Electronic Supplementary Material for

Near-Infrared fluorogenic switches for detection of Hg(II) ions: Applications in real samples and living cells

Asaithambi Gomathi,[a] and Periasamy Viswanathamurthi,[a,*]

[a]*Department of Chemistry, Periyar University, Salem, Tamil Nadu, India. 636011*

*To whom correspondence should be addressed, e-mail:*viswanathamurthi@gmail.com*

Fax: +91 427 2345124

Experimental section

Cell culture

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C under an atmosphere of 5% CO_2. Cells were plated on 18 mm glass cover slips and allowed to adhere for 24 h.

Cytotoxicity assay

The methyl thiazolyl tetrazolium (MTT) assay was used to measure the cytotoxicity of probe towards HeLa cells. The cells were seeded into a 96-well cell-culture plate. Various concentrations (0, 20, 40, 60, 80 and 100 μM) of NIRM probe were added to the wells. The cells were incubated at 37°C under 5% CO_2 for 24 h. 10 μL MTT (5 mg/mL) was added to each well and incubated at 37°C under 5% CO_2 for 4 h. Multiskan GO microplate reader was used to measure the absorbance at 510 nm for each well. The viability of cells was calculated according to the following equation: Cell viability (%) = (mean of absorbance value of treatment group) / (mean of absorbance value of control group).

Cell imaging

HeLa cells were initially cultured in a tissue culture flask containing DMEM medium supplemented with 10% FBS, penicillin (100 μg mL^{-1}) and streptomycin (100 μg mL^{-1}) in a CO_2 incubator. Prior to imaging studies, the cells were seeded into a 6 well plate and grown in DMEM medium at 37°C till 80% confluence in CO_2 incubator. Subsequently, the cells were

washed thrice with sterile phosphate buffered saline (PBS), incubated with 10 μM of NIRM probe in DMEM at 37°C for 30 min in a CO_2 incubator. The cells were again washed with sterile PBS to remove excess probe and their images were acquired using a fluorescence microscope (Eclipse Ti-U, Nikon, USA) with a filter that allowed green light emission. The cells were subsequently incubated with sterile PBS separate set with 30 μM Hg^{2+} for 1 h. The images of the cells were acquired with a fluorescence microscope.

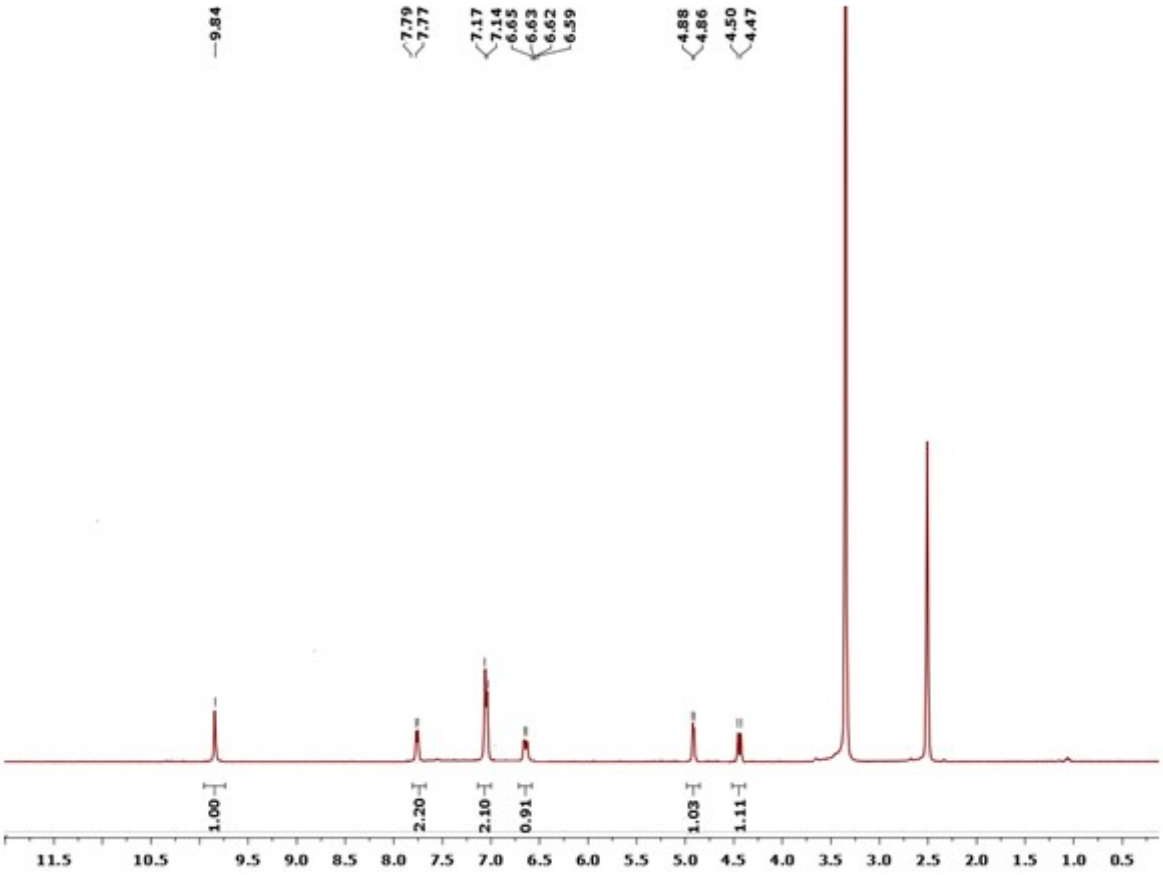


Fig. S1. ^{1}H-NMR spectrum of compound 1.

