

Analog Computation by DNA Strand Displacement Circuits

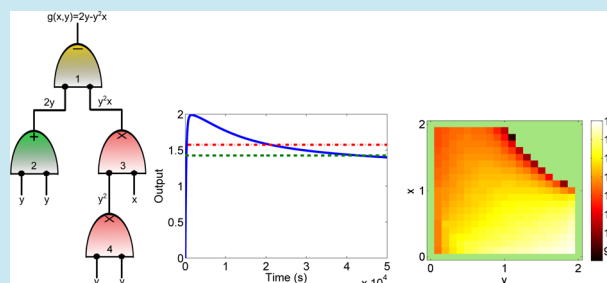
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S Supporting Information

ABSTRACT: DNA circuits have been widely used to develop biological computing devices because of their high programmability and versatility. Here, we propose an architecture for the systematic construction of DNA circuits for analog computation based on DNA strand displacement. The elementary gates in our architecture include addition, subtraction, and multiplication gates. The input and output of these gates are analog, which means that they are directly represented by the concentrations of the input and output DNA strands, respectively, without requiring a threshold for converting to Boolean signals. We provide detailed domain designs and kinetic simulations of the gates to demonstrate their expected performance. On the basis of these gates, we describe how DNA circuits to compute polynomial functions of inputs can be built. Using Taylor Series and Newton Iteration methods, functions beyond the scope of polynomials can also be computed by DNA circuits built upon our architecture.

KEYWORDS: DNA nanoscience, molecular programming, DNA computing, analog computation, self-assembly, DNA nanotechnology



Programmability and versatility are essential properties of deoxyribonucleic acid (DNA).¹ On the basis of these properties, diverse research has been done to develop DNA systems with designated functions.^{2–7} One important problem is to devise DNA circuits to perform designated computation which can be digital or analog, and excellent work has been done on constructing DNA circuits for both digital and analog computation.^{2,3,7–13}

Digital circuits process information encoded by binary bits, where each bit has two possible values “0” and “1”. These binary values are decided by a threshold of a physical quantity (e.g., voltage for electrical analog machines or position for mechanical analog machines), where if a physical quantity is above the threshold, it is 1, otherwise, it is 0. Digital circuits have their advantages. For example, they are less prone to offsets than analog circuits, and design of error-correction schemes for digital circuits is better developed.^{14–17} Remarkable work has been done on digital DNA circuits,^{2,3,7} and one major achievement is the construction of a digital DNA circuit in vitro that computes square roots of 4-bit numbers.³

In contrast, analog circuits process information directly encoded by a physical quantity. In general, practical analog computation systems operate within certain ranges of input analog values (input ranges). Moreover, there generally is a trade-off between their computation precision versus their input ranges. These ranges can be tuned to adjust computation precision. Analog circuits have their advantages over digital circuits. For example, analog circuits require much fewer gates to conduct numerical computation up to a given accuracy compared to their digital counterparts.¹⁴ Elementary arithmetic operations such as addition, subtraction, and

multiplication are computed by single gates in analog circuits, whereas digital circuits require multiple gates for each elementary arithmetic operation. It means that analog circuits consume less resources than corresponding digital circuits¹⁴ and this property makes analog circuits useful in resource-limited environments (e.g., in living cells¹⁸). Furthermore, for some applications, analog circuits can be more robust than digital circuits.¹⁹ Excellent work has been done on inventing general schemes to implement arbitrary chemical reaction networks (CRNs) with DNA strand displacement.^{10,13} Analog DNA circuits with continuous dynamics (e.g., controllers, oscillators) have been developed on the basis of these schemes.^{8,9,11,12}

1.2. Overview of Analog Computation. There is an extensive history of analog computation.²⁰ In general, an analog computer has components (arithmetic gates) that compute basic arithmetic operations such as addition, subtraction, and multiplication. These operations may be done by various physical means, such as cog-based mechanical devices or electrical circuits, etc. Typically, the range of values (e.g., the range of mechanical positions in a mechanical analog computer or voltage range in an electrical analog computer) computed by a given precision by an analog arithmetic gate is restricted, due to various physical constraints, but typically this is not a limitation, since the values can be scaled to arbitrary ranges. Hence the precision of each arithmetic gate is ultimately limited by underlying physical constraints on the

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devices that implement the arithmetic gate, but the precision can typically be tuned by adjusting the input ranges. All known analog computers (including in particular our proposed DNA-based analog circuits) appear to have some sort of trade-off between precision of analog values computed and the operating range of values over which the analog computations are done. Analog devices can offer some advantages (such as requiring a smaller number of gates) over computing devices whose gates can only execute Boolean operations. The same issues of advantages (e.g., in number of gates) and limitations (e.g., in the range of values, but released via rescaling) appear in the DNA-based analog circuits of this paper. Analog circuits for arithmetic operations compute the outputs whose values are determined by the inputs. Analog circuits with continuous dynamics generate outputs that are designated functions in the time domain.

1.3. Potential Applications of Analog DNA Circuits.

Analog DNA circuits can be used as analog control devices^{11,12} in which real values are sensed and analog computation provides controlling output. For example, control of chemical reaction networks requires both sensing of concentrations of input molecules and after analog computation, controlling concentrations of output molecules. Also, in applications known as “DNA doctor”,²¹ in which various nucleic acid sequence concentrations (e.g., concentrations of mRNAs) are sensed, and an analog computation can be used to determine the amount of a drug molecule that should be released. Prior devices for control of chemical reaction networks and DNA doctor²¹ applications have been limited to finite-state control, and analog DNA circuits will allow much more sophisticated analog signal processing and control. DNA robotics have allowed devices to operate autonomously (e.g., to walk on a nanostructure) but also have been limited to finite-state control. Analog DNA circuits can allow molecular robots to include real-time analog control circuits to provide much more sophisticated control than offered by purely digital control. Many artificial intelligence systems (e.g., neural networks and probabilistic inference) that dynamically learn from environments require analog computation,¹⁴ and analog DNA circuits can be used for back-propagation computation of neural networks and Bayesian probabilistic inference systems.

1.4. Prior Molecular-Scale Analog Computation.

There is considerable excellent prior work on engineering nucleic acid systems^{8–12,22–24} (both using enzymes and enzyme-free) and cells^{18,25,26} to do complex dynamics. For example, the following ingenious prior systems have been demonstrated to compute time-varying cyclic signals: (a) a nucleic acid system using enzymes,²² (b) a DNA-based system using only hybridization reactions,⁸ (c) and a cell-based system.²⁵ Prior work on analog DNA systems has mainly focused on developing DNA circuits with continuous dynamics.^{8,9,11,12} There is still a need to develop an architecture to construct analog DNA circuits for arithmetic computation. In principle, the general schemes to implement arbitrary chemical reaction networks (CRNs) with DNA strand displacement^{10,13} can serve as such an architecture, but there needs to be more detailed investigation.

1.5. Our Contribution. In this paper, we present an architecture to construct DNA circuits for arithmetic computation in an analog fashion. There are three elementary gates in our architecture: addition, subtraction, and multiplication gates. For each gate, we first propose its high-level

chemical reaction network analogy and then give our DNA implementation. Each gate is evaluated by simulation using Language for Biochemical Systems (LBS).²⁷ DNA circuits to compute polynomial functions of inputs can be built on the basis of these gates. By Taylor Series and Newton Iteration methods, some functions beyond the scope of polynomials can also be computed by DNA circuits built upon our architecture.

