Colloids and Surfaces B: Biointerfaces 169 (2018) 305–312

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

# Graphene oxide-assisted Au nanoparticle strip biosensor based on GR-5 DNAzyme for rapid lead ion detection

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ARTICLE INFO

*Keywords:*
GR-5 DNAzyme
Graphene oxide-assisted
Strip biosensor
Lead ion
Au nanoparticle

ABSTRACT

This study has reported that a GR-5 DNAzyme based lead ion strip biosensor could exhibit an enhanced specificity with the assistance of graphene oxide (GO). This enhancement results from the specific π-stacking interaction between the ribose rings of the nucleobases and the carbon hexagons in GO which can reduce the false positive interference by removing unhybridized ssDNA during the annealing of GR-5 DNAzyme. Meanwhile, conjugate pad was sprayed with two kinds of AuNP-DNA probes, and nitrocellulose membrane test zone and control zone were immobilized with two kinds of biotin-DNA probes, respectively. The limit of detection of this strip biosensor was estimated to be about 0.05 nM (S/N = 3) and 1 nM (with naked eyes) with a linear range from 0.01 to 100 μM. Furthermore, the strip biosensor exhibited excellent selectivity toward $Pb^{2+}$ in the presence of other divalent metal ions. For real soil samples, the obtained recoveries were in the range from 91.5% to 113.1%.

## 1. Introduction

Lead pollution that is caused by industrial activities and urbanizations has attracted increasing concern, because it may cause severe adverse effects on human marrow hematopoietic system, cardiovascular system, immune system, nervous system and digestive system [1–3]. As a central nervous system poison, it had worse on children [3]. Most of lead that released into the environment will enter the soil, and then enter the human body through foods. Many countries have made provision for lead level in the environment and food. Therefore, it is critical to develop a simple, fast, low cost and portable lead detection device with high sensitivity and selectivity. The commonly used lead detection methods include graphite furnace atomic absorption spectrometry [4,5], fluorescence spectroscopy [6–9], ultraviolet spectrophotometry [10–12], electrochemical analysis [13–16], inductively coupled plasma mass spectrometry [17,18] and dithizone colorimetric method [19], etc. Though these methods can provide good enough performance in lead detection, they always require expensive equipment and very experienced professionals. Furthermore, most of these equipments and operation processes have to be conducted only at laboratory and are not adaptive for in field scenarios.

On the other hand, the lateral flow test strip is a convenient, low cost and prompt approach that have been successfully applied to a wide range of analytes such as biomolecules, chemical contaminants, heavy metals [20–23]. Various molecules including antibody, aptamer…, have been employed as receptors in test strip because of their high affinity and specificity to their corresponding ligands. Recently, DNAzymes were reported to been utilized to construct test strip for the detections of metal ions including $Pb^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Mn^{2+}$ and $Cu^{2+}$ [24–26]. DNAzymes are a class of DNA fragments that can own various catalytic functions including cleavage of nucleic acid, cleavage of phosphoramidate bond, igation of nuclei acid, formation of RNA branch or lariat, formation of nucleopeptide bond, DNA phosphorylation, DNA adenylation, DNA depurination, and porphyrin metallation. Among these DNAzymes, 8–17 DNAzyme can make specific response to $Pb^{2+}$ in which the substrate strand of 8–17 DNAzyme can be cleaved at rA [12,27–34]. Zeng et al. [24] sprayed conjugate pad with one AuNP-DNA probe, binding cleaved fragment H1-H2-biotin through a series of toehold-mediated strand displacement reaction, achieved the detection of lead ion. However, further studies found besides lead ion, 8–17 DNAzyme was also active for $Zn^{2+}$, $Mn^{2+}$, $Cr^{2+}$ and $Co^{2+}$, which meaned the interfere problem. Recently, Lu et al. [35] found that GR-5 DNAzyme had higher selectivity for lead ion. The results showed this DNAzyme had ~40,000 times selectivity for $Pb^{2+}$ over $Zn^{2+}$, which

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https://doi.org/10.1016/j.colsurfb.2018.05.020
Received 4 September 2017; Received in revised form 3 May 2018; Accepted 9 May 2018

**Table 1**
Sequences of oligonucleotides used in the study.

| Name | Sequence (5'-3') |
|---|---|
| Substrate strand (GR-5S) | GGTCTCACTATrAGGAAGAGATGATGTCTGTCAGATGTAG |
| DNAzyme strand (GR-5E) | GACATCATCTCTGAAGTAGCGCCGCCGTATAGTGAGACC |
| AuNP-DNA probe a' | GACATCATCTCT-$(CH_2)_6$-SH |
| AuNP-DNA probe c' | SH-$(CH_2)_6$-GGTCTCACTATA |
| Biotin-DNA probe a | AGAGATGATGTCAAAAAA-biotin |
| Biotin-DNA probe c | biotin-AAAAAATATAGTGAGACC |
| Biotin-DNA probe b' | biotin-AAAAAACTACATCTGACA |
| Biotin-DNA probe d' | CGGCGCTACTTCAAAAAA-biotin |

has been greatly improved than ~160 times of 8–17 DNAzyme. Therefore, it can be expected GR-5 DNAzyme may exhibit a better performance in the application of test strip based $Pb^{2+}$ detection.

For strip biosensor, there are two important issues that need to be addressed, one is how to improve the sensitivity, the other is how to exclude false-positive results. For $Pb^{2+}$ test strips based on DNAzyme, the previous studies usually immobilized one corresponding complementary sequence on strip that directed at one cleaved small fragment of DNAzyme, the remaining fragments did not play any role in the chromatographic process. In this work, it is the first time to detect lead ions using two different AuNP-DNA probes on conjugate pad and two different biotin-DNA probes on test zone (TZ) and other two biotin-DNA probes on control zone (CZ), which were all complementary with different part of the cleaved small DNA fragments respectively. This method improved the utilization efficiency of the cleaved fragments, meanwhile acquired higher detection sensitivity.

