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A G-quadruplex based label-free fluorescent biosensor for lead ion

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ABSTRACT

Many Pb^{2+} biosensors based on Pb^{2+}-specific RNA-cleaving DNAzyme have been developed in the past years. However, many of them have limited practical use because of high cost (e.g., enzymes), complicated processing and the use of unstable molecules (e.g., RNA). In this study, a novel label-free fluorescent biosensor for Pb^{2+} was proposed based on Pb^{2+}-induced allosteric G-quadruplex (PS2.M). In the presence of K^+, N-methyl mesoporphyrin IX (NMM) could bind to K^+-stabilized G-quadruplexes, giving rise to high fluorescence. On addition of Pb^{2+}, Pb^{2+} competitively binded to K^+-stabilized G-quadruplexes to form more compact DNA folds. The Pb^{2+}-stabilized G-quadruplexes did not bind to NMM, which resulted in fluorescence decrease. This allowed us to utilize PS2.M for quantitative analysis of Pb^{2+} using the NMM–G-quadruplex system by convenient "mix-and-detect" protocol. The fluorescence emission ratio (F_0/F) showed a good linear response toward Pb^{2+} over the range from 5.0 nM to 1.0 μM with a limit of detection of 1.0 nM. This proposed biosensor was simple and cost efficiency in design and in operation with high sensitivity and selectivity. We validated the practicality of this biosensor for the determination of Pb^{2+} in lake water samples.

1. Introduction

The development of accurate, sensitive on-site chemo- and/or biosensors for lead ions (Pb^{2+}) is very important. For Pb^{2+} is one of the most toxic heavy metal ions and poses a severe risk to human health and the environment (Needleman, 2004). Since Lu's group demonstrated the first deoxyribozyme-based biosensor for Pb^{2+} (Li and Lu, 2000), the development of Pb^{2+} sensors with Pb^{2+}-specific functional DNA molecules has drawn great research interest worldwide. So far, two types of functional DNA molecules have been applied to design Pb^{2+} sensors. One is RNA-cleaving DNAzyme (such as 8–17 DNAzyme and GR-5 DNAzyme). The DNAzyme is composed of a substrate strand and an enzyme strand. Both strands are DNA, except that the substrate strand contains a single RNA linkage (ribonucleoside adenosine (rA)) that serves as the cleavage site. In the presence of Pb^{2+}, the substrate strand is cleaved into two pieces by the enzyme strand. Over the past 10 years, many highly sensitive and selective Pb^{2+} sensors based on Pb^{2+}-specific RNA-cleaving DNAzyme have been developed via colorimetric (Liu and Lu, 2003, 2004), fluorescent (Kim et al., 2011; Lan et al., 2010; Wang et al., 2009; Zhang et al., 2010; Zhao et al., 2011b; Wen et al., 2011), electrochemiluminescent (Zhu et al., 2009), electrochemical (Shen et al., 2008; Xiao et al., 2007; Yang et al., 2010) and scattering (Miao et al., 2011; Sun et al., 2011) techniques. Nevertheless, many of these systems have limited practical use because of, for example, high cost (e.g., enzymes), complicated processing and the use of unstable molecules (e.g., RNA).

As a substitute, Pb^{2+}-induced allosteric G-quadruplex oligonucleotide is the other functional DNA molecules for sensing Pb^{2+}. G-quadruplexes are unique higher-order structures, in which G-rich nucleic acid sequences form stacked arrays of G-quartets connected by Hoogsteen-type base pairing. However, compared with RNA-cleaving DNAzyme, less attention has been paid to G-quadruplexes for the design of Pb^{2+} biosensors (Li et al., 2010a,b, 2011a,b; Liu et al., 2009). For example, Wang's group (Li et al., 2010a) utilized the excellent efficiency of Pb^{2+} for stabilizing G-quadruplexes to construct a Pb^{2+}-driven DNA molecular device based on a DNA duplex–quadruplex exchange. However, the limit of detection for Pb^{2+} was only 20 nM. In addition, the need of hemin as cofactor (Li et al., 2010b, 2011a) or fluorophore to be labeled (Liu et al., 2009) might limit their application. So, the design of convenient and inexpensive approaches for the sensitive and selective detection of Pb^{2+} with rapid, easy manipulation is still in great demand.

