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Enzyme-free strip biosensor for amplified detection of Pb^{2+} based on a catalytic DNA circuit†

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A simple and enzyme-free strip biosensor for the amplified detection of Pb^{2+} has been constructed based on a catalytic DNA circuit. This assay is ultrasensitive, enabling the visual detection of Pb^{2+} concentrations as low as 10 pM without instrumentation.

Lead (Pb^{2+}) is a major environmental pollutant accompanied by severe health risks,[1] and thus, its ultrasensitive and quantitative detection is highly desirable for environmental protection, as well as disease prevention and treatment. In recent years, several exquisite sensors have been developed for Pb^{2+} detection using fluorescent, colorimetric, electrochemical, and Raman scattering methods.[2] Although these assays are effective, most of them necessitate the use of costly apparatus, require experienced operators, and lack portability, which limit the applications in field monitoring and point-of-care diagnostics. Hence, the development of an on-site, inexpensive, and rapid method for Pb^{2+} detection is urgently needed.

Recently, strip biosensors have received considerable interest because of their portability, user-friendly format, long-term stability, short assay time, and cost-effectiveness.[3] Moreover, the strip platform minimizes the requirements for highly qualified personnel and eliminates complex analysis procedures involving expensive equipment. The qualitative analysis is easy to perform by visually observing the color intensity of the red band on the test zone, and the quantitative data can be obtained by recording the optical responses with a handheld "strip reader". Our group and others have successfully developed strip biosensors for the detection of nucleic acids,[4a,b] proteins,[4c] cancer cells,[4d] and small molecules.[4e,f] Lu and co-workers have constructed an easy-to-use dipstick test for Pb^{2+}.[5] However, the detection limit of 0.5 μM is below the maximum level (72 nM) of Pb^{2+} in drinking water permitted by the U.S. Environmental Protection Agency (EPA). An even higher sensitivity is required if the strip biosensor for Pb^{2+} is to be used in clinical or environmental samples to ensure a large signal-to-noise ratio.

One way to improve the sensitivity is through signal amplification. Various amplification strategies have been developed, such as polymerase chain reaction (PCR),[6a] rolling circle amplification (RCA),[6b] polymerase-based isothermal strand displacement,[6c] and nicking endonuclease/exonuclease-mediated signal amplification.[6d,e] Although these techniques have made significant progress toward sensitivity improvement, all of them require protein enzymes, which increase the experimental cost and complexity. As an alternative, some enzyme-free methods have become increasingly attractive for signal amplification, such as hybridization chain reaction,[7a,b] entropy-driven catalysis,[7c] and catalyzed hairpin assembly.[7d–f] The unique feature of these techniques is that they are inherently modular, easy to scale up, and do not require perishable protein enzymes. However, the application of an enzyme-free amplified sensor for heavy metal ion monitoring has not been reported to date. Herein, for the first time, we developed a simple and enzyme-free strip biosensor for the amplified detection of Pb^{2+} based on a catalytic DNA circuit.

We employed the Pb^{2+}-specific 8–17 DNAzyme as the molecular recognition element for the detection of Pb^{2+}. The substrate strand (17DS) containing a ribonucleoside adenosine (rA, indicated in red) was extended at the 5′-end to 35 *mer* oligonucleotide without changes to the original DNAzyme. The DNAzyme strand 17E was hybridized with the substrate strand 17DS in solution first (Scheme 1A, left). In the presence of Pb^{2+}, the substrate strand 17DS was cleaved into two fragments at the rA position. The cleaved product at the 5′-end (named strand C, indicated in blue) of 17DS is released due to decreased affinity to 17E (Scheme 1A, right). The released strand C was designed as an input to trigger the amplified detection of Pb^{2+} using an enzyme-free DNA circuit (Scheme 1B). DNA is represented as directional lines, with the arrow denoting the 3′ end and the square denoting the 5′ end. To concisely describe the functions of the circuit, DNA strands are conceptually subdivided into functional domains that act as a unit in nucleic acid hybridization, branch migration, and displacement. Domains are represented here by numbers; a starred domain denotes a domain complementary in sequence to the domain without a star (for example, domain 1* is complementary to domain 1). In the presence of Pb^{2+}, the released strand C first

