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Supporting Information

A novel water-soluble naphthalimide-based turn-on fluorescent probe for mercury ion detection in living cells and zebrafish

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1. Materials and instrumentations

All the chemicals were from commercial suppliers and used without further purified. ^{1}H NMR spectra was recorded by Bruker AV-400 spectrometer with chemical shift served as ppm (The probe dissolved in DMSO-d_6, TMS as internal standard). High resolution mass spectra (HRMS) was recorded by using an LC-MS 2010A (Shimadzu) instrument. Fluorescence emission spectra was obtained on a Horiba FluoroMax-4 spectrophotometer with excitation wavelength of 400 nm. The slit width was 5.0 nm for both excitation and emission. The respond fluorescence images of cells to Hg^{2+} were obtained on an Olympus FV1000-IX81 confocal fluorescence microscope.

2. Determination of the detection limit of NIML

The detection limit was calculated based on the fluorescence titration. The detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

$$\sigma = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{n-1}}$$

Where σ is the standard deviation of blank measurement, $\bar{x}$ is the mean of the blank measures, x_i is the value of blank measures, n is the number of tested blank measures (n = 5), and k is the slope between the fluorescence intensity ratios versus the concentrations of Hg^{2+}.

3. General method

The fluorescence data of all these probes were recorded in the aqueous solution (HEPES, 5 mM, pH = 7.4). Unless otherwise stated, the emission of probe **NIML** at 540 nm was measured after adding Hg^{2+} in solution for 6-7 min at room temperature,

and slit widths: $d_{ex} = d_{em} = 5$ nm

4. Effect of pH on probe NIML performance

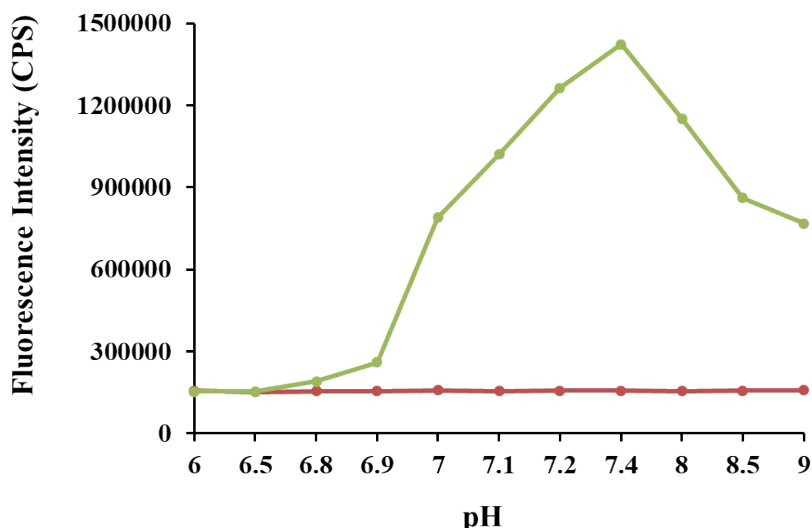

Figure S1. Recognition of Hg^{2+} (7 μM) by **NIML** (10 μM) at different pH conditions in the aqueous solution (10 mM HEPES, pH = 7.4) at 25 °C. $\lambda_{ex} = 400$ nm, $\lambda_{em} = 540$ nm.

5. Cytotoxicity assays

The cell viability of HeLa cells, treated with probe **NIML**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan) assay. Briefly, HeLa cells, seeded at a density of 1 × 106 cells·mL^{-1} on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 12 h. Then the live HeLa cells were incubated with various concentrations (0, 5,10 and 20 μM) of probe **NIML** suspended in culture medium for 4 h. Subsequently, CCK-8 solution was added into each well for 2 h.

