# Label-free aptamer-based sensor using abasic site-containing DNA and a nucleobase-specific fluorescent ligand†

Zhiai Xu, Kotaro Morita, Yusuke Sato, Qing Dai, Seiichi Nishizawa and Norio Teramae*

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**A label-free adenosine sensor with emissive response is designed based on an AP site-containing aptamer/DNA duplex and a small fluorescent molecule 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND).**

Aptamers, short nucleic acids isolated from *in vitro* experiments named as SELEX (systematic evolution of ligands by exponential enrichment),[1] have attracted much attention over the past decade because of their high affinity and specificity to various targets including metal ions, small molecules, proteins, and whole cells.[2] High affinity and specificity of aptamers toward targets are generally achieved by a combination of molecular shape complementarity, hydrogen-bonding, and stacking interactions.[3] Such binding events typically involve target-induced structural changes of aptamers, resulting in the formation of unique secondary structures responsible for the target binding, and the internal- or stem–loop structures are representative of active sites for binding. The binding events can be read out by using the structural changes of aptamers based on optical and electrochemical measurements in combination with chromogenic, fluorogenic or redox-active molecules.[2,4] Among these methods, fluorescence detection has been widely adopted because of its simplicity, sensitivity, and quick response. Many design principles for fluorescence signaling of the recognition events have been proposed, such as labeling fluorophores to aptamers, labeling both of fluorophores and quenchers to aptamers and oligonucleotides complementary to a part of aptamers, respectively.[5] Label-free probe design was also proposed by using intercalators or positively charged polymers as signal reporters.[6]

We have recently reported a relatively simple class of DNA duplex aptamers,[7] where an abasic or apurinic/apyrimidinic (AP) site was utilized as an active cavity for binding events. While the naturally occurring AP site is one of the most common forms of DNA damages, we have intentionally incorporated such a lesion site, but in a chemically stable fashion, into the DNA duplex to detect a specific nucleobase in a DNA strand. A DNA strand containing an AP site (AP-DNA) is hybridized with a target DNA strand so as to place the AP site opposite the target nucleobase, by which a hydrophobic cavity is provided for a fluorescent ligand to recognize a nucleobase through a pseudo-base pairing.[8] In the present study, we report on a new design logic for a label-free aptamer-based fluorescent sensor. An AP site was designed to be situated within an aptamer/DNA duplex, and a small fluorescent ligand was used as a signal reporter for target sensing as schematically shown in Fig. 1.

To demonstrate the feasibility of the principle shown in Fig. 1, a 32-mer DNA:
5′-*AGAGA* *A**C**CTG GG*GGA GTATT GCGGA GGAAG GT-3′ (AA1) and a 12-mer AP-DNA strand: 5′-CCCAG **X**TTCT CT-3′ (AC2, where **X** denotes the AP site having a propyl linker (Spacer C3)) were used. AA1 is composed of the reported 27-mer adenosine aptamer[10] (underlined bases) and an additional AGAGA sequence at the 5′-end. AC2 is complementary to a part of the AA1 sequence (bases written in italic), and it can form a duplex with AA1 so as to place the AP site facing toward the cytosine base (the base written in bold) in the AA1 strand. A fluorescent ligand, 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) has been reported to bind selectively to cytosine (Fig. 2, inset) with high affinity at the AP site.[11] Accordingly, ATMND is expected to strongly bind to cytosine opposite the AP site in the AA1/AC2 duplex to form the AA1/AC2/ATMND complex, resulting in quenching of its fluorescence. Upon addition of adenosine, it is expected that an aptamer/adenosine complex forms with releasing ATMND from the AA1/AC2/ATMND complex if the binding affinity for the aptamer/adenosine complex is higher than that for the aptamer/AP-DNA duplex. This structure-switching[5c] from the aptamer/AP-DNA duplex to the aptamer/adenosine complex will cause the emissive response of ATMND fluorescence.

