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A quencher-free DNAzyme beacon for fluorescently sensing uranyl ions via embedding 2-aminopurine

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ABSTRACT

DNAzyme-based fluorescent probes have provided valuable protocols for detecting uranium, one of the most common radioactive contaminants in the environment, with ultra-high selectivity and sensitivity. Designing novel DNAzyme beacons to update the mode of fluorescence reporting and/or quenching will continuously enhance "turn-on" sensing performance as well as promote actual application of the biological probes. In this work, we developed a novel quencher-free DNAzyme beacon by embedding fluorescent 2-aminopurine for rapid detection of uranyl ion. 2-aminopurine is able to substitute adenine and keep strong fluorescence in single-stranded DNA whereas being quenched in the hybridized double-stranded DNA by the base-stacking interaction. The combination of such trait of 2-aminopurine and cleavage reaction of DNAzyme in the presence of target co-factors possesses two main advantages for ion sensing: simplicity for avoidance of extra quencher groups and high performance because of superiority of DNAzyme essence. The experimental conditions including embedding site, pH and salt concentration of buffer solutions, and the amount ratio of enzyme strand to substrate strand used to form DNAzymes were systematically optimized to inspire the highest performance of the biological beacon. Thus, a detection limit of 9.6 nM, a wide linear range from 5 nM to 400 nM (R^2 = 0.997), and selectivity of more than 400 000-fold over other metal ions were achieved by the novel DNAzyme probes. The highly sensitive, selective and quencher-free DNAzyme probes accommodated a simple and cost-efficient alternative to current fluorescent counterparts, holding a great potential for further application in practical ion assay.

1. Introduction

Uranium has been one of the most common radioactive pollutants in the environment especially along with the development of uranium mining, nuclear power plants, and other medical or industrial application, which raises concerns about the nuclear contamination to the whole environment (Brugge and Buchner, 2011; Cantaluppi and Degetto, 2000; Xiao et al., 2017). In aqueous solution uranium mostly exists in the stable form of uranyl ion (UO_2^{2+}), which is highly bioavailable to pose risks to human health by disturbing function of organs such as skeleton, kidneys, lungs, and livers (Craft et al., 2004; Fisenne et al., 1988; Wilson et al., 2014). Although techniques based on radiospectrometry and mass spectrometry have been widely used to identify UO_2^{2+} with high precision, they are inevitably laborious and time-consuming (Faulques et al., 2015; Li et al., 2016). Nowadays for no matter environmental contamination treatment or earlier clinical diagnosis, sensors capable of qualitatively and quantitatively determining the toxic ions are intensively necessary to establish fast, simple, and accurate analysis, which has been promoting the development of various UO_2^{2+}-specific probes including inorganic acids (Harvey et al., 2018; Ye et al., 2017), organic compounds (Elabd and Attia, 2015; Jain et al., 2005; Shamsipur et al., 2015; Shu et al., 2015), biological beacons (Roozbahani et al., 2017; Zhu and Zhang, 2014) and the other sensing strategies (Chen et al., 2018; Dutta and Kumar 2016).

DNAzyme is one of the most outstanding metal ion probes, which was firstly reported in 1994 (Breaker and Joyce, 1994) and generally comprises two strands, substrate strand and enzyme strand. The catalytically active DNA is able to strongly and specifically bind a metal ion and then induce the cleavage of substrate strand, thereby providing a promising platform to facilitate different sensing mechanisms (Gong et al., 2015; Mei et al., 2003). Attracted by the interests of promoting DNAzyme into practical application in the field of ion detection, two aspects of attempts have been implemented in recent years: enriching the "probe base" so as to cover as many metal ions as possible and

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exploring novel sensing mechanisms to achieve as high detecting performance as possible. For the former, a series of ion-specific DNAzymes have been obtained via *in vitro* selection process in the presence of corresponding metal cofactors such as Pb^{2+} (Breaker and Joyce, 1994; Li and Lu, 2000), Cu^{2+} (Carmi et al., 1996), Zn^{2+} (Santoro et al., 2000), Hg^{2+} (Nolan and Lippard, 2003), Mg^{2+} (Cheng et al., 2014), Cd^{2+} (Huang and Liu, 2014), Co^{2+} (Bruesehoff et al., 2002; Mei et al., 2003), Mn^{2+} (Wang and Silverman, 2003), and UO_2^{2+} (Liu et al., 2007). For the latter, various sensing mechanisms on the basis of electrochemical (Xiao et al., 2007; Yun et al., 2016), colorimetric (Lee et al., 2008; Liu and Lu, 2004a), surface enhanced resonance spectroscopic (Moskovits, 2005) and fluorescent (Wernette et al., 2007; Xiao et al., 2015) designations have been proposed. Fueled by the progresses in these two aspects, several sensing methods of uranyl ion were established (Lee et al., 2008; Lin et al., 2011; Schlücker, 2014; Yun et al., 2016; Zhou et al. 2014, 2016), of which detection based on fluorescence "turning-on" was impressive for the simplicity and sensitivity. Despite the high performance, most DNAzyme-based probes in such sensing system to date need to label one fluorophore and at least one quencher at the 5′ and 3' ends of DNAzyme in order to fluorescently signal the catalytic cleavage reaction (Liu and Lu, 2007; Liu and Lu, 2003; 2004b). Actually, the labeling procedures not only increase the costs of probe preparation but also complicate the whole detection process, thus limiting the practical application of fluorescent DNAzymes on large scale (Xiao et al., 2015).

