

## Electronic Supplementary Information

### Fast and sensitive fluorescent probe for hydrogen sulfide ratiometric detection in mitochondria

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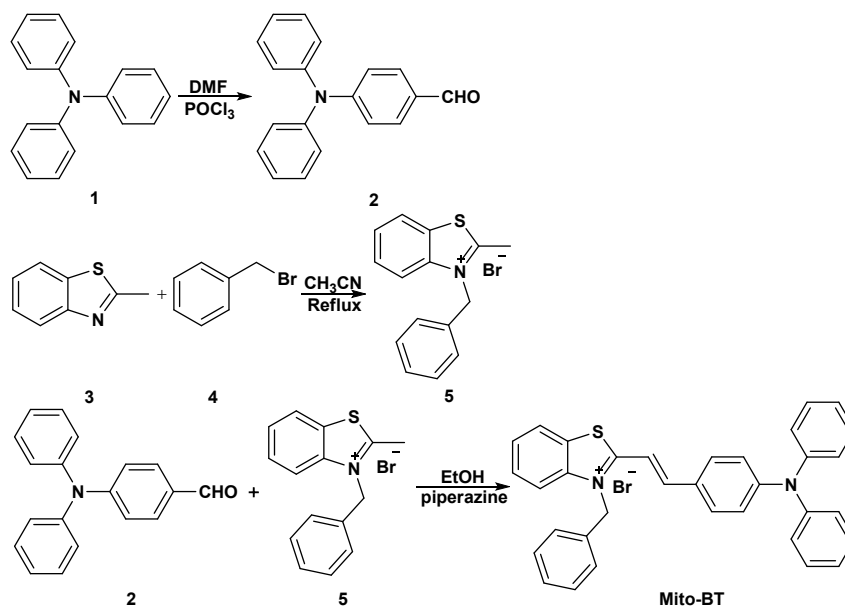
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#### Synthetic procedures of dye and intermediates



**Scheme S1.** Synthesis of intermediates and **Mito-BT**.

#### (1) 2, the synthesis of 4-(diphenylamino)benzaldehyde.<sup>S1</sup>

POCl<sub>3</sub> (4.0 mL) was dropped slowly into DMF (16 mL) at 0 °C and stirred for another 2 h at room temperature. To the above solution was added a dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>)

solution of triphenylamine (7.5 g, 31 mmol). After the mixture was refluxed for 10 h  $\text{CH}_2\text{Cl}_2$  was removed. The residue was poured into water (500 mL) and the yellow solid was collected by suction filtration. Product was obtained by column chromatography (7.2 g, yield 86%).

**(2) 5, the synthesis of 3-benzyl-2-methylbenzothiazolium bromide salt.**

Benzyl bromide (0.85g, 5 mmol) was added under nitrogen to 2-methyl benzothiazole (0.90g, 6 mmol) with stirring at 60-70 °C for 3 h. The mixture was then cooled, the precipitate filtered off, and washed with ether to give 3-benzyl-2-methylbenzothiazolium bromide, 3-benzyl-2-methylbenzothiazolium bromide salt 1a was obtained as white solid as crude product (2.6 g, yield 81%).

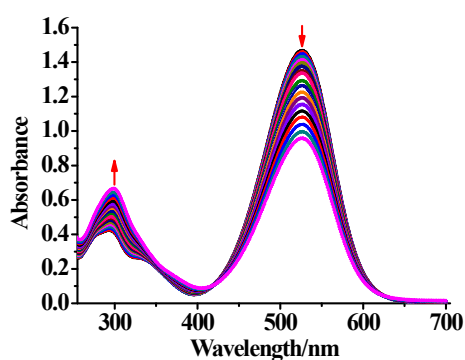
**(3) the synthesis of Mito-BT.**

**Mito-BT** was conveniently synthesized via the condensation of compound **5** with **2** (Scheme 1). Compound **5** (1.4 g, 4.4 mmol), compound **2** (1.0 g, 3.6 mmol), triethylamine (3 drops) were mixed in an absolute ethanol solution (20 mL). The solution was refluxed under nitrogen for 24 h, and then cooled down. The precipitate was collected, washed with diethyl ether, then dried, giving **Mito-BT** as a violet solid (1.49 g, 82.4%).  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  8.43 (d,  $J = 7.7$  Hz, 1H), 8.24 (d,  $J = 15.4$  Hz, 1H), 8.14 (d,  $J = 8.2$  Hz, 1H), 7.95 (dd,  $J = 21.5, 12.1$  Hz, 3H), 7.75 (dt,  $J = 15.2, 7.2$  Hz, 2H), 7.43 (t,  $J = 7.8$  Hz, 4H), 7.36 (dd,  $J = 14.7, 6.6$  Hz, 5H), 7.30 – 7.17 (m, 8H), 6.88 (d,  $J = 8.8$  Hz, 2H), 6.24 (s, 2H). HR-MS (ESI):  $m/z$ , calcd for  $\text{C}_{34}\text{H}_{27}\text{N}_2\text{S}^+$  495.1889, found 495.1885.

