Electronic Supplementary Information (ESI)

For

Radon detection based on lead-induced conformational change of aptamer T30695

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1. Experimental details

Materials.

All oligonucleotides used in this study were synthesized by Bioengineering (Shanghai) Co., Ltd., using denaturing polyacrylamide gel electrophoresis (ULTRAPAGE) for purification. The oligonucleotides include guanine-rich oligonucleotide strand T30695, the five complementary strands of T30695 (C-16, C-15, C-14, C-13, C-12), a nucleotide sequence R made up of random oligonucleotides, and its complementary strand C-R. Table 1 shows the nucleotide sequences of each strand.

Table S1 Sequences of oligonucleotide

Name	Sequences
T30695	5'-GGGTGGGTGGGTGGGT-3'
C-16	5'-ACCCACCCACCCACCC-3'
C-15	5'-CCCACCCACCCACCC-3'
C-14	5'-CCCACCCTCCCACCC-3'
C-13	5'-CCCACCCTCCCACC-3'
C-12	5'-CCACCCTCCCACC-3'
R	5'-ATCCAACTGCTACAGG-3'
C-R	5'-CCTGTAGCAGTTGGAT-3'

Fluorescent PG(2000×) dye stored in dimethyl sulfoxide was purchased from Molecular Robes Company and stored in the dark at -20 °C. Prior to usage, the dye was diluted with ultrapure water to 10×. A mixed cellulose membrane with a pore size of 0.8 μm and a diameter of 90 mm was purchased from Milipore Corporation. A standard Pb^{2+} solution of 100 μg/mL was purchased from the China Institute of Metrology. Prior to usage, the solution was diluted with ultrapure water to the appropriate concentrations. Glacial acetic acid (GR) was purchased from Tianjin Chemical Reagent Factory. Tris at least 99.9% purity was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Bi $(NO_3)_3 \cdot 5H_2O$, was acquired from Guangdong Guanghua Sci-Tech Co., Ltd., and it had a purity of at least 99%.

This study was conducted in a radon chamber at The University of South China. The experiments utilized a F-4500 fluorescence spectrophotometer (Hitachi, Japan), with a scanning speed of 1,200 nm/min. The excitation wavelength was 502 nm, and the photomultiplier tube (PMT) voltage was 700 V. The slit widths for excitation is

5nm and The slit widths for excitation is 10 nm. We also used a circular dichroism MOS-500 instrument (France Bio-logic Company, France), with a scanning speed of 30 nm/min and a scanning range of 225 to 350 nm. We used an AB204-S analytical balance (Mettler-Toledo Instruments Co., Ltd.) and a pH-metre PB-20 (Sartorius Company), respectively, for weight and pH measurements. Centrifugation was performed with a TD5K-III type benchtop centrifuge (Changsha East Wang Experimental Instrument Co., Ltd.).

Collection of Lead Decayed from Radon

In order to collect the lead that resulted from radon decay, we used a cylindrical disposable plastic petri dish with a diameter of 70 mm and a height of 16 mm. We added 10 mL of 0.2% acetic acid as the absorbent. The top of the plate was sealed with a Milipore mixed cellulose microporous membrane. The plate was then exposed to a radon chamber for cumulative passive sampling. After sampling, the final volume was topped up to 10 mL using 0.2% acetic acid.

Fluorescence Detection With Pb^{2+}

We added 50 μL of Tris-HAC (pH=7.0, 40 mM), 20 μL of T30695 (100 nM), and an appropriate amount of standard lead solution to a series of 2 mL Centrifuge tubes. The mixtures were incubated at room temperature for 90 min. Then, 28 μL of C-16 (the complementary strand of T30695) and 22 μL of PG (10 ×) were added to the reaction, topped up with ultrapure water to yield a final volume of 200 μL. The reaction was incubated in the dark at room temperature for 12 min. The excitation wavelength was 502 nm, and the emission wavelength was 525 nm. The fluorescence intensity of each tube was measured as F. A blank value of F_0 was measured with ultrapure water instead of the standard lead solution. The change in fluorescent intensity was calculated as $\Delta F = F_0 - F$.

Detection of Radon

The sampling absorbent was a HAC solution with a pH below 7.0. In order to prepare a sample buffer system, the sample's pH was adjusted to 7.0 using 1 M Tris. We used 50 μL of the sample solution to detect radon. For the sample blank experiment, a Tris-HAC buffer solution was used without the sample.

2. Optimization of Experimental Conditions

Optimization of complementary strand

Because this study involves the formation of a double helix structure by T30695

and its complementary strand, the degree of binding between the two strands and the stability of the double helix structure are central to the overall experimental stability. We designed five complementary strands labeled C-16, C-15, C-14, C-13, and C-12 based on the number of bases that can be paired with T30695 (see Table 1). different complementary strands were added, and measured the fluorescence intensity of Experimental tube and blank tube. As shown in Fig. 1A, the fluorescence intensity decreased as the number of complementary nucleotides decreased between C-16 to C-12. The strongest fluorescence intensity and the maximum difference in fluorescence were achieved when the complementary strand C-16 was added. Therefore, we identified C-16 as the optimal complementary strand for the experiment.

