

Supporting Information

Selective red-emission detection for mercuric ion in aqueous solution and live cells using fluorescent probe based on unnatural peptide receptor

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1. Synthesis of **1**.

1 was synthesized in solid phase synthesis with Fmoc chemistry (Scheme S1, Supporting Information).¹ Fmoc protected amino acid (0.3 mmol, 3 equiv) was assembled on Rink Amide MBHA resin (0.1 mmol) for the synthesis of **1**. Cyanostilbene ((E)-2-(2-(2-(benzo[d]thiazol-2-yl)-2-cyanovinyl)-5(diethylamino) phenoxy)acetic acid) fluorophore was synthesized according to the previous reported procedure.² The deprotection of alloc group of the side chain of Dap was carried out by the following literature procedure.³ Methanesulfonyl chloride (92.9 μ L, 1.2 mmol), Triethylamine (168 μ L, 1.2 mmol) in DMF (3 mL) were added into the resin and the resulting solution was mixed for 4 h at room temperature. Deprotection and cleavage of the compounds from the resin was achieved by treatment with a mixture of TFA/H₂O (95:5, v/v) at room temperature for 4 h. After filtration and washing of the resin by TFA, a gentle stream of nitrogen was used to remove the excess TFA. The crude was triturated with diethyl ether chilled at -20 °C and then centrifuged at 3000 rpm for 10 min at -10 °C.

2. Preparation of aqueous buffered solutions with various pHs.

Aqueous buffered solutions at different pH were prepared in distilled water using the following chemicals. (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was used for the buffered solutions at pH ranging from 7.0 to 8.0. 2-(N-morpholino)ethanesulfonic acid (MES) was used for the buffered solutions at pH ranging from 3.9 to 6.5. N-cyclohexyl-2-aminoethanesulfonic acid (CHES) was used for the buffered solution at pH ranging from 8.5 to 11.5.

3. Determination of detection limit.

The detection limit of **1** to Hg²⁺ was calculated based on a fluorescence titration. To determine the S/N ratio, the fluorescence emission intensity at 535 nm of 2 μ M of **1** in aqueous solutions was measured 10 times, and the standard deviation of the blank measurements was determined. Three separate measurements of the emission intensity at 535 nm were measured in the presence of increasing Hg²⁺ concentrations, and the mean emission intensity at 535 nm was plotted as a function of the Hg²⁺ concentration to determine the slope. The detection limit was calculated using the following equation:

$$\text{Detection limit} = 3\sigma/m$$

where σ is the standard deviation of the intensity at 535 nm of **1** in the absence of Hg²⁺, and m is the slope of the emission intensity ratio (I_{600}/I_{535}) of 5 μ M of **1** as a function of the Hg²⁺ concentration.⁴

4. Determination of dissociation constant. The dissociation constant was calculated based on the titration curve of the probe with metal ion.⁵ The fluorescence signal, F , is related to the equilibrium concentration of the complex (HL) between host (H) and metal ion (L) by the following expression:

$$F = F_0 + \Delta F \times [\text{HL}]$$

$$[\text{HL}] = 0.5 \times [\text{K}_D + L_T + H_T - \{(-\text{K}_D - L_T - H_T)^2 - 4 L_T H_T\}^{1/2}]$$

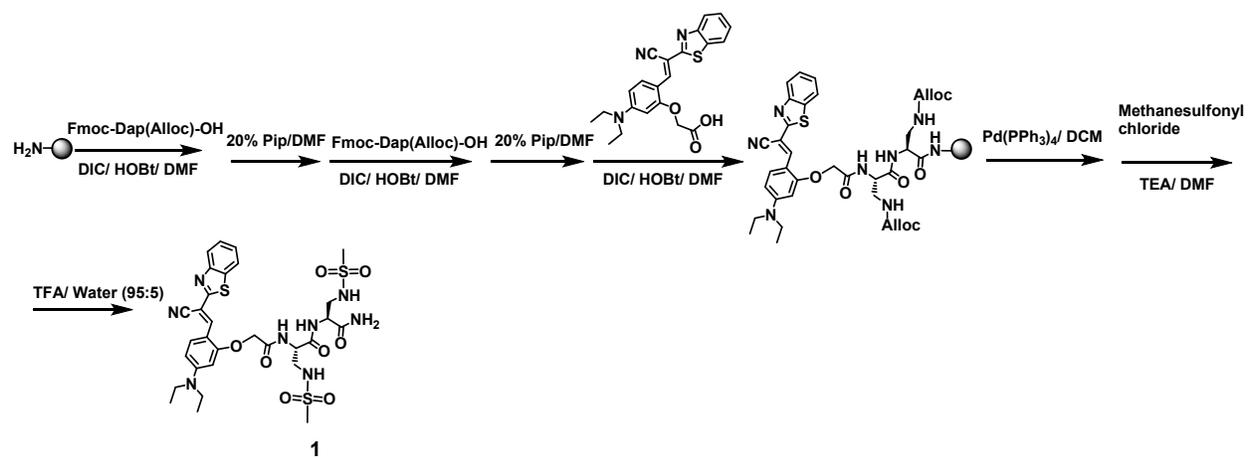
where F_0 is the fluorescence of the probe only and ΔF is the change in fluorescence due to the formation of HL, L_T and H_T are total concentrations of metal ion (L) and host (H), respectively.

5. Cell toxicity.

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. All cells were supplemented with an antibiotic antimycotic solution (100 units ml⁻¹ penicillin, 0.1 mg ml⁻¹ streptomycin, and 0.25 mg ml⁻¹ amphotericin B), and grown at 37 °C in standard cell culture conditions (5% CO₂, 95% humidity). Cell imaging experiments were performed with a Olympus CKX53 fluorescent microscope (Olympus Inc., Center Valley, PA, U.S.A.) with 20 objective lens. Excitation at 460 nm was carried out. HeLa cells were attached to the plate 24 h before study. After cells were treated with 2 μM of **1** containing 1% DMSO for 30 min at 37 °C and then washed twice with HEPES. The green fluorescence of the cells was confirmed and then the cells were further incubated in 10 μM Hg(ClO₄)₂ in HEPES for 30 min. Cells were washed three times with HEPES and confocal fluorescent microscopy was recorded for them.

The cytotoxicity was assessed by WST-1 solution assay. HeLa cells (1 × 10³) were seeded in each well of a 96-well plate and incubated for 18 h at 37°C in a humidified incubator containing 5% CO₂ in air. After incubation, cells were treated with DMSO (0.1%) as a control vehicle and the indicated concentration of the chemicals for 24 h. After incubation, 20 μl of WST-1 solution was added to each well for 4 h. Then, the visible absorbance at 460 nm of each well was quantified using a microplate reader. Non- treated cells were used as a control and incubated in the same conditions for the same time. The relative cell viability (%) was calculated by the following equation.

$$\%Cell Viability = \frac{(Optical\ density\ of\ sample)}{(Optical\ density\ of\ control)} \times 100\%$$



