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Supporting Information

Cyclodextrin as water-soluble host of azobenzene-based pH probe enables long-term monitoring

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Contents

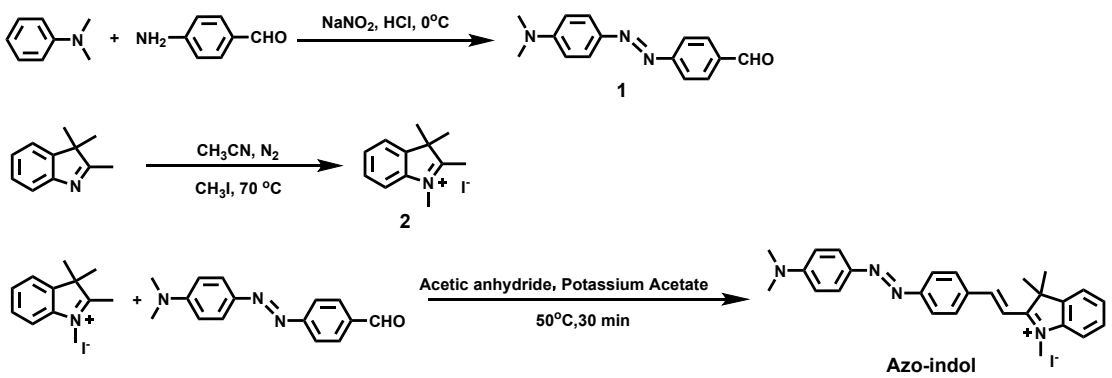
1. Experimental Proc edures

1.1 Materials and general methods

Materials: All reagents were purchased from commercial sources and used without further purification. All other chemical reagents of analytical grade were used directly without further purification. Deionized water was used to prepare all aqueous solutions. Phosphate buffer solutions were all purchased from KGI Bio.

Instruments: The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ultra Shield Plus 400 MHz NMR instrument at 298 K using deuterated solvents. Chemical shifts are given in ppm, and are referenced against external Me_4Si (^{1}H, ^{13}C). Mass spectra were obtained on a Bruker autoflex matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer. The UV-visible absorption spectra were obtained with a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. PBS buffer solvents with different pH values were measured by a Sartorius PB-10 standard pH meter.

1.2 Synthesis and characterization



Scheme S1. Synthetic routes of Azo-indol.

Synthesis and characterization of 1: Add 20 ml of deionized water to a round-bottomed flask and cool to 0 °C in an ice brine mixture, then add 0.4 ml of concentrated hydrochloric acid to the flask, stir vigorously and cool to 0 °C. P-aminobenzaldehyde (67 mg, 0.5 mmol) was dissolved and dispersed in approximately 110 mL of acetone, which was added dropwise to the round-bottomed flask through a constant-pressure dropping funnel over a period of 30 min with vigorous stirring and kept at 0 °C. The solution was then added to the round-bottomed flask at a constant pressure. $NaNO_2$ (37 mg) was dissolved in 2 mL of deionized water, cooled to 0 °C, and added dropwise. A small amount of urea was added to the solution to destroy the small amount of HNO_3 in the solution. N,N-dimethylaniline (63.0 μL, 0.5 mmol) was dissolved in 3 mL of glacial acetic acid, mixed well, and then added drop by drop to the round-bottomed bottle through a constant-pressure funnel with vigorous stirring for about 10 min. Continue to add 15-20 mL of saturated aqueous sodium acetate to the solution at the end of the shift. When the reaction was complete, the reaction solution was filtered through a

Brewer's funnel to obtain a red precipitate. The crude product was purified by column chromatography (SiO_2, dichloromethane/petroleum ether) and dried under vacuum to give compound 1 as an orange-red solid. (Yield=90%). ^{1}H NMR (400 MHz, $CDCl_3$-d_6) δ 10.08 (s, 1H), 8.12 – 7.86 (m, 6H), 6.80 (d, J = 9.3 Hz, 2H), 3.16 (s, 6H).

Synthesis and characterization of 2: A stirrer was added to a 250 mL round-bottomed flask bottle, a condenser tube was put on to seal it, vacuum was applied, N_2 was added, and finally an N_2 balloon was tied for protection. 2,3,3-Trimethyl-3H-indole (1 mL, 6.28 mmol), iodomethane (0.4 mL, 6.28 mmol) and a small amount of acetonitrile were slowly added to a round-bottomed flask via a syringe, and the reaction was carried out for 18 hours at 70 °C. After the reaction is complete, bring to room temperature, add excess petroleum ether (about 150 mL) and stir at room temperature for 3-4 hours. The crude product was then filtered through a Brinell funnel. The crude product was washed with petroleum ether and a small amount of acetonitrile and purified three times repeatedly to give the compound as a pale pink solid, which was dried under vacuum.(Yield=92%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (d, J = 8.8 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.67 – 7.59 (m, 2H), 3.97 (s, 3H), 2.77 (s, 3H), 2.54 – 2.48 (m, 3H), 1.53 (s, 6H).

Synthesis and characterization of Azo-indol: Compound 1 (30 mg, 0.12 mmol), Compound 2 (26 mg, 0.12 mmol) and CH_3COOK (11 mg) were added to a 100 mL round bottom flask bottle, evacuated and N_2 balloon was tied to keep nitrogen. Then acetic anhydride (7 ml) was added to it. React at 50°C overnight reaction. When the reaction was finished, saturated aqueous sodium bicarbonate was added to the reaction solution and the pH was adjusted to 7-8 and tested by pH paper. The crude product was obtained by filtration through a Brewer's funnel. The crude product was purified by column chromatography (SiO_2 , dichloromethane/petroleum ether/methanol) and dried under vacuum to give compound 3 as a purple-black solid (Yield=60%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, J = 16.3 Hz, 1H), 8.38 (d, J = 8.6 Hz, 2H), 7.90 (dd, J = 28.9, 8.9 Hz, 6H), 7.75 (d, J = 16.4 Hz, 1H), 7.69 – 7.60 (m, 2H), 6.88 (d, J = 9.3 Hz, 2H), 4.19 (s, 3H), 3.11 (s, 6H), 1.82 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 182.05 (s), 155.49 (s), 153.67 (s), 152.30 (s), 144.15 (s), 143.46 (s), 142.31 (s), 135.56 (s), 132.23 (s), 129.97 (s), 129.48 (s), 126.03 (s), 123.36 (s), 122.88 (s), 115.75 (s), 113.90 (s), 112.21 (s), 52.69 (s), 35.10 (s), 25.76 (s). MS (MALDI-TOF-MS): calcd. For $[C_{27}H_{29}N_4]^+$409.56 $[M]^+$; found 409.42 $[M]^+$.

