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Highly Sensitive and Selective Optical Detection of Lead (II) Using a Label-Free Fluorescent Aptasensor

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Abstract: Lead (Pb) is a heavy metal that does great harm to human bodies through its accumulation in the food chain. It remains a challenge to use environment-friendly and biocompatible materials to sensitively detect lead in organisms and aquatic ecosystems. To achieve highly sensitive and selective Pb^{2+} detection, a sensor based on ultra-sensitive double-stranded DNA (dsDNA) specific dye PicoGreen and label-free oligonucleotides was reported. The principle of this method is that the structural change of Pb^{2+} induced G-rich thrombin aptamer from random coil to G-quadruplex, which prevented its binding to its complementary sequences to form dsDNA and caused the fluorescent intensity decrease with PicoGreen. The results showed that this method satisfied the requirement on the maximum residue limit (MRL) of Pb^{2+} and could detect Pb^{2+} at the lowest concentration of 1 ng/mL within a dynamic range of six orders of magnitude. Since aptamer was highly specific, this method showed high Pb^{2+} selectivity against eight other metals. Finally, the proposed assay was successfully validated by determining Pb^{2+} in water samples.

1. Introduction

Heavy metal ions have great impacts on ecosystems and may seriously endanger human health because of their pollution. Among heavy metals, lead is significantly harmful to human health by damaging various human organisms such as the nervous system, hematopoietic stem cells in the bone, digestive system, and male reproductive system. During the sensitive development period of the nervous system, children's brains are especially sensitive to lead. Researches showed that mental retardation in

children was correlated with the increase of lead contamination. When blood lead levels in children augmented by every 10 mg/100 mL, children's intelligence test scores decreased by 6-8[1]. Therefore, the United States generally considered blood lead poisoning when children's lead content reached 0.1 μg/mL compared with previously 0.25 mg/mL[2]. In this case, monitoring the Pb^{2+} level in aqueous solutions is an important task.

As the most common techniques for Pb^{2+} detection, atomic absorption spectroscopy (AAS)[3], reversed-phase high-performance liquid chromatography (RP-HPLC)[4], inductively coupled plasma optical emission spectroscopy (ICP-OES)[5] and inductively coupled plasma mass spectrometry (ICP-MS)[6] have been developed. Nevertheless, these techniques require sophisticated and expensive equipment and materials, and using them is time-consuming.

To address these limitations, aptamers selected in vitro using the SELEX method are used as new recognition molecules for analytical applications. As single-stranded DNAs or RNAs, aptamers are highly specific to small-molecule drugs, peptides, cells and proteins with high affinity within a large range of target ligands[7, 8]. Compared with conventional antibodies, aptamers possess prominent advantages, such as high binding capability, excellent stability, wide target scope, simple synthesis and easy modification[4]. TBA (5'-GGTTGGTGTGGTTGG-3')[9] is aptamer dedicated to Pb^{2+}, which has a small dissociation constant within the nanomolar range and is not prone to bind with other heavy metal ions.

Recently, some Pb^{2+}-based aptamer sensors have been reported to detect Pb^{2+} with induced allosteric G4 structures[10-13]. Liu and his co-workers proposed a new sensitive method to detect Pb^{2+} with TBA where the 5' and 3' termini were labeled by a fluorophore and quencher, respectively[14]. Li et al. developed an electrochemical sensing platform for sensitive and selective detection of lead based on conformational switching of Pb^{2+}-induced G-rich DNA with the crystal violet functioning as a G4-binding indicator[11]. However, DNA probes in these methods require an additional immobilization technique or DNA-labeling process, which is complex, time-dependent and cost-ineffective. Therefore, it is very important to develop a simple, convenient and inexpensive sensor for sensitive monitoring of Pb^{2+} in the environment and food.

Here, we reported a simple, sensitive and selective fluorescent approach for Pb^{2+} detection based on aptamer in the absence of any label. The method is dependent on dsDNA's conformational changes and light-emitting properties of PicoGreen. On this sensing platform, the PicoGreen dye transduces fluorescence signals with its high specificity for dsDNA. This approach was proved to be more sensitive, more specific, simpler and cheaper than earlier techniques.

