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Biochemical Logic Circuits Based on DNA Combinatorial Displacement

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ABSTRACT DNA, as an excellent nano-engineering material, contributes to a new computing model, namely, DNA computing. This model is a type of biological computing, which takes advantage of the high density and high parallelism of molecules. One of the current methods of implementing DNA computing is to construct DNA circuits, among which the toehold-mediated DNA strand displacement technique is an important method. The hybridization of toehold domains provides the start position and accelerates the branch migration process. Toehold-based DNA combinatorial displacement is a practical method for designing and implementing DNA circuits. In this paper, we designed and simulated a multiplexer using the DNA combinatorial displacement mechanism to verify its practicability. Additionally, we improved and optimized the existing logic INHIBIT gate by leveraging the DNA combinatorial displacement mechanism so that the DNA strands in the entire chemical reaction network (CRN) system are capable of coexisting in large quantities. Moreover, we applied this improvement to the demultiplexer. Our method provides more capabilities to larger and more complicated DNA integrated circuits.


INDEX TERMS Combinatorial strand displacement, logic gate, CRN, DNA computing.

I. INTRODUCTION

Due to their remarkable programmability and specificity, DNA molecules have become one of the most important materials for building nano-molecular machines with rich functions [1], [2]. DNA nanotechnology has been used in a wider range of fields [3], [4], including DNA computing [5]–[7], molecular machine construction [1], [8], [9], and disease diagnosis [10]–[12]. By manipulating DNA strand techniques, an increasing number of DNA nanodevices [13]–[15] are being implemented, including biochemical sensors [16]–[18], calculators [19]–[21], timers [22], molecular probes [23]–[25], and nanomachines [26]–[28]. One important technology to implement these applications is DNA strand displacement technology. Since the introduction of toehold-mediated chain replacement technology by Yurke *et al.* [29], biochemical technology based strand displacement has been greatly developed. The toehold method

tremendously accelerates the process of branch migration and provides a reliable support for large biochemical reaction circuits. The introduction of toeholds guides a wide variety of biochemical reaction networks, including entropy-driven reactions and DNA-catalyzed networks [30], DNAzyme-related logic circuits [31], nonlinear hybridization chain reactions [32], DNA logic gate platform for miRNA analysis [33] and deoxyribozyme-based molecular automaton [34].

However, after nearly two decades of development, the traditional toehold-mediated strand displacement method is unable to satisfy the increasing divergence of applications. Existing DNA strand-based methods, such as using deoxyribozymes to implement operations and dynamic analysis [35] and using restriction enzymes to build a molecular cryptosystem for images [36], are all combinations of diverse tools. Upgrading and adjusting the basic DNA strand displacement methods will push DNA computing technology to new heights. Yang *et al.* [37] proposed a toehold allosteric technique, which improved the basic toehold reaction structure. In the novel structure, changes in the short strand involved

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in further hybridization structure can lead to a long-strand hybridization so that a short strand is allowed to guide the long strand for displacement. The junction structure proposed by Sun *et al.* [38] improved the energy change process during the toehold-mediated strand displacement reaction, such that when the toehold is hybridized, it exceeds the energy limits. This novel structure significantly reduces the leakage of continuous displacements on a single long strand of DNA in the traditional manner. Bath and Turberfield [39] introduced a combinatorial displacement strategy, which, compared with these two improvements, changed the toehold-mediated strand displacement process to a larger extent. The proposed combination technique separates the toehold domain from the specific domain such that these two domains can be recombined. It can be used for Boolean operations and complex DNA logic circuits. This provides strong programmability for biochemical reaction networks that are composed of DNA molecules and makes a positive contribution to the development of molecular programming methods. Using this combinatorial displacement strategy, Niu and Zhang *et al.* [40], [41] built AND, OR, INHIBIT, and XOR logic gates and designed logic cascaded circuits by using these logic gates. They implemented a 4-to-2 encoder that simplified the previous 4-to-2 encoder designed by using basic DNA strand displacement technology. Although these logic gates can be implemented effectively, the input molecules of the INHIBIT gate have certain limitations. When the logic gate is cascaded into more complex circuits, the hybridization of the complementary single strands affects the validity of the input signal, which consumes the input signal before it reaches the target.

