Electronic Supplementary Material (ESI) for Analyst.
This journal is © The Royal Society of Chemistry 2022

DNA Tile Self-assembly Driven by Antibody-mediated Four-way Branch Migration

(Supplementary Information)

Xingdi Cui, [a] Yuan Liu, [b] Qiang Zhang *[a, b]

a Key Laboratory of Advanced Design and Intelligent Computing, Dalian University, Ministry of Education, Dalian 116622, China. Email: axingxing_77@163.com
b School of Computer Science and Technology, Dalian University of Technology, Dalian 116024, China. Email: zhangq@dlut.edu.cn

Contents

S1. Antibody-mediated four-way branch migration mechanism 1
Effect of antibody characteristics on reaction rate 2
Tuning of antibody-mediated four-way branch migration kinetics 5
S2. Self-assembly strategy of DNA tiles 6
The second improvement scheme 6
Combination of improvement schemes 1 and 2 6
The third improvement scheme 7
S3. DNA sequence 8

S1. Antibody-mediated four-way branch migration mechanism

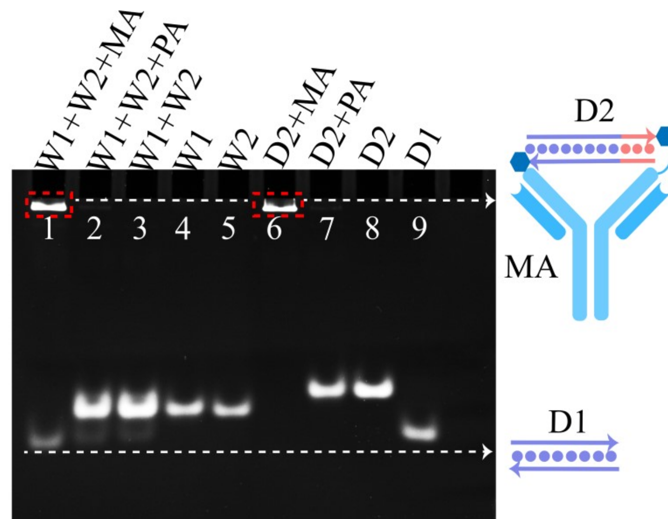

Figure S1. Polyacrylamide gel electrophoresis analysis of antibody-mediated four-way branch migration mechanism. The input and substrate of each lane are marked on the top of the gel. MA stands for monoclonal antibody and PA stands for polyclonal antibody. This gel electrophoresis analysis was stained with GelRed. ([W1]=[W2]=167nM, [MA]=[PA]=250nM).

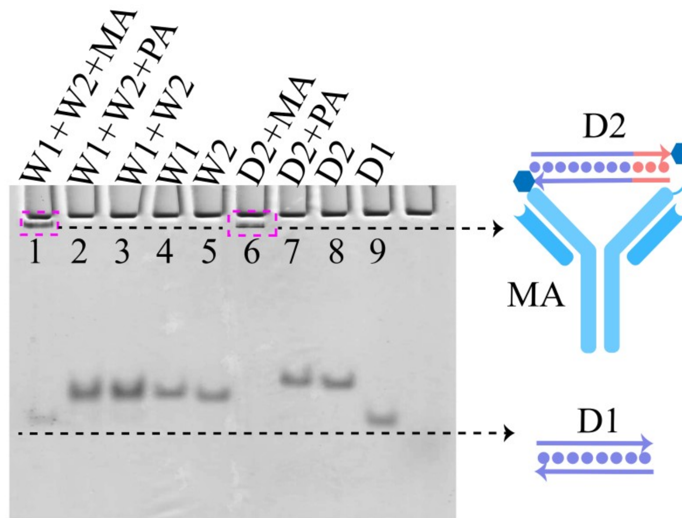

Figure S2. Polyacrylamide gel electrophoresis analysis of antibody-mediated four-way branch migration mechanism. The input and substrate of each lane are marked on the top of the gel. MA stands for monoclonal antibody and PA stands for polyclonal antibody. ([W1]=[W2]=200nM, [MA]=[PA]=600nM).

