Electronic Supplementary Information

An Efficient Target-Intermediate Recycling Amplification Strategy for Ultrasensitive Fluorescent Assay of Intracellular Lead Ion

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Experimental Section

Chemicals and Materials. Phosphate buffer solutions (PBS) were composed of 0.1 M K_2HPHO_4, 0.1 M NaH_2PO_4, and 0.1 M KCl and the pH was adjusted to 8.0. TM buffer solutions (pH 8.0) were prepared by 20 mM Tris and 50 mM $MgCl_2$ standard stock solutions. $Pb(NO_3)_2$, NaCl, $MgCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$, $NiCl_2 \cdot 6H_2O$, $FeCl_3$, $CoCl_2$, $HgCl_2$, $CaCl_2$, $MnCl_2$ were purchased from Sigma Chemical Co.(St. Louis, MO, USA). All custom-synthesized DNAs (Table S1) were purchased form Dingguo Biological Technology Co., Ltd. (Chongqing, China). All the other chemicals were of analytical grade and used without further purification. Ultrapure water with resistance of 18.2 MΩ/cm was used in all of the experiments.

Table S1. The DNA sequences employed in the target-intermediate recycling amplification strategy.

Name	Sequence (5'-3')
Substrate	CAC TAT AT(rA) GGA AGA GA
DNAzyme	TCT CTT CCC CGA GCC GGT CGA AAT ATA GTG
Hairpin	TCT CTT CCC CCA CTA TAT TTC GAC CGG CTC GGG GAA GAG A
P_1	CGA CCG GCT CGG GGA AGA GA-BHQ-1
P_2	ROX-TTT TCT CTT CCC CGA GCC GGT CGA AAT ATA GTG GGG GAA GAG A

Apparatus. All fluorescence measurements were carried out with a F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). All pH measurements were carried out with a pH-3C digital pH-meter (Shanghai LeiCi Device Works, Shanghai, China). Gel Doc XR+ System(Bio-Rad, California, USA) was used to take images of gels.

Native polyacrylamide gel electrophoresis (PAGE). Firstly, 10 μL of each sample were mixed with 2 μL of 6×loading buffer. And then, 10 μL of mixture was transferred into the gel electrophoresis system, respectively. Electrophoresis was performed in 1×TBE (pH 8.0) at a 120 V constant voltage for 90 min. The gels were then stained with ethidium bromide for 20 min, followed by photographing with the Gel Doc XR+ System.

Fluorescence Measurements. The emission spectra were recorded by a F-7000 fluorescence spectrophotometer with 549 nm excitation, and fluorescence data were collected from 590 nm to 700 nm. The slit widths of the both the excitation and emission were set as 5 nm, and the PMT voltage was set as 950 V.

Cell culture and cell lysis buffer preparation. MB231 and A549 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum containing different concentration of Pb^{2+}. The cells were maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2). After 24 h cultivation, cells were collected in the exponential phase and washed twice with sterile PBS. After that, 10^6 cells were resuspended in 1 mL of ice-cold sterile water and stored in 2 mL EP tube. Then, the cells were repeatedly frozen and thawed to prepare cell lysis buffer. The cell lysis was used immediately for detection or frozen at -80 °C.

Measurement Procedure. All the oligonucleotides used in this work were dissolved to final concentration of 100 μM by TM buffer solutions, and stored at -20 °C for later use. Before use, hairpin was heated at 90 °C for 5 min and slowed cooled down to indoor temperature to form stem-loop structure. Following, DNAzyme (1 μM),

Substrate (1 μM), Hairpin (1.5 μM), P_1 (1 μM), and P_2 (1 μM) were mixed with TM buffer. And then, samples with different concentration of Pb^{2+} was added to the mixture to a total volume of 50 μL. The mixture was incubated at 37 °C for 120 min. Eventually, the fluorescence of resulting solutions was measured by fluorescence spectrophotometer.

