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Supplementary Information

An α-naphtholphthalein-derived colorimetric fluorescent chemoprobe for the portable and visualized monitoring of Hg^{2+} by the hydrolysis mechanism

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Experiment section

Chemicals and Materials

All reagents and solvents were used as received and without any further purification. α-Naphtholphthalein were purchased from Huaxia Reagent, China. Other solvents such as acetic acid (AcOH), methanol (MeOH), dimethyl sulfoxide (DMSO), Diaminomaleonltrile, hexamethylenetetramine, were rendered from Aladdin, China. MTT was purchased from Beyotime, China. Mercury(II) perchlorate trihydrate ($Hg(ClO_4)_2 \cdot 3H_2O$), zinc ($ZnCl_2$), magnesium ($MgCl_2$), calcium ($CaCl_2 \cdot 2H_2O$), cobalt ($Co(NO_3)_2 \cdot 6H_2O$), iron ($FeCl_2 \cdot 4H_2O$), nickel ($NiCl_2 \cdot 6H_2O$), stannum ($SnCl_2 \cdot 2H_2O$), cadmium ($Cd(NO_3)_2 \cdot 4H_2O$), lithium (LiCl), sodium ($NaNO_3$), lead ($Pb(NO_3)_2$), iron ($MnCl_2 \cdot 4H_2O$), vanadium ($VOSO_4 \cdot xH_2O$) and copper ($CuCl_2 \cdot 2H_2O$) salts purchased from Accustandard (J.K. Chemicals and Industries, China). Milli-Q (Millipore Corp., Bedford, MA, USA) double distilled water was used for the whole experimental works. Phosphate buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), penicillin, fetal bovine serum (FBS), Penicillin-Streptomycin Solution and other cell culture relevant chemicals were received from Hyclone (USA).

Instruments

^{1}H NMR and ^{13}C NMR spectra were collected by a Bruker ARX 400 MHz spectrometer. High-resolution mass spectra (HRMS) were measured on a GCT Premier CAB 048 mass spectrometer operating in MALDI-TOF mode. UV-vis absorption spectra were recorded on a Rarian 50 Conc UV-Vis spectrophotometer. Fluorescence emission spectra were recorded on Edinburgh FS5 fluorescence spectrophotometer. The pH values were recorded by desktop pH meter on METTLER, Switzerland S220-B. The RGB value was picked using the smartphone with the App (ColorAssist 2.4, FTL app, Inc). Fluorescence imaging experiments were operated with confocal laser scanning microscope (LSM 900 with Airyscan2, Zeiss, Germany).

Synthesis of NS

A mixed solution of α-Naphtholphthalein (2.0944 g, 5.01 mmol) and hexamethylenetetramine (HMTA) (2.1148 g, 15.09 mmol) in trifluoroacetic acid (TFA) (40 mL) was refluxed for 6 hours (TLC monitoring). After the reaction solution was cooled to room temperature, the HCl solution (100 mL, 1 mol/L) was added, vigorously stirring for half an hour, and then extracted with dichloromethane (100

mL). The organic layer was washed with water thrice and saturated brine once, and was dried over sodium sulfate. With the removal of dichloromethane, the orange solid was obtained. The final product was purified by column chromatography with a mixture of dichloromethane/ethanol (100/1, *v/v*), NS: Yield 68 %; HRMS, m/z: calcd. for $C_{30}H_{18}O_6$ 474.11034, found 474.11094.

Synthesis of PM

Mixed solution of methanol and dichloromethane (1/1, *v/v*) of intermediate NS (0.4934 g, 1.04 mmol) was added dropwise to a solution of diaminomaleonitrile (DAMN)(0.2697 g, 2.49 mmol) in 10 mL of methanol in the presence of one drop concentrated sulfuric acid (Scheme 2). After the reaction was refluxed and stirred for 6 h, the brown-colored product was precipitated. The obtained product was filtered, washed with methanol and dichloromethane, and then dried in vacuum. Yield: 78%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.41 (t, 4H, NH_2, J = 8 Hz), 7.50 (t, 2H, Phen-H, J = 8 Hz), 7.73 (t, 2H, Phen-H, J = 8 Hz), 7.82-7.89 (m, 4H, Phen-H), 7.98-8.12 (m, 4H, Phen-H), 8.36 (d, 2H, Phen-H, J = 8 Hz), 8.57(s, 2H, NCH), 12.25(s, 2H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): 163.65, 153.05, 152.60, 150.83, 148.70, 148.62, 135.71, 134.26, 131.93, 128.90, 127.90, 127.42, 123.79, 123.12, 121.05, 113.97, 111.13, 110.43, 108.26, 104.21, 97.47, 43.51, 12.22; HRMS, m/z: calcd. for $C_{38}H_{22}N_8O_4$ 654.17640, found 654.17774.

