Supporting Information

Probing fluctuations in sulfur dioxide and viscosity levels during mitochondrial dysfunction using a dual-response fluorescent probe with good water solubility

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1. Experimental Section

Materials and Instruments. All commercial chemicals were purchased from suppliers and used as received. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker 400 spectrometer. The mass spectra were obtained using a Bruker Daltonics micr-OTOF-Q II spectrometer. Emission spectra were recorded on a Hitachi F-7000 fluorometer, and UV-vis absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer. A Leici PHS-3C pH meter was used for pH measurements. Fluorescence imaging experiments were conducted using an Olympus FV3000 inverted microscope. HeLa cells were obtained from Xiangya Hospital at Central South University, China.

Detection Limit. The detection limit was calculated according to the following equation:

$$\text{Detection limit} = 3\sigma/k$$

where σ is the standard deviation of the blank measurement, and k is the slope between the fluorescence intensity (F_{477nm}) and SO_2 concentration.

Cytotoxicity Test. Exponential growth Hela cells were cultured in a high-glucose Dulbecco's modified Eagle medium (DMEM) at 37°C and a 5% CO_2 atmosphere. Hela cells were seeded in 96-well plates at 10^4 well^{-1} and incubated for 24 h, then different concentrations (0 μM, 5.0 μM, 10.0 μM, 15.0 μM, and 20.0 μM) of **MBI** was added into 96-well plates and incubated for 24 h. The cell viability was detected by MTT assay following a standard protocol.

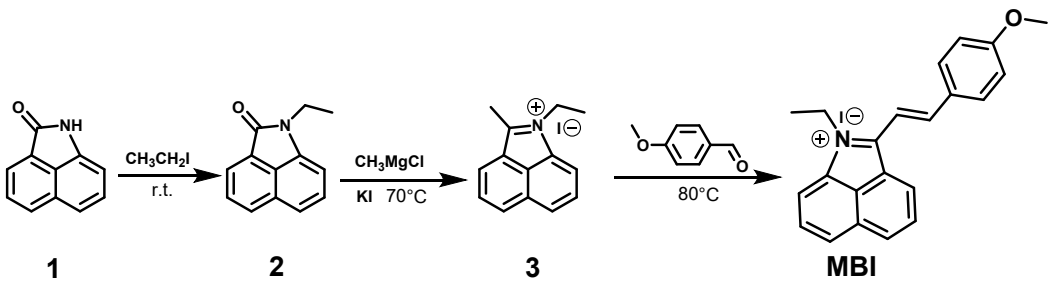
Colocalization experiments. HeLa cells were incubated with probe **MBI** (10.0 μM) in the culture medium for 30 min at 37 ℃, and then the cells were washed with PBS three times. Commercial fluorescent dyes including Mito-tracker Green, ER-tracker Green, and Lyso-tracker Green (1.0 μM) were added and co-incubated for another 30 min, and cell imaging was then carried out after washing the cells with PBS three times. Green channel: λ_{abs} = 405 nm, λ_{em} = 500-550 nm; Red channel: λ_{abs} = 488 nm, λ_{em} = 570-670 nm.

Fluorescence Imaging of SO_2 in Living Cells. For fluorescence imaging of exogenous SO_2 in living cells, HeLa cells were divided into two groups: the first group were incubated with probe **MBI** (10.0 μM) only for 30 min; the second group were pre-treated with probe **MBI** (10.0 μM) for 30 min, and then incubated with Na_2SO_3 (100.0 μM) for another 30 min. For fluorescence imaging of endogenous SO_2 in living cells, HeLa cells were divided into two groups: the first group were incubated with probe **MBI** (10.0 μM) only for 30 min; the second group were pre-treated with probe **MBI** (10.0 μM) for 30 min, and then incubated with GSH (500.0 μM) and $Na_2S_2O_3$ (250.0 μM) for another 30 min. After washing three times with PBS buffer, the imaging experiments were carried out. Cyan channel: λ_{ex} = 405 nm, λ_{em} = 450-500 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 570-670 nm.

Fluorescence Imaging of Viscosity in Living Cells. HeLa cells were divided into two groups: the first group were incubated with probe **MBI** (10.0 μM) only for 30 min; the second group were pre-treated with probe **MBI** (10.0 μM) for 30 min, and then incubated with nystatin (10.0 μM) for another 30 min. After washing three times with PBS buffer, the imaging experiments were carried out. Cyan channel: λ_{ex} = 405 nm, λ_{em} = 450-500 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 570-670 nm.