N-methyl mesoporphyrin IX (NMM) is an unsymmetrical anionic porphyrin characterized by a pronounced structural selectivity for G-quadruplexes but not for duplexes, triplexes or single-stranded forms (Arthanari et al., 1998). It is weakly fluorescent

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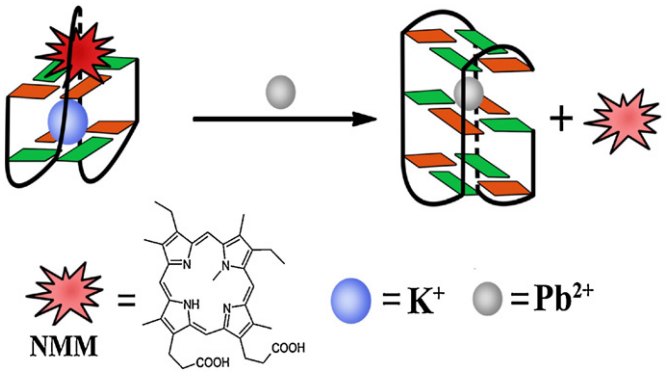
by itself, but exhibits a dramatic fluorescence enhancement upon binding to quadruplex DNA. However, similar properties were not at all evident in the presence of duplex, triplex or single-stranded nucleic acid structures (Hu et al., 2010, 2011; Qin et al., 2010; Zhao et al., 2011a). This allows us to utilize NMM as a fluorescent probe to indicate the formation of the G-quadruplex structure and monitor its conformational change. G-quadruplexes are four-stranded DNA structures that are generally stabilized by monovalent cations such as K^+ and Na^+ (Sen and Gilbert, 1990). In comparison with K^+, Pb^{2+} has a higher efficiency for stabilizing G-quadruplexes due to the formation of more compact DNA folds (Li et al., 2009, 2010b; Kotch et al., 2000). Inspired by this fact, we supposed that Pb^{2+} could competitively bind to K^+-stabilized G-quadruplexes, resulting in sharp changes in their structures and functions. For this reason, herein we utilized NMM to serve as a fluorescence reporter and a widely used G-quadruplex (i.e., PS2.M) as the recognition element to develop a novel label-free sensitive fluorescent biosensor for Pb^{2+} detection.



Scheme 1. Schematic of utilizing Pb^{2+}-induced allosteric G-quadruplex (PS2.M) for label-free fluorescent detection of Pb^{2+}.

2. Experimental

2.1. Chemicals

DNase- and RNase-free water treated by 0.1% diethylpyrocarbonate (DEPC) and PAGE purified G-rich oligonucleotide (PS2.M: GTGGGTAGGGCGGGTTGG) were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Pb^{2+} standard solution (1000 μg/mL) was purchased from General Research Institute for Nonferrous Metals of China. Tris(hydroxymethyl)aminomethane (Tris) was bought from Sinopharm Group Co. Ltd. (Shanghai, China). NMM was acquired from J&K Scientific Ltd. (Beijing, China). All other chemicals were of analytical grade. The water used was purified by Millipore Milli-Q (18 MΩ/cm).

Stock solution of PS2.M (10 μM) was prepared by DNase- and RNase-free water to prevent the degradation of DNA by enzyme. The stock solution of NMM (1.8 mM) was prepared in dimethyl sulfoxide (DMSO), stored in darkness at −20 °C. Before use, the oligonucleotide solution and NMM solution were diluted to required concentration with Tris–HCl buffer (10 mM, pH 7.6) and water, respectively. The concentration of NMM was measured on a Lambda 750 spectrophotometer (PE, USA) by absorbance at 379 nm assuming an extinction coefficient of $1.45 \times 10^5\ M^{-1}\ cm^{-1}$ (Hu et al., 2010, 2011; Zhao et al., 2011a).

2.2. DNA melting experiments

The UV melting curves of G-quadruplex DNA (0.13 μM) in 10 mM Tris–HCl buffer (pH 7.6) containing 3.3 mM KCl were recorded on a Lambda 750 spectrophotometer equipped with a temperature-controlled water bath. The absorbance was always monitored at 295 nm, which is the characteristic absorption of the quadruplex structures (Mergny et al., 1998). In a typical experiment, 1 mL of sample solution was added into a 1-cm path length quartz cuvette, and then covered with a layer of paraffin oil to prevent evaporation. The solution was held at 15 °C for 2 min, and then slowly heated to 60 °C with a rate of 0.5 °C/min. The background of the buffer solution was subtracted from the collected data. The resulting melting curves were analyzed to obtain the melting temperature (T_m) via the first derivative.

