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Electronic Supplementary Information for:

Near-Infrared Fluorescence Probe for Hydrogen Peroxide Detection: Design, Synthesis, and Application in Living Systems

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Table of contents

1. Synthesis of probe 1

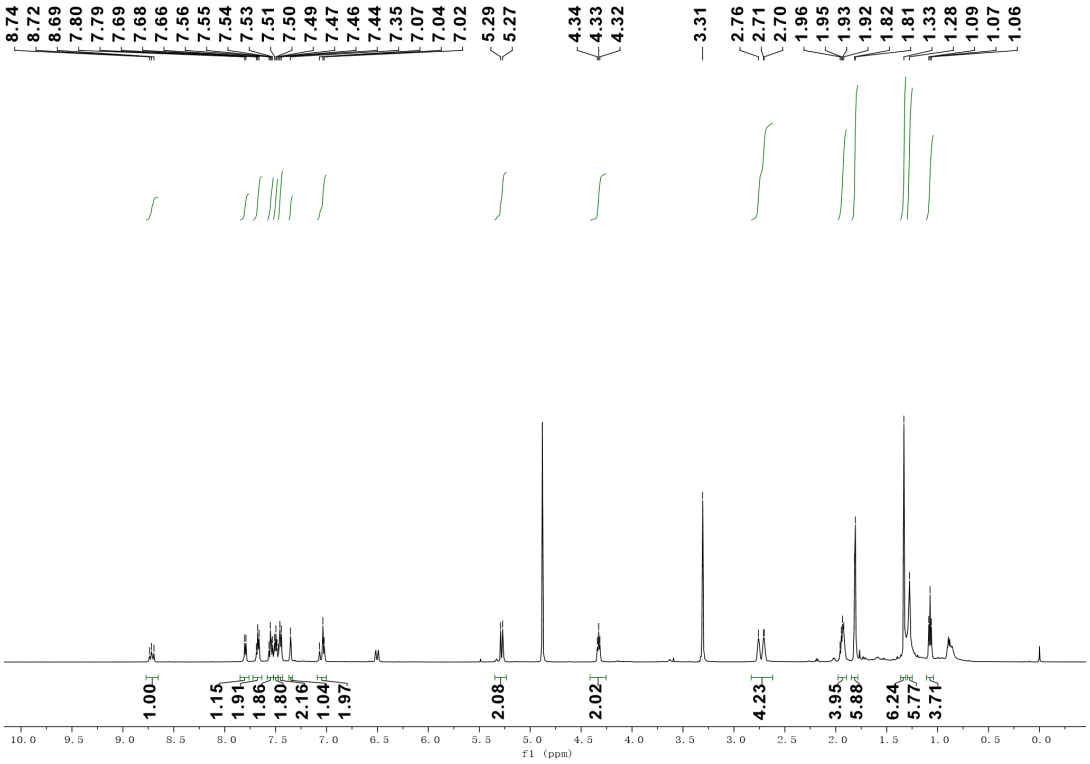

Fig. S1 ^{1}H NMR spectrum of **1** in CD_3OD.

