Electronic Supplementary Information

A fluorescent probe with restricted intramolecular rotation-induced emission for label-free detection of mercury ions

Shengliang Li,[#,a,b] Hua Deng,[#,a] Weipeng Cao,[a] Chunqiu Zhang,[a] Shubin Jin,[a] Xiangdong Xue,[a] Jinchao Zhang,[c] Feng Li,[b] Guozhang Zou*[a] and Xing-Jie Liang*[a]

[a] *Laboratory of Nanomedicine and Nanosafety, Division of Nanomedicine and Nanobiology, National Center for Nanoscience and Technology of China, and CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Chinese Academy of Sciences, Beijing, 100190, China. Fax: +86-010-55 62656765; Tel: +86-010-82545630; E-mail: zougz@nanoctr.cn and liangxj@nanoctr.cn.*

[b] *Department of Neurobiology and Anatomy, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China*

[c] *College of Chemistry & Environmental Science, Chemical Biology Key Laboratory of Hebei Province, Hebei University, Baoding, China*

 # Contributed equally to this work

* Corresponding authors:

Prof. Guozhang Zou, Tel.: +86 10 82545530(O); fax: +86 10 62656765. E-mail addresses: zougz@nanoctr.cn.

Prof. Xing-Jie Liang Tel.: +86 10 82545569(O); fax: +86 10 62656765. E-mail addresses: liangxj@nanoctr.cn.

Experimental details

Materials and instruments

The oligonucleotides were synthesized by Beijing Sunbiotech Co. Ltd (Beijing, China). Ultrapure water (18.2 MΩ cm-1) produced by a Milli-Q system (Millipore Co., USA) was used in all the experiments. All chemicals used were of analytical grade. UV-Vis absorption spectra were measured with a Lambda 950 UV/Vis/NIR spectrophotometer (Perkin-Elmer, USA). The fluorescence spectra were obtained in a microcell with 1 cm path length using a Perkin-Elmer LS55 luminescence spectrometer. Circular dichroism spectra were determined using a J-810-CD spectropolarimeter (JASCO, Japan) equipped with a thermostated cell holder.

Synthesis and characterization of CWQ-11

The carbazole-based cyanine probe CWQ-11 was prepared as previously described with slight modifications.[1,2] Briefly, a catalytic amount of piperidine was added dropwise to a mixture of 1,4-dimethyl-pyridinium iodide (10 mmol), 9-pentyl-carbazolyl-3,6-dialdehyde (4 mmol) in ethanol (30 ml). The resulting mixture was allowed to reflux for 48 h and stirred overnight. The reactant was cooled to room temperature. After filtration and recrystallization using methanol, the desired product, a reddish-yellow solid, was obtained by vacuum drying. The chemical structure of CWQ-11 was confirmed by ^{1}H NMR, mass spectrometry and HPLC analysis. ^{1}H NMR (400 MHz, DMSO-d6, ppm): 0.83 (t, 3H, J = 6.6 Hz), 1.30 (m, 4H), 1.82 (m, 2H), 4.26 (s, 6 H), 4.49 (t, 2 H, J = 6.8 Hz), 7.54 (d, 1 H, J= 16.4 Hz), 7.80 (d, 2 H, J = 8.4 Hz), 7.96 (d, 2 H, J= 8.4 Hz), 8.21-8.25 (m, 6 H), 8.62 (s, 2 H), 8.83 (d, 4 H, J = 6.8 Hz).

CWQ-11/T_{33}-based sensor for Hg^{2+}.

For detection of mercury ions, concentrations of Hg^{2+} varying from 0 to 1 μM were added to obtain the limit of detection and metal ion response range of the CWQ-11/T_{33} sensor. A stock solution of CWQ-11 (2 μM) was prepared in ultrapure water. Aliquots of this CWQ-11 (200 nM) and the T_{33} oligonucleotide (200 nM) were added separately to 5 mM Tris-HCl (pH 7.4) solutions containing Hg^{2+} (0–1000 μM), to give final volumes of 300 μL. After incubation at ambient temperature for 5 min, the fluorescence spectra were measured at an excitation wavelength of 465 nm and an emission range from 485 to 800 nm with the excitation and emission slit widths set at

5 nm.

