Electronic Supporting Information

A Reusable Biosensor for Detecting Mercury (II) at Subpicomole Level Based on “Turn-on” Resonance Light Scattering

Qiaoli Yue,¹ Tongfei Shen,¹ Junting Wang,² Lei Wang,¹ Shuling Xu,¹ Haibo Li,¹ Jifeng Liu¹*

¹ Shandong Provincial Key Laboratory of Chemical Energy Storage and Novel Cell Technology, Department of Chemistry, Liaocheng University, Liaocheng 252059, China
² Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA

Corresponding author:
Prof. Jifeng Liu
Tel & Fax, +86-635-8239001;
Email, liujifeng111@gmail.com
Experimental section

Apparatus

All RLS measurements were carried on a LS 55 spectrofluorometer (Perkin Elmer, USA) with a 1.0 cm length sample cell. The absorption spectra were recorded with a Lambda 25 spectrophotometer (Perkin Elmer, USA). The digital photos were captured with Nikon 4500 digital camera (Tokyo, Japan). The circular dichroism (CD) measurements were obtained on a J-810 CD spectrometer (Jasco, Japan). Atomic fluorescence measurements were performed on a PF6 atomic fluorescence spectrophotometer (AFS) (Persee, China). Transmission electron microscope (TEM) images were obtained on a JEM-2100 electron microscope operating at 200 kV (JEOL Ltd., Japan).

Materials

20 and 50 nm sized iron oxide MNPs coated with streptavidin were purchased commercially (Micromod, GmbH Germany). The sequences of all biotin labeled oligonucleotides used in the present work shown in Table 1 were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. Tris (hydroxymethyl)-aminomethane (Tris) and 3-(N-morpholino)propanesulfonic acid (MOPs) were purchased from Fluka (Buchs, Switzerland). NaNO₃, NaH₂PO₄, Na₂HPO₄ and other common metal salts used were obtained from Beijing Chemical Works (Beijing, China). All chemicals were used without further purification and deionized water (≥18.2 MΩ) was prepared using a Millipore water system.
Procedures for Hg\textsuperscript{2+} Sensing

MNPs coated with streptavidin (0.1 mg ml\textsuperscript{-1}) was treated directly in 10 mM MOPs solution by dilution from stock solution to 2.0 ml. 4 µl of 0.1 µM linker 1 and 0.1 µM linker 2 were added to 1.0 ml magnetic particle dispersion, respectively. The final concentrations for linker 1 and linker 2 were both at 0.2 nM in titration experiments for Hg\textsuperscript{2+}. And then they were incubated for about 20 mins at room temperature to form composite of MNPs and DNA linkers (denoted as MNPs@linker 1 and MNPs@linker 2, respectively). To the mixture of the dispersions of MNPs@linker 1 and MNPs@linker 2, a series of Hg\textsuperscript{2+} standard solutions (1.0-80.0 pM Hg\textsuperscript{2+}) were added to form MNPs@linker 1–Hg\textsuperscript{2+}–MNPs@linker 2. Stock solutions of other metal ions used for interference study were prepared in water with a similar procedure.

The RLS spectra were recorded by scanning simultaneously the excitation and emission monochromators of the spectrofluorometer in the wavelength range of 300.0-700.0 nm, with the same starting wavelength and same scanning speed (\(\Delta\lambda = 0\) nm). In the procedure, the slits of both the excitation and emission monochromators were kept at 2.5 nm. RLS intensity was measured at 459.0 nm.

Optimization of conditions

Effect of MNPs

Note that strong RLS occurred when the nanoparticles aggregated.\textsuperscript{1} To optimize the conditions, 20 and 50 nm MNPs were employed to test the effect of MNPs size on the detection of Hg\textsuperscript{2+}, with their TEM images illustrated in Fig. S2. From the TEM images, it can be observed that
the aggregation degree of MNPs after Hg$^{2+}$ addition was obvious and the aggregations were near-spherical with the size about 270 nm for both 20 and 50 nm MNPs. It can be deduced that the value of $\Delta$RLS from monodispersion nanoparticles to aggregations for 20 nm MNPs was larger than that of 50 nm. The following experiments can also confirm this deduction. The linear responses of the relative RLS intensity versus [Hg$^{2+}$] were made using 20 and 50 nm MNPs, respectively. As illustrated in Fig. S3A, it can be seen that the responses of RLS intensity to [Hg$^{2+}$] were both in a good linearity for the system using 20 and 50 nm MNPs. Furthermore, the sensitivity for Hg$^{2+}$ detection using 20 nm MNPs was higher than that of 50 nm MNPs (due to the larger slope value).