In this paper, based on the idea of combinatorial displacement, we proposed an improved INHIBIT gate, which can greatly reduce the mutual interference between the CONTROL and INPUT signals. This improved INHIBIT gate was served for the demultiplexer. To verify the application ability of combinatorial displacement mechanism in cascaded circuits, we further designed and simulated a multiplexer, which can be used to explore more potential applications.

II. COMBINATORIAL STRAND DISPLACEMENT

A. MECHANISM OF COMBINATORIAL DISPLACEMENT

In the process of a basic DNA strand displacement, an invading strand is usually a single-stranded DNA molecule with a special domain (called a toehold) that can trigger branch migration by binding to a corresponding domain on a double-stranded DNA; finally, the incumbent strand of the double-stranded DNA will be replaced by the invading strand. Based on this strategy, the combinatorial displacement mechanism involves two regions, the invading strand's toehold domain and the displacing domain. As shown in Figure 1, the two single strands can connect with each other via a pair of complementary subchains (linking domains) and form a new structure (displacing complex). The displacing complex can react rapidly with a double-stranded DNA molecule with a

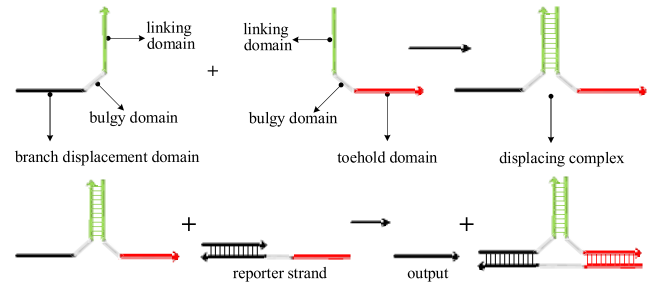


FIGURE 1. The mechanism of combinatorial displacement of DNA strands [39].

TABLE 1. Logic gates based on combinatorial displacement mechanism [40], [41].

	INPUT1	INPUT2 (CONTROL)	SUBSTRATE
AND			
OR			
INHIBIT			

reporter structure to generate an output strand, which can be used to carry desired information, participate in the downstream reaction, and complete the cascade function. This replacement process is difficult to perform when either of the chains of the displacing complex is missing. The feasibility of the mechanism is proved in [39] by implementing a polyacrylamide gel electrophoresis and the formation of different DNA structures can be seen clearly in the gel. This method can not only realize the basic functions of the strand displacement but also can be designed for new applications, including Boolean matrix operations, logic gates, and cascaded circuits.

B. LOGIC GATES STRUCTURED BY COMBINATORIAL DISPLACEMENT

Based on the combinatorial displacement mechanism, logic gates such as AND, OR, XOR, and INHIBIT have been proposed. Here, we introduce three of them in detail. These three will be used in the design of our mechanism; see Table 1 for their structures.

In the AND gate, if and only if the two single strands of INPUT1 and INPUT2 coexist can they form a displacing complex, complete the displacement reaction with the substrates, and output the result "1". In any other case, the displacing complex cannot be formed, and the output is "0". In the substrate composition of the OR gate, two single strands are designed in advance. When either INPUT1 or INPUT2 is input, a displacing complex can be formed with the substrate; then, the combinatorial displacement reaction is completed, and output the result "1". In the INHIBIT gate, the single strand representing the INPUT1 and the single strand representing the CONTROL are designed to be

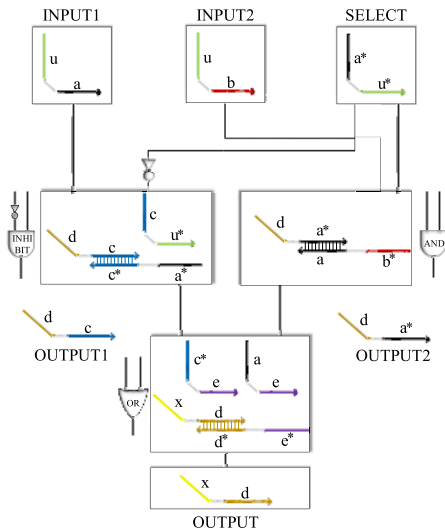


FIGURE 2. Schematic diagram of the multiplexer structure.

completely complementary, so as soon as the CONTROL is input into the system (i.e., the CONTROL is “1”), the function of the INPUT1 is suppressed by a rapid complementary hybridization reaction, and the output is “0” regardless of what the INPUT1 is (“0” or “1”). When the CONTROL is “0”, INPUT1 can form a displacing complex with the substrate, complete the combinatorial displacement reaction, and output “1”.

