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Triple functional DNA–protein conjugates: Signal probes for Pb^{2+} using evanescent wave-induced emission

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ABSTRACT

We describe here a Pb^{2+}-dependent DNAzyme-based evanescent wave-induced emission (EWIE) biosensing platform using triple functional DNA–protein conjugates as signal probes for Pb^{2+} detection. Upon reaction with Pb^{2+}, the substrate strand is cleaved, releasing an invasion fragment, which is then hybridized with the complementary DNA strand immobilized on magnetic beads, while dissociating of the original hybridized signal probes. The signal probes, consisting of a streptavidin moiety and a Cy5.5 labeled DNA moiety, act simultaneously as signal conversion, signal recognition and signal report elements. Detection of the signal probes is accomplished by first adsorbing to the desthiobiotin-modified optical fiber, followed by fluorescence emission induced by an evanescent field. A linear calibration was obtained from 20 nM to 800 nM with a detection limit of 1 nM. The optical fiber system is robust enough for 250 sensing cycles and can be stored at room temperature over one month. These results demonstrate that application of DNA–streptavidin conjugates has been extended to DNAzyme-based biosensors, maintaining activity, specificity, regeneration and long-term storage ability.

1. Introduction

Rapid detection of specific heavy metal ion has received much attention in scientific research. Of particular interest has been the detection of lead ion (Pb^{2+}), a dangerous pollutant which causes adverse health effects in people, including renal malfunction, delayed physical/mental development, blood–brain barrier, and kidney disease (Chow et al., 2005; Needleman, 2004; Sane et al., 2013). Various instrumental analytical techniques for Pb^{2+} detection have been developed, such as AAS (Ucar et al., 2014), ICP-AES (Hao et al., 2015), and ICP-MS (Palmer et al., 2006). However, these widely-used instrumental techniques are rather complex, requiring sophisticated instrument and professional personnel, which prevent their further application. Therefore the development of new sensing techniques for Pb^{2+} quantification is a hot topic in analytical chemistry in recent years (Deibler and Basu, 2013).

Among newly developed sensors, DNAzyme-based biosensors are particularly attractive (Lan and Lu, 2012; Li and Kong, 2013). DNAzymes, a series of functional oligonucleotides, are able to catalyze several types of reactions and are highly special for metal cofactors such as Pb^{2+} ions (Li et al., 2014), Mg^{2+} ions (Elbaz et al., 2010), UO_2^{2+} ions (Liu et al., 2007), and Zn^{2+} ions (Qian et al., 2014), etc. DNAzymes are stable, low cost, and have selectivities that are comparable with that of antibodies (Li et al., 2000), thus exhibiting strong competitiveness in sensing applications (Willner et al., 2008). Over the past two decades, various DNAzyme-based biosensors for Pb^{2+} using different signal transduction modes have been developed, including colorimetry (Liu and Lu 2003), fluorescence (Zhao et al., 2011), surface plasmon resonance (SPR) (Pelossof et al., 2012), surface-enhanced Raman scattering (SERS) (Wang and Irudayaraj, 2011) and electrochemistry (Xiao et al., 2007). These DNAzyme-based biosensors require simpler sensing instrument and less professional personnel than traditional instrumental techniques.

Many reported DNAzyme-based biosensors operate in homogeneous solution phase. However, moving to surface-based sensors have several inherent advantages. First, surface-based sensors are more amenable to washing, thus minimizing the background noises that challenge most homogeneous sensors (Swearingen et al., 2005). Second, signal enrichment via solid-phase binding is technically easier to achieve, which can lead to ultrasensitive detections. Third, the surface-based sensors exhibit the potential of miniaturization of multifunctional integrated devices, which pave the way toward multi-target sensing platform. Up to now, numerous surface-based sensors have been developed (Zhang and Da, 2010), among them, of particular interest is the evanescent wave induced emission (EWIE) sensing platform (Fig. S1), which is portable, biological compatible, and highly sensitive with a background suppression design (Xiong et al., 2014; Zhong et al., 2014).

