

Electronic Supplementary Information

A fast-responding fluorescent turn-on sensor for sensitive and selective detection of sulfite anions

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Experimental Section

Materials and reagents: 9-(Chloromethyl)anthracene, potassium carbonate (K_2CO_3), sodium salts of anions (I^- , F^- , Cl^- , Br^- , HCO_3^- , SO_4^{2-} , PO_4^{3-} , NO_3^- , NO_2^- , $B_4O_7^{2-}$, CO_3^{2-} , HPO_4^{2-} , SCN^-) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. 4-Formylbenzoic acid, benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), dimethylsulfoxide (DMSO for HPLC), N,N-dimethylformamide (DMF), anhydrous ethylenediamine, triethylamine (TEA), ammonia, monohydrate citric acid and dichloromethane (DCM) were obtained from Alfa Aesar. Beer (Pearl River®) was made by Guangzhou Zhujiang Brewery Group Co. Ltd., and red wine (Great Wall®) was made by Cofco Wines & Spirits Co. Ltd. (China). Rainwater was collected locally. The water used in this study was the triple-distilled water which was further treated by ion exchange columns and then by a Milli-Q water purification system.

Synthesis of N¹-(anthracen-9-ylmethyl)ethane-1,2-diamine: 9-(Chloromethyl)anthracene (0.15 g, 0.66 mmol) and K_2CO_3 (0.456 g, 3.3 mmol) were suspended in DMF (8 mL), the mixture was cooled to 0 °C with ice bath. Then ethylenediamine (0.88 mL, 13.2 mmol) was dropped to the mixture with syringe. The reaction mixture was heated to 55 °C for 20 h under nitrogen atmosphere. After cooling to room temperature, the DMF and potassium carbonate was removed by extraction with dichloromethane/deionized water, the organic phase was dried with anhydrous Na_2SO_4 and then purified on a silica gel column using methanol: ammonia = 20 : 1 (v/v) as eluent to obtain faint yellow viscous liquid (0.144 g, 87.5%). 1H -NMR (400 MHz, DMSO- d_6 , δ ppm): 8.54 (s, 1H),

8.41-8.43 (d, 2H), 8.07-8.09 (d, 2H), 7.49-7.58 (m, 4H), 4.63 (s, 2H), 2.78-2.79 (t, 2H), 2.71-2.72 (t, 2H). MS (ESI): m/z 250.2 $[M]^+$.

Synthesis of N-(2-((anthracen-9-ylmethyl)amino)ethyl)-4-formylbenzamide (the probe):

4-Formylbenzoic acid (0.06 g, 0.4 mmol), PyBOP (0.208 g, 0.4 mmol) and TEA (0.061 mL, 0.44mmol) were dissolved in dry dichloromethane (5 mL) and stirred for 15 min. Afterwards, TEA (0.061 mL, 0.44mmol) together with the above-obtained N¹-(anthracen-9-ylmethyl)ethane-1,2-diamine (0.1 g, 0.4 mmol) dissolved in dry dichloromethane (5 mL) was added and stirred at room temperature for 24 h under nitrogen atmosphere. Then the reaction mixture was extracted between dichloromethane and saturated sodium bicarbonate, 10% (wt%) citric acid solution, 5% (wt%) sodium chloride solution and deionized water respectively. The organic phase was dried with anhydrous Na₂SO₄ and purified by flash chromatography (ethyl acetate : dichloromethane = 10:1, v/v) to obtain the probe (0.105 g, 68.4%). ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 10.08 (s, 1H), 8.65 (s, 1H), 8.55 (s, 1H), 8.45-8.48 (d, 2H), 8.07-8.09 (d, 2H), 8.00-8.05 (d, 4H), 7.51-7.54 (m, 4H), 4.70 (s, 2H), 3.47-3.53 (t, 2H), 2.96-3.02 (t, 2H), 1.99 (s, 1H). MS (APCI): m/z 381.6 $[M-H]^+$.

