Supplementary data for

A Novel Ratiometric Fluorescent Probe for Rapid Response of Hydrogen Peroxide in Living Cells

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1. Materials and Synthesis details.

All commercial chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified before use. Liver cancer cell line HepG2 was purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma-Aldrich. Other medium components were obtained from Sigma-Aldrich too. Fetal bovine serum (FBS) was obtained from Hyclone Laboratories Inc. Hydrogen peroxide 30% was bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Fluorescence imaging of HepG2 cells were obtained using Olympus FV1000 confocal fluorescence microscope. ^{1}H NMR and ^{13}C NMR spectra were performed on Bruker Ascend 400 NMR spectrometer. Electrospray ionization mass spectra (ESI-MS) were collected on Agilent 6460 Triple Quadrupole LC/MS instrument. Absorbance spectra were recorded on Shimadzu UV-2450 UV-visible spectrophotometer. Fluorescence emission and excitation spectra were measured using Hitachi F-7000 spectrofluorometer. All UV/Vis and fluorescence titration experiments were performed using 10 μM of **JNY-1** in a mixed buffer solution of DMF: PBS (30:70, v/v, pH=7.4, 10 mM) with varying concentrations of analytes at room temperature.

1.1 Synthesis details

The synthesis routine of **JNY-1** is shown at Scheme 1.

This procedure was adapted from a known literature.[28,29] To a 100 mL three-neck round-bottom flask were added fluorescein (1.7 g, 5.0 mmol) and MeOH (3 mL) at room temperature and the whole system was then cooled to 0 ^{o}C. A mixture of NaOH aqueous solution (15 g, 50%) and 15-crown-5 (20 μL) was added within 5 min. The resulting mixture was stirred for 10 min, and then allowed to warm gradually in an oil bath. $CHCl_3$ (4 mL) was added dropwise while the reaction temperature was maintained at 55 °C. The reaction mixture was further stirred for 10 h at this temperature, and then cooled to room temperature. The mixture is acidified with H_2SO_4 (1 mL, 10 M), and the purple-black precipitate appeared. This solid was filtered and dried and purified by flash column chromatography (DCM/EtOAc = 85:15) on silica gel to afford the crude monoaldehyde-functionalized fluorescein, which could be recrystallized in ace-tone

(10 mL) to give pure sample (252 mg, 14%)
as a pale-yellow solid.

Synthesis and Characterization of Compound 1.To a solution of monoaldehyde-functionalized fluorescein (720 mg, 2.0 mmol) and diethyl malonate (365 μL, 2.4 mmol) in dry EtOH (40 mL) were added piperidine (6 drops) and glacial acetic acid (2 drop) at room temperature. Then, the resulting mixture was heated gradually to 80 °C and reflux for 6h. The above mixture was cooled to room temperature, followed by filtering and crystallization. The product was then washed with EtOH (40 mL) and dried in vacuum to afford the desired compound 1 as a solid (592 mg, 65%). ^{1}H NMR (DMSO-d_6, 400 MHz; ppm): δ10.39 (s, 1H), 9.01 (s, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.81 (td, J= 7.2, 0.8 Hz, 1H), 7.75 (td, J = 8.0, 0.8 Hz, 1H), 7.31 (d, J =7.6 Hz, 1H), 7.14 (dd, J = 13.6, 9.2 Hz, 2H), 6.96 (t, J = 1.2 Hz,1H), 6.65 (d, J = 1.2 Hz, 2H), 4.35 (q, J = 7.2 Hz, 2H), 1.36 (t,J = 7.2 Hz, 3H). 13C NMR (DMSO-d_6, 100 MHz; ppm): δ168.5, 162.5, 159.9, 155.6, 155.4, 152.3, 150.8, 147.7, 141.7,135.9, 133.8, 130.5, 129.1, 125.7, 124.9, 124.1, 117.6, 114.3,113.8, 112.2, 108.9, 107.4, 102.7, 81.5, 61.6, 14.1. ESI-MS: m/z 457.3 $[M + H]^+$