1.3 Determination of apparent pKa

The pKa values were extracted from the absorption spectra of Azo-indol and Azo-indol + CD in buffer solutions of pH 1.0 – 13.0, respectively, according to the following equation 1, which fitted the absorbance and pH data.

$$A_{obs} = \frac{A_{AH+}^{\infty}}{(1 + 10^{pH - pKa})} + \frac{A^{\infty}}{(1 + 10^{pKa - pH})}$$

equation 1

1.4 Titration of H^+ or OH^- through ^1H NMR and MALDI-TOF

Sample solution 1 of 2×10^{-2} M was prepared by dissolving the sample Azo-indol in 0.5 ml of deuterated DMSO, sample solution 2 of 1 M was prepared by dissolving concentrated sulfuric acid in 0.5 ml of deuterated D_2O, and sample solution 3 of 2 M was prepared by dissolving sodium hydroxide in 0.5 ml of deuterated D_2O. Solution 2 was added dropwise to solution 1 by a pipette gun at room temperature 25 °C with a molar equivalence ratio of H^+ to Azo-indol of 2:1 and mixed thoroughly for 10 min before being tested by NMR spectroscopy. The molar equivalence ratio of H^+ to Azo-indol was continuously increased to reach a steady state where the NMR peak displacement no longer changed and the test was stopped. Similarly, the reaction mechanism of OH^- with Azo-indol was verified by adding solution 3 dropwise to solution 1 via a pipette gun to control the molar equivalence ratio of OH^- to Azo-indol.

Sample solution 1 of 2×10^{-2} M was prepared by dissolving sample Azo-indol in 0.5 ml methanol; sample solution 2 of 1 M was prepared by dissolving concentrated hydrochloric acid in 0.5 ml deionized water; sample solution 3 of 2 M was prepared by dissolving sodium hydroxide in 0.5 ml deionized water. Solution 2 was added dropwise to solution 1 by a pipette gun at room temperature 25 °C with a molar equivalence ratio of H^+ to Azo-indol of 1:1 and mixed thoroughly for 10 min before being tested by a MALDI-TOF mass spectrometer. Similarly, the reaction mechanism of OH^- with Azo-indol was verified by adding solution 3 dropwise to solution 1 via a pipette gun to control the molar equivalence ratio of OH^- to Azo-indol.

1.5 Measurement of reversible pH response

Sample solution 1 of 10^{-3} M was prepared by dissolving sample Azo-indol in DMSO; sample solution 2 of 10^{-2} M was prepared by dissolving concentrated hydrochloric acid in deionized water; and sample solution 3 of 10^{-2} M was prepared by dissolving sodium hydroxide in deionized water. Sample solution 1 was diluted to a test concentration of 2×10^{-5} M was added to the cuvette, and sample solution 2 was added to ensure that the molar equivalence ratio of H^+ to Azo-indol was 10:1, and left to stabilize for 10 min and then the absorption spectrum was tested by UV-3600. Sample solution 3 was then added to ensure that the molar equivalence ratio of OH^- to Azo-indol was 10:1, and the absorption spectra were tested after 10 min of stabilization. The suboperation was repeated 10 times and the absorption spectra were tested separately.

1.6 Measurement of responsive specificity

A 10^{-3} M sample solution was prepared by dissolving the sample Azo-indol in DMSO, and a 10^{-3} M sample solution was prepared by dissolving different kinds of ionic salts in deionized water. The anionic and cationic response of Azo-indol was tested by diluting the sample solution to the test concentration of 2×10^{-5} M and adding it to the cuvette at room temperature of 25°C. The absorption spectra were tested by dropping the solutions containing different ionic salts into the cuvette and mixing them well for 5 min.

1.7 pH responsive measurement of Azo-indol after interaction with CD

Samples Azo-indol and *α-, β- and γ-CD* were dissolved in DMSO, and molar equivalent ratios of 1:1, 1:4, 1:7, 1:10, 1:13, 1:16, and 1:20 were prepared by controlling the amount of CD added to the samples Azo-indol + *α*-CD; Azo-indol + *β*-CD, and Azo-indol + *γ*-CD respectively Solution. At room temperature of 25 °C, mixing and stirring were performed for 12 h to make Azo-indol interact with CDs completely. The UV-visible absorption spectra of Azo-indol + CDs at different pH values were tested by adding different equivalent ratios of Azo-indol + CDs solutions to previously prepared PBS buffers of different pH values.

2. Supplementary Figures

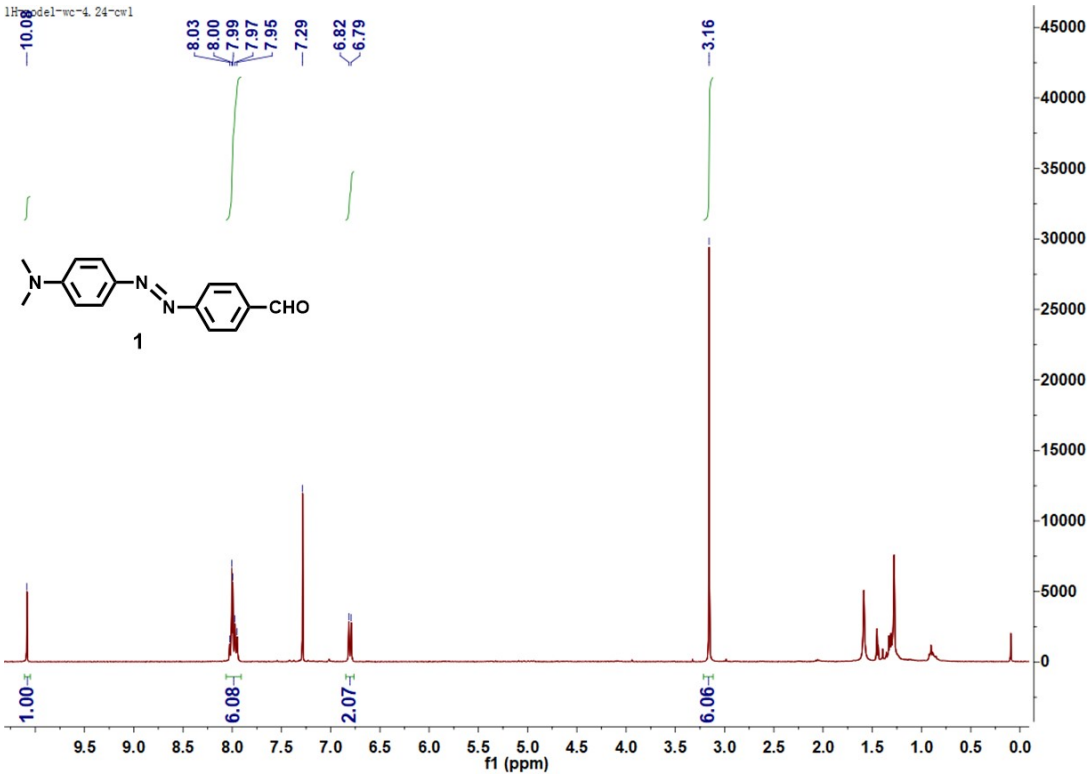


Fig. S1 ^{1}H NMR spectrum of **1** in $CDCl_3$.

