Electronic Supplementary Information

A Simple Lateral Flow Biosensor for Rapid Detection of Lead (II) Ions Based on G-quadruplex Structure-switching

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Experimental section

Reagents and chemicals

$HAuCl_4 \cdot 3H_2O$, trisodium citrate, Tween-20, sodium dodecyl sulfate (SDS), tris (hydroxymethyl) aminomethane (Tris), dimethylsulfoxide (DMSO), bovine serum albumin (BSA), Pb $(Ac)_2$, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-methyl mesoporphyrin IX (NMM) was purchased from J&K Scientific Ltd. (Beijing, China). The stock solution of NMM was prepared in DMSO and stored in darkness at -20°C before use. Nitrocellulose (NC) membrane was purchased from Shantou ealon (Shantou, China). Fiberglass and absorbent paper were purchased from Shanghai Kinbio (Shanghai, China). The adhesive plate was purchased from Shanghai Jiening biotechnology co. LTD. All buffer solutions used in this study were prepared in our lab. Oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biological Engineering Technology (Shanghai, China) and listed in Table S1.

Table S1. Sequences of oligonucleotides used in this work

Name	Sequences (from 5' to 3')
DNA 1-2	domain 1 domain 2 TCGGGTGTGGGTG GGTGGTGGTGGTTGTGGTGGTGGTGG
DNA 3	CACCC ACACC CGATT TTTTT TTTTT-SH
DNA 4	CCACC ACCAC CACAA CCACC ACCAC CACAA
DNA 5	TCGGG TGTGG GTGTC GGGTG TGGGTG

Instrumentation

Circular dichroism (CD) spectra were measured on a chirascan spectropolari-meter that was purchased from Applied Photophysics Ltd. (Great Britain). Flurescence measurements were carried out on a fluorescence spectrophotometer (PerkinElmer LS55). Peak areas of the red bands on the test zone and control zone were measured by a hand-held strip reader (DT1030, Shanghai Kinbio, Shanghai, China).

Fluorescence measurements

500 μL of Tris-HAc (10 mM, pH 7.4) was added into each of the nine eppendorf tubes. DNA 1-2 and NMM was then added into each tube to reach the final concentration of 1 μM and 4 μM, respectively. Pb^{2+} was added into each tube to reach the final concentration of 1 μM, 1.5 μM, 2 μM, 2.5 μM, 3 μM, 3.5 μM, 4 μM, 5 μM, and 8 μM, respectively. After incubation at RT for 10 min, fluorescent intensity was performed on the fluorescence spectrophotometer. The excitation

wavelength and emission wavelength of NMM is 399 nm and 610 nm, respectively.

Circular dichroism (CD) measurements

DNA 1-2 was mixed with various metal ions in Tris-HAc (10 mM, pH 7.4) buffer for 5 min to reach a final volume of 200 uL at room temperature (RT). CD measurements were carried out on a spectropolarimeter equipped with a programmable temperature-control unit in 15 min. The spectra from 320 to 220 nm were obtained in 1 mm path length cuvettes and averaged from 3 scans with the reaction buffer background subtracted.

Preparation of AuNP-DNA3 conjugates

AuNP with a diameter of 15 nm was prepared using citrate reduction method.[1] Briefly, AuNP with a diameter of 15 nm was prepared using citrate reduction method. All glassware used in this preparation was thoroughly cleaned, rinsed in doubly distilled water, and oven-dried prior to use. In a 500 mL round-bottom flask, 100 mL of 0.01% $HAuCl_4$ in doubly distilled water was brought to a boil with vigorous stirring. To this solution was added 4.5 mL of 1% trisodium citrate. The solution turned deep blue within 20 s, and the final color changed to wine-red occurred 60 s later. Boiling was continued for an additional 10 min, the heating device was removed, and the colloid solution was stirred for another 15 min. The resulting AuNP solution was stored in dark bottles at 4℃.

The AuNP-DNA3 conjugates were obtained using the previously reported method.[2] Briefly, 4 mL of AuNP solution was concentrated to 1 mL by centrifugation at 13.4×10^3 g for 20 min. Then 1.0 OD of thiolated DNA 3 was added to 1 mL of concentrated AuNP solution and shaken gently overnight at 4℃. The solution was subjected to "aging" by adding 110 μL of 100 mM phosphate buffer (pH 7.0, containing 1% sodium dodecyl sulfate and 1.5 M NaCl) and kept at 4℃for 12 h. AuNP-DNA3 solution was centrifuged (13.4×10^3 g, 20 min) and rinsed three times with rinsing buffer (20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20, 10% sucrose and 0.1% NaN_3) to remove any unbound DNA3. The AuNP-DNA3 pellets were resuspended in 100 μL of rinsing buffer and then stored at 4℃ before use.

