Electronic Supplementary Material (ESI) for Analytical Methods.


**Supplementary Information (SI)**

# A mitochondrial and lysosomal targeted ratiometric probe for detecting intracellular $H_2S$

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*Materials and instruments*

3-aminophenol, 2-bromo-3-chloropropane, 2,4-dihydroxybenzaldehyde, acetacetic ester, N,N-dimethylformamide, acetic ether, dichloromethane , anhydrous ethanol, anhydrous methanol, petroleum ether, dimethyl sulfoxide, methyl sulfonic acid, $NaHCO_3$, $POCl_3$, $K_2CO_3$, $Na_2SO_4$, $NaHSO_3$, NaHS, NaOH, NaF, NaCl, NaBr, NaI, KCl, $CaCl_2$, $AlCl_3$, $ZnCl_2$, $FeCl_2$, $FeCl_3$, $SnCl_2$, $Pb(NO_3)_2$, CuCl, $CuCl_2$, $MgCl_2$, AgCl, $Ni(NO_3)_2$, $MnCl_2$, $Co(NO_3)_2$, $Cd(NO_3)_2$, $Na_3PO_4$, $Na_2HPO_4$, $NaH_2PO_4$, $Na_2CO_3$, $NaBF_4$, NaI, $NaN_3$, NaSCN, $NaNO_3$, $NaHSO_4$, $Na_2C_2O_4$, $NaNO_2$, Ala, Arg, Asp, Cys, Gln, Glu, Gly, GSH, Hcy, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Val, $H_2O_2$.

Human serum was purchased from Yilaisa Biotechnology Co., Ltd. (Jiangsu, China).

Simulate urine environment with NaCl 8 g, urea 20 g, $MgSO_4$ (hexahydrate) 0.8 g, $CaCl_2$ (dihydrate) 0.3 g, was diluted with distilled water to 1 L.

Dual-beam UV-vis spectrophotometer (TU-1901), fluorospectrophotometer (F-4600), pH meter (PHS-2F), 400 MHz NMR spectrometer (AVIII HD 400), 600 MHz NMR spectrometer (AVIII HD 600), high-resolution mass spectrometer (IonSpec4.7), rotary evaporator (RE-2000B), electronic analytical balance (FA2004), vacuum drying oven (DZF-6020), constant temperature magnetic stirrer (85-2), ultrasonic cleaner (SB-100D), circulating water vacuum pump (SHB-3), vacuum oil pump (2XZ-4), digital camera (D3300), portable UV analyzer (ZF-5).

*Synthesis of product B1*

3-aminophenol (2.05 g, 18.785 mmol), $NaHCO_3$ (6.3125 g, 75.14 mmol) and 1-bromo-3-chloropropane (11.83 g, 75.14 mmol) were added to a 100 mL round bottom flask and were evenly dissolved in DMF (30 mL). Then the reaction mixture was stirred at 70 ℃ for 12 h until the reaction was completed. After cooling to room temperature, the reaction solution was poured into ice water. The solution was extracted with ethyl acetate, and then washed with water. The remaining solution was dried by $Na_2SO_4$ and further purified by silica gel column chromatography ($V_{PE}$ : $V_{EA}$ = 20 : 1) to get white solid B1 (900 mg, yield: 25.29 %).

$^1$H NMR (600 MHz, DMSO-$d_6$) δ 8.63 (s, 1H), 6.48 (d, $J$ = 8.0 Hz, 1H), 6.00 (d, $J$ = 8.0 Hz, 1H), 3.03 – 2.97 (m, 4H), 2.57 (t, $J$ = 6.5 Hz, 2H), 2.51 (d, $J$ = 6.3 Hz, 2H), 1.86 – 1.80 (m, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 153.0, 143.6, 126.1, 112.0, 107.7, 103.0, 49.6, 49.1, 26.7, 22.1, 21.5, 21.0.

*Synthesis of product B2*

2 mL of dried DMF was placed in a round neck flask and stirred in an ice-water bath for 15 min. Then 0.5 mL of $POCl_3$ was added dropwise to the flask and stirring for 30 min. The product B1 (189.2 mg, 1 mmol) from the previous step dissolved in DMF was added dropwise to the reaction system and stirred at room temperature for 1 h. Then, the temperature was raised to 100°C for 1 h and the heating was stopped. After cooling to room temperature, 10 mL of water was added to the reaction flask while the pH of the reaction system was adjusted to 6-8 with saturated potassium carbonate solution, and stirring was continued for 1 h. After the reaction was completed, it was extracted with $CH_2Cl_2$, and then the organic phase was washed with saturated brine, and dried overnight with anhydrous sodium sulfate. After silica gel column chromatography, a light yellow solid product is obtained. (154 mg, yield: 70.9%).