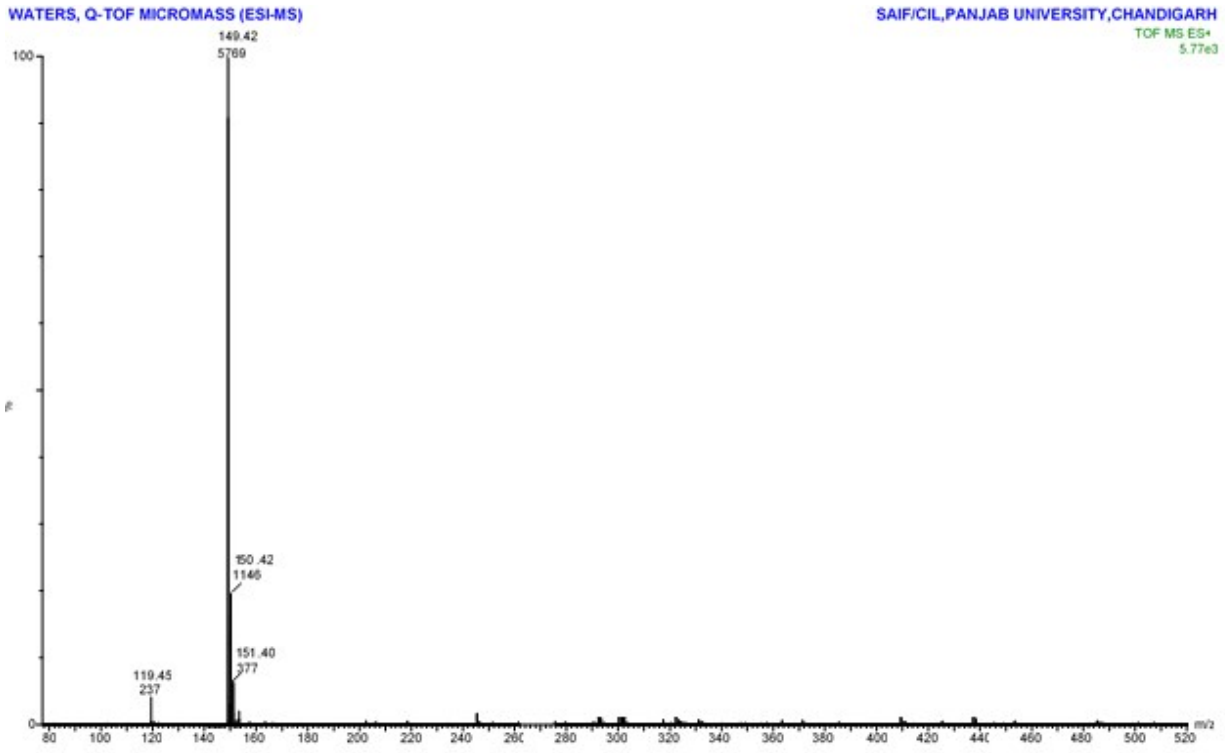


Fig. S2. Mass spectrum of compound 1

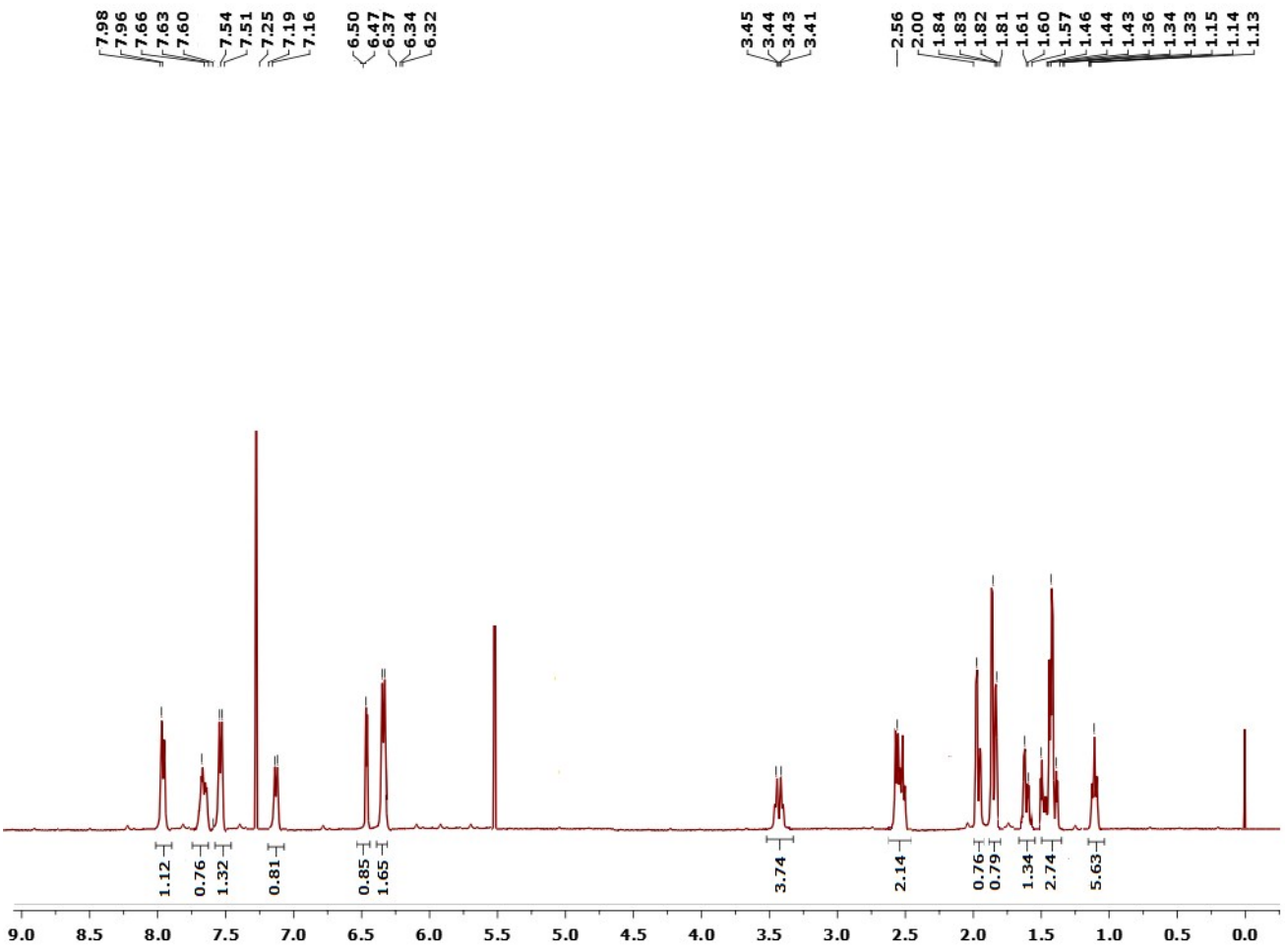


Fig. S3. ^{1}H-NMR spectrum of compound 3.

