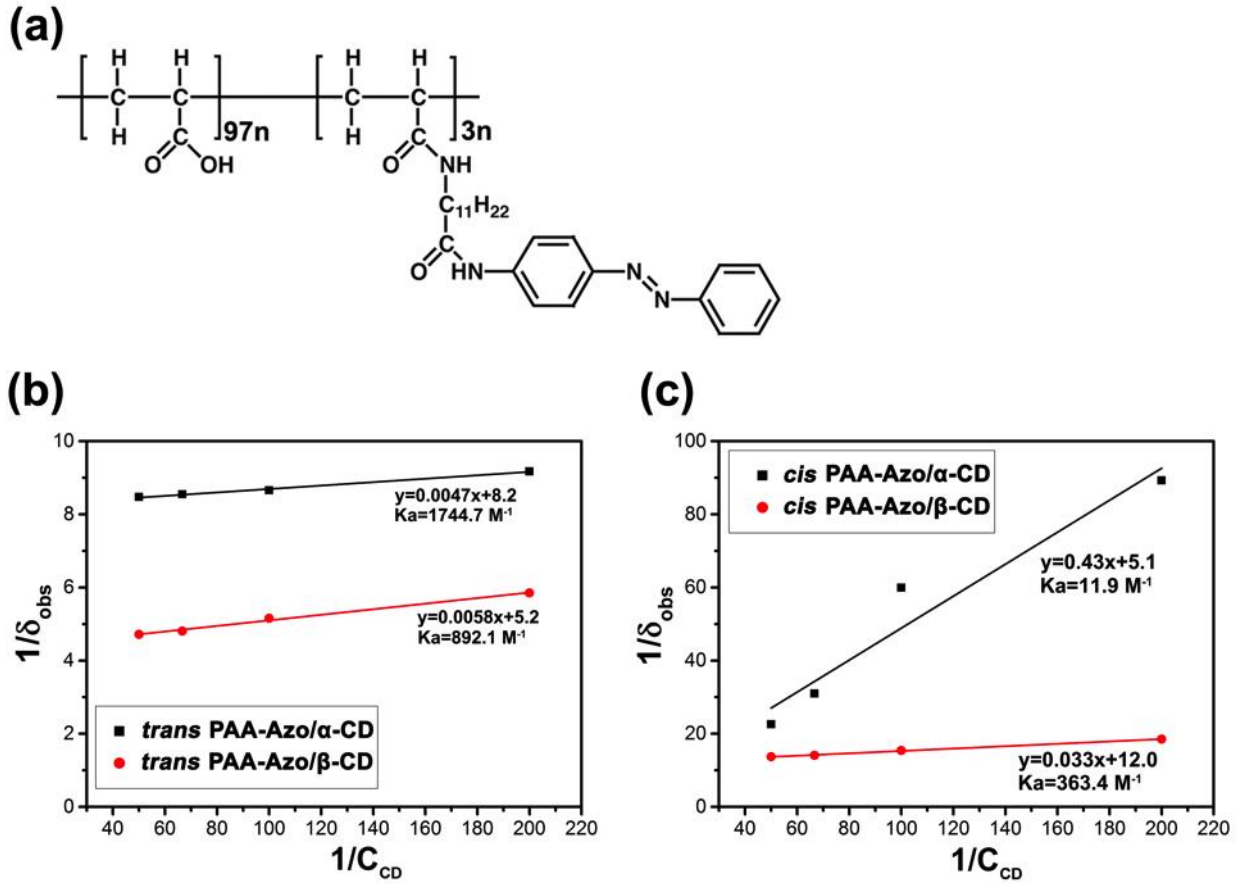

Figure S21. (a) Chemical structure of PAA-Azo. (b) Association constants between *trans* PAA-Azo and CDs. (c) Association constants between *cis* PAA-Azo and CDs.

7. Red-light-responsive hydrogels

Hydrogel preparation. Solutions of PAA-mAzo (0.27 mL, 3 wt% in PBS buffer, pH 8) and PAA-β-CD (0.1 mL, 3 wt% in PBS buffer, pH 8) were mixed in the dark. The supramolecular hydrogel was formed spontaneously within 10 min.