2.3. Fluorescence measurement for Pb^{2+}

To 300 μL Tris–HCl working buffer (10 mM, 2 mM KCl, pH 7.6) containing 0.13 μM PS2.M oligonucleotide and different concentration of Pb^{2+} was added 10 μL NMM solution (18 μM), 30 min later the fluorescence assay was carried out by a Cary fluorophotometer (Varian, USA).

3. Results and discussion

3.1. Principle of sensing Pb^{2+}

Pb^{2+}-stabilized G-quadruplexes have been reported to have shorter M—O and O—O bonds than those stabilized by K^+ (Kotch et al., 2000), which indicates that Pb^{2+} has higher efficiency than K^+ at stabilizing G-quadruplexes (Kotch et al., 2000; Li et al., 2009). It is envisioned that Pb^{2+} could competitively bind to K^+-stabilized G-quadruplexes, resulting in sharp changes in their structures and functions. Here, we utilized K^+-stabilized PS2.M to sense Pb^{2+} by fluorescence technique. The basic design of this biosensor for Pb^{2+} is shown in Scheme 1. In the presence of K^+, PS2.M folded into a unimolecular G-quadruplex (Li et al., 2010b), which was allowed to bind NMM, giving rise to high fluorescence. On addition of Pb^{2+}, Pb^{2+} could competitively bind to K^+-stabilized G-quadruplexes to form more compact DNA folds. The Pb^{2+}-stabilized G-quadruplexes did not bind with NMM, which resulted in fluorescence decrease.

To validate that Pb^{2+} has higher efficiency than K^+ at stabilizing G-quadruplexes, the UV melting curves of PS2.M stabilized by different cations were recorded. As shown in Fig. S1 (see supporting information), the T_m value of PS2.M increased by 10 °C (from 32 °C to 42 °C) on addition of Pb^{2+}, which may rationally interpret why Pb^{2+} can competitively bind to PS2.M even in the presence of 3300-fold K^+.

Since Pb^{2+} has higher efficiency than K^+ at stabilizing G-quadruplexes, we hypothesized that the Pb^{2+}-stabilized G-quadruplexes did not bind with NMM due to the conformational change. To test the hypothesis, fluorescence spectroscopy was utilized to reveal the binding of G-quadruplexes to NMM, reflected by the fluorescence signal change. Fig. 1 shows the fluorescence spectra (excited at λ = 399 nm) of NMM in different condition. NMM was weakly fluorescent by itself (curve 1 in Fig. 1), but exhibited a dramatic fluorescence enhancement upon binding to K^+-stabilized G-quadruplexes (curve 2 in Fig. 1). On addition of nano-mole level of Pb^{2+}, the fluorescence decreased (curve 3 in Fig. 1) due to the formation of Pb^{2+}-stabilized G-quadruplexes, which suggested NMM was released from the G-quadruplexes owing to the conformational change. A similar rule was also obtained by Dong' group that Pb^{2+} induced K^+-stabilized G-quadruplex (PS2.M) to undergo a conformational change and released hemin from the G-quadruplexes, accompanied by a decrease in the peroxidase-like catalytic activity of K^+-stabilized G-quadruplexes (with hemin as a co-factor) (Li et al., 2009, 2010b). Hence, PS2.M could be chosen to serve as the

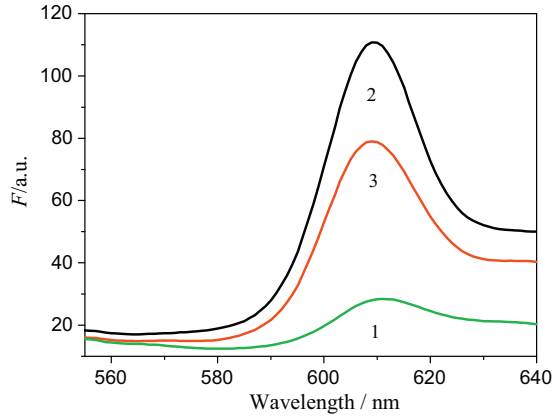


Fig. 1. Fluorescence spectra of 10 mM Tris–HCl (pH 7.6) containing 0.6 μM NMM (curve 1), 0.6 μM NMM + 0.13 μM PS2.M + 3.3 mM K^+ (curve 2), 0.6 μM NMM + 0.13 μM PS2.M + 3.3 mM K^+ + 100 nM Pb^{2+} (curve 3). The fluorescence spectra were taken after incubating the mixture for 30 min.

