A simple and label-free sensor for mercury(II) detection in aqueous solution by malachite green based on resonance scattering spectral assay

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Electronic Supplementary Information

(Including Experimental details, Optimization of sensing conditions, Equation, Supplementary figures, scheme and table)

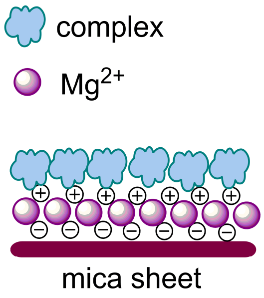
Experimental details:

(1) Reagents and chemicals

Hg^{2+} standard solution (1 mg/mL) was purchased from Merck Co., Inc. (Germany). Malachite green (MG) was obtained from Sigma-Aldrich (Milwaukee, WI, USA). The aptamer for Hg^{2+} is reference to previous literatures with little modification[1, 2], and was synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). Its sequence is 5'-GCTTTCTTCTTTCTTCCCCCCTTGTTTGTTGTTTGC-3'. Before use, the Hg^{2+} aptamer sample was dissolved in 50 mM N-(2-hydroxyethyl) piperazine-N-2-ethanesulfonic acid (HEPES) buffer solution of pH 7.2. Unless otherwise mention, all other reagents were analytical grade and used without further purification or treatment. Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout.

(2) Instrumentation and sample pre-treatment

A model of F-4500 fluorescence spectrophotometer (Hitachi, Japan) was used to record the RS intensity, with the excited slit of 10 nm and emission slit of 2.5 nm, and PMT voltage of 700 V. A model of J-815 CD spectrometer (Jasco, Japan) was employed to characterize the structure change of Hg^{2+} aptamer. Multimode Nanoscope IIIa scanning probe microscope (Digital Instrument Inc., NY. USA) was used to observe the images of Hg^{2+} aptamer-MG-Hg^{2+} complex. A suitable volume of $MgCl_2$ solution (10 mM) was added onto the fresh split surface of mica sheet, and it was placed at room temperature for 5 min to absorb Mg^{2+} ions through electrostatic interaction. Then an amount of sensing solution was added onto the above modified mica sheet, and it was placed at room temperature for 10 min to absorb objects, followed by removing the excess of salts (including Cl^- ions and free Mg^{2+} ions) by distilled water. The whole mica sheet was air dried before scanning probe microscope (SPM) observation, and the scheme of mica sheet for SPM is showed below.



(3) Procedure of Hg^{2+} determination

An appropriate volume of 100 µM Hg^{2+} aptamer and 1 mM MG stock solution were added into a 2 mL plastic tube, diluted to 450 µL with ultrapure water. Then a 50 µL Hg^{2+} solution with varying concentration was added, mixed thoroughly and incubated for 10 min. The blank sample was added 50 µL ultrapure water instead Hg^{2+} solution. The RS spectra were recorded by means of synchronous scanning excited wavelength λ_{ex} and emission wavelength λ_{em} (λ_{ex}-λ_{em}=$\triangle\lambda$=0) on fluorescence spectrophotometer. The RS intensity at 611 nm (I) and the blank solution without Hg^{2+} (I_0) were recorded. The value of $\triangle I = [I - I_0]/I_0$ was calculated.

Optimization of sensing conditions:

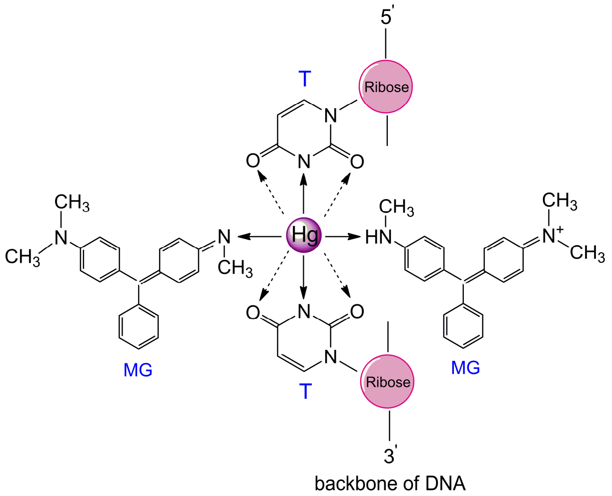
To optimize the label-free sensing system for Hg^{2+} ion detection, the varying concentrations of MG and Hg^{2+} aptamer were investigated. Fig. S5 shows that too many of MG molecules decreased the RS intensity, and 10 µM of MG was enough to the sensing system. Moreover, Hg^{2+} ion is usually dissociated to $Hg(OH)^+$ or $Hg(OH)_2$ in aqueous solution (Equation), and adding an amount of nitric acid will be beneficial to maintain the divalent mercuric ion, which promotes the formation of complex and causes an increase in the RS intensity. However, high concentration of nitric acid will degrade Hg^{2+} aptamer and lead to a decline in the RS intensity (Fig. S6). The effect of Hg^{2+} aptamer is presented in Fig. S7, and the results exhibits that 2 µM of Hg^{2+} aptamer is suitable for the sensing system. Too many of Hg^{2+} aptamer enlarged the particle diameter of complex (Fig. S8(A)), and some of them even formed bigger micelles through the intermolecular interaction of complex (Fig. S8(B)). Once the particle diameter exceed excited wavelength, some energy of excitation would lose by reflection and cause a decrease in the RS intensity. In the optimized conditions, the RS intensity of sensing system was increased by 37-fold after addition of 50 µM Hg^{2+} ions (Fig. S7). Unless otherwise mention, the following experiments were done at 10 µM of MG and 2 µM of Hg^{2+} aptamer.

Equation:

$$Hg^{2+} + H_2O \leftrightarrow HgOH^+ + H^+ \qquad (1)$$

$$HgOH^+ + H_2O \leftrightarrow Hg(OH)_2 + H^+ \qquad (2)$$

Supplementary figures, scheme and table:



Scheme S1 Supposed structure of Hg^{2+} aptamer-MG-Hg^{2+} complex. The complex is maintaining by T-Hg^{2+}-T mismatched base pair and the coordinate bonds between the N of MG molecules and Hg^{2+}.

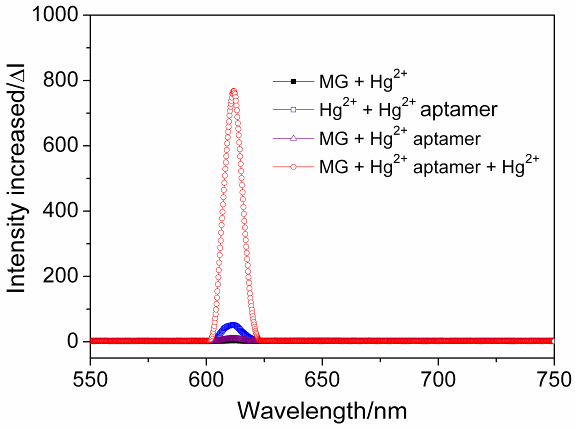


Fig. S1 RS spectra of the sensing system with different substrates. The RS intensity was recorded at excited slit of 10 nm and emission slit of 1 nm.

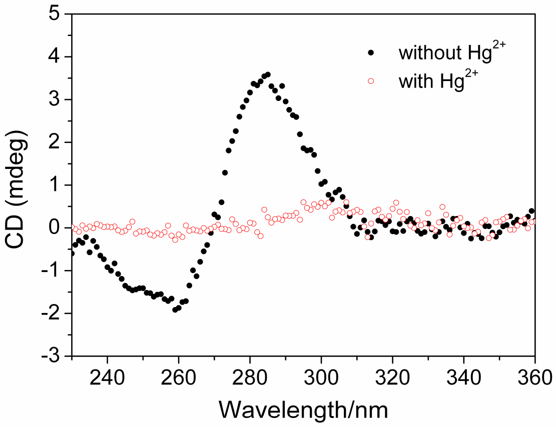


Fig. S2 RS spectra of the sensor in the absence of and after the addition of 50 μM Hg^{2+} ions for 10 min.

