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Label-free Catalytic and Molecular Beacon Containing an Abasic Site for Sensitive Fluorescent Detection of Small Inorganic and Organic Molecules

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ABSTRACT

In this work, two methods with complementary features, catalytic and molecular beacon (CAMB) and label-free fluorescent sensors using abasic site, have been combined into new label-free CAMB sensors that possess advantages of each method. The label-free method using dSpacer-containing molecular beacon makes CAMB more cost-effective and less interfering to the catalytic activity, while the CAMB allows the label-free method to use true catalytic turnovers for signal amplifications, resulting in a new label-free CAMB sensor for Pb^{2+} ion, with a detection limit of 3.8 nM while maintaining the same selectivity. Furthermore, by using CAMB to overcome the label-free method's limitation of the requiring excess enzyme strand, a new label-free CAMB sensor using aptazyme is also designed to detect adenosine down to 1.4 μM, with excellent selectivity over other nucleosides.

INTRODUCTION

Metal ions and small organic molecules can be beneficial or toxic in biology and environment and such effects depend highly on the identity and quantity of the metal ions and small organic molecules. For example, cobalt is a known essential element in biology that is part of vitamin B_{12}. At high concentration, however, cobalt can be a carcinogen.[1] Therefore, sensors that can detect and quantify these metal ion and organic molecule targets have been developed for various applications.[2-6] In comparison with instrument analysis such as inductively coupled plasma (ICP) and mass spectrum, these sensors have the advantage that no complicated instrument or sample pretreatment is needed. Until recently antibody-based assays are a major methodology that is general enough for the development of sensors for many targets. However, antibodies have been shown to be not very effective in detecting metal ions and small molecules and in applications under non-physiological conditions. To overcome this challenge, sensors based on catalytic nucleic acids (ribozyme and DNAzymes)[7,8], aptamers[9] and aptazymes (a combination of aptamers and catalytic nucliecaicds),[10] collectively called functional nucleic acids (FNAs), are emerging as an alternative method to antibodies for sensing small molecules. These FNAs were obtained through in vitro selection[7,8] or systematic evolution of ligands by exponential enrichment (SELEX)[9,11] from random nucleic acid libraries containing 10^{14} or more sequences. Because in principle any molecule or metal ion can be introduced as the target into the selection process to yield FNAs that are specific to the target, FNAs are considered as a general platform for the development of sensors for a broad range of targets, including metal ions, organic molecules, proteins, nucleic acids, and even cells and viruses.[7-9,11-24] Therefore, by transforming the recognition event between the FNAs and their targets into physically detectable signals,[17,23,24] many FNA sensors have been successfully developed, such as colorimetric,[25-33] fluorescent,[13,19,26,34-38] electrochemical[39-41] and magnetic sensors[42-44]. Among them, fluorescent FNA sensors are particularly

interesting and have been intensely researched because of their simple instrumentation, facile operation, high sensitivity and visible signals.

Among the fluorescent FNA sensors, catalytic beacons have been successfully used as a general platform for the development of sensors for a broad range of metal ions, such as Pb^{2+},[8,13] Mg^{2+},[45] Zn^{2+},[21] Co^{2+},[15,46] UO_2^{2+},[19] and Cu^{2+} [47-49]. The catalytic beacon normally consists of a quencher-labeled DNAzyme (or ribozyme) hybridizing with a fluorophore-labeled substrate of the DNAzyme, thus the DNAzyme has both the roles of quencher and catalyst. Upon the addition of a specific metal ion as the cofactor of the DNAzyme, the cleavage of the substrate catalyzed by the DNAzyme will perturb DNA hybridization and dramatically increase the distance between the quencher and fluorophore, resulting in the enhancement of fluorescence signal. However, to ensure low background fluorescence before the target-induced cleavage of substrate, the quencher-labeled DNAzyme is usually added in excess to minimize the free fluorophore-labeled substrate in solution because the quenching of fluorophore is via the DNA duplex formation between the DNAzyme and its substrate. Therefore, the enzymatic reaction is usually single turnover. Although multiple turnover catalytic reaction of DNAzymes can improve the sensor's sensitivity via signal amplification as protein enzymes or a DNAzyme with peroxidase activity,[50-56] it is quite challenging for the catalytic beacons,[13,19,49,57] either the first[13] or the second generation,[19,49,57] to realize such amplification without sacrificing low background fluorescence. Recently, an improved sensor design called catalytic and molecular beacons (CAMBs)[58] was developed by Zhang et al. through the combination of both catalytic beacons[13,19,49,57] and molecular beacons (MBs).[59,60] In their design, the substrate of the DNAzyme was actually a MB, and the DNAzyme no longer had to take the role of quencher. Therefore, with high quenching efficiency maintained by the substrate itself, the concentration of DNAzyme used could be lower than that of the substrate, and signal amplification via multiple turnover reactions was achieved in this way. In addition, the CAMB design could also facilitate the development of aptazyme sensors based on catalytic beacons for the sensitive

detection of a broader range of targets other than metal ions.