Effect of antibody characteristics on reaction rate

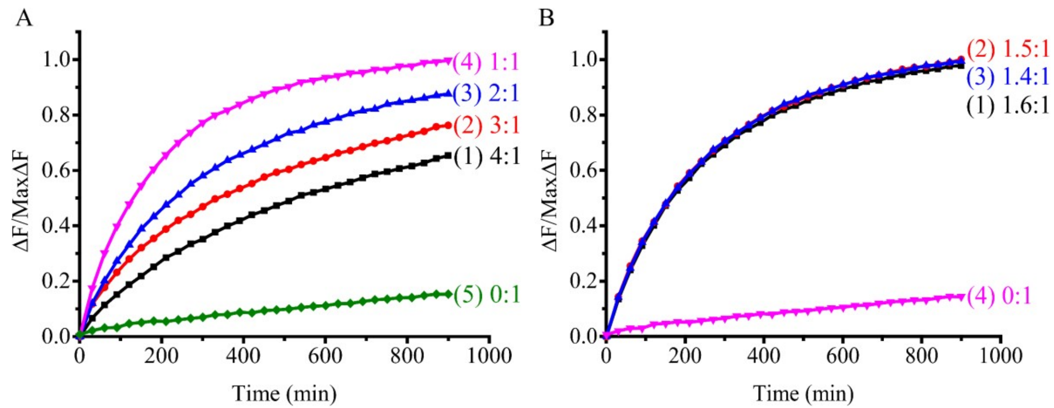

Figure S3. Kinetic traces of different ratio of antibody to substrate concentration. On the right is the concentration ratio of antibody to substrate represented by each curve. Except for the different concentration ratios, all other reaction conditions remain the same. The reaction substrates are W1 and W2, and the monoclonal antibody is the input. ([W1]=[W2]=100nM).

Figure S3A is the fluorescence kinetic analysis of the reaction when the concentration ratio is 0:1 to 4:1 (integer multiples). It can be seen from the figure that when the concentration of antibody and substrate is 1:1, the reaction rate is the fastest, and the promotion effect of the antibody is the optimal, as the concentration of the antibody increases, the promotion effect gradually decreases. Figure S3B shows the experimental results when the concentration ratio is 1.4:1 and 1.6:1. The results show that there is no obvious difference in the fluorescence signal of the three concentration ratios, and the reaction rate is basically the same.

