Electronic Supplementary Material (ESI) for Analyst.

An activatable near-infrared hemicyanine-based probe for selective detection and imaging Hg^{2+} in living cells and animals

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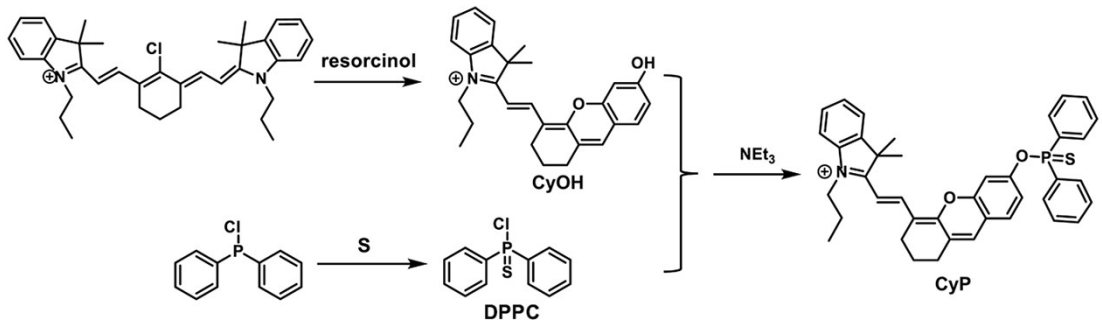
Synthesis for compound CyOH: Grinded potassium carbonate (64 mg, 0.58 mmol) and resorcinol (84 mg, 0.58 mmol) were stirred in 4 mL CH_3CN at 55 °C under N_2 atmosphere for 15 minutes, before IR-780 (100 mg, 0.19 mmol) dissolved in 4 mL CH_3CN was added dropwise into the mixture. The reaction mixture was then stirred for 3 h. The residue was then diluted with DCM and washed with water for three times. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was then purified with silica gel chromatography (DCM/CH_3OH, v:v= 25:1) to obtain pure CyOH (55 mg, 73.33%).^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.33 (d, *J* = 13.9 Hz, 1H), 7.37 – 7.30 (m, 4H), 7.23 (d, *J* = 8.8 Hz, 1H), 7.17 (t, *J* = 7.4 Hz, 1H), 7.04 – 6.98 (m, 2H), 6.94 (s, 1H), 5.91 (d, *J* = 13.9 Hz, 1H), 3.97 (t, *J* = 7.5 Hz, 2H), 2.70 (t, *J* = 6.1 Hz, 2H), 2.61 (d, *J* = 6.1 Hz, 2H), 1.93 – 1.85 (m, 4H), 1.70 (s, 6H), 1.05 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (500 MHz, Chloroform-*d*) δ 161.98, 157.04, 142.64, 140.50, 138.93, 129.25, 128.52, 124.34, 122.29, 115.34, 115.18, 109.85, 103.19, 97.77, 48.85, 45.64, 28.60, 28.44, 24.33, 20.89, 20.57, 11.69. HRMS: m/z calcd for $[C_{28}H_{30}NO_2]^+$ = 412.2271; found: 412.2275.

Synthesis for compound DPPC [1]

Chlordiphenylphosphin (500 mg, 2.27 mmol) and elemental sulfur (800 mg, 25 mmol) was heated to 130 °C. At this temperature, the reaction mixture was stirred for 6.5 h and then allowed to cool to room temperature. Clear yellowish oil was obtained. Then the product was purified with silica gel chromatography (PE) to obtain pure diphenylphosphinothioic chloride (compound DPPC) (574 mg, 100%).^{31}P NMR (500

MHz, Chloroform-*d*) δ 80.19. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.05 – 7.93 (m, 4H), 7.62 – 7.56 (m, 2H), 7.54– 7.50 (m, 4H). ^{13}C NMR (500 MHz, Chloroform-*d*) δ 135.89, 135.12, 132.59, 132.57, 131.04, 130.94, 128.75, 128.63. GCMS: m/z calcd for $[C_{12}H_{10}ClPS]^{+}$ =251.9929; found: 252.0700.

Synthesis for probe CyP: **CyOH** (50 mg, 0.12 mmol) was dissolved in dichloromethane (DCM) (10 mL) with triethylamine (50 μL). Compound DPPC (300.0 mg, 1.2 mmol) was added into the mixture at 0 °C and stirred for 30 min. The residue was diluted with DCM and washed with water for three times. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was concentrated and purified after silica gel chromatography (DCM/CH_3OH, v:v =20:1) to obtain pure **CyP** (67 mg, 90% yield). ^{31}P NMR (500 MHz, Chloroform-*d*) δ 84.33. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.66 (dt, *J* = 13.7, 6.5 Hz, 1H), 8.14 – 8.04 (m, 4H), 7.92 (s, 1H), 7.75 (dd, *J* = 7.4, 1.3 Hz, 1H), 7.69 – 7.63 (m, 3H), 7.63 – 7.56 (m, 5H), 7.56 – 7.51 (m, 1H), 7.42 – 7.35 (m, 1H), 7.25 – 7.19 (m, 1H), 7.16 (q, *J* = 2.1 Hz, 1H), 7.07 (m, 1H), 6.61 (dt, *J* = 15.2, 2.1 Hz, 1H), 4.40 (t, *J* = 7.4 Hz, 2H), 2.78 – 2.65 (m, 4H), 1.95 (m, 4H), 1.80 (d, *J* = 2.5 Hz, 6H), 1.09 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 178.48, 159.95, 152.96, 152.72, 146.27, 142.02, 141.47, 133.93, 133.05, 132.62, 132.59, 131.46, 131.36, 129.80, 129.38, 128.82, 128.71, 128.18, 127.81, 122.42, 119.11, 119.08, 118.98, 115.49, 113.46, 109.42, 109.38, 106.33, 106.28, 50.88, 47.74, 29.39, 28.17, 24.34, 21.64, 20.23, 11.55. HRMS: m/z calcd for $[C_{40}H_{39}NO_2PS]^{+}$ = 628.2432; found: 628.2442.



Scheme S1. The synthetic route of the probe **CyP**.