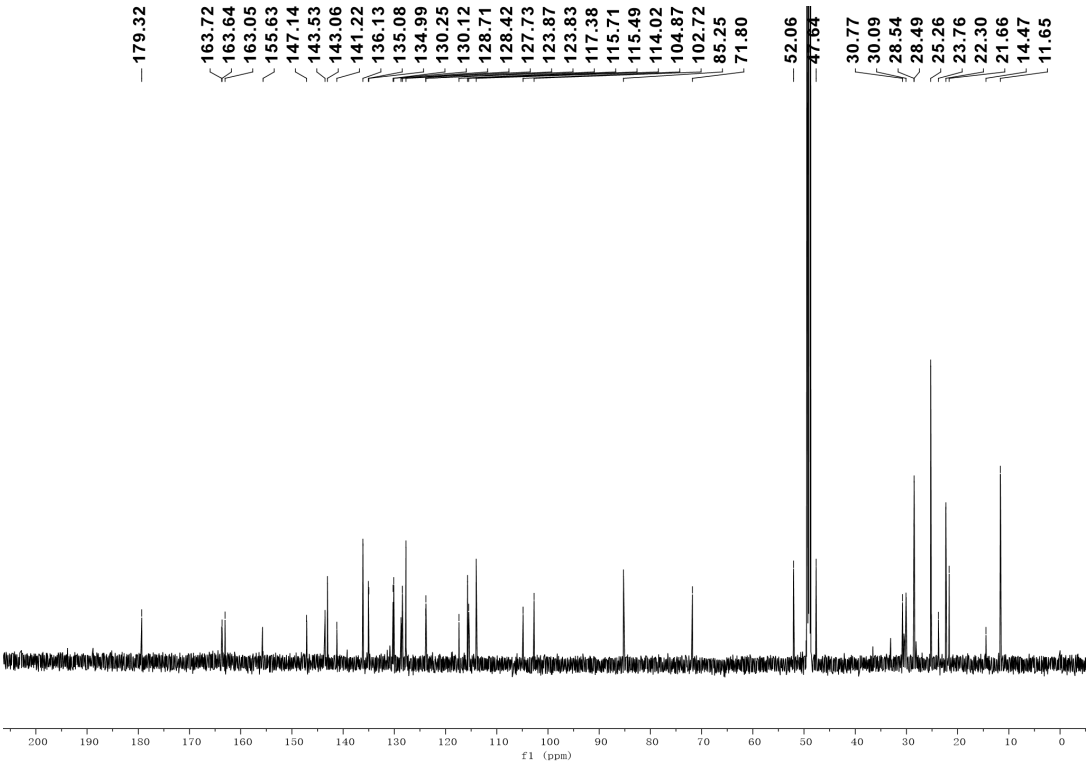

Fig. S2 ^{13}C NMR spectrum of **1** in CD_3OD.