Loading BSA into the hydrogel and red-light-controlled BSA release. As shown in Figure S29, the BSA loaded supramolecular hydrogel was prepared at the bottom of the cuvette. BSA was dissolved in PBS buffer (pH=8) with the concentration of 1 mg/mL. PAA-mAzo and PAA-β-CD solutions were prepared as mentioned above. Then, 135 μL of PAA-mAzo, 50 μL of PAA-β-CD and 15 μL of BSA solutions were mixed at the bottom of the cuvette in the dark. BSA-loaded supramolecular hydrogel was formed spontaneously within 10 min. The BSA loaded hydrogel was washed with PBS buffer for several times to remove non-trapped proteins.

For the photo-controlled protein release, 3.5 mL of PBS solution (pH=8) was added into the cuvette. The hydrogel was irradiated by the LEDs from the side of the cuvette (Figure S22 (a)). The concentration of the released BSA was monitored using fluorescence spectroscopy (Figure S22 (b)-(f)). The red-light-controlled BSA release in deep tissue was investigated by placing a piece of pork tissue between the light source and the cuvette (Figure S23).

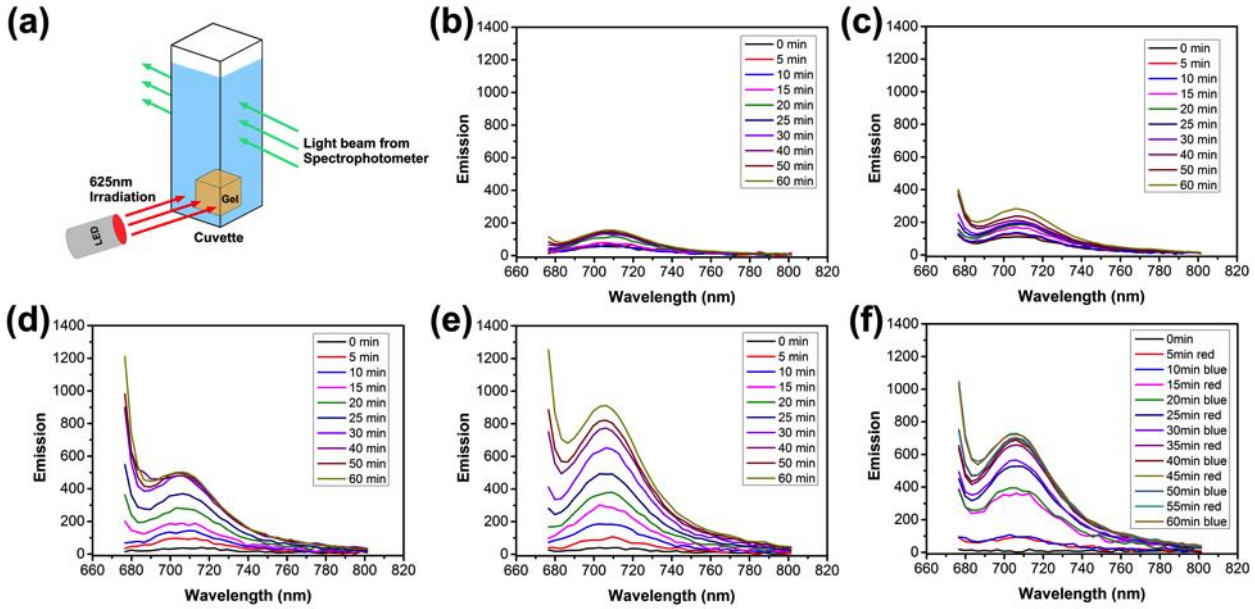

Figure S22. (a) Schematic illustration of red-light-induced protein release. Fluorescence spectra (λ_{ex} = 640 nm) of the BSA-loaded hydrogel in the dark (b), under 60 mW/cm^2 470-nm blue light (c), 15 mW/cm^2 625-nm red light (d) and 60 mW/cm^2 625-nm red light (e) irradiation for different time. The amount of released BSA is shown in Figure 3c in the main manuscript. (f) Fluorescence spectra (λ_{ex} = 640 nm) of the BSA-loaded hydrogel under alternate 625-nm red light (60 mW/cm^2) and 470-nm blue light (60 mW/cm^2) irradiation for different time. The amount of released BSA is shown in Figure 3d in the main manuscript.