2. RESULTS

2.1. Preliminaries of Analog DNA Gates. **2.1.1. Toehold-Mediated Strand Displacement.** Toehold-mediated strand displacement is the primary mechanism for the proposed architecture. A domain-level illustration of toehold-mediated strand displacement is shown in Figure 1. We call

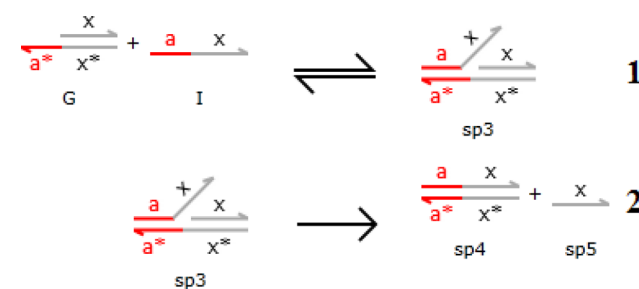


Figure 1. A domain-level illustration of toehold-mediated strand displacement. Reaction 1 is a reversible reaction, where the forward reaction and the reverse reaction are called toehold binding and toehold unbinding, respectively. Reaction 2 is called branch migration.

the a^* domain in G a toehold, where a *domain* is a sequence of nucleotides. The arrow in a DNA strand indicates the 3' end. In reaction 1, the a domain in I hybridizes to the a^* domain in G, where the asterisk symbol means complementary domain. We call this step *toehold binding*. Usually, the toehold domain is short (less than 10 nucleotides), so the toehold binding reaction is reversible and we call the reverse reaction *toehold unbinding*. Once the toehold binding step is complete, reaction 2 can happen where the x domain in I (called *branch migration domain*) competes with the sp5 strand in G on binding to the x^* domain. Eventually, strand I wins and displaces sp5. We call this step *branch migration*. In the diagrams of this paper, we may combine these two steps into a single step for simplicity.

2.1.2. Related Concepts. Here we introduce some basic concepts to define our analog DNA gates.

Input Range. Each analog gate is assigned an input range, within which the inputs of a gate should lie if they are to be computed within the required precision. Hence if one of the inputs of an arithmetic gate is outside its input range, the arithmetic operation may not be computed within the required precision. Specifically, in our DNA-based analog computation architecture, the input range of an analog DNA gate is the range of concentration within which its input DNA strands should occur, in order for the gate to operate correctly. The input ranges are not particular to our analog DNA gates, and typically any analog gate has an input range. Our analog DNA gates have the advantage that the input range can be tuned easily by programming the concentrations of components in a gate.

Valid Output Range. Analog gates process continuous physical quantities as input and provide continuous output. This makes analog gates suffer more from two things compared to digital gates: signal fluctuation and signal degradation. Continuous information is more sensitive to noise and more difficult to restore than discrete information encoded by binary bits. Therefore, due to noise, the output of an analog gate cannot remain at the theoretically correct value for given inputs. There will always be an error, which usually tends to grow larger over time due to signal degradation. To evaluate analog gates, we define the term *valid output range*: Given inputs x_1 and x_2 , a gate that conducts arithmetic operation \otimes , and $0 < r < 1$ which describes the error, the valid output range is $((1 - r) * (x_1 \otimes x_2), (1 + r) * (x_1 \otimes x_2))$ for inputs x_1 and x_2 . The output lying within this range is considered correct. The time that the output stays within the valid output range is used to quantify the performance of the gate under these inputs. The value of r does not really influence the execution of a gate and it is just a parameter for interpreting the performance.

Note that all known analog systems, like electronic and mechanical analog systems, have restricted input ranges. Fluctuation and degradation of output signal is also a common problem for all known analog systems, which necessitates a valid output range. These properties are unavoidable for all analog systems,¹⁴ not only for our DNA-based analog system.

2.2. Abstractions of the Gates. The abstractions in Figure 2 will be used to describe the functions and

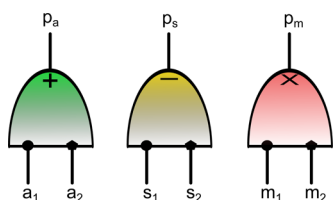


Figure 2. Abstractions of our analog DNA gates: (left) addition gate; (middle) subtraction gate; (right) multiplication gate.

mechanisms of the gates. For example, the addition gate: The inputs are a_1 and a_2 , and the output is p_a . The plus symbol $+$ denotes addition. To distinguish a_1 and a_2 , different symbols are assigned to the two input ports: a circle to a_1 , and a pentagon to a_2 . The two inputs need to be distinguished because they play different roles in the mechanism of the addition gate. In other words, their corresponding input DNA strands go through different reaction pathways during the computation process, as explained in the following detailed description of the gates.

2.3. Addition Gate. **2.3.1. The Chemical Reaction Network To Inspire Our Addition Gate.** Our addition gate is inspired by the following chemical reaction network:



The addition gate computes $p_a = a_1 + a_2$ (see abstraction in Figure 2). Ia1 and Ia2 are input chemical species to the gate, where their initial concentrations $[\text{Ia1}]_0$, $[\text{Ia2}]_0$ represent the two inputs a_1 and a_2 , respectively, which means $a_1 = [\text{Ia1}]_0$ and $a_2 = [\text{Ia2}]_0$. The addition gate is composed of chemical

species A1 and A2. To have an input range of $(0, r_a)$ which means $a_1, a_2 \in (0, r_a)$, we let the initial concentrations be $[\text{A1}]_0 = [\text{A2}]_0 = r_a$. Oa is the output chemical species of this gate and its concentration at equilibrium $[\text{Oa}]_\infty$ represents p_a . As shown in reactions 1a and 1b, all Ia1 and Ia2 will eventually be transformed to Oa and then $[\text{Oa}]_\infty = [\text{Ia1}]_0 + [\text{Ia2}]_0$ which means $p_a = a_1 + a_2$. There are no constraints on rate constants k_1 and k_2 .

2.3.2. DNA Implementation of Our Addition Gate. As shown in Figure 3, Ia1 and Ia2 are two input DNA strands.

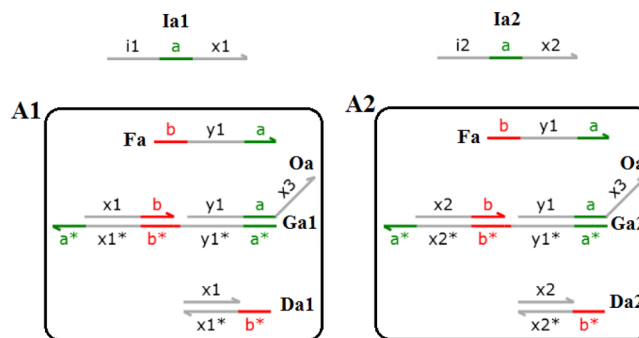


Figure 3. DNA design of our addition gate. Each of A1 and A2 in the original chemical reaction network is implemented by three DNA species (e.g., A1 is implemented by Fa, Ga1 and Da1). Initial concentrations are $[\text{Ga1}]_0 = [\text{Da1}]_0 = [\text{Ga2}]_0 = [\text{Da2}]_0 = r_a$ and $[\text{Fa}]_0 = 2r_a$ because it is in both A1 and A2, where $(0, r_a)$ is the input range. The gray domains are branch migration domains of twenty nucleotides in length. The color domains are toeholds of five nucleotides in length. All DNA figures are drawn by Visual DSD²⁸ in this paper.

