A near-infrared and mitochondria-targeted fluorescence probe for ratiometric monitoring of sulfur dioxide derivatives in living cells

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1. Energy transfer efficiency was obtained based on the following equality:

\begin{equation}
    E = 1 - \frac{F_{DA}}{F_D}
\end{equation}

In this equation, $E$ represents the FRET energy transfer efficiency in probe SNB. $F_{DA}$ represents fluorescence intensity of donor moiety in probe SNB. $F_D$ represents fluorescence intensity of the donor alone.

2. Detection limit calculation:

\begin{equation}
    \text{L}_{OD} = 3\sigma/k
\end{equation}

Where, $\sigma$ represents the standard deviation of the blank solutions without the addition of sulfur dioxide derivatives, and $k$ represents the slope of titration linear relationship.

**Scheme S1** Synthesis route of the acceptor.

**Fig. S1-4** The $^1$H NMR, $^{13}$C NMR, IR and MS spectra of probe SNB.
**Fig. S5** The overlap between the emission band of the donor and the absorbance band of the acceptor.

**Fig. S6** The energy transfer efficiency.

**Fig. S7** The MS of the mixture of SNB and HSO₃⁻/SO₃²⁻.

**Fig. S8** The ¹H NMR of the mixture of SNB and HSO₃⁻/SO₃²⁻.

**Fig. S9** Selectivity of SNB toward HSO₃⁻/SO₃²⁻ by UV-Vis spectra.

**Fig. S10** The time dependent of the SNB response to HSO₃⁻/SO₃²⁻.

**Fig. S11** The pH dependence of the SNB for the detection of HSO₃⁻/SO₃²⁻.

**Fig. S12** The toxicity analysis of SNB in HeLa cells.

**Fig. S13** The photo-stability analysis of SNB in bioimaging.

**Fig. S14** The photo-stability analysis of SNB in daylight.

**Fig. S15** The thermostability analysis of SNB in temperature cycle experimental.

**Table S1** The comparison of probe SNB with other probes.

![Scheme S1](image)

**Scheme S1** Synthesis route of the acceptor

The acceptor of probe SNB was prepared according to previous report [S1]. p-Dimethylaminobenzaldehyde (178.4 mg) and 1,4-dimethylquinolin-1-ium iodide (286.3 mg) were dissolved into EtOH (20.00 mL) under the catalysis of piperdine (0.50 mL) and kept refluxing 6 h. Then, the mixture was purified by column chromatography (DCM : MeOH = 15:1) to give the acceptor in 62% yield.
Fig. S1 The $^1$H NMR spectrum of probe SNB.

Fig. S2 The $^{13}$C NMR spectrum of probe SNB.
Fig. S3 The MS spectrum of probe SNB.

Fig. S4 The IR spectrum of probe SNB.
Fig. S5 The overlap between the emission band of the donor and the absorbance band of the acceptor. (5 μM; Fluorescence spectra: $\lambda_{ex} = 420$ nm, speed: 1200 nm/s)

Fig. S6 The energy transfer efficiency. (5 μM, $\lambda_{ex} = 420$ nm, speed: 1200 nm/s, slit: 5/5)
Fig. S7 MS of the mixture of SNB and HSO$_3^-$/SO$_3^{2-}$ (DMSO/water mixed solution).
**Fig. S8** The $^1$H NMR of the mixture of SNB and HSO$_3^-$/SO$_3^{2-}$ (DMSO-$d_6$ and D$_2$O mixed solution).
**Fig. S9** Selectivity of SNB toward HSO$_3^-$/SO$_3^{2-}$ by UV-Vis spectra.

**Fig. S10** The time dependent of the SNB response to HSO$_3^-$/SO$_3^{2-}$. (5 μM, $\lambda_{\text{ex}}$ = 420 nm, speed: 1200 nm/s, slit: 5/5)
Fig. S11 The pH dependence of the SNB for the detection of HSO$_3^-$/SO$_3^{2-}$.

Fig. S12 The toxicity analysis of SNB for HeLa cells. Hela cells were cultivated with Dulbecco's modified Eagle's medium (DMEM) which contains supplement of 10% FBS (Fetal Bovine Serum) in the carbon dioxide incubator with an atmosphere of 5% CO$_2$ and 95% air at 37 °C. HeLa cells were placed to a 96-well plate in the concentration of 40000 per mL for 24 h, and then incubated with probe SNB (0, 0.1, 1, 5 and 10 μM) for 6 h, respectively. After that SRB assay was conducted to measure the viability of cells.
**Fig. S13** The photo-stability analysis of SNB in bioimaging. HeLa cells were pre-treated with SNB (1 μM) for 1 h. First line: fluorescence images at the blue channel (450-610 nm); Second line: fluorescence images at the red channel (610-700 nm); Third line: images at bright field; Forth line: merged images of the first and second lines. ($\lambda_{ex} = 405$ nm)

**Fig. S14** The photo-stability analysis of SNB in daylight.
The thermostability analysis of SNB in temperature cycle experimental. Firstly, the fluorescence spectra of probe SNB were recorded at 37 °C, then heated to 60 °C and cold down 37 °C to measure fluorescence, 5 times cycles.

Table S1 The comparison of probe SNB with other probes

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<th>Probe</th>
<th>Response ions</th>
<th>Ratiometric</th>
<th>Detection limit</th>
<th>Targeted subcellar</th>
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**Reference**