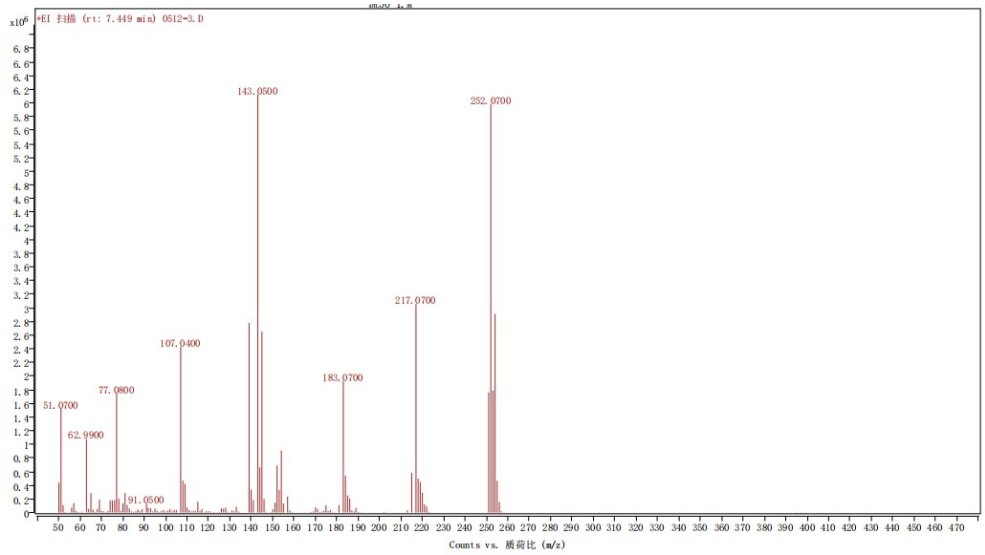


Figure S1. The GC-MS of compound DPPC.

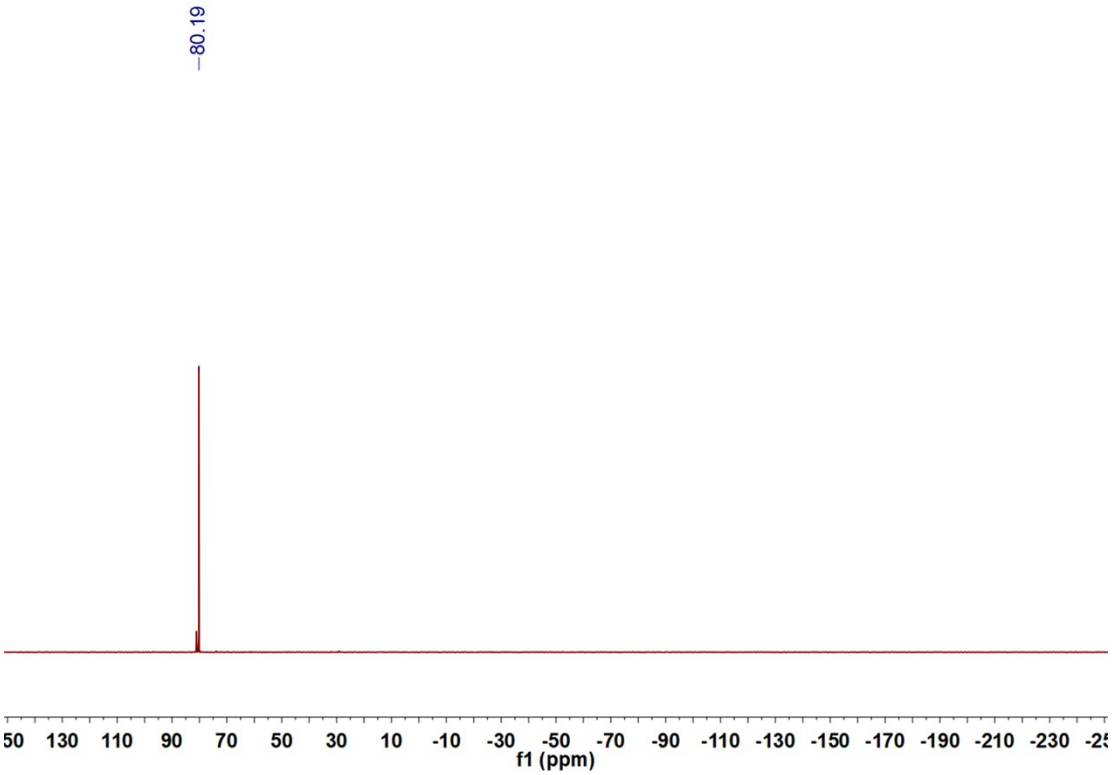


Figure S2. The ^{31}P NMR of DPPC in $CDCl_3$.

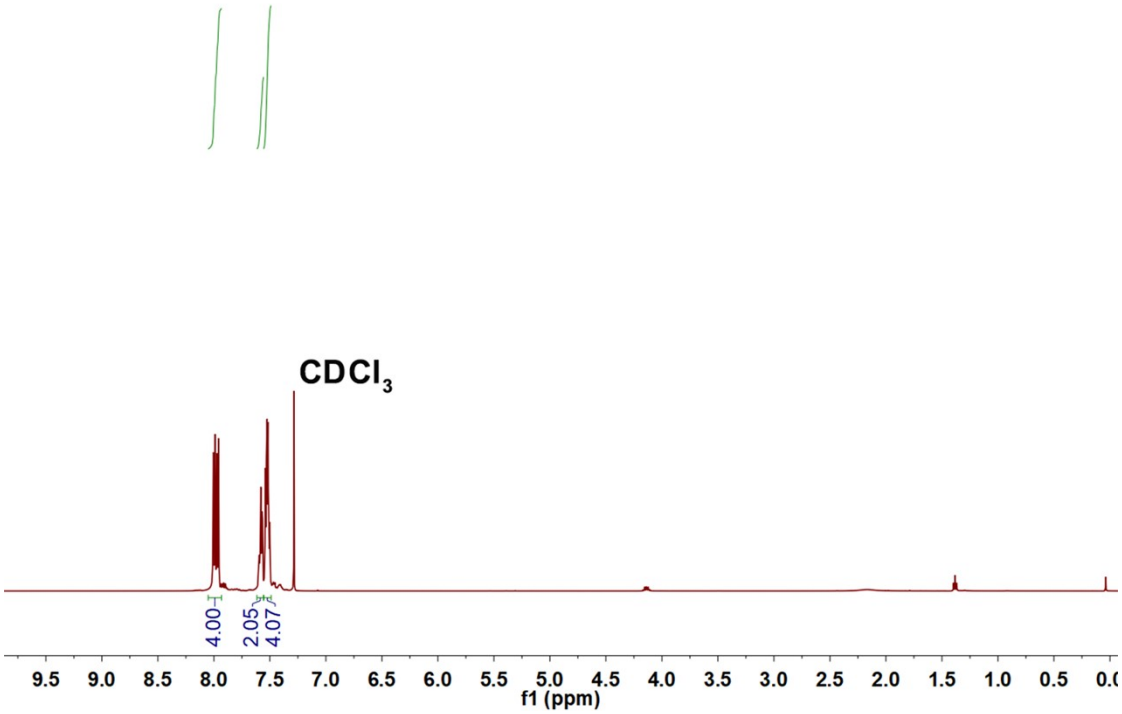


Figure S3. The ^{1}H NMR of DPPC in $CDCl_3$.