Selectivity measurements

To test the specificity of this system, the interactions of CWQ-11/T_{33} with different metal ions were examined. Solutions containing CWQ-11 (200 nM) and the T_{33} oligonucleotide (200 nM) were mixed with individual metal ions at 1 μM. The fluorescence intensity was recorded at an excitation wavelength of 465 nm and an emission range from 485 to 800 nm after incubation for 5 minutes at RT.

Circular dichroism spectroscopy

300 μL 5 mM Tris-HCl solutions (pH 7.4) containing T_{33} (500 nM) and CWQ-11 (500 nM) was mixed with Hg^{2+} (0–15 μM). After incubation for 5 min, the circular dichroism spectra were determined using a J-810-CD spectropolarimeter (JASCO, Japan) equipped with a thermostated cell holder, using a 0.1 cm quartz cell. Spectra of 100 JM TPE-Q19 were collected by averaging three scans.

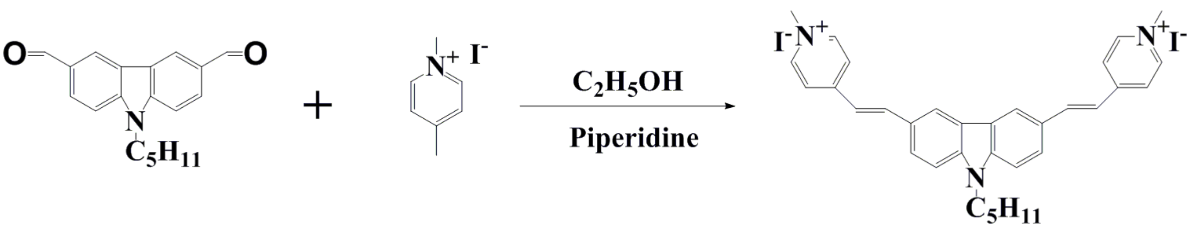
Analysis of real water samples

To further study the activity of the CWQ-11/T_{33} sensor system, we used the sensor system to test metal ions in real water samples from different sources. Samples of tap water and lake water were collected from our laboratory and Kunming lake of the Summer Palace in Beijing, respectively. And the real water samples were filtered through an ultrafiltration membrane before detection. Moreover, standard solutions of 1 μM Hg^{2+} were spiked into water samples for quantification analysis. The protocol for mercury ions detection was similar to that described above. In order to confirm the results from this sensor system, the concentrations of Hg^{2+} in real samples were analyzed by ICP-MS.

References

1 Coe, B. J.; Harris, J. A.; Asselberghs, I.; Clays, K.; Olbrechts, G.; Persoons, A.; Hupp, J. T.; Johnson, R. C.; Coles, S. J.; Hursthouse, M. B.; Nakatani, K., Quadratic Nonlinear Optical Properties of N-Aryl Stilbazolium Dyes. *Advanced Functional Materials,* 2002**,** ***12***, 110-116.

2 Meulenaere,E. D.; Chen, W.-Q.; Cleuvenbergen, S. V.; Zheng, M.-L.; Psilodimitrakopoulos, S.; Paesen, R.; Taymans, J.-M.; Ameloot, M.; Vanderleyden, J.; Loza-Alvarez, P.; Duan, X.-M.; Clays, K. Molecular engineering of chromophores for combined second-harmonic and two-photon fluorescence in cellular imaging. *Chem. Sci.*, 2012, ***3***, 984-995.



Scheme S1. Synthesis of the aromatic-based cyanine probe CWQ-11.