In this work, we introduced a novel quencher-free fluorescent DNAzyme beacon by embedding 2-aminopurine (2-AP) into designed site of substrate strand rather than labelling fluorophores at the ends of DNA structure. 2-AP is a fluorescent analogue of adenosine (A), which can pair with thymine (T) and form stacking in DNA duplex structure without affecting the stability of the genetic molecules (Somsen et al., 2005). Interestingly, 2-AP emits a strong fluorescent signal as a free deoxynucleotide or in single-stranded DNA, whereas its fluorescence can be significantly quenched in double-stranded DNA because of the base-stacking interaction (Mitsis and Kwagh, 1999; Rachofsky et al., 2001; Somsen et al., 2005). The combination of such trait of 2-AP and cleavage reaction of DNAzyme in the presence of UO_2^{2+} is highly consistent with fluorescent sensing of turn-on mechanism, meanwhile, avoiding labelling extra quenchers. Accordingly, we constructed a quencher-free UO_2^{2+} DNAzyme beacon by substituting an adenosine with 2-AP in the previously reported UO_2^{2+} sensor 39S/39E. As designed, the fluorescence was highly quenched with 2-AP embedded in duplex structure, while in the presence of UO_2^{2+} its fluorescence recovered upon the destruction of duplex structure (cleavage of DNAzyme), thereby achieving a quencher-free signal-on sensing process. In order to reach the highest sensing performance, we also optimized a series of experimental conditions including substitution sites of 2-AP, pH and NaCl concentration of buffer solutions, and the ratio between substrate strands and enzyme strands used to form DNAzymes. Subsequently, we tested the sensitivity and selectivity of the 2-AP-based DNAzyme beacon in the process of sensing UO_2^{2+}, achieving a detection limit of 9.6 nM, a wide linear range from 5 nM to 400 nM ($R^2 = 0.997$), and selectivity of more than 400 000-fold over other metal ions. These results indicate that the proposed sensing protocol for UO_2^{2+} can also realize simple, rapid and precise detection without expensive instruments or sophisticated procedures, and, to an extent, rival those traditional fluorophore-quencher-labelled DNAzyme. Impressively, this novel DNAzyme beacon is especially advantageous for the fluorescence quenching resulting from stacking interaction of 2-AP with adjacent bases rather than additional quenchers. Furthermore, considering that 2-AP is embedded in DNA sequences and consequently protected by adjacent bases, the probe is expected to be more resistant to interference. Therefore, the detection platform offers a significant potential for further and larger application in uranium detection and analysis.

2. Experimental section

2.1. Materials and reagents

All DNA samples were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China) and purified with HPLC. The pristine sequences of enzyme strand (39E) and substrate strand (39S) were listed as follows:

39S: 5′ ACTCACTATrAGGAAGAGATGGACGTGA 3';
39E: 5′ CACGTCCATCTCTGCAGTCGGGTAGTTAAACCGACCTTCAG ACATAGTGAGT 3'.

2.2. Procedures for ion sensing and optimization of detection conditions

2.2.1. Sensor preparation and uranyl ion detection

Typically, 50 μL of 50 μM 2-AP-based 39S (2-AP-39S) and 50 μL of 75 μM 39E were annealed in a buffer containing 300 mM NaCl and 50 mM MES at a pH of 5.5 by heating the mixture to 95 °C for 2 min and then cooling to 4 °C in 1 h. The resulting solution was diluted 20 fold by the same buffer. 400 μL of as-prepared sensor was transferred to a quartz cuvette with a 0.5 cm path length on each side and placed in a fluorometer (PTI QM-40; Horiba, Canada). The temperature of the fluorometer was set at 25 °C and all of detection in this work was at the same temperature. The excitation was set at 315 nm and emission at 365 nm. Kinetics upon addition of 40 μL 10 × UO_2^{2+} stock solution was monitored. In the analyzed solution, the initial 2-AP-39S was 2.5 μM, 39E was 3.75 μM.

2.2.2. Optimization of 2-AP embedding site

A series of DNAzyme with different embedding sites of 2-AP were synthesized and used to prepare the above sensors (Fig. 2a). In the detection procedure, 8 μM UO_2^{2+} stock solution was added to obtain UO_2^{2+} concentration of 800 nM in the sample, while the DNAzyme (of each site-based type) was kept as 2.5 μM in the buffer containing 50 mM MES and 300 mM NaCl at a pH of 5.5. And the DNAzymes were prepared through hybridization of enzyme strands and substrate strands with ratio of 1.5:1.

2.2.3. Optimization of buffer pH

A series of buffer of different pH were prepared as above and used in sensor preparation and subsequent detection. The pH used here included 5.5, 6.3, 7.0, 7.8, 8.5 (For pH of 7.0, 7.8 and 8.5, the buffer was prepared with 50 mM Tris acetate instead of MES). 8 μM UO_2^{2+} stock solution was added and UO_2^{2+} concentration in the sample was 800 nM, while the DNAzyme was kept as 2.5 μM. All of the buffers contained 300 mM NaCl and DNAzymes were prepared through hybridization of enzyme strands and substrate strands with ratio of 1.5:1.