The influences of MNPs concentration and size were tested, respectively. 0.02, 0.04, 0.1, and 0.2 mg ml$^{-1}$ MNPs were used to study the effect of MNPs concentration. In the procedure, $\Delta$RLS was recorded after addition of 1.0, 5.0, 10.0, and 20.0 pM Hg$^{2+}$ using the four MNPs samples, respectively (Fig. S3B). It can be observed that $\Delta$RLS increased with increasing Hg$^{2+}$ concentration ([Hg$^{2+}$]). In addition, the difference of $\Delta$RLS was not significant for the system using 0.1 and 0.2 mg ml$^{-1}$ MNPs when the concentration of Hg$^{2+}$ was more than 5.0 pM, and 0.1 mg ml$^{-1}$ MNPs was used for the following experiments.

Effect of buffer composition and pH

The RLS signal response to [Hg$^{2+}$] was influenced by the acidity of the solution. Four buffer systems (10 mM, pH 7.4), Tris-HCl, MOPs, sodium acetate, and sodium phosphate were tested in this work. The values of $\Delta$RLS for the solutions buffered with sodium acetate, sodium phosphate, Tris-HCl, and MOPs in the presence of 40 pM Hg$^{2+}$ were 169, 216, 302, and 517, respectively. Thus, MOPs buffer solution was selected in this work.
Fig. S4 depicts the pH dependence of ∆RLS on addition of Hg$^{2+}$ ions. The system pH was then adjusted respectively to 6.5, 7.4, and 8.5 by adding standard NaOH or HNO$_3$ to MOPs buffer solutions. The responses of RLS intensity to [Hg$^{2+}$] were tested in these three systems and the sensitivity was highest at pH 7.4. It can be explained that at a pH below 7.0, protonation of the nitrogen atoms on the thymine base reduces its affinity with Hg$^{2+}$ ions, while at relatively higher pH, Hg$^{3+}$ ions may hydrolysis, reducing its complex with DNA. So MOPs with pH 7.4 was used in our work.

**Effect of NaNO$_3$ concentration**

In the present system, NaNO$_3$ was employed for adjusting the ion strength. Fig. S5 illustrated the dependence of the relative RLS increase versus [Hg$^{2+}$] ranging from 5.0 to 80.0 pM in the presence of NaNO$_3$ with different concentrations. The relationships between relative RLS intensity and Hg$^{2+}$ concentration were linear for 0, 0.01, and 0.1 M NaNO$_3$ except 1.0 M NaNO$_3$. The change of RLS intensity is the largest over the range of Hg$^{2+}$ concentration using 0.1 M NaNO$_3$. It can be deduced that the magnetic biosensor for Hg$^{2+}$ detection is appropriate in the medium of 0.1 M NaNO$_3$.

**Effects of DNA strands and the position of T-T mismatches**

The formation of T-Hg$^{2+}$-T complexes can induce aggregation events of MNPs, resulting in measurable changes of RLS intensity. For comparison, four strands of ssDNA with fourteen bases containing 6 T bases were selected as illustrated in Table 1. In the presence of Hg$^{2+}$, linker 1 and linker 2 (L1), and linker 3 and linker 4 (L2) can hybridize with 6 T-T mismatches, respectively. The RLS spectra and calibration of Hg$^{2+}$ were tested using L1 and L2 attached on MNPs, respectively. By comparison, the system using L1 for capture Hg$^{2+}$ ions gave much lower LOD
(Fig. 2), and the system for L2 showed wider range of linear response (Fig. S6). It can be rationalized that system L2 with continuing thymine bases at one end is much easier to dissociate than system L1. To verify the assumption and the formation of T-Hg$^{2+}$-T, melting experiments and circular dichroism (CD) measurements of L1 and L2 in the presence and absence of Hg$^{2+}$ were carried out. Fig. S7 showed the thermally induced transition profiles of the DNA duplexes containing consecutive T-T mispairs. In this procedure, free-labeled DNA sequences (Table 1) were used. In the presence of Hg$^{2+}$, the duplexes were significantly stabilized, depending on the position of the T-T mispairs. It can be observed that the melting temperature ($T_m$) for L1’ and L2’ alone were at 43 °C and 40 °C, respectively. They increased to 59 °C for L1’ and 54 °C for L2’ in the presence of Hg$^{2+}$, indicating that the T-T mispairs in DNA duplexes capture Hg$^{2+}$ and the complex of T-Hg$^{2+}$-T is stable. $T_m$ of L1’ in the presence of Hg$^{2+}$ is higher than that of L2’, which suggests that L1’ is much more stable than L2’ in the presence of Hg$^{2+}$.

