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Self-paired dumbbell DNA -assisted simple preparation of stable circular DNAzyme and its application in Pb^{2+} sensor

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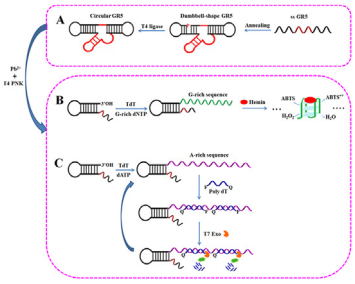
HIGHLIGHTS

- A simple and common strategy to preparation stable circular DNAzyme is developed based on the self-paired dumbbell DNA.
- Exploiting this stable circular DNAzyme, two colorimetry and fluorescence sensing system for Pb^{2+} detection are developed.
- Those two sensing systems could work well over a wide range of temperature, salt concentration, and pH.
- Those two sensing platform hold high sensitivity with detection limit as low as 0.085 nM and 0.0015 nM.

GRAPHICAL ABSTRACT



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ABSTRACT

During its development in recent decades, DNAzyme has become a promising candidate for application in biosensor field. However, it still suffers from the problem of thermodynamic and biological instability such as nuclease digestion, which limits its applications in complex samples. Here we have presented a simple and common strategy to resolve this problem by engineering the linear DNAzyme into a circular shape DNAzyme based on the integration of substrate and enzyme parts into one single-stranded sequence. This circular DNAzyme system is indeed endowed with excellent stability due to the stable intramolecular double-stranded formation and extraordinary resistance to nuclease digestion due to the closed structure. We demonstrated that this circular DNAzyme system gained excellent stability and could active under conditions across a broader range of temperature, salt concentrations, and pH. Depending on this circular DNAzyme, combing with Terminal deoxynucleotidyl transferase (TdT)-generated G-quadruplexes, a label free colorimetric sensing platform for Pb^{2+} quantitation was developed, and a detection limit of 0.085 nM was achieved. Then the enzyme digestion cycle amplification was introduced to further improve the sensitivity of the sensing system, an ultralow detection limit of 0.0015 nM for this fluorescence method was achieved. Based on the two sensing platforms, ultrasensitive

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analysis of Pb^{2+} in environmental water and food samples was successfully realized. It is anticipated that this stable circular DNAzyme design will be helpful for trace detection in complex samples.

1. Introduction

DNAzymes are artificial short single/double-stranded nucleic acid molecules which own targets identification ability and catalytic activity [1]. Since the first isolation in 1994 [1–3], a large number of DNAzymes that can catalyze RNA/DNA cleavage, splicing, RNA ligation and so on have been screened out [4–8] and widely applied in multiple research fields [9–15]. Compared to natural protein enzymes, DNAzymes have many advantages those making them be applied more widely. First, DNAzymes can be screened out through *in vitro* SELEX (systematic evolution of ligands by exponential enrichment) process [16–19], so in theory corresponding DNAzyme can be screened for any substrate. Second, DNAzymes can be easily functionalized with desired chemical tags, which range from optical-/electro-labels to even nanomaterials [20–23]. Third, the screened DNAzymes can be obtained in a large quantity and low cost via nucleic acids synthetic methods. Fourth, DNAzymes can be denatured and renatured many times without loss of their activity [24–26], making them remain stable property even under harsh conditions. Finally and most importantly, DNAzymes show high specificity for corresponding targets, and the enzyme activity is closely related to the concentration of the target [27–29]. These properties make DNAzyme an excellent candidate for applications in diverse fields including analytical chemistry, chemical biology, biosensing, and so on [8,9,12,30,31]. However, most DNAzymes screened are still suffered from the issues of thermodynamic instability and biological instability [32,33], limiting their application in complex samples and harsh conditions. Therefore, developing a more stable DNAzyme is still an urgent need for its further application in detection.

Nowadays, many studies have been done in engineering linear DNAzyme into a circular formation to gain improved stability. Comparing to the linear form, circular nucleic acid demonstrated to possess amazing features including excellent thermodynamic stability, biological stability and structural rigidity because of the stable intramolecular duplex helix and the complete resistance to nuclease digestion [34]. Recently, Breaker et al. obtained a circular DNAzyme from *in vitro* selection which showed exceedingly resistance to hydrolysis [35]. Zhang group constructed a circular DNAzyme which can be applied in ions detection in complex samples [36]. By engineering Mg^{2+} and Zn^{2+}-dependent RNA-cleaving DNAzymes into a two-ring DNA catenane, Willner group proposed a new kind of DNAzyme displays stable and switchable catalytic properties [37]. Wang group reported a versatile self-sufficient Zn^{2+} DNAzyme which integrates the substrate chain and enzyme part and realized its application in cell [38]. In these methods, two oligonucleotide strands were often required for the preparation of circular DNAzyme, and just one oligonucleotide strand (substrate strand or enzyme strand) was cyclized. So the DNAzyme systems usually consisted of intermolecular substrate/enzyme duplexes, which may still suffer from low stability in harsh conditions. In addition, the linear oligonucleotide strands existed in the system would inevitably generate background signal due to the nonspecific hybridization or extension.