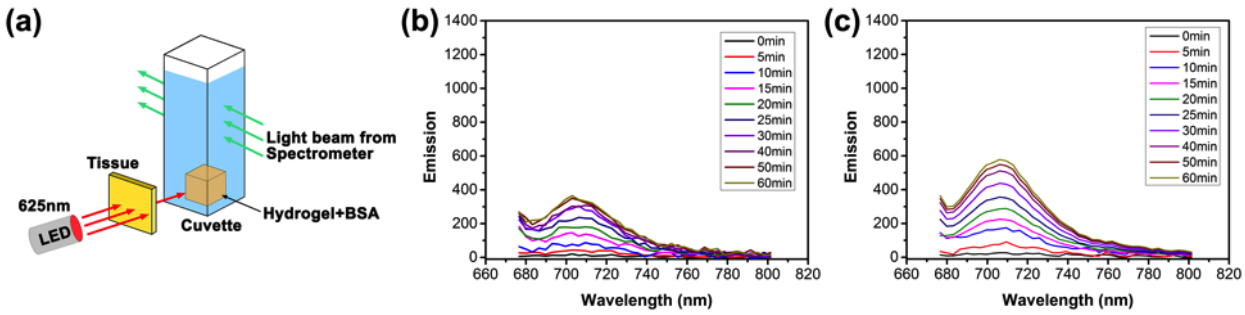

Figure S23. (a) Schematic illustration of red-light-induced BSA release in deep tissue. Fluorescence spectra (λ_{ex} = 640 nm) of the BSA-loaded hydrogel under 15 mW/cm^2 (b) and 60 mW/cm^2 (c) 625-nm red light irradiation for different time when a piece of tissue was placed between the hydrogel and the LED. The amount of released BSA is shown in Figure 3f in the main manuscript.

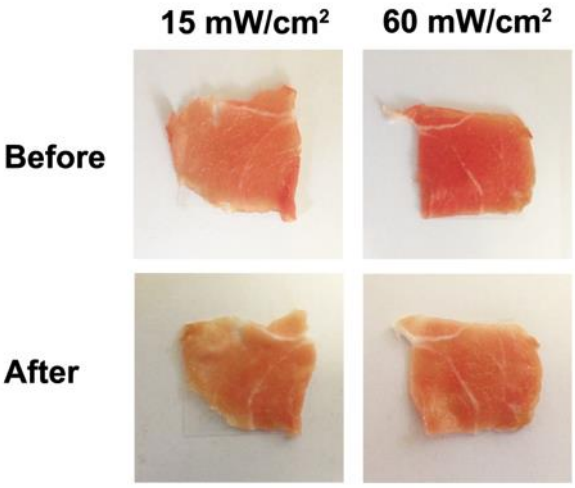

Figure S24. Tissue before and after red light irradiation for 60 min. The used light intensity is much lower than the maximum permissible exposure of skin at 625 nm (200 mW/cm^2). No burn wound was observed. The obvious burn wound is reported by our former researches.[4]

