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Supporting Information

A water-soluble and highly specific fluorescent probe for imaging thiophenols in living cells and zebrafish

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1. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence spectra of free probe **1** were measured by five times and its standard deviation was obtained. To gain the slope, the fluorescence intensity ratio at 492 nm was plotted as the increasing concentrations of thiophenol. So the detection limit was calculated with the following equation (1):

$$\text{Detection limit} = 3\sigma/k \quad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of thiophenol.

2. Cytotoxicity assays

The cell viability of RAW 264.7 macrophage cells, treated with probe **1**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, RAW 264.7 macrophage cells, seeded at a density of 1 × 106 cells·mL-1 on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 12 h. Then the live RAW 264.7 macrophage cells were incubated with various concentrations (0, 5, 10, 20, and 30 μM) of probe **1** suspended in culture medium for 6 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

3. Imaging studies of live cells

The RAW 264.7 macrophage cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and incubated under an atmosphere containing 5% CO_2 at 37 °C humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin-streptomycin. Before imaging by confocal fluorescence microscope, probe **1** (10 μM) was used as a bioimaging reagent to incubate RAW 264.7 macrophage cells for 30 min, then removed culture medium and washed with phosphate-buffered

saline for three times. After that, these cells were further incubated upon addition of 50 μM 2-aminothiophenol for 30 min then imaged at room temperature.

4. Imaging studies of zebrafish

Healthy male and female zebrafish (AB stain) were maintained in different tanks with a 14 h light / 10 h dark cycle at 28 °C. Then, sexually mature zebrafish were selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation in the morning. After sterilizing and cleaning, the fertilized eggs were added to zebrafish embryo culture water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator at 28 °C. The 4-day-old zebrafish were incubated with 10 μM probe **1** for 20 min, and then washed with culture water to remove the remaining probe. After that, the zebrafish were further incubated with 2-aminothiophenol (10 μM) for 20 min and imaged by confocal fluorescence microscope.

5. The HRMS data for reaction product of probe 1 with thiophenol

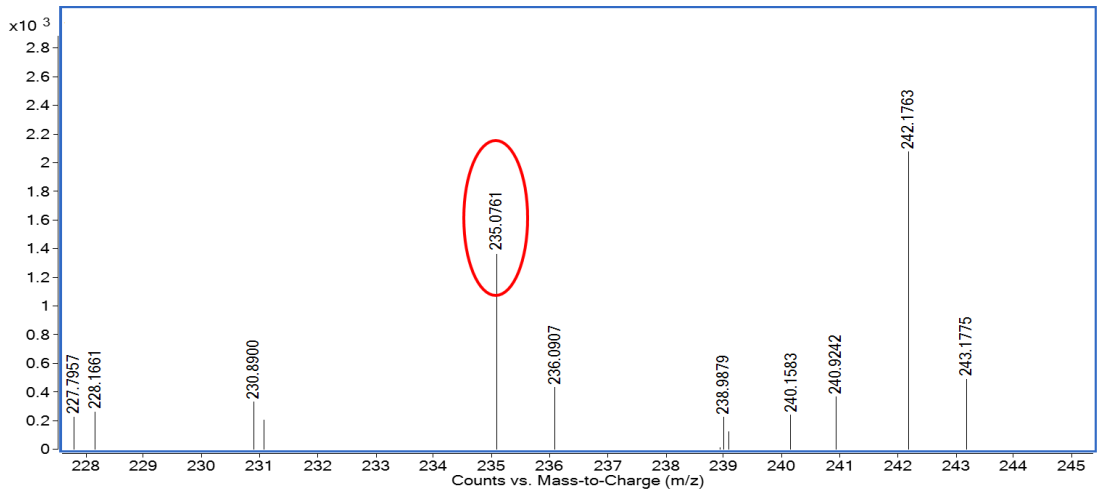

Figure S1. The HRMS data for reaction product of probe **1** with thiophenol.

6. The absorption spectra of probe 1 at different concentrations in the aqueous solution

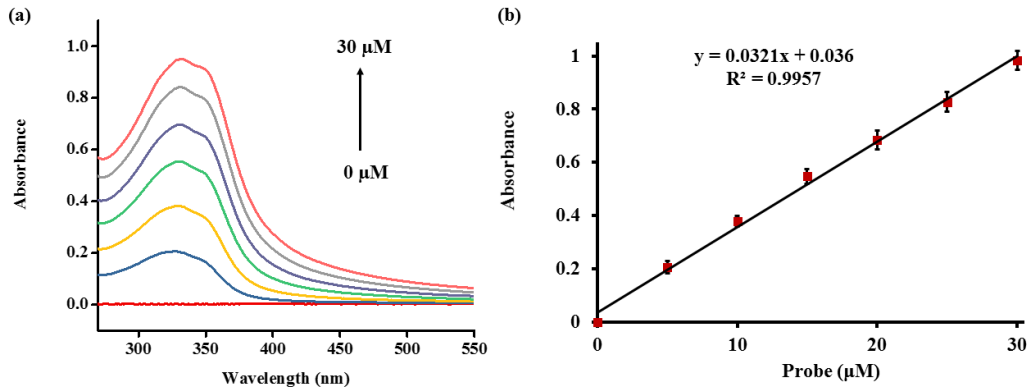

Figure S2. (a) The absorption spectra of different concentration probe **1** (0-30 μM) in the PBS solution (5 mM, pH = 7.4). (b) The absorbance of different concentration probe **1** as a function of probe concentrations (0-30 μM).

7. The absorption responses of probe 1 in the presence of thiophenol under the aqueous solution

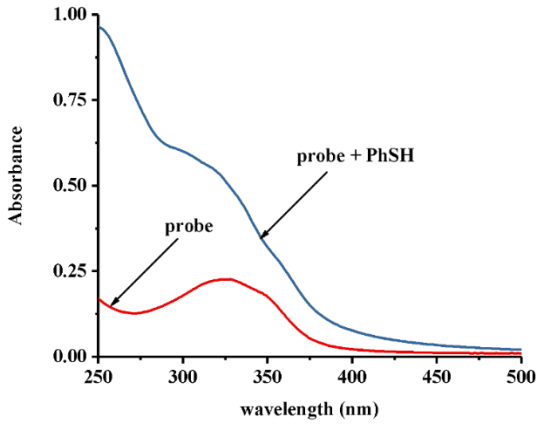

Figure S3. Absorption responses of probe **1** (10 μM) in the presence of 2-aminothiophenol (50 μM) under the PBS solution (5 mM, pH = 7.4) at 25 °C.

8. Effects of pH on the recognition of probe 1 for thiophenol

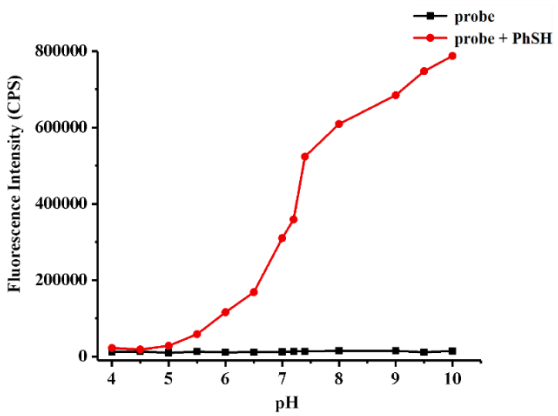

Figure S4. The fluorescence intensities at 492 nm of probe **1** (5 μM) in the absence and presence of thiophenol (50 μM) at the varied pH values.