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Short communication

## Potentiometric aptasensing based on target-induced conformational switch of a DNA probe using a polymeric membrane silver ion-selective electrode



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## ABSTRACT

In this article, we introduce a general, sensitive, facile, and label-free potentiometric assay based on metal-mediated DNA base pairs. A nucleic acid with one adenosine-5'-triphosphate (ATP) binding sequence (aptamer) in the middle and two cytosine(C)-rich sequences at the lateral portions was employed as a model. A rigid hairpin structure can be formed in the presence of  $Ag^+$  ions, in which the C residues of the spatially separated nucleotides are linked by the ions. The strong interaction between Ag<sup>+</sup> ions and cytosines forms a stable C-Ag<sup>+</sup>-C structure, which could reduce the concentration of silver ions released from the polymeric membrane silver ion-selective electrode (ISE) at the samplemembrane interface and decrease the potential response. In the presence of its target, the aptamer (the loop sequence of the probe) binds specifically to the target via reaction incubation. Such target-binding induced aptamer conformational change prevents the formation of C-Ag<sup>+</sup>-C structure, leaving more silver ions at the sample-membrane interface, which can be detected by the silver ISE. ATP can be quantified in the range of 0.5-3.0 µM with a detection limit of 0.37 µM. The relative standard deviation for 5  $\mu$ M ATP is 5.5%. For the proposed method, the combination of using ion fluxes of silver ions as modulating reagents and as signal reporters greatly simplifies the detection procedures. In addition, by changing the binding sequence in the middle of the probe, the present detection method will be able to explore new applications of ISE for the detection of a large variety of targets.

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## 1. Introduction

There has been significant interest in the development of metal–DNA base pairs for electron transfer, DNA-based nanostructures and sensing applications (Clever et al., 2007; Okamoto et al., 2009). Some metal ions can selectively bind to native or artificial bases in DNA duplexes to form metal-mediated base pairs (Yang et al., 2009a, 2009b). Among these metals,  $Ag^+$  is capable of selectively coordinating cytosine (C) bases and forms a stable C–Ag<sup>+</sup>–C complex, while Hg<sup>2+</sup> is shown to specifically bridge thymine (T) bases (Ono et al., 2011, 2008; Miyake et al., 2006). By using silver or mercury ions mediated conformational changes of DNA probes, highly selective and sensitive sensors for metal ions (Ueyama et al., 2002; Nagatoishi, et al., 2005; Ono and Togashi, 2004; Cai et al., 2011), amino acids (Lee et al., 2008), glutathione (Xu and Hepel, 2011), adenosine triphosphate (Wang et al., 2010), DNA (Wang et al., 2009; Yang, et al., 2009a, 2009b), logic gates (Li et al., 2011; Xie et al., 2012) and redox environments have been developed (Miyake and Ono, 2005). However, these systems need additional signal reporters such as AuNPs (Kanayama et al., 2011), AgNPs (Li et al., 2009), quantum dots (Freeman et al., 2009), DNAzyme substrates (Li et al., 2009), redox tags or indicators (Yan et al., 2012; Wu et al., 2010), electrochemiluminescence intercalators (Tang et al., 2010), and fluorescence-quencher pairs (Wen et al., 2010) to transduce the conformational change of a DNA probe upon recognition of a given target, thus adding more complexity, cost and overall assay time. Very recently, biosensors based on a liquid crystal and a resonant oscillator have been reported for  $Hg^{2+}$  and  $Ag^+$ , respectively (Yang et al., 2013; Park et al., 2013), which allow the measurements to be done in a label-free way. Herein, we introduce a general, sensitive, facile, and label-free potentiometric assay based on metal-mediated DNA base pairs. In this method, silver ions not only specifically interact with the C-C mismatch in the DNA probe but also serve as a transducer, thus eliminating the addition of other signal reporters.