8. NMR spectra of new compounds

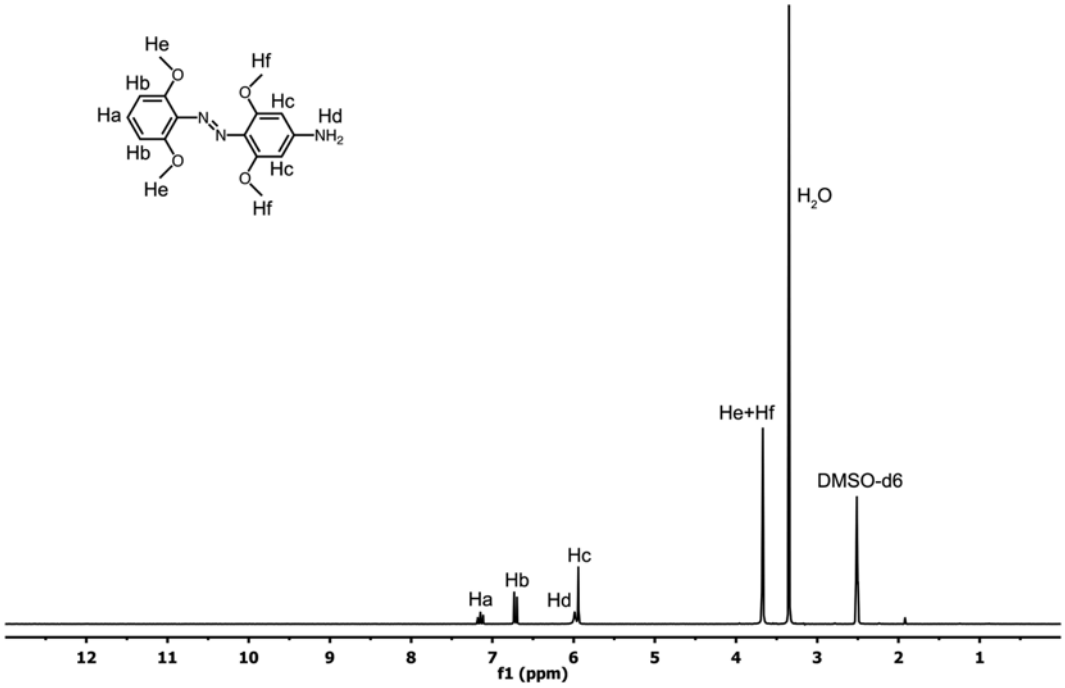

Figure S25. ^{1}H-NMR (250 MHz, 298K, DMSO-d_6) spectrum of 3.

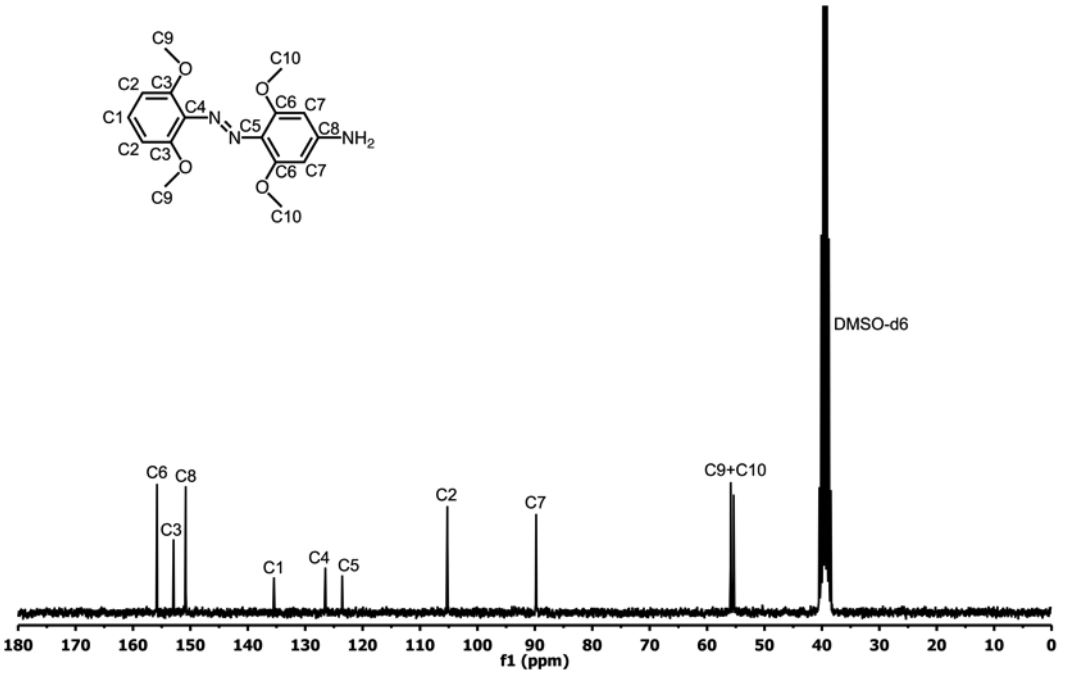

Figure S26. ^{13}C-NMR (63 MHz, 298K, DMSO-d_6) spectrum of 3.