Fluorescence Imaging of SO_2 and Viscosity during Cell Apoptosis Induced by Cisplatin. HeLa cells were divided into two groups: the first group were incubated with probe **MBI** (10.0 μM) only for 30 min; the second group were pre-treated with probe **MBI** (10.0 μM) for 30 min, and then incubated with cisplatin (10.0 μM) for another 30 min. After washing three times with PBS buffer, the imaging experiments were carried out. Cyan channel: λ_{ex} = 405 nm, λ_{em} = 450-500 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 570-670 nm.

2. Synthesis of Probe MBI



Scheme S1. Synthesis route for probe **MBI**.

Synthesis of compounds 2 and 3. The synthesis of compounds **2** and **3** was referred to the literature that has been reported.[1]

Synthesis of probe MBI. Compound **3** (100.0 mg, 0.31 mmol) and anisaldehyde (42.0 mg, 0.31 mmol) were dissolved in 5.0 mL absolute ethanol and the mixture was refluxed for 6 h under argon protection. After the reaction, the solvent was removed under reduced pressure and 20 mL of ethyl acetate was added. Stirring was continued at 80 °C for 1 h, followed by sonication for 30 min. The resulting solution was then cooled to room temperature to produce a precipitate, which was filtered and dried to obtain probe **MBI** as black solid (105.0 mg, 71.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (t, J = 6.1 Hz, 1H), 8.84 (dd, J = 16.0, 4.8 Hz, 1H), 8.72 (t, J = 6.3 Hz, 1H), 8.36 (dt, J = 14.4, 6.2 Hz, 2H), 8.29 (dd, J = 8.7, 5.8 Hz, 2H), 8.16 (t, J = 7.7 Hz, 1H), 7.94 (dt, J = 23.3, 9.2 Hz, 2H), 7.21 (s, 2H), 4.93 – 4.80 (m, 2H), 3.93 (d, J = 3.7 Hz, 3H), 1.60 – 1.49 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.28, 161.97, 153.79, 137.08, 135.52, 133.72, 131.36, 130.27, 129.21, 128.23, 127.83, 127.41, 119.30, 115.44, 112.70. HRMS (ESI) m/z：*calcd* for $C_{22}H_{20}NO^{+}[M]^{+}$, 314.15; found, 314.1502.

3. Spectroscopic Property

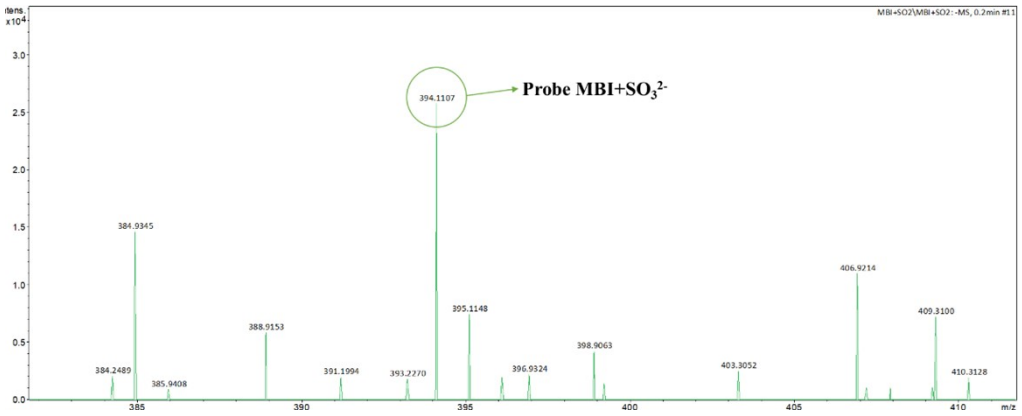


Fig. S1 HRMS spectrum of the reaction mixture of probe **MBI** and SO_2.

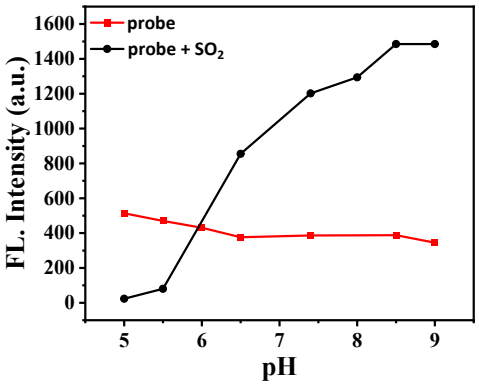


Fig. S2 pH influence on the fluorescence intensity of **MBI** (10.0 μM) at 477 nm with and without SO_2 (2.0 μM) in water. Excitation wavelength: 405 nm.

