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Supplementary Information

Light-up fluorophore–DNA aptamer pair for label-free turn-on aptamer sensors

Teru Kato, Ippei Shimada, Ryota Kimura and Masumi Hyuga

Graduate School of Bionics, Computer and Media Sciences, Tokyo University of Technology,

1404-1 Katakura, Hachioji, Tokyo 192-0982, Japan

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Experimental details

Reagents and chemicals

Dapoxyl sulfonyl ethylenediamine (SEDA) and dapoxyl sulfonic acid sodium salt (SA) were purchased from Invitrogen. NHS-activated Sepharose 4 Fast Flow and glycogen were acquired from GE Healthcare Life Sciences and Roche Life Science, respectively. *Ex Taq* (*Taq* DNA polymerase) and ATP were purchased from Takara. Human α-thrombin and fetal bovine serum were from Haematologic Technologies and Gibco, respectively. All other reagents were of analytical grade and were purchased from Wako Pure Chemical, Tokyo Chemical Industry, or Sigma-Aldrich. The ssDNAs were synthesized by standard phosphoramidite chemistry and purified by high-performance liquid chromatography, except for the PCR primers, which were purified by solid-phase extraction using a reversed-phase resin.

Preparation of dapoxyl SEDA-immobilized gel

To 1.5 mL of NHS-activated Sepharose 4 Fast Flow, 0.3 μmol of dapoxyl SEDA was coupled according to the manufacturer's instructions in 1.5 mL of coupling buffer (0.2 M $NaHCO_3$/NaOH, 0.5 M NaCl, pH 8.3) containing 10% DMF (v/v).

SELEX procedure

SELEX was performed as previously described with minor modifications.[1] For each round of selection, a 76mer fluorescently labeled ssDNA with a random insert of 40 nucleotides (5′-FAM-GTACCAGCTTATTCAATT-N_{40}-AGATAGTATGTTCATCAG-3′, 0.5 nmol for the first round of selection) was denatured in 0.25 mL of binding buffer (20 mM Tris/HCl, 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, pH 7.6) at 95°C for 5 min and then allowed to cool to room

temperature for 30 min. The folded ssDNA was loaded onto a column packed with 0.3 mL of dapoxyl SEDA-immobilized gel. After equilibration for 30 min, the column was washed with 5 mL of the binding buffer. The remaining ssDNA was recovered from the column using urea/TE solution (3.5 M urea, 40 mM Tris/HCl, 10 mM EDTA) by incubation for 10 min at 80°C, collected, and then precipitated with ethanol in the presence of 15 μg of glycogen. The amount of 5′-FAM-labeled ssDNA was fluorescently monitored using an Ultra Evolution fluorescence microplate reader (TECAN). The collected ssDNA was amplified by PCR and subsequently used as the input for the next round of selection.

Negative selection was also performed before positive selection rounds 2–6 and 9. In the negative selection, the folded ssDNA in 0.25 mL of binding buffer was loaded onto a column with 0.3 mL of NHS-activated Sepharose 4 Fast Flow that had been deactivated with blocking buffer (0.5 M ethanolamine, 0.5 M NaCl, pH 8.0). After 30 min, the ssDNA was recovered by elution with 0.6 mL of binding buffer and then subjected to positive selection using the column with dapoxyl SEDA-immobilized gel.

PCR was conducted using the primers 5′-FAM-GTACCAGCTTATTCAATT-3′ and 5′-biotin-CTGATGAACATACTATCT-3′. Fifty μL of each PCR mixture contained 1 unit of *Ex Taq* DNA polymerase, 25 pmol of each primer, and 10 nmol of each dNTP. The thermal cycling consisted of heating at 94°C for 1 min, 46°C for 1 min, and 72°C for 1 min. The amplified biotinylated double-stranded DNA was precipitated with ethanol and bound to the NeutrAvidin agarose resin (Thermo Fisher) for the generation of ssDNA. In the first round of selection, 0.6% of the loaded ssDNA library was retained on the dapoxyl SEDA-immobilized column. A significant increase in binding to dapoxyl SEDA was observed in the subsequent rounds, and after 9 rounds of selection, 32% of the loaded ssDNA was retained on the column. PCR-amplified double-stranded DNA from the 9th-round library was cloned into pGEM-T

vector (Promega) for sequencing by the dideoxy methods.

Fluorescence measurements

The aptamers were denatured in a similar manner to the ssDNA library and then mixed with dapoxyl and occasionally with the analyte. Fluorescence excitation and emission spectra were collected using a Jasco FP-6200 fluorescence spectrophotometer. Fluorescence emission data of multiple samples were collected using an Ultra Evolution fluorescence microplate reader (TECAN). Fluorescence enhancement by the aptamer sensors was evaluated after 10 min of incubation with dapoxyl and the analyte. All measurements of the dapoxyl dyes were conducted in the binding buffer containing 0.025% DMSO, except the measurements of fluorescence quantum yields, which were conducted in the binding buffer containing 0.25% DMSO.

Determination of dissociation constants and quantum yields

We determined the dissociation constants (K_ds) for dapoxyl–aptamer complexes by measuring the increase in fluorescence as a function of increasing aptamer concentration in the presence of a fixed concentration of dapoxyl SEDA or dapoxyl SA (25 nM). Average values from three independent experiments were fitted to a quadratic equation for 1:1 complexation by a nonlinear regression analysis using the Kaleidagraph software.[2] The fluorescence quantum yields were determined in the presence of excess DAP-10-42 according to the literature method;[3] Lucifer yellow, which has a quantum yield of 0.21 in water, was used as a standard.[4]

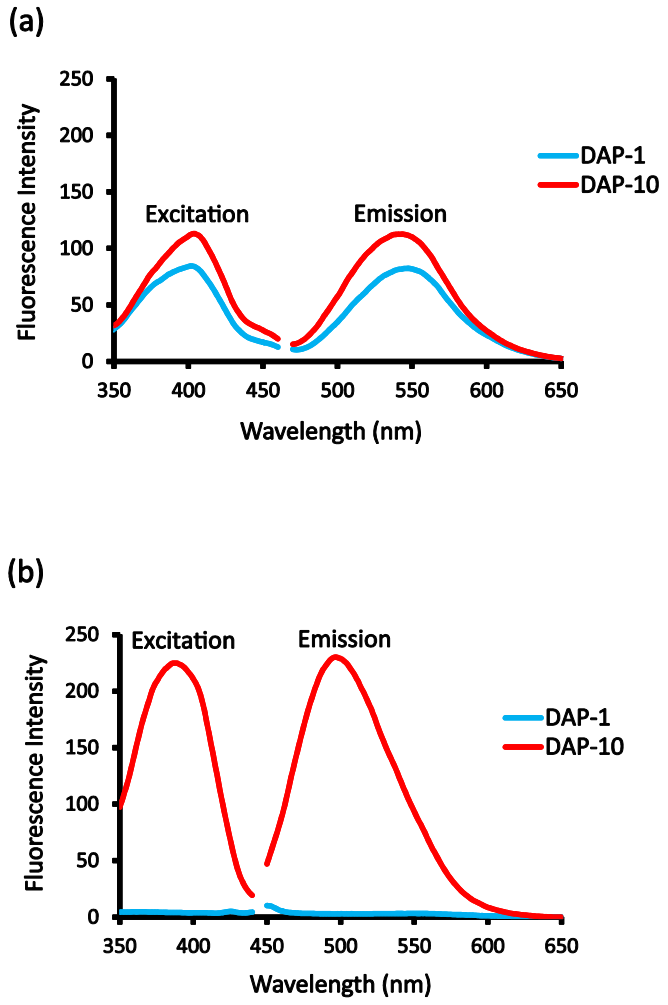

Fig. S1 Excitation and emission spectra of dapoxyl SEDA (a) and dapoxyl SA (b) in the presence of DAP-1 (250 nM, blue) or DAP-10 (250 nM, red). The excitation spectra of 250 nM dyes were measured with emission at 540 nm for dapoxyl SEDA or at 500 nm for dapoxyl SA. The emission spectra of 250 nM dyes were measured with excitation at 400 nm for dapoxyl SEDA or at 390 nm for dapoxyl SA.

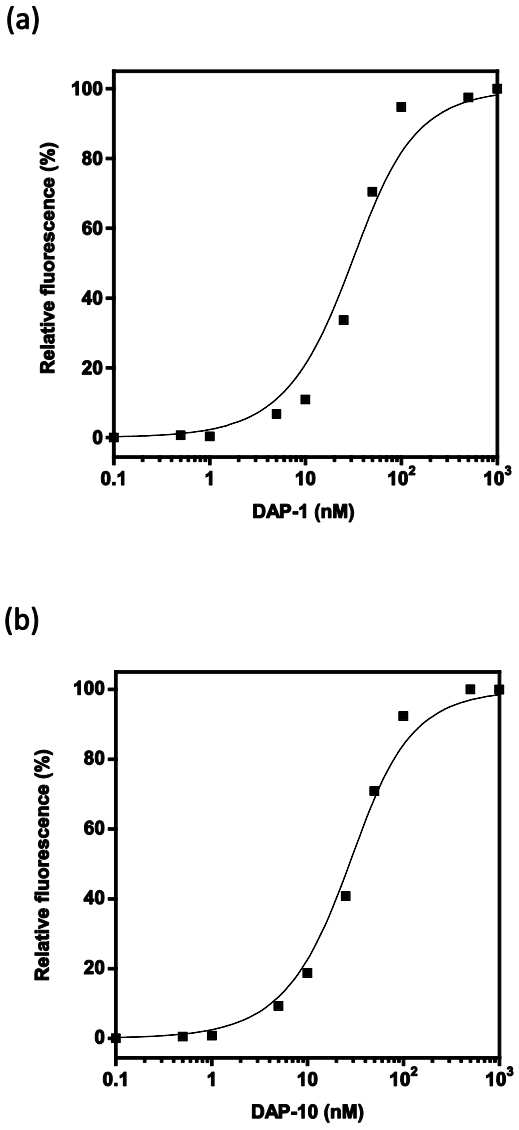

Fig. S2 Determination of Dissociation constants (K_d) of DAP-1 and DAP-10 for dapoxyl SEDA. K_ds of DAP-1 (a) and DAP-10 (b) for dapoxyl SEDA were calculated by measuring the increase in fluorescence (excitation at 400 nm and emission at 540 nm) when increased concentrations of DNA aptamers were incubated with a fixed concentration of dapoxyl SEDA (25 nM). The K_d values were 17.7 ± 3.9 nM for DAP-1 and 14.9 ± 2.0 nM for DAP-10.

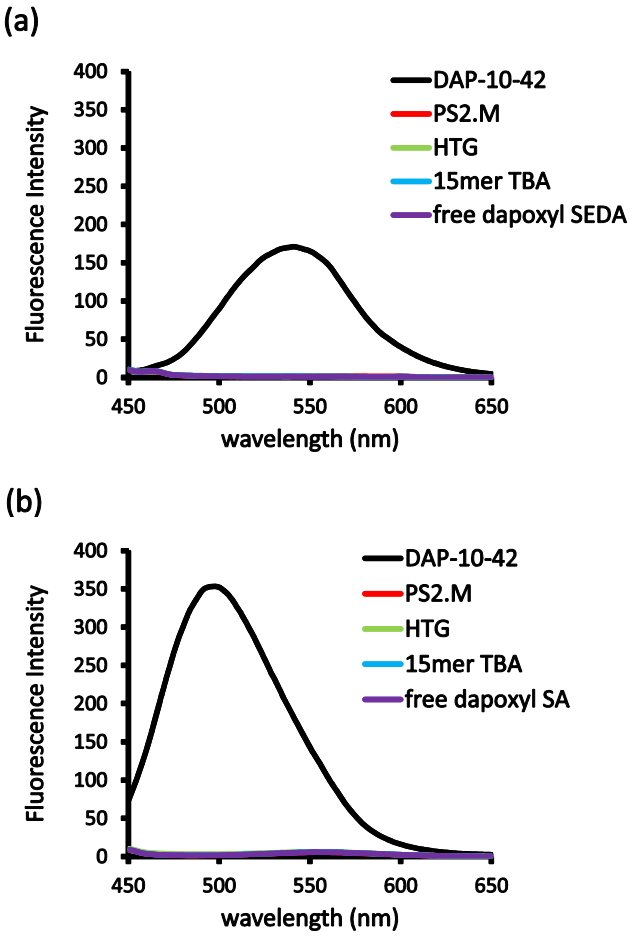

Fig. S3 Fluorescence emission spectra of dapoxyl SEDA (a) and dapoxyl SA (b) in the absence (purple) and presence of DAP-10-42 or typical G-quadruplex-forming DNAs (250 nM). Black: DAP-10-42; red: PS2.M (5′-GTG GGT AGG GCG GGT TGG-3′, 18mer)[5]; green: HTG (5′-AGG GTT AGG GTT AGG GTT AGG G-3′, 22mer)[6]; blue: 15mer thrombin-binding aptamer (TBA, 5′-GGT TGG TGT GGT TGG-3′)[7]. Fluorescence measurements of 250 nM dapoxyl dyes were conducted with an excitation wavelength of 400 nm for dapoxyl SEDA or 390 nm for dapoxyl SA.

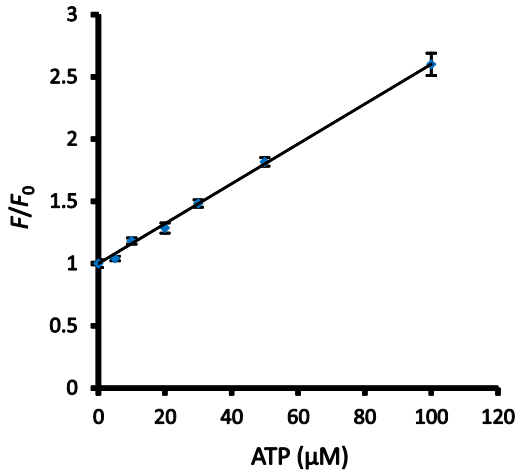

Fig. S4 Linear relationship between the fluorescence enhancement (F/F_0) of 250 nM dapoxyl SA and concentration of ATP. Fluorescence measurements in the presence of 250 nM A1-stem-3 were conducted using the excitation and emission wavelengths of 390 and 500 nm, respectively. Error bars indicate the standard deviation of five experiments at each ATP concentration.

Table S1 Sequences of dapoxyl-binding DNA aptamers. The PCR primer regions are underlined. The numbers in parentheses indicate the sums of the individual sequences among 31 clones.

Clone	Sequence (5′ to 3′)
DAP-1 (27/31)	GTACCAGCTTATTCAATTAACCACATACGTTCGGGTTTTGGTAGGGTAGGGGCTTGTCAGATAGTATGTTCATCAG
DAP-10 (4/31)	GTACCAGCTTATTCAATTACGGGGGAGGGTGTGTGGTCTTGCTTGGTTCGTATTGGTTAGATAGTATGTTCATCAG

Table S2 Sequences and fluorescence enhancement abilities of truncated aptamers derived from DAP-10. The fluorescence enhancement (F.E.) of dapoxyl SEDA (250 nM) by truncated aptamers (250 nM) is reported as a percentage of the fluorescence intensity (excitation at 400 nm and emission at 540 nm) in the presence of truncated aptamers normalized to that in the presence of DAP-10. The PCR primer regions are underlined. Complementary sequences at both ends of DAP-10-42 are shown in italics.

Aptamer	Sequence (5′ to 3′)	F.E. (%)
DAP-10	GTACCAGCTTATTCAATTACGGGGGAGGGTGTGTGGTCTTGCTTGGTTCGTATTGGTTAGATAGTATGTTCATCAG	100
DAP-10-40	ACGGGGGAGGGTGTGTGGTCTTGCTTGGTTCGTATTGGTT	49
DAP-10-58	GTACCAGCTTATTCAATTACGGGGGAGGGTGTGTGGTCTTGCTTGGTTCGTATTGGTT	19
DAP-10-42	*CAATTACG*GGGGGAGGGTGTGTGGTCTTGCTTGGTT*CGTATTG*	150
DAP-10-31	*CG*GGGGAGGGTGTGTGGTCTTGCTTGGTT*CG*	47
DAP-10-27	GGGGAGGGTGTGTGGTCTTGCTTGGTT	1

Table S3 Properties of dapoxyl–DAP-10-42 complexes. Extinction coefficients and quantum yields were measured in the binding buffer containing 0.25% DMSO.

Fluorophore	Excitation maximum (nm)	Emission maximum (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	K_d (nM)
Dapoxyl SEDA	359	561	9,990 (at 400 nm)	0.00086	-
Dapoxyl SEDA bound to DAP-10-42	402	540	10,200 (at 400 nm)	0.621	7.6 ± 1.2
Dapoxyl SA	354	558	5,700 (at 390 nm)	0.025	-
Dapoxyl SA bound to DAP-10-42	391	497	12,000 (at 390 nm)	0.866	24.8 ± 1.9

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