Native Polyacrylamide Gel Electrophoresis (PAGE). PAGE was employed to demonstrate the feasibility of proposed target-intermediate recycling signal amplification strategy. As shown in Figure S1, lane 1 and lane 2 represented the Substrate and DNAzyme, respectively. The hybridization of DNAzyme and Substrate was shown in lane 3. Lane 4 was the hybridization of DNAzyme and Substrate with 1 μM Pb^{2+}, and the sample was incubated for 120 min at 37 °C before the PAGE test. Lane 5, lane 6 and lane 7 was the Hairpin, P_1 and P_2, respectively. Lane 8 was the hybridization of P_1 and P_2. Lane 9 was the mixture of Hairpin, P_1 and P_2. Land 10 was the mixture of DNAzyme, Hairpin, P_1 and P_2, the PAGE result showed that the hybridization of Hairpin and P_2 could be obtained due to the DNAzyme, the DNAzyme could open the Hairpin and triggered the stranded replacement reaction to generate the hybridization of Hairpin and P_2. Lane 11 and lane 12 was the detection system without and with the Pb^{2+}, the hybridization of Hairpin and P_2 could be clearly observed in land 12 compared to lane 11.

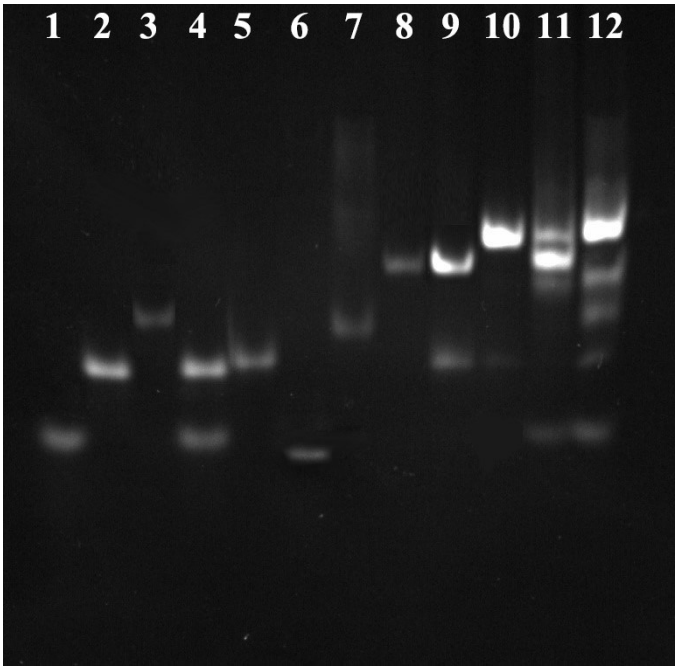

Figure S1. Native 16% PAGE analysis of the target-intermediate recycling signal amplification based fluorescent assay. The concentrations of the oligonucleotide strands were all 2 μM.

A kinetics study was carried out to monitor the time-dependent emission of ROX at 603nm after the reaction was initiated through the addition of various amounts of Pb^{2+} (Figure S2A). The rate of fluorescence enhancement ratio (F - F_0 / F_0 per minute) showed an approximately linear relationship with Pb^{2+} concentration ($c_{Pb^{2+}}$) in 0 – 1 μM range as $F - F_0 / F_0 = 0.0001\, c_{Pb^{2+}} + 1.0074$ (Figure S2B), and the $R = 0.9865$. For Pb^{2+} measurements, the rate ($F - F_0 / F_0$ per minute) within 24-30 min after Pb^{2+} addition was recorded rather than fluorescence intensity at a specific time point. The fluorescence enhancement ratio was gradually increased along with the increasing Pb^{2+} concentration, and the excellent sensitivity of our proposed assay was further illustrated