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Ion fluxes across polymeric membranes have been extensively investigated for large improvement of lower detection limits of polymeric membrane ion-selective electrodes (ISEs) (Sokalski et al., 1997) and for the development of new potentiometric sensing modes (Shvarev and Bakker, 2003; Bhakthavatsalam et al., 2006). Currently, the applications of ISEs have evolved to provide a promising transducer for biosensing (Chumbimuni-Torres et al., 2006; Wu et al., 2009). Recently, we have developed a promising detection configuration that makes use of outward ion fluxes through ISE membranes, i.e. the fluxes in the direction of sample solution, to provide controlled-release of substrates for in situ potentiometric biosensing of enzymes and their inhibitors (Ding and Oin, 2009a, 2009b). In the present work, ion-fluxes released from the inner solution of a polymeric membrane silver ISE are used to generate silver ions at the sample-membrane interface, which could transduce the aptamer-target binding events into potentiometric signals. To this end, a nucleic acid containing an aptamer (a specific target binding sequence) in the middle and a cytosine (C)-rich sequence at either of the lateral portions was designed. Scheme 1 illustrates the mechanism of the proposed sensing protocol. A rigid hairpin structure can be formed in the presence of Ag<sup>+</sup> ions, in which the C residues of the spatially separated nucleotides are linked by the ions. The strong interaction between Ag<sup>+</sup> ions and cytosine forms a stable  $C-Ag^+-C$  structure, which could reduce the concentration of silver ions released at the sample-membrane interface and thus decrease the potential response of the polymeric membrane silver ISE (Scheme 1A). In the presence of its target, the aptamer (the loop sequence of the probe) binds specifically to the target via reaction incubation. Such a target binding-induced aptamer conformational change prevents the formation of the C-Ag<sup>+</sup>-C structure, thus leaving more silver ions at the samplemembrane interface, which can be potentiometrically measured by the silver ISE (Scheme 1B). For the proposed method, the combination of using ion fluxes of silver ions as modulating reagents and as signal reporters greatly simplifies the detection procedures.

### 2. Materials and methods

#### 2.1. Membrane preparation

In this work, *o*-xylene-bis-(N,N-diisobutyldithio carbamate) was used as an ionophore for the present electrode (Szigeti et al., 2006). The silver ion-selective membranes contained 0.47 wt% sodium tetrakis[3,5 bis(trifluoromethyl)phenyl]borate, 0.53 wt% ionophore, 56.0 wt% 2-nitrophenyl octyl ether and 43.0 wt% high molecular weight poly(vinyl chloride). The preparation details are available in the Supporting Information.

## 2.2. EMF measurements

All the measurements of potentiometric detections were carried out at  $20 \pm 2$  °C using a CHI 760C electrochemical



**Scheme 1.** Schematic diagram of potentiometric aptasensing based on the ion fluxes of a silver ion-selective electrode.

workstation (Shanghai Chenhua Apparatus Corporation, China) with an ion-selective electrode (ISE) and a Hg/Hg<sub>2</sub>Cl<sub>2</sub> with double junction reference electrode. Measurements of EMF were performed with stirring in the galvanic cell:SCE/1.0 M LiOAc/sample solution/ISE membrane/inner solution/AgCl/Ag.

#### 2.3. ATP detection

In this work, 0.18  $\mu$ M of the probe was chosen as a compromise between the response range and sensitivity. For ATP determination, the ISE potential was first measured in 3-(N-morpholino) propane sulfonate (MOPS) buffer of pH 7.0 containing 0.18  $\mu$ M DNA to obtain a baseline. Then, 0.18  $\mu$ M of the probe was mixed with ATP at various concentrations in MOPS buffer. After incubation at room temperature for 30 min, the electrode was in contact with the solution to obtain the response. The potential difference between the baseline and the potential measured at 8 min after sample addition was used for the quantification of ATP.