For interference of false-positive results, in the studies of lead ion detection strip based on DNAzyme, the two single strands of DNAzyme (DNAzyme strand and substrate strand) were usually synthesized separately and then hybridized to form a certain structure. During the hybridization process, complete reaction of those two single strands could not be achieved, there was always remaining single strand in solution, it would combine with complementary probes on the strip and cause false positive result, so how to overcome this interference is very important. In recent years, carbon nanotube, graphene oxide (GO) and other nano-carbon materials have increasingly been applied in biological field. GO is used to combine with gold nanoparticles to increase the electrochemical signal to enhance the detection sensitivity [36,37]. In our study, we use the characteristic that the surface unsaturated carbon atom of GO can form a large π bond with the bases of single-stranded DNA by non-covalent π stacking interaction, which can make GO bind single-stranded DNA closely; however, the interaction of GO and double-stranded DNA is very weak [38–41]. Based on this mechanism, herein, we used GO to process the sample after the hybridization of the two single strands, it was found free single-stranded DNA could be cleared absolutely, and the procedure had no effect on enzyme activity. The results also confirmed that this approach reduced interference of false positive result effectively. Compared with other strips for the detection of $Pb^{2+}$ [24,25], the design in our study could make full use of the substrate strand and enzyme strand by employing dual AuNP-DNA probes and could reduce the false positive by introducing GO. After applying the above two novel points, high sensitivity and no false positive results of strip for lead ion detection was realized, and the detection limit was 0.05 nM (S/N = 3) and 1 nM (with naked eyes), which could meet the testing requirement of real samples. In addition, the single testing cost of strip was about 1.8 RMB, indicating it had a very good application potential.

## 2. Experimental section

### 2.1. Apparatus

Conjugate pad, TZ and CZ were obtained with HM3030 dispenser (Shanghai Kinbio Tech., China), strips were cut by a programmable strip cutter (Shanghai Kinbio Tech.,China), the normalized intensities of the red bands on the TZ and CZ were recorded by strip image analyzer (Shanghai Kinbio Tech. Co., Ltd. Shanghai, China). UV–vis absorption spectra were performed with UV 2450 spectrophotometer (Shimadzu, Japan), ultrapure water (≥18.2 MΩ cm) was produced by the Milli-Q system (Millipore, USA).

### 2.2. Materials and solutions

Chloroauric acid ($HAuCl_4{\cdot}4H_2O$), sodium citrate, sodium chloride, trisodium phosphate, Tween 20, sucrose, polyethylene glycol octylphenol ether (Triton X-100), sodium dodecyl sulfate, polyethylene glycol (PEG 4000), trisodium phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, Tris(hydroxymethyl)aminomethane, glacial acetic acid, boric acid, edetate disodium ($EDTA_2Na$), silver nitrate, ethanol, nitric acid, formaldehyde, sodium carbonate, acrylamide, bisacrylamide, ammonium persulfate, *N,N,N′,N′*-tetramethylethylenediamine, sodium hydroxide, mercuric chloride, barium chloride, zinc chloride, copper nitrate, cobalt chloride, nickel sulfate, manganese chloride and calcium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); 4-hydroxyethyl piperazine acetic acid (HEPES), lead nitrate, cadmium nitrate were purchased from Aladdin (Shanghai, China); GO (2 mg/mL, 50–200 nm) was acquired from XF Nanotechnology Co., Ltd. (Nanjing, China); streptavidin-biotin (1 mg) was purchased from BBI Life Sciences Corporation (Shanghai, China). All chemicals were of analytical-reagent grade; absorbent paper (SX42), glass fiber (CB06), PVC plastic adhesive backing (SMNF31-25) were obtained from Shanghai Kinbio Tech. Co., Ltd (Shanghai, China); nitrocellulose membrane (CN140) was purchased from Sartorius (Goettingen, Germany); the sequences of the oligonucleotides used in this study were shown in Table 1. All sequences were synthesized and purified (HPLC) by Sangon Biotech Co., Ltd. (Shanghai, China).

### 2.3. Preparation of Au nanoparticle

Au nanoparticle (AuNP) was prepared by a previously reported trisodium citrate reduction method [42,43]. Briefly, 100 mL $HAuCl_4$ solution (0.01%) was heated to boiling and then added 2 mL trisodium citrate solution (1%) rapidly. The color of solution turned from yale yellow to wine-red, and after heating for another 10 min, the solution was slowly cooled to room temperature, filtered with a 0.22 μm