III. APPLICATION OF COMBINATORIAL DISPLACEMENT TO LOGIC CIRCUITS

A. MULTIPLEXER

To demonstrate the efficacy of the combinatorial displacement mechanism, we constructed a multiplexer using the logic gates described above.

As shown in Figure 2, the multiplexer is a cascade circuit consisting of an INHIBIT gate, an AND gate, and an OR gate. It has three inputs: INPUT1, INPUT2 and SELECT. The signals of INPUT1 and INPUT2 are transmitted to the INHIBIT gate and the AND gate, respectively, while the signals of SELECT are transferred to both the INHIBIT gate and the AND gate. In the INHIBIT gate, when SELECT is present, INPUT1 is rapidly depleted by the extremely rapid reaction triggered by the hybridization of completely complementary base-pairing, which leads to no INPUT1 signals are accepted by the INHIBIT gate and hence no output signal OUTPUT1 is produced (i.e., OUTPUT1 = “0”). When SELECT is vacant, INPUT1 generates a displacing complex with the substrate, completes the process of combinatorial displacement, and produces the output signal OUTPUT1 (i.e., OUTPUT1 = “1”). In the AND gate, if and only if both INPUT2 and SELECT exist, a displacing complex can be formed, and the combinatorial displacement is completed, producing the output signal OUTPUT2 (i.e., OUTPUT2 = “1”). In the absence of either of these two inputs, the combinatorial displacement process cannot be finished, resulting in OUTPUT2 = “0”.

TABLE 2. The truth table of the multiplexer.

INPUT1	INPUT2	SELECT	OUTPUT
0	0	0	0
0	1	0	0
1	0	0	1
1	1	0	1
0	0	1	0
0	1	1	1
1	0	1	0
1	1	1	1

TABLE 3. The DNA sequences for the multiplexer.

domains	sequences
a	AGATTTAGTAGGACGA
b	TAGGAACACGAACAGA
c	TGTATCTTGTCCGTTTC
d	CATCCCTACCCTTAAC
e	TAGAACAAGACACAGC
u	ACAGGACAGGGATAGC
x	CCCAATCAAAGCCACG

Finally, the output signals OUTPUT1 of the INHIBIT gate and OUTPUT2 of the AND gate are transmitted, as the input signals, into the next stage and are cascaded to the OR gate to generate the final output signal OUTPUT. In this stage, when any one of input signals (OUTPUT1 and OUTPUT2) is present, a displacing complex is generated with the single strand designed in the substrate, and thus, the combinatorial displacement process is completed and OUTPUT = “1”. OUTPUT is “0” if and only if both OUTPUT1 and OUTPUT2 are “0”. The truth table corresponding to the output signal and the three input signals (INPUT1, INPUT2, and SELECT) is shown in Table 2.

Figure 3 shows the simulation results. The inputs of INPUT1, INPUT2 and SELECT corresponding to their outputs in different cases are demonstrated, wherein the ratio of the concentration of the input signal to the concentration of the substrate is 5:1. By referring to [39], the rate constant of the formation of displacing complex used in the simulation is $10^6 M^{-1} s^{-1}$, and the rate constant of the toehold-mediated strand-displacement process in which the displacing complex invades the substrate is $4 \times 10^4 M^{-1} s^{-1}$. Using this method of overdosing, the effectiveness of the output is guaranteed by completely consuming all of the substrates of the gates.

The DNA sequences we use in the simulation experiment for the multiplexer are depicted in Table 3, in which the bulgy domain is TT. All the sequences are designed by NUPACK.

