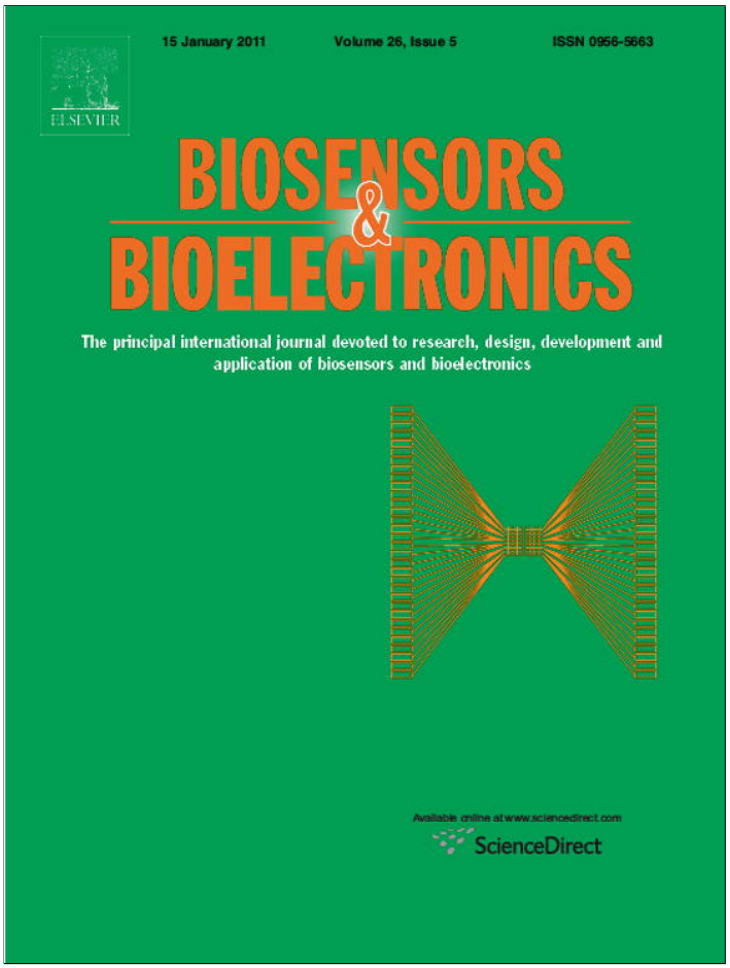

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Short communication

Ultrasensitive and selective detection of mercury(II) in aqueous solution by polymerase assisted fluorescence amplification

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ABSTRACT

A new label-free Hg^{2+} ions assay with polymerase assisted fluorescence amplification was developed. In this approach, a single strand template probe containing a mercury ion recognition sequence (MRS) which is composed of two thymine (T)-rich functional areas separated by a spacer of random bases was designed. In this sensing system, upon addition of Hg^{2+} ions, the complexation of Hg^{2+} ions with the MRS yielded a hairpin complex through T–Hg^{2+}–T base pairs. The formation of the hairpin structure initiated the replication of the template probe in the presence of the polymerase/dNTPs. Then the replication products of long duplex DNA probes were stained with a sensitive reagent SYBR Green I (SG). As a result, SG integrated with the duplex DNA probes and induced a distinguishable fluorescence enhancement in response to Hg^{2+} ions triggered replication reaction. Due to the fluorescence amplification step, this method exerted substantial enhancement in sensitivity and could be used for rapid and selective detection of low picomolar Hg^{2+} ions. It is expected that this cost-effective fluorescence sensor might hold considerable potential in the detection of Hg^{2+} ions in real environmental samples.