2.2.4. Optimization of salt concentration of buffer

A series of buffer containing 50 mM, 100 mM, 300 mM, 800 mM and 1.5 M NaCl were prepared as above and used in sensor preparation and subsequent detection. 8 μM UO_2^{2+} stock solution was added and UO_2^{2+} concentration in the sample was 800 nM, while the DNAzyme was kept as 2.5 μM. All of the buffers were at pH of 5.5 and DNAzymes were prepared through hybridization of enzyme strands and substrate strands with ratio of 1.5:1.

2.2.5. Optimization of ratio of enzyme to substrate

A series of sensors of different enzyme to substrate ratios were prepared as above and used in subsequent detection. The enzyme to substrate ratios used here included 1:1, 1.2:1, 1.5:1, 1.7:1 and 2:1. 8 μM UO_2^{2+} stock solution was added and UO_2^{2+} concentration in the sample was 800 nM, while the DNAzyme (of each ratio-based type) was kept as 2.5 μM. All of the buffers were at pH of 5.5 and contained salt of 300 mM.

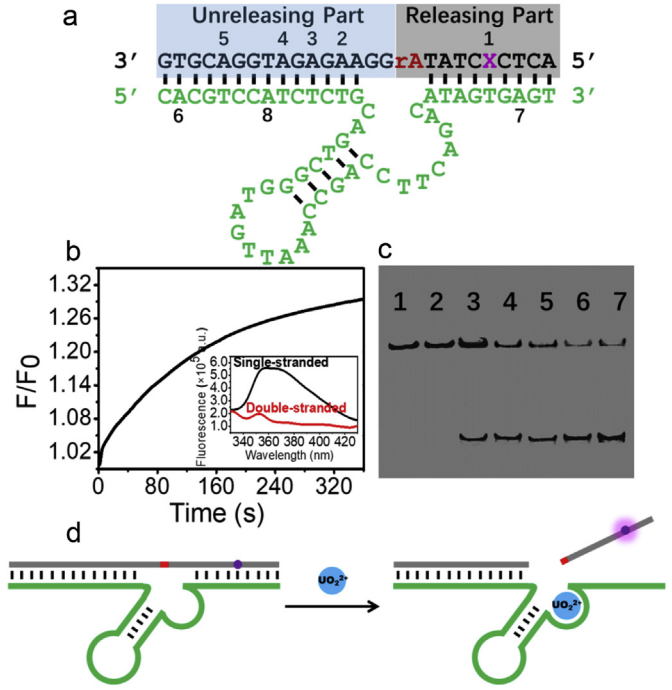

Fig. 1. a. The secondary structure of the 2-AP-embedded DNAzyme based on UO_2^{2+}-specific 39E/39S. rA and X denote ribo-adenosine (cleavage site) and 2-AP, respectively. The structure is also marked by 1 – 8 (eight designed sites used for embedding 2-AP), deep color (proposed releasing part) and light color (proposed unreleasing part). **b.** Fluorescence enhancement of 2-AP-based DNAzyme (2.5 μM) after addition of 800 nM UO_2^{2+}. **Inset:** fluorescence intensity of only substrate strands and hybridized DNAzymes with the same concentration (2.5 μM) at 365 nm (excited at 315 nm). **c.** PAGE gel electrophoresis analysis of the 2-AP DNAzyme. Lane 1, substrate oligonucleotides alone with 200 nM UO_2^{2+}; Lane 2, the 2-AP DNAzymes without UO_2^{2+}; Lane 3–7, 5 min, 10 min, 15 min, 30 min and 60 min, respectively, after 200 nM UO_2^{2+} addition to 2.5 μM 2-AP-based DNAzyme. **d.** Signal generation scheme of the UO_2^{2+} catalytic beacon based on embedding 2-AP. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.2.6. Sensitivity test

Sensors were prepared as above and the concentration were uniformly set as 2.5 μM under the optimized conditions. The fluorescence recovery was monitored in the presence of UO_2^{2+} with concentration as 2 μM, 800 nM, 400 nM, 300 nM, 200 nM, 100 nM, 80 nM, 40 nM, 15 nM, and 5 nM, respectively.

2.2.7. Selectivity test

Sensors were prepared as above and the concentration were uniformly set as 2.5 μM under the optimized conditions. In the subsequent detection, metal stock solutions were added. The metal ions used here included: Ca^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Mg^{2+}, VO^{2+}, Pb^{2+}.

2.2.8. Gel-based activity assay

2-AP-39S and 39E were annealed in a buffer containing 300 mM NaCl and 50 mM MES at a pH of 5.5. The 2-AP-39S was 2.5 μM and 39E was 3.75 μM. After taking a 5 μL aliquot out as a zero time point, UO_2^{2+} was added to a final concentration of 200 nM to the remaining solution, and aliquots were taken out at designated time points. All aliquots were quenched with a stop buffer containing 90% formamide, 1 mM EDTA and 50 mM Tris acetate at a pH of 8.5. A sample containing only the substrate and UO_2^{2+} was also prepared. The cleaved and uncleaved substrates were separated by 20% PAGE, and the gel was analyzed by a fluorescence imager (WD-9413B; Liuyi, Beijing) by exciting at 302 nm.

