Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information for

Pyrrolo-dC based fluorescent aptasensors for the molecular recognition of targets

Taihua Li, Rongzhan Fu, and Hyun Gyu Park*

Department of Chemical and Biomolecular Engineering, KAIST, 335 Gwahak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea, E-mail: hgpark@kaist.ac.kr, Phone: +82-42-350-3932, +82-42-350-3972 and Fax: +82-42-350-3910.

Experimental Section

Materials

All DNA oligonucucleotides including pyrrolo-deoxycytosine (pyrrolo-dC)-incorporated DNA were synthesized and HPLC purified by Genotech Co. (Daejeon, Korea). Sequences of the oligonucleotides are listed in Table S1 and S2. The ATP, GTP, bovine serum albumin (BSA), human α-thrombin, and PDGF-BB were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals were of at least analytical reagent grade and used without further purification. Aqueous solutions were prepared with doubly distilled water.

Preparation of ATP, thrombin, and PDGF aptasensors

For ATP assay, ATP aptamer and competitor with/without UFB probe were diluted to 0.16 μM in TE buffer (pH 8.0) containing 300 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 10 mM Tris-HCl and heated at 65℃ for 5 min. After the addition of ATP, the solution was incubated at 30℃ for 30 min followed by the fluorescent measurement.

For thrombin and PDGF detection, corresponding aptamers, competitors, and UFB probes were diluted to 0.5 μM in the binding buffer (thrombin in pH 8.0 TE buffer with 1mM $MgCl_2$, 0.1 mM EDTA and 10 mM Tris; PDGF in pH 7.4 PBS buffer with 10 mM PB, 138 mM NaCl, 3 mM $MgCl_2$ and 3 mM $CaCl_2$) and heated at 65℃ for 5 min. After the addition of thrombin and PDGF, the solutions were incubated at 45℃ for 30 min and at 30℃ for 1 hr, respectively followed by the fluorescent measurement.

Fluorescence measurement

The fluorescence intensities of all samples were recorded by using a RF-5301 PC (Spectrofluorophotometer, Shimadzu, Japan) with excitation at 350 nm and emission at 455 nm.

Table S1. Sequence information for aptamers, competitors, and the universal fluorescent base (UFB) probe used in this study.

Target	Aptamer (5'-3')	Competitor (5'-3')	UFB probe (5'-3')
ATP[a]	ACCTGGGGGAGTATTGCGGAGGAAGGT	TTTTTTACXTTXCTXCGCAAT	
ATP[b]	ACCTGGGGGAGTATTGCGGAGGAAGGT	***AGAGAGAGAGAG***AGACCTTCCTCCGCAATACTCC**CTCTCTCTCTCT**	
Thrombin[b]	GGTTGGTGTGGTTGG	***AGAGAGAGAGAG***AGCCAACCACACCAACC**CTCTCTCTCTCT**	***CTXTXTXTCT***
PDGF[b]	CAGGCTACGGCACGTAGAGCATCACCATGATCCTG	***AGAGAGAGAGAG***AGCAGGATCATGGTGATGCT**CTCTCTCTCTCT**	

[a] The sequences for aptamer and competitor used in the first strategy for ATP assay in this study.
[b] The sequences for aptamer, competitor, and UFB probe used in the advanced strategy for the target detection in this study.
X represents pyrrolo-dC (PdC).
The underlined sequence of the aptamer is complementary to the underlined sequence of the corresponding competitor.
The bold letters in the competitor sequence indicate the bases that form a stem and the bold italic letters of the competitor indicate the bases that hybridize with the UFB probe in the absence of target molecule in the advanced strategy.

Table S2. Sequence information for mutant aptamers, their corresponding competitors, and UFB probe used in mutant assay of the second strategy.

Target	Mutant Aptamer (5'-3')	Competitor (5'-3')	UFB probe (5'-3')
ATP	ACCTGGGGCAGTATTGC GGAGCAAGGT	***AGAGAGAGAGAG***AGACCTTGCTCCGCA ATACTGC**CTCTCTCTCTCT**	***CTXTXTXTCT***
Thrombin	GCTTGGTGTCGTTGG	***AGAGAGAGAGAG***AGCCAACGACACCAA GC**CTCTCTCTCTCT**	
PDGF	CAGGCTACGGCACGTAGC GATTCACCATGATCCTG	***AGAGAGAGAGAG***AGCAGGATCATGGTG AATCG**CTCTCTCTCTCT**	

X represents pyrrolo-dC (PdC).
The underlined sequence of the mutant aptamer is complementary to the underlined sequence of the corresponding competitor.
The bold letters in the competitor sequence indicate the bases that form a stem and the bold italic letters of the competitor indicate the bases that hybridize with the UFB probe in the absence of target molecule in the advanced strategy.
The red colored letters in the mutant aptamer sequence indicate the point mutated bases as compared to the target aptamers.