Despite of the advantages, CAMB, catalytic beacon, and most other fluorescent FNA sensor designs generally require a fluorophore and a quencher labeled at 5' and 3' ends of the FNAs to show a fluorescence switch upon the interaction between the sensors and their targets. The covalent labeling is not only expensive and complicated but also possible to reduce the activity of the FNAs.[61,62] In contrast, label-free approaches that utilize unmodified nucleic acids are more cost-effective and can better preserve the FNAs' activity. Although intercalating dyes have been used as the external fluorophores of label-free fluorescent FNA sensors for the detections of different targets,[63-65] the intercalation of the fluorophores to the FNA duplex is random and the binding site can hardly be controlled, which may hinder rational design of the FNA sensors. Some other techniques, such as the introduction of an additional aptamer that can bind external fluorophores,[66,67] have been developed for the controlled binding of fluorophores to FNAs, but the conjugation of the aptamers to FNA sensors may also make the rational design difficult. Previously, Teramae's group has successfully developed a Spacer C3-based approach for the label-free detection of organic molecules by fluorescent aptamer sensors.[68,69] We have also developed a general methodology for the design of label-free fluorescent FNA sensors for a broad range of targets, such as metal ions and organic molecules, by using either a dSpacer (an abasic site containing a tetrahydrofuranyl residue) or a vacant site in the FNAs.[70,71] The external fluorophore binds specifically to the dSpacer or vacant site, and it is straightforward to introduce the dSpacer or vacant site to the FNA sensors. Despite these advantages, these label-free methods have not taken advantage of the catalytic turnover for signal amplification to improve sensitivity. The need to use excess enzyme strand to ensure DNA hybridization also prevents its application for even broader range of targets.

Interestingly, we note that the above label-free method and the CAMB method complement each other very well, as the disadvantages of the label-free method is exactly the advantages of the CAMB method, while advantages of label-free method can help overcome the limitation of labeled CAMB

method reported before. Therefore, in this work, we take the advantages of both the CAMB sensor design[58] and the label-free approach using a dSpacer,[70] and report the development of label-free fluorescent CAMB sensors for the detection of metal ions (Pb^{2+}) and organic molecules (adenosine) with high sensitivity and selectivity.

MATERIALS AND METHODS

The fluorophore 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) was purchased from Ryan Scientific Inc. (Mt. Pleasant, SC). Lead acetate, human serum, and all other chemicals for buffer were commercially available from Sigma-Aldrich Chemical Co. (St. Louis, MO) and were used without further purification. All solutions were prepared by Milli-Q water (resistance >18 MΩ·cm). The oligonucleotides used in this work were custom synthesized by Integrated DNA Technology Inc. (Coralville, IA) with the sequences as follows:

Pb^{2+}-Dependent DNAzymes

17E (5+5) 5′-TCTTCTCCGAGCCGGTCGAAATAGT-3′

17E (5+6) 5′-TCTTCTCCGAGCCGGTCGAAATAGTG-3′

17E (6+6) 5′-CTCTTCTCCGAGCCGGTCGAAATAGTG-3′

17E (6+7) 5′-CTCTTCTCCGAGCCGGTCGAAATAGTGT-3′

17E (7+7) 5′-TCTCTTCTCCGAGCCGGTCGAAATAGTGT-3′

17E (8+8) 5′-ATCTCTTCTCCGAGCCGGTCGAAATAGTGTG-3′

Substrate MBs (each strand with a single RNA nucleotide (rA) in the Middle, X represents the dSpacer):

MB1 (with 7 base pairs at the stem loop)

3′-GTCCCGTAAAAAAAGTAGAGAAGGrATATCACACAAAAAAAAACGXGAC-5′

MB2 (with 8 base pairs at the stem loop)

3′-CGTCCCGTAAAAAAAGTAGAGAAGGrATATCACACAAAAAAAAACGXGACG-5′

Control substrate MB without dSpacer:

MB3

3′-GTCCCGTAAAAAAAGTAGAGAAGGrATATCACACAAAAAAAAACGGGAC-5′

Aptazymes (based on an adenosine aptamer and a Mg^{2+}-dependent 10-23 DNAzyme):

AAP1 (6+6, 3bp)

5′-CTCTTCAGCGATCTAGGGGGAGTATTGCGGAGGATAGCACCCATGTTAGTGT -3′

AAP2 (7+7, 2bp)

5′-TCTCTTCAGCGATCTGGGGGAGTATTGCGGAGGAAGCACCCATGTTAGTGTG -3′

AAP3 (7+7, 3bp)

5′-TCTCTTCAGCGATCTAGGGGGAGTATTGCGGAGGATAGCACCCATGTTAGTGTG -3′

AAP4 (8+8, 2bp)