sensing element for label-free fluorescent detection of Pb^{2+} when using NMM as a fluorescent reporter.

3.2. Optimization of sensing conditions

The fluorescence intensity was first studied as a function of incubation time to investigate the kinetics of the formation of NMM–G-quadruplex complexes in the presence of different cations. As illustrated in Fig. S2 (see supporting information), the fluorescence intensities were increased until the incubation time reached 30 min in the absence or presence of Pb^{2+}. This indicated that it took about 30 min to form stable NMM–G-quadruplex complexes. Thus, an incubation time of 30 min was employed in the next experiments.

A further study was performed to investigate the pH-dependence of fluorescence response ($\Delta F = F_0 - F$) of NMM–G-quadruplex complexes in the presence of Pb^{2+} (see Fig. S3 in supporting information). The fluorescence response (ΔF) increased from pH 7.0 and reached a maximum at pH 7.6, followed by a decrease to pH 8.0. Accordingly, a buffer solution of pH 7.6 was selected.

Fluorescence titration experiments were then performed to obtain the maximal fluorescence signal of NMM–G-quadruplex complexes. The fluorescence signal was enhanced markedly as the NMM concentration was increased, and little change was observed when the concentration exceeded 0.6 μM (Fig. 2a). The fluorescence signal increased promptly as the K^+ concentration was increased, when K^+ concentration was higher than 2.0 mM, the signal was no more increase (Fig. 2b). Therefore, 0.6 μM of NMM and 2.0 mM of K^+ were used to ensure a good signal-to-background ratio.

3.3. Sensitivity and selectivity

The fluorescence signals toward different concentration of Pb^{2+} were measured under the optimal conditions. The fluorescence intensity decreased with increasing the concentration of Pb^{2+} (Fig. 3). The inset in Fig. 3 depicts the emission intensity ratio (F_0/F) plotted against the concentration of Pb^{2+}, showing a good linear response toward Pb^{2+} over the range from 5.0 nM to 1.0 μM ($R^2 = 0.998$). The limit of detection for Pb^{2+} was estimated to be 1.0 nM based on 3 s rule. Relative to other G-quadruplexes-based sensors, this biosensor provided better or comparable sensitivity toward Pb^{2+} ions (see Table S1 in supporting information). The maximum contamination level for Pb^{2+} in drinking water defined by the