Scheme S1. Synthetic scheme of **1**

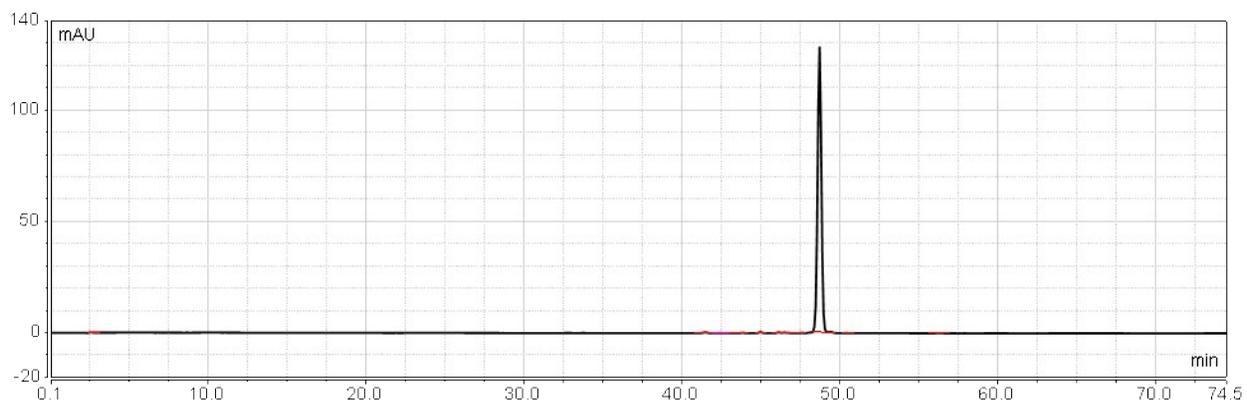


Figure S1. HPLC Chromatogram of **1**

*** [High Resolution] Orbitrap Mass Spectrometer (Model : LTQ Orbitrap XL / Company : ThermoFisher Scientific)

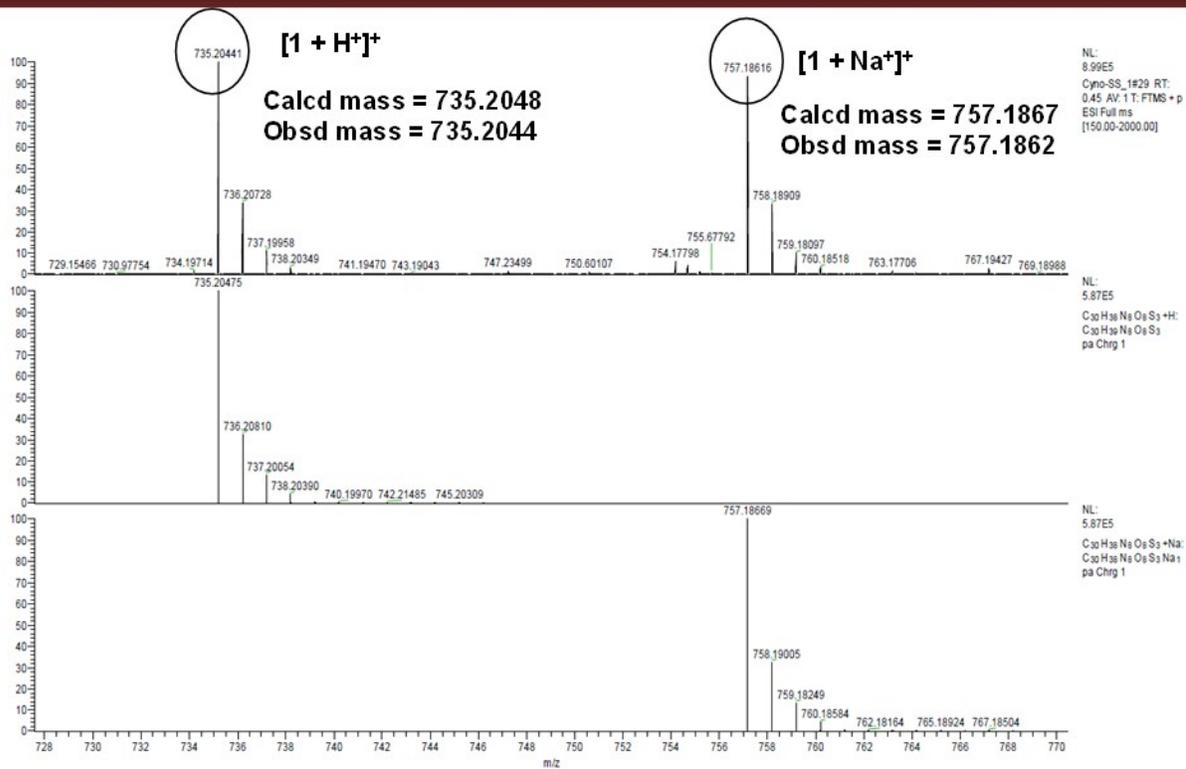


Figure S2. HRMS data of 1

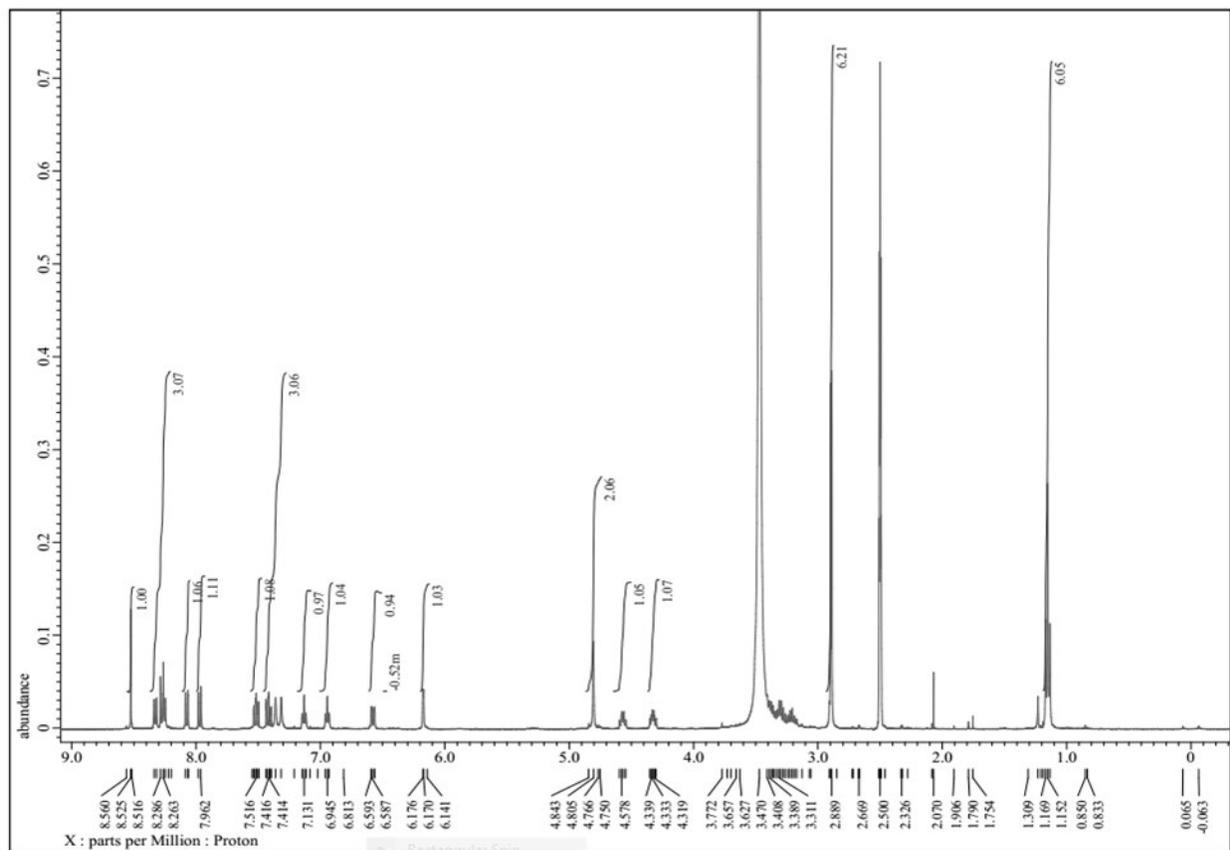


Figure S3. ^1H NMR of 1

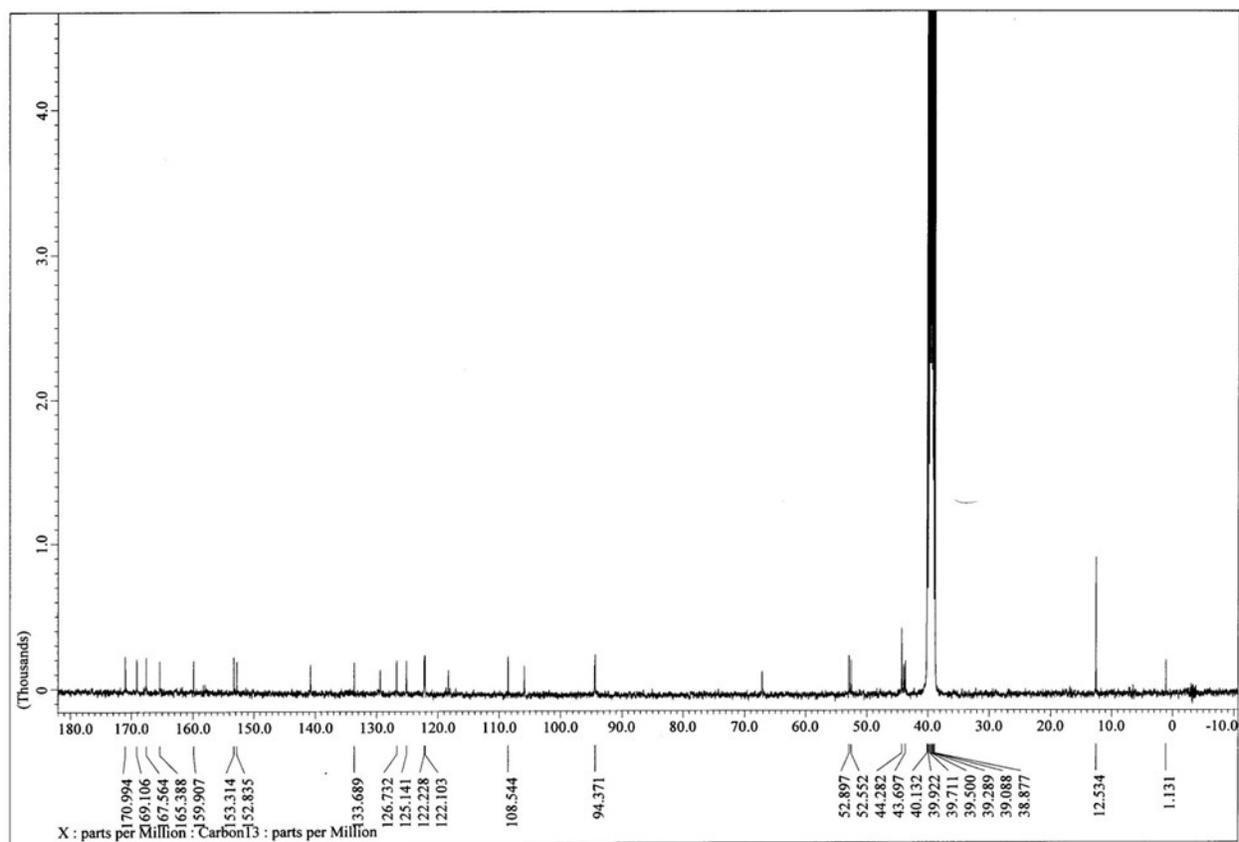


Figure S4. ^{13}C NMR of **1**

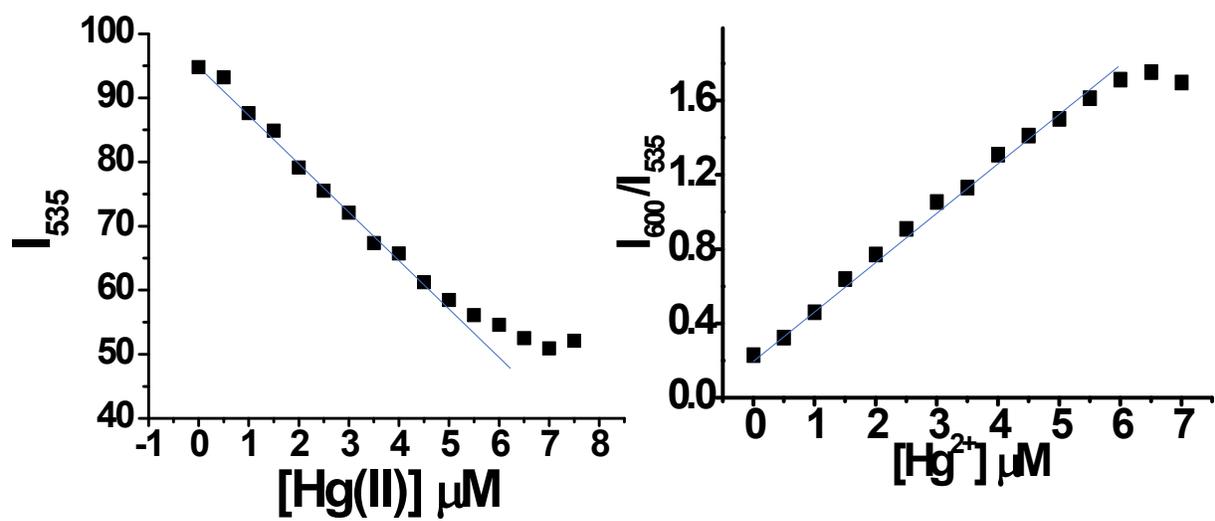


