Supporting Information

A Color Turn-On Fluorescent Probe for Real-Time Detection of Hydrogen Sulfide and Identification of Food Spoilage

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1. Materials and methods

4-Bromo-1,8-naphthalic Anhydride (98%), 2-propynylamine (98%), copper sulfate pentahydrate (98%), were purchased from Energy Chemical. N-Hydroxysuccinimide (98%) and L-ascorbic acid (99%) were purchased from Innochem. Azide Poly (ethylene glycol) 2000 (Azido PEG, Mₐ = 2.0 kg/mol) were purchased from Sigma-Aldrich. Benzenesulfonyl chloride (98%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Gases including hydrogen sulfide (H₂S), hydrogen (H₂), oxygen (O₂), carbon monoxide (CO), carbon dioxide (CO₂) and sulfur dioxide (SO₂) were
purchased from Dalian Special Gases Co., Ltd. All other chemicals and solvents were purchased from Energy Chemical or Innochem. Milli-Q water with a resistivity of 18.2 MΩ·cm was used in this study. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker NMR spectrometer operated in Fourier transform mode (400 M or 500 M). High-resolution Mass spectrometric data was detected on a time of flight mass spectrometer (Agilent 1260-6230). Absorption spectra were measured on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). Fluorescence spectra were performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). Excitation and emission slit widths (slit: 5/5 nm) were modified to adjust the fluorescence intensity to a suitable range.

2. Synthesis of probe 1

![Scheme S1. Route for the synthesis of probe 1.](image-url)
2.1 Synthesis of 6-bromo-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (M1)

4-Bromo-1,8-naphthalic Anhydride (5 g, 18.05 mmol, 1 eqv.) and 2-propynylamine (1.09 g, 19.85 mmol, 1.1 eqv.) were dissolved in 100 mL dry ethanol under argon atmosphere. The mixture was stirred for 3 h at 70 °C. After reaction, the mixture was cooled down to room temperature and then poured into water (250 mL) to get the precipitate. The solid filtered and washed by Milli-Q water for three times, followed by air-drying at room temperature to get M1 as a white solid (5.3 g, 94%). The product was used directly for the next step.

2.2 Synthesis of 6-hydroxy-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (M2)

M1 (4.0 g, 12.73 mmol, 1 eqv.), N-hydroxysuccinimide (1.61 g, 14.01 mmol, 1.1 eqv.) and potassium carbonate (K₂CO₃) (5.81 g, 42.02 mmol, 3.3 eqv.) were dissolved in DMSO (60 mL) under argon atmosphere. The mixture was stirred at 80 °C for 3 h. After reaction, the mixture was cooled down to room temperature. The pH of the solution was adjusted to 3 by carefully adding HCl solution (1 M). The solid was precipitated and filtered, which was washed by Milli-Q water for three times. The crude production was air-dried and was purified by column chromatography using petroleum ether/ethyl acetate (2:1-1:1) as the eluent (2.8 g, 88%). ¹H NMR (400 MHz, DMSO) δ (ppm): 11.96 (s, 1H), 8.72 – 8.49 (m, 2H), 8.39 (dd, J = 14.4, 8.2 Hz, 1H), 7.78 (dt, J = 12.8, 8.1 Hz, 1H), 7.18 (t, J = 8.8 Hz, 1H), 4.76 (s, 2H), 3.10 (t, J = 2.4 Hz, 1H). ¹³C NMR (400 MHz, DMSO) δ. 171.0, 162.9, 162.2, 160.7, 133.9, 131.4, 129.3, 125.7, 122.4, 121.4, 112.1, 110.1, 79.6, 72.7, 28.8. HRMS: [M-H]⁺ 250.0510; found 250.0506.