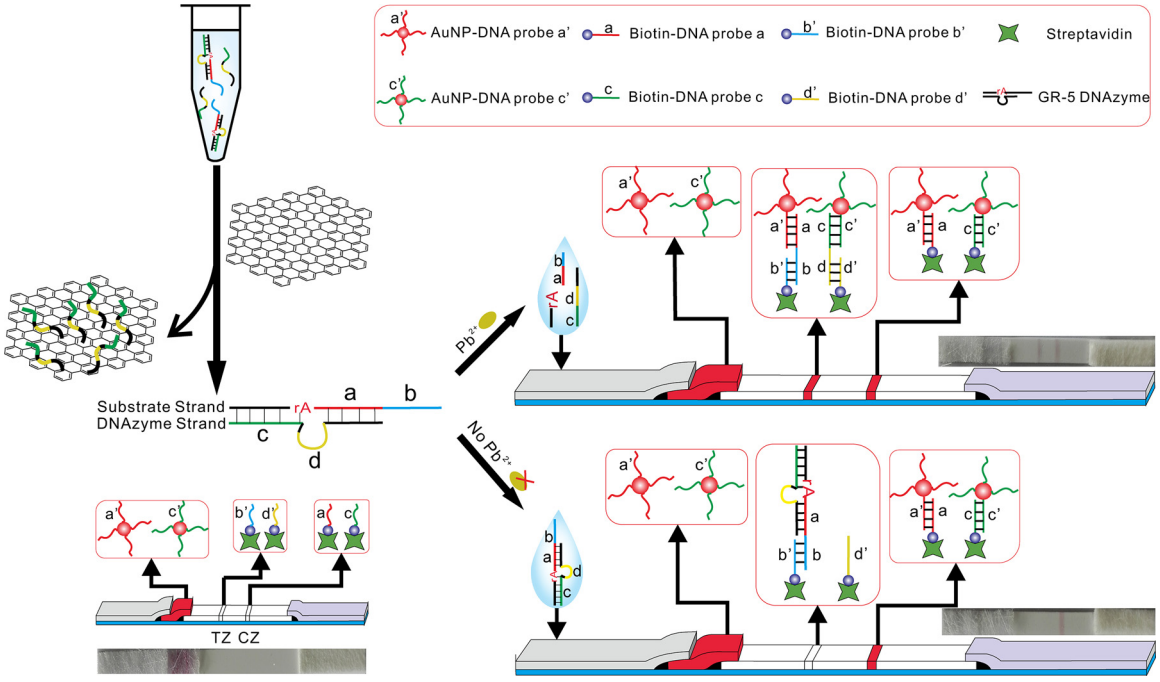


**Fig. 1.** Schematic representation of the strip biosensor for detection of lead ion based on GR-5 DNAzyme.

Millipore syringe filter to remove large particle, the filtrate was stored in refrigerator at 4 °C for future experiments.

### 2.4. Preparation of AuNP-DNA probe

AuNP-DNA probes were prepared according to the protocol reported previously with slight modifications [44]. The 45 μL thiolated DNA a′ and c′ (100 μM) were added to 14 mL AuNP solution, respectively. After reacting for 24 h, PBS (0.1 M) and SDS (10%) were added to obtain the final concentration of 9 mM and 0.1%, respectively. The mixture was placed on an orbital shaker for 30 min and then aged for 2 days by addition of 2 M NaCl (final concentration of 0.2 M). The solution was centrifuged at 4 °C for 25 min with 12000 rpm, the supernatant was discarded to remove non-modified DNA probes, and the red precipitates were resuspended in 1 mL buffer (20 mM $Na_3PO_4$, 5% BSA, 0.25% Tween-20 and 10% sucrose, pH 7.4) according to literature [45], this procedure was repeated three times. Finally, the red precipitates were resuspended in 700 μL buffer. The obtaining AuNP-DNA probe c' and a' were stored at 4 °C for future use.

### 2.5. Preparation of the strip

The areas of strip from left to right were sample pad, conjugate pad, nitrocellulose membrane, absorption pad according to the direction of migration. All the parts were assembled on the PVC plastic adhesive backing. Each part was overlapped 2 mm to ensure migration of the sample solution during testing process. The sample pad was obtained from the glass fiber and soaked with the buffer (4% Triton X-100, 1% BSA, 2% glucose, 2% PEG 4000, 100 mM boric acid and 0.1% SDS, pH 8.0), then it was dried at 37 °C for 1 h and stored at room temperature. The conjugate pad was prepared by spraying 4 μL/cm AuNP-DNA probe c' and a' mixed solution onto the processed glass fiber with the HM3030 dispenser. Then it was dried at 37 °C for 1 h and stored at 4 °C. 15 μL biotinylated DNA probe b' (100 μM) and 15 μL probe d' (100 μM), 15 μL biotinylated DNA probe a and 15 μL biotinylated DNA probe c were mixed with 20 μL streptavidin (1 mg/mL), respectively. The mixed sulutions were reacted at room temperature for 2 h. Then the streptavidin-biotinylated DNA probe b', d' (SA-biotin-DNA b', d') and the streptavidin-biotinylated DNA probe a, c (SA-biotin-DNA a, c) were dispensed on the TZ and CZ of the nitrocellulose membrane with the HM3030 dispenser, respectively. The distance between the TZ and CZ was around 5 mm. The membrane was then dried at 37 °C for 12 h and stored at 4 °C in desiccator.

### 2.6. Preparation of assay solution

The DNAzyme strand (GR-5E) and substrate strand (GR-5S) of GR-5 DNAzyme were dissolved in 5 × SSC (pH 7.4) buffer to reach a final concentration of 150 nM and 100 nM, respectively. The mixed solution was heated in 95 °C water bath for 5 min and allowed to cool slowly to room temperature for 3 h. GO (2 mg/mL) was added to the above solution at a final concentration of 62.5 μg/mL, and the mixure was incubated for 1 h and centrifuged for 10 min with 10500 rpm to remove the GO precipitation, the supernatant was collected as the assay solution.

### 2.7. Polyacrylamide gel electrophoretic analysis

The above GR-5 DNAzyme mix solution, assay solution alone or with different concentrations of $Pb^{2+}$ (10 μM, 100 nM, 1 nM and 0.05 nM) for 1 h. The used volume of the solution was 30 μL. The samples were applied to a polyacrylamide (PAGE) gel (20% polyacrylamide, 30% acrylamide, 29:1 acrylamide/bisacrylamide) to separate GR-5 DNAzyme, DNAzyme strand or substrate strand. The electrophoresis was carried in 1 × tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, pH 8.0) at 90 v for 1 h. The gel was stained with silver nitrate, then photographed with camera.

### 2.8. $Pb^{2+}$ detection procedure

The $Pb^{2+}$ standard solution with different concentration was added to the assay solution and incubated for 1 h, then the mixture was dropped on the sample pad of the strip for assay. After 5 min, 5 × SSC buffer was added to the sample pad to wash the strip. The characteristic red bands at TZ and CZ of strip could be observed by naked eyes after another 15 min resulted from the accumulation of AuNPs, and the normalized intensities were recorded by strip image analyzer. Soil sample was digested and spiked with different concentrations of $Pb^{2+}$ to carry out the recovery experiments.