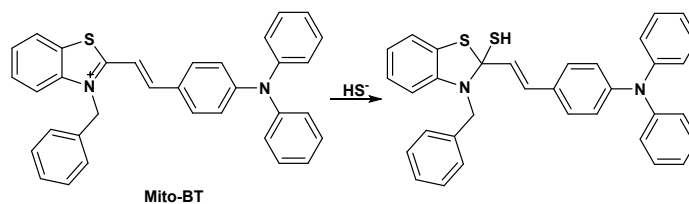
**Table S1.** Spectral properties of **Mito-BT** in various solvents.

Solvents	$\lambda_{\text{abs}}^{\text{a}}$	$\lambda_{\text{em}}^{\text{b}}$	$\Delta\lambda^{\text{c}}$	$\Phi_{\text{f}}^{\text{d}}$	$\varepsilon^{\text{e}}$
C <sub>6</sub> H <sub>5</sub> CH <sub>3</sub>	300, 532	654	122	0.011	15700, 43700
Dioxane	303, 527	651	124	0.239	14300, 39000
CH <sub>3</sub> CN	295, 522	620	98	0.061	14500, 45700
CH <sub>3</sub> CH <sub>2</sub> OH	295, 531	649	118	0.058	13700, 48500
CH <sub>3</sub> OH	292, 527	643	116	0.065	14200, 50600
DMF	297, 520	635	115	0.108	15400, 40200
DMSO	298, 518	645	127	0.073	14300, 41700
H <sub>2</sub> O	297, 515	663	148	0.014	12900, 40600

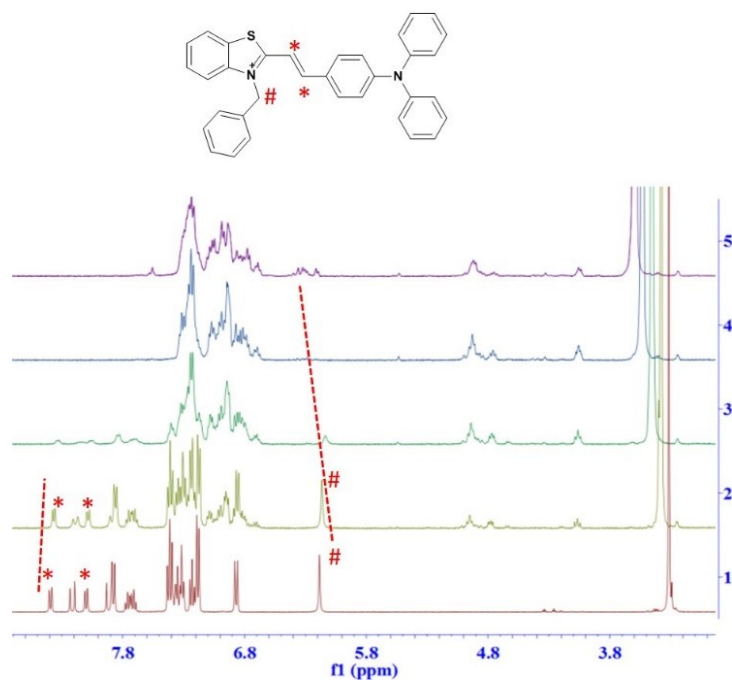
[a] The two absorption peaks of **Mito-BT** (nm). [b] The emission peak of **Mito-BT** (nm), excited at 460 nm. [c] Stokes shift of **Mito-BT** (nm). [d] Fluorescence quantum yields of **Mito-BT**. [e] mol<sup>-1</sup> cm<sup>-1</sup> L.



