Supporting Information

1, 8-Naphthalimide-based Fluorescent Sensor with High Selectivity and Sensitivity for Hg^{2+} in Aqueous Solution and Living Cells

Daying Liu, *[a,b] Hualing Zhu, [a] Jun Shi, [a] Xinxin Deng, [a] Tingting Zhang, [a] Ye Zhao, [a]

Pengpeng Qi, [a] Guangming Yang, [b] Huarui He *[c]

[a] College of Basic Science, Chemistry Experiment Teaching Center, Tianjin Agricultural University, Tianjin, China

[b] Department of Chemistry, Key Laboratory of Advanced Energy Materials Chemistry (Ministry of Education), Nankai University, Tianjin, China

[c] Heowns Biochem Technologies LLC, Tianjin, China

1. Materials, Measurement and Methods

Unless otherwise noted, all materials were obtained from Heowns Biochem Technologies LLC and were used without further purification. Flash chromatography was carried out on silica gel (300-400 mesh). ^{1}H NMR spectra were recorded using Varian 300 MHz. Fluorescence spectra were measured on the Gangdong F-280 fluorometer.

2. Synthesis

The compounds **1** were synthesized according to a previously reported procedure [1].

Synthesis of 2

A suspension of 0.5 g (0.96 mmol) of compound **1**, 0.2 g (1.05 mmol) of methyl 6-(chloromethyl)picolinate, 0.32 mL (1.92 mmol) N, N-diisopropylethylamine and 0.16 g (0.96 mmol) KI in DMF (10 mL) was heated at 90°C for 18h under nitrogen atmosphere. The progress was monitored by TLC (PE: EA=1:2). After the reaction was complete, the mixture was cooled and poured into water. The resultant precipitate was filtered, dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4, filtered and evaporated to get crude product, which was purified by flash column chromatography, to

* Correspondence author. *E-mail address*: liudaying03@163.com (D. Y. Liu)

* Correspondence author. *E-mail address*: huarui.he@heowns.com (H. R. He)

afford product 85 mg. ^{1}H NMR ($CDCl_3$) δ 8.46 (dd, J = 7.3, 0.9 Hz), 8.36 (d, J = 8.4 Hz), 7.94 (dd, J = 7.7, 0.9 Hz), 7.81 (d, J = 8.4 Hz), 7.70 (t, J = 7.7 Hz), 7.46 (t, J = 7.8 Hz), 6.98 (d, J = 8.4 Hz), 6.65 (d, J = 8.5 Hz), 6.55 (d, J = 8.5 Hz), 5.31 (s), 5.26 (d, J = 4.9 Hz), 4.65 (s), 4.49 (s), 4.04 (q, J = 7.1 Hz), 3.95 (s), 3.52 (dd, J = 12.0, 6.6 Hz), 2.90 (t, J = 6.7 Hz), 1.97 (s), 1.47 (s), 1.20 (d, J = 7.1 Hz). MS (+ESI): Calc. for MH^+, 671.28; Found, 671.3； Calc. for MNa^+, 693.28; Found, 693.3.

Synthesis of 3

A suspension of 100 mg (149.09 μmol) of compound **2**, 66 μL (596.34 μmol) of ethyl bromoacetate, 0.2 mL (1.19 mmol) N, N-diisopropylethylamine and 99 mg (596.34 μmol) KI in DMF (1 mL) was heated at 110°C for 20h under nitrogen atmosphere. The progress was monitored by TLC (PE: EA=1:2). After the reaction was complete, the mixture was cooled and poured into water. The resultant precipitate was filtered, dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4, filtered and evaporated to get crude product, which was purified by flash column chromatography, to afford product 10 mg, which HPLC purity meets the requirement of Fluorescence Measurement. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 – 8.54 (m), 8.45 (d, J = 8.4 Hz), 8.04 – 7.99 (m), 7.89 (dd, J = 6.5, 1.7 Hz), 7.76 (t, J = 7.7 Hz), 7.65 – 7.51 (m), 7.09 (d, J = 8.6 Hz), 6.74 (d, J = 8.5 Hz), 6.59 (d, J = 8.7 Hz), 5.39 (s), 5.32 – 5.26 (m), 4.85 (s), 4.25 – 4.18 (m), 4.03 (s), 3.60 (dd, J = 12.1, 6.7 Hz), 2.98 (t, J = 6.7 Hz), 1.55 (s), 1.26 (d, J = 7.1 Hz). MS (+ESI): Calc. for MH^+, 757.32, Found, 757.4; Calc. for MNa^+, 779.3; Found, 779.2。