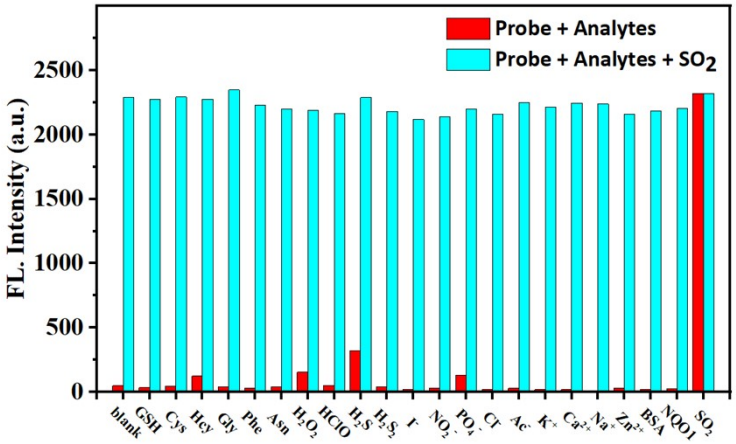


Fig.S3 Fluorescence intensity of probe **MBI** (10.0 μM) at 477 nm in the absence and presence of SO_2 (2 μM) with the co-existence of relevant species (200.0 μM), including GSH, Cys, Hcy, Gly, Phe, Asn, H_2O_2, HClO, H_2S, H_2S_2, I^-, NO_2^-, PO_4^-, Cl^-, Ac^-, K^+, Ca^{2+}, Na^+, Zn^{2+}, BSA, and NQO1. Excitation wavelength: 405 nm.

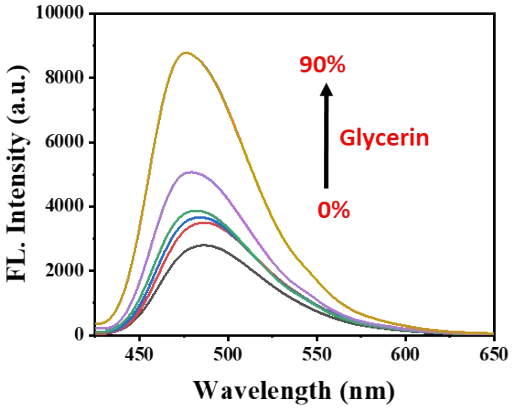


Fig.S4 Fluorescence spectra of probe **MBI** (10.0 μM) in the presence of SO_2 (2.0 μM) under different viscosity conditions. Excitation wavelength: 405 nm.

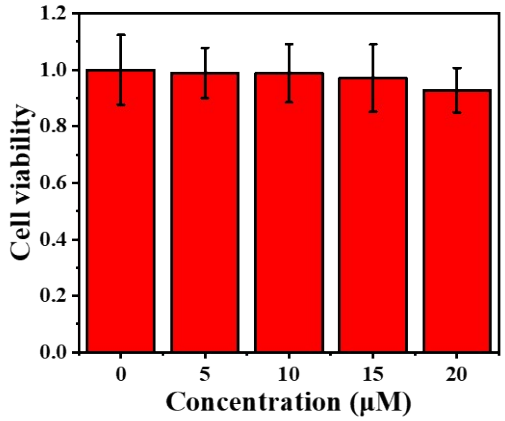


Fig.S5 MTT assay of Hela cells incubated with different concentrations of probe **MBI** for 24 h.

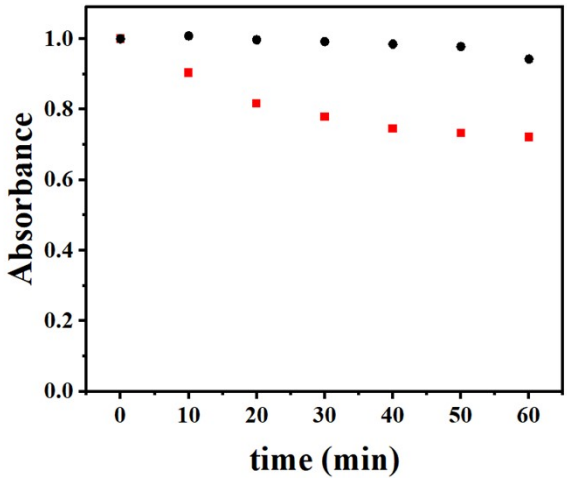


Fig. S6 Time-dependent absorbance changes of probe **MBI** under dark (black line) and a 480-490 nm LED light irradiation (red line) in water.

