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A novel detection of radon based on its decay product inducing conformational changes of an aptamer probe

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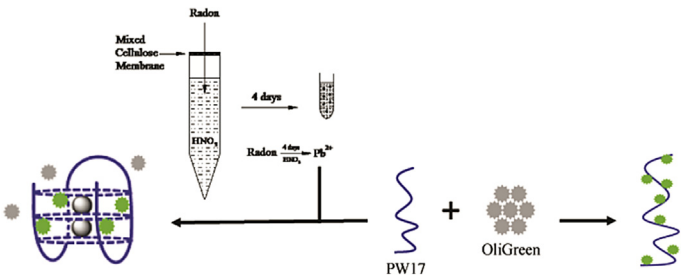
HIGHLIGHTS

- The label-free fluorescence sensor for detection of radon.
- This microscale experiment without radiation damage to experimenters and with less harm to environment.
- It provides a sensitive, low cost and simple strategy for radon accumulated concentration and lead ion detection.

GRAPHICAL ABSTRACT



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ABSTRACT

This study proposes a novel method for the detection of inert gas radon using a label-free, specific, fluorescence-sensing aptamer in the context of PW17-OG system. This method utilizes the cyanine dye OliGreen (OG) as a signal reactor and the aptamer PW17 as a fluorescent identification probe. When OG integrates into the free curling PW17, a strong fluorescence signal is generated. After radon decays, the long lived naturally occurring radon progeny Pb being disposed and introduced to the system. Lead ions induce PW17 to form a stable G-quadruplex, thereby inhibiting the interaction between OG and PW17 and resulting in a reduction of the fluorescence intensity. The fluorescence intensity show a good linear relationship with lead ion and the radon concentration (D), thereinto, We fitted linear regression of radon concentration in the range of 0.92–4.22 ($\times 10^4$ Bqhm^{-3}) to receive a good relationship between ΔF and the concentration of radon with the detection limit of 1963 Bqhm^{-3}. This method has been successfully applied for detecting standard cumulative concentration of radon and the detection limit reached the national standard of China. This sensitive method can exclude radiation damage in field testing, furthermore, it explores a new field in biological analysis using an aptamer to detected inorganic, gaseous, and radioactive materials.

1. Introduction

The radioactive noble gas ^{222}radon is produced by the decay of ^{226}radium within the natural decay chain of ^{238}uranium [1]. The following ^{222}Rn (half-life 3.82 d) decay chain is ^{218}Po (3.05 min), ^{214}Pb (26.8 min), ^{214}Bi (19.7 min), ^{214}Po (1.64×10^{-4} s), and ^{210}Pb (21 y) [2]. Radon is commonly found in all rocks and soil [3] and the byproducts can cause substantial damage to the respiratory, hematopoietic, and digestive systems; in severe cases, it may lead to lung cancer [4]. Humans have constant exposure to radon, therefore, the sensitive and reliable detection of radon concentrations is of particular interest to public health.

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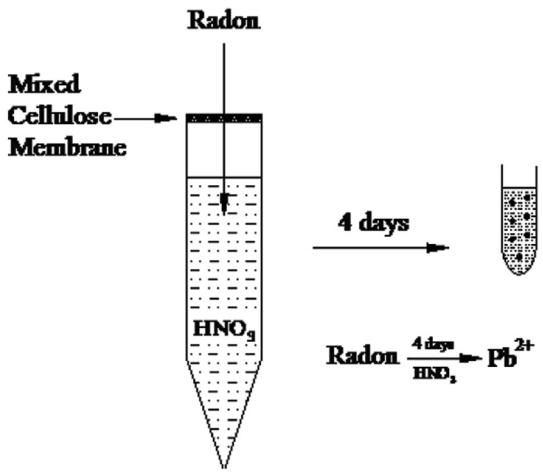
Currently traditional methods for detecting α and β-rays generated from radon radiation [5] include activated charcoal absorption, α-track etch detection, and electret probing [6,7]. However, these strategies are limited by various factors including the unwieldy size and exorbitant price of the required equipment, a relatively protracted period of detection, and health risks to personnel carrying out the experiments. Radon is persistent continues to damage to the human body, as a result, an urgent need exists for a new method that simplifies the operation of detecting the accumulative doses of radon. This method have solved those problem above, and may have the potential for application. The sampling device with small size is easy to transport, and it can be mailed to the tested area directly after subsequent improvements.

