Supporting Information

A carbonothioate-based far-red fluorescent probe for specific detection of mercury ions in living cells and zebrafish

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1. General procedure for analysis

The stock solution of probe **CBRB** was prepared in ethanol. After a series of preliminary experiments, the fluorescence intensities/spectra of probe **CBRB** were obtained by $\lambda_{ex}/_{em}$ = 520/625 nm and 5 nm of slit. All measurements were made after analytes addition 20 minutes.

2. Imaging studies of living 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 **CBRB** (10 μM) was used as a bioimaging reagent to incubate RAW 264.7 macrophage cells for 20 min, then removed culture medium and washed with phosphate-buffered saline for three times. After that, these cells were further incubated upon addition of 20 μM Hg^{2+} for 30 min then imaged at room temperature. The excitation wavelength was 546 nm.

3. 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℃. 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℃. The 6-day-old

zebrafish were incubated with 10 μM probe **CBRB** for 20 min, and then washed with culture water to remove the remaining probe. After that, the zebrafish were further incubated with 20 μM Hg^{2+} for 30 min and imaged by confocal fluorescence microscope.

4. Characteristic spectra of probe CBRB towards Hg^{2+}

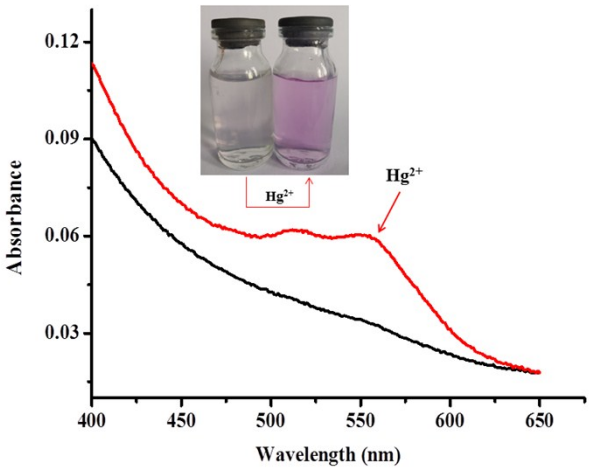

Figure S1. Absorption spectra of probe **CBRB** (20 μM) upon addition of Hg^{2+} (20 μM) in 5 mM HEPES (pH 7.4) aqueous solution. The illustration shows the change of color for adding Hg^{2+} to probe **CBRB**.

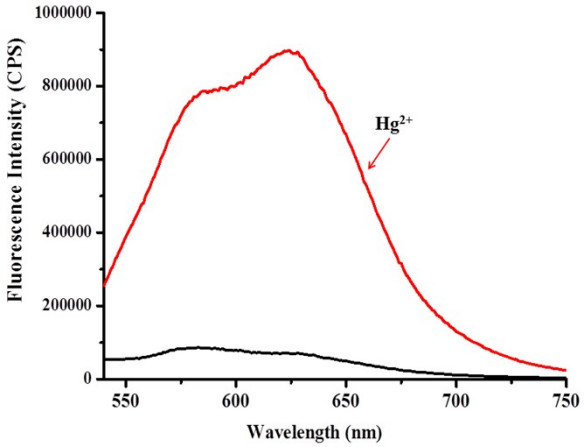

Figure S2. Fluorescence spectra of probe **CBRB** (5 μM) in the presence of 5 μM Hg^2 under aqueous solution containing 5 mM HEPES (pH 7.4).

5. Effects of pH on probe CBRB and its responses towards Hg^{2+}

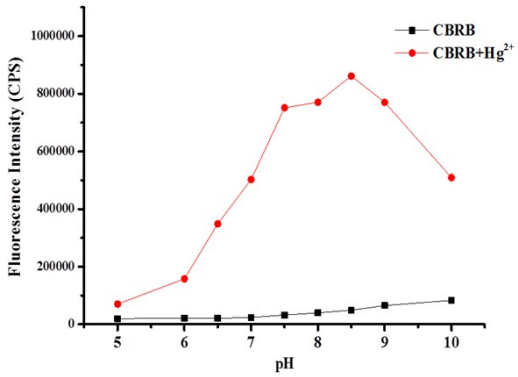

Figure S3. Fluorescence responses (625 nm) of probe **CBRB** (5 μM) and after addition of 5 μM Hg^{2+} in 5 mM HEPES aqueous solution as a function of different pH values.