First, the fluorescence behavior of ATMND was examined in the presence of the AA1/AC2 duplex. As shown in Fig. S1 (ESI†), ATMND exhibits significant quenching of its fluorescence upon addition of the AA1/AC2 duplex containing an AP site opposite cytosine (Fig. S1(d), ESI†), whereas less than 10%

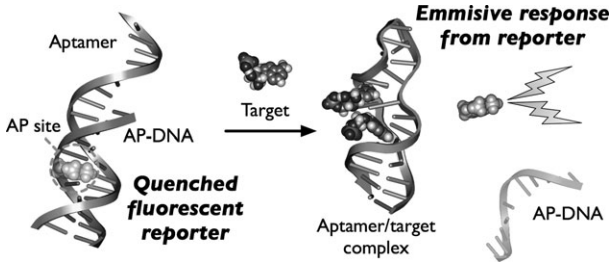


**Fig. 1** Schematic illustration of the label-free aptamer-based sensor composed of an aptamer, AP-DNA, and a fluorescent reporter molecule. The 3D structure of aptamer/target is drawn from the Protein Data Bank (PDB #1AW4[9]).

*Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-8578, Japan. E-mail: teramae@mail.tains.tohoku.ac.jp; Fax: +81-22-795-6552; Tel: +81-22-795-6549*

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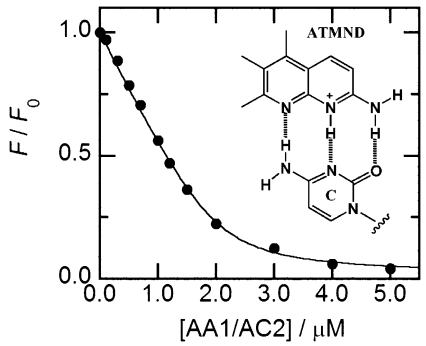


**Fig. 2** Non-linear regression analysis of the fluorescence titration curve based on a 1 : 1 binding isotherm model. [ATMND] = 2 μM, [sodium cacodylate] = 10 mM, [NaCl] = 300 mM, [EDTA] = 1.0 mM, pH 7.0. Inset: possible binding structure of the 1 : 1 complex between ATMND and cytosine (C) bases.[11]

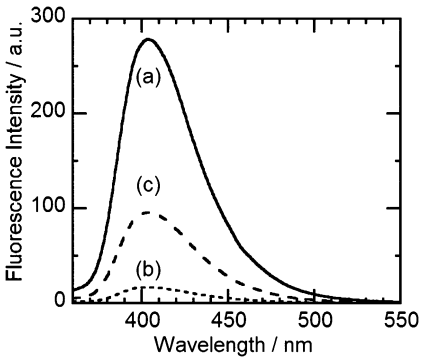


**Fig. 3** Fluorescence spectra of the AA1/AC2/ATMND complex in the absence (b) and presence (c) of 100 μM adenosine. Curve (a) is the fluorescence spectrum of ATMND alone. [ATMND] = 2 μM, [duplex] = 4 μM, other solution conditions are the same as given in Fig. 2.

decrease in the fluorescence intensity of ATMND is observed in the presence of single-stranded AA1 or AC2 (Fig. S1(b) and (c), ESI†). From the fluorescence titration curve shown in Fig. 2, the binding affinity of ATMND with the AA1/AC2 duplex was estimated. The titration curve was analyzed by nonlinear least-squares regression based on a 1 : 1 binding isotherm model:[8a]

$$F/F_0 = \{1 + kK_{11}[D]\}/\{1 + K_{11}[D]\} \quad (1)$$

where $F$ and $F_0$ are the fluorescence intensities of ligand in the presence and absence of the AA1/AC2 duplex, respectively, and $k$ ($= k_{11}/k_L$) represents the ratio of proportionality constants connecting the fluorescence intensities and concentrations of the species (1 : 1 complex, $k_{11}$; free ligand, $k_L$). $K_{11}$ is the 1 : 1 binding constant for complexation of a ligand with the AA1/AC2 duplex. The free duplex concentration, [D], can be related to known total concentrations of duplex ($D_0$) and ligand ($L_0$), by the following equation:[8a]

$$D_0 = [D] + \{L_0K_{11}[D]\}/\{1 + K_{11}[D]\} \quad (2)$$

Together, eqn (1) and (2) describe the system.