Over the past decades, the discovery of aptamers has shown tremendous potential and versatility in the analytical and bioanalytical fields [8]. By focusing on the decay product generated from radon radiation and these findings, our group pioneered a new approach to detecting radon using biological analysis. The potential contamination was prevented from entering the tube by covering mixed cellulose membrane with 0.8 μm pore which can block 99.9% of the already exists radon progeny in the laboratory air, allowing only radioactive particles and gas molecules into the samples [9,10], and achieving highly purified samples as a result.

We were putting the sampling equipment in radon chamber; the equipment were exposed under the standard concentration of radon. During the sampling of radon, standard concentration of radon in radon chamber diffused into the sampling equipment. By controlling the exposure time to getting the corresponding standard cumulative concentration of radon. After radon collects, the samples would have gone through a half-life cycle to further stabilizing the sample system and gathering more considerable stable daughter Pb to oxidizing to Pb^{2+}(Scheme 1).

Meanwhile, according to the previous reports [11–13], We selected the oligonucleotide PW17, which was enriched with guanine and used as an identification probe, with OG acting as the signal reactor. The cyanine dye OliGreen (OG) is scarcely fluorescent because its quantum yield is less than one percent [14]; however, when it binds to single-strand oligonucleotide, its fluorescence is amplified 1000 times [15]. Although OG interacts strongly with free single-strand aptamers, its fluorescence intensity changes as the aptamer structure transforms [14–16]. We presumed that the interactions between OG and PW17 produce a strong fluorescence (Scheme 2). When radon samples or Pb^{2+} is introduced to the reaction, it causes the formation of a stable G-quadruplex structure from unbound state, thereby inhibiting the interaction between PW17 and OG. The loss of this interaction results in a significant reduction in fluorescence intensity. Based on non-labeled fluorescent intensity, this method successfully achieves sensitive detection of lead and radon in the environment. The present study explores a new field in biological analysis by using aptamers to detect non-metallic radioactive gases.



Scheme 1. The principle diagram of the sample processing.

2. Experimental

2.1. Apparatus

This study was conducted in a radon chamber at South China University's Institute of Nuclear Industry No. 6. The experiments utilized an F-4500 fluorescence spectrophotometer (Hitachi, Japan), with 1200 nm/min scanning speed, 5 nm and 10 nm slits for excitation and emission wavelength respectively. We also used a circular dichroism JASCO-815 instrument (JASCO Corporation, Japan), with a scanning speed of 200 nm/min.

2.2. Reagents

PAGE-purified oligonucleotide: PW17 (5′-GGGTAGGGCG GGTTGGG-3′); R (5′-ATCGAAATTCGCATCGG-3′) was purchased from Sangon Biotech Ltd. (Shanghai, China), and diluted with ultrapure water to a concentration of 100 μM and stored at 4 °C until needed. The OG dye used (Molecular Robes Corporation) has a defined concentration of 2000×, and it was stored at −20 °C.

The standard lead solution used (China Institute of Metrology) was diluted with ultrapure water to a concentration of 100 μg/L of Pb^{2+} in the working solution. Trisaminomethane (Tris) with a purity of at least 99.9%, purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., was used to form a Tris-HNO_3 buffer as the same with radon samples and pH of 7.0. Premium grade nitric acid was purchased from Sinopharm Chemical Reagent Ltd. Co. $Bi(NO_3)_3 \cdot 5H_2O$, acquired from Guangdong Guanghua Sci-Tech Co., Ltd., had a purity of at least 99%. All reagents were diluted with ultrapure water and stored at 4 °C.

2.3. Procedure

2.3.1. Preparing of samples

We added 14 mL of 0.2%HNO_3 to a series of 15 mL centrifugation tubes, which were covered with a mixed cellulose membrane (Merck Millipore Ltd.) with an aperture of 0.8 μm and fixed with adhesive tape. The tubes were then exposed to radon laboratory for sampling. By controlling the exposure time to getting the corresponding standard cumulative concentration of radon. After sample retrieval, the tubes were sealed at room temperature for four days before measurement. Four days later, the sample mixed with Tris buffer to reach a pH level of 7.0 before measurement.

