# Supporting Information

# Fluorescent τ-Probe: Quantitative Imaging of Ultra-trace Endogenous Hydrogen Polysulfide in Cells and in Vivo

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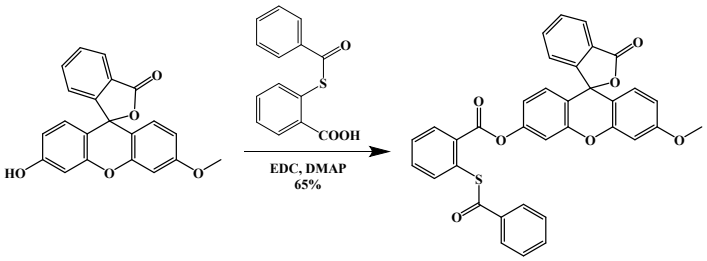
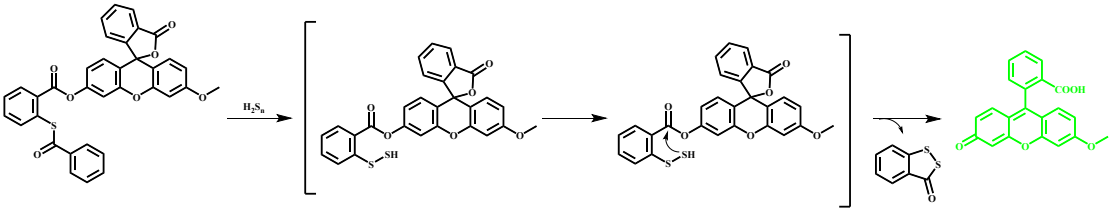
## 1.Detailed synthesis procedure and design strategies of τ-probe