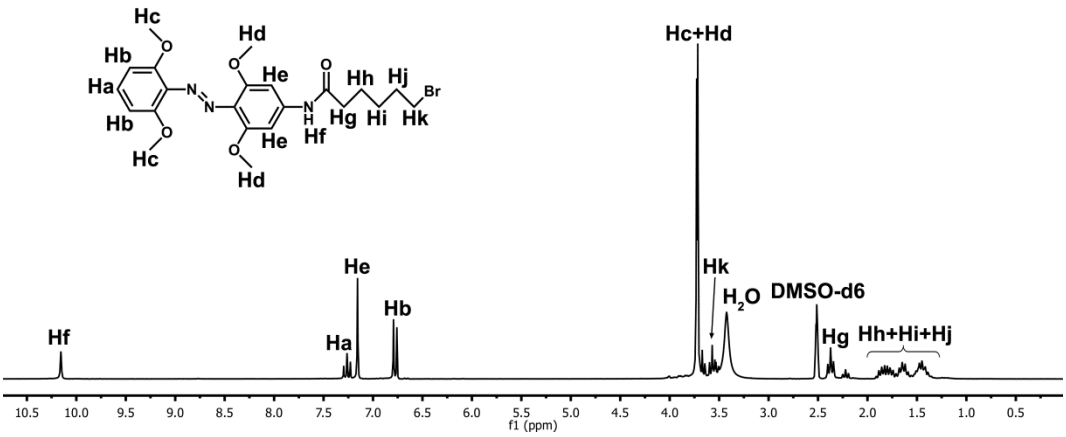

Figure S27. ^{1}H-NMR (250 MHz, 298K, DMSO-d_6) spectrum of 5.

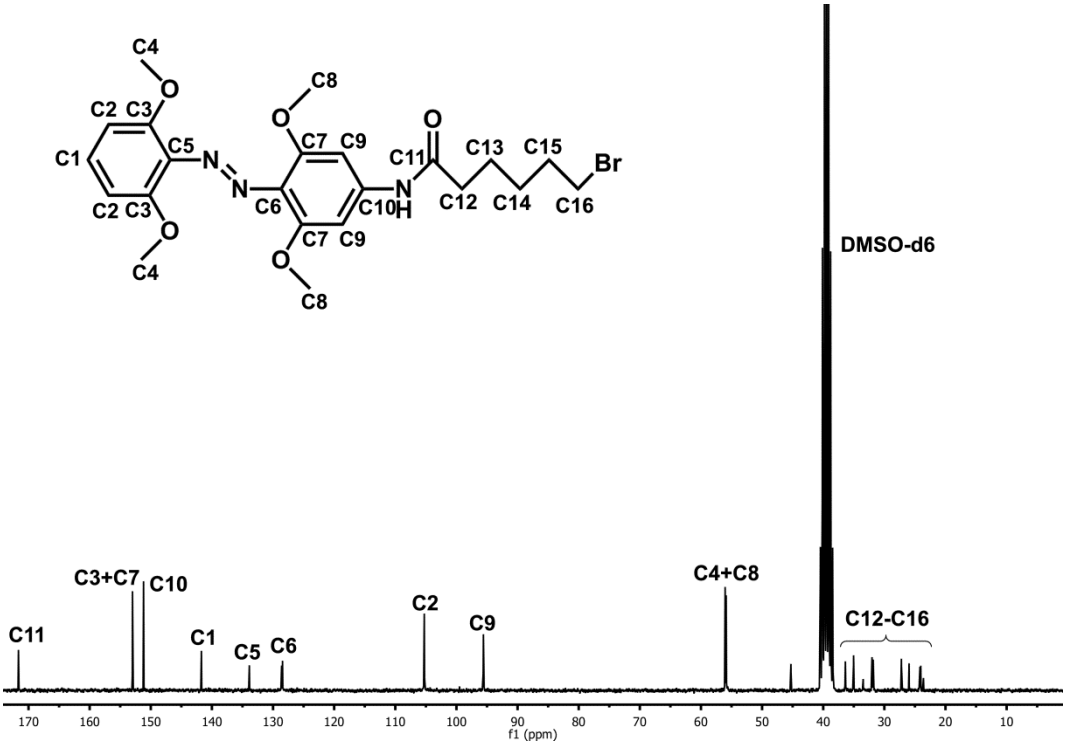

Figure S28. ^{13}C-NMR (63 MHz, 298K, DMSO-d_6) spectrum of 5.