3. Results and discussion

3.1. Detection of UO_2^{2+} ions using 2-AP-embedded DNAzyme

As illustrated in Fig. 1a, a 2-AP-embedded DNAzyme was designed and prepared on the basis of uranyl-specific DNAzyme (39S/39E) structure, which also comprised two strands: substrate strand containing ribo-adenosine (rA) as cleavage site and 2-AP (X) as signal emitter, and enzyme strand including a highly conserved bulge structure as catalytic core. The feasibility of the whole sensing mechanism was basically determined by key factors related to fluorescence "off" and "on". For the turning-off, the quenching effect of 2-AP fluorescence (at 365 nm and excited at 315 nm in this work) through stacking interaction in the duplex DNA structure was verified by the comparison between fluorescence intensity of only substrate strands and that of hybridized DNAzyme of the same concentration (2.5 μM). Nearly 400% decrease of intensity in Fig. 1b ***Inset*** indicated that fluorescence "turning-off" effect was able to be achieved in this protocol, which was also consistent with the results of similar research elsewhere (Liao et al., 2016). In the following research we would optimize various experimental conditions related to the strength of the two complementary strands, which determined rigidity of the base-stacking interaction, to enhance the "off" effect. For the turning-on, the presence of co-factor UO_2^{2+} (800 nM) rendered substrate strand (in the buffer 50 mM MES, 300 mM NaCl, pH 5.5) being catalytically cleaved into two fragments and released (Fig. 1d), along with which the fluorescence intensity of the system gradually increased due to destruction of stacking interaction between 2-AP and adjacent bases. The kinetic curve shown in Fig. 1b reflected that fluorescence of the system would increase by a degree of 30% in 350 s, basically indicating a turn-on effect. Obviously, enhancement of the fluorescence intensity in our system was not remarkable as those in fluorophore-quencher-labelled counterparts. This might be limited by the feature of 2-AP as signal reporter embedded in DNA strands, which was also observed in a similar sensing research (Liao et al., 2016). The above results were further confirmed by PAGE gel electrophoresis analysis shown Fig. 1c: in the first lane containing

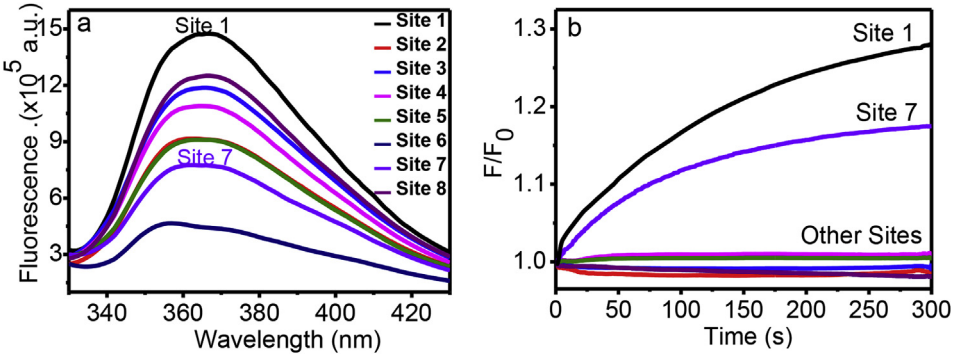

Fig. 2. Optimization of embedding site. **a.** Fluorescence intensity of substrate strands with 2-AP embedded in different sites at the same concentration (2.5 μM). **b.** Fluorescence enhancement of DNAzymes with 2-AP being embedded in different sites upon addition of 800 nM UO_2^{2+}. All of the DNAzymes was at the same concentration of 2.5 μM.

