Electronic Supplementary Information

For

Easy Design of Logic Gates based on Aptazymes and Noncrosslinking Gold Nanoparticle Aggregation

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Experimental Section

General. Reagents and solvents were purchased from standard suppliers and used without further purification. PCR primers and templates were synthesized and OPC-purified by Operon Biotechnologies (Tokyo, Japan), Hokkaido System Science (Sapporo, Japan) or Invitrogen (Tokyo, Japan). Thiol-oligodeoxynucleotide (thiol-probe-DNA) was synthesized and DTT-reduced by Takara Bio (Ohtsu, Japan). Quantification of these oligonucleotides and aptazymes was performed with the spectrophotometer GeneQuant from GE Healthcare (Buckinghamshire, UK). A colloidal solution containing 9.0×10^{10} particles/mL AuNPs with a diameter of 40 nm was purchased from BBInternational (Cardiff, UK). UV-vis absorbance spectra of AuNPs were obtained with NanoVue from GE Healthcare (Buckinghamshire, UK). The aggregation degree was calculated as follows: Aggregation degree (%) = $[(A_{529}^{ref} - A_{529}^{obs}) / A_{529}^{ref}] \times 100$, wherein A_{529}^{ref} and A_{529}^{obs} are the absorbances at 529 nm of only AuNP and observed AuNP solution after calculation, respectively.

Preparation of Probe-DNA-Tethered AuNPs. Preparation of probe-DNA-tethered AuNPs was performed as described previously.[1] The DTT-reduced thiol-probe-DNA (5'-SH-$(CH_2)_6$-d(CTC TTC GAT AAC GGG CAA CC)-3') was purified with ethanol precipitation. A 5-nmol sample of the purified thiol-probe-DNA was mixed with 1 mL of the AuNP solution without salt at 50˚C for 24 h. Then, Tris-HCl (pH 7.6) and NaCl were added to the mixture to final concentrations of 10 mM and 100 mM, respectively. After additional incubation of the mixture at 50˚C for 41 h, AuNPs were centrifuged at 10,000 rpm for 15 min to remove unreacted thiol-probe-DNA. The centrifuged AuNPs were redispersed into 1 mL of 10 mM Tris-HCl (pH 7.6) and 100 mM NaCl and re-centrifuged at 10,000 rpm for 15 min to remove remaining unreacted thiol-probe-DNA. The obtained probe-DNA-tethered AuNPs were redispersed into 50 μL of 10 mM Tris-HCl (pH 7.6) and 100 mM NaCl containing 0.01% Tween 20.

This probe-DNA-tethered AuNP solution was stored at 4˚C and used within 1 week. The surface coverage was estimated from the amounts of unreacted thiol-probe-DNA (17 pmol/cm^2; 500 probe-DNA per particle). In calculations for the logic gates, 1 μL of the probe-DNA-tethered AuNPs (strand concentration of the probe-DNA was 1 μM) was 9-fold diluted with 8 μL of the buffer solution A1 (125 mM Tris-HCl (pH 7.6) and 1.25 M NaCl) for the YES, OR and AND gates, or 8-fold diluted with the buffer solution A2 (143 mM Tris-HCl (pH 7.6) and 1.43 M NaCl) for the NOT gate.

Preparation of DNA Templates for Aptazymes. DNA templates for aptazymes were prepared by polymerase chain reactions (PCR). The PCR was carried out using PrimeSTAR Max DNA Polymerase from Takara Bio (Ohtsu, Japan) according to the manufacturer's instructions in 50 μL of a reaction mixture containing 50 pmol of a forward primer with a T7-promoter sequence, 50 pmol of a reverse primer, and 5 fmol of a synthetic template. The sequences of these primers and templates are summarized below. Next, 48 μL of the resulting solution was purified with a GFX column from GE Healthcare (Buckinghamshire, UK) to be dissolved into 48 μL of nuclease-free water.

PCR Primers and Templates for Preparation of DNA Templates for Aptazymes.

(T7-promoter sequence is underlined.)

DNA template for a theophylline-dependent aptazyme in the YES and OR gates

Forward primer: 5'-d(GTA ATA CGA CTC ACT ATA GGG CGA CCC TGA TGA GCC TGG ATA C)-3'

Reverse primer: 5'-d(CTC TTC GAT AAC GGG CAA CCT ACG GCT TTC AC)-3'

Template: 5'-d(TGA TGA GCC TGG ATA CCA GCC GAA AGG CCC TTG GCA GTT AGA CGA AAC GGT GAA AGC CGT AGG TT)-3'

DNA template for a cGMP-dependent aptazyme in the OR gate

Forward primer: 5'-d(GTA ATA CGA CTC ACT ATA GGG CGA CCC TGA TGA G)-3'

Reverse primer: 5'-d(CTC TTC GAT AAC GGG CAA CCT ACG GCT TTC AC)-3'

Template: 5'-d(GGG CGA CCC TGA TGA GCC CTG CGA TGC AGA AAG GTG CTG ACG ACA CAT CGA AAC GGT GAA AGC CGT AGG T)-3'

DNA template for a theophylline-dependent aptazyme in the AND gate

Forward primer: 5'-d(GTA ATA CGA CTC ACT ATA GGG CGA CCG GGC ATG GAC TGA TGA G)-3'

Reverse primer: 5'-d(GAT AAC GGG CAA CCC TTC TCC CGG CAC GGA TAC GGC TTT CAC)-3'

Template: 5'-d(GGC ATG GAC TGA TGA GCC TGG ATA CCA GCC GAA AGG CCC TTG GCA GTT AGA CGA AAC GGT GAA AGC CGT ATC CGT)-3'

DNA template for a cGMP-dependent aptazyme in the AND gate

Forward primer: 5'-d(GTA ATA CGA CTC ACT ATA GGG AGA AGC TGA TGA G)-3'

Reverse primer: 5'-d(TCC GTG CCG GGA GAA GTA CGG CTT TCA C)-3'

Template: 5'-d(GGA GAA GCT GAT GAG CCC TGC GAT GCA GAA AGG TGC TGA CGA CAC AT C GAA ACG GTG AAA GCC GTA CTT)-3'

DNA template for a theophylline-dependent aptazyme in the NOT gate

Forward primer: 5'-d(GTA ATA CGA CTC ACT ATA GGG CCG TTA TCC TGA TGA G)-3'

Reverse primer: 5'-d(GGT TGC CCG TTA TCT ACG GCT TTC AC)-3'

Template: 5'-d(GGC CGT TAT CCT GAT GAG CCT GGA TAC CAG CCG AAA GGC CCT TGG CAG TTA GAC GAA ACG GTG AAA GCC GTA GAT A)-3'

Preparation of Aptazymes. Aptazymes were obtained by run-off transcription of the DNA templates prepared as above using a MEGAshortscript T7 Kit from Applied Biosystems (Tokyo, Japan). Ten microliters of a reaction mixture containing 4 μL of the purified DNA template was incubated at 37˚C for 2 h. To the reaction mixture was added 1 unit of the TURBO DNase included in the kit, and the mixture was incubated at 37˚C for an additional 15 min. The transcribed aptazymes were simply purified with Microcon YM-30 from Millipore (Tokyo, Japan) and a QIAquick Nucleotide Removal Kit from QIAGEN (Tokyo, Japan). Concentrations of the purified aptazymes were determined by their absorbance at 260 nm.

Illustration of Proposed Secondary Structures of Aptazymes and Probe-DNA-tethered AuNP.

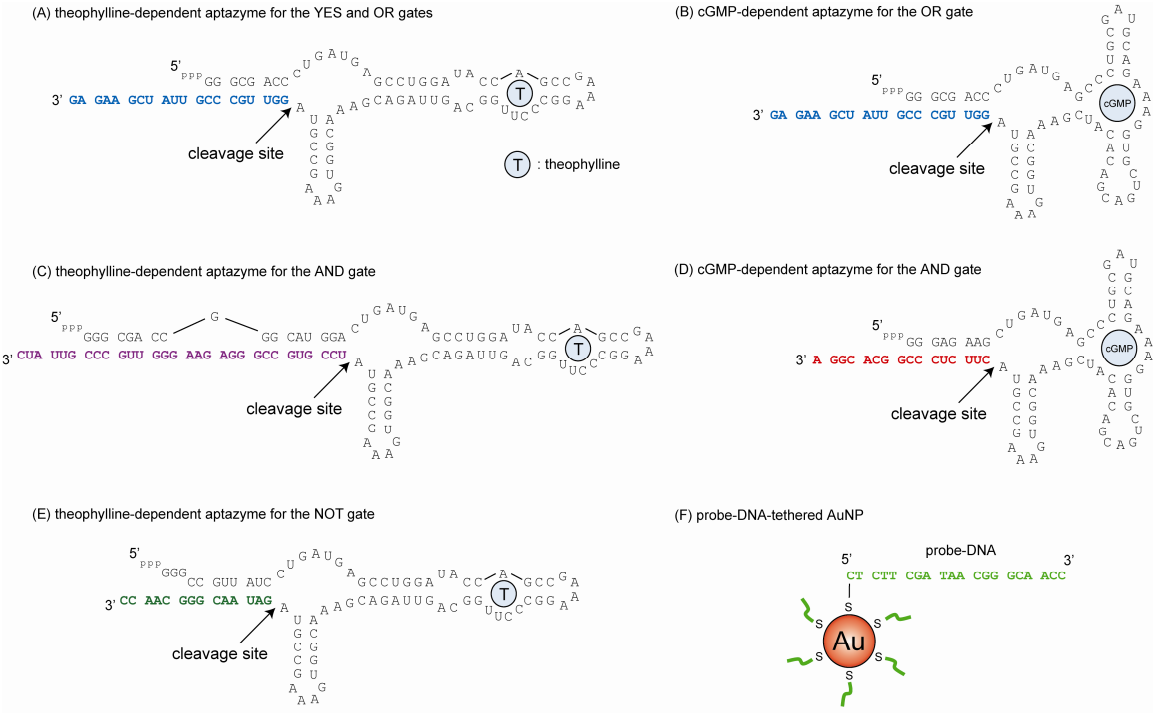

Figure S1. Illustration of proposed secondary structures of various aptazymes in the ON state (A~E)[2] and the probe-DNA-tethered AuNP (F) in the logic gates.

Calculations for Various Logic Gates. Ten microliters of reaction mixture containing 5 μM each of in vitro-transcribed theophylline-dependent aptazyme and/or cGMP-dependent aptazyme for the corresponding gate (YES, OR, or AND) in the buffer solution B (50 mM Tris-HCl (pH 7.6) and 20 mM $MgCl_2$) was incubated at 23˚C for 5 min (or 30 min for the AND gate) in the absence or presence of 1 mM each of input molecules (theophylline and/or cGMP), and then 1 μL of the aptazyme reaction solution was added to 9 μL of the 9-fold-diluted probe-DNA-tethered AuNPs. After the mixture was warmed at 55˚C for 5 min to separate the cleaved RNA from the aptazymes and to make it form duplexes with the probe-DNA, calculation results (i.e. colors of the AuNP solutions) were observed with the naked eye. Photographs and UV-vis spectra of the AuNP solutions were taken by a camera and measured with a spectrometer, respectively.

Design of the NOT gate. A NOT gate that requires little ingenuity was also designed (Figure S2). Although there are some ways to construct a NOT gate, we employed a way to use the same probe-DNA-tethered AuNPs as used in the YES, OR, and AND gate. This NOT gate is made up of the AuNPs, an aptazyme for one input (theophylline), and an aggregation-inducing DNA (ai-DNA, 14 mer) (Figure S2A). In the absence of theophylline, the ai-DNA hybridizes to the probe-DNA on AuNPs with a blunt end at the AuNP surface terminus to induce AuNP aggregation (Figure S2A, left). The amounts of ai-DNA were optimized so that the AuNPs barely aggregated. As a result of the optimization, althogh 15 nM ai-DNA was sufficient to induce AuNP aggregation in the absence of the aptazyme, 50 nM ai-DNA was required in the presence of 500 nM aptazyme. This difference is probably due to some of the ai-DNA hybridizing to the aptazyme before self-cleavage. In contrast, in the presence of theophylline, the ai-DNA hybridized more of the cleaved RNA (14 mer) from the aptazyme than the probe-DNA because of the tighter binding of 14 bp RNA-DNA (i.e. the cleaved RNA and the ai-DNA) than 14 bp DNA-

DNA (i.e. the probe-DNA and the ai-DNA) or 8 bp RNA-RNA (i.e. the cleaved RNA and the aptazyme) interduplex. It should be noted that 8 bp RNA-RNA intraduplex before the self-cleavage is much more stable. Because the amounts of ai-DNA were optimized as above, small amounts of duplex formation between the ai-DNA and the cleaved RNA leave the probe-DNA-tethered AuNPs dispersed (Figure S2A, right). Figures S2B and S2C show the calculation results for this NOT gate, which are ideal responses. In this NOT gate, although one hybridization switch must be designed, the once-designed switch can be shared with other aptazymes.

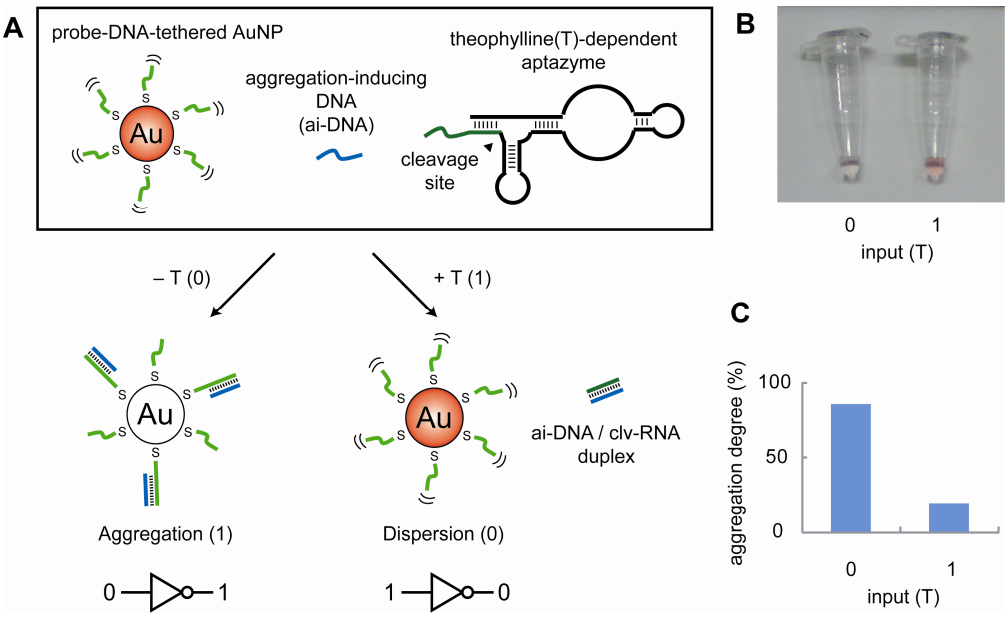

Figure S2. The NOT gate. (A) Illustration of the designed strategy of the NOT gate. (B,C) Calculation results for the NOT gate. Photograph of AuNP solutions (B) and their aggregation degree (C) in the absence (input = 0) or presence (input = 1) of 1 mM theophylline.

Calculations for the NOT gate. Calculations for the NOT gate were slightly different from those for the other gates. Ten microliters of reaction mixture containing 5 μM of in vitro-transcribed theophylline-dependent aptazyme for the NOT gate in the buffer solution B was incubated at 23˚C for 5

min in the absence or presence of 1 mM theophylline, and then 1 µL of the aptazyme reaction solution and 1µL of 500 nM ai-DNA (5'-d(GGT TGC CCG TTA TC)-3') was added to 8 µL of the 8-fold-diluted AuNPs. Detection of AuNP aggregation was performed as in the other gates.

UV-Vis Absorbance Spectra.

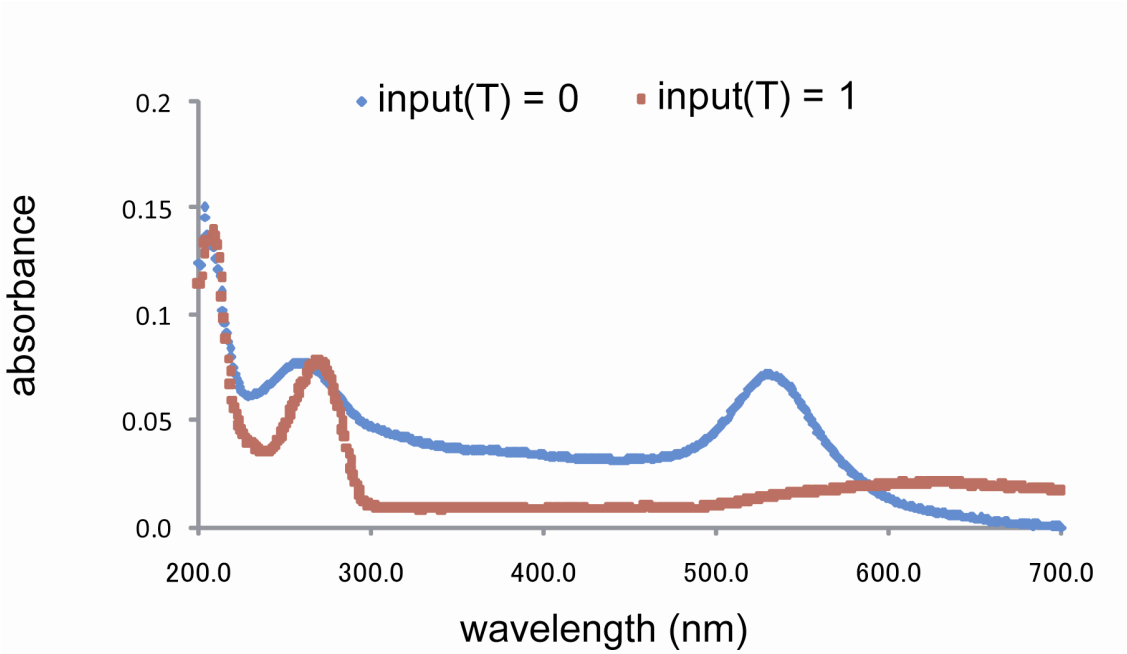

Figure S3. Absorbance spectra of the YES gate.

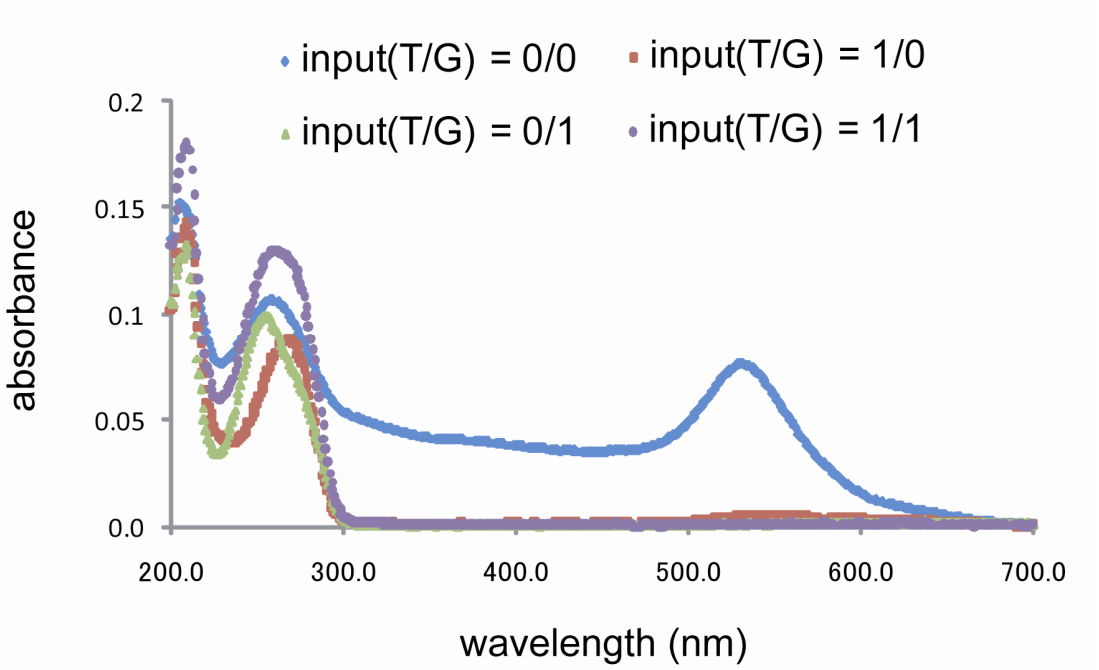

Figure S4. Absorbance spectra of the OR gate.

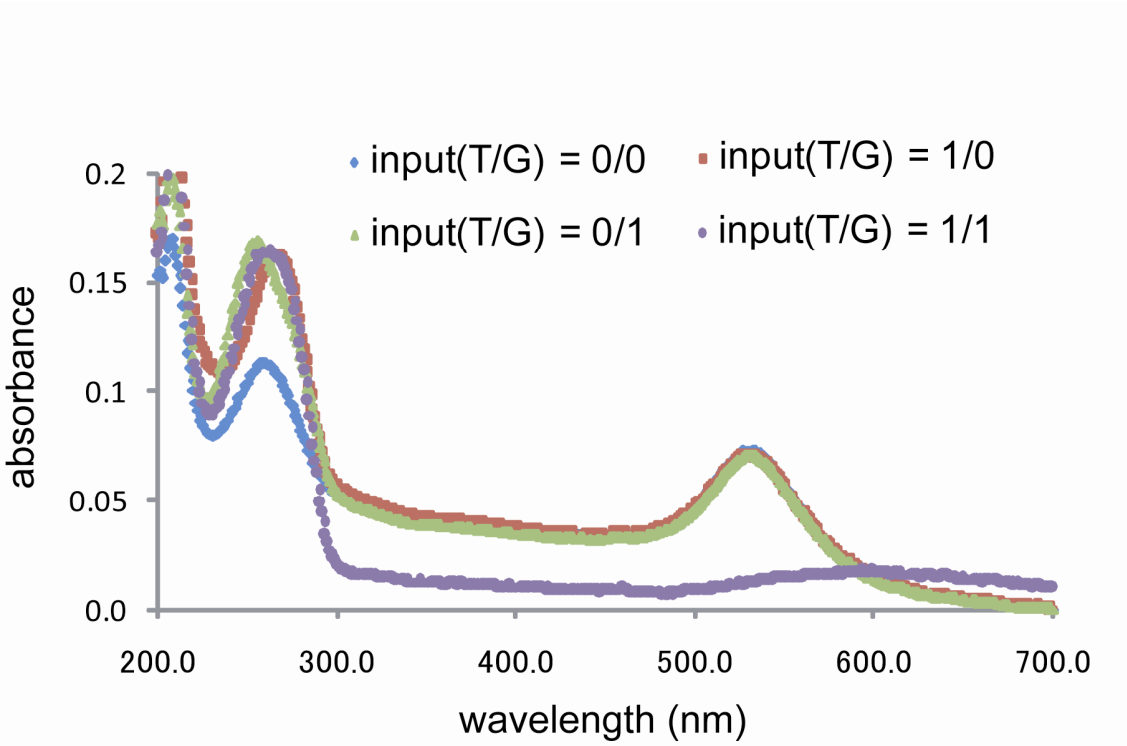

Figure S5. Absorbance spectra of the AND gate.

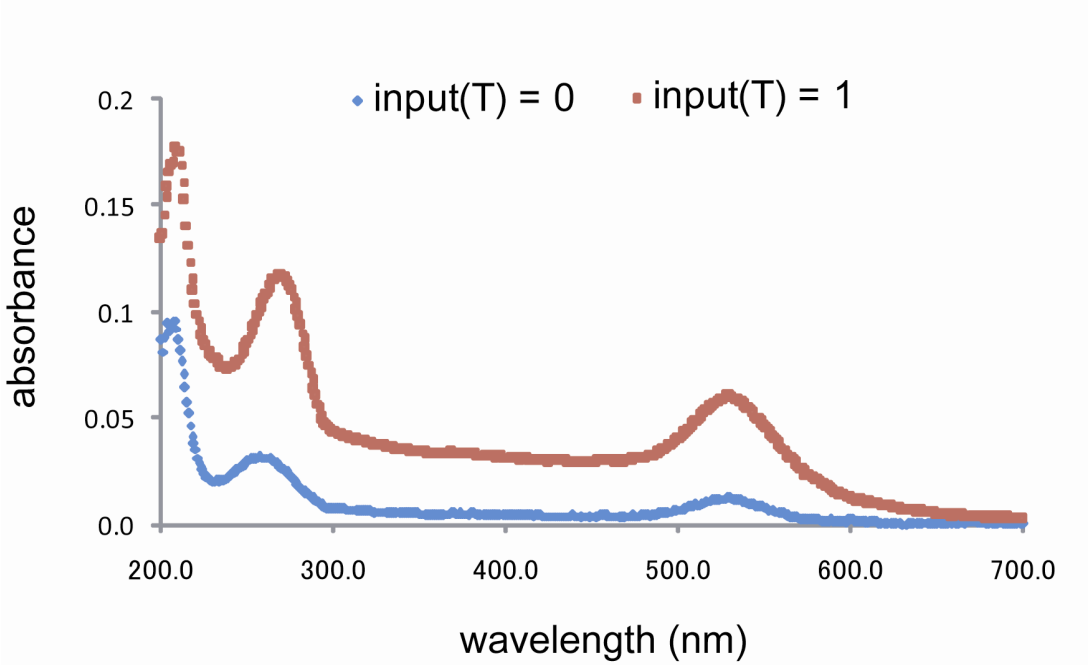

Figure S6. Absorbance spectra of the NOT gate.

References.

1 A. Ogawa and M. Maeda, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 6517

2 Secondary structures of aptazymes in the ON state were proposed by reference to the following reports: G. A. Soukup, G. A. M. Emilsson and R. R. Breaker, *J. Mol. Biol.*, 2000, **298**, 623; ; G. A. Soukup, E. C. DeRose, M. Koizumi and R. R. Breaker, *RNA*, 2001, **7**, 524.