# Electronic Supplementary Information

# For

# A Chemodosimeter Approach to Fluorescent Sensing and Imaging of Inorganic and Methylmercury Species

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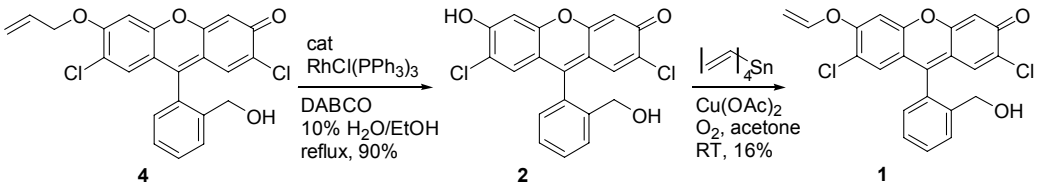
## General Methods

$^{1}H$ and $^{13}C$ NMR spectra were measured with a Bruker DPX-300 and DRX-500. Coupling constants (*J* value) are reported in Hertz. The chemical shifts are shown in ppm. UV absorption spectra were obtained using a HP 8453 UV/Vis spectrophotometer. Fluorescence spectra were recorded on a Photon Technical International Fluorescence system.

## Synthesis of probe 1:




Compound **4** was synthesized according to literature procedure.[1,2]

**Compound 4**: $^{1}H$ NMR ($CDCl_3$, 300 MHz, 293K): $\delta$ 7.37−7.40 (m, 2H), 7.26−7.30 (m, 1H), 6.89 (s, 1H), 6.87 (s, 1H), 6.84 (m, 1H), 6.83 (s, 1H), 6.73 (s, 1H), 5.98 (ddt, *J* = 17.4, 10.2, 5.1 Hz, 1H), 5.47 (ddt, *J* = 17.4 ,3.0, 1.5 Hz, 1H), 5.32 (ddt, *J* = 10.2, 3.0, 1.5 Hz, 1H), 5.28 (s, 2H), 4.63 (ddt, *J*= 5.1, 1.5, 1.5 Hz, 2H); $^{13}C$ NMR ($CDCl_3$, 75 MHz, 293K): $\delta$ 155.0, 152.5, 150.5, 150.0, 144.3,139.1, 132.5, 130.0, 129.3 (two carbons), 129.1, 124.2, 121.5, 118.8, 118.6, 118.5, 117.7, 116.1, 104.1,101.9, 83.6, 72.8, 70.3.

**Synthesis of compound 2**[1]: To a solution of allyl ether **4** (0.200g, 0.47 mmol) in 7 mL of 10 % $H_2O$ in EtOH (by volume) was added $RhCl(PPh_3)_3$ (0.430 g, 0.047 mmol) and DABCO (0.026g,

0.24 mmol). Then the reaction mixture was refluxed for 8 h. The reaction mixture was cooled to room temperature and the solvent was evaporated under *vacuo*. The residue obtained was purified by silica gel chromatography (eluent: 5% MeOH in $CH_2Cl_2$) to get fluorescein **2** as an orange solid (0.163g, 90%):

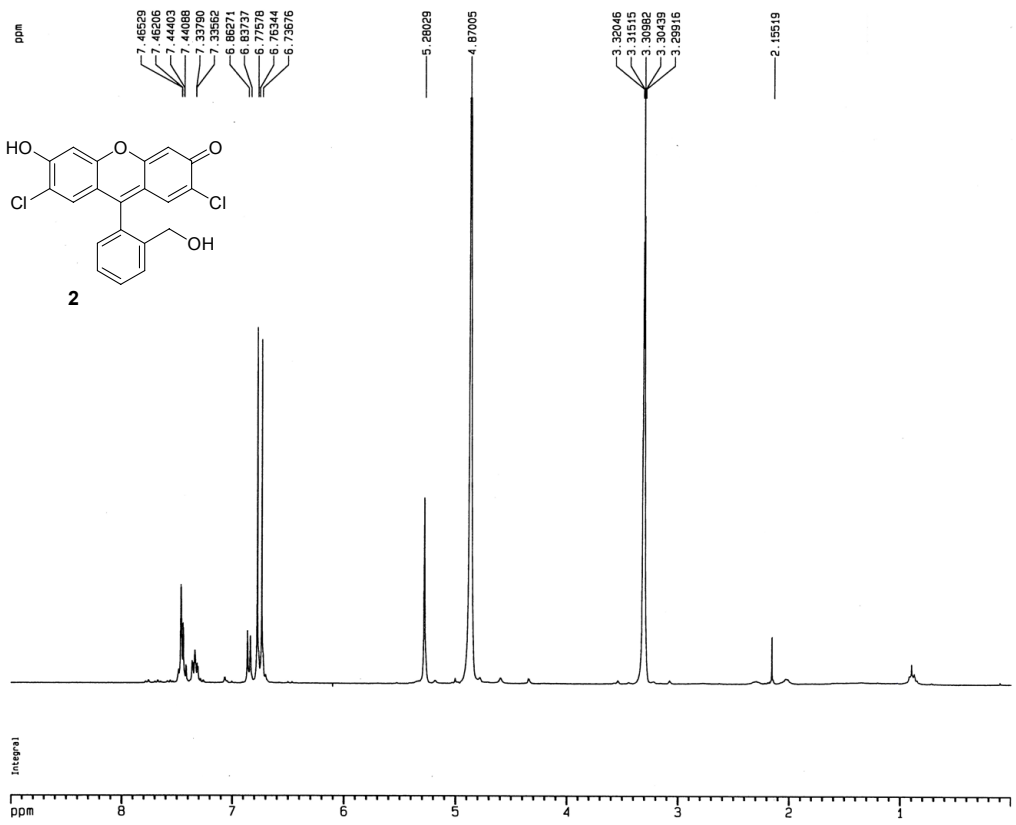
$^1$H NMR ($CD_3OD$, 300 MHz, 293 K): $\delta$ 5.28 (s, 2H), 6.74 (s, 2H), 6.78 (s, 2H), 6.85 (d, $J$ = 7.5 Hz, 1H), 7.32−7.37 (m, 1H), 7.42−7.47 (m, 2H). $^{13}$C NMR ($CD_3OD$, 75 MHz, 293K): $\delta$ 155.6, 151.4, 145.7, 140.4, 130.8, 130.1, 124.8, 122.5, 118.3, 117.8, 104.7, 84.9, 73.3.