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One challenge in developing surface-based sensors is to establish a regenerable sensing surface and keep the biological activities of DNA strands. Aiming at this problem, we use special designed DNA–protein conjugates as signal probes to skirt the problem of direct DNA-immobilizing protocols. Because functional DNAs are selective toward nucleic acid relative events (Breaker, 2002; Seetharaman et al., 2001), while the coupled proteins may serve as amplifying or catalytic units (Sacca and Niemeyer, 2011), DNA–protein conjugates are especially useful in analytical chemistry for target recognition, signal transformation and signal amplification (Tran et al., 2013; Zhou et al., 2014b). Examples include DNA-directed immobilization (DDI) (Wacker et al., 2004), biochip technologies (Niemeyer, 2007), active biocatalytic cascades (Freeman et al., 2009), signal amplifying designs (Mor-Piperberg et al., 2010), and signal transforming using glucose meters (Xiang and Lu, 2012). We once reported a highly reusable aptasensor using DNA–protein conjugates (Wang et al., 2015). Here we incorporate DNAzyme-based designs into the EWIE sensing platform using DNA–streptavidin (STV) conjugates as signal probes. The DNA–STV conjugates work simultaneously as signal conversion, recognition, and report elements, acting as a beneficial complement to the design of a reusable Pb^{2+} biosensor with high sensitivity and selectivity. Besides, DNAzymes provide a further facet of selectively evolved nucleic acids while reducing the cost. The whole detection is rapid and could be conducted at room temperature. In this work, the dethiobiotin-modified sensing surface could be regenerated for about 250 times with less than 6% signal attenuation. To the best of our knowledge, this study is the first to report a reusable EWIE sensor using DNA–protein conjugates and DNAzymes, and expands the scope of application of DNA–protein conjugates to DNAzyme-based sensing.

2. Experimental section

2.1. Materials

Carboxyl-coated magnetic beads (Dynabeads® MyOne™ Carboxylic Acid, Catalog Code: 65012, 1.0 μm in average diameter) were purchased from Invitrogen Dynal AS (Oslo, Norway). Amicon-3K/50K centrifugal filters were purchased from Millipore Inc. (Billerica, MA). Magnetic separation stand (twelve-position) was purchased from Promega (Madision, WI, USA). Glutaraldehyde, desthiobiotin, streptavidin (STV), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), 3-aminopropyl-triethoxysilane (APTS), and Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Other chemicals used for buffers and solvents were purchased from J&K, Inc. All solutions were prepared using molecular biology grade USP sterile purified water (RNase-, DNase- and protease- free).All oligonucleotides were purchased from Takara Biotechnology (Dalian, China) Co., Ltd. The oligonucleotides were annealed at 90 °C for 30 min in buffers before use. See Supplementary information for DNA sequences, buffers and the optical fiber used in this work.

2.2. Synthesis of signal probes: DNA–STV conjugation

Various methods for covalent/noncovalent coupling of synthetic DNA oligomers to proteins have been described (Niemeyer, 2007). While a number of methods for conjugating DNA with proteins are based on the remarkable biomolecular recognition of biotin by the homotetrameric proteins avidin or streptavidin (Xiang and Lu, 2013), it may introduce unnecessary interference to our sensing system, since the STV moiety of the conjugate is designed to be captured by the desthiobiotin-modified optical fiber, better not to react with biotin in advance.

Therefore the signal probes (DNA–STV conjugates) used in this work were synthesized by conjugating signal probe (Sp) DNA and STV using the heterobifuntional linker, sulfo-SMCC (Xiang and Lu, 2011). Add 0.7 μl of 1 M sodium phosphate buffer (pH 5.5) and 0.7 μL of 30 mM TCEP in sterile purified water to 10 μL of 1 mM Sp DNA (mercapto-terminated) in sterile purified water, then the obtained mixture was kept at room temperature. 1 h later, the mixture was purified by Amicon-3K using buffer A by 8 times. Meanwhile, 0.4 mg sulfo-SMCC and 3 mg STV were added to 150 μL of buffer A. After votexing for 5 min, the solution was rotated for 1 h at room temperature. After that, the excess insoluble sulfo-SMCC was removed by centrifugation, then the obtained clear solution was collected and purified by Amicon-50K using buffer A by 8 times. The purified solution of sulfo-SMCC-activated STV was mixed with the above solution of Sp DNA. The resulting solution was kept at room temperature for 48 h. To remove unreacted thiol-DNA-Cy5.5, the solution was purified by Amicon-50K 8 times using buffer A. The prepared signal probes were preserved in buffer D at 4 °C until use, with the final protein content diluted to 0.5 mg/mL.

2.3. Evanescent wave-induced emission (EWIE) biosensing platform

Fig. S1 presents the schematic of the EWIE biosensing platform which has been proposed as previously described with a moderate modification (Long et al., 2008). Briefly, all samples were pumped into a glass flow cell through a flow delivery system. An optical fiber was embedded inside the cell, along which the incident laser (635 nm) propagated via total inner reflection, thus generating an evanescent field at the fiber surface. The surface of the optical fiber could be functionalized with different molecules or nanomaterials according to actual experimental needs, such as antigens (Long et al., 2010), antibodies (Koets et al., 2014), DNA strands (Long et al., 2011), and other nanomaterials (Liu and Tan, 1999). In order to build a reusable sensing surface, a desthiobiotin-modified fiber was used in this work, see Supplementary information for its preparation. Once being functionalized, the surface was able to capture signal probes (fluorophores modified) within the generated evanescent field. Naturally, fluorophores within this evanescent field would be excited, then the emitted fluorescence was further collected by the same optical fiber, passed through an optical fiber bundle, filtered subsequently by a band pass filter and detected by photodiodes through a lock-in amplifier. One built-in computer worked for the control of delivery system, data acquisition and auto-processing, all mentioned above.

