Electronic Supplementary Information

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

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Experimental Section

Chemicals and materials

Streptavidin, $HAuCl_4 \cdot 3H_2O$, trisodium citrate, Tween-20, sodium dodecyl sulfate (SDS), tris-(hydroxymethyl)aminomethane (Tris), bovine serum albumin (BSA), and $Pb(Ac)_2$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glass fiber (CFSP001700) and nitrocellulose membranes (HF12002XSS, HF18002XSS, and HF24002XSS) were purchased from Millipore (Billerica, MA). Other common chemicals were analytical reagent grade and were used as received. All solution was prepared with ultrapure water (18.2 MΩ/cm) from a Millipore Milli-Q water purification system (Billerica, MA). Environmental water sample was taken from a nearby lake and filtered through a 0.2 μm membrane to remove the insoluble impurities.

Oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and listed in Table S1.

Table S1 Sequences of oligonucleotides used in the present work[a]

Name	Sequences (from 5' to 3')
DNAzyme strand (17E)	CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
Substrate strand (17DS)	^{3*}CGACATCT ^{2*}ACCTAGCA ^{1*}CTCACTAT rAGGAAGAGATG
H1	^{1}CATGATAGTGAG ^{2}TGCTAGGT ^{3}AGATGTCG ^{4*}CCATGTGTAGA
	^{3*}CGACATCT ^{2*}ACCTAGCA ^{5*}CCTTGTCA ^{6*}TAGAGCAC
H2	^{3}AGATGTCG ^{4}TCTACACATGG ^{3*}CGACATCT ^{2*}ACCTACGA ^{4*}CCATGTGTAGA-Biotin
C_4	^{3*}CGACATCT ^{2*}ACCTAGCA ^{1*}CTCA
C_6	^{3*}CGACATCT ^{2*}ACCTAGCA ^{1*}CTCACT
C_8	^{3*}CGACATCT ^{2*}ACCTAGCA ^{1*}CTCACTAT
C_{10}	^{3*}CGACATCT ^{2*}ACCTAGCA ^{1*}CTCACTATCA
C_{12}	^{3*}CGACATCT ^{2*}ACCTAGCA ^{1*}CTCACTATCATG
DNA probe 1	SH-AAAAAAAAAAAA ^{6}GTGCTCTA ^{5}TGACAAGG
DNA probe 2	Biotin-^{5*}CCTTGTCA ^{6*}TAGAGCAC

[a]rA denotes adenosine ribonucleotide while all others are deoxyribonucleotides. Domains are named by numbers and complementarity is denoted by asterisks. DNA probe 1 was used to conjugate with AuNPs and dispensed on the conjugate pad of the strip. DNA probe 2 was dispensed on the control zone of the strip.

Preparation of gold nanoparticles (AuNPs)

AuNPs with average diameter 15 ± 3.5 nm were prepared according to the reported methods with slight modifications.[1] All glassware used in this preparation was thoroughly cleaned in aqua regia (three parts HCl and one part HNO_3), rinsed in doubly distilled water, and oven-dried prior to use. In a 500 mL round-bottom flask, 250 mL of 0.01% $HAuCl_4$ in doubly distilled water were

brought to boil with vigorous stirring, followed by the addition of 10 mL of 1% trisodium citrate. The solution turned deep blue within 20 s, and the final color changed to wine-red after 60 s. Boiling was pursued for an additional 10 min; the heating source was removed, and the colloid solution was stirred for another 15 min. The resulting AuNPs solution was stored in dark bottles at 4 ^{o}C and used to prepare the DNA-AuNPs conjugate.

Preparation of DNA-AuNPs conjugates

Conjugation reactions were carried out by adding 100 μL of thiolated DNA (DNA probe 1, 1.0 OD) to 900 μL of the 10-fold concentrated AuNPs solution. After standing at 4 ^{o}C for 24 h, the solution was subjected to "aging" by the addition of NaCl up to a concentration of 150 mM, and a certain quantity of 1% sodium dodecyl sulfate (SDS) was added to reach a final concentration of 0.01%. The solution was allowed to stand for another 24 h at 4 ^{o}C, and the excess of reagents was removed by centrifugation for 20 min at 12000 rpm. The supernatant was discarded, and the red pellets were resuspended in 1 mL of buffer (pH 7.5) containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20, and 10% sucrose. The resulting AuNPs-DNA probe 1 conjugate solution was stored at 4 ^{o}C before further use.

