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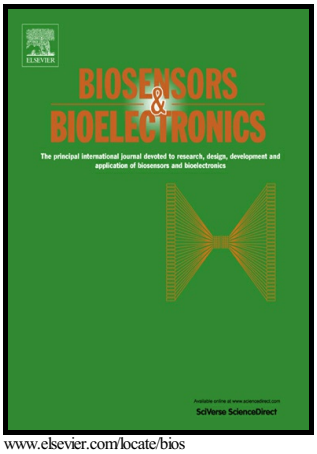

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Practical, highly sensitive, and regenerable evanescent-wave biosensor for detection of Hg^{2+} and Pb^{2+} in water

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Abstract

A "turn-on" evanescent-wave fiber biosensor based on functional nucleic acids was developed for on-site detection of Hg^{2+} and Pb^{2+} in environmental water samples. A two-step strategy was conducted to take advantage of the rapid binding of T–T mismatches with Hg^{2+} and the cleavage of a substrate by 8–17 DNAzyme with Pb^{2+} in solution, as well as sensitive DNA detection on a liquid–solid interface based on evanescent wave. The biosensor demonstrated high sensitivity (with a detection limit of 22 pM for Hg^{2+} and 20 nM for Pb^{2+}) and selectivity without any signal amplification, rapid detection (within 13 min per test), low cost (10–20 Chinese yuan per sample), and multiple-cycle regeneration (at least 18 times). The method was also successfully applied on Hg^{2+} and Pb^{2+} detection in real environmental water samples.

Keyword: Fluorescent biosensor, Functional nucleic acids, Heavy metal ions, On-site, Regeneration

1. Introduction

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Heavy metal pollution has caused serious harm to the environment and human health, and has become a worldwide environmental problem (Duruibe et al. 2007; Liu et al. 2011). Rapid industrialization and urbanization over the last two decades have worsened heavy metal pollution, particularly in developing countries such as China. Between 2009 and 2011, more than 30 major heavy-metal poisoning incidents occurred in mainland China, and thousands of people, including hundreds of children, were poisoned and suffered irreversible harms (Zhang et al. 2011).In these heavy metal pollution incidents, lead and mercury ions were two of the most common pollutants (He et al. 2013).

Hg^{2+} and Pb^{2+} ions interfere with a variety of body processes; these ions are toxic to many organs and tissues, including the heart, bones, intestines, kidneys, and reproductive and nervous systems, particularly causing potentially permanent learning and behavior disorders in children (Wikipedia.org 2015a, b). These heavy metals cause serious health risks to humans even in small doses (de la Riva et al. 2002; Leopold et al. 2010; Leopold et al. 2009; Sanders et al. 2012). According to the Minister of Health of the People's Republic of China (MOH), the maximum tolerable levels of Hg^{2+} and Pb^{2+} in drinking water were 0.001 mg/L (5 nM) and 0.01 mg/L (48 nM) (China 2007), respectively, which are more stringent than the standards defined by the US Environmental Protection Agency (EPA) (10 nM for Hg^{2+} and 72 nM for Pb^{2+}) (EPA 2014). Although conventional methods, such as atomic adsorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS), can detect Hg^{2+} and Pb^{2+} at very low concentrations, they are difficult to use for on-site applications because of their poor portability and high cost (Batista et al. 2011; Jarzyńska and Falandysz 2011; Leopold et al. 2009; Louie et al. 2012; Rodrigues et al. 2011). In Tsinghua University, a single heavy metal ion analysis usually costs as much as 10–30

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US dollars (60–200 Chinese yuan) with different instruments. Furthermore, in the sudden heavy metal pollution, these instruments are difficult to detect pollutants in real time. Therefore, exploring a new simple, fast, cheap and real-time heavy metal detection technology is important (Ligler 2008; Martín-Yerga et al. 2013).

Exploring approaches for portable detection of Hg^{2+} and Pb^{2+} is an ongoing challenge for researchers. Many researchers have attempted to develop different technologies for functional nucleic acid (FNA) sensing based on fluorescence (Du et al. 2012; Lan et al. 2010; Liu et al. 2012; Wang et al. 2008a), electrochemistry (Shi et al. 2014; Xiao et al. 2007b), and other techniques (Chen et al. 2014; Liu et al. 2007; Teh et al. 2014; Wang et al. 2008b). For Hg^{2+} detection, the most widely used FNA is thymine (T)-rich DNA, which forms T–Hg^{2+}–T mismatches (Li et al. 2016). For Pb^{2+} detection, the RNA-cleaving Pb^{2+}-specific 8–17 DNAzyme is the main FNA used (Liu et al. 2009). Wang et al. reported a "turn-on" fluorescent sensor for Hg^{2+} based on T–Hg^{2+}–T mismatch. In the presence of Hg^{2+}, the long T-rich DNA strand with a quencher forms a hairpin structure and releases a short complementary strand with a fluorophore, which previously hybridized with the long T-rich strand; the biosensor showed high sensitivity with a low detection limit of 3.2 nM (Wang et al. 2008a). By contrast, Liu et al. reported that the 8–17 DNAzyme can cleave the substrate with Pb^{2+} and subsequently cause a significant increase in fluorescence intensity by releasing the cleaved DNA strands with a fluorophore. The detection limit for Pb^{2+} was below 10 nM at room temperature (Liu et al. 2012). Many works have been focused on the development of this type of biosensors in homogeneous solution to improve its performance in terms of sensitivity and signal output, thereby demonstrating that biosensors are promising for Hg^{2+} and Pb^{2+} detection in aqueous solutions.