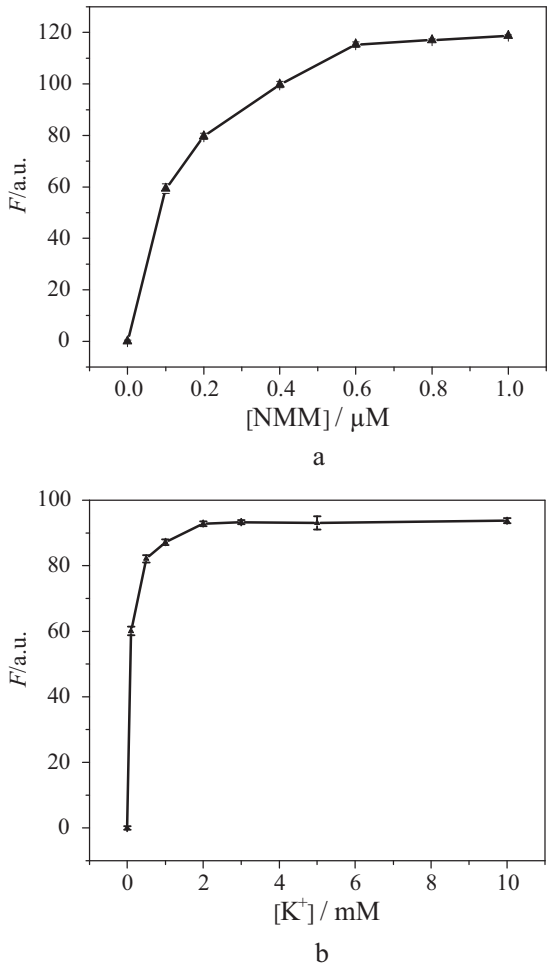


Fig. 2. (a) Fluorescence titration of PS2.M with NMM. Experiments were carried out in a 10 mM Tris–HCl (pH 7.6) buffer containing 0.13 μM PS2.M + 3.3 mM K^+. (b) Fluorescence titration of PS2.M with K^+. Experiments were carried out in a 10 mM Tris–HCl (pH 7.6) buffer containing 0.13 μM PS2.M + 0.6 μM NMM. The background fluorescence of free NMM was subtracted from the sample solution. The excited and emission wavelength was set at 399 nm and 610 nm, respectively.

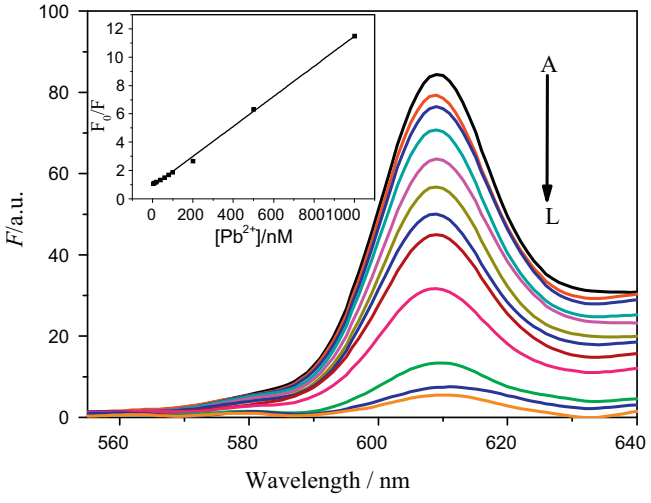


Fig. 3. Fluorescence spectra of 10 mM Tris–HCl (pH 7.6) containing 0.6 μM NMM, 0.13 μM PS2.M and 2.0 mM K^+ in the presence of 0 nM (A), 5 nM (B), 10 nM (C), 20 nM (D), 40 nM (E), 60 nM (F), 80 nM (G), 100 nM (H), 200 nM (I), 500 nM (J), 1000 nM (K), 2000 nM (L) Pb^{2+}. F_0 and F are the fluorescence intensity in the absence and presence of Pb^{2+}, respectively. The excited and emission wavelength were set at 399 nm and 610 nm, respectively. The background fluorescence of free NMM was subtracted from the sample solution.

Table 1
Analysis Pb^{2+} in lake water samples by the proposed biosensor.[a]

Sample	Original value (nM)	Pb^{2+} added (nM)	Pb^{2+} found (nM)	Recovery (%)
Lake water	9.3	20.0	27.7	92.0
		60.0	63.5	90.3
		100.0	103.0	93.7

[a] The results were mean value of three experiments.

U.S. Environmental Protection Agency is 72 nM, so the proposed Pb^{2+} biosensor is sufficient for Pb^{2+} monitoring in the environment.

The selectivity of this biosensor was evaluated by testing the fluorescence emission ratio (F_0/F) in the presence of individual metal ion. As shown in Fig. 4a, although the concentration of other tested ion was larger than that of Pb^{2+}, only Pb^{2+} caused a considerable large fluorescence emission ratio. We further challenged this biosensor with 80 nM Pb^{2+} in the coexistence of other metal ion. As shown in Fig. 4b, the response of this biosensor to Pb^{2+} was almost unaffected by the coexistence of other tested metal ion. These results indicated this biosensor was highly selective for Pb^{2+}.