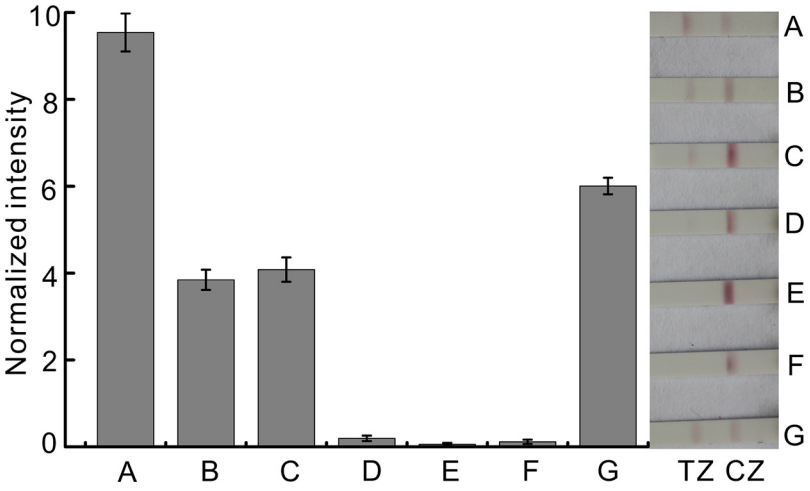


**Fig. 2.** The image of strip biosensor under different detection conditions. (A-C): GR-5 DNAzyme + GO + $Pb^{2+}$; (D) GR-5 DNAzyme + GO; E: $Pb^{2+}$; F: blank; G: GR-5 DNAzyme. Conjugate pad was immobilized with AuNP-DNA probe c' and a' (A, D, E, F and G), or only with AuNP-DNA probe c' (B), or only with AuNP-DNA probe a' (C). Concentration of $Pb^{2+}$: 1 μM. Concentration of sprayed Au nanoparticle: 4 μL/cm. The assay buffer: 5 × SSC.

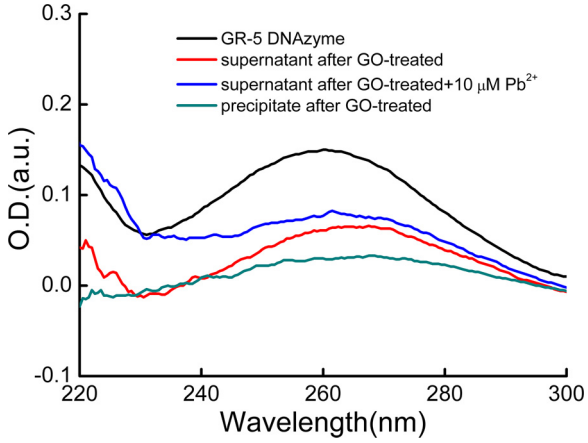


**Fig. 3.** UV–vis spectra of GR-5 DNAzyme under different treatment conditions.

## 3. Results and discussion

### 3.1. The strategy of the proposed strip biosensor

In this paper, we have designed a GO-assisted strip biosensor based on GR-5 DNAzyme for the detection of lead ion. As shown in Fig. 1, the conjugate pad was immobilized with AuNP-DNA probe c' and a' mixed solution, the TZ and CZ of nitrocellulose membrane were immobilized with two biotin-DNA probes, respectively. In the presence of $Pb^{2+}$, the GO treated GR-5 DNAzyme solution was cleaved at rA position. While the solution migrated along the strip, one end of free 'ab' DNA sequence hybridized with AuNP-DNA probe a' immobilized on the conjugate pad, when this Au nanoparticle migrated to TZ, the other end of free 'ab' DNA sequence would hybridize with biotin-DNA probe b' on TZ to form a sandwich structure. Similarly, free 'cd' DNA sequence would hybridize with AuNP-DNA probe c' on conjugate pad and biotin-DNA probe d' on TZ to form another sandwich structure, thus AuNPs were accumulated on TZ which would appear visible red band, after these, the excess AuNP-DNA probes would hybridize with biotin-DNA of CZ.

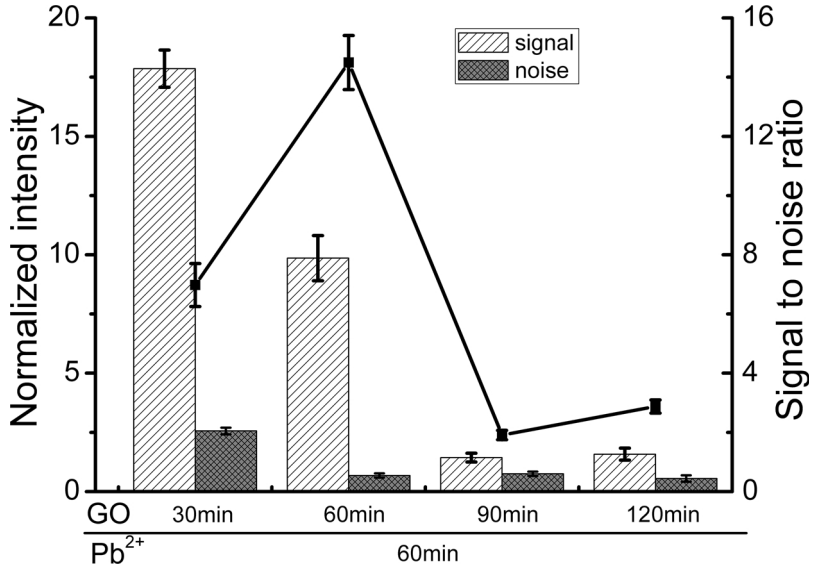


**Fig. 4.** The treatment of GO with different reaction times. The noise values were obtained without the addition of $Pb^{2+}$. Error bars were obtained from three parallel experiments. The other conditions were same as Fig. 2A.

