Supporting Information

A specific amino-based fluorescent probe for mercury ions detection in water samples, cells and zebrafish

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1. Materials and instruments

Except for otherwise noted, chemical reagents were received from commercial vendor and employed without further purification. Absorption spectra were carried out using a UV-3101PC spectrophotometer. Fluorescence emission spectra were performed using a Horiba FluoroMax-4 spectrophotometer. The slit width was 5.0 nm for both excitation and emission. High resolution mass spectra (HRMS) were obtained by LC-MS2010A instrument. ^{1}H data were obtained by Bruker AV-400 NMR spectrometer. Fluorescence imaging of Hg^{2+} in live cells and zebrafish were carried out on an Olympus FV1000-IX81 confocal fluorescence microscope.

2. Synthesis of compound 1

Terephthalaldehyde (1.21 g, 9 mmol), 9,10-phenanthroquinone (0.624 g, 3 mmol), and ammonium acetate (4.61g, 60 mmol) were dissolved in glacial AcOH (30 mL). After stirring for 40 min at the 100 ℃, the hot solution was cooled to room temperature. And then the resultant yellow solid was obtained by filtration and washed with acetate acid, dilute sodium hydrogen carbonate solution, and deionized water. The yellow solid was dried under reduced vacuum, and then purified by silica gel column chromatography using acetone as eluent to afford the pure product compound **1** (610 mg, 63%).

3. Preparation of the testing solution

The probe **TD-Hg** (4.1 mg) was dissolved with 10 mL dimethyl sulfoxide

(DMSO) to obtain probe stock solution. Then, a 50 μL probe solution and 1 mL HEPES (100 mM, pH = 7.4) were dropped in the mixture (Ethanol: water = 4:6). Finally, the testing solution was obtained.

4. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The detection limit (DL) was calculated as follows:

$$DL = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of Hg^{2+}. The fluorescence spectra of free probe were measured by five times and its standard deviation was obtained.

5. The absorption spectra of the probe TD-Hg for Hg^{2+}

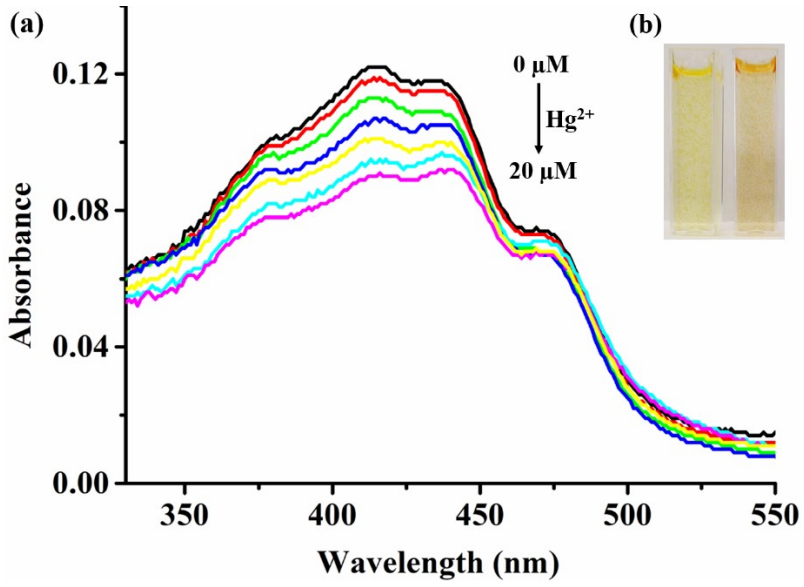

Figure S1. (a) Absorption spectra of probe **TD-Hg** (5 μM) with different concentrations of Hg^{2+} (0-20 μM). (b) Color change of probe **TD-Hg** (20 μM) before (left) and after (right) the addition of Hg^{2+} (40 μM).