The data can be fitted well with the equations and $k$ value of 0.013 and the dissociation constant $K_d$ of 0.13 μM ($K_d = 1/K_{11}$ were obtained. Although ATMND does not covalently bind to DNA, it binds with high binding affinity to the AA1/AC2 duplex by pseudo-base pairing with cytosine opposite the AP site probably *via* three hydrogen bonds (Fig. 2, inset) as proposed by Nakatani *et al.*[11] and Sato *et al.*[12]

Second, the response of the system upon addition of adenosine was examined. As shown in Fig. 3, fluorescence of ATMND (2 μM) is quenched strongly in the presence of the AA1/AC2 duplex (4 μM) (from (a) to (b)), and its fluorescence intensity recovers greatly to 5.8-fold after adding 100 μM adenosine (curve (c)). The increase in fluorescence intensity upon addition of adenosine can be assigned to release of ATMND from the AA1/AC2 duplex. This indicates that interaction between adenosine and the AA1/AC2/ATMND complex causes effective structure-switching from the AA1/AC2 duplex to the AA1/adenosine complex.

As control experiments, mutated AA1 sequences were used to examine the response for adenosine. AA1s (5′-AGAGA ACCTG GG-3′) has a shortened sequence of AA1 and is composed of a 12-mer sequence of AA1 from the 5′-side. AA1s is fully complementary to AC2 except the cytosine base opposite the AP site. AA1m14 (5′-AGAGA ACCTG GGGAA GTATT GCGGA GGAAG GT-3′) and AA1m27 (5′-AGAGA ACCTG GGGGA GTATT GCGGA G**A**AAG GT-3′) have the equal length of sequence with AA1 but one guanine base is replaced by adenine (designated as **A** in bold) at the 14th and 27th base positions, respectively. In addition, AA1mm (5′-AGAGA ACCTG GGG**A**A GTATT GCGGA G**A**AAG GT-3′) with two mutated bases at the 14th and 27th base positions was examined for the detection of adenosine. When adenosine was added to these mutated-AA1/AC2/ATMND systems, no recovery of fluorescence was observed (Fig. S2, ESI†), indicating that the aptamer sequence in the AA1 strand is responsible for the recognition of adenosine in our method and the results are consistent with those given in the literatures.[9,10]

Further, the effect of the sequence length of AP-DNA on the fluorescence recovery of the AA1/AP-DNA/ATMND complex upon addition of adenosine was also examined by using AC3 (5′-GCAAT ACTCC CCCAG **X**TTCT CT-3′) and AC4 (5′-ACCTT CCTCC GCAAT ACTCC CCCAG **X**TTCT CT-3′) as AP-DNA. AC3 and AC4 are partially and fully complementary to AA1, respectively. Both of them have an AP site at the same 7th base position from the 3′-side as 12-mer AC2, but the sequence lengths are 22-mer and 32-mer for AC3 and AC4, respectively. Quenching efficiency of ATMND was larger for AA1/AC3 (93%) and AA1/AC4 (95%) duplexes than that for AA1/AC2 duplex (78%) as listed in Table S1, ESI† (2 μM ATMND and 2 μM DNA duplex). However, no recovery of ATMND fluorescence appeared upon addition of adenosine. This observation can be ascribed to the stronger affinity of the aptamer sequence (AA1) with longer AP-DNA (AC3 and AC4), which prevents complexation of adenosine with AA1.

As for the response time to detect adenosine based on aptamer-based sensors, it was reported that 90 min or 3 h was needed to obtain a stable response for the detection of

 

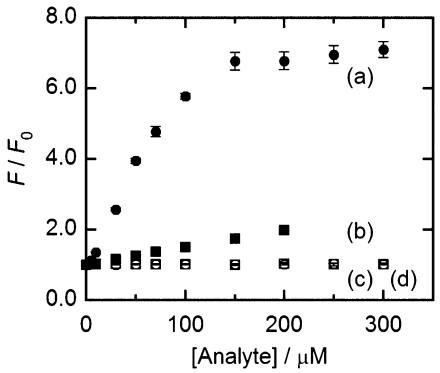


**Fig. 4** Fluorescence intensity change of the AA1/AC2/ATMND complex upon addition of nucleotides. (a) adenosine, (b) guanosine, (c) cytidine, and (d) thymidine. [ATMND] = 2 μM, [duplex] = 4 μM, other solution conditions are the same as given in Fig. 2. $F$ and $F_0$ denote the fluorescence intensities with and without adenosine, respectively. Error bar in (a) is the standard deviation obtained from the three independent repeated measurements.