Construction of the strip biosensor

The strip biosensor consists of five components: adhesive plate, sample pad, conjugate pad, NC membrane, and absorbent pad. The size of adhesive plate is 6 cm wide and 30 cm long. The sample pad (1.7 cm × 30 cm) was made from fiberglass and saturated with a buffer (pH 8.0)

containing 1% BSA, 2% Triton, 2% PEG 4000, 20 mM Tris-Ac, and 50 mM NaAc. Then it was dried and stored in the humidity below 25% at 25℃. The conjugate pad (0.8 cm × 30 cm) was prepared by dispensing AuNP-DNA3 solution (9 mL cm^{-1}) onto the fiberglass pad with the dispenser, then drying it in the humidity below 25% at 25℃, and stored in a desiccator at 4℃. NC membrane (2.5 cm × 30 cm) was used to immobilize DNA 4 (100 μM, 33 μL) and DNA 5 (100 μM, 33 μL) at different locations to form test zone and control zone, respectively. DNA 4 and DNA 5 was dissolved in PBS buffer before used. The distance between the test zone and control zone is 5 mm. The DNA probe loaded membrane was then dried at 37℃ for 1 h and stored in a desiccator at 4℃. The adsorbent pad was cut into 1.8 cm wide and 30 cm long. Finally, the sample pad, conjugate pad, NC membrane, and absorbent pad were attached along the long axis of an adhesive plate with an overlap of 2-3 mm in the order to allow the sample to flow. Then they were cut into 4 mm-wide strips using a strip cutter (Shanghai Kinbio, shanghai, China) and stored under dry conditions.

Analytical procedure

DNA 1-2 was mixed with various concentrations of Pb^{2+} in Tris-HAc (10 mM, pH 7.4) buffer to reach a final volume of 100 μL. After incubation at RT for 5 min, the mixture was then loaded onto the sample pad. Ten minutes later, the red bands on the test zones were observed by reference to a colorimetric card, and the peak areas of the red bands on the test zone were recorded by the portable strip reader.

Recovery experiment

The practical application of the strip biosensor was demonstrated by applying it to detect Pb^{2+} in real freshwater samples (tap water and lake water). The samples were filtered with a 0.22 μm filter membrane before use. Pb^{2+} content in the tap water and lake water was too low to be detected by this biosensor. Recovery experiments were performed by spiking different amounts (2 nM, 20 nM, 50 nM) of Pb^{2+} into tap water and lake water, respectively.

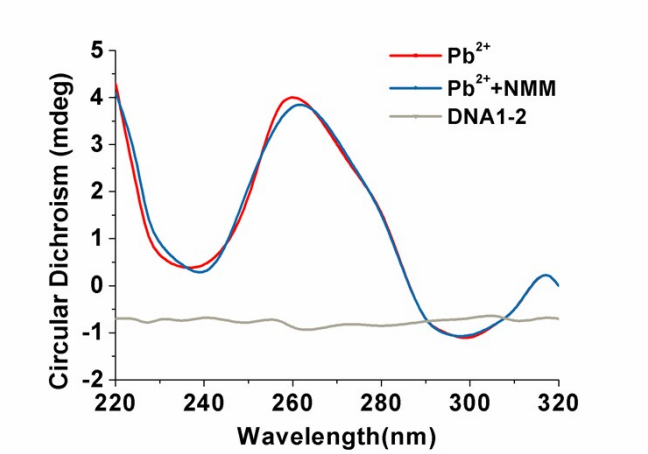

Figure S1. CD spectra measurement of G-quadruplex formation with or without NMM. The concentration of Pb^{2+}, DNA1-2, and NMM is 2 μM.