5′-ATCTCTTCAGCGATCTGGGGGAGTATTGCGGAGGAAGCACCCATGTTAGTGTGT -3′

Kinetic fluorescence studies. For a standard measurement of Pb^{2+} using label-free catalytic molecular beacon (CAMB) and 8-17 DNAzyme, 492.5 μL buffer A (25 mM HEPES at pH 7.0 and 200 mM NaCl), 2.5 μL ATMND solution (100 μM), 2.5 μL substrate MB (200 μM), and 2.5 μL 17E DNAzyme (200 μM) were added sequentially into a 1.5 mL microcentrifuge tube, after vortexing the tube was allowed to stand at ambient condition for 1 min. The solution was then transferred to a cuvette in a FluoroMax-P fluorimeter (HORIBA Jobin Yvon Inc., Edison, NJ) and the temperature was controlled at 5 °C for 10 min to allow the temperature to reach equilibrium. After that, 5 μL Pb^{2+} stock solution of different concentrations was added to the cuvette and followed by vortexing, then time-dependent fluorescent measurement at $\lambda_{ex/em}$ = 358/405 nm was immediately started. Typically, the rate of fluorescence enhancement ($\Delta F/F_0$ per minute) within 5-8 min after Pb^{2+} addition was calculated for all the measurements. However, when the concentration of Pb^{2+} reached to micromolar level, the cleavage reactions were extremely fast, in such a case, only the rates for the first 60 s after Pb^{2+} addition were calculated.

In a standard adenosine measurement, 492.5 μL buffer B (25 mM HEPES at pH 7.2, 5 mM $MgCl_2$ and 100 mM NaCl), 2.5 μL ATMND solution (100 μM), 2.5 μL substrate MB (200 μM), and 2.5 μL aptazyme AAP (200 μM) were added sequentially into a 1.5 mL microcentrifuge tube. After vortexing, the tube was allowed to stand at ambient condition for 1 min. The solution was then transferred to a cuvette in the fluorimeter with a constant temperature control at 5 °C for 10 min to allow the temperature to reach equilibrium. A 5 μL of adenosine stock solution in buffer B was then added to the above mixed solution followed by vortexing and time-dependent fluorescent measurement at $\lambda_{ex/em}$ = 358/405 nm was immediately initiated. The rate of fluorescence enhancement ($\Delta F/F_0$ per minute) within 5-8 min after the addition of adenosine was calculated for all the measurements.

Preparation of cell lysate. Hela cells (Human epithelial carcinoma cell line) were used for preparing cell lysate. The lysis solution was prepared with 50 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholic acid and protease inhibitor at the ratio of 1:100. Hela Cells were washed with PBS and detached from the flask by using 0.25% trypsin, and then centrifuged at 900 rpm for 3 minutes. Cells (10^6) were lysed in 500 uL of the lysis solution and incubated on ice for 20 min on a shaker. The lysates were then centrifuged at 17000 x g at 4 ºC for 30 min. The supernatant was removed and stored at -20 ºC for further use.

Pb^{2+} detection in cell lysate. The as prepared cell lysate was condensed and redissolved in buffer A to make a 20% cell lysate solution. The detection procedure of Pb^{2+} in this diluted cell lysate solution is the same as mentioned above.

Adenosine detection in human serum. Human serum was diluted in buffer C (25 mM HEPES at pH 7.2, 10 mM $MgCl_2$ and 100 mM NaCl) to produce a 10% serum sample. Then, 485 μL serum sample, 5 μL ATMND solution (100 μM), 5 μL substrate MB2 (200 μM), and 5 μL aptazyme AAP2 (200 μM) were added sequentially into a 1.5 mL microcentrifuge tube. After vortexing, the tube was allowed to stand at ambient condition for 1 min and transferred to a cuvette and kept at 5 ºC for 10 min. Finally, the

fluorescence intensity at $\lambda_{ex/em}$ = 358/405 nm was recorded.

RESULTS AND DISCUSSION

Design of the label-free CAMB sensor for the detection of Pb^{2+} based on 8-17 DNAzyme. To demonstrate our label-free CAMB sensor design can be a general methodology for the development of sensors to detect metal ions, we first chose the Pb^{2+}-specific 8-17 DNAzyme[8] as a model to construct the label-free CAMB sensor for the detection of Pb^{2+}, which is a toxic heavy metal ion to human health and regulated by the U.S. Environmental Protection Agency (EPA) to be 15 ppb (72 nM) as the maximum contamination level in drinking water. The sensor design used in this study was a novel integration of the CAMB design reported by Zhang et al.[58] and our previous report using an abasic site-containing DNAzyme for label-free fluorescent detections.[70] As shown in Figure 1a, instead of engineering the 8-17 DNAzyme binding arm with an abasic site, we extended both ends of the substrate strand (black) of the DNAzyme to form a substrate molecular beacon (MB); in this case, the abasic site was within the stem region of the MB, and no chemical modification to either the DNAzyme (green) or the hybridization part of substrate strand was needed. Through complementary hydrogen bonding toward the opposite cytosine nucleobase and π-π stacking effects from the flanking guanine nucleobases, the external fluorophore ATMND could bind to the abasic site in the hybridized stem region of the MB, resulting in the quenching of its fluorescence. Upon addition of target metal ion Pb^{2+}, the DNAzyme would catalyze the cleavage of the MB substrate, leading to the dehybridization of MB stem region (owing to the significant decrease in its melting temperature); and then releasing ATMND into solution with its recovered fluorescence. Therefore, the concentration of Pb^{2+} could be monitored by the fluorescence enhancement of ATMND.