Figure S5. The linear range of the ratiometric response at 600 nm/535 nm and that of the turn-off response at 535 nm in aqueous buffer solution (10 mM HEPES buffer, pH 7.4) containing 1% DMSO.

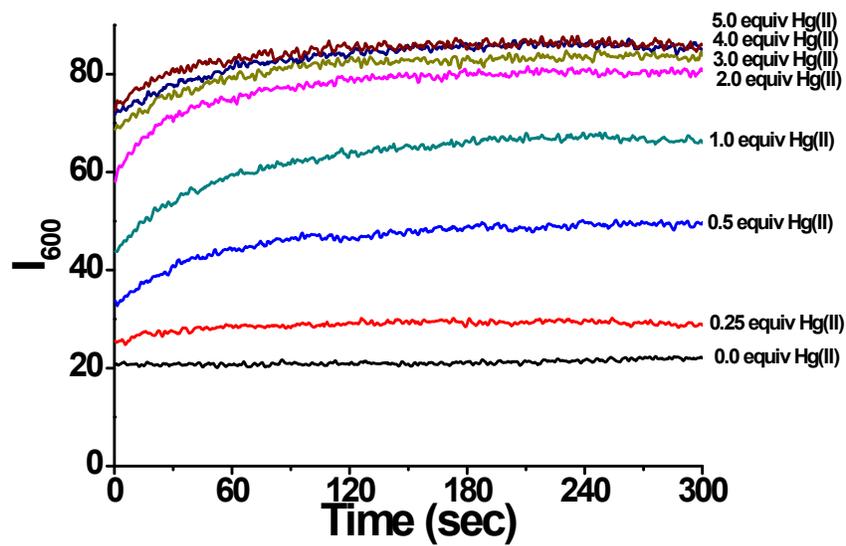


Figure S6. After addition of Hg^{2+} ions into the solution containing **1** ($2 \mu\text{M}$), time-dependent emission intensity was measured in aqueous buffer solution (10 mM HEPES buffer, pH 7.4) containing 1% DMSO.