Quantum yield measurement

Quinine sulphate (Φ_s = 0.54, 0.1 M H_2SO_4) was used as standard reference to determine the fluorescence quantum yield of **PM** and NS. The quantum yields of these three compounds in THF/H_2O (1:1, *v/v*, 10 mM, HEPES, pH 7.4) were calculated by the following equation：

$$\Phi_x = \Phi_s(A_sF_x / A_xF_s)(n_x^2/n_s^2)$$

where, A_x and A_s are the absorbance of the sample and the reference, respectively. F_x and F_s are the corresponding relative integrated fluorescence intensities at the excitation wavelength (350 nm) respectively, and n is the refractive index of the solvent. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

General UV–vis and fluorescence spectra studies

A stock solution of **PM** ($2x10^{-3}$ M) was prepared in DMSO. Then, it was diluted to 10 μM for UV-vis and fluorescence studies with a mixture of THF/H_2O (1:1, *v/v*, 10 mM, HEPES, pH 7.4) at 25 °C. The solutions of metal cations (e. g. Zn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Ag^{+}, Ni^{2+}, Sn^{2+}, Cd^{2+}, Li^{+}, Na^{+}, Pb^{2+}, Mn^{2+}, VO^{2+}, Cu^{2+}, Hg^{2+}) were prepared in deionized water. Fluorescence tests were performed by seperately adding the appropriate amount of metal cation to 3 mL of a **PM** solution using micropipette.

Limit of detection calculation (LOD)

Based on the linear fitting in Figure 2B, the limit of detection (LOD) was calculated using the formula of 3σ/slope, where σ is the standard deviation of the fluorescent intensity of **PM** without metal ion and measured 10 times under the same condition. The corresponding slope was obtained after linear fitting the titration curves in Figure 2b.

Theoretical calculations

Theoretical studies were performed using Gaussian 09[1] supported by GaussView 5.0[2]. Molecular orbitals (HOMOs/ LUMOs) and energy levels of **PM** and NS were calculated by B3LYP/6-31G(d) functions of DFT method.

RGB analysis for Hg^{2+}detection[3]

Here we tried to develop a portable smartphone-assisted intelligent platform and selected the smartphone iPhone XR containing the App *ColorAssist* to obtain RGB values. Firstly, the silica dishes were fitted with 5 μM **PM** solution and then were added with the different Hg^{2+} concentration (0 μM, 0.5 μM, 1.0 μM, 2.0 μM, 3.0 μM, 4.0 μM, 5.0 μM, 6.0 μM, 7.0 μM, 8.0 μM, 9.0 μM, 10 μM) respectively. The smartphone camera was placed in front of each sample under the fixed distance of 20 cm. In the process of each measurement, the test samples should be placed directly below a portable 365 nm UV lamp to ensure the perpendicular light for every sample, which was beneficial for the proportional relationship between the Hg^{2+} concentration and RGB values. At last, the RGB values of each test sample were measured by the APP *ColorAssist*, and they were transmitted to a computer for determining relative linear relationships by use of Origin (version: OriginPro 2021).

Detection of real water samples

Water samples were collected from different sources, such as i) river water (from Dasha river near Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences), ii) bay water (from the Shenzhen Bay, GuangDong, China) and iii) tap water (from the lab). Before experiments, each of the raw water samples was filtered through 0.45 μm filter paper to remove the suspended particle and then each of the samples were intoxicated with standard Hg^{2+} solutions to prepare the Hg^{2+} spiked real water samples. After the sample preparation, 10 μM of **PM** in THF/H_2O (1:1, *v/v*, 10 mM, HEPES, pH 7.4) was treated with those water specimens and followed by their fluorescence spectra were recorded. The recovery study has also been carried out for the assessment of the reliability of **PM**.

Preparation of reduced newborn-calf serum

New born-calf serum samples were purchased from Hyclone (USA) and stored at -20ºC before use. A newborn-calf serum solution obtained from a commercial source was added with excess triphenylphosphine(TPP) to reduce disulfides to free thiols in the presence of sulfosalicylic acid. Aliquots of the newborn-calf serum solution after treatment with HEPES buffer (10 mM, pH 7.0) were then added directly to the **PM**-Hg^{2+} system (3 mL).

Cytotoxicity assay

The viability was determined by using MTT assay which is based on the reduction of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, yellow in color) into formazan (blue color) by mitochondrial succinate dehydrogenase. Dispense 100 μL of Hela cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 h at 37 °C in a humidified incubator with 5% CO_2. Add 10 μL of various concentrations of **PM** (5 μM, 10 μM, 20 μM, 30 μM, 50 μM, 70 μM, 100 μM) into the culture media in the plate. After incubating the plate for 24 h in the incubator, the cell medium was exchanged with fresh medium (100 μL), and then 20 μL of the MTT (5 mg/mL) solution was then added. Medium was removed after the incubation period of 4 hours followed by the addition of 100 μL of DMSO to dissolve the formazan crystals. Absorbance was taken at 600 nm by an ELISA Plate Reader (Biotek Synergy HT). Untreated cells were taken as control. All the experiments were performed in triplicate. Cell viability was determined by using given formula: Cell viability (%) = *Absorbance of treated cells / Absorbance of untreated cells*. The experimental steps of

cell viability of HeLa cells at different concentrations of NS are the similar as above.

