Employing an ICT-FRET Integration Platform for the Real-Time Tracking of SO$_2$ Metabolism in Cancer Cells and Tumor Models

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1. General Information

1.1 Materials
All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05mol/L Na₂HPO₄ water solution and 0.05mol/L KH₂PO₄ water solution with the volume ratio 4:1. Bisulfite and various analytes were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All chemicals and solvents used were of analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

1.2 Instruments
TLC analysis was performed using precoated silica plates. Ultraviolet–visible (UV–vis) spectra were recorded on U-3900 UV-Visible spectrophotometer. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ¹H NMR and ¹³C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI-MS was measured with a Thermo Scientific Q Exactive. The cells imaging experiments were measured by a Zeiss LSM710 Airyscan confocal laser scanning microscope. The ratiometric images were obtained by the image analysis software Image Pro-plus 6.0. The imaging assays of living body were performed in Perkinelmer In Vivo Imaging System.

1.3 Bio-imaging

**Cell Culture and Imaging.** The cells were grown in Dulbecco’s Modified Eagle’s medium supplemented with 12% Fetal Bovine Serum and 1% antibiotics at 37 °C in humidified environment of 5% CO₂. Cells were plated on 6-well plate and allowed to adhere for 24 h. Before the experiments, cells were washed with PBS 3 times.

**SiRNA Transfection.** Sulfite oxidase (SUOX) protein expression in HepG2 cells was knocked out using SUOX-specific siRNA (GenePharma, Suzhou, China) according to the literature report.¹

**Mice and tumor model.** All the animal experiments were performed by following the protocols approved by Radiation Protection Institute of Drug Safety Evaluation Center in China (Production license: SYXK (Jin) 2018-0005). Balb/c type mouse (6-8 weeks, male) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Experiments were conducted according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Tumor-bearing mice were obtained by subcutaneous injection of HepG2 cells. The images were recorded in a dual emission mode, Blue channel: \( \lambda_{em} = 520 \text{ nm} (\lambda_{ex} = 430 \text{ nm}) \), Red channel: \( \lambda_{em} = 660 \text{ nm} (\lambda_{ex} = 605 \text{ nm}) \).
2. Experimental Section

2.1 Scheme S1. Synthesis route of probe Mito-CM-BP.

2.2 Synthesis and characterization

Compound 3 and CM was prepared according to our previous reported works.\(^2\)

Synthesis of Compound BP

Compound BP was prepared according to the literature reported work.\(^3\) Briefly, 4-piperazinoacetophenone (0.408 g, 2 mmol) and 4-(diethylamino)-2-hydroxybenzaldehyde (0.386 g, 2 mmol) was slowly added to the concentrated H\(_2\)SO\(_4\) (10 mL). The mixture was further heated at 90 °C for 8 h, after cool down to room temperature the mixture was added slowly to ice-water (200 mL), then 1 mL HClO\(_4\) was added and the result suspension was precipitated and filtered off to wash with water, and dried in vacuum. The product was purified by column chromatograph with dichloromethane and methanol (v:v = 10:1) to give compound 3 as a purple black powder (0.62 g, 67.3 %).

Synthesis of probe Mito-CM-BP.

To a solution of CM (0.156 g, 0.5 mmol), were added 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCl, 0.26 g, 2 mmol) and 1-Hydroxybenzotriazole (HOBt, 0.38 g, 2 mmol), and the resulting mixture was stirred in dried DMF (15 mL) at 0 °C under N\(_2\) for 30 min. Then, compound CM (0.184 g, 0.4 mmol) and triethylamine (0.2 mL) were sequentially added. The resulting mixture was stirred at room temperature for 24 h, and then poured into water and washed with cold water to afford a black solid. This powder was purified by column chromatography on silica (methanol/dichloromethane = 1:15 v/v) to afford probe Mito-CM-BP (0.105 g, 34.3% yield). \(^1\)H NMR (600 MHz, DMSO) \(\delta\) 8.69 (s, 1H), 8.63 (d, \(J = 8.4\) Hz, 1H), 8.28 (d, \(J = 8.7\) Hz, 2H), 7.98 (d, \(J = 8.5\) Hz, 1H), 7.90 (d, \(J = 9.2\) Hz, 1H), 7.77 (s, 1H), 7.60 (d, \(J = 8.8\) Hz, 1H), 7.35 (d, \(J = 9.7\) Hz, 1H), 7.30 (s, 1H), 7.18 (d, \(J = 8.9\) Hz, 2H), 6.83 (d, \(J = 8.7\) Hz, 1H), 6.64 (s, 1H), 3.79 (s, 4H), 3.73 (s, 4H), 3.67 (d, \(J = 6.9\) Hz, 4H), 3.52 (d, \(J = 6.7\) Hz, 4H), 1.23 (t, \(J = 6.7\) Hz, 6H), 1.16 (t, \(J = 6.8\) Hz, 6H). \(^{13}\)C NMR (151 MHz, DMSO) \(\delta\) 148.39 (s), 143.66 (s), 132.24 (s), 131.26 (s), 117.29 (s), 117.25 – 116.77 (m), 114.20 (s), 110.94 (d, \(J = 7.2\) Hz), 108.67 (s), 108.14 (s), 102.93 (s), 97.05 (s), 96.47 (s), 45.65 (s), 44.99 (s), 12.87 (s). ESI-MS: [M + H]\(^{+}\) Calcd. For 656.3231, Found 656.3234
3. Spectrometric Studies