Their initial concentrations $[\text{Ia1}]_0$ and $[\text{Ia2}]_0$ represent a_1 and a_2 , respectively. Oa, which is the DNA strand composed of domains $y1-a-x3$ (S' to $3'$ direction) is the output DNA strand and its concentration at equilibrium $[\text{Oa}]_\infty$ represents p_a . Each of A1 and A2 is implemented by three DNA species, where a DNA species is a DNA strand or complex. The DNA reactions in the addition gate are shown in Figures 4 and 5. The relationship between the original chemical reaction network and DNA reactions is shown in Table 1. The simulation to demonstrate this DNA implementation is in section 2.7.

2.4. Subtraction Gate. **2.4.1. The Chemical Reaction Network To Inspire Our Subtraction Gate.** Our subtraction gate is inspired by the following chemical reaction network:



The subtraction gate computes $p_s = s_1 - s_2$, where $s_1 > s_2$ is required (see abstraction in Figure 2). Is1 and Is2 are input chemical species to the gate, where their initial concentrations $[\text{Is1}]_0$, $[\text{Is2}]_0$ represent the two inputs s_1 and s_2 , respectively, which means $s_1 = [\text{Is1}]_0$ and $s_2 = [\text{Is2}]_0$. The subtraction gate is composed of chemical species S. To have an input range of $(0, r_s)$ which means $s_1, s_2 \in (0, r_s)$, we let the initial concentrations be $[\text{S}]_0 = r_s$. S' is an intermediate product. The concentration of Is1 at equilibrium $[\text{Is1}]_\infty$ represents p_s . As shown in reactions 2a and 2b, Is2 and Is1 cancel each other in pairs which leaves $[\text{Is1}]_\infty = [\text{Is1}]_0 - [\text{Is2}]_0$ concentration of Is1, and then we have $p_s = s_1 - s_2$. If $s_1 \leq$

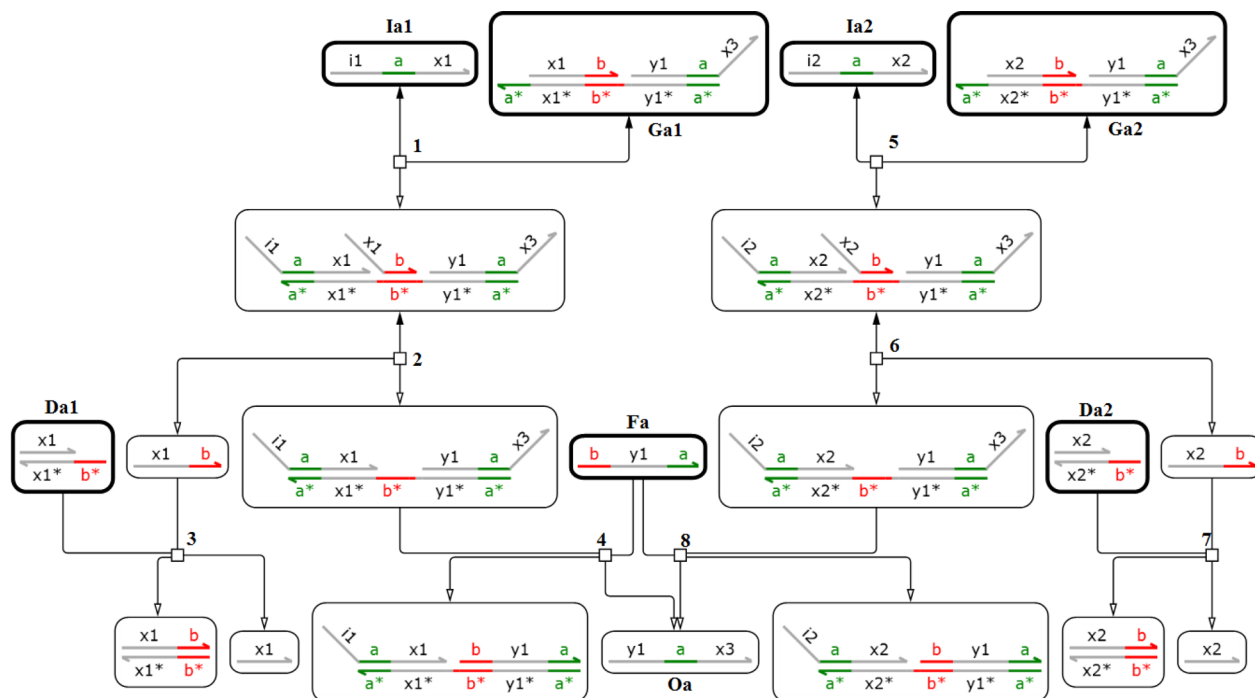


Figure 4. DNA reactions diagram in the addition gate. Each reaction is assigned a number. Edges without arrows represent reactants. Edges with hollow arrows represent products. In reversible reactions, hollow arrows represent the products of the forward reactions and solid arrows represent the products of the reverse reactions.