Cell culture and cell imaging

HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) contained with 10% fetal bovine serum (FBS) and 5% Penicillin-Streptomycin Solution in a 37 ˚C humidified incubator environment with 5% CO_2. The fluorescence bioimaging of **PM** in HeLa cells was carried out as follows: A group of living cells was divided into three groups and seeded in 8-well flat-bottomed plates for 24 h at 37 ˚C under 5% CO_2. The one group of Hela cells were incubated only with probe **PM** (10 μM) for 20 mins and washed with PBS buffer (pH = 7.4) three times, and then imaged as control. The other two groups of cells were initially incubated with probe **PM** (10 μM), and then with Hg^{2+} (10 μM, 30 μM) for 30 mins. In all cases, HeLa cells were imaged after washing the cells with PBS buffer and images were recorded using confocal laser scanning microscope (LSM 900 with Airyscan2, Zeiss, Germany). The experimental steps of confocal fluorescence imaging analysis of resistance to Cys/GSH (20, 100 μM) of **PM** (10 μM) for Hg^{2+} (20 μM) detection in living HeLa cells are the similar as above.

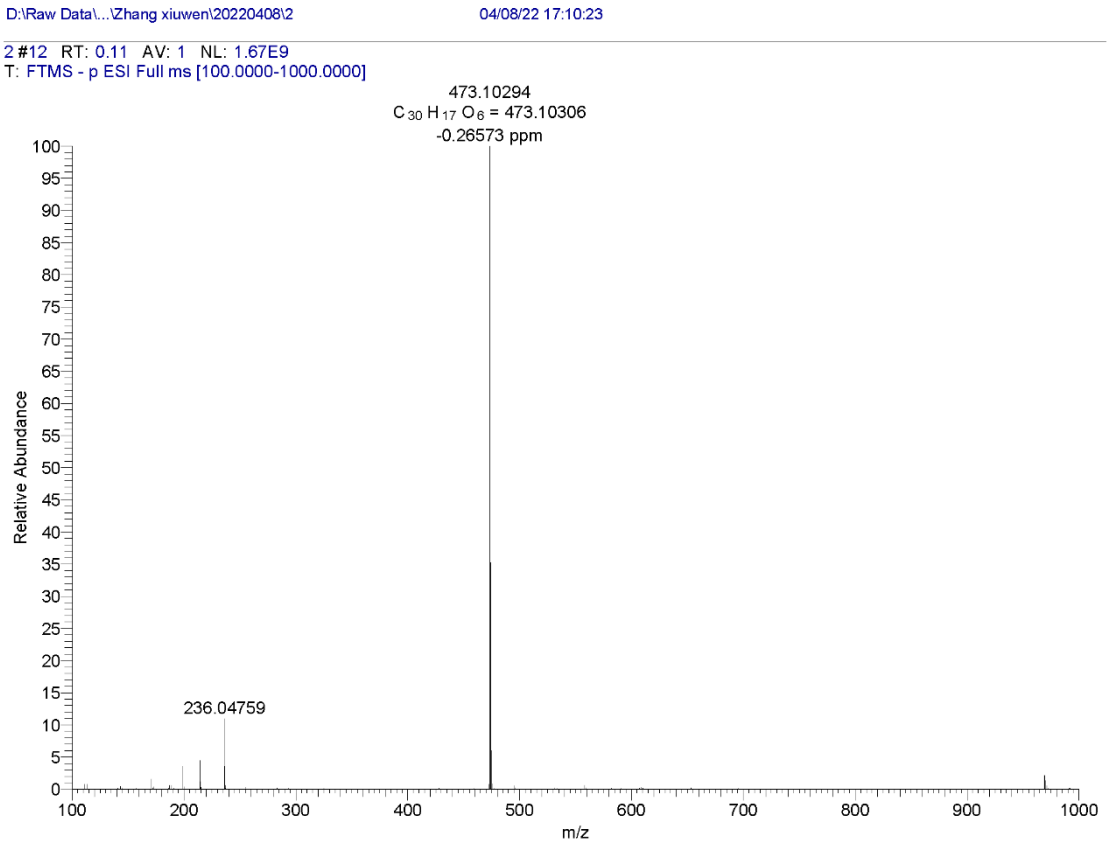


Fig. S1. HRMS spectrum of NS.