Measurements: ¹H NMR spectrum was recorded on a Bruker Avance 400MHz NMR Spectrometer. Mass spectra were obtained through Bruker Esquire HCT Plus mass spectrometer. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer with excitation wavelength being 370 nm. UV-vis spectra were recorded on a Hitachi U-3010 UV-vis Spectrophotometer.

For sulfite testing, the probe was dissolved in DMSO solution to prepare the stock

solution (10^{-3} M). Stock solutions of SO_3^{2-} and various other ions (10^{-3} M) were prepared by dissolving their salts in pH 7.0 HEPES buffer. The test solution was prepared by adding the requisite amounts of stock solutions together, and then diluting with pH 7.0 HEPES buffer, the final solvent was pH 7.0 HEPES buffer containing DMSO (2%, v/v). The test solution was stirred for 1 minute, then the fluorescence spectra was recorded.

In the food and medicine industries, several sulfite agents (for example, sodium metabisulfite, sodium sulfite, and sodium hydrogen sulfite) have been used as preservatives. These sulfite agents are all chemically equivalent since they are converted to the same ionic species (SO_3^-) when they are dissolved in aqueous solution and the pH is close to neutrality, because under this condition only sulfite is present.

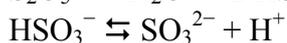
(1) Sodium hydrogen sulfite (or sodium bisulfite, NaHSO_3):

HSO_3^- is a weakly acidic species with a pK_a of 7.0. Its conjugate base is the sulfite ion, SO_3^{2-} :



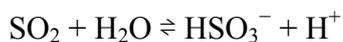
When the pH is close to neutrality (around 7.0), hydrogen sulfite (or bisulfite) ions exist in the form of sulfite ions.

(2) sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$):



Sodium metabisulfite (pK_a around 1.8) quickly dissociates to the bisulfite anion in aqueous solution, when the pH is close to neutrality (around 7.0), hydrogen sulfite (or bisulfite) ions exist in the form of sulfite ions.

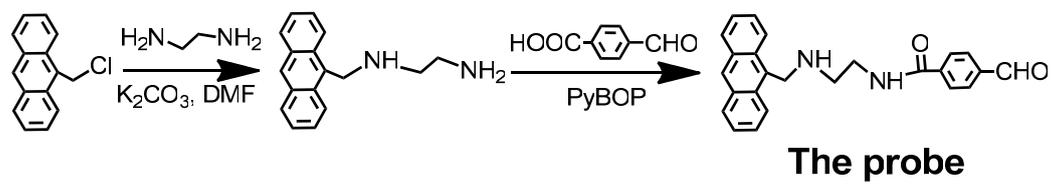
(3) Sulfur dioxide dissolves in water:



Sulfur dioxide is fairly soluble in water, when it dissolves in water, sulfurous acid H_2SO_3 forms, this elusive acid only exists in solution, and the conjugate base of this elusive acid is bisulfite (or hydrogen sulfite). When the pH is close to neutrality (around 7.0), hydrogen sulfite (or bisulfite) ions exist in the form of sulfite ions.

References:

1. W. H. Sperber, M. P. Doyle, *Compendium of the Microbiological Spoilage of Foods and Beverages*, Springer, New York, **2009**.
2. L. Pizzoferrato, G. D. Lullo, E. Quattrucci, *Food Chemistry*. **1998**, *63*, 275-279.
3. R. M. D. Machado, M. C. F. Toledo, E. Vicente, *Eur. Food Res. Technol.* **2009**, *229*, 383-389.



Scheme 1. Synthesis route for the probe.