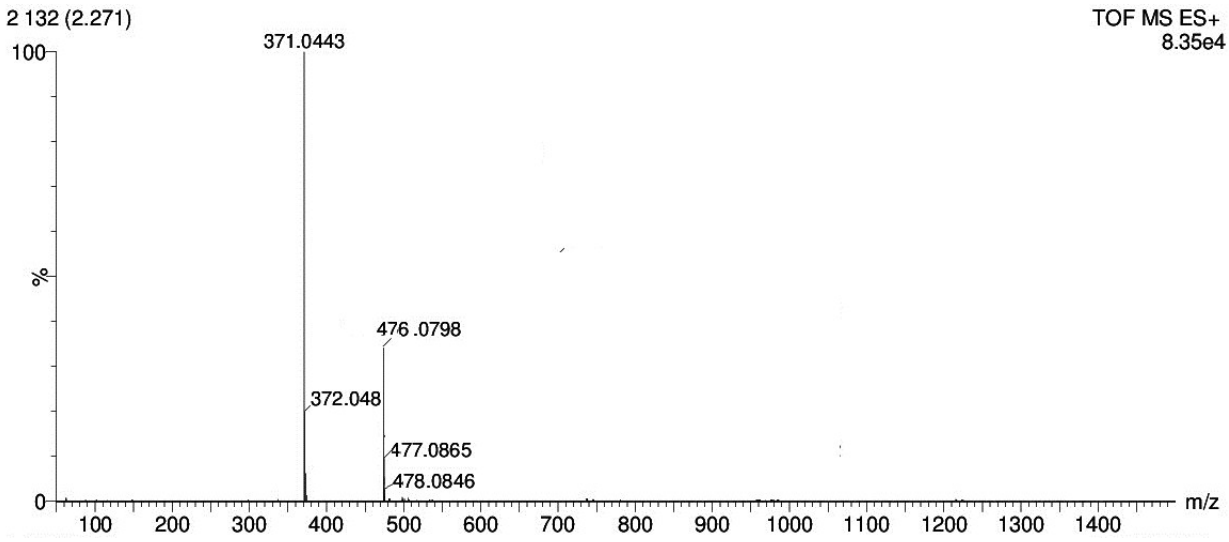


Fig. S4. Mass spectrum of compound 3.

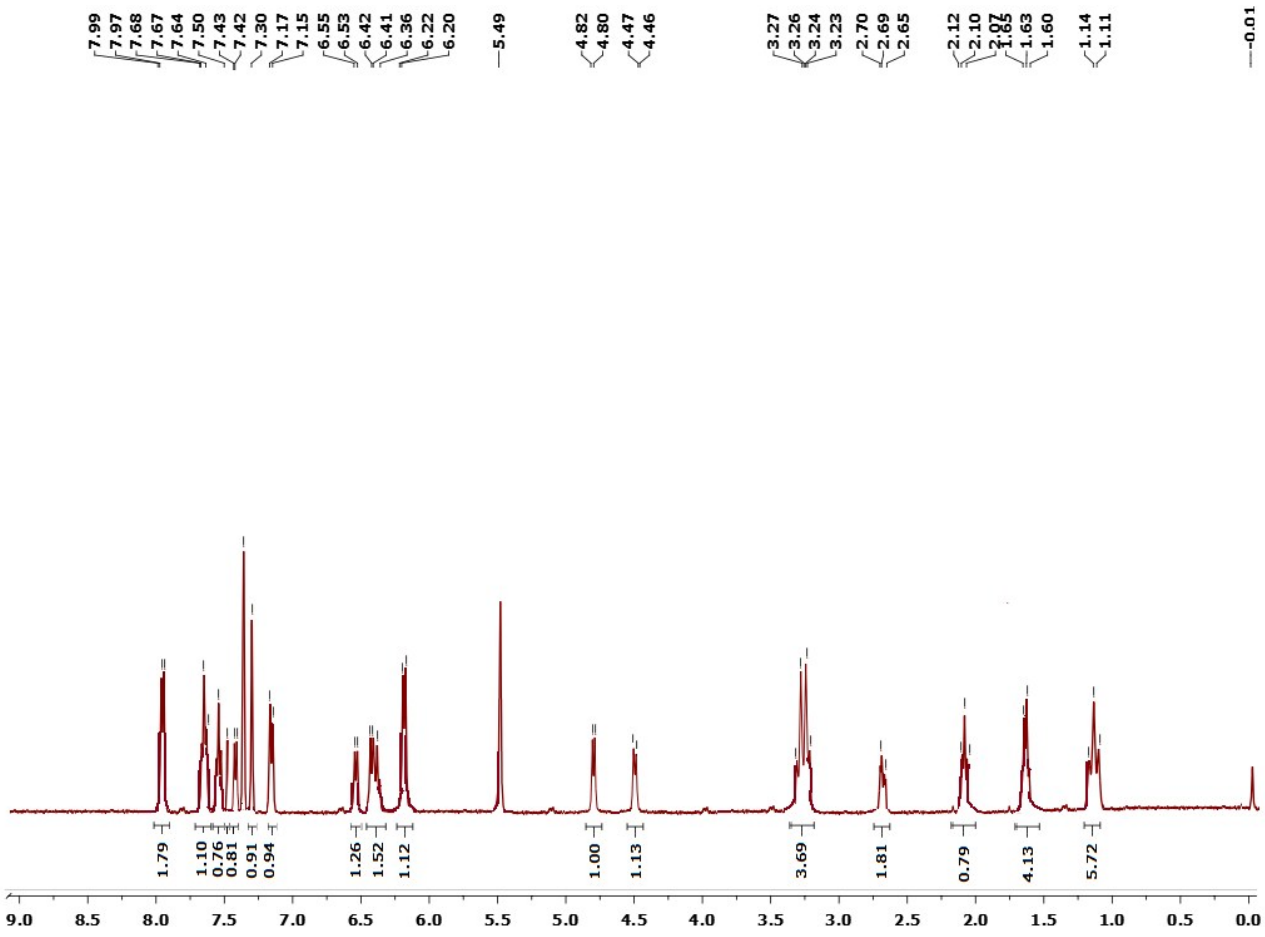


Fig. S5. ^{1}H-NMR spectrum of NIRM.