To check whether the MB stem region could form a stable duplex that was essential for the binding of ATMND to the abasic site, the fluorescence spectra of ATMND in the absence and presence of MB1,

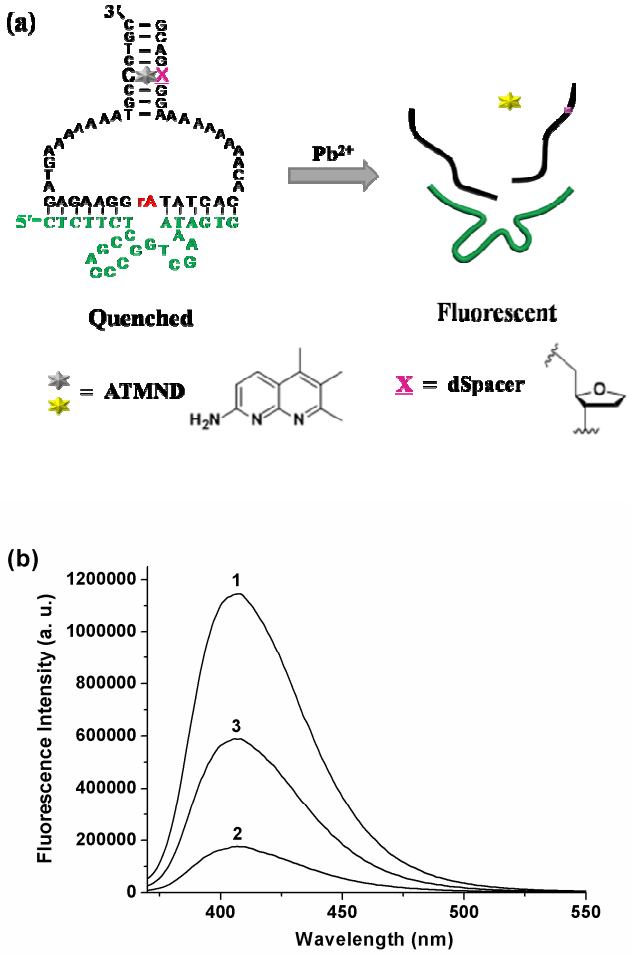


Figure 1. (a) Schematic illustration of fluorescent detection of Pb^{2+} by the label-free CAMB sensor. (b) Fluorescence emission spectra of ATMND in the absence and presence of 17E (6+6) - MB2 for Pb^{2+} detection, which were collected in buffer A (25 mM HEPES at pH 7.0 and 200 mM NaCl): 1, ATMND (0.5 μM); 2, ATMND (0.5 μM), 17E (6+6) (1.0 μM) and MB2 (1.0 μM); 3, ATMND (0.5 μM), 17E (6+6) (1.0 μM), MB2 (1.0 μM) and Pb^{2+} (1.0 μM) after 15 min reaction. The excitation wavelength was 358 nm. All spectra were recorded at 5 °C.

which contained a stem of 7 base pairs and a dSpacer, were first collected (Figure S1 in supporting information). When alone in buffer solution or with DNAzyme 17E (6+6), ATMND (0.5 μM) exhibited intensely blue fluorescence emission with a band centered at 405 nm. Upon further addition of MB1, the

fluorescence emission of ATMND was quenched by 56 %, which can be attributed to the binding of ATMND to the abasic site in the hybridized MB stem region, because the control strand without any abasic site in its stem region could not induce any quenching of the fluorescence.

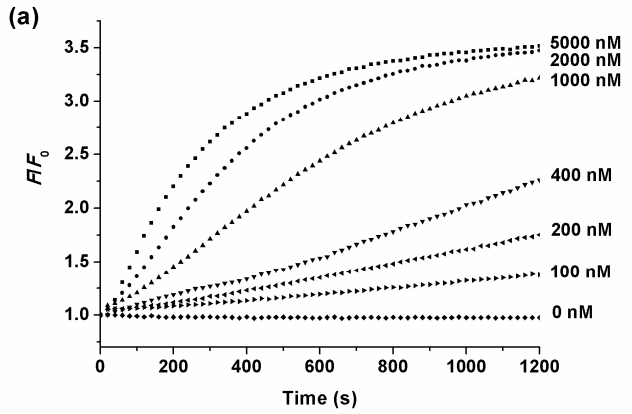
To achieve higher quenching efficiency with even lower fluorescence background, we added an extra G-C base pair to the MB1 (the modified sequence is designated as MB2) to enhance the stability of stem region, therefore, facilitate the binding of ATMND to the abasic site. As expected, MB2 showed a greater quenching efficiency and the fluorescence of ATMND was quenched by more than 85% (see Figure 1b). Other MB substrates with even longer stem lengths were not tested because the melting temperature of their stem region (9 bases or more) were calculated to be more than 40 ºC in buffer solution in 200 mM NaCl, which would be difficult to be dehybridized by the Pb^{2+}-induced cleavage reaction under the experimental conditions. Therefore, MB2 was chosen as the substrate molecular beacon design and used in further investigations.