1. Introduction

Mercury ion (Hg^{2+}) is a highly toxic heavy metal ion and the most stable form of inorganic mercury. Hg^{2+} contamination is widespread and originates mainly from coal-burning power plants, oceanic and volcanic emissions, gold mining, and waste combustion. Hg^{2+} ions can accumulate in vital organs and cause cellular toxicity, which leads to variety of adverse health effects, such as damaged DNA, inhibited ligand–receptor interactions, disabled normal functions of the liver and kidney, disrupted immune system homeostasis, and even death (Bolger and Schwetz, 2002; Harris et al., 2003; Zheng et al., 2003). According to the US Environmental Protection Agency (EPA) standard, the maximum allowable level (MAL) for Hg^{2+} in drinking water is 10 nM (2.0 parts per billion (ppb)). Concerns over toxic exposure to Hg^{2+} ions have motivated exploration of new methods for monitoring aqueous Hg^{2+} ions. To work toward this goal, a number of methods for the detection of Hg^{2+} ions have been developed during the past years. Of these existing methods, atomic absorption/emission spectroscopy is the gold standard technique for detecting Hg^{2+} ions (Cizdziel and Gerstenberger, 2004). However, sophisticated instrumentation and complicated sample preparation processes are the limiting factors for its practical application. Other methods for assaying Hg^{2+} ions include inductively coupled plasma mass spectrometry (Jitaru and Adams, 2004), conjugated polymers (CP) (Kim and Bunz, 2006), DNAzymes (Liu and Lu, 2007; Hollenstein et al., 2008), organic chromophores (Yang et al., 2005; Nolan and Lippard, 2007; Avirah et al., 2007; Lee et al., 2007a; Liu et al., 2008a) and fluorophores (Huang and Chang, 2006). Problems encountered by these methods include the cross-sensitivity toward other metal ions, weak fluorescence enhancement factors, short emission wavelengths and time-consumption.

It is well-known that Hg^{2+} ions can selectively bind in between two DNA thymine (T) bases and promote these T–T mismatches to form stable T–Hg^{2+}–T base pair (Katz, 1952; Miyake et al., 2006). Hence, the methods for the analysis of Hg^{2+} ions, which were using the high specificity of mercury ion recognition sequence (MRS) have been elegantly developed (Ono and Togashi, 2004; Lee et al., 2007b; Liu et al., 2007; He et al., 2008; Lee and Mirkin, 2008; Li et al., 2008; Liu et al., 2008; Wang and Liu, 2008; Liu, 2008; Wang et al., 2008; Ye and Yin, 2008; Li et al., 2009; Liu et al., 2009; Ren and Xu, 2009; Wu et al., 2009; Xu et al., 2009; Yu et al., 2009; Zhang et al., 2009). Unfortunately, most of these sensors can hardly reach the available sensitive standard of MAL. Thus, the development of an ultrasensitive, environmentally friendly, yet simple method for Hg^{2+} ions detection is needed.

As we know, specific fluorometric dyes as powerful tools for quantitative analysis have been studied extensively for three decades (Labarca and Paigen, 1980). SYBR Green I (SG) is the most frequently used fluorometric dye for melocular detection because

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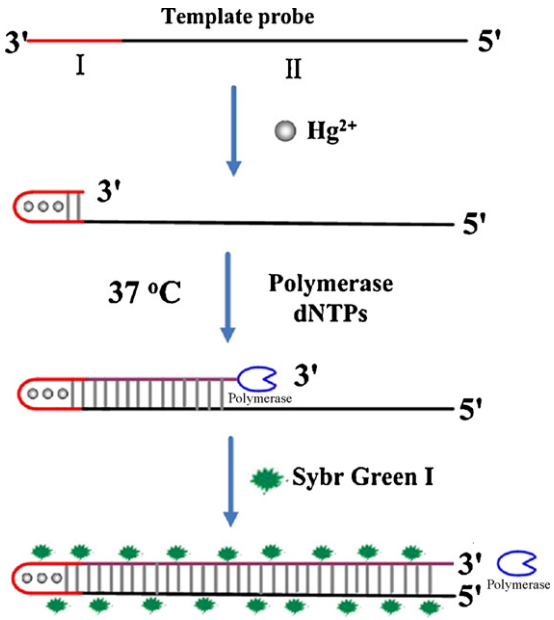
of its favorable photo physical properties, temperature stability, and high sensitivity (Webster et al., 2001; Diggle et al., 2003). Recent studies have revealed different affinities of SG integrate with single stranded DNA (ssDNA) and double-stranded DNA (dsDNA) (Zipper et al., 2004). SG interacts with ssDNA through electrostatic interactions, while it binds to dsDNA through both intercalation and minor groove binding. Hence, SG shows weak fluorescence upon binding to ssDNA, and exhibits enhancement at least 11-fold fluorescence intensity upon binding to dsDNA. The feature allows the label-free detection of DNA hybridization events. Motivated by this property of MRS and SG, Liu and his co-workers have successfully expanded the application of SG to detect Hg^{2+} ions in environmental samples (Liu et al., 2008; Wang and Liu, 2008).