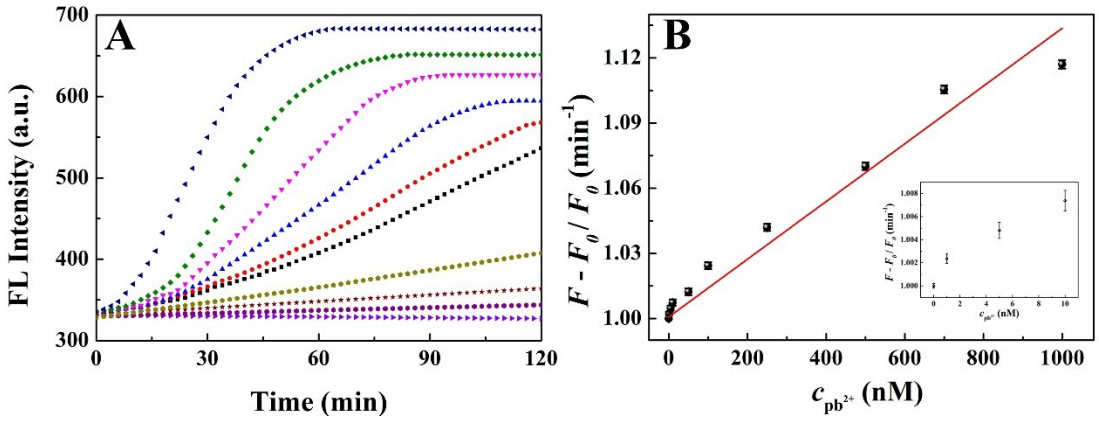

Figure S2. (A) Kinetics of fluorescence enhancement, and (B) relationship between fluorescence enhancement rate and Pb^{2+} concentrations. Fluorescence enhancement rate was calculated within 24-30 min after Pb^{2+} addition.

Kinetics study of the DNAzyme (Figure S3) and Hairpin (Figure S4) was also carried out. As the Figure S3 and Figure S4 showed, the fluorescent response was gradually increased along with the increasing DNAzyme and Hairpin, respectively. Therefore, the contribution of DNAzyme and Hairpin used in our proposed assay were important as the target Pb^{2+}.

The rate of fluorescence enhancement ratio (F - F_0 / F_0 per minute) showed an approximately linear relationship with DNAzyme concentration (c_{DNAzyme}) at least in 0 – 0.8 μM range as $F - F_0 / F_0 = 0.0002 c_{\mathrm{DNAzyme}} + 1.0089$ (Figure S3B), and the R = 0.9852. The rate ($F - F_0 / F_0$ per minute) was calculated within 24-30 min after DNAzyme addition.

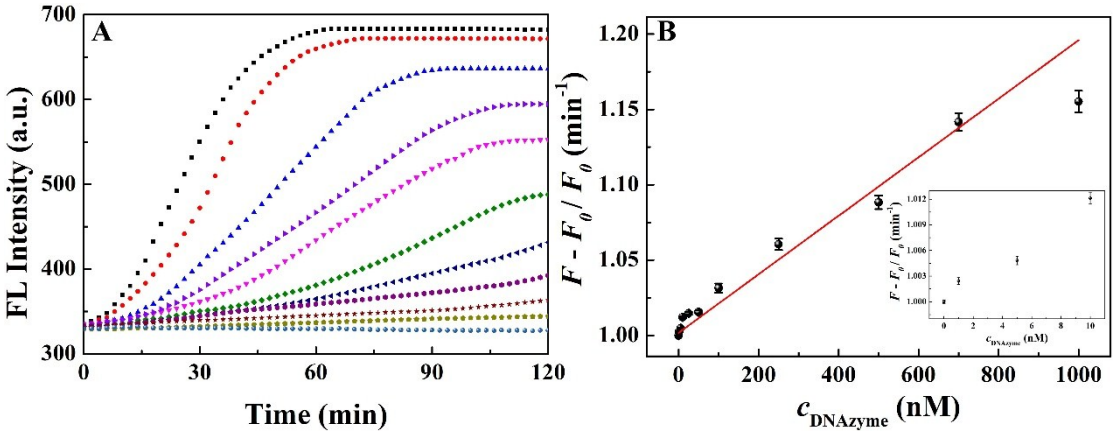

Figure S3. (A) Kinetics of fluorescence enhancement, and (B) relationship between fluorescence enhancement rate and DNAzyme concentrations. Fluorescence enhancement rate was calculated within 26-34 min after Pb^{2+} addition.