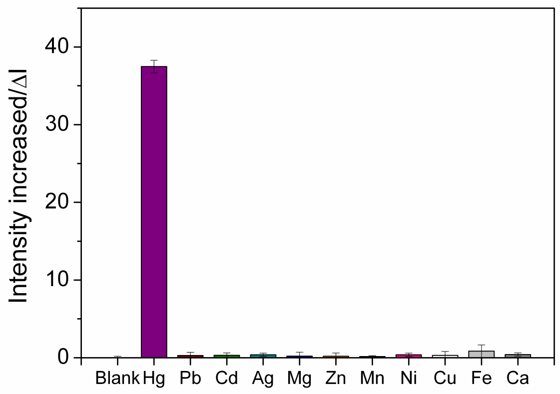


Fig. S3 Specificity of the label-free RS sensor for Hg^{2+} detection. The concentrations of metal ions were all 50 µM. Except for silver ion with monovalent, all the other metal ions were divalent.

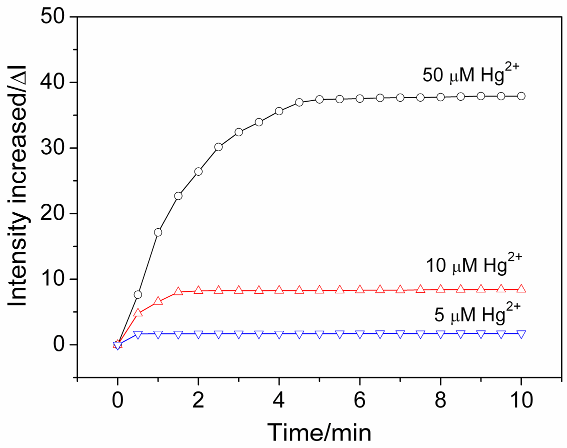


Fig. S4 Kinetics of the RS intensity increase in the presence of different concentrations of Hg^{2+} ions.

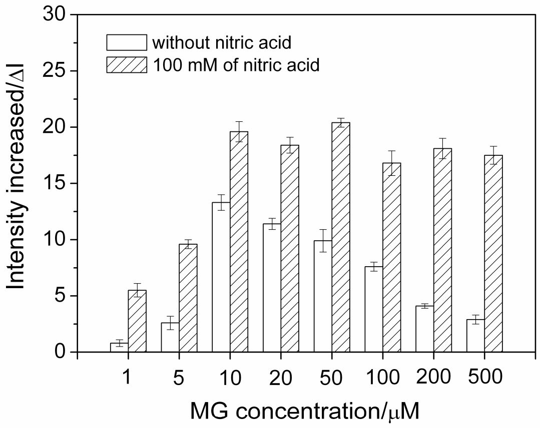


Fig. S5 Effect of MG concentrations on the RS intensity of complex. Experimental conditions: 1 µM Hg^{2+} aptamer and 50 µM Hg^{2+} ions.

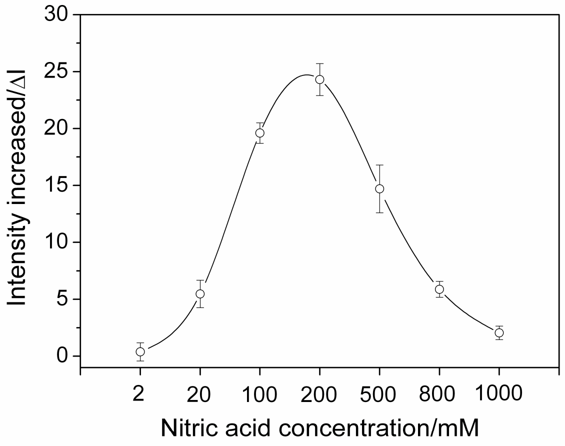


Fig. S6 Effect of nitric acid concentrations on the RS intensity of complex. Experimental conditions: 10 μM MG, 1 μM Hg^{2+} aptamer and 50 μM Hg^{2+} ions.