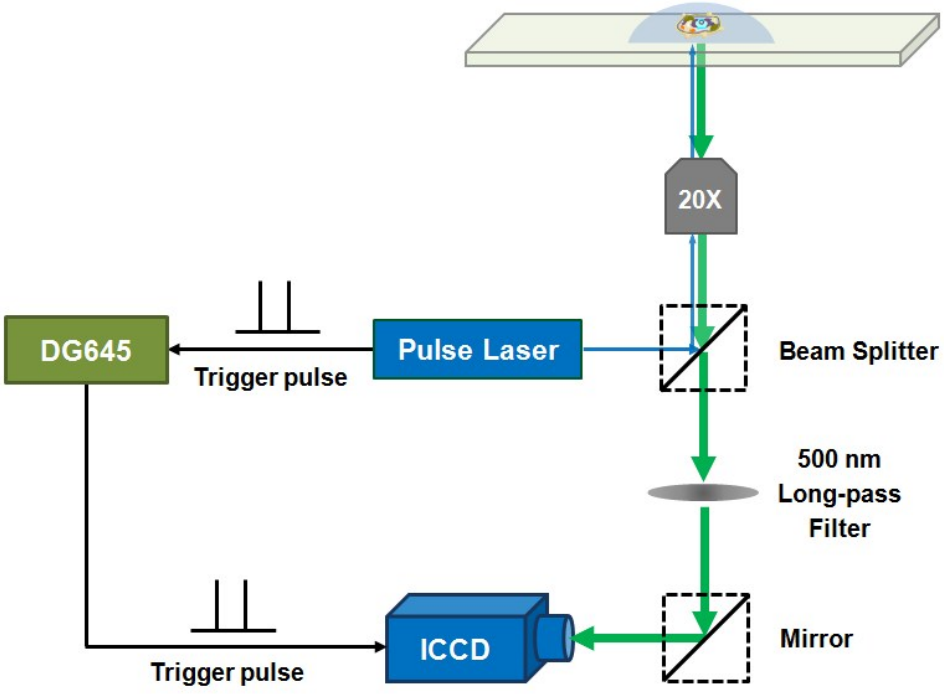



**Scheme S1.** The synthesis procedure of τ-probe.




**Scheme S2.** Reaction path of τ-probe and $H_2S_n$.

## 2. Schematic diagram of fluorescence lifetime microscope




**Scheme S3.** Schematic diagram of time-gated transient microscope for fluorescence lifetime imaging.

## 3. Characterizations of τ-probe

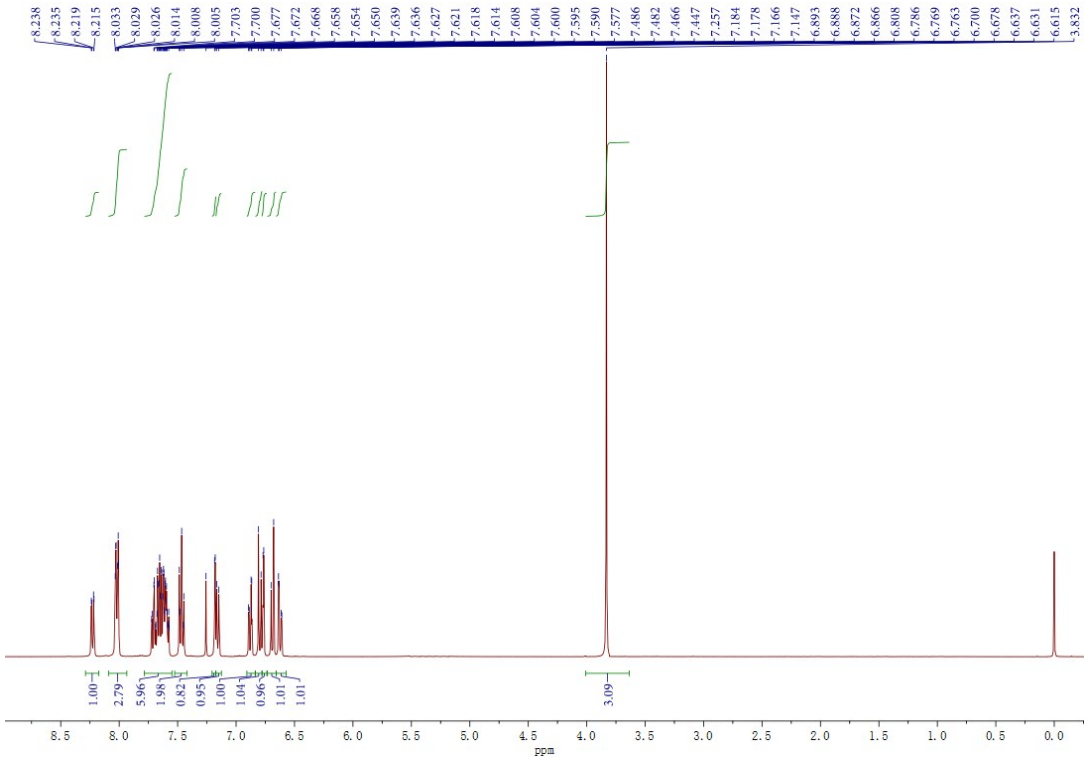