### 3. Results and discussion

For the proposed strategy, the design of the DNA probe is essential. One basic requirement is that the target–aptamer binding constant should be larger than the association constant between Ag<sup>+</sup> ions and the mismatched C bases in the probe (Wang et al., 2010). In this case, the envisaged target can unfold the C–Ag<sup>+</sup>–C structure, thus causing a conformational switch of the DNA probe, which can be monitored by the change in the ISE membrane potential. Herein, a nucleic acid with an adenosine–5′triphosphate (ATP) binding sequence in the middle, 3 cytosine bases at either of the lateral portions and a poly T linker connecting these two parts was employed as a model (Wang et al., 2010). The probe sequence is as follows: 5′–CACCTGGGG-GAGTATTGCGGAG GAAGGT(T)<sub>31</sub>CACCTC-3′, for which a 27-mer aptamer with a binding constant of  $1.67 \times 10^5$  M<sup>-1</sup> for ATP is included (Nutiu and Li, 2003).

To confirm the rationality of the target-specific oligonucleotide probe, real-time potential responses of the silver ISE upon additions of the probe and its target molecule were investigated. Further information about the electrode and its characteristics is available in the supporting information. The activity of Ag<sup>+</sup> released at the membrane surface was 0.51 (  $\pm$  0.02)  $\mu$ M obtained by calibrating with a series of Ag<sup>+</sup> solutions at higher concentrations (see Fig. S1 in the Supporting Information). For comparison, a 65 mer DNA without mismatched C bases was chosen as a control probe. The probe sequence is as follows: 5'-TATTTGGGG-GAGTATTGCGGA GGAAGGT(T)<sub>31</sub>CATTTT-3'. As shown in Fig. 1A, the ISE potential response decreases rapidly with the addition of the designed ATP probe, indicating the efficient interaction between Ag<sup>+</sup> ions and the nucleic acid probe. The consumption of the silver ions released at the membrane surface is mainly caused by the strong interaction between Ag<sup>+</sup> ions and C-C mismatches in the DNA probe.

Previous reports have shown that the ATP-aptamer binds specifically to the target molecule via reaction incubation, which induces a change in the aptamer conformation and rigidity to the folded quadruplex structure (Ding et al., 2012). In the presence of ATP, the binding of ATP by the loop sequence of probe unfolds the  $C-Ag^+-C$  structure through the formation of a quadruplex structure. Therefore, more free silver ions are generated which can be detected potentiometrically via the silver ISE. As can be seen from Fig. 1A, the potential tends to increase gradually in the presence of ATP. The observed response is related to the reaction between ATP and the binding sequence in the probe, which results in the



**Fig. 1.** Potentiometric responses of the ISE for successive additions of (A) 0.18  $\mu$ M DNA probe and 10  $\mu$ M ATP and (B) 0.18  $\mu$ M control probe and 10  $\mu$ M ATP. Inset: potential responses of the ISE for successive additions of (A) 0.18  $\mu$ M DNA probe and 10  $\mu$ M CTP, UTP, or GTP.

release of silver ions from the probe. In addition, the potential increase is caused exclusively by ATP when compared to other nucleoside triphosphates such as cytidine-5'-triphosphate (CTP), guanosine-5'-triphosphate (GTP) and uridine-5'-triphosphate (UTP) (see inset in Fig. 1A).

Notably, a potential decrease was also observed for the control probe (Fig. 1B). The potential decrease of the control probe can be attributed to the coordinating interaction of the DNA bases with the Ag<sup>+</sup> ions (Zinchenko et al., 2008). In addition, the control probe contains one C base so that Ag<sup>+</sup> could directly bind to the C-C mismatched base pairs in the heteroduplexes. Indeed, a similar phenomenon was observed for thermodynamic analysis of the specific interaction between the C-C mismatch base pairs and Ag<sup>+</sup> (Torigoe et al., 2005). As compared to the response with DNA probe, a much smaller potential decrease can be obtained with the control probe. This is probably due to the strong interaction between Ag<sup>+</sup> ions and C–C mismatch in the DNA probe, thus causing rapid potential responses. Moreover, ATP binding-induced conformational change of the control probe could not change binding sites for Ag<sup>+</sup> in nucleic acids. Indeed, the recorded potential of the electrode will not increase (Fig. 1B). Thus, the mismatched C bases at the lateral portions, which render the formation of a folded structure in the presence of Ag<sup>+</sup>, match with our rational design of the probe and facilitate the aptamer–target binding readout for potentiometric aptasensing assays. It should be noted that the cytosine contents at the lateral portions and the length of the linker in the sequence determine the stem stability of the probe in the presence of Ag<sup>+</sup>. Therefore, the method has the flexibility of modulating the association constant between Ag<sup>+</sup> and mismatched C bases in the probe by alteration of cytosine contents at the lateral portions and the length of linker. Besides, the binding sequence in the middle of the probe can also be designed by using different aptamers for detection of a large variety of targets.