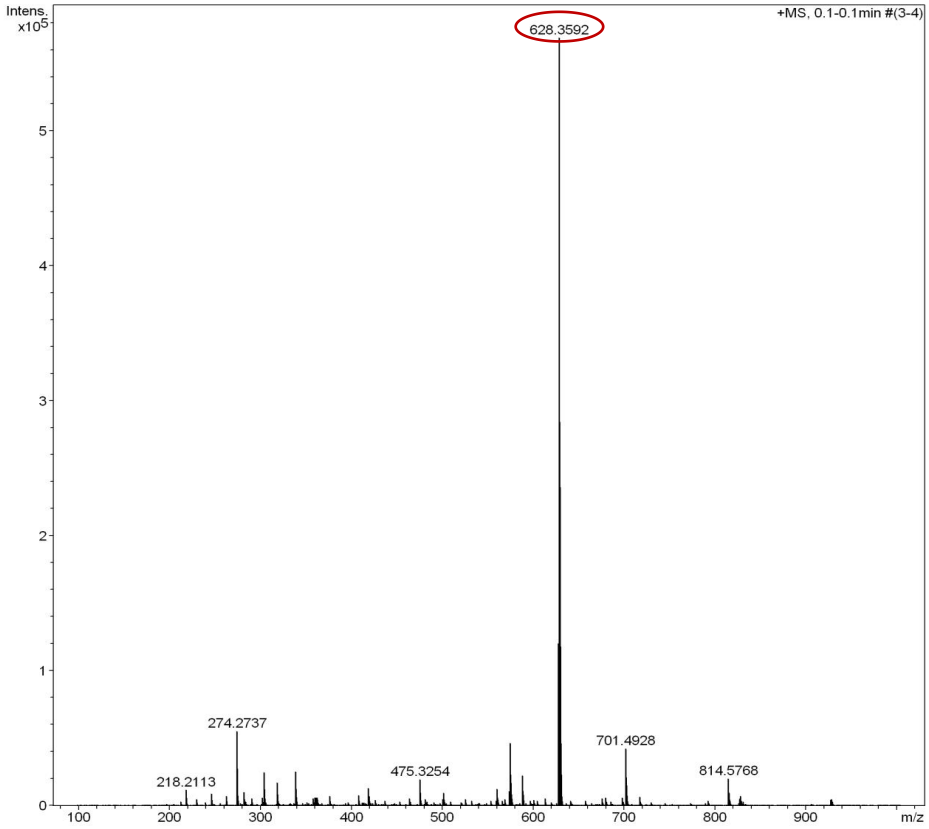

Fig. S3 ESI-MS of the probe **1**

2. Electrospray ionization mass spectrum of the reaction solution of 1

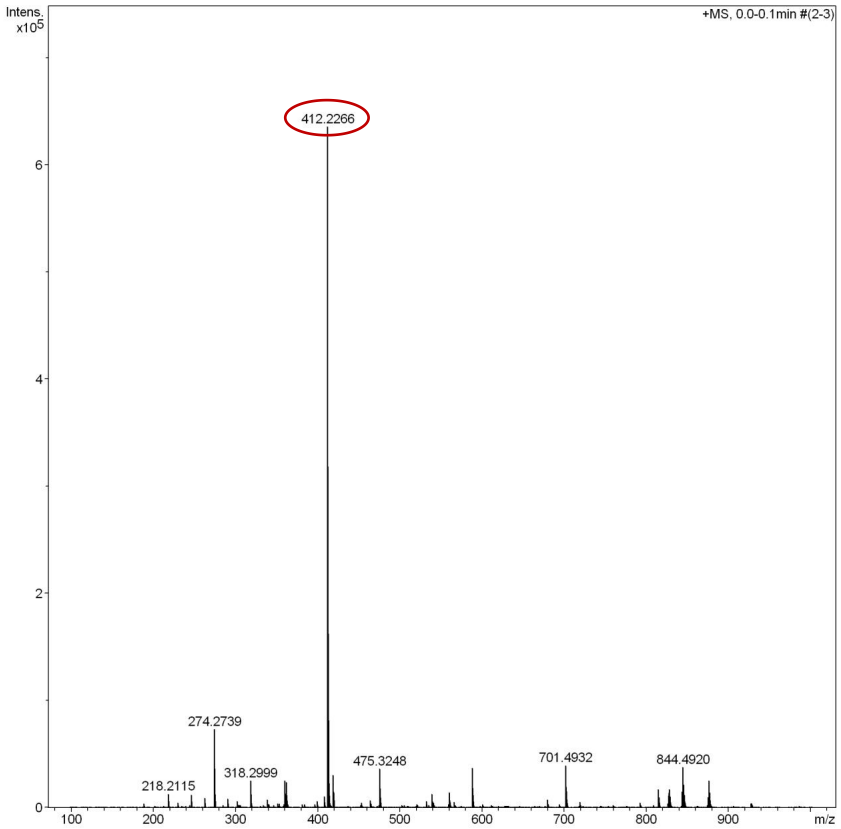

Fig. S4 ESI-MS of the reaction solution of **1** (20 μM) with H_2O_2 (80 μM).