A recent review suggested that immobilizing DNA probes can enhance repeatability and stability

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of biosensors (Borisov and Wolfbeis 2008). Numerous laboratories have developed electrochemical DNA biosensors for Hg^{2+} and Pb^{2+} detection because of the ease of attaching DNA to electrodes (Kong et al. 2009; Xiao et al. 2007a). Kong et al. developed an ultrasensitive electrochemical biosensor by fixing T-rich DNA on gold electrodes; this biosensor was successfully regenerated three times and achieved a detection limit of 0.5 nM (Kong et al. 2009). Xiao et al. demonstrated a convenient measurement method for detecting Pb^{2+} based on immobilized 8–17 DNAzyme. The biosensor had a detection limit of approximately 0.3 μM (62 ppb) and required a long time (30 min) to cleave the substrate strands on electrodes (Xiao et al. 2007a). Unfortunately, electrochemical biosensors for Hg^{2+} and Pb^{2+} are difficult to use for on-site monitoring (Botasini et al. 2013; Liu et al. 2008; Martín-Yerga et al. 2013). Fluorescence is an attractive alternative because of its high sensitivity, high selectivity, and rapid detection. Chip-based fluorescent biosensors have attracted considerable attention in recent years because the chip on which DNAs are stabilized can be used many times after regeneration; this reusability is useful for on-site monitoring (Abel et al. 1996; Jung et al. 2001). However, few studies have reported the detection of Hg^{2+} and Pb^{2+} by immobilized DNA on a chip as fluorescent biosensors. Long et al. developed a “turn-off” mode fiber biosensor using evanescent wave to excite fluorescent signal, which showed a detection limit of 2.1 nM for Hg^{2+}(Long et al. 2011). Nevertheless, the inherent limitation of the “turn-off” mode may result in false-positive signals from sample matrix. Similar to the methods of Xiao and Liu, Du et al. and Liu et al. immobilized T-rich DNA and 8–17 DNAzyme, respectively, on chips, and developed fluorescent chip-based sensors, which worked at a “turn-on” mode with a detection limit of 8.6 nM for Hg^{2+} and 1 nM for Pb^{2+}, but the detection required two time-consuming steps of DNA hybridizations (Du et al. 2012; Liu et al. 2012). The urgent demand for biosensors with higher

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sensitivity, rapid detection (i.e., within 10 min), and low cost has been suggested in a recent publication (Shen et al. 2014).

Based on the above studies, the detection of Hg^{2+} based on T–T mismatch and the detection of Pb^{2+} based on DNAzyme can release a free single-stranded DNA (ssDNA) molecule. Motivated by this finding, we found that these two metal ions could be detected using a similar method based on the released ssDNA. In this study, we reported a fiber-based fluorescent biosensor for "turn on" detection of Hg^{2+} and Pb^{2+} by evanescent wave. The biosensor achieved high sensitivity and selectivity without any signal amplification, rapid detection (within 7 min for Hg^{2+} and 13 min for Pb^{2+} per test), and multiple-cycle regeneration (at least 18 times). The method was also successfully applied for Hg^{2+} and Pb^{2+} detection in real environmental water samples. To the best of our knowledge, this study is the first to achieve such short detection time, numerous regeneration times, and suitable detection limits. We speculate that this detection strategy is practical for the rapid detection of Hg^{2+} and Pb^{2+} in aqueous solutions.

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2. Experimental section

2.1 Reagents

Glycine, 3-aminopropyltriethoxysilane (APTS), and glutaraldehyde (GA) were purchased from Sigma-Aldrich (Shanghai, China). Tris(hydroxymethyl) aminomethane (Tris), acetic acid (HAc), and sodium nitrate ($NaNO_3$) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The standard solutions containing mercury or lead dissolved in 1 M HNO_3 were purchased from the National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials (http://www.ncatn.com/indexen.htm). The mercury and lead concentration in standard solution was

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1,000 ± 7 μg/mL, corresponding to 5 ± 0.035 μmol/mL. The concentration of mercury ion and lead ion was confirmed by ICP-MS, respectively. All salts, namely, $Cu(NO_3)_2$, $Pb(NO_3)_2$, $MnCl_2$, $AlCl_3$, $FeCl_2$, $MgCl_2$, $CdCl_2$, $Hg(NO_3)_2$, $AgNO_3$, $Zn(NO_3)_2$, and $FeCl_3$ were analytical grade and used without further purification.