To confirm that the presence of Pb^{2+} could result in the cleavage of MB2 and subsequent release of ATMND into solution, 1 μM of Pb^{2+} was added to a buffer solution containing 0.5 μM ATMND, 1 μM 17E (6+6) and 1 μM MB2. A more than 3-fold fluorescence enhancement was observed after 15 min (Figure 1b). These results suggested that the label-free CAMB sensor design in our study did not perturb the original activity of the 8-17 DNAzyme, which was crucial for the fluorescence enhancement.

To optimize the sensing performance, the effect of the arm lengths on both sides of cleavage site rA in the 8-17 DNAzyme strand was also investigated. The CAMB DNAzyme contained 8 base pairs (not count the G-T wobble pair) on both 3' and 5' side of cleavage site rA was called 17E (8+8) was first tested. Decreasing them to 7 base pairs on both sides (17E (7+7)) resulted in 168% increase of fluorescent intensity (Figure S2). Encouraged by this finding, we tried to decrease the stem lengths even further and found that the fluorescent increase reached maximum for 17E (6+6), after which the intensity started to decrease for 17E (6+5). When the stem length was decreased even further to 17E

(5+5), almost no fluorescent increases was observed, suggesting that, even though the shorter arm length could facilitate the dehybridization of the substrate MB2 stem after cleavage, if the arm length was shorter than 6 bases at either side, it would weaken the hybridization between the substrate and DNAzyme, and hence, reduce the efficiency of the DNAzyme-catalyzed reaction. Therefore, the optimal arm length of the DNAzyme was determined to be 6 bases on each side of the cleavage site. In addition, the effect of ionic strength on the rate of fluorescence enhancement was also investigated. The rate increased when the Na^+ concentration was raised from 100 to 200 mM, but further raising of the Na^+ concentration to 300 mM resulted in a decrease of the rate (see Figure S3 in the Supporting Information). According to the above results, the 17E (6 + 6) DNAzyme with 6 bases on each arm in a buffer solution containing 200 mM NaCl was selected for further Pb^{2+} sensing experiments.

Under the above optimized condition, kinetics studies were carried out to monitor the time-dependent fluorescence response of ATMND at 405 nm, after the reaction was initiated by the addition of different



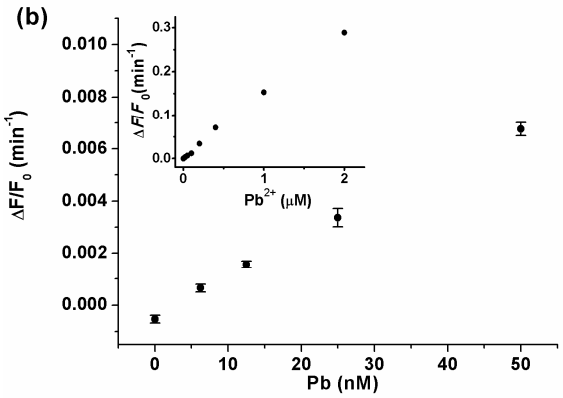


Figure 2. (a) Kinetics of fluorescence enhancement by Pb^{2+}-induced catalytic reaction. (b) Relationship between the fluorescence enhancement rates and Pb^{2+} concentrations. Excitation and emission are at $\lambda_{ex}/\lambda_{em}$ = 358/405 nm. Condition: 0.5 μM ATMND, 1 μM 17E (6+6), 1 μM MB2 in Buffer A (25 mM HEPES pH 7.0, 200 mM NaCl) at 5 °C.

concentrations of Pb^{2+} (Figure 2a). Instead of using fluorescence intensity at a certain time point as a measure of sensing performance, the rate of fluorescence enhancement ($\Delta F/F_0$ per minute) within 5 - 8 min after the addition of Pb^{2+} was recorded, because the rate measurement is much more resistance to fluctuations in the background fluorescence of the samples than intensity measurement. As displayed in Figure 2b, the rate of fluorescence enhancement ($\Delta F/F_0$ per minute) showed an approximately linear relationship with Pb^{2+} concentration ($C_{Pb}{}^{2+}$) in the range of 0 - 2 μM as $\Delta F/F_0\,(\text{min}^{-1}) = 0.146 \times C_{Pb}{}^{2+}$ (μM). Our method based on the label-free CAMB sensor here was very sensitive to Pb^{2+} with a detection limit measured by $3\sigma_b$/slope (σ_b, standard deviation of the blank samples) to be 3.8 nM, which was comparable or even lower than some previously reported labeled and label-free fluorescent methods,[13,57,72-74] and considerably lower than the EPA-defined maximal contamination level of Pb^{2+} (72 nM) in drinking water. In addition, this method maintained an excellent selectivity for Pb^{2+} over other divalent metal ions, such as Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Hg^{2+} and Cd^{2+} (Figure 3).