2. Experimental

2.1 Reagents and chemicals

Pb^{2+} aptamer has a sequence 5'-GGT TGG TGT GGT TGG-3'[9], and its complete complementary strand is 5'-CCA ACC ACA CCA ACC-3'. All involved

oligonucleotide sequences were synthesized and then purified using HPLC by Shanghai Sangon Biotechnology Co., Ltd.

The 10-fold concentrated fluorescent dye PicoGreen (PG) was obtained from Invitrogen (Carlsbad, USA). Lead nitrate, as well as $Pb(NO_3)_2$, LiCl, $CaCl_2$, $Cu(SO_4)_2$, $Zn(NO_3)_2$, $MgCl_2$, KCl, $AgNO_3$, NaCl, and $HgNO_3$ (Lot#BCBJ9926V) were from Sigma-Aldrich (CA, USA). Other standard chemicals (analytical grade) were purchased from Beijing Chemical Reagent Company. Ultrapure water was purified by the Milli-Q system (Millipore, Bedford, USA). An LS-55 Fluorescence Spectrometer (Perkin-Elmer, Norwalk, CT) was used to scan fluorescence intensities at excitation and emission lengths of 480 nm and 520 nm, respectively, and the results were recorded with a Tecan Infinite 200 multifunctional microplate reader (Tecan Austria GmbH, Austria), where the excitation and emission lengths were 480 nm and 520 nm, respectively. Agilent ICP-MS 5000 (Agilent Technologies Inc., USA) was used to analyze water samples to validate the assay.

2.2 Fluorescent detection of Pb^{2+}

To detect Pb^{2+} using this approach, 25 μL of 0.5 μM aptamer containing Pb^{2+} that was dissolved in water at different concentrations was added to microplate wells and subsequently incubated at room temperature for 10 min. Afterwards, 25 μL of 0.5 μM complementary strand dissolved in water and 10 μL of 10× PG (10 mM Tris-HCl; pH 8.0) were both added to the wells with the samples. Following 3 min incubation, the fluorescence intensities of the samples were scanned with the aforementioned

LS-55 Fluorescence Spectrometer and recorded by the multifunctional reader. The experiment was performed in triplicate to obtain average values.

2.3 Detection of Pb^{2+} in real samples

Tap water, mineral water (Ganten) and purified water (Wahaha) were all used as real samples to verify the practicability of this sensing system. Tap water was prepared in our laboratory, while other samples were purchased from a supermarket. They were all directly used for Pb^{2+} detection in the analysis methods described as above.

3. Results and discussion

3.1 Design of the principle

PG is specific double-stranded dye used during quantitative assay of dsDNA in a solution. This dye does not fluoresce if it is released free. As a signal probe, the PG dye can detect dsDNA whose concentration is so low as 25 pg/mL[15]. The sensing mechanism of Pb^{2+} detection is illustrated in Fig. 1. The figure shows that the aptamer and complementary strand form a hybrid without Pb^{2+}, and then PicoGreen is inserted into the dsDNA duplex, resulting in strong fluorescent intensities. However, when Pb^{2+} exists, the structure of the aptamer is changed from ssDNA to a G-quadruplex that binds to the target, forming the aptamer-target complex. Upon addition of Pb^{2+}, no or few aptamer sequences are present to form dsDNA, and then PicoGreen fails to induce fluorescence. Therefore, Pb^{2+} can be quantitatively analyzed when the changes in the fluorescence intensity of the PG dye are monitored, which adheres to the

principle of the negative correlation between the amount of Pb^{2+} and the intensity of fluorescence.

