RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

ARTICLE TYPE www.rsc.org/xxxxxx | XXXXXXXX

An Exonuclease III-aided ‘‘Turn-on’’ Fluorescence Assay for Mercury Ions Based on Graphene Oxide and Metal-Mediated “Molecular Beacon”

Ying Zhou, Xiao-Jing Xing, Dai-Wen Pang, and Hong-Wu Tang*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, State Key Laboratory of Virology, and Wuhan Institute of Biotechnology,Wuhan University, Wuhan, 430072, P. R. China

*Corresponding author: Hong-Wu Tang. Tel: +86 27 68756759, Fax: +86 27 68754067, E-mail: hwtang@whu.edu.cn.

[a] These authors contributed equally to this work.

A novel fluorescence “turn-on” strategy, which is based on the formation of Hg^{2+}-mediated molecular-beacons (MBs), the preferable cleavage capacity of exonuclease III to double-stranded DNA compared to single-stranded one, and the remarkable difference in the binding ability of graphene oxide (GO) with single-stranded DNA and the mononucleotides, is designed for Hg^{2+} assay. The Hg^{2+}-mediated base pairs facilitate the dye labeled MBs to fold into a hairpin structure which is more likely to be digested by exonuclease III, and obvious increase in the fluorescence intensity is observed after incubating with GO due to the weak affinity of the product-mononucleotides to GO. A fluorescent ‘‘turn-on’’ method based on graphene oxide and exonuclease III was designed for Hg^{2+} assay. The introduction of GO greatly increases the signal-to-background ratio, and the sensitivity is significantly improved due to the amplified capibility of exonuclease III. Under the optimal conditions, Hg^{2+} is detected specifically and sensitively with a detection limit of 0.83 nM. Compared with the reported Hg^{2+} assay methods, the proposed strategy is simple, cost effective and selective, which might provide a new platform for developing a sensitive Hg^{2+} biosensor. Mercury level in the blood is an important indicator of mercury poisoning in clinical study. To testify the possibility of this method for the assay of Hg^{2+} in real samples, detection of Hg^{2+} in 1 % human serum was investigated and satisfactory results were obtained, which suggest that this method has great potential for bioanalysis.

Introduction

Mercury ions, which represent a widespread highly toxic contaminant in aquatic ecosystems, pose severe risk for human health and the environment.[1] Mercury exposure can cause a number of adverse health effects, such as damage in the brain, nervous system, immune system, kidney, and many other organs.[2] The concern for the worldwide spread of mercury pollution and its damage to human health make the development of efficient, selective and sensitive methods for the detection of Hg^{2+} urgent. In the past decades, many methods, including atomic fluorescence spectroscopy,[3] inductively coupled plasma mass spectrometry(ICP-MS),[4] selective cold vapor atomic fluorescence spectrometry,[5] have been developed. However, most of them are rather expensive, complex, and not suitable for on-site analyses. The past few years have witnessed great progress in the development of optical and electrochemical techniques for the detection of Hg^{2+} based on small molecules,[6,7] DNAzymes,[8] oligonucleotides,[9] polymers[10] and functional nanoparticles.[11] Despite these progress, some limitations, which include insufficient resolution in solution, relatively low selectivity among other metal ions, the need to use organic solvents or sophisticated synthesis of the probe still exist. Therefore, a simple sensor with high sensitivity and selectivity for facile on-site and real-time mercury detection is still needed.

Recently, synthetic oligodeoxyribonucleotides (ODNs) containing artificial bases have been used to form metal-mediated base pairs in which hydrogen bonds in Watson–Crick (W–C) type base pairs in natural DNA are replaced by metal–base bonds.[12] There is no need to time-consuming organic synthesis for the formation of metal-mediated base pairs which makes its process simpler compared with base pair formation. Hairpin-DNA molecular- beacons(MBs), which are dually labeled single-stranded oligonucleotide with a loop-and-stem structure and become fluorescent upon binding to a complementary sequence or target as a result of the disruption of the stem−loop structure and an increased distance between the fluorophore and quencher tagged at either end,[13] have been applied as promising probes as they not only provide a variety of analytical signal transduction options but also have better selectivity than linear DNA probes. It is proven that the DNA incorporating one or more metal-mediated base pairs shows higher stability than natural DNA.[14] Previous research have reported that Hg^{2+} can specifically insert into two DNA thymine bases and facilitates these T–T mismatches to form stable base pairs.[15] This special property opens a new way for utilizing T-containing oligonucleotides for the development of Hg^{2+}-mediated MBs for Hg^{2+} detection. Some T–Hg^{2+}–T based Hg^{2+} detection systems include fluorescence biosensors,[16] gold nanoparticle-based colorimetric method,[17-19] anodic stripping voltammetry,[20] amperometric biosensor based on electrically contacted enzyme and electrochemical DNA structure switching sensors,[21, 22] have been reported. Despite these developments, most of them might suffer relatively small signal-to-background ratio. It should be still desirable to develop a detection system that is not only simple, sensitive but also selective and reliable.