3. Fluorescence kinetic curves of 1 reacting with H_2O_2

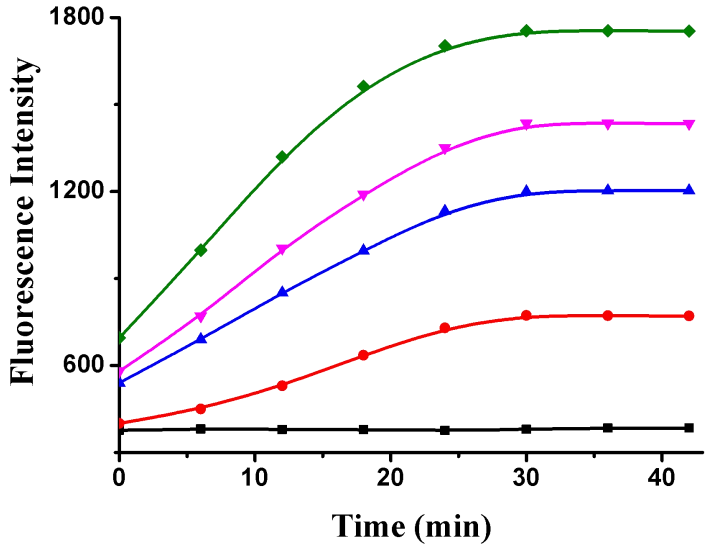

Fig. S5 Plots of fluorescence intensity of **1**(10 μM) vs.the reaction time in the presence of varied concentrations of H_2O_2 (from bottom to top): 0 (control), 10, 40, 60 and 80 μM.The measurements were performed at 37 °C in 10 mM PBS (pH 7.4) with λex/em= 670/708 nm.

4. Effects of pH and temperature

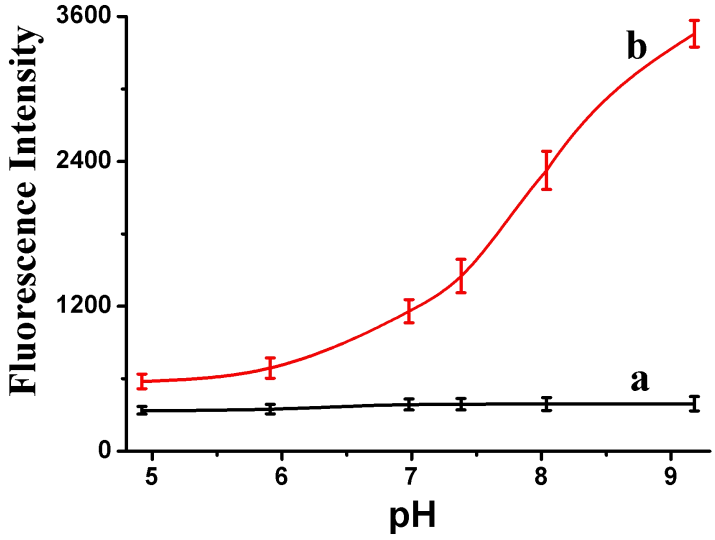

Fig. S6 Effects of pH on the fluorescence of 10 μM **1** (a) before and (b) after reaction with H_2O_2 (60 μM). The results are the mean ± standard deviation of three separate measurements; λex/em= 670/708 nm.

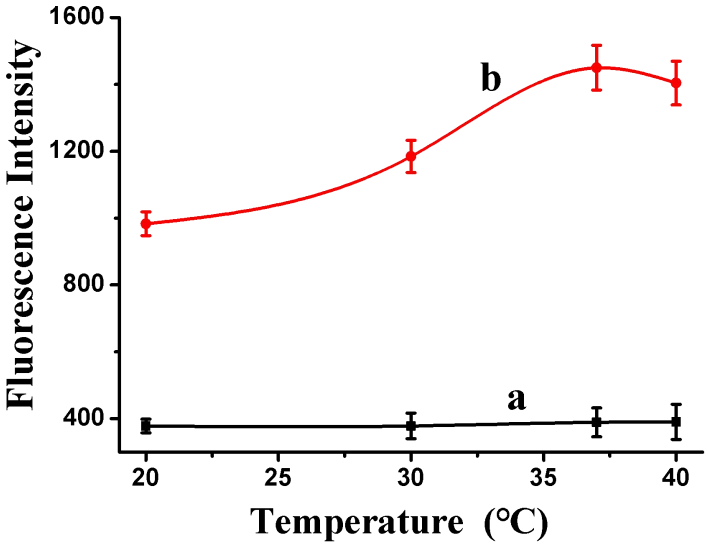

Fig. S7 Effects of temperature on the fluorescence of 10 μM **1** (a) before and (b) after reaction with H_2O_2 (60 μM).The results are the mean ± standard deviation of three separate measurements; λex/em= 670/708 nm.