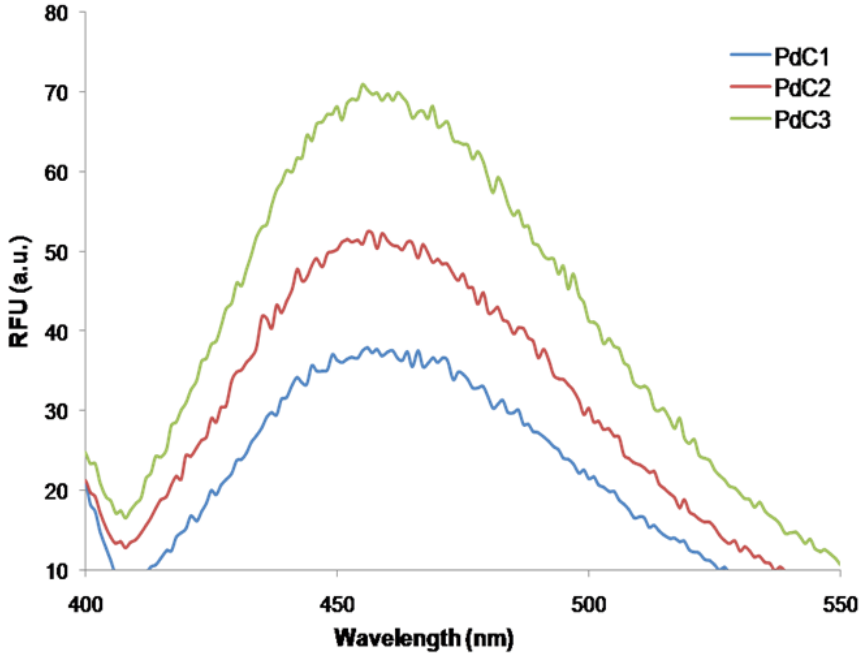

Fig. S1 Comparision of the fluorescence spectra of three different oligonucleotides containing different numbers of PdC. PdC1: 5'-TCCTCAGAAGTTTATGCA**X**T-3'; PdC2: 5'-TCCT**X**AGAAGTTTATGCA**X**T-3'; PdC3: 5'-TC**X**T**X**AGAAGTTTATGCA**X**T-3'. X represents PdC. Spectra were recorded at room temperature in a buffer containing 500 mM KCl, 15 mM $MgCl_2$ and 100 mM Tris-HCl (pH 8.0) with excitation at 350 nm.

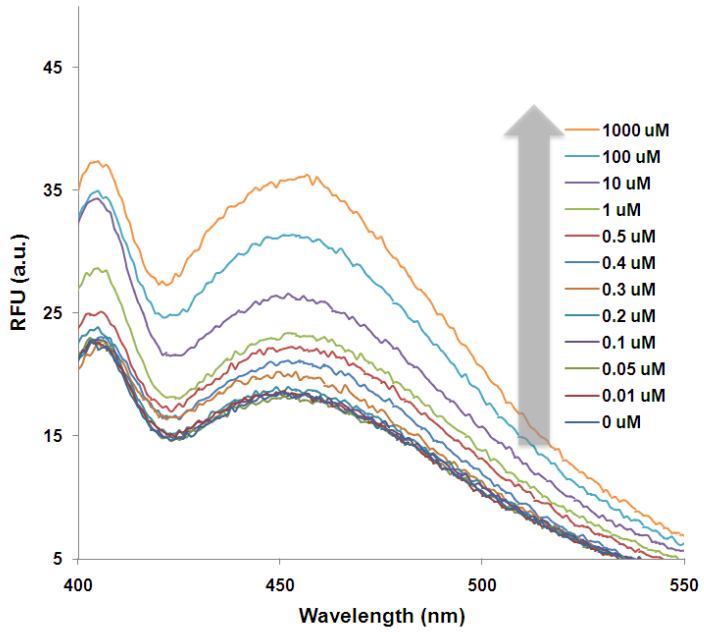

Fig. S2 Fluorescence spectra of ATP aptamer/competitor complex upon addition of ATP at 0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 10, 100, and 1000 μM. The spectra were recorded at room temperature in a buffer containing 300 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 10 mM Tris (pH 8.0) with excitation at 350 nm.