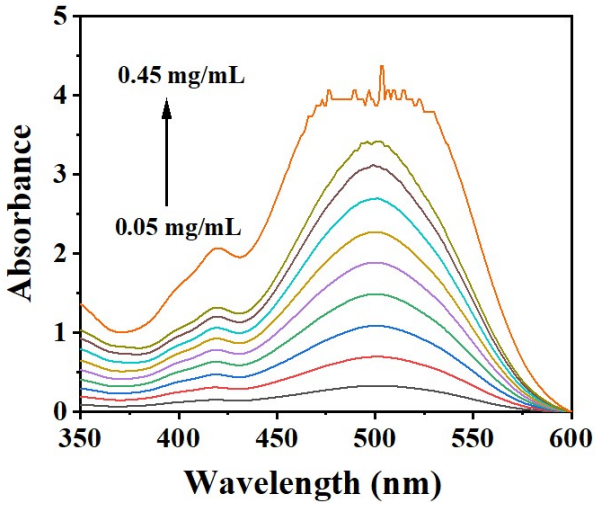


Fig. S7 UV-vis absorption spectra of different concentrations of probe **MBI**.

Table S1. Fluorescent quantum yield of probe **MBI** in water containing different proportion of glycerol.

Proportion of glycerol in water	0%	30%	50%	80%
Fluorescent quantum yield (φ)	0.003	0.018	0.032	0.187

4. ^{1}H NMR, ^{13}C NMR and HRMS

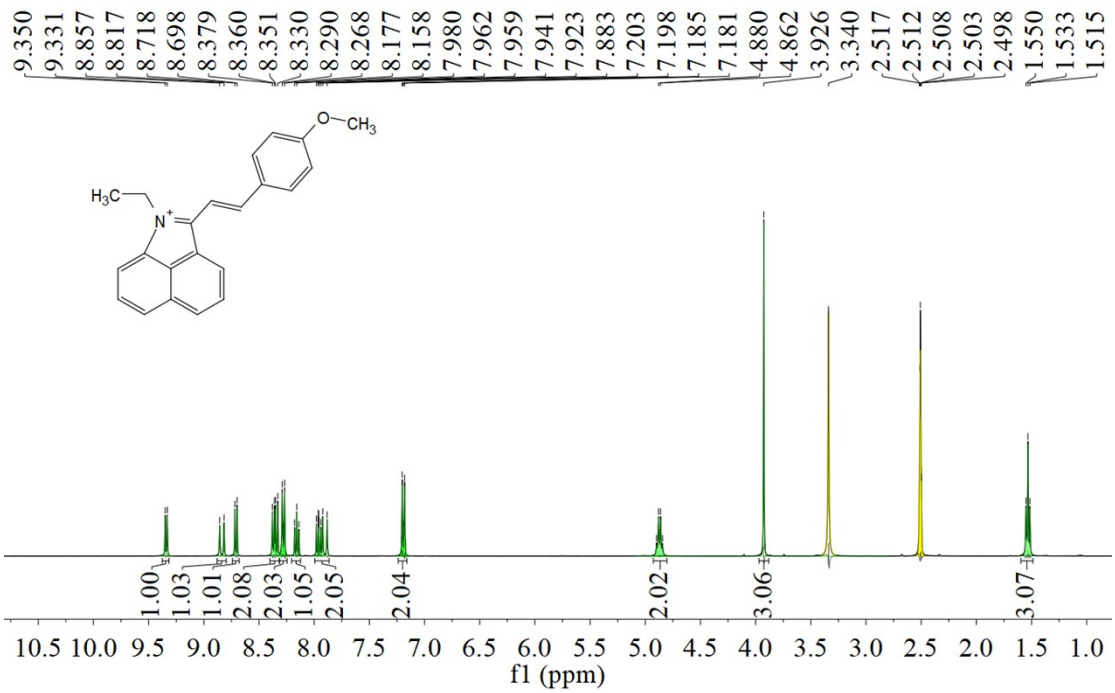


Fig.S8 ^{1}H NMR spectrum of probe **MBI** in DMSO-d_6.