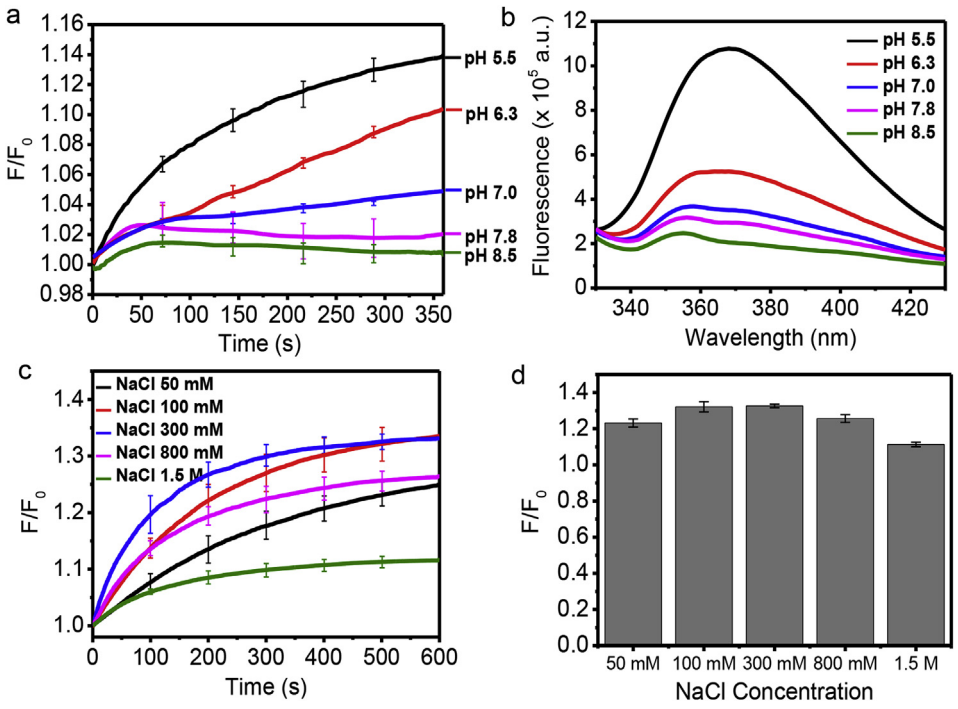

Fig. 3. Optimization of experimental conditions. Fluorescence kinetics of 2-AP-based DNAzyme (2.5 μM) in the presence of UO_2^{2+} (800 nM) in buffer solutions with **a.** pH range from 5.5 to 8.5 and **c.** NaCl concentration range from 50 mM to 1.5 μM. **b.** Fluorescence intensity of substrate oligonucleotides alone in buffer solutions with pH range from 5.5 to 8.5. **d.** Statistical results of five repeated experiments for each salt concentration to evaluate corresponding homogeneity and error bar of 300 mM was smallest.

only the substrate oligonucleotides and 200 nM UO_2^{2+}, no cleavage happened; for the 2-AP DNAzymes in the absence of UO_2^{2+} in the second lane, only the dissociated substrate strand was observed and no cleaved products were found; and upon addition of 200 nM UO_2^{2+} to the 2.5 μM 2-AP DNAzymes, a cleaved product band was observed and its intensity increased with time (lane 3–7 represent 5 min, 10 min, 15 min, 30 min and 60 min, respectively, after UO_2^{2+} addition to 2-AP-based DNAzyme). The gel-based assays indicated that the fluorescence enhancement was indeed a result of DNAzyme-catalyzed cleavage and consequently the embedding of 2-AP into the DNAzyme was feasible to detecting uranyl ions through signal turning on.

3.2. Optimization of embedding site

According to a number of researches previously (Rachofsky et al., 2001), the fluorescence of 2-AP is susceptible to its local environment when located into a oligonucleotide. It means that choosing an optimal embedding site in the skeleton of DNA for 2-AP to keep its fluorescence as high as possible after release is necessary for the fact that there are multiple adenosines in 39S/39E DNAzyme. Additionally, the designed site should also be able to form complete base-stacking interaction to ensure the quenching effect and keep far away from highly conserved catalytic core to facilitate the cleavage reaction. In the light of these principles, we selected eight sites (denoted as Site 1 to 8 in Fig. 1a) in both substrate and enzyme strands to embed 2-AP and corresponding fluorescence of each strand (2.5 μM) was shown in Fig. 2a, which obviously suggested that 2-AP in the Site 1 would generate strongest fluorescence signal. We also monitored the kinetics of fluorescence enhancement for different sites upon addition of UO_2^{2+} to further evaluate the choice of embedding position. The comparison of the eight kinetic curves demonstrated that Site 1 was still the best (Fig. 2b) and, in the meanwhile, provided a new approach to study the releasing mechanism of DNAzyme cleavage products. As shown in Fig. 1a, only when 2-AP was embedded into the 5' fragment of substrate (Site 1) or its complimentary part of enzyme strand (Site 7), an increase in fluorescence was observed. For other embedding sites, the kinetics seemed to be inhibited. That is, the catalytically cleaved substrate strand was consisted of one releasing part and another unreleasing part, the former of which would dissociate while the latter kept hybridized. The kinetics difference between Site 7 and Site 1 could attribute to two reasons: firstly, the ambient environment of Site 1 was much better to keep strong fluorescence of 2-AP, which had been demonstrated above; and secondly, the free part of enzyme strand after cleavage, as the extended sequence of catalytic core area, was much more flexible to form coil or crinkle states than complementary fragment of substrate strand, thus interfering signal emitting. In consideration of both the fluorescence intensity and the intrinsic releasing mechanism, we chose Site 1 on the substrate strand as the 2-AP embedding site.

3.3. Optimization of detection conditions

It has been addressed that factors related to fluorescence “off” and “on” of 2-AP embedded in the DNAzyme are definitive for the final sensing performance, which intrinsically concentrates into conditions that influence hybridizing strength of the two strands (2-AP-39S and 39E). Here all of the detection was conducted at 25 °C, a temperature favored by most practical applications. Generally, for a DNA with constant nucleotides and structure *in vitro* at room temperature, pH and salt concentration of buffer solution have predominant impact on the hybridizing strength (Tan and Chen, 2006, 2008). However, for our system the optimization is not facile due to two complex and even contrary facts: DNAzyme will be more stable in weak alkaline environment while both the stability of UO_2^{2+} and fluorescence of 2-AP embedded in oligonucleotide require acidity (Brown et al., 2009; Moulin et al., 1995); and high salt concentration will enhance hybridizing strength to facilitate fluorescence turning off whereas affect subsequent turning on by impeding the release of cleavage fragments. In addition, amount ratio of enzyme to substrate can also play a significant role in fluorescence turning-off process.