We set the concentration of T30695 at 10 nM and added different concentrations of C-16 to measure the fluorescence intensity in tubes with either 30 nM of lead or no lead. These conditions allowed us to investigate the influence of the amount of C-16 on the value of ΔF. As Fig. 1B shows, ΔF increased gradually as the concentration of C-16 ranged between 8 and 14 nM. When the concentration of C-16 exceeded 14 nM, ΔF began to decline. Therefore, we determined the optimal concentration of C-16 to be 14 nM.

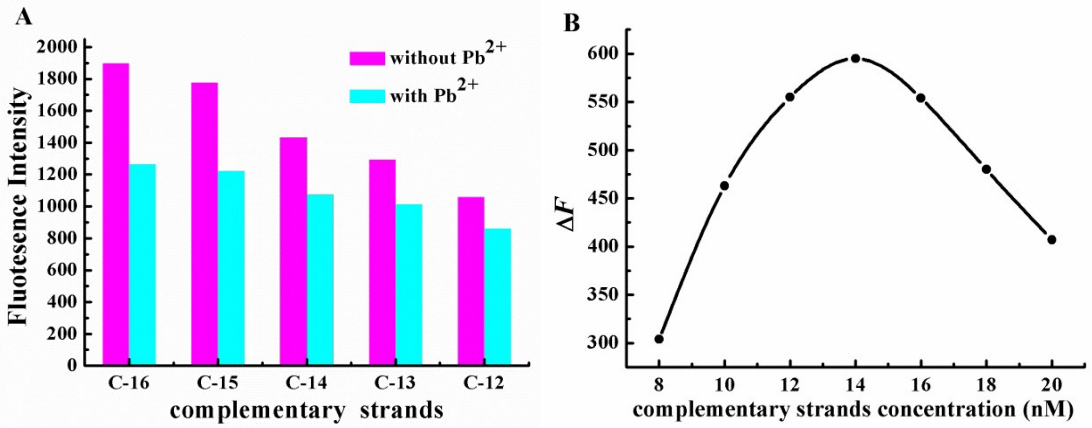

Fig. S1. Selection of complementary strandsand andoptimization of complementary strand concentration. (A) 10 nM T30695 incubating with 0nM Pb^{2+} (pink) or 30nM Pb^{2+} (blue) in Tris–HAc buffer(pH=7.0) at 25℃ for 90min, adding 10nM different complementary strands and 1× PG , incubating in 25℃ for 12min. (B) 10 nM T30695 incubating with 30nM Pb^{2+} in Tris–HAc buffer(pH=7.0) at 25℃ for 90min, adding different concentration of C-16 and 10×PG 20μL, incubating in 25℃ for 12min.

Optimization of pH

The the pH level of the buffer system of the sensor can also substantially impact the experimental outcome. The solubility of the oligonucleotide chain decreases when

the system is too acidic, and Pb^{2+} can precipitate and form $Pb(OH)_2$ if the system is basic. Therefore, we investigated the impact of the pH level in this experiment by varying it between 6.0 and 7.75. As Fig. 2A shows, we determined the optimal pH of the buffer to be 7.0.

Optimization of PG

In this study, we also optimized the amount of PG required. As Fig. 2B shows, ΔF increased gradually as the added volume of PG (10×) ranged between 8 and 20 μL. When the amount added exceeded 20 μL, ΔF stabilized. Therefore, we chose to add 22 μL of PG at 10× working concentration.

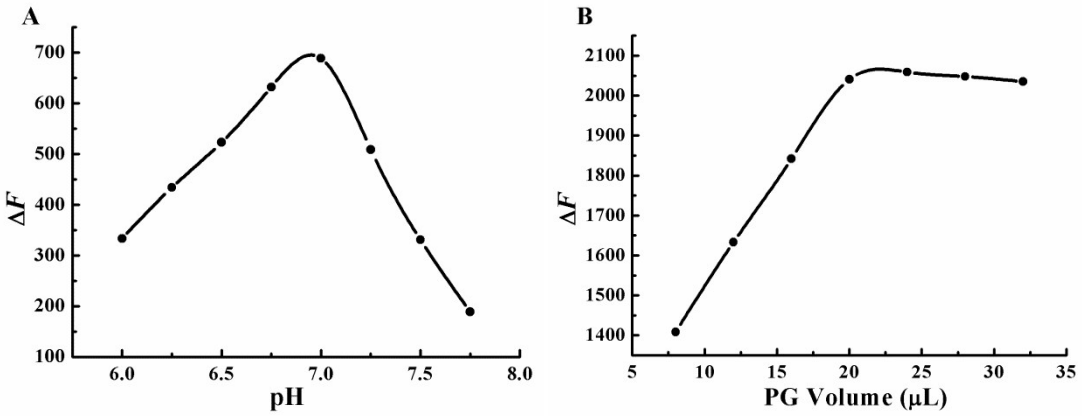

Fig. S2. Optimization of pH and PG volume.(A) 10 nM T30695 incubating with 30nM Pb^{2+} in Tris–HAc buffer with different pH at 25℃ for 90min, adding 14nM C-16 and 1× PG , incubating 12min.(B) 10 nM T30695 incubating with 30nM Pb^{2+} in Tris–HAc buffer(pH=7.0) at 25℃ for 90min, adding 14nM C-16 and different volume of PG , incubating in 25℃ for 12min.