B. IMPROVED INHIBIT GATE

In subsection III-A, we use the basic logic gates to design a multiplexer, which is a typical chemical reaction circuit. In the multiplexer, INPUT1 and SELECT can coexist in one solution, but they only cowork at the INHIBIT gate. These two inputs do not work simultaneously in any other part. Then, the following problem arises: when the two strands must function together in another region to participate in a

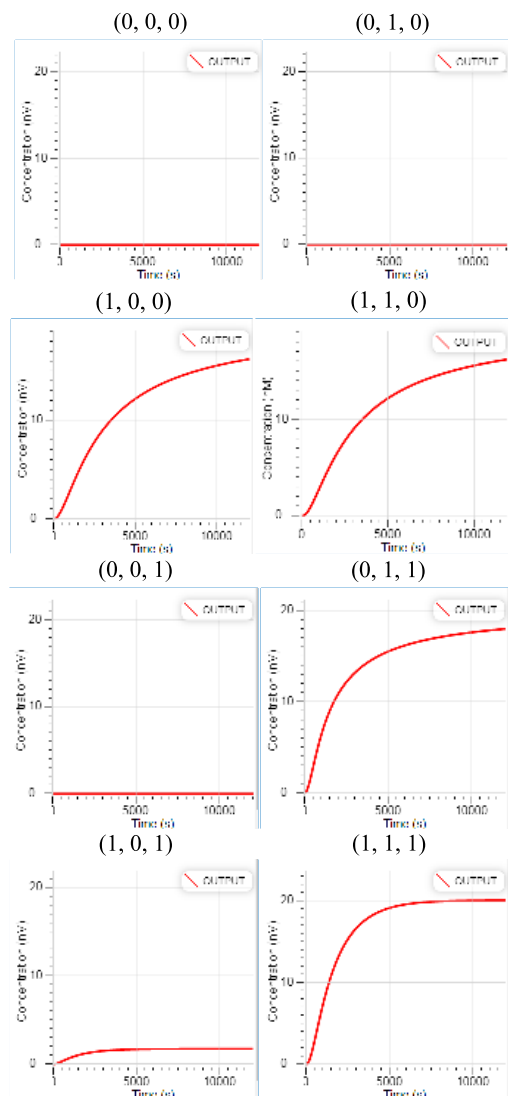


FIGURE 3. The multiplexer simulation results for various operating conditions with different inputs. The inputs corresponding to INPUT1, INPUT2, and SELECT are marked above figures. For example, (0, 1, 0) means the case that INPUT1 = “0”, INPUT2 = “1”, and SELECT = “0”. [INPUT1] = [INPUT2] = [SELECT] = 100nM and the concentration of each logic gate is 20nM.

certain reaction as the input, it is difficult for INPUT1 and SELECT to participate in the next circuit due to their fast binding characteristics.

As reported in Table 1, in the design of the INHIBIT gate, the strand that represents INPUT1 and the strand that represents the CONTROL are completely complementary. This fully complementary single-strand hybridization reaction is so rapid that when both inputs are present, INPUT1 is consumed before the combinatorial displacement reaction with the substrate. This design can effectively guarantee the function of the INHIBIT gate but greatly reduce this possibility when either of the two input strands must function in other reactions. When the two inputs are present at the same time, it is difficult for one of the strands to participate in other parts

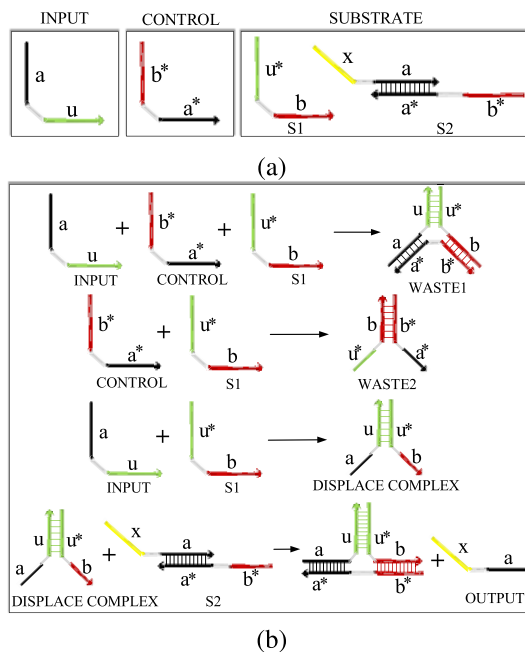


FIGURE 4. (a) A schematic of the improved INHIBIT gate. (b) All possible reactions in the INHIBIT gate.

of the biochemical reaction networks before being consumed. In short, it is difficult for the two input strands to coexist to form complex circuits.