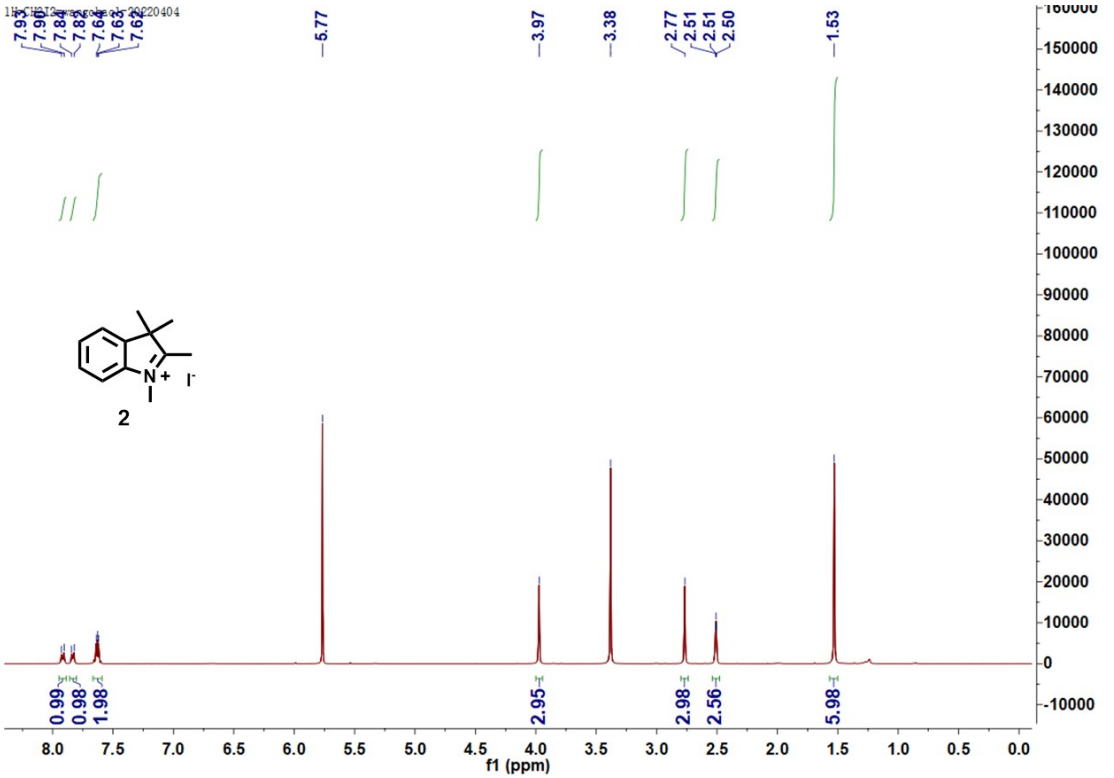


Fig. S2 ^{1}H NMR spectrum of **2** in DMSO-d_6.

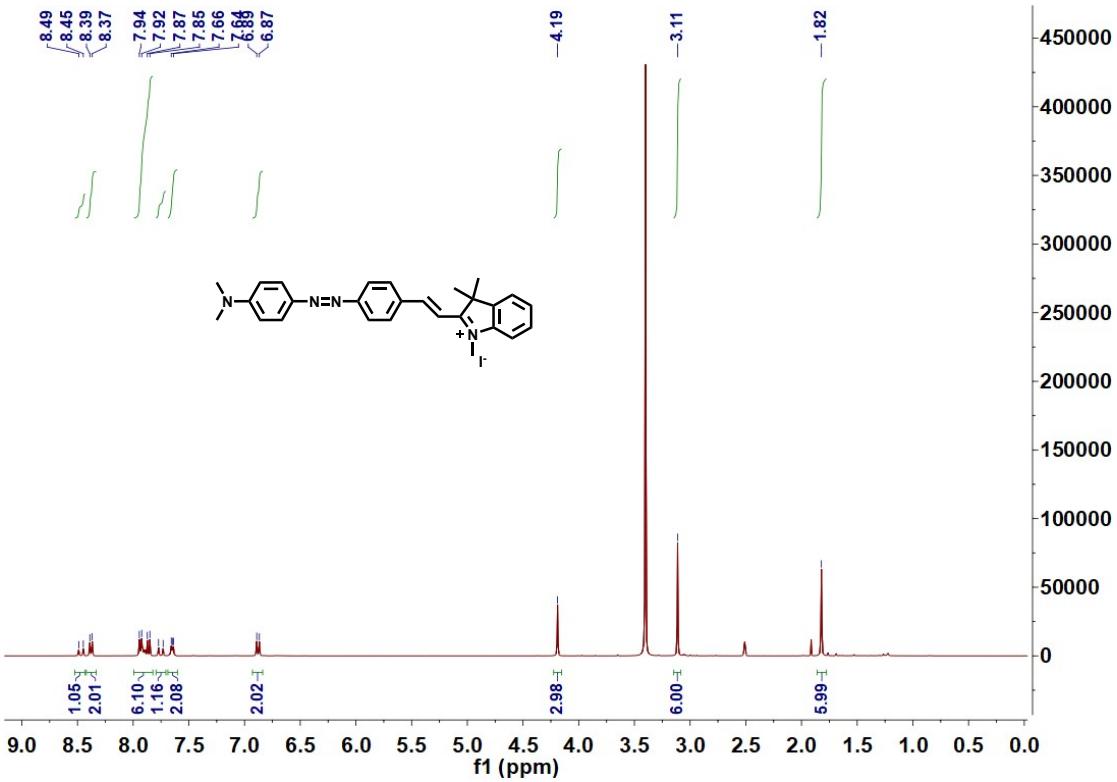


Fig. S3 ^{1}H NMR spectrum of Azo-indol in DMSO-d_6.