Graphene oxide (GO), a single-atom-thick two-dimensional nanosheet prepared by acid exfoliation of graphite,[23] has attracted great interests in biological applications owing to its unique characteristics such as good water-solubility, versatile surface modification and superior fluorescence quenching ability.[24, 25] Furthermore, GO was reported to interact with single-stranded DNA (ssDNA) by π–π stacking interactions between nucleotide

bases and GO but hardly interact with rigid double-stranded DNA (dsDNA) , or aptamer-target complexes. [26] Nowadays, graphene oxide (GO)-based sensing platforms are being widely applied for the detection of various analytes attributing to its unique ability of absorbing DNA species as well as its super quenching capacity for a wide range of fluorophores.[27] In our previous report,[28] we have developed a novel fluorescence turn-on strategy for the biothiols assay, which is based on the resistance of metal-mediated molecular-beacons (MBs) toward nuclease digestion and the specific cleavage capacity of exonuclease I towards single-stranded DNA compared to double-stranded one. We found that GO shows remarkable difference in the binding ability toward single-stranded DNA and mononucleotides, and thus displays different fluorescence quenching capacity. Exonuclease III (Exo III) is an enzyme with specific exodeoxyribonuclease activity for dsDNA in the direction from 3′ to 5′.[29] Inspired by our previous study, taking advantage of the specific affinity between Hg^{2+} and T–T mismatches, the preferable cleavage capacity of exonuclease III to double-stranded DNA compared to single-stranded one, and the superb fluorescence quenching ability of GO, we present an Exo III-aided ''turn-on'' fluorescence detection method for Hg^{2+}. This strategy relies on the principle that Exo III shows different cleavage capacity for single-stranded DNA and double-stranded one in the absence and presence of Hg^{2+} and the preferential binding ability of GO to single-stranded DNA (ssDNA) over mononucleotides. As Hg^{2+} could be released from the T−Hg^{2+}−T hybrid by Exo III, facilitates the formation of a new T−Hg^{2+}−T hybrid, which may lead to this conformation-dependent enzymatic activity triggered by metal ions a signal-amplified strategy.

Experimental section

Chemicals and materials

Graphite powder, sulfuric acid, potassium persulfate, phosphorus pentoxide, hydrogen peroxide, mercuric nitrate, and potassium permanganate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade or of the highest purity available. The oligonucleotides were purchased from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China), their sequences are listed in Table S1. GelRed Nucleic Acid Gel Stain (10000 ×) was purchased from Biotium, Inc (USA). Agarose was purchased from Invitrogen Corporation (USA). 6 × loading buffer, 20 bp DNA ladder marker and Exo III, were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The buffer solutions used in this work are as follows: 10 ×Exo III Buffer are consisted of 500 mM Tris-HCl (pH 8.0), 50 mM $MgCl_2$, 10 mM DTT.

Apparatus

A Hitachi F-4600 fluorescence spectrophotometer (Hitachi Company, Tokyo, Japan) was used to record the fluorescence spectra and measure the fluorescence intensity. The sample cell was a 700 μL quartz cuvette. The fluorescence intensity was monitored by exciting the sample at 480 nm and measuring the emission at 520 nm. The slits for excitation and emission were set at 5 nm and 10 nm, respectively. The digital image of the gel was captured with the AlphaImager IS-2200 (Alpha Innotech, CA, USA).

Synthesis of graphene oxide

Graphite oxide was synthesized from natural graphitic powder according to Hummer's method and our previous report (Hummers and Offeman, 1958; Xing et al., 2012). The resulting mixture was filtered and washed with 10% HCl aqueous solution and deionized water. Finally, the as-synthesized product was purified by dialysis for one week, dried in a desiccator and then dispersed in water under sonication for 5 h.