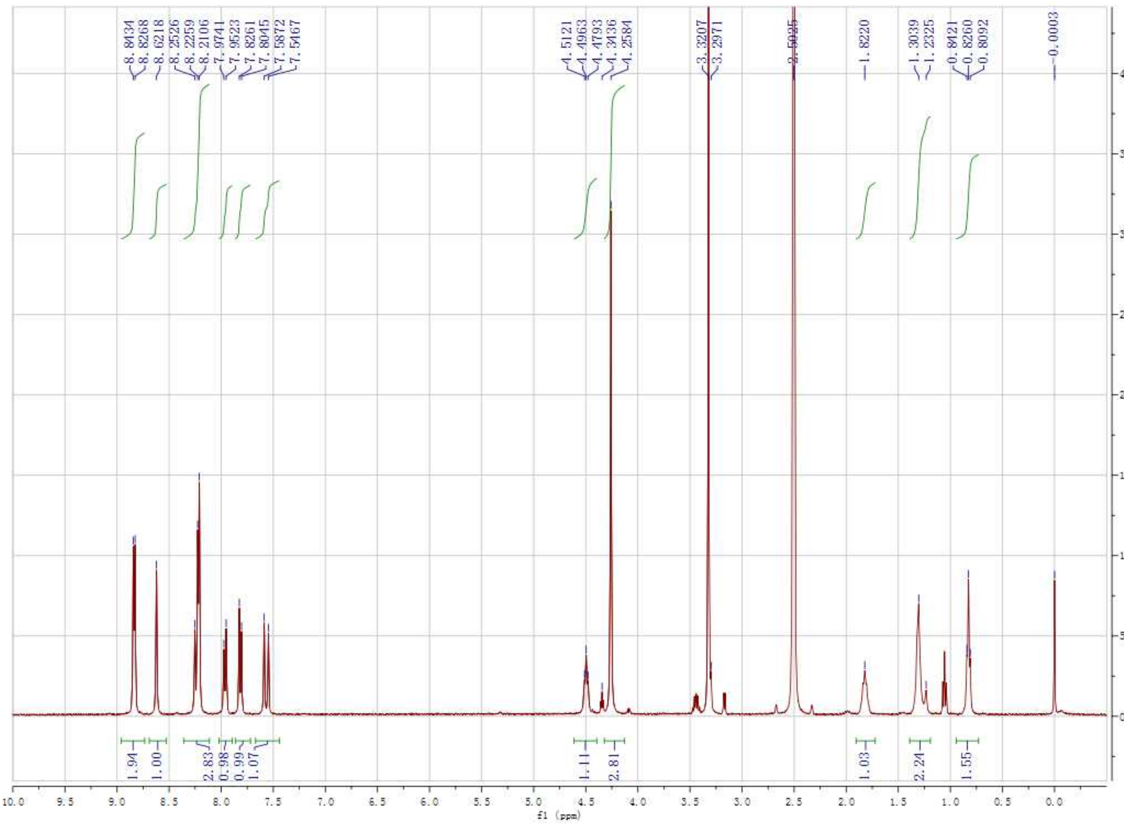


Fig. S2 Characterization of CWQ-11 by ^{1}H NMR (DMSO–d6)

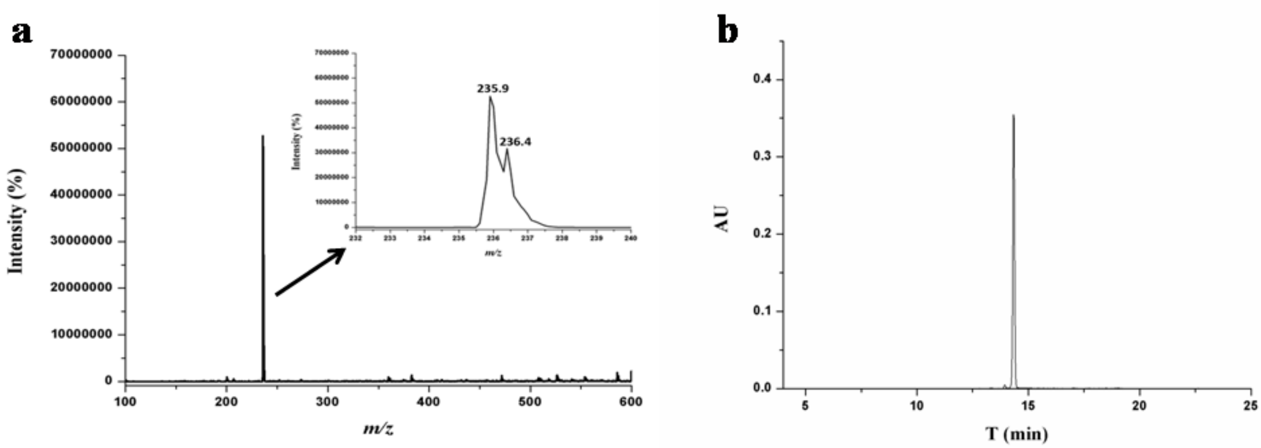


Fig. S3 Characterization of CWQ-11 by MALDI-TOF-MS (a) and HPLC (b)