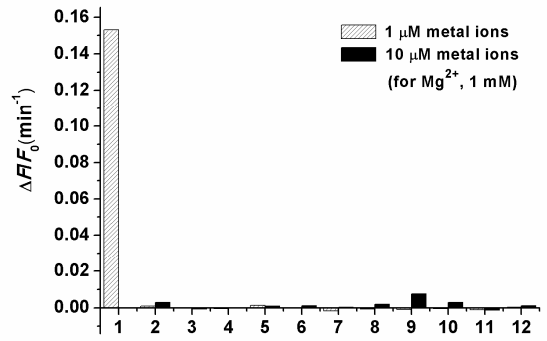


Figure 3. Selectivity of the label-free CAMB sensor for Pb^{2+} detection over other divalent metal ions: 1, 1 μM Pb^{2+}; 2, Mg^{2+}; 3, Ca^{2+}; 4, Sr^{2+}; 5, Ba^{2+}; 6, Mn^{2+}; 7, Fe^{2+}; 8, Co^{2+}; 9, Zn^{2+}; 10, Ni^{2+}; 11, Hg^{2+}; 12, Cd^{2+}. Condition: 0.5 μM ATMND, 1 μM 17E (6+6), 1 μM MB2 in Buffer A (25 mM HEPES pH 7.0, 200 mM NaCl) at 5 °C.

Design of the label-free CAMB sensor for the detection of adenosine based on an adenosine aptazyme. In comparison with the reported label-free fluorescent methods based on Spacer C3, dSpacer or vacant site,[68-71] the current design of label-free CAMB sensor not only takes advantage of multiple turnover DNAzyme reactions for signal amplification, but also makes it possible for rational design of aptazyme[10] sensors for a broader range of targets beyond metal ions. The previously reported label-free methods based on Spacer C3, dSpacer or vacant site generally required excess amount of DNAzyme than its substrate in order to minimize the amount of un-hybridized substrate in solution, because the presence of un-hybridized substrate strand could inhibit the fluorescence enhancement induced by the target-induced catalytic reactions and lower the sensitivity of the sensor or cause false negative results. As a result, when the DNAzyme sensor is directly transformed into an aptazyme one, the excess aptazymes in solution can bind targets but have no hybridized substrates to cleave for the production of fluorescence enhancement, resulting in lower sensitivity and even false negative results. In contrast, in our current label-free CAMB sensor design, the DNAzyme strand does not need to be added in excess to ensure the efficient DNA hybridization with its substrate, because the presence of un-hybridized

substrate does not affect the signal enhancement induced by the target.

Because of the above advantageous feature and encouraged by the success in the Pb^{2+} sensor design, we explored further application of this label-free CAMB system to aptazyme sensors for the detection of broader range of targets beyond Pb^{2+} ions, such as organic molecules. Figure 4 depicts the sensing strategy for the label-free fluorescence detection of adenosine in this work using an allosteric DNAzyme (aptazyme)[54] based on a Mg^{2+}-dependent 10-23 DNAzyme[45] and an adenosine aptamer[16] because it has been testified that the hairpin structure in its catalytic core can be modified with its catalytic activity preserved. As in the case of the Pb^{2+} sensor above, the fluorophore ATMND could bind to the abasic site in the hybridized MB stem region of the substrate, and lead to its fluorescence quenching. After the addition of the aptazyme strand, the formation of the substrate-aptazyme duplex could not induce the cleavage of the substrate and the subsequent release of ATMND from the stem region, unless the target

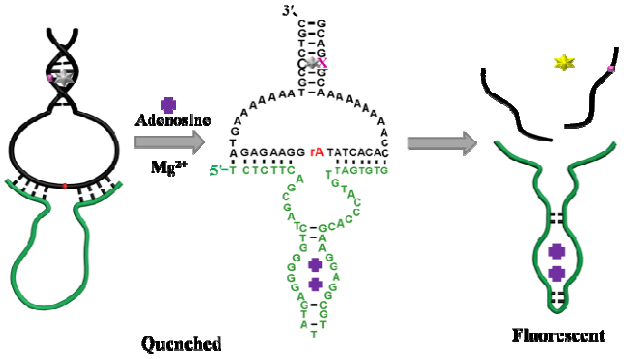


Figure 4. Schematic illustration of fluorescent detection of adenosine by an aptazyme based on 10-23 DNAzyme using the label-free CAMB sensor.