$^1$H NMR (600 MHz, DMSO-$d_6$) δ 11.85 (s, 1H), 9.36 (s, 1H), 6.98 (s, 1H), 3.28 (dd, $J$ = 12.2, 7.3 Hz, 4H), 2.62 (t, $J$ = 6.2 Hz, 2H), 2.54 (t, $J$ = 6.4 Hz, 2H), 1.83 (dd, $J$ = 12.1, 6.1 Hz, 4H).$^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 191.6, 158.4, 149.2, 130.9, 113.4, 110.0, 104.2, 49.5, 49.1, 26.5, 21.0, 19.9, 19.2.

*Synthesis of product B3*

B2 (217 mg, 1 mmol) was dissolved in 5 mL of absolute ethanol, and ethyl acetate (260 mg, 2 mmol) and piperidine (80 μL, 0.81 mmol) were added to the reaction solution. Under the protection of argon, it was heated to reflux for 5 h and then cooled to room temperature. Filtered with suction and washed with n-hexane to obtain an orange solid (205.6 mg, yield: 72.57%).

$^1$H NMR (600 MHz, $CDCl_3$) δ 8.33 (s, 1H), 6.96 (s, 1H), 3.34 (dd, $J$ = 12.0, 6.5 Hz, 4H), 2.88 (t, $J$ = 6.4 Hz, 2H), 2.76 (t, $J$ = 6.2 Hz, 2H), 2.67 (s, 3H), 2.01 – 1.94 (m, 4H).$^{13}$C NMR (151 MHz, $CDCl_3$) δ 196.0, 161.3, 153.8, 148.8, 147.8, 127.8, 114.9, 108.0, 105.6, 50.4, 50.0, 30.6, 27.4, 21.2, 20.2, 20.1.

*Synthesis of product HABA*

B3 (283.4 mg, 1 mmol) and 2,4-dihydroxybenzaldehyde (165.74 mg, 1.2 mmol) were dissolved in methanesulfonic acid and stirred at 90 °C for 6 h. After the reaction is completed, it is cooled to room temperature, the reaction solution is added dropwise to ice brine, and a precipitate is formed. The precipitate is suction filtered and washed with water. The resulting crude product is separated by silica gel column chromatography to obtain a dark green solid (240 mg, yield: 62.11%).

$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 8.86 (s, 1H), 8.75 (d, $J$ = 9.1 Hz, 1H), 8.43 (d, $J$ = 9.1 Hz, 1H), 7.99 (d, $J$ = 8.7 Hz, 1H), 7.27 (dd, $J$ = 22.3, 12.4 Hz, 4H), 3.55 (d, $J$ = 4.5 Hz, 4H), 2.76 – 2.65 (m, 4H), 1.92 (s, 4H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 167.7, 167.0, 158.3, 157.5, 153.5, 153.2, 149.9, 145.3, 132.4, 130.0, 123.2, 119.6, 117.2, 114.6, 112.0, 106.4, 102.8, 50.9, 26.8, 19.4.

*Preparation of test solution*

HABA was diluted to 1 mM with chromatographic grade DMSO. Deionized water was used to prepare various cation and anion stock solutions at a concentration of 0.01 M, the concentration of $H_2S$ was 0.05 M. UV-vis and the fluorescence spectra of HABA were obtained in DMSO-$H_2O$ (v/v, 3:7) solution (10 μM, 3 mL). The excitation wavelength for fluorescence measurement was 440 nm, and the slit widths were 5 nm and 2.5 nm; the excitation wavelength for fluorescence measurement was 630 nm, and the slit widths were 10 nm and 10 nm.