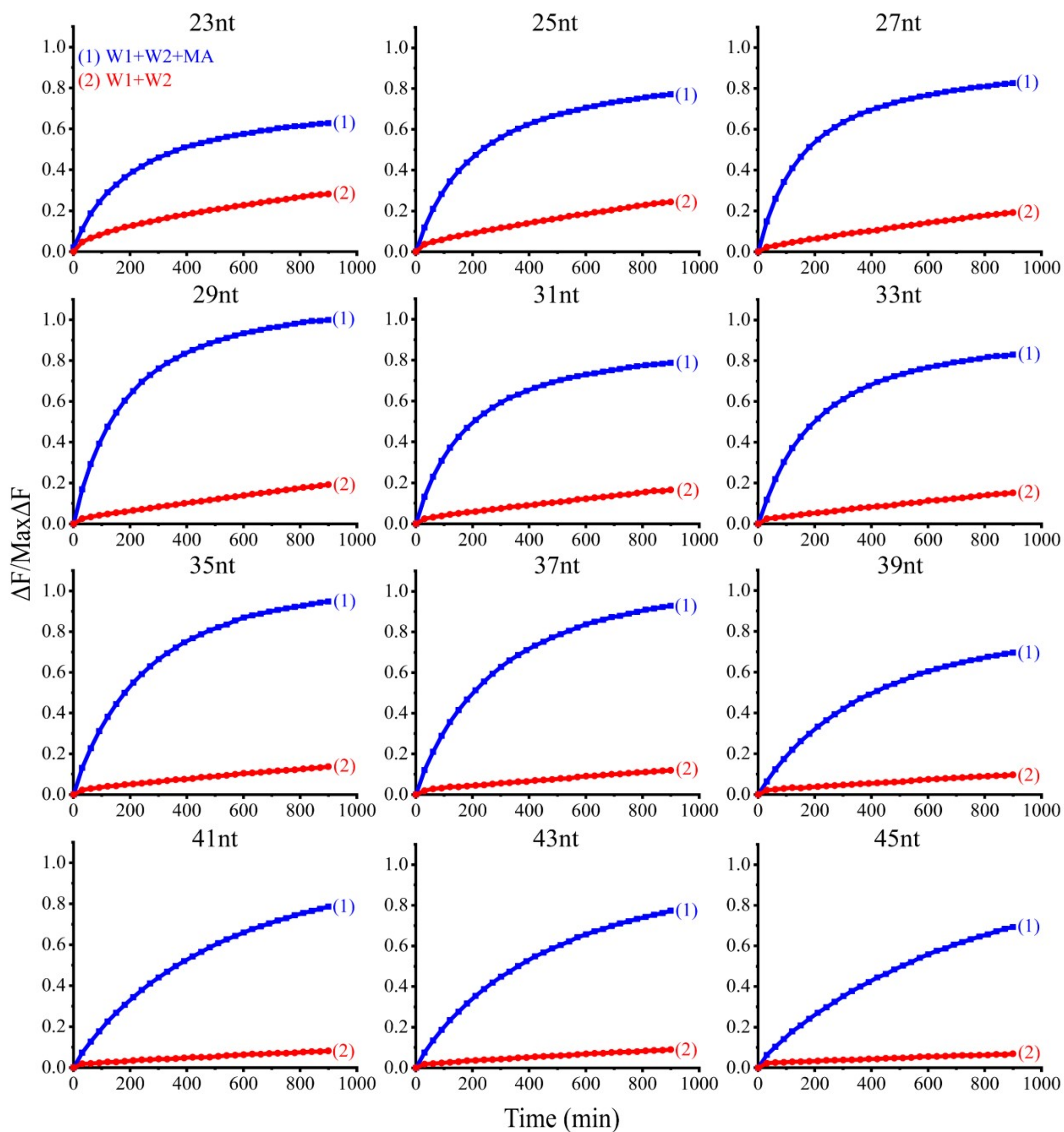

Figure S4. Kinetic traces of different substrate strand lengths. Each curve represents input with W1 and W2 as substrates, curve 1 represents antibody as input and curve 2 represents no input. ([W1]=[W2]=100nM, [MA]=150nM).

