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A reusable biosensor for detecting mercury(II) at the subpicomolar level based on "turn-on" resonance light scattering†

Qiaoli Yue,[a] Tongfei Shen,[a] Junting Wang,[b] Lei Wang,[a] Shuling Xu,[a] Haibo Li[a] and Jifeng Liu*[a]

A reusable sensing strategy employing magnetic nanoparticles and thymine-containing aptamers was developed to detect Hg^{2+} in real water samples based on "turn-on" resonance light scattering. The LOD was as low as 500 fM (S/N = 3), and the present sensor can be repeatedly used for at least seven cycles.

The mercury(II) ion (Hg^{2+}) is the most stable and toxic form of inorganic mercury and can accumulate in vital organs and tissues. Monitoring Hg^{2+} is important because it is one of the persistent contaminants in the environment and has deleterious effects on human health.[1] The traditional techniques for monitoring mercury in environmental samples such as atomic absorption/emission spectroscopy require expensive and sophisticated instrumentation and/or complicated sample preparation.[2] Recently, several "turn-on" spectroscopic methods for Hg^{2+} detection have been reported, such as colorimetry,[3] fluorescence,[4] in which Hg^{2+} induced the color changes of gold nanoparticles or fluorescence changes of rhodamine B. These methods were constructed based on the colorimetric or fluorometric signal enhancement, among which the lowest limit of detection (LOD) for Hg^{2+} was about 10.0 nM.

The emerging approach to detect Hg^{2+} ions involves the use of a thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) complex. T–Hg^{2+}–T coordination chemistry has been developed in recent years, owing to the high stability and selectivity.[5] For example, a fluorescence resonance energy transfer process based on Hg^{2+} induced DNA-folding and colorimetric systems based on DNA-nanoparticle conjugates were applied for the detection of Hg^{2+}, with the LOD of 40 nM,[5a] 100 nM,[5b] and 1.0 μM,[5c] respectively. Although these methods can give good selectivity, they cannot be repeatedly used.

Resonance light scattering (RLS) based assays have been used to sensitively detect metal ions,[6] bacteria,[7] peptides[8] and proteins,[9] owing to the strong dependence of signal intensity on the aggregation or assembly of samples. RLS methods facilitate the procedure for monitoring analytes. The focus of this work is to design a sensitive and reusable biosensor to detect Hg^{2+} employing magnetic nanoparticles (MNPs) as a RLS probe. T-containing ssDNA (linker 1 and linker 2 as listed in Table 1) was functionalized on the MNPs. In the presence of Hg^{2+}, linker 1 and linker 2 hybridized with each other, which induced the aggregation of MNPs to form MNPs@linker 1–Hg^{2+}–MNPs@linker 2 (Scheme 1). The digital photos show that MNPs@linker 1–Hg^{2+}–MNPs@linker 2 formed and aggregated in the presence of Hg^{2+} ions (Fig. S1 in ESI†). In addition, the aggregation of MNPs induced by T–Hg^{2+}–T complexes was further confirmed by TEM observation (Fig. S2 in ESI†).

To optimize the conditions, the influences of MNPs concentration and size, buffer composition and pH, $NaNO_3$ concentration, and DNA strands and the position of T–T mismatches were tested, respectively (as shown in ESI†). It can be illustrated that the aggregation degree was more obvious and the sensitivity for Hg^{2+} detection using 20 nm MNPs was much higher than that of 50 nm MNPs. The specific reaction between T bases and Hg^{2+} was better in MOPS buffer solution with pH 7.4 containing 0.1 M $NaNO_3$.

In addition, as control experiment, the effect of Hg^{2+} on the aggregation of MNPs@linker 1 and MNPs@linker 2 was

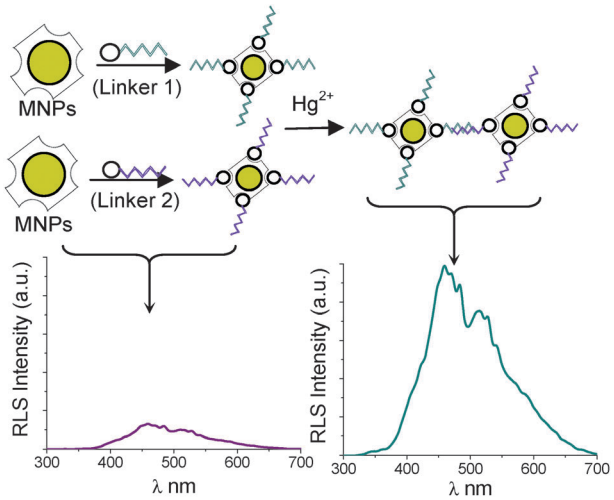
[a] *Shandong Provincial Key Laboratory of Chemical Energy Storage and Novel Cell Technology, Department of Chemistry, Liaocheng University, Liaocheng 252059, China. E-mail: liujifeng111@gmail.com; Fax: +86-635-8239001; Tel: +86-635-8239001*

