Supplementary Material (ESI) for Chemical Communications

Supporting Information for

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

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Experimental

Reagents: ATMND was purchased from Enamine (Kiev, Ukraine). All of the DNA samples were custom synthesized and HPLC purified (>97 %) by Nihon Gene Research Laboratories Inc. (Sendai, Japan). Adenosine was purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). The other reagents were commercially available analytical grade and were used without further purification. The concentration of DNA, adenosine, thymidine, cytidine and guanosine were determined from the molar extinction coefficient at 260 nm[1] (AA1: 5'-AGAGA ACCTG GGGGA GTATT GCGGA GGAAG GT-3'; ε = 341160 M^{-1} cm^{-1}, AA1s: 5'-AGAGA ACCTG GG-3'; ε = 128400 M^{-1} cm^{-1}, AA1m14: 5'-AGAGA ACCTG GGGAA GTATT GCGGA GGAAG GT-3'; ε = 342420 M^{-1} cm^{-1}, AA1m27: 5'-AGAGA ACCTG GGGGA GTATT GCGGA GAAAG GT-3'; ε = 342420 M^{-1} cm^{-1}, AA1mm: 5'-AGAGA ACCTG GGGAA GTATT GCGGA GAAAG GT-3'; ε = 343680 M^{-1} cm^{-1}, AC2: 5'-CCCAG **X**TTCT CT-3'; ε = 94320 M^{-1} cm^{-1}, AC3: 5'-GCAAT ACTCC CCCAG **X**TTCT CT-3'; ε = 189360 M^{-1} cm^{-1}, AC4: 5'-ACCTT CCTCC GCAAT ACTCC CCCAG **X**TTCT CT-3'; ε = 271200 M^{-1} cm^{-1}, where **X** denotes the abasic site (a propyl residue, Spacer C3)). Water was deionized (≥18.0 MΩ cm specific resistance) by an Elix 5 UV Water Purification System and a Milli-Q Synthesis A10 system (Millipore Corp., Bedford, MA).

Instruments and measurements: A JASCO model V-570 UV–vis spectrophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan) was used to measure absorption spectra. Fluorescence spectra was measured with a JASCO model FP-6500 spectrofluorophotometer equipped with a thermoelectrically temperature controlled cell holder (Japan Spectroscopic Co. Ltd., Tokyo, Japan). Fluorescence measurements were carried out at 5°C using a 3 × 3 mm quartz cell. The sample solutions containing DNA were annealed before measurements: heated at 75°C for 10 min, and then gradually cooled down to 5°C (3°C min^{-1}), after which the solution temperature was raised again to 20°C. Excitation wavelength for ATMND was set at 350 nm.

1. J. D. Puglisi, I. Tinoco, Jr., *Method Enzymol.* 1989, **180**, 304.

Table S1. Quenching efficiency and fluorescence recovery of ATMND for aptamer/AP-DNA duplexes.

Duplex[a)]	Sequences[b)]	Q.E.[c)]	F / F_0[d)]
AA1/AC2	5′-AGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3′ 3′-TCTCTT**X**GACCC-5′	78%	2.9
AA1/AC3	5′-AGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3′ 3′-TCTCTT**X**GACCCCCTCATAACG-5′	93%	1.0
AA1/AC4	5′-AGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3′ 3′-TCTCTT**X**GACCCCCTCATAACGCCTCCTTCCA-5′	95%	1.0

a) AC2, AC3, and AC4 are AP site-containing DNAs (AP-DNA). [ATMND] = 2 μM, [DNA duplex] = 2 μM.

b) In the sequence of AP-DNAs, **X** denotes the AP site (Spacer C3).

c) Q.E. means the quenching efficiency of ATMND given by the ratio of the fluorescence intensities for free ATMND to that for the AA1/AP-DNA/ATMND complex.

d) F_0 and F are the fluorescence intensities of ATMND in the AA1/AP-DNA/ATMND system without and with adenosine (100 μM), respectively.

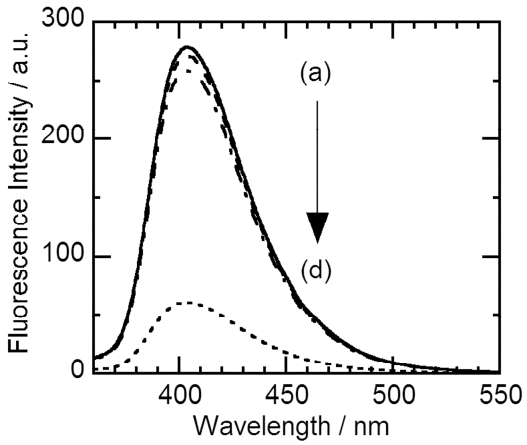

Fig. S1. Fluorescence spectra of ATMND in the absence and presence of DNA strands. (a) ATMND alone, (b) AC2/ATMND, (c) AA1/ATMND, (d) AA1/AC2/ATMND. [ATMND] = 2 μM, [DNA] = 2 μM. [sodium cacodylate] = 10 mM, [NaCl] = 300 mM, [EDTA] = 1.0 mM, pH 7.0.