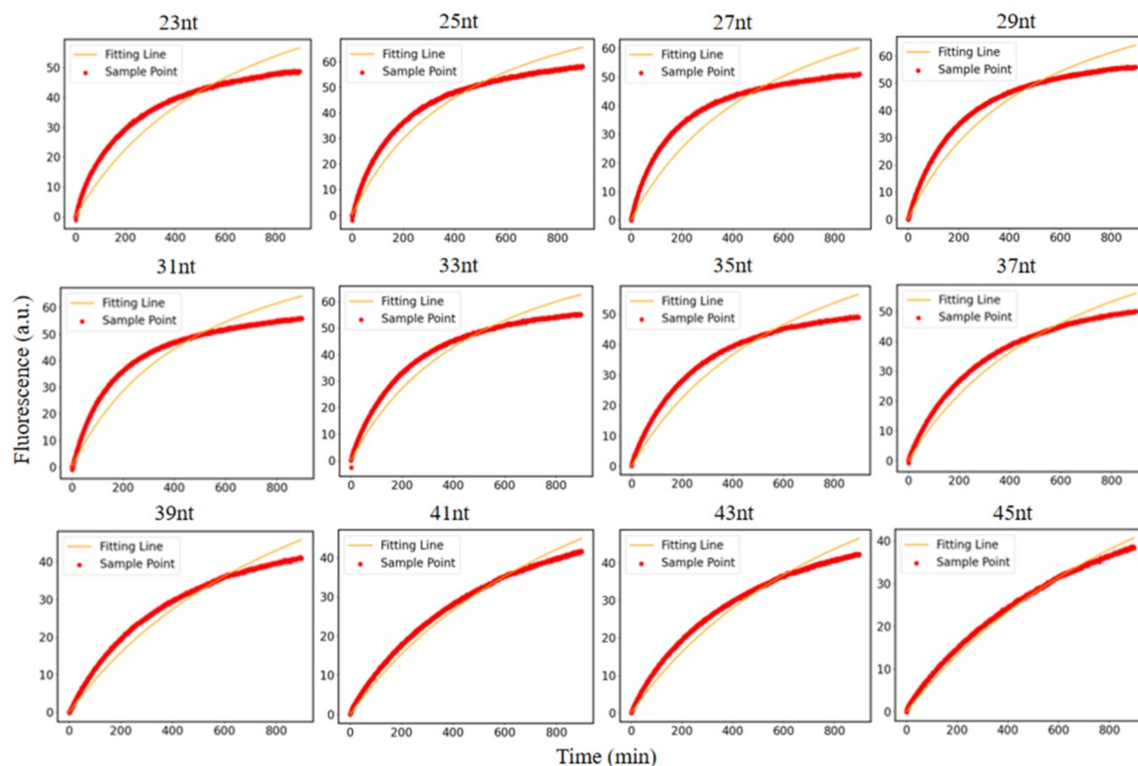

Figure S5. Fluorescence traces of different substrate strand lengths. The red curve represents the addition of W1, W2 and MA, and MA represents the monoclonal antibody. The orange curve represents the reaction simulation traces when the rate constant is K_v. The reaction rate constant is different for different substrate strand lengths. ([W1]=[W2]=100nM, [MA]=150nM).

Tuning of antibody-mediated four-way branch migration kinetics

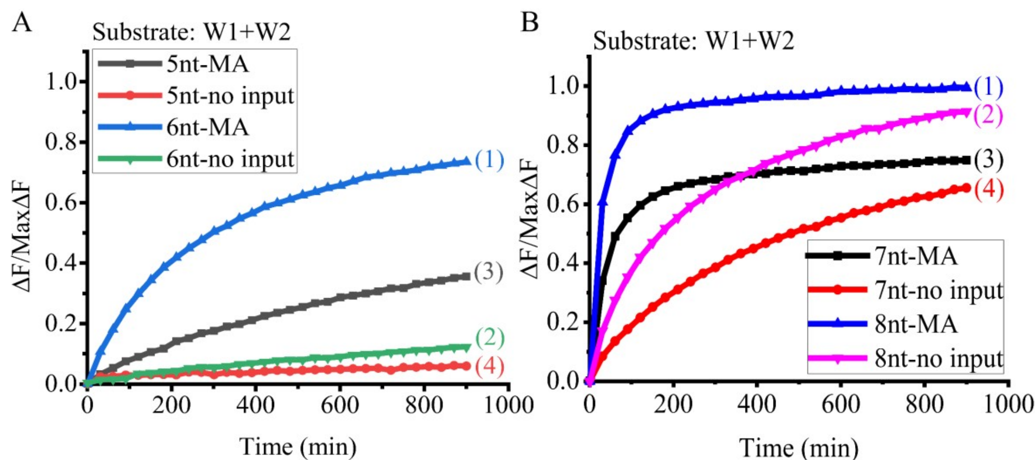

Figure S6. Fluorescence kinetic analysis of different toehold lengths. (A) Kinetic traces of toehold length at 5nt and 6nt. (B) Kinetic traces of toehold length at 7nt and 8nt. Each curve represents inputs with W1 and W2 as substrates, and different inputs are added. MA represents monoclonal antibody. ([W1]=[W2]=100nM, [MA]=150nM).

In order to further optimize the reaction rate of the four-way branch migration mechanism, fluorescence kinetics analysis was performed on the four-way branch migration with different toehold lengths. When the toehold length is 5 nucleotides (Figure S6A), even with antibody input, the reaction rate is very slow (curve 3). When the toehold length is 7 nucleotides or 8 nucleotides (Figure S6B), the fluorescence signal in the presence of antibody is obviously enhanced (curves 1 and 3), and quickly reaches an equilibrium state, while the fluorescence signal in the absence of antibody is also obviously enhanced (curves 2 and 4), and finally tend to be consistent with the fluorescence value when the antibody is present. This shows that when the toehold length is greater than or equal to 7 nucleotides, the initial state of the reactant is not stable, which causes the four-way branch migration reaction to proceed at a rapid rate and reach an equilibrium state. Only when the length of toehold is 6 nucleotides (Figure S6A), the fluorescence signal is obviously enhanced when the antibody is input (curve 1), but it has no obvious change when the antibody is not present (curve 2). This shows that when the toehold length is 6 nucleotides, the promotion effect of the antibody is optimal, which makes the four-way branch migration proceed at a faster reaction rate. Therefore, the above experimental results show that 6 nucleotides is the optimal toehold length of antibody-mediated four-way branch migration mechanism.