Preparation of the strips

The strip consists of four components: sample pad, conjugate pad, nitrocellulose membrane, and absorption pad. All the components were mounted on a common backing layer (typically an inert plastic, e.g., polyester). The sample pad (17 mm x 30 cm) was made from glass fiber (CFSP001700, Millipore) and saturated with a buffer (pH 8.0) containing 0.25% Triton X-100, 0.02 M Tris-HCl, and 0.15 M NaCl. Then, it was dried at 37 °C for 2 h and stored in desiccators at room temperature. The conjugate pad (8 mm x 30 cm) was prepared by dispensing 5 μL AuNPs-DNA probe 1 conjugate solution onto the glass fiber pad with the dispenser HM3030 (Shanghai Kinbio Tech. Co., Ltd. Shanghai, China). The pad was dried at room temperature and stored in a desiccator at 4 ^{o}C. The test zone and control zone on the nitrocellulose membrane (25 mm x 30 cm) were prepared by dispensing a concentration of 1.2 mg/mL streptavidin and 1.2 mg/mL streptavidin-biotinylated DNA probe 2 solutions, respectively. To facilitate the immobilization of DNA probe 2 on the nitrocellulose membrane, streptavidin was used to react with the biotinylated control DNA probe 2 to form the streptavidin-biotin DNA probe 2 conjugates.[1,2] The conjugates were then dispensed on the control zone of the nitrocellulose membrane with the HM3030 dispenser. The distance between the test zone and control zone was around 5 mm. The membrane was then dried at 37 °C for 1 h and stored at 4 °C in a dry state. Finally, the sample pad, conjugate pad, nitrocellulose membrane, and absorption pad were assembled on a plastic adhesive backing (60 mm x 30 cm), and each part overlapped 2 mm to ensure migration of the solution through the strip during the assay. Strips with a 4 mm width were cut by using a programmable strip cutter ZQ2000 (Shanghai Kinbio Tech. Co., Ltd. Shanghai, China).

Analytical procedure

The DNA solutions were heated at 90 ^{o}C for 10 min to dissociate any intermolecular interaction, and gradually cooled to room temperature. 50 nM DNAzyme substrate stand (17DS) was first incubated with 150 nM DNAzyme stand (17E) in 20 mM Tris-Ac buffer (containing 150 mM NaAC, pH 7.4) at room temperature for 20 min to form the DNAzymes. The Pb^{2+} solution was then added into the resulting DNAzymes and incubated for 40 min which led to the maximum cleavage of the DNAzyem substrate strand (17DS) at the rA position. Finally, 200 nM H1 and 300 nM H2 were added, and the mixture was incubated for 90 min at room temperature. The mixed sample solution (total volume, 80 μL) was applied to the sample pad of the strip for assay. After waiting for 5 min, another 20 μL Tris-Ac buffer was added to the sample pad to wash the strip. Accumulation of AuNPs on the test and control zones produced the characteristic red bands. Visual detection of Pb^{2+} was simply realized by observing the color on the test zone of the strip. The red bands were visualized within 10 min. The intensities of the red bands on the test and control zones were recorded by using the portable "strip reader" equipped with the "GoldBio strip reader" software (Shanghai Kinbio Tech. Co., Ltd. Shanghai, China), which could search the red bands in a fixed reaction area automatically and then figure out parameters such as peak height and area integral.

For real samples analysis, 10 μL of lake water samples spiked with different concentrations of Pb^{2+} was added to the DNAzymes solution. Other procedures were the same as described above.

To probe the effect of the toehold length on the sensing performance of the DNA circuit, 10 nM C_4, C_6, C_8, C_{10}, or C_{12} (with the toehold lengths of 4, 6, 8, 10, or 12 nt, respectively) was incubated with 200 nM H1 and 300 nM H2 to initiate the DNA circuit.

Safety Considerations

Because Pb^{2+} and most of the tested heavy metal ions are highly toxic and have deleterious effects on human health, all experiments involving in heavy metal ions should be performed with protective gloves. The waste solutions that contain heavy metal ions should be collectively reclaimed to avoid polluting the environment.

Effect of temperature

The reaction temperature of the catalytic DNA circuit affects the amount of duplex DNA (H1-H2-biotin) generated in the presence of Pb^{2+}. We compared the signal-to-noise (S/N) ratio of the strip for 10 nM Pb^{2+} at different temperatures (25°C, 37°C, and 45°C) (Fig. S1). It was found that the S/N ratio decreased with the increase of the temperature from 25°C to 45°C. The S/N ratio loss at a high temperature would be attributed to the conformation change of hairpin probes (H1 and H2). At high temperatures, the stem-loop structures of H1 and H2 were opened and produced straight-chain oligonucleotides which induced a hybridization reaction between H1 and H2 to form duplex DNA (H1-H2-biotin) even in the absence of Pb^{2+}, resulting in a high background signal. Therefore, catalytic DNA circuit was performed at room temperature (~25 °C).