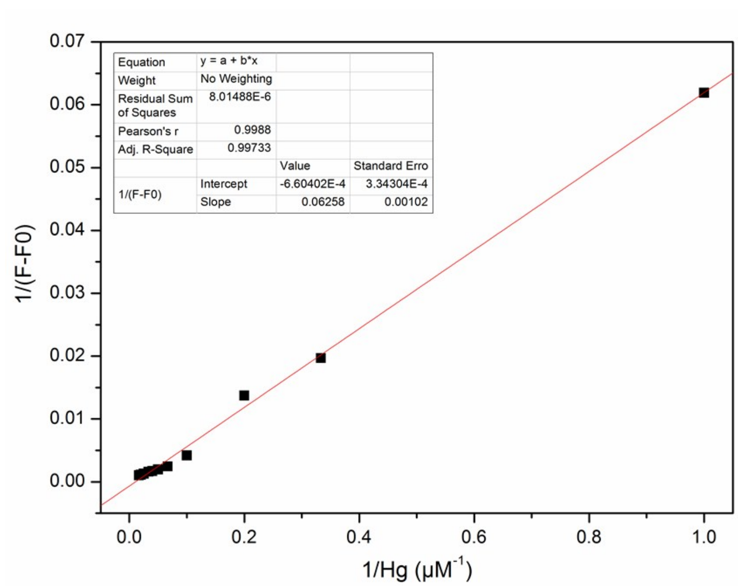
Synthesis of 4

0.5mL of trifluoroacetic acid (TFA) was added into a solution of 9 mg (11.89 μmol) of compound **3** in CH_2Cl_2 (3 mL). The resulting solution was stirred at room temperature for about 30 min when the TLC indicated that most of compound **3** were gone. The mixture was then diluted with 1:1 CH_2Cl_2:MeOH (10 mL) and the solvent was evaporated. The residue was dissolved in 10 ml methanol and evaporated again. This process was repeated for 6 times to remove TFA thoroughly, then placed on oil pump for 30 min to dry the product completely, afforded 9 mg yellow solid, which was used directly on to the next step without further purification.

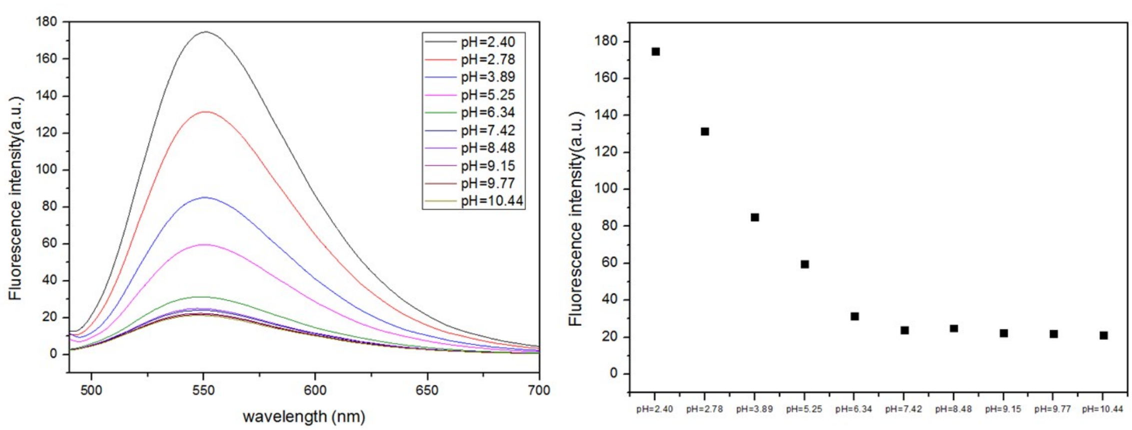
Synthesis of Sensor Hg: Hydrolysis of 4

To a solution of 9 mg (11.89 μmol) compound **4** in dry methanol (2 mL) was added NaOH (24 mg, 0.58 mmol) in 2 mL of water. The resulting solution was stirred at 40 °C for 8h. The progress was monitored by TLC (DCM: MeOH=10:1). After the reaction was complete, the mixture was cooled and used to do fluorescence response directly.