In order to optimize the sensing performances, there were three important changes on the flow cell used in this system (see Fig. S2, Supplementary information for the design of the flow cell). On the basis of the original merits of this system, *i.e.*, reduced optical components and easy optical alignment (Hao et al., 2014; Zhou et al., 2014a), this new EWIE biosensing platform was designed to have better sealing property, less reagents consumption, and ease to standardized production, thus enabling better detection performances for test samples.

2.4. Procedures for Pb^{2+} detection using the EWIE sensing platform

2.4.1. Sensor preparation

A 0.1 mL portion of 10 mg/mL solution of carboxyl magnetic beads (MBs) in a microtube was placed in a magnetic separation stand for about 1 min. Then the clear solution was discarded. The use of magnetic beads was advantageous because of their chemical stability, ease of surface modification, and convenience for separation (Centi et al., 2007; Jinghua et al., 2014). 40 mg of EDC was added to 2 mL buffer B containing 5.8 nM amine-modified DNA.

After gently mixing, the mixture was immediately incubated with the abovementioned 1 mg MBs at room temperature for 24 h to allow the formation of MB–DNA conjugates. Therefore, uncoupled ssDNA could be easily removed from the system using a magnetic separation stand. Then, unreacted amino groups on the surface of MBs were blocked using buffer C at 68 °C for 4 h. After that, the MBs were washed twice using buffer C to remove excess biotin–DNA. Later, 350 μL of signal probes (about 0.5 mg/mL) in buffer D was added to the solution and mixed well at room temperature for 30 min. Excess signal probes were washed off by buffer D three times. The signal probes were restored for further use by condensing the washing solutions using an Amicon-50K. In each test, 100 μL MB solution was used after removal of solvent.

2.4.2. Pb^{2+} detection using the EWIE biosensing platform

Add 2 μL DNA substrate (100 μM) and 2.5 μL DNAzyme (100 μM) to 100 μL buffer E containing various amount of lead ions in a microtube. The mixture was set to rotate at room temperature for 30 min. Then, 2 μL of quench buffer was added to the mixture to end the reaction. 100 μL buffer D was mixed with the solution, and the resulting solution was added to the MBs residue, then mixed at room temperature. 30 min later, after removal of the MBs, another 150 μL buffer D was added to the clear solution. Finally, the solution was tested using the EWIE biosensing platform.

At the very beginning of the EWIE measurement, the laser was switched on and signal acquisition began. Typically, each test was carried out by four steps (if not specified, the pump speed was 1.8 mL/min) :

1) 450 μL sample solution was delivered to the flow cell (15 s); 2) The pump automatically turned off for 1 min, while the evanescent wave induced fluorescence signals were recorded; 3) Wash buffer was pumped into the flow cell for 1 min to wash off the captured signal probes and regenerate the sensing surface; 4) PBS buffer was pumped into the flow cell for 30 s or more as a model buffer to ensure a stable baseline. The whole EWIE detection procedure takes less than 5 min.

3. Results and discussion

3.1. Experimental principle and sensing scheme

Overall, this sensing scheme contains three signal conversions. The schematic diagram of the methodology is shown in Fig. 1. The original Pb^{2+}-dependent 8–17 DNAzyme sequence (Breaker and Joyce, 1994; Brown et al., 2003) was used with minor base changes in the substrate binding arms (shown in dark red), which would have little effect on the catalytic activity or metal ion selectivity. Besides, the substrate (in blue) was extended by 9 nucleotides (nt) in the 5′-end to reduce the interruption of non-hybridized substrates (Fig. 1a) (Zhang et al., 2010). Owing to the property that DNAzymes required specific cofactors to catalyze reactions, the DNA substrates would be cleaved at the single RNA site (in red) by DNAzyme in the presence of Pb^{2+} as shown in Fig. 1a. After the cleavage, the right duplex length decreased to 9 nt pairs with a melting temperature at 20.99 °C. Therefore this 19-nt arm of the substrates could no longer form a stable duplex with the DNAzymes under the given reaction condition, and thus was released into the solution. At this point, the concentration of this released 19-nt oligonucleotide reflected the concentration of lead ion, which could be regarded as the first signal conversion.