Test of the General Feasibility of This Approach

The digestion of MB by Exo III in the absence and presence of Hg^{2+} were investigated, respectively. In detail, 2 μL of MB (10 μM) without and with 1 μL of Hg^{2+} (91.5 μM) were firstly mixed at 25 ºC for 1 h, respectively. Then 6 U Exo III was added to incubate with above solution in 1 × Exo III buffer at 25 ºC for 15 min. Finally, all the reacted samples were heated to 80 °C for 10 min to inactivate Exo III and stop the digestion reaction. 6 μL of each reacted sample was mixed with 1.2 μL 6 × loading buffer and was loaded onto a 3.5 % agarose gel mixed with GelRed. Following electrophoresis in 0.5 % TBE buffer at 100 volt for 60 min, the gel image was captured with the AlphaImager IS-2200.

General Confirmation of Amplified Mechanism of Exo III

To confirm the hypothesized mechanism of Hg^{2+} releasing and cyclical reacting with MB, a gel electrophoresis experiment was conducted. 2 μL of MB (10 μM) with 1 μL of Hg^{2+} (3.66 μM) were firstly mixed at 25 ºC for 1 h in two microcentrifuge tubes, respectively. Then 6 U Exo III was added to incubate with above solution in 1 × Exo III buffer at 25 ºC for 10 min and 70 min. 2 μL of MB (10 μM) with 1 μL of Hg^{2+} (91.5 μM) were incubated with 6 U Exo III for 10 min after 1 h mixture at 25 ºC. Each reacted sample was heated to 80 °C for 10 min to inactivate Exo III and stop the digestion reaction. 6 μL of each reacted sample was mixed with 1.2 μL 6 × loading buffer and was loaded onto a 3.5 % agarose gel mixed with GelRed. Following electrophoresis in 0.5 % TBE buffer at 100 volt for 60 min, the gel image was captured with the AlphaImager IS-2200.

Study of Exo III Activity on DNA Duplex with T−Hg^{2+}−T Base Pairs

3.33 μM DNA 2 and 7.625 μM Hg^{2+}, 1.67 μM DNA 1 , 1.67 μM DNA 2 and 7.625 μM Hg^{2+}, 1.67 μM DNA 1, 1.67 μM DNA 2 and 7.625 μM Hg^{2+},1.67 μM DNA 3, 1.67 μM DNA 1 and 7.625 μM Hg^{2+}, 1.67 μM DNA 4 , 1.67 μM DNA 1 and 7.625 μM Hg^{2+}, 1.67 μM DNA 5 ,1.67 μM DNA 1 and 7.625 μM Hg^{2+}, 1.67 μM DNA 6, 1.67 μM DNA 1 and 7.625 μM Hg^{2+} were first mixed at 25 ºC for 1 h, respectively. Then 6 U Exo III was added to incubate with five of samples in 1 × Exo III buffer at 25 ºC for 15 min. The other two samples were incubated with 1 × Exo III buffer of same volume. Finally, all the reacted samples were heated to 80 °C for 10 min to inactivate Exo III and stop the digestion reaction. 6 μL of each reacted sample was mixed with

1.2 μL 6 × loading buffer and was loaded onto a 3.5 % agarose gel mixed with GelRed. Following electrophoresis in 0.5 % TBE buffer at 100 volt for 60 min, the gel image was captured with the AlphaImager IS-2200.

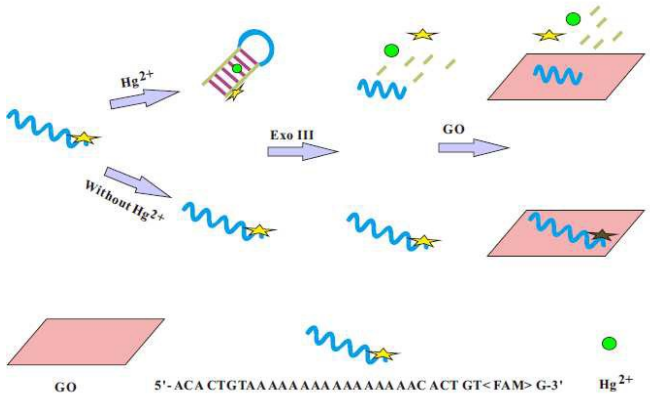
Detection procedure

For Hg^{2+} assay, a solution containing 10 μL of the probe stock solution was incubated with different concentrations of Hg^{2+} solution at 25 ℃ for 1 h, then 0.5 U of Exo III was added into above mixture solution. After the mixture was incubated for 15 min at 25 ℃, 10 μg/mL of GO was added and incubated for another 30 min at room temperature. Then the fluorescence emission spectra were recorded immediately for excitation at 480 nm. The same procedures were repeated in the presence of other metal ions instead of Hg^{2+} to assess the selectivity.Analytical procedure.