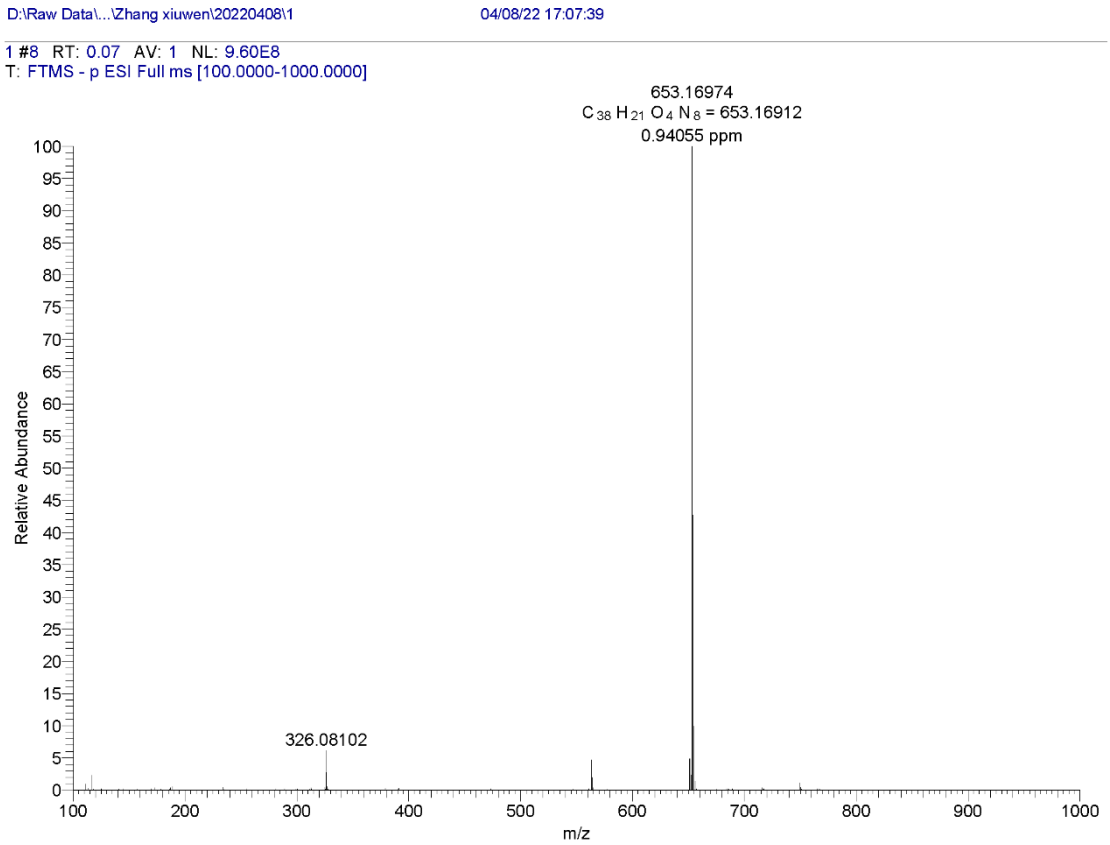


Fig. S2. HRMS spectrum of **PM.**

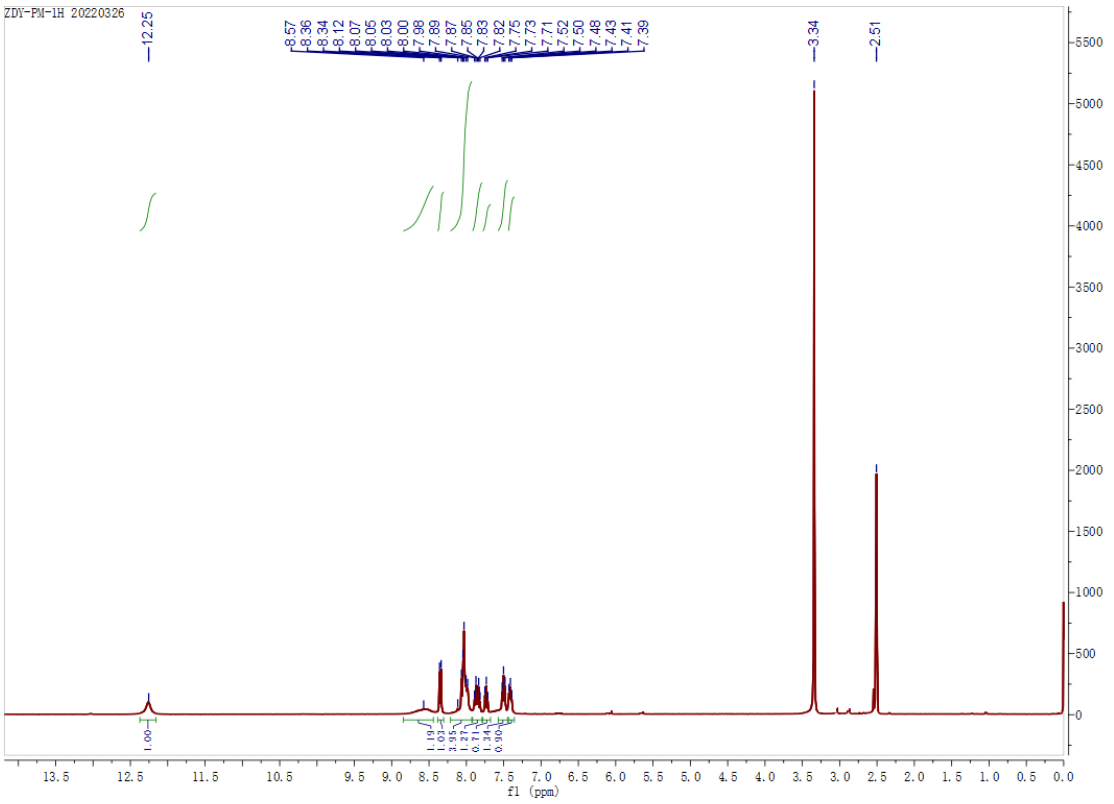


Fig. S3. ^{1}H NMR spectrum of **PM** in DMSO-*d*$_{6}$.