Inspired by these previous theories, we exploited a strategy based on polymerase assisted fluorescence amplification which can analyze Hg^{2+} ions with a high specificity and an enhanced sensitivity. The results reveal that the developed sensor could be applied to monitor the existence of ultra low concentration of Hg^{2+} ions with high selectivity.

2. Materials and methods

2.1. Materials

2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, 99%) was obtained from Sigma (St. Louis, MO, USA). $Hg(ClO_4)_2$ was purchased from Alfa Aesar (Royston, England). Tris–HCl, $MgCl_2$, and dithiothreitol (DTT) were all obtained from BBI (Kitchener, Canada). These reagents were of analytical grade. Water (≥18.2 MΩ) used throughout the experiments was generated by a Milli-Q water purification system (Millipore, Bedford, MA, USA). The polymerase Klenow fragment exo- and deoxynucleotide solution mixture dNTPs were ordered from MBI (Fermentas, USA). SYBR Green (10,000×) was purchased from Invitrogen and diluted with dimethyl sulfoxide (DMSO) to a concentration of 20× before use. The probe DNA (5′-TA ACAAAATAAATACAATAAATAAATAAAATAACAATACCCTCAGCACAA CCCCAGATTCTTTCTTCCCTTGTTTGTTTCTGGGG-3′) was received from Shanghai Sangon (Shanghai, China).



Scheme 1. Schematic description of the polymerase-based fluorescence amplification strategy for Hg^{2+} ions detection.