Results and discussion

Working principle

Scheme 1 illustrates the principle of this strategy. The detection system consists of a dye labeled single-stranded oligonucleotide with a T-T mismatch in the region of stem (5'-ACA CTGTAA AAA AAA AAA AAC ACT GT⟨FAM⟩ G-3') (MB), GO, and Exo III. Hg^{2+}, a widespread highly toxic contaminant in aquatic ecosystems, was chosen as a model system to demonstrate the proof-of-concept of our approach. In the absence of Hg^{2+}, the base-pair binding energy is not sufficient to fully hybridize under room temperature, leading to the probe adopts a random coil structure which shows resistance to the digestion of Exo III andsubsequently, it is absorbed onto the GO surface after being incubated with GO due to the strong binding strength between single-stranded DNA and GO, resulting in a significant quenching of the fluorescence.



Scheme.1 Schematic illustration of GO/ Exo III based sensing strategy.

In the presence of Hg^{2+}, T−Hg^{2+}−T base pairs are formed, and the linear probe transforms into a hairpin structure. This conformational switch triggers the occurrence of nuclease digestion of Exo III from the 3′ terminus of the nucleotide probe. No obvious fluorescence change is observed after the introduction of GO due to the weak affinity of the product mononucleotides to GO. Therefore, Hg^{2+} detection could be easily obtained by monitoring the fluorescence signal enhancement. As Hg^{2+} could be released from the T−Hg^{2+}−T hybrid by Exo III, and it is involved in the formation of new T−Hg^{2+}−T hybrids, which may lead to this conformation-dependent enzymatic activity triggered by metal ions a signal-amplified method.

Test of the General Feasibility of This Approach

The fluorescence intensities of MB under different conditions were measured to testify the general feasibility of this approach and the corresponding fluorescence spectra are shown in Fig. 1. The initial fluorescence from MB (curve a) is greatly quenched to 4.9 % in the presence of 10 μg/mL GO (curve d), resulting from the strong binding of MB with GO and the high quenching ability of GO. When Exo III digestion is included in the assay steps, just as expected, only a slight fluorescence signal change is observed (curve c), indicating the poor cleavage capacity of Exo III to the single stranded DNA. However, when Hg^{2+} is added, the assay system shows dramatically higher fluorescence intensity (curve b). The enhanced fluorescence suggests the occurrence of nuclease digestion where MB is digested into mononucleotides exhibiting a weak affinity to GO surface. These observations confirm that the stem-loop structure formed by the coordination of T-Hg^{2+}-T is more likely to be digested by Exo III than the random coil ssDNA, and this strategy can be used for Hg^{2+} assay.

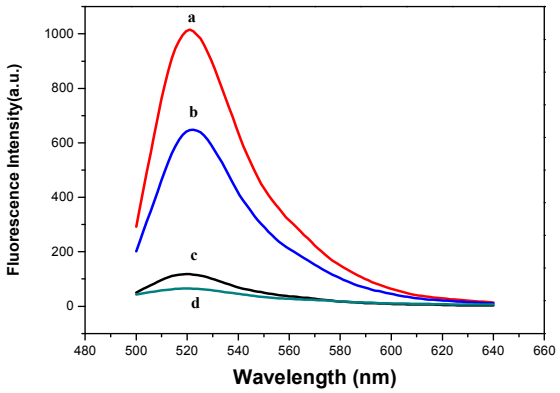


Fig. 1 Fluorescence intensity of MB under different conditions: (a) MB; (b) MB + Hg^{2+} + Exo III + GO; (c) MB + Exo III + GO; (d) MB + GO.

At the same time, gel electrophoresis was used to further testify the feasibility of this approach. The results are shown in Fig. 2. As can be seen in Fig. 2A, only the band in lane 3 corresponding to MB/Hg^{2+}/Exo III complex disappears. From Fig. 2B which was taken under ultraviolet light, yellow band of FAM labelled on MB can be clearly seen. This indicates that MB was digested by Exo III in the presence of Hg^{2+}. The gel electrophoresis results further prove the existence of Hg^{2+} prompts the enzyme reaction of Exo III.