Next, the second signal conversion was shown in Fig. 1b. In a separate solution, the DNA section of the synthesized signal probes (in green) could hybridize to an amine DNA (in black) that linked to carboxyl MBs. We noticed that the released 19-nt oligonucleotide (in blue) is longer in matched base pairs with the amine DNA (duplex length: 18) than the DNA section of the synthesized signal probes (duplex length: 12), therefore the former could compete with signal probes in hybridizing with the amine DNA, thus inducing the release of the signal probes. Now the amount of signal probes reflected the concentration of the released 19-nt oligonucleotides. The released signal probes were collected using magnetic separation.

Subsequently, the third signal conversion was shown in Fig. 1c. The obtained solution containing signal probes was pumped into the flow cell of the EWIE biosensing platform. Inside the flow cell, a dethiobiotin-modified fiber would capture the STV-conjugated signal probes via affinity interaction. Moreover, the signal probes were labeled with Cy5.5 fluorophores, which would emit their unique fluorescence within the evanescent field, finally resulting in a detectable signal recorded by a built-in computer. After that, the sensing surface was regenerated before the next measurement. So far the original concentration of Pb^{2+} had been converted into the evanescent wave induced fluorescent signal. In contrast, in the absence of Pb^{2+}, the uncleaved substrates remained hybridized to DNAzymes to form a stable duplex and could not cause the release

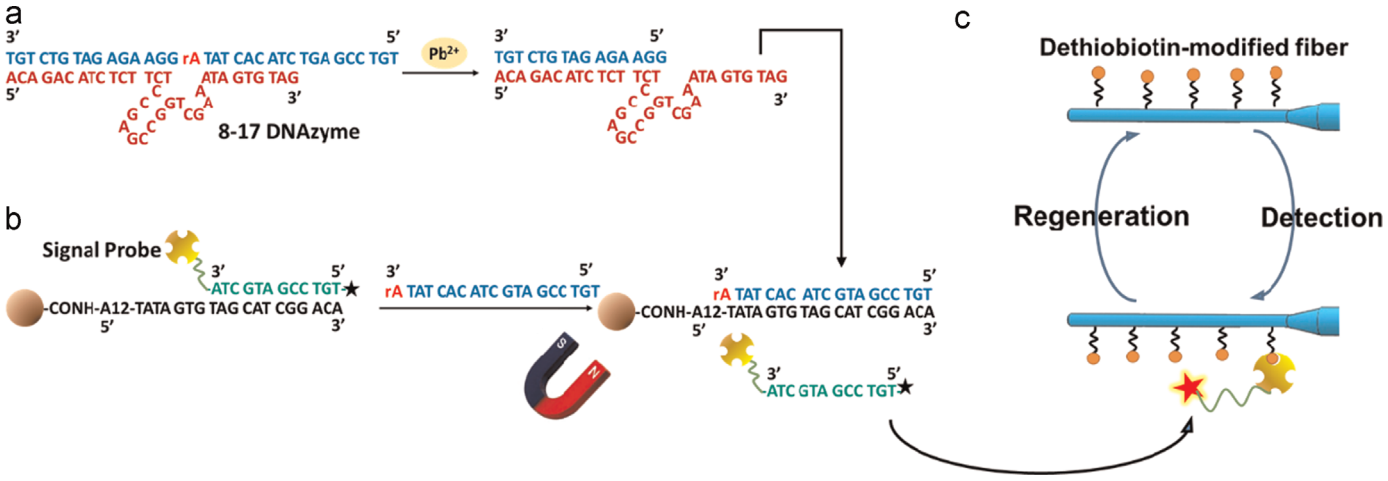

Fig. 1. Proof-of-concept competitive assay for the detection of Pb^{2+} by functional MBs and DNA–STV conjugates using EWIE biosensing platform. **(a)** Pb^{2+}-induced cleavage of the DNA substrate (blue) by the 8–17 DNAzyme (Pb^{2+}-dependent, shown in dark red). The reaction yields the cleaved ssDNA product (short strand in blue, not shown in Fig.1c) as the competitive DNA, shown in Fig.1b. **(b)** Release of DNA–STV conjugates (signal probes) from magnetic beads by the competitive DNA. The signal probes consist of three units: ssDNA strand (green), Cy5.5 fluorophores (black star) and streptavidin (yellow). **(c)** Adsorption of released signal probes by the desthiobiotinylated optical fiber for further measurement using the EWIE platform.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