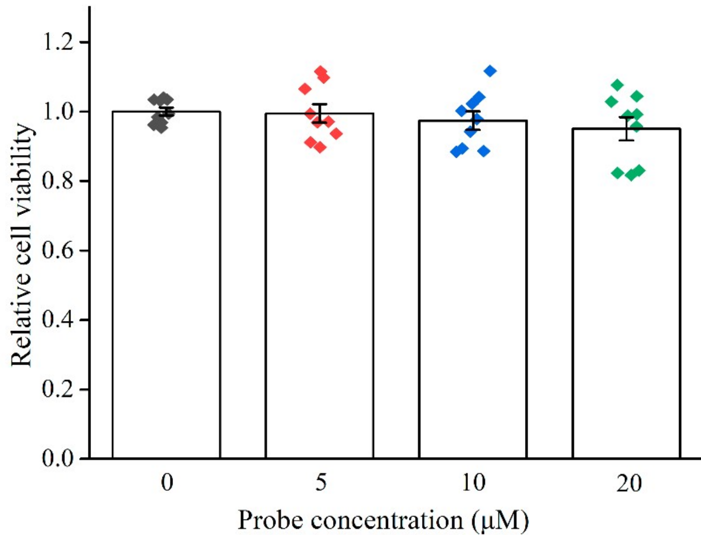

Figure S2. Cell viability with different concentration probe **NIML**.

6. Culture of living cells

The HeLa cells were cultured in high-glucose (4.5 g/L) Dulbecco's modified Eagle's medium (DMEM) and incubated under a humidified atmosphere containing 5% CO_2 at 37 °C for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin-streptomycin.

Blank group of cells (HeLa) were incubated with **NIML** (20 μM) for 20 min, and then washed with culture water for three times before fluorescence bioimaging by confocal fluorescence microscope; control group of cells were incubated with **NIML** (20 μM) for 20 min, third group of cells were incubated with **NIML** (20 μM) for 20 min, then treated with Hg^{2+} (20 μM) for 20 minutes before fluorescence bioimaging.

7. zebrafish imaging experiments

Healthy male and female zebrafishs (AB stain) were maintained in different tanks with a 14 h light / 10 h dark cycle at 28 °C. Then, sexually mature zebrafish were

selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation in the morning. After sterilizing and cleaning, the fertilized eggs were added to zebrafish embryo culture water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator at 28 °C.

8. HRMS proofs for recognition mechanism of NIML

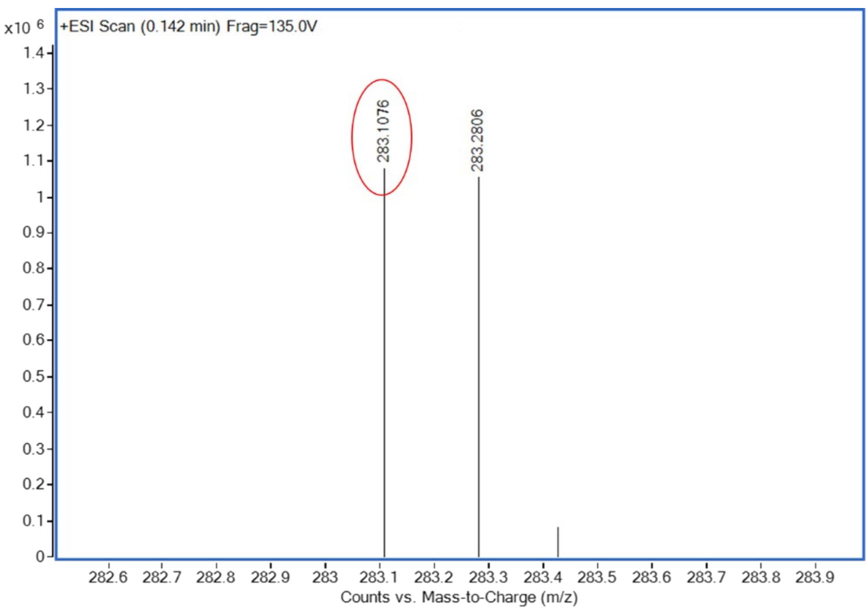

Fig. S3. HRMS data of probe **NIML**.