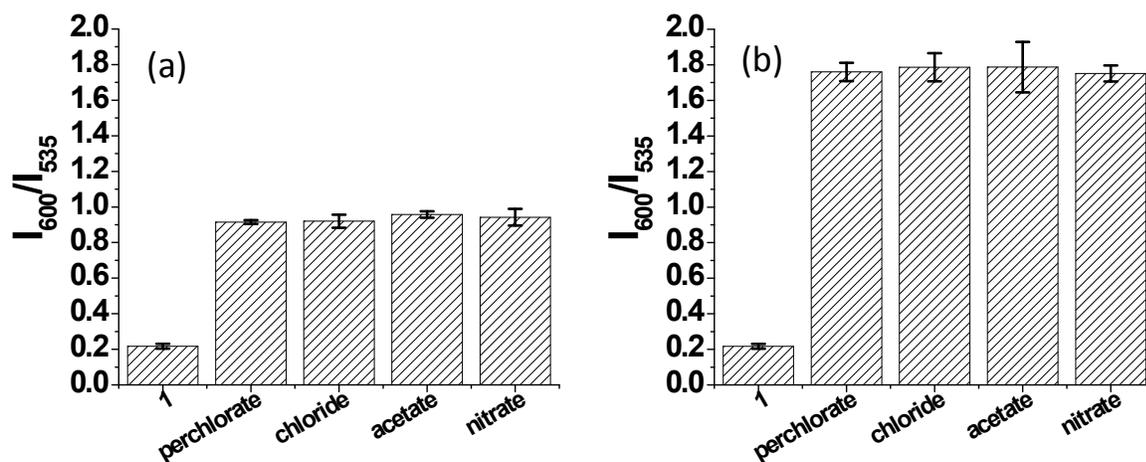


Figure S7. The emission intensity of **1** (2 μM) at 600 nm/535 nm induced by various Hg(II) salt (HgCl_2 , $\text{Hg}(\text{ClO}_4)_2$, $\text{Hg}(\text{OAc})_2$ and $\text{Hg}(\text{NO}_3)_2$) at (a) 2.5 μM and (b) 10 μM in aqueous buffer solution (10 mM HEPES buffer, pH 7.4) containing 1% DMSO.

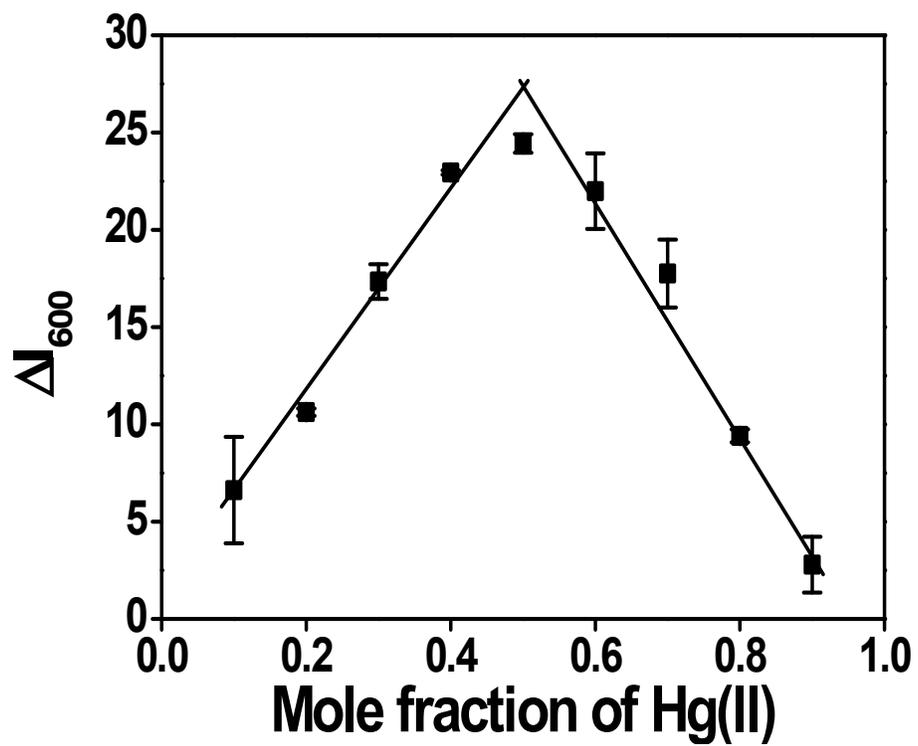


Figure S8. Job's plot for **1** with Hg^{2+} . The total $[\mathbf{1}] + [\text{Hg}^{2+}] = 5 \mu\text{M}$.

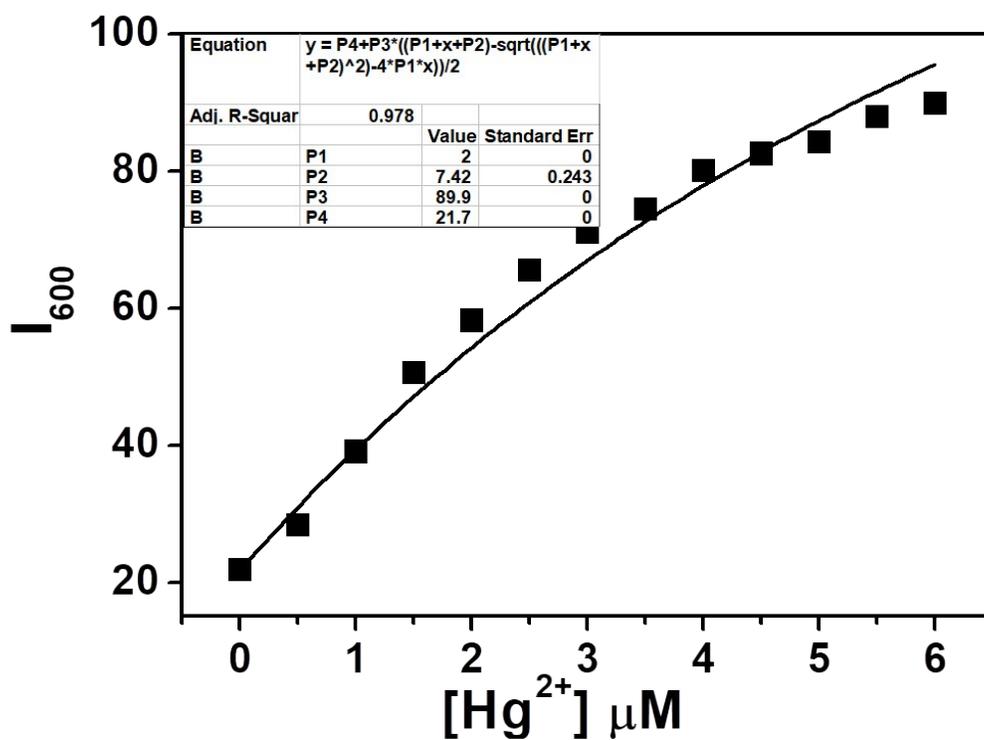


Figure S9. Non-linear least square fitting of the intensity as a function of Hg^{2+} by a 1:1 complex model.