The CD spectra of system L1’ and L2’ alone exhibit a positive Cotton effect at 277.0 nm and a negative Cotton effect at 254.0 nm (Fig. S8), which correspond to base stacking and the helicity, respectively. When Hg$^{2+}$ was added to the system L1’ or L2’, however, the positive Cotton effect becomes much weaker while the negative Cotton effect is enhanced, and either positive or negative Cotton effects get accompanying red-shift with increasing [Hg$^{2+}$]. The CD spectra changes after addition of Hg$^{2+}$ can be ascribed to the formation of Hg$^{2+}$-DNA complexes via T-Hg$^{2+}$-T coordination chemistry. Additionally, it can also be observed that the changes in CD spectra for system L1 in the presence of Hg$^{2+}$ are more remarkable than that of system L2, which is consistent with the result of melting experiment test. Therefore, to achieve a high sensitivity for Hg$^{2+}$ detection, system L1 composed of linker 1 and linker 2 was selected.
References


Fig. S1 Digital images for the mixture of MNPs@linker 1 (A) and MNPs@linker 2 (B) before and after the addition of 1.0 μM Hg^{2+}. The concentration of streptavidin modified MNPs was 0.1 mg ml^{-1}. 
Fig. S2 TEM images of the mixture of MNPs@linker 1 and MNPs@linker 2 for 20 nm (A) and 50 nm MNPs (C), and the responsive aggregation induced by 80.0 pM Hg$^{2+}$ (B, D). MNPs 0.1 mg ml$^{-1}$. The scale bars were 200 nm for A and B, and 1.0 µm for C and D.
Fig. S3 Influences of MNPs size (A) and concentration (B) on the response of RLS intensity toward the concentration of Hg^{2+} in the range of 1.0-80 pM. The error bars were obtained by three separated measurements.
**Fig. S4** The effect of pH on the linear response between relative RLS intensity and concentration of Hg$^{2+}$ in the range of 1.0-80 pM. The error bars were obtained by three separated measurements.
**Fig. S5** The effect of concentration of NaNO₃ on the relationship of RLS intensity versus the concentration of Hg²⁺ in the range of 5.0-80.0 pM. The error bars were obtained by three separated measurements.
**Fig. S6** RLS spectra of the MNPs@linker 3 and MNPs@linker 4 mixture in the presence of varying concentration of Hg$^{2+}$ (μM) (A) and the plots of the relative RLS intensity versus the concentration of Hg$^{2+}$ (B). For RLS spectra from curve 1 to curve 13 were 10 mM MOPs (curve 1), and the mixture of MNPs@linker 3 and MNPs@linker 4 in the absence (curve 2) and presence of Hg$^{2+}$ (curve 3-13) with the concentration at 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, and 100.0 μM, respectively. The inset displays the linear response between the relative RLS intensity and the concentration of Hg$^{2+}$ ranging from 0.05 to 2.0 μM. The error bars were obtained by three separated measurements.
**Fig. S7** Relative absorbance at 260 nm vs. temperature for the mixture of oligonucleotide DNA fragments with the DNA sequences of 5'-TATTTTATTTTATA-3' and 5'-TATATTTTATTTTA-3' (A), and 5' -TATATATATTTTTT-3' and 5' -ATATATATTTTTTT-3' (B) in the absence of Hg$^{2+}$ (black curve), and in the presence of 60 μM Hg$^{2+}$ (red curve). Each solution obtained 5 μM oligomers in 10 mM MOPS, 0.1 M NaNO$_3$, pH 7.4.
Fig. S8 The CD spectra of system L1’ (A) and system L2’ (B) in the absence (black curves) and presence of Hg$^{2+}$ ions with different concentrations (red to cyan curves). Concentrations: L1’, L2’ 10 μM; NaNO₃, 0.1 M; pH 7.4.
Fig. S9 RLS spectra of the MNPs@linker 1 (A) and MNPs@linker 2 (B) in the presence of varying concentration of Hg$^{2+}$ (0-100 pM). Curves 1-8, [Hg$^{2+}$] was 0, 1.0, 2.0, 5.0, 10, 20, 50, 100 pM, respectively. MNPs 0.1 mg ml$^{-1}$. 

Electronic Supplementary Material (ESI) for Chemical Communications
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Table S1 Determination of Hg$^{2+}$ in water samples using the proposed method and AFS$^a$

$^a$ The concentration of Hg$^{2+}$ at pM level; $^b$ Mean of three separated measurements; $^c$ RSD, relative standard deviation. In the tap water sample, Pb$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Cd$^{2+}$ and Cu$^{2+}$ were added.

Concentration: MNPs, 0.1 mg ml$^{-1}$; linker 1 and linker 2, 0.12 μM; MOPs 10 mM; NaNO$_3$ 0.1 M; pH 7.4.