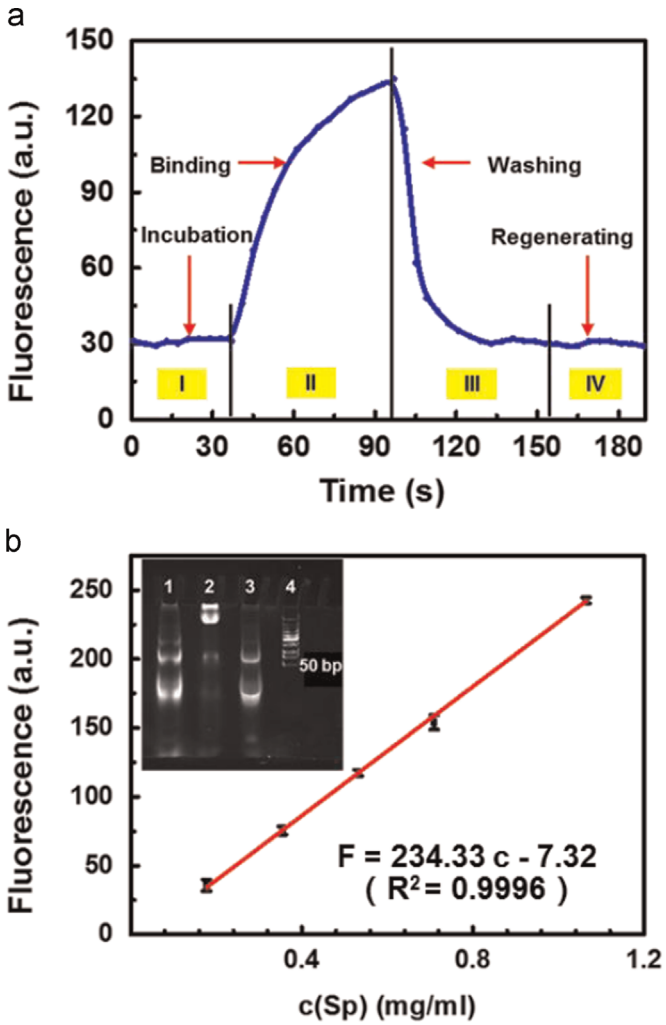

Fig. 2. (a) A typical signal trace during a complete test cycle based on the EWIE biosensing platform. (b) Evanescent wave-induced fluorescence signal responses to various concentrations of signal probes (Sp). Inset: PAGE characterization of Sp (DNA–STV conjugates). Native PAGE (4–20% gradient gel) of images for the conjugates. Lane 1: Sp DNA and STV with linker (unpurified conjugates); lane 2: after the removal of free DNA (purified DNA–STV conjugates); lane 3: Sp DNA (24 bp, 5'-Cy5.5-modified); lane 4: Marker (DNA ladder). Note: under the condition of electrophoresis, disulfide bonds are usually formed from the oxidation of thiol (–SH) groups, which explains the second band in lane 1 and lane 3.