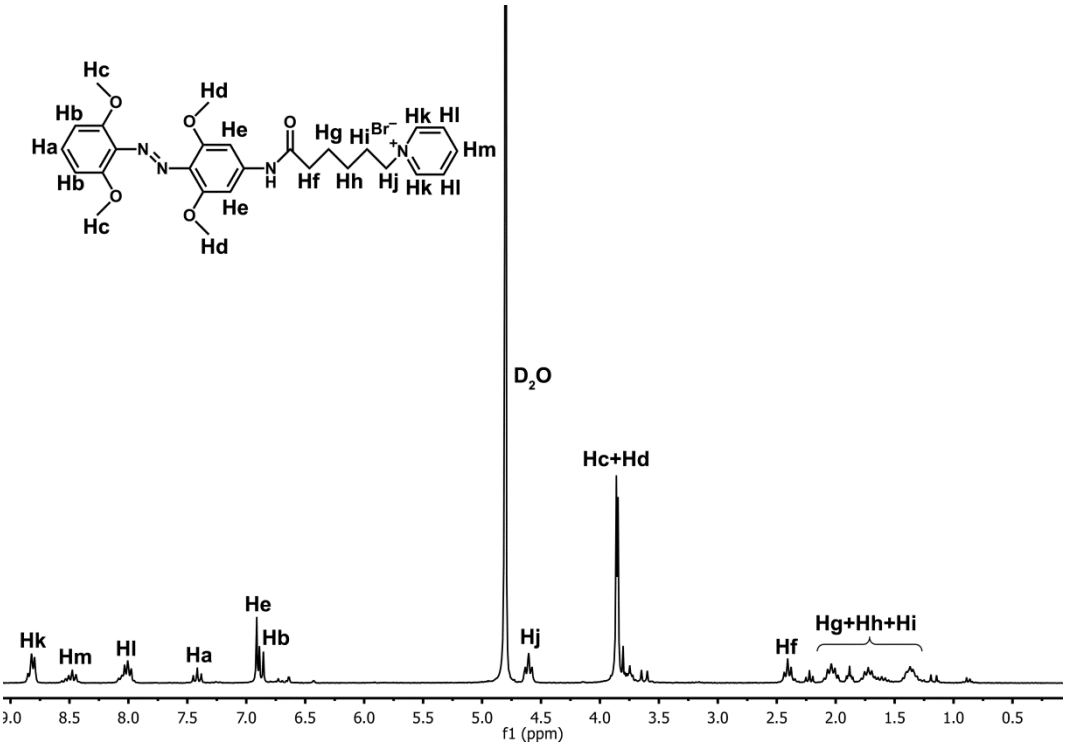

Figure S29. ^{1}H-NMR (250 MHz, 298K, D_2O) spectrum of mAzo-Py.