**Fig. S1** $^{1}$H NMR spectrum of τ-probe.

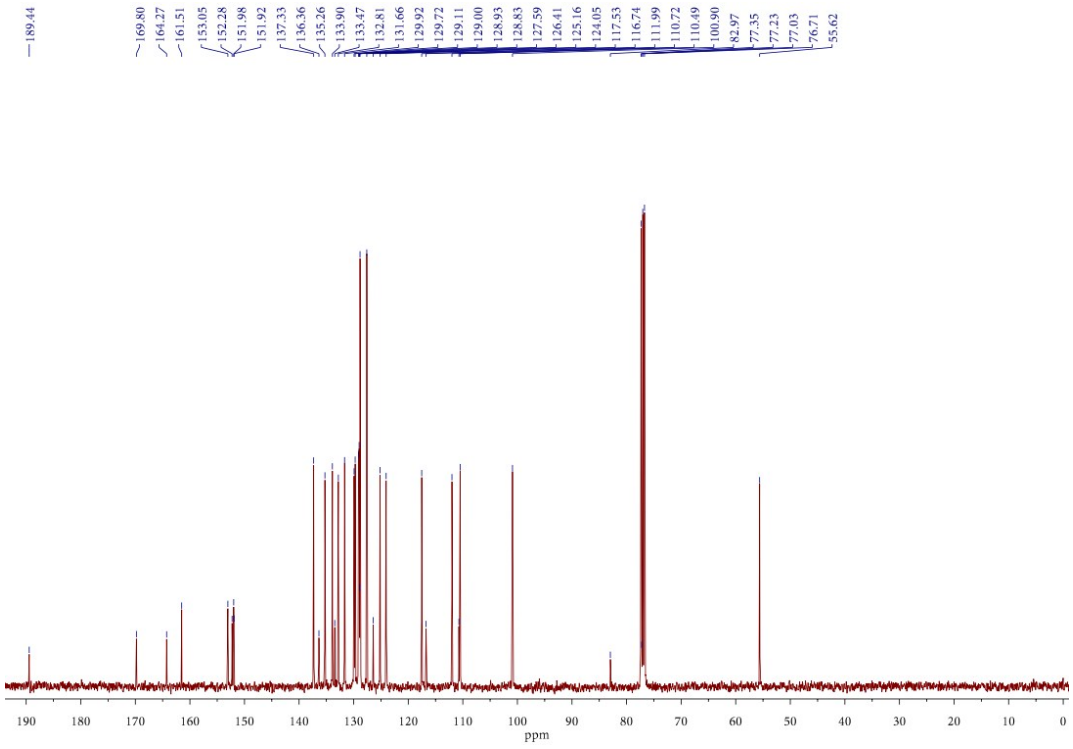


**Fig. S2** $^{13}$C NMR spectrum of τ-probe.

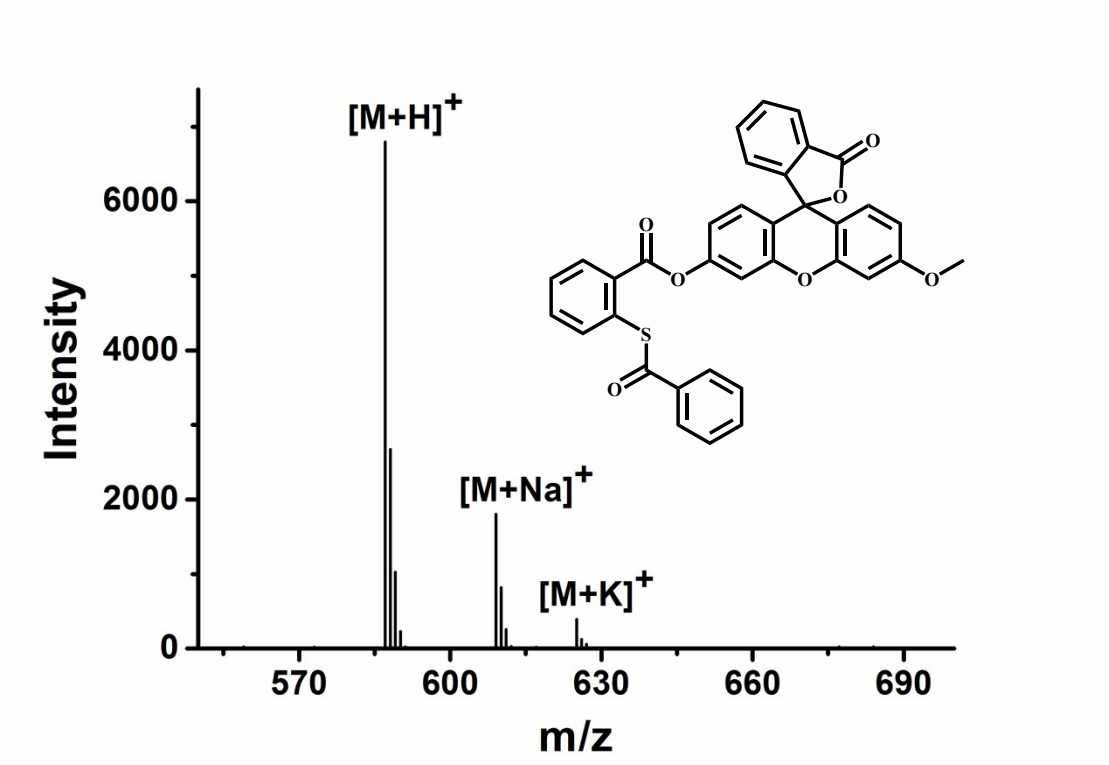



**Fig. S3** Mass spectrum of τ-probe, LC-MS (ESI$^+$): m/z $C_{35}H_{23}O_7S^+$, calcd. 587.1168, found [$M^+$] 587.1169.