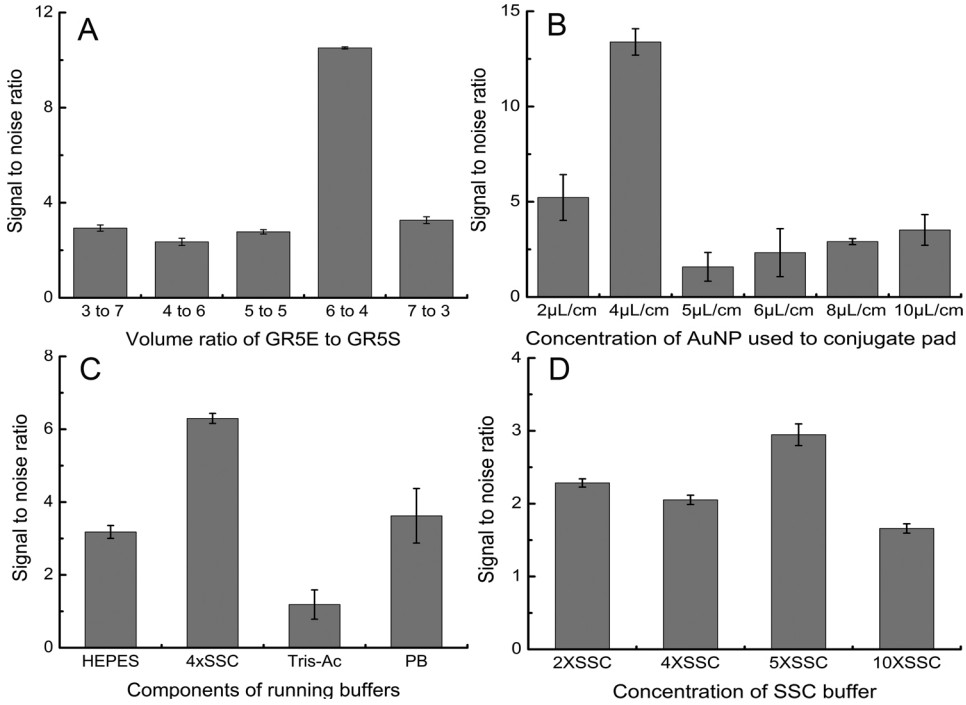


**Fig. 5.** The effect of volume ratio of AuNP-DNA probe c'–a' (A), concentration of AuNP used to conjugate pad (B), different running buffers (C) and different concentration of buffer (D). Concentration of $Pb^{2+}$: 1 μM. Error bars were obtained from three parallel experiments.

In the absence of $Pb^{2+}$, GR-5 DNAzyme kept stable duplex structure, the DNA sequence 'a' and 'c' were both occupied and couldn't hybridize with AuNP-DNA probes on conjugate pad, indicating the sandwich structure couldn't be formed, and AuNPs could not be accumulated on the TZ, thus the TZ would not appear characteristic red band. However, the AuNP-DNA probe a' and c' would continue to arrive at the CZ, and hybridize with the biotin-DNA probe a and c, respectively.

### 3.2. Characterization of nanoparticle

Au nanoparticle was the basic element of the strip. Au nanoparticle and AuNP-DNA probe were characterized by UV–vis spectroscopy firstly. It was found that the absorption peak of Au nanoparticle was about 520 nm (see Fig. S1A in Supplementary material), and the Au nanoparticle concentration was about 0.8 nM with 21 nm diameter according to the literature report [46]. After the Au nanoparticle was modified with thiolated DNA, the maximum absorption peak had a red shift and decreased intensity compared with those of Au nanoparticle (see Fig. S1A in Supplementary material), which was attributed to the increasing hydration radius of Au nanoparticle after the modification of thiolated DNA on surface and the loss of Au nanoparticle during the purification process, respectively. One interesting phenomenon was observed. After the addition of NaCl to 0.2 M during the preparation of probe, the visible color of Au nanoparticle solution did not change (see Fig. S1A, inset b in Supplementary material), while the aggregate occurred in bare Au nanoparticle solution (see Fig. S1A, inset c in Supplementary material), indicating that the thiol-DNA on Au nanoparticle surface provided protection for Au nanoparticle. Then, we used concentrated AuNP-DNA probe (see Fig. S1B in Supplementary material) with a concentration of about 14 nM for subsequent experiments.

### 3.3. The proposed strip biosensor under different experimental conditions

#### 3.3.1. Effect of different kinds of AuNP-DNA probes

The strip biosensor was investigated under different experimental conditions after the preparation with AuNP-DNA probe. Firstly, the two detection results obtained by one kind of AuNP-DNA probe and two kinds of AuNP-DNA probes immobilized on conjugate pad respectively were compared. It was observed that the band intensity of TZ with AuNP-DNA probe a' and c' (Fig. 2A) on conjugate pad was significantly greater than that of only one single probe AuNP-DNA probe c' (Fig. 2B) or AuNP-DNA probe a' (Fig. 2C) on conjugate pad, when the GO treated GR-5 DNAzyme solution for assay. It was indicated that the conjugate pad sprayed with two AuNP-DNA probes could utilize DNA fragments sufficiently to improve the sensitivity of lead ion detection. In the subsequent experiments, the conjugate pad was all sprayed with two kinds of AuNP-DNA probes. When the assay solution did not contain GR-5 DNAzyme except $Pb^{2+}$ (Fig. 2E), there was no visible color in TZ and only visible color in CZ, just like blank buffer (Fig. 2F). This meant the AuNP-DNA probe couldn't be immobilized at TZ, and had to migrate to CZ for hybridization.

#### 3.3.2. Reducing false-positive results with GO

UV–vis spectra of GR-5 DNAzyme under different treatment conditions were examined. GO-treated GR-5 DNAzyme was centrifuged to determine the precipitate and the supernatant, respectively. The results showed that the UV–vis absorption of GO-treated GR-5 DNAzyme supernatant was decreased, indicating single-strand was absorbed on GO precipitate (Fig. 3), and UV–vis spectra proved the amount of GR-5E adsorbed on GO by calculating was 50.8 nM (see Fig. S2 in Supplementary material). It was also found there was a weak peak in UV–vis absorption of GO-treated GR-5 DNAzyme precipitate (Fig. 3), which belonged to the adsorbed GR-5E. When GO-treated GR-5 DNAzyme was cleaved by $Pb^{2+}$, the UV–vis absorption was increased (Fig. 3), this was because hyperchromic effect of single-strand DNA. After that, the effect of GO for the strip biosensor was investigated, obvious red band could be found at the TZ when GO was not adopted to treat assay solution containing GR-5 DNAzyme without the addition of $Pb^{2+}$ (Fig. 2G), indicating that there was a false positive result. However, after treating the assay solution with GO, the signal of TZ was extremely low and there was no visible color with naked eyes (Fig. 2D), indicating that the pretreatment with GO greatly reduced the false positive result caused by free single stranded DNA effectively. Based on these results, three conclusions could be drawn: 1) the assay solution must contain GR-5