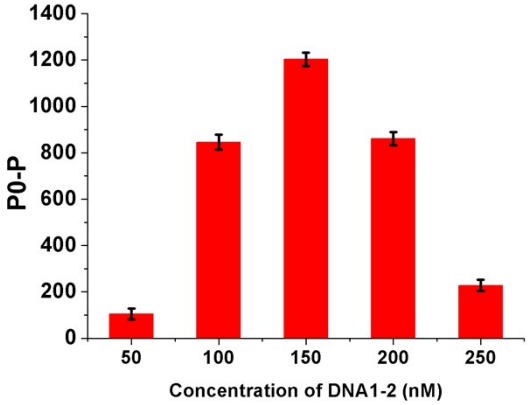

Figure S2. Effect of DNA1-2 concentration was investigated. P0: peak areas of negative samples of the red bands on the test zone, P: peak areas of positive samples of the red bands on the test zone. The concentration of Pb^{2+} is 100nM.

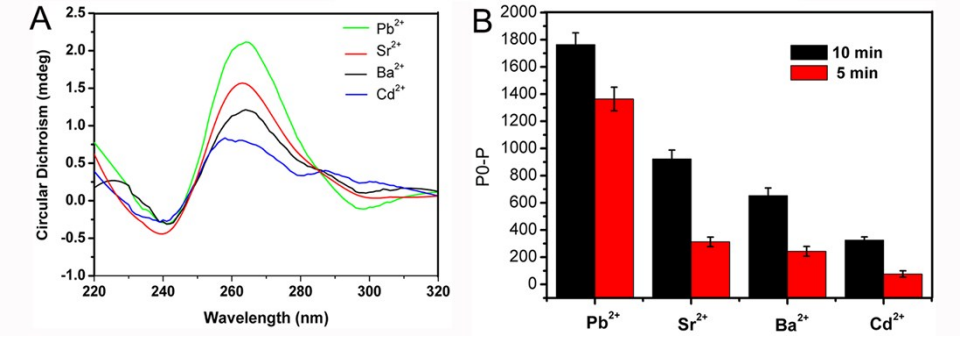

Figure S3. (A) The CD spectra of DNA1-2 (1μM) with Pb^{2+}, Sr^{2+}, Ba^{2+} and Cd^{2+} (1μM). (B) The corresponding (P0-P) values examined by this biosensor. The concentration of DNA1-2 is 150 nM. The concentration of Pb^{2+}, Sr^{2+}, Ba^{2+} and Cd^{2+} is 1μM.

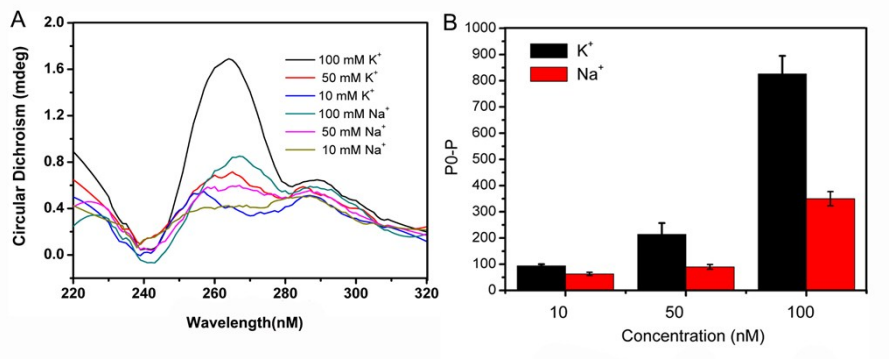

Figure S4. Different concentrations (10 mM, 50 mM, and 100 mM) of Na^+ and K^+ were examined by (A) CD spectra measurement and (B) this biosensor under optimal experimental conditions. The concentration of DNA 1-2 is 1μM for CD spectra measurement and 150 nM for this biosensor.

Table S2. Detection limits of various techniques for Pb^{2+} detection

Technique	Detection time	LOD
Colorimetric biosensor	20 min	15nM,[3] 50 nM[4]
	15 min	2 μM[5]
	25 min	2 μM[6]
	5 min	30 μM[7]
	120 min	0.5 μM[8]
	30 min	1 μM[9], 0.1 μM[10]
	20-25 min	100 nM[11]
Lateral flow biosensor	160 min	10 pM[12]

Table S3. Recovery experiment of Pb^{2+} in tap water and lake water

Samples	Pb^{2+} (nM) (n=3)		Recovery (%)
	spiked	detected	
Tap water	2	1.85±0.04	92.5
	20	21.4±0.72	107
	50	51.7±1.22	103.4
Lake water	2	1.76±0.07	88
	20	18.1±0.66	90.5
	50	53.2±1.43	106.4

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