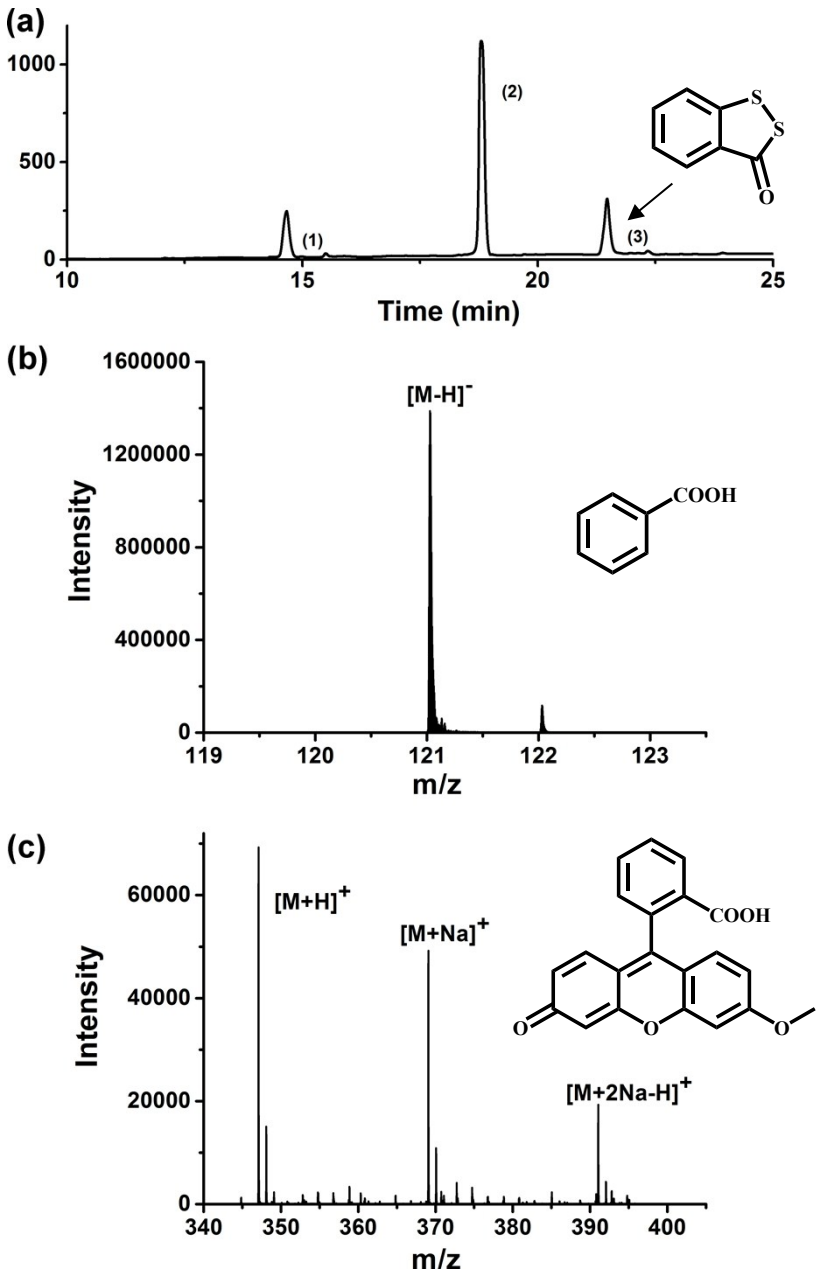



**Fig. S4** (a) HPLC trace of τ-probe (10 μM) treated with $Na_2S_4$ (20 μM) in phosphate buffer (10 mM, pH 7.4)/methanol solution mixture (2:1, v/v) with 3 vol % dimethyl sulfoxide (DMSO). (b) MS of product (1) in (a) at retention time of 14.67 min, confirming formation of benzoic acid from the reaction between τ-probe and $Na_2S_4$. m/z $C_7H_6O_2^-$, calcd. 121.0295, found [$M^-$] 121.0306. (c) MS of product (2) in (a) at retention time of 18.80 min, confirming formation of 3-O-Methylfluorescein from the reaction between τ-probe and $Na_2S_4$. m/z $C_{21}H_{14}O_5^+$, calcd. 347.0914, found [$M^+$] 347.0909. Product (3) in (a) was speculated as 3H-1,2-benzodithiol-3-one, which was lacking both acidic and basic functional groups present a great challenge to ionize and detect using electrospray ionization (ESI)-MS.

**4. Additional figures**

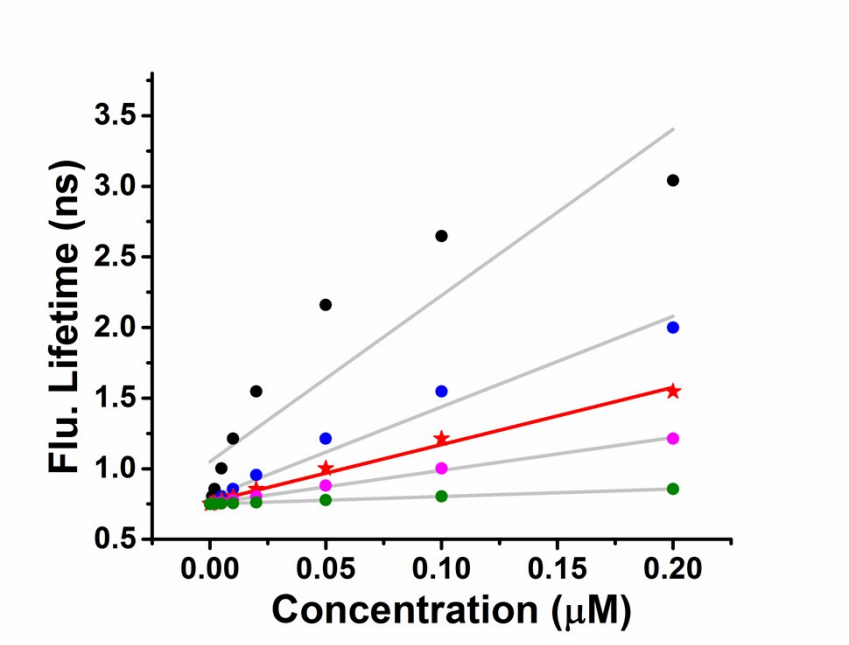



**Fig. S5** Fluorescence lifetime theoretical curves of τ-probe at different concentrations. The curves from up to down represented the concentration of τ-probe at 1, 5, 10, 20, and 100 μM.