[b] *Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA*

† Electronic supplementary information (ESI) available: Experimental section, digital photos of MNPs and MNPs@linker 1-Hg^{2+}–MNPs@linker 2, effects of MNPs, buffer composition and pH, $NaNO_3$ concentration, and the position of T–T mismatches, DNA sequences used in this work, and results for determination of Hg^{2+} in water samples using the present method and proposed AFS. See DOI: 10.1039/c3cc38488h

Table 1 DNA sequences used in the present work

Name		Sequences
L1	Linker 1	5′-Biotin-TATTTTATTTTATA-3′
	Linker 2	5′-Biotin-TATATTTTATTTTA-3′
L2	Linker 3	5′-Biotin-TATATATATTTTTT-3′
	Linker 4	5′-Biotin-ATATATATTTTTTT-3′
L1′	Free-labeled 1	5′-TATTTTATTTTATA-3′
	Free-labeled 2	5′-TATATTTTATTTTA-3′
L2′	Free-labeled 3	5′-TATATATATTTTTT-3′
	Free-labeled 4	5′-ATATATATTTTTTT-3′



Scheme 1 Schematic representation of a magnetic sensor for Hg^{2+} detection using T–Hg^{2+}–T complexes by the RLS method.

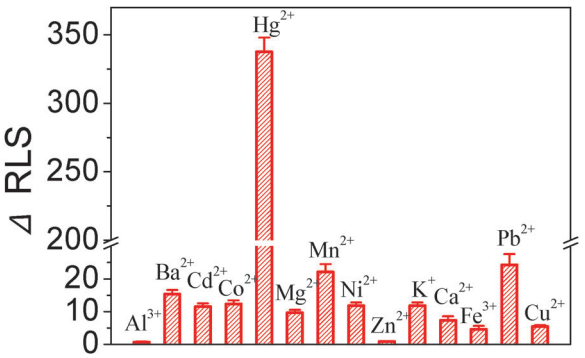


Fig. 1 The variation of RLS intensity (459.0 nm) upon the addition of different metal ions. Concentrations: 0.1 mg ml^{-1} MNPs, 0.2 nM ssDNA, and 40 pM Hg^{2+}. All other metal ions are 1.0 μM. $NaNO_3$ 0.1 M, and pH 7.4. The error bars were obtained from three separate measurements.

monitored, respectively. As shown in Fig. S9 (ESI†), in the presence of 1.0–100 pM Hg^{2+} the RLS intensity of MNPs@linker 1 and MNPs@linker 2 increased from 97 to 122 and from 111 to 146 at 459.0 nm, respectively. It was suggested that the combination of MNPs@linker 1 and MNPs@linker 2 was appropriate for Hg^{2+} detection rather than only one of them.

Under optimum conditions, the selectivity was determined by test RLS signal changes of MNPs that occurred within 5 min after separately adding the following metal ions (1 μM): Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, K^{+}, Ca^{2+}, Fe^{3+}, Pb^{2+} and Cu^{2+}, and 40 pM Hg^{2+} to RLS probes. Only Hg^{2+} caused the notable change in RLS intensity of the system, and this technique showed excellent selectivity for Hg^{2+} over alkali, alkaline, and other heavy transition-metal ions (Fig. 1). Anions including Cl^{-}, Br^{-}, F^{-}, SO_4^{2-}, and CO_3^{2-} (all sodium salts) were also tested, and showed no interference with the detection of Hg^{2+} (data not shown).

The increment of RLS intensity at 459.0 nm was fitted with the equation $\Delta RLS = 129.05 + 10.85C$ (Hg^{2+}, pM) over the concentration ranging from 1.0 to 80.0 pM (Fig. 2A and B), with a correlation coefficient of 0.9971 and an LOD (S/N = 3) of 500 fM. Compared with other sensors for monitoring Hg^{2+} based on the specific interaction of T–Hg^{2+}–T complexes by adopting colorimetry, fluorescence, LSPR and electrochemistry methods, the proposed system showed the advantage of the highest sensitivity (Table 2).