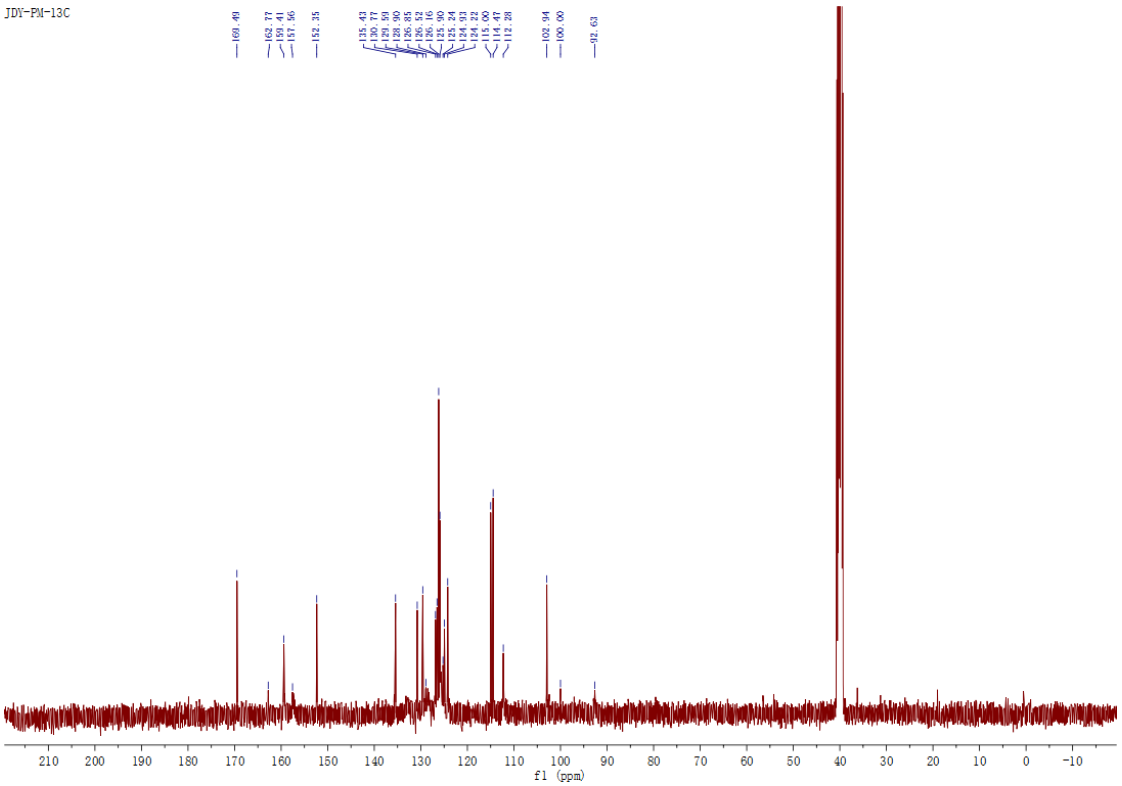


Fig. S4. ^{13}C NMR spectrum of **PM** in d_6-DMSO.

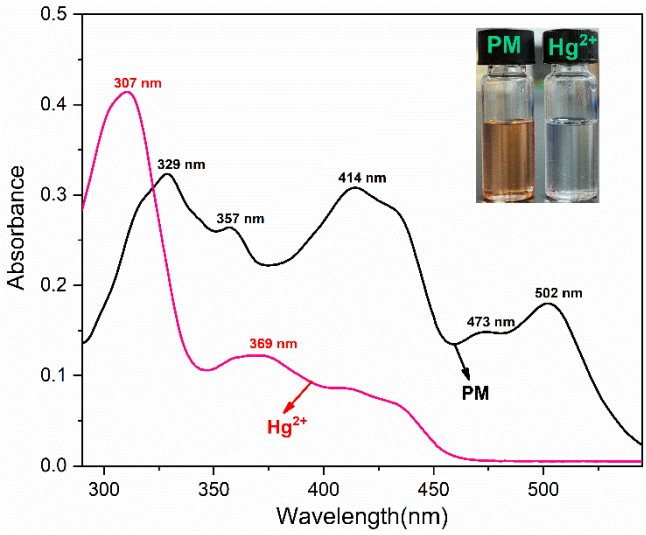


Fig. S5. Absorption spectra of **PM** and **PM**-Hg^{2+} in DMSO/H_2O mixture (Inset: colorimetric pictures of **PM** and **PM**-Hg^{2+} solutions under daylight).