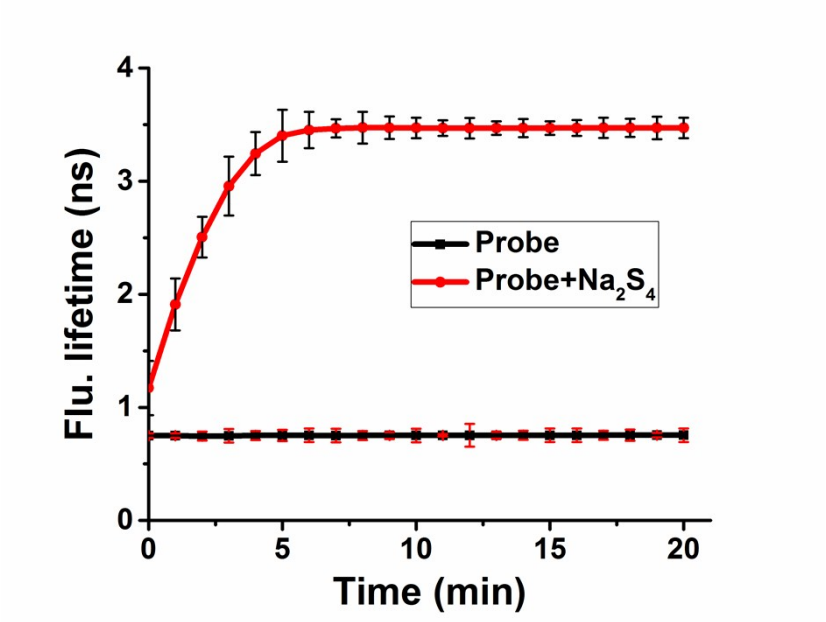



**Fig. S6** Fluorescence lifetime kinetic studies of 10 μM τ-probe and τ-probe upon addition of 20 μM $Na_2S_4$. The study of τ-probe is shown in black curve, while τ-probe upon addition of $Na_2S_4$ in red curve. The reactions were carried out for 30 min at room temperature. All experiments were repeated at least three times. Error bars are standard deviation.

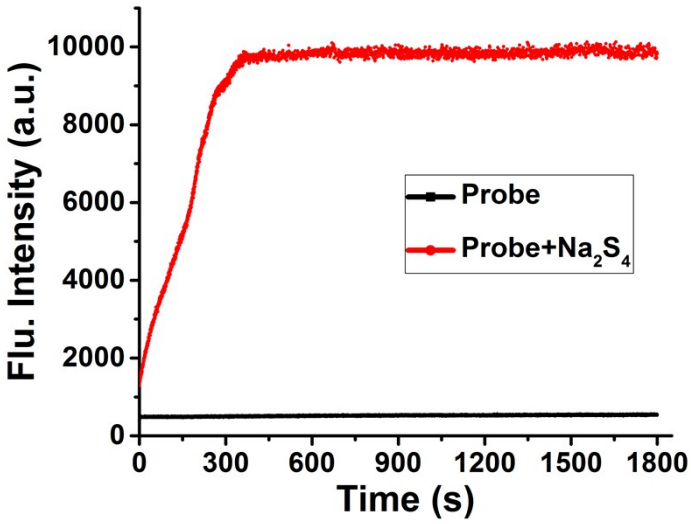



**Fig. S7** Fluorescence intensity kinetic studies of 10 μM τ-probe and τ-probe upon addition of 20 μM $Na_2S_4$. The study of τ-probe is shown in black curve, while τ-probe upon addition of $Na_2S_4$ in red curve. The reactions were carried out for 30 min at room temperature.

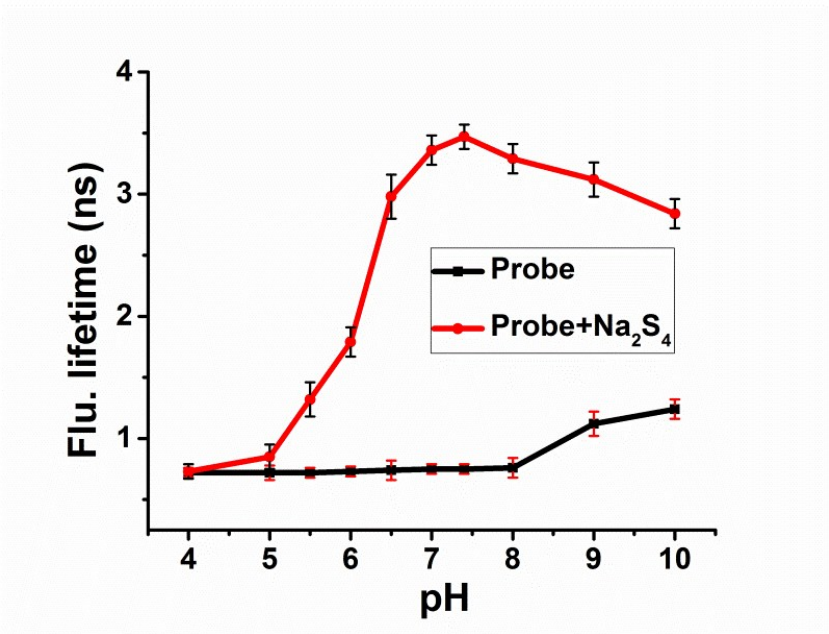



**Fig. S8** The acidity stability of 10 μM τ-probe (black) and τ-probe upon addition of 20 μM $Na_2S_4$ (red) in various buffer solutions of different pH for 30 min at room temperature. All experiments were repeated at least three times. Error bars are standard deviation.