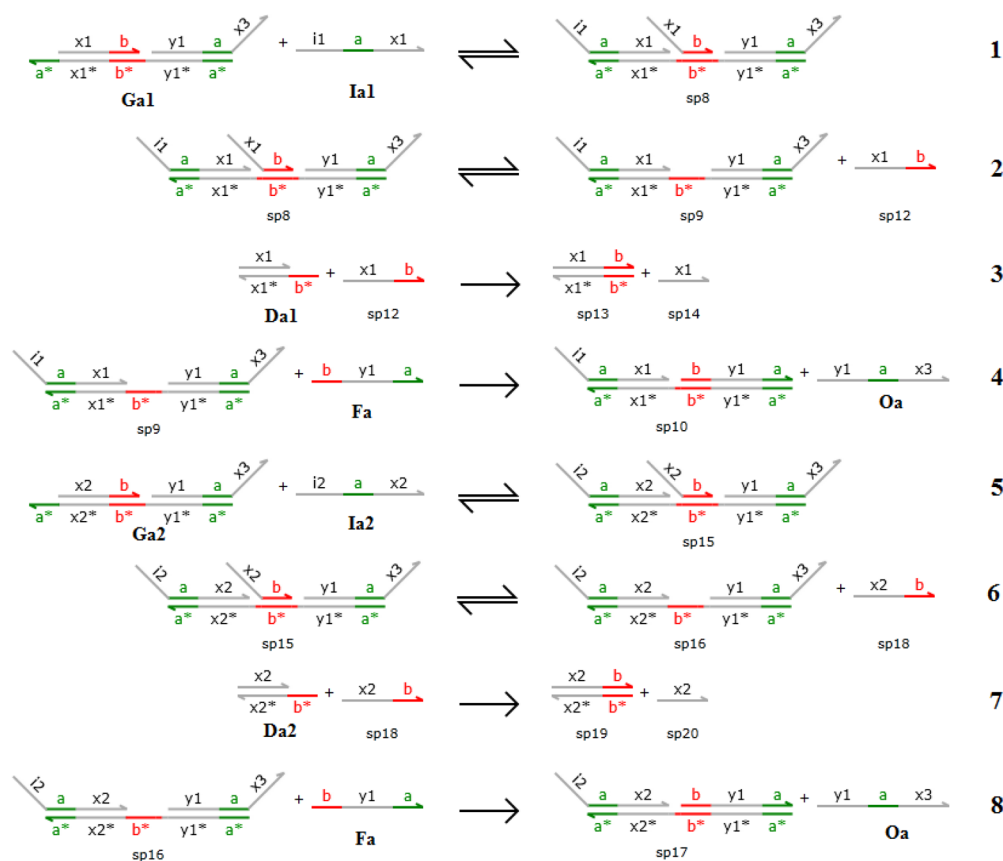


Figure 5. A list of the DNA reactions in the addition gate. Each reaction is assigned a number, which is consistent with Figure 4. The rate constants in such lists in this paper are discussed in section 4.

s_2 , all I_{s1} will be consumed and the result is just 0. There are no constraints on rate constants k_1 and k_2 .

2.4.2. DNA Implementation of Our Subtraction Gate. As shown in Figure 6, I_{s1} and I_{s2} are two input DNA strands.

Table 1. Relationship between the Original Chemical Reaction Network and DNA Reactions in the Addition Gate^a

abstract chemical reaction	DNA reactions
1a	1, 2, 3, and 4
1b	5, 6, 7, and 8

^aEach abstract chemical reaction is implemented by several DNA reactions. The DNA reactions corresponding to the numbers are shown in Figures 4 and 5.

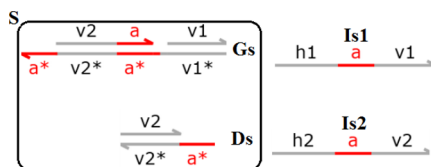
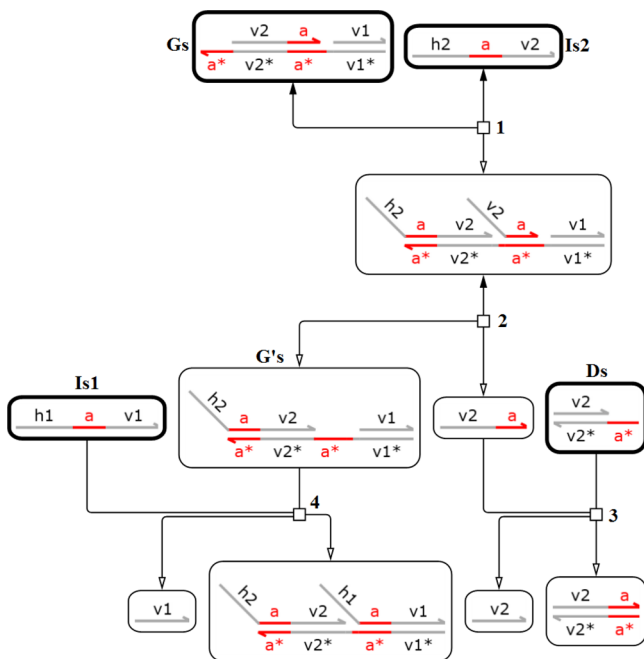
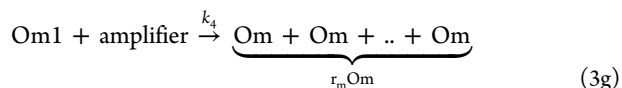
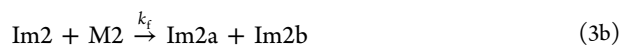


Figure 6. DNA design of subtraction gate. S in the original chemical reaction network is implemented by two DNA complexes Gs and Ds. Initial concentrations are $[Gs]_0 = [Ds]_0 = r_s$, where $(0, r_s)$ is the input range. The gray domains are branch migration domains of twenty nucleotides in length. The color domains are toeholds of five nucleotides in length.

Their initial concentrations $[Is1]_0$ and $[Is2]_0$ represent s_1 and s_2 , respectively. The concentration of Is1 at equilibrium $[Is1]_\infty$ represents p_s . S in the original chemical reaction network is implemented by two DNA complexes Gs and Ds. The DNA reactions in the subtraction gate are shown in Figures 7 and 8. The relationship between the original chemical reaction network and DNA reactions is shown in Table 2. The simulation to demonstrate this DNA implementation is in Section 2.7.

2.5. Multiplication Gate. 2.5.1. The Chemical Reaction Network To Inspire Our Multiplication Gate. Our multiplication gate is inspired by the following chemical reaction network:

**Figure 7.** DNA reactions in the subtraction gate. Each reaction is assigned a number.

The multiplication gate computes $p_m = m_1 m_2$ (see abstraction in Figure 2). Im1 and Im2 are input chemical species to the gate, where their initial concentrations $[Im1]_0$, $[Im2]_0$ represent the two inputs m_1 and m_2 , respectively, which means $m_1 = [Im1]_0$ and $m_2 = [Im2]_0$. The multiplication gate is composed of chemical species M1, M2, M3, Gm4 and “amplifier”. To have an input range of $(0, r_m)$ which means $m_1, m_2 \in (0, r_m)$, we let the initial concentrations be $[M1]_0 = [M2]_0 = [M3]_0 = [Gm4]_0 = [\text{amplifier}]_0 = r_m$. Om is the output chemical species of this gate and its concentration at equilibrium $[Om]_\infty$ represents p_m . All other chemical species are intermediate products. For the reaction rate constants, we have a requirement that $k_s \ll k_f, k_1, k_2$.

We now describe how the chemical reaction network will perform multiplication in the ideal situation. Note that all the following description is not a mathematical proof for the performance of the chemical reaction network, and we just expect or conjecture that the chemical reaction network in the ideal situation will perform this way. There is no explicit solution to the ordinary differential equations of this chemical reaction network. To demonstrate our design, we will do a stochastic model-checking for fixed numbers of molecules for this chemical reaction network. Also, in section 2.7, we will show the simulation results of the DNA implementation of our multiplication gate.

The ideal situation is an extreme case of the requirement $k_s \ll k_f, k_1, k_2$, where reactions 3b, 3c and 3d finish first, then reaction 3a starts to produce Im1a, and then reactions 3e and 3f start to work. It means that before reactions 3a, 3e, and 3f start to work, reactions 3b, 3c, and 3d have finished and made the concentration ratio between G'm3 and Gm4 be

$$\frac{[G'm3]}{[Gm4]} = \frac{[Im2]_0}{r_m - [Im2]_0} = \frac{m_2}{r_m - m_2}$$

Let the reaction volume be V . Reaction 3b produces $[Im2]_0 V$ amount of Im2a, and then reaction 3c produces $[Im2]_0 V$ amount of G'm3. Reaction 3b also produces $[Im2]_0 V$ amount of Im2b, and then reaction 3d consumes $[Im2]_0 V$ amount of Gm4, which means that $[Gm4]_0 V - [Im2]_0 V$ amount of Gm4 is left. Therefore, the concentration ratio between G'm3 and Gm4 will be $\frac{[Im2]_0 V}{[Gm4]_0 V - [Im2]_0 V} = \frac{m_2}{r_m - m_2}$.