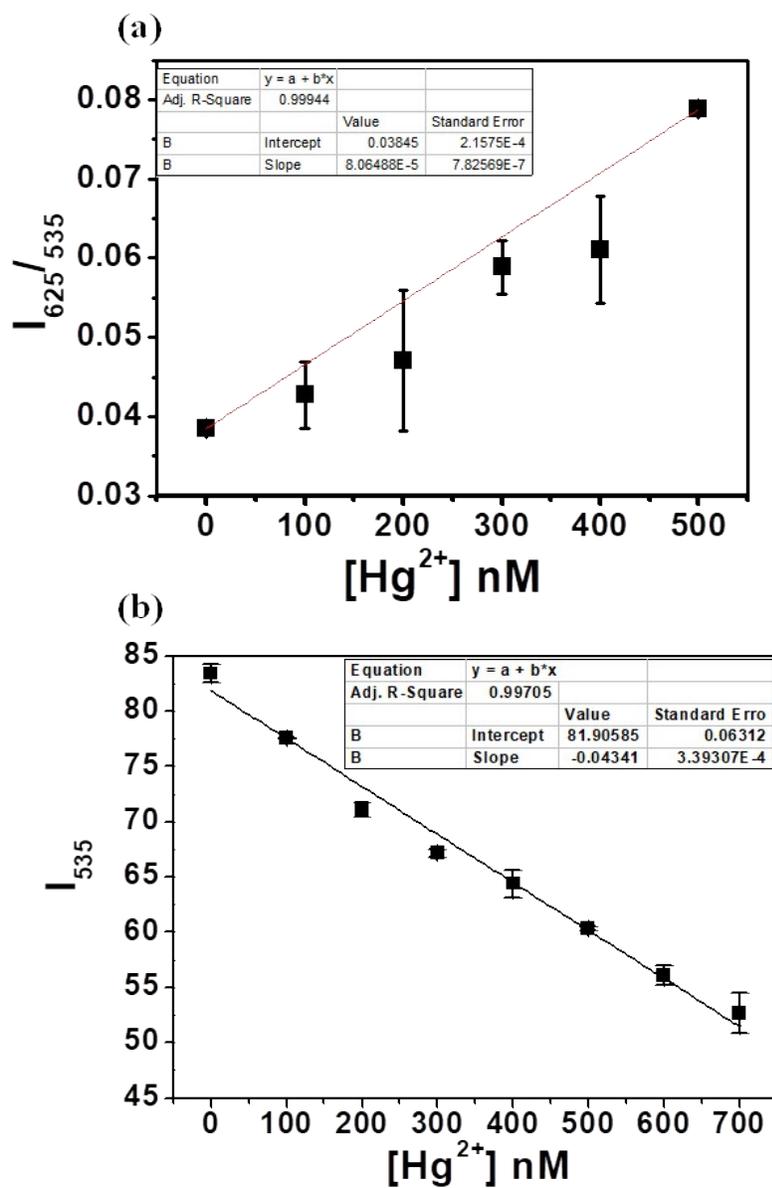


Figure S10. Linear intensity ratio change and intensity changes of **1** (2 μM) as a function of Hg^{2+} in aqueous buffered solution (10 mM HEPES, pH 7.4) containing 1 % DMSO.

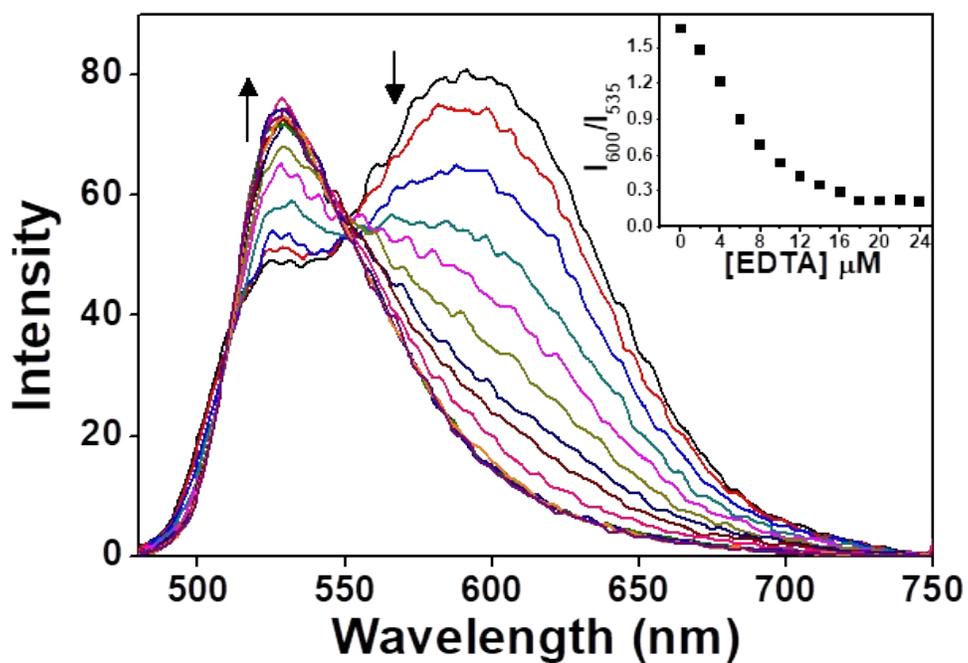


Figure S11. Fluorescence emission spectra of **1** (2 μM) with Hg^{2+} (4 equiv) in the presence of increasing concentration of EDTA in aqueous buffered solution (10 mM HEPES, pH 7.4) containing 1 % DMSO ($\lambda_{\text{ex}} = 470 \text{ nm}$, slit = 12/10 nm).

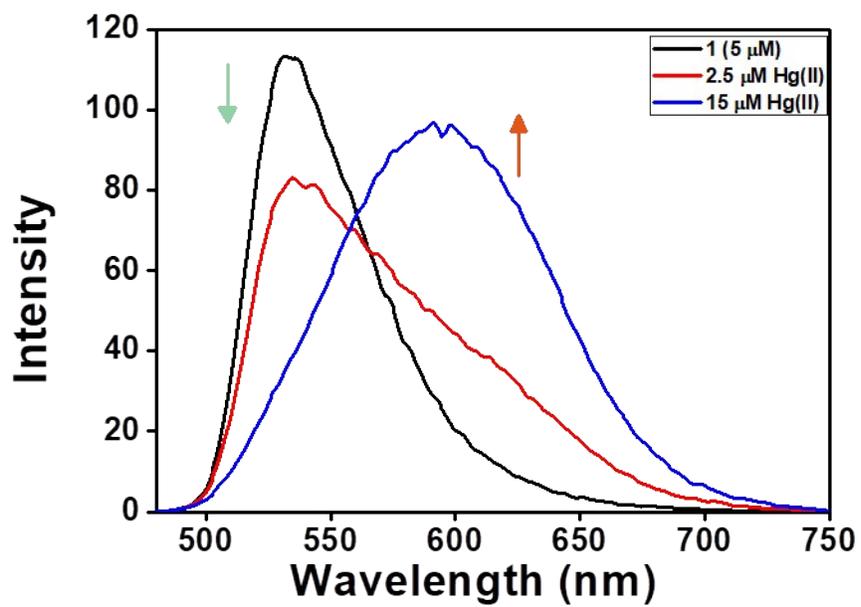


Figure S12. Fluorescence emission spectra of **1** (5 μM) with increasing concentration of Hg^{2+} in aqueous buffered (1 mM HEPES buffer, pH 7.4) solution containing 5 % DMSO ($\lambda_{\text{ex}} = 470$ nm).