5. Cytotoxicity assay

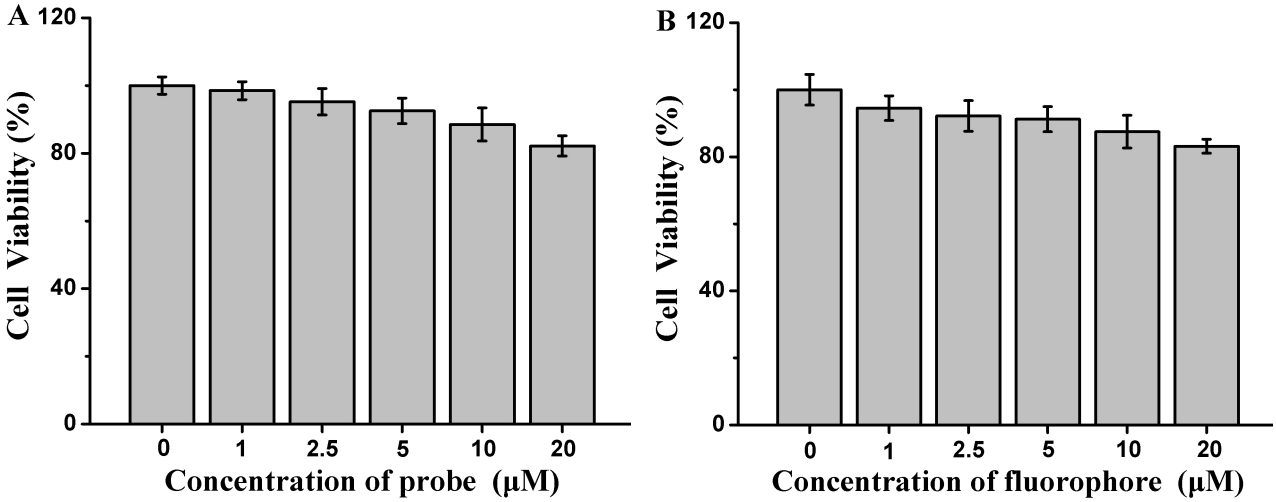

Fig. S8 Effects of (A) probe **1** and (B) fluorophore **2** with varied concentrations (10 μM) on the viability of HepG2 cells. The viability of the cells without probe **1** or fluorophore **2** is defined as 100%. The results are the mean ± standard deviation of six separate measurements.

6. Relative pixel intensity measurements in HepG2 cells and mice

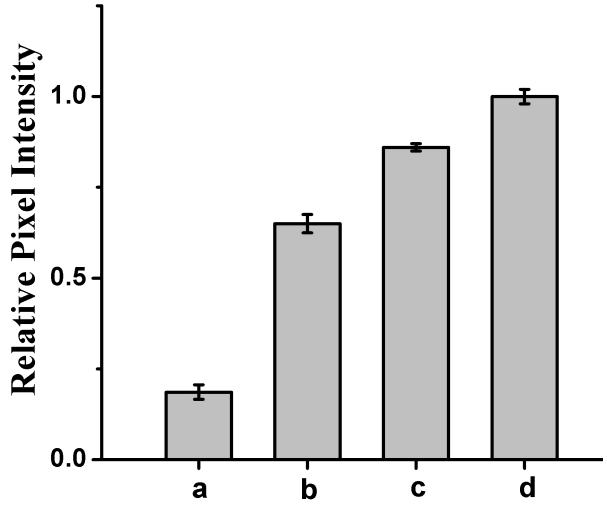

Fig. S9 Relative pixel intensity measurements obtained from the images of HepG2 cells: (a) HepG2 cells were incubated with only 10 μM probe **1** for 30 min. On the basis of the (a) group, then the HepG2 cells were incubated with 50 μM H_2O_2 for (b) 10 min. (c) 20min. (d) 30min. The results are the mean ± standard deviation of three separate measurements.