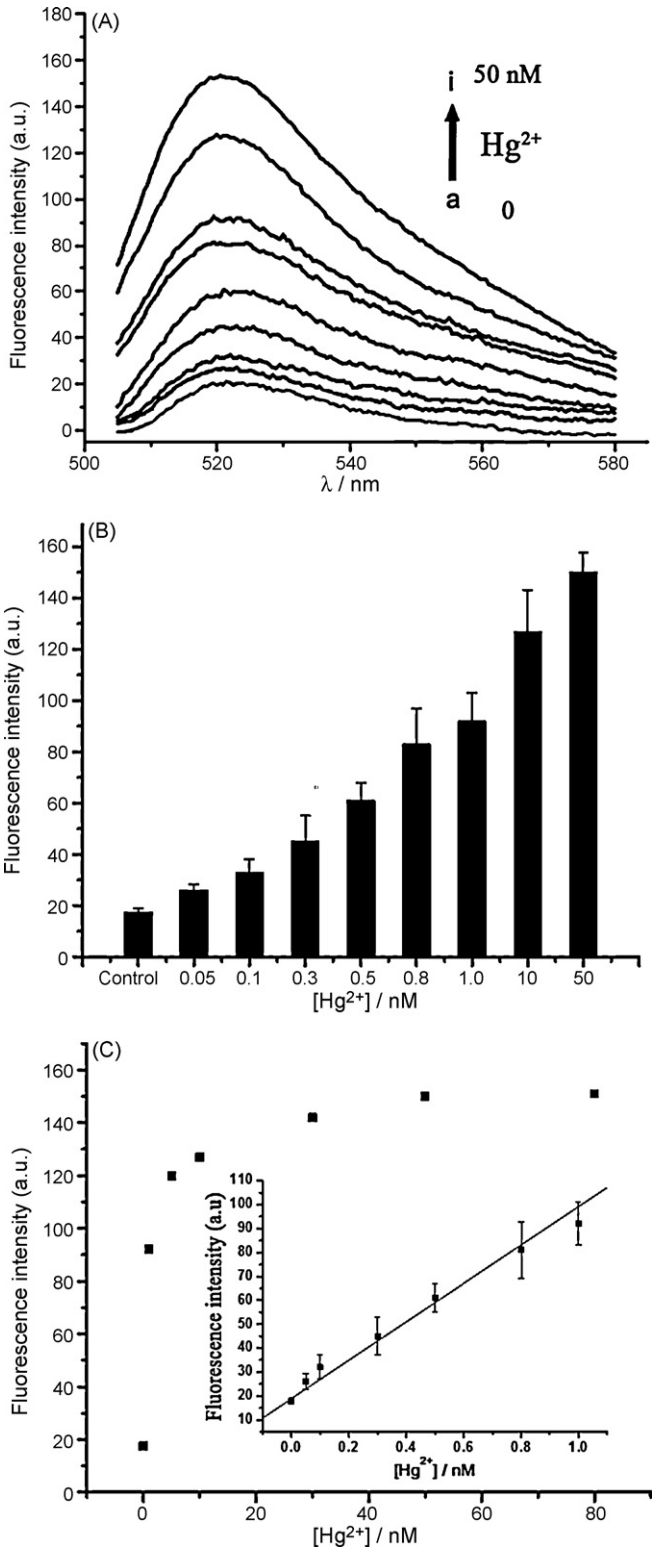


Fig. 1. (A) Fluorescence spectra of this sensing system in the absence and presence of different concentrations of Hg^{2+} ions: a, control; b, 50 pM; c, 100 pM; d, 300 pM; e, 500 pM; f, 800 pM; g, 1 nM; h, 10 nM; i, 50 nM. (B) The quantitative fluorescence intensity at 522 nm vs. $[Hg^{2+}]$. (C) The value of fluorescence intensity as a function of the concentration of Hg^{2+} ions from 0 to 50 nM. Inset: linear correlation of fluorescence intensity vs. Hg^{2+} ions concentration (from 50 pM to 1 nM). Assay protocols were same as above optimized conditions.