2.3.2. Measurement of Pb^{2+} concentration

Pb^{2+} working solution was added to a series of centrifugation tubes to yield a gradient solution of final concentration from 0 to 35 μg/L with 20 μL of 1 μM PW17, 75 μL of Tris-HNO_3, and filled each tube with distilled water to 180 μl. The mixture were incubated at room temperature for 30 min before the addition of 20 μl of 20 × OG dye. Fluorescence intensity was measured at an excitation wavelength of 500 nm and an emission wavelength of 524 nm. A standard curve using different Pb^{2+} concentrations and a linear regression equation was calculated based on the findings for the fluorescence intensity.

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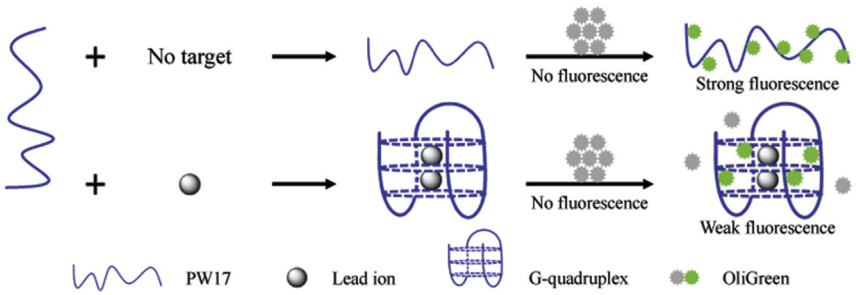

Scheme 2. Schematic diagram of Pb^{2+}-induced conformational changes of oligonucleotide for fluorescence detection of Lead ion.

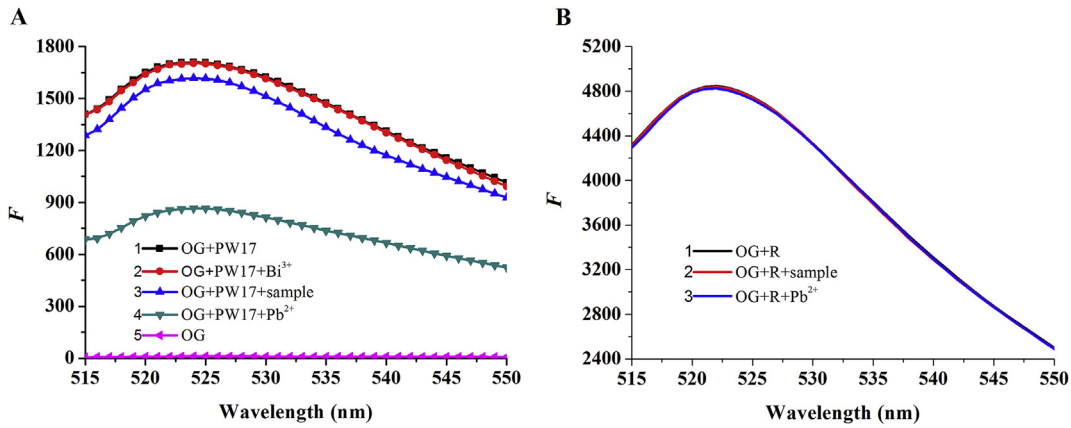

Fig. 1. Fluorescence spectra of different measurement systems.

2.3.3. Measurement of radon concentration

To determine the radon concentration, 75 μL of each sample solution was measured for different radon concentration and fluorescence intensity, according to the Pb^{2+} concentration detection method described above. A curve for fluorescence intensity versus radon concentration was plotted based on the measurements.

3. Result and discussion

3.1. Experiment principles

In this paper, we propose a new approach to detecting radon. When the target is added to the system, it may causes the conformational changes of free PW17 to formation stable G-quadruplex structure, thereby inhibiting the interaction between PW17 and OG (Scheme 2). To prove this hypothesis, we have done a series of experiments.