**Synthesis of probe 1:** Anhydrous $Cu(OAc)_2$ (0.046 g, 0.39 mmol) was added to a solution of **2** (0.100 g, 0.26 mmol) in dry acetone (7 mL). The mixture was purged with dry $O_2$ in a septum-capped flask via a balloon fitted with a needle. Tetravinyltin (70 µL, 0.39 mmol) was then added to the reaction mixture. It was stirred at room temperature for 96 h. The resulting dark red mixture was poured into aqueous 25% $NH_4OAc$ (10 mL). After 10 min of stirring, the aqueous layer was extracted with EtOAc. Then combined organic layers were washed with brine, dried ($Na_2SO_4$), and concentrated. The residue was subjected to column chromatography (eluent: 10% EtOAc in Hexane), affording vinyl ether **1** as a pale yellow solid (17 mg, 16%): mp 180 °C; $^1$H NMR ($CDCl_3$, 500 MHz, 293K): $\delta$ 4.63 (dd, $J$ = 2.0, 12.0 Hz, 1H), 4.92 (dd, $J$ = 2.0, 13.5 Hz, 1H), 5.36 (s, 2H ), 6.61-6.65 (m, 1H), 6.88-6.94 (m, 4H), 6.99 (s, 1H), 7.31-7.34 (m, 1H), 7.41−7.44 (m, 2H).$^{13}$C NMR ($CDCl_3$, 125 MHz, 293K): $\delta$ 72.5, 82.9, 97.4, 103.6, 105.5, 115.6, 118.2, 119.0, 120.2, 121.1, 123.6, 128.6, 128.7, 128.8, 130.0, 138.5, 144.0, 147.2, 149.4, 150.0, 151.9, 152.7; HRMS: m/z calcd. for $C_{22}H_{15}O_4Cl_2$ $(M + H)^+$ 413.0347 found 413.0345.

## References

1. F. Song, L. Garner, K. Koide, *J. Am. Chem. Soc.* 2007, **129**, 12354-12355
2. B. A. Sparano, S. P. Shahi, K. Koide, *Org. Lett.* 2004, **6**, 1947-1949