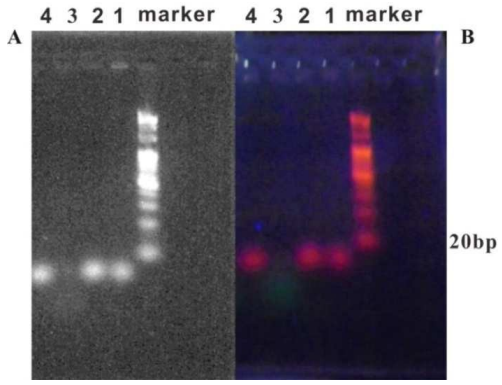


Fig. 2 Image of the gel electrophoresis to verify the feasibility of this method, Lane 1:3.3 μM MB only; Lane 2: 3.3 μM MB with 15.25 μM Hg^{2+}; Lanes 3 and 4: 3.3 μM MB treated with 6 U Exo III without Hg^{2+}(lane 4) and with 15.25 μM Hg^{2+} (lane 3).

To generally confirm the hypothetical amplified mechanism of this method, a gel electrophoresis experiment was conducted (Fig. S1). The result shows that Exo III performs the same cleavage effect on the system with low concentration of Hg^{2+} compared with the system with relatively high concentration of Hg^{2+} after a much longer incubation time, which implies that the cyclic reaction may occur.

To investigate the influence of T−Hg^{2+}−T base pairs on the activity of Exo III, five different DNA containing different numbers of T−T mismatches at different sites with DNA 2 were introduced. Each of these DNA was incubated with an excessive amount of Hg^{2+} to ensure that each T−T mismatched pair has one Hg^{2+} incorporated. The Exo III digestion of these DNA duplexes was analyzed by gel electrophoresis. As shown in Fig. S2, T−Hg^{2+}−T base pairs have no obvious inhibiting effect on exonuclease activity.

Optimization of experiment conditions

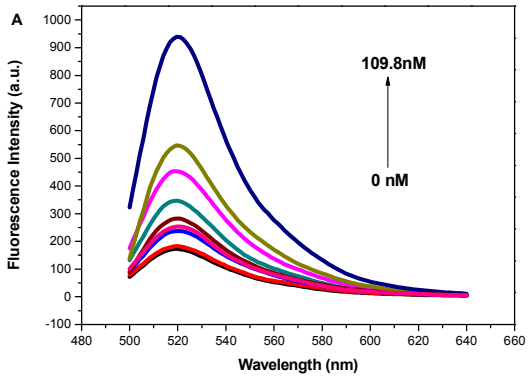
In an attempt to obtain an optimal assay condition and achieve a high sensitivity, the concentration of GO and Exo III, the enzymatic reaction time were investigated. According to the results, 10 μg/mL GO (Fig. S3) and 0.5 U Exo III (Fig. S4) were used in the following experiment. The enzymatic reaction time was set at 15 min (Fig. S5).

The reaction temperature is an important aspect involved in the detection system. A relatively low temperature may lead to a high background signal caused by intramolecular base pairing of MB and a poor activity of Exo III, while too high temperature may not be beneficial to the stability of Hg^{2+}-induced hairpin strand. So we need to find a suitable temperature which is conducive to the formation of hairpin structure as well as ensuring a low background signal. Fig. S6 shows different fluorescence intensities of the detection system under different temperatures. A downward trend of fluorescence intensity in the system without Hg^{2+} can be seen under relatively low temperature which may be caused by the dissociation of intramolecular pairing base of MB, and more and more MBs adopt a random coil structure which is resistant to the digestion of Exo III. In the system with Hg^{2+}, fluorescence intensity changes slowly at the beginning stage. During this period, two processes may be involved. Firstly, some intramolecular pairing bases of MB dissociated slowly with the increasing of temperature which result in the resistance to the digestion of Exo III. On the other hand, the formation of hairpin structure mediated by Hg^{2+} facilitates the cleavage reaction which contributes to the increasing of fluorescence intensities. These two complementary effects made the fluorescence intensities remain stable. While under the temperature between 15ºC and 25ºC, an obvious increase of fluorescence signal can be observed which shows the formation of more hairpin structure mediated by Hg^{2+}. However, with the continuous growth of temperature, the hairpin structure of MB will be opened with the approaching of its melting temperature, at the same time, a high background signal was shown due to the enhanced Exo III activity, as a result, the decrease of F/F_0 value can be observed (Inset, Fig. S6). We choose 25ºC as the ultimate enzyme reaction temperature after the comprehensive analysis of the above results.