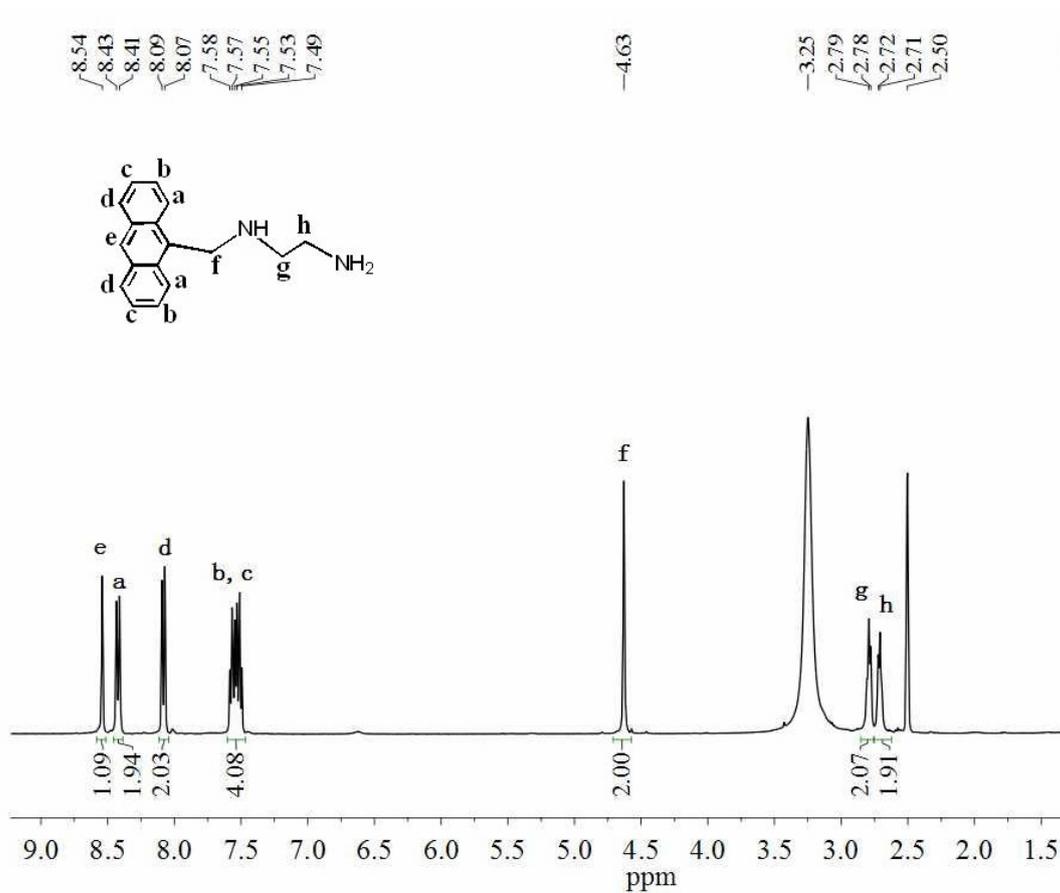


Fig. S1. ¹H NMR spectrum of N¹-(anthracen-9-ylmethyl)ethane-1,2-diamine in DMSO-d₆.

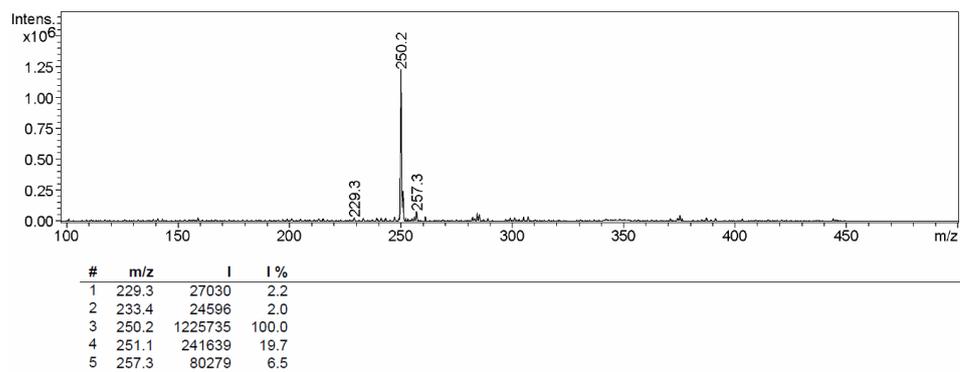


Fig. S2. Mass spectrum of N¹-(anthracen-9-ylmethyl)ethane-1,2-diamine.
MS (ESI): m/z 250.2 [M]⁺