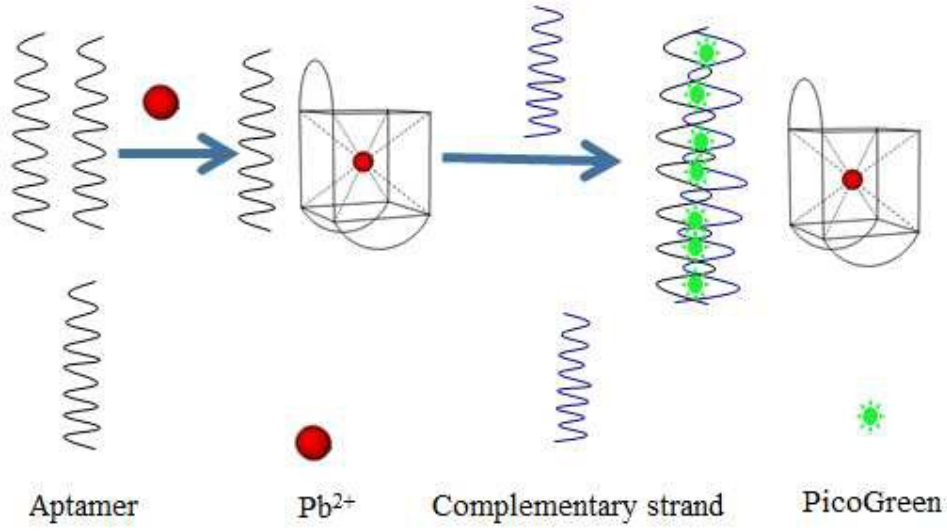

Fig. 1. Configurations and measurement principles of label-free fluorescent aptamer-based PG assay for Pb^{2+} detection.

3.2 PG amount optimization

In this strategy, the amount of PG was optimized before performing Pb^{2+} assay. Different volumes (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μL) of 10× PG solutions were added into the mixture after the reactions. As shown in Fig. 2, with the increase of the PG volume from 0 to 20 μL, the fluorescence intensity gradually increases, and when PG increases continuously from 10 to 20 μL, the fluorescence intensity reaches the peak level and does not change obviously. Thus, 10 μL of 10× PG solution was selected for the following experiments.

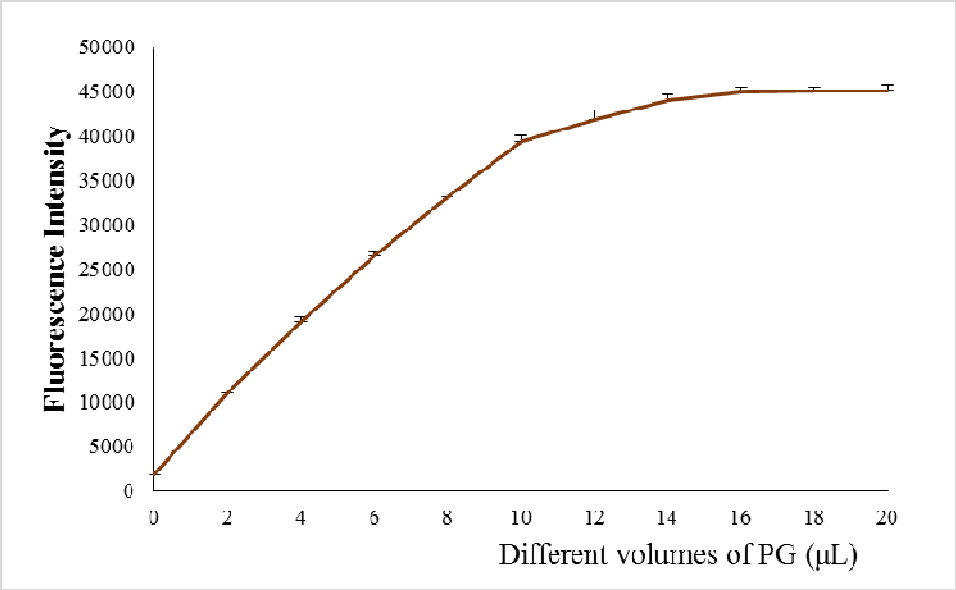

Fig. 2. The fluorescence intensity changes depending on the volumes (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μL) of 10× PG solutions.

3.3 Aptasensor reaction time optimization

In this study, the method is dependent upon the change of the PG's fluorescence intensity. Therefore, the fluorescence decrease ascribing solely to the specific Pb^{2+}-aptamer interaction is an important factor. Before Pb^{2+} was analyzed, time required for reaction between aptamer and Pb^{2+} to form a G4 structure was optimized. As shown in Fig. 3, the fluorescent intensity increased for the first 5 min due to the binding of free aptamer to the complementary strand. Then, the fluorescent intensity showed different changes from 5 to 20 min for different concentrations of Pb^{2+} due to the competition for aptamer between the aptamer/Pb^{2+} complex and aptamer/complementary strand DNA duplex. Most aptamers bind to the complementary sequences at low concentrations of Pb^{2+} within 10 min, and the

increasing fluorescent intensity plays a major role. For a high concentration of Pb^{2+}, most aptamer binds to Pb^{2+}, and the decrease of the fluorescence intensity plays a major role even at the beginning of the reaction. Generally, about 10 min was selected as the dsDNA reaction time because it demonstrated the greatest discrepancy in the fluorescence intensity with Pb^{2+} of various concentrations. This optimized reaction time provided high sensitivity.