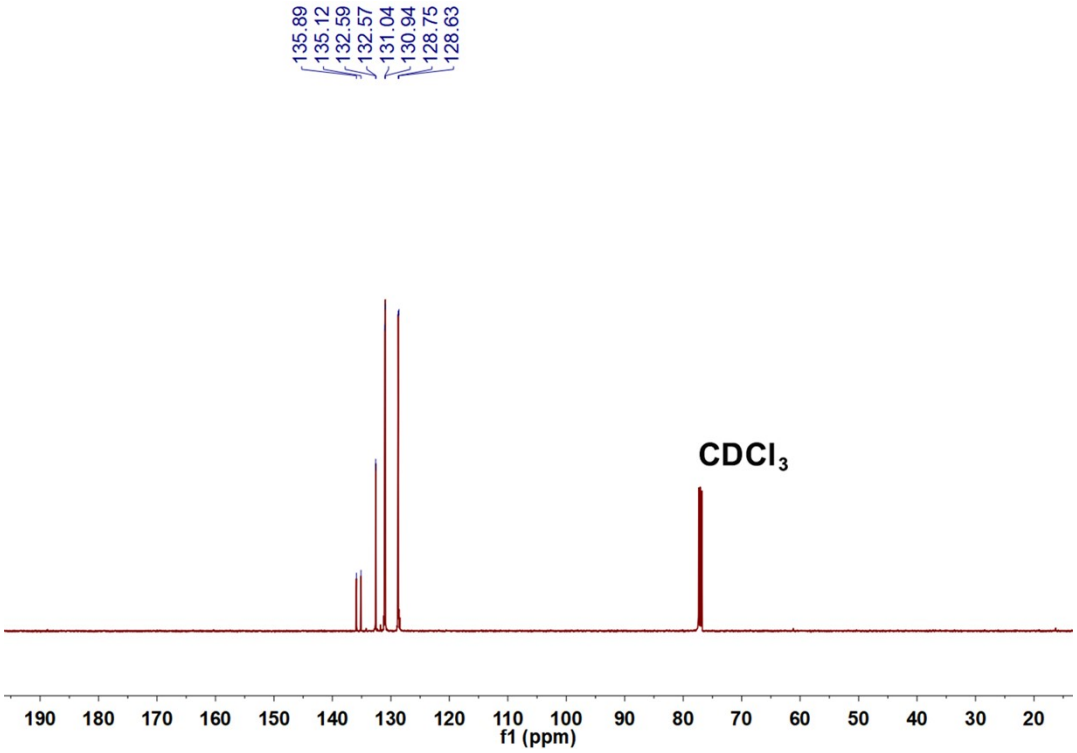
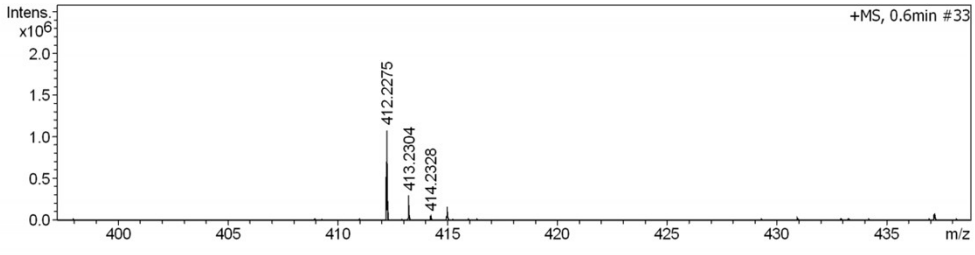


Figure S4. The ^{13}C NMR of DPPC in $CDCl_3$.

Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1350 m/z	Set Collision Cell RF	700.0 Vpp	Set Divert Valve	Waste



#	m/z	Res.	S/N	I	I %	FWHM
1	412.2275	29891	1809.9	1079064	100.0	0.0138
2	413.2304	20659	488.1	290988	27.0	0.0200
3	414.2328	18325	77.0	45964	4.3	0.0226

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma		Score	rdb	e¯ Conf	N-Rule
412.2275	1	C28H30NO2	412.2271	-0.9	21.0	1	100.00	14.5	even	ok

Figure S5. The HR-MS of **CyOH**.

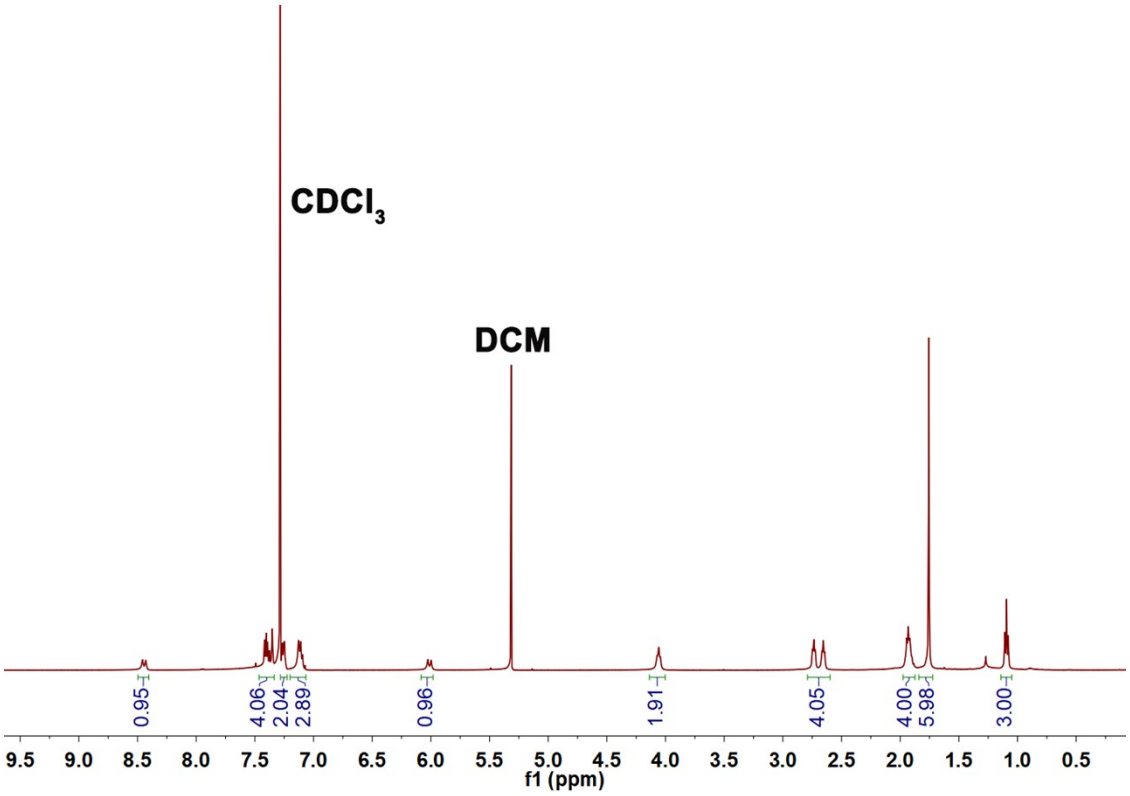


Figure S6. The ^{1}H NMR of **CyOH** in $CDCl_3$.