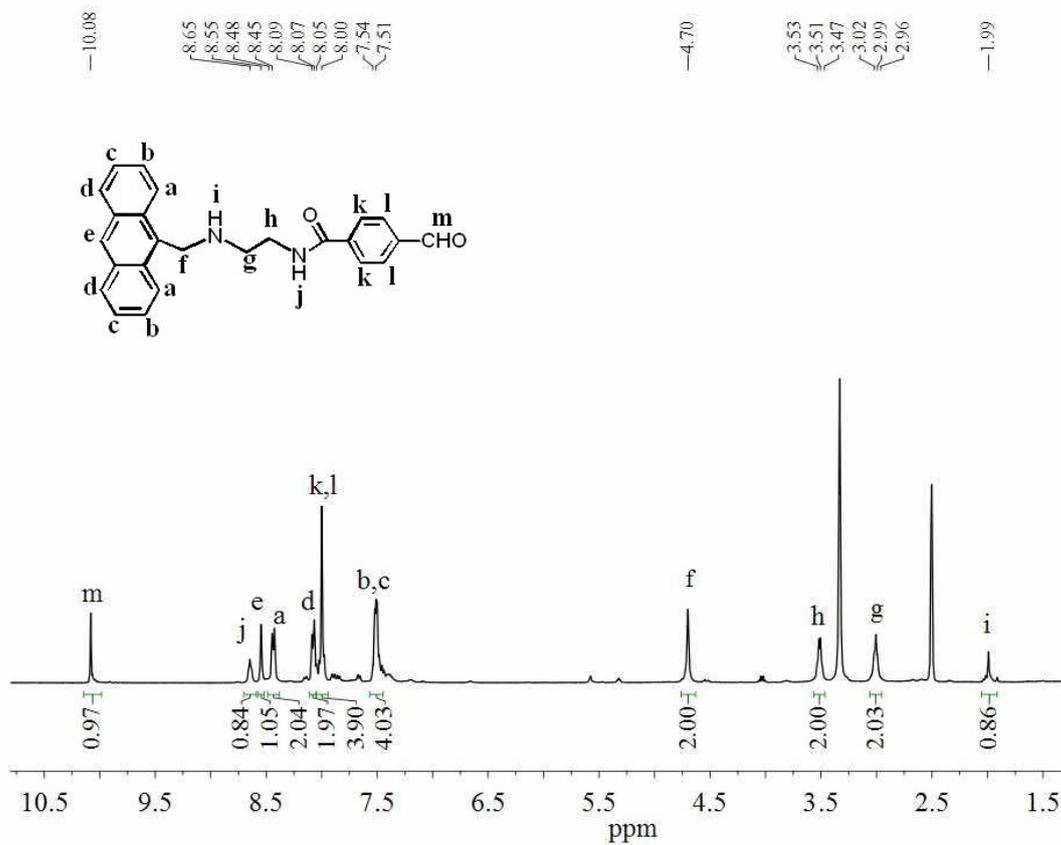


Fig. S3. ^1H NMR spectrum of N-(2-((anthracen-9-ylmethyl)amino)ethyl)-4-formylbenzamide (the probe) in DMSO-d_6 .