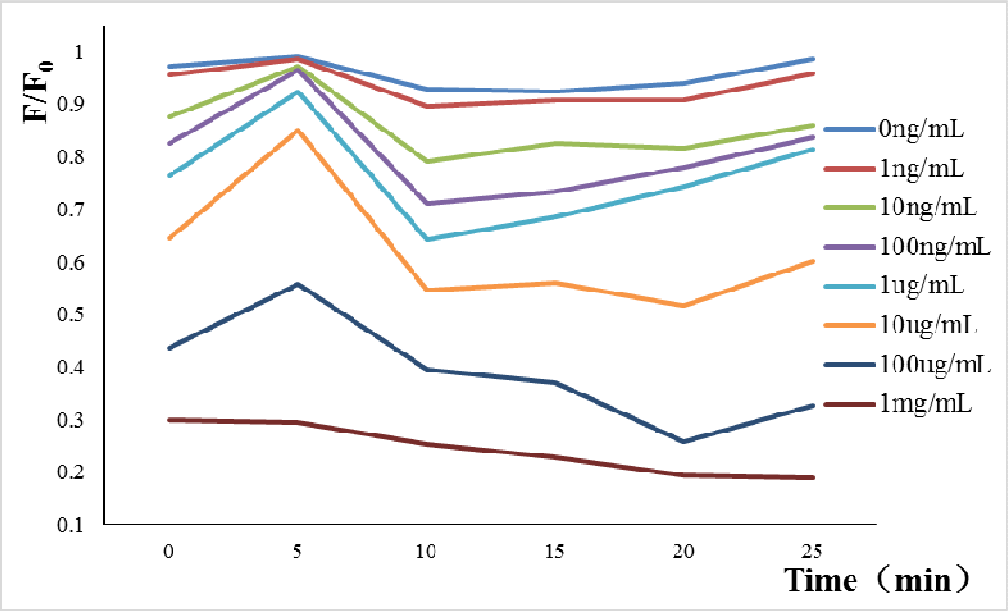

Fig. 3. Optimization on the reaction time of aptamer and Pb^{2+} to form a G4 structure after the addition of aptamer into Pb^{2+} of different concentrations.

3.4 Sensitivity evaluation for the label-free fluorescent aptasensor

With the experimental conditions optimized, the changes in the fluorescence intensity were obtained by using different concentrations of Pb^{2+}, which facilitated sensitivity evaluation for the proposed sensing platform. The results shown in Fig. 4 revealed a concentration-dependent response with a series of measured concentrations

of Pb^{2+}. When Pb^{2+} was unavailable, the sensor system provided excessively high fluorescence because the aptamer probes were chiefly hybridized with complementary probes and the PG intercalation caused an exceptionally increase in fluorescence emission. Figure. 4 (A) shows that with the increasing concentrations of Pb^{2+} from 1 ng/mL to 1 mg/mL, the fluorescence intensity is gradually decreased because of the decrease in DNA duplexes and intercalated PG. As shown in Figure. 4 (B), the decrease of fluorescence signals represented by F/F_0 is proportional to the Pb^{2+} concentration, indicating that quantitative acquisition of the Pb^{2+} concentration can be performed. With six or more orders of magnitude, Pb^{2+} can be detected at even the lowest concentration of 1 ng/mL, and under the conditions, the fluorescent intensity was decreased by about 10% compared to the blank sample. Compared with Pb^{2+} sensors reported by other literatures, which utilize fluorescence, the aptasensor in this work exhibits high sensitivity and cost-effective (Table 1).