Given that reactions 3e and 3f have the same rate constant, we expect that the Im1a produced by reaction 3a will be distributed to reactions 3e and 3f according to the concentration ratio between Gm4 and G'm3 that we calculated above. This means that $\frac{m_2}{m_2 + (r_m - m_2)}$ portion of

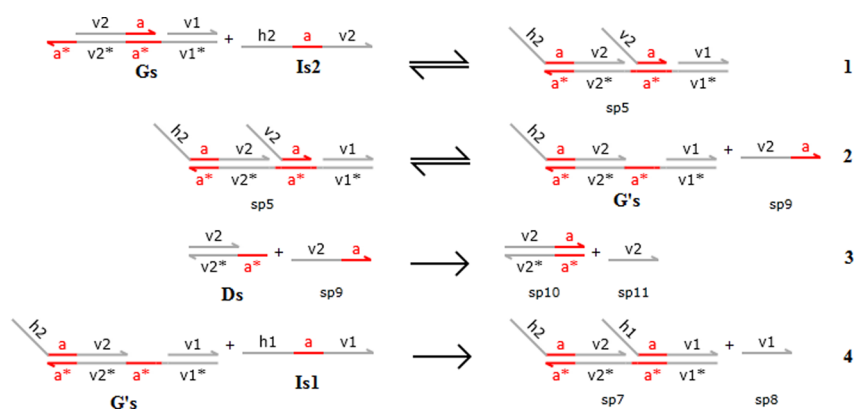


Figure 8. A list of the DNA reactions in the subtraction gate. Each reaction is assigned a number, which is consistent with Figure 7.

Table 2. Relationship between the Original Chemical Reaction Network and DNA Reactions in the Subtraction Gate^a

abstract chemical reaction	DNA reactions
2a	1, 2, and 3
2b	4

^aEach abstract chemical reaction is implemented by one or several DNA reactions. The DNA reactions corresponding to the numbers are shown in Figures 7 and 8.

Im1a will be consumed by reaction 3f and then the concentration of Om1 at equilibrium is

$$[Om1]_{\infty} = m_1 \frac{m_2}{m_2 + (r_m - m_2)} = \frac{m_1 m_2}{r_m}$$

if we ignore reaction 3g. By adding an amplification reaction, 3g, we have the concentration of Om at equilibrium be

$$[Om]_{\infty} = r_m \frac{m_1 m_2}{r_m} = m_1 m_2 = [Im1]_0 [Im2]_0$$

where we use $[Om]_{\infty}$ to represent the output of our multiplication gate.

To support our design, we conducted a stochastic model-checking for fixed numbers of molecules for the chemical reaction network in the ideal situation. To simplify the simulation, we ignore reactions 3b, 3c, and 3d (also 3g). Instead, we directly set up the concentration ratio $\frac{[G'm3]_0}{[Gm4]_0}$ and check how Im1a is distributed between reactions 3e and 3f. Here we use the same notation for the number of molecules as the notation for concentration. There is no constraint on the relationship between k_s and k_3 , and then we just let $k_s = k_3 = 0.01 \text{ s}^{-1}$. Note that in stochastic simulation of chemical reaction networks, s^{-1} is the unit of rate constants in bimolecular reactions. The simulation is done by Language for Biochemical Systems (LBS)²⁷ in a beta version of Visual GEC.²⁹

First, we check the expected number of Om1 molecules at equilibrium, and we expect that

$$E[[Om1]_{\infty}] = [Im1]_0 \frac{[G'm3]_0}{[G'm3]_0 + [Gm4]_0}$$

where $E[\cdot]$ denotes expectation. Let $[M1]_0$ be 30 (number of molecules), $[Im1]_0$ be 10. We vary $[G'm3]_0$ and $[Gm4]_0$. The numbers (of molecules) we choose here do not have special

meanings, and it is just that we have to give specific numbers for a stochastic model check. To speed up the simulation, we choose small numbers. The simulation results are shown in Table 3. In all cases, the results show that $E[[Om1]_{\infty}]$ is as

Table 3. Simulation Results for Checking the Expected Number of Om1 molecules at equilibrium ($E[[Om1]_{\infty}]$)^a

$E[[Om1]_{\infty}]$	$[G'm3]_0$	$[Gm4]_0$
7.5	30	10
6.67	20	10
5	10	10
3.33	10	20
2.5	10	30

^aIn all cases, the results show that

$$E[[Om1]_{\infty}] = [Im1]_0 \frac{[G'm3]_0}{[G'm3]_0 + [Gm4]_0}$$

expected. We believe that $E[[Om1]_{\infty}]$ in this stochastic model is the value of $[Om1]_{\infty}$ in the deterministic model when there are large number of molecules in the system.

Next, we check $\frac{\text{Var}[[Om1]_{\infty}]}{E[[Om1]_{\infty}]}$, where $\text{Var}[\cdot]$ denotes variance. Let $[M1]_0$ be 30. Let both $[G'm3]_0$ and $[Gm4]_0$ be 30. We vary $[Im1]_0$ and expect that $\frac{\text{Var}[[Om1]_{\infty}]}{E[[Om1]_{\infty}]}$ becomes smaller when $[Im1]_0$ gets larger. The simulation results are shown in Table 4. The results are consistent with what we expect. It implies that larger inputs to our multiplication gate yield relatively smaller errors in the outputs.

Table 4. Simulation Results for $\text{Var}[[Om1]_{\infty}]/E[[Om1]_{\infty}]$ ^a

$[Im1]_0$	5	10	15	20	25	30
$\frac{\text{Var}[[Om1]_{\infty}]}{E[[Om1]_{\infty}]}$	0.43	0.29	0.23	0.18	0.15	0.13

^aThe results show that $\frac{\text{Var}[[Om1]_{\infty}]}{E[[Om1]_{\infty}]}$ becomes smaller when $[Im1]_0$ gets larger.

In a realistic DNA system, we know that we cannot have the ideal situation which is the extreme case of the requirement $k_s \ll k_f, k_1, k_2$, so computation errors are expected.

