

## Supporting Information

### **A dichlororesorufin-based colorimetric and fluorescent probe for ultrasensitive detection of mercury ions in living cells and zebrafish**

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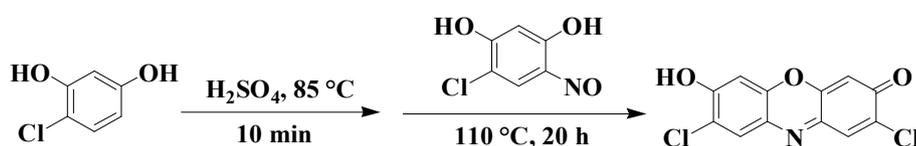
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## 1. Synthesis of compound 1

4-Chlororesorcinol (1.45 g, 10.0 mmol) was dissolved in 10 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and heated to 85 °C with stirring for 10 min. Then 4-chloro-6-nitrosoresorcinol (1.74 g, 10.0 mmol) was added gradually. The mixture was then heated to 110 °C with stirring for 20 h, cooled to room temperature and poured with stirring into 300 mL of ice-water. After 10 min, the precipitate was filtered, washed with water, and dried under air suction to afford pure compound **1** as a dark brown powder in >99% yield (2.83 g).



**Figure S1.** The synthetic route of compound **1**.

## 2. General procedure for analysis

The stock solution of probe **R-Hg** (1 mM) was prepared in tetrahydrofuran. The preliminary experiments showed that test conditions of fluorescence intensities/spectra of probe **R-Hg** were obtained in  $\lambda_{\text{ex/em}} = 530/595$  nm and 2/3 nm of slit. Moreover, the test system was in aqueous solution containing 5 mM HEPES (pH 7.4) at room temperature. All data were recorded after adding analytes 60 minutes. On account of the above investigated conditions, the specific properties of probe **R-Hg** could be studied through further experiments.

## 3. Preparation of 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<sub>2</sub> at 37 °C

humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin–streptomycin. Cells were seeded on dish for fluorescence microscopic imaging by laser confocal fluorescence microscope.

The cell viability of RAW 264.7 macrophage cells, treated with probe **R-Hg**, was assessed by a cell counting kit-8 (CCK-8). Briefly, RAW 264.7 macrophage cells, seeded at a density of  $1 \times 10^6$  cells·mL<sup>-1</sup> on a 96-well plate, were maintained at 37 °C in a 5% CO<sub>2</sub> / 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 **R-Hg** 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.

#### **4. Preparation 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<sub>2</sub>, 0.16 mM MgSO<sub>4</sub>) and cultured in illumination incubator at 28°C.

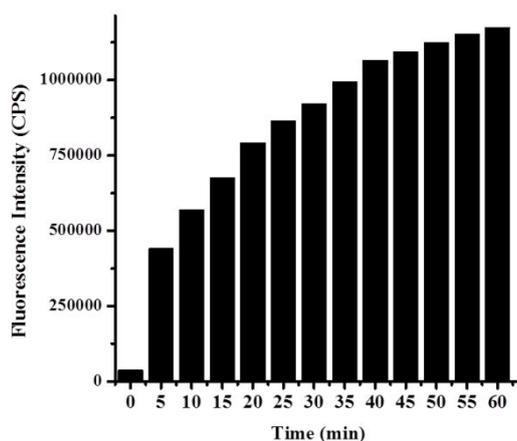
#### **5. Determination of the detection limit**

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of probe **R-Hg** was measured by eight times and the standard deviation of blank measurement was achieved. To gain the slope, the fluorescence intensity at 595 nm was plotted as a concentration of Hg<sup>2+</sup>. So the detection limit was calculated with the following equation (1):

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

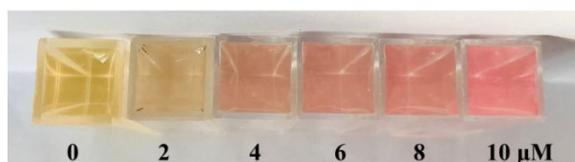
Where  $\sigma$  is the standard deviation of blank measurement,  $k$  is the slope between the fluorescence intensity versus  $\text{Hg}^{2+}$  concentration.

## 6. Time-dependence of detecting $\text{Hg}^{2+}$



**Figure S2.** Fluorescence intensities of probe **R-Hg** ( $5 \mu\text{M}$ ) at  $595 \text{ nm}$  over time in the presence of  $\text{Hg}^{2+}$  ( $5 \mu\text{M}$ ).

## 7. The color variations of probe solution in the presence of $\text{Hg}^{2+}$



**Figure S3.** The color changes of probe **R-Hg** ( $10 \mu\text{M}$ ) solution after the addition of  $\text{Hg}^{2+}$  ( $0, 2, 4, 6, 8, 10 \mu\text{M}$ ).