adenosine or ATP in the electrochemical measurements[13a,b] based on the analyte-induced structure-switching from an aptamer/DNA duplex to an aptamer/analyte complex. In the detection using an ion-selective field-effect transistor method, the response time was reported as *ca.* 4 min.[5g] In our method, as shown in Fig. S3(a), ESI† upon addition of adenosine, the fluorescence intensity of the AA1/AC2 system rapidly increases and reaches to nearly three-fold within several minutes. The response is very fast and the fluorescence intensity of ATMND is very stable for more than one hour.

As shown in Fig. 4, fluorescence intensity increases linearly as the concentration of adenosine increases from 5 to 100 μM and the dynamic response range covers the concentration range from 5 to 150 μM (plot (a)). The system shows highly noticeable response to adenosine, while no response to cytidine and thymidine (plot (c), (d)) and a slight response to guanosine (plot (b)) in the concentration range examined. The response to guanosine can be ascribed to the binding of guanosine to cytosine opposite the AP site in the AA1/AC2 duplex, because slight fluorescence recovery was also observed when adding guanosine to the AA1s/AC2/ATMND complex. At the concentration range below 10 μM, the present system exhibits a distinct fluorescence recovery for adenosine over guanosine (Fig. S4, ESI†).

Although aptamer-based sensors for adenosine with high sensitivity have been reported based on electrochemical measurements,[13] it is known that the response is slow and immobilization and wash steps are needed in inhomogenous assays compared to the homogenous assay. The detection limit of the present method for adenosine is 2 μM (Fig. S5, ESI†) and it is superior to the reported aptamer-based optical detection methods, such as crosslinked (300 μM)[14a] and non-crosslinked (10 μM)[14b] gold nanoparticles, quantum dots (50 μM),[14c] and DNAzyme-based colorimetric assay (6 μM).[14d]

In summary, a label-free aptamer-based adenosine sensor with an emissive response is developed. The duplex formed by an aptamer and an oligonucleotide containing an AP site offers a versatile platform for the detection of adenosine in combination with a fluorescent ligand, ATMND, which binds to a specific nucleobase (cytosine) opposite the AP site. Since we found adenine-selective green fluorescent ligand for the detection of single nucleotide polymorphisms,[8d] the present method could be easily extended to use a variety of nucleobase-specific fluorescence ligands. It should be noted that the method could be in principle applicable to various targets by using their aptamer sequences and AP site-containing DNA strands.