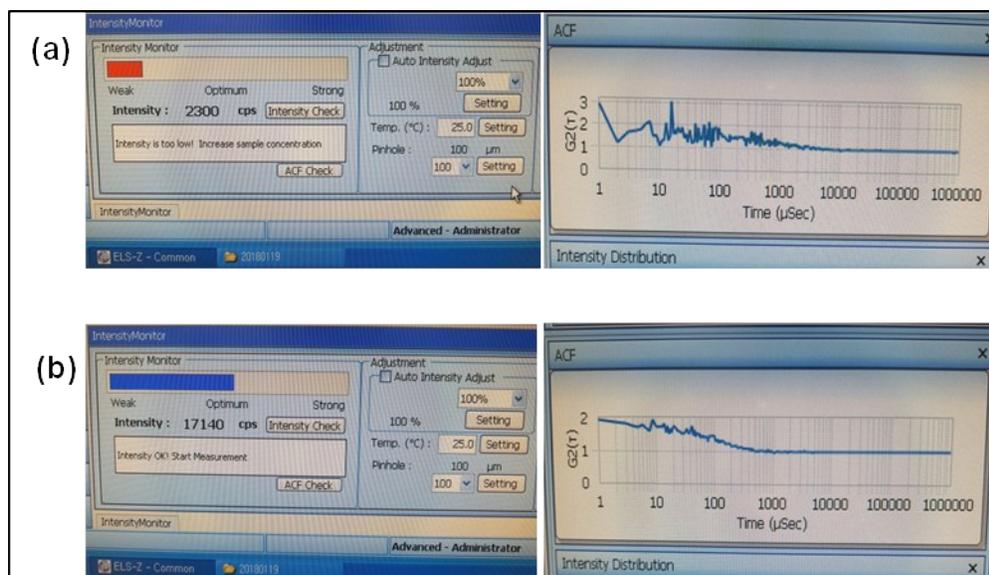


Figure S13. Particle size analysis of **1** (5 μM) in the (a) absence and (b) presence of Hg^{2+} (15 μM) in aqueous buffered solution (1 mM HEPES, pH 7.4) containing 5 % DMSO.

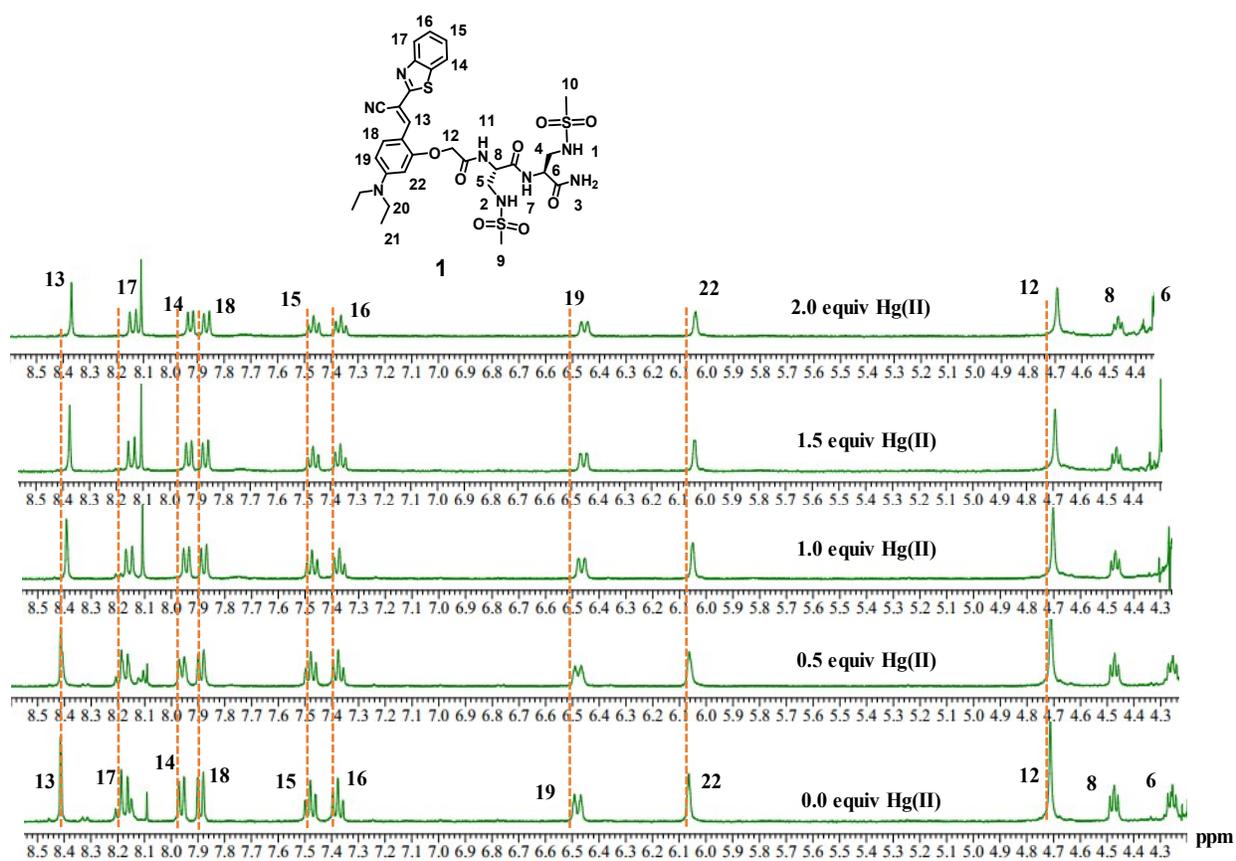


Figure S14. Partial ^1H NMR spectra (400 MHz) of **1** (4 mM) with Hg^{2+} in $\text{DMSO-d}_6/\text{D}_2\text{O}$ (v/v = 7:3) containing 4 mM ammonium formate at 25 °C.