In response to this problem, we modify the existing INHIBIT gate and overcome the difficulty of coexistence of the strands of INPUT1 and CONTROL by redesigning the domains of the input strands and the substrate, as shown in Figure 4 (a). The single strand in the input position and the single strand in the control position of our design do not have a complete complementary structure but rather are only partially complementary. In this case, when the input signals INPUT and CONTROL are input simultaneously, the product after the reaction between them can continue to participate in other reactions, while they will completely hybridize with S1 in the substrate to form a stable product, preventing the occurrence of combinatorial displacement reactions. As shown in Figure 4 (b), INPUT, CONTROL, and S1 have domains a, b, u and their corresponding fully complementary domains a*, b*, u*. Therefore, when INPUT = “1” and CONTROL = “1”, these three strands can rapidly hybridize with each other to generate a stable trigeminal structure WASTE1 (in this case, the OUTPUT = “0”, since no displacing complex is generated). When CONTROL = “0” and INPUT = “1”, the INPUT and S1 can generate a displacing complex, complete the combinatorial displacement reaction with S2 in the substrates, and finally generate the OUTPUT (i.e., OUTPUT = “1”). When CONTROL = “1” and INPUT = “0”, the complex (WASTE2) generated by the CONTROL and S1 can only react very slowly with S2, and the OUTPUT is also “0”. When CONTROL = “0” and INPUT = “0”, nothing happens, and the OUTPUT is “0”.

TABLE 4. Truth table of the improved INHIBIT gate.

INPUT	CONTROL	OUTPUT
0	0	0
0	1	0
1	0	1
1	1	0

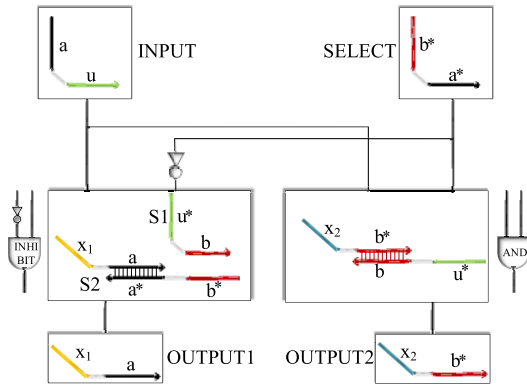


FIGURE 5. Schematic diagram of the demultiplexer structure.

The truth table corresponding to this improved INHIBIT gate is shown in Table 4.

This improved method can avoid single strands disturbing the CONTROL signal and the INPUT signal. Therefore, they can coexist to a large extent in the solution, complete a more complex biochemical reaction network, and build a larger integrated circuit. To prove the feasibility of this improvement, we utilize the improved INHIBIT gate to construct a demultiplexer in which the two inputs of the INHIBIT gate should cooperate in another gate in the same system.

C. DEMULTIPLEXER

To demonstrate the effectiveness and versatility of this improvement, we designed and simulated a demultiplexer with the improved INHIBIT gate. As shown in Figure 5, the demultiplexer has two inputs, which are simultaneously transmitted to the two logic gates and hybridize with each other at the same time to produce output signals. This simultaneity and coexistence put new demands on the two input signals. If the two inputs, INPUT and SELECT, are quickly exhausted, then the AND gate cannot function. The improved INHIBIT gate solves this problem.

The demultiplexer has two inputs (INPUT and SELECT) and two outputs (OUTPUT1 and OUTPUT2). When neither the SELECT signal nor the INPUT signal is present (that is, INPUT = "0" and SELECT = "0"), the INHIBIT gate and the AND gate do not receive any input signal and they produce no output signal (OUTPUT1 = "0" and OUTPUT2 = "0"). When the INPUT signal is vacant while the SELECT signal is present (that is, INPUT = "0" and SELECT = "1"), it has that no displacing complex is formed in both INHIBIT gate and AND gate and also no output signal is generated (OUTPUT1 = "0" and OUTPUT2 = "0"). When the

TABLE 5. The truth table of the demultiplexer.