The fluorescence spectrophotometer results displayed in Fig. 1A confirm the principle. In isolation, OG is weakly fluorescent (curve 5, top to bottom), which shows a fluorescence intensity close to 0. However, a strong fluorescence is produced when OG binds to PW17 (Curve 1). However, Pb^{2+} induces aptamer PW17 forming a G-quadruplex structure and inhibiting the interaction between PW17 and OG. Consequently, the fluorescence intensity decreases significantly (Curve 4). The data demonstrate that the Pb^{2+} and PW17 form a highly sensitive combination. When the radon sample solution was added to this system, the resulting shown in Curve 3, was highly similar to Curve 4, and the fluorescence was greatly reduced as well. From the above, these data suggest that Pb^{2+} was present in the sample solution and induced the conformational changes of PW17.

During radon's process of decaying to form long life daughter, ^{214}Bi is formed as an intermediate. We performed a comparative experiment with equal amounts of Pb^{2+} and Bi^{3+} and found similar fluorescence intensity in the systems, with overlapping curves shown in Fig. 1A. This result indicates that trace Bi^{3+} cannot induce the conformational change of PW17 from free state, not does it interfere with the measurement. The data confirm that the reduced fluorescence intensity measurements in the system are caused by Pb^{2+}.

When the negative control oligonucleotide R replaced PW17 in the experiment, all three measurement systems showed the same strong fluorescence, as indicated by the three overlapping curves in

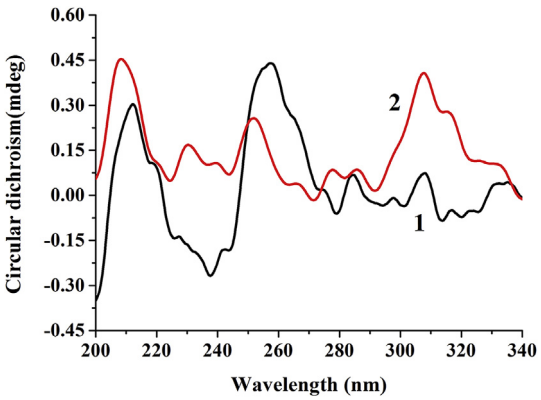

Fig. 2. Circular dichroism spectra of 20 mM Tris–HNO_3 (pH 7.2) containing 4 μM PW17 (curve 1), 4 μM PW17 + 0.2 μg/L Pb^{2+} (curve 2).

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Fig. 1B. This lack of change confirms that Pb^{2+} only can induce the conformational change from specific oligonucleotide sequence. The data also prove that the change in fluorescence intensity is associated with both the metal ions and the oligonucleotide's structure, and that change is not due to the intrinsic fluorescence-quenching property of heavy metals [17].

3.2. Characteristics of circular dichroism

The circular dichroism (CD) spectra is utilized to confirm our principle. As shown in Fig. 2, the addition of a small amount of Pb^{2+} transformed Curve 1 into Curve 2. A positive peak appears in Curve 2 around 310 nm are consistent with the antiparallel G-quadruplex structure [18–20]. This result further supports the correlation analysis described in Section 3.1 that Pb^{2+} is responsible for inducing the conformational changes of PW17 from free state to G-quadruplex, and inhibits OG binding to PW17, resulting in weakened fluorescence intensity.

3.3. Optimization of experimental conditions

In order to achieve a more stable and sensitive system, optimization was performed for each experimental condition. Firstly, we worked to fixed amounts of PW17 and OG, as shown in Fig. 3A and B. Based on the comparison among fluorescence values and the stability of the experiment, the optimal final concentration of PW17 and amount of OG in the plateau were determined to be 100 nM and 20 μl, respectively.