mith-55-166-Rh, 300b, 17-4-08, MeOD

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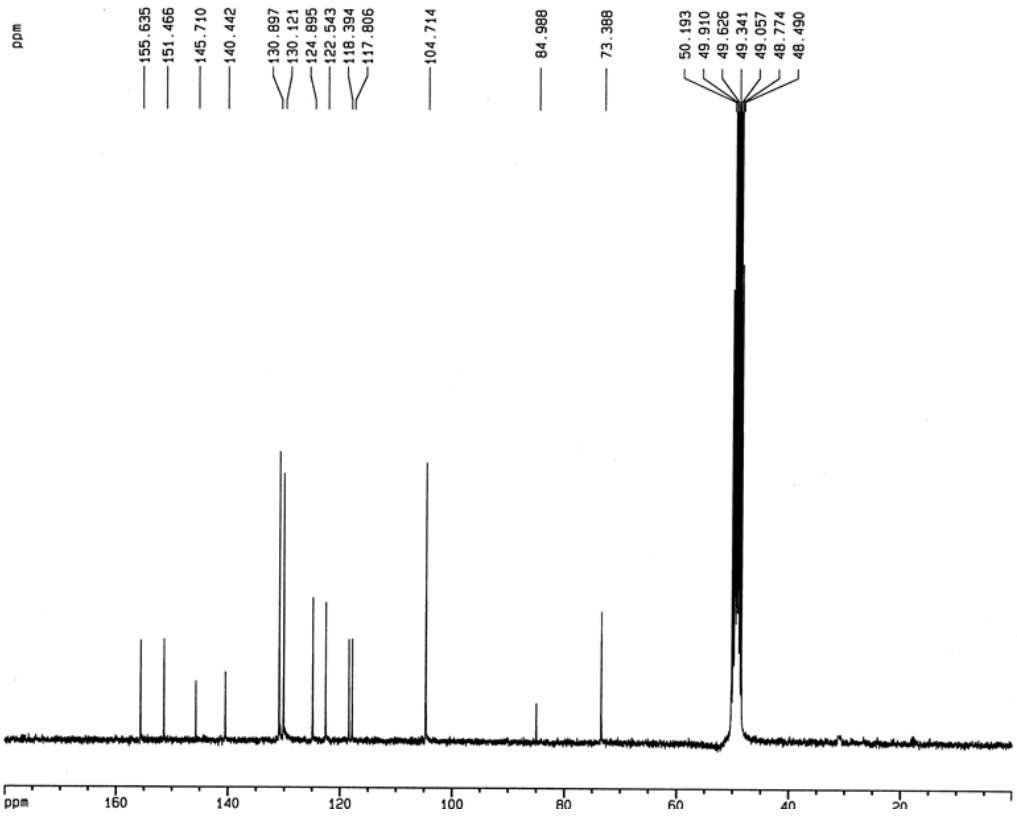
mith-55-166-OH, 300b, 17-4-08, MeOD,

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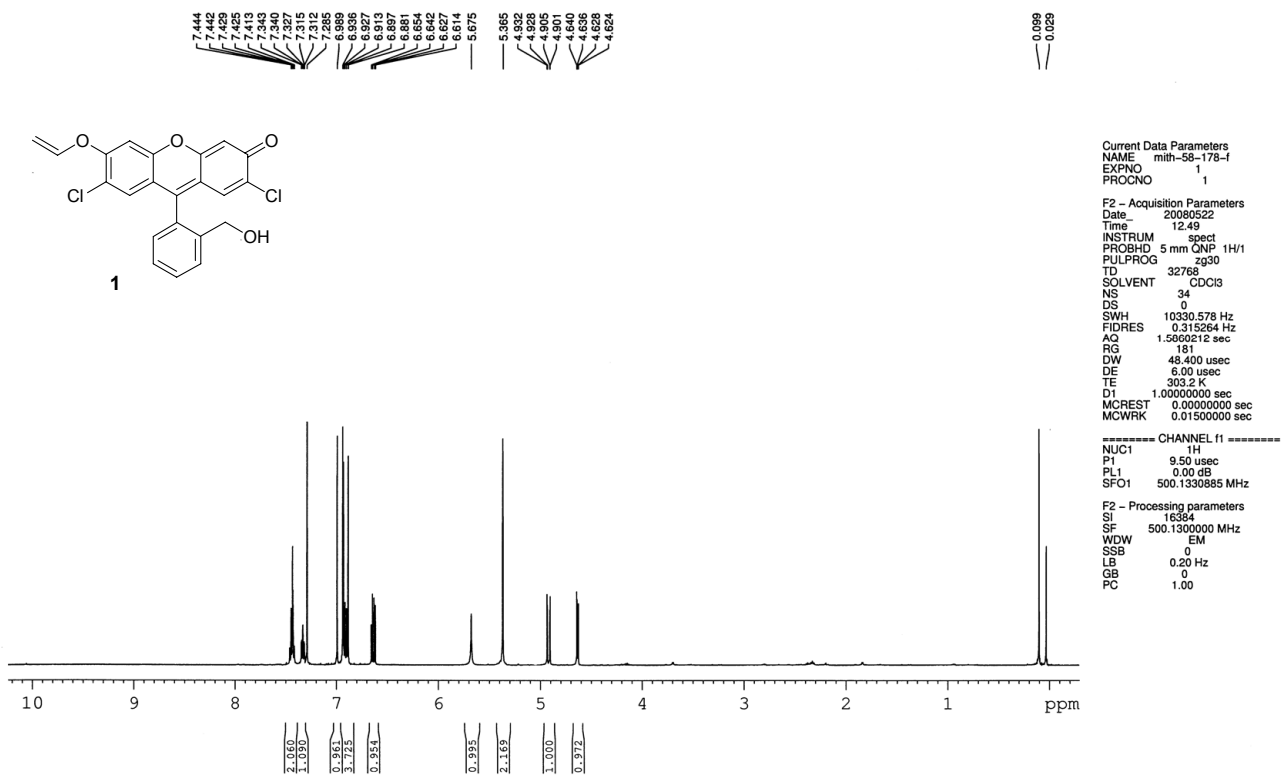