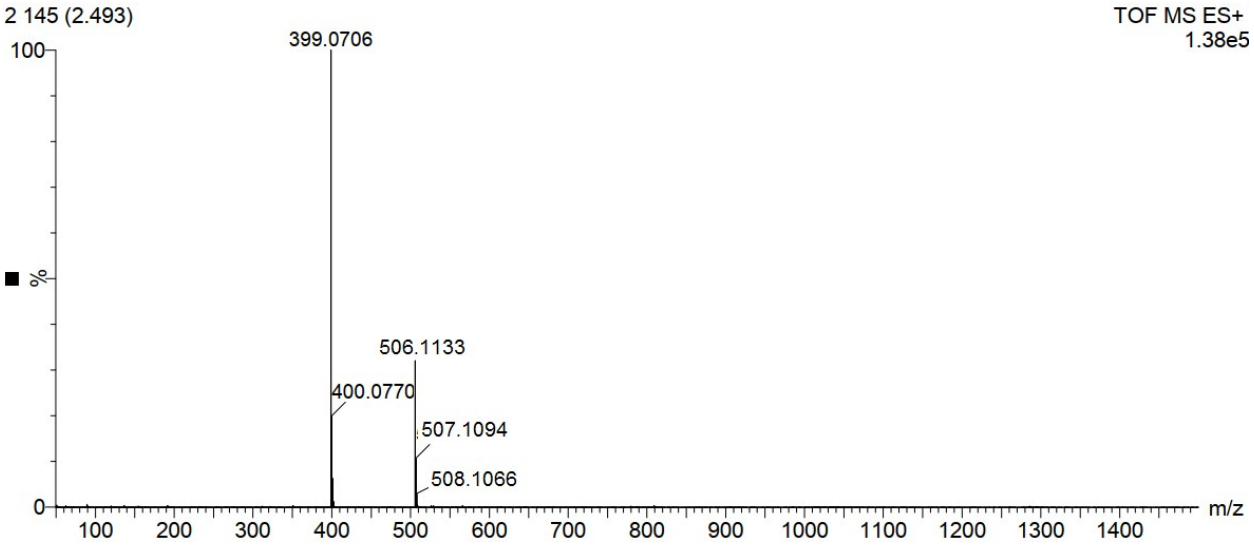


Fig. S6. Mass spectrum of probe NIRM.

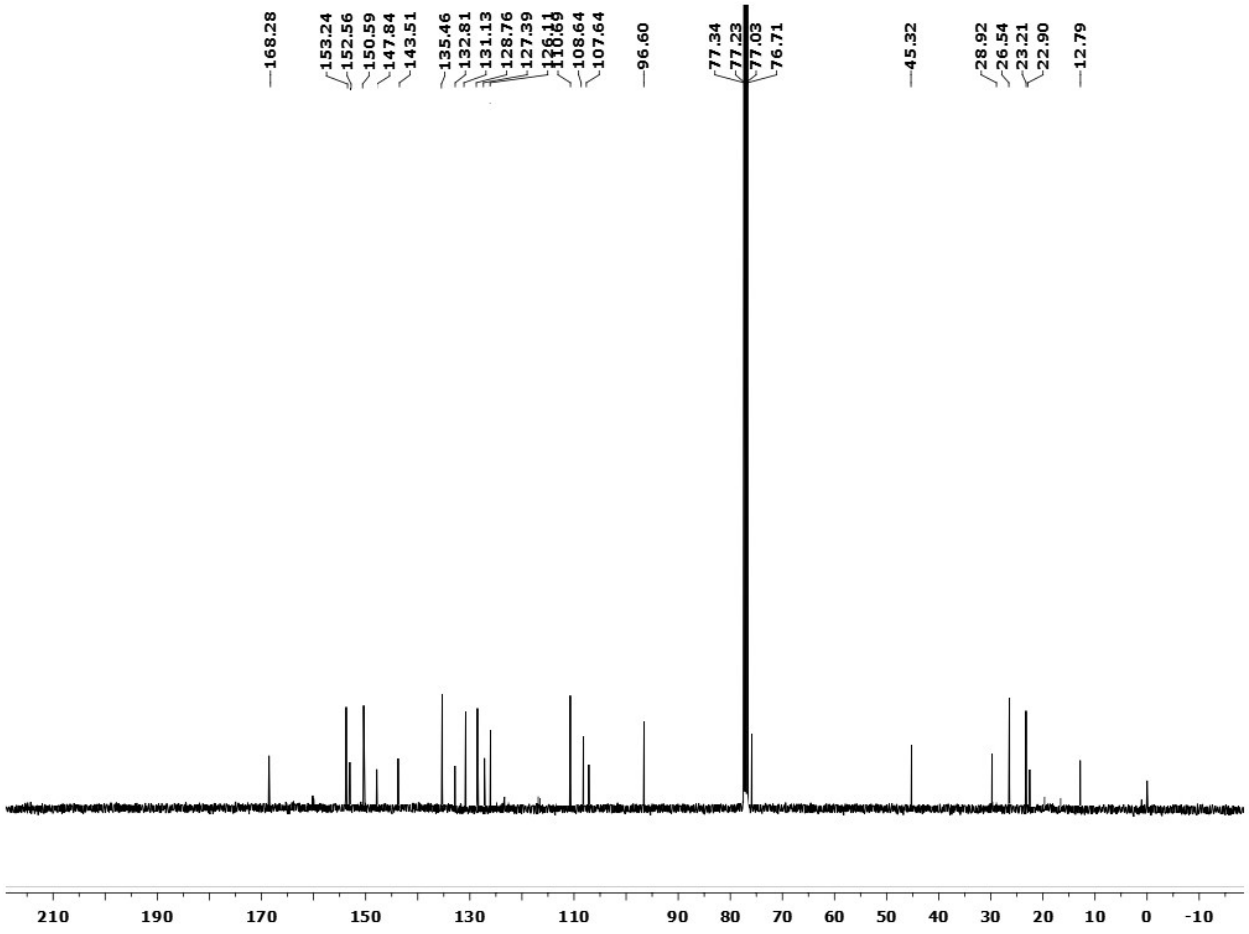


Fig. S7. ^{13}C-NMR spectrum of probe NIRM.