INPUT	SELECT	OUTPUT1	OUTPUT2
0	0	0	0
0	1	0	0
1	0	1	0
1	1	0	1

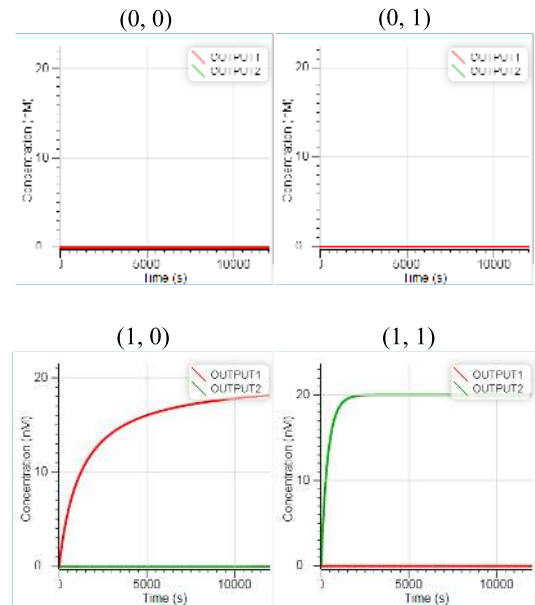


FIGURE 6. The demultiplexer simulation results for various operating conditions with different inputs. The input signals corresponding to (INPUT, SELECT) is marked directly above each figure. For example, (1, 0) means the case that INPUT = "1", SELECT = "0". [INPUT] = [SELECT] = 100nM and the concentration of each logic gate is 20nM.

INPUT signal is present while the SELECT signal is vacant (that is, INPUT = "1" and SELECT = "0"), the INPUT signal can pass through the INHIBIT gate smoothly and complete the combinatorial displacement process together with the substrate, but the displacing complex cannot be generated in the AND gate. Therefore, INHIBIT gate can generate an output signal (OUTPUT1="1") while the AND gate does not generate any output signal (OUTPUT2="0"). When both of the INPUT signal and SELECT signal are present, the SELECT signal suppresses the INPUT signal in the INHIBIT gate and no output signal is produced (OUTPUT1 = "0"), while in the AND gate, the SELECT signal hybridizes with the INPUT signal to form a displacing complex, which completes the combinatorial displacement process and produces an output signal (OUTPUT2 = "1"). The corresponding truth table is presented in Table 5.

DNA strands of each part of the demultiplexer are shown in Figure 5; in the improved mechanism of the INHIBIT gate, INPUT and SELECT are no longer fully complementary, and only domain a is fully complementary. In order to consume these two input signals completely, domain u* and domain b are integrated into a single-stranded DNA among the substrates. Thus, if and only if INPUT and SELECT

TABLE 6. The DNA sequences for the multiplexer.

domains	sequences
a	GGACGGGATGAGATGA
b	CAGGAACAATACAGGC
x_1	CCTCACTTCACTCCCA
x_2	ACCCTCACCTTTACTA
u	GTGAGTTTGATTTGAG

exist will they collectively exhaust the single strand S1 in the substrate in the INHIBIT gate. Once the S1 is exhausted, although the remaining INPUT can exist in a large amount, the displacing complex cannot be formed in the INHIBIT gate, which ensures the normal operation of the INHIBIT gate. When the signal SELECT is absent, the signal INPUT together with S1 can form the displacing complex, complete the combinatorial displacement process, and generate the output signal (OUTPUT1). In the AND gate, INPUT and SELECT can easily form a displacing complex and generate the output signal (OUTPUT2) with the substrate of the AND gate. It can be observed that this design can ensure the normal operation of the demultiplexer and avoid the exhaustion of the INPUT and SELECT at the same time.

The simulation results are shown in Figure 6, in which the tuple (x, y) above each figure denotes the corresponding inputs of INPUT and SELECT (that is, INPUT = “ x ” and SELECT = “ y ”) and the curves in each figure represent the output results of OUTPUT1 (red) and OUTPUT2 (green).

The DNA sequences we use in the simulation experiment for the demultiplexer are depicted in Table 6, in which the bulgy domain is TT. All the sequences are designed by NUPACK.

IV. DISCUSSION

The combinatorial displacement mechanism effectively expands the toolbox of DNA strand displacement technology, which greatly enriches the biochemical reaction networks designed by means of the DNA strand displacement technique. This mechanism has excellent cascading characteristics, and it can be effectively utilized to design and implement a variety of integrated biochemical reaction circuits. We first used this mechanism to simulate a multiplexer with three logic gates and two cascades through Visual DSD. This directly proves that the logic gates designed by the combinatorial displacement mechanism have good compatibility.