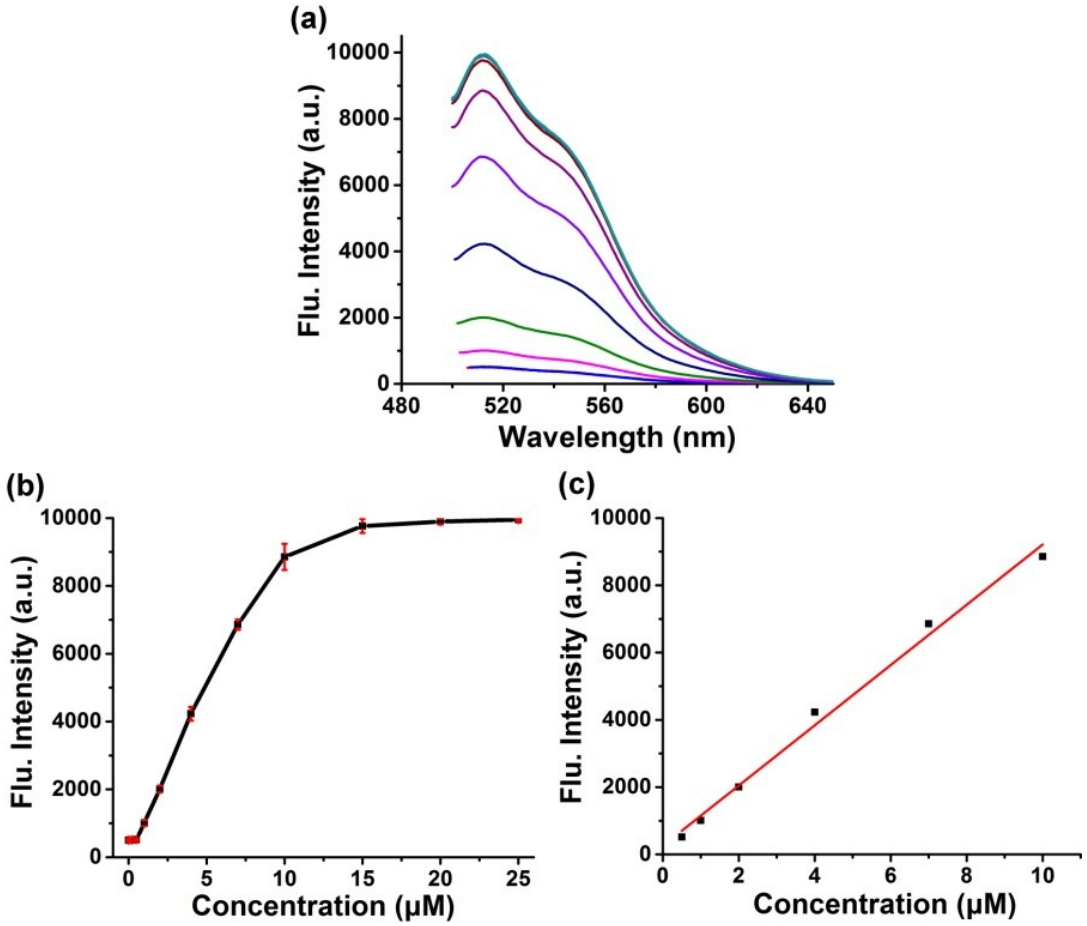



**Fig. S9** (a) Fluorescence intensity of τ-probe (10 μM) upon addition of $Na_2S_4$ in varied concentrations (0, 0.5, 1, 2, 4, 7, 10, 20, and 25 μM for curves from bottom to top, respectively). The curves at the concentration of 0 and 0.5 μM were superposed. (b)The relationship between the fluorescence intensity of τ-probe at 515 nm and the concentration of $Na_2S_4$ (0, 0.1, 0.2, 0.5, 1, 2, 4, 7, 10, 20, and 25 μM for dots from left to right, respectively). Changes at 0.5μM and below were not detectable. (c) Linear relationship between the fluorescence intensity of τ-probe at 515 nm and the concentration of $Na_2S_4$ (from 0.5 to 10 μM). The excitation wavelength was 488 nm. All experiments were repeated at least three times. Error bars are standard deviation. Reactions were carried out for 30 min at room temperature.