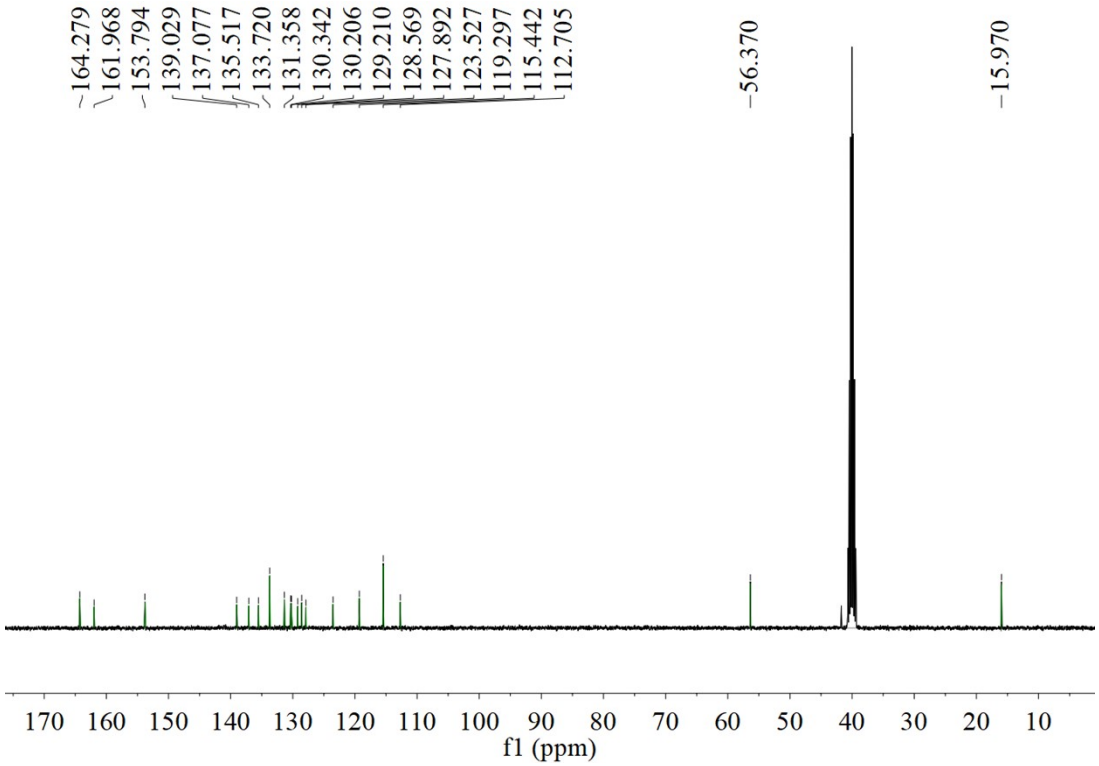


Fig. S9 ^{13}C NMR spectrum of probe **MBI** in DMSO-d_6.

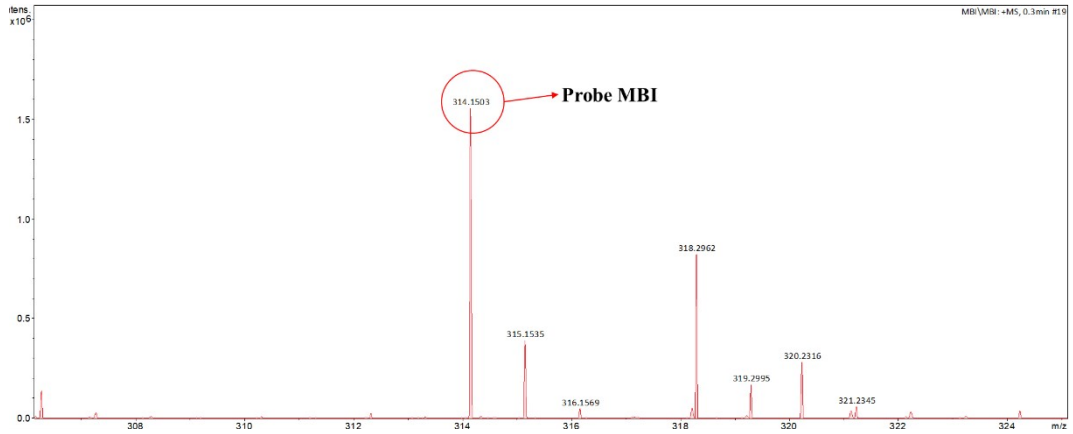


Fig.S10 HRMS spectrum of **MBI**.

5. References

1. D. Yao, Y. Wang, R. Zou, K. Bian, P. Liu, S. Shen, W. Yang, B. Zhang and D. Wang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 4276-4284.