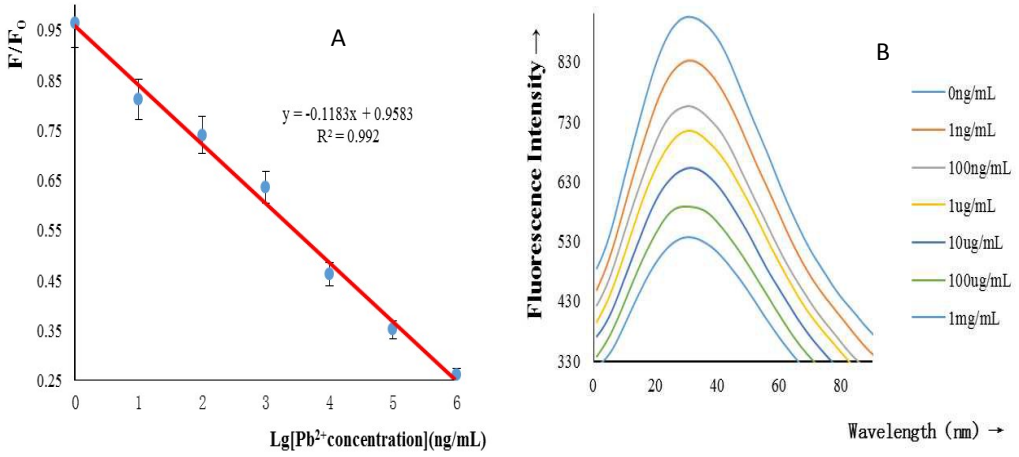

Fig. 4. Label-free fluorescent aptamer-based PG fluorescent assay for detection of Pb^{2+}. (A) The fluorescence intensity changes correlated with the logarithm of Pb^{2+}

concentrations within the range of 1 ng/mL to 1 mg/mL. (B) Fluorescence spectra of the solution at various concentrations of Pb^{2+}.

Table 1. Analytical performance comparison between different Pb^{2+} biosensors based on oligonucleotides

Probe	Detection method	Linear range	Reference
8-17DNAzyme/polythiophene	Fluorescence	10 nM	[16]
G quadruplex/NMM	Fluorescence	5 nM	[17]
G quadruplex/multi-walled carbon nanotube	Fluorescence	20 nM	[18]
GR-5DNAzyme/fluorophore carboxyfluorescein	Fluorescence	1 nM	[19]
8-17DNAzyme/fluorophore carboxyfluorescein	Fluorescence	3.0×10^{-9} to 5.0×10^{-6} (mol/mL)	[20]
G-quadruplex DNAzyme	Colorimetry and chemiluminescence	0 to 3.2×10^{-8} (mol/mL)	[21]
8-17DNAzyme/Ru(bpy)32+	ECL	18 nM	[22]
C−AuNPs	Fluorescence	10 nM	[23]
DNAzyme	Fluorescence	530 pM	[24]
G-quadruplex	Fluorescence	1 ng/mL	This work

3.5 Specificity confirmation

To study the selectivity of Pb^{2+}, 10 μg/mL of metal ions, including Ag^{+}, Cu^{2+}, Li^{+}, Zn^{2+}, Na^{+}, Ca^{2+}, Mg^{2+}, K^{+} and Hg^{2+} were used to perform control experiments. As shown in Fig. 5, Pb^{2+} causes a significant decrease in fluorescence signals while other metal ions have an eligible effect on fluorescent intensity. This sensing method is highly selective because aptamer is highly affinitive to Pb^{2+} against many other metal ions.