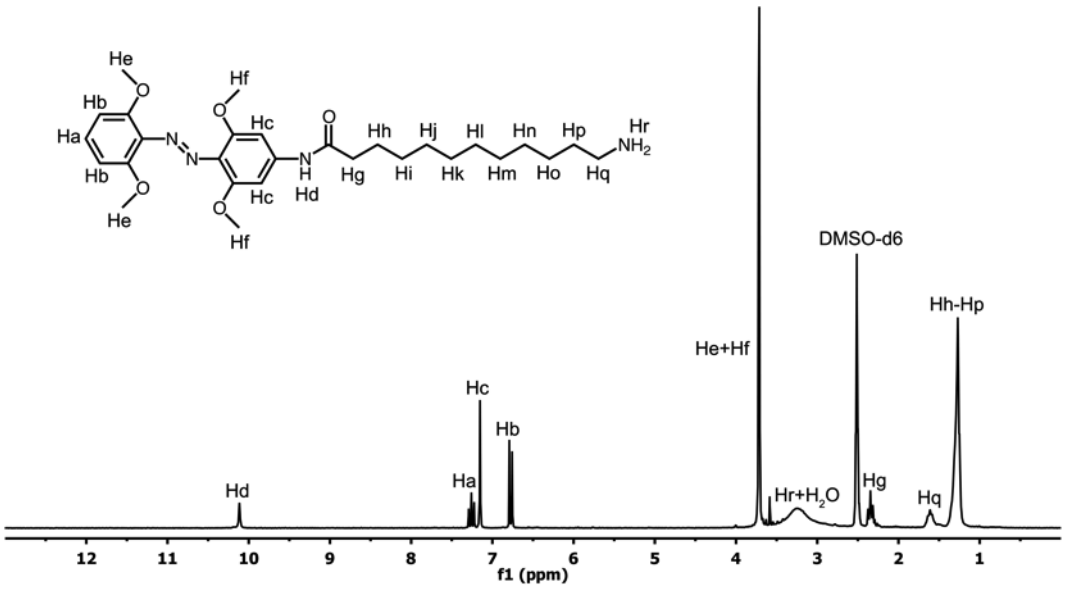

Figure S30. ^{1}H-NMR (250 MHz, 298K, DMSO-d_6) spectrum of 11.

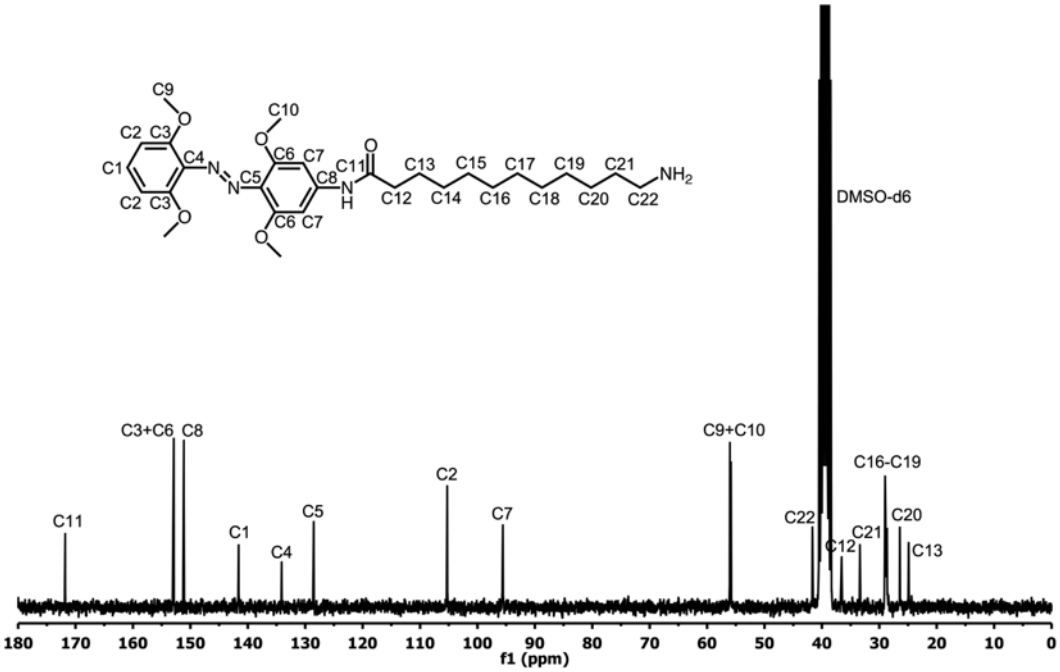

Figure S31. ^{13}C-NMR (63 MHz, 298K, DMSO-d_6) spectrum of 11.

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[2] S. Tamesue, Y. Takashima, H. Yamaguchi, S. Shinkai, A. Harada, *Angew. Chem. Int. Ed.* **2010**, *49*, 7461-7464.

[3] Y. Zhou, D. Wang, S. Huang, G. Auernhammer, Y. He, H.-J. Butt, S. Wu, *Chem. Commun.* **2015**, *51*, 2725-2727.

[4] Z. Chen, W. Sun, H.-J. Butt, S. Wu, *Chem. Eur. J.* **2015**, *21*, 9165-9170.