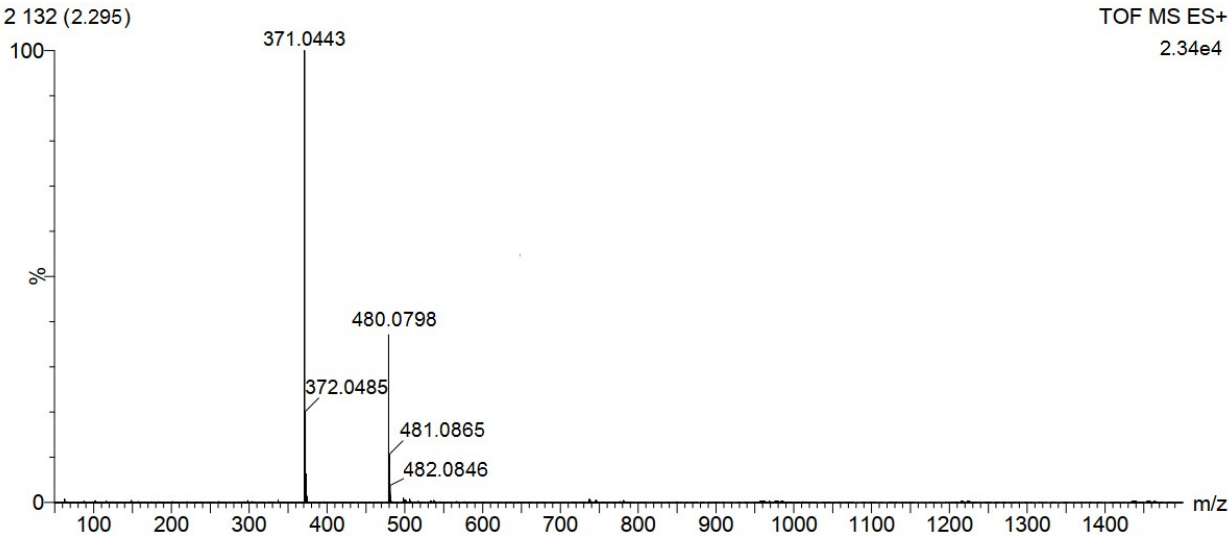


Fig. S8. Mass spectrum of control compound NIC.

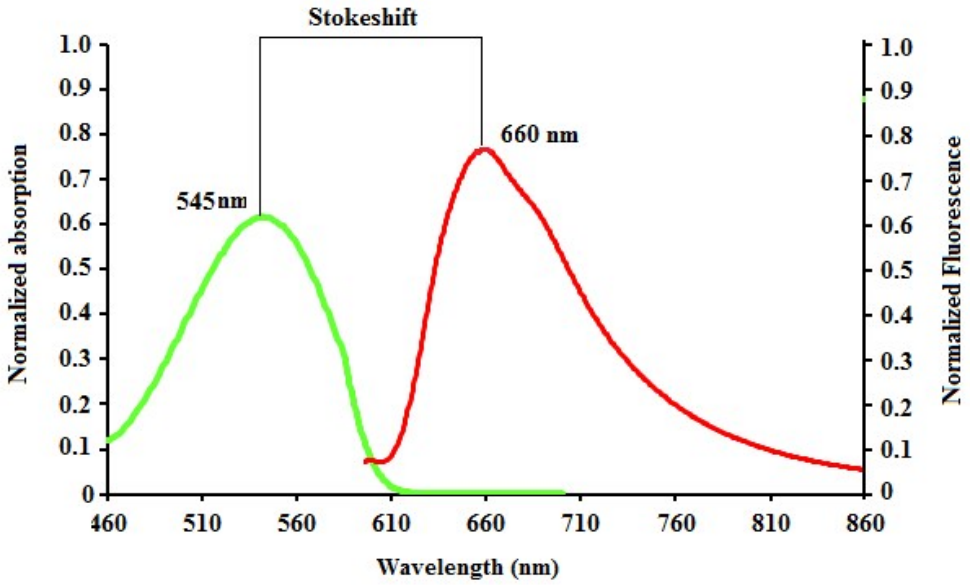


Fig. S9. UV-Visible spectra of probe NIRM (10 μM) and emission spectra of probe NIRM treated with 30 μM solution of Hg^{2+} in Ethanol–H_2O (2:8 v/v 50 mM HEPES buffer solution) at pH 7.4