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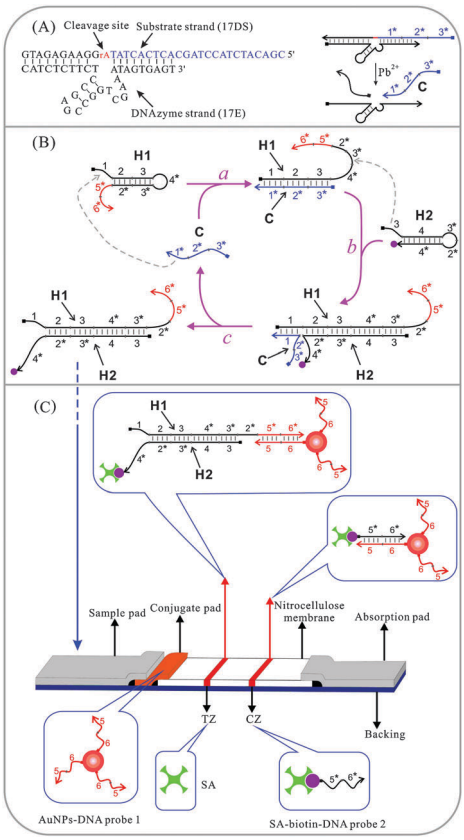

Scheme 1 (A) The secondary structure of the Pb^{2+}-specific 8–17 DNAzyme (left). The substrate is cleaved into two fragments in the presence of Pb^{2+} (right). The cleavage site is indicated in red (rA). (B) The cleaved DNA strand C (indicated in blue) is used as an input to trigger the enzyme-free DNA circuit for the amplified detection of Pb^{2+} through a series of toehold-mediated strand displacement reactions (*a*, *b*, and *c*). Toehold binding is shown by dotted gray lines with arrows. 5′ and 3′ termini of DNA strands are shown as squares and arrows, respectively. (C) Schematic illustration of the strip biosensor for visual detection of the formed duplex DNA (H1–H2-biotin). AuNP–DNA probe 1 is dispensed on the conjugate pad. SA and SA–biotin–DNA probe 2 are dispensed on the TZ and CZ, respectively. SA, streptavidin; TZ, the test zone; CZ, the control zone.

uses domain 1* as a toehold to bind domain 1 of hairpin H1, potentially initiating a branch migration (toehold-mediated stand displacement reaction) to open H1, leading to the formation of a C–H1 intermediate where the domain 3* of H1 is open (Scheme 1B, reaction *a*). Then, domain 3 of hairpin H2 can hybridize to the newly opened toehold 3* of H1, again initiating a branch migration to open H2 and form the C–H1–H2 complex (Scheme 1B, reaction *b*). The last step of the catalytic cycle is the spontaneous dissociation of strand C from the H1–H2 duplex (Scheme 1B, reaction *c*). During this process, the released strand C can act as a catalyst to trigger the catalytic assembly of another pairs of H1 and H2. In this enzyme-free DNA circuit, C catalyzes the formation of the duplex H1–H2 through toehold-mediated strand displacement reactions. H2 is modified with a biotin group and the formed output (H1–H2-biotin) is then applied on the sample pad of a strip for visual detection.