To study the reusability of the biosensor for the determination of Hg^{2+}, 80 pM L-cysteine was added into the MNPs@linker 1–Hg^{2+}–MNPs@linker 2 dispersions (40 pM Hg^{2+}) to release Hg^{2+} and the MNPs were collected by applying an external magnetic field. Because the aggregation-induced RLS intensity enhancement was caused by the formation of T–Hg^{2+}–T complexes between T-containing ssDNA on the MNPs surface and Hg^{2+}, one might expect that a strong Hg^{2+} ion chelator, cysteine, might be able to prevail over thymine and thus redisperse the aggregation.[10] However, after addition of L-cysteine (80 pM) into the aggregated MNPs@linker 1–Hg^{2+}–MNPs@linker 2 system, the RLS intensity of MNPs coated with ssDNA was restored completely as shown in Fig. 2C.

Real samples including tap water, river water, and lake water samples were analyzed using the present sensor system. River water and lake water samples were obtained from Tuhai River in Liaocheng and the lake in the campus of Liaocheng University, respectively. The samples collected were first filtered through a 0.22 μm membrane, then centrifuged for 10 min at

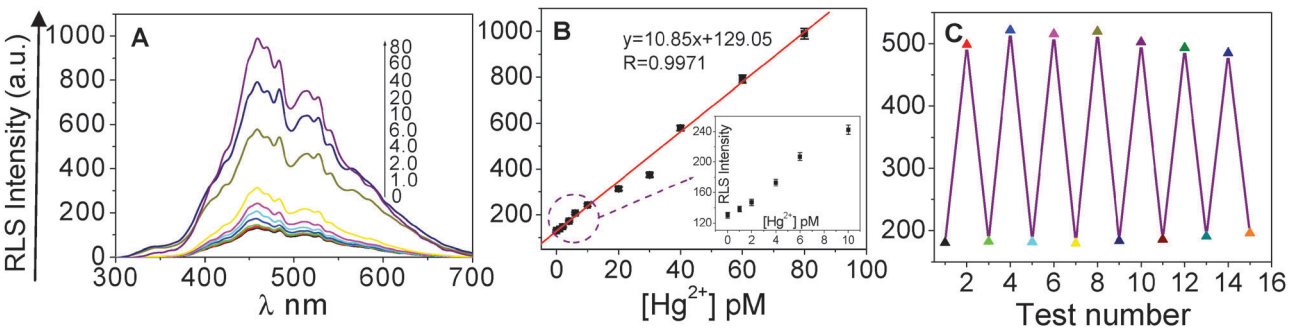


Fig. 2 RLS spectra of the MNPs@linkers in the presence of Hg^{2+} with varying concentrations (pM) (A), the plots of the relative RLS intensity *versus* the concentration of Hg^{2+} (B), and the RLS intensity of MNPs@linkers after enhancing by Hg^{2+} ions (40 pM) can be recovered by the addition of L-cysteine (80 pM) and applying an external magnetic field (C). The RLS intensity was recorded at 459.0 nm for B and C. The inset displays the linear response between the relative RLS intensity and the concentration of Hg^{2+} ranging from 0 to 10.0 pM. The error bars were obtained from three separate measurements.

Table 2 Comparison of the sensitivity of different Hg^{2+} sensors based on T–Hg^{2+}–T complexes on different probes

Ref. no.	Method	Probe	Linear range (μM)	LOD (nM)
6*b*	LSPR scattering	GNPs	0.04–0.6	1.0
11	Colorimetry	GNPs	0.5–5.0	250
12	Fluorescence	—	0.01–0.2	5.0
13	Fluorescence	GNPs	0.096–6.4	40
14	Electrochemistry	GNPs	0–0.1	0.5
15	Fluorescence polarization	GNPs	0.01–1000	10
16	Fluorescence	GNPs	0.05–2.5	25
17	Fluorescence	Silver clusters	0.01–0.3	10
Present work	RLS	MNPs	1.0×10^{-6}–8.0×10^{-5}	5.0×10^{-4}

10 000 rpm and detected according to the general procedure with three replicates. The results averaged from three determinations are summarized in Table S1 (in ESI†).

The present assay displays a high selectivity for Hg^{2+} against a background of competing analytes. Moreover, the results show a good agreement with that determined by the AFS method.

In conclusion, we have demonstrated a novel RLS method, and the present method provides a reusable but sensitive technique to detect Hg^{2+} at room temperature. The present sensor utilizes a combination of the advantages of functionalized MNPs due to their easy magnetic separation and the specific interaction of thymine and Hg^{2+} to form T–Hg^{2+}–T complexes selectively. When Hg^{2+} was added the MNPs modified with ssDNA containing T bases can aggregate. The particles aggregation resulted in the enhancement of RLS. The proposed method is applicable to the determination of Hg^{2+} in both standard solutions and water samples in the presence of excess of other metal ions, with a LOD down to 500 fM *via* common standard working curves. The strategy is potentially applicable to the determination of any other metal ions able to produce the aggregation of MNPs.

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