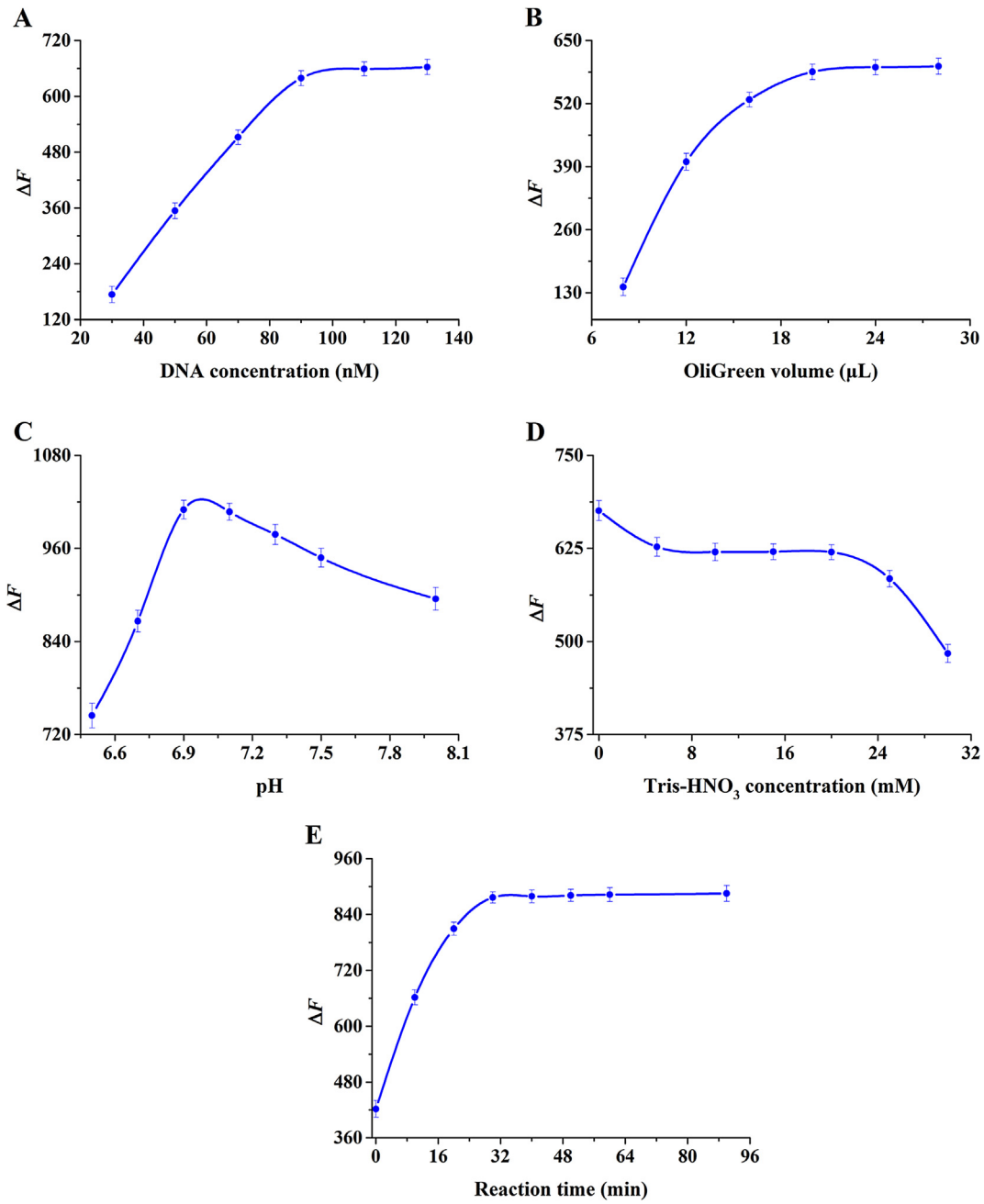

Fig. 3. Optimization of experiment: Influence of PW17 (A), OG (B), pH (C), buffer (D) and reaction time (E) on relative fluorescence intensity.

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Processing the radon sample solutions requires HNO_3 as a solvent to dissolve the collected radon progeny, lead. However, not only do strong acidic conditions affect the dye, but they may also cause hydrolysis of the oligonucleotide and other commonly used basic solutions containing K^+ ions and Na^+ ions are known to induce the formation of G-quadruplex [21]; therefore, we selected the Tris solution, which has milder effects as an experimental buffer to adjust the sample solution's pH. Fig. 3C shows that the systemic fluorescence intensity measurement varied with the change of pH. The Tris concentration in the measurement systems also impacted the experimental outcome, as shown in Fig. 3D. The fluorescence intensity decreased with increasing concentrations of Tris, stabilizing when the Tris concentration ranged between 5 and 20 mM. Therefore we chose the Tris solution with suitable concentration to maintain the pH of the measurement system at 7.0.