2.5.2. DNA Implementation of Our Multiplication Gate. As shown in Figure 9, Im1 and Im2 are two input DNA strands. Their initial concentrations $[Im1]_0$ and $[Im2]_0$

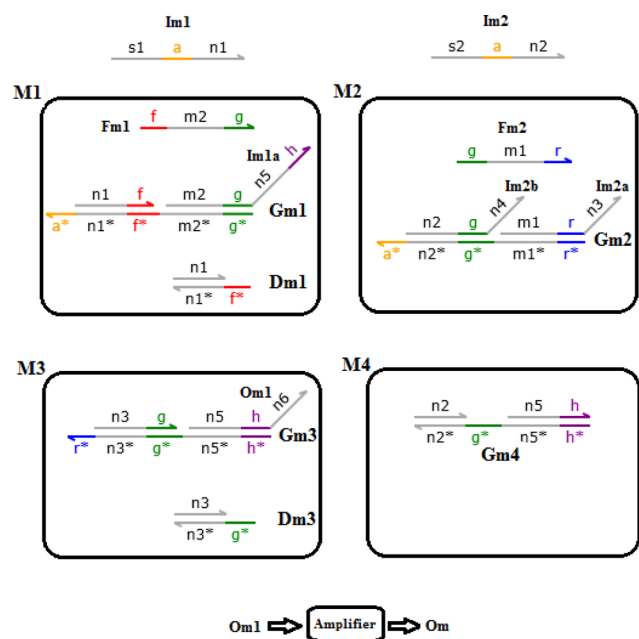


Figure 9. DNA design of our multiplication gate. Each of M1, M2, and M3 in the original chemical reaction network is implemented by several DNA species. Initial concentrations are $[Fm1]_0 = [Gm1]_0 = [Dm1]_0 = [Fm2]_0 = [Gm2]_0 = [Gm3]_0 = [Dm3]_0 = [Gm4]_0 = r_m$, where $(0, r_m)$ is the input range. The gray domains are branch migration domains of twenty nucleotides in length. The color domains are toeholds of five nucleotides in length.

represent m_1 and m_2 , respectively. Om is the output DNA strand and its concentration at equilibrium $[Om]_\infty$ represents p_m . Each of M1, M2, and M3 is implemented by several DNA species, in which a DNA species is a DNA strand or complex. The design of the amplifier is shown in Figure 15. The DNA reactions in the multiplication gate are shown in Figures 10, 11, 12, 13, and 14. The relationship between the original chemical reaction network and DNA reactions is shown in Table 5.

To make reaction 3a much slower than reactions 3b, 3c, and 3d ($k_s \ll k_f, k_1, k_2$), we slow down DNA reaction 1 (forward), in which domain n1 of Im1 is modified such that there are mismatches when Im1 displaces sp17, and then the toehold-mediated strand displacement is slowed down. In the simulation of this paper, we let the strand displacement be slowed down by 2 orders of magnitude. To make reactions 3e and 3f have the same rate constant, we let the two toehold-mediated strand displacement reactions 12 and 13 have the same toehold g and branch migration domain n5-h (5' to 3' direction). The simulation to demonstrate this DNA implementation is in section 2.7.

2.5.3. Related Work on DNA Multiplication Gate. Zhang et al.³⁰ proposed a DNA-based amplifier that has fixed gain. The concentration of the product of their amplifier at equilibrium is αI_0 , where I_0 is the initial concentration of the input DNA strand and constant α is the gain of the amplifier. Genot et al.³¹ also developed a method by which they can multiply a number (represented by the concentration of a DNA strand) by a constant. Both their work is a preliminary step toward designing a multiplication gate. They can multiply the input by a constant. The input is represented by the concentration of an input DNA strand and the constant is encoded by the concentrations of the components of their

amplifiers. Lakin et al.³² developed a two-input multiplier but their multiplier is not autonomous, which means that, in order to make the multiplier work as desired, the input DNA strands to the multiplier need to be separately added into the solution manually at designated times.

2.6. Leak Reactions. Thus, far, we have only described the designed reactions of the three gates. Here we discuss potential leak reactions (unintended reactions). As shown in Figure 17, the main leak reaction (reaction 1) in the addition gate is caused by strand Fa, where Fa is able to displace Oa even in the absence of the input strand Ia1. This kind of leak reaction also happens between Ga2 and Fa in the addition gate. The reason why this can happen is that the base pairs in the circled part of Ga1 can be temporarily broken and create a toehold for Fa. This kind of leak is typical in systems based on DNA strand displacement. To reduce the leak, we can use some design techniques such as manipulating sequence design, incorporating clamps, etc.^{2-4,6,7,33-35} Both the subtraction and multiplication gates suffer from the same leak mechanism. The leak (reaction 2) in the subtraction gate is that Is1 can invade Gs and displace the v1 domain even if there is no Is2. The leak in the multiplication gate is caused by strands Fm1 and Fm2 (reactions 3 and 4). In the amplifier of the multiplication gate, there is also such leak (reaction 5).

The leak reactions will cause errors for the gates. As we said in the description of the gates, we use the concentration of the output strand of a gate at equilibrium to represent the output of this gate, if we do not consider leaks. If we want to evaluate the performance of a gate under particular inputs, we just need to check the percentage difference between the output of the gate (concentration of the output strand at equilibrium) and the theoretically correct result.

With leaks, we cannot use the concentration of the output strand at equilibrium to evaluate the performance of a gate, because the leak reactions will keep generating output until it reaches the maximum output value that a gate can provide. Instead, we use the time that the output stays within the valid output range (see its definition in section 2.1.2) to quantify the performance of a gate under particular inputs. In this paper, we use $r = 0.05$ to define the valid output range (see the meaning of r in section 2.1.2).

2.7. Simulation Results of the Gates. For each gate, we performed the simulation for three input ranges (0,1), (0,2), and (0,4). Later, we will use gates within these input ranges to build analog DNA circuits to compute polynomials. The model of simulation is discussed in section 4.

Figures 18, 19, and 20 shows an example for each gate for input ranges (0,1), (0,2), and (0,4), respectively, to give an intuitive sense of how our gates execute. The ranges between the red dotted line and green dotted line are the valid output ranges. The output stays in the valid output range for a long period in each case. The summary of the gates' performance is shown in Figures 21, 22, and 23, and they show how long the output stays within the valid output range for each combination of inputs. We observe that the output stays in the valid output range for a longer period when the inputs are larger because the influence of leak is relatively smaller for larger inputs. The simulated reaction time is 7.2×10^5 seconds (200 h) and "simulated reaction time" is for how long we predict the reaction by simulation.

2.8. Strategy To Construct Analog DNA Circuits. Our analog gates are modular, which means that the input and output strands have the same motif. This property confers