**Figure S1:** (a) The overlap between emission spectrum of CM (orange line, $E_x=488$ nm) and absorption spectrum of BP (red line) in PBS (0.01 M, 10% DMSO, pH 7.4); (b) the overlap between emission spectrum of CM-GSH (blue line, $E_x=405$ nm) and absorption spectrum of BP (red line) in PBS (0.01 M, 10% DMSO, pH 7.4), (c) the fluorescence intensity of CM in the absence ($F_D$: orange line) and presence ($F_{DA}$: red line) of the BP with excitation wavelength at 488 nm in PBS (0.01 M, 10% DMSO, pH 7.4), (d) the fluorescence intensity of CM-GSH in the absence ($F_D$: blue line) and presence ($F_{DA}$: red line) of the BP with excitation wavelength at 405 nm in PBS (0.01 M, 10% DMSO, pH 7.4).
Figure S2: (a) UV-vis absorption and spectra of **CM** (10.0 μM) in PBS (0.01 M, 10% DMSO, pH 7.4). (b) The fluorescence spectral changes of **CM** (10.0 μM) in PBS (0.01 M, 10% DMSO, pH 7.4) with excitation wavelength at 488 nm. (c) UV-vis absorption and spectra of **CM** (10.0 μM) after treatment with GSH (2 mM) in PBS (0.01 M, 10% DMSO, pH 7.4). (d) The fluorescence spectral changes of **CM** (10.0 μM) after treatment with GSH (2 mM) in PBS (0.01 M, 10% DMSO, pH 7.4) with excitation wavelength at 405 nm. (e) UV-vis absorption and spectra of **BP** (10.0 μM) in PBS (0.01 M, 10% DMSO, pH 7.4). (f) The fluorescence spectral changes of **BP** (10.0 μM) in PBS (0.01 M, 10% DMSO, pH 7.4) with excitation wavelength at 590 nm. (g) UV-vis absorption and spectra of probe **Mito-CM-BP** (10.0 μM) in PBS (0.01 M, 10% DMSO, pH 7.4). (f) The fluorescence spectral changes of probe **Mito-CM-BP** (10.0 μM) in PBS (0.01 M, 10% DMSO, pH 7.4) with excitation wavelength at 488 nm.
Figure S3: Proposed mechanism of probe Mito-CM-BP for GSH and SO₂ detection.
Figure S4: The cytotoxicity test of Mito-CM-BP.

Figure S5: (a) Time-dependent relative fluorescence changes images of probe Mito-CM-BP with HepG 2 cells. (b) Time-dependent average gray value of red channel from 0-40 min.
**Figure S6:** Confocal fluorescence images of HepG 2 and SW480 cells stained with Mito-CM-BP and MitoTracker Green.

**Figure S7:** In vivo fluorescence imaging of GSH with Mito-CM-BP in living mice.
Figure S8: In vivo fluorescence imaging of SO₂ with Mito-CM-GSH-BP in living mice.
4. Characterization

Figure S9: Structure characterization of Mito-CM-BP.

$^1$H-NMR spectrum of Mito-CM-BP in DMSO-$d_6$. 
$^{13}$C-NMR spectrum of Mito-CM-BP in DMSO-$d_6$
REFERENCES