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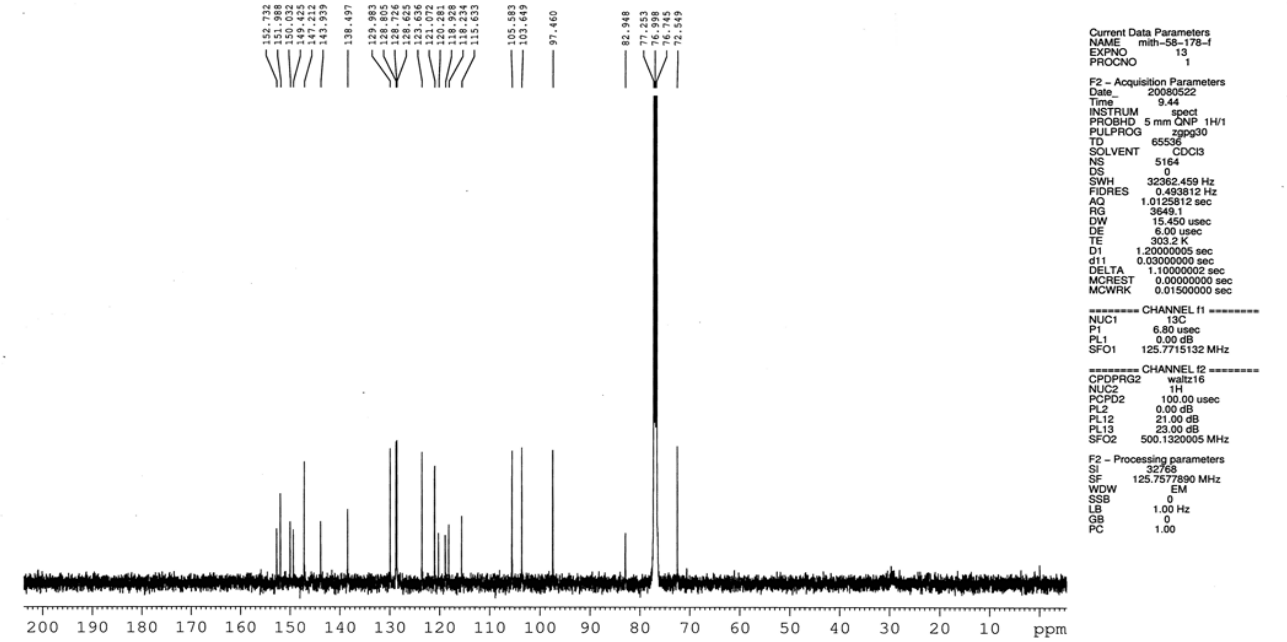

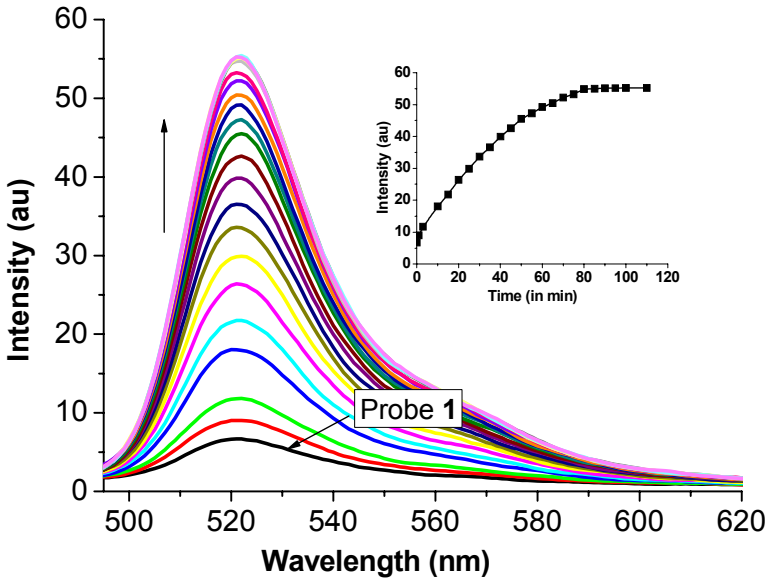


***Fig. S1*** Time-dependent fluorescence intensity profile for a 1:1 mixture of probe **1** and $CH_3HgCl$ in PBS buffer solution containing 5% DMSO at pH 7.4. Inset: a plot of fluorescence intensity as a function of time (excitation at 480 nm; the intensity was estimated by the peak height at $\lambda_{ex}$ = 520 nm).