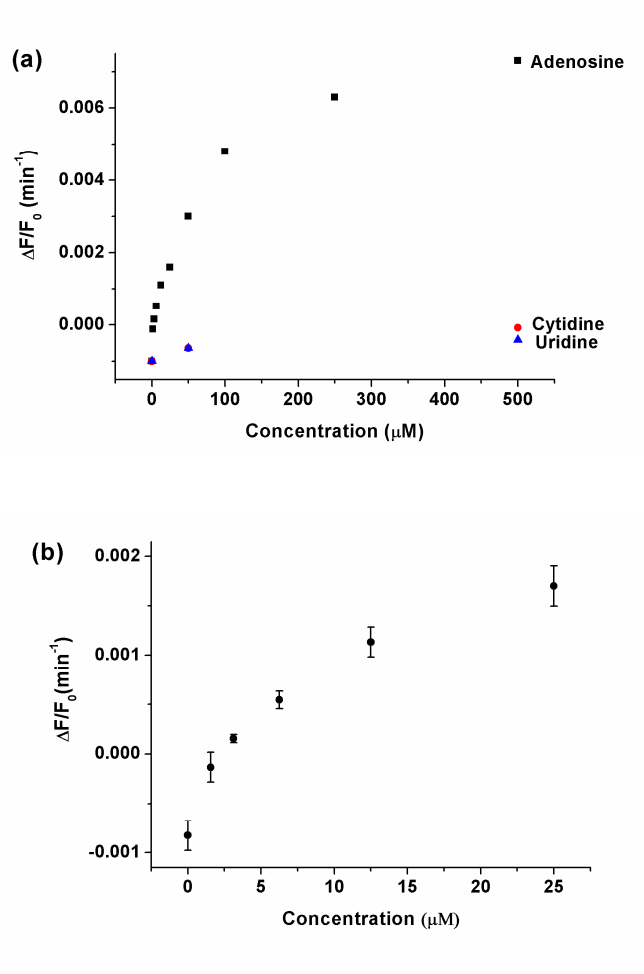


Figure 5. (a) Relationship between the rate of fluorescence enhancement and the concentration of adenosine, uridine and cytidine. (b) Results of adenosine detection in the range of 0-25 μM. Excitation and emission are at: $\lambda_{ex}/\lambda_{em}$ = 358/405 nm. Condition: 0.5 μM ATMND, 1 μM APP2, and 1 μM MB2 in Buffer B (25 mM HEPES at pH 7.2, 5 mM $MgCl_2$, 100 mM NaCl) at 5 °C.

adenosine was present in the Mg^{2+}-containing buffer; while, in the presence of both adenosine and Mg^{2+}, a fluorescence enhancement signal was generated through releasing of ATMND into the buffer. Aptazymes with different arm lengths or complementary parts were first tested to optimize the sensing performance of the adenosine sensor. As shown in Figure S4, the aptazymes AAP2 with 7 base pairs on both 3' and 5' side of cleavage site rA and 2 complementary base pairs in the loop were selected for adenosine detection.

In a solution containing 0.5 μM ATMND, 1 μM AAP2 and 1 μM MB2, upon the addition of 5 mM Mg^{2+}, little fluorescence enhancement was observed (see Figure S5 in Supporting Information), indicating that the aptazyme was inactive without adenosine as the target. In the presence of adenosine, however, a clear fluorescence enhancement was observed, suggesting that the aptazyme was activated by adenosine and led to the release of ATMND from MB stem duplex into solution by the catalytic cleavage of the substrate. As shown in Figure 5, the rate of fluorescence enhancement ratio ($\Delta F/F_0$ per minute) increased with increasing the concentration of adenosine and reached a plateau at ~ 500 μM. A detection limit, measured by $3\sigma_b$/slope (σ_b standard deviation of the blank samples) of 1.4 μM was obtained, which is comparable or even lower than most reported fluorescent sensors for adenosine without signal amplification or the previous label-free methods using a Spacer C3, dSpacer, or vacant site.[68-71] This low detection limit is due to the fact that the activated aptazyme could catalyze multiple turnover cleavage of the substrate MBs for signal amplification and the rate measurement in this work was much less vulnerable to fluctuations in the background fluorescence than the intensity measurement used in the reported works. The selectivity of this method toward adenosine over other nucleosides such as uridine and cytidine were also investigated. Little fluorescence enhancement of the label-free CAMB sensor was observed for these control compounds even in concentration up to 500 μM, indicating that the sensor was very selective to adenosine. Guanosine was not tested for its low solubility in aqueous solution which hindered preparation of its stock solution.

Application of the Sensors in biological relevant Samples. To explore the sensors' potential applications in biological relevant sample analysis, the label-free fluorescent CAMB sensors were used to detect Pb^{2+} in cell lysate and adenosine in human serum, respectively. As shown in Figure 6, the

calibration curves were obtained using 20% cell lysate and 10% serum samples with different concentrations of analyte. The detection limits of 9.2 nM for Pb^{2+} and 6.7 μM for adenosine are close to those obtained in buffer. These results suggested the successful detection of Pb^{2+} and adenosine in biological relevant samples by using the label-free fluorescent CAMB sensors.