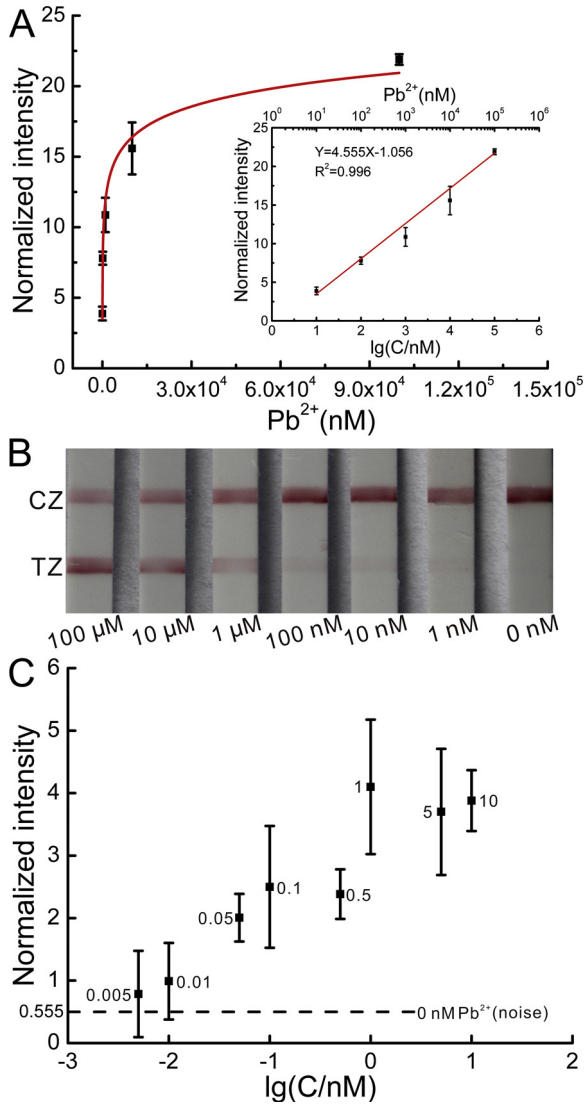


**Fig. 6.** Plots of the band intensity of TZ with the concentrations of $Pb^{2+}$ (A), Inset: the calibration curve of normalized intensity and logarithm of $Pb^{2+}$ concentration. The images of strip biosensor at different $Pb^{2+}$ concentrations (B). The normalized intensity of TZ at low $Pb^{2+}$ concentrations (C). Error bars were obtained from three parallel experiments. The other conditions were same as Fig. 2A.

DNAzyme and $Pb^{2+}$, GO treatment can reduce false positive results; 2) conjugate pad containing two kinds of AuNP-DNA probes can get better sensitivity for strip biosensor; 3) the color of TZ is indeed the result of the interaction between the $Pb^{2+}$ and GR-5 DNAzyme. It also demonstrates that it is feasible to detection $Pb^{2+}$ by this detection principle.

### 3.3.3. Effect of GO with different treatment times

Since the DNAzyme strand GR-5E and substrate strand GR-5S of GR-5 DNAzyme were involved in hybridizing with complementary AuNP-DNA probes, the AuNP-DNA probe c' could hybridize with DNA sequence c of GR-5E, and AuNP-DNA probe a' could hybridize with DNA sequence a of GR-5S. However, if GR-5E and GR-5S couldn't be hybridized entirely, the remaining free single-stranded DNA would bind to AuNP-DNA probes at conjugate pad, then go to TZ, and this could cause false positive result. It was well known that GO could be adsorbed by single-stranded DNA closely rather than double-stranded DNA. So in the experiment, the assay solution was heated for denaturation and annealed to form double-stranded GR-5 DNAzyme, after that, GO was introduced to adsorb remaining free single-stranded DNA to exclude false positive result, then $Pb^{2+}$ was added for cleavage. So, different treatment time of GO on detection result was investigated. It was found that signal values and noise values were high with false positive results when the treatment time was 30 min (Fig. 4), indicating that free single-stranded DNA had not yet been completely adsorbed on GO, leading to the high noise value. Accompanying with the increase of treatment time with GO, the noise values were decreased sharply and false positive results could be reduced when it was longer than 30 min (Fig. 4). It was found that the signal values was decreased dramatically accompanied by the reduction of signal to noise ratio when the treatment time was more than 60 min (Fig. 4), this might be due to the unpaired DNA sequence b extending from GR-5S, and it would adsorb on GO after the binding of free single-stranded DNA, resulting in the slight adsorption of a small amount of GR-5 DNAzyme on GO, so decreased the concentration of cleaved DNA sequence a and c, and caused the reduction of effective signal. Treatment time of 60 min with GO could obtain high signal value and signal to noise ratio while reducing false positive results, hence, this treatment time was chosen in following experiments.

### 3.4. Optimization of experimental conditions

The volume ratio of the two kinds of AuNP-DNA probe (c' and a') on conjugate pad was investigated. The two AuNP-DNA probes have different hybridization capacity and stability with corresponding biotin-DNA probe on CZ and corresponding cleaved fragments from GR-5 DNAzyme. So it is important to optimize the volume ratio of AuNP-DNA probe c' (for GR5E) and a' (for GR5S). The results showed when the volume ratio (GR5E to GR5S) was lower or greater than 6–4, the signal to noise ratio (Band intensity in TZ with and without $Pb^{2+}$ represents the signal and noise, respectively) would be decreased significantly (Fig. 5A), thus only the GR5E and GR5S in ratio of 6–4 could achieve the maximum signal to noise ratio.