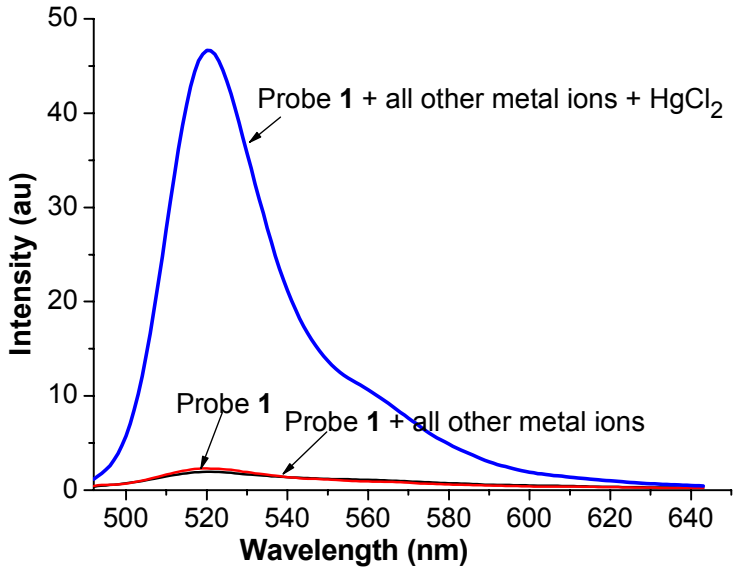


***Fig. S2*** Comparison of fluorescence response of probe **1** (5.0 μM) toward $Hg^{2+}$ and all other metal ions ($Cr^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Co^{2+}$, $Mn^{2+}$, $Ni^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Ba^{2+}$, $Cd^{2+}$, $Fe^{3}$+, $Ag^{+}$, and $Hg^{2+}$), acquired in the PBS buffer solution containing 5% DMSO (excitation at 480 nm). An equimolar amount of each metal ion was added to the probe.

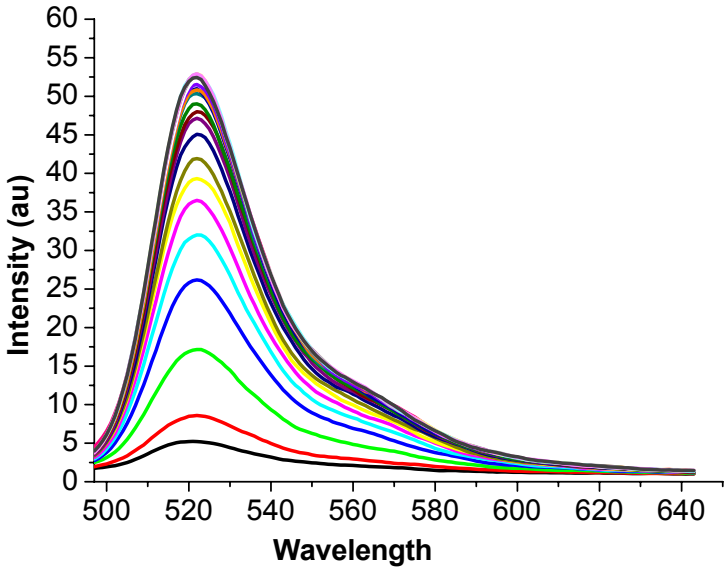


***Fig. S3*** Time-dependent fluorescence intensity profile (1−180 min) for a 2:1 mixture of probe **1** (5μM) and $Hg(OAc)_2$ in PBS buffer solution containing 5% DMSO at pH 7.4.

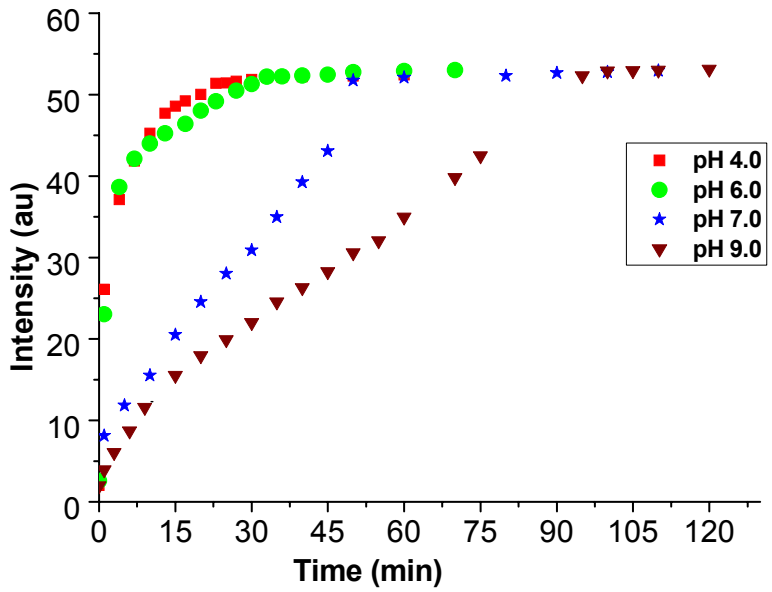


***Fig. S4*** Time-dependent fluorescence intensity profile for a 2:1 mixture of probe **1** and $HgCl_2$ at different pH (excitation at 480 nm; the intensity was estimated by the peak height at $\lambda$ = 520 nm). Interestingly, the fluorescence increased faster at low pH. This result suggests that hydrolysis of the hemiacetal intermediates is promoted at lower pH, plausibly through protonation at the aryl ether oxygen.