The length of incubation time exerts substantial influence on fluorescence intensity. Prolonged reactions of Pb^{2+}-induced G-quadruplex increased and occurring a decrease of the amount of free curling PW17 in the solution, leading to reduced fluorescence intensity and enhanced ΔF because of the lower number of single strands available for OG binding. The ΔF reached its maximum value after 30 min. Based on these findings, 30 min was selected as the incubation time for the following experiment, as illustrated in Fig. 3E.

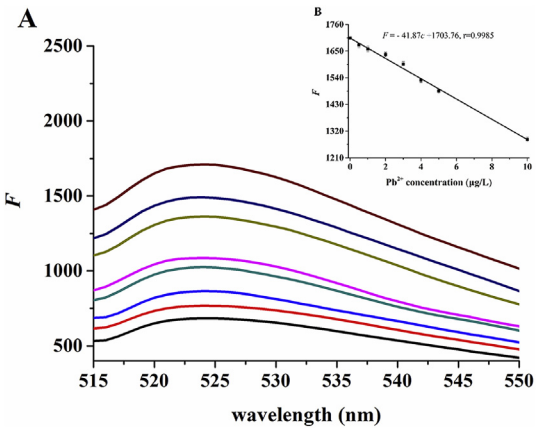

Fig. 4. (A) The impact of Lead ion's concentration on fluorescence spectrum (c_{Pb2+} = 0, 5, 10, 15, 20, 25, 30 and 35 × 10^{-6} g/L) and its standard curve (B) (c_{Pb2+} = 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 10.0 × 10^{-6} g/L).

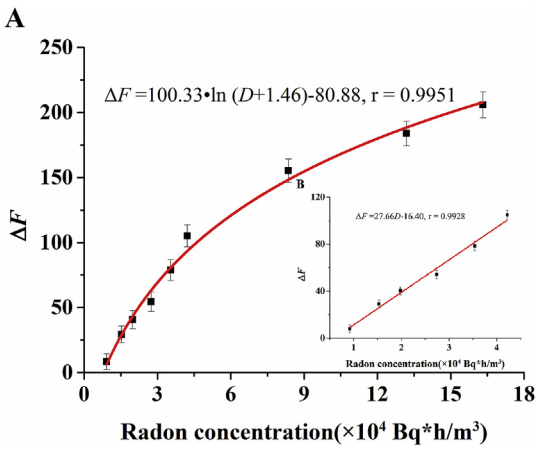

Fig. 5. The relationship between ΔF and the concentration of radon.

3.4. Standard curve, sensitivity, and the detection limit

Under the optimized conditions, fluorescence intensity reduced gradually with incremental increases in Pb^{2+} concentration. Fig. 4A shows the correspondence of the standard fluorescence intensity curve measured at 524 nm and the different concentration of Pb^{2+}. A good linear relationship appeared between the probe and lead ion in the low concentration range of 0.5–10 μg/L (Fig. 4B). The limit of quantitative determination of 129.69 ng/L was according to the 3σ/slope rule.

3.5. Measurement of radon radiation and mathematical quantitative model

Different Radon concentration were obtained in the radon laboratory using the acquisition method described previously. Using radon samples instead of Pb^{2+} standard solution, the fluorescence intensity were measured as described.