In order to obtain optimal pH, we monitored the fluorescence kinetics of 2-AP-based DNAzyme in the presence of UO_2^{2+} in buffer solutions with pH range from 5.5 to 8.5 (Fig. 3a). At pH values higher than 5.5, the reactivity of the sensing system decreased and almost vanished at pH above 7.8. It should attribute to the formation and domination of hydrolyzed uranyl species such as $UO_2(OH)^+$ in alkaline media, which was not in favor of catalysis (Brown et al., 2009; Moulin et al., 1995), and the fact that fluorescence of 2-AP embedded in oligonucleotide would decrease along with the increase of pH (Fig. 3b).

Buffers with pH below 5.5 were neglected due to the instability of DNA structure in such acidic environment, which can be deduced by the report that the 39S/39E DNAzyme was completely inactive at pH 3.5 (Brown et al., 2009). Similar operation was executed to optimize salt concentration of buffer solution. As shown in Fig. 3c ion detection was less efficient within buffer containing salt higher than 300 mM or lower than 100 mM, which, however, was due to distinct reason. Higher salt concentrations leading to the stronger base pair interaction restrained the release of cleavage products so as to suppress fluorescence recovery. At lower salt concentrations, DNAzyme, containing non-hybridized area, might be not capable to ensure enough stability of structure required by the catalytic reaction. For each salt concentration, we repeated same experiment 5 times to evaluate corresponding homogeneity as shown in Fig. 3d. The comparison of error bars derived from the statistical results suggested that the salt concentration of 300 mM was favored to keep stability and efficiency of DNAzyme probes. Interestingly, the optimal pH and salt concentration are identical with those of the *in vitro* selection that screened out this 39S/39E DNAzyme (Liu et al., 2007), further indicating that the substitution of 2-AP do not influence the catalytic property of DNAzyme.

Theoretically, substrate strand carrying 2-AP can be hybridized more completely to enhance the fluorescence "off" state in the presence of excessive enzyme strands. By varying the amount ratios of enzyme to substrate from 1:1 to 2:1 we actually observed the predicted trend through testing the fluorescence intensity (Fig. 4a), which nevertheless displayed another ranking regarding for sensing performance in the experiments of kinetics monitoring (Fig. 4b). Ratios of 1.2:1 and 1.5:1 would be prone to give the highest sensing efficiency while 1:1 and 1.7:1 the middle, and 2:1 the lowest. In fact excess amount of enzyme oligonucleotides will capture and bind uranyl ions to decrease the effective concentration, as observed in research conducted by Brown et al.(Brown et al., (2009), while insufficient amount may not give birth to enough DNAzymes, both of which can affect the sensing efficiency. We repeated same experiment for 5 times and the statistical analysis proposed ratio of 1.5:1 the best rather than 1.2:1 for the capability to ensure homogeneity of parallel sensing (Fig. 4c). In conclusion we finally compromise those contradictions through a series of attempt and obtained preferred conditions for our sensing system.

3.4. Sensitivity and selectivity of 2-AP-embedded DNAzyme probes

Under optimized conditions, we evaluated the sensitivity of the proposed DNAzyme beacon (2.5 μM) by monitoring the fluorescence recovery kinetics at varying UO_2^{2+} concentrations (Fig. 5a). We observed that higher UO_2^{2+} concentration would result in larger fluorescence enhancement with increasing rate, and that for each concentration, generally, the kinetics curve would experience rapid climbing firstly and then gradually slowing down to a plateau. To quantify UO_2^{2+}, the initial rates of fluorescence enhancement in the time window of 1–2 min were plotted as the function of UO_2^{2+} concentration (Fig. 5b), from which the apparent dissociation constant for the metal ion was calculated to be 339 nM that was better than that of quencher-needed DNAzyme sensors (Liu and Lu, 2007). This larger apparent dissociation constant suggested a weaker affinity of DNAzyme to UO_2^{2+} which might arise from competition of uranyl ion binding between excess enzyme strands and the nucleic beacons (Brown et al., 2009). Furthermore, a linear relationship (Fig. 5b ***Inset***) was observed over a wide range of 5 nM–400 nM, with a correlation equation of $y = 1.607 \times 10^{-4}x - 0.002$ ($R^2 = 0.997$). The detection limit was estimated to be 9.6 nM (3σ/slope), rivaling those achieved by the most sensitive analytical instruments (Abbasi, 1989; Boomer and Powell, 1987; Huff and Bowers, 1990). And the 130 nM limit of UO_2^{2+} in drinking water suggested by the World Health Organization is well within this sensor's linear detection range. What should be noted is that in order to exclude the aberrancy of the rates extracted in only one test we repeated same experiments for each concentration of UO_2^{2+} for 5 times (Figs. 5c and 6a–i) and corresponding slopes calculated statistically were used to establish subsequent analysis. To test the selectivity of this sensor, the fluorescence enhancement upon addition of 11 competing metal ions at different concentrations was monitored. The corresponding rates of fluorescence enhancement were calculated to be compared with that of UO_2^{2+} (Fig. 5d). At all three concentrations (100 μM, 1 mM, 10 mM), none of the metals exhibited a response higher than that of 40 nM UO_2^{2+}. This result indicated that the proposed sensor had over 400 000-fold selectivity over other competing metal ions.