Synthesis and Characterization of Probe **JNY-1**.2,3,4,5,6-pentafluorobenzene-1-sulfonyl chloride (266 mg, 1.0 mmol) was added dropwise to a solution of compound 1 (246 mg, 0.5 mmol) and Et_3N (83 μL, 0.6 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. Stirring was continued at this temperature for a half-hour, and then the resulting mixture was further stirred for 10 h at room temperature. Water (10 mL) was added to the mixture, followed by extraction with CH_2Cl_2 (15 mL x 3). The combined organic phase was dried by anhydrous Na_2SO_4. The solvent was removed by evaporation, and the residue was purified by flash column chromatography (petroleum ether/EtOAc = 1:1) on silica gel to afford probe 2 as a white solid (274 mg, 81%).^{1}H NMR ($CDCl_3$-d, 400 MHz; ppm) δ 9.10 (s, 1H), 8.08 (d, J = 6.8 Hz, 1H), 7.73 (p, J = 7.4, 6.8 Hz, 2H), 7.45 (d, J = 2.3 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 7.07 (d, J = 3.1 Hz, 2H), 7.01 (d, J = 8.8 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 4.50 (q, J = 7.1 Hz, 2H), 1.48 (t, J = 7.1 Hz, 3H).^{13}C NMR ($CDCl_3$-d,100 MHz; ppm) δ 168.40, 162.90, 156.45, 155.68, 152.03, 150.88, 149.68, 147.65, 142.11, 135.84, 133.45, 130.76, 130.24, 125.88, 125.72,

123.73, 119.00, 118.43, 118.30, 114.19, 113.19, 110.96, 107.88, 80.22, 62.43, 14.30.

ESI-MS: m/z 687.3 $[M + H]^+$

2. Structure characterizations of probe JNY-1

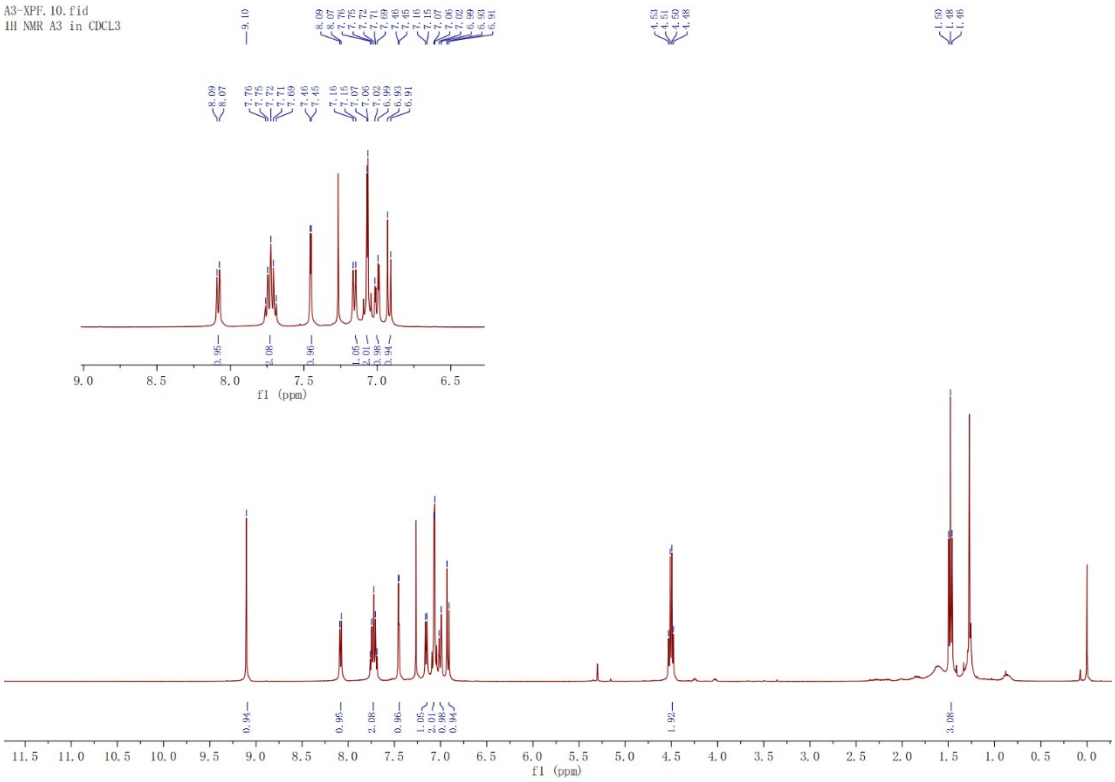

Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) of **JNY-1**.