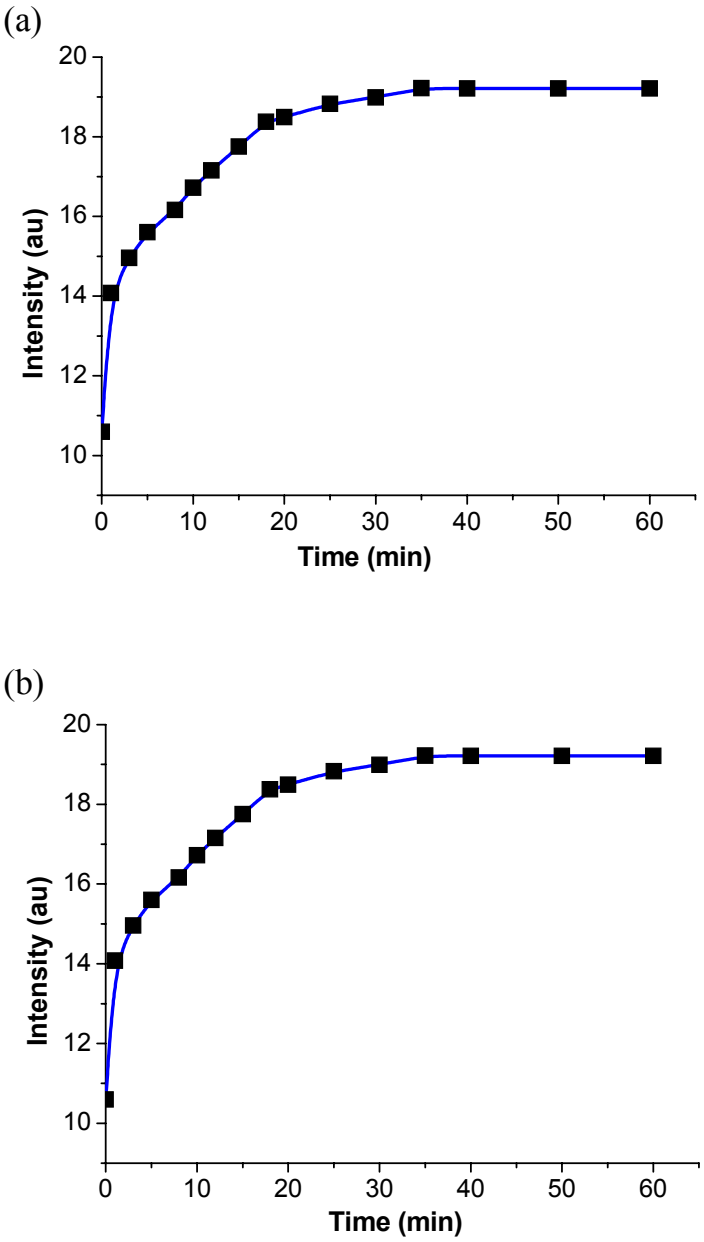


***Fig. S5*** A plot of the fluorescence intensity change as a function of the reaction time, measured for a 10:1 mixture of probe **1** (10 $\mu$M) with (a) $HgCl_2$ and (b) $CH_3HgCl$ in the PBS buffer (pH 7.4) containing 5% DMSO (excitation at 480 nm; the intensity was estimated by the peak height at $\lambda$= 520 nm).

