A simple fluorescent assay for lead(II) detection based on lead(II)-stabilized G-quadruplex formation

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Supplementary Information

Experimental section

Materials and Instruments

Oligonucleotides that supposed to bind with Pb^{2+} to form G-quadruplex according to previous reports were applied in this assay[1-4], and a random sequence was also used as a negative control. All the oligonucleotides were labeled with 6-carboxyfluorescein (FAM) at the 5' end and synthesized by Invitrogen Biotechnology Co., Ltd. (Shanghai, China), and the sequences are as follows:

TBAA	5'-FAM-GGAAGGTGTGGAAGG-3'	(15 bp)
T30695	5'-FAM-GGGTGGGTGGGTGGGT-3'	(16 bp)
PW17	5'-FAM-GGGTAGGGCGGGTTGGG-3'	(17 bp)
PS2.M	5'-FAM-GTGGGTAGGGCGGGTTGG-3'	(18 bp)
Random DNA	5'-FAM-ATCGAATTCCCGATGG-3'	(16 bp)

Tris (Tris-(hydroxymethyl)aminomethane), acetic acid and cationic compounds such as nitrates of K^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Fe^{3+} and sulfates of Fe^{2+}, Mn^{2+}, Cu^{2+} were obtained from commercial sources and used without further purification. Standard solution (1 mg/mL, 1000 ppm) of Pb^{2+}, Hg^{2+} and Cd^{2+} were purchased from Merck Co., Inc. (Germany) and used after diluted to appropriate concentration with ultrapure water. Ultrapure water that utilized to prepare all aqueous solutions was from a Millipore-MilliQ (Milli-Q plus, Millipore Inc, Bedford, MA, USA) system.

An F-4500 fluorescence spectrophotometer (Hitachi, Japan) was used to record the fluorescence intensity, with the response time of 0.5 s, PMT voltage of 700 V, scan speed of 1200 nm/min, excitation wave length of 480 nm and excitation and emission slits of 10 nm. Time scan style was operated when studied the kinetics of fluorescence quenching, with a scan time of 1200 s, excitation wave length of 480 nm and emission wave length of 520 nm.

A J-815 CD spectrometer (Jasco, Japan) was employed to characterize the structural changes of the oligonucleotides. The optical chamber (1 cm path length, 1 mL volume) was deoxygenated with dry purified nitrogen (99.99%) before use and kept the nitrogen atmosphere during

experiments. Three scans (100 nm/min) from 200 to 320 nm at 1 nm intervals were accumulated and averaged. The background of the buffer solution was subtracted from the CD data.

A Thermostatic incubating device (Eppendorf, China) was used to carry out quenching experiments at various temperatures.

Selection of lead-binding oligonucleotides

Different oligonucleotides with a same final concentration (25 nM) were dissolved in Tris-acetate buffer (10 mM, pH 8.0) individually, and then Pb^{2+} of various concentrations was added. Blank sample for each oligonucletide was carried out by replacing Pb^{2+} with ultrapure water. After incubated 5 min at room temperature, fluorescence intensity of each sample was measured and the quenching ratio, $(F_0\text{-}F)/F_0$ was calculated, where F_0 stands for the fluorescence intensity of FAM in the absence of Pb^{2+} and F for the fluorescence intensity of FAM after addition of Pb^{2+}. The oligonucleotide which responded with a largest quenching ratio was chose for subsequent study.

Kinetics of fluorescence quenching

Firstly fluorescence intensity of each sample containing 25 nM T30695 was measured and then various ions were added individually. The fluorescence intensity of each ion-treated sample was re-measured promptly within 5 seconds.

Investigation of the quenching mechanism

Quenching experiments at various temperatures (300 K, 310 K, 320 K and 330 K) and different Pb^{2+} concentrations (0 ppb, 6 ppb, 15 ppb, 40 ppb, 100 ppb and 200 ppb) were carried out in Tris-acetate buffer (10 mM, pH 8.0) for 5 min and the fluorescence intensities in the absence and presence of Pb^{2+} were recorded as F_0 and F, respectively. Stern-Volmer plot was generated by plotting F_0/F against Pb^{2+} concentrations.

Sensitivity and selectivity of the detection of Pb^{2+}

2.5 μL, 5 μM T30695 was firstly added into Tris-acetate buffer (10 mM, pH 8.0) with appropriate volume, and then various concentrations (from 0.5 to 200 ppb) of Pb^{2+} were introduced into the above solution, the total volume of the final solution was fixed at 500 μL. After incubated 5 min at room temperature, the fluorescence intensity was measured.