4. Conclusions

In conclusion, we proposed a novel quencher-free DNAzyme beacon based on embedding 2-aminopurine into a designated site of substrate strand for fluorescent detection of uranyl ions. The intrinsic fluorescence of 2-AP can be quenched by the base-pair interaction in DNA duplex structure and recover along with the catalytic cleavage reaction, which provides a facile signal turn-on protocol for ion sensing without extra quencher groups and thereby significantly simplifying the probe preparation and subsequent detection. In order to enhance the performance of such DNAzyme beacons we systematically optimized a series of experimental conditions including embedding site, pH and NaCl concentration of buffer, and ratio of enzyme strand to substrate strand. Under the optimized conditions a promising quencher-free DNAzyme probe of uranyl ion with a detection limit of 9.6 nM, a wide linear range from 5 nM to 400 nM ($R^2 = 0.997$), and selectivity of more than 400, 000-fold over other metal ions was obtained. Moreover, due to the steric protection from adjacent bases of 2-AP, this probe is expected to be more rigid against interference. Therefore, the probe holds a great potential for practical application for the capability of detecting its specific metal ions through rapid catalytic cleavage reaction with high sensitivity, outstanding selectivity and remarkable capability of real-time monitoring. Obviously, there still be huge space for the 2-AP-based

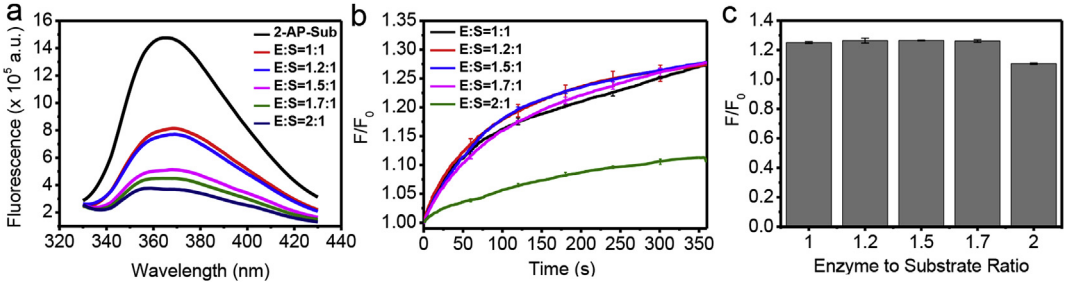

Fig. 4. Optimization of amount ratio of enzyme to substrate. **a.** Fluorescence intensity of substrate oligonucleotides alone (2.5 μM) and DNAzymes prepared through hybridization with different ratios of enzyme strands and substrate strands. **b.** Fluorescence kinetics of DNAzymes (2.5 μM) with different enzyme-to-substrate ratios upon the addition of UO_2^{2+} (800 nM). **c.** Statistical results of five repeated experiments for each ratio to evaluate corresponding homogeneity and error bar of ratio value of 1.5 was smallest.

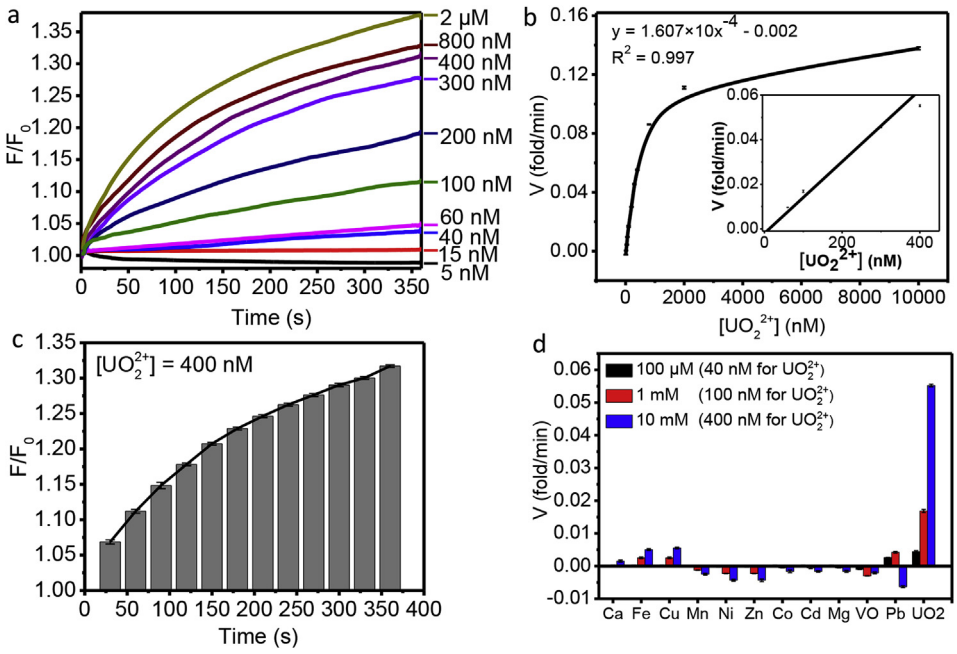

Fig. 5. Sensitivity and selectivity of 2-AP-embedded DNAzyme probes. **a.** Fluorescence recovery kinetics at varying UO_2^{2+} concentrations. **b.** Initial rates of fluorescence enhancement in the time window of 1–2 min plotted as the function of UO_2^{2+} concentration. Inset: The linear relationship over a wide range of 5 nM–400 nM, with a correlation equation of $y = 1.60723 \times 10^{-4}x - 0.00201$ ($R^2 = 0.997$), from which the detection limit was estimated to be 9.6 nM (3σ/slope). **c.** Statistical results collected from five same experiment of fluorescence enhancement of 2-AP-embedded DNAzyme (2.5 μM) in the presence of 400 nM UO_2^{2+}. **d.** Statistical rates of fluorescence enhancement of 2-AP-embedded DNAzymes in the presence of 11 competing metal ions with concentrations of 100 μM, 1 mM, and 10 mM, respectively, to characterize the selectivity of this sensor.