6. Cytotoxicity assays of cells and behavioral analysis of zebrafish

The cell viability of HeLa cells, treated with probe **TD-Hg**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, HeLa 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 HeLa cells were incubated with various concentrations (0, 5 and 10 μM) of probe **TD-Hg** suspended in culture medium for 7 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

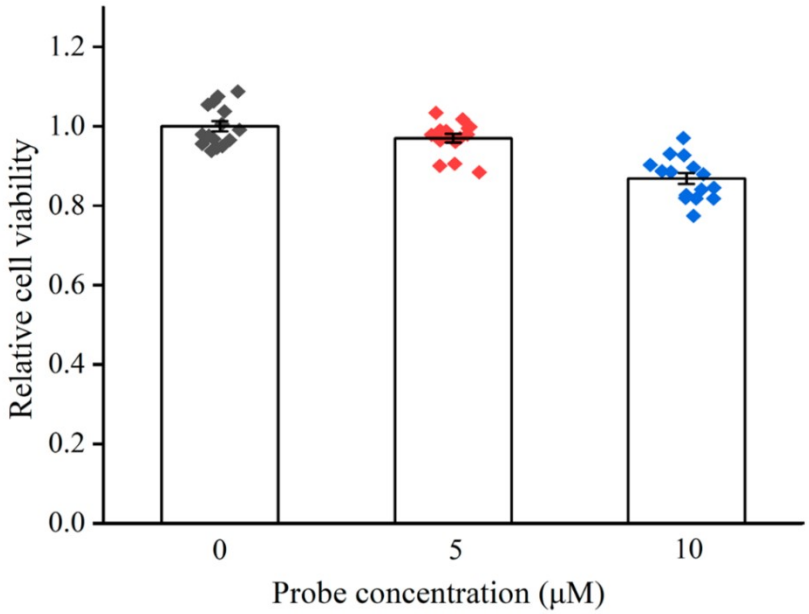

Figure S2. Cells viability with different concentration of probe **TD-Hg**.

Use and handling of zebrafish was strictly in accordance with the Institutional Animal Care and Use Committees of Qilu University of Technology. Ethical approval was granted by the Institutional Animal Care and Use Committees of Qilu University of Technology. Behavioral analysis was conducted to assess the biocompatibility of **TD-Hg**. The normal zebrafish larvae at 132 hpf (hour post-fertilization) were

randomly divided into two well plates and exposed to different concentrations of **TD-Hg** (0, 10 μM). Then the zebrafish larvae were cleaned in bathing medium and placed into 48-well plates (one larva per well) at 144 hpf. After a 30 min acclimation period, the locomotor activity of each larva was monitored for 10 min in a silent room using an automated computerized video-tracking system, and the detailed track was recorded with Zebralab software.