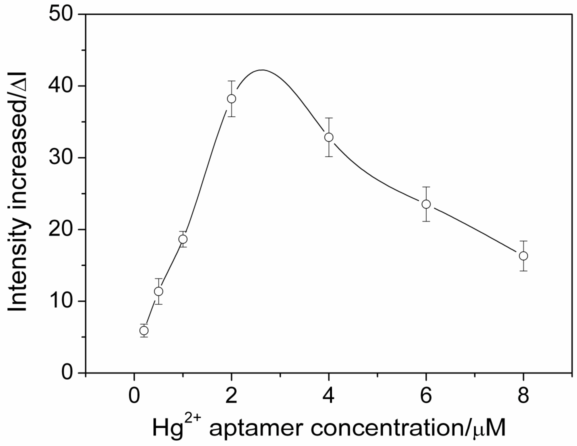


Fig. S7 Effect of Hg^{2+} aptamer concentrations on the RS intensity of complex. Experimental conditions: 10 μM MG and 50 μM Hg^{2+} ions.

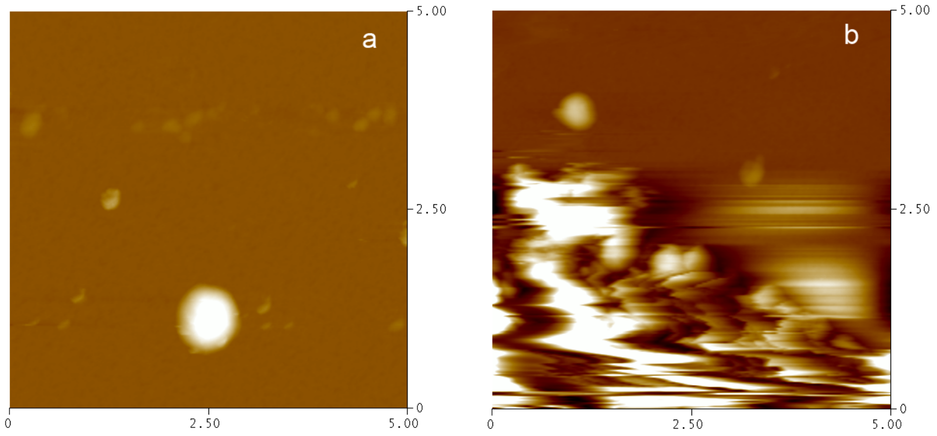


Fig. S8 Scanning probe microscope of Hg^{2+} aptamer-MG-Hg^{2+} complex at 6 μM of Hg^{2+} aptamer. (a) The particle diameter of Hg^{2+} aptamer-MG-Hg^{2+} complex is about 676 nm. (b) Some of the complex even formed bigger micelles through intermolecular interaction.

Table S1 Determination of Hg^{2+} ions in water samples

Samples	Mean found (μM)	Mean recovery (%)	RSD (%)
Hg^{2+} (0.1)[a], Pb^{2+} (0.1), Cd^{2+} (0.2), Fe^{2+} (0.1), Mn^{2+} (0.1), NO_3^- (0.8), SO_4^{2-} (0.2)	0.105	105	1.2
Hg^{2+} (0.3), Cd^{2+} (0.2), Mg^{2+} (0.1), Ag^+ (0.2), Ca^{2+} (0.1), NO_3^- (1.2), SO_4^{2-} (0.1), Cl^- (0.2)	0.312	104	1.8
Hg^{2+} (0.5), Pb^{2+} (0.2), Ni^{2+} (0.3), Cu^{2+} (0.3), Zn^{2+} (0.3), NO_3^- (1.4), SO_4^{2-} (0.6) , Cl^- (0.6)	0.523	105	1.6

[a] Concentration (μM) of ions added.

Reference

1. Z. L. Jiang, G. Q. Wen, Y. Y. Fan, C. N. Jiang, Q. Y. Liu, Z. Huang and A. H. Liang, *Talanta*, 2010, **80**, 1287-1291.
2. T. Li, S. J. Dong and E. Wang, *Anal Chem*, 2009, **81**, 2144-2149.