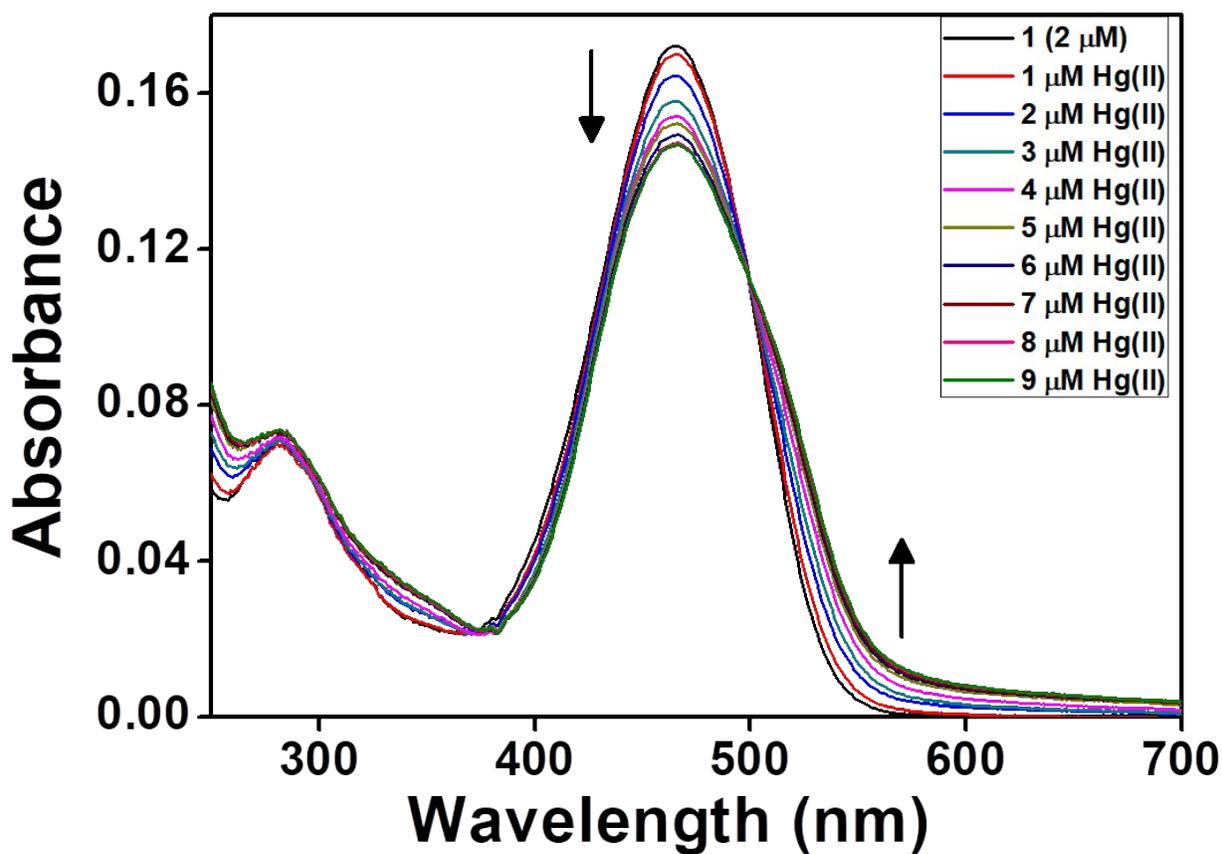


Figure S15. UV-vis absorption spectra of **1** (2 μM) upon the addition of Hg²⁺ in aqueous buffered solution (10 mM HEPES, pH 7.4) containing 1 % DMSO.

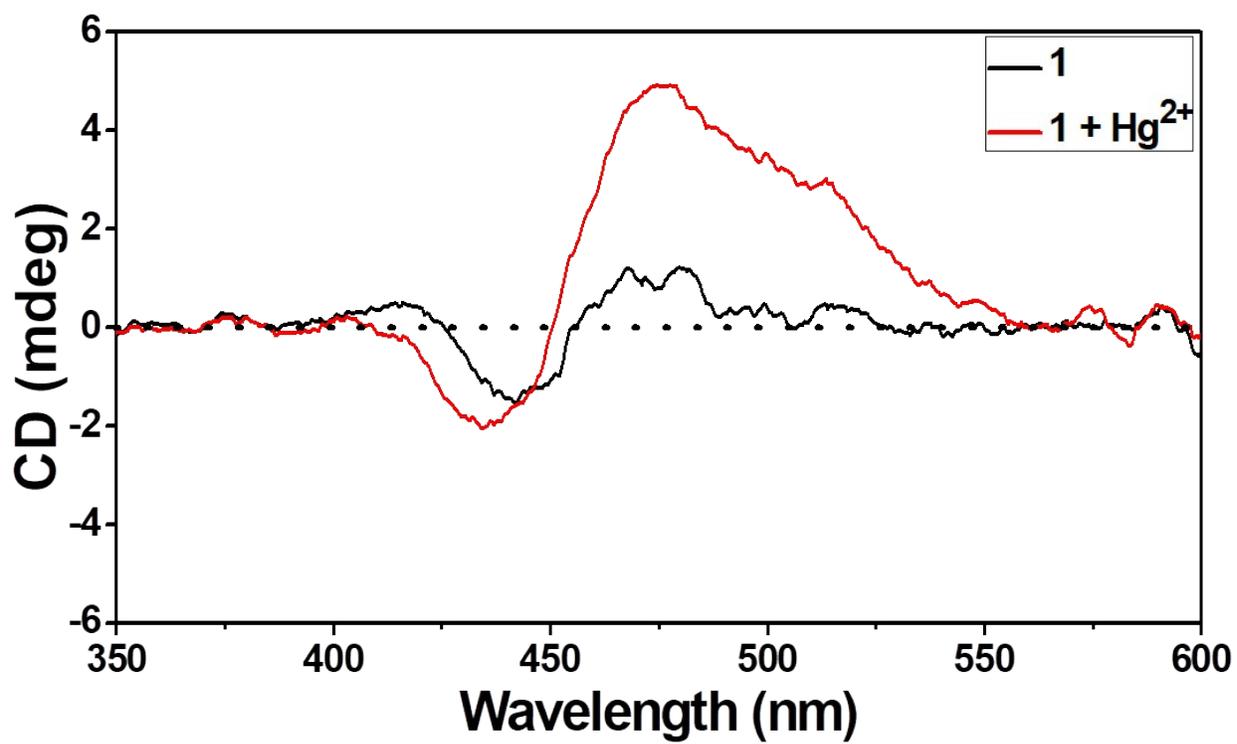


Figure S16. CD-spectra of **1** (10 μ M) in the absence and presence of Hg²⁺ (4 equiv) in aqueous buffered solution (1 mM HEPES, pH 7.4) containing 20 % CH₃CN.