All HPLC-grade oligonucleotides were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The DNAs for the detection of mercury are as follows. The aminated DNA sequence was 3′-NH_2-$(CH_2)_{12}$-TTTT TTTT TTTT TT-5′ (TS). The labeled DNA sequence was 5′-BHQ2-TTTT TTTT TTTT TT-3′ (TB). The complementary fluorescently labeled DNA sequence was 5′-AAAA AAAA AAAA AA-Cy3-3′ (AF). The DNAs for the detection of lead are as follows. The aminated DNA sequence was 5′-NH_2-T_6-ATA GTG AGT-3′ (17PS). The labeled DNA sequence was 5′-ACAGACATCATCTCTGAAGTAGCGCCGCCGTATAGTGAGT-BHQ2-3′ (17EB). The complementary fluorescently labeled DNA sequence was 5′-Cy3-ACTCACTATrA GGAAGAGATGATGTCTGT-BHQ-3′ (17SF).

The control DNA sequence was 5′-TCG GCT TAG CCT-Cy3-3′. The oligomer samples were dissolved in buffer consisting of 10 mM Tris–HAc and 50 mM $NaNO_3$, with pH 7.2. The 100 μM stock solutions of each DNA were prepared by dissolving DNA in buffer. DNA stock solutions were stored at −25 °C until use.

Equal volumes of TB (50 nM) or 17EB (50 nM) and their corresponding complementary DNA AF (50 nM) or 17SF (50 nM) were mixed and heated in a dry bath at 90 °C for 5 min. The mixtures were then slowly cooled to room temperature to ensure complete hybridization. The hybridized DNA was subsequently stored at 4 °C.

A stock solution of 1 μM Hg^{2+} and Pb^{2+} was prepared by diluting standard mercury and lead

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solutions in 0.5 M HNO_3, respectively. All other solutions were prepared in ultrapure water with 18.2 MΩ.

2.2 Evanescent wave all-fiber biosensor platform

The portable evanescent wave all-fiber biosensor platform was used for detection, as described in an earlier paper (Long et al. 2011). A more detailed introduction could find in support material and shown in Figure 1S.

2.3 Immobilization of DNA onto fiber optical sensor surface

The tapered fiber was particularly fabricated as the optical sensor, as previously described (Long et al. 2008). The fiber was initially cleaned with a piranha solution (H_2SO_4/H_2O_2, 3:1 [v/v]) and then immersed in dehydrated toluene mixed with 2% (v/v) APTS for 60 min for amination. Afterward, the fiber was washed three times with toluene and washed three times with ethanol, and then dried in an oven at 200 °C for 60 min (Halliwell and Cass 2001). Subsequently, the aminated fiber was immersed in 4.0% (v/v) GA solution for 1 h, washed with ultrapure water, and dried with N_2. After these preparations, the fiber was immersed in 250 μL solution composed of 125 nM TS or 125 nM 17PS in 50 mM Tris–HCl and 100 mM NaCl (pH 7.4) at 4 °C overnight to immobilize TS or 17PS onto the fiber surface. The fiber surface was then dipped in 20 mM glycine solution for 1 h to block the remaining aldehyde sites (Razumovitch 2009). All reactions were performed at room temperature unless otherwise indicated.

2.4 Mercury or lead ion analysis

Different volumes of stock metal ion solutions were mixed in 300 μL buffer with 25 nM AF + TB or 17EB + 17SF oligomer hybrids to react for 8 min. The mixture was pumped into the chamber at a rate of 300 μL/min for 24 s and let the probes to bind with TS or 17PS. Meanwhile, the fluorescence

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signal was recorded until no change was detected in the signal intensity. The sensor surface was regenerated by flushing with the following solutions in sequence: ultrapure water for 24 s, 30% urea and 0.2% sodium dodecyl sulfate (SDS) solution (twice, each time for 24 s and a 24 s interval between washes), ultrapure water for 24 s and buffer solution for 24 s.

2.5 Selectivity test

To investigate the practical potential for environmental water samples, we studied the selectivity of the biosensor by examining anti-interference effects of 12 common types of monovalent (Ag^{+}), divalent (Ca^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+},Mn^{2+}, Mg^{2+}, and Pb^{2+}(or Hg^{2+})), and trivalent (Fe^{3+}, Al^{3+}) metals on our sensor at 10 μM. The salt solutions at the specified concentrations were prepared and were then used to detect the signal intensities, which were in turn compared with that obtained from Hg^{2+} solution at 10 nM concentrations. Similar to the selectivity tests of Hg^{2+}, the fluorescent signal value of 10 μM Pb^{2+} was compared with the rest of the metal ions.