Sensitivity and selectivity of the detection system

The quantitative detection of Hg^{2+} using this method under the optimized experimental conditions was evaluated by monitoring the fluorescence change under different concentrations of Hg^{2+}. As shown in Fig. 3A, by increasing the concentration of Hg^{2+}, the fluorescence intensity dramatically increases, implying that more and more MB is digested by Exo III. Furthermore, the fluorescence intensity shows a clear linear dependence on Hg^{2+} concentration in the range of 1.83-109.8 nM (Fig. 3B). The linear regression equation is y =7.39 x + 199.33 with the correlation coefficient of 0.9776. The limit of detection (LOD) based on the $3\sigma/S$ calculation (σ is the standard deviation for the blank solution, and S is the slope of the calibration curve) is about 0.83 nM, an improved sensitivity compared to some reported fluorescence methods (Table1). The satisfying sensitivity of our method may be mainly attributed to the low background signal induced by GO and amplified signal by Exo III.

Considering the structural similarity of some other metal ions, the selectivity of the detection was investigated by substituting the Hg^{2+} in the system with various metal ions of the same concentration of Hg^{2+} (3.66 μM), such as Mg^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Cd^{2+} and Mn^{2+}. No significant increase in fluorescence intensity is observed in comparison to that of Hg^{2+} (Fig. 3C). These results indicate that the proposed strategy has high selectivity for Hg^{2+} which is attributed to the specific T−Hg^{2+}−T base pairing and the preferential activity of Exo III.



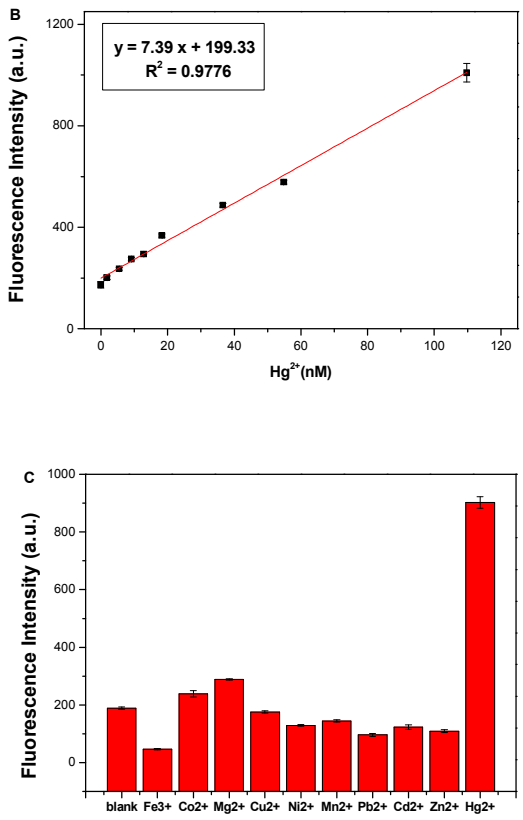


Fig. 3 (A) Fluorescence spectra of the assay system in the presence of different concentrations of Hg^{2+}. Bottom to top: 0, 1.83, 5.49, 9.15, 12.81, 18.3, 36.6, 54.9, 109.8nM. (B) Linear relationship between the fluorescence intensity and Hg^{2+} concentrations. (C) Fluorescence intensity of the assay system in the presence of different metal ions.

Table 1 Comparison of the linear range and detect limit for Hg^{2+} by other methods

Method	Linear range	LOD	References
Oligodeoxyribonucleotide(ODN)-based	40-100nm	40 nM	9
DNA/single-walled carbon nanotubes based	0.5–5.0μM	250 nM	30
Non-crosslinking aggregation of double-stranded	45-720nM	10 nM	31
Application of a DNA-based luminescence switch-on method	0.03-2.5μM	500 nM	32
Gold nanoparticle-based colorimetric and "turn-On" fluorescent probe	96-6400nM	40 nM	33

The detection of Hg^{2+} in human serum

In order to evaluate the practicability of the proposed method, the detection of Hg^{2+} in human serum was performed, and the detection process was the same as that in the pure buffer. Fig. 4A shows the fluorescence intensities of MB in the presence of different concentrations of Hg^{2+} in 1 % human serum. A good linear relationship with a calibration equation $y = 3.64\,x + 313.37$ (R^2 = 0.9819) is still obtained in 1 % human serum (Fig. 4B). These results demonstrate the potential value of the proposed method for detecting Hg^{2+} in real samples.