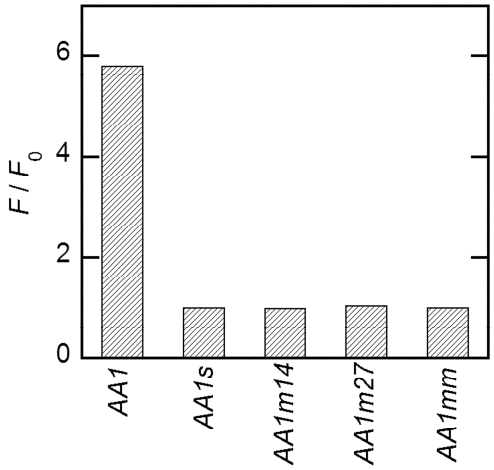

Fig. S2. Fluorescence response of the aptamer/AC2/ATMND systems. Sequences of aptamers (AA1, AA1s, AA1m14, AA1m27, and AA1mm) are given in Experinmetal in this Supporting Information. [adenosine] = 100 μM, [ATMND] = 2 μM, [duplex] = 4 μM, [sodium cacodylate] = 10 mM, [NaCl] = 300 mM, [EDTA] = 1.0 mM, pH 7.0.

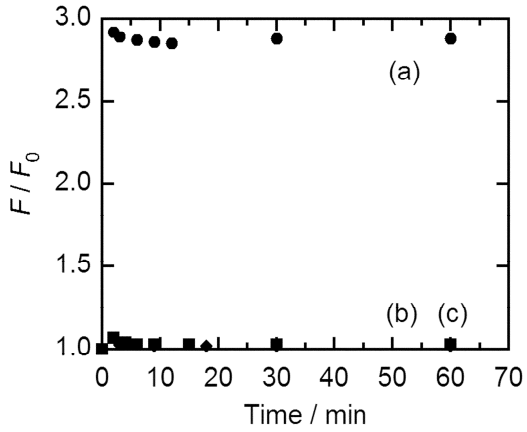

Fig. S3. Time-dependent fluorescence change of the AA1/AP-DNA/ATMND system upon addition of 100 μM adenosine. AP-DNAs are (a) AC2, (b) AC3, (c) AC4. [ATMND] = 2 μM, [duplex] = 2 μM, [sodium cacodylate] = 10 mM, [NaCl] = 300 mM, [EDTA] = 1.0 mM, pH 7.0.

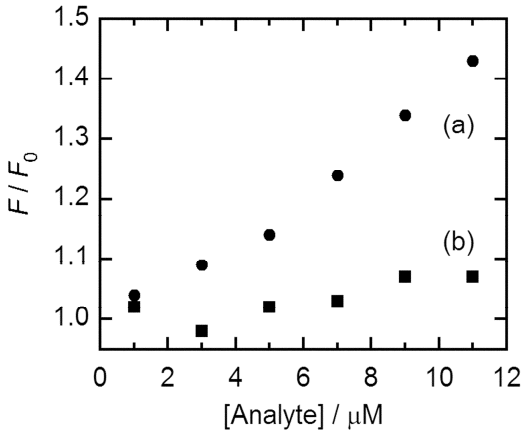

Fig. S4 Fluorescence intensity change of the AA1/AC2/ATMND complex upon addition of nucleotides. (a) adenosine, (b) guanosine. [ATMND] = 2 μM, [duplex] = 4 μM, [sodium cacodylate] = 10 mM, [NaCl] = 300 mM, [EDTA] = 1.0 mM, pH 7.0.

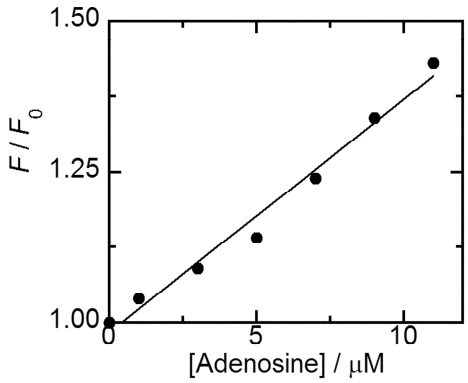

Fig. S5 The linear fitting analysis for the determination of detection limit (LOD).

Linear fitting equation: y = 0.98414 + 0.0386x (R = 0.99097)

The standard deviation of blank was determined from three independent repeated measurements:

$F_0(1)$	$F_0(2)$	$F_0(3)$	mean	S_{blank}
1.02	0.984	0.994	1.00	0.0186

LOD = $3 \times S_{blank}/0.0386$= 1.55 μM

Based on these data, we estimated the LOD as 2 μM.