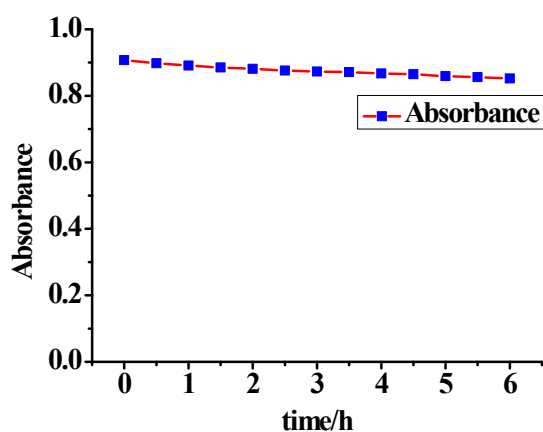
**Fig. S1.** Absorption spectra of **Mito-BT** (30  $\mu\text{M}$ ) in water-ethanol (v/v=1:4) upon titration with HS<sup>-</sup> (0–60  $\mu\text{M}$ ).



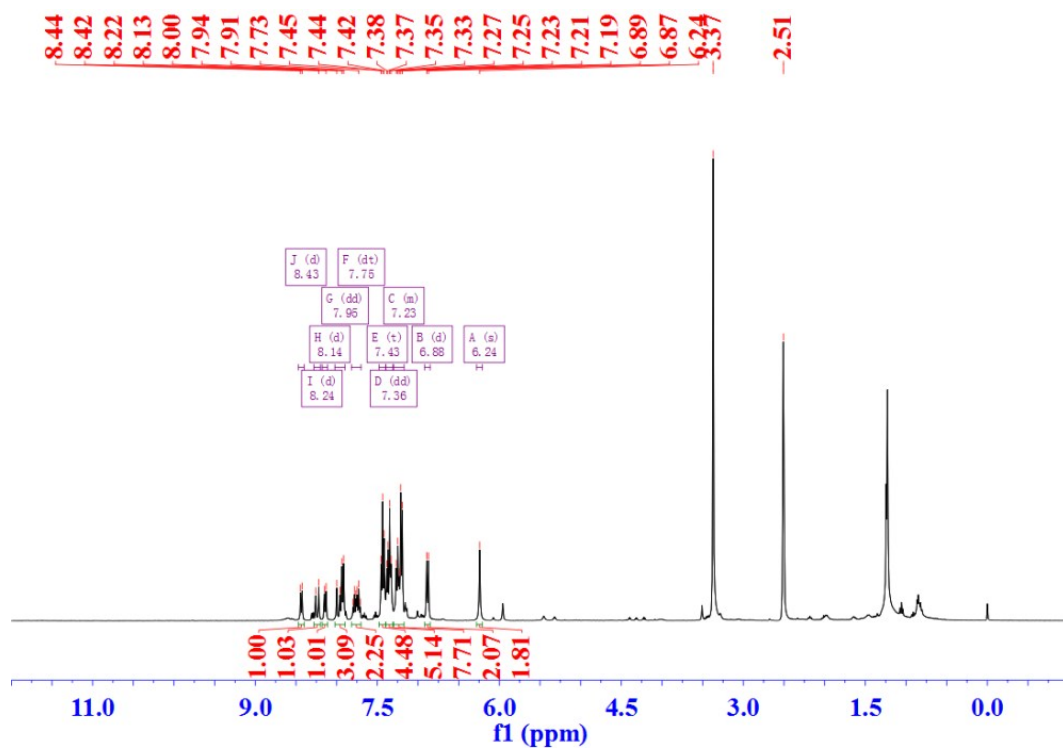
**Fig. S2.** Proposed H<sub>2</sub>S sensing mechanism of **Mito-BT**.



**Fig. S3.** The sensing mechanism of **Mito-BT** for  $\text{HS}^-$ .  $^1\text{H}$  NMR spectral change of **Mito-BT** (10 mM) in the absence and presence (2, 3, 4, 5) of  $\text{HS}^-$ , the gradient of  $\text{HS}^-$  is 2 mM. The  $^1\text{H}$  NMR titration experiment was performed for examining the sensing mechanism of **Mito-BT** to  $\text{HS}^-$ . **Mito-BT** was dissolved in DMSO to get a mixed solution of 10 mM. Then, a solution of  $\text{HS}^-$  in  $\text{D}_2\text{O}$  was added into the above solution in a gradient of 2 mM.



**Fig. S4.** The photofading experiment of **Mito-BT** (30  $\mu\text{M}$ ) in DMSO under 1000 W iodine-tungsten lamp.



$^1\text{H}$  NMR (400 MHz) spectra of **Mito-BT** in  $\text{d}^6\text{-DMSO}$ .

#### References:

S1: P. Xue, P. Chen, J. Jia, Q. Xu, J. Sun, B. Yao, Z. Zhang, R. Lu, *Chem. Commun.*, 2014, **50**, 2569.