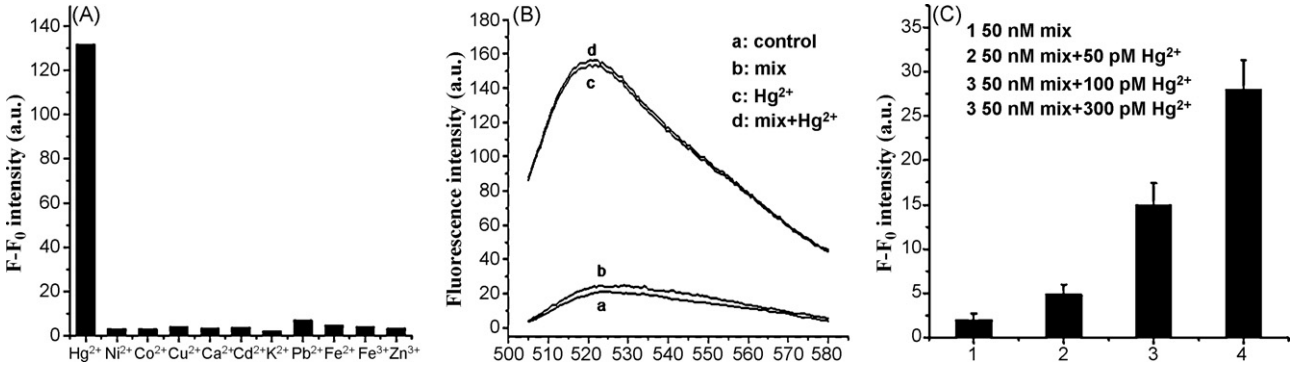


Fig. 2. (A) The difference in fluorescence intensity change ($F - F_0$) upon the addition of 50 nM Hg^{2+} ions and different metal ions with concentration of 1 μM. (B) Fluorescence spectra of sensoring systems containing template probe (20 nM) buffer, polymerase (10 units) and dNTPs (0.1 mM) as control (curve a), control with 10 types of mixture metal ions (2 μM mix) (curve b), control with Hg^{2+} ions (50 nM) (curve c) and Hg^{2+} ions (50 nM) with 10 types of mixture metal ions (2 μM mix) (curve d). (C) Analysis of Hg^{2+} ions in high ion background (50 nM mix) with fluorescence intensity discrimination $F - F_0$: (1) 50 nM mix; (2) 50 pM Hg^{2+} ions and 50 nM mix; (3) 100 pM Hg^{2+} ions and 50 nM mix; 300 pM Hg^{2+} ions and 50 nM mix.

2.2. Equipments and data analysis software

Fluorescence analysis was used by Perkin Elmer LS-55 (Indiana, USA). Regression analysis of the calibration data was performed by use of OriginPro 7.5.

2.3. Analysis of samples

The optimum detection system was prepared with the template probe DNA (20 nM) and different concentrations of Hg^{2+} ions in buffer (HEPES (10 mM), Tris–HCl (20 mM), $MgCl_2$ (10 mM), DTT (1 μM), pH 7.9). The use of DTT in buffer solution is to protect the thiohydroxy in polymerase and to insure the normal function of polymerase. After intensive mixing, the samples were incubated at room temperature for 10 min. Next, polymerase (10 units) and dNTPs (0.1 mM) were added to the solutions, which were incubated at 37 °C for 10 min. Then the samples were treated with 3 μL of SG (20×) and detected at 6 min later using a fluorescence spectrometer. To evaluate the sensitivity and detection range of this sensing system, and to confirm the selectivity of this method, assay protocols were the same as above mentioned.

3. Results and discussion

3.1. Sensing strategy

The sensing system was depicted in Scheme 1. A probe comprised of two domains was designed. Region I (marked in red) is the MRS that is composed of two T-rich functional areas separated by a spacer of random bases. On the other hand, due to the SG's characteristic of sequence-specificity, Region II (marked in black) is an optimum sequence that contains more dA and dT bases and used as the template for polymerase extension reaction (Zipper et al., 2004). Upon addition of Hg^{2+} ions into this sensing system, the MRS folds into a hairpin structure at 3′-end with Hg^{2+}-mediated base pairs. The formation of the hairpin structure initiates the replication of the template probe in the presence of the polymerase/dNTPs. As a result, the formed long duplex DNA probes were stained with a sensitive reagent SG, and then induced a distinguishable fluorescence enhancement.

3.2. The sensibility and detection range of designed sensing system

In our study, we evaluated the sensitivity of the Hg^{2+} ions fluorescence sensor under optimized conditions. Fig. 1A illustrates fluorescence spectra of sensing system after addition of different concentrations of Hg^{2+} ions (0–50 nM). In absence of Hg^{2+} ions, SG interacted with designed ssDNA probe and exhibited weak fluorescence due to its discrimination property of intercalation (excited at 494 nm, Fig. 1A (curve a)). With the increase of the Hg^{2+} ions concentration (up to 50 nM), an obvious change of the fluorescence spectrum of the sensing system appeared. The emission at 523 nm distinctly increased, and the maximum emission of the sensor gradually blue-shifted to 521 nm. This phenomenon is related to the change of oligonucleotide conformation, and it implied that more and more dsDNAs are appearing in solution (Zipper et al., 2004). We estimated that, induced by Hg^{2+} ions, Region I would generate a hairpin structure and then trigger the replication process. The hypothesis was further proved by gel electrophoresis experiments (Fig. S1). We noted that a new nucleic acid band with large molecular weight was appearing, and the colour of this band deepened in magnitude with respect to the concentration of Hg^{2+} ions. This result confirms the expectations from outlined in Scheme 1. Besides, we plotted fluorescence intensity at 522 nm against different Hg^{2+} ions concentration to quantitatively reflect the sensitivity of the Hg^{2+} ions fluorescence sensor (Fig. 1B). As shown in Fig. 1C, the assay range and calibration curves of this method were established. When the solution was treated with high concentration of Hg^{2+} ions (>50 nM), a plateau effect was achieved. A linear correlation ($R^2 = 0.993$) was obtained between the value of fluorescence intensity and the concentration of Hg^{2+} ions over the range 0–1.0 nM (Fig. 1C, inset). Then the limit of detection (LOD) for Hg^{2+} ions at a signal-to-noise ratio (S/N) of 3 was estimated to be 40 pM. To our knowledge, the sensibility of this method was obviously enhanced at least 10-fold over the previous reported fluorescence biosensors (Liu et al., 2008; Wang and Liu, 2008).