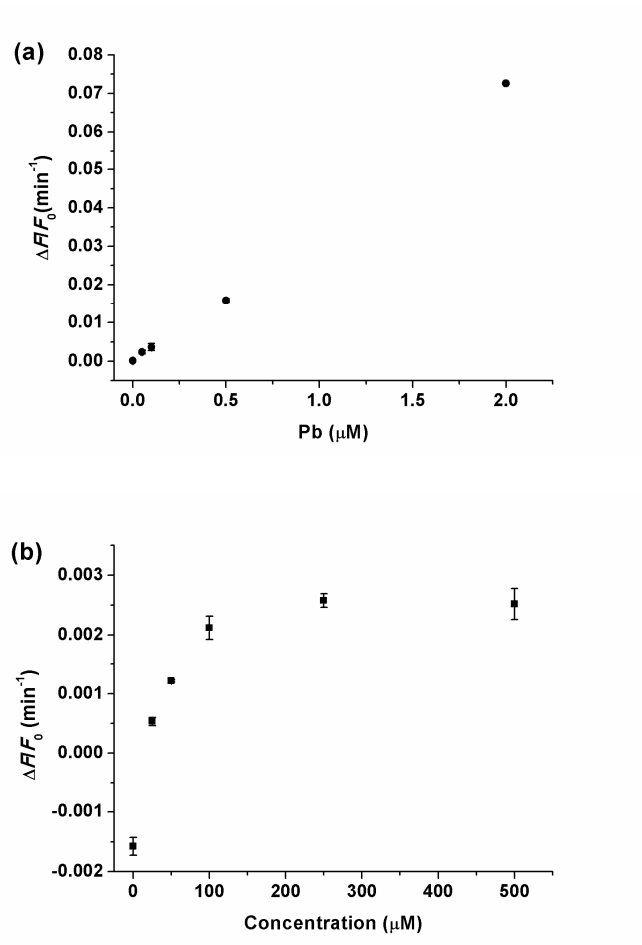


Figure 6. (a) Relationship between the fluorescence enhancement rates and Pb^{2+} concentrations in 20% cell lysate. (b) Relationship between the fluorescence enhancement rates and adenosine concentrations in 10% human serum. Excitation and emission are at $\lambda_{ex}/\lambda_{em}$ = 358/405 nm.

CONCLUSION

In summary, label-free fluorescent catalytic and molecular beacon (CAMB) sensors have been successfully developed for the detection of metal ions and organic molecules in this work. The sensor

design has both the advantages of the label-free sensors that are low-cost and well preserve the activity of the functional nucleic acids (FNAs) and that of the CAMB sensors which exhibit signal amplification via multiple turnover reactions and ease for the rational design of aptazymes. By using the 8-17 DNAzyme and the aptazyme of adenosine based on 10-23 DNAzyme as examples, our methodology can successfully detect Pb^{2+} and adenosine with high sensitivity and selectivity. This label-free fluorescent CAMB approach could further facilitate the application of other FNA sensors for a wide range of analytes.

AUTHOR INFORMATION

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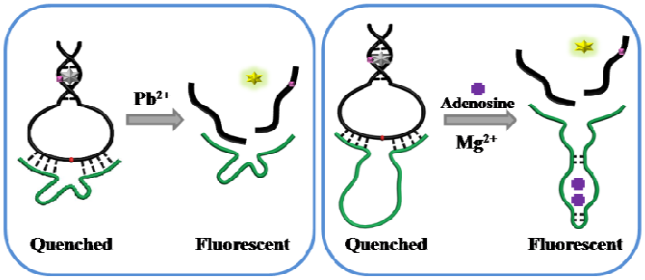

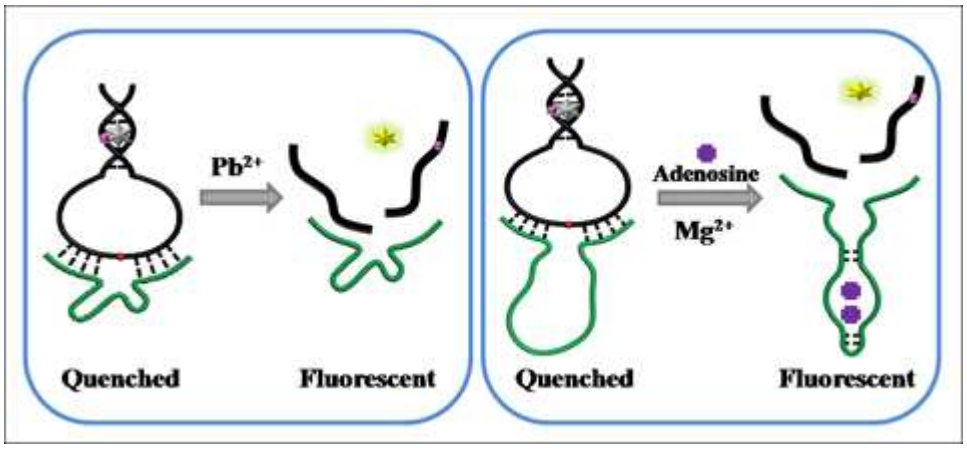

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