## Figures

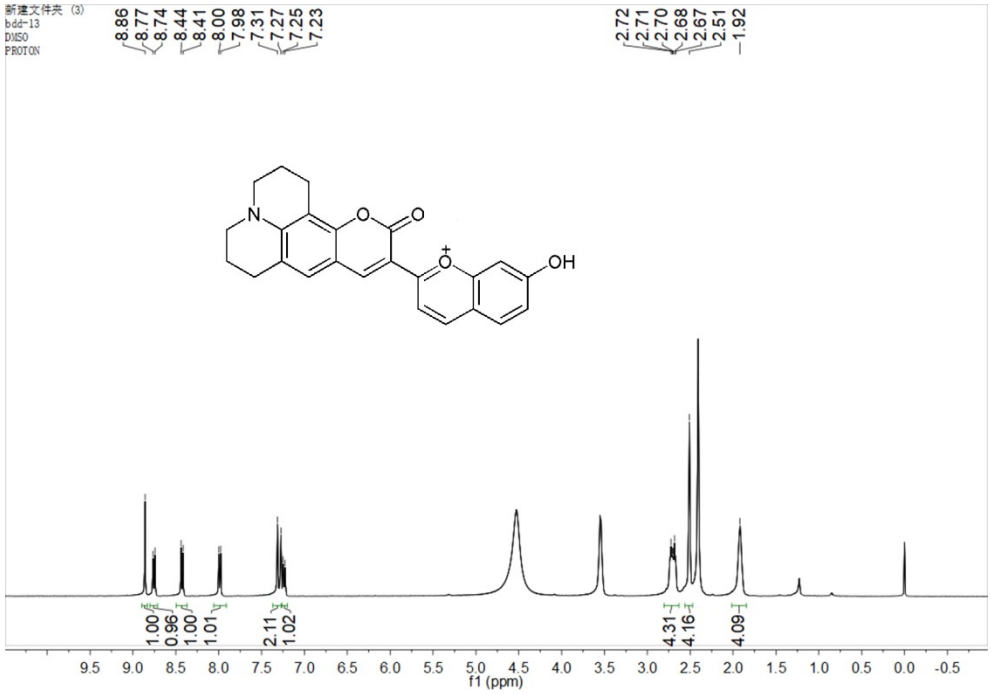


**Fig. S1.** $^{1}$H NMR spectrum of HABA.

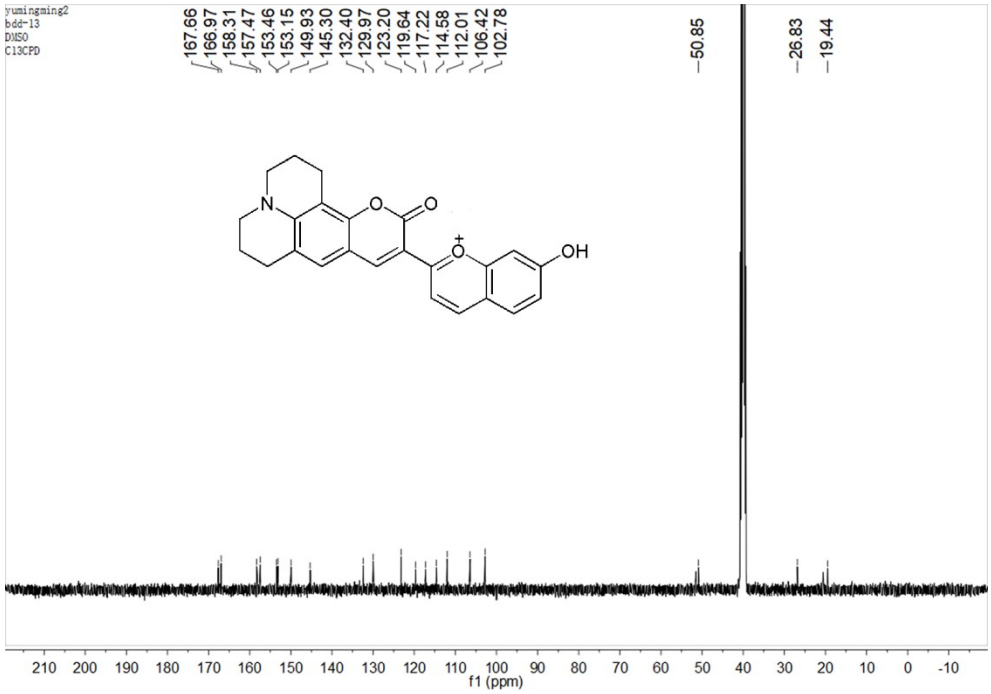


**Fig. S2.** $^{13}$C NMR spectrum of HABA.

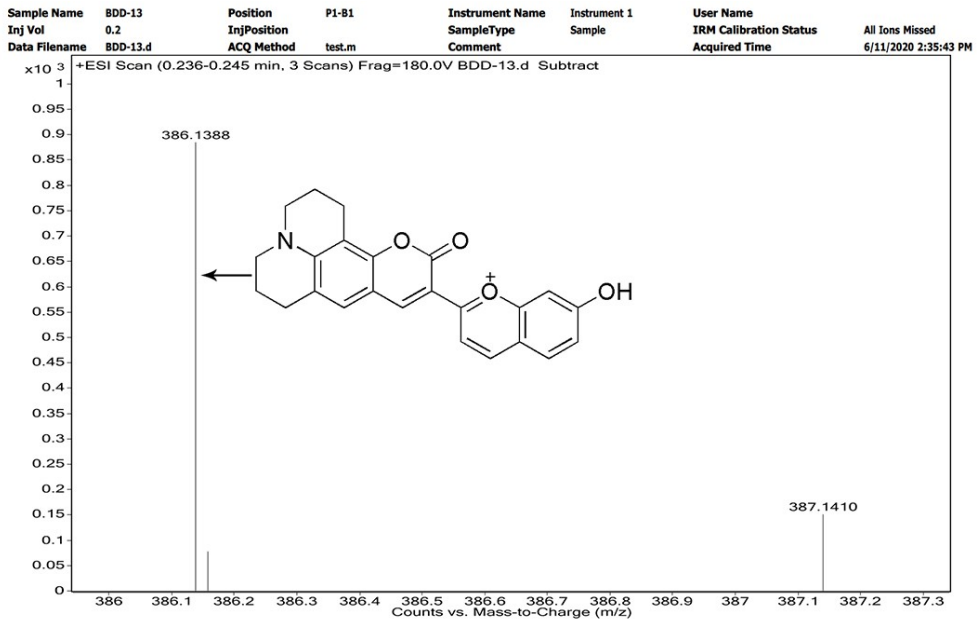


**Fig. S3.** High-resolution mass spectrum of HABA.

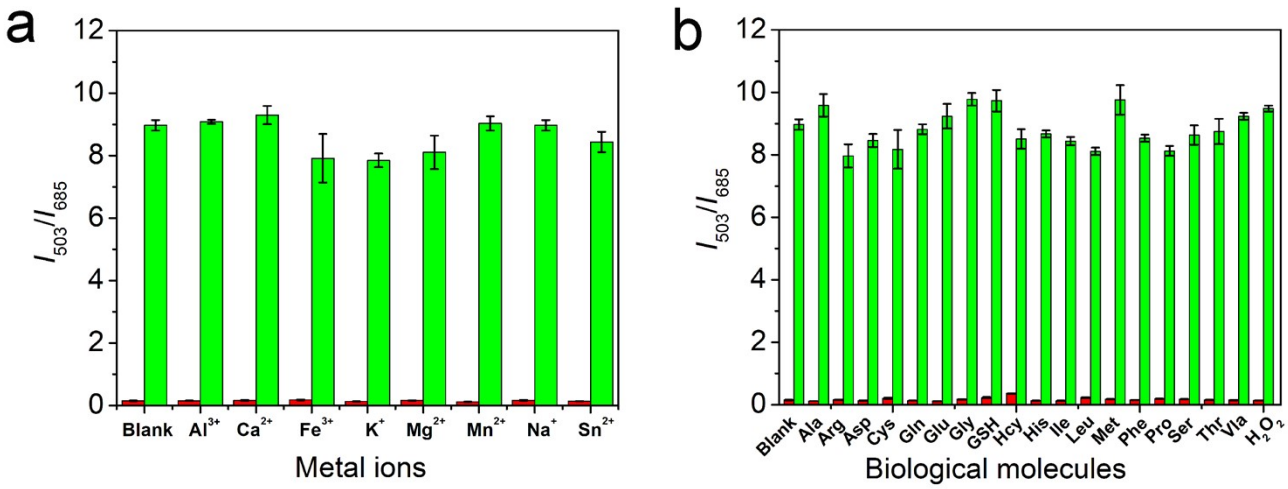


**Fig. S4.** (a) Emission spectral responses of HABA ($V_{PBS}$:$V_{DMSO}$ =7:3, $1\times10^{-5}$ mol/L) in the presence of indicated metal ions (260 μmol/L) ($Al^{3+}$, $Ca^{2+}$, $Fe^{3+}$, $K^{+}$, $Mg^{2+}$, $Mn^{2+}$, $Na^{+}$, $Sn^{2+}$) at $I_{503}/I_{685}$ (red bars) and emission spectral responses of HABA toward $H_2S$ (260 μmol/L) at $I_{503}/I_{685}$ under the presence of above metal ions (green bars). (b) Emission spectral responses of HABA ($V_{PBS}$:$V_{DMSO}$ =7:3, $1\times10^{-5}$ mol/L) in the presence of indicated biological small molecules (260 μmol/L) (Ala, Arg, Asp, Cys, Gln, Glu, Gly, GSH, Hcy, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Val, $H_2O_2$) at $I_{503}/I_{685}$ (red bars) and emission spectral responses of HABA toward $H_2S$ (260 μmol/L) at $I_{503}/I_{685}$ under the presence of above biological small molecules (green bars), $\lambda_{ex}$=440 nm and 630 nm.