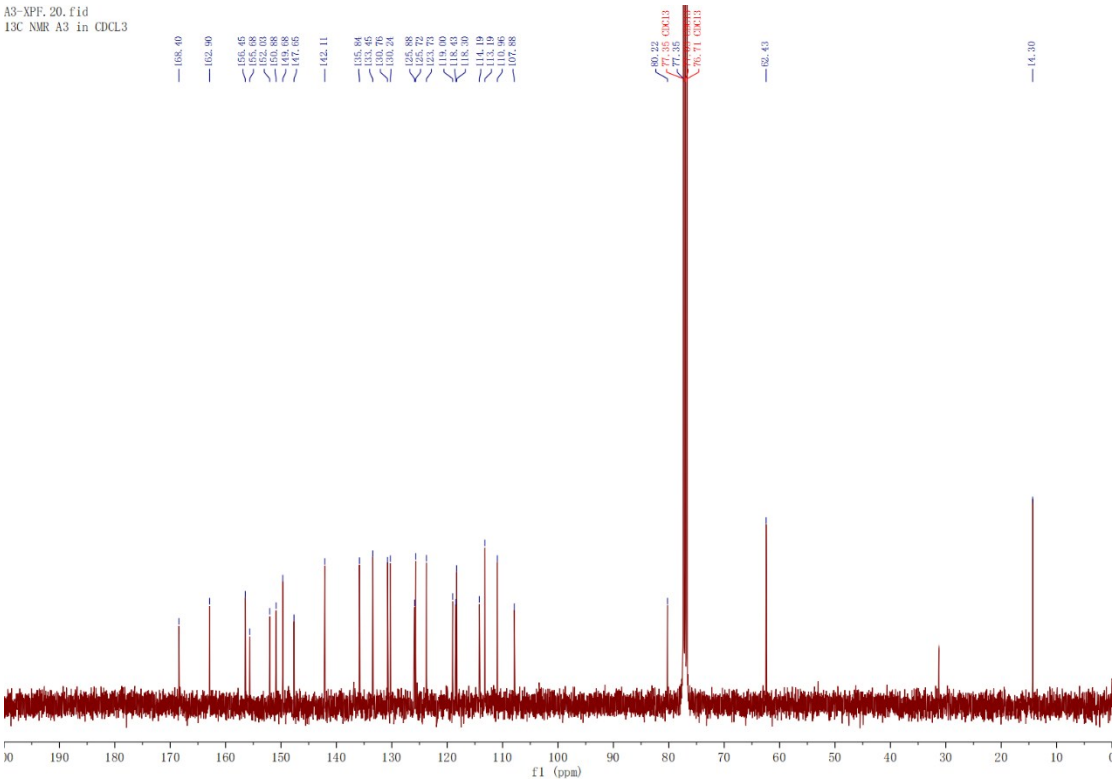

Figure S2. ^{13}C NMR (100 MHz, $CDCl_3$-*d*) of **JNY-1**.