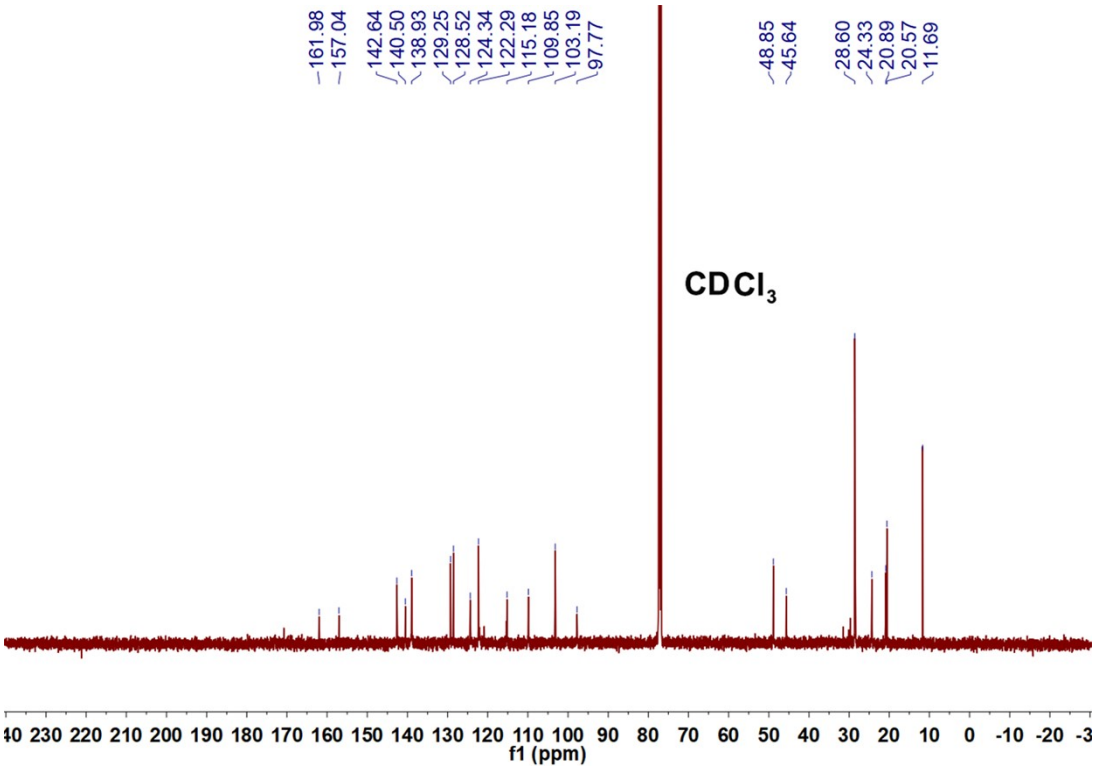
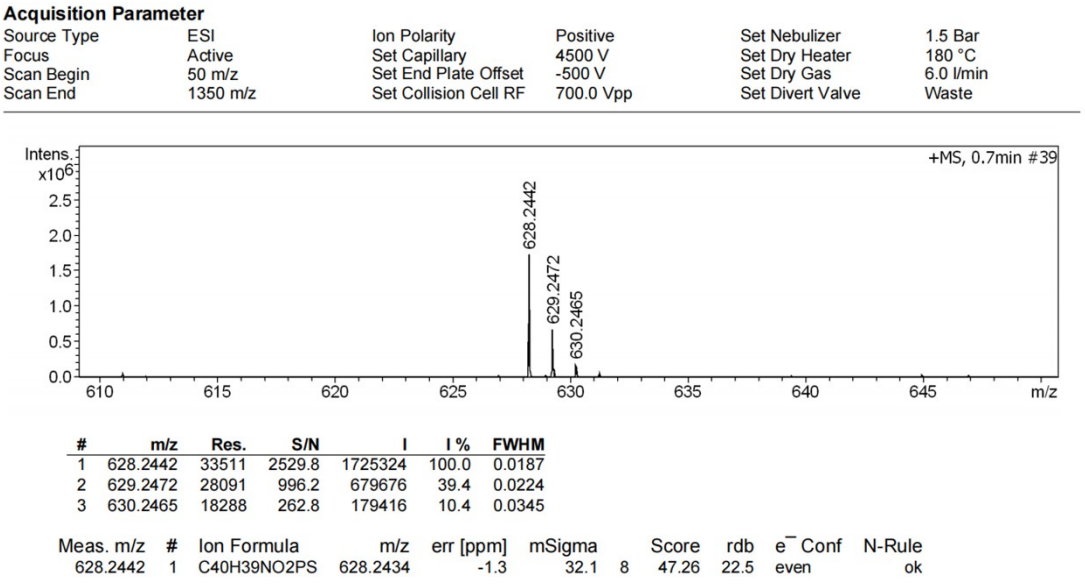


Figure S7. The ^{13}C NMR of **CyOH** in $CDCl_3$.



Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1350 m/z	Set Collision Cell RF	700.0 Vpp	Set Divert Valve	Waste

#	m/z	Res.	S/N	I	I %	FWHM
1	628.2442	33511	2529.8	1725324	100.0	0.0187
2	629.2472	28091	996.2	679676	39.4	0.0224
3	630.2465	18288	262.8	179416	10.4	0.0345

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma		Score	rdb	e⁻ Conf	N-Rule
628.2442	1	C40H39NO2PS	628.2434	-1.3	32.1	8	47.26	22.5	even	ok

Figure S8. The HR-MS of **CyP**.