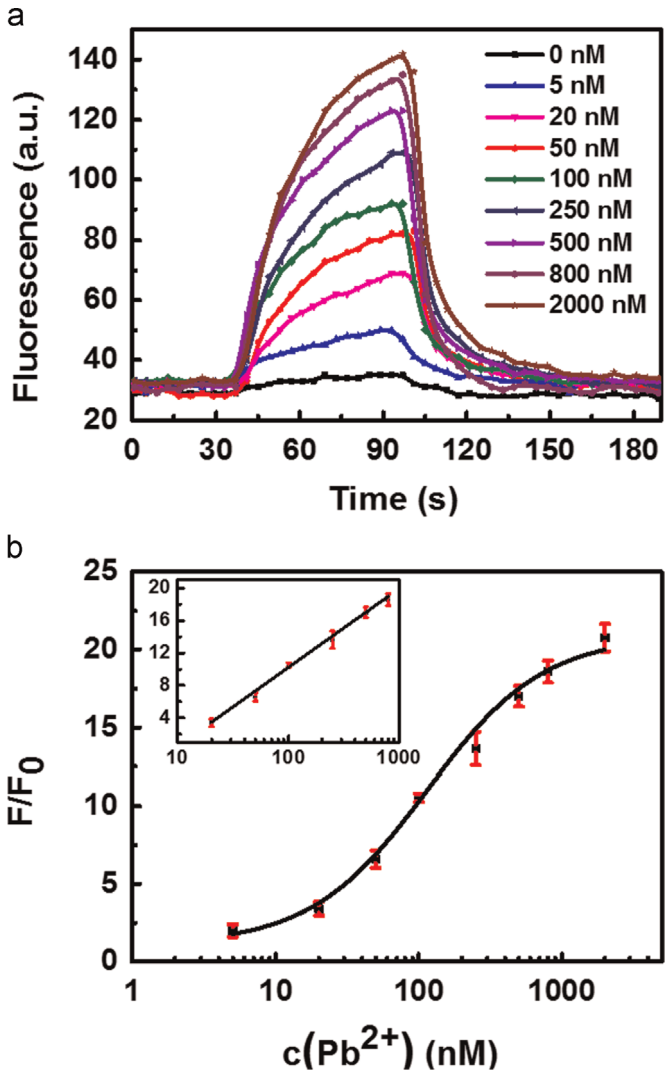

Fig. 3. (a) Original EWIE biosensing platform signal traces observed in the flow of Pb^{2+} sample solution at various concentrations over the dethiobiotin-modified fiber sensor surface. (b) Variations of fluorescence intensity ratio, F/F_0, with the concentration of Pb^{2+} and logarithmic calibration plot to detect Pb^{2+} using the EWIE system (F: the average fluorescence intensity of three samples; F_0: the average fluorescence intensity of nine blank samples). The error bars in the figure correspond to the standard deviation of the data points in triplicate experiments. The inset presents the linear range in nanomolar concentrations of Pb^{2+}.

of signal probes, resulting in no fluorescent signal production using the EWIE biosensing platform.

3.2. Functionality of the signal probes

As mentioned above, to further optimize the sensing performance, particularly for the improvement of regenerate ability of the DNAzyme-based sensing surface, special designed DNA–streptavidin (STV) conjugates were applied in this system. Prior to Pb^{2+} detection, we tested the possibility of using a desthiobiotin-modified optical fiber to measure signal probes. A typical evanescent wave-induced fluorescence signal was shown in Fig. 2a, which exhibited the dynamic process based on the affinity interaction between desthiobiotin and streptavidin. Besides, sections I–IV in Fig. 2a represented the signal traces corresponding to the abovementioned four steps. The obtained signal indicated that upon injection of signal probes there was a time-dependent increase in the fluorescent emission of Cy5.5. Furthermore, the evanescent wave-induced fluorescence signal responses to various concentrations of signal probes were shown in Fig. 2b. Considering the relatively moderate concentrations, linear fitting was adopted. In this range, R-square of the fitting equation is 0.9996, suggesting the signal probes are potential and feasible in sensing applications. In addition, native PAGE was carried out to demonstrate the purified solution contained mainly DNA – STV conjugates and little unreacted DNA or STV composites (Fig. 2b, inset).

3.3. Detection performance of the EWIE biosensor

3.3.1. Detection range

As shown in Fig. 3a, since DNAzymes were sensitivity towards their target ions, when the concentrations of Pb^{2+} present in the samples increased from 0 to 2000 nM, the final fluorescence readout in the EWIE biosensing platform increased accordingly until reaching a plateau. Calibration curve was measured by relating net fluorescence intensity ratios(F/F_0) to corresponding Pb^{2+} concentrations, and was fitted with a four-parameter logistic model (Fig. 3b). The detection limit was found to be 1 nM, as defined by $3\sigma_b$/slope (σ_b is the standard deviation of nine blank

samples), with a linear range of at least 20–800 nM ($F/F_0 = 9.68916 \times \log[c(Pb^{2+})] + 0.38715$, $R^2 = 0.992$). According to the regulations introduced by US EPA, the maximum permitted levels of Pb^{2+} in drinking water was 72 nM, and this detection limit is much lower than the regulated level. Moreover, the analytical performances of different optical DNAzymes-based assays developed for Pb^{2+} analysis are summarized and the sensitivity of this proposed method is competitive compared to these techniques (see Table S1). In addition, the proposed Pb^{2+} sensor based on the DNAzymes and conjugates has several unique advantages. First, the proposed Pb^{2+} sensor is much simpler and faster (5 min per assay, including measurement and regeneration). Second, the bioassay based on the proposed EWIE biosensing platform is low-cost (~ 8 dollars per sample), which is attractive for its further applications. Another important feature of the DNA–protein conjugate-based biosensing system is quite stable and highly regenerated, one optical fiber could be kept using for at least one month with an attenuation less than 6% (Fig. 4a), allowing for at least 250 sensing cycles (see details as follows).