3.3. The selectivity of this assay

To test the selectivity of this novel method for Hg^{2+} ions detection, control experiments were executed to evaluate whether other environmentally relevant metal ions performed the similar function as the Hg^{2+} ions in the current biosensor. As revealed in Fig. 2A, one can find that only the Hg^{2+} ions samples (50 nM) show a significant higher fluorescence value change $F - F_0$ (where the value of F_0 and F represent the fluorescence intensity of the template probe DNA, SG, polymerase, dNTPs and buffer solution before and after the addition of metal ions, respectively) relative to the competing metal ions samples (1 μM) at identical conditions. In addition, the sensor was examined with the mixed metal ions. Fig. 2B describes

that the ten types of nonspecific ions with high concentration (2 μM mix) also give weak response to the sensor in contrast to mixed ions with Hg^{2+} ions (50 nM). The fluorescence spectra had changed noteworthily in shape and intensity after addition of Hg^{2+} ions. Furthermore, in order to provide quantitative evidence that the sensibility pattern is still feasible in a complex system, samples with co-existence of an ultra low concentration of Hg^{2+} ions and a remarkable high ion background of other metal ions were tested. Fig. 2C indicates that Hg^{2+} ions can be easily analyzed with the S/N ratio greater than 3 at a concentration of 50 pM, even in the presence of other metal ions at 1000-fold than the concentration of the analyte. These results clearly demonstrate that the sensor which is based on the ability of Hg^{2+} ions to form the stable structure of T–Hg^{2+}–T complexes is highly specific.

4. Conclusions

In summary, by using Hg^{2+} ions induced T–Hg^{2+}–T structure formation and by taking advantage of polymerase assisted SG emission fluorescence amplification, we have successfully introduced a simple, but ultrasensitive and selective assay for Hg^{2+} ions monitoring. Our method achieves the following virtues for analytical recognition. Firstly, the template probe containing T–T mismatch pair selectively bind the Hg^{2+} ions as an acceptor, thus this method has excellent specificity even in the high concentration (1000-fold) of other metal ions. Secondly, our strategy does not require the oligonucleotide labeled with any signaling chemical groups. The "signal-on" and label-free method could be rapid completed within half an hour. Lastly, due to the effect of fluorescence amplification step, our strategy obtained an extremely high sensitivity (40 pM). In view of these advantages, this high-efficient designed fluorescence sensor might hold considerable potential for the application of Hg^{2+} ions screening from a wide range of biological, toxicological, and environmental samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.03.013.