As shown in Fig. 5, the change in fluorescence becomes greater as the concentration of Pb^{2+} increases, which occurs as the radiation intensity of radon strengthens. Pb^{2+} induced the formation of the free curling PW17 to an increased amount of G-quadruplex, resulting in a reduced quantity of single strands for OG to binding. Hence, the sample solution's fluorescence intensity dropped and the ΔF was increased. The radon concentration increment rate coincided with prolonged exposure time, while the reduction in the fluorescence intensity became less obvious as the time lapse. A Logarithmic growth relationship was observed between ΔF and the radon concentration in the range of 0.92–16.32 (×10^4 Bqhm^{-3}) (Fig. 5A). We fitted linear regression of radon concentration in the range of 0.92–4.22 (×10^4 Bqhm^{-3}) to receive a good relationship between ΔF and the concentration of radon with the detection limit of 1963 Bqhm^{-3} (Fig. 5B). According to the ICRP Publication 65 [22], the action level related to radon concentration for remedial measures in dwellings is 200–600 Bqm^{-3}, and the corresponding annual accumulated exposure dosage of radon is about

Table 1
Determination results of Pb^{2+} in the samples (n = 6).

Sample	c_{Rn} (Bqhm^{-3})	F (a.u.)	Found (μg/L)	Added (μg/L)	Total found (μg/L)	RSD (%)	Recovery (%)
1	0.92 × 10^4	1695.07	0.21	1	1.27	3.62	106.11
2	1.53 × 10^4	1674.13	0.71	1	1.74	3.34	103.33
3	1.98 × 10^4	1662.83	0.98	1	2.00	3.06	102.67
4	2.74 × 10^4	1649.01	1.31	1	2.30	2.57	99.65
5	3.54 × 10^4	1624.72	1.89	1	2.86	2.63	97.01
6	4.22 × 10^4	1598.35	2.52	1	3.53	2.42	101.40
7	8.36 × 10^4	1548.18	3.72	1	4.70	2.44	98.89
8	13.19 × 10^4	1519.51	4.40	1	5.37	2.36	97.67
9	16.32 × 10^4	1497.51	4.93	1	5.90	2.45	97.33

'Found', 'Added' and 'Total found' refers to the final concentration of Pb^{2+}.

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Table 2
Determination results of radon in the samples (n = 6).

Sample	Method	Found (10^3 Bqhm^{-3})	RSD (%)	*t*
1	TM	9.88	2.96	2.12
	RAD7	9.86	2.88	
2	TM	12.59	2.70	2.10
	RAD7	12.58	2.78	

Theoretical t (5, 95%) = 2.228; TM: this method.

8.76–26.28 × 10^5 Bqhm^{-3}. Alpha particle track measurement as a standard method for the accumulative doses of radon, with the detection limit of 2100 Bqhm^{-3}. Therefore, the sensor proposed here is applicable for radon monitoring in the environment.

Table 1 shows that spiked recoveries were 97.01%–106.11%, suggesting that the proposed probe could be applied for the sensitive and reliable analysis of radon samples. Table 2 shows radon concentration was measured by using RAD7 radon detectors and this method in parallel. The results were analyzed by using *t*-test. Within the 95% confidence level, the t-values do not exceed the theoretical value, which indicates that there is no statistical difference between the two methods. It is obvious that the proposed method is reliable and practical.

4. Conclusions

In summary, we developed a novel and sensitive method of radon detection using a label-free fluorescent sensor without radiation damage during in the testing. By taking advantage of a mixed cellulose membrane with a 0.8 μm aperture to cover the collection tube's opening to blocking any other interfering factors. This method based on the decay product, explores a new field in measurement of radon accumulated concentration by biological analysis. To fulfill the potential application in future, our group focus on the improvement of efficiency and stability of sampling.