S2. Self-assembly strategy of DNA tiles

The second improvement scheme

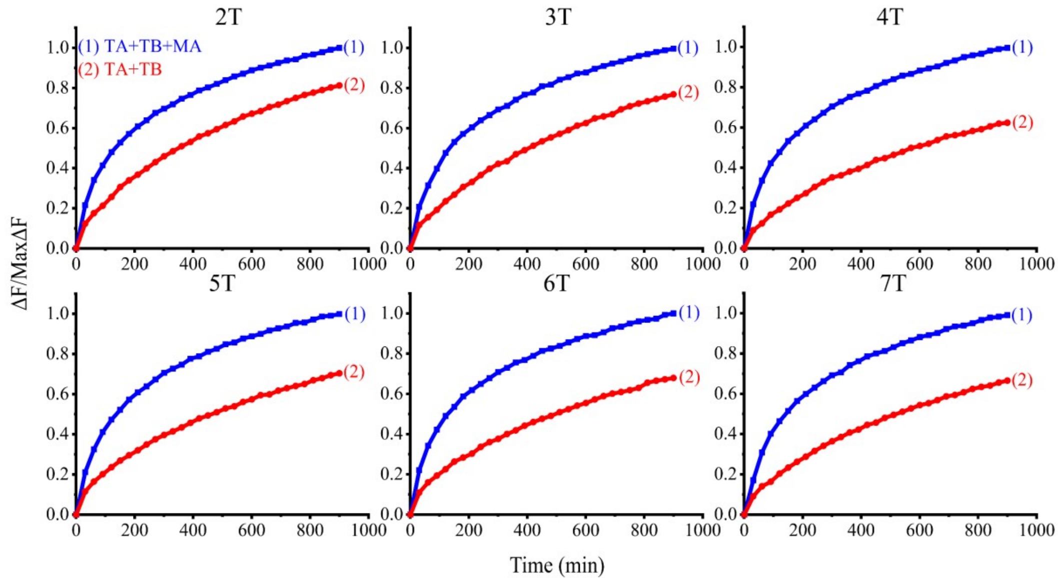

Figure S7. Kinetic traces of modifying T loops with different number of T bases. Each curve represents input with TA and TB as substrates, curve 1 represents antibody as input and curve 2 represents no input. ([TA]=[TB]=100nM, [MA]=150nM).

Combination of improvement schemes 1 and 2

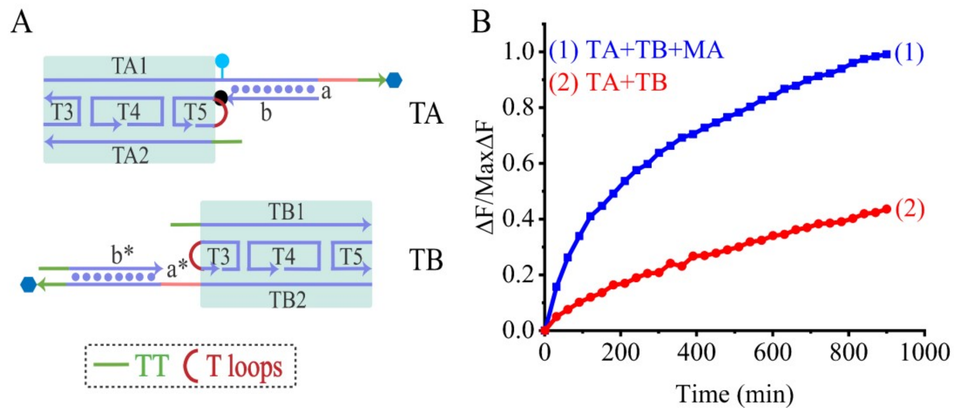

Figure S8. (A) Schematic of tiles combining improvement schemes 1 and 2. (B) Kinetic traces of the combination of improvement schemes 1 and 2. The input of each curve has been marked in the figure, and MA represents monoclonal antibody. ([TA]=[TB]=100nM, [MA]=150nM).

We combine the first scheme with the four-nucleotide T loops in the second scheme to optimize the tile connection scheme, as shown in Figure S8A. The results of the fluorescence experiment are shown in Figure S8B. The fluorescence signal is reduced when there is no antibody input (curve 2). It shows that combining the two schemes further destroys the π-π stacking.