2.6 Sensitivity test in environmental water samples

The practical potential of the biosensor for environmental water samples was assessed by spiking Hg^{2+} or Pb^{2+} in water from Dan Jiang Kou reservoir (Hubei, China), which will be delivered to Beijing as drinking water. The water sample was filtered through a 0.22 μm membrane to prepare Hg^{2+} or Pb^{2+} solution. And the concentration of Hg^{2+} and Pb^{2+} in standard solution was confirmed by ICP-MS. Samples with various concentrations of Hg^{2+} or Pb^{2+} were prepared in environmental water using the exact method as those in ultrapure water (described above).

2.7 Detection of Hg^{2+} and Pb^{2+}

The detection processes of Hg^{2+} and Pb^{2+} were similar, taking the detection of mercury as an example. The aqueous reaction involved adding T–T hybrid to Hg^{2+} solution or environmental water

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sample with Hg^{2+} contaminant and then incubating for 8 min to ensure complete reaction of Hg^{2+} with T–T hybrid.

The reaction mixture was injected into the chamber, and the designed two-step detection occurred. The regeneration process was added. The signal profile shown in Figure 2S was divided into two main sections, namely, (I) and (II), and six small segments. The two sections corresponded to the liquid–solid reaction process (containing 1 segment) and regeneration process (containing 2, 3, 4, 5, and 6 segments).

The first segment of profile was obtained after pumping mixed solution (composed of hybridized AF with TB and Hg^{2+}) into the chamber. In this segment, the signal intensity continued to increase rapidly with reaction time, indicating that the generated unfolded AF in the solution was captured with TS probes, and the fluorescent label of Cy3 on AF targets was excited by evanescent wave. When signal intensity reached the equilibrium point (EP), the value of the signal intensity at that moment was recorded, which was positively correlated with Hg^{2+} concentration, as illustrated in the reaction equations shown in Figure 1(a). Furthermore, a specific unfolded strand with 12 bases (control DNA) was tested to hybridize TS, which failed to generate a signal, indicating that the strain with 12 bases did not interfere with the detection, as reported in previous studies (Du et al. 2012; Kong et al. 2009).

The second segment reflected the addition of the ultrapure water into the chamber (i.e., the first step of regeneration) to remove Hg^{2+} in solution. The generated increase in the intensity of the profile was attributed to the unfolding of portion of unreacted DNA, consequently yielding free AF located in the evanescent wave field and excited.

The third segment of the profile corresponded to the flushing of the reaction chamber with 30%

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(w/w) urea solution. As a common eluent for DNA, urea can melt and separate tightly folded DNA. Furthermore, aside from acting as an eluent, urea displays weak alkalescence, and Hg^{2+} may be complexed by OH^- (Zhang et al. 2010). The signal intensity rapidly decreased through the influence of urea.

In general, urea can only melt portions of DNA molecules (Shelton et al. 1999). SDS as an anion surfactant can strongly interact with DNA to cleave the hydrogen bonds between the bases on DNA molecules and to separate nucleic acid chains. SDS solution (0.2% [w/w]) was used to flush the sensor chamber to elute the remaining hybridized DNA on the optical sensor surface so that the background signal decreases. As expected, the signal intensity continued to decrease during the SDS solution flushing step, as shown in the fourth segment of profile.

Flushing the chamber again with ultrapure water to eliminate the residue of SDS on the surface weakly reduced the signal intensity owing to the change of refractive index, as reflected in the fifth segment in the profile. The signal intensity remained constant in the final segment, which corresponded to the buffer flushing process.

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3. Results and discussion

3.1 Schematic of the turn-on evanescent wave biosensor for Hg^{2+} and Pb^{2+} detection

Detection in homogeneous solutions is usually difficult for sensor regeneration required by on-site monitoring, and detection on a liquid–solid interface is time-consuming because of steric resistance and electrostatic repulsion. Therefore, a two-step strategy was developed to take advantage of the rapid binding of T–T mismatches with Hg^{2+} and the cleavage of the substrate by 8–17 DNAzyme with Pb^{2+} in solution, as well as the sensitive DNA detection on a liquid–solid interface based on

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evanescent wave. In our design, the detection of Hg^{2+} and Pb^{2+} was implemented by detecting DNA instead, as depicted in Scheme 1. The proposed detection procedure was composed of two steps. In the first step, T-rich DNA hybridized with complement strand or 8–17 DNAzyme with substrate strand in buffer solution, after which heavy metal ions were added and reacted with hybridized DNA for 8 min. In the second step, the above mixture was pumped into a reaction chamber, where the captured DNA strand was stabilized on an optical fiber to hybridize the free ssDNA labeled by the fluorophore, followed by excitation via evanescent wave to generate a detectable fluorescence signal.

