Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Allosteric control of a DNA-hydrolyzing deoxyribozyme with short oligonucleotides and its application in DNA logic gates

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Experimental section

Oligonucleotides. Oligonucleotides were purchased form Sigma Genosys and purified by denaturing PAGE. The sequences of oligonucletides are listed in Table S1. DNA was eluted from the gel by crush-soaking in a buffer containing 10 mM Tris-HCl (pH 7.5 at 23 °C), 200 mM NaCl, and 1 mM EDTA, followed by precipitation with ethanol. The substrate strands were then radiolabeled with [γ-^{32}P] ATP (Perkin Elmer) using T4 polynucleotide kinase (New England Biolabs). The 5′ ^{32}P-labeled RNAs were isolated and purified by denaturing PAGE as described above.

Deoxyribozyme cleavage assays. The assay was carried out according to the method described by Gu *et al* (*J. Am. Chem. Soc.*, 2013, 135, 9121). Trace amount of 5′ ^{32}P-labeled substrate strands (8 nM, ~1000 cpm) were mixed with enzyme strands (333 nM) and cofactor strands (333 nM) in 30 μL solution containing 50 mM HEPES (pH 7.05 at 23°C), 100 mM NaCl after incubation at 90°C for 3 min, followed by cooling on ice for 5 min, and then incubation at 37°C for 5 min. For the construction of deoxyribozyme-based logic gates, each strand was mixed as shown in Table S2 and annealed as described above.

Cleavage reactions were initiated by adding another 30 μL of a solution containing 50 mM HEPES (pH 7.05 at 23 °C), 100 mM NaCl, 40 mM $MgCl_2$, and 4 mM $ZnCl_2$. After 10 min, 5 μL of the sample was removed and added to 5 μL stop solution (90% formamide, 30 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol). Samples were separated by denaturing 15% PAGE and visualized/quantified by FLA9000 Image Scanner (FUJIFILM). Values for k_{obs} were determined from the negative slopes of the natural log of fractions of uncleaved substrate plotted against time.

Table S1 Oligonucleotides used in this study

		sequence (5'-3')
cofactor effect (all)	cofactor	AUCCUCGAAGUGUUAAUCUGGUAGGUUGUG
cofactor effect (14-8)	substrate	CTCGTGATGCAGACGTTGAAGGATTATCTACACTTCGAG
cofactor effect (14-8)	enzyme	CCTACCAGATTAAGATAATCTAGTTGAGCTGTCTGCATCACGAG
cofactor effect (14-5)	substrate	CTCGTGATGCAGACGTTGAAGGATTAACACTTCGAG
cofactor effect (14-5)	enzyme	CCTACCAGATTATAATCTAGTTGAGCTGTCTGCATCACGAG
cofactor effect (14-2)	substrate	CTCGTGATGCAGACGTTGAAGGAACACTTCGAG
cofactor effect (14-2)	enzyme	CCTACCAGATTATCTAGTTGAGCTGTCTGCATCACGAG
cofactor effect (11-5)	substrate	GTGATGCAGACGTTGAAGGATTAACACTTCGAG
cofactor effect (11-5)	enzyme	CCTACCAGATTATAATCTAGTTGAGCTGTCTGCATCAC
cofactor effect (10-5)	substrate	TGATGCAGACGTTGAAGGATTAACACTTCGAG
cofactor effect (10-5)	enzyme	CCTACCAGATTATAATCTAGTTGAGCTGTCTGCATCA
cofactor effect (9-5)	substrate	GATGCAGACGTTGAAGGATTAACACTTCGAG
cofactor effect (9-5)	enzyme	CCTACCAGATTATAATCTAGTTGAGCTGTCTGCATC
cofactor effect (8-5)	substrate	ATGCAGACGTTGAAGGATTAACACTTCGAG
cofactor effect (8-5)	enzyme	CCTACCAGATTATAATCTAGTTGAGCTGTCTGCAT
AND gate, INHIBIT gate	substrate (1), (4)	CTCGTGATGCACTCGTTGAAGGTTTAACACTTCGAG
AND gate	Input 1	CCTACCAGATTATAAACTAGTTGAGCTGAGTGCATCA
AND gate	Input 2	ATCCTCGAAGTGTTAATCTGGTAGGTTGTG
OR gate, XOR gate	substrate (2), (6)	CTCGTGATGCCATCGTTGAAGGTATAACATGACTC
OR gate	substrate (3)	TACCGTAGTGCCTCGTTGAAGGTCAATATTGACTC
OR gate	Input 3	TGTTATACTAGTTGAGCTGATGGCATCACGAG
OR gate	Input 4	ATATTGACTAGTTGAGCTGAGGCACTACGGTA
INHIBIT gate	Input 5	CTCGAAGTGTTAAACCTTCAACG
INHIBIT gate	Input 6	TGTTAAACTAGTTGAGCTGAGTGCATCACGAG
XOR gate	substrate (5)	GAGTCATGTTATACGTTGAAGGATGGCATCACGAG
XOR gate	Input 7	GCTCCAGATGCTATGGGAGTCATGTTATACTAGTTGAGCTGATGGCATCACGAGTAGTGCATATCATATG
XOR gate	Input 8	CATATGATATGCACTACTCGTGATGCCATCTAGTTGAGCTGTATAACATGACTCCCATAGCATCTGGAGC

Table S2 Concentration of the oligonucleotides used for the logic gate construction

Gate		conc. (nM)
AND	Substrate (1)	~8
	Input 1	333
	Input 2	333
INHIBIT	Substrate (4)	~8
	Input 5	333
	Input 6	333
OR	Substrate (2)	~8
	Substrate (3)	~8
	Input 3	333
	Input 4	333
XOR	Substrate (5)	~8
	Substrate (6)	~8
	Input 7	50
	Input 8	50

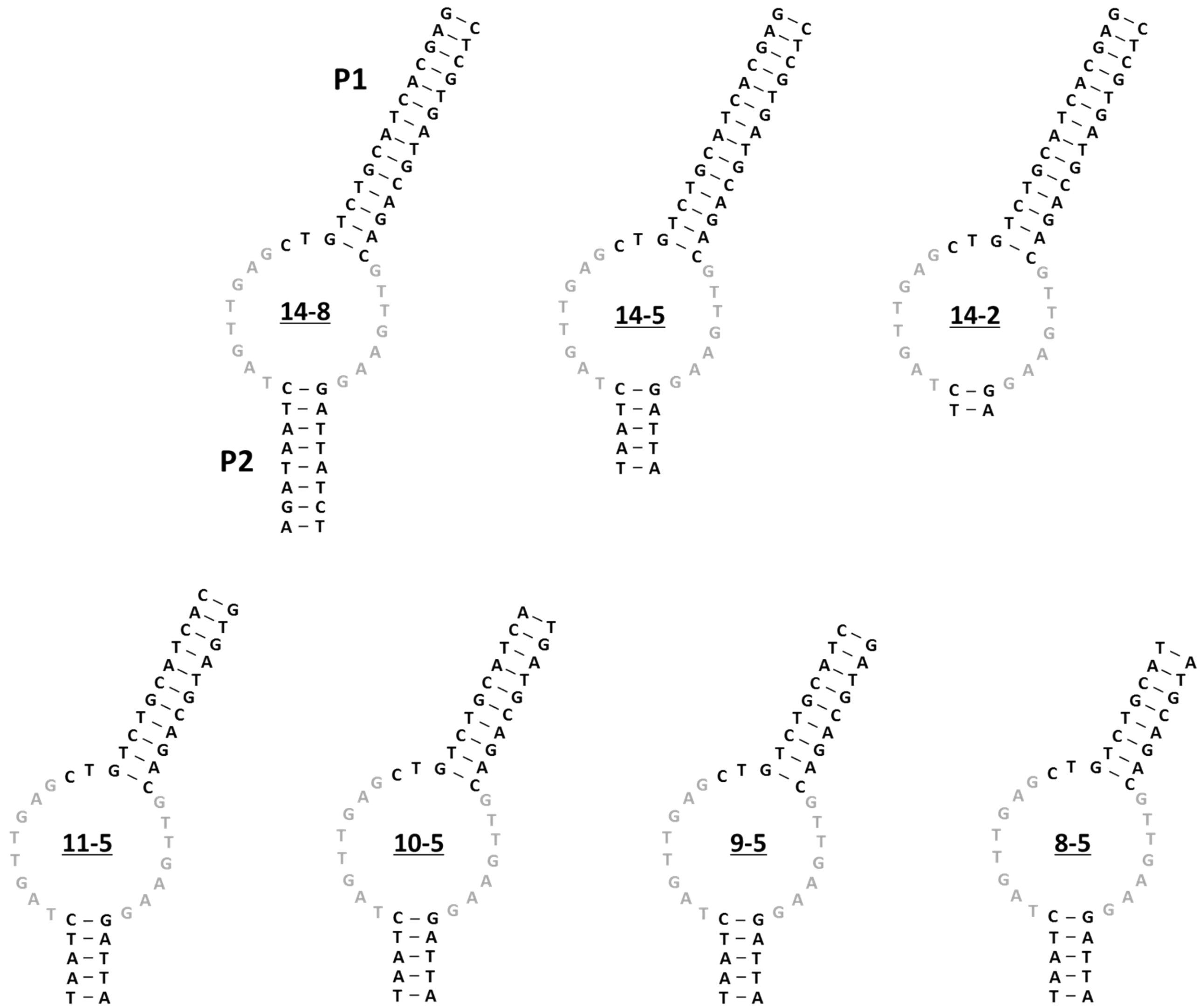

Fig. S1 Sequences of the P1 and P2 stems for the cofactor effect study (Figure 2).