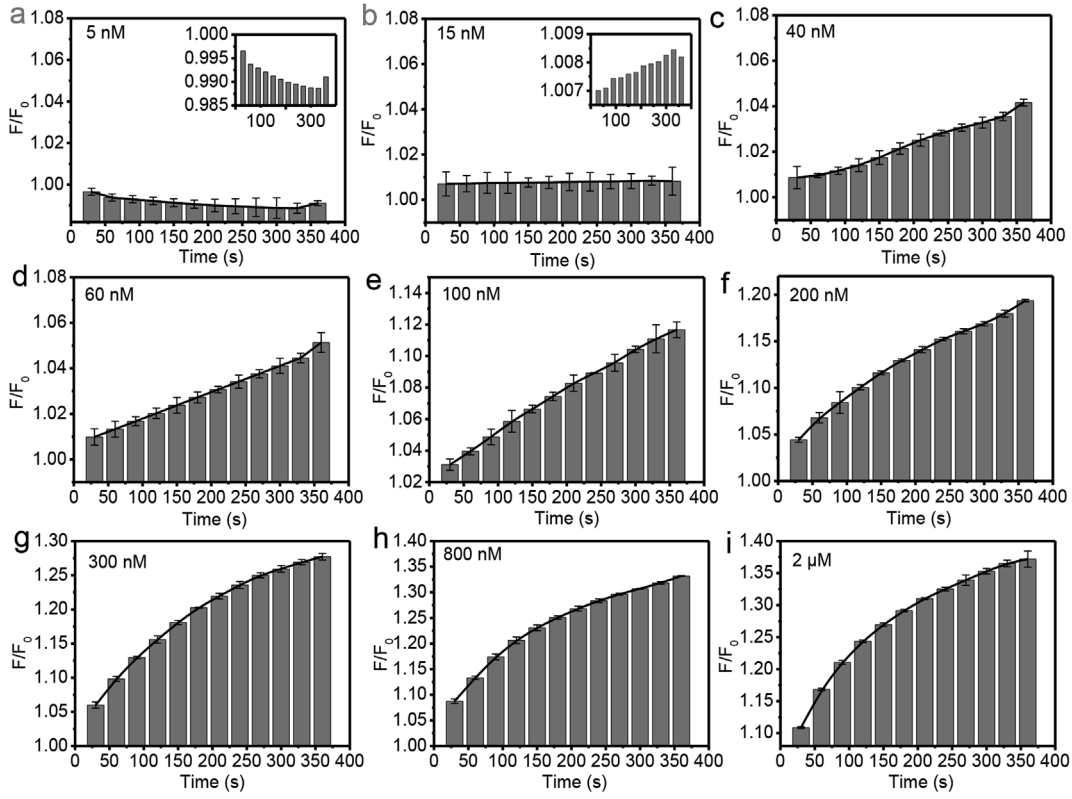

Fig. 6. Statistical results collected from five same experiment of fluorescence enhancement of 2-AP-embedded DNAzyme (2.5 μM) in the presence of UO_2^{2+} at various concentrations. a. 5 nM (Inset: fluorescence intensity decreased because of enlarged solution volume in the case of the amount of UO_2^{2+} beyond the detection limit), b. 15 nM (Inset: initial rates of fluorescence enhancement in the time window of 0–350 s), c. 40 nM, d. 60 nM, e. 100 nM, f. 200 nM, g. 300 nM, h. 800 nM, i. 2 μM.

DNAzyme beacons to progress compared with traditional probes especially in aspect of fluorescence enhancement, which could probably be limited by the feature of 2-AP as signal reporter embedded in DNA strands. However, we definitely believe that it can be promoted through further attempts toward decreasing background fluorescence such as optimizing sequence of non-catalytic area and updating the protocol of 2-AP embedding. Continuous development of this quencher-free DNAzyme probe based on 2-AP will allow for a wider application in on-site and real-time environmental monitoring for uranium in a more facile and cost-efficient way.

Declaration of interest statement

The authors (Xiaolong Wang, Rui Zeng, Shengnan Chu, Wei Tang,

Na Lin, Jun Fu, Jiangrong Yang, Bo Gao) of manuscript "*A Quencher-Free DNAzyme Beacon for Fluorescently Sensing Uranyl Ions via Embedding 2-Aminopurine*" declare no competing financial interests.

CRediT authorship contribution statement

Xiaolong Wang: Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Methodology, Supervision, Project administration. **Rui Zeng:** Data curation, Investigation, Writing - original draft, Methodology. **Shengnan Chu:** Investigation. **Wei Tang:** Resources, Validation. **Na Lin:** Resources, Validation. **Jun Fu:** Funding acquisition, Project administration. **Jiangrong Yang:** Funding acquisition. **Bo Gao:** Funding acquisition.

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