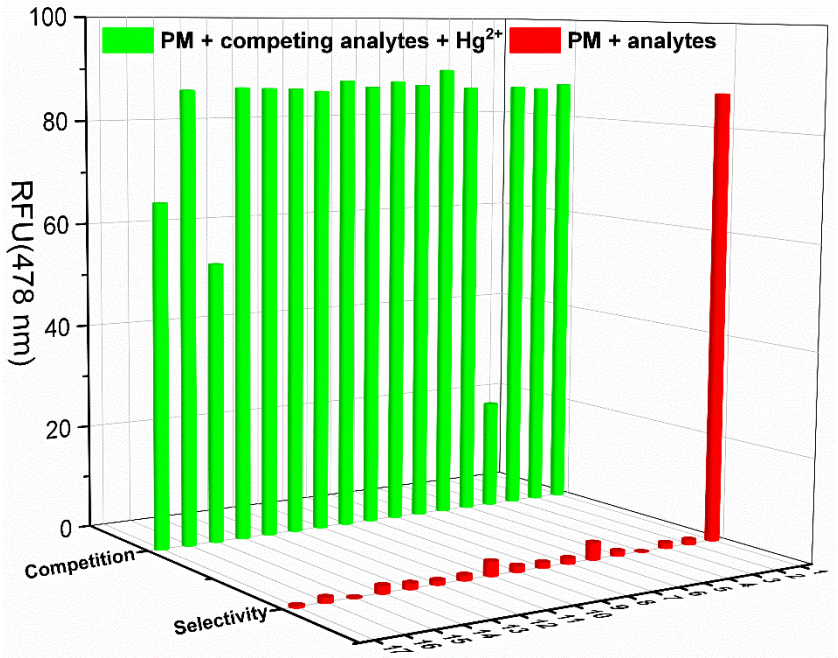


Fig. S6. Relative fluorescence intensity change of **PM** (10 μM) with Hg^{2+}(100 μM) in the presence of competing analytes; (1. Hg^{2+} 2. Zn^{2+} 3. Mg^{2+} 4. Cu^{2+} 5. Co^{2+} 6. Sn^{2+} 7. Ni^{2+} 8. Cd^{2+} 9. Li^{+} 10. Na^{+} 11. VO^{2+} 12. Mn^{2+} 13. Pb^{2+} 14. Ca^{2} 15. Fe^{2+} 16. Ag^{+} 17. Fe^{3+}, 300 μM).

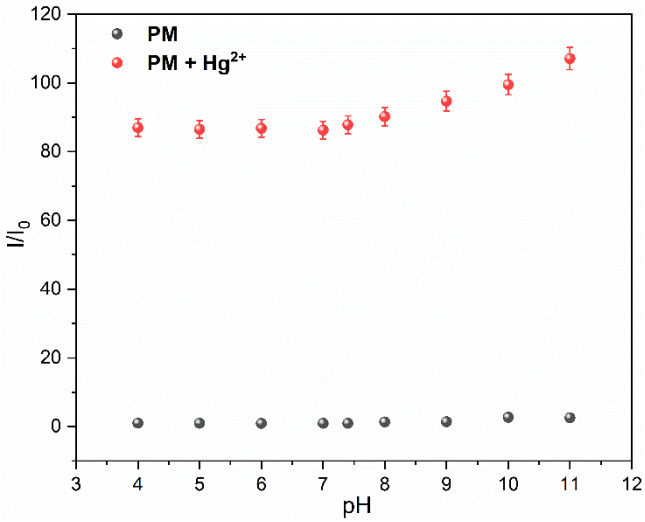


Fig. S7. The changes in intensity at 478 nm of **PM** (10 μM) and **PM** with 10 equiv of Hg^{2+} at different pH value.