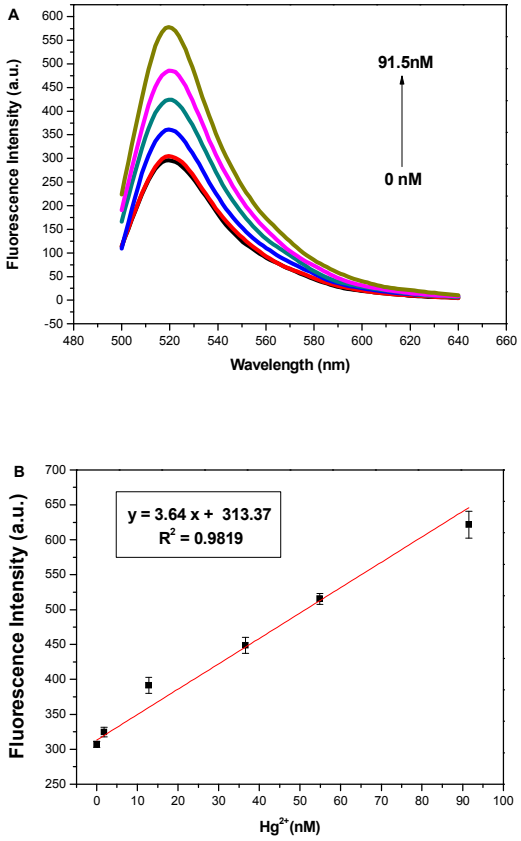


Fig. 4 (A) Fluorescence spectra of the assay system in the presence of different concentrations of Hg^{2+} in 1% human serum. Bottom to top: 0, 1.83, 12.81, 36.6, 54.9, 91.5nM. (B) Linear relationship between fluorescence intensity and Hg^{2+} concentrations in 1 % human serum.

Conclusions

In summary, we have proposed a novel and sensitive fluorescence turn-on strategy for the assay of Hg^{2+} by employing metal-mediated MB, GO and Exo III. An obvious improvement on the selectivity of Hg^{2+} analysis was shown in this strategy. The design mechanism is mainly based on the preferential activity of Exo III to dsDNA compared to ssDNA, and the remarkable difference in the affinity of GO with ssDNA and mononucleotides. A dye labeled single-stranded oligonucleotide with a T-T mismatch was designed for the detection of Hg^{2+}. Under the optimal conditions, Hg^{2+} could be detected specifically and sensitively with a limit of 0.83 nM. More importantly, the proposed method was also applied to the determination of Hg^{2+} in human serum with satisfactory results, which suggests that this strategy has great potential for bioanalysis. Furthermore, this new approach will greatly contribute to the application of metal-mediated base pairs-based biosensors in biochemical and

biomedical studies as long as the collective problems involved in the application of GO could be properly addressed.

Acknowledgements

We gratefully acknowledge the support of the National Basic Research Program of China (973 Program, nos. 2011CB933600 and 2006CB933100), the National Natural Science Foundation of China (21275110), and the Fundamental Research Funds for the Central Universities (2042014kf0246)