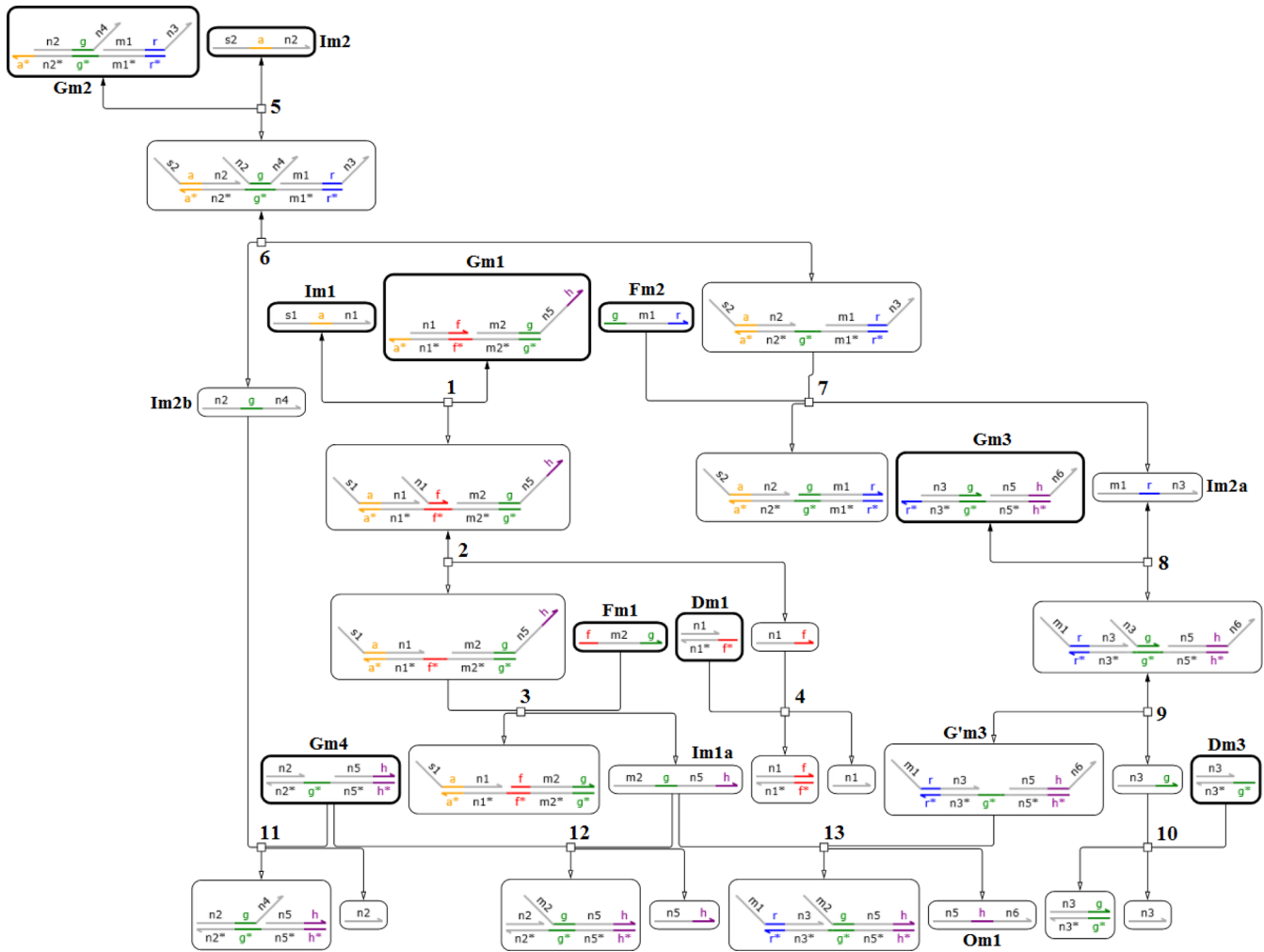


Figure 10. DNA reactions in the multiplication gate. Each reaction is assigned a number.

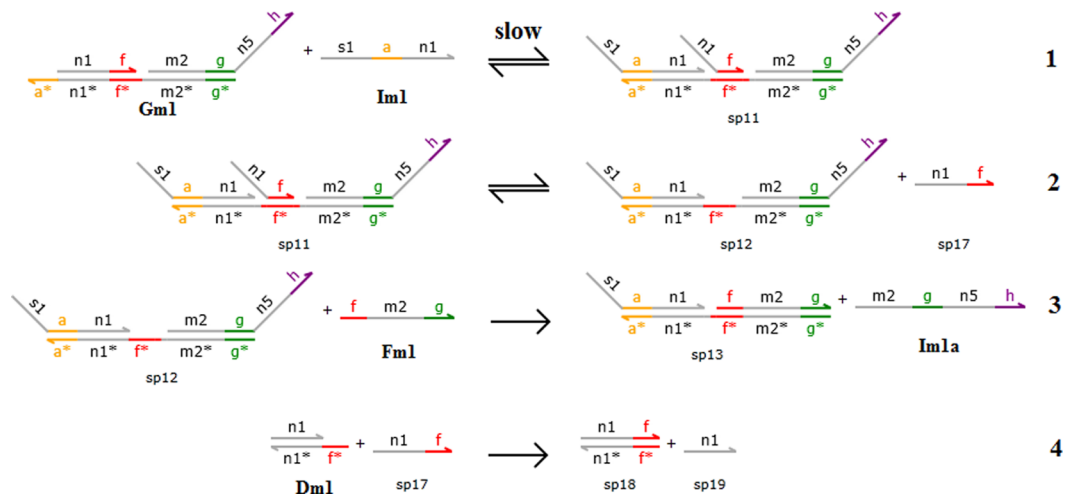


Figure 11. A list of the DNA reactions in the multiplication gate (part 1). Each reaction is assigned a number, which is consistent with Figure 10. Reaction 1 (forward) is slowed down by modifying domain *n1* of Im1 such that there are mismatches when Im1 displaces sp17.

scalability to circuits built by our gates. The simplest strategy of building circuits is connecting the gates together by programming the branch migration domains' sequences in the output strands such that these strands can find their designated downstream gates. The concern for this strategy is that when we designed and simulated the single gates, we

assumed that the inputs were "static" which means that they were fully prepared at the moment that the gates started to work. However, when a gate is part of a circuit, its input may be "dynamic", which means that the inputs are dynamically (or gradually) produced by other gates, and this may influence the performance of our gates.

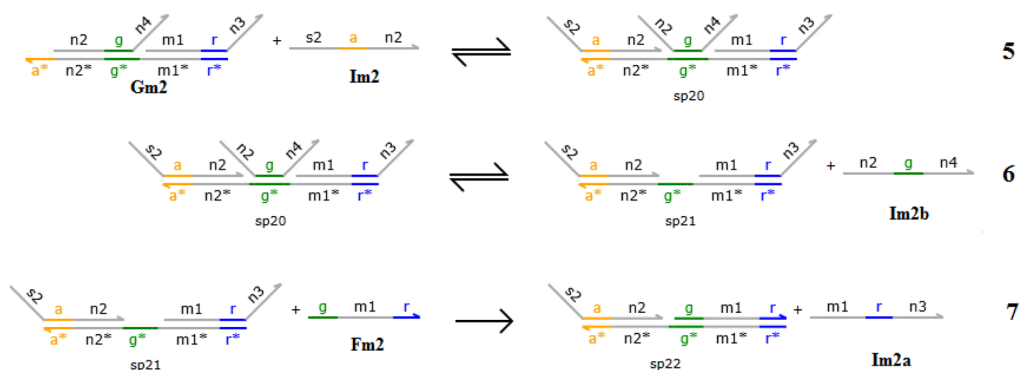


Figure 12. A list of the DNA reactions in the multiplication gate (part 2). Each reaction is assigned a number, which is consistent with Figure 10.

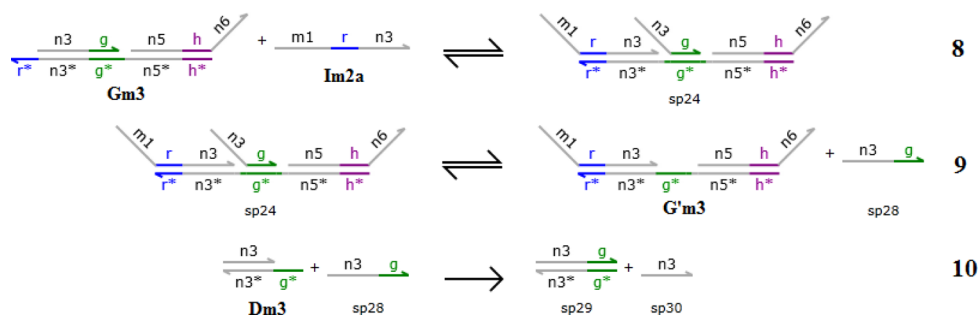


Figure 13. A list of the DNA reactions in the multiplication gate (part 3). Each reaction is assigned a number, which is consistent with Figure 10.