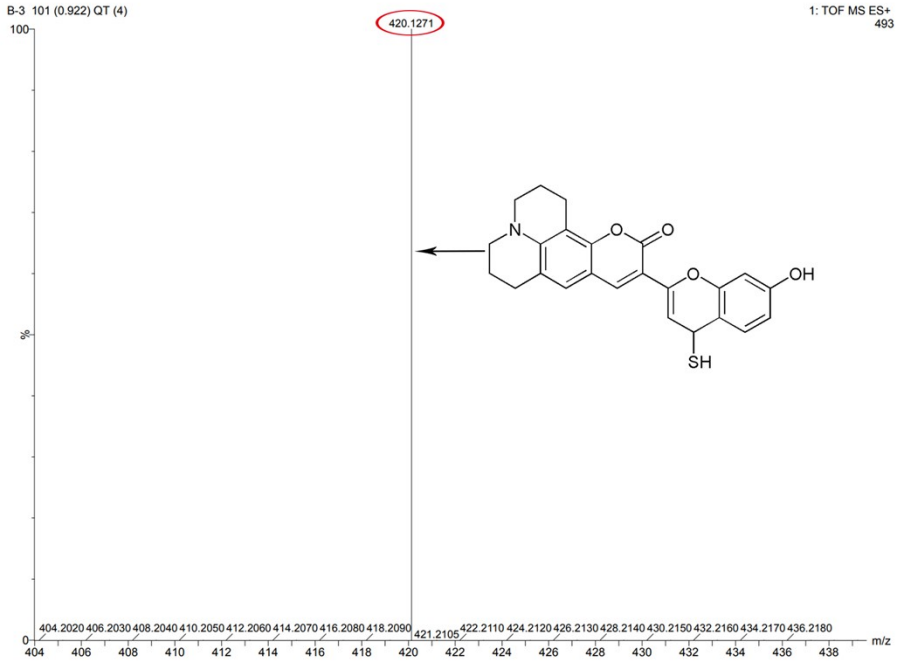


**Fig. S5.** High-resolution mass spectrum of probe HABA reacted with $H_2S$.

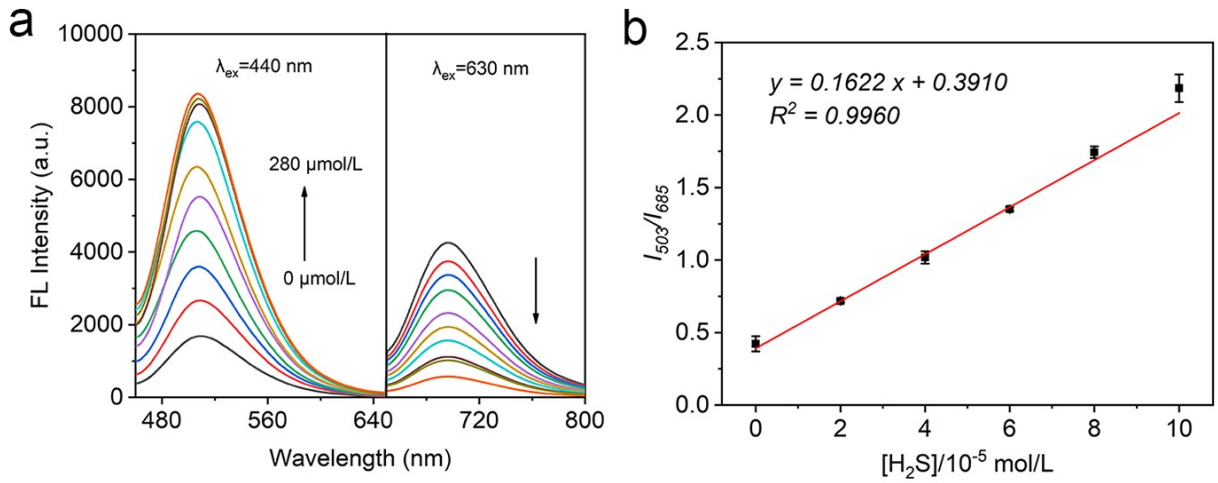


**Fig. S6.** (a)The fluorescence emission spectra of HABA upon addition of different concentrations of $H_2S$ in human serum. (c) The linear relationship between fluorescence intensity ratio ($I_{503}/I_{685}$) and concentrations of $H_2S$ from 0-100 μmol/L.

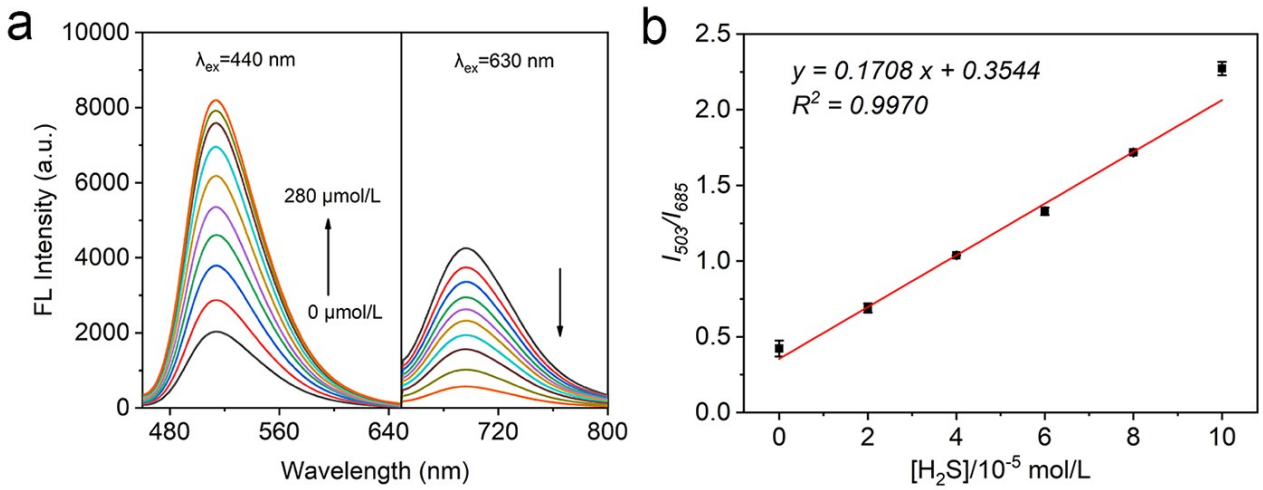


**Fig. S7.** (a) The fluorescence emission spectra of HABA upon addition of different concentrations of $H_2S$ in urine fluids. (b) The linear relationship between fluorescence intensity ratio ($I_{503}/I_{685}$) and concentrations of $H_2S$ from 0-100 μmol/L.

**Table S1. The cytotoxicity of HABA.**

| Concentration of HABA (μM) | 0.01 | 0.08 | 0.63 | 1.25 |
|---|---|---|---|---|
| Cell viability | 0.987 | 0.997 | 0.987 | 0.871 |

**Table S2. Comparison of various fluorescent probes employed for H2S recognizing.**

| probe | Detection mode | Detection condition | Response time | LOD | pH ranges | Ref |
|---|---|---|---|---|---|---|
| NFOS | Off-on | PBS/DMSO | 10 min | 0.5 μM | 6.8-8 | [S1] |
| DN-DM | Off-on | EtOH-HEPES | 30 min | 3.9 μM | 3-11 | [S2] |
| DN-DA | Off-on | EtOH-HEPES | 20 min | 9.6 μM | 4-9 | [S2] |
| H-LDS | Off-on | EtOH-PBS | 12 min | 0.57 μM | 4-10 | [S3] |
| P1 | Off-on | PBS/DMSO | 15 min | 1.06 μM | 6-8 | [S4] |
| HABA | Ratiometic | PBS/DMSO | 8 min | 1.11 μM | 6-10 | This work |

**References**

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S4 J. Yang, C. Yin, Y. Wen, Y. Zhang and F. Huo, *New J. Chem.*, 2020, **44**, 7091.