Given that circular nucleic acids can also initiate intramolecular self-hybridization structure, a simple, universal, and effective circular DNAzymes preparation method might be developed by combining the substrate strand and enzyme strand into one dumbbell shaped strand. Herein, we take one of the most popular RNA-cleaving DNAzyme GR5 [39,40] as an example, to verify the above hypothesis. An easier alternative circular DNAzyme preparation assay was developed by integrating the substrate and enzyme parts into one dumbbell shaped GR5 DNAzyme system. After analyzing the T_m and digestion resistibility of the circular GR5 DNAzyme, this proposed circular GR5 DNAzyme has been found to own excellent stability even in extreme environments. To confirm potential applications of the circular GR5 DNAzyme in biosensing, two sensing platforms were designed for Pb^{2+} quantitation. The closed circular DNAzyme could avoid nonspecific hybridization and extension effectively, conferring the sensing system with the virtues of high sensitivity and strong anti-interference. Those two sensing systems were successfully applied for the quantitation of Pb^{2+} in real samples such as river water, vegetable, and cereal samples. This work indeed paved an easy and versatile way to prepare DNAzyme with high stability. We expect this method can make some contributions to promoting the application of DNAzyme in the biosensing field.

2. Experimental section

2.1. Pb^{2+} colorimetric biosensor based on circular GR5 DNAzyme and TdT-synthesized G-quadruplexes

Firstly, different concentrations of Pb^{2+} were added into the reaction system containing 20 μL of circular GR5 (1 μM). After a quick vortex, 20 U T4 polynucleotide kinase (T4 PNK) was added to convert the 2′, 3′ cyclic phosphate 3′ termini into 3′-OH, this reaction was performed at 37 °C for 30 min. Next, 2 μL of dNTP (100 mM, 40% dATP and 60% dGTP), 20 U of Terminal deoxynucleotidyl transferase (TdT) and 1 × TdT buffer was added into above system to extension G-rich sequence, the total volume of this mixture was 50 μL. After a complete vortex, this extension reaction was performed at 37 °C for 60 min.

Afterward, 40 μL reaction system containing 1 × HEPES buffer (pH 6.4), 20 mM KCl, 200 mM NaCl, and 0.5 mM hemin were added into above extension products to promote the formation of G-quadruplex DNAzyme. And this binding reaction was performed at 25 °C for 30 min.

Then, 5 μL (2,2′-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid) diammonium salt ($ABTS^{2-}$, 3.2 mM) and 5 μL H_2O_2 (2.2 mM) were added into the above G-quadruplex DNAzyme products. The reaction between $ABTS^{2-}$ and H_2O_2 was performed at room temperature for 4 min. Finally, the UV absorption spectrum of the reaction product $ABTS^{-}$ was recorded at 420 nm.

2.2. Pb^{2+} biosensor based on circular GR5 DNAzyme and TdT/T7 exo-aided recycling amplification reaction

In this sensing system, the processes of circular GR5 cleavage reaction and the 2′, 3′ cyclic phosphate hydroxylation reaction are the same as that of above colorimetric sensor. Next, 2 μL of dATP (100 mM), 20 U of TdT and 1 × TdT buffer was added into above system to extension A-rich sequence, the total volume of this mixture was 50 μL. After a complete vortex, this extension reaction

was performed at 37 °C for 60 min, followed by heat deactivation of TdT enzyme at 80 °C for 10 min. Afterward, 50 μL reaction solution containing 1 μM Poly-dT probe, 10 U of T7 Exo, and 1 × NEBuffer 4 was added into above A-rich sequence products to trigger the amplification reaction. The amplification reaction was carried out at 25 °C for 30 min. Finally, the emission spectra from 500 to 580 nm were monitored with excitation at 492 nm.