The concentration of AuNP used in the conjugate pad would affect the color of TZ directly, the lower AuNP concentration would reduce the sensitivity of the strip biosensor, and in contrast, the higher AuNP concentration might cause false positive result. The results showed that 4 μL/cm AuNP concentration had maximum signal to noise ratio (Fig. 5B), the decrease of signal to noise ratio at higher concentration was attributed to the increasing background signal, so this concentration was chosen for the next experimental condition.

The buffer type and ionic strength are the factors that can affect the sensitivity of the strip biosensor, appropriate buffer and ionic strength are beneficial to the hybridization of AuNP-DNA probe with biotin-DNA probe, and it would minimize the nonspecific hybridization and increase the sensitivity of the strip biosensor. The results showed that the

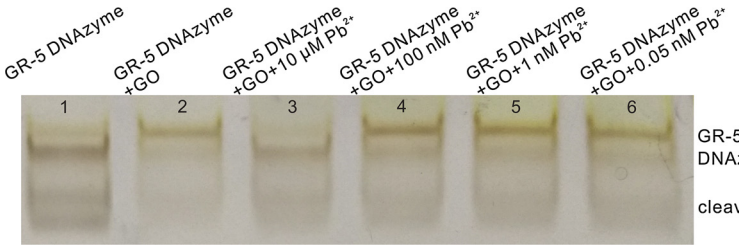


**Fig. 7.** Native polyacrylamide gel (20%) analysis. Lanes 1–6: GR-5 DNAzyme, GR-5 DNAzyme + GO, GR-5 DNAzyme + GO + 10 μM $Pb^{2+}$, GR-5 DNAzyme + GO + 100 nM $Pb^{2+}$, GR-5 DNAzyme + GO + 1 nM $Pb^{2+}$, GR-5 DNAzyme + GO + 0.05 nM $Pb^{2+}$. Gel band from top to bottom: GR-5 DNAzyme (double strand), DNAzyme strand (single strand), cleaved substrate strand.

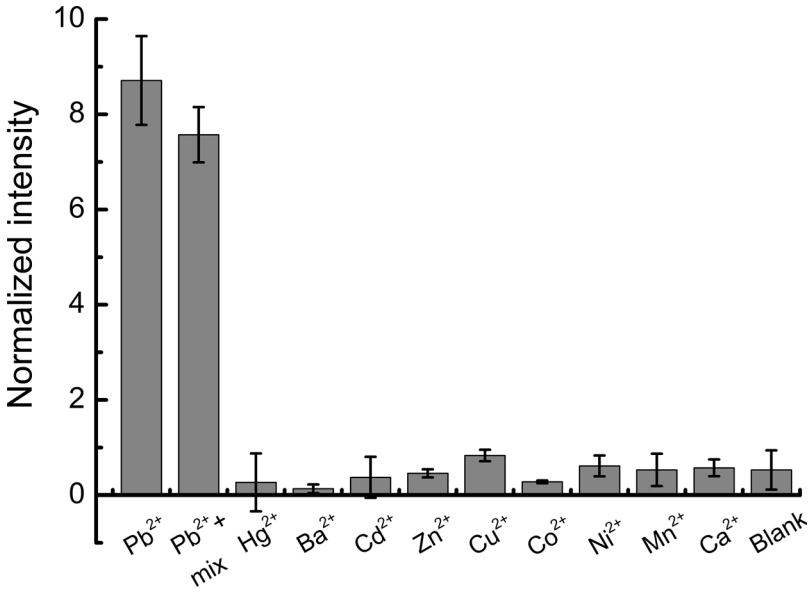


**Fig. 8.** Selectivity of the strip biosensor for 500 nM $Pb^{2+}$ over other 5 μM competing divalent metal ions. $Pb^{2+}$ + mix: the mixed sample consisting of $Pb^{2+}$ and other metal ions. Error bars were obtained from three parallel experiments. The other conditions were same as Fig. 2A.

**Table 2**
Recovery experiments of $Pb^{2+}$ in soil samples diluted 100-fold.

| Samples | Original (nM) | Added (nM) | Found (nM) | Recovery (%) |
|---|---|---|---|---|
| 1 | 19.1 | 0 | 21.6[a] ± 2.8[b] | 113.1 |
| 2 | 19.1 | 10 | 30.1[a] ± 1.3[b] | 110.3 |
| 3 | 19.1 | 20 | 38.8[a] ± 2.8[b] | 98.3 |
| 4 | 19.1 | 40 | 56.3[a] ± 4.5[b] | 93.0 |
| 5 | 19.1 | 60 | 74.0[a] ± 6.2[b] | 91.5 |

[a] Mean values of three measurements.
[b] Standard deviation.

common nucleic acid hybridization buffer, SSC had the highest signal to noise ratio (Fig. 5C). Since appropriate ionic strength (NaCl concentration) plays vital role for hybridization of DNA, it can reduce the repulsion between DNA molecules to promote the hybrid process. It was found that 5 × SSC was the optimal ionic strength (Fig. 5D), the signal to noise ratio was reduced accompanied with the decrease of SSC concentration (5 × SSC to 2 × SSC), and this might be due to the increase of background signal. The rise of the concentration of SSC (5 × SSC to 10 × SSC) also led to the decrease of signal to noise ratio, the reduced signal was the main reason for this phenomenon. Therefore, the subsequent experiments would be carried out under the above optimized conditions.