The third improvement scheme

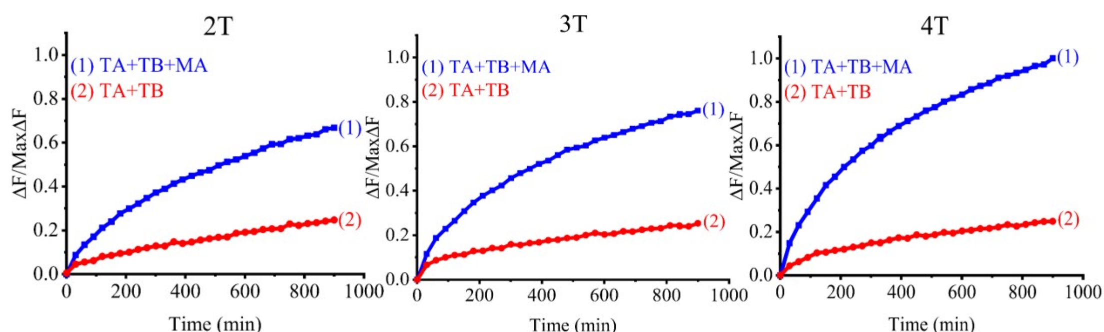

Figure S9. Kinetic traces of different number of T bases. The input of each curve has been marked in the figure, and MA represents monoclonal antibody. ([TA]=[TB]=100nM, [MA]=150nM).

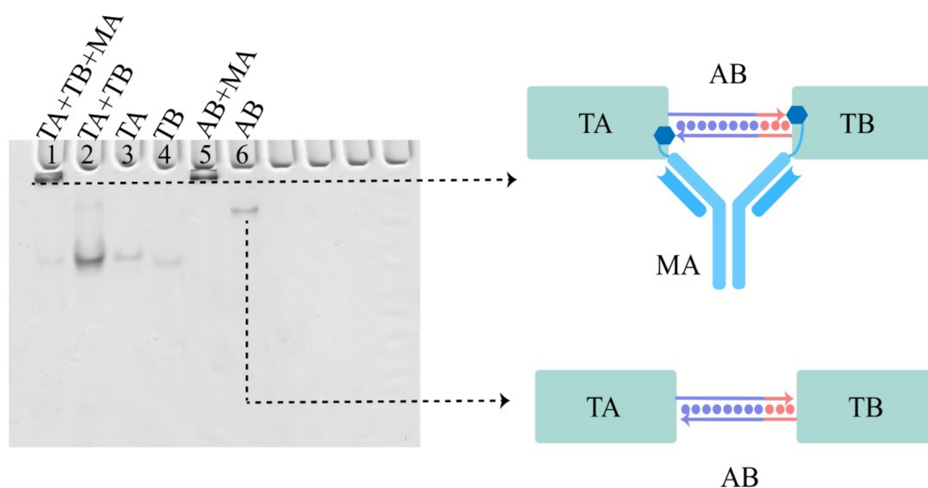

Figure S10. Polyacrylamide gel electrophoresis analysis when adding four T bases. The input and substrate of each lane are marked on the top of the gel. MA represents monoclonal antibody. ([TA]=[TB]=100nM, [MA]=150nM).

Figure S9 shows the results of the fluorescence experiment with different numbers of T bases. It can be observed from the figure that the improvement effect is better when four T bases are added. The results of the polyacrylamide gel electrophoresis experiment when adding four T bases are shown in Figure S10. Lanes 3 and 4 represent the substrates TA and TB involved in the reaction, lanes 5 and 6 are references to the products. In lane 1, with the antibody as input, it can be observed that the complex structure AB combined

with the antibody is formed at the corresponding position, and there is almost no substrate residue. This shows that the antibody promotes the strand displacement reaction, which makes the two tiles connected together and the reaction is relatively complete. However, when the antibody is not added (lane 2), the production of the complex structure AB cannot be clearly seen, and the obvious substrate residue can be observed, indicating that the strand displacement reaction basically does not occur when there is no antibody input, the tiles are not connected together. This is consistent with the results of the fluorescence experiment.

S3. DNA sequence

We use NUPACK to analyze all the DNA sequences to reduce undesired hybridization between DNA strands. DNA sequences are shown in Table S1 and S2.

