Electronic Supplementary Information

for

Transcription Monitoring Using Fused RNA with a Dye-Binding Light-Up Aptamer as a Tag: A Blue Fluorescent RNA

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CONTENTS:

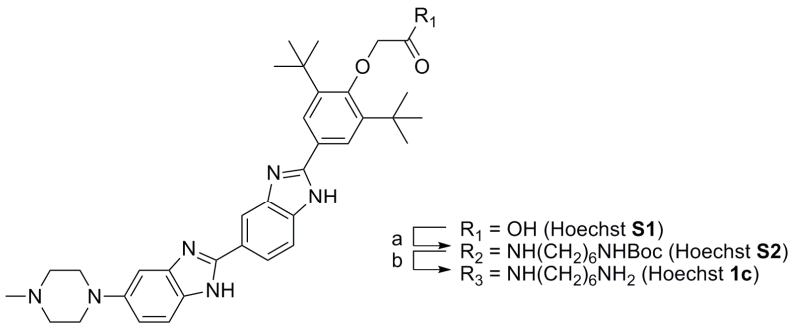
1. General

Reagents and solvents were purchased from standard suppliers and used without further purification. DNA oligomers were purchased from Gene Design Inc. (Japan). Sequencing was carried out using Applied Biosystems 3130/3130xl Genetic Analyzer (Applied Biosystems). Fluorescence spectra were measured with RF-5300PC spectrofluorophotometer (Shimadzu). SPR analyses were performed with a BIAcore X system (GE Healthcare). Fluorescence intensities in transcription monitoring were measured with a Wallac 1420 ARVOsx (PerkinElmer Life Sciences).

2. Synthesis of Hoechst derivatives

Hoechst derivatives 1, 1a, and 1b. Hoechst derivatives **1**, **1a**, and **1b** were prepared according to our previous report.[1]

Hoechat derivative 1c (*N*-(6-aminohexyl)-2-(2,6-di-*tert*-butyl-4-(5-(4-methylpiperazin-1-yl)-1*H*,1'*H*-2,5'-bibenzo[*d*]imidazol-2'-yl)phenoxy)acetamide).



Scheme S1 Synthesis of Hoechst derivatives. *Reagents*: (a) $NH_2(CH_2)_6NHBoc$, EDCI, NHS, DIEPA; (b) 4N HCl in ethyl acetate, 49% in two steps.

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 6.4 mg, 0.033 mmol), *N*-hydroxysuccinimide (NHS, 3.9 mg, 0.034 mmol), and diisopropylethylamine (DIPEA, 4.4 mg, 0.034 mmol) were added to a solution of Hoechst **S1**·TEAA salt[1] (2 mg, 0.0027 mmol) in dry DMF (800 μL). The resulting mixture was stirred under N_2 at room temperature. After stirring for 1 h, $H_2N(CH_2)_6NHBoc$ (7.3 mg, 0.034 mmol) was added, and the mixture was stirred under N_2 for additional 10 h at room temperature. The crude product was purified by HPLC and lyophilized to give Hoechst **S2**. **S2** was then dissolved in 1/1 mixture of CH_3OH/4N HCl in ethyl acetate. The mixture was stored for 2 h at room temperature and the organic solvent was removed. The crude product was purified by HPLC and lyophilized to give Hoechst **1c** in a yield of 49%. The concentration of **1c**, when needed, was determined by use of the known extinction coefficient of Hoechst 33342. MALDI-TOF *m*/*z* calcd. for $[M + H]^+$ 693.46, found: 693.28.

3. In Vitro Selection.

dsDNA template for ssRNA pool. dsDNA pool containing 31 randomized nucleotides were prepared by PCR amplification of ssDNA template 5'-GGT GAT CAG ATT CTG ATC CAN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNT GAA GCT TGG ATC CGT CGC-3' using primers 5'-GTA ATA CGA CTC ACT ATA GGG TGA TCA GAT TCT GAT CCA-3' and 5'-GCG ACG GAT CCA AGC TTC A-3', wherein promoter region for T7 RNA polymerase is underlined.

In vitro selection. *In vitro* selection was carried out by referring conventional protocols with slight modifications.[1,2] ssRNA pool that containing 31 randomized nucleotides was transcribed from the dsDNA template using a T7 MEGAshortscript kit (Ambion). Transcribed RNA (10 nmol for the initial and second rounds, 1 nmol for the third and subsequent rounds) was dissolved in 150 μL of 1× PBS (pH 7.4, 10 mM phosphate buffer containing 138 mM NaCl and 2.7 mM KCl) and then heated at 60 °C for 3 min, cooled to room temprature and left for 10 min. Negative selection was performed before each positive selection. In the negative selection, the pre-annealed RNA library was applied to streptavidin-coated magnetic beads (1 mg, pre-washed with 1× PBS) and incubated for 15 min at room temprature under mild shaking. The recovered RNA, which was not bound to the streptavidin-coated magnetic beads, was then subjected to positive selection. The recovered RNA solution was incubated with Hoechst **1b**-immobilized magnetic beads[1] (0.1 mg, pre-washed with 1× PBS) for 15 min at room temprature under mild shaking. After the beads were washed three times with 1000 μL of wash buffer (1× PBS (pH 7.4, 10 mM phosphate buffer containing 138 mM NaCl and 2.7 mM KCl) containing 2.5 mM $MgCl_2$ and 0.05% Tween20), they were detached from the magnetic beads with 400 μL of elution buffer (40 mM Tris-HCl pH 8.0 containing 0.02% Tween20, 10 mM EDTA, and 3.5 M urea) by 30 min incubation at room temprature. The recovered RNA was purified by 2-propanol precipitation, followed by ethanol precipitation. Collected RNA was reverse transcribed and amplified using a PrimeScript One Step RT-PCR Kit (TaKaRa). Amplified dsDNA was used for the next round.

Cloning and Sequencing. The RT-PCR products of enriched 8th round pool were ligated into a pDrive Cloning Vector (Qiagen) and cloned into EZ Competent Cell (Qiagen) according to the manufacture's manual. Plasmid DNAs were isolated and sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit and Applied Biosystems 3130/3130xl Genetic Analyzer (Applied Biosystems).

4. Preparation of RNA aptamers

The dsDNA templates for RNA aptamers were prepared by PCR amplification using synthetic template and primers. RNA-aptamers were transcribed from the dsDNA templates using a T7 MEGAshortscript kit (Ambion), and purified by 8% PAGE containing 7 M urea.

5. Fluorescence measurement

Fluorescence spectra were obtained at 25 °C. Hoechst derivatives (200 nM) were dissolved in the binding buffer (1× PBS (pH 7.4, 10 mM phosphate buffer containing 138 mM NaCl and 2.7 mM KCl) containing 2.5 mM $MgCl_2$) in the presence (200 nM), or absence of aptamers. The solutions were excited at 345 nm, and the emissions were monitored in the range of 360-650 nm wavelength. Fluorescence enhancement (I_{on}/I_{off}) refers to the ratio of intensities of a Hoechst derivative (excitation at 345 nm, emission at 470 nm) in the presence (I_{on}) and the absence (I_{off}) of an aptamer after subtracting background fluorescence intensity of the buffer.

6. Surface plasmon resonance analysis

Hoechst **1b** was immobilized on a sensor chip SA (streptavidin) in a continuous flow of HBS-N buffer (pH 7.4, 10 mM HEPES containing 150 mM NaCl) at a flow rate of 10 μL min^{-1}. A solution (60 μL) of Hoechst **1b** (1.0 μM) was injected onto the streptavidin-coated gold surface, and nonspecifically bound materials were washed off with a flush of 100 mM NaOH. Binding experiments with various concentrations of aptamer II (30 μL) were performed at 25 °C in a continuous flow of running buffer (1× PBS containing 2.5 mM Mg^{2+}) at a flow rate of 20 μL min^{-1}. Kinetic analyses were performed with BIAevaluation 3.1 software.

7. Transcription monotoring

Preparation of dsDNA template for transcription of luciferase mRNA fused with an aptamer tag. dsDNA encoding the RNA-tag domain, which contains five aptamer sequences, was PCR amplified using template 5'-GTA TCG CAG TCC CAG GTT CGT TTT CGA AGG GAC TGT TTT TTA CTG ACC AGG TTC GTT TTC GAA GGT CAG TTT TTT TGT CGG GCA GGT TCG TTT TCG AAG CCC GAC CTT GAG GAC GGA CAG G-3', forward primer 5'-ATC GCC GTG TAA TTC TAG ACT GAG GCA GGT TCG TTT TCG AAG CCT CAG GTA TCG CAG TCC CAG G-3', and reverse primer 5'-GTC TGC TCG AAG CGG CCG GCC GAC GGA CTT CGA AAA CGA ACC TGT CCG TCC TCA AG-3'. The amplified dsDNA was inserted into the Xba I-Fse I site immediately downstream of the stop codon in the pGL3-Control Vector (Promega) and cloned using *E.coli* DH5α.

dsDNA template for T7 transcription of luciferase mRNA having the RNA-tag domain was PCR amplified using this vector as template, forward primer 5'-<u>GTA ATA CGA CTC ACT ATA</u> GGC ATT CCG GTA CTG-3', and reverse primer 5'-GTT GTT AAC TTG TTT ATT GCA GCT TAT AAT GG-3' (promoter region for T7 RNA polymerase is underlined). The amplified dsDNA was purified using GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) or QIAquick PCR Purification Kit (Qiagen) according to the manufacture's manual. dsDNA template for transcription of control luciferase mRNA was prepared by PCR amplification using the original pGL3-Control Vector as template.

Tanscription monitoring. mRNAs were transcribed from the dsDNA-templates (6.8 ng μL^{-1}) in 50 μL of transcription buffer containing 7.5 mM NTPs (T7-MEGAscript, Ambion), and 2.5 μM Hoechst **1c**.

Hoechst **1c**: After incubation, 5 μL of the reaction solution was diluted with 145 μL of binding buffer (1× PBS (pH 7.4, 10 mM phosphate buffer containing 138 mM NaCl and 2.7 mM KCl) containing 2.5 mM $MgCl_2$, pre-heated at 37 °C). Fluorescence intensities were measured by Wallac 1420 ARVOsx multilabel counter using ex/em =355/460 filter set.

RiboGreen: After incubation, 2 μL of the reaction solution was diluted with 98 μL of water. 2 μL of the diluted solution was mixed with 10 μL of 20× TE buffer, 100 μL of RiboGreen (1/200 diluted), and 88 μL of water. Fluorescence intensities were measured by Wallac 1420 ARVOsx multilabel counter using ex/em =485/535 filter set.

8. Fig. S1

(a)

	I_{on}/I_{off}
class I (18/32)	
(18) 5'GGGTGATCAGATTCTGATCCAGGAATTCAAGCGTTAGATCGCAGACTGTGGATGAAGCTTGGATCCGTCGC3'	5.1
class II (3/32)	
(3) GGGTGATCAGATTCTGATCCAGGTTACCAAGCAGGTTCGGCCTCGTCTGAGGTGAAGCTTGGATCCGTCGC	13.1
class III (4/32)	
(2) GGGTGATCAGATTCTGATCCAGTCTTGTCCAAGCGTTGAATCCCTGGGGTGATGAAGCTTGGATCCGTCGC	3.3
(1) GGGTGATCAGATTCTGATCCACTGTGTTCCAGGCGTTGTACCTTTCGGATGGTGAAGCTTGGATCCGTCGC	3.1
(1) GGGTGATCAGATTCTGATCCACGTCCAGGCGTTGAATCGACTTCGGTGCTGATGAAGCTTGGATCCGTCGC	10.8

(b)

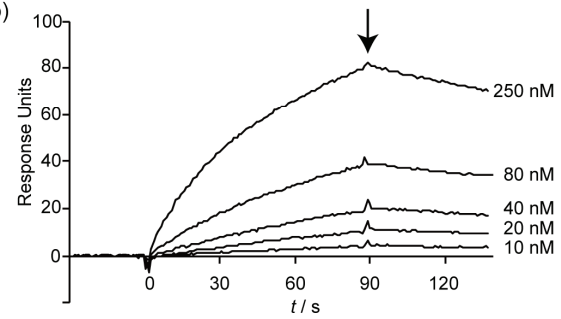


Fig. S1 (a) The sequences of aptamer classes I-III. The numbers in parentheses indicate the sum of the individual sequences among 32 clones. The primer sites are underlined. On the right side, the fluorescence enhancements (I_{on}/I_{off} at 470 nm, ex. = 345 nm) of Hoechst **1a** (200 nM) with each aptamers (200 nM) are shown. (b) Display of the SPR sensorgrams showing the affinity of aptamer II for Hoechst **1b**. Various concentrations of aptamer II (10-250 nM) were injected (total = 30 μL, t = 90 s) at a flow rate of 20 μL min^{-1} over a sensor chip SA pre-coated with Hoechst **1b**. The arrow indicates the end of injection.

9. Fig. S2

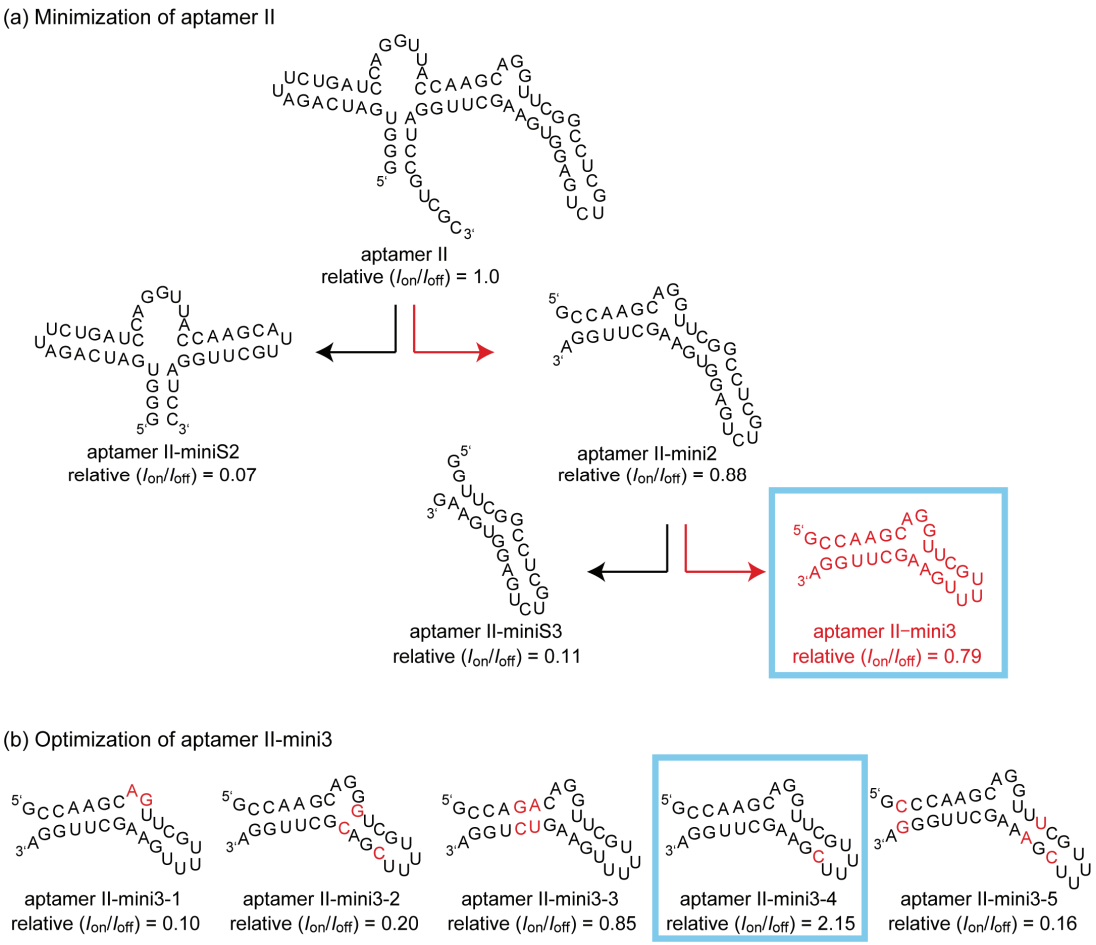


Fig. S2 Aptamer II and (a) minimized/(b) optimized aptamers. The relative fluorescence enhancement compared to that of Hoechst **1a**/aptamer II pair (relative (I_{on}/I_{off})) is shown at the bottom of each structure, where (I_{on}/I_{off}) of the Hoechst **1a**/aptamer II pair is set at 1.0. The fluorescence intensities (excitation = 345 nm and emission = 470 nm) of Hoechst derivative **1c** (200 nM) with or without aptamer (200 nM) were measured in the binding buffer (1× PBS containing 2.5 mM $MgCl_2$) at 25 °C.

10. Fig. S3

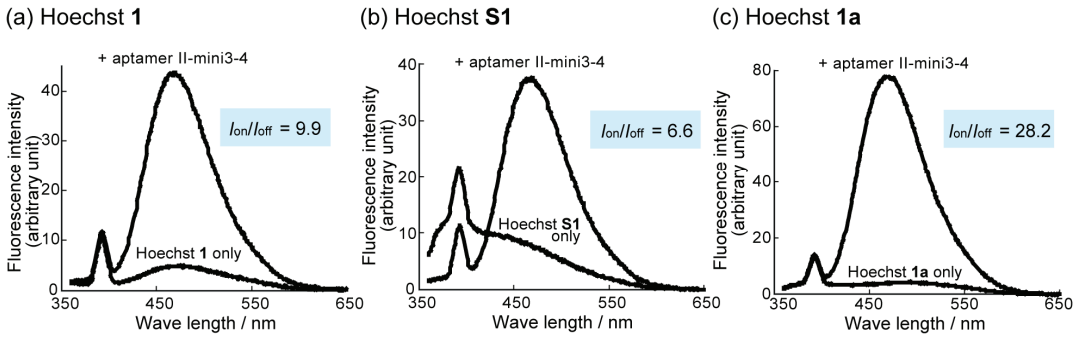


Fig. S3 Fluorescence spectra of (a) Hoechst **1**, (b) Hoechst **S1**, and (c) Hoechst **1a** (200 nM) in the absence and presence of aptamer II-mini3-4 (200 nM). Fluorescence spectra (excitation at 345 nm) were measured in the binding buffer (1× PBS containing 2.5 mM $MgCl_2$) at 25 °C. Fluorescence enhancement (I_{on}/I_{off}) refers to the ratio of intensities of a Hoechst derivative (excitation at 345 nm, emission at 470 nm) in the presence (I_{on}) and the absence (I_{off}) of an aptamer after subtracting background fluorescence intensity of the buffer.

11. Fig. S4

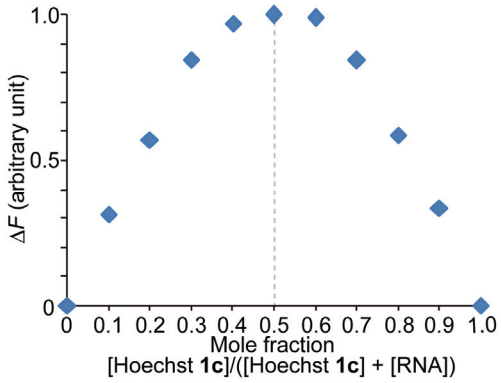


Fig. S4 Job plot analysis for binding of Hoechst **1c** to aptamer II-mini3-4. Change in fluorescence intensity (ΔF, excitation at 345 nm and emission at 470 nm) is plotted against molar fraction of the Hoechst **1c**. Fluorescence spectra were recorded in the binding buffer (1× PBS containing 2.5 mM $MgCl_2$) at 25 °C using a constant value for [Hoechst **1c**] + [RNA] = 0.1 μM.

12. References

1. S. Sando, A. Narita and Y. Aoyama, *ChemBioChem*, 2007, **8**, 1795-1803.
2. G. Werstuck and M. R. Green, *Science*, 1998, **282**, 296-298.