### 3.5. Performance of the proposed strip biosensor

Under the optimal experimental conditions, the performance of the strip biosensor with different concentrations of $Pb^{2+}$ was examined. With the increasing of $Pb^{2+}$ concentration, the band intensity of TZ was also increasing (Fig. 6B), and it exhibited a good linear response against the concentration over the range from 0.01–100 μM ($R^2$ = 0.996) (Fig. 6A). Furthermore, it could be also concluded that the limit of detection of the strip biosensor for $Pb^{2+}$ was 0.05 nM (S/N = 3) (Fig. 6C) and 1 nM (with naked eyes) (Fig. 6B). In order to directly observe the effect of GO adsorption and DNAzyme cleavage, the native polyacrylamide gel electrophoresis analysis was investigated. GR-5 DNAzyme was formed by denaturation and annealing of substrate strand (GR-5S) and DNAzyme strand (GR-5E) at the molar ratio of 1: 1.5, thus GR-5 DNAzyme had two clear bands, which were GR-5 DNAzyme (double strand) and DNAzyme strand (single strand), respectively (Fig. 7, lane 1), while bottom band might be caused by the insufficient purification of the DNA sequence. GO-treated GR-5 DNAzyme had one obvious band and one very weak band (lane 2), indicating that the free single-strand DNA was almost adsorbed. However, after cleavage by $Pb^{2+}$, the GR-5 DNAzyme band became very weak while DNAzyme strand and cleaved substrate strand were enhanced (lane 3). When the $Pb^{2+}$ concentration was reduced, DNAzyme and cleaved substrate strand band were weakened (lanes 3–5). When the $Pb^{2+}$ concentration was 1 nM and 0.05 nM (lane 5 and 6), the bands

**Table 3**
Comparison of $Pb^{2+}$ detection methods based on DNAzyme.

| $Pb^{2+}$ detection method | LOD | real sample | reference |
|---|---|---|---|
| fluorescence generation by enzymatic cleavages of DNAzyme immobilized on the AuNPs | 5 nM | tap water | [43] |
| fluorescence detection by induced enhancement of DNAzyme catalysis | 0.4 nM | soil and salt water | [44] |
| turn-on fluorescence using lead(II)-driven DNA molecular device | 5 nM | N/A | [6] |
| fluorescence detection using fluorescence resonance energy transfer and DNAzyme | 10 nM | N/A | [45] |
| colorimetric detection by using gold nanoparticles and DNAzyme | 3 nM | N/A | [24] |
| colorimetric detection using accelerated color change of gold nanoparticles assembled by DNAzymes | N/A | N/A | [12] |
| amplified surface plasmon resonance and electrochemical detection using the $Pb^{2+}$-dependent DNAzyme and hemin/G-quadruplex | 5 fM/1 pM | mineral water | [14] |
| electrochemical detection using DNAzyme functionalized gold nanoparticles to enhance the sensitivity | 0.028 nM | N/A | [46] |
| dual AuNP-DNA probes strip biosensor based on GR-5 DNAzyme and graphene oxide | 0.05 nM | soil | in this study |

had no change and was same as the GO-treated GR-5 DNAzyme (lane 2), while 0.05 nM $Pb^{2+}$ concentration was just the limit of detection.

### 3.6. The selectivity of the proposed strip biosensor

To determine selectivity performance of this strip biosensor for $Pb^{2+}$, several environmentally divalent metal ions (such as $Hg^{2+}$, $Ba^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Mn^{2+}$ and $Ca^{2+}$) were examined with a concentration of 5 μM when $Pb^{2+}$ was 500 nM. It was observed that the response of lead ion was far greater than those of other ions, even though the concentration of other metal ions was 10-fold higher than $Pb^{2+}$ (Fig. 8). The response to the mixed solution of $Pb^{2+}$ with other all those divalent metal ions was close to that of individual $Pb^{2+}$, indicating that this strip biosensor had high selectivity for $Pb^{2+}$, which could be well applied to the detection of $Pb^{2+}$ in real samples.

### 3.7. Spiked and real sample detection

In order to demonstrate the application potential of the strip biosensor for $Pb^{2+}$ detection in real samples, soil sample from the urban area was adopted. After digestion, filtration and 100 times dilution, the soil sample was spiked with different concentrations of $Pb^{2+}$ using the standard addition method, and it was also determined by ICP-MS. $Pb^{2+}$ concentration in soil samples was calculated as 19.1 nM, the experimental results were shown in Table 2, it was found that the recoveries were obtained with range of 91.5% to 113.1% for all samples, indicating that the proposed method had very good practicability for lead ions detection in real samples. Many DNAzyme $Pb^{2+}$-sensing platforms had high sensitivity [47–50], these methods are presented in Table 3. Compared with the other $Pb^{2+}$-sensing platforms, the GR-5 DNAzyme-based strip biosensor also had high sensitivity, the introduced GO could reduce false positive interference, while achieving visual detection without professional equipment and meeting the actual sample detection. However, the formation of the GR-5 DNAzyme double-strand required denaturation and annealing, which cost much time and we hoped that the next step can improve this step to shorten the time.

## 4. Conclusions

In summary, GO-assisted AuNP strip biosensor based on GR-5 DNAzyme was developed for highly sensitive and rapid $Pb^{2+}$ detection. The conjugate pad was immobilized with two kinds of AuNP-DNA probes which were bound to the two small DNA fragments cleaved from the GR-5 DNAzyme. This improved the utilize efficiency of the cleaved fragments and acquired higher detection sensitivity. The addition of GO excluded false positive results by adsorbing the free single-stranded DNA. The strip biosensor had high sensitivity with a 0.05 nM LOD (S/N = 3) and 1 nM (with naked eyes) and a dynamic range from 0.01 to 100 μM $Pb^{2+}$, which could satisfy the detection of trace amount of $Pb^{2+}$ in the real sample. Similarly, metal ions–specific DNAzyme (e.g. $Na^{+}$-spectific NaA43 DNAzyme; $Mg^{2+}$-dependent E5 DNAzymes and $Cu^{2+}$-dependent PSCu10 DNAzyme) [27,51–53] containing substrate strand and enzyme strand could be used for the metal ions detection using dual AuNP-DNA probes by adding a sequence to its original sequence. Certainly, since both the substrate strand and enzyme strand were used to bind dual AuNPs-DNA probes, it was necessary to add GO to adsorb the excess free single strand to reduce the false positive.

## Acknowledgements

This work was supported by the National Key Research and Development Program of China (2017YFA0700402), the National Natural Science Foundation of China (Grant nos. 81471697, 81771878), the Natural Science Foundation of Hubei Province (2017CFB413), Applied Basic Research Program of Wuhan City (2016060101010044), the Fundamental Research Funds for the Central Universities (Hust, 2016YXMS253, 2017KFXKJC002). We also thank the Analytical and Testing Center (HUST) for the help of measurement.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.colsurfb.2018.05.020.

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