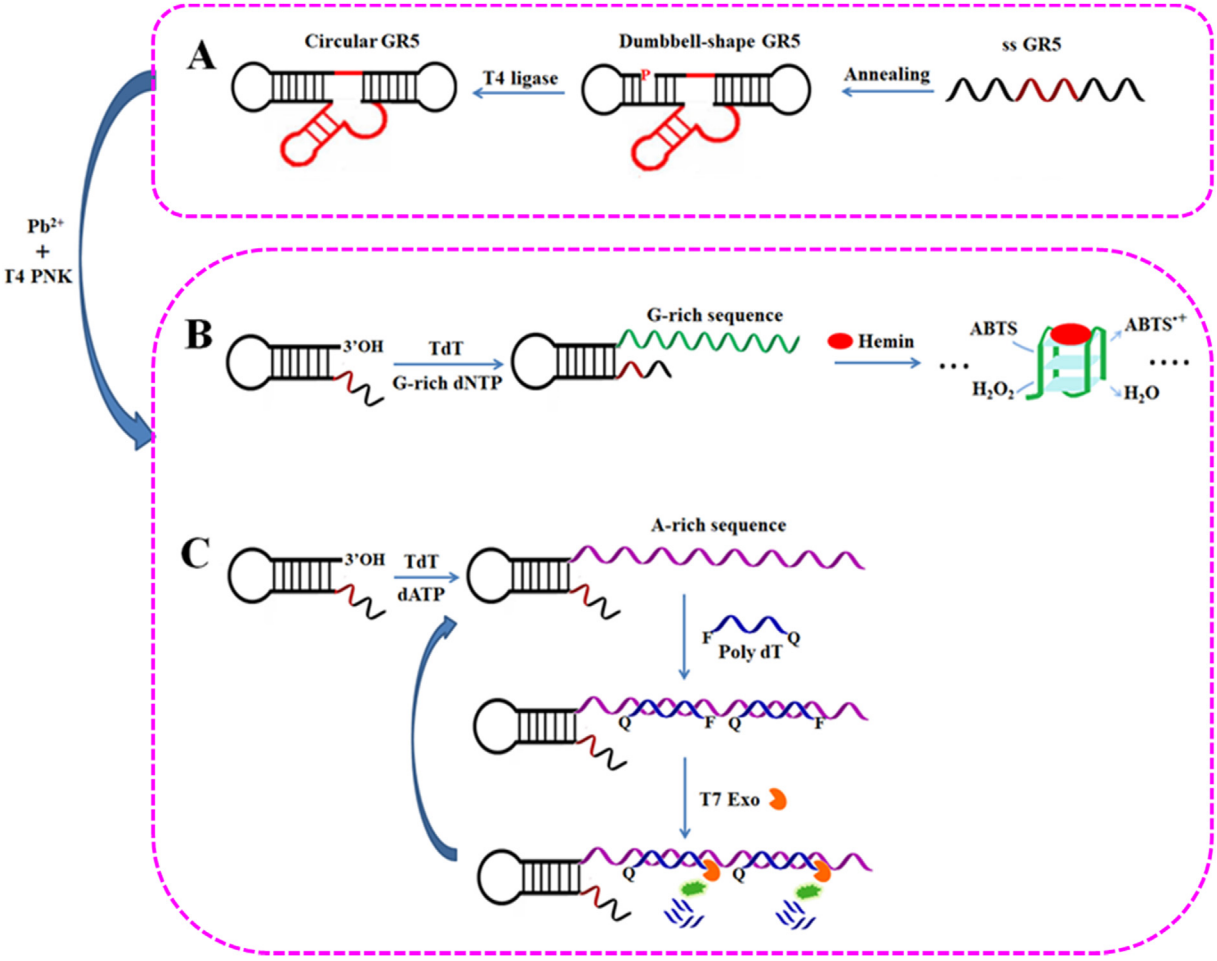
3. Results and discussion

3.1. Principle of circular DNAzyme preparation and Pb^{2+} sensing

Herein, we take one of the most popular RNA-cleaving DNAzyme GR5 as an example to verify the above hypothesis. The circular GR5 DNAzyme was prepared via simple ligation of ssDNA. As shown in Scheme 1A, the substrate and enzyme parts were integrated into one dumbbell shaped GR5 DNAzyme strand (DS-GR5) so that only one single-stranded DNA with a phosphorylated 5′-terminal (ssGR5, Table S1) was used. The dumbbell shaped GR5 DNAzyme was sealed into circular GR5 DNAzyme through intramolecular ligation of the 5′-P and 3′-OH ends under the conduct of T4 DNA ligase. Besides low cost and easy-to-preparation characters, this circular DNAzyme could also be prepared in large quantity and used at any time, which gives great help to shorten the length of the experiment.

Based on the high dependence of GR5 catalytic activity on cofactor Pb^{2+}, two simple Pb^{2+} biosensors were obtained. As shown in Scheme 1B, a colorimetric Pb^{2+} sensing platform was developed based on Terminal deoxynucleotidyl transferase (TdT)-generated G-quadruplexes. In the presence of Pb^{2+}, the substrate part of the circular GR5 will be cleaved at the ribonucleotide site, generating phosphate 3′ termini which can be subsequently hydroxylated by T4 PNK. In the presence of dNTP pool (60% dGTP and 40% dATP), the 3′-hydroxyl-end could be elongated to form randomly arrayed G-quadruplexes, helped along by TdT polymerase [41]. Subsequently, these randomly arrayed G-quadruplexes can bind to hemin to form peroxidase-mimicking G-quadruplex DNAzyme. In this case, the oxidation-reduction reaction between $ABTS^{2-}$ and H_2O_2 was catalyzed, resulting in a color change. In the absence of Pb^{2+}, the circular DNAzyme could not break and there are no any termini to extension, which guaranteed ultralow background signal. And Pb^{2+} can be reused during the reaction, that is to say one Pb^{2+} may participate in the cleaving of multiple circular DNAzymes, so high sensitivity of this method is ensured.

Furthermore, by adding a short Poly-dT fluorescence probe and T7 Exo, the sensing strategy could be easily combined with TdT/T7 Exo-aided recycling amplification, so more sensitive sensor can be obtained (Scheme 1C). Similarly, in this system with dATP pool, long Poly-dA tail can be prepared under the action of TdT. When Poly-dT was added into the system, it could bind to the Poly-dA tail and form double-stranded DNA, resulting in the absolute quenching of fluorophores. In the presence of T7 Exo, the 5′-terminal of



Scheme 1. Mechanism of circular GR5 preparation and its application in Pb^{2+} detection: (A) Circular GR5 preparation based on ssDNA GR5 (B) Pb^{2+} detection based on circular GR5 and TdT-synthesized G-quadruplexes (C) Pb^{2+} biosensor based on circular GR5 and TdT/T7 Exo-aided recycling amplification.