The strip biosensor consists of four components: sample pad, conjugate pad, nitrocellulose membrane, and absorption pad (Scheme 1C). A 5′-thiol-modified DNA probe 1 (complementary to domains 5* and 6* of H1, indicated in red) is conjugated with gold nanoparticles (AuNPs), and the conjugates (AuNP–DNA probe 1) are dispensed on the conjugate pad. Streptavidin and streptavidin–biotin–DNA probe 2 (complementary to DNA probe 1) are dispensed on the nitrocellulose membrane to form the test and control zone, respectively. The sample solution containing the formed duplex DNA (H1–H2-biotin) is applied on the sample pad. The solution migrates by capillary action, passes the conjugate pad, and then rehydrates the AuNP–DNA probe 1 conjugates. The domains 5* and 6* of the H1–H2-biotin duplex hybridize with the DNA probe 1 of the AuNP–DNA probe 1 conjugates to form the H1–H2-biotin–AuNP– DNA probe 1 complex and continue to migrate along the strip. The hybrids are captured on the test zone by the reaction between biotin and the pre-immobilized streptavidin. The accumulation of AuNPs on the test zone is visualized as a characteristic red band. The excess of AuNP–DNA probe 1 conjugates continue to migrate and are captured on the control zone by the hybridization events between DNA probe 1 and DNA probe 2, thus forming a second red band. In the absence of Pb^{2+}, 17DS was not cleaved and domain 1* of C was occluded by the complementary region of 17E. Therefore C should be catalytically inactive. The two hairpins H1 and H2 do not interact with each other to initiate the amplification reaction. In this case, no red band was observed on the test zone due to the failure of capturing the AuNP–DNA probe 1 conjugate on the test zone. A red band is always formed on the control zone of the strip to confirm the proper functioning of the test.

To evaluate the sensitivity and dynamic range of the enzyme-free amplified strip biosensor, different concentrations of Pb^{2+} were examined under optimal experimental conditions (Fig. S1 and S2, ESI†). As shown in Fig. 1A, the color intensities of the red bands on the test zone increased with increasing Pb^{2+} concentration. In the absence of Pb^{2+}, there was no red band observed on the test zone. Their corresponding optical responses were further confirmed by recording the intensities (peak areas) of the red bands with the aid of a portable "strip reader" (Fig. 1B). The resulting calibration curve shows that the peak areas are proportional to the logarithm of Pb^{2+}

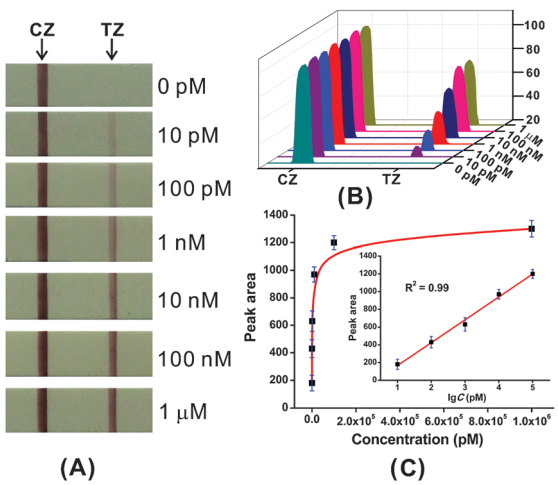

Fig. 1 (A) Photo images of the strip with different concentrations of Pb^{2+}. The readout was recorded with a digital camera. (B) Their corresponding optical responses. The peak areas were recorded with a strip reader. (C) Plots of the optical responses of the strip *vs.* the concentration of Pb^{2+}. Inset: calibration curve of the peak areas *vs.* the logarithm of Pb^{2+} concentration. The error bars represent the standard deviation of three independent measurements.

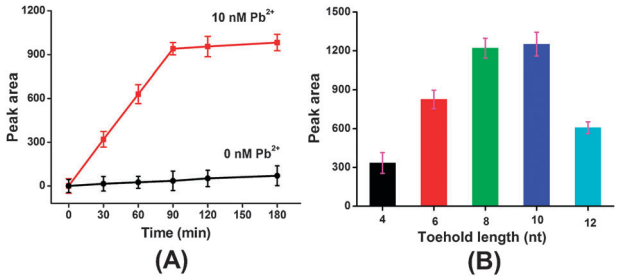

Fig. 2 (A) Effect of reaction time of the DNA circuit on the signal amplification. (B) Effect of toehold length on the reaction rate of the DNA circuit. Five catalyst-DNA strands (C_4, C_6, C_8, C_{10}, and C_{12}, listed in Table S1, ESI†) were chosen to initiate the DNA circuit. Their corresponding toehold lengths are 4, 6, 8, 10, and 12 nt, respectively. *C* = 10 nM, H1 = 200 nM, H2 = 300 nM, reaction time = 90 min. The error bars represent the standard deviation of three independent measurements.