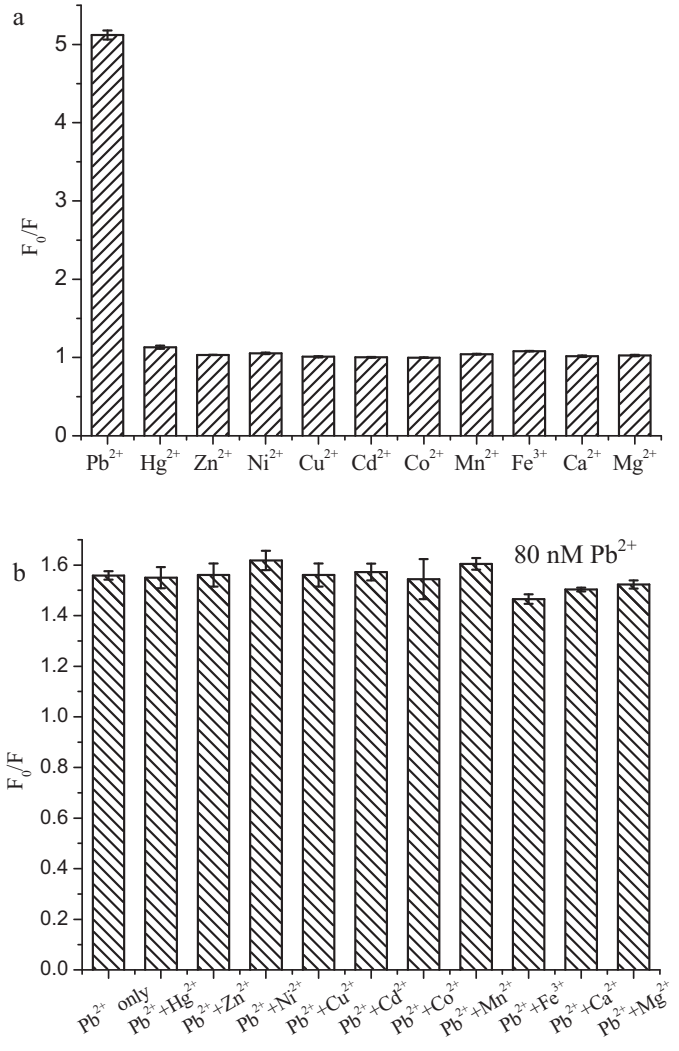


Fig. 4. Interference study. (a) The fluorescence emission ratio (F_0/F) in the presence of individual metal ion (500 nM Pb^{2+}, 500 nM Hg^{2+}, 1 μM Zn^{2+}, 1 μM Ni^{2+}, 1 μM Cu^{2+}, 3 μM Cd^{2+}, 5 μM Co^{2+}, 10 μM Mn^{2+}, 10 μM Fe^{3+}, 100 μM Ca^{2+}, 100 μM Mg^{2+}). (b) The fluorescence emission ratio (F_0/F) in the presence of 80 nM Pb^{2+} (the first bar) and at the coexistence of 80 nM Pb^{2+} with 100 nM Hg^{2+}, 1 μM Zn^{2+}, 1 μM Ni^{2+}, 1 μM Cu^{2+}, 1 μM Cd^{2+}, 1 μM Co^{2+}, 10 μM Mn^{2+}, Fe^{3+}, 100 μM Ca^{2+}, 100 μM Mg^{2+}, respectively.

3.4. Application

To demonstrate the application potential of this biosensor in environmental analysis, we applied PS2.M to analyze real freshwater samples (lake water). The lake water samples were filtered by a 0.22 μm filter. The same procedure was conducted for measurements by using lake water samples instead of Pb^{2+} standard solution. The results are listed in Table 1, indicating that the proposed biosensor can be applied to analyze Pb^{2+} in environmental samples.

4. Conclusions

In summary, we have demonstrated a quadruplex-based fluorescent biosensor for Pb^{2+} by convenient "mix-and-detect" protocol with high sensitivity and selectivity. This biosensor could detect as low as 1 nM of Pb^{2+} by simply mixing PS2.M DNA, NMM, K^+ and Pb^{2+}. The G-quadruplex DNA was low cost and stable as comparing with RNA-cleaving DNAzyme. The fluorescence reporter NMM was commercially available and cheap. The assay could be accomplished by using a common fluorophotometer. No sophisticated experimental technique or any chemical modification of DNA was required, which offers the advantages of simplicity in design and in operation and cost efficiency from an application standpoint. Our proposed biosensor could be applied to analyze Pb^{2+} in environment.

Acknowledgments

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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.2012.02.031.

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