Poly-dT probe can be digested into mononucleotides, generating recovered fluorescence signal. In this sensing system, one poly-dA sequence could be recycled and hybridized with multiple Poly-dT probes, thus further improving the sensitivity and making ultra-sensitive detection in complex system possible.

3.2. Stability of circular DNAzyme

It is well known that DNA double helix hybrid structure is susceptible to external conditions such as temperature, pH, and ionic strength and so on, which are inevitable in on-site environments. Therefore, good thermodynamic stability is prerequisite for the successful applications of DNAzyme in point-of-care and on-site detection. In order to investigate the thermodynamic stability of circular GR5 DNAzyme, melting curves under different pH and ionic strength were measured. As shown in Fig. 1A, with the increase of the concentration from 0 to 500 mM of NaCl, the T_m value of circular GR5 increased from 50 °C to 60 °C, which is much higher than room temperature 37 °C. Similarly, Fig. 1B showed that the T_m of the structure could keep higher than 50 °C within the range of pH from 5.0 to 9.0. In addition, when the complementary sequence (Table S1) is exactly the same with circular DNAzyme, the T_m values of linear DNAzyme has been measured under the same concentration. As shown in Fig. S1 and Table S2, the results demonstrated that circular DNAzyme indeed hold better thermodynamics stability. These results demonstrated that this circular GR5 can maintain stable double helix structure even in harsh conditions, suggesting great feasibility and applicability in on-site monitoring fields.

To investigate the biological stability, we compared the resistance capabilities of circular GR5 DNAzyme to different nuclease including Exo I and Exo III, which are all important nuclease that can digest ssDNA/dsDNA into mononucleotides. As shown in Fig. 1C, due to the protection from enclosed circular structures that without exposed terminals, circular DNAzyme showed a robust resistibility to nuclease and complex biological matrix. For the circular DNAzyme, there is still an obvious band (Lane 3 and Lane 4) after being treated with Exo I and Exo III. On the contrary, for the linear DNAzyme, there is no band after the treatment by 10U Exo I + 10U Exo III (Lane 2) within 60 min incubation, demonstrating that linear GR5 DNAzyme was completely degraded. These results strongly supported the excellent biostability of circular DNAzyme compared to unclosed linear DNAzyme, demonstrating a great promise for applications of the circular system in complex biological samples.

The resistance ability to nuclease degradation of circular DNAzyme was further verified by UV–vis absorption spectra based on TdT-synthesized G-quadruplexes (Scheme 1B). As shown in Fig. 1D, after treating with Exo I + Exo III, the unclosed DS-GR5 giving an ultra-low absorbance which is almost the same as the absorbance of free Hemin, suggesting that there is no G-quadruplexes produced. In contrast, when the circular GR5 was incubated with three nucleases (Exo I + Exo III) simultaneously, there is a sharp enhanced absorption signal, accompanied by the green color change of the solution. Notably, the conformation of G-quadruplexe that generated through TdT extension was characterized by the circular dichroism (CD) spectroscopy (Fig. S2). Besides this, the catalytic performance of circular GR5 DNAzyme was investigated by electrophoresis experiment. As shown in Fig. S3, result demonstrated this circular GR5 DNAzyme presented similar