Table S1. Sequence of four-way branch migration

Length (nt)	Strand Name		DNA Sequence (5' to 3')
23	S		FAM- CTCTTCCTACCGTCCTCAGTCAC
	S*		GAGGACGGTAGGAAGAG-BHQ1
	L		CTCTTCCTACCGTCCTC
	L*		GTGACTGAGGACGGTAGGAAGAG
25	S		FAM- CTCTCTTCCTACCGTCCTCAGTCAC
	S*		GAGGACGGTAGGAAGAGAG
	L		CTCTCTTCCTACCGTCCTC
	L*		GTGACTGAGGACGGTAGGAAGAGAG
27	S		FAM- CCCTCTCTTCCTACCGTCCTCAGTCAC
	S*		GAGGACGGTAGGAAGAGAGGG
	L		CCCTCTCTTCCTACCGTCCTC
	L*		GTGACTGAGGACGGTAGGAAGAGAGGG
29	S		FAM- TACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*		GAGGACGGTAGGAAGAGAGGGTA
	L		TACCCTCTCTTCCTACCGTCCTC
	L*		GTGACTGAGGACGGTAGGAAGAGAGGGTA
31	S		FAM- CGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*		GAGGACGGTAGGAAGAGAGGGTACG
	L		CGTACCCTCTCTTCCTACCGTCCTC
	L*		GTGACTGAGGACGGTAGGAAGAGAGGGTACG
33		S	FAM- GCCGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	Toehold:6	S*	GAGGACGGTAGGAAGAGAGGGTACGGC
		L	GCCGTACCCTCTCTTCCTACCGTCCTC
		L*	GTGACTGAGGACGGTAGGAAGAGAGGGTACGGC
	Toehold:5	S*	TGAGGACGGTAGGAAGAGAGGGTACGGC
		L	GCCGTACCCTCTCTTCCTACCGTCCTCA
	Toehold:7	S*	AGGACGGTAGGAAGAGAGGGTACGGC
		L	GCCGTACCCTCTCTTCCTACCGTCCT
	Toehold:8	S*	GGACGGTAGGAAGAGAGGGTACGGC
		L	GCCGTACCCTCTCTTCCTACCGTCC
35	S		FAM- TAGCCGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*		GAGGACGGTAGGAAGAGAGGGTACGGCTA

	L	TAGCCGTACCCTCTCTTCCTACCGTCCTC
	L*	GTGACTGAGGACGGTAGGAAGAGAGGGTACGGCTA
37	S	FAM- CCTAGCCGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*	GAGGACGGTAGGAAGAGAGGGTACGGCTAGG
	L	CCTAGCCGTACCCTCTCTTCCTACCGTCCTC
	L*	GTGACTGAGGACGGTAGGAAGAGAGGGTACGGCTAGG
39	S	FAM- CTCCTAGCCGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*	GAGGACGGTAGGAAGAGAGGGTACGGCTAGGAG
	L	CTCCTAGCCGTACCCTCTCTTCCTACCGTCCTC
	L*	GTGACTGAGGACGGTAGGAAGAGAGGGTACGGCTAGGAG
41	S	FAM- GACTCCTAGCCGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*	GAGGACGGTAGGAAGAGAGGGTACGGCTAGGAGTC
	L	GACTCCTAGCCGTACCCTCTCTTCCTACCGTCCTC
	L*	GTGACTGAGGACGGTAGGAAGAGAGGGTACGGCTAGGAGTC
43	S	FAM- CAGACTCCTAGCCGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*	GAGGACGGTAGGAAGAGAGGGTACGGCTAGGAGTCTG
	L	CAGACTCCTAGCCGTACCCTCTCTTCCTACCGTCCTC
	L*	GTGACTGAGGACGGTAGGAAGAGAGGGTACGGCTAGGAGTCTG
45	S	FAM- GACAGACTCCTAGCCGTACCCTCTCTTCCTACCGTCCTCAGTCAC
	S*	GAGGACGGTAGGAAGAGAGGGTACGGCTAGGAGTCTGTC
	L	GACAGACTCCTAGCCGTACCCTCTCTTCCTACCGTCCTC
	L*	GTGACTGAGGACGGTAGGAAGAGAGGGTACGGCTAGGAGTCTGTC