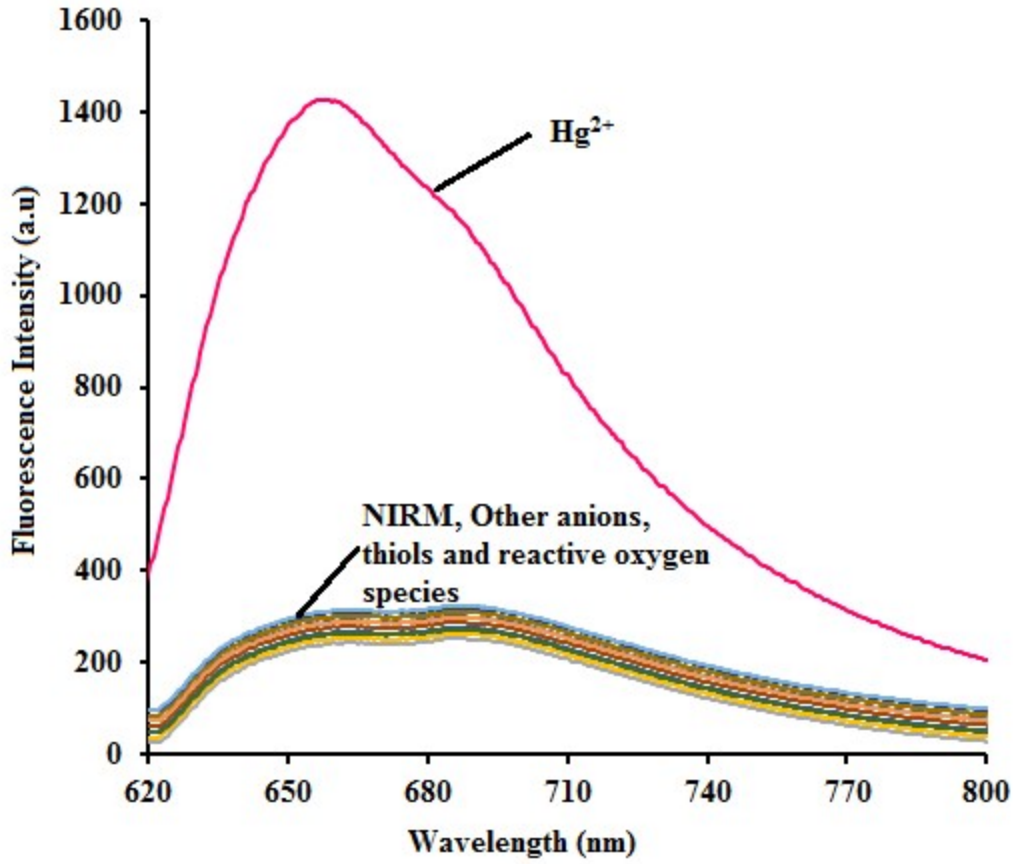


Fig. S10. Fluorescence responses of NIRM (10 μM) in Ethanol–H_2O (2:8 v/v 50 mM HEPES buffer solution) at pH 7.4. with 30 μM of various anions, biothiol and reactive oxygen species (Cl^-, Br^-, I^-, CN^-, CO_3^{2-}, HCO_3^-, CH_3COO^-(OAc), SO_4^{2-}, F^-, SCN^-, HSO_3^- NO_3^{2-}, SH^-, Cys, HCy, GSH, H_2O_2, HClO, $NO^{\bullet}$, $OH^{\bullet}$, O_2^-).

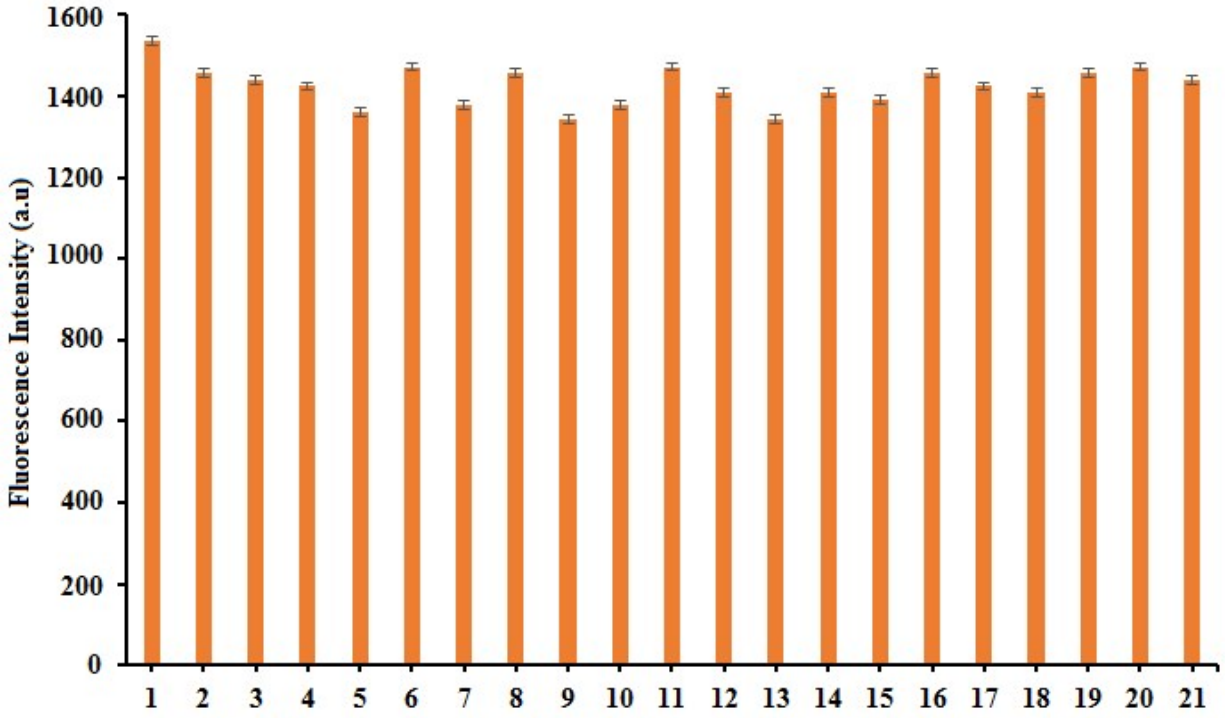


Fig. S11. Fluorescence response of NIRM (10 μM) at 660 nm in the presence of 30 μM of Hg^{2+} and one different other species (30 μM). (1=Cl^-, 2=Br^-, 3=I^-, 4=CN^-, 5=CO_3^{2-}, 6=HCO_3^-, 7=CH_3COO^-(OAc), 8=SO_4^{2-},9= F^-, 10=SCN^-, 11=HSO_3^-, 12=NO_3^{2-}, 13=SH^-, 14=Cys, 15=HCy, 16=GSH, 17=H_2O_2, 18=HClO, 19=$NO^{\cdot}$ 20=$OH^{\cdot}$, 21=O_2^-).