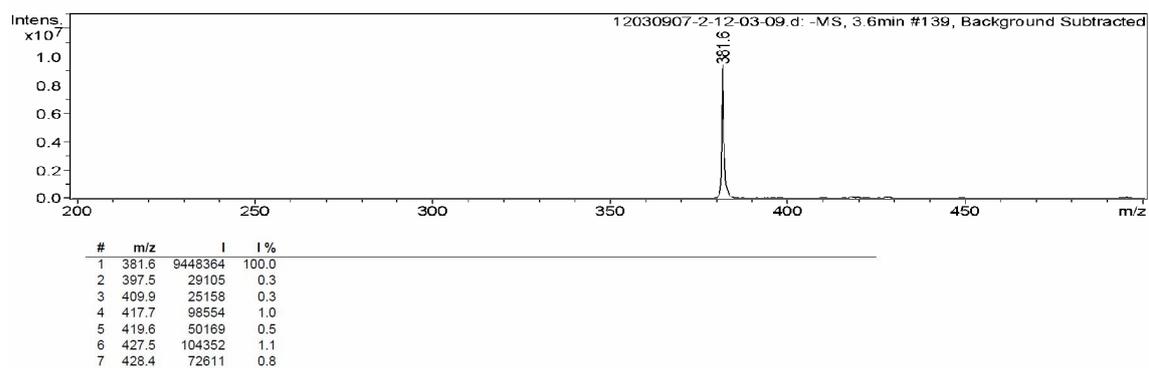


Fig. S4. Mass spectrum of N-(2-((anthracen-9-ylmethyl)amino)ethyl)-4-formylbenzamide (the probe).

MS (APCI): m/z 381.6 [M-H]⁺

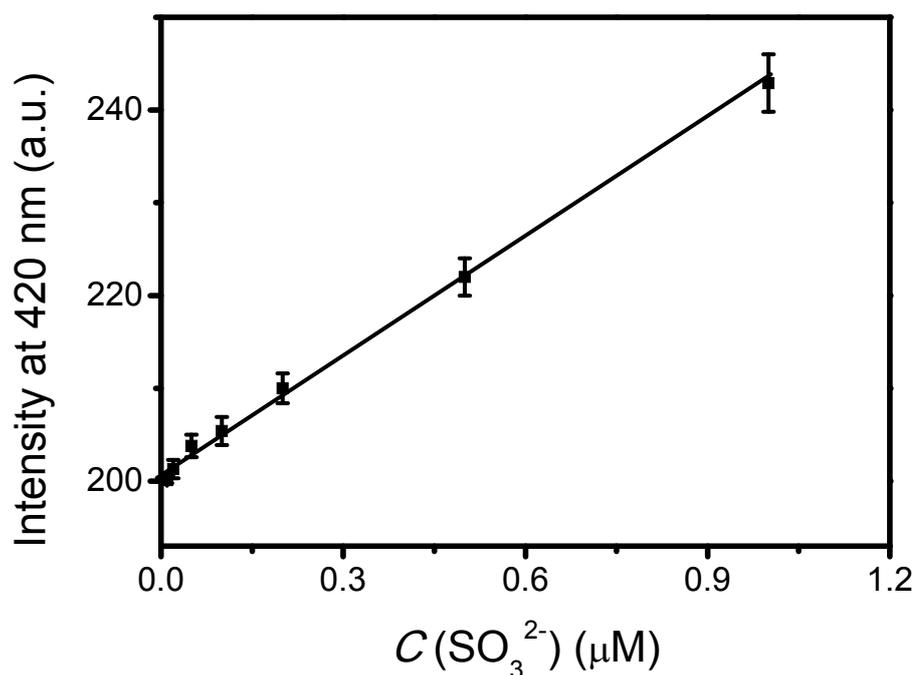


Fig. S5. The fluorescence intensity ratio of the probe (1×10^{-5} M) as a function of sulfite concentration in pH 7.0 HEPES buffer containing DMSO (2%, v/v). Sulfite concentration: 0 - 1.0 μM (the lower concentration part). ($\lambda_{\text{exc}} = 370$ nm).

The method for determining the detection limit:

First the calibration curve was obtained from the plot of fluorescence intensity, I_{420} , as a function of the analyte concentration (sulfite). The regression curve equation was then obtained for the lower concentration part.

The detection limit = $3 \times \text{S.D.} / k$

where k is the slope of the curve equation, and S.D. represents the standard deviation for the fluorescence intensity of the probe solution in the absence of sulfite.

$$I_{420} = 200.36 + 42.3 \text{ E6} [\text{sulfite}] \quad (R = 0.998)$$

$$\text{LOD} = 3 \times 0.14 / 42.3 \text{ E6} = 10 \text{ nM}$$

References:

V. Thomsen, D. Schatzlein and D. Mercurio, *Spectroscopy* 2003, **18**, 112-114.

A. D. McNaught and A. Wilkinson, *IUPAC Compendium of Chemical Terminology*, 1997.

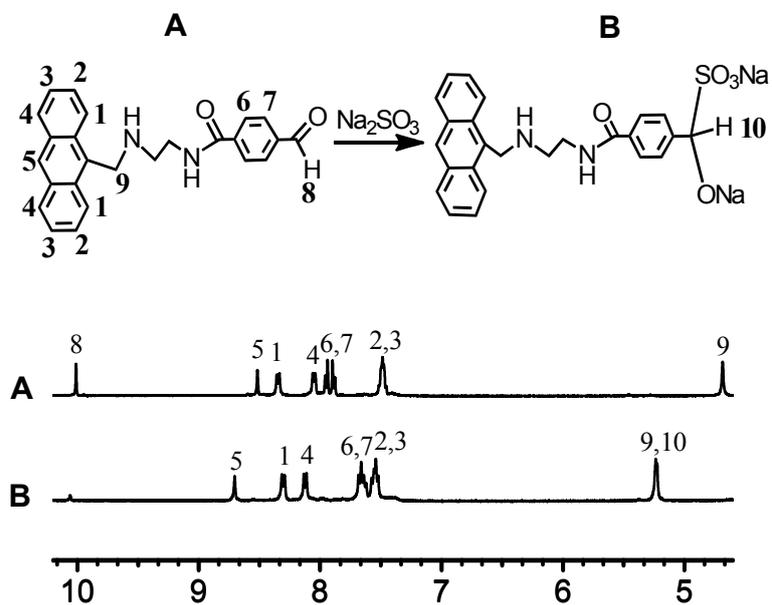


Fig. S6. Partial ¹H NMR spectra of the probe compound (4 mM) before and after addition of sulfite (10 equiv) in D₂O/DMSO-d₆ (v/v, 1 : 1).

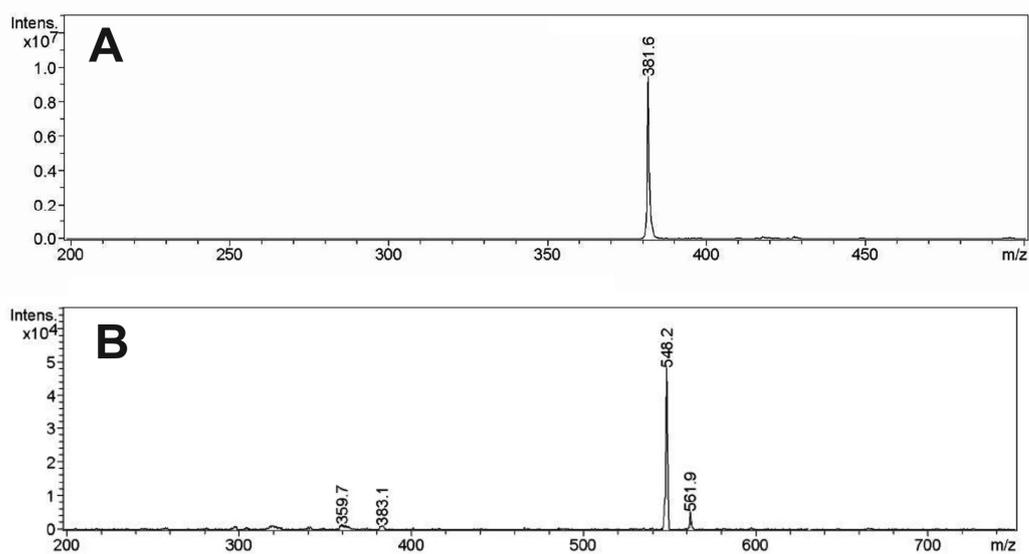


Fig. S7. Mass spectra of the as-prepared probe compound (A) and the chemical formed upon addition of excessive amount of sulfite anion (B). The signals at m/z 381.6 are $[\text{probe}]^+$, while the signals at m/z 548.2 are $[\text{probe} + \text{Na}_2\text{SO}_3 + \text{K}]^+$.