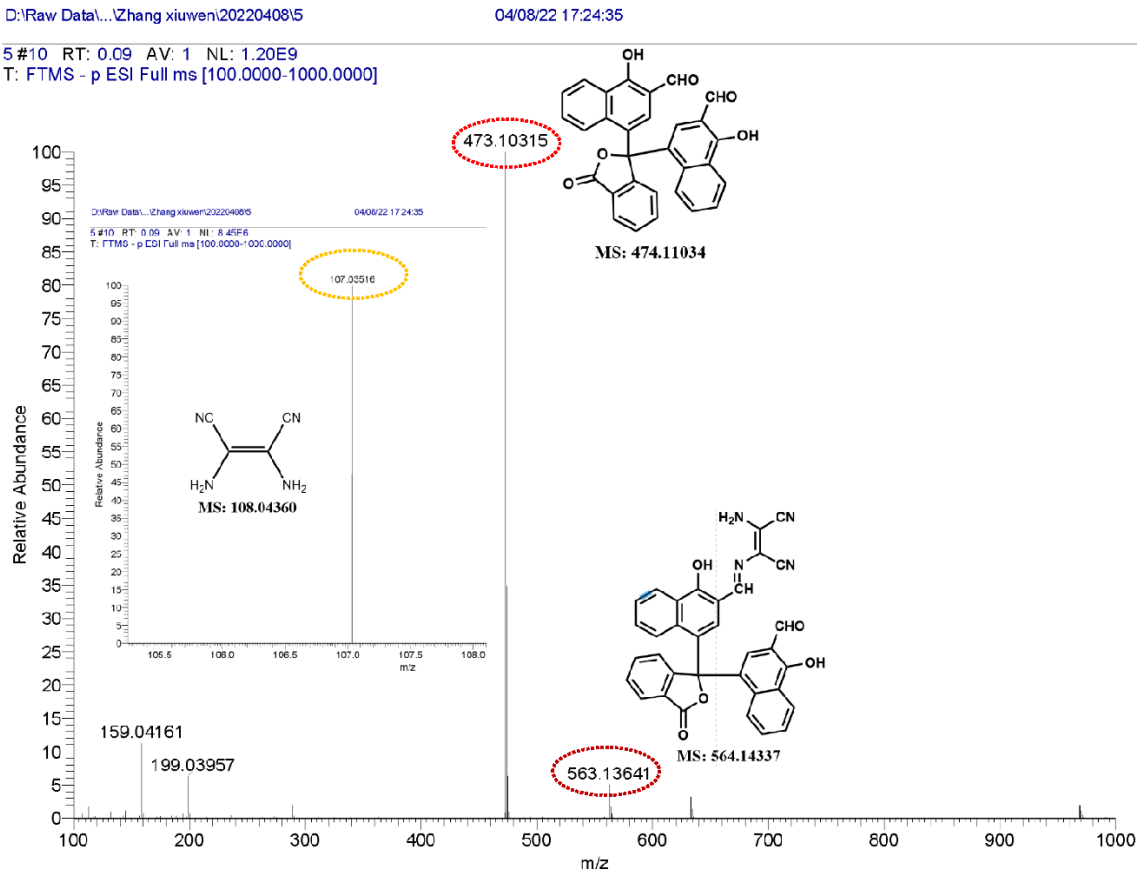


Fig. S8. HRMS spectrum of **PM** in the presence of 2 equiv. of Hg^{2+}.

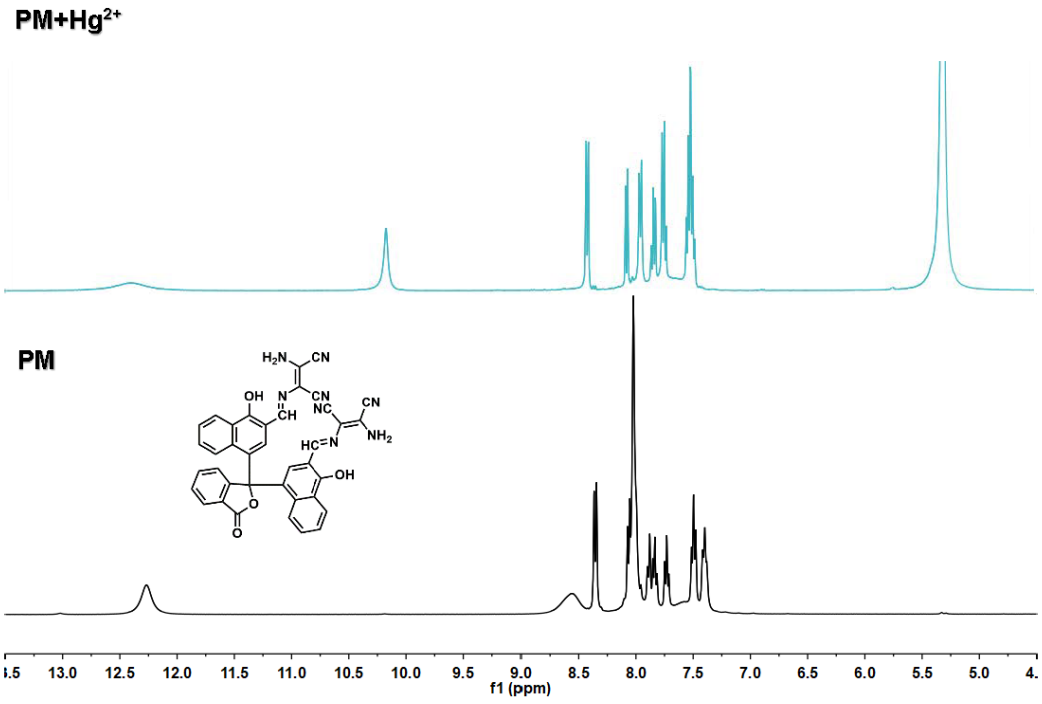


Fig. S9. ^{1}H NMR spectra of the probe **PM** with Hg^{2+} ions in DMSO-d_6.