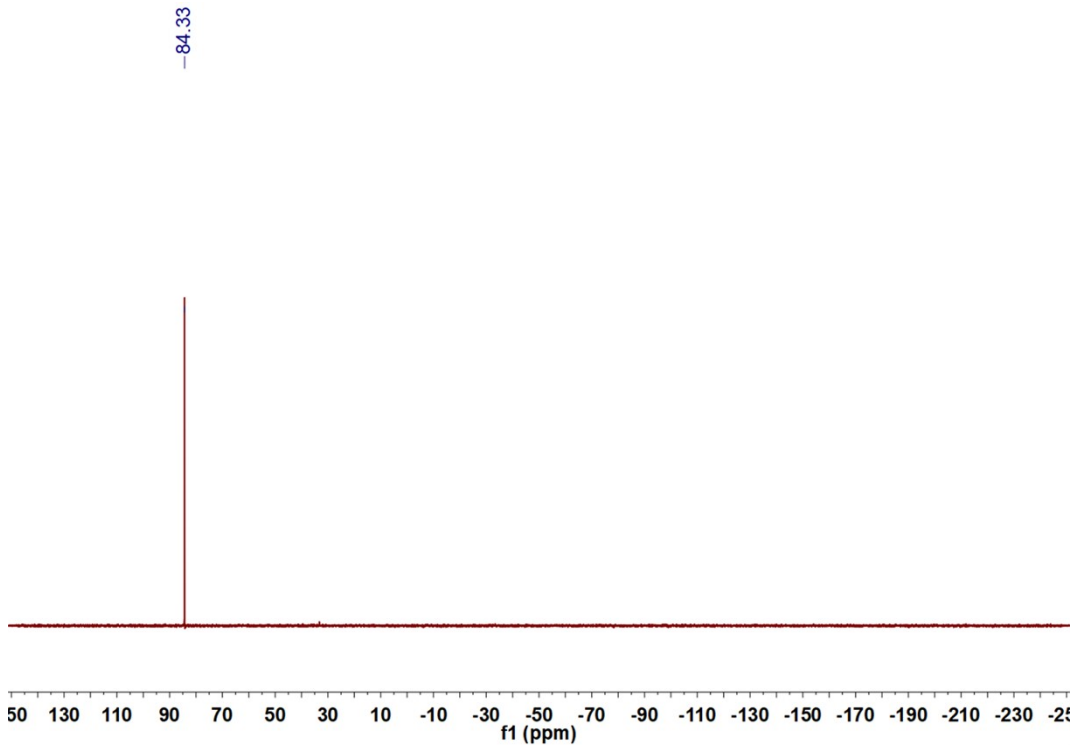


Figure S9. The ^{31}P NMR of **CyP** in $CDCl_3$.

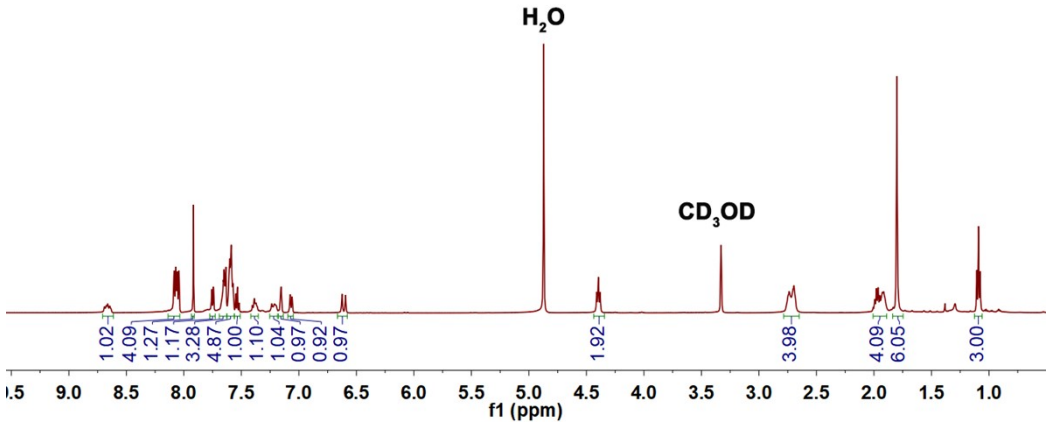


Figure S10. The ^{1}H NMR of **CyP** in $CDCl_3$.

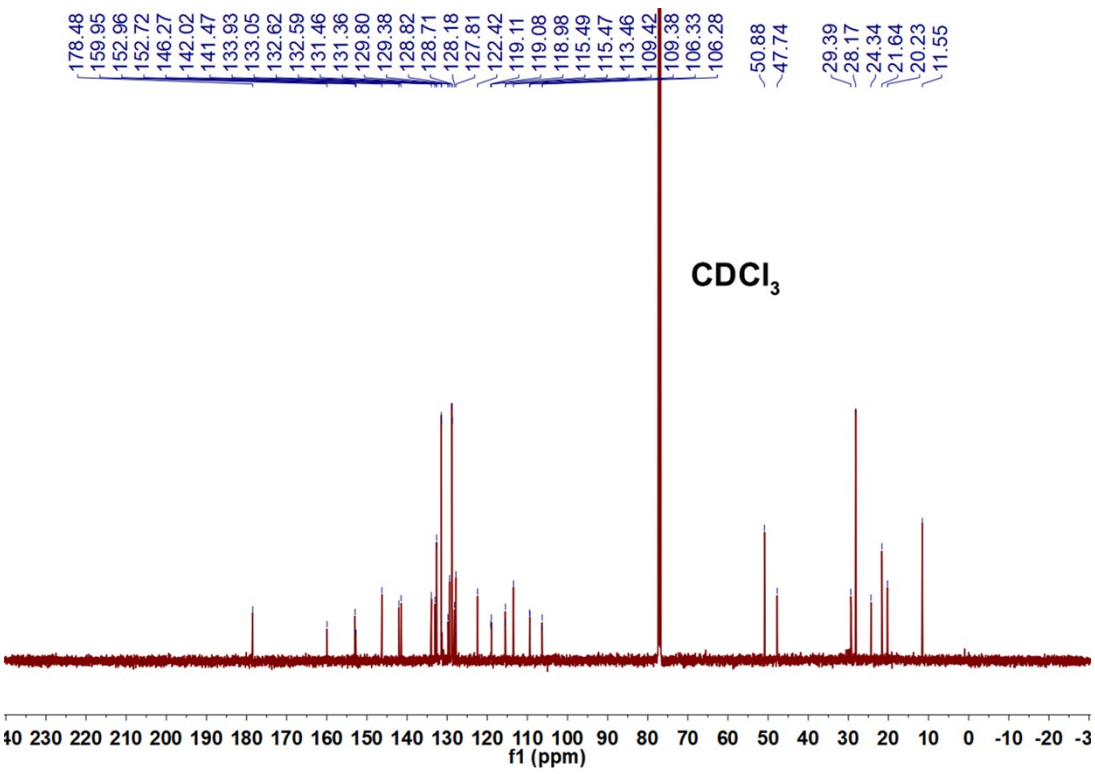


Figure S11. The ^{13}C NMR of **CyP** in $CDCl_3$.