References

Avirah, R.R., Jyothish, K., Ramaiah, D., 2007. Org. Lett. 9, 121–124.
Bolger, P.M., Schwetz, B.A., 2002. New Engl. J. Med. 347, 1735–1736.
Cizdziel, J.V., Gerstenberger, S., 2004. Talanta 64, 918–921.
Diggle, C.P., Bentley, J., Kiltie, A.E., 2003. Nucleic Acids Res. 31, e83.
Harris, H.H., Pickering, I.J., George, G.N., 2003. Science 301, 1203–11203.
He, S., Li, D., Zhu, C., Song, S., Wang, L., Long, Y., Fan, C.H., 2008. Chem. Commun., 4885–4887.
Hollenstein, M., Hipolito, C., Lam, C., Dietrich, D., Perrin, D.M., 2008. Angew. Chem., Int. Ed. 47, 4346–4350.
Huang, C.C., Chang, H.T., 2006. Anal. Chem. 78, 8332–8338.
Jitaru, P., Adams, F.C., 2004. J. Chromatogr. A 1055, 197–207.
Katz, S.J., 1952. J. Am. Chem. Soc. 74, 2238–2245.
Kim, I.B., Bunz, U.H.F., 2006. J. Am. Chem. Soc. 128, 2818–2819.
Labarca, C., Paigen, K., 1980. Anal. Biochem. 102, 344–352.
Lee, M.H., Cho, B.K., Yoon, J., Kim, J.S., 2007a. Org. Lett. 9, 4515–4518.
Lee, J.S., Han, M.S., Mirkin, C.A., 2007b. Angew. Chem., Int. Ed. 46, 4093–4096.
Lee, J.S., Mirkin, C.A., 2008. Anal. Chem. 80, 6805–6808.
Li, D., Wieckowska, A., Willner, I., 2008. Angew. Chem., Int. Ed. 47, 3927–3931.
Li, T., Dong, S.J., Wang, E.K., 2009. Anal. Chem. 81, 2144–2149.
Liu, X.F., Tang, Y.L., Wang, L.H., Zhang, J., Song, S.P., Fan, C.H., Wang, S., 2007. Adv. Mater. 19, 1471–1474.
Liu, J., Lu, Y., 2007. Angew. Chem., Int. Ed. 46, 7587–7590.
Liu, L., Zhang, G.X., Xiang, J.F., Zhang, D.Q., Zhu, D.B., 2008a. Org. Lett. 10, 4581–4584.
Wang, J., Liu, B., 2008. Chem. Commun., 4759–4761.
Liu, B., 2008. Biosens. Bioelectron. 24, 756–760.
Liu, C.W., Hsieh, Y.T., Huang, C.C., Lin, Z.H., Chang, H.T., 2008. Chem. Commun., 2242–2244.
Liu, C.W., Lin, Y.W., Huang, C.C., Chang, H.T., 2009. Biosens. Bioelectron. 24, 2541–2546.
Miyake, Y., Togashi, H., Tashiro, M., Yamaguchi, H., Oda, S., Kudo, M., Tanaka, Y., Kondo, Y., Sawa, R., Fujimoto, T., Machinami, T., Ono, A., 2006. J. Am. Chem. Soc. 128, 2172–2173.
Nolan, E.M., Lippard, S.J., 2007. J. Am. Chem. Soc. 129, 5910–5918.
Ono, A., Togashi, H., 2004. Angew. Chem., Int. Ed. 43, 4300–4302.
Ren, X., Xu, Q.H., 2009. Langmuir 25, 29–31.
Wang, H., Wang, Y.X., Jin, J.Y., Yang, R.H., 2008. Anal. Chem. 80, 9021–9028.
Webster, J.R., Burns, M.A., Burke, D.T., Mastrangelo, C.H., 2001. Anal. Chem. 73, 1622–1626.
Wu, D.H., Zhang, Q., Chu, X., Wang, H.B., Shen, G.L., Yu, R.Q., 2009. Biosens. Bioelectron. 25, 1025–1031.
Xu, X.W., Wang, J., Jiao, Kui., Yang, X.R., 2009. Biosens. Bioelectron. 24, 3153–3158.
Yang, Y.K., Yook, K.J., Tae, J., 2005. J. Am. Chem. Soc. 127, 16760–16761.
Ye, B.C., Yin, B.C., 2008. Angew. Chem., Int. Ed. 47, 8386–8389.
Yu, C.J., Cheng, T.L., Tseng, W.L., 2009. Biosens. Bioelectron. 25, 204–210.
Zhang, X.R., Li, Y., Su, H.R., Zhang, S.S., 2009. Biosens. Bioelectron. 25, 1338–1343.
Zheng, W., Aschner, M., Ghersi-Egea, J.F., 2003. Toxicol. Appl. Pharmacol. 192, 1–11.
Zipper, H., Brunner, H., Bernhagen, J., Vitzthum, F., 2004. Nucleic Acids Res. 32, e103.