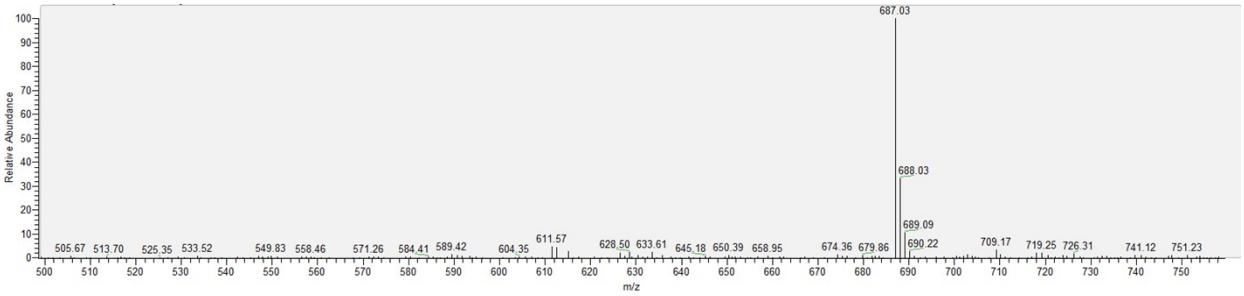

Figure S3. ESI MS spectra of **JNY-1**

3. UV-vis spectra of JNY-1 upon addition of H_2O_2.

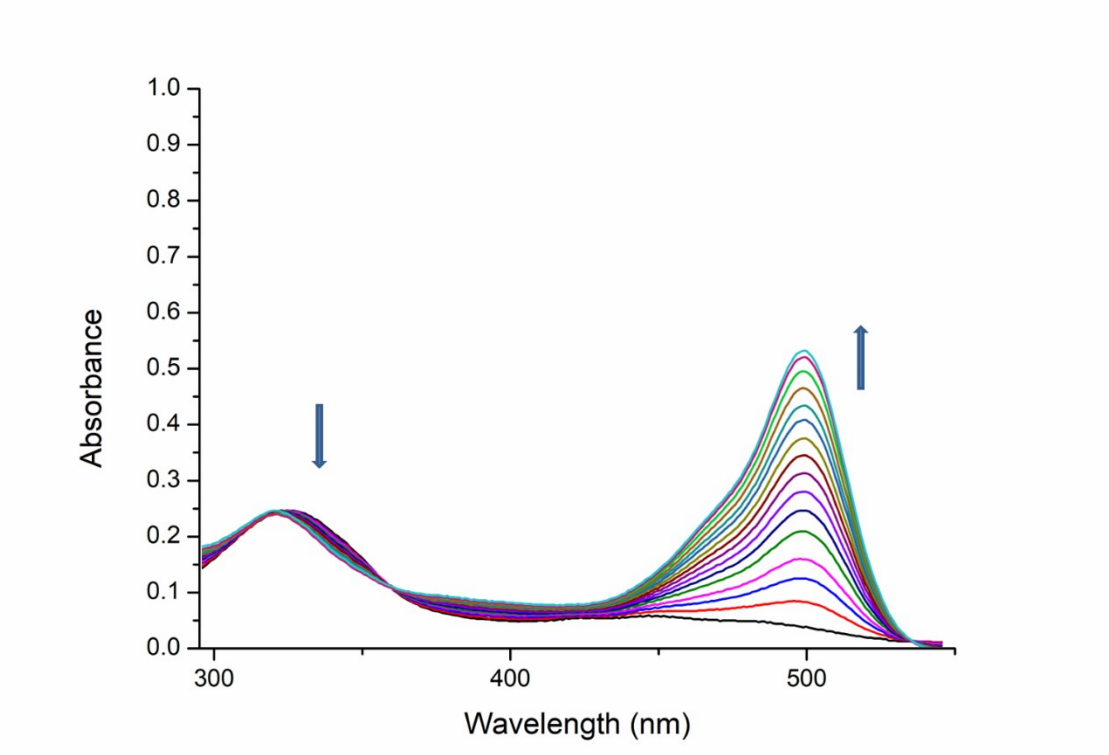

Figure S4. UV-vis spectra of **JNY-1** upon addition of H_2O_2.