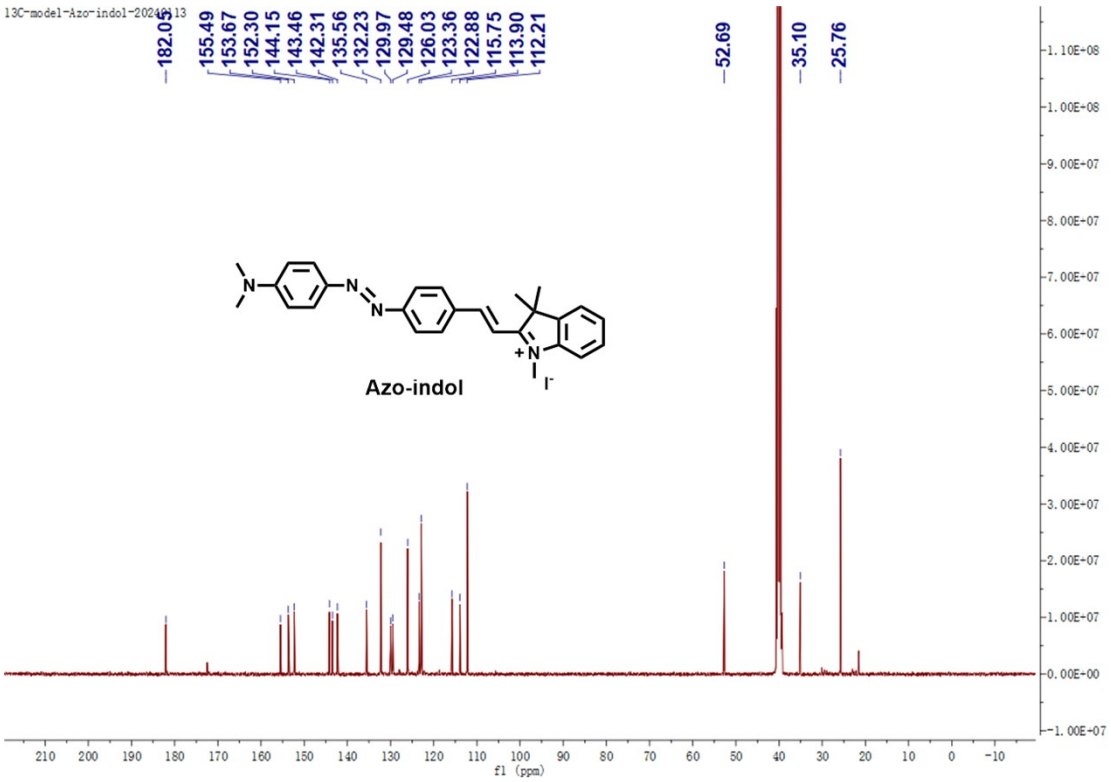


Fig. S4 ^{13}C NMR spectrum of Azo-indol in DMSO-d_6.

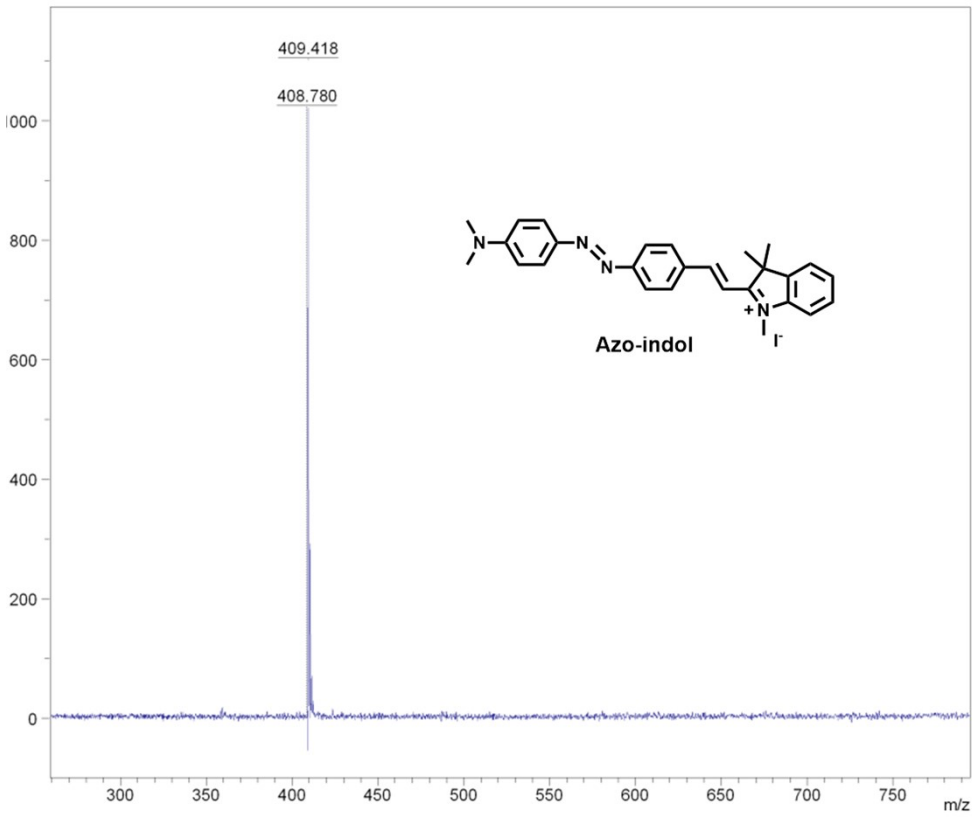


Fig. S5 MALDI-TOF mass spectrum of Azo-indol.

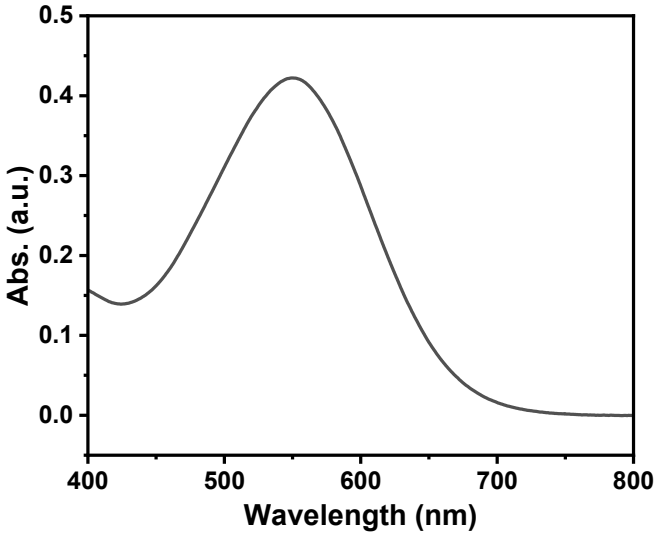


Fig. S6 Absorption spectra of Azo-indol (10^{-5} M) in DMSO.

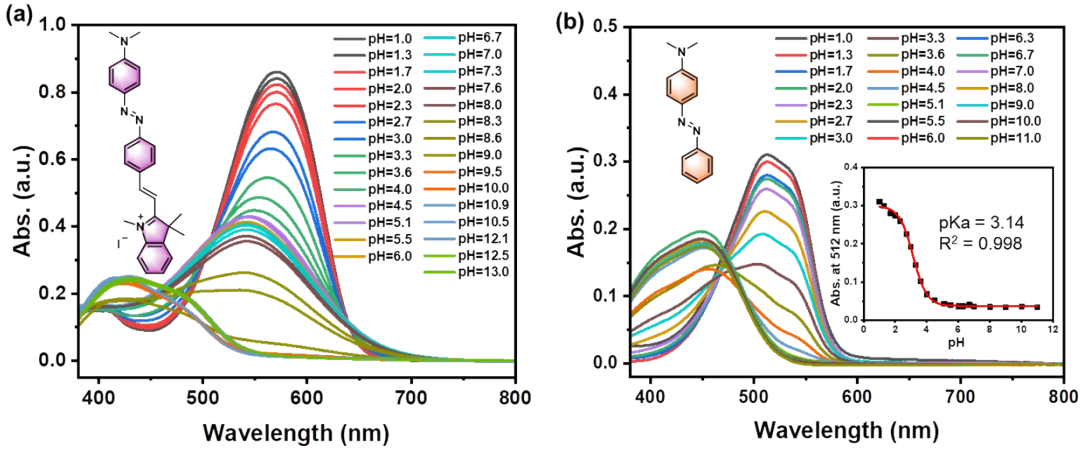


Fig. S7 (a) Absorption spectra of Azo-indol (10^{-5} M) at pH value ranging from 1.0 to 13.0 in PBS buffer. (b) Absorption spectra of of MY (10^{-5} M) at pH value ranging from 1.0 to 11.0 in PBS buffer. Insert: Corresponding absorbance at 512 nm at different pH values and fitting plots of apparent pKa.