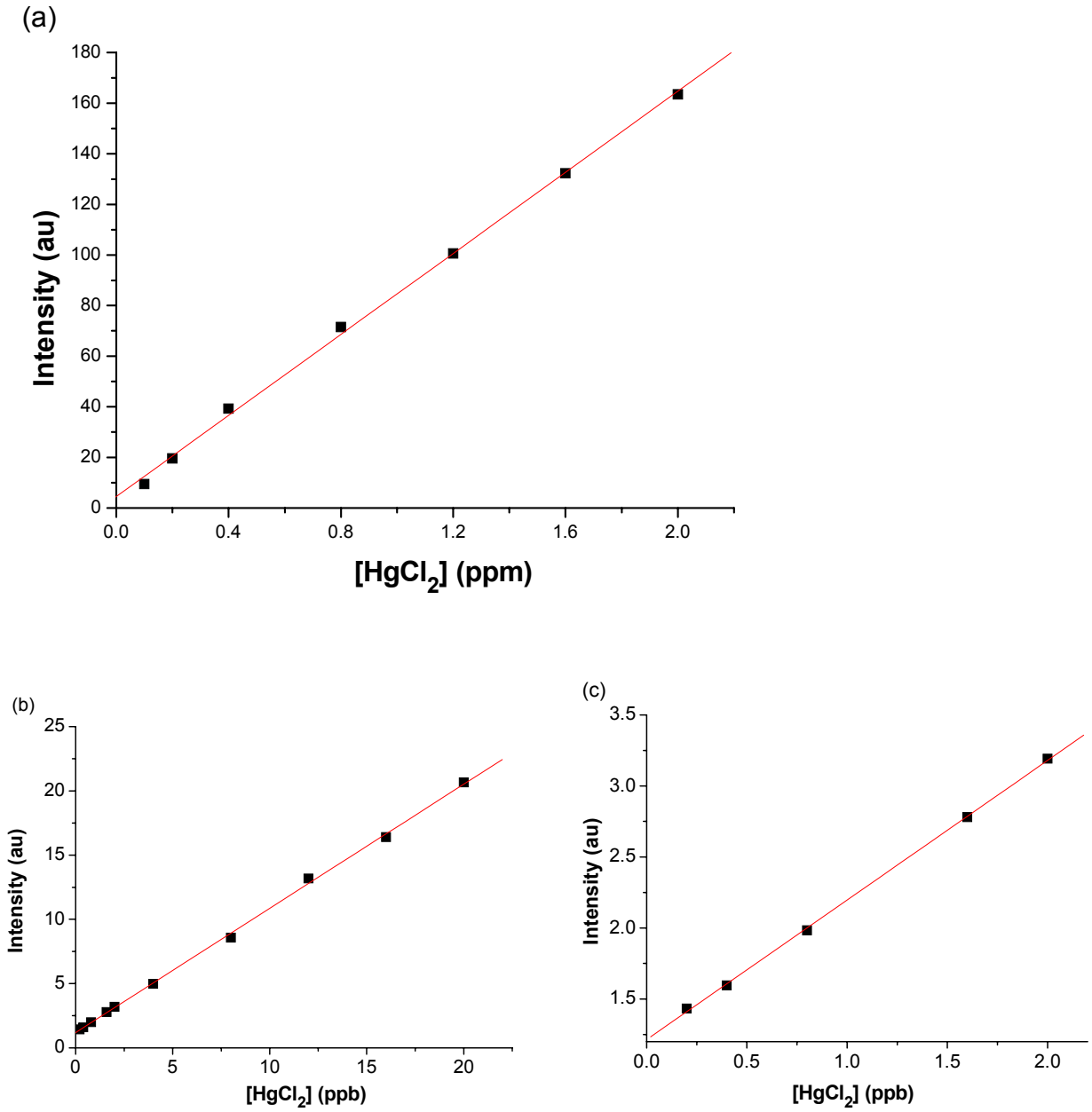


***Fig. S6*** (a) A plot of fluorescence intensity depending on the concentration of $HgCl_2$ in the range of 0.1 – 2 ppm. Each measurement was done after 1 h of mixing for a 2:1 mixture of probe **1** and $HgCl_2$ in the PBS buffer (pH 7.4) containing 5% DMSO (excitation at 480 nm; the intensity was estimated by the peak height at $\lambda$ = 520 nm). (b) A region between the 0.2 – 20 ppb [$HgCl_2$]. (c) An expanded region of graph (b) between 0.2 – 2.0 ppb [$HgCl_2$].

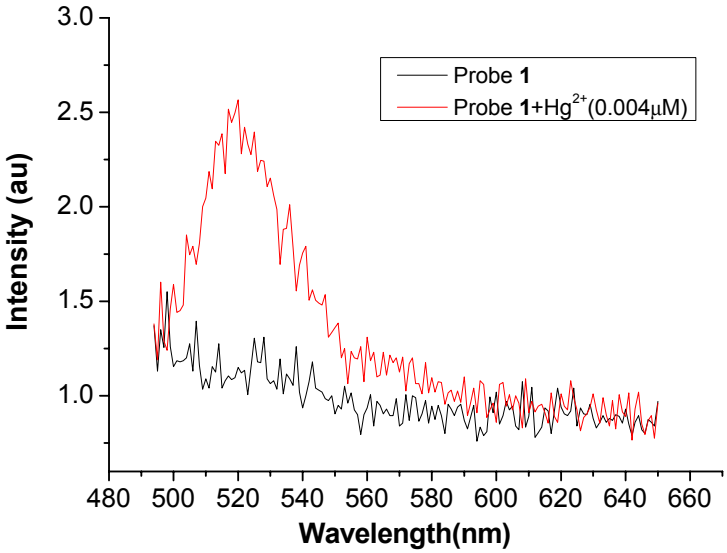


***Fig. S7*** a) Fluorescence spectra of probe **1** upon addition of $HgCl_2$ (0.004 μM = 0.8 ppb) after 1h showing a signal-to-background ratio is more than three.

The measurement shows that the fluorescence intensity changes linearly depending on the concentration of $HgCl_2$ (0.2 – 2000 ppb) examined. On the basis of this result and the data for the signal-to-noise ratio of three, the detection limit of probe **1** is estimated to be below 1 ppb level.

**Imaging of mammalian cells incubated with $CH_3HgCl$ and probe 1.** HeLa cells (A549) were seeded in a 6-well plate at a density of $10^4$ cells per well in culture media (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS)). After 24 h, the cells were incubated with 50 μM of probe **1** in culture media for 30 min at 37 °C. After washing with PBS to remove the remaining probe, the cells were further treated with 100 μM of $CH_3HgCl$ in culture media for 30 min. The cells incubated with both $CH_3HgCl$ and probe **1** were imaged by fluorescence microscopy (Stemi 2000-C, ZAISS, Germany).

