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Electronic Supporting Information
for

Aptazyme-based molecular device that converts a small-molecule input to an RNA output

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Contents:

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Experimental Section:

Materials

All the DNA oligonucleotides were synthesized and OPC-purified by Sigma Aldrich Japan (Hokkaido, Japan) or by Operon Biotechnologies (Tokyo, Japan). DNA oligonucleotides > 50 nucleotides long were purified using additional denaturing polyacrylamide gel electrophoresis with a denaturing gel containing 8% polyacrylamide (acrylamide:bisacrylamide, 29:1) and 8.0 M urea. Ultrapure DNase/RNase-free distilled water was purchased from Invitrogen (Carlsbad, CA, USA). Bst DNA polymerase was purchased from Takara (Tokyo, Japan). KOD plus DNA polymerase and Thermo RNA polymerase were purchased from TOYOBO (Tokyo, Japan). RQ DNase I and SYBR Gold Nucleic Acid Gel Stain were purchased from Invitrogen. Flavin mononucleotide sodium salt (FMN) was purchased from Wako (Osaka, Japan). All other chemicals were of analytical grade and used without further purification. Band intensity was measured using an FLA-5100 fluoroimage analyzer (FUJI Film, Tokyo, Japan).

***In vitro* transcription of the aptazyme**

The DNA template for aptazyme transcription was obtained using polymerase chain reaction (PCR). PCR was performed using KOD plus DNA polymerase according to the manufacturer's instructions in 300 μL reaction solution. The reaction solution contained 7.5 pmol forward and reverse templates (Table S1) and 90 pmol forward and reverse primers (Table S1). The PCR product was purified by phenol extraction and ethanol precipitation. The precipitated product was dissolved in 60 μL distilled water. The aptazyme was obtained by *in vitro* transcription of DNA templates by using T7 RNA polymerase. The *in vitro* transcription was performed in 150 μL reaction solution containing 40 mM Tris-HCl (pH 8.0), 14 mM $MgCl_2$, 1 mM Tris (2-carboxyethyl) phosphine (TCEP), 3 mM of each NTP, 0.5 units RNase inhibitor, 2.5 units T7 RNA polymerase, and 60 μL purified DNA template in solution. KOH was added to adjust the pH of the solution to 7.8. After incubation at 37°C for 2 h, the solution was incubated at 37°C for an additional 15 min with 1 unit of RQ DNase I. The transcribed aptazyme was purified using a denaturing gel containing 8% polyacrylamide (acrylamide:bisacrylamide, 29:1) and 8.0 M urea. The concentration of the purified aptazyme was determined by absorbance at 260 nm.

Device transcription assay

Device reactions were performed by incubation at 50°C for 60 min. The reaction buffer for the device contained 40 mM Tris-HCl (pH 8.0), 50 mM NaCl, 33.8 mM $MgCl_2$, 2.0 mM TCEP, 0.20 mM of each dNTP, 2.0 mM adenosine triphosphate (ATP), 2.0 mM guanosine triphosphate (GTP), 2.0 mM cytidine triphosphate (CTP), 1.825 mM uridine triphosphate (UTP), and 0.175 mM fluorescein-12-UTP. The concentrations of the aptazyme and DNA subunit 1 were 1.0 μM and 0.50

μM, respectively. In the control reaction for producing the appropriate output RNA, 1.0 μM of cIII DNA (Table S1), which had the complementary sequence to domain III, was used as the primer to produce double-stranded promoter. The concentrations of Bst DNA polymerase and Thermo T7 RNA polymerase were 0.80 and 1.25 units/μL, respectively. The reaction was stopped with an equal volume of gel loading buffer (2× GLB) containing 8.0 M urea, 10% (v/v) glycerol, 50 mM ethylenediaminetetraacetic acid (EDTA), and 0.05% (w/v) bromophenol blue (BPB). Next, 8.0 μL of reaction solution was applied to a denaturing gel containing 20% polyacrylamide (acrylamide:bisacrylamide, 29:1) and 8.0 M urea. Because of the high amount of output RNA, the control reaction sample was diluted to 1:20 before applying to the gel. Tris-borate-EDTA buffer (89 mM Tris-HCl, 89 mM boric acid, and 2 mM EDTA, pH 8.3) was used as the running buffer for electrophoresis. The gel was run at a constant voltage of 250 V at constant temperature of 50°C. After electrophoresis, the fluorescein-labeled output RNA in the gel was observed using an FLA-5100 fluoroimage analyzer.

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Table S1. Oligonucleotide sequences used in this study

Strand name	DNA sequence
DNA subunit 1	5′-TGCGGCGTAACTATCTCTGATCTCCCTATAGTGAGTCGTAT TACGGCGGCCGTTT-3′
cIII DNA	5′-GAAACGGCCGCC-3′
Forward template for the FMN-dependent aptazyme	5′-**GGTAACACGCATATGTAATACGACTCACTATA**GGCCGT AGGTTGGAAACGACCCTGATGAGCCTTAGGATATGCTTCGG CAGAAGGACGTCGAAACGGCTCGG-3′
Reverse template for the FMN-dependent aptazyme	5′-GGGAACCGTTTCGACGTCCTTCTGCCGAAGCATATCCTAA GGCTCATCAGGGTCGTTTCCAACCTACG-3′
Forward primer for the FMN-dependent aptazyme, cleavage mutant, and interaction mutant	5′-GGTAACACGCATATGTAATACGACTC-3′
Reverse primer for the FMN-dependent aptazyme and cleavage mutant	5′-GGCGGCCGTTTCGACGTCCTTCTG-3′
Forward template for the cleavage mutant	5′-**GGTAACACGCATATGTAATACGACTCACTATA**GGCCGT AGGTTGGAAACGACCCTGGTGAGCCTTAGGATATG-3′
Reverse template for the cleavage mutant	5′-GGGAACCGTTTCGACGTCCTTCTGCCGAAGCATATCCTAA GGCTCACCAGGGTCGTTTCCAACCTACG-3′
Forward template for the interaction mutant	5′-**GGTAACACGCATATGTAATACGACTCACTATA**GGCCGT AGGTTGGAAACGACCCTGATGAGCCTTAGGATATG-3′
Reverse template for the interaction mutant	5′-GGGAACCGTTTCGACGTCCCTCTGCCGAAGCATATCCTAA GGCTCATCAGGGTCGTTTCCAACCTACG-3′
Reverse primer for the interaction mutant	5′-GGCGGCCGTTTCGACGTCCCTCTG-3′
TAMRA-labeled DNA probe	5′-TGCGGCGTAACTATCTCTGATCTCCC-3′-TAMRA
Complementary DNA subunit 1	5′-AAACGGCCGCCGTAATACGACTCACTATAGGGAGATCAG AGATAGTTACGCCGCA-3′

The **bold letters** indicate the T7 promoter sequence

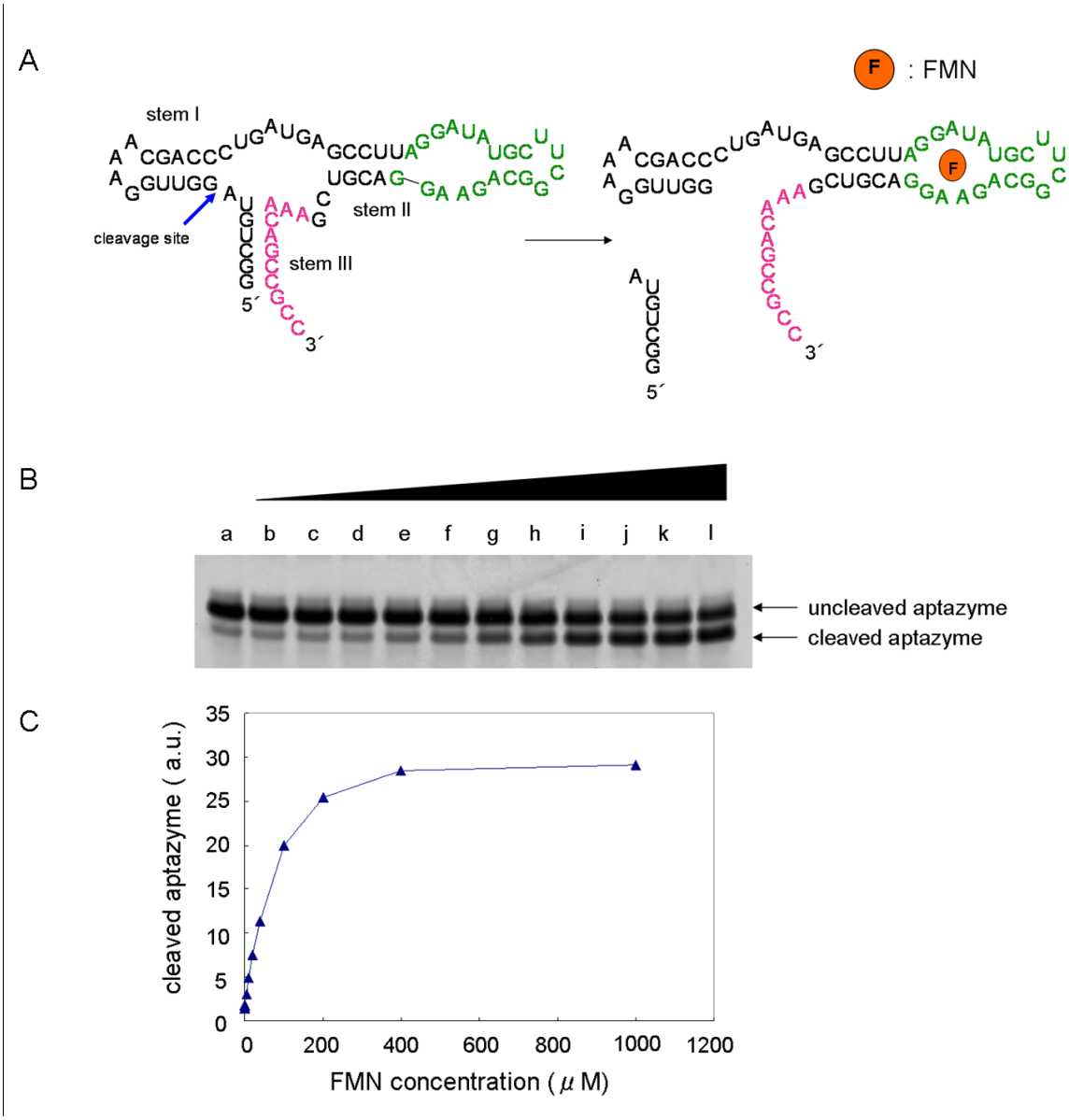

Figure S1. Design and analysis of the aptazyme

A) Design of the FMN-dependent aptazyme

Based on the flavin mononucleotide (FMN)-dependent aptazyme constructed by Soukup *et al.*,[1] an aptazyme suitable for our purpose was constructed. By circular permutation, the 5′ and 3′ termini that had been on the stem I of the parent aptazyme were moved to stem III. The GAAA loop that had been on stem III of the parent aptazyme was moved to stem I. Moreover, a 3-nucleotide (nt) overhang sequence—GCC—was added to the 3′ terminus to stabilize the hybridization between the aptazyme and DNA subunit 1. The resultant aptazyme had a 6-nt stem III. Green nucleotides represent the anti-FMN aptamer domain. Pink nucleotides represent the sequence that hybridized with DNA subunit 1. The cleavage site is indicated by a blue arrow.

Binding of an FMN molecule to the aptamer domain changed the conformation of the aptazyme into

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a self-cleaving form. In the cleaved aptazyme, a fragment dissociates from its complementary sequence on the aptazyme.

B) Gel analysis of the self-cleaved aptazyme in the presence of different FMN concentrations (μM): (a) 0, (b) 0.04, (c) 0.4, (d) 1.0, (e) 4.0, (f) 10, (g) 20, (h) 40, (i) 100, (j) 200, (k) 400, and (l) 1,000. The reaction solution for the aptazyme was incubated at 50°C for 60 min. The reaction buffer contained 40 mM Tris-HCl (pH 8.0), 50 mM NaCl, 33.8 mM $MgCl_2$, 2.0 mM Tris (2-carboxyethyl) phosphine (TCEP), 0.20 mM each dNTP, and 2.0 mM NTP. The concentration of the aptazyme was 1.0 μM. The reactions were stopped with an equal volume of 2× gel loading buffer containing 8.0 M urea, 10% (v/v) glycerol, 50 mM ethylenediaminetetraacetic acid (EDTA), and 0.05% (w/v) bromophenol blue. Next, 8.0 μL of the solution was applied to a denaturing gel containing 8.0% polyacrylamide (acrylamide:bisacrylamide, 29:1) and 8.0 M urea. Tris-borate-EDTA buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA, pH 8.3) was used as the running buffer for electrophoresis. The gel was run at a constant voltage of 250 V in a chamber set at 50°C. After electrophoresis, the gel was stained with SYBR Gold Nucleic Acid Gel Stain and observed on an FLA-5100 fluoroimage analyzer.

C) A plot of the intensity of the cleaved aptazyme band as a function of the input FMN concentration

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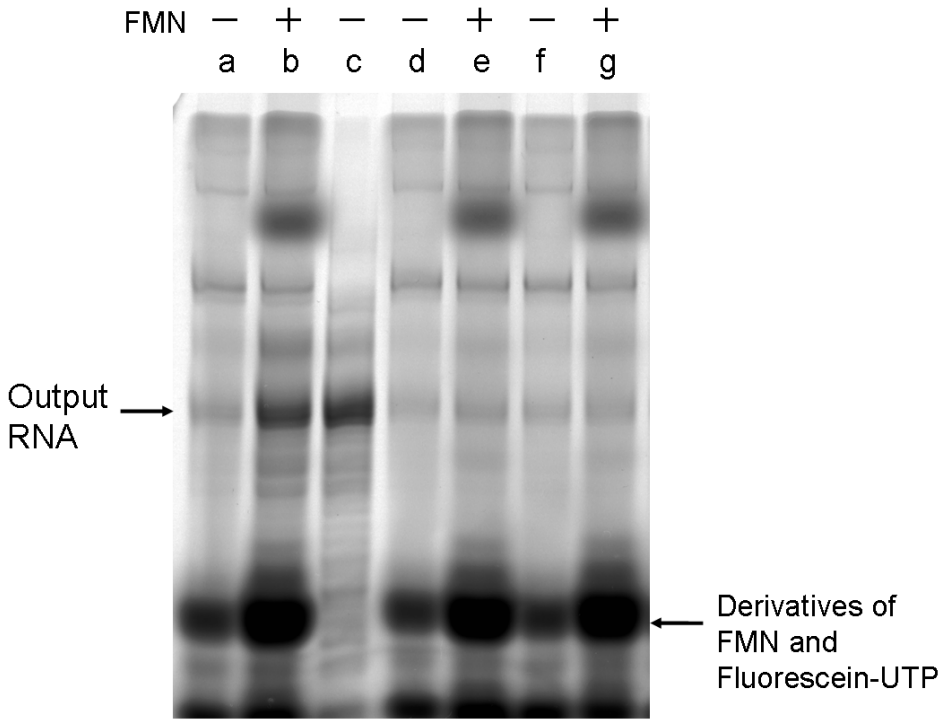

Figure S2. Full image of the gel showing the operation of the device dependent on the flavin mononucleotide (FMN) input: (a) no input for the FMN-dependent aptazyme, (b) 400 μM FMN for the FMN-dependent aptazyme, (c) control reaction to produce the appropriate output RNA, (d) no input for the cleavage mutant, (e) 400 μM FMN for the cleavage mutant, (f) no input for the interaction mutant, and (g) 400 μM FMN for the interaction mutant.

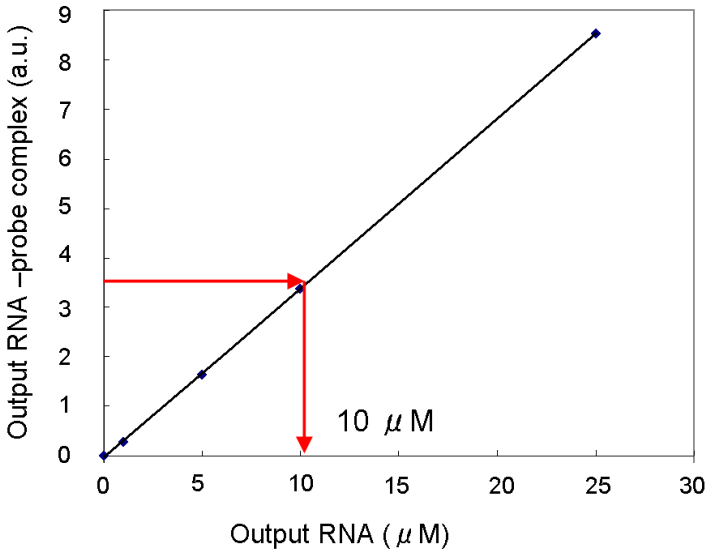

Figure S3. Quantification of the output RNA generated by the device in response to flavin mononucleotide (FMN) input.

To determine the concentration of output RNA produced in response to FMN input, a standard curve of the output RNA-DNA probe complex with the respective output RNA concentrations was constructed. To make the standard curve, serially diluted solutions of purified output RNA were mixed with 25 μM TAMRA-labeled DNA probe (Table S1) that specifically hybridizes with the output RNA. The purified output RNA was prepared by *in vitro* transcription under reaction conditions similar to that for the production of the aptazyme. For the *in vitro* transcription, we used 0.50 μmol of the double-stranded DNA subunit 1; the Complementary DNA subunit 1 strand was used as the template for output RNA. After incubation at room temperature for 10 min, equal volume of 2× gel loading buffer containing 10% (v/v) glycerol and 0.05% (w/v) bromophenol blue (BPB) was added to the solutions. Next, 5.0 μL of the solution was applied to a non-denaturing gel containing 20% polyacrylamide (acrylamide:bisacrylamide, 29:1). Gel electrophoresis was performed as described in Figure S1B, except that it was run at room temperature. After electrophoresis, the band intensities of the output RNA-DNA probe complexes in the gel were measured using an FLA-5100 fluoroimage analyzer. The band intensities of the output RNA-DNA probe complexes were plotted against the output RNA concentration (black line). The red lines highlight the concentration of output RNA produced by the device transcription assay in the presence of 400 μM FMN. From this result, the concentration of output RNA generated by the device was determined to be 10 μM.

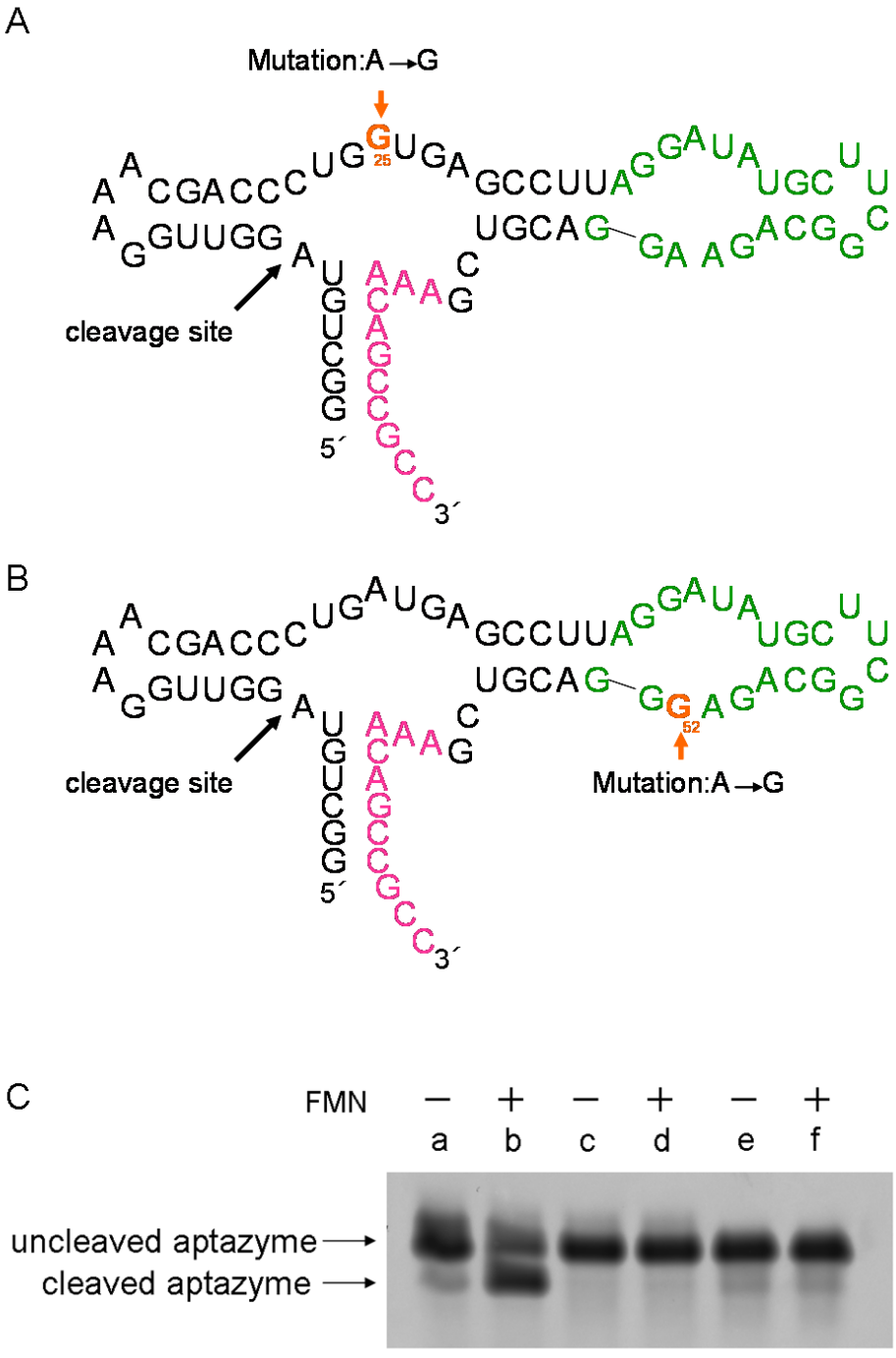

Figure S4. Design and analysis of aptazyme mutants

A) Design of a cleavage mutant of the flavin mononucleotide (FMN)-dependent aptazyme

To construct an aptazyme mutant lacking the self-cleavage activity, we introduced a mutation into the catalytic pocket of the hammerhead ribozyme. We substituted A for G at position 25 of the

aptazyme because this substitution is known to abolish the cleavage activity of a hammerhead ribozyme.[2]

B) Design of an interaction mutant of the FMN-dependent aptazyme

To construct a mutant aptazyme lacking the binding activity to FMN, we introduced a mutation in the FMN-binding aptamer. We substituted A for G at position 52 of the aptazyme because it is one of the conserved nucleotides of the FMN-binding aptamer.[3]

C) Gel analysis of the self-cleaved aptazyme mutants in the absence and presence of FMN input: (a) no input for the FMN-dependent aptazyme, (b) 400 μM FMN for the FMN-dependent aptazyme, (c) no input for the cleavage mutant, (d) 400 μM FMN for the cleavage mutant, (e) no input for the interaction mutant, and (f) 400 μM FMN for the interaction mutant.

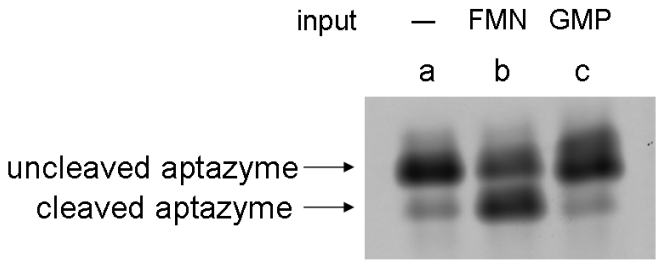

Figure S5. Gel analysis of the self-cleaved aptazyme in the presence of a control input: (a) no input, (b) 400 μM flavin mononucleotide (FMN), and (c) 400 μM guanine monophosphate (GMP)

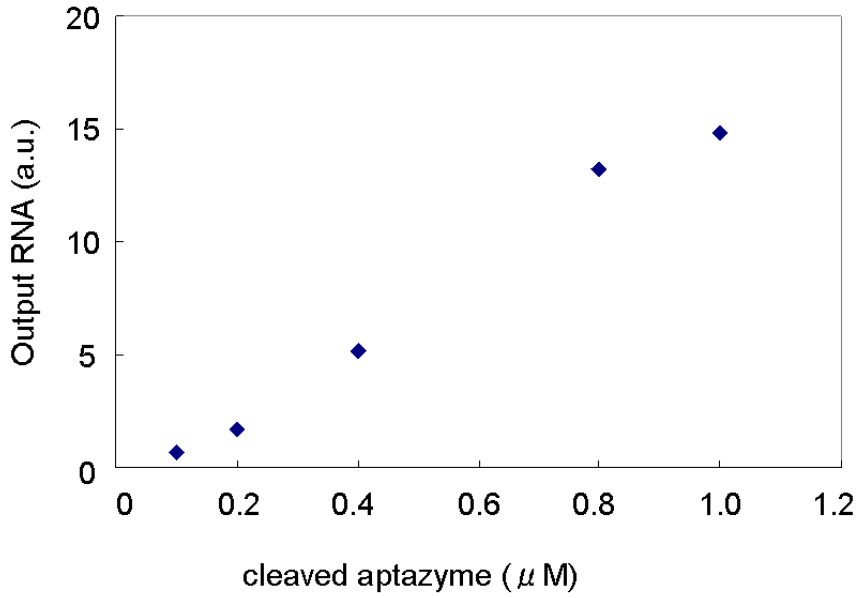

Figure S6. The output RNA generated in response to varying concentrations of cleaved aptazyme in the absence of flavin mononucleotide (FMN).

Output RNA generation in response to the input of cleaved aptazyme, instead of FMN, was measured. The reaction conditions were the same as those of the device transcription assay described previously. As the concentration of the cleaved aptazyme increased, the amount of the output RNA increased.

References

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