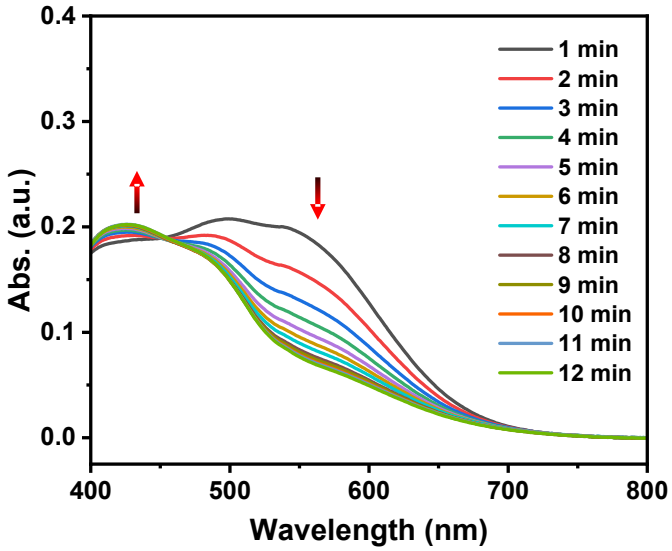


Fig. S8 Absorption spectra of Azo-indol (10^{-5} M) after added into PBS buffer (pH=9.2) in 12 min.

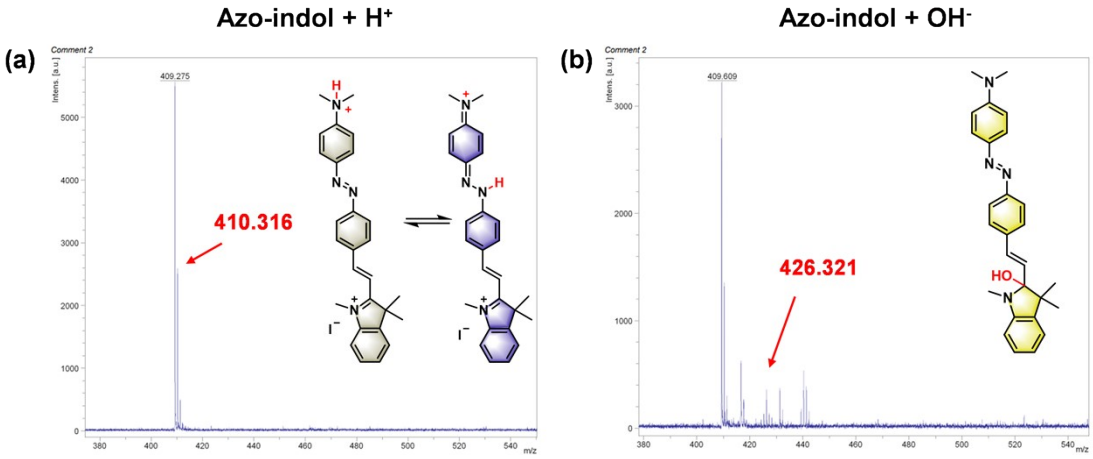


Fig. S9 MALDI-TOF mass spectrum of Azo-indol after reaction with (a) 2 eq H^+ or (b) 10 eq OH^-.

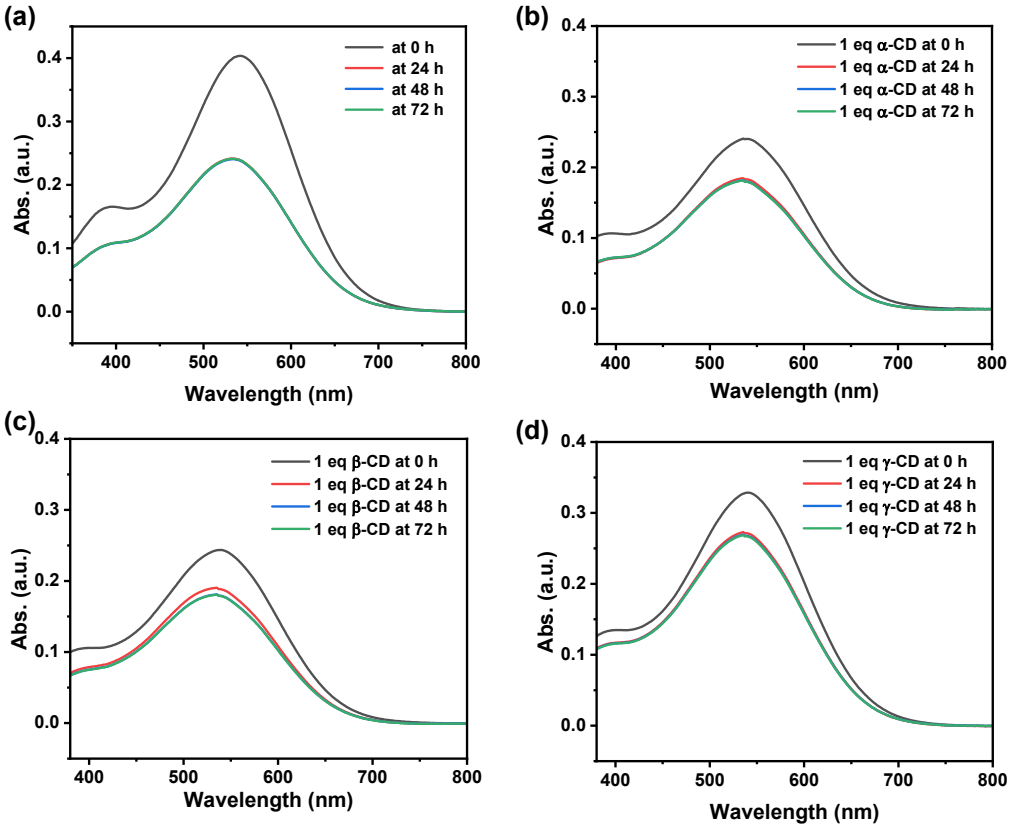


Fig. S10 Absorption spectra of Azo-indol (10^{-5} M) (a) before and (b,c,d) after interaction with 1 eq of α-CD, β-CD or γ-CD in PBS buffer (pH 6.7) during 72 h.