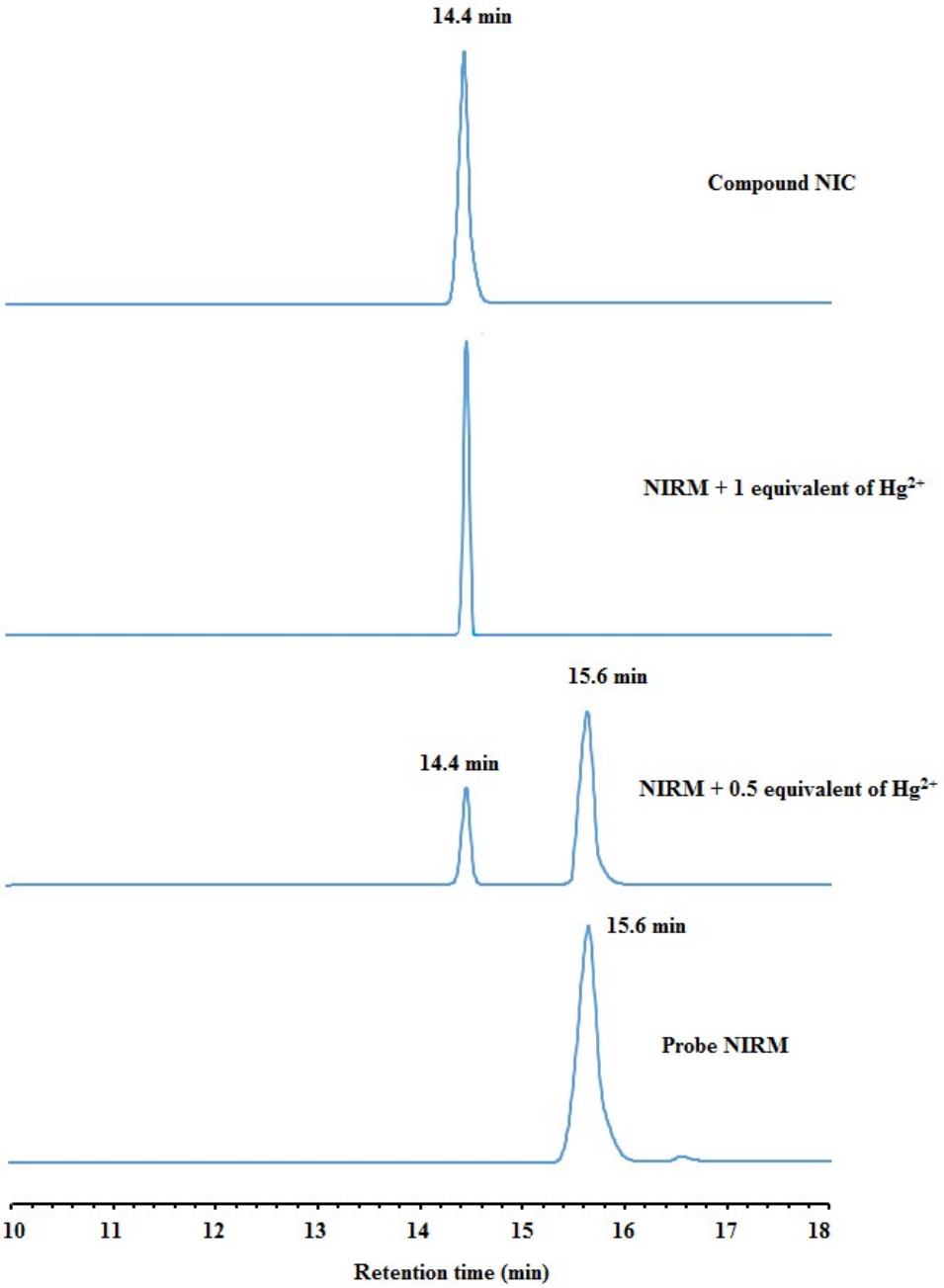


Fig. S12. HPLC chromatogram study of NIRM, NIC and NIRM treated with Hg^{2+}.