3.2 Detection of Hg^{2+} using the two-step method

We initially hybridized the TB strain (labeled with BHQ2 quencher) and AF strain (labeled with Cy3 fluorescence) in buffer to detect Hg^{2+} [Figure 1(a)]. Upon adding Hg^{2+}, AF was unfolded from T–A pairs to form free AF along with the formation of T–Hg^{2+}–T. The above mixture was subsequently passed onto an optical fiber where the probe strand TS was stabilized, and the free AF was captured, followed by excitation via evanescent wave to generate detectable fluorescence signals. After the detection was completed, the biosensor was regenerated by cleaning up the probe with denaturing solutions for another round of detection.

The detected signal profiles of Hg^{2+} solutions at 0, 0.41, 0.83, 1.67, 3.34, 5, 6.67, and 10 nM concentrations are shown in Figure 1(b). The signal intensity increased linearly with Hg^{2+} concentration until reaching the plateau. Higher concentrations of Hg^{2+} generated more free AF (because of the formation of T–Hg^{2+}–T complexes), which was subsequently captured by the immobilized TS on the fiber surface, resulting in a greater rate of signal enhancement. The increase in signal enhancement rate at high Hg^{2+} concentrations demonstrated the apparent “turn-on” mode of the detection strategy. The time required to hybridize AF and TS adequately on the optical fiber

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surface was gradually extended with the increase in Hg^{2+} concentration, e.g., 50 s for 0.41 nM Hg^{2+} solution and 250 s for 10 nM. The followed flushing steps usually needed 200 s to ensure that the biosensor was adequately cleaned up for regeneration. The entire measurement process, including the hybridization on the liquid–solid interface and the cleanup of the sensor with urea and SDS solutions, was completed within 7 min, which is shorter than the time required for detecting Hg^{2+} by several other approaches (Du et al. 2012; Kong et al. 2009). This short time required for detecting each sample was attributed to the T–T mismatch reaction occurring in solution instead of on the liquid–solid interface. Less steric resistance led to a more rapid reaction after adding Hg^{2+}. A positive linear relationship ($y = 56.83x + 13.01$, $R^2 = 0.99$) was found between the signal intensity and Hg^{2+} concentration over a range of 0.41–10 nM, as shown in Figure 1(c). The detection limit of the biosensor was as low as 22 pM (S/N = 3), which is much lower than that determined by other methods based on electrochemical detection (Kong et al. 2009). The melting temperature (Tm) of the duplex formed by TB and AF was approximately 35 °C in 50 mM Na^+ solution by mFold (Markham and Zuker 2005), which increased by several degrees Celsius by attaching the fluorescent label Cy3 in AF (Liu and Lu 2003). This temperature indicated that the hybridization of TB and AF was stable at room temperature and allowed a low background signal to achieve low detection limit without ~~any~~ employing a signal amplification procedure.

When Hg^{2+} concentration was increased to 15 nM, the signal profile was approximately identical with that obtained at 10 nM (data not shown), suggesting that the linear range of the method was between 22 pM and 10 nM. Hg^{2+} concentration in environmental water samples ranges from 0.02 nM to 15 nM (Streško et al. 1994), a range that is covered by the detection limit of our sensor, suggesting a strong practical potential of our biosensor.

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3.3 Detect Pb^{2+} using the two-step method

The detection of Pb^{2+} based on DNAzyme is shown in Figure 2. Similar to Hg^{2+} detection, we initially hybridized 17 EB strains labeled with BHQ2 quencher and 17 SF strains labeled with Cy3 fluorescence. Subsequently, 17EB catalyzed the cleavage of its substrate 17SF through the addition of Pb^{2+}, and partly complementary substrate strands were released, which could hybridize with fixed probe strands 17PS on the optic fiber. The rest of the procedure was consistent with the detection of Hg^{2+}.