6. The ^{1}H and ^{13}C NMR spectra of probe CBRB

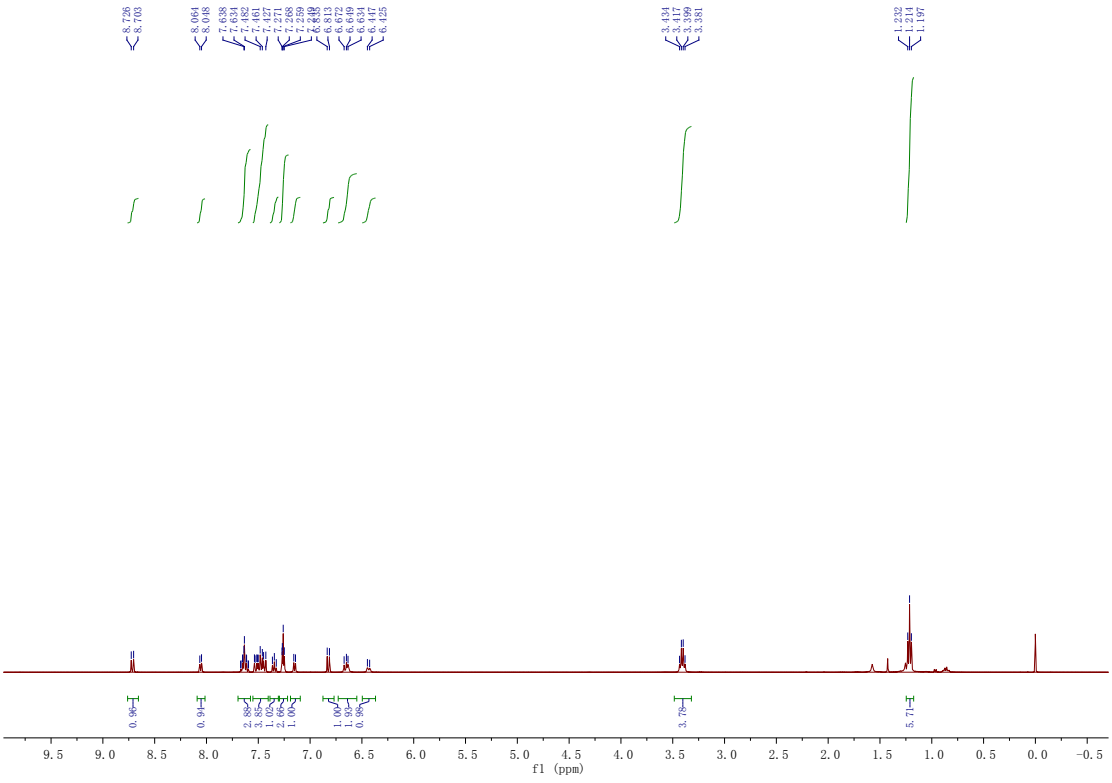

Figure S4. The ^{1}H NMR spectra of probe **CBRB**

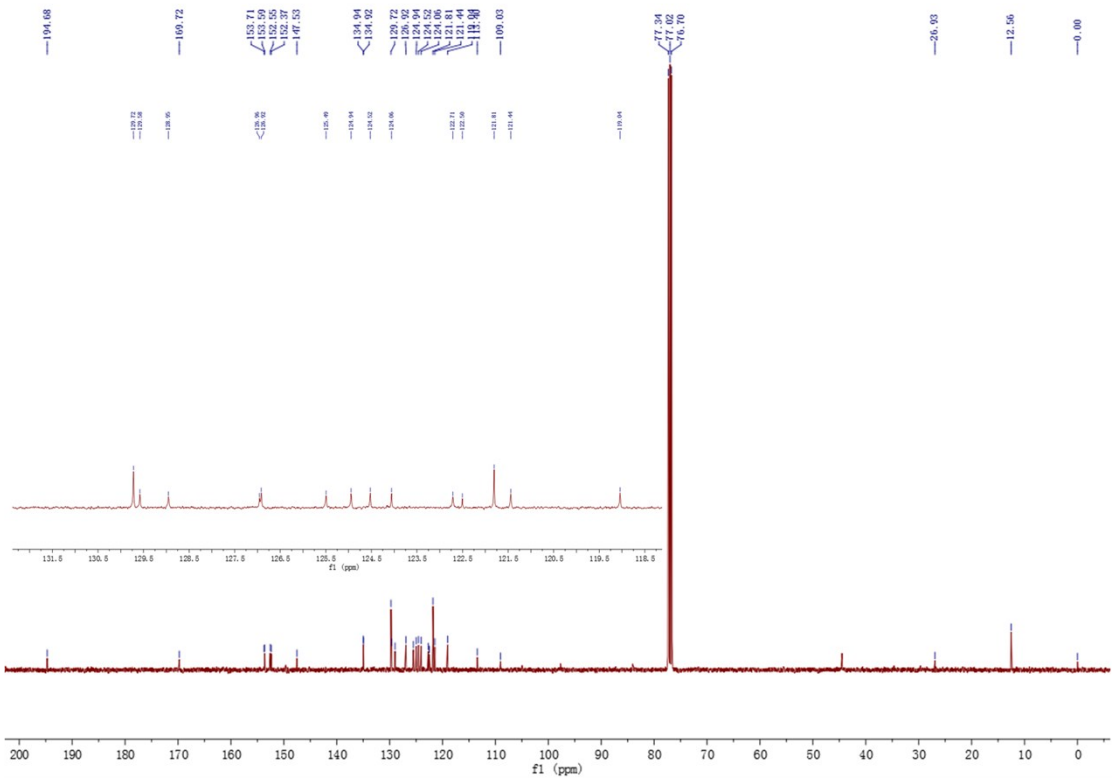

Figure S5. The ^{13}C NMR spectra of probe **CBRB**

7. The HRMS of probe CBRB and reaction products of probe and Hg^{2+}

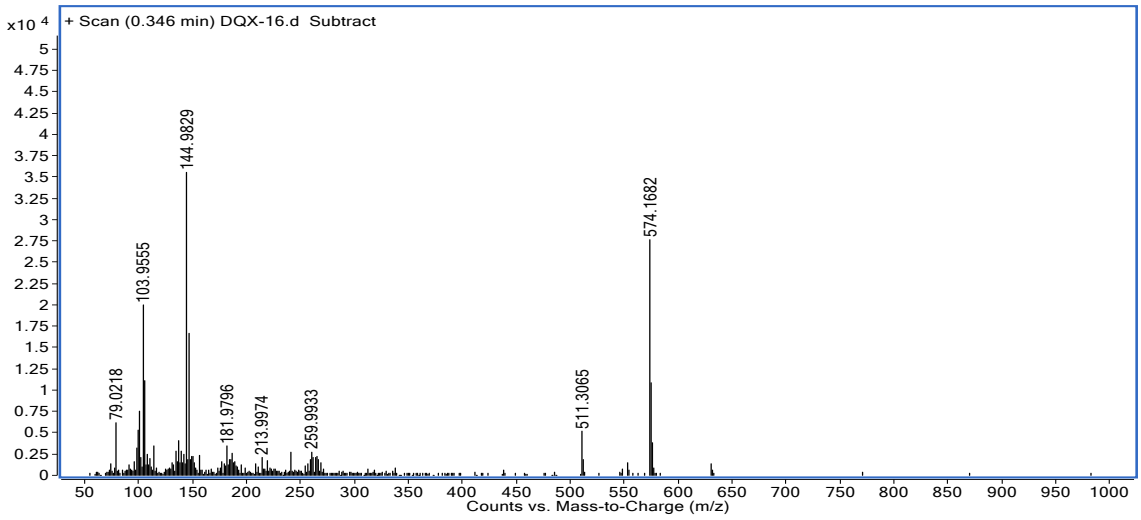

Figure S6. The HRMS of probe **CBRB**

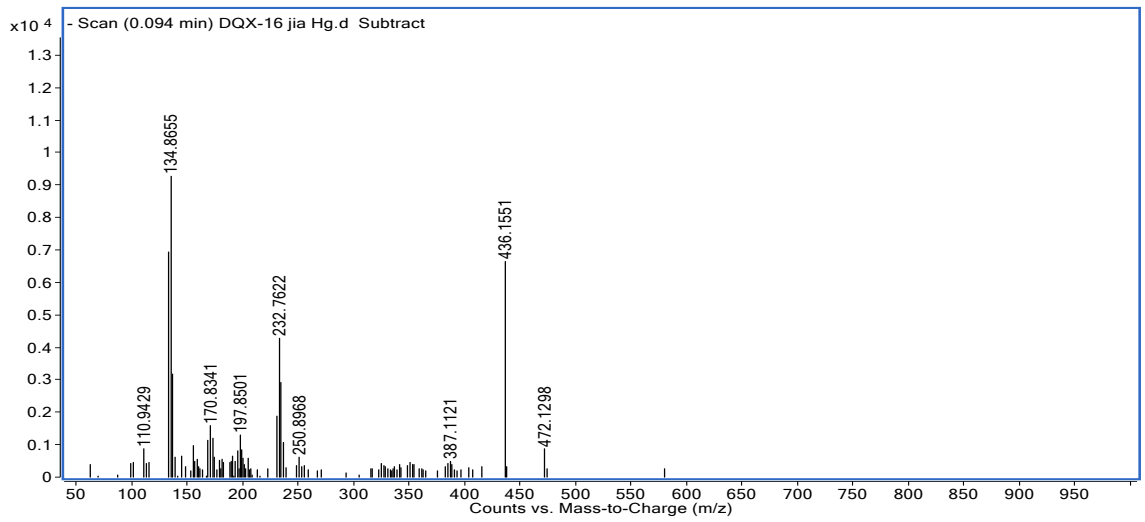

Figure S7. The HRMS of reaction products of probe and Hg^{2+}

8. HPLC chromatogram of probe CBRB in the absence and presence of Hg^{2+}

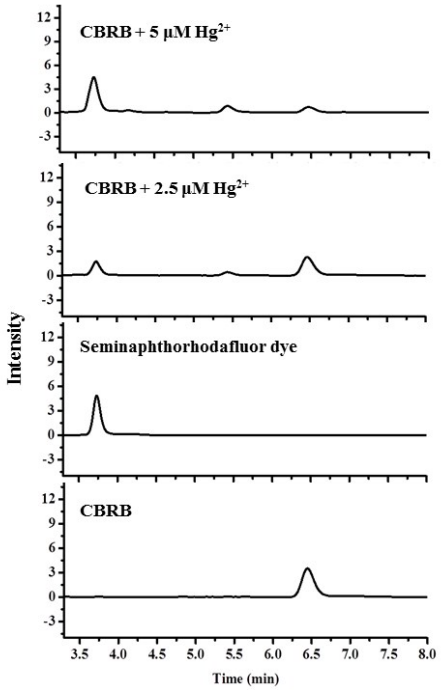

Figure S8. HPLC chromatogram traces of probe **CBRB** before or after the treatment of Hg^{2+} for 20 min. The peak corresponding to **CBRB** decreased in an Hg^{2+}-dose independent way accompanied by the simultaneous increase of a new peak whose structure was elucidated by HRMS analysis to be the seminaphthorhodafluor dye.

9. The cytotoxicity assays of probe CBRB

The cell viability of RAW 264.7 macrophage cells, treated with probe **CBRB**, was assessed by a cell counting kit-8 (CCK-8). Briefly, RAW 264.7 macrophage cells seeded at a density of 1×10^6 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 **CBRB** 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.

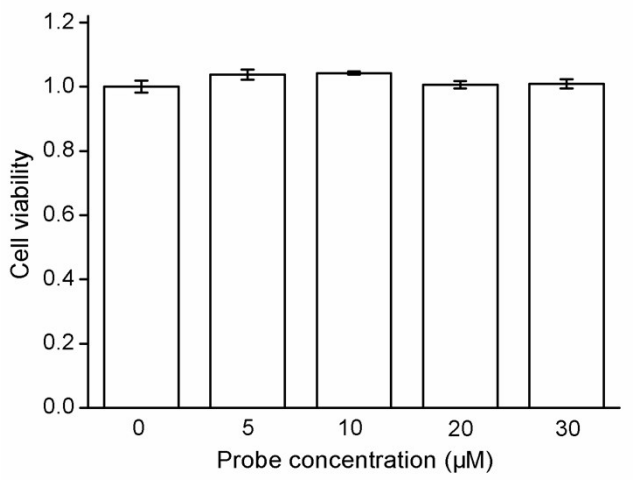

Figure S9. Cytotoxicity assays of probe **CBRB** at different concentrations for RAW 264.7 macrophage cells.