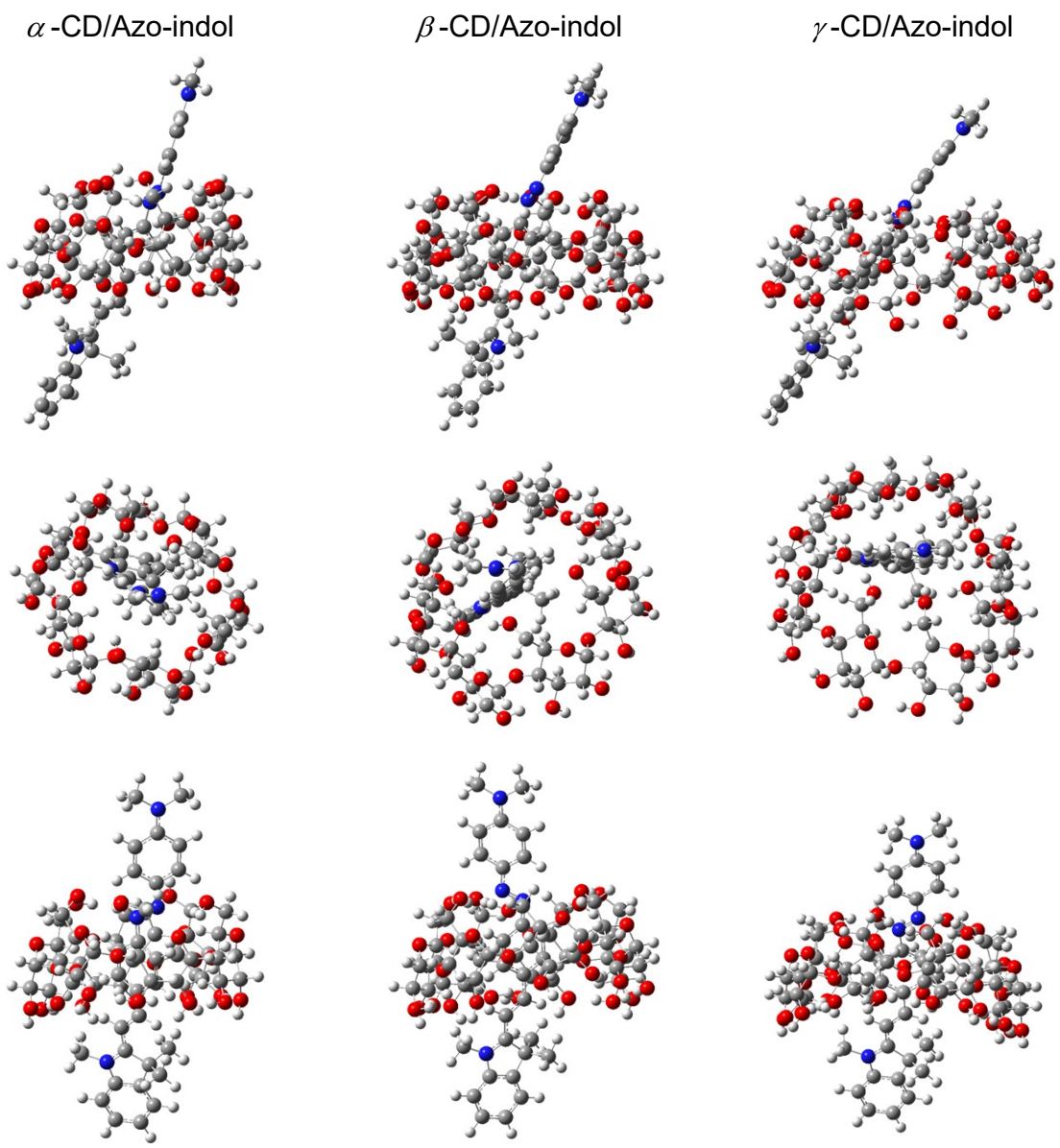


Fig. S11 Models of the DFT calculated structure of the energy-minimized CDs/Azo-indol complexes in water at room temperature, which were determined at the B3LYP/6-31G(d) level with Gaussian 16.

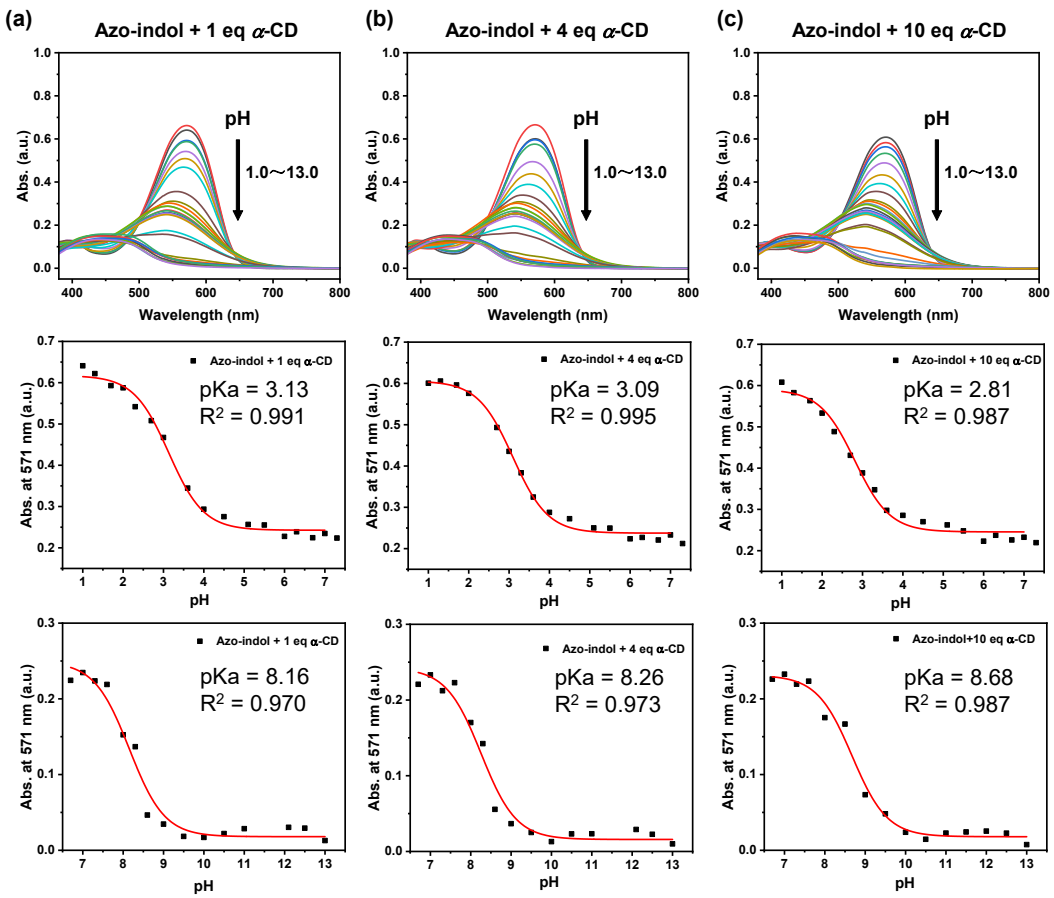


Fig. S12 Absorption spectra of Azo-indol (10^{-5} M) after interaction with (a) 1 eq α-CD, (b) 4 eq α-CD or (c) 10 eq α-CD in PBS buffer with the pH value ranging from 1.0 to 13.0 and corresponding absorbance at 571 nm at different pH values and fitting plots of apparent pKa.