2.3 Synthesis of 1,3-dioxo-2-(prop-2-yn-1-yl)-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl 2,4-dinitrobenzenesulfonate (M3)

M2 (100 mg, 0.4 mmol, 1 eqv.) was dissolved in 3 mL dry dichloromethane (DCM) and then the mixture was cooled down to 0 °C in an ice water bath. At this temperature, benzenesulfonyl chloride (111 mg, 0.48 mmol, 1.2 eqv.) was added to the above mixture. Then the mixture was increased to room temperature and was stirred for 3 h. Fine yellow
powders appeared and were filtered from the solution. The product was purified by column chromatography using DCM as the eluent (2.8 g, 88%) (185 mg, 96%). $^1$H NMR (400 MHz, DMSO) δ 9.16 (d, $J = 2.2$ Hz, 1H), 8.64 – 8.58 (m, 2H), 8.52 (d, $J = 8.1$ Hz, 1H), 8.39 (dd, $J = 12.6$, 8.6 Hz, 2H), 7.97 (t, $J = 7.9$ Hz, 1H), 7.71 (d, $J = 8.1$ Hz, 1H), 4.78 (d, $J = 2.2$ Hz, 2H), 3.16 (t, $J = 2.3$ Hz, 1H). $^{13}$C NMR (400 MHz, DMSO) δ 162.2, 161.6, 151.7, 148.6, 148.0, 133.7, 132.0, 131.5, 130.4, 128.8, 127.7, 124.8, 122.1, 121.5, 121.3, 120.0, 79.0, 73.1, 29.1. HRMS: [M-H]$^+$ 480.0143; found 480.0133.

2.4 Synthesis of probe 1
Azido PEG2000 (218 mg, 0.11 mmol, 1.05 eqv.), copper sulfate pentahydrate (10 mg, 0.041 mmol, 0.4 eqv.), and L-ascorbic acid (10 mg, 0.052 mmol, 0.5 eqv.) were dissolved in Milli-Q water (2 mL). The solution was bubbled with argon before use. M3 (50 mg, 0.1 mmol, 1 eqv.) was dissolved in DMF and was added dropwise into the above-prepared mixture under argon protection. The reaction mixture was stirred under argon at room temperature for ~ 3 h until M3 was completely reacted (monitoring via TLC). After that, DCM (10 mL) was added to the mixture, which was washed by water and dried with anhydrous magnesium sulfate. The filtrate was evaporated under reduced pressure. The product was purified by column chromatography. The solvent was evaporated and the product was obtained as yellow powders (100 mg, 85%). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.74 (d, $J = 2.2$ Hz, 1H), 8.67 (d, $J = 7.3$ Hz, 1H), 8.61 (d, $J = 8.1$ Hz, 1H), 8.55 (dd, $J = 8.6$, 2.2 Hz, 1H), 8.46 (d, $J = 8.0$ Hz, 1H), 8.30 (d, $J = 8.6$ Hz, 1H), 7.81 (t, $J = 7.9$ Hz, 2H), 7.67 (d, $J = 8.1$ Hz, 1H), 5.49 (s, 2H), 4.49 (t, $J = 5.1$ Hz, 2H), 3.88 – 3.44 (m, 180H), 3.38 (s, 3H), 1.84 (s, 8H). $^{13}$C NMR (500 MHz, CDCl$_3$) δ 162.7, 162.1, 151.7, 148.5, 148.1, 142.5, 133.8, 131.9, 131.4, 130.5, 128.9, 128.8, 127.8, 127.6, 124.8, 123.6, 122.5, 121.9, 121.4, 120.1, 86.3, 73.9, 69.8, 58.0, 49.3, 35.3. The MS of the compound was also measured (Figure S9).
Figure S1. $^1$H NMR spectrum of M2 (400 MHz, DMSO-d$_6$).

Figure S2. $^{13}$C NMR spectrum of M2 (400 MHz, DMSO-d$_6$).
Figure S3. $^1$H NMR spectrum of M3 (400 MHz, DMSO-d$_6$).

Figure S4. $^{13}$C NMR spectrum of M3 (400 MHz, DMSO-d$_6$).
Figure S5. $^1$H NMR spectrum of probe 1 (500 MHz, CDCl$_3$).

Figure S6. $^{13}$C NMR spectrum of probe 1 (500 MHz, CDCl$_3$).
Figure S7. High-resolution mass spectrum of M2.