Acknowledgements

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References

[1] A. Schmidt, M. Schlueter, M. Melles, M. Schubert, Continuous and discrete on-site detection of radon-222 in ground-and surface waters by means of an extraction module, Appl. Radiat. Isotopes 66 (2008) 1939–1944.
[2] J. Rickards, J.-I. Golzarri, G. Espinosa, A Monte Carlo study of radon detection in cylindrical diffusion chambers, J. Environ. Radioact. 101 (2010) 333–337.
[3] M.E. Kitto, M.A. Torres, D.K. Haines, T.M. Semkow, Radon measurement of natural gas using alpha scintillation cells, J. Environ. Radioact. 138 (2014) 205–207.
[4] M.T. Duran, A.R. Ravina, I.P. Lamelas, V.L. Fernandez, J.A. Arca, C.M. Martinez, C.P. Alvarez, F.J.G. Barcala, O.C. Añon, A.G. Gomez, Residential radon and lung cancer histological types in never smokers, Eur. Respir. J. 44 (2014) P2736.
[5] J. Irlinger, M. Wielunski, W. Rühm, Thoron detection with an active Radon exposure meter—First results, Rev. Sci. Instrum. 85 (2014) 022106.
[6] B. Nodari, M. Caldara, V. Re, L. Fabris, Radon Fast Detection and Environmental Monitoring with a Portable Wireless System, Advances in Sensors and Interfaces (IWASI), 2015 6th IEEE International Workshop on, IEEE, 2015, pp. 254–259.
[7] A. Sorimachi, H. Takahashi, S. Tokonami, Influence of the presence of humidity, ambient aerosols and thoron on the detection responses of electret radon monitors, Radiat. Meas. 44 (2009) 111–115.
[8] H. Zhao, Y.-S. Wang, X. Tang, B. Zhou, J.-H. Xue, H. Liu, S.-D. Liu, J.-X. Cao, M.-H. Li, S.-H. Chen, An enzyme-free strategy for ultrasensitive detection of adenosine using a multipurpose aptamer probe and malachite green, Anal. Chim. acta 887 (2015) 179–185.
[9] X. Ma, Study on the Performance of Radioactive Aerosol Sampling Filter, University of South China, Hengyang, 2012.
[10] X. Ma, Q. Tang, Y.-H. Liu, Z.-D. Wu, Tests on the sampling performance of three kinds of filters for α-radioactive aerosol sampling, Nucl. Electron. Detect. Technol. 32 (2012) 116–119.
[11] Y. Wang, J. Wang, F. Yang, X. Yang, Spectrophotometric detection of lead (II) ion using unimolecular peroxidase-like deoxyribozyme, Microchim. Acta 171 (2010) 195–201.
[12] T. Li, S. Dong, E. Wang, G-quadruplex aptamers with peroxidase-like DNAzyme functions: which is the best and how does it work? Chem. An Asian J. 4 (2009) 918–922.
[13] W. Zhai, C. Du, X. Li, A series of logic gates based on electrochemical reduction of Pb 2+ in self-assembled G-quadruplex on the gold electrode, Chem. Commun. 50 (2014) 2093–2095.
[14] C.-W. Liu, C.-C. Huang, H.-T. Chang, Control over surface DNA density on gold nanoparticles allows selective and sensitive detection of mercury (II), Langmuir 24 (2008) 8346–8350.
[15] C.-C. Huang, H.-T. Chang, Aptamer-based fluorescence sensor for rapid detection of potassium ions in urine, Chem. Commun. (2008) 1461–1463.
[16] C.-K. Chiang, C.-C. Huang, C.-W. Liu, H.-T. Chang, Oligonucleotide-based fluorescence probe for sensitive and selective detection of mercury (II) in aqueous solution, Anal. Chem. 80 (2008) 3716–3721.
[17] K. Rurack, Flipping the light switch 'ON'—the design of sensor molecules that show cation-induced fluorescence enhancement with heavy and transition metal ions, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 57 (2001) 2161–2195.
[18] T. Li, E. Wang, S. Dong, Potassium– lead-switched G-quadruplexes: a new class of DNA logic gates, J. Am. Chem. Soc. 131 (2009) 15082–15083.
[19] I. Smirnov, R.H. Shafer, Lead is unusually effective in sequence-specific folding of DNA, J. Mol. Biol. 296 (2000) 1–5.
[20] I.V. Smirnov, F.W. Kotch, I.J. Pickering, J.T. Davis, R.H. Shafer, Pb EXAFS studies on DNA quadruplexes: identification of metal ion binding site, Biochemistry 41 (2002) 12133–12139.
[21] H. Sun, X. Li, Y. Li, L. Fan, H.-B. Kraatz, A novel colorimetric potassium sensor based on the substitution of lead from G-quadruplex, Analyst 138 (2013) 856–862.
[22] International Commission on Radiological Protection, Protection Against Radon-222 at Home and at Work. ICRP Publication 65, Pergamon Press, Oxford, 1994.