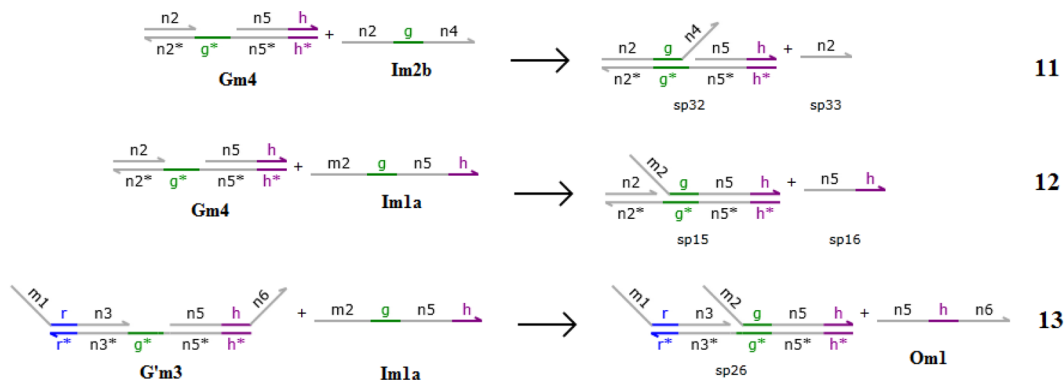


Figure 14. A list of the DNA reactions in the multiplication gate (part 4). Each reaction is assigned a number, which is consistent with Figure 10.

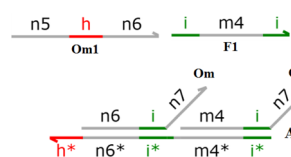


Figure 15. Design of a $2 \times$ amplifier, where one Om1 strand generates two Om strands. We can choose r_m as a power of 2. For $r_m = 2^n$, we just need to connect n layers of such $2 \times$ amplifiers in series to get a $2^n \times$ amplifier. The maximum possible input of layer-0 (represented by $[Om1]$) is $\frac{r_m}{r_m} = r_m = 2^n$ as the calculation in section 2.3.1 with $m_1 = m_2 = r_m$. Therefore, the maximum possible input of layer i is 2^{n+i} ($0 \leq i \leq n - 1$). In layer i , we let the initial concentrations of the DNA strand F and the DNA complex A be 2^{n+i} , such that the amplifier can afford the maximum possible input. The reactions in the amplifier are shown in Figure 16. The two Om strands are identical in terms that only the $i-n7$ (5' to 3' direction) part in the two strands matters in further reactions.

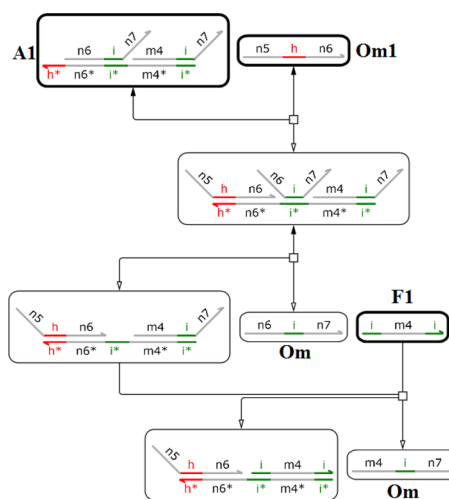


Figure 16. DNA reactions in a $2 \times$ amplifier.

Table 5. Relationship between the Original Chemical Reaction Network and DNA Reactions in the Multiplication Gate^a

abstract chemical reaction	DNA reactions
3a	1, 2, 3, and 4
3b	5, 6, and 7
3c	8, 9, and 10
3d	11
3e	12
3f	13

^aEach abstract chemical reaction is implemented by one or several DNA reactions. The DNA reactions corresponding to the numbers are shown in Figures 10, 11, 12, 13, and 14.

This concern is common for all kinds of analog systems. Analog systems have the property that they do not restore the signal for each stage and only the output signal is robust. We have some methods to mitigate this problem in our architecture. The addition gate is not influenced much by this issue because it is essentially just a transducer and it does not matter much how the inputs arrive. For the subtraction gate, if either input comes earlier, it can simply wait for the other one. Eventually, the cancellation between Is1 and Is2 will be finished, and the remaining Is1 will serve as output strands. For the multiplication gate, we make input strand Im2 be prepared in a “static” fashion such that the desired concentration ratio between G'm3 and Gm4 is formed as early as possible. Im1 can be prepared in a “dynamic” fashion by another gate, and this also gives more time to the formation of the concentration ratio. For example, in Figure 25, for all multiplication gates, the Im2 input strands (the input on the right) are prepared in a “static” fashion, not dynamically (or gradually) produced by another gate.

Note that one issue for the subtraction gate is that input strand Is1 can skip this gate to react with a downstream gate because Is1 serves both as input and output strands (e.g., in Figure 24). This issue can be solved by using only one subtraction gate in a circuit and placing it at the output port of the circuit (e.g., Figure 27), such that its output strand

does not interact any downstream gate. For example, a polynomial function $f(x) = 3x^4 - 2x^3 + 5x - 1$ can be transformed to $f(x) = (3x^4 + 5x) - (2x^3 + 1)$, which only has one subtraction operation. Since we require $s_1 > s_2$ in our subtraction gate, circuits constructed by our architecture can only accept x such that $f(x) = (3x^4 + 5x) - (2x^3 + 1) > 0$. This transformation does not increase the number of gates needed and the only difference is that the circuit needs two subtraction gates and one addition gate for the original polynomial, but it needs one subtraction gate and two addition gates for the transformed polynomial.

In the following sections, we will first present the construction of analog DNA circuits to compute polynomial functions of inputs. We show two examples: a circuit to compute

$$f(x) = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!}$$

for $0 < x < 1$, and a circuit to compute $g(x,y) = 2y - y^2x$, for $x,y \in (0,2)$ and $g(x,y) > 0$. Then we use these two circuits to construct circuits that compute nonpolynomial functions by strategies such as Taylor Series and Newton Iteration.

2.9. An Analog DNA Circuit To Compute $f(x) = 1 + x + x^2/2! + x^3/3!$ for $0 < x < 1$. Figure 25 shows an analog DNA circuit to compute

$$f(x) = 1 + x + x^2/2! + x^3/3!$$

for $0 < x < 1$. Each gate is assigned a number for the convenience to describe the circuit. If the output strand of a gate serves as the input strand of another gate, we put a wire to connect the corresponding output and input ports, which means that we unify the input and output DNA strands such that these two gates can communicate. Wires that have a gate only at one end indicate the inputs or the output of the circuit. Each wire is assigned a formula which describes an input of the circuit or what the subcircuit under the wire computes. The input ranges of the gates are chosen according to the upper bound of the inputs that a gate may encounter in the circuit.