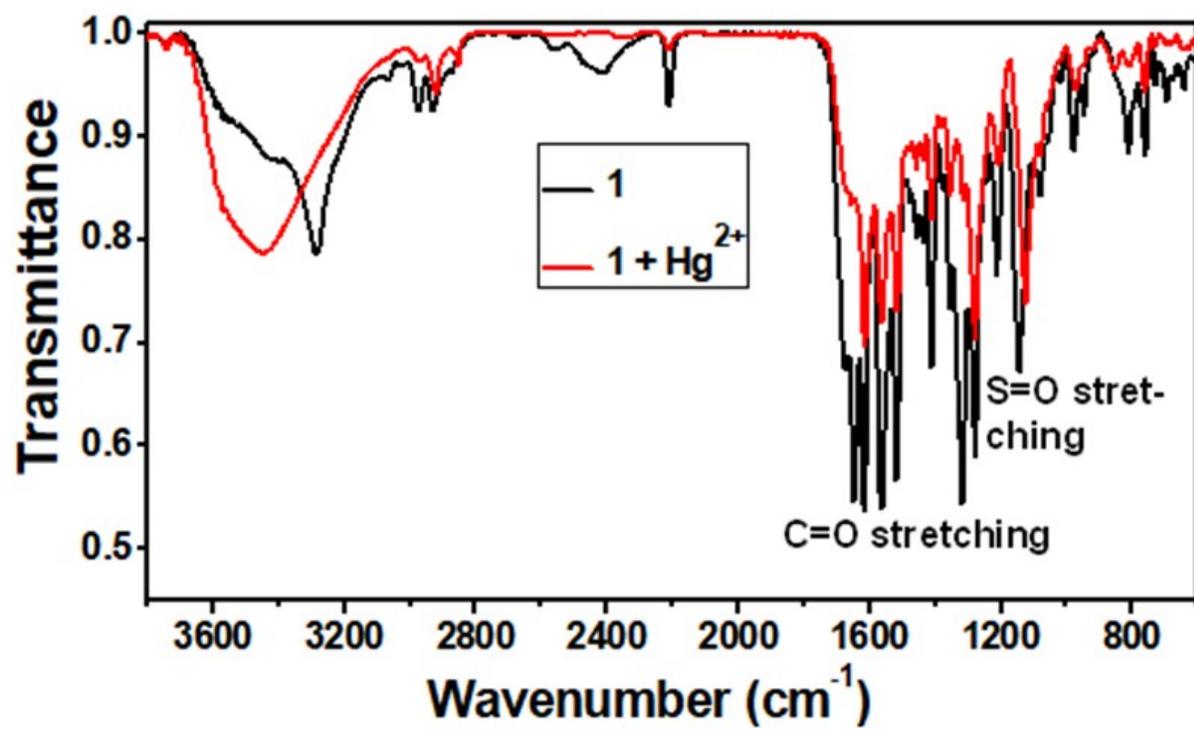


Figure S17. FT-IR spectra of **1** in the absence and presence of Hg²⁺

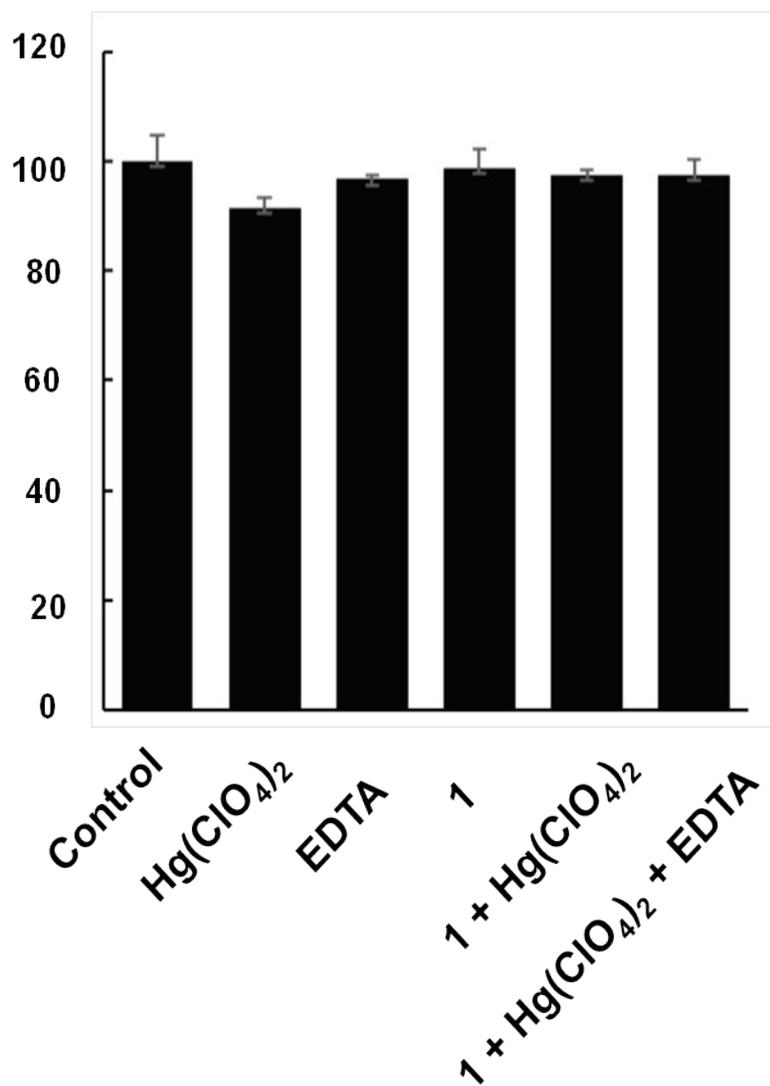


Figure S18. WST-1 assay for the viability of HeLa cells in DMEM 10% FBS treated with **1**, **1** + Hg(ClO₄)₂ and **1** + Hg(ClO₄)₂ + EDTA for 24 h. The results are based on three separate WST-1 assays. The concentration of **1**, Hg(ClO₄)₂ and EDTA is 2 μM, 10 μM and 20 μM, respectively.

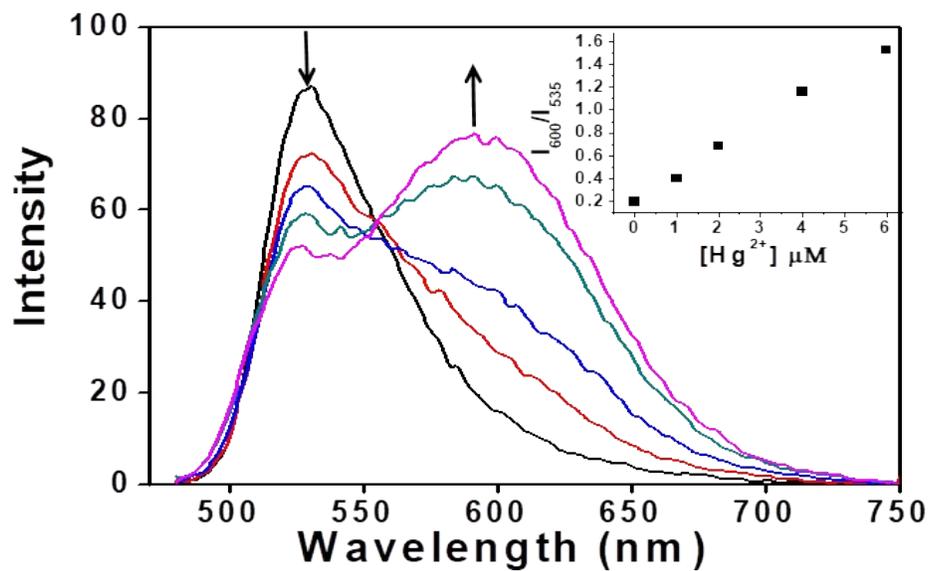


Figure S19. Detection of Hg²⁺ (0~6 μM) in tap water samples (50% v/v tap water, 10 mM HEPES, pH 7.4, 1% DMSO) using **1** (2 μM).

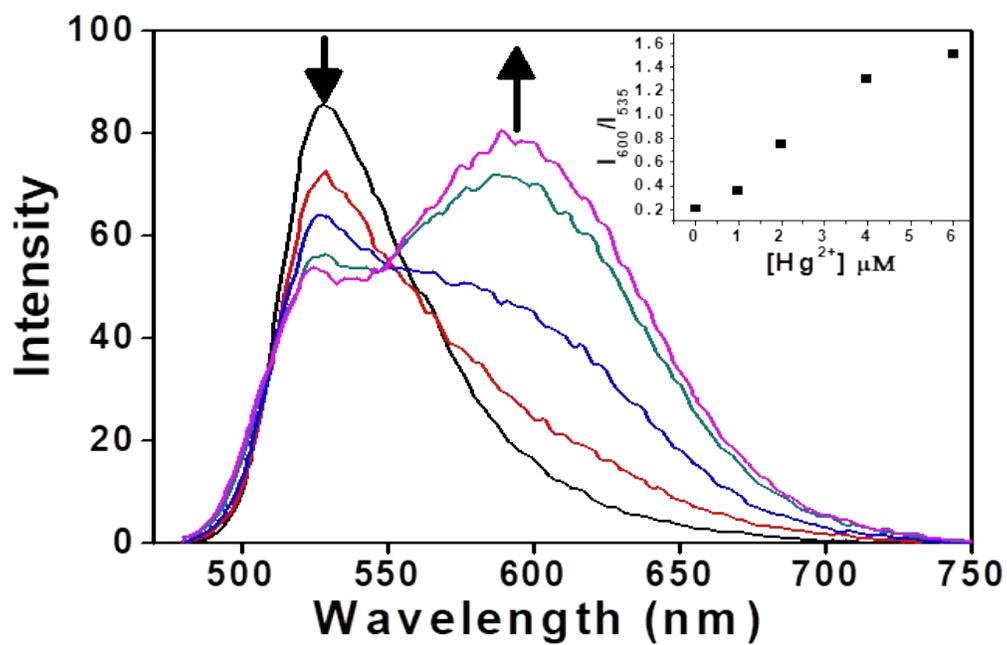


Figure S20. Detection of Hg²⁺ (0~6 μM) in ground water samples (50% v/v ground water, 10 mM HEPES, pH 7.4, 1% DMSO) using 1 (2 μM).

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