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## Notes and references

1 (*a*) C. Tuerk and L. Gold, *Science*, 1990, **249**, 505; (*b*) A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818.
2 See recent reviews and references therein: (*a*) N. O. Fisher, T. M. Tarasow and J. B.-H. Tok, *Curr. Opin. Chem. Biol.*, 2007, **11**, 316; (*b*) I. Willner and M. Zayats, *Angew. Chem., Int. Ed.*, 2007, **46**, 6408; (*c*) T. Mairal, V. C. Özalp, P. L. Sánchez, M. Mir, I. Katalkis and C. K. O'Sullivan, *Anal. Bioanal. Chem.*, 2008, **390**, 989.
3 T. Hermann and D. J. Patel, *Science*, 2000, **287**, 820.
4 N. de-los-Santos-Alvarez, M. J. Lobo-Castanon, A. J. Miranda-Ordieres and P. Tunon-Blanco, *Trends Anal. Chem.*, 2008, **27**, 437.
5 (*a*) S. D. Jhaveri, R. Kirby, R. Conrad, E. J. Maglott, M. Bowser, R. T. Kennedy, G. Glick and A. D. Ellington, *J. Am. Chem. Soc.*, 2000, **122**, 2469; (*b*) M. N. Stojanovic, P. de Prada and D. W. Landry, *J. Am. Chem. Soc.*, 2001, **123**, 4928; (*c*) R. Nutiu and Y. Li, *J. Am. Chem. Soc.*, 2003, **125**, 4771; (*d*) E. J. Merino and K. M. Weeks, *J. Am. Chem. Soc.*, 2003, **125**, 12370; (*e*) S. Nagatoishi, T. Nojima, B. Juskowiak and S. Takenaka, *Angew. Chem., Int. Ed.*, 2005, **44**, 5067; (*f*) N. Rupcich, R. Nutiu, Y. Li and J. D. Brennan, *Angew. Chem., Int. Ed.*, 2006, **45**, 3295; (*g*) B. Shiyahovsky, D. Li, Y. Weizmann, R. Nowarski, M. Kotler and I. Willner, *J. Am. Chem. Soc.*, 2007, **129**, 3814; (*h*) T. Tang, P. Mallikaratchy, R. Yang, Y. Kim, Z. Zhu, H. Wang and W. Tan, *J. Am. Chem. Soc.*, 2008, **130**, 11268.
6 (*a*) H. A. Ho and M. Leclerc, *J. Am. Chem. Soc.*, 2004, **126**, 1384; (*b*) Y. X. Jiang, X. H. Fang and C. L. Bai, *Anal. Chem.*, 2004, **76**, 5230; (*c*) B. Li, H. Wei and S. J. Dong, *Chem. Commun.*, 2007, 73.
7 (*a*) N. B. Sankaran, S. Nishizawa, T. Seino, K. Yoshimoto and N. Teramae, *Angew. Chem., Int. Ed.*, 2006, **45**, 1563; (*b*) M. Li, Y. Sato, S. Nishizawa, T. Seino, K. Nakamura and N. Teramae, *J. Am. Chem. Soc.*, 2009, **131**, 2448.
8 (*a*) K. Yoshimoto, S. Nishizawa, M. Minagawa and N. Teramae, *J. Am. Chem. Soc.*, 2003, **125**, 8982; (*b*) K. Yoshimoto, C.-Y. Xu, S. Nishizawa, T. Haga, H. Satake and N. Teramae, *Chem. Commun.*, 2003, 2960; (*c*) C. Zhao, Q. Dai, T. Seino, Y.-Y. Cui, S. Nishizawa and N. Teramae, *Chem. Commun.*, 2006, 1185; (*d*) B. Rajendar, S. Nishizawa and N. Teramae, *Org. Biomol. Chem.*, 2008, **6**, 670; (*e*) Z. Ye, B. Rajendar, Q. Dai, S. Nishizawa and N. Teramae, *Chem. Commun.*, 2008, 6588.
9 C. H. Lin and D. J. Patel, *Chem. Biol.*, 1997, **4**, 817.
10 D. E. Huizenga and J. W. Szostak, *Biochemistry*, 1995, **34**, 656.
11 H. Suda, A. Kobori, J. Zhang, G. Hayashi and Kazuhiko Nakatani, *Biorg. Med. Chem.*, 2005, **13**, 4507.
12 Y. Sato, S. Nishizawa, K. Yoshimoto, T. Seino, T. Ichihashi, K. Morita and N. Teramae, *Nucleic Acids Res.*, 2009, **37**, 1411.
13 (*a*) Z.-S. Wu, M.-M. Guo, S.-B. Zhang, C.-R. Chen, J.-H. Jiang, G.-L. Shen and R.-Q. Yu, *Anal. Chem.*, 2007, **79**, 2933; (*b*) X. Zuo, S. Song, J. Zhang, D. Pan, L. Wang and C. Fan, *J. Am. Chem. Soc.*, 2007, **129**, 1042; (*c*) B. Li, Y. Du, H. Wei and S. Dong, *Chem. Commun.*, 2007, 3780.
14 (*a*) J. Liu and Y. Lu, *Angew. Chem., Int. Ed.*, 2006, **45**, 90; (*b*) W. Zhao, W. Chiuman, M. A. Brook and Y. Li, *ChemBioChem*, 2007, **8**, 727; (*c*) J. Liu, J. H. Lee and Y. Lu, *Anal. Chem.*, 2007, **79**, 4120; (*d*) N. Lu, C. Shao and Z. Deng, *Chem. Commun.*, 2008, 6161.