4. Effect of pH.

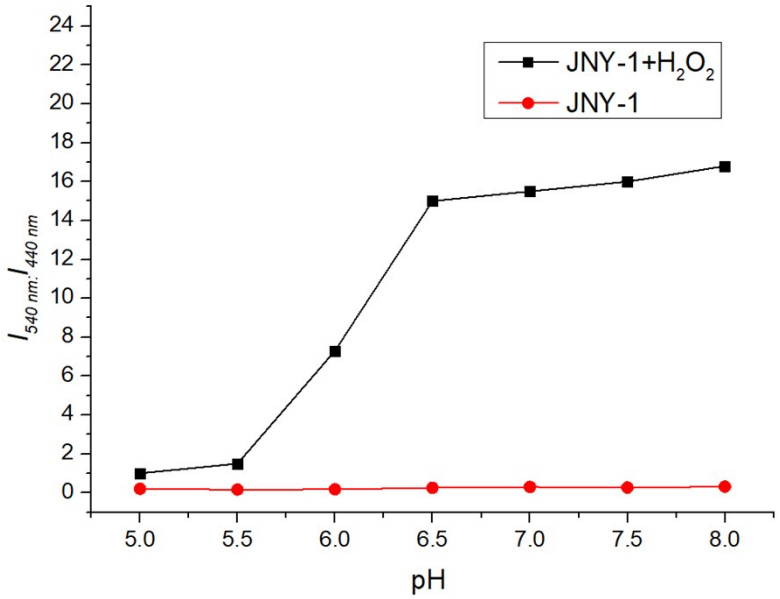

Figure S5. pH effect on the I_{540}/I_{440} of **JNY-1** (10 μM) in the absence and presence of H_2O_2 (100 μM).

5. Color changes of probe upon addition of H_2O_2.

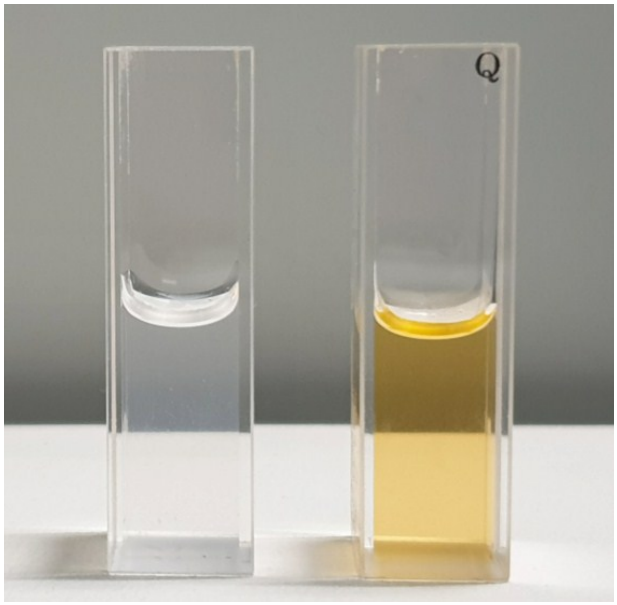

Figure S6. Color changes of probe **JNY-1** (10 μM) upon addition of H_2O_2 (100 μM)

6. Emission color changes of probe upon addition of H_2O_2

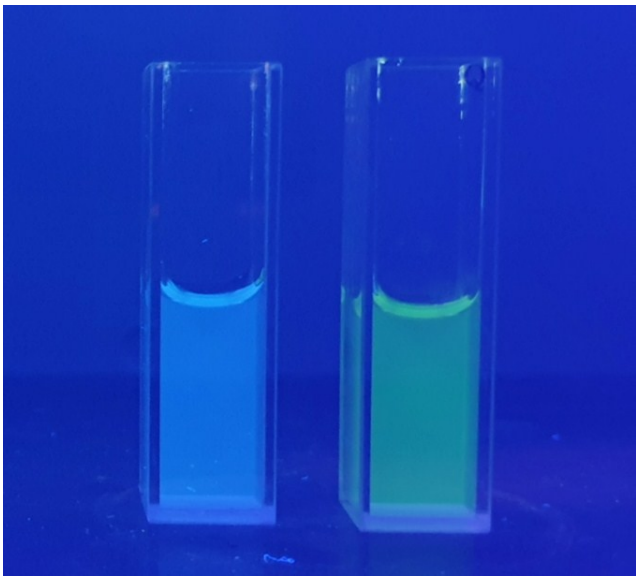

Figure S7. Emission color changes of probe **JNY-1** (10 μM) upon addition of H_2O_2 (100 μM)