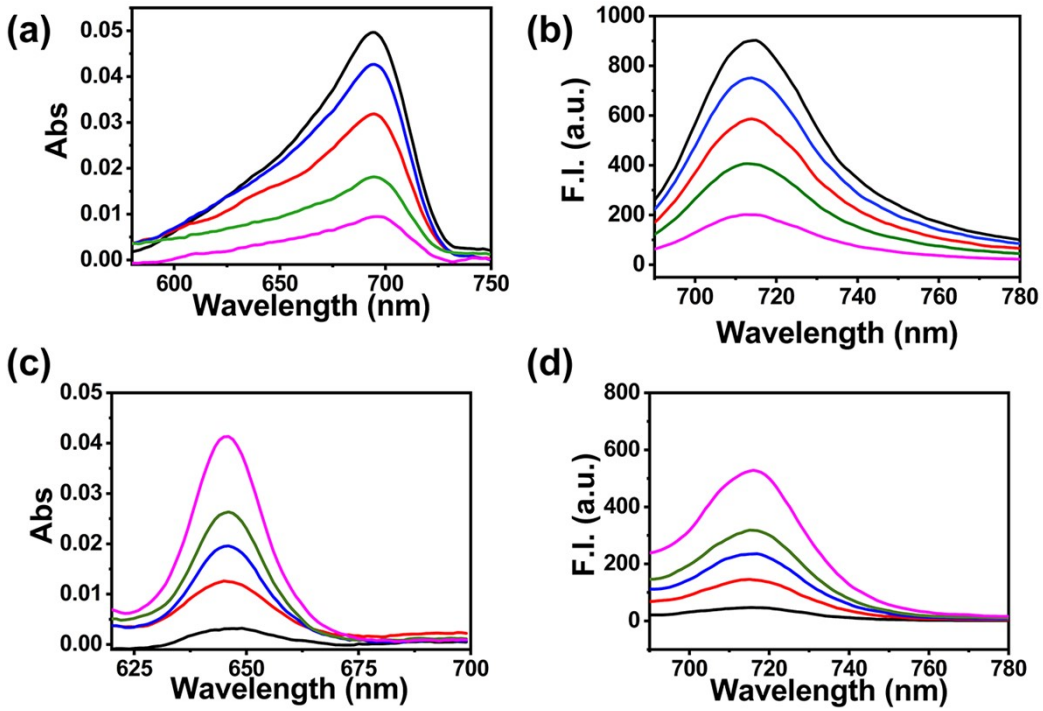


Figure S12. Detection of fluorescence quantum yield of **CyOH**. (a-b) The absorbance and fluorescence spectra of **CyOH** in CH_3OH. (c-d) The absorbance and fluorescence spectra of TCPP (Φ=0.25) in DMSO[2]. Both probes were detected by the same excitation wavelength (645 nm) and slit width (10 nm).

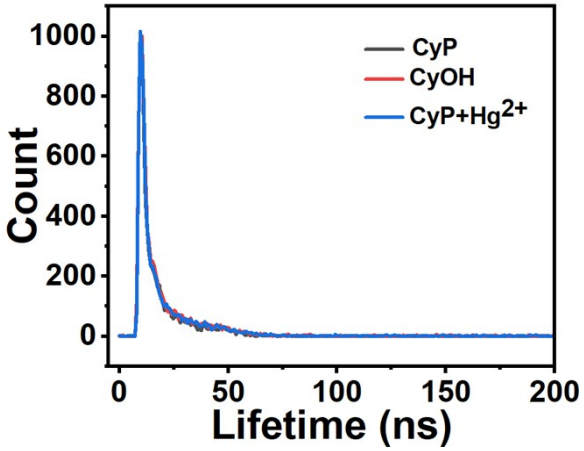


Figure S13. The fluorescence lifetime of probe CyP (10 μM), CyOH (10 μM) and CyOH (10 μM) + Hg^{2+}(100 μM).

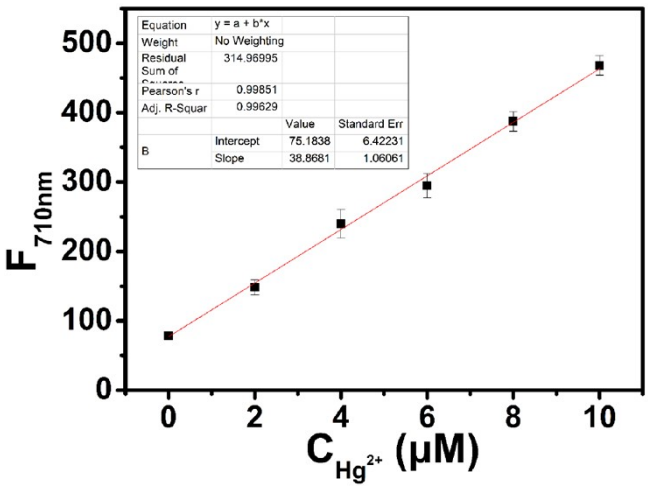


Figure S14. Linear fitting of **CyP** (10 μM) incubated with different concentrations of Hg^{2+} (0~10 μM).

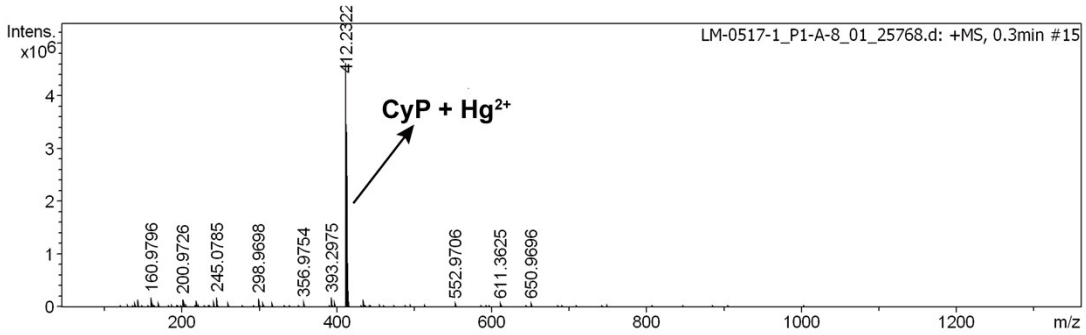


Figure S15. The mass spectra of the solution of **CyP** incubated with Hg^{2+}.