Subsequently, we made some improvements to the traditional INHIBIT gate to solve the CONTROL signal and INPUT signal interference. We turned the two complementary strands into partial complements; this improvement allows multiple inputs to coexist in the same system. When both INPUT and CONTROL signals are present, the single-stranded DNA in the substrate of the INHIBIT gate can be exhausted by generating a stable trimer with two inputs, thereby preventing the formation of a displacing complex and invalidating the combinatorial displacement. This effectively avoids the phenomenon of rapid polymerization due to

complete complementarity and thus an inability to transmit the input signals to other logic gates.

Finally, we designed a demultiplexer in which multiple-input signals interfere before they are simultaneously input into different logic gates. This interference is effectively avoided by using a modified version of the INHIBIT gate. In the improved system, since the two input signals are partially complementary, they can operate simultaneously in different logic gates by reacting with different substrates. The simulation system demonstrated the effectiveness of this application and further proved that the combinatorial mechanism has the potential to form larger-scale integrated circuits and can ultimately achieve different computing functions.

Compared with the traditional INHIBIT gate, the improved INHIBIT gate has good versatility, compatibility and expandability. However, the formation of the complex with the trigeminal structure in this INHIBIT gate is not as fast as the formation of a double chain, which reduces the response speed of the entire system. It is very efficient to apply traditional INHIBIT gates when conditions permit. Thus, in the multiplexer, we did not use the improved INHIBIT gate in order to increase the stability of the entire system. In other cases, such as to avoid a large amount of interference in a short period of time, it is necessary to use the improved INHIBIT gate. The demultiplexer is a typical example. The application of the improved INHIBIT gate enables the demultiplexer to be implemented based on a combinatorial displacement mechanism.

The improved INHIBIT gate increases the possibility to establish complicated cascade circuits. Besides, in the combinatorial displacing mechanism, the product of the upstream can work as the input of the downstream, since they have the similar structure. For example, in the multiplexer, the output signals of the INHIBIT gate and AND gate can serve as the input signal of OR gate. Based on this characteristic, the output signal of OR gate can also serve as the input signal of INHIBIT gate, as well as AND gate. Thus, theoretically, it is possible to utilize this strategy to create larger scale circuits, such as 4-1 multiplexer and 8-3 encoder.

The length of the toehold is a key factor in designing and manufacturing the DSD multiplexer system. The length and the sequence of the toehold can adjust the reaction rate within six orders of magnitude. Practically a single toehold length adjustment is in a small scale. Studies on the DNA renaturation process show that the length of toehold must be at least 3 base pairs, to ensure a stable toehold binding. The research on the relationship between the replacement rate and the toehold length shows that when the toehold length reaches 6 base pairs, the replacement rate is saturated, and further increase of the toehold length does not improve the replacement rate.

This work was focused on the improvement of the traditional logic gates in theory. Observe that the more complex regular relationships, the more leakages will be formed. There are many reasons leading to the leakage during the

simulation, including the unreasonable structure of the logic gates, the unsuitable domain sequences, and the unsuitable concentration of strands, etc. In practice, the leakages may be attributed to the insufficient protection of input strands or reaction temperature. There are some strategies that can be used for reducing the leakage, such as concentration control, toehold clamps and cancellation. In this situation, logic cascade will be ceased in short steps. The DNA sequences and toehold regions are also important to implement experiments. Hence, DNA sequences should be carefully designed in further study.

In the further, we will improve our logic gates to realize the fractional representation. By adjusting the concentration of the inhabit, input and control strands, the value of the molecular strands can be fractional even minus. The specific value of output strands is determined by the reaction relationship and ratio between the input strands. In this way, complex mathematical formula can be realized and various of functions can be achieved. Furthermore, we will devote ourselves to designing more types of logic gates to realize more complex biochemical reaction circuits, such as four- or five-layer cascade circuits, complex mathematic formulae and even neural networks. We hope that DNA circuits combined with this mechanism can be more widely applied to DNA computing, disease diagnosis, biochemical sensing and other fields.

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