3. Benesi–Hildebrand plot (fluorescence intensity at 550 nm)

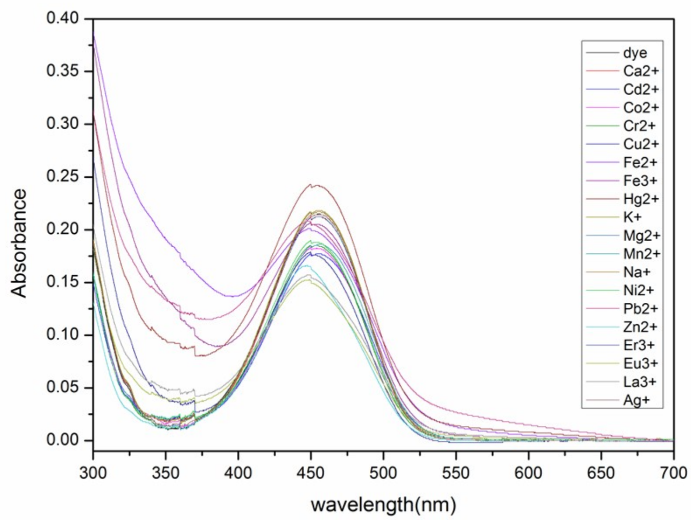


4. The pH values influence

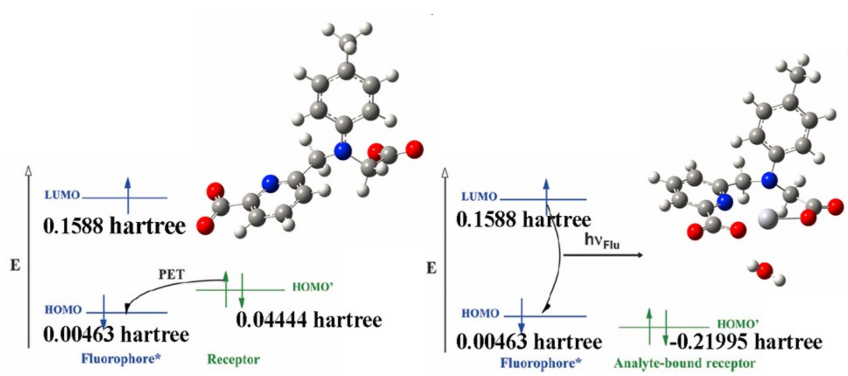


The pH value of the environment around the fluorescent probe usually shows somewhat of an effect on its performance toward target metal ion due to the protonation or deprotonation reaction for the fluorophore in the basic condition. The effects of pH on the fluorescence response of the new fluorescent sensor were therefore investigated. In the section of lower pH value from 6.0 to 2.0, the fluorescence intensity increased with decreasing pH value, which might be caused by the protonation of the sensor, and thus, its binding capability was lower with the metal ions. In a wide range of pH from 6.5 to 10.5, acidity does not affect the fluorescence intensity of the new fluorescent sensor. In other words, there is no need for strict control of the pH value of sample solution, which is convenient for practical applications of the proposed probe in actual water samples and living cells.

5. UV-vis titration of probe before and after addition of various metal ions

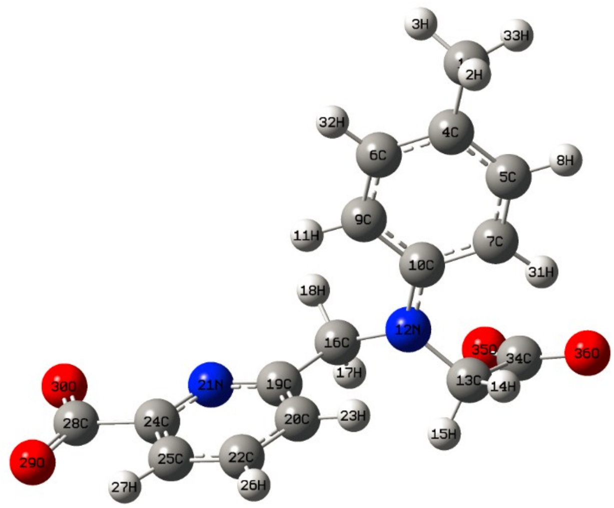


6. DFT calculations information about the bond distances and angles, the electron transformation between HOMO and LUMO orbit.