The fluorescent biosensor was incubated with different concentrations of Pb^{2+} (ranging from 0 μM to 10 μM) to evaluate its biosensitivity. The schematic of the two-step method for Pb^{2+} detection based on DNAzyme is described in Figure 2(a). Although the detection based on DNAzyme was different from that based on T–T mismatch, we found that the two-step method could be used in Pb^{2+} detection and had good versatility. The fluorescence signals of the sensing solution as a function of Pb^{2+} concentration are shown in Figure 2(b) and 2(c). With the addition of more lead ions, the fluorescent signals also increased, thereby demonstrating the characteristics of the "turn-on" detection mode. The time required to capture the released substrate strains was gradually extended, e.g., roughly 300 s for 3.34 μM Pb^{2+} solution and 600 s for 10 μM Pb^{2+} solution. As previously mentioned, the complete regeneration of the biosensor usually required 200 s. The entire measurement process, including the capture on the fiber and the regeneration of the sensor, was completed within 13 min, which is shorter than the time required for other biosensors to detect Pb^{2+} (Liu et al. 2012; Xiao et al. 2007a). When the Pb^{2+} concentration was increased to more than 10 μM, the fluorescence signals were no longer enhanced, thereby suggesting that the linear range of the method was approximately 0–10 μM. These results indicate a positive linear relationship

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($y = 64.78x + 117.54$, $R^2 = 0.98$) between the signal intensity and Pb^{2+} concentration, as shown in Figure 2(d). The detection limit of the biosensor was as low as 20 nM (S/N = 3), which was much lower than the toxicity level of Pb^{2+} in drinking water, as defined by MOH and US EPA (48 nm and 72 nM, respectively). These results indicate the potential use of this biosensor for sensitive detection of Pb^{2+} in environmental water samples.

3.4 Sensitivity and selectivity

The selectivities of the biosensor for Hg^{2+} and Pb^{2+} detection were also investigated. In Figure S3 (a), results demonstrated the high selectivity of the biosensor to a 10 nM Hg^{2+} sample. The influences of the 12 metal ions were negligible (< 5% signal of 10 nM Hg^{2+}) at a 10 μM level, with some even at 100 μM. In the presence of a high-concentration mixture of the interference metal ions and Hg^{2+}, the fluorescence signal decreased slightly. As results of the increase of ionic strength, the speed of hybridization accelerated and hybridization reaction was not complete in fiber (Razumovitch 2009). Fortunately, the detection results were only slightly affected. This finding indicates that desirable selectivity of our biosensor for Hg^{2+} was obtained, and this accounted for the selectivity (over 20,000-fold) to Hg^{2+} over other metal ions at the same concentration (Lan et al. 2010). Similar to the anti-inference results of Hg^{2+}, all the other metal ions exhibited insignificant interferences on the biosensor for Pb^{2+} detection, as shown in Figure S3 (b).

3.5 Reusability and stability

Reusability included detection and regeneration process. In addition, multiple-cycle regeneration is equivalent to the ability of the biosensor to be reused multiple times. We used a covalent way to link DNA while using mild detergents, such as urea and SDS, to effectively ensure the stability of the fixed probes. The signal intensities of the measurements for 10 nM Hg^{2+} samples over 18 times of

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cycle regeneration remained approximately identical (with SD < 5%), as illustrated in Figure S4. The detection of Hg^{2+} was implemented by detecting DNA instead; thus, we expected to gain the same results for the detection of Pb^{2+}. We found that the optical fiber fixed Pb^{2+} probes can be used hundreds of times by using similar methods. The good regeneration property of our sensor was attributed to the fact that the probes were covalently bonded on the optical fiber.

3.6 Detection of Hg^{2+} and Pb^{2+} in environmental water samples

Considerable efforts have been made in investigating the practical potential of many biosensors by detecting environmental water samples with known Hg^{2+} and Pb^{2+} concentrations, whereas Hg^{2+} and Pb^{2+} are generally found in a wide range of concentrations in environmental water samples. Thus, we measured the environmental water samples from Dan Jiang Kou reservoir (Hubei, China) spiked with Hg^{2+} and Pb^{2+} ions as shown in Figures 3(a) and 3(b). The concentrations of Hg^{2+} and Pb^{2+} in standard solution were confirmed by ICP-MS. Similar to that shown in the water samples with buffers, the signal intensities of Hg^{2+} and Pb^{2+} in the environmental water samples also showed a positive linear relationship (Hg^{2+}: $y = 46.38X - 29.98$, $R^2 = 0.98$; Pb^{2+}: $y = 44.02X + 82.21$, $R^2 = 0.98$). Compared with ultrapure water samples under identical Hg^{2+} and Pb^{2+} concentration conditions, the signal intensity of environmental water samples was lower, indicating that several materials in the environmental water could reduce the binding of Hg^{2+} and Pb^{2+} with DNA. For example, humic acid, which is commonly present in environmental water can adsorb free Hg^{2+} and Pb^{2+} ions in water and decrease available Hg^{2+} and Pb^{2+} for sensor detection (Khwaja et al. 2006; Shahid et al. 2012). Overall, the detection results obtained from DI water samples and real environmental water samples were almost identical. Tables 1S and 2S show the recent reports about the detection of Hg^{2+} and Pb^{2+} based on FNAs biosensor. Our biosensor and the biosensors from

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previous studies exhibit similar sensitive (Pb^{2+} slightly worse), and the detection time and method of ease of use had a significant advantage. All results demonstrate a strong potential for the biosensor to be applied on-site.

