Supplementary Information for

A Zero-Backgroud Flurescent Probe for Hg^{2+} Designed via the "Covalent-Assembly" Principle

Linlin Song,[†] Zuhai Lei,[†] Baoyan Zhang,[†] Zhiping Xu,[†]* Zhong Li,[†]* and Youjun Yang[†]*

[†]Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Meilong Road 130, Shanghai 200237, China

* E-mail: youjunyang@ecust.edu.cn,
* E-mail: zhipingxu@ecust.edu.cn,
* E-mail: zhongli@ecust.edu.cn.

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General methods

Chemicals including 1,2-ethanedithiol, PTSA, HEPES, perchlorate salts of various metal ions including Hg^{2+}, Mn^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, Fe^{2+} and Ag^{+} used in this work were purchased from Energy Chemicals, Ltd. Solvents of analytical grade including CH_2Cl_2, EtOAc, Petroleum Ether and toluene were purchased from Titan Scientific. All chemicals and solvents were used without further purification. Bottled water from Watsons was used for preparation of sample and buffer solutions. DMSO from Lingfeng Chemicals was used in preparation of the stock solution of **Hg570** and subsequent spectroscopic studies.

The ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Bruker AV-400 spectrometer. Chemicals shifts were referenced to the residue solvent peaks and given in ppm. HRMS was acquired on a Micromass GCT spectrometer. UV-Vis absorption spectra were acquired on a SHIMADZU UV-2600 UV-vis spectrophotometer. Fluorescence emission spectra were acquired on a PTI-QM4 steady-stead fluorimeter equipped with a 75 Watt Xenon arc-lamp and a R928 PMT. The excitation and emission slits were set to 2 nm and all emission spectra were corrected with respect to the PMT sensitivity at different wavelengths. Fluorescence titration studies were performed by addition of an aliquot of the stock solution of Hg^{2+} or other metal ions into a **Hg570** solution (10 μM) in HEPES solution (10 mM at pH = 7.4) with 5% DMSO using a micro-syringe.

The relative fluorescence quantum yield of **Pyronin B** was measured at room temperature with rhodamine B ($\phi = 0.65$) as the reference.[1]

Synthetic procedures and Compound Characerization

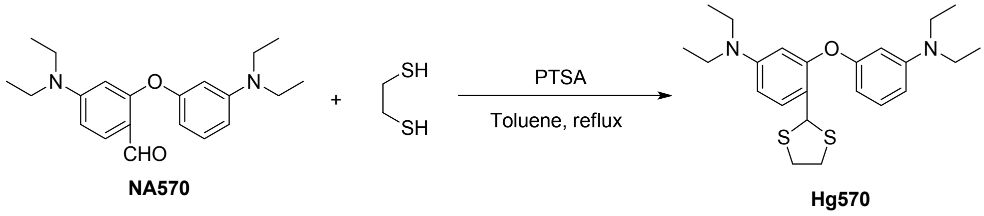


Figure S1. The synthetic scheme of **Hg570**.

Synthesis of 3-(3-(diethylamino)phenoxy-4-(1,3-(dithiolan-2-yl)-N,N-diethylaniline (Hg570). **NA570**[2] (0.5 g, 1.47 mmol), 1,2-Ethanedithiol (0.14mL, 1.67 mmol) and p-toluenesulfonic acid (25.3 mg, 0.147 mmol) were heated to reflux in 20 mL of toluene with constant stirring for 10 minutes. Then the mixture was cooled to room temperature before mixed with deionized water (100 mL) and extracted with CH_2Cl_2 (30 mL × 3). The CH_2Cl_2 layer was dried with MgSO4 before the solid was filtered off. The filtrate was concentrated under reduced pressure to give a viscous residue, which was purified by a flash column using petroleum ether and EtOAc (20:1, v/v) as the eluent to afford **Hg570** (0.56g, white solid) in a 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, *J* = 8.8 Hz, 1H), 7.11 (dd, *J* = 8.1, 8.1 Hz, 1H), 6.49 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.40 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.38 (t, *J* = 2.4 Hz, 1H), 6.21 (d, *J* = 2.3 Hz, 1H), 6.20 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.04 (s, 1H), 3.517-3.441 (m, 2H), 3.34 (q, *J* = 7.0 Hz, 4H), 3.34-3.26 (m, 2H), 3.28 (q, *J* = 7.1 Hz, 4H), 1.17 (t, *J* = 7.0 Hz, 6H), 1.11 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ159.3, 154.6, 149.3, 148.6, 129.9, 117.6, 108.1, 106.3, 104.2,102.9 101.5, 76.7, 49.5, 44.4, 39.8, 12.6. HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{33}N_2OS_2$ at 417.2034, found 417.2037.

