Electronic Supplementary Information

An ESIPT-based ratiometric fluorescent probe for the imaging of nitroxyl in living cells

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The method for the statistical analysis in MTT assay

All data were obtained from at least three separate experiments and the results were expressed as mean±S.D. Data were analyzed for statistical significance by one-way ANOVA, and pb0.05 was considered statistically for the indication of significant difference. The IC\textsubscript{50} values were obtained through the Probit regression model between inhibition ratio and concentration. There are five concentration gradients and each concentration gradient corresponds to one inhibition ratio. A fitting line Y=A+BX is gained, plotted by log(concentration) on the horizontal axis and probit of inhibition ratio on the vertical axis. Then log(IC\textsubscript{50}) is got by calculating the value of X when Y is half of probit of inhibition ratio. Next, we can get the value of IC\textsubscript{50} by log(IC\textsubscript{50}). We need to get three values of IC\textsubscript{50}, and then calculate the mean and standard deviation.

Equation:

\[
\text{IC}_{50}\text{Average} = \frac{(\text{IC}_{50_A} + \text{IC}_{50_B} + \text{IC}_{50_C})}{3}
\]

\[
\text{Standard deviation} = \left\{ \frac{[(\text{IC}_{50_A} - \text{IC}_{50}\text{Average})^2 + (\text{IC}_{50_B} - \text{IC}_{50}\text{Average})^2 + (\text{IC}_{50_C} - \text{IC}_{50}\text{Average})^2]}{3} \right\}^{0.5}
\]
The method for administration of the probe for cellular experiments

HeLa cells were seeded in a 96-well plate in culture media and allowed to adhere for 24 h. The culture medium was then removed, and the cells were washed once with 1 mL of phosphate-buffered saline (PBS). HeLa cells were placed in 1 mL of PBS and were incubated with 5 μM probe 1 (Stock solution of probe was prepared by dissolving probe 1 in DMSO) for 30 min at 37 °C. After washing the cells three times with PBS to remove the excess probe, the cells were placed in 1 mL of PBS and treated with 100 μM AS for 20 min. Finally, the cells were carefully washed three times with PBS and mounted on the microscope. The fluorescence images were recorded with a Leica TCS SP5 II Confocal Laser Scanning Microscope.
Fig. S1 Absorption spectra of free probe 1 (10 μM) and the reference HBT (10 μM).

Fig. S2 $^1$H NMR spectra of (A) the standard HBT and (B) isolated product of probe 1 with HNO in $d_6$-DMSO.
**Fig. S3** Change of $F_{460}/F_{380}$ of probe 1 (2 μM) towards AS (15 μM) with time (from 0 to 10 min).

**Fig. S4** Fluorescence responses of probe 1 (2 μM) to testing species (1 mM) in the presence of AS (20 μM): 1, just AS; 2, Na$_2$S; 3, Cys; 4, GSH; 5, H$_2$O$_2$; 6, K$^+$; 7, Fe$^{3+}$; 8, NO; 9, NO$_2^-$; 10, KO$_2$; and 11, Na$^+$. 
**Fig. S5** HeLa cytotoxicity assays at different concentrations of probe 1.

**Fig. S6** Fluorescence response ($F$) of probe 1 (2 μM) in pH 7.4 PBS buffer/CH$_3$CN (7:3, v/v) in the presence of AS (30 μM) and Fluorescence response ($F$) of probe 1 (2 μM) in pH 7.4 PBS buffer/CH$_3$CN (9:1, v/v) in the presence of AS (30 μM).
Fig. S7 ESI-MS spectrum of probe 1.

Fig. S8 $^1$H NMR spectrum of probe 1 in $d_6$-DMSO.
**Fig. S9** $^{13}$C NMR spectrum of probe 1 in $d_6$-DMSO.

**Fig. S10** $^{31}$P NMR spectrum of probe 1 in $d_6$-DMSO.
Fig. S11 $^1$H NMR spectrum of the isolated product of probe 1 with HNO in $d_6$-DMSO.

Fig. S12 $^{13}$C NMR spectrum of the isolated product of probe 1 with HNO in $d_6$-DMSO.
Fig. S13 $^1$H NMR spectrum of HBT in $d_6$-DMSO.