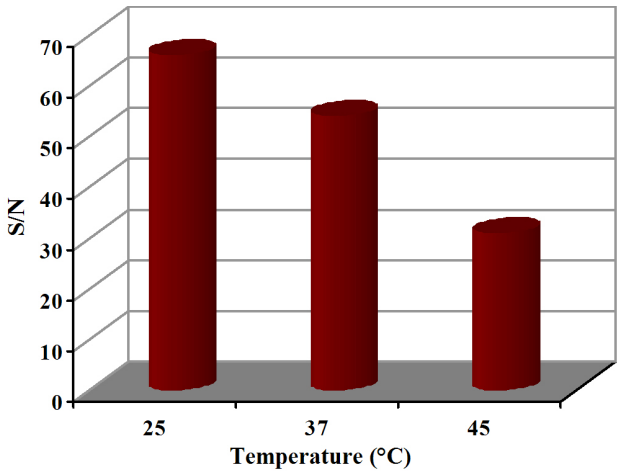

Fig. S1 Effect of the reaction temperature of the catalytic DNA circuit on the S/N ratio of the strip. Pb^{2+} concentration: 10 nM; reaction time: 90 min.

Optimization of experimental parameters

The experimental parameters (e.g., the amount of AuNPs-DNA probe 1 conjugate, the concentration of streptavidin immobilized on the test zone, and the type of nitrocellulose membrane) that affect the sensitivity and reproducibility of the strip test were optimized. The optimizations were performed by varying one experimental condition and keeping constant conditions for other parameters. Control experiments (in the absence of Pb^{2+}) were conducted at the same conditions. The performance of the strip was evaluated by comparing the signal-to-noise (S/N) ratio.

In the current study, the AuNPs-DNA probe 1 conjugates were immobilized on the conjugate pad of the strip by physical adsorption. The accumulations of AuNPs on the test and control zones were visualized as characterized red bands which could be used for the visual detection of Pb^{2+}. The intensities of the red bands depended on the captured AuNPs-DNA probe 1 conjugates on the test and control zones which, in turn, corresponded to the amount of conjugates on the conjugate pad. Fig. S2A shows the effect of different amount of AuNPs-DNA probe 1 conjugates on the S/N ration for the assay of 10 nM Pb^{2+}. It was found that the S/N ratio of the assay increased upon raising the volume of AuNPs-DNA probe 1 conjugates from 3 μL to 5 μL; further increasing the volume caused a decrease of the S/N ratio, which was ascribed to the increased nonspecific adsorption and background signal. Thus, 5 μL of AuNPs-DNA probe 1 conjugates was dispensed on the conjugate pad when assembling a strip.

Another factor taken into account for the parameter optimization is the amount of streptavidin immobilized on the test zone of the strip. Streptavidin was used to capture the formed H1-H2-biotin-AuNPs-DNA probe 1 complex via the specific streptavidin-biotin reaction. Streptavidin with different concentrations ranging from 0.4 mg/mL to 2 mg/mL was dispensed on the test zone. The performance of the strip was evaluated by comparing the S/N ratio in the presence of 10 nM Pb^{2+} (Fig. S2B). It was found that the S/N ratio increased up to 1.2 mg/mL streptavidin; a further concentration increase resulted in a decrease of the S/N ratio, which may be caused by the increased nonspecific adsorption of AuNPs-DNA probe 1 on the test zone. Therefore, 1.2 mg/mL streptavidin solution was used to prepare the strip test zone.

The response of the strip biosensor is also relevant to the nitrocellulose membrane materials. Sandwich-type AuNPs-DNA probe 1-H1-H2-biotin-SA complex was formed on the strip, and the reaction time, which depends on the migration time of buffer in the nitrocellulose membrane, plays an important role in affecting the sensitivity and reproducibility of the strip test. The migration time of sample solution differs with different nitrocellulose membrane. Three kinds of nitrocellulose membranes (HF12002XSS, HF18002XSS, and HF24002XSS) were used to prepare the strip. According to the manufacturer's instructions, the migration time of the buffer in HF12002XSS, HF18002XSS, and HF24002XSS membranes are 120, 180, and 240 sec, respectively. Fig. S2C presents the responses of 10 nM Pb^{2+} on the strips prepared with the above nitrocellulose membranes. It can be seen that the S/N ratio of the strip fabricated by HF24002XSS is significantly higher than those prepared with HF12002XSS and HF18002XSS. It indicates that a relatively long migration time is helpful in improving the sensitivity of the strip. The whole assay time with HF24002XSS is around 10 min. So the HF24002XSS nitrocellulose membrane was used to prepare the strip.