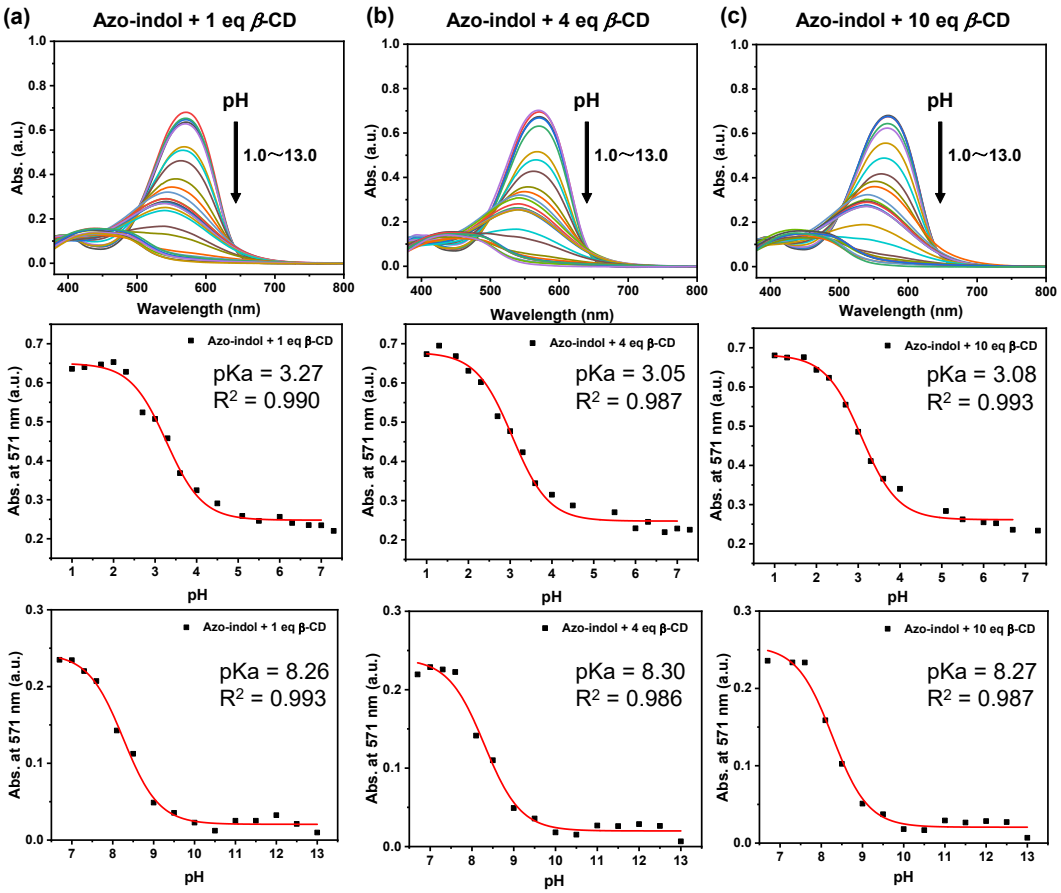


Fig. S13 Absorption spectra of Azo-indol (10^{-5} M) after interaction with (a) 1 eq β-CD, (b) 4 eq β-CD or (c) 10 eq β-CD in PBS buffer with the pH value ranging from 1.0 to 13.0 and corresponding absorbance at 571 nm at different pH values and fitting plots of apparent pKa.

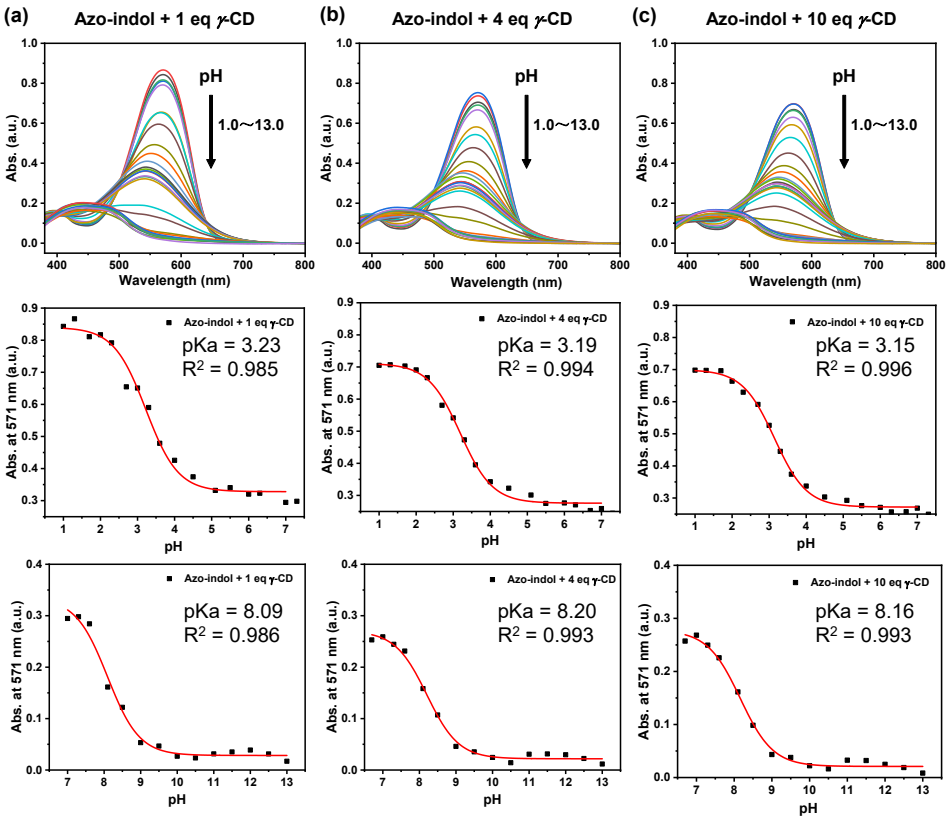


Fig. S14 Absorption spectra of Azo-indol (10^{-5} M) after interaction with (a) 1 eq γ-CD, (b) 4 eq γ-CD or (c) 10 eq γ-CD in PBS buffer with the pH value ranging from 1.0 to 13.0 and corresponding absorbance at 571 nm at different pH values and fitting plots of apparent pKa.