Based on the aforementioned results, the single cost of Pb^{2+} or Hg^{2+} detection using our biosensor only amounts to 10–20 yuan, which is roughly 1/10–1/6 of the cost of using traditional methods. In fact, with the use of more inexpensive fluorophores, a single analysis may cost lesser. Thus, our biosensor is suitable for monitoring serious heavy metal pollutions in developing countries.

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4. Conclusion

We developed and characterized an evanescent wave fiber-based fluorescent biosensor for the "turn-on" detection of Hg^{2+} and Pb^{2+} based on T–T mismatch-containing DNA and DNAzyme. The biosensor is rapid, highly sensitive and selective, low cost, and robustly regenerable for Hg^{2+} and Pb^{2+} detection. The evanescent wave-excited fluorescence signals achieved the standards outlined by MOH and US EPA without the use of signal amplification. The biosensor is ready for a new analysis after 7 min for Hg^{2+} detection and 13 min for Pb^{2+} detection. Thus, the biosensor is suitable for emergency use. The same probe fiber can be reused for dozens of times without decreasing performance, and the cost of each test is approximately 1/6–1/10 of that by traditional methods. In particular, the biosensor is convenient to use and can be easily used without training. Moreover, the application of the biosensor for Hg^{2+} and Pb^{2+} detection in environmental water samples was successfully achieved, suggesting its great potential for on-site environmental monitoring.

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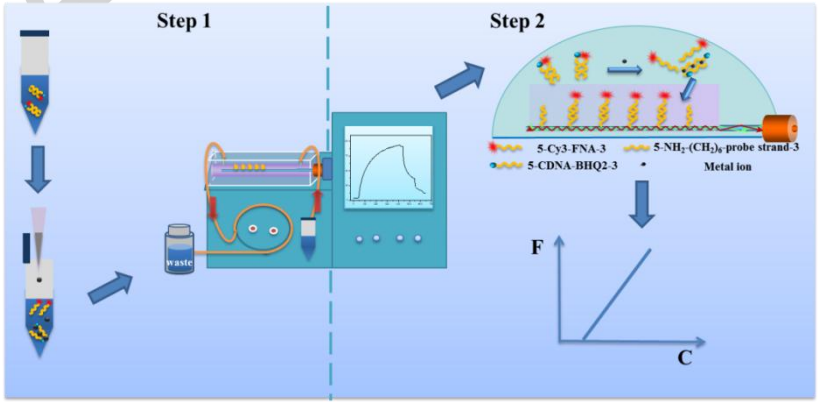

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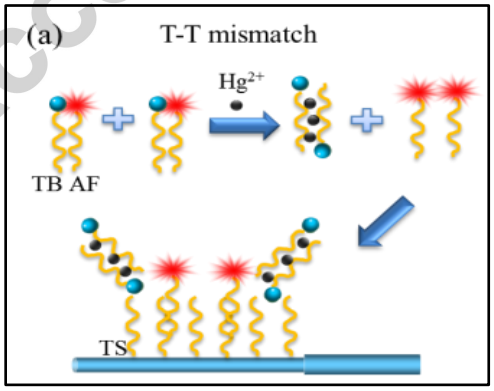
Scheme 1. Schematic of the two-step turn-on mode biosensor for Hg^{2+} and Pb^{2+} detection



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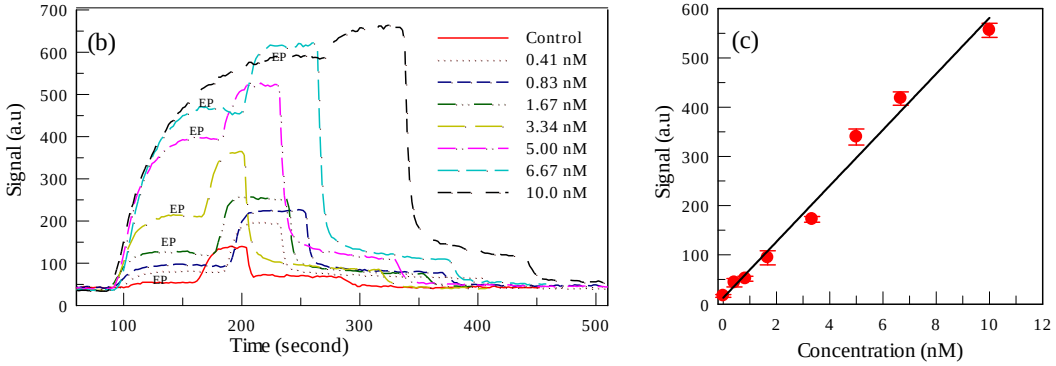

Figure 1. Sensitivity of the biosensor for Hg^{2+} detection. (a) Schematic of detection principle of Hg^{2+}. (b) Signal profiles of Hg^{2+} within the contraction range of 0–10.0 nM. (c) Plot showing positive linear relationship between signal intensity and Hg^{2+} concentration over the range of 0–10.0 nM .