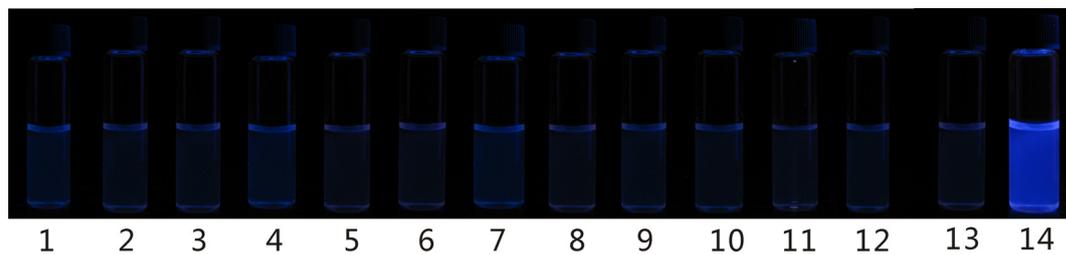


Fig. S8. Photographs of the probe solution (1.0×10^{-5} M, in pH 7.0 HEPES aqueous solution containing DMSO (2%, v/v)) in the presence of various anions (1×10^{-4} M) respectively under 365 nm UV light (fluorescence change).

1: I⁻; 2: F⁻; 3: Cl⁻; 4: Br⁻; 5: HCO₃⁻; 6: SO₄²⁻; 7: PO₄³⁻; 8: NO₃⁻; 9: NO₂⁻; 10: B₄O₇²⁻; 11: CO₃²⁻; 12: HPO₄²⁻; 13: SCN⁻; 14: SO₃²⁻

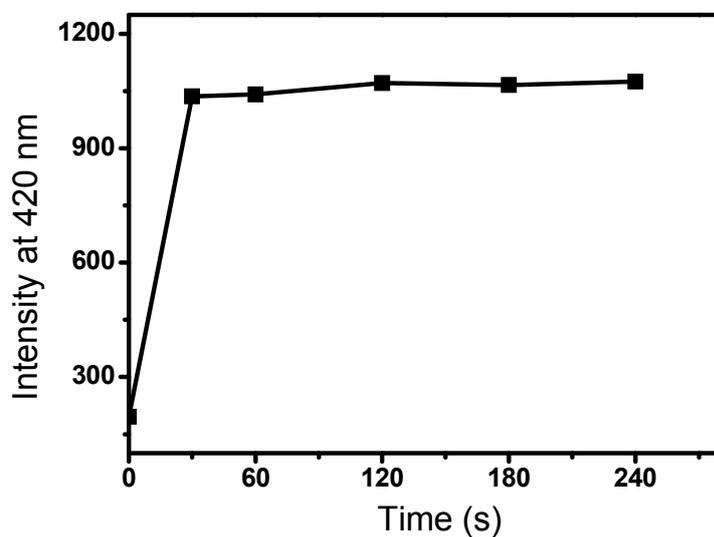


Fig. S9. Fluorescence intensity at 420 nm as a function of time for the probe (1×10^{-5} M, in pH 7.0 HEPES aqueous solution containing DMSO (2%, v/v)) in the presence of sulfite ($100 \mu\text{M}$). The time-scan measurement of the fluorescence intensity was conducted immediately after the anion was added into the sensor solution ($\lambda_{\text{exc}} = 370$ nm).