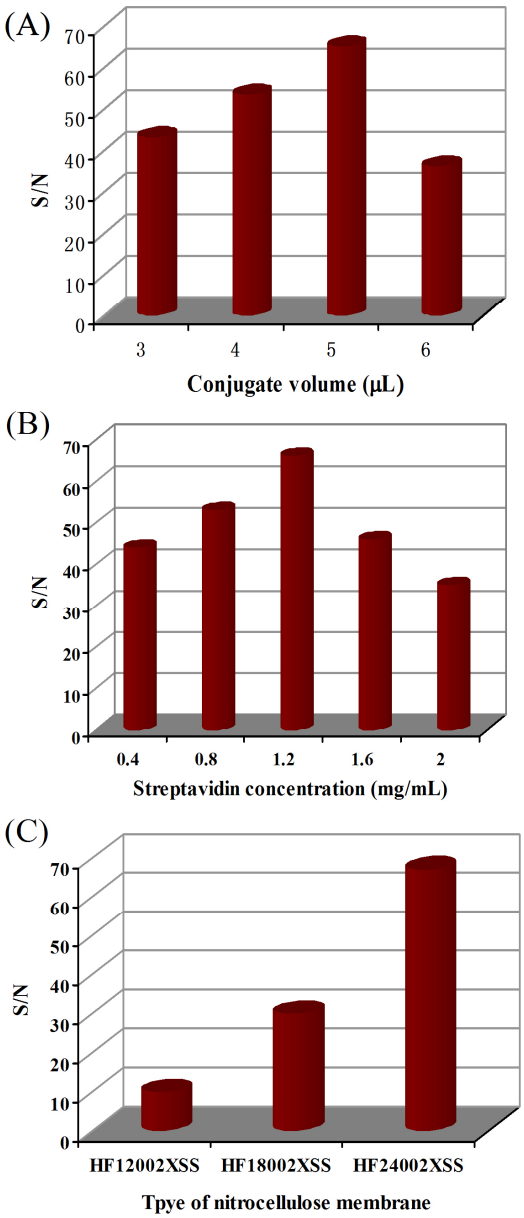

Fig. S2 (A) Effect of the volume of the AuNPs-DNA probe 1 conjugate on the S/N ratio of the strip. (B) Effect of streptavidin concentration (immobilized on the test zone) on the S/N ratio of the strip. (C) Effect of nitrocellulose membrane type on the S/N ratio of the strip. Pb^{2+} concentration: 10 nM; volume of the sample solution: 80 μL. All experiments were performed at room temperature.

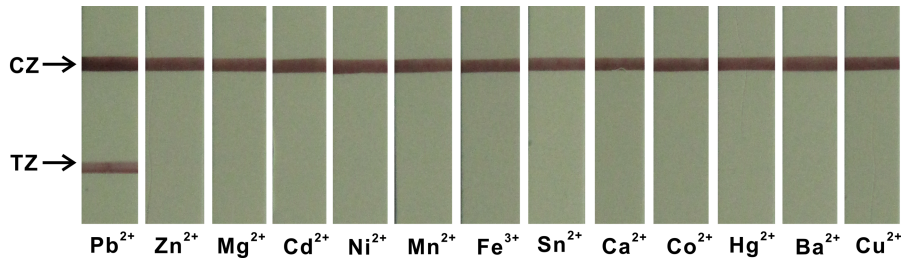

Fig. S3 Selectivity of the strip for Pb^{2+} over other competing metal ions. The concentration was 10 nM for Pb^{2+} and 500 nM for other metal ions. The incubation time for all metal ions was 90 min.

Table S2 Recovery experiments of Pb^{2+} in lake water samples

River water	Pb^{2+} spiked (nM)	Pb^{2+} founded[a] (nM)	Recovery (%)
1	1	0.88 ± 0.4	88
2	10	9.7 ± 1.5	97
3	100	106.3 ± 5.6	106.3

[a]Average of five determinations ± standard deviation.

References

1 X. Mao, Y. Ma, A. Zhang, L. Zhang, L. Zeng and G. Liu, *Anal. Chem*., 2009, **81**, 1660.

2 X. Mao, H. Xu, Q. Zeng, L. Zeng and G. Liu, *Chem. Commun*., 2009, 3065.