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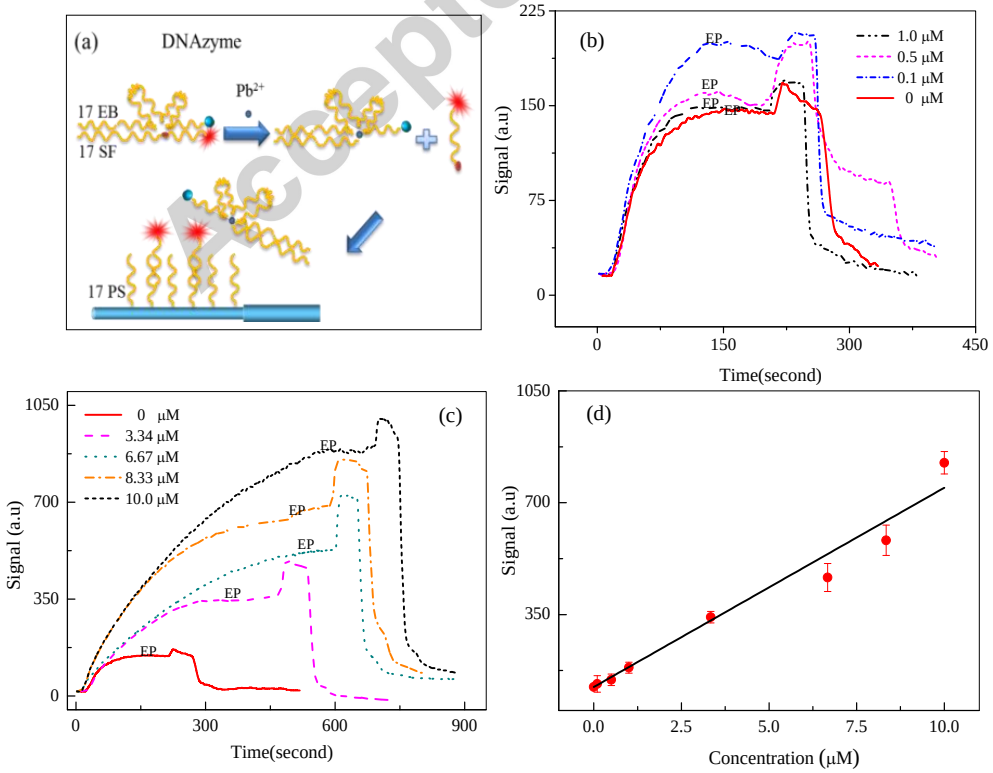

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Figure 2. Sensitivity of the biosensor for Pb^{2+} detection. (a) Schematic of the two-step method for Pb^{2+} detection based on DNAzyme. (b) Fluorescent signal changes of the sensing solutions treated with 0, 0.1, 0.5, and 1.0 μM Pb^{2+}. (c) Fluorescent signal changes of the sensing solutions treated with 0, 3.34, 6.67, 8.34, and 10 μM Pb^{2+} (d) Calibration curves of the biosensor.

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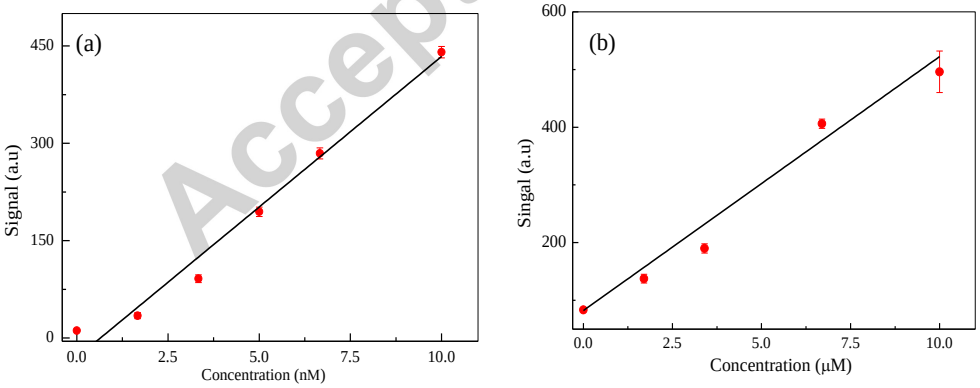

Figure 3. Signal intensity versus metal ion concentration. (a) Hg^{2+} and (b) Pb^{2+}