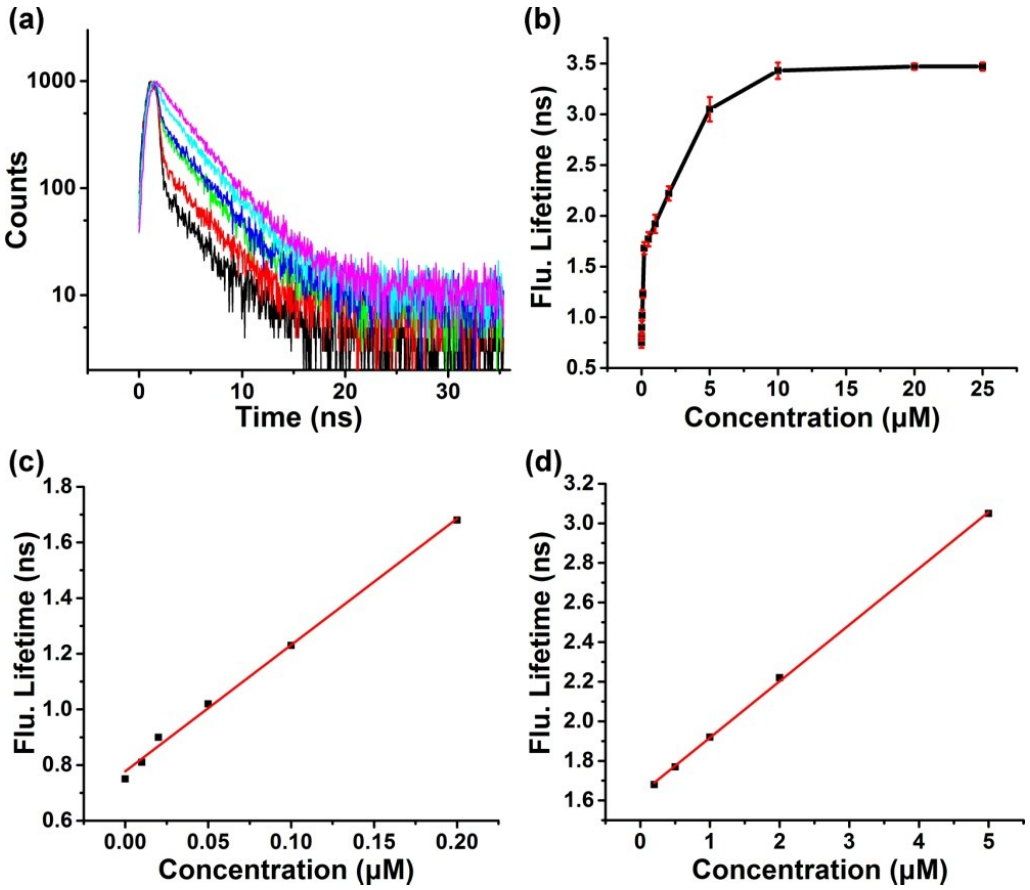



**Fig. S10** (a) Fluorescence lifetime of τ-probe (10 μM) upon addition of $Na_2S_4$ with various concentrations (0, 0.02, 0.2, 1, 5, and 20 μM for curves from bottom to top, respectively). (b) Fluorescence lifetime of τ-probe (10 μM) upon addition of $Na_2S_4$ with various concentrations (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, and 25 μM for dots from left to right, respectively). (c) Linear relationship between the fluorescence lifetime of τ-probe and the concentration of $Na_2S_4$ (from 0 to 0.2 μM). (d) Linear relationship between the fluorescence lifetime of τ-probe and the concentration of $Na_2S_4$ (from 0.2 to 5 μM). The excitation wavelength was 488 nm. All experiments were repeated at least three times. Error bars are standard deviation. Reactions were carried out for 30 min at room temperature.

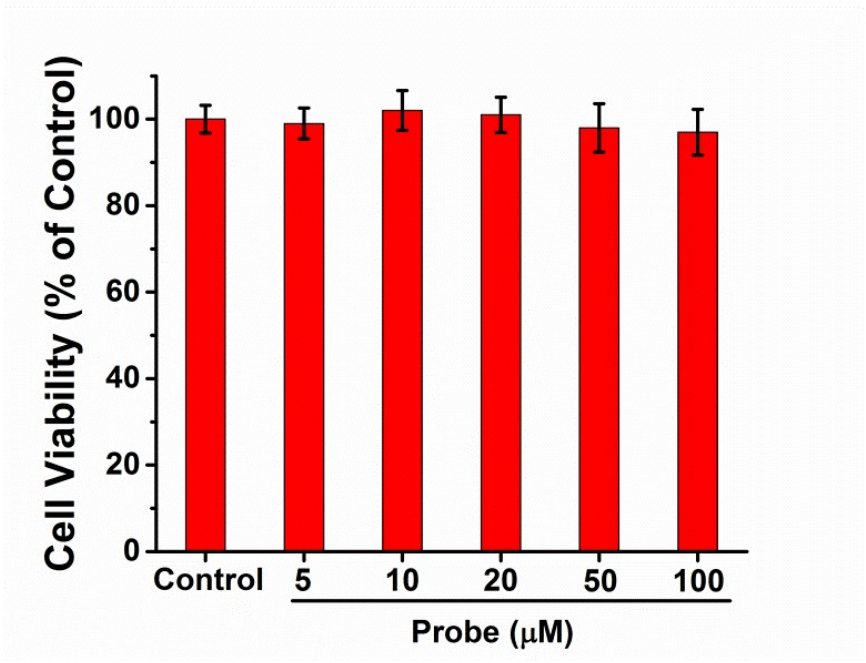



**Fig. S11** Effects of τ-probe at varied concentrations on the viability of HeLa cells. The cytotoxicity of τ-probe to HeLa cells was evaluated by MTT assay. The viability of the cells without τ-probe is defined as 100%. The results are expressed as the mean ± standard deviation of five separate measurements.