9. ^{1}H NMR and ^{13}C NMR Spectrums of NIML

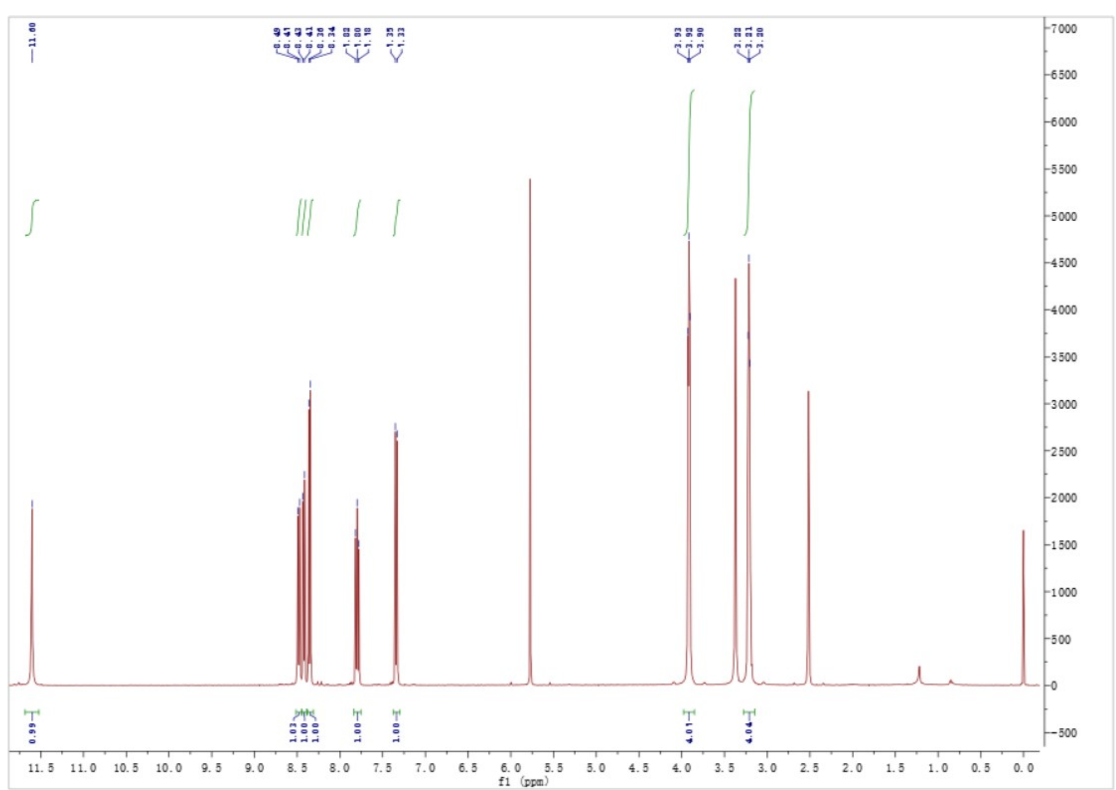

Fig. S4. ^{1}H NMR Spectrum of **NIML**

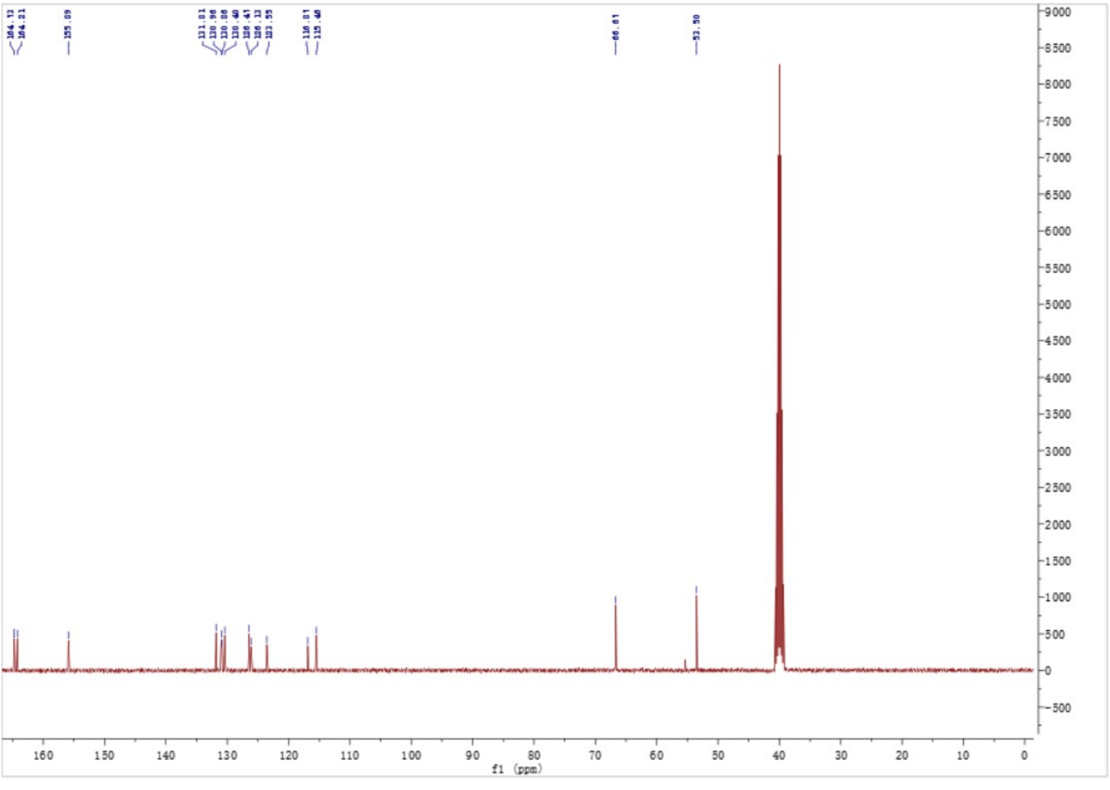

Fig. S5. ^{13}C NMR Spectrum of **NIML**

10. The contrast of fluorescence intensity between the probe NIML and control

structure

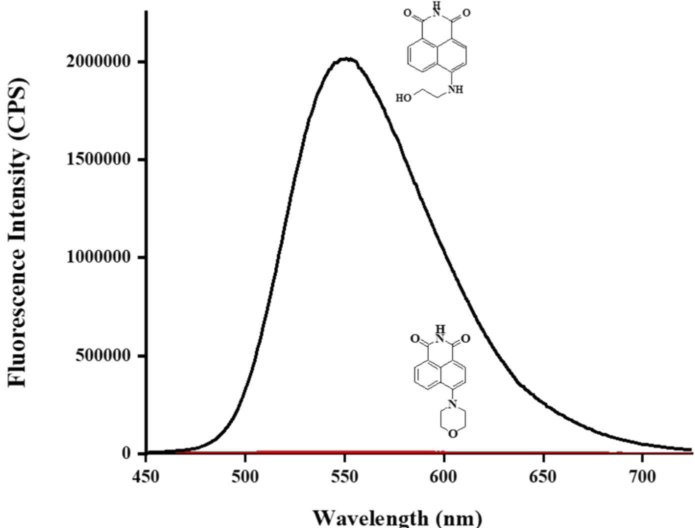

Figure S6. The contrast of fluorescence intensity between the probe **NIML** (5 μM) and control structure (5 μM). Black line: control structure, red line: probe **NIML**. Slit: 3 nm

11. Comparison of fluorescent probes for Hg^{2+}

Table S1. Comparison of fluorescent probes for Hg^{2+}

Probe structures	Probe type	Stokes shift (nm)	Detection limit	Solvent system	Application	References
	Turn off	83	0.83μM	CH_3CN/H_2O, 4/1	Cell imaging, water samples	*Talanta*, 2019, **200**, 494-502.
	Turn on	57	5.6 nM	CH_3OH/H_2O, 1:99	-	*J. Photoch. Photobio. A*, 2019, **378**, 85-93.
	Turn on	96	40 nM	$EtOH/H_2O$, 2:8,	Cell imaging	*ACS Omega*, 2020, **5**, 18176-18184.

	Ratiometric	93	14.7 nM	DMSO/H_2O, 1:99	Water samples	*J. Photoch. Photobio. A*, 2020, **391**, 112354.
	Turn on	90	14.7 nM	CH_3CN/H_2O, 4/1	Cell imaging	*J. Mol. Liq.*, 2022, **348**, 118448.
	Turn on	140	8.8 nM	Aqueous solution	Cell imaging, zebrafish imaging, water samples	This work

12. The statement of live subjects (zebrafish)

All experiments were performed in compliance with relevant laws or guidelines, and all experiments followed institutional guidelines. Use and handling of animals were strictly in accordance with the Institutional Animal Care and Use Committees of Qilu University of Technology whose guidelines were based on the US National Institutes of Health (NIH). Ethical approval was granted by the Institutional Animal Care and Use Committees of Qilu University of Technology.