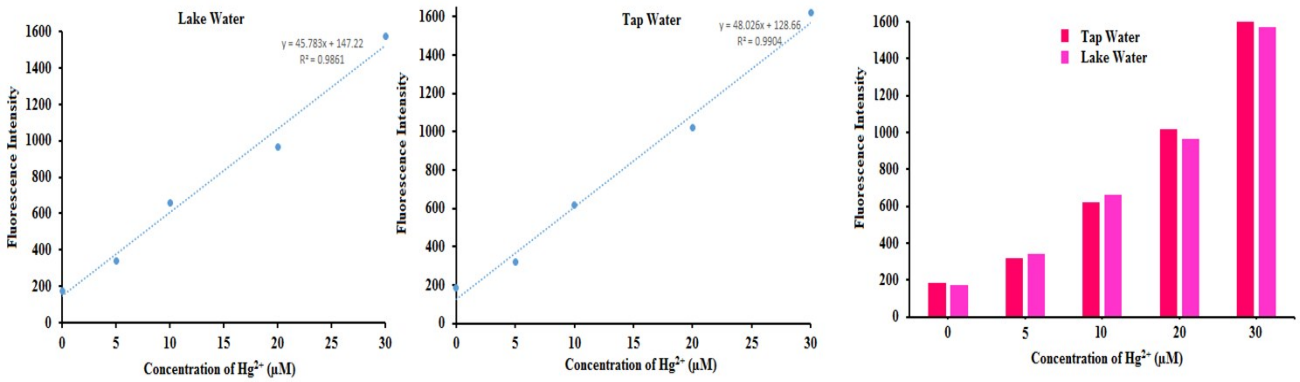


Fig. S13. Real sample analysis of Hg^{2+} in tap and lake water

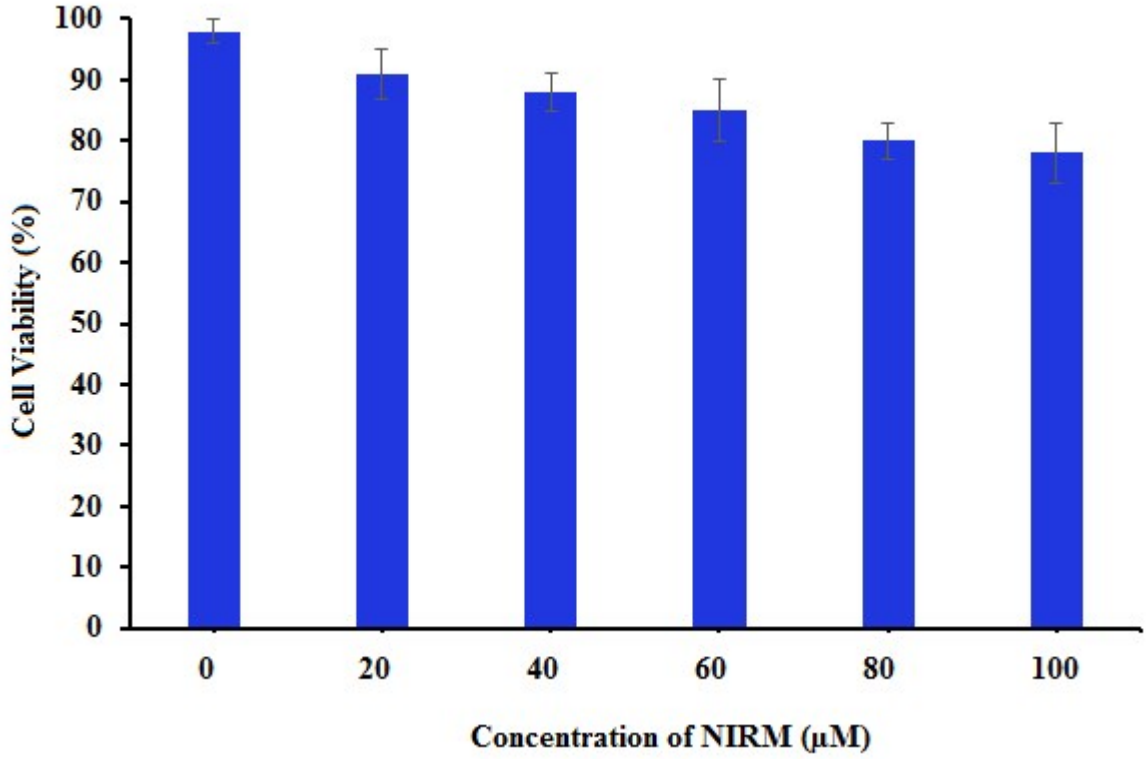


Fig. S14. MTT assay of probe NIRM.

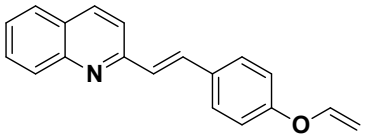
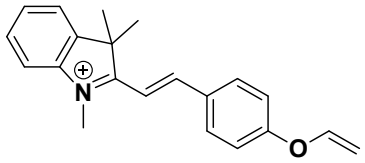
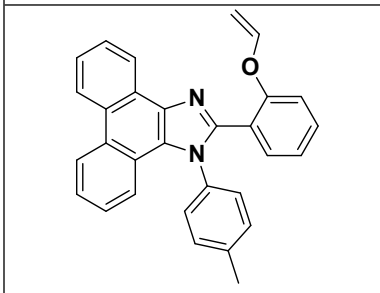
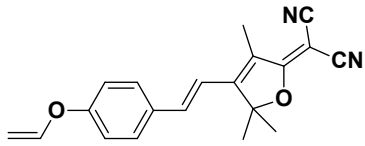
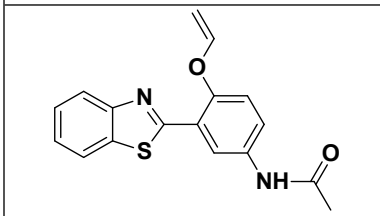
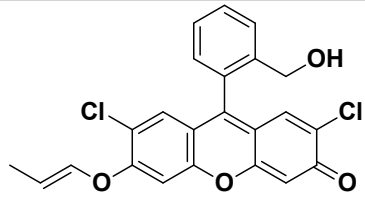
Table S1. Determination of Hg^{2+} in real samples.

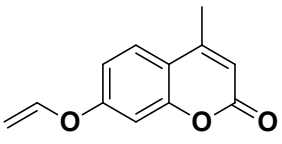
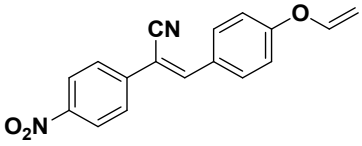
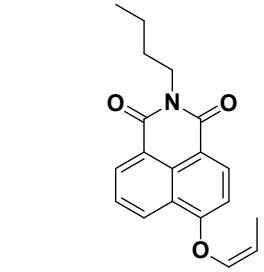
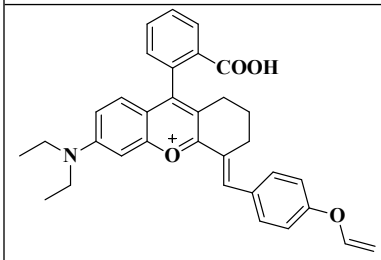
Sample	Spiked amount of Hg^{2+} (μM)	Found Amount of Hg^{2+} Mean[a] ±SD[b] (μM)	Recovery (%)
Tap Water 1	0	-	-
2	5	4.75 ± 0.03	95
3	10	9.83 ±0.08	98.3
4	20	19.81 ±0.06	99.05
5	30	29.63 ±0.05	98.7
Lake Water 1	0	-	-
2	5	4.85 ±0.1	97
3	10	9.89 ±0.05	98.9
4	20	19.91 ±0.08	99.5
5	30	29.92 ±0.12	99.7

[a] Mean of five determination

[b]SD, standard deviation

Table S2. Comparison of present probe with reported probes based on the vinyl ether group.

Probe	LOD	Quantum yield	Response time	Stoke shift	Imaging application	Ref
	1×10^{-7} M	–	3 min	-	–	1
	-	0.018	1 min	-	HeLa Cells	2
	7.8 nM	-	60 min	120 nm	HeLa Cells	3
	0.008μM	-	40 min	-	HeLa Cells	4
	20 ppb	0.2	30 min	-	-	5
	20 nM	-	60 min	-	-	6

	0.12 μM	-	10 min	-	-	7
	37 nM	-	-	-	-	8
	0.045 μM	-	30 min	-	-	9
	3.2 nM		10 min	>110 nm	HeLa	THIS WORK

References

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