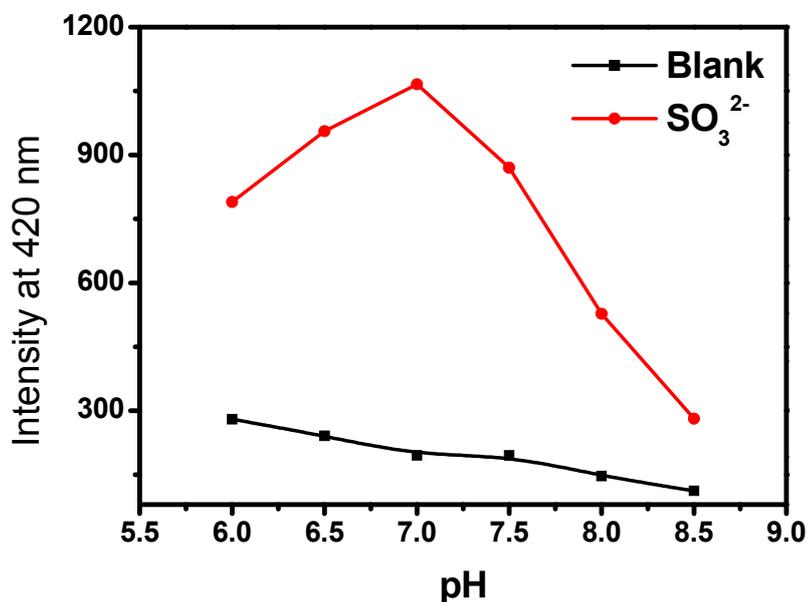


Fig. S10. Fluorescence intensity at 420 nm of the probe solution (1.0×10^{-5} M, in pH 7.0 HEPES aqueous solution containing DMSO (2%, v/v)) in the absence and in the presence of sulfite anions (1×10^{-4} M) as a function of pH (λ_{exc} : 370 nm).

pH 6.0 and pH 6.5: PBS buffer solutions; pH 7.0, 7.5, 8.0 and 8.5: HEPES buffer solutions.

To confirm the suitable pH range for sulfite anion sensing, the effect of pH on the fluorescence intensity for the probe in the absence or presence of 100 μ M sulfite anions was investigated experimentally and the results are given in Figure S7. In the absence of sulfite anions, for the probe solution, no remarkable fluorescence emission was observed in the pH range from 6.5 to 7.5, suggesting that the spectroscopic property of the probe is stable at this pH range; In the presence of sulfite anions, a remarkable increase in fluorescence intensity was observed between pH 6.5 and 7.5, indicating in this pH range the sulfite anions can react with the probe. It also can be found that, the solution exhibits high fluorescence intensity which remained relatively high from pH 6.5 to pH 7.5. These data confirm that the probe could serve as a fluorescent turn-on sensor for sulfite under neutral pH environment.

Table S1 Determination of sulfite in beer and red wine.

Sample	Determined sulfite (μM)	Added sulfite (μM)	Combined sulfite (μM)	Measured (μM)	Recovery (%)
Beer	7.9 ^(a)	-	-	-	-
		20.0	27.9	27.0	96.8
		10.0	17.9	17.6	98.3
		5.0	12.9	13.4	103.9
		2.0	9.9	10.2	103.0
		1.0	8.9	8.7	97.8
Red wine	2.1 ^(b)	-	-	-	-
		20.0	22.1	22.2	100.5
		10.0	12.1	11.5	95.0
		5.0	7.1	7.3	102.8
		2.0	4.1	4.0	97.6
		1.0	3.1	3.0	96.8

Note: (a) The final concentration of beer in the test solution is 10-fold diluted. The determined sulfite level in the diluted beer sample without spiking sulfite is 7.9 μM , which means the sulfite concentration in undiluted beer sample is ca. 79 μM (or 6.3 mg/kg), in compliance with China National Standard for Food Additives (GB2760-2011).

(b) The final concentration of red wine in the test solution is 30-fold diluted. The determined sulfite level in the diluted red wine sample without spiking sulfite is 2.1 μM , which means the sulfite concentration in undiluted beer sample is ca. 63 μM (or 5.0 mg/kg), in compliance with China National Standard for Food Additives (GB2760-2011).

Table S2. Determination of sulfite in rainwater.

Sample	Determined sulfite (μM)	Added sulfite (μM)	Combined sulfite (μM)	Measured (μM)	Recovery (%)
Rain water	5.7 ^(a)	-	-	-	-
		20.0	25.7	25.2	98.1
		10.0	15.7	15.5	98.7
		5.0	10.7	10.8	100.9
		2.0	7.7	7.6	98.7
		1.0	6.7	6.8	101.5

Note: (a) The final concentration of rainwater in the test solution is 3-fold diluted. The determined sulfite level in the diluted rainwater sample without spiking sulfite is 5.7 μM , which means the sulfite concentration in undiluted rainwater is ca. 17.1 μM .