References

1 K .Yoshizawa, E. B. Rimm, J. S. Morris, V. L. Spate, C. C. Hsieh, D. Spiegelman, M. J. Stampfer, W. C. Willett, *N. Engl. J. Med.,* 2002, **347**, 1755-1760.
2 H. H. Harris, I. J. Pickering, G. N. George, *Science*, 2003, **301**, 1203-1203.
3 J. L. Gómez-Ariza, F. Lorenzo, T. García-Barrera, *Anal. Bioanal. Chem*., 2005, **382**, 485-492.
4 B. M. W. Fong, T. S. Siu, J. S. K. Lee, S. Tam, *J. Anal. Toxicol.,* 2007, **31**, 281-287.
5 N. Bloom, W. F. Fitzgerald, *Anal. Chim., Acta.,* 1988, **208**, 151-162.
6 J. H. Huang, Y. F. Xu, *J.Org. Chem*., 2009, **74**, 2167-2170.
7 Y. K. Yang, K. J. Yook, J. Tae, *J. Am., Chem., Soc.,* 2005, **127**, 16760-16761.
8 J. M. Thomas, R. Ting, D. M. Perrin, *Org. Biomol., Chem.*, 2004, **2**, 307-312.
9 A. Ono, H. Togashi, *Angew. Chem.Int. Edit.*, 2004, **43**, 4300-4302.
10 C. D. Geary, I. Zudans, A. V. Goponenko, S. A. Asher, S. G. Weber, *Anal. Chem*., 2005, **77**, 185-192.
11 C. C. Huang, , Z. Yang, K. H. Lee, H. T. Chang, *Angew. Chem*.,2007, **119**, 6948-6952.
12 E. Meggers, P. L. Holland, W. B. Tolman, F. E. Romesberg, P. G. Schultz, *J. Am., Chem. soc*., 2000, **122**, 10714-10715.
13 S. Tyagi, D. P. Bratu, F. R. Kramer, *Nat. Biotechnol*., 1998, **14**, 303-308.
14 Y. X. Wang, J. S. Li, H. Wang, J. Y. Jin, J. H. Liu, K. M. Wang, , W. H. Tan and R. H, Yang, *Am. Chem.,* 2010, **82**, 6607-6612.
15 J. Anichina, Z. Dobrusin, D. K. Bohme, *J. Phys.Chem*., 2010, **114**, 15106-15112.
16 B. C. Ye, B. C. Yin, *Angew. Chem. Int. Edit.*, 2008, **47**, 8386-8389.
17 X. J. Xue, F. Wang, X. G. Liu, *J. Am. Chem. Soc*., 2008, **130**, 3244-3245.
18 X. W. Xu, J. Wang, K. Jiao, X. R. Yang, *Biosens. Bioelectron.*, 2009, **24**, 3153-3158.
19 J. S. Lee, M. S. Han, C. A. Mirkin, *Angew. Chem*.., 2007, **119**, 4171-4174.
20 A.Mandil, L. Lidrissi, A. Amine, *Microchim. Acta*.,2010, **170**, 299-305.
21 S. J. Liu, H. G. Nie, J. H. Jiang, G. L. Shen, R. Q. Yu, *Anal. Chem*., 2009, **81**, 5724-5730.
22 Z. Q. Zhu, Y. Y. Su, J. Li, D. Li, J. Zhang, S. P. Song, Y. Zhao, G. X. Li, C. H. Fan, *Anal. Chem*., 2009, **81**, 7660-7666.
23 K. S. Kim, Y. Zhao, H. Jang, S. Y. Lee, J. M. Kim, K. S. Kim, J. H. Ahn, P. Kim, J. Y. Choi, B. H. Hong, *Nature*, 2009, **457**, 706-710.
24 Z. Liu, J. T. Robinson, X. M. Sun, H. J. Dai, *J. Am. Chem. Soc*., 2008, **130**, 10876-10877.
25 N. Mohanty, V. Berry, *Nano. Lett*., 2008, **8**, 4469-4476.
26 S. J. He, K. K. Liu, S. Su, J. Yan, X. H. Mao, D. F. Wang, Y. He, L. J. Li, S. P. Song, C. H. Fan, *Anal., Chem*., 2012, **84**, 4622-4627.
27 J. R. Zhang, W. T. Huang, W. Y. Xie, T. Wen, H. Q. Luo, N. B. Li, *Analyst*, 2012, **137**, 3300-3305.
28 X. J. Xing, Y. Zhou, X. G. Liu, D. W. Pang, H. W. Tang, *Small*, 2014, **10**, 3412–3420.
29 H. J. Lee, Y. Li, A. W. Wark, R. M. Corn, *Anal. Chem.*, 2005, **77**, 5096-5100.
30 S. Y. Niu, Q. Y. Li, R. Ren, K. C. Hu, *Anal. Lett*., 2010, **43,** 2432-2439.
31 N. Kanayama, T. Takarada, M. Maeda, *Chem.Commun.*, 2011, **47**, 2077-2079.
32 H. Z. He, K. H. Leung, W. C. Fu, D. S. H. Chan, D. L, Ma, *Environ. Res. Lett.,* 2012, **7**, 044032.
33 H. Wang, Y. X. Wang, J. Y. Jin, R. H. Yang, *Anal. Chem.,* 2008, **80**, 9021-9028.