Electronic Supplementary Information

For

Multiple-input and visible-output logic gates using signal-converting DNA machines and gold nanoparticle aggregation

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Experimental Section

General. DNA polymerase (Klenow fragment, exo-), nicking enzyme (Nb. BbvCI) and dNTPs (deoxyribonucleotide triphosphates) mix were purchased from New England Biolabs Japan (Tokyo, Japan) and used without further purification. 1st input DNAs were synthesized by Life Technologies Japan (Tokyo, Japan). 2nd input DNAs for exogenous addition and DNA machine templates were synthesized and OPC-purified by Operon Biotechnologies (Tokyo, Japan). Thiol-ssDNA was synthesized and OPC-purified by Tsukuba Oligo Service (Tsukuba, Japan). Quantification of these ssDNAs was performed with a spectrophotometer (GeneQuant 1300, 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 a spectrophotometer (BioSpec-nano, Shimadzu, Kyoto, Japan). The aggregation degree was calculated as follows: aggregation degree (%) = $[(A_{529}^{ctrl} - A_{529}) / A_{529}^{ctrl}] \times 100$, where A_{529}^{ctrl} is the absorbance at 529 nm of AuNP solutions without input ssDNAs.

Preparation of ssDNA-Tethered AuNPs. Preparation of ssDNA-tethered AuNPs was performed as described.[1] 5 nmol of DTT-reduced thiol-ssDNA x (5'- TTTCTCTTCGATAACT-$(CH2)_6$-SH-3') or y (5'-SH-$(CH_2)_6$-TGTGTATGGGTGTAG-3') was purified with ethanol precipitation and dissolved into 50 μL of a TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA). Then, 10.8 μL of 1 M Tris-HCl (pH 7.6) and 21.6 μL of 5 M NaCl were added to 48 μL of the purified thiol-ssDNA. To 80.4 μL of the mixture was added a pre-incubated mixture of 1 mL of the AuNP colloidal solution and 1 μL of 2.3 mM dATP. After incubation of the mixture at 50˚C for 3 h and at 4˚C for 1 h, the resultant ssDNA-tethered AuNPs was centrifuged at 10,000 rpm (8,609 rcf) for 15 min to remove unreacted thiol-ssDNA. The centrifuged ssDNA-tethered AuNPs was redispersed into 1 mL of a dispersion solution (10 mM Tris-

HCl (pH 7.6) and 100 mM NaCl, 0.01% Tween 20) and re-centrifuged for 15 min to remove the remaining unreacted thiol-ssDNA. The obtained ssDNA-tethered AuNPs was redispersed into ~50 μL of the dispersion solution. The amount of ssDNA on AuNPs was estimated by DTT displacement. A mixture of 4 μL of the ssDNA-tethered AuNPs and 1 μL of 100 mM DTT was incubated at room temperature until the DTT displaced the tethered ssDNA to completely aggregate AuNPs (overnight). The concentrations of released ssDNA in the supernatant were measured with a Qubit Fluorometer (ssDNA assay) from Invitrogen (Tokyo, Japan), wherein DTT-treated thiol-ssDNAs were used for calibration curves. 3 μL of the x-AuNP and y-AuNP solution mixture containing ~2 pmol each of ssDNA x and y were mixed with 5 μL of a 2× detection buffer (22.2 mM Tris-HCl (pH 7.6) and 555.6 mM NaCl) to prepare 8 μL of an AuNP detection solution just before use.

Detection of 1st input ssDNAs by Logic Gates. A mixture containing 2 pmol each of DNA machine templates, 5 U of Klenow fragment (exo-), 5-20 U of Nb.BbvCI, 2 nmol each of dNTPs, and 2 pmol each of 1st input ssDNAs and/or 100 pmol of Hg^{2+} (DNA machine templates and 1st inputs were premixed and pre-incubated at room temperature for 1 h only in the detection of Hg^{2+}) was incubated at 37ºC (or 4ºC for some negative control experiments) for 30 min in a 1× NEBuffer 2 (10 μL) from New England Biolabs. Then, 1 μL of the DNA machine reaction solution (or 1× NEBuffer 2 for the foundational AND gate) was added to a mixture of 1 μL of 2 (2.5 for [(A OR B) AND C], 1.5 for [A AND (NOT B) AND C]) pmol each of exogenous 2nd inputs or water and 8 μL of the AuNP detection solution. The mixture was warmed at 50˚C for 5 min and the temperature was gradually lowered to 20˚C at a rate of 0.5˚C/min to precisely and smoothly crosslink AuNPs with DNA machine-derived and/or exogenously-added 2nd input ssDNAs. Incidentally, this annealing step was omitted in the detection of

Hg^{2+}. After further incubation at room temperature for 2 h, photographs of the AuNP solution were taken by a digital camera and UV-vis spectra thereof were measured with a spectrometer.

Sequences of ssDNAs

(All sequences are written from 5' (left) to 3' (right).)

- 1st input ssDNAs

(Randomly selected 22-23 mer ssDNAs)

A: TGGAGTGTGACAATGGTGTTTGA

B: TGAGGTAGTAGGTTGTATGGTT

C: TGGAATGTAAAGAAGTATGTAA

D: TTAAGGCACGCGGTGAATGCCA

- 2nd input ssDNAs

(A part of the Nb.BbvCI recognition site is underlined.)

a: <u>TGAGG</u> CTGCTATGACTGATG A GTTATCGAAGAGAAA

b: <u>TGAGG</u> CATCAGTCATAGCAG T TCAGAGTCAGTTCAG ATC

c: <u>TGAGG</u> CTACACCCATACACT A CTGAACTGACTCTGA CAA

anti-b: <u>TGAGG</u> ATCTGAACTGACTCTGA A CTGCTATGACTGATG

(The ssDNAs b and c have three extra nucleotides at their 3' end to inhibit their extension on their partially complementary 2nd input ssDNAs.)

- DNA machine templates

(The anti-1st input is italicized, the anti-nicking site is underlined, and the anti-2nd-input is bold.)

A→a:
TTTCTCTTCGATAACTCATCAGTCATAGCAGCCTCAGC*TCAAACACCATTGTCACACTCCA*

B→b:
GATCTGAACTGACTCTGAACTGCTATGACTGATGCCTCAGC*AACCATACAACCTACTACCTCA*

C→c:
TTGTCAGAGTCAGTTCAGTAGTGTATGGGTGTAGCCTCAGC*TTACATACTTCTTTACATTCCA*

D→c:
TTGTCAGAGTCAGTTCAGTAGTGTATGGGTGTAGCCTCAGC*TGGCATTCACCGCGTGCCTTAA*

E→c:
TTGTCAGAGTCAGTTCAGTAGTGTATGGGTGTAGCCTCAGCTTCCCCA*GATTCTTTCTTCCCTTGTTTGTTTC*

B→a:
TTTCTCTTCGATAACTCATCAGTCATAGCAGCCTCAGC*AACCATACAACCTACTACCTCA*

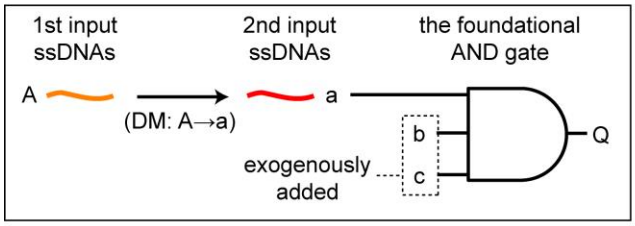
C→a:
TTTCTCTTCGATAACTCATCAGTCATAGCAGCCTCAGC*TTACATACTTCTTTACATTCCA*

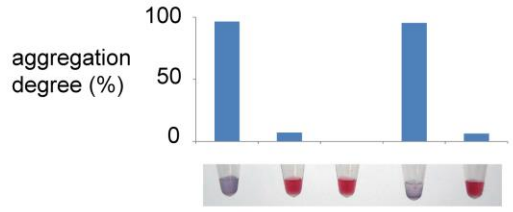
B→anti-b:
CATCAGTCATAGCAGTTCAGAGTCAGTTCAGATCCTCAGC*AACCATACAACCTACTACCTCA*

C→b:
GATCTGAACTGACTCTGAACTGCTATGACTGATGCCTCAGC*TTACATACTTCTTTACATTCCA*

Figures.

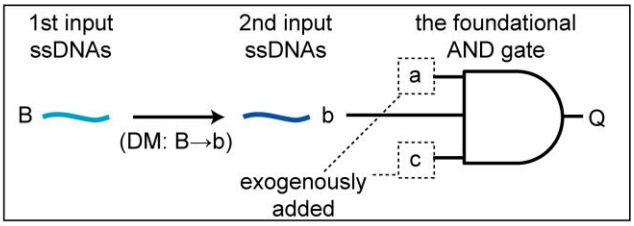
A

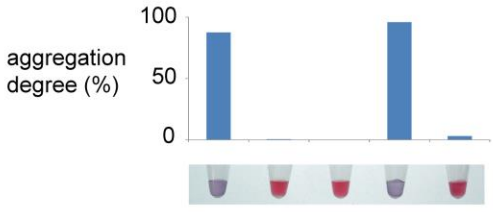




lane		1	2	3	4	5
1st input	A	+	–	–	–	+
DMR temp (ºC)		37	37	37	37	4
exo-add 2nd input	a	–	–	–	+	–
	b	+	+	–	+	+
	c	+	+	–	+	+
output		T	F	F	T	F

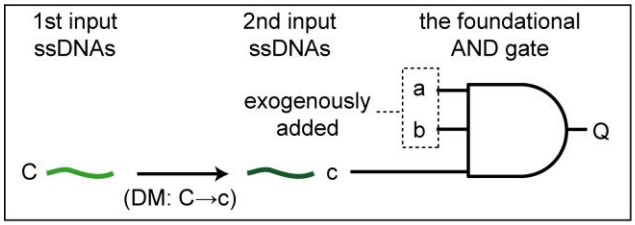
B

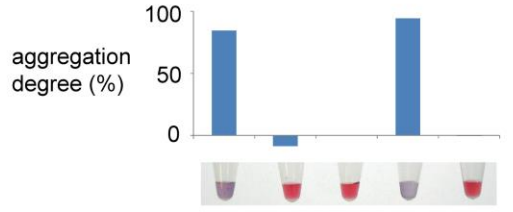




lane		1	2	3	4	5
1st input	B	+	–	–	–	+
DMR temp (ºC)		37	37	37	37	4
exo-add 2nd input	a	+	+	–	+	+
	b	–	–	–	+	–
	c	+	+	–	+	+
output		T	F	F	T	F

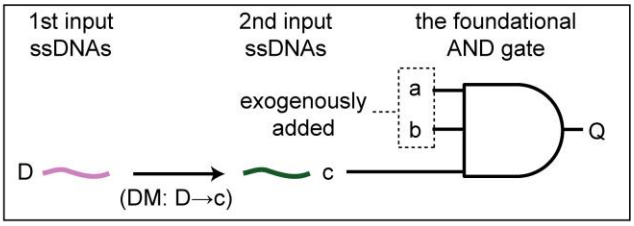
C

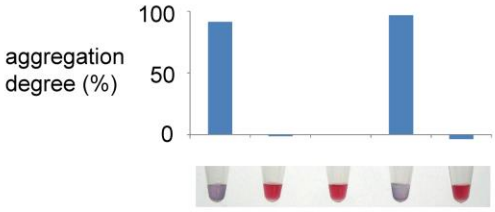




lane		1	2	3	4	5
1st input	C	+	–	–	–	+
DMR temp (ºC)		37	37	37	37	4
exo-add 2nd input	a	+	+	–	+	+
	b	+	+	–	+	+
	c	–	–	–	+	–
output		T	F	F	T	F

D





lane		1	2	3	4	5
1st input	D	+	–	–	–	+
DMR temp (ºC)		37	37	37	37	4
exo-add 2nd input	a	+	+	–	+	+
	b	+	+	–	+	+
	c	–	–	–	+	–
output		T	F	F	T	F

Figure S1. Verification of operation of each DNA machine (A: A→a; B: B→b; C: C→c; D: D→c) used in three-input AND gates by using the AuNP-based foundational AND gate. The aggregation degree was calculated as follows: aggregation degree (%) = $[(A_{529}^{ctrl} - A_{529}) / A_{529}^{ctrl}] \times 100$, where A_{529}^{ctrl} is the absorbance at 529 nm of AuNP solutions without any input and exogenous ssDNA (lane 3 in each Figure). The term "DMR temp" represents the temperature of the DNA machine reaction.

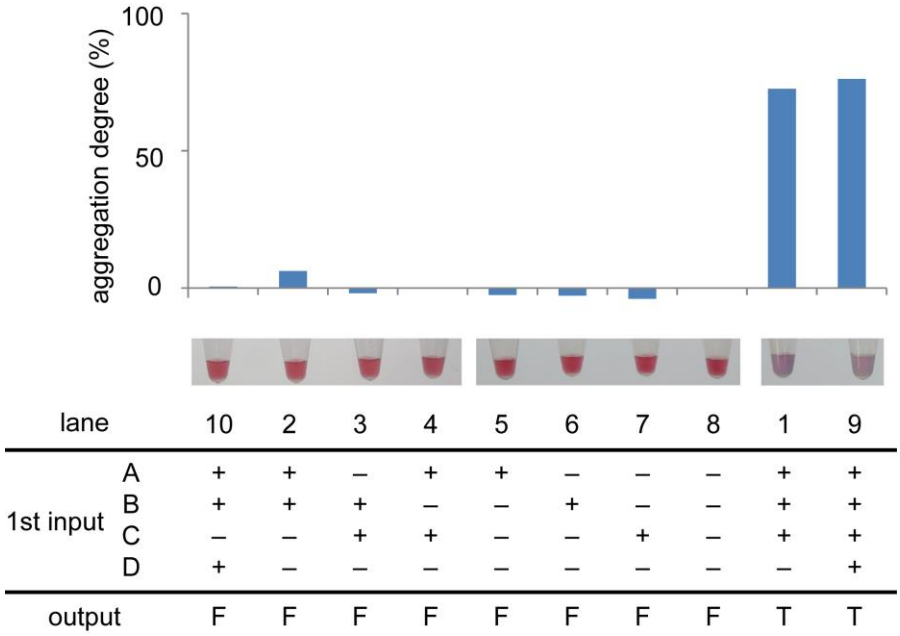
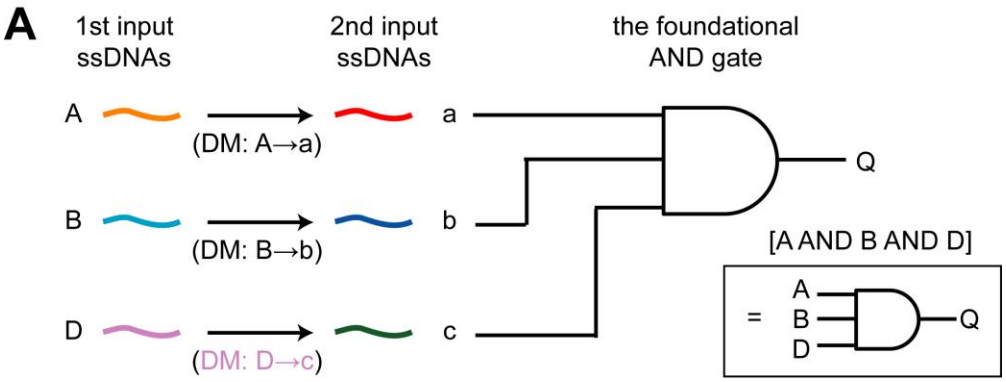
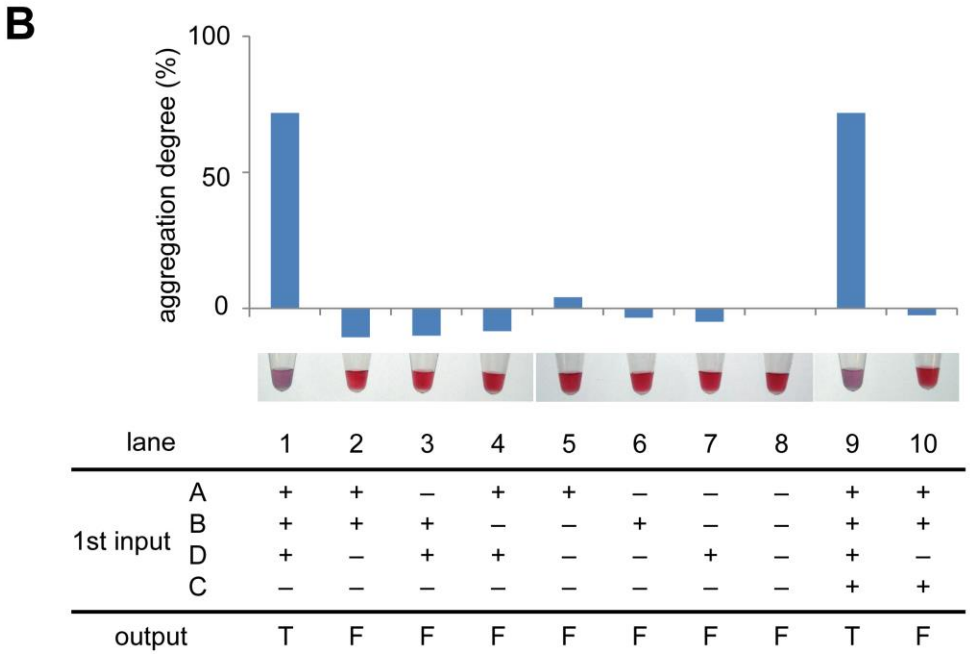


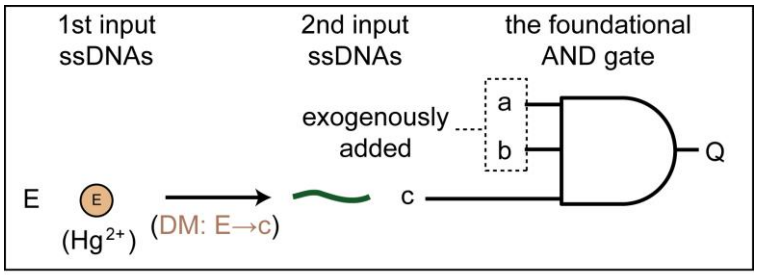
Figure S2. The effect of a foreign input ssDNA D on the [A AND B AND C] gate. The ssDNA D did not affect both of dispersion (lane 10 vs 2) and aggregation (lane 9 vs 1) of AuNPs in the gate. Each lane was numbered according to other graphs.

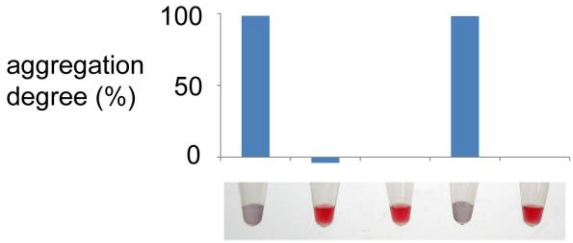




	lane	1	2	3	4	5	6	7	8	9	10
1st input	A	+	+	–	+	+	–	–	–	+	+
	B	+	+	+	–	–	+	–	–	+	+
	D	+	–	+	+	–	–	+	–	+	–
	C	–	–	–	–	–	–	–	–	+	+
output		T	F	F	F	F	F	F	F	T	F

Figure S3. The [A AND B AND D] gate. (A) Schematic illustration of the gate. (B) Operation of the gate, which agreed with the ideal truth table.





lane		1	2	3	4	5
1st input	E	+	–	–	–	+
DMR temp (ºC)		37	37	37	37	4
exo-add 2nd input	a	+	+	–	+	+
	b	+	+	–	+	+
	c	–	–	–	+	–
output		T	F	F	T	F

Figure S4. Verification of operation of the E→c DNA machine used in the [A AND B AND E] gate (Fig. S5).

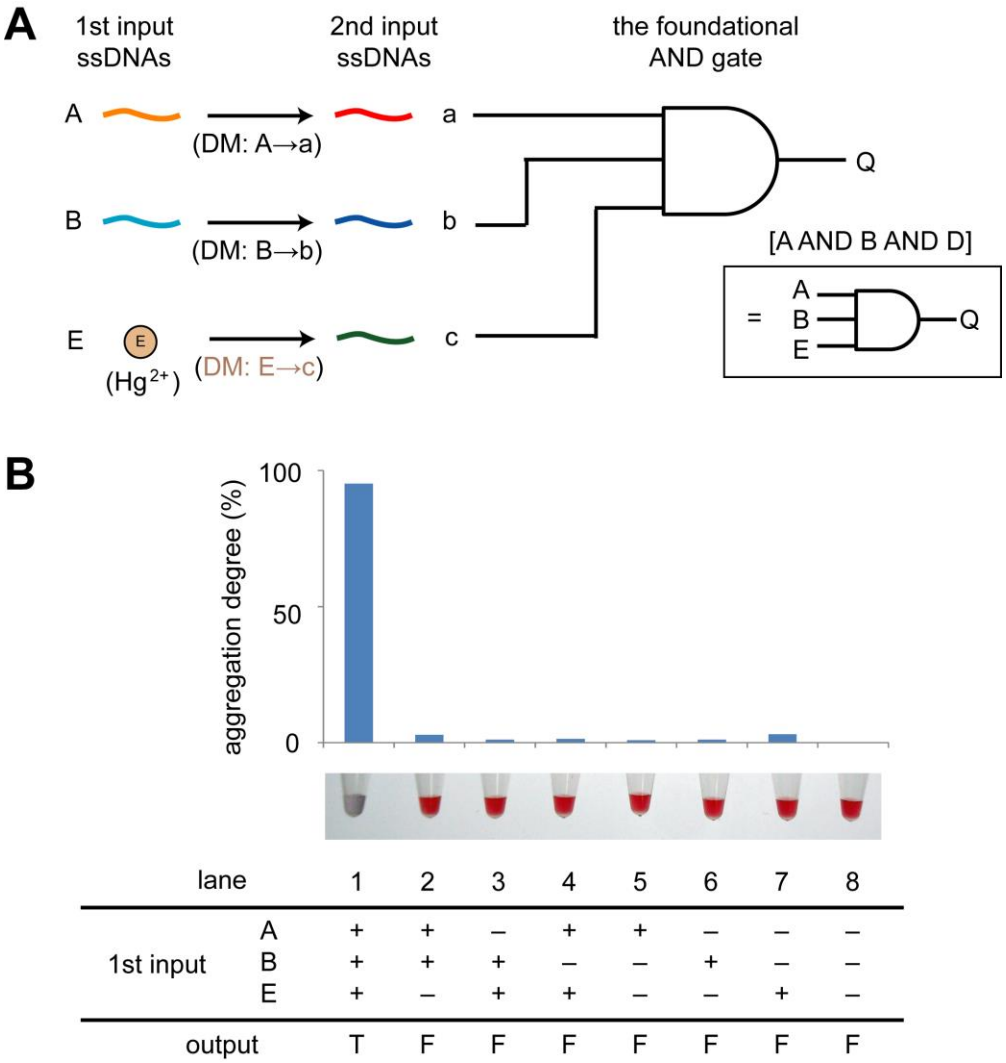


Figure S5. The [A AND B AND E] gate. (A) Schematic illustration of the gate. (B) Operation of the gate, which agreed with the ideal truth table.

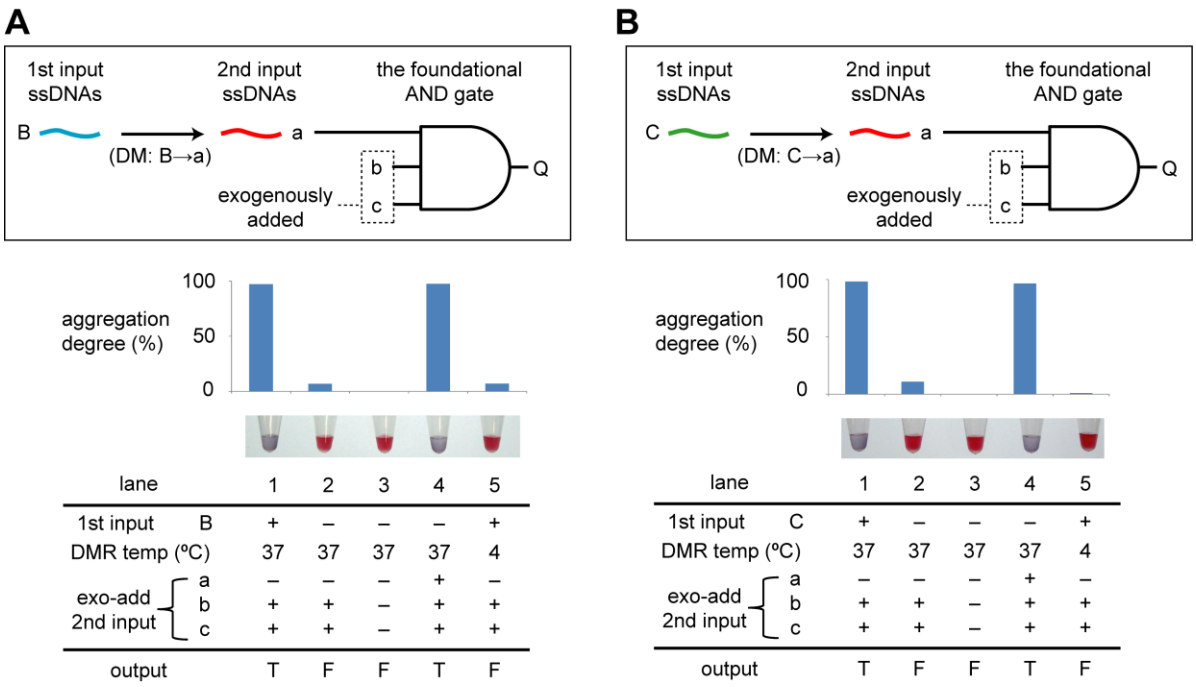
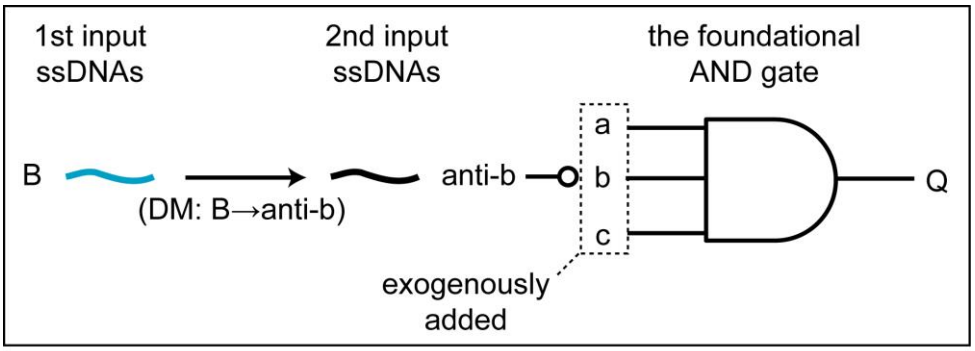
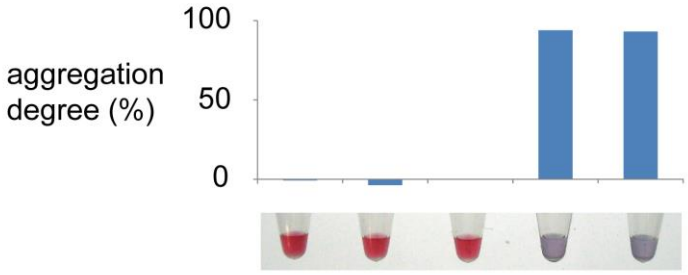


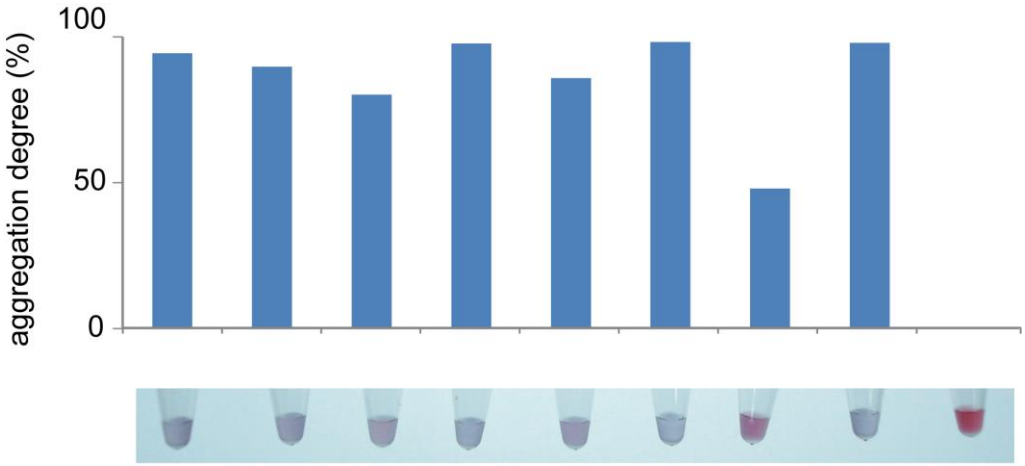
Figure S6. Verification of operation of each DNA machine (A: B→a; B: C→a) used in the [A OR B OR C] gate or the [(A OR B) AND C] gate.





lane		1	2	3	4	5
1st input	B	+	–	–	–	+
DMR temp (°C)		37	37	37	37	4
exo-add 2nd input	a	+	+	–	+	+
	b	+	+	–	+	+
	c	+	+	–	+	+
	anti-b	–	+	–	–	–
output		F	F	F	T	T

Figure S7. Verification of operation of the B→anti-b DNA machine used in the [A AND (NOT B) AND C] gate.



lane		1	2	3	4	5	6	7	8	9
2nd input	a	++	+	++	++	+	++	+	+	–
(+: 200 nM)	b	++	++	+	++	+	+	++	+	–
(++: 1 μM)	c	++	++	++	+	++	+	+	+	–
output		T	T	T	T	T	T	T	T	F

Figure S8. Performance of the foundational AND gate with various ratios of 2nd inputs. Although these results indicate that the ratio of 2nd inputs slightly affects the aggregation degree, the ratio of 2nd inputs produced from DNA machines in logic gates can be adjusted by optimizing concentrations of each DNA machine and/or exogenous 2nd input, depending on the situation.

References.

(1) Ogawa A, *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 155.