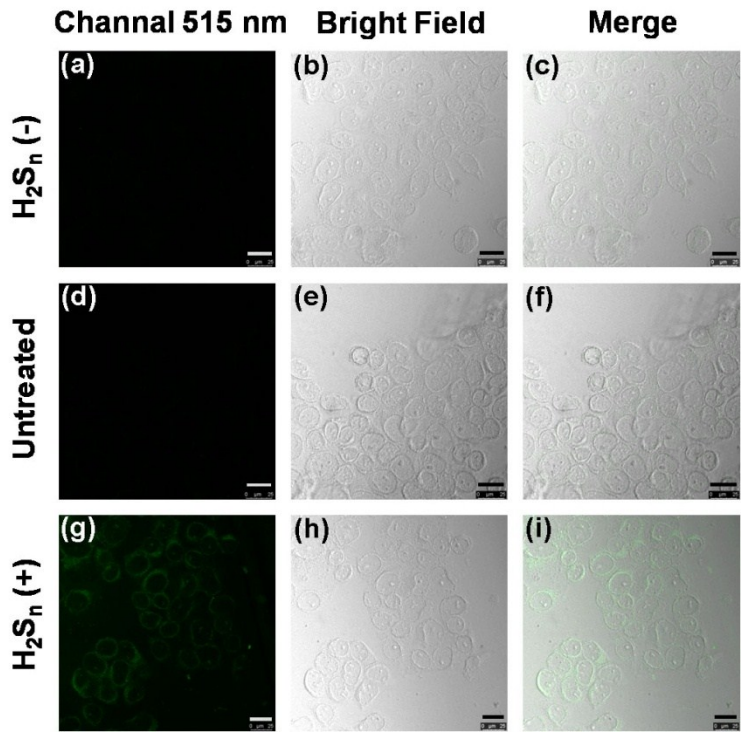



**Fig. S12** Confocal microscopy images show the difference in fluorescence intensity signal of the different treated HeLa cells incubated with 10 μM τ-probe for 30 min. HeLa cells in (a-c) treated with 100 μM N-ethylmaleimide (NEM) for 15 min to eliminate endogenous $H_2S_n$. Native HeLa cells in (d-f), and HeLa cells in (g-i) treated with 20 μM $Na_2S_4$ for 30 min to increase intracellular $H_2S_n$. Different imaging channels are displayed horizontally for each sample (from left to right): channel 515 nm (505−550 nm), bright field, and merge images. The excitation of τ-probe was at 488 nm, and the collected range of emission was at 505−550 nm. Scale bars in all images are 25 μm.

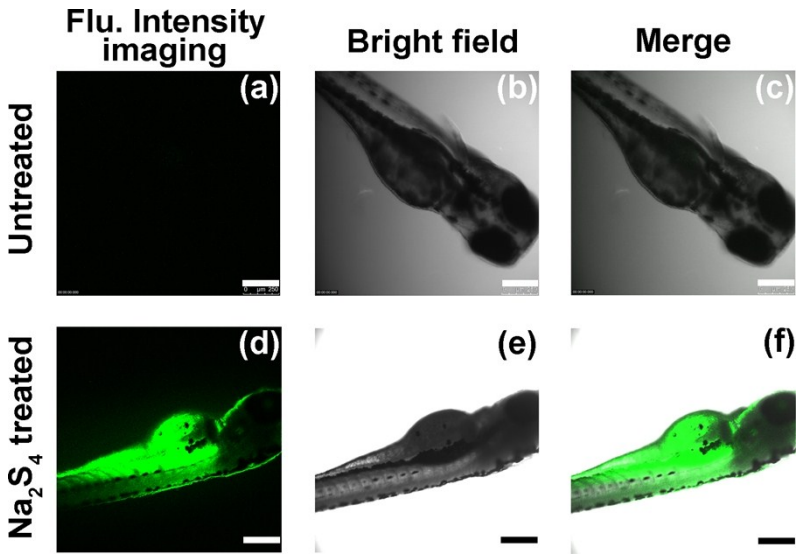



**Fig. S13** Confocal microscopy images show the difference in fluorescence intensity signal of the different treated zebrafishes incubated with 10 μM τ-probe for 30 min. Zebrafish in (a-c) was untreated. Zebrafish in (d-f) treated with 20 μM $Na_2S_4$ for 30 min to increase intracellular $H_2S_n$. After washing with PBS solution, the τ-probe was incubated. Different imaging channels are displayed horizontally for each sample (from left to right): channel 515 nm (505−550 nm), bright field, and merge images. The excitation of τ-probe was at 488 nm, and the collected range of emission was at 505−550 nm. Scale bars in all images are 250 μm.