As illustrated above, the target-induced release of silver ions from the probe can be used for real-time potentiometric assays. However, the potential response is rather small probably due to the poor reversibility of the electrode. The silver ions released at the sample–membrane interface are largely consumed by the efficient interaction with DNA in the sample solution, which could prolong the recovery time of the electrode. Indeed, our previous report has shown that the recovery time of the electrode is rather long and the reversibility of the electrode was poor for high concentration measurements based on the zero-current release system (Guo et al., 2008). For subsequent experiments, ATP was



**Fig. 2.** (A) Potentiometric responses of the ion-selective electrode to 0  $\mu$ M DNA probe (a) and 0.18  $\mu$ M DNA probe in the presence of 5  $\mu$ M (b), 2.3  $\mu$ M (c), 1.5  $\mu$ M (d), 1.0  $\mu$ M (e), 0.5  $\mu$ M (f), and 0  $\mu$ M (g) ATP in 0.01 M MOPS. (B) Calibration curve of the ISE for detection of ATP. Error bars represent one standard deviation for three measurements.

first incubated with the probe in MOPS buffer at room temperature for 30 min. The electrode was then in contact with the solution to obtain the response. In the presence of ATP, the ATP binding-induced conformational change of the probe can prevent the formation of stable  $C-Ag^+-C$  structures, thus increasing the potential response as monitored by the silver ISE.

As shown in Fig. 2, with increase in the ATP concentration, the potential gradually increased, reaching a plateau when the concentration of ATP exceeded 10 µM. Based on the potential difference between the baseline and the potential measured at 8 min after sample addition. ATP can be quantified in the range of 0.5-3.0  $\mu$ M with a detection limit of 0.37  $\mu$ M (3 $\sigma$ ) (Fig. 2B). Compared with other methods for ATP detection, the proposed potentiometric method is comparable to or better than other aptamer based detection methods (Zuo et al., 2009; Yoshizumi et al., 2008). Experiments also revealed that ATP binding-induced conformational change of the control probe could not lead to an obvious potential change between the baseline and the potential after sample addition (see Fig. S2 in the Supporting Information). This is probably due to the target induced conformational change which could not change binding sites for Ag<sup>+</sup> in nucleic acids. Assuming the interaction process between ATP and the aptamer meets the requirements of the Langmuir isotherm for a fixed concentration of aptamer (Sun et al., 2010), a binding constant of  $4.5 \pm 1.4 \times 10^5$  M could be obtained by recasting the data of Fig. 2B (see Fig. S3 in the Supporting Information). This value is in good agreement with those reported before (Wang et al., 2005). It should be noted that the assay sensitivity and dynamic range can be adjusted by altering the probe concentration or silver ion fluxes according to methods mentioned before (Ding and Qin, 2009b).

## 4. Conclusions

In summary, we have developed a general, facile, and labelfree potentiometic aptasensing assay based on metal-mediated DNA base pairs. This novel assay is simple in design, avoiding the addition of other signal reporters. In addition, by changing the binding sequence in the middle of the probe, the present detection method will be able to explore new applications of ISE for the detection of a large variety of targets. For practical applications, the interferences present in complex matrixes such as plasma, urine, and environmental samples can be eliminated by using probe-modified magnetic nanoparticles.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.01.052.

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