To determine the selectivity of the fluorescent assay, different metal ions, including K^+, Na^+, Ag^+, Zn^{2+}, Cd^{2+}, Mn^{2+}, Mg^{2+}, Cu^{2+}, Ca^{2+}, Ni^{2+}, Hg^{2+}, Fe^{2+} and Fe^{3+}, at a concentration of 15 ppb or higher (50 ppb or 200 ppb), were added to the sensor solution individually and the change in the fluorescence intensity was monitored.

Supplementary figures and table:

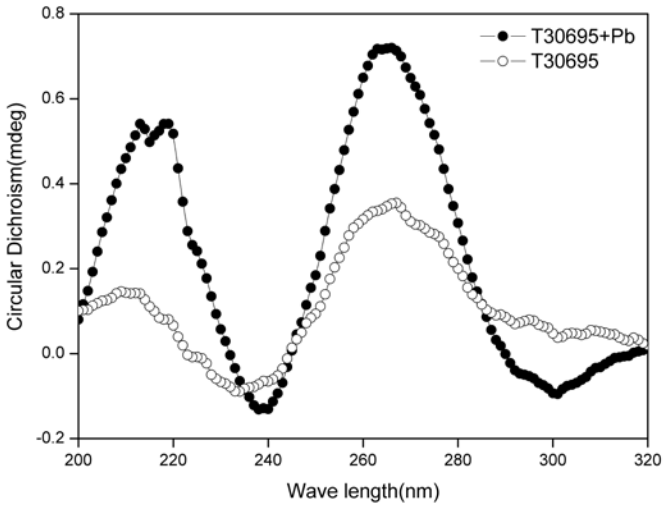

Fig. S1 CD spectra of 1 μM T30695 in the absence and in the presence of 4 ppm Pb^{2+}. The lead (II) ion treatment reaction was performed in Tris-acetate buffer (10 mM, pH 8.0) for 5 min at room temperature.

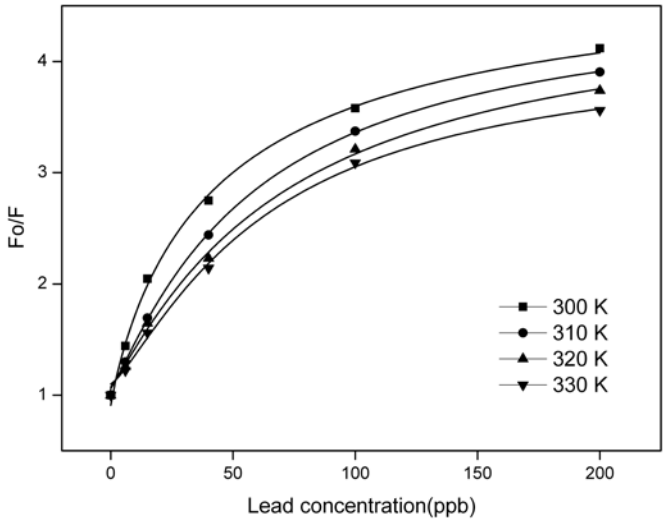

Fig. S2 Stern-Volmer curves for fluorescence quenching by Pb^{2+} of different concentrations (0 ppb, 6 ppb, 15 ppb, 40 ppb, 100 ppb and 200 ppb) at four different temperatures (300 K, 310 K, 320 K and 330 K). The concentration of T30695 is 25 nM.

Table S1 Results of Competitive assays

Samples	Mean found (ppb)	Mean recovery (%)	RSD (%)
Pb^{2+} (15)[a], K^{+} (30), Ni^{2+} (50), Ca^{2+} (100), Fe^{3+} (20), NO_3^{-} (264.2)[b], SO_4^{2-} (34.3)	14.3	95.3	4. 14
Pb^{2+} (50), Cd^{2+} (50), Mg^{2+} (100), Ag^{+} (50), Mn^{2+} (100), Cu^{2+} (100), NO_3^{-} (630.5), SO_4^{2-} (325.6)	54.2	108.4	3.75
Pb^{2+} (100), Hg^{2+} (30), Na^{+} (100), Fe^{2+} (50), Zn^{2+} (200), NO_3^{-} (729.4), SO_4^{2-} (85.7)	110.8	110.8	6.69

[a] Final concentration (ppb) of ions added,

[b] Concentration of anion was calculated from that of corresponding cation.

References

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