3.3.2. Regeneration

Owing to the reversible recognitions between STV and dethiobiotin molecules, those bound signal probes could be washed off easily from the dethiobiotin-modified fiber using 0.5% SDS (pH 1.9) solution. To evaluate the regeneration ability of this dethiobiotin-modified sensing surface, about every other 50 detections,

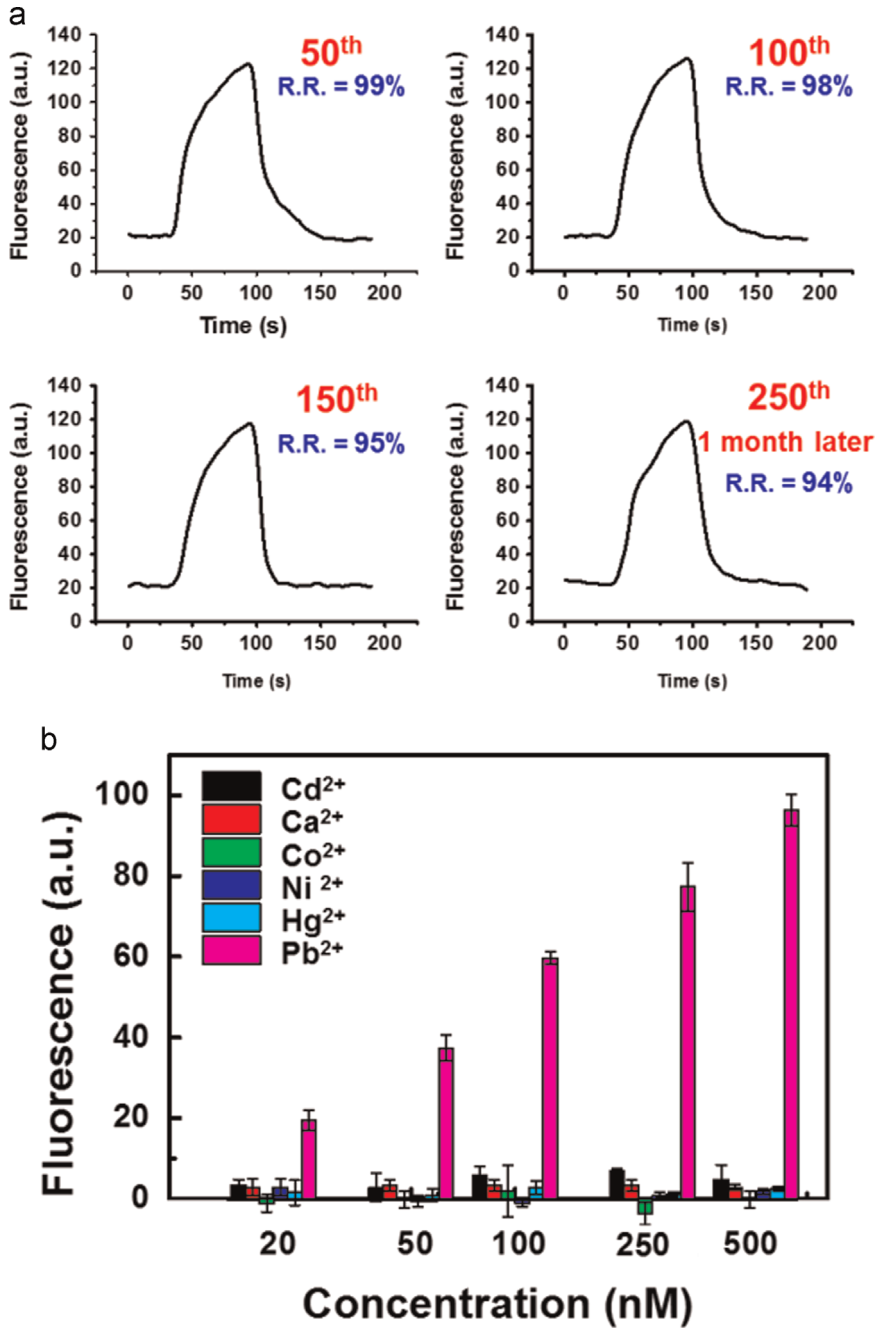

Fig. 4. (a) Representative standard signal traces obtained at different times using one optical fiber (all being treated with 500 nM Pb^{2+}). The number of regeneration cycles (in red) and signal recoveries rates (R.R., in blue) of all measured samples were labeled in the figure, respectively. (b) Comparison of sensor signals observed upon the addition of with lead (II) ion and five other metal ions which are common seen in environmental waste waters. Each data value is the average of at least two independent experimental results.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Determination of Pb^{2+} in three different water samples ($n=3$).

Sample	Pb^{2+} (nM)		Recovery (%)
	Spiked	Detected mean[a] ± SD[b]	
Bottled purified water	3	2.73 ± 0.72	91
	150	142.15 ± 0.33	95
Tap water	3	2.67 ± 0.27	89
	150	135.89 ± 0.65	91
Mineral spring water	3	3.22 ± 0.33	107
	150	152.01 ± 0.96	101

[a] Mean value of three individual determinations.
[b] Standard deviation.

standard tests were conducted using the same sample. Four representative standard signal traces are shown in Fig. 4a, the signal recoveries of all measured samples ranged from 94% to 99%. During the whole test procedure, the fiber was reused for at least 250 times and still maintained good performance. So the proposed sensing method is applicable for Pb^{2+} detection with enough accuracy and reusability.