concentration in the range 10 pM to 100 nM (Fig. 1C). The red band on the test zone is quite visible even at 10 pM Pb^{2+}, which can be used as the threshold for the visual determination of Pb^{2+} without instrumentation. This detection limit of our strategy is four orders of magnitude lower than the DNAzyme-based dipstick test for Pb^{2+} without signal amplification.[5] Compared to previously reported colorimetric,[2f] fluorescent,[2a] and electrochemical[2g] DNAzyme sensors for Pb^{2+}, the new strip system developed here also possesses higher sensitivity. Such an attractive detection limit of our assay protocol can be primarily attributed to the continuous turnover capability of the catalytic DNA circuit in the presence of Pb^{2+}. As the maximum contamination level of Pb^{2+} in drinking water was defined by the United States Environmental Protection Agency (EPA) to be 72 nM, this strip is promising for on-site monitoring of Pb^{2+} with its superior detection limit and wide dynamic range.

The process of signal amplification was strongly affected by the reaction time of the catalytic DNA circuit. As shown in Fig. 2A, the optical response elevates gradually with increasing reaction time within 90 min in the presence of 10 nM Pb^{2+} and keeps a constant level after that, which indicates that the equilibrium is reached. However, the background signal (in the absence of Pb^{2+}) also increases slowly. In order to obtain the best signal-to-background ratio, 90 min was selected as the optimum reaction time.

The reaction rate of the toehold-mediated stand displacement primarily depends on the length of the toehold. As shown in Fig. 2B, increasing the toehold lengths from 4 to 8 nt, the peak area of the red band on the test zone of the strip increased accordingly, and further increasing the toehold length (10 nt) does not significantly accelerate the reaction. While the 12 nt toehold leads to a much lower rate, the slower catalysis by C_{12} arises from the nearly irreversible toehold binding. In general, toehold binding should be strong enough to efficiently initiate strand displacement and weak enough to spontaneously dissociate and regenerate the catalyst strand.[7e,f,8] Hence, 8 nt toehold was used in our DNA circuit design.

To validate the specificity of the strip for Pb^{2+} analysis, several other divalent metal ions are tested as a control. As shown in Fig. S3, ESI†, 10 nM Pb^{2+} could produce a bright red band on the test zone, while all other metal ions at concentrations of 500 nM did not yield a red band on the test zone. The results demonstrate that our developed strip exhibits a high selectivity to Pb^{2+} over other competing metal ions. The practical application of our amplified strip sensor is demonstrated by applying it to detecting Pb^{2+} in real freshwater samples (lake water). The samples collected were simply filtered, and found that the Pb^{2+} content in the lake water was too low to be detected by the sensor. The recovery experiments with spiked Pb^{2+} were carried out and the analytical results are shown in Table S2 (ESI†). Satisfactory recovery values between 88 and 106.3% for Pb^{2+} were obtained for the recovery, which confirmed that the proposed sensor was applicable for practical Pb^{2+} detection in environmental samples.

In summary, we have successfully constructed, for the first time, an enzyme-free strip biosensor for the amplified detection of Pb^{2+} using a catalytic DNA circuit for signal transduction. The reaction mechanism is based on a toehold-mediated nucleic acid hybridization, branch migration, and displacement. In comparison with the previously reported enzyme-based strips,[4a,b,6a] the whole signal amplification process does not require any protein enzyme, making the system more simple and cost effective. The generated signals (red bands on the test zone of the strip) can be unambiguously read out by the naked eye. The amplified strip is ultrasensitive for Pb^{2+} detection, with a detection limit of 10 pM, which is four orders of magnitude better than the previously reported strip without signal amplification.[5] The strip is robust and can be applied to environmental samples with excellent selectivity. In order to reduce the reaction time and move the strip much closer to field monitoring, future work will focus on developing an even faster DNA circuit with accelerated catalytic ability.

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