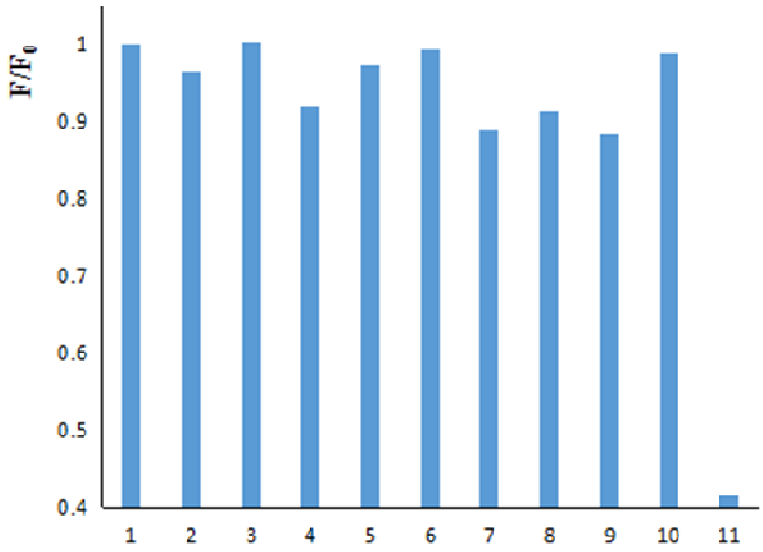

Fig. 5. Specificity test against ifferent metal ions (each at 10 μg/mL). 1: background; 2: Ag^{+}; 3: Cu^{2+}; 4: Li^{+}; 5: Zn^{2+}; 6: Na^{+}; 7: Ca^{2+}; 8: Mg^{2+}; 9: K^{+}; 10: Hg^{+}; 11: Pb^{2+}.

3.6 Analysis of water samples

To explore practical applications of the proposed fluorescent system, trace Pb^{2+} in tap water samples was determined. Table 2 showed that the Pb^{2+} level in tap water was sufficiently low so that it failed to be detected using the sensor. Interestingly, an obvious increase was observed in real signals when Pb^{2+} at certain concentrations was added into the samples. The developed assay was validated by analyzing water samples in comparison with ICP-MS analysis. As shown in Table 2, there was no significant difference between the two methods. Thus, this approach shows potential applications in determination of Pb^{2+} in water samples.

Table 2. Results obtained in analysis of water samples with the label-free fluorescent aptasensor method and ICP-MS.

	Spiked (ng/mL)	ICP-MS (ng/mL)	Fluorescent Aptasensor	
			Mean(ng/mL)	RSD%
Tap water	0	0.9	-0.22	1.33%
	30	32.8	31.7	2.56%
	100	87.9	94.5	0.53%
Purified water (Wahaha)	0	-0.77	-1.3	2.09%
	30	27.9	28.3	1.43%
	100	102	107	0.98%
Mineral water (Ganten)	0	-1.6	-1.4	0.73%
	30	23.7	27.8	1.39%
	100	96	97.9	1.79%

4. Conclusion

For the purpose of Pb^{2+} detection, we have designed a simplified, fast, highly selective and highly sensitive sensor in this paper. This sensing system is developed according to the principle that when binding to its targets, aptamer with its structure changed leads to the decrease in DNA duplexes of aptamer/complementary sequences, and further the decrease in the fluorescent intensity of PG inserted into the dsDNA duplex. Compared with other fluorescent aptasensors for Pb^{2+}, the proposed fluorescent aptasensor avoids DNA modification, becoming a simple and cost-effective analysis method for Pb^{2+}. Importantly, the linear dynamic range was found from 1 ng/mL to over 1 mg/mL, and the detection sensitivity for Pb^{2+} was found to be 1 ng/mL. Furthermore, the entire detection procedure could be completed in less than 30 min. Therefore, the proposed method is simple, fast, highly sensitive and easy-to-read for Pb^{2+} analysis and can be utilized in future detection on site in environmental waters.

Acknowledgements

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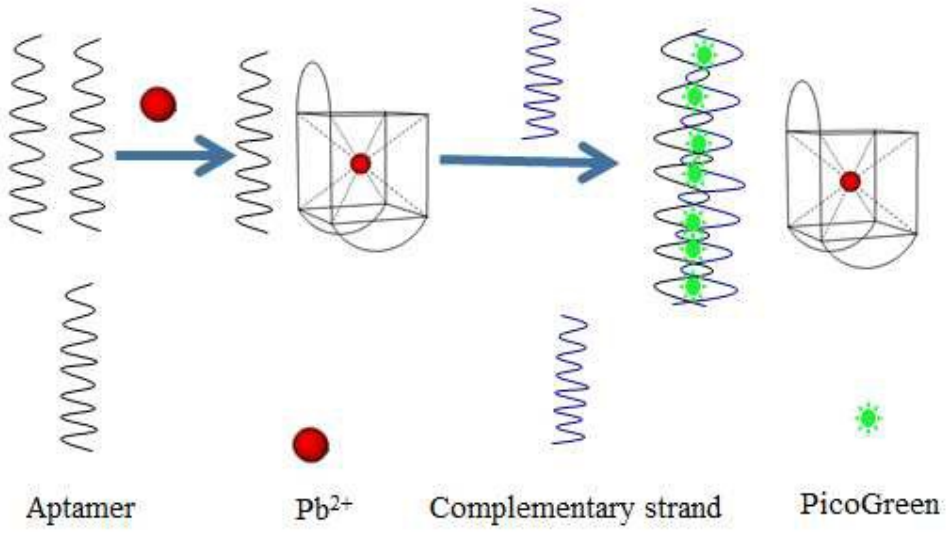
References