Structure of **Sensor-**Hg^{2+} complex estimated by density functional theory calculations

	HOMO orbit / hartree	LUMO orbit / hartree
receptor	0.04444	0.19022
Hg^{2+}	-1.19340	-0.75066
Hg^{2+} complex	-0.21995	-0.07540



Bond	length		Angle	Degree		Dihedral	Degree	
	Ligand	Hg complex		Ligand	Hg complex		Ligand	Hg complex
H2 -C1	1.102	1.095	H3 -C1-H2	106.692	107.885	C5- C4-C1-H3	155.445	-165.941
H3 -C1	1.097	1.097	C4 -C1-H 3	111.430	111.420	C7- C5-C4-C1	178.481	-179.043
C4 -C1	1.511	1.510	C5- C4-C1	121.661	121.585	H8- C5-C4-C1	-2.957	0.763
C5- C4	1.403	1.400	C6- C4-C1	121.703	121.711	C9- C6-C4-C1	-178.147	179.213
C6- C4	1.401	1.398	C7- C5-C4	122.598	122.138	C10- C9-C6-C4	-0.208	-0.021
C7- C5	1.390	1.393	H8- C5-C4	119.113	119.550	H11- C9-C6-C4	178.154	179.263
H8- C5	1.090	1.087	C9- C6-C4	122.215	121.783	N12- C10-C9-C6	178.465	178.698
C9- C6	1.391	1.395	C10- C9-C6	121.023	120.867	C13- N12-C10-C9	-171.150	168.040
C10- C9	1.418	1.405	H11- C9-C6	119.192	118.217	H14- C13-N12-C10	45.403	-51.188
H11- C9	1.084	1.083	N12- C10-C9	121.813	120.924	H15- C13-N12-C10	161.078	-169.164
N12- C10	1.376	1.437	C13- N12-C10	119.930	115.426	C16- N12-C10-C9	21.501	30.363
C13- N12	1.468	1.474	H14- C13-N12	108.824	107.607	H17- C16-N12-C10	142.584	155.728
H14- C13	1.096	1.095	H15- C13-N12	106.544	112.047	H18- C16-N12-C10	28.975	39.277
H15- C13	1.096	1.096	C16- N12-C10	121.130	115.243	C19- C16-N12-C10	-94.233	-84.648
C16- N12	1.458	1.471	H17- C16-N12	106.798	107.625	C20- C19-C16-N12	-11.386	152.865
H17- C16	1.095	1.095	H18- C16-N12	110.277	111.183	N21- C19-C16-N12	169.365	-28.990
H18- C16	1.095	1.099	C19- C16-N12	115.853	115.265	C22- C20-C19-C16	-178.503	178.263
C19- C16	1.529	1.528	C20- C19-C16	121.713	121.752	H23- C20-C19-C16	1.938	-2.314
C20- C19	1.398	1.397	N21- C19-C16	115.286	117.884	C24- N21-C19-C16	178.667	-179.622
N21- C19	1.346	1.335	C22- C20-C19	118.114	118.868	C25- C22-C20-C19	-0.148	0.742
C22- C20	1.397	1.396	H23- C20-C19	119.851	120.090	H26- C22-C20-C19	-179.988	179.995
H23- C20	1.085	1.086	C24- N21-C19	119.003	121.791	H27- C25-C22-C20	179.828	179.194
C24- N21	1.346	1.341	C25- C22-C20	119.063	119.719	C28- C24-N21-C19	179.447	-178.105
C25- C22	1.389	1.394	H26- C22-C20	120.117	119.959	O29- C28-C24-N21	176.824	4.858
H26- C22	1.090	1.086	H27- C25-C22	123.861	120.318	O30- C28-C24-N21	-3.472	-174.965
H27- C25	1.084	1.083	C28- C24-N21	120.539	118.389	H31- C7-C5-C4	175.364	179.523
C28- C24	1.568	1.301	O29- C28-C24	113.414	116.738	H32- C6-C4-C1	1.273	-0.975

Reference: (1) Daying Liu, Jing Qi, Huarui He et al., Anal. Methods, 2014, 6, 3555-3559.