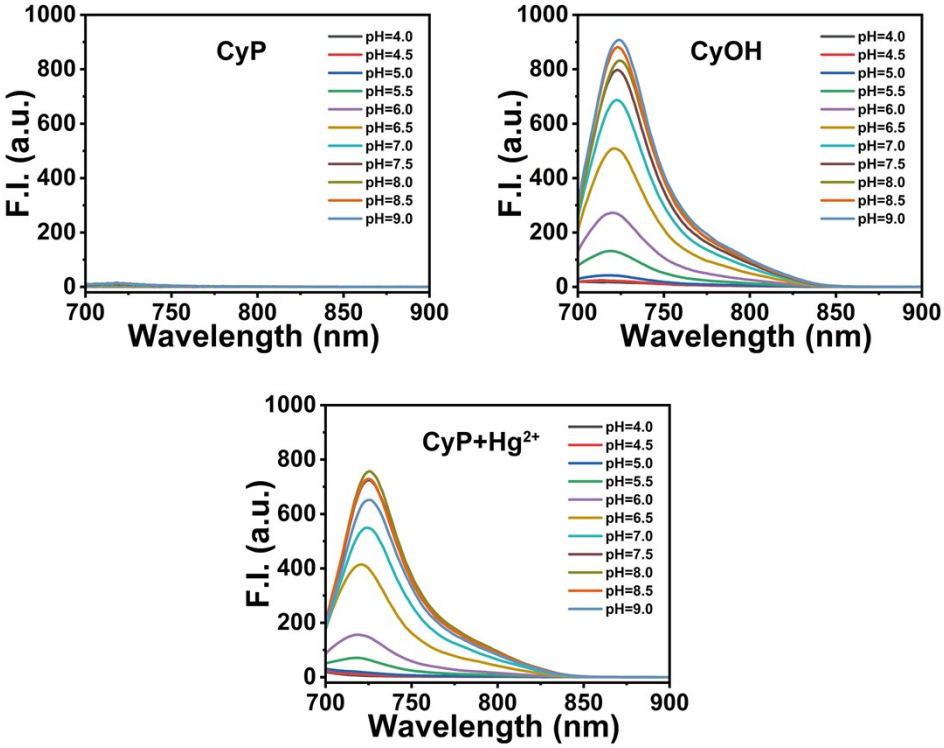


Figure S16. Fluorescence intensity of **CyP** (10 μM), **CyOH** (10 μM), **CyP** (10 μM) with Hg^{2+} in solution of different pH.

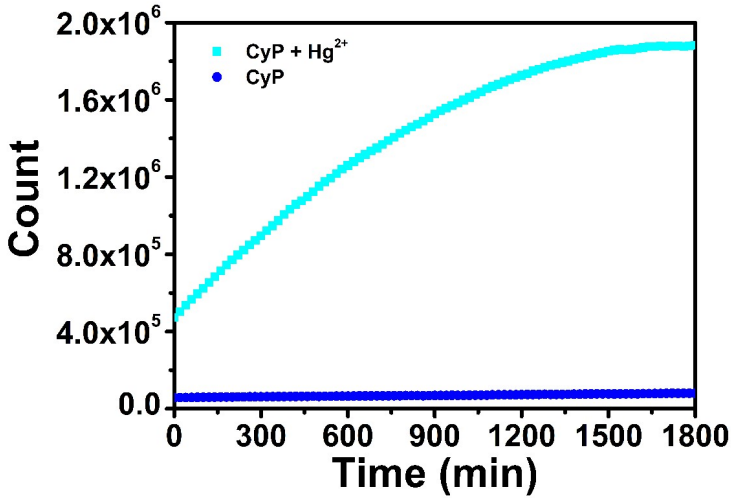


Figure S17. Fluorescence intensity-time relationship of **CyP** without Hg^{2+} and with Hg^{2+}.

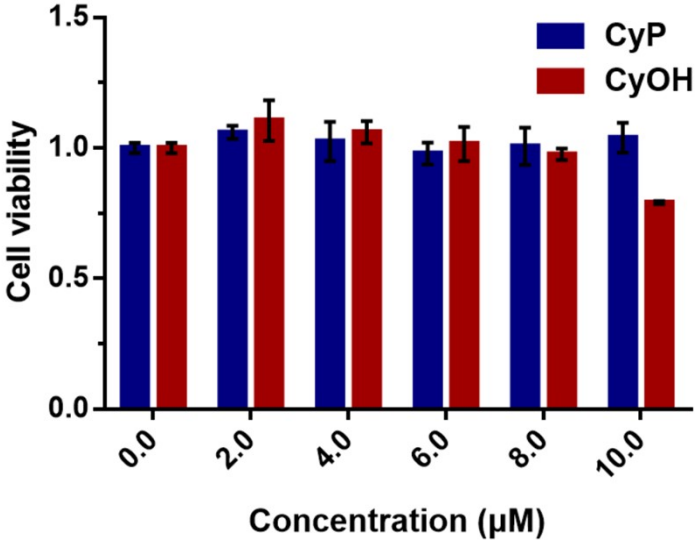


Figure S18. The cytotoxicity test for **CyOH** and **CyP** at different concentration (0-10 μM).

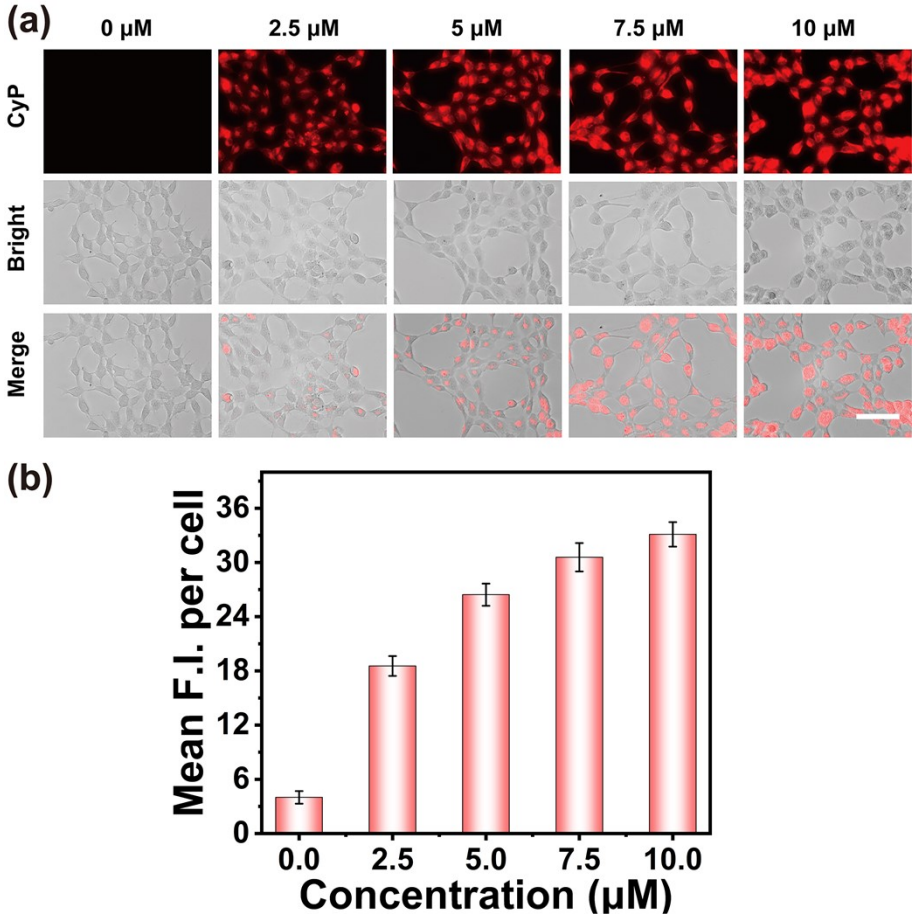


Figure S19. (a) Cell images of SHSY-5Y cells pre-incubated Hg^{2+} (20 μM) for 30 min, then added **CyP** (0, 2.5, 5, 7.5 or 10 μM) for 20 min. (b) Mean fluorescence intensities of SHSY-5Y cells in the presence of different concentrations of **CyP**.