1. A. M. Chen, K. N. Dietrich, J. H. Ware, J. Radcliffe and W. J. Rogan, *Environ Health Persp*, 2005, 113, 597-601.
2. *Kansas medicine: the journal of the Kansas Medical Society*, 1992, 93, 358-359.
3. Y. Liu, Z. C. Liu, Y. Wang, J. D. Dai, J. Gao, J. M. Xie and Y. S. Yan, *Microchim Acta*, 2011, 172, 309-317.
4. G. Yang, C. M. Zhang, Q. F. Hu and J. Y. Yin, *J Chromatogr Sci*, 2003, 41, 195-199.
5. M. D. A. Korn, J. B. de Andrade, D. S. de Jesus, V. A. Lemos, M. L. S. F. Bandeira, W. N. L. dos Santos, M. A. Bezerra, F. A. C. Amorim, A. S. Souza and S. L. C. Ferreira, *Talanta*, 2006, 69, 16-24.
6. A. Schutz, I. A. Bergdahl, A. Ekholm and S. Skerfving, *Occup Environ Med*, 1996, 53, 736-740.
7. C. Tuerk and L. Gold, *Science*, 1990, 249, 505-510.
8. A. D. Ellington and J. W. Szostak, *Nature*, 1990, 346, 818-822.
9. F. W. Kotch, J. C. Fettinger and J. T. Davis, *Org Lett*, 2000, 2, 3277-3280.
10. C. W. Liu, C. C. Huang and H. T. Chang, *Anal Chem*, 2009, 81, 2383-2387.
11. F. Li, Y. Feng, C. Zhao and B. Tang, *Chem Commun*, 2011, 47, 11909-11911.
12. L. Q. Guo, D. D. Nie, C. Y. Qiu, Q. S. Zheng, H. Y. Wu, P. R. Ye, Y. L. Hao, F. F. Fu and G. N. Chen, *Biosens Bioelectron*, 2012, 35, 123-127.
13. T. Li, S. J. Dong and E. K. Wang, *J Am Chem Soc*, 2010, 132, 13156-13157.
14. C. W. Liu, C. C. Huang and H. T. Chang, *Anal Chem*, 2009, 81, 2383-2387.
15. A. I. Dragan, J. R. Casas-Finet, E. S. Bishop, R. J. Strouse, M. A. Schenerman and C. D. Geddes, *Biophys J*, 2010, 99, 3010-3019.
16. X. Chen, H. L. Guan, Z. K. He, X. D. Zhou and J. M. Hu, *Anal Methods-Uk*, 2012, 4, 1619-1622.
17. X. B. Fu, F. Qu, N. B. Li and H. Q. Luo, *Analyst*, 2012, 137, 1097-1099.
18. S. E. Wang and S. H. Si, *Anal Methods-Uk*, 2013, 5, 2947-2953.
19. X. H. Zhao, R. M. Kong, X. B. Zhang, H. M. Meng, W. N. Liu, W. Tan, G. L. Shen and R. Q. Yu, *Anal Chem*, 2011, 83, 5062-5066.
20. X. B. Zhang, Z. D. Wang, H. Xing, Y. Xiang and Y. Lu, *Anal Chem*, 2010, 82, 5005-5011.
21. T. Li, E. Wang and S. Dong, *Anal Chem*, 2010, 82, 1515-1520.
22. D. Y. Liu, Y. Y. Xin, X. W. He and X. B. Yin, *Analyst*, 2011, 136, 479-485.
23. Y. S. Wu, F. F. Huang and Y. W. Lin, *Acs Appl Mater Inter*, 2013, 5, 1503-1509.
24. Y. Guo, J. T. Li, X. Q. Zhang and Y. L. Tang, *Analyst*, 2015, 140, 4642-4647.



Configurations and measurement principles of label-free fluorescent aptamer-based PG assay for Pb^{2+} detection.