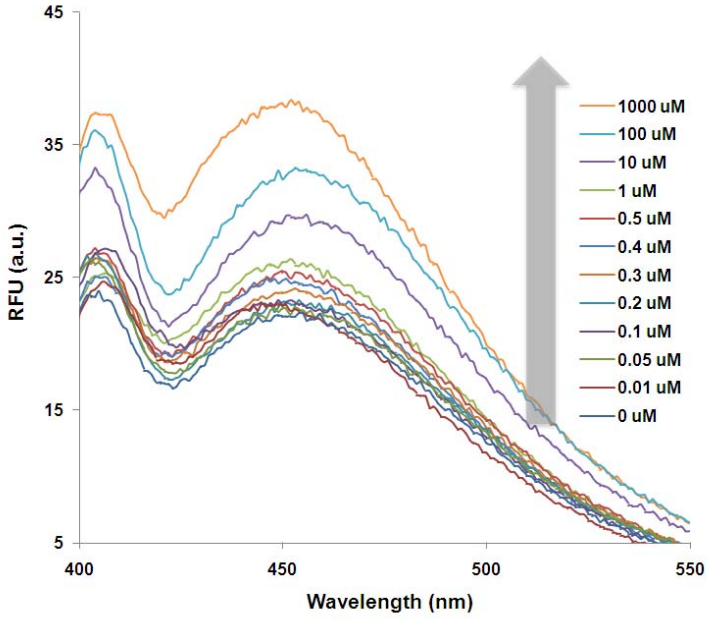

Fig. S3 Fluorescence spectra of the aptamer/competitor/UFB probe complex upon the addition of ATP at 0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 10, 100, and 1000 μM. The spectra were recorded at room temperature in a buffer containing 300 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 10 mM Tris (pH 8.0) with excitation at 350 nm.

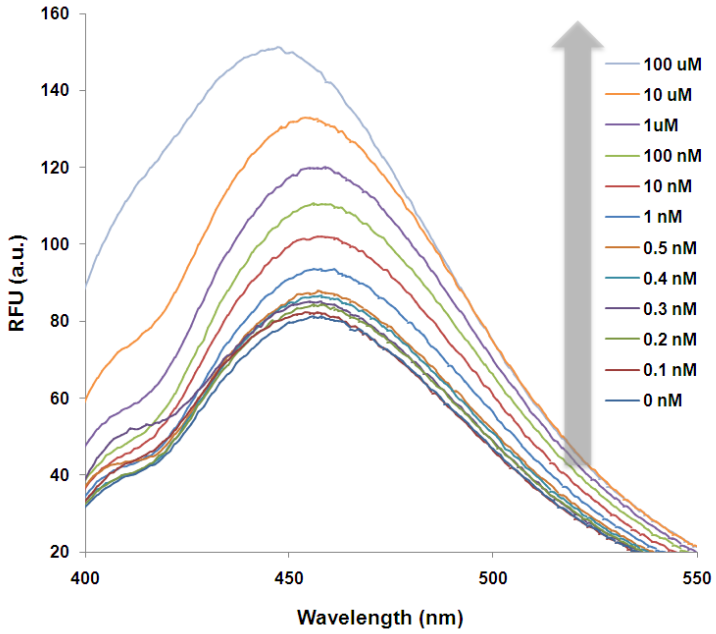

Fig. S4 Fluorescence spectra of aptamer/competitor/UFB probe complex upon addition of α-thrombin at 0, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 10, 100, 1000, 10000, and 100000 nM. The spectra were recorded at room temperature in a buffer containing 1 mM $MgCl_2$, 0.1 mM EDTA and 10 mM Tris (pH 8.0) with excitation at 350 nm.

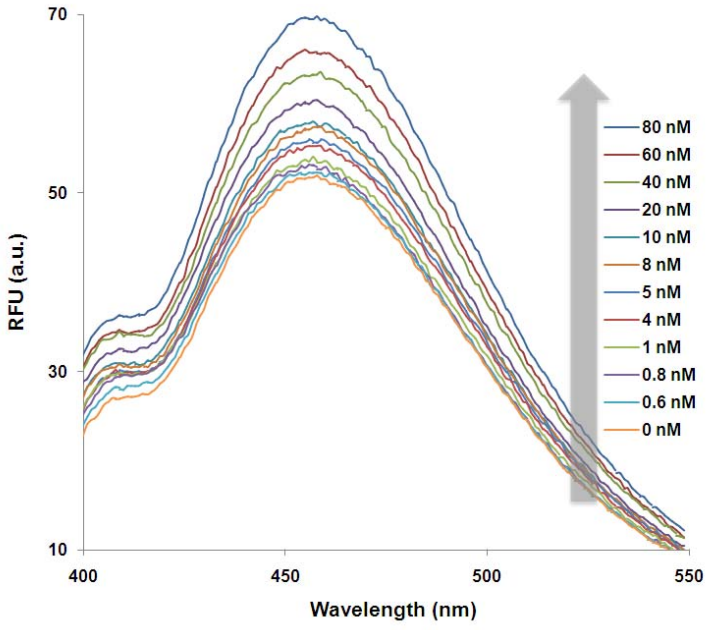

Fig. S5 Fluorescence spectra of aptamer/competitor/UFB probe complex upon addition of PDGF-BB at 0, 0.6, 0.8, 1, 4, 5, 8, 10, 20, 40, 60, and 80 nM. The spectra were recorded at room temperature in a PBS buffer (pH 7.4) containing 10 mM phosphate, 138 mM NaCl, 3 mM $MgCl_2$ and 3 mM $CaCl_2$ with excitation at 350 nm.

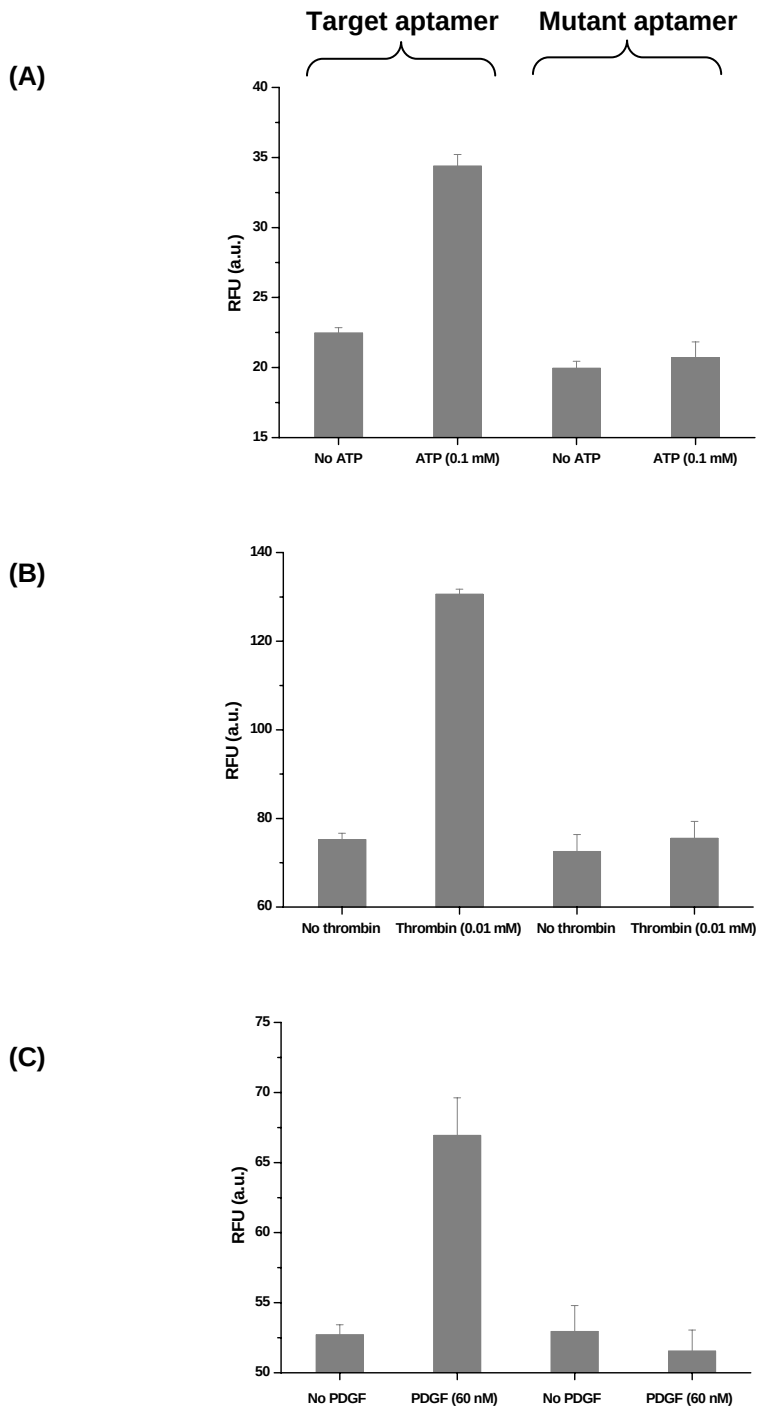

Fig. S6 Control assays of mutant aptamers. (A) ATP; (B) Thrombin; (C) PDGF. Target aptamers (Table S1) were compared with their inactive mutants (Table S2) for fluorescence signaling capability in the absence and presence of their targets (ATP: 0.1 mM; Thrombin: 0.01 mM; PDGF: 60 nM). The experiments were performed in triplicate in the same way as described for Figure 1B, 2A and 2B. Each data point represents the average value of three independent experiments with error bars indicated.