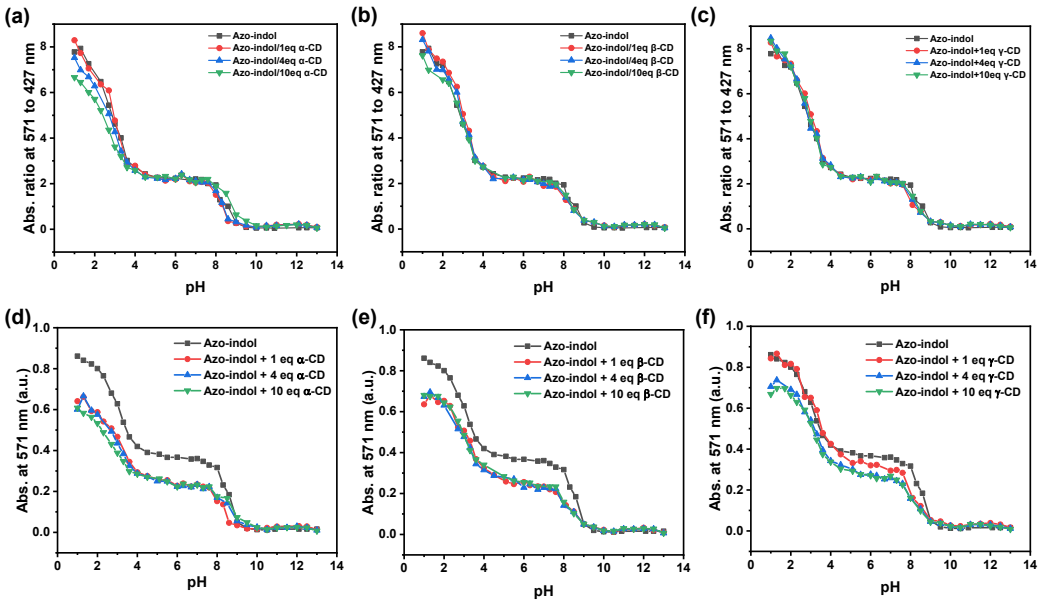


Fig. S15 (a,b,c) Absorption ratio at 571 nm to 427 nm and (d,e,f) absorbance at 571 nm of Azo-indol (10^{-5} M) after interaction with 1 eq, 4 eq or 10 er of α-CD, β-CD or γ-CD in PBS buffer at different pH values.

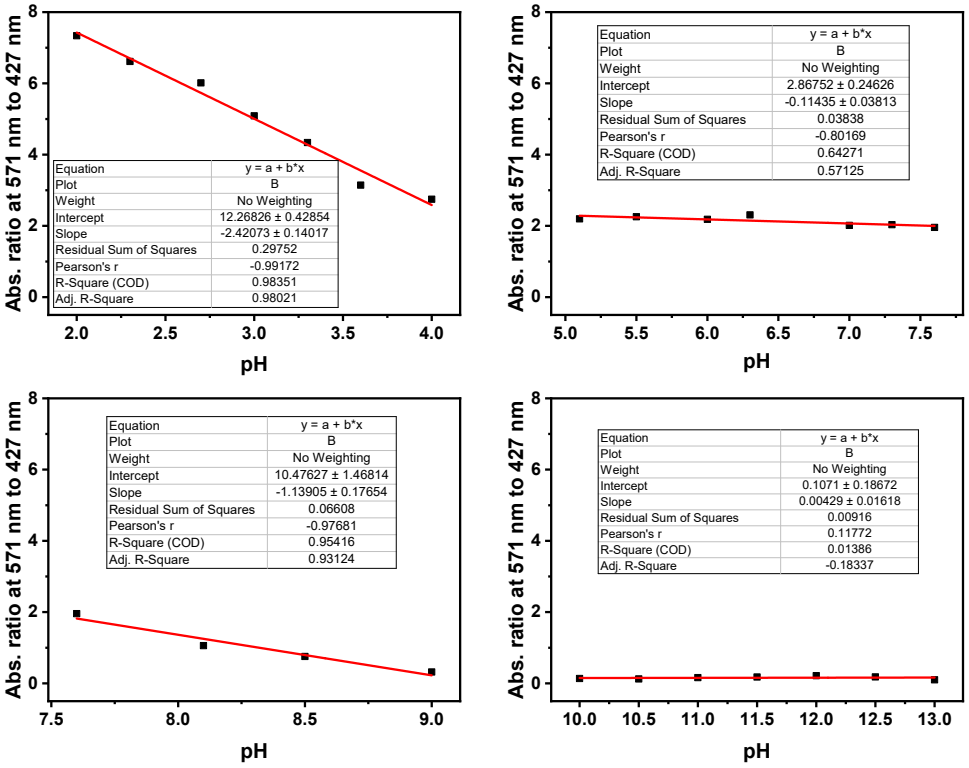


Fig. S16. Linear fitting curves of the ratio of absorbance at 571 nm to 427 nm of γ-CD/Azo-indol (10^{-5} M) in the pH range of 2.0~4.0, 5.1~7.6, 7.6~9.0 and 10.0~13.0.

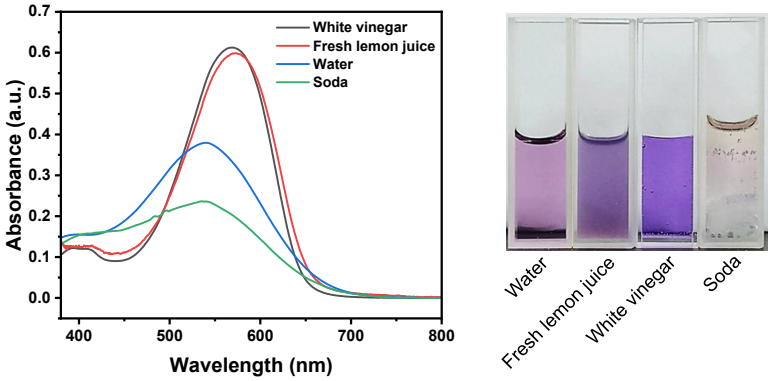


Fig. S17. Absorption spectra and corresponding photographs of γ-CD/Azo-indol (10^{-5} M) in white vinegar, fresh lemon juice, water and soda.

Table S1. Apparent pKa values of Azo-indol (10^{-5} M) before and after interaction with 1 eq, 4 eq or 10 er of α-CD, β-CD or γ-CD.

	p*K*a	R-Square	p*K*a	R-Square
Azo-indol	3.02	0.998	8.46	0.987
Azo-indol + 1.0 eq α-CD	3.13	0.991	8.16	0.970
Azo-indol + 4.0 eq α-CD	3.09	0.995	8.26	0.973
Azo-indol + 10.0 eq α-CD	2.81	0.987	8.68	0.987
Azo-indol + 1.0 eq β-CD	3.27	0.990	8.26	0.993
Azo-indol + 4.0 eq β-CD	3.05	0.987	8.30	0.986
Azo-indol + 10.0 eq β-CD	3.08	0.993	8.27	0.987
Azo-indol + 1.0 eq γ-CD	3.23	0.985	8.09	0.986
Azo-indol + 4.0 eq γ-CD	3.19	0.994	8.20	0.993
Azo-indol + 10.0 eq γ-CD	3.15	0.996	8.16	0.993