Combined with an automated fluid and data handling system, reusable biosensors had several advantages compared to their disposable counterparts, such as fewer working steps and shorter operating time to obtain a reliable result. Besides, the obtained calibration curve can be applied for a series of subsequent measurements (Abel et al., 1996), which will allow an increase in the detection speed and signal reproducibility compared to traditional methods. Most importantly, the reusability of biosensor is the premise of realizing automatic and on-line monitoring for pollutants, which is critical for certain circumstances, such as environmental monitoring.

3.3.3. *Selectivity*

To evaluate the selectivity of this method, the difference of fluorescence intensities for Pb^{2+} detection and five other metal ions, including Ni^{2+}, Co^{2+}, Ca^{2+}, Cd^{2+} and Hg^{2+}, were compared. In order to evaluate the selectivity more accurately and more comprehensively, each metal ion mentioned above was analyzed at five different concentrations from 20 nM to 500 nM. As indicated in Fig. 4b, in contrast to significant linear response as observed for Pb^{2+}, other metal ions showed very low interference to the Pb^{2+} detection. Hence, the results showed high selectivity toward Pb^{2+} over other test metal ions because of the specific function of 8–17 DNAzyme.

3.3.4. *Determination of Pb^{2+} in water samples*

The Pb^{2+} detection performances in tap water and drinking water samples were evaluated. Three different water samples were prepared. Non-contaminated commercial mineral spring water and bottled purified water were purchased from the local supermarket, and tap water was also chosen as a type of water sample. Firstly, the water samples were gently mixed with Pb^{2+} standard solution, making final Pb^{2+} concentrations at two levels (3.0 nM and 150 nM) for the recovery experiments. After that, 90 μL water sample was mixed with 10 μL 10 × buffer A, and then the obtained 100 μL solution was treated using the same protocol as above for the Pb^{2+} detection in designed buffer. The detection performance of this technique for the water samples spiked with Pb^{2+} is presented in Table 1. Bottled purified water samples, which were considered to contain fewer impurities, exhibited the best recovery results both in the case of low Pb^{2+} concentration and high Pb^{2+} concentration. Moreover, due to the presence of various mineral components, detection results obtained from the mineral spring water sample is slightly higher than other samples. The average recoveries were found to vary from 89% to 107%, with the satisfactory variations that the average relative standard deviation was no more than 7%. Therefore, the developed DNAzyme-based biosensing technique showed satisfactory accuracy for Pb^{2+} detection in real water samples.

4. Conclusions

As there is a push for robust, sensitive and selective methods to detect Pb^{2+} on site, platforms for Pb^{2+} determinations must possess the merit of laboratory analytical methods, and be portable, rugged, regenerable, and robust enough for long-time unattended use. Working toward this goal, this work demonstrates the applicability of triple functional DNA–protein conjugates as signal probes for rapid and selective Pb^{2+} detection based on an evanescent wave-induced emission biosensing platform. The signal probes were sensitive towards Pb^{2+} ions in the linear range of 20-800 nM with a detection limit at 1 nM, and also were selective for Pb^{2+} ions over other competitive metal ions. Additionally, since the surface-bound desthiobiotins can be reused for at least 250 times, the possibility exists of storing the sensing surfaces of the optical fiber for long-term use. This is a necessity, if the fiber-based designs are to be incorporated as the active elements of DNAzyme or functional nucleic acid-based sensor structures for field use. Preliminary results indicate that this is possible. Furthermore, the application of this sensing strategy for the detection of nanomolar Pb^{2+} ions in three water sample was demonstrated. And most significantly, the application of DNA–STV conjugates has been extended to DNAzyme-based biosensors, no longer confined to the structure-switching of aptamers as demonstrated before.

Although all of the experiments discussed here were performed on an optical-fiber based EWIE biosensing platform, it is reasonable to believe the biosensing detections using functional DNA-based transducers and signal probes derived from DNA–protein conjugates can be ported to other solid surfaces, such as the planar waveguide platform, using EWIE or other techniques like surface enhanced Raman scattering (SERS) and local surface plasmon resonance (LSPR). The major challenge that remains, then, is to incorporate these flow-through devices into one hybrid microfluidic system for efficient multi-target sensing. Research en route to this goal is ongoing.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.06.003.

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