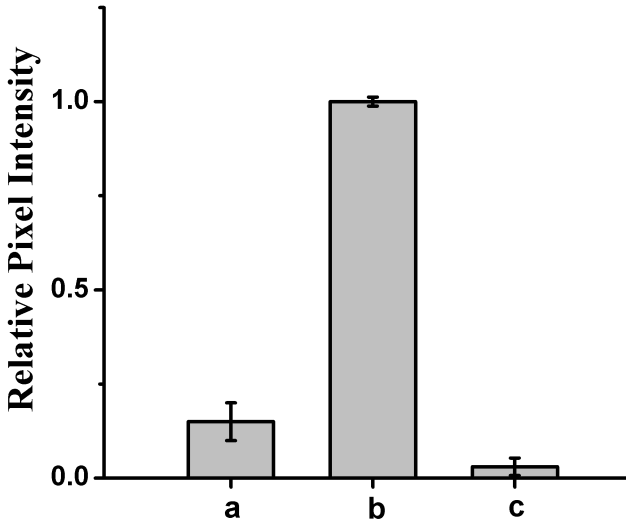

Fig. S10 Relative pixel intensity measurements obtained from the images of HepG2 cells: (a) HepG2 cells incubated with only probe **1** (10μM) for at 37℃ for 30 min; (b) HepG2 cells incubated with rotenone (100 nM) at 37 ℃ for 30 min and then further incubated with probe **1** (10 μM) for 30 min; (c) HepG2 cells incubated with rotenone (100 nM) at 37 ℃ for 30 min, then incubated with NAC (20 mM) for another 30 min, and further incubated with probe **1** (10 μM) for 30 min. The strongest fluorescence intensity from the image of cells incubated with rotenone (100 nM) at 37 ℃ for 30 min and then further incubated with probe **1** (10 μM) for 30 min is defined as 1.0. The results are the mean ± standard deviation of three separate measurements.

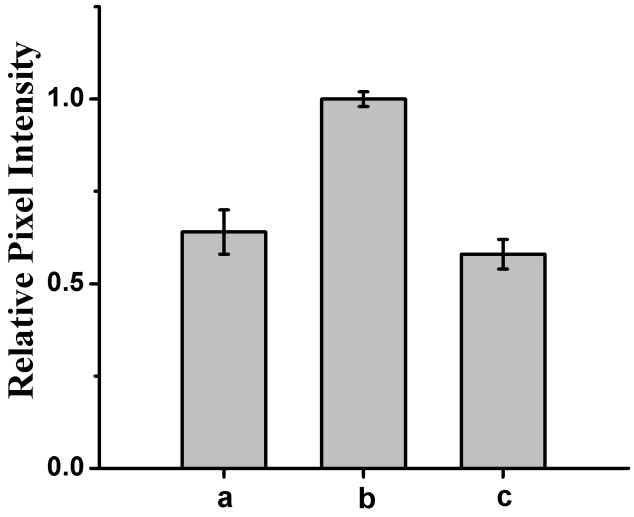

Fig. S11 Relative pixel intensity measurements obtained from the images of mice: (a) The mouse was given an injection of Probe **1** (200 μM, 100 μL) into the peritoneal cavity; (b) The mouse was pretreated with rotenone (2.5 mM, 100 μL) and followed by injection of Probe **1** (200 μM, 100 μL) at the same region after 1 h; (c) The mouse was successively treated with rotenone (2.5 mM,100 μL) for 1 h, NAC (20 mM, 100 μL) for 1 h, and Probe **1** (200 μM, 100 μL) at the same position. The strongest fluorescence intensity from the image of the mouse was pretreated with rotenone (2.5 mM, 100 μL) and followed by injection of Probe **1** (200 μM, 100 μL) at the same region after 1 h is defined as 1.0. The results are the mean ± standard deviation of three separate measurements.