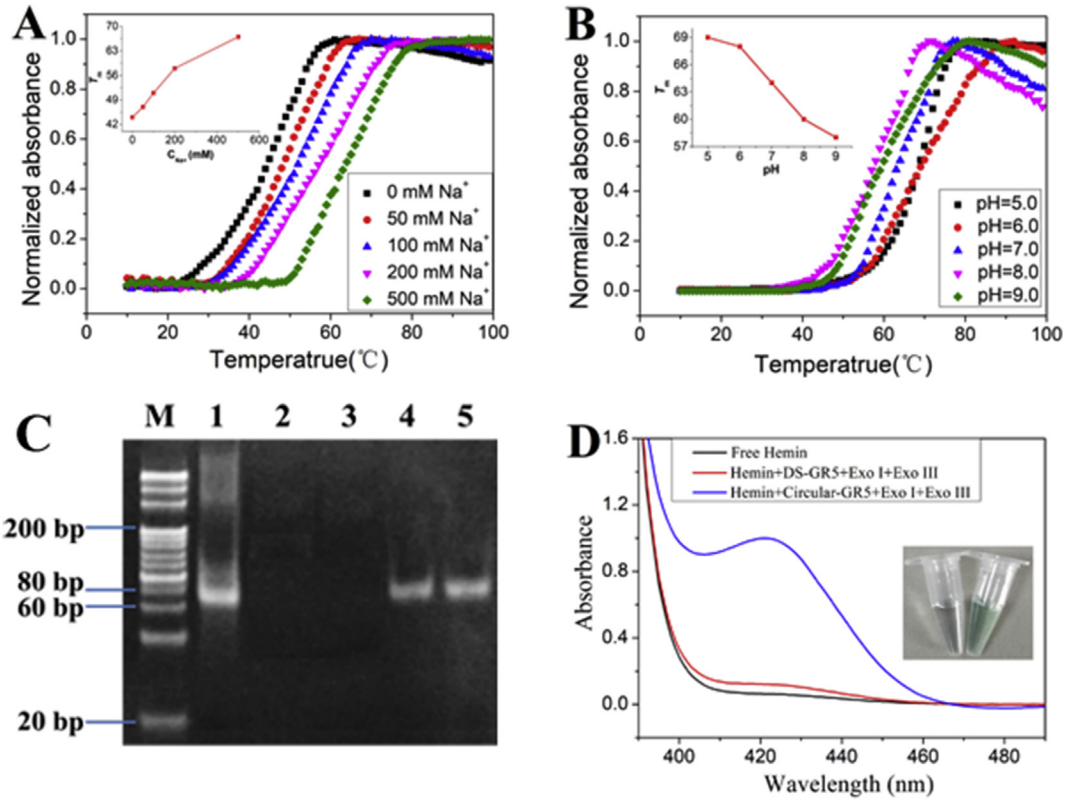


Fig. 1. Stability of the circular GR5 (A) Melting curves of the circular GR5 under different ionic conditions and (B) different pH, the inserts show pH or Na^+ concentration dependent T_m change of the circular GR5. (C) Electrophoretic assay for biostability of circular GR5. Lane M is the DNA marker. Lane 1: DS-GR5; Lane 2: unclosed DS-GR5 + Exo I + Exo III; Lane 3: circular GR5; Lane 4: circular GR5 + Exo I + Exo III. (D) UV–vis absorption spectra change of the DS-GR5/circular GR5 sensing system after treatment with multiple nucleases. The inserts show the photographic images of the reaction systems catalyzed by free Hemin or G-quadruplex/Hemin.

catalytic properties as linear DNAzyme under identical experimental conditions. All these results demonstrated that circular GR5 DNAzyme could indeed gain significant biostability without any influence on its catalytic activity, demonstrating a great promise for applications of the circular DNAzyme system in complex biological samples.

In addition, in order to prove the universality of this method for preparation of circular DNAzyme, take Zn^{2+} 8−17 DNAzyme (Table S1) as another model, a circular DNAzyme was built (Fig. S4). Then the stability and catalytic activity of this circular 8−17 DNAzyme was investigated by the same way as that of circular GR5. As shown in Figs. S5 and S6, and Table S3, Melting curves, PAGE electrophoresis, and UV−vis absorption spectra results revealed that this circular 8−17 DNAzyme not only has better stability but also has the same catalytic activity compared to linear 8−17 DNAzyme, demonstrating that our proposed circular DNAzyme preparation method is a universal means.

3.3. Circular DNAzyme based colorimetric biosensor for Pb^{2+} detection

In order to verify the potential application of circular GR5 DNAzyme, based on the high dependence on cofactor Pb^{2+} of its catalytic activity and TdT-synthesized G-quadruplexes, a simple Pb^{2+} sensor was developed (Scheme 1B). Secondly, in order to verify the suitability for on-site use of the sensing system, the sensor performance of Pb^{2+} was investigated under different conditions. Three temperatures (4 °C for common refrigerated storage temperature; 25 °C for room temperature and 37 °C for physiologically relevant temperature) and three pH values (5.0 for acidity, 7.0 for neutrality, and 9.0 for alkalinity) were selected as representatives. In the presence of 20 nM Pb^{2+}, the sensing system displayed the same increased absorbance values at 420 nm under different conditions (Fig. 2A), suggesting that the proposed Pb^{2+} sensor can work well in complicated conditions which may cross over a wide range of temperature and pH.

To further validate the feasibility of the proposed sensor, we employed PAGE to monitor the reaction process. In the presence of Pb^{2+}, a new band located around 400 bp was observed (Fig. 2B, lane 3), indicating that the circular DNAzyme has been cleaved, and a long DNA chain has been extended. On the contrary, the band of products showed no significant difference from the original circular GR5 in the absence of Pb^{2+} (Fig. 2B, lane 1&2), indicating that the Pb^{2+} was a key initiator for the GR5 cleavage process. Moreover, these results also demonstrated that the circular structure DNAzyme based sensing system can effectively avoiding the unspecific amplification because of the lacking of exposed 5′ or 3' terminal, ensuring ultralow background signal.

Then under optimized conditions (Figs. S7-S9 and 5 U T4 PNK, 20 U TdT, 1 min Pb^{2+} cleavage and 50 min TdT amplification time), the sensitivity of the Pb^{2+}sensor was investigated. For the colorimetric assay, the absorbance at 420 nm was real time monitored under different concentrations of Pb^{2+} (Fig. 2C), the absorption intensity progressively increased with Pb^{2+} concentration (Fig. 2D). A linear relationship ($A = 0.093 + 0.296C$, $R^2 = 0.9989$, where A is the absorbance intensity and C is the Pb^{2+} concentration) was obtained between the absorption intensity and the concentration of Pb^{2+} in a range from 0.2 to 1.0 nM with an estimated detection limit as low as 0.085 nM (Fig. 2E). The detection limits of the above two assays are all about 1000 times lower than 72 nM which was defined by EPA as the safety limit in drinking water [42]. The high sensitivity may be largely attributed to the completely closed circular GR5 DNAzyme, which lacked exposed 5′ or 3' terminals and ensured ultralow background signal.