1. Kubin, R.F.; Fletcher, A.N. *J. Lumin.* **1982**, *27*, 455-462.
2. Lei, Z.; Yang, Y. *J. Am. Chem. Soc.*, **2014**, *136*, 6594-6597.

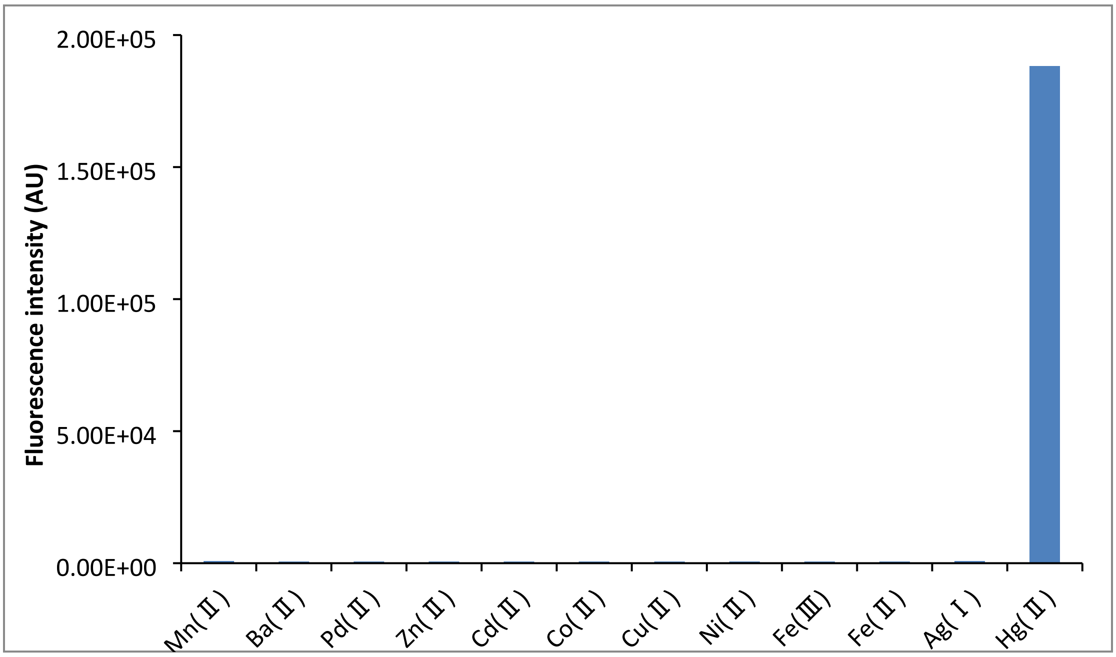


Figure S2. Selectivity study of **Hg570** (10 μM) in HEPES buffer (10 mM with pH = 7.4) with 5% DMSO as co-solvent, against various potentially interfering transition metal ions. Hg(II) was at 9 μM, all metal ions was at 100 μM.