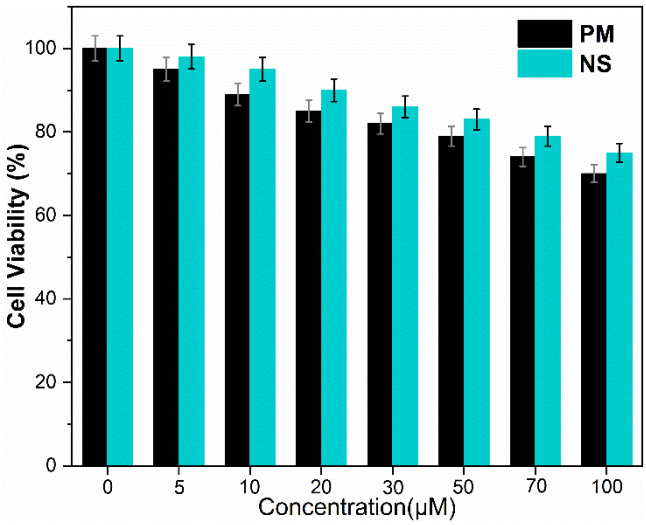
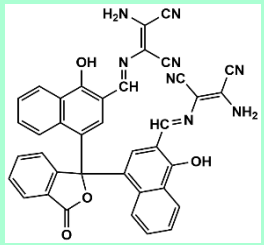
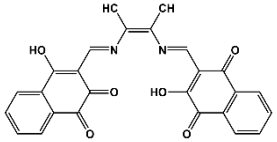
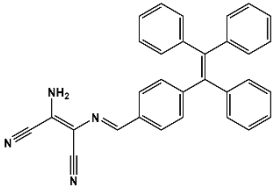
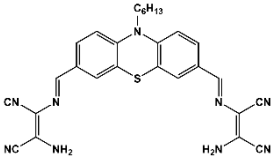
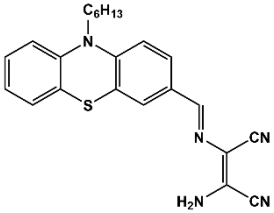
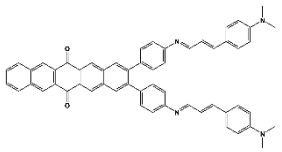
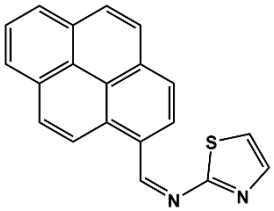
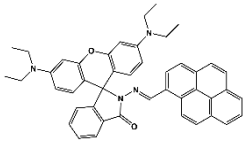
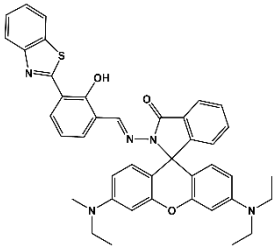
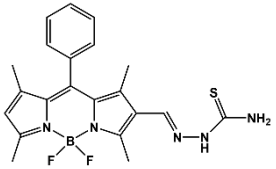


Fig. S10. Cell viability of HeLa cells at different concentrations (0, 5, 10, 20, 30, 50, 70, 100 μM) of **PM** (black bar) and NS (cyan bar) by using MTT method. The data is shown as the mean ± SD, n = 3.

Table S1. The comparison of analytical performance of this work with other previous work about the detection of Hg^{2+}.

Chemoprobes	Analyte	$\lambda_{ex}/\lambda_{em}$ (nm)	Stokes shift (nm)	Detection mode	Sensing mechanism	Response time	LOD (nm)	Analytical applications	Imaging application	Ref.
	Hg^{2+}	380/478	98	colorimetric/ fluorimetric	hydrolysis	30 min	20.0	Portable analysis/ Real water	living cells	This work
	Hg^{2+}	/	/	colorimetric	coordination	30 min	110	Paper strip	/	4
	Hg^{2+}	394/600	206	colorimetric/ fluorimetric	coordination	/	19.4	/	living cells	5
	Hg^{2+}	450/575	125	colorimetric/ fluorimetric	coordination	/	35	/	living cells	6
	Hg^{2+}	425/550	75	colorimetric/ fluorimetric	coordination	/	17.8	/	living cells	7

	Hg^{2+}	380/462	182	fluorimetric	hydrolysis	5 min	2500	/	living cells	8
	Hg^{2+}	355/417	62	colorimetric/ fluorimetric	hydrolysis	/	270	Real water	/	9
	Hg^{2+}	365/452 515/576	87 61	fluorimetric	hydrolysis	40 min	19100	Real water	/	10
	Hg^{2+}	360/535	175	fluorimetric	coordination	/	15	/	living cells	11
	Hg^{2+}	485/510	125	colorimetric/ fluorimetric	coordination	72 s	370	/	/	12

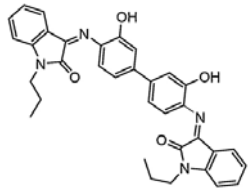
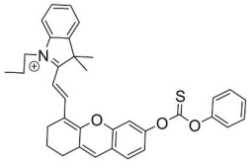
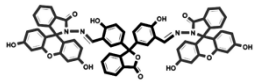
	Hg^{2+}	/	/	colorimetric	coordination	60 min	3930	Paper strip	/	13
	Hg^{2+}	588/708	120	colorimetric	coordination	3 min	180	/	living cells/ zebrafish	14
	Hg^{2+}	350/520	170	fluorimetric	combination	10 s	1160	Real water	/	15

Table S2. Detection of Hg^{2+} in actual water samples by the portable detection platform (RGB analysis).

Sample	Add (μM)	Detect (μM)	Recovery rate (%)	RSD (%) (n=3)
River Water[i]	0	No detected	-	-
	1.0	0.90	90.07	4.81
	3.0	2.80	93.21	2.83
	5.0	4.92	98.34	0.96
	10.0	10.38	103.78	1.76
Bay Water[ii]	0	No detected	-	-
	1.0	0.92	92.09	3.48
	3.0	2.83	94.28	2.75
	5.0	4.85	97.01	2.34
	10.0	9.82	98.22	1.35
Tap Water[iii]	0	No detected	-	-
	1.0	1.02	102.09	2.15
	3.0	2.96	98.67	1.02
	5.0	5.03	100.60	0.68
	10.0	10.10	101.03	0.93

i) River water was collected from Dasha river near Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences.
ii) Bay water was collected from the Shenzhen Bay, GuangDong, China.
iii) Tap water was collected from our lab.

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[6] K.M. Vengaian, C.D. Britto, K. Sekar, G. Sivaramanb, S. Singaravadivel, Fluorescence “on-off-on”

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