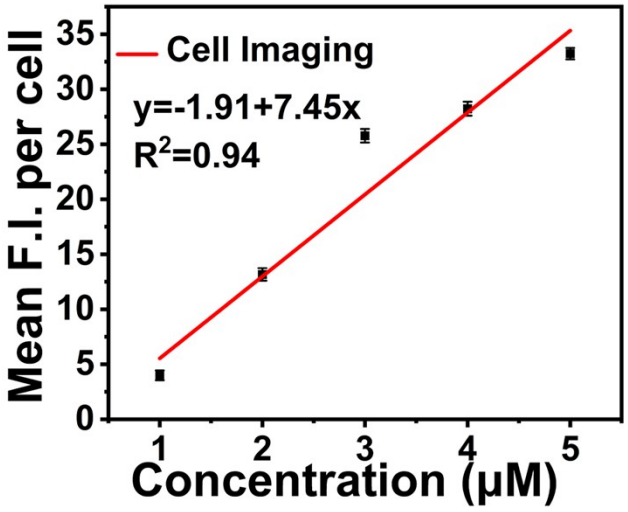


Figure S20. Linear analysis of mercury ion concentration and mean fluorescence intensity of cells in cell imaging.

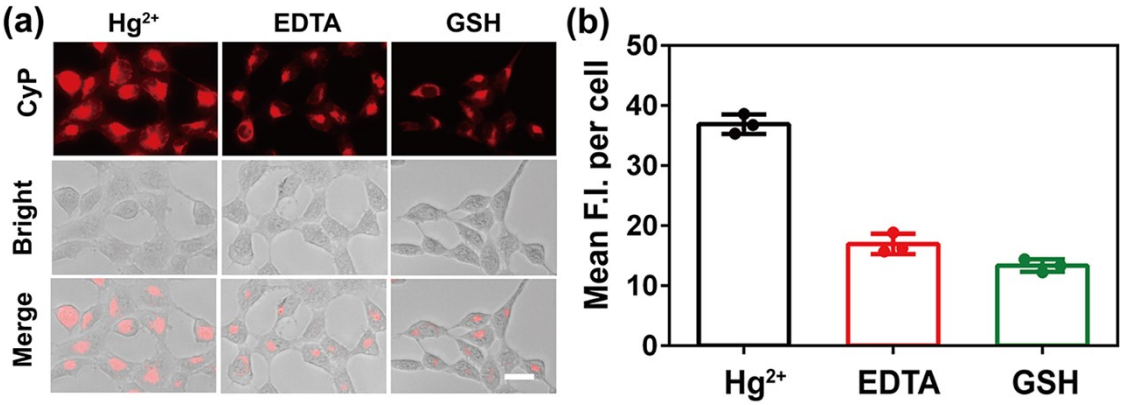


Figure S21. (a) SHSY-5Y cells incubated with Hg^{2+} (20 μM) for 30 min and EDTA (40 μM) and GSH (40 μM) for 1 h, respectively. (b) Mean fluorescence intensities of SHSY-5Y cells of panel a.

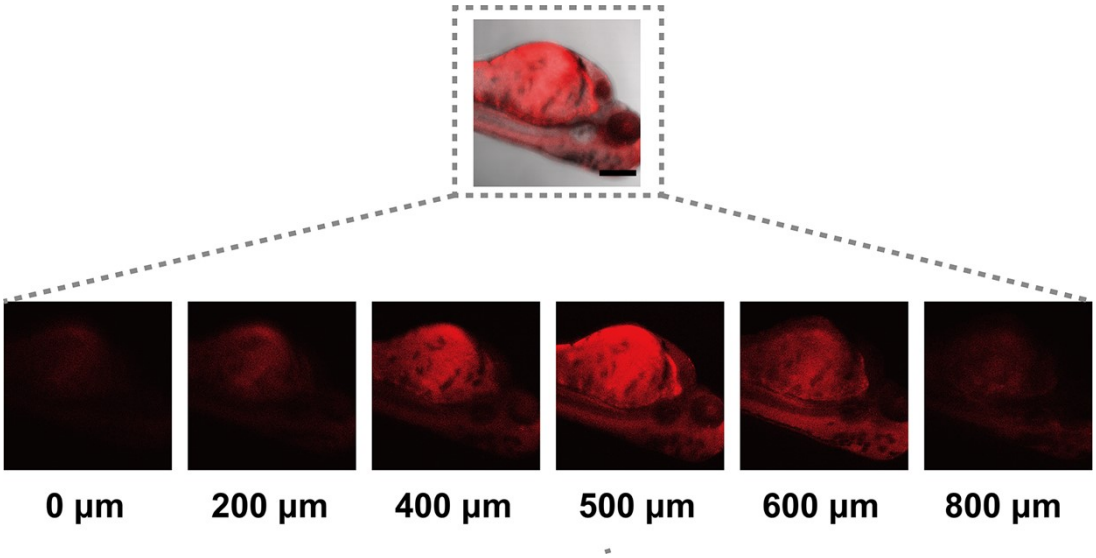
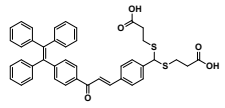
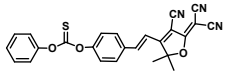
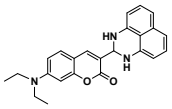
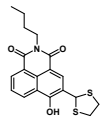
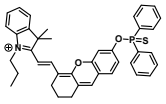


Figure S22. Z-scanned confocal fluorescence images of zebrafish incubated with Hg^{2+} (40 μM) for 30 min, and then incubated with **CyP** (10 μM) for 20 min (Scar bar 250 μm).

Table S1. Comparison of different fluorescence methods for detecting Hg^{2+}.

Method	Media	Detection limit/nM	λ_{em} (nm)	Cell image	Animal image
Tang et al. (2019)		$4.16X10^3$	537	No	No
Xu et al.(2018)		17.0	604	No	No
Wu et al. (2019)		$1.08x10^3$	480	Yes	Yes
Tian et al. (2020)		40.0	510	Yes	No
This work		54.8	710	Yes	Yes

References

1 J. Wagner, M. Ciesielski, C. A. Fleckenstein, H. Denecke, F. Garlichs, A. Ball and M. Doering, Org. Process Res. Dev., 2013, **17**, 47-52.

2 P. G. Mahajan, N. C. Dige, B. D. Vanjare, A. R. Phull, S. J. Kim, S. K. Hong and K. H. Lee, J Fluoresc., 2018, **28**, 871-882.