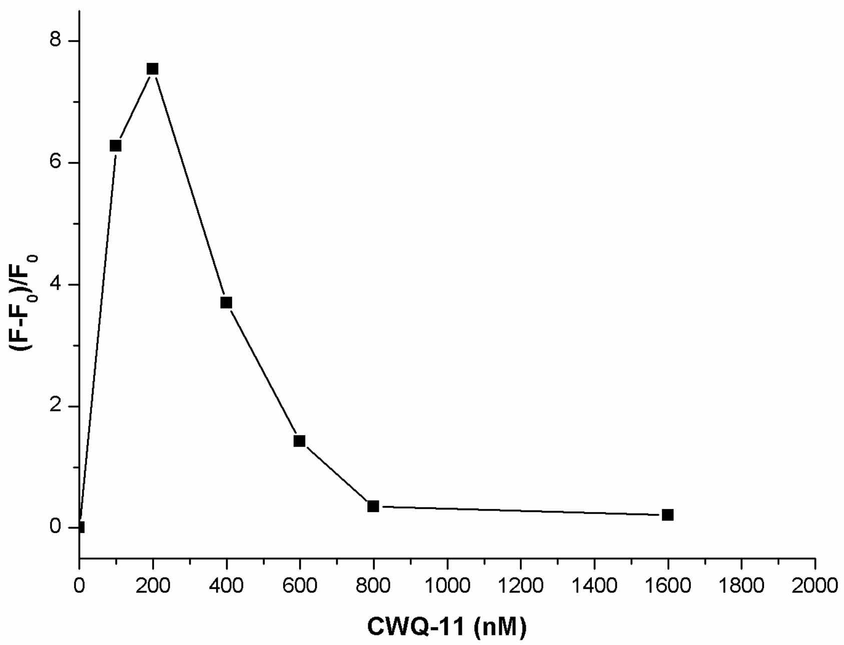


Fig. S4 Values of relative fluorescence $[(F-F_0)/F_0]$ of 5 mM Tris-HCl solutions (pH 7.4) containing T_{33} (200 nM) and CWQ-11 at various concentrations.

$$y = 0.068x + 0.166 \qquad R^2 = 0.973$$

Fig. S5 The linear equation of the fluorescence $[(F-F_0)/F_0]$ at 580 nm and the Hg^{2+} concentration over the range of 0–100 nM

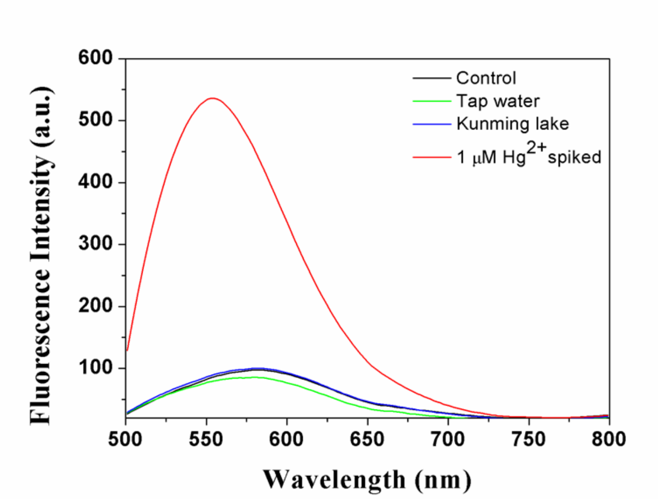


Fig. S6 Determination of Hg^{2+} in tap water and lake water samples with the CWQ-11/T_{33} sensor system

Samples	Tap water	Lake water
Hg^{2+} (nM)	0.29	0.33

Fig. S7 Determination of Hg^{2+} concentrations in real water samples by ICP-MS analysis.