**Imaging of zebrafish incubated with $CH_3HgCl$ and probe 1.** The three-day-old zebrafish was maintained in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM $KH_2PO_4$, 0.05 mM $Na_2HPO_4$, 0.7 mM $NaHCO_3$, $10^{-5}$% methylene blue; pH 7.5). The three-day-old zebrafish was incubated with 50 μM of probe **1** in E3 media for 30 min at 28 °C. After washing with PBS to remove the remaining probe, the zebrafish was further treated with 100 μM of $CH_3HgCl$ in E3 media for 30 min at 28 ºC. The zebrafish was imaged by fluorescence microscopy.

**Images of zebrafish organs treated with $CH_3HgCl$ and probe 1.** Adult zebrafish (three-month-old with identifiable organs) was exposed to 200 nM of $CH_3HgCl$ in E3 media for 24 h, washed with PBS three times, and then incubated with 50 μM of probe **1** for 30 min. The treated zebrafish was dissected to isolate tissues and organs that were then examined by using fluorescence microscopy. Fluorescence intensities of isolated tissues and organs were analyzed by using Image Pro Plus version 5.1 software.

**Real-time monitoring of $HgCl_2$ and $CH_3HgCl$ in A549 cells.** A549 cells were incubated with 50 μM sensor in culture media for 30 min. After washing with PBS to remove the remaining sensor, $HgCl_2$ or $CH_3HgCl$ was added to the cells in culture media, and fluorescence intensity was continuously monitored by using a fluorescence microplate reader (SpectraMax GeminiEM, Molecular Devices).

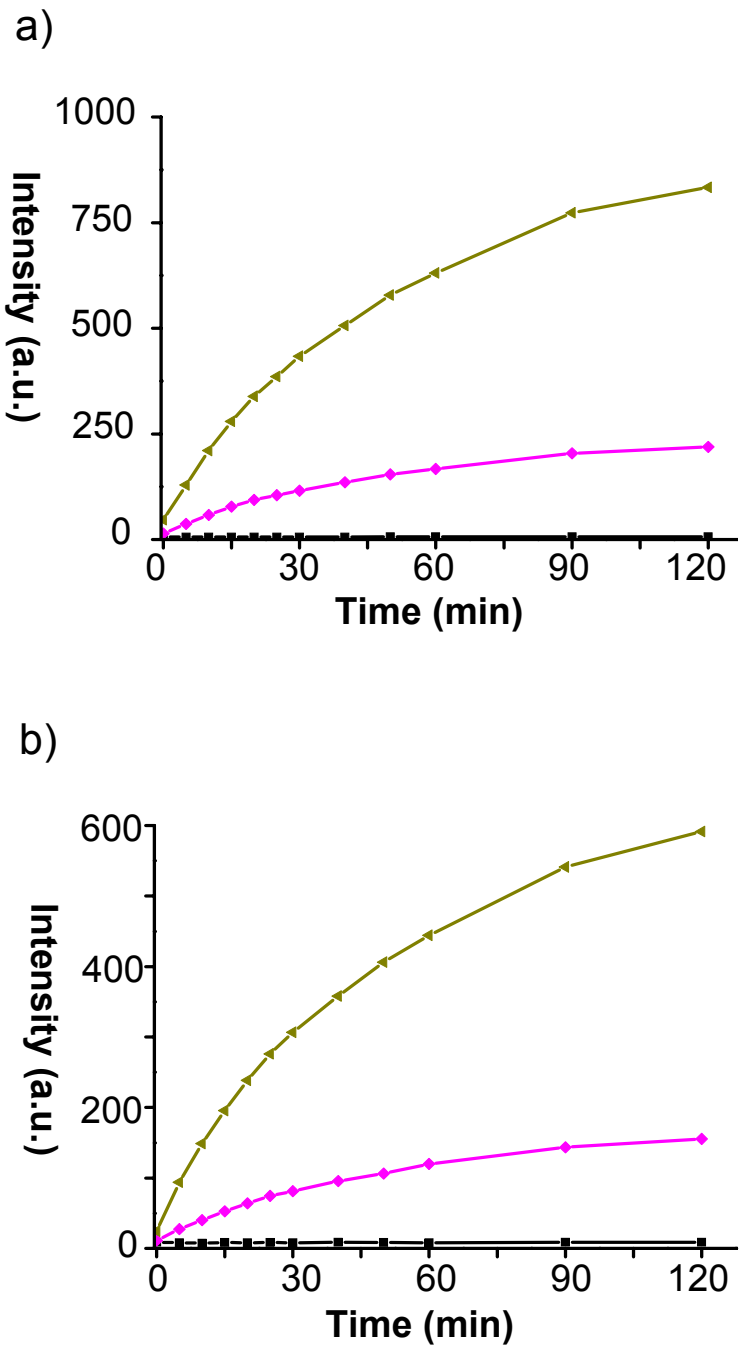


***Fig. S8*** Real-time monitoring of (a) $HgCl_2$ and (b) $CH_3HgCl$ uptake by A549 cells (concentrations of ions: ▲: 250 μM, ◆: 100 μM, ■: 0 μM).