Figure S8. High-resolution mass spectrum of M3.
3. DFT calculations
The molecular geometries (the atomic standard orientations after geometry optimization of ground state and excited state have been given in Supporting Information) and the molecular orbital levels were investigated using density functional theory (DFT) and time-dependent density functional theory (TDDFT) calculations, respectively. Becke’s three-parameter hybrid exchange functional with the non-local correlation provided by Perdew 86 (B3P86 functional) [2] and the triple-ζ valence quality with one set of polarization functions (TZVP) basis set [3] were chosen throughout. All calculations on electronic structures were carried out using the Gaussian 09 program suite [4].

4. Sensing property of probe 1 for anions
To examine the selectivity of the probe to H2S. The potential property of the probe for sensing anions was studied. Probe 1 was dissolved in 3 mL distilled water to obtain the probe aqueous solutions (5 µM), and then NaxA (Ax-= Cl−, Br−, HCO3−, HSO3−, SO42−, CNS−, COO−and NO3−) was added to above probe aqueous solutions, respectively, and the concentration of anions was 1 mM. Meanwhile, the absoption and fluorescent emission spectra of the probe-anions were measured.
5. Determination of the detection limit

The detection limit was calculated based on the method reported in previous literature [51]. The fluorescence emission spectrum of probe 1 was measured by three times and the standard deviation of blank measurement was achieved. The fluorescence intensity at 670 nm was plotted as a concentration of \( \text{H}_2\text{S} \). The detection limit was calculated by using \( \text{detection limit} = 3\sigma/k \): where \( \sigma \) is standard deviation of blank measurement, \( k \) is the slope between the fluorescence intensity versus \( \text{H}_2\text{S} \) concentration.


For practical applications, we made the probe 1 into a test paper. Firstly, we cut the filter paper into a test paper strip. Then, the test paper was put into the prepared probe solution (DMSO, 5 mM, 50 µL). Noted that each test paper contains the same amount of probe. The test paper was then dried in the drying oven at 60 °C. Finally, the test paper was obtained in reserve.

7. The \( \text{H}_2\text{S} \) gas detection by using test paper of probe 1.

The \( \text{H}_2\text{S} \) gas with a concentration of 500 ppm (a mixture with air) are commercially available and \( \text{H}_2\text{S} \) with other concentrations (25 ppm and 100 ppm) was prepared by quantitatively diluting the commercial \( \text{H}_2\text{S} \) gas with air. Before performing the gas detection by using the probe test paper, \( \text{H}_2\text{S} \) gases were inserted into the glass bottles and flowed for 30s to make sure the air was removed and replaced with \( \text{H}_2\text{S} \) gas. After that the test paper of probe 1 was inserted into the glass bottle for sensing.
Figure S10. Frontier molecular orbital (MO) of M3 (A) and M2 (B) calculated with density functional theory/time-dependent density functional theory (DFT/TDDFT) using Gaussian 09.
Figure S11. High-resolution mass spectrum of probe 1 after reaction with H$_2$S. $[\text{M-H}]^+$ 250.0506; found 250.0506. The result indicates that the product after reaction with H$_2$S compound is M3.

Figure S12. UV-vis spectra of probe 1 (5 µM) in the presence of NaSH (50 µM) and other anions (Cl$^-$, Br$^-$, HCO$_3^-$, HSO$_3^-$, SO$_4^{2-}$, CNS$^-$, COO$^-$ and NO$_3^-$, 200 µM) in PBS (pH 7.4). Measured after 2 min of mixing.
Figure S13. Fluorescence spectra of probe 1 (5 µM) in the presence of NaSH (50 µM) and other anions (Cl\(^{-}\), Br\(^{-}\), HCO\(_3\)^{-}, HSO\(_3\)^{-}, SO\(_4\)^{2-}, CNS\(^{-}\), COO\(^{-}\) and NO\(_3\)^{-}, 200 µM) in PBS (pH 7.4). Measured after 2 min of mixing. Excitation: 450 nm.

Figure S14. Fluorescence responses of M2 (5 µM) and M3 (5 µM) to various pH in PBS buffer (0.1 M, pH 7.4).
Figure S15. Egg spoilage Identification by using probe text paper: (a) control group; (b) fresh egg without shell; (c) the egg shell was broken and stored for 3 days at room temperature. The egg without shell was put into the glass bottle and then the text paper was inserted.

References: