Electronic supplementary information

Engineering chemical reaction modules via programming assembly of DNA hairpins

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S1: Materials and Methods

Materials:

The DNA oligonucleotides used in this project were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). Individual DNA oligonucleotides were dissolved in 10 mM Tris buffer (pH=7.4, 300 mM NaCl, 5 mM $MgCl_2$).

Methods:

DNA sequence design: Random DNA sequences composed of A, T, C, and G were designed with the help of software NUPACK[1] to avoid secondary structures.

DNA oligonucleotides: The concentrations of single-stranded DNA (ssDNA) were calculated on the basis of the molar extinction coefficient of DNA. The absorbance of DNA at 260 nm was measured using a Cary 300 UV-Vis spectrophotometer.

Preparation of DNA hairpins: The ssDNA strands for each sample were prepared with nominal stoichiometry at 10 μM. For the preparation of stable hairpin nanostructures, the DNA samples were processed using following annealing procedures: 95°C for 10 min, and then slow cooling to room temperature. The final DNA samples were stored at 4°C for further use.

Preparation of double-stranded signal complex: The ssDNA strands for each sample were prepared with nominal stoichiometry at 10 μM. The reporter strand and quencher strand were mixed at a 1:1 molar ratio, maintained at 95°C for 10 min, and then slowly cooled to room temperature. The final DNA samples were stored at 4°C for further use.

Typical procedure for engineering DNA-only chemical reactions: Three kinds of DNA hairpins mix at a 1:1:1 molar ratio, and then the inputted strands with varied quantity were added. The excess double-stranded signal complex was added to the

solutions. All experiments were performed at 25 °C. Signal changes were monitored by measuring the fluorescence intensity on the Fluorescence Spectrophotometer F-7000. The excitation and emission wavelengths were 480/518 nm for FAM, and 580/610 nm for Texas Red in the study.

S2: Supporting Figures

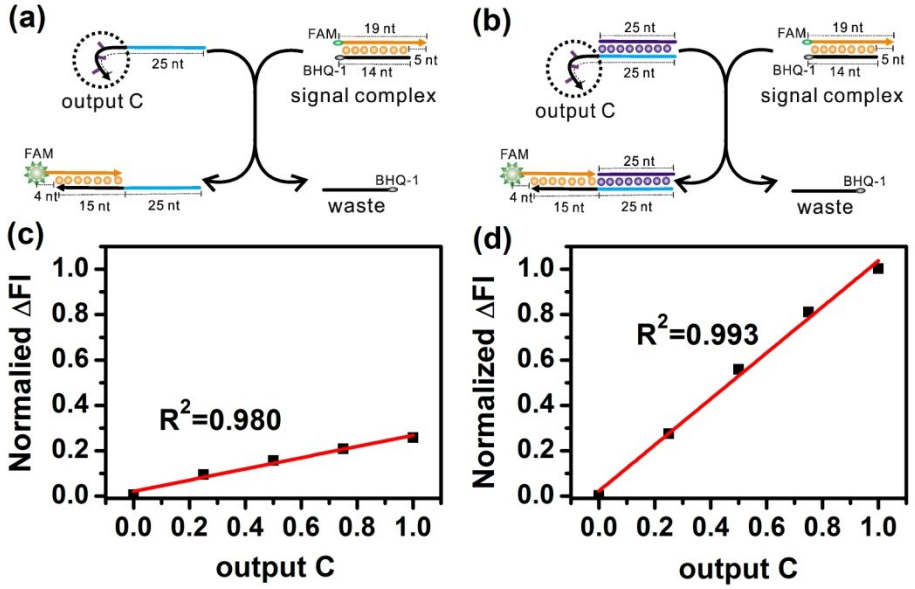

Fig. S1. The variation of fluorescent signal after the accomplishment of DNA displacement reactions between the signal complex with different amounts of output C, which was conjugated with (a, c) single-stranded and (b, d) double-stranded DNA domains. The concentration of signal complex is 312.5 nM, and the 1× [output C] = 125 nM. The 3′-terminal of the DNA sequence is indicated with an arrow.

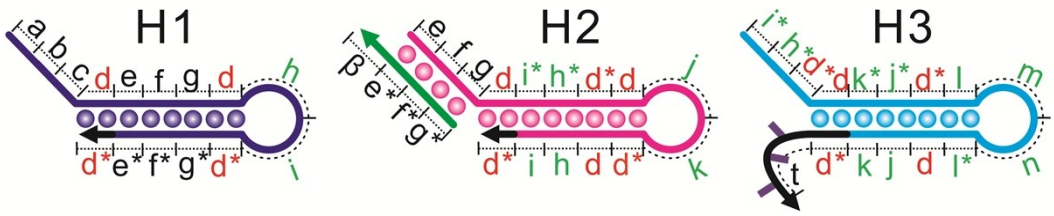

Fig. S2. DNA hairpins used for constructing non-catalytic reaction $A + B \rightarrow C$. In our following experiments, the functions that the outputted strand endows on other hairpins have been tested. In addition, the function of outputted sequence in triggering downstream reaction is decided not only by the region of inactive tail (t) but also its adjacent segment (d*), which worked as an invading domain. For avoiding the sophisticated procedure in design and decrease the cost in buying new sets of oligonucleotides while programming the other hairpins with outputted strand, all domains at the 3′-terminal of hairpins were designed with the same sequences. In our project, the length of segments a, b, c, d, e, f, g, h, i, j, k, l, m, n, and their complementary segments are 4 nt; the length of β is 10 nt. The 3′-terminal of the DNA sequences is labeled with an arrow.

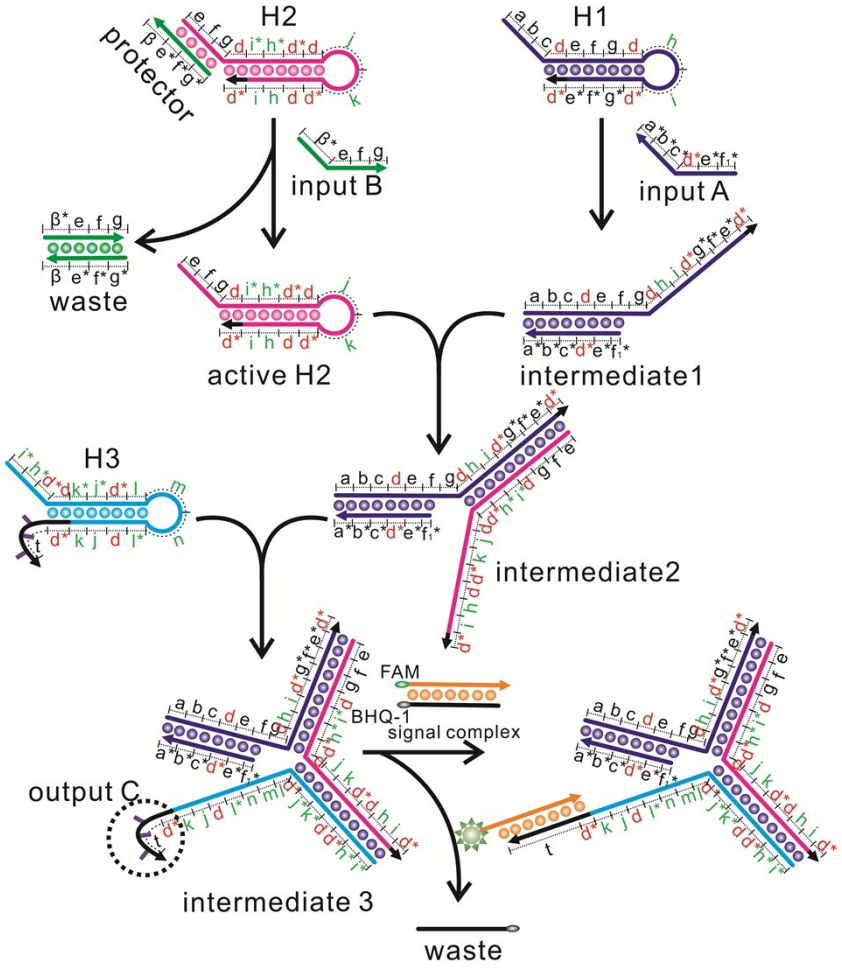

Fig. S3. Schematic representation for the non-catalytic reaction $A + B \rightarrow C$. First, the input A strand invades the free toehold domain (a-b-c) on H1 and initiates the DNA branch migration reactions.[2] After the input A is fully hybridized with its complementary segments a-b-c-d-e-f1, the hairpin structure of H1 is disrupted, resulting in intermediate 1. Meanwhile, the protector strand (β-e*-f*-g*) on H2 is peeled off by input B because the DNA strand displacement reaction (DSDR) started from the free region β on the protector, forming active H2 with a sticky toehold domain. Second, a new DNA displacement reaction is initiated between the active H2 with intermediate 1, on which the free sequences e*-f*-g* worked as binding sites for sticky toehold domain (e-f-g) on active H2. After the free segments e*-f*-g*-d*-i-h-d on intermediate 1 are fully hybridized with their complementary sequences on H2, the hairpin structures of H2 is broken down, and intermediate 2 is formed. Third, the newly exposed segments on intermediate 2 react with the sticky toehold domain (i*-h*-d*) on H3, resulting the opening of H3 and producing intermediate 3. With the appearance of exposed adjacent domain d* connecting to the inactive tail region (t) on intermediate 3, the functional tail d*-t, treated as output C, is obtained. As a result, the capacity of tails for triggering downstream reactions is activated. The quantification of output C is achieved by monitoring the fluorescent signal, which is attributed to the replacement of BHQ-1-modified quencher strands from the FAM-functionalized reporter strands. The 3′-terminal of the DNA sequences is indicated with an arrow.

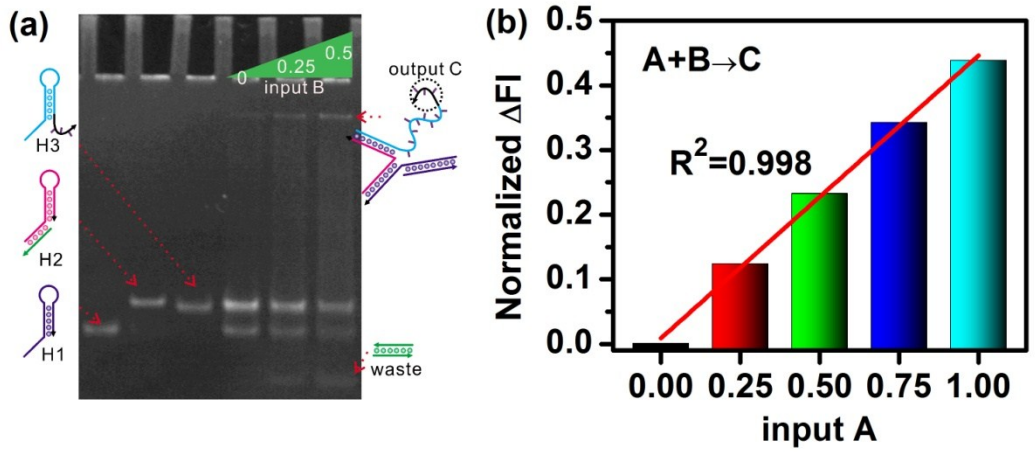

Fig. S4. (a) The 10% PAGE gel analysis for the non-catalytic reaction to various input B concentrations. (b) Fluorescence responses of non-catalytic reaction to various input A concentrations were recorded at 2.5 h. 1× [input A] = 125 nM. [H1] = [H2] = [H3] = 125 nM

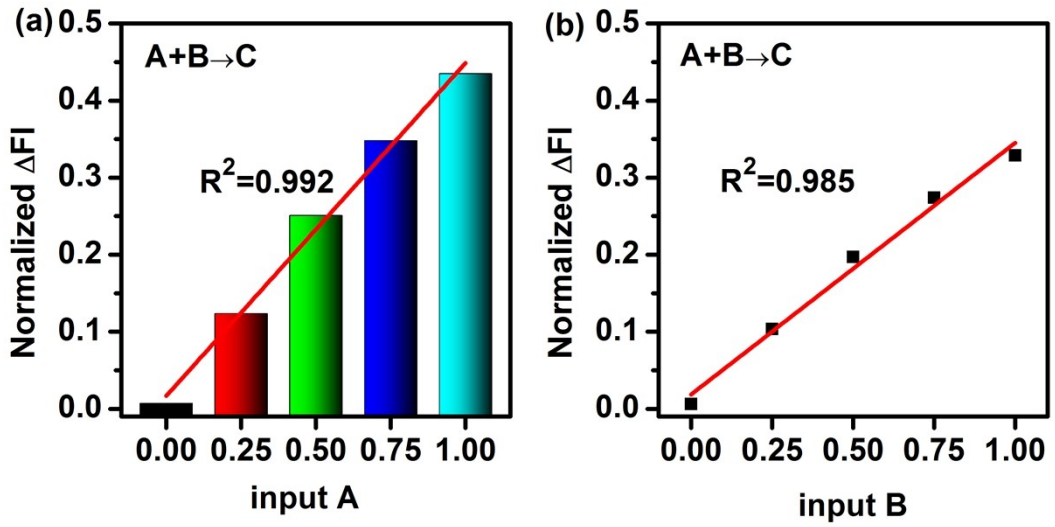

Fig. S5. Fluorescence changes of non-catalytic reaction $A + B \rightarrow C$ were collected after 24h. The experiments were performed with varied input A (input B), 1× input B (input A) and 2.5× signal complex, the 1× [input B] = 125 nM. [H1] = [H2] = [H3] = 125 nM

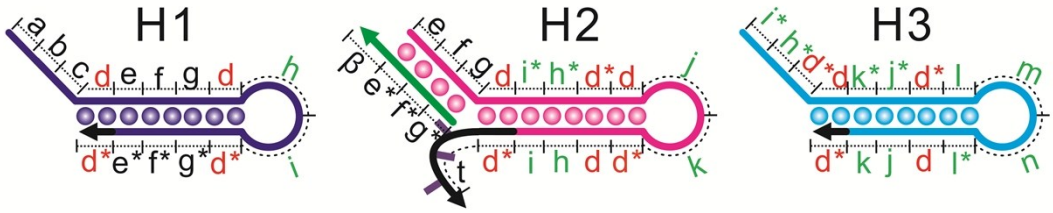

Fig. S6. Engineering the non-catalytic reaction $A + B \rightarrow C$ by programming the output C on hairpin H2. The 3′-terminal of the DNA sequences is indicated with an arrow.

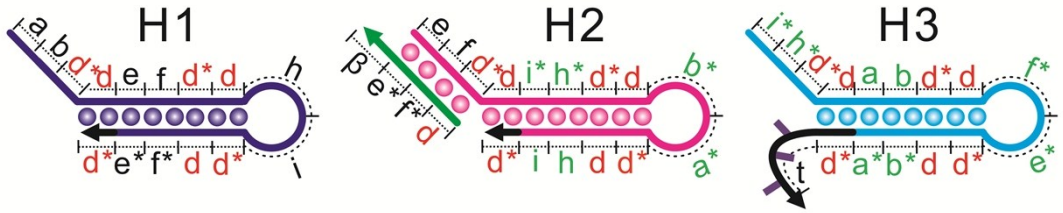

Fig. S7. DNA hairpins used in the catalytic reaction $B \rightarrow C$. The DNA sequences of three hairpins were designed with the help of NUPACK, which are displayed in Table S3. In our project, the length of segments a, b, d, e, f, h, i, and their complementary segments are 4 nt; the length of β is 10 nt. The 3′-terminal of the DNA sequences is indicated with an arrow.

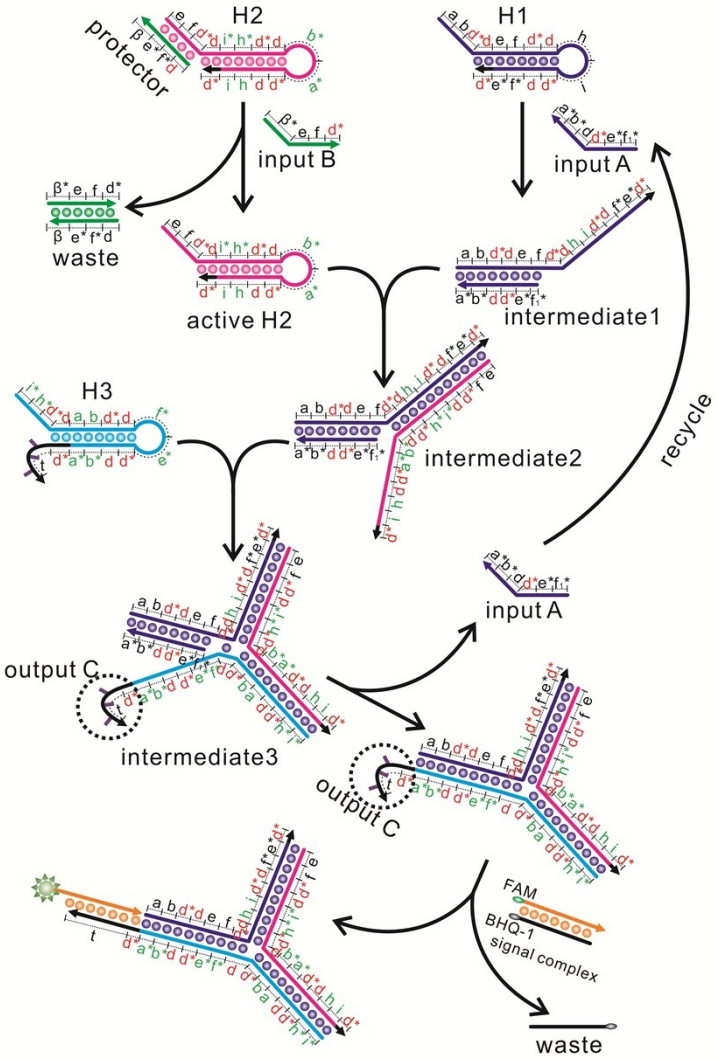

Fig. S8. Schematic representation for the catalytic reaction $B \rightarrow C$. Similar to the processes in Fig. S3, the input A is introduced to open hairpin H1, and the input B is used to active the function of hairpin H2. The resulting products are intermediate 1 and active H2, correspondingly. Then, a new

DNA displacement reaction is initiated between the active H2 with intermediate 1, on which the free sequences e*-f*-d invade the sticky toehold domain (e-f-d*) on active H2. After the free segments, e*-f*-d-d*-i-h-d, on intermediate 1 are fully hybridized with their complementary sequences on H2, the hairpin structures of H2 is broken down and intermediate 2 is formed. Third, the newly exposed segments on intermediate 2 react with the sticky toehold domain (i*-h*-d*) on H3, resulting in the opening of H3 and producing intermediate 3. Herein, the intermediate 3 stays at the unstable state as the existence of potential strand displacement reactions between the free segments, a*-b*-d-d*-e*-f* on disrupted H3 with the complex of input A and its complementary strand, resulting in DNA junction and releasing the input A into solution. Once the adjacent domain (d*) conjugating to inactive tail (t) is exposed, the functional output C is obtained, displacing the quencher strands from signal complex and resulting in fluorescence signal. The 3′-terminal of the DNA sequences is indicated with an arrow.

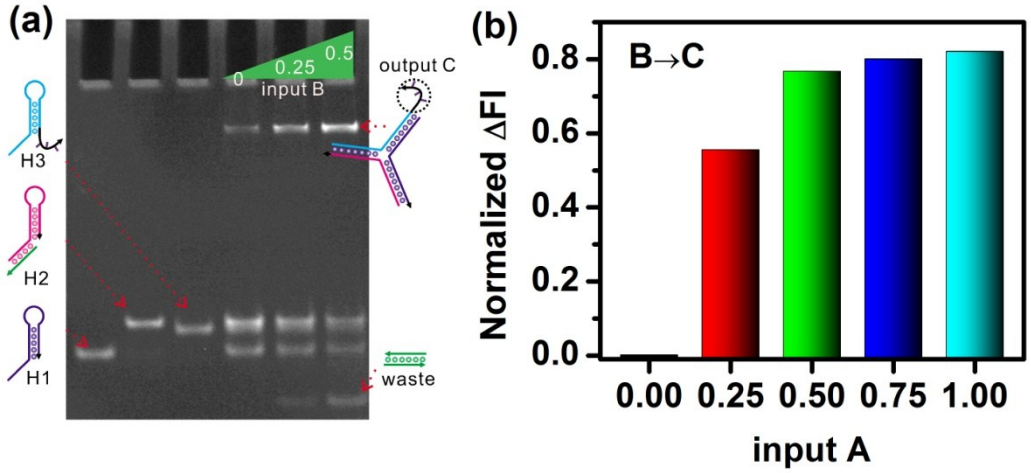

Fig. S9. (a) The 10% PAGE gel analysis for the catalytic reaction to various input B concentrations. (b) Fluorescence responses of catalytic reaction to various input A concentrations were recorded at 2.5 h. 1× [input A] = 125 nM. [H1] = [H2] = [H3] = 125 nM

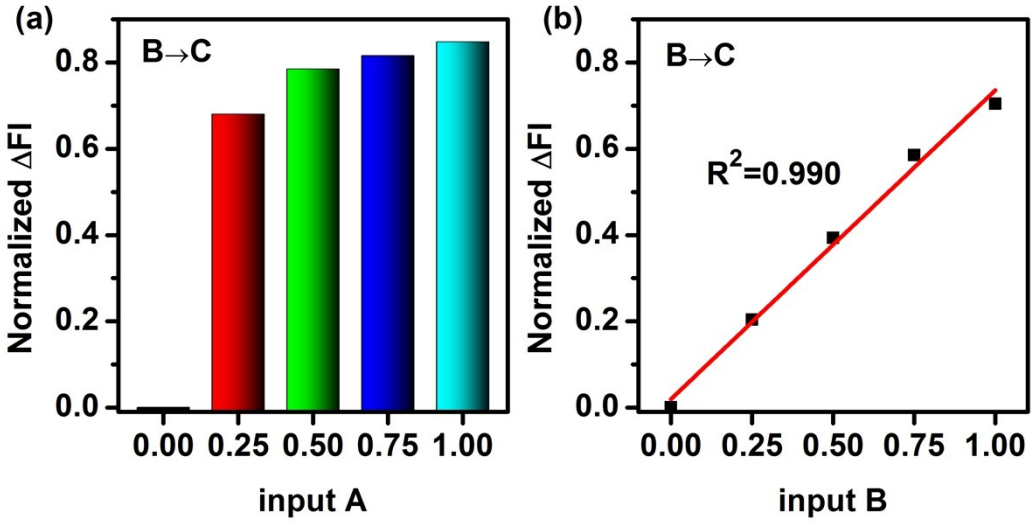

Fig. S10. The fluorescence changes of the catalytic reaction $B \rightarrow C$ are collected after 24h. The

experiments were performed with varied input A (input B), 1× input B (input A), 2.5× signal complex, and the 1× [input B] = 125 nM. [H1] = [H2] = [H3] = 125 nM

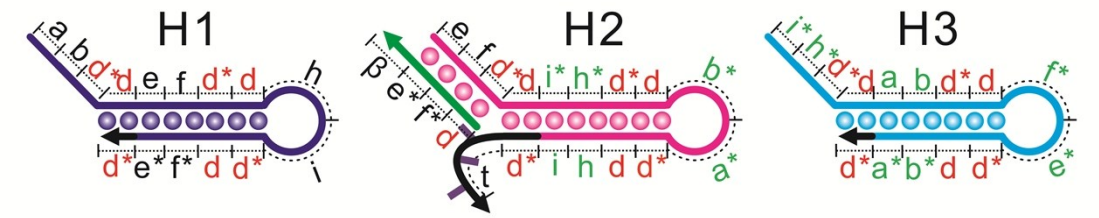

Fig. S11. Engineering the catalytic reaction $B \rightarrow C$ by programming the output C on hairpin H2. The 3′-terminal of the DNA sequences is indicated with an arrow.

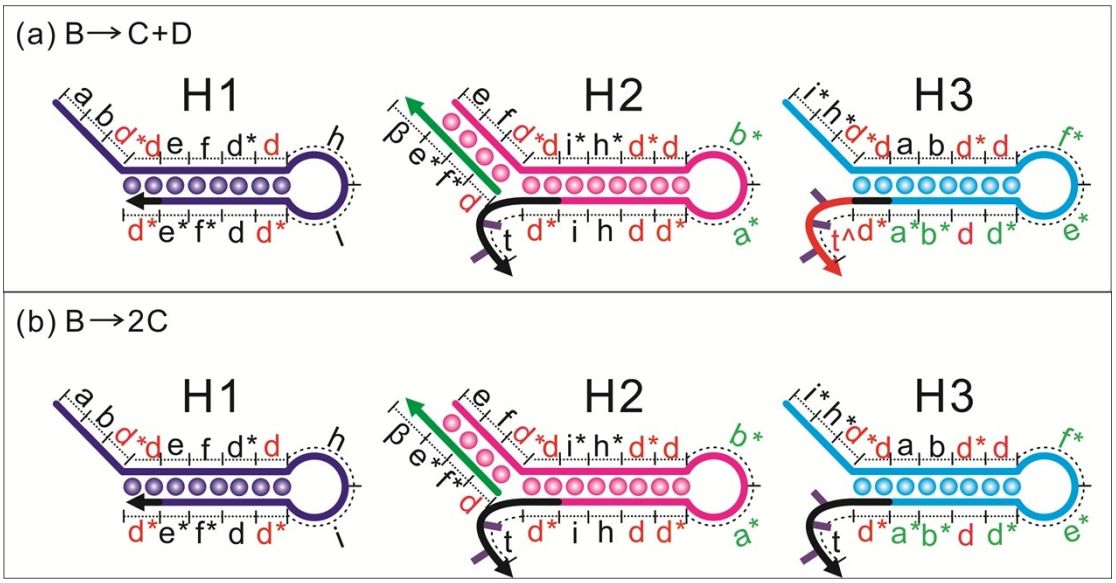

Fig. S12. DNA hairpins used for constructing the catalytic decomposition reactions with diverse outputs. The 3′-terminal of the DNA sequences is indicated with an arrow.

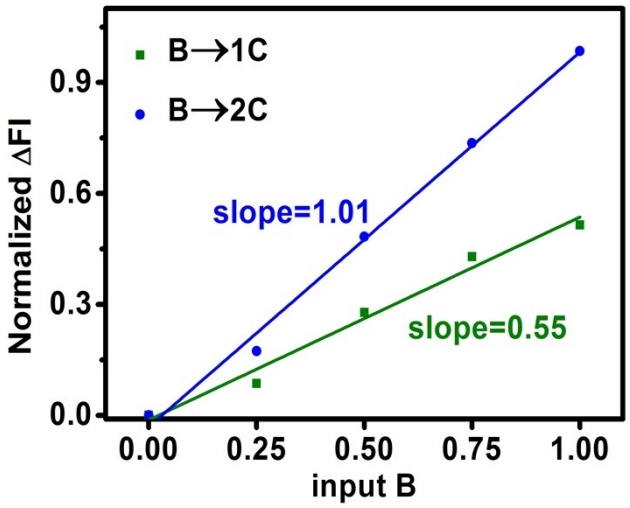

Fig. S13. The calculation of the slopes of fitting curves of the catalytic decomposition reaction $B \rightarrow \mathrm{m}C$.

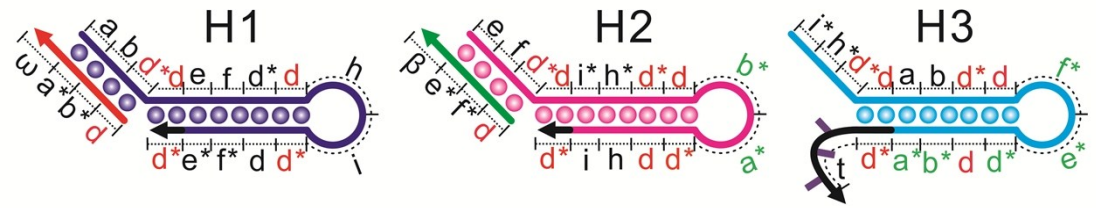

Fig. S14. DNA hairpins used for constructing the catalytic combinational reactions $B + C \rightarrow D$ with multiple inputs. The 3′-terminal of the DNA sequences is indicated with an arrow.

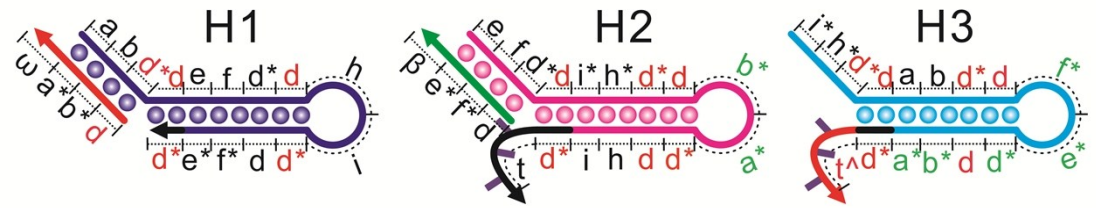

Fig. S15. DNA hairpins used for constructing the catalytic metathetical reactions $B + C \rightarrow D + E$ with multiple inputs and diverse outputs. The 3′-terminal of the DNA sequences is indicated with an arrow.

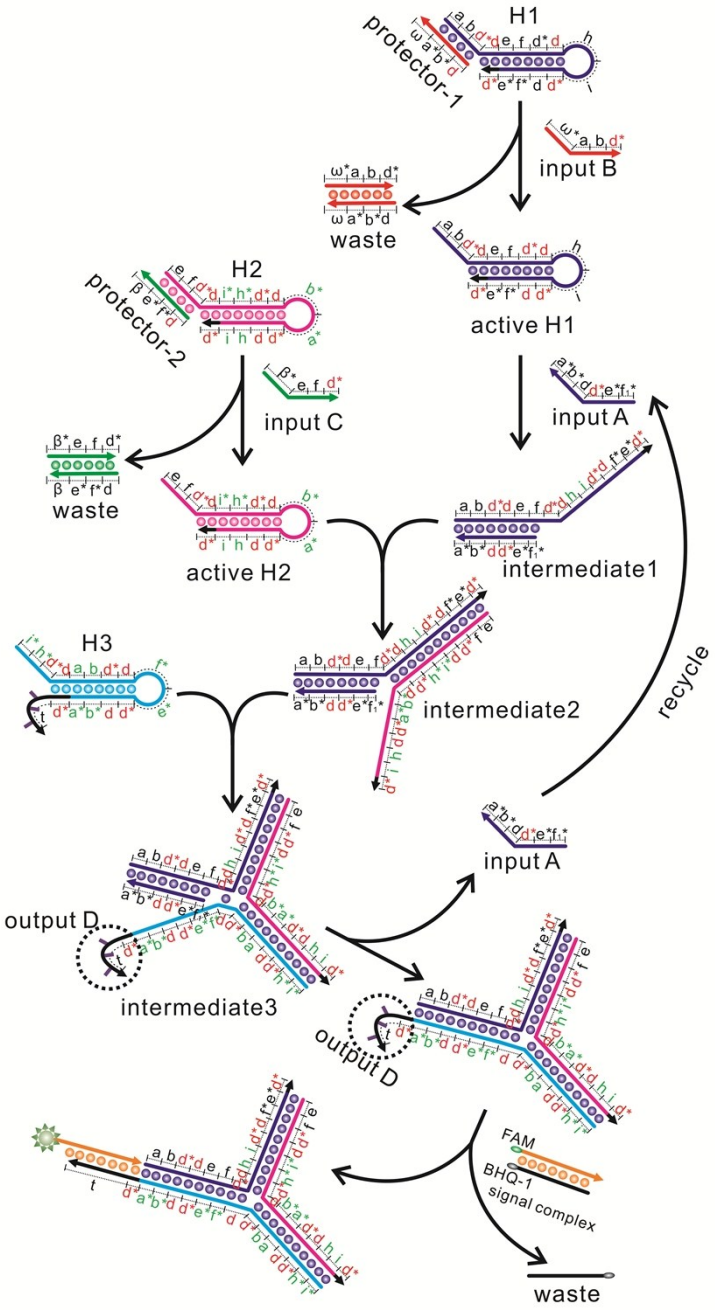

Fig. S16. Schematic representation for the catalytic combinational reaction $B + C \rightarrow D$. The input B and input C are used to activate hairpin H1 and H2 through peeling off the protector strands from the corresponding toehold domains. The following details are the same as the description in Fig. S8. The 3′-terminal of the DNA sequences is indicated with an arrow.

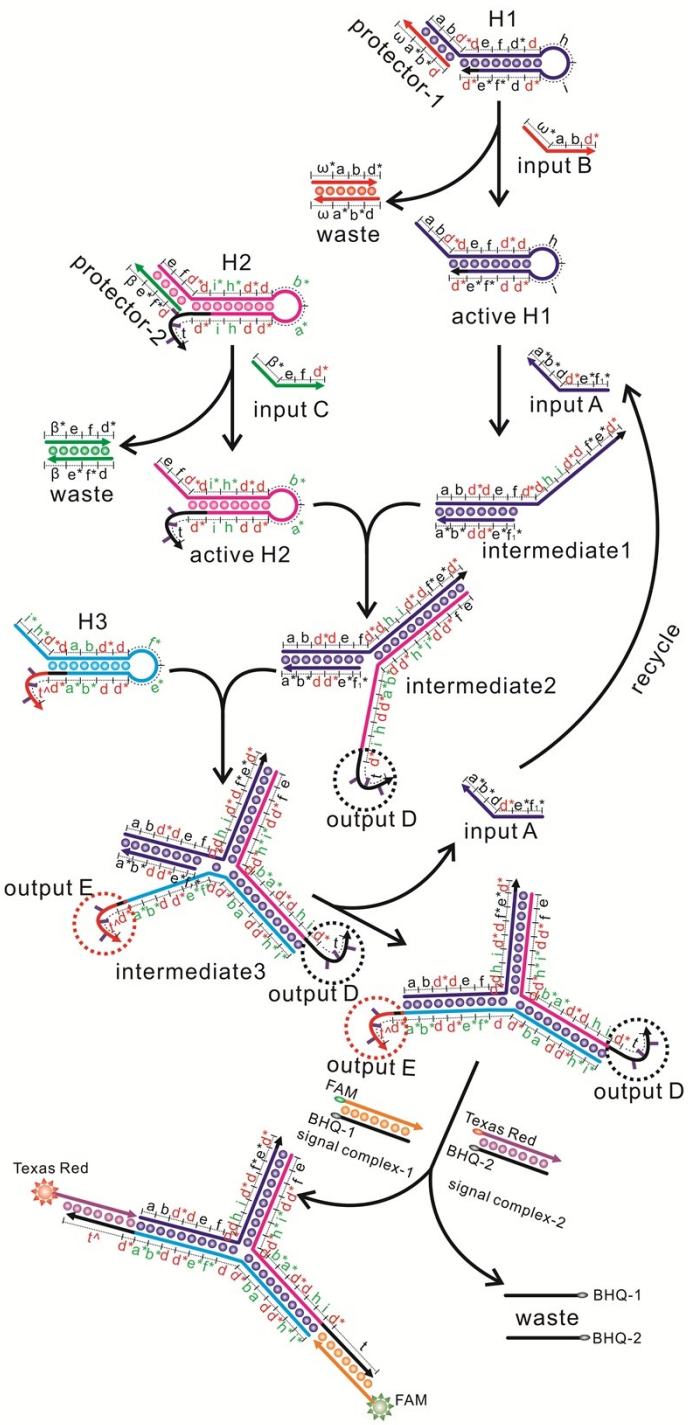

Fig. S17. Schematic representation for the catalytic metathetical reaction $B+C \rightarrow D+E$. Hairpins H2 and H3 are conjugated with different outputted sequences. After the accomplishment of the series of DNA strand displacement reactions, complete DNA junction terminating with two kinds of functional tails is obtained. The d*-t is labeled as output D, which is used for releasing the FAM-modified strand from the signal complex-1. In addition, d*-t^ is treated as output E, which is used for initiating the strand displacement reaction with signal complex-2 and resulting in the fluorescence signal of Texas Red. The 3′-terminal of the DNA sequences is indicated with an arrow.

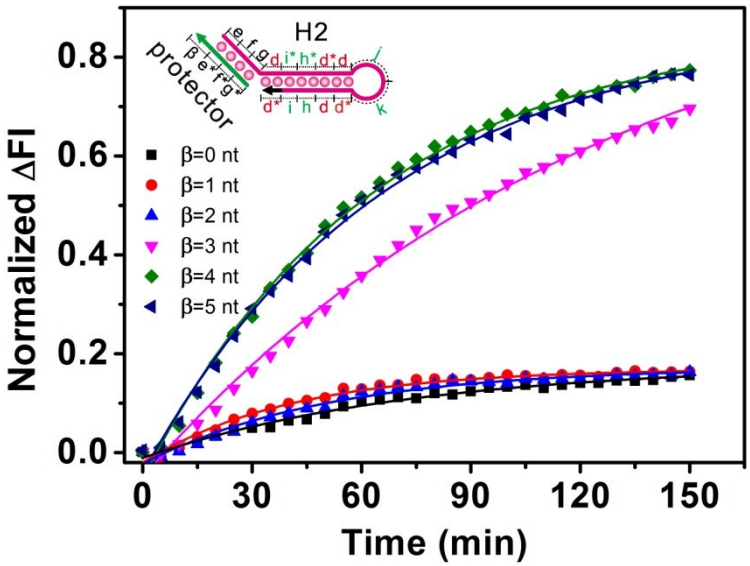

Fig. S18. The kinetic studies of catalytic reaction $B \rightarrow C$ with varied toehold length of protector strand on H2.

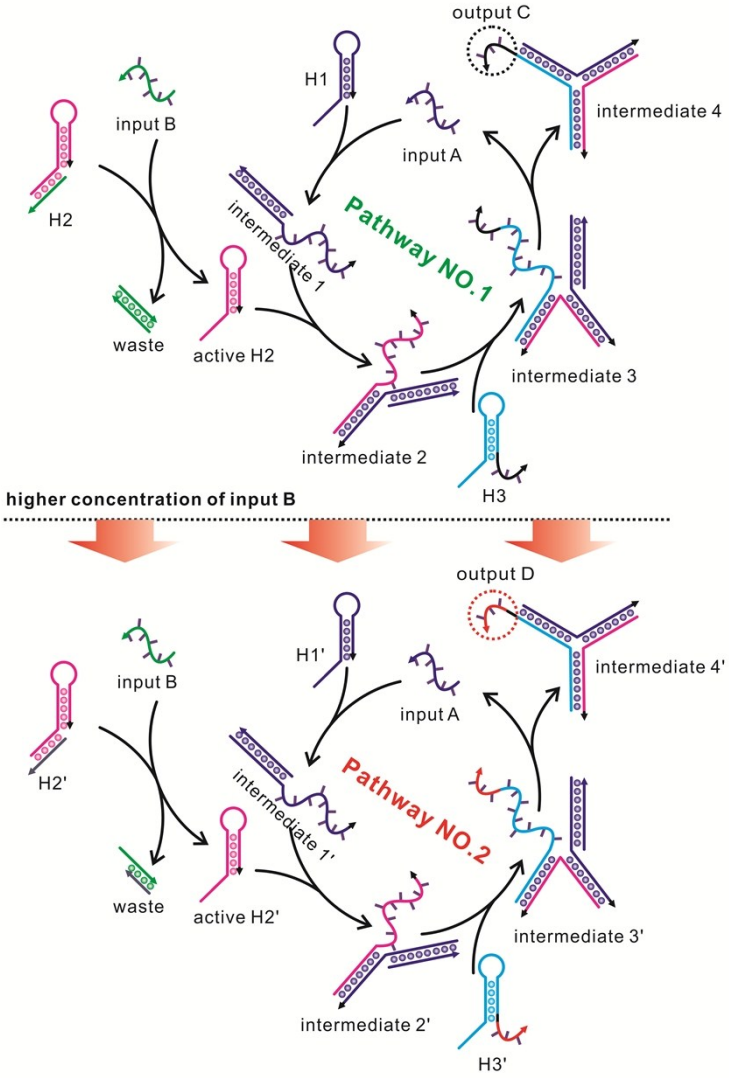

Fig. S19. The mechanism of warning controller that constructed with multiple catalytic reaction modules.

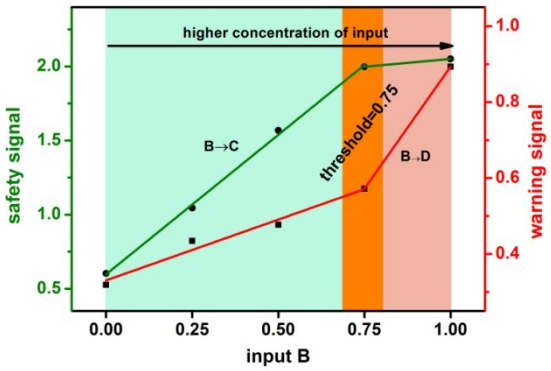

Fig. S20. Programming the operation of warning controller with varied threshold values.

S3: Supporting Tables

Table S1: DNA sequences used for constructing the non-catalytic reaction $A + B \rightarrow C$. (All sequences started from the 5′-terminal)

hairpin H1	TATCATTCGAAGCTTCGCTCTGATGAAGCTTCTCGCACATGAAGCTTCATCAG AGCGAAG
hairpin H2	GCTCTGATGAAGCTTCATGTGCGAGAAGCTTCGAATGATAGAAGCTTCTCGCA CATGAAG
tailed-hairpin H2	GCTCTGATGAAGCTTCATGTGCGAGAAGCTTCGAATGATAGAAGCTTCTCGCA CATGAAGACAGACGACAA
hairpin H3	ATGTGCGAGAAGCTTCTATCATTCGAAGACTCCTGTGTCCGAGTCTTCGAATG ATAGAAG
tailed-hairpin H3	ATGTGCGAGAAGCTTCTATCATTCGAAGACTCCTGTGTCCGAGTCTTCGAATG ATAGAAGACAGACGACAA
protector strand	CTTCATCAGAGCCACCTAGTTG
input A	TCAGAGCGAAGCTTCGAATGATA
input B	CAACTAGGTGGCTCTGATGAAG
reporter strand	FAM-AATCTTGTCGTCTGTCTTC
quencher strand	CAGACGACAAGATT-BHQ-1

Table S2: DNA sequences used for constructing the catalytic decomposition reactions $B \rightarrow C + \mathrm{D}$. (All sequences started from the 5′-terminal)

hairpin H1	TATCATTCGAAGCTTCGCTCTGATGAAGCTTCTCGCACATGAAGCTTCATCAG AGCGAAG
tailed-hairpin H2	GCTCTGATGAAGCTTCATGTGCGAGAAGCTTCGAATGATAGAAGCTTCTCGCA CATGAAGACAGACGACAA
second-tailed-hairpin H3	ATGTGCGAGAAGCTTCTATCATTCGAAGCTTCATCAGAGCGAAGCTTCGAATG ATAGAAGCGAAAACCAAG
protector strand	CTTCATCAGAGCCACCTAGTTG
input A	TCAGAGCGAAGCTTCGAATGATA
input B	CAACTAGGTGGCTCTGATGAAG
reporter strand	FAM-AATCTTGTCGTCTGTCTTC
quencher strand	CAGACGACAAGATT-BHQ-1
second-reporter strand	Texas Red-ATCTCTTGGTTTTCGCTTC
second-quencher strand	GAAAACCAAGAGAT-BHQ-2

Table S3: DNA sequences used for constructing the catalytic reaction $B \rightarrow \mathrm{m}C$. (All of the sequences started from the 5′-terminal)

hairpin H1	TATCATTCGAAGCTTCGCTCTGATGAAGCTTCTCGCACATGAAGCTTCATCAG

	AGCGAAG
hairpin H2	GCTCTGATGAAGCTTCATGTGCGAGAAGCTTCGAATGATAGAAGCTTCTCGCA CATGAAG
tailed-hairpin H2	GCTCTGATGAAGCTTCATGTGCGAGAAGCTTCGAATGATAGAAGCTTCTCGCA CATGAAGACAGACGACAA
hairpin H3	ATGTGCGAGAAGCTTCTATCATTCGAAGCTTCATCAGAGCGAAGCTTCGAATG ATAGAAG
tailed-hairpin H3	ATGTGCGAGAAGCTTCTATCATTCGAAGCTTCATCAGAGCGAAGCTTCGAATG ATAGAAGACAGACGACAA
protector strand	CTTCATCAGAGCCACCTAGTTG
input A	TCAGAGCGAAGCTTCGAATGATA
input B	CAACTAGGTGGCTCTGATGAAG
reporter strand	FAM-AATCTTGTCGTCTGTCTTC
quencher strand	CAGACGACAAGATT-BHQ-1

Table S4: DNA sequences used for constructing the catalytic combinational reactions $B + C \rightarrow D$. (All sequences started from 5′-terminal)

hairpin H1	TATCATTCGAAGCTTCGCTCTGATGAAGCTTCTCGCACATGAAGCTTCATCAG AGCGAAG
hairpin H2	GCTCTGATGAAGCTTCATGTGCGAGAAGCTTCGAATGATAGAAGCTTCTCGCA CATGAAG
tailed-hairpin H3	ATGTGCGAGAAGCTTCTATCATTCGAAGCTTCATCAGAGCGAAGCTTCGAATG ATAGAAGACAGACGACAA
protector-1 strand	CTTCGAATGATATAACCTGAAT
protector-2 strand	CTTCATCAGAGCCACCTAGTTG
input A	TCAGAGCGAAGCTTCGAATGATA
input B	ATTCAGGTTATATCATTCGAAG
input C	CAACTAGGTGGCTCTGATGAAG
reporter strand	FAM-AATCTTGTCGTCTGTCTTC
quencher strand	CAGACGACAAGATT-BHQ-1

Table S5: DNA sequences used for constructing the catalytic metathetical reactions $B + C \rightarrow D + E$. (All sequences started from 5′-terminal)

hairpin H1	TATCATTCGAAGCTTCGCTCTGATGAAGCTTCTCGCACATGAAGCTTCATCAG AGCGAAG
tailed-hairpin H2	GCTCTGATGAAGCTTCATGTGCGAGAAGCTTCGAATGATAGAAGCTTCTCGCA CATGAAGACAGACGACAA
second-tailed-hairpin H3	ATGTGCGAGAAGCTTCTATCATTCGAAGCTTCATCAGAGCGAAGCTTCGAATG ATAGAAGCGAAAACCAAG
protector-1 strand	CTTCGAATGATATAACCTGAAT
protector-2 strand	CTTCATCAGAGCCACCTAGTTG

input A	TCAGAGCGAAGCTTCGAATGATA
input B	ATTCAGGTTATATCATTCGAAG
input C	CAACTAGGTGGCTCTGATGAAG
reporter strand	FAM-AATCTTGTCGTCTGTCTTC
quencher strand	CAGACGACAAGATT-BHQ-1
second-reporter strand	Texas Red-ATCTCTTGGTTTTCGCTTC
second-quencher strand	GAAAACCAAGAGAT-BHQ-2

Table S6: The DNA sequences used for constructing warning controller. (All of the sequences started from 5′-terminal)

hairpin H1	AATATCATTCCTACTCATTCTCTTGAAGGAATGAGAATGAGTAGGAA
hairpin H2	GATGCTACTCATTCTCATTCCTTCAAAGGAATGATATTGAAGGAATGAGAA
tailed-hairpin H3	TCATTCCTTCAATATCATTCCTGAGAATGAGTAGGAATGATATTGAAGACAGA CGACAA
hairpin H1′	AATATCATTCCTACTCATTCTCTTGAAAGTGTGAGAATGAGTAGGAA
hairpin H2′	GATGCTACTCATTCTCACACTTTCAAAGGAATGATATTGAAAGTGTGAGAA
second-tailed-hairpin H3′	TCACACTTTCAATATCATTCCTGAGAATGAGTAGGAATGATATTGAAACGAAA ACCAAG
protector strand	TGAGTAGCATCATCATATCCA
second protector strand	TGAGTAGCATCATC
input A	AGAATGAGTAGGAATGATATT
input B	TGGATATGATGATGCTACTC
reporter strand	FAM-AATCTTGTCGTCTGTCTTC
quencher strand	CAGACGACAAGATT-BHQ-1
second-reporter strand	Texas Red-ATCTCTTGGTTTTCGTTTC
second-quencher strand	GAAAACCAAGAGAT-BHQ-2

References:

1. Zadeh, J. N.; Steenberg, C. D.; Bois, J. S.; Wolfe, B. R.; Pierce, M. B.; Khan, A. R.; Dirks, R. M.; Pierce, N. A., NUPACK: Analysis and design of nucleic acid systems. *Journal of Computational Chemistry* **2011,** *32*, 170-173.

2. Zhang, D. Y.; Turberfield, A. J.; Yurke, B.; Winfree, E., Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA. *Science* **2007,** *318*, 1121-1125.