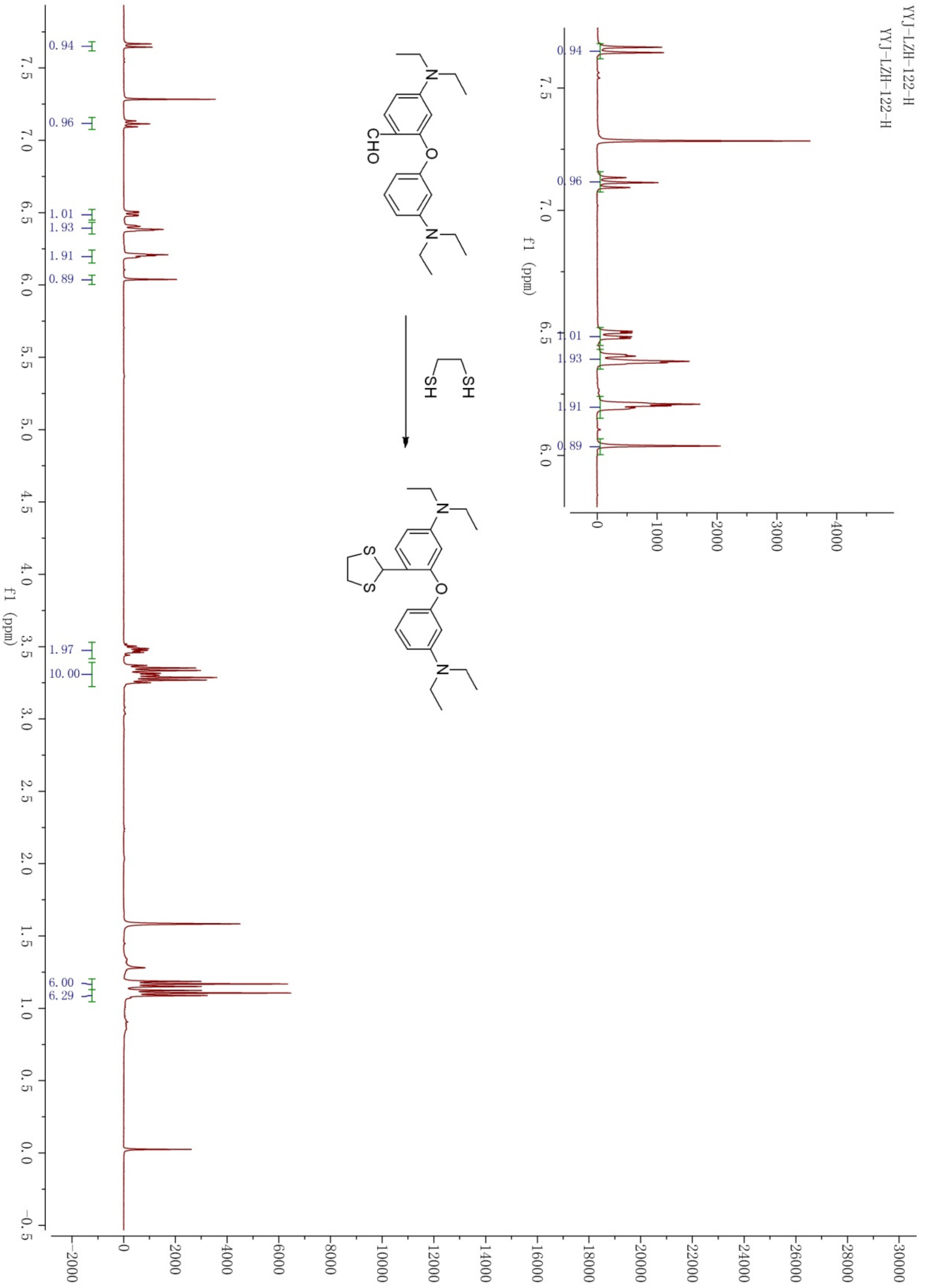


Figure S3: ^{1}H-NMR of compound **Hg570**

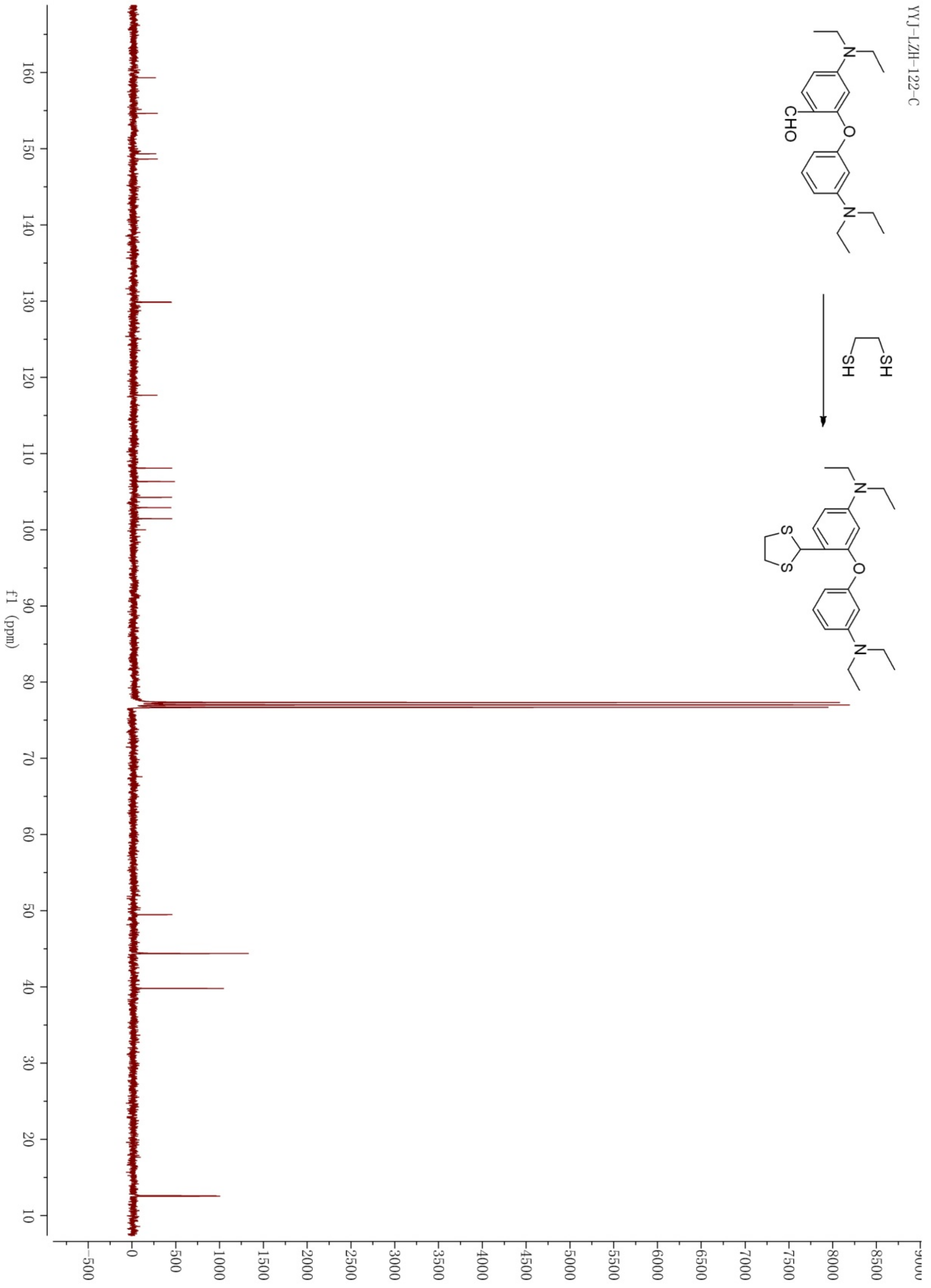
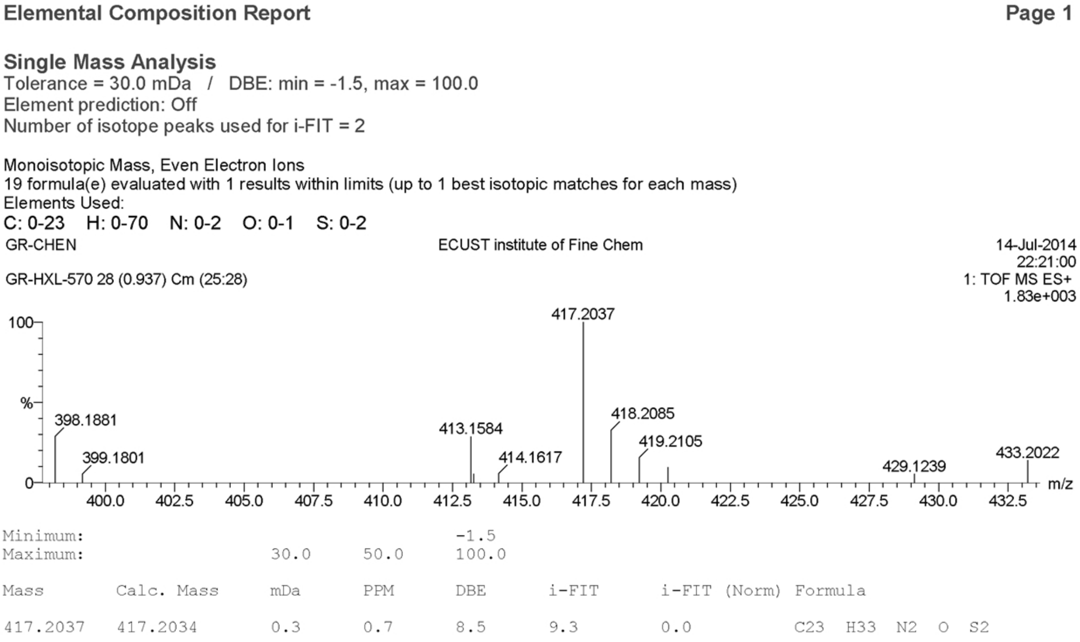


Figure S4: ^{13}C-NMR of compound **Hg570**



Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-23 H: 0-70 N: 0-2 O: 0-1 S: 0-2

GR-CHEN ECUST institute of Fine Chem 14-Jul-2014 22:21:00
GR-HXL-570 28 (0.937) Cm (25:28) 1: TOF MS ES+ 1.83e+003

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Minimum:                                    -1.5
Maximum:                30.0      50.0      100.0

Mass       Calc. Mass   mDa       PPM       DBE       i-FIT      i-FIT (Norm) Formula

417.2037   417.2034     0.3       0.7       8.5       9.3        0.0          C23  H33  N2  O  S2
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Figure S5: HR-MS of compound **Hg570**