Optimization of incubation time

We examined the ideal incubation time for the formation of G-quadruplex. As Fig. 3A shows, when the incubation time was less than 90 min, ΔF increased gradually. When the incubation time exceeded 90 min, the $Pb2^{+}$ were fully integrated with T30695. The T30695 also became unstable, possibly due to prolonged incubation at room temperature, resulting in a decrease in ΔF after 90 min. Therefore, we chose 90 min as the optimal incubation time for Pb^{2+} and T30695. Fig. 3B shows the optimization of the incubation time for T30695, C-16, and PG. Between 3 and 12 min, the remaining T30695 that had not formed G-quadruplex was bound to C-16, allowing PG to be embedded in the double helix. As a result, ΔF increased. After 12 min, the competitive binding of C-16 and Pb^{2+} emerged, causing the dissociation of previously formed G-quadruplex, which then formed a new duplex with C-16.

Consequently, ΔF started to fall. Based on these results, we determined the optimal incubation time of T30695, C-16, and PG to be 12 min.

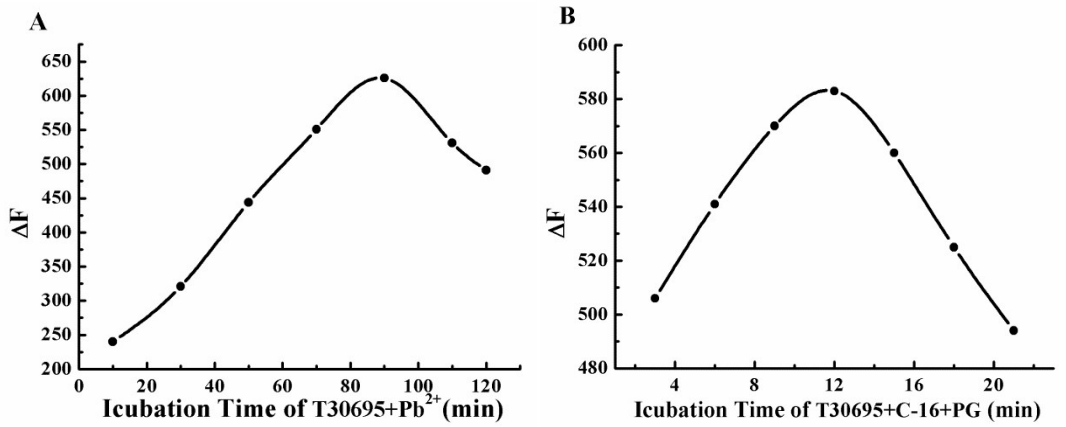

Fig. S3. Optimization of incubation time.(A) 10 nM T30695 incubating with 30nM Pb^{2+} in Tris–HAc buffer(pH=7.0) at 25℃ for different time, adding 14nM C-16 and 1.1× PG, incubating in 25℃ for 12min.(B) 10 nM T30695 incubating with 30nM Pb^{2+} in Tris–HAc buffer(pH=7.0) at 25℃ for 90min, adding 14nM C-16 and 1.1× PG, incubating in 25℃ for different time.

3. Supplementary Figures

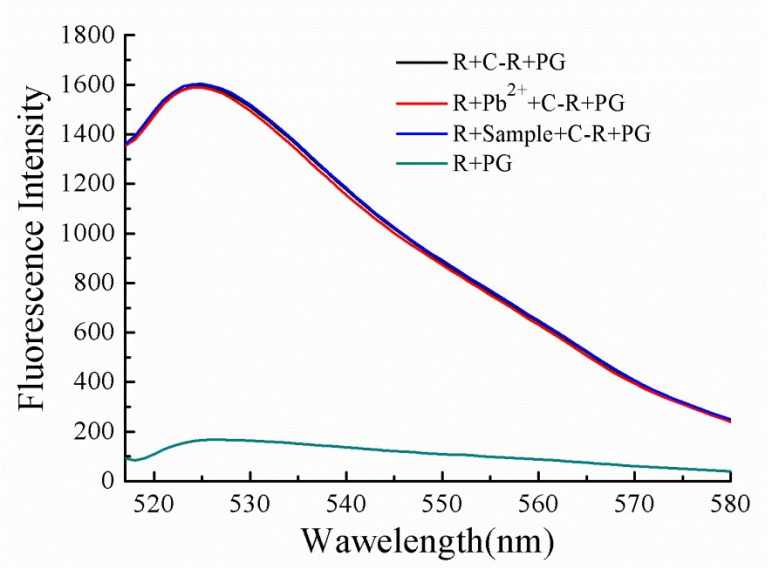

Fig. S4 Fluorescence emission spectra of PG in Tris-Hac (10mM, pH 7.0) buffer solution under different conditions. The concentrations of T30695, C-16, Pb^{2+}, Sample , PG are 10nM, 14nM, 60nM, 50μL and 1×, respectively.