Table S2. Sequence of self-assembly strategy of DNA tiles

Scheme Name	Strand Name	DNA Sequence (5' to 3')
Initial scheme	TA1	ACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAAGCAGT ACCCTCTCTTCCTACAGTATTCAGTGTG
	TA2	TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAAT
	T3	ATTCTACTCGTGGATCTATGGT
	T4	AGAATTGCGTCGTGGTTGCTAGGTCTCGCTATCACCGATGTG
	T5	TTCTGGTTTCACCTTAACGATA
	b	GAATACTGTAGGAAGAGAGGGTACTGC
	TB1	ACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAA
	TB2	TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAATCACAC TGAATACTGTAGGAAGAGAGGGTACTGC
	b*	GCAGTACCCTCTCTTCCTACAGTATTC
Improvement scheme 1	TA1	ACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAAGCAGT ACCCTCTCTTCCTACAGTATTCAGTGTGTT
	TA2	TTTATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAAT
	T3	ATTCTACTCGTGGATCTATGGT

	T4		AGAATTGCGTCGTGGTTGCTAGGTCTCGCTATCACCGATGTG
	T5		TTCTGGTTTCACCTTAACGATATT
	b		GAATACTGTAGGAAGAGAGGGTACTGC
	TB1		TTACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAA
	TB2		TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAATCACAC TGAATACTGTAGGAAGAGAGGGTACTGCTT
	b*		TTGCAGTACCCTCTCTTCCTACAGTATTC
Improvement scheme 2	TA1		ACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAAGCAGT ACCCTCTCTTCCTACAGTATTCAGTGTG
	TA2		TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAAT
	T3		ATTCTACTCGTGGATCTATGGT
		2T	CTCGTGGATCTATGGTTTATTCTA
		3T	CTCGTGGATCTATGGTTTTATTCTA
		4T	CTCGTGGATCTATGGTTTTTATTCTA
		5T	CTCGTGGATCTATGGTTTTTTATTCTA
		6T	CTCGTGGATCTATGGTTTTTTTATTCTA
		7T	CTCGTGGATCTATGGTTTTTTTTATTCTA
	T4		AGAATTGCGTCGTGGTTGCTAGGTCTCGCTATCACCGATGTG
	T5		TTCTGGTTTCACCTTAACGATA
		2T	ACGATATTTTCTGGTTTCACCTTA
		3T	ACGATATTTTTCTGGTTTCACCTTA
		4T	ACGATATTTTTTCTGGTTTCACCTTA
		5T	ACGATATTTTTTTCTGGTTTCACCTTA
		6T	ACGATATTTTTTTTCTGGTTTCACCTTA
		7T	ACGATATTTTTTTTTCTGGTTTCACCTTA
	b		GAATACTGTAGGAAGAGAGGGTACTGC
	TB1		ACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAA
	TB2		TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAATCACAC TGAATACTGTAGGAAGAGAGGGTACTGC
	b*		GCAGTACCCTCTCTTCCTACAGTATTC
Improvement scheme 3	TA1		ACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAAGCAGT ACCCTCTCTTCCTACAGTATTCAGTGTGTT
	TA2		TTTATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAAT
	T3		ATTCTACTCGTGGATCTATGGT
	T3(4T loops)		CTCGTGGATCTATGGTTTTTATTCTA
	T4		AGAATTGCGTCGTGGTTGCTAGGTCTCGCTATCACCGATGTG
	T5		TTCTGGTTTCACCTTAACGATA
	T5(4T loops)		ACGATATTTTTTCTGGTTTCACCTTA

	b		GAATACTGTAGGAAGAGAGGGTACTGC
	TB1		TTACCATAGATCCTGATAGCGAGACCTAGCAACCTGAAACCAGAA
	TB2	2T	TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAATTTCACACTGAATACTGTAGGAAGAGAGGGTACTGC
		3T	TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAATTTTCACACTGAATACTGTAGGAAGAGAGGGTACTGC
		4T	TATCGTTAAGGACGACGCAATTCTCACATCGGACGAGTAGAATTTTTCACACTGAATACTGTAGGAAGAGAGGGTACTGC
	b*		TTGCAGTACCCTCTCTTCCTACAGTATTC

The red highlights indicate toehold a and a *.

The technique for coupling digoxin to the modified amino group on oligonucleotide was EDC/NHS.