Supporting Information

An ultrasensitive ratiometric fluorescent probe for the detection of Hg^{2+} and its application in cell and zebrafish

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1. Materials and instrumentations

All the chemicals were from commercial suppliers and used without further purified. ^{1}H NMR spectra was recorded using Bruker AV-400 spectrometer with chemical shift served as ppm (TMS as internal standard). High resolution mass spectra (HRMS) were recorded using a LC-MS 2010A (Shimadzu) instrument. Fluorescence emission spectra were obtained on a Horiba FluoroMax-4 spectrophotometer with a excitation wavelength of 450 nm. The respond fluorescence images of cells and zebrafish to Hg^{2+} were obtained on an Olympus FV1000 confocal fluorescence microscope.

2. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectra of probe **CP-Hg** were measured ten times and the standard deviation of blank measurement was obtained. To gain the slope, the fluorescence intensity ratios of F_{575}/F_{490} were plotted as the increasing concentrations of Hg^{2+}. The detection limit was calculated with the following equation (1):

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

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity ratios versus the concentrations of Hg^{2+}.

3. Preparation of the testing medium

The probe **CP-Hg** (4.7 mg) was dissolved with 10 mL ethanol to obtain probe stock solution. Then, 50 μL probe solution and 0.5 mL HEPES (100 mM, pH7.4) were dropped in the distilled water, and then added water until 10 mL. Finally, the

testing medium HEPES (5 mM, pH7.4) buffer solution was obtained.

4. Cell culture

The RAW 264.7 macrophage cells were cultured in high-glucose (4.5 g/L) Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin and incubated under a humidified atmosphere containing 5% CO_2 at 37 °C for 24 h.

5. Cytotoxicity assays

The cell viability of RAW 264.7 macrophage cells treated with probe **CP-Hg** was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan) assay. 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 **CP-Hg** suspended in culture medium for 24 h. Subsequently, CCK-8 solution was added into each well and incubated for 2 h, and the absorbance at 450 mm for each well was obtained.

6. Bioimaging application in cell

Control group was captured directly using confocal fluorescence microscope (FV1000, Olympus Corporation, Tokyo, Japan). The second group was incubated with **CP-Hg** (10 μM) for 20 min, and then washed with culture medium for three times before fluorescence bio-imaging. The third group was incubated with **CP-Hg** (10 μM) for 20 min, and then treated with Hg^{2+} (1 μM) for 10 min. After washing the

cells with culture medium, images were harvested and resized using the software Photoshop CS3 (Adobe Systems, San Jose, CA, USA).

7. Culture 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±0.5 °C. Then, sexually mature zebrafish were selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation. After being sterilized and cleaned, the fertilized eggs were added into zebrafish embryo culture medium (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator at 28±0.5 °C. Care and handling of zebrafish assuredly adhered to the Institutional Animal Care and Use Committees of Qilu University of Technology.

8. Behavioral test with zebrafish

Locomotion activity of larvae was recorded using Zebrabox video-track system (Viewpoint, Lyon, France) in Biology Institute, Qilu University of Technology (Shandong Academy of Sciences). Behavioral test was conducted to evaluate the safety of **CP-Hg**. The normal zebrafish larvae at 72 hpf (hour post fertilization) were randomly divided into five plates and exposed to various concentrations of **CP-Hg** (0, 5, 10, 20, 30 μM) dissolved in the bathing medium. The larvae were transferred and maintained in a 14 h light (~1000 lux): 10 h dark (LD) cycle at 28±0.5 °C with changing fresh **CP-Hg** solution daily. Then the zebrafish larvae were cleaned in bathing medium and placed into a 48-well plate (one larva per well) at 120 hpf. After a 30 min acclimation period, the locomotor activity of each larva was monitored for

20 min in a silent room using an automated computerized video-tracking system (Viewpoint, Lyon, France), and the detailed track was recorded with Zebralab software (Viewpoint). All the behavioral data including swimming duration and movement distance from zebralab were further processed by computer programs Excel (Microsoft, USA) and OriginPro 8 (OriginLab, USA). Additionally, to reduce possible diurnal factors on level of locomotor activity, all behavioral tests were performed at zeitgeber time 6-8 (ZT6-8).

9. Bioimaging application in zebrafish

The 5-day-old zebrafish were incubated with **CP-Hg** (10 μM) for 20 min. the residual probe was wash away using the culture medium, and then a confocal laser scanning biological microscope was used to view the sections and acquire the images. The second group of zebrafish was treated with Hg^{2+} (0.5 μM) for 10 min followed by incubating with **CP-Hg** (10 μM) for 20 min. Then confocal fluorescence microscopy was used to observe and capture data.