7. Characterization of the probe TD-Hg

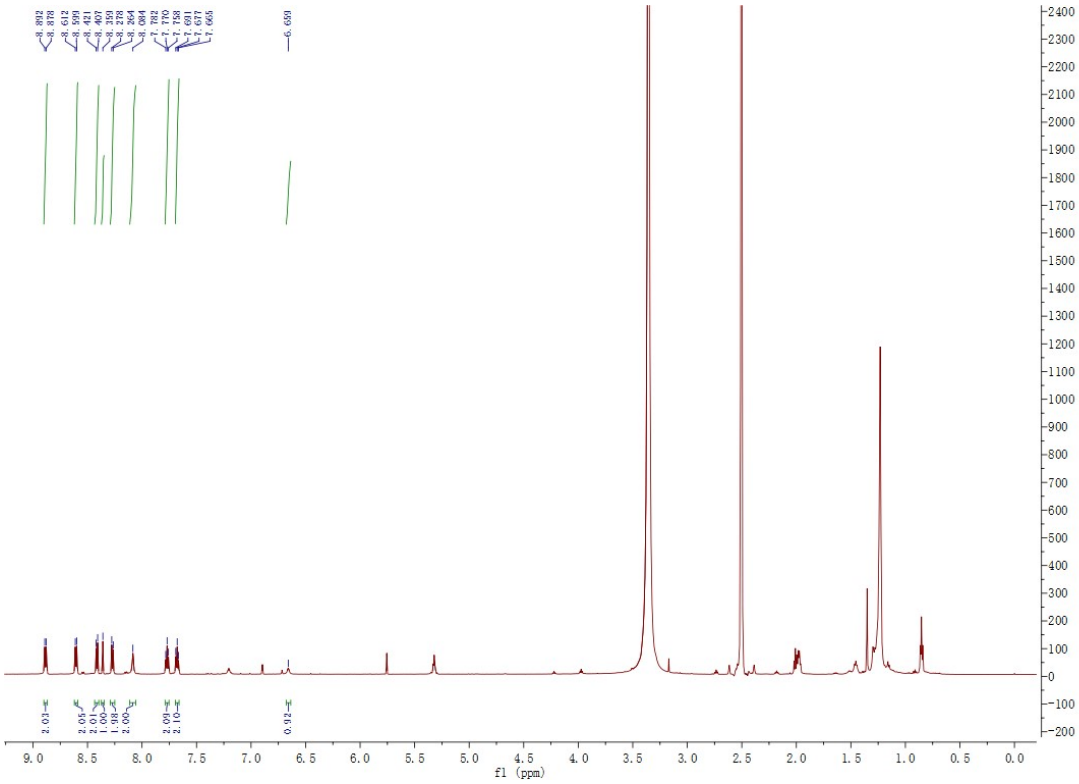

Figure S3. ^{1}H-NMR data of probe **TD-Hg**.

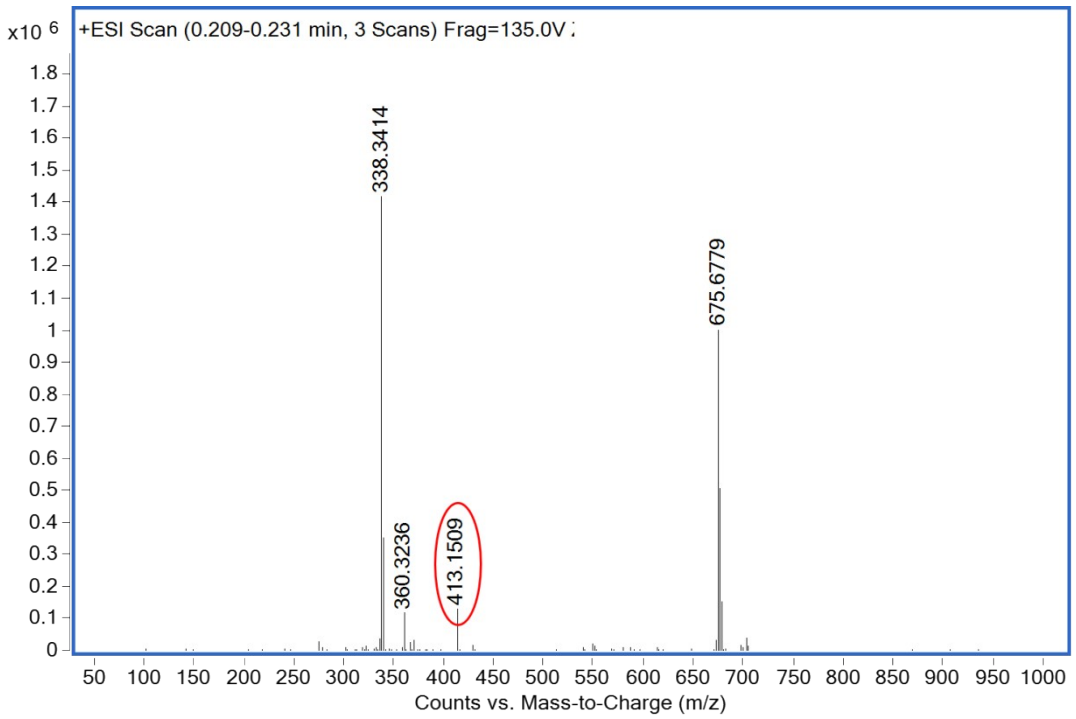

Figure S4. HRMS data of probe **TD-Hg**.