The rate of fluorescence enhancement ratio (F - F_0 / F_0 per minute) showed an approximately linear relationship with DNAzyme concentration (c_{Hairpin}) in 0 – 1.5 μM range as $F - F_0 / F_0 = 0.0001 c_{\text{Hairpin}} + 1.0074$ (Figure S4B), and the $R = 0.9865$. The rate ($F - F_0 / F_0$ per minute) was calculated within 24-30 min after Hairpin addition.

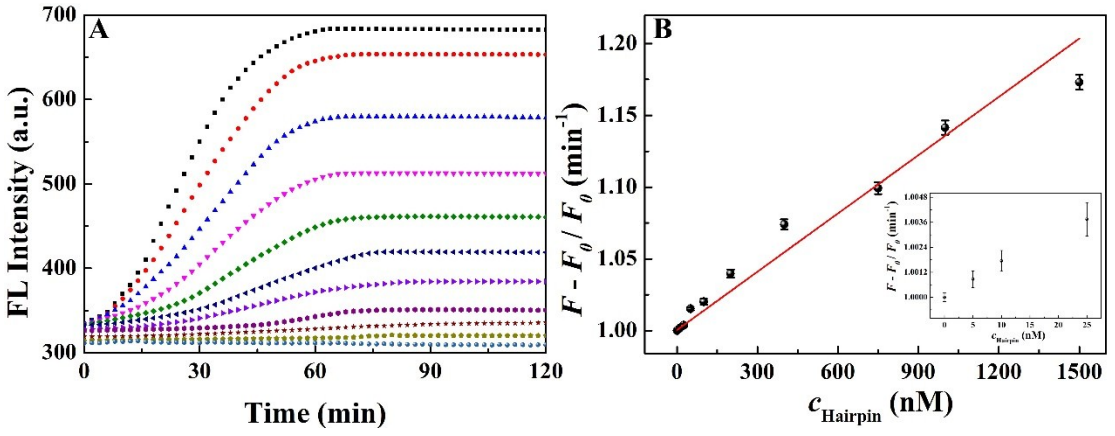

Figure S4. (A) Kinetics of fluorescence enhancement, and (B) relationship between fluorescence enhancement rate and Hairpin concentrations. Fluorescence enhancement rate was calculated within 26-34 min after Pb^{2+} addition.

To evaluate the applicability of the proposed fluorescent assay, its performances were compared with that of several Pb^{2+} detection methods, including electrochemiluminescent, electrochemical and fluorescent assay (Table S2). Apparently, a lower detection limit was obtained with our proposed fluorescent assay based on the target-intermediate recycling amplification, indicating the well performance and potential application of the proposed assay for Pb^{2+} detection. Moreover, the applicability of the proposed assay is better than electrochemiluminescent and electrochemical methods. Normally, sophisticated fabrication of the electrode was necessary in electrochemiluminescent and electrochemical methods, making these methods limited by complex operations and time-consuming procedures. For our proposed fluorescent assay, all the oligonucleotides was mix in buffer with detection samples, and the concentration of Pb^{2+} could be calculated simply based on the fluorescence response from fluorescence spectrophotometer. Additionally, the reactions in our proposed assay were performed in a homogenous system, which was beneficial to generate higher efficient reaction, and then achieve higher detection sensitivity.

Table S2. Comparison of Various Pb^{2+} Detection Methods.

Detection method	LOD	Linear range	Ref
ICP-MS	0.07 ng g^{-1}	0.2 - 100 ng g^{-1}	1
ICP-MS	0.007 μg L^{-1}	0.02 - 5.0 μg L^{-1}	2
Electrochemiluminescent Assay	70 nM	100 - 10000 nM	3
Electrochemical Assay	300 nM	500 - 10000 nM	4

Electrochemical Assay	1.0 nM	5 - 100 nM	5
Fluorescent Assay	4.0 nM	10 - 1000 nM	6
Fluorescent Assay	20 nM	100 - 4000 nM	7
Fluorescent Assay	5.0 nM	20 - 1000 nM	8
Fluorescent Assay	0.3 nM	1 - 1000 nM	this work

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