To evaluate the specificity of the proposed method, this proposed sensing system was simultaneously applied to detect different metal ions. Various metal ions such as Ca^{2+}, Mn^{2+}, Cd^{2+}, Ba^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Cr^{3+} were analyzed under the same condition. As shown in Fig. 2F, in the presence of 20 nM Pb^{2+}, a significant enhanced absorbance signal is observed, while other metal ions could generate little or no UV absorbance signal even with a 500-fold increase in concentration. Moreover, a mixture of other ions has almost no effect on the absorbance signal, indicating that this Pb^{2+} sensor holds good specificity and strong anti-interference capability. In addition to the high dependence of GR5 catalytic activity on cofactor Pb^{2+}, this excellent specificity is largely due to the stable structure of circular DNAzyme, which can efficiently avoid the nonspecific breakage and digestion.

3.4. Circular DNAzyme based fluorescence signal amplifier for Pb^{2+} detection

Furthermore, with the help of a short Poly-dT fluorescence probe and T7 Exo, a more sensitive sensing system was developed based on the combination with enzyme digestion cycle amplification (Scheme 1C). Firstly, the feasibility of this sensing platform for Pb^{2+} detection was verified by fluorescence analysis. As shown in Fig. S10, the blank control without Pb^{2+} exhibited almost negligible fluorescence signal change. On the contrary, a significantly enhanced fluorescence signal was observed in the presence of 10 nM Pb^{2+}, demonstrating that the circular GR5 was cleaved and T7 Exo aided enzyme digestion cycle amplification reaction was successfully triggered. The high ratio of signal to noise (S/N) between these two systems with or without Pb^{2+} suggested the feasibility of this method for Pb^{2+} sensitive detection. In addition, when the cleavage reaction of circular GR5 performed under different temperature and pH conditions, the sensing system displayed the same increased fluorescence values at 517 nm (Fig. 3A), suggesting that this Pb^{2+} sensor can work well in complicated conditions which may cross over a wide range of temperature and pH.

Under optimized conditions (Figs. S11-S12 and 5 U T7 Exo and 400 nM Poly-dT), the sensitivity of this sensor was evaluated by recording the fluorescence spectrum of this sensing system containing varying amounts of Pb^{2+} (Fig. 3B). The fluorescence intensity at 517 nm progressively increases with Pb^{2+} concentration. The linear regression equation was described as $F = 7.68 + 3017.71C$ ($R^2 = 0.9953$) with the detection limit of 0.0015 nM (Fig. 3C). Compared with the colorimetric method without cycle amplification, the sensitivity was enhanced by one order of magnitude above colorimetric method. By comparison with others Pb^{2+} sensors based on traditional linear DNAzyme, it is found that the proposed two sensors could give comparable or higher sensitivity under a broader range condition (Table S4). Similarly, the specificity of this sensor was evaluated by recording the fluorescence responses of the sensor to various metal ions including Ca^{2+}, Mn^{2+}, Cd^{2+}, Ba^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Cr^{3+} under the same condition. As shown in Fig. 3D, the experiment results indicated that this sensor exhibited a high selectivity for Pb^{2+} than the tested analogues.

3.5. Pb^{2+} detection in real samples

In order to investigate the practicability of the sensor for Pb^{2+} detection in practical samples, we explored the possibility of utilizing the above two sensing system for Pb^{2+} analysis in river water, vegetables (cabbage) and cereal (rice) samples. The standard addition experiment was performed by adding the Pb^{2+} with known concentrations (10, 50, and 100 nM) to the real samples, and ICP-MS was conducted as a confirmatory method. The experimental data obtained from the proposed biosensor were matched

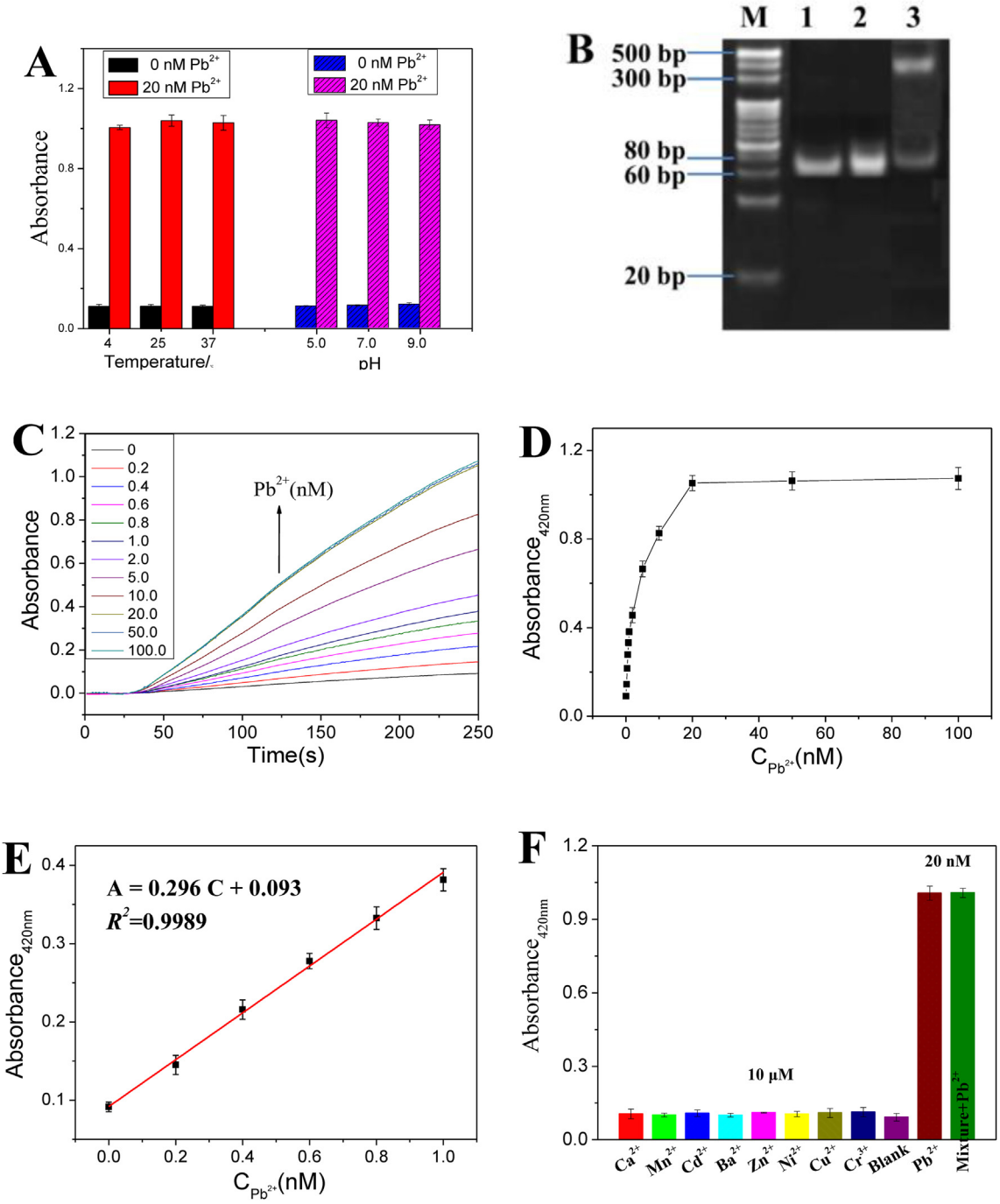


Fig. 2. Pb^{2+} biosensor based on circular GR5 and TdT-synthesized G-quadruplexes. (A) UV–vis absorption signal at 420 nm of the sensing system in the absence or presence of Pb^{2+} under different temperature or pH. The other reaction conditions are the same as section 2.1 except the temperature and pH; (B) PAGE electrophoresis analysis of the reaction process: Lane M: the DNA marker; Lane 1: circular GR5; Lane 2 and Lane 3: circular GR5 based sensing system in the absence and presence of Pb^{2+}; (C) Time-dependent absorbance changes at 420 nm under different concentrations of Pb^{2+}. (D) The absorbance at 4 min was plotted as a function of the Pb^{2+} concentrations. (E) The linear relationship between the UV–vis intensity and the concentration of Pb^{2+} from 0 to 1 nM. (F) Selectivity of the proposed Pb^{2+} colorimetric sensor. [Pb^{2+}] = 20 nM; the concentration of other metal ions is 10 μM. Error bars show the standard deviation of three experiments.

well with that from ICP-MS method (Table 1), suggesting that the biosensor can work well as a convenient tool for on-site monitoring of Pb^{2+} in different samples. It was found that the recoveries are ranged from 99.0% to 103.0%, respectively. Those results demonstrated the proposed sensing systems not only possess good accuracy and satisfactory reliability but also show great potential of application in complex samples.

4. Conclusion

In this paper, we have introduced a circular DNAzyme system which could be prepared by self-template ligation of single-stranded DNA. This circular DNAzyme was endowed with excellent stability and could remain catalytic activity even under harsh conditions. Exploiting this circular DNAzyme system, we developed two simple, sensitive platforms to detect Pb^{2+} based on colorimetry

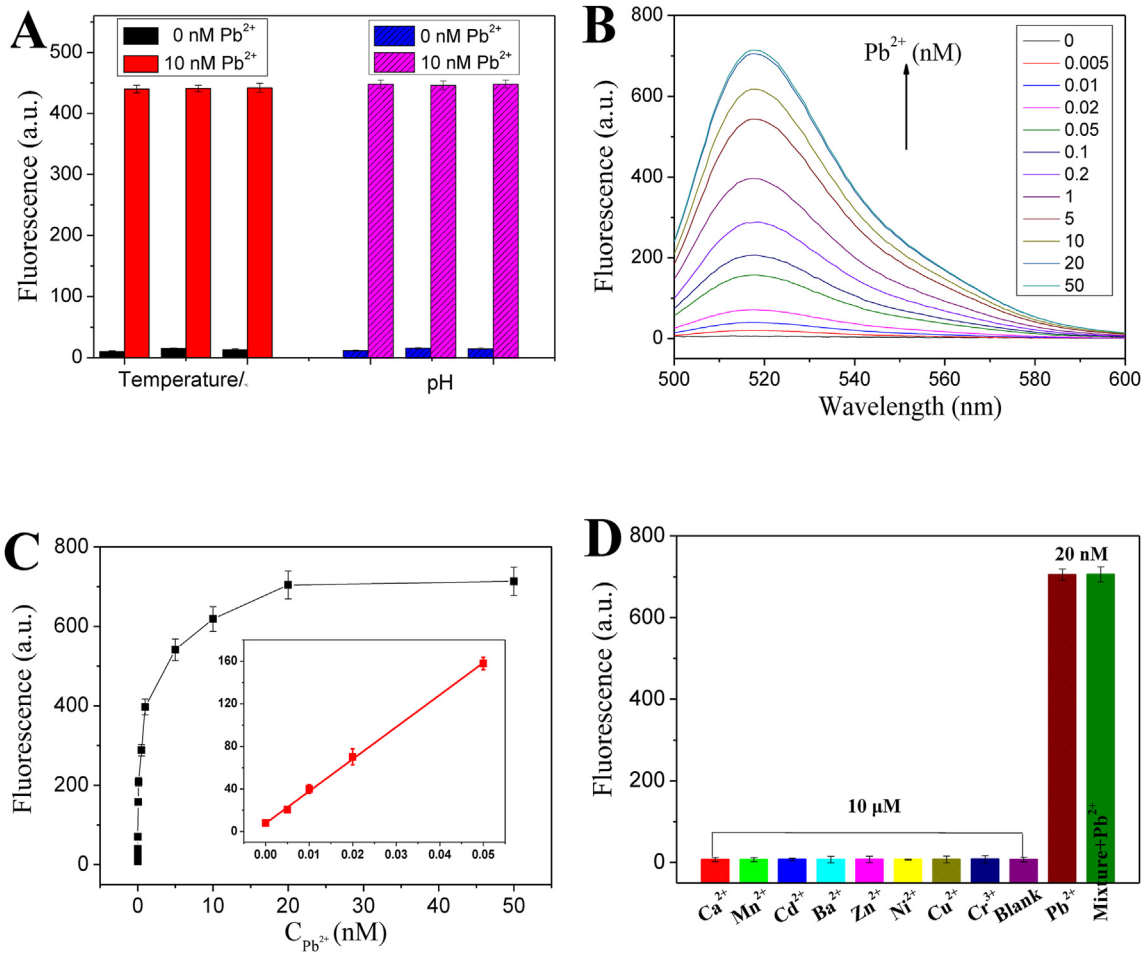


Fig. 3. Pb^{2+} biosensor based on circular GR5 and TdT/T7 Exo-aided amplification reaction. (A) Fluorescence signal at 517 nm of the sensing system in the absence or presence of Pb^{2+} under different temperature or pH. (B) Fluorescence spectra of the sensing systems containing different concentrations of Pb^{2+}. (C) Pb^{2+} concentration-dependent change in the fluorescence intensity at 517 nm. Insert demonstrate a relationship between the fluorescence intensity the concentration of Pb^{2+}. (D) Selectivity of the proposed Pb^{2+} colorimetric sensor. [Pb^{2+}] = 20 nM; the concentration of other metal ions is 10 μM. Error bars show the standard deviation of three experiments.

Table 1
Detection Pb^{2+} in real samples based on this sensing system.

Sample	[Pb^{2+}] added(nM)	[Pb^{2+}] found (nM)		ICP-MS (nM)	Recovery (%)
		Colorimetric sensor	Fluorescence sensor		
River water	0	0.36 ± 0.03	0.43 ± 0.04	ND	/
	50	51.23 ± 4.63	50.86 ± 3.61	50.63 ± 0.83	101.7–102.4
	100	101.56 ± 6.24	100.98 ± 3.48	101.21 ± 4.12	100.6–101.2
Cabbage	0	10.65 ± 0.03	11.34 ± 0.05	11.33 ± 0.04	/
	50	61.14 ± 4.63	62.44 ± 3.15	60.33 ± 0.48	101.0–102.2
	100	113.56 ± 7.67	112.98 ± 5.38	111.21 ± 3.12	101.6–103.0
Rice	0	15.6 ± 0.06	16.1 ± 0.08	16.33 ± 0.05	/
	50	66.28 ± 4.63	65.94 ± 6.56	66.43 ± 4.83	99.7–101.4
	100	114.91 ± 7.24	115.13 ± 5.38	116.08 ± 8.12	99.0–99.3

and fluorescence methods. These finds would have a positive effect on multifarious aspects: (1) self-template ligation of single-stranded DNA provides a novel method to prepare a more stable DNAzyme system; (2) since the circular DNAzyme can be prepared in large quantity and suitable for long-term storage, so it has a potential application in on-site detection of target in complex samples; (3) the completely closed feature of circular DNAzyme would effectively avoid nonspecific hybridization and extension, ensuring the sensing system with extremely low background signal; (4) Using the same design ideas, the techniques that can naturally be extended to prepare other functional DNAzyme by simple changing the enzyme sequence. Overall, we here indeed paved an easy and versatile way to prepare highly stable DNAzyme system, which demonstrated a broad prospect application in sensitive, portable and rapid on-site detection of target in complex samples.

CRediT authorship contribution statement

Hongxin Jiang: Conceptualization, Methodology, Investigation, Writing – original draft. **Pingping Ji:** Visualization, Investigation. **Yaping Xu:** Investigation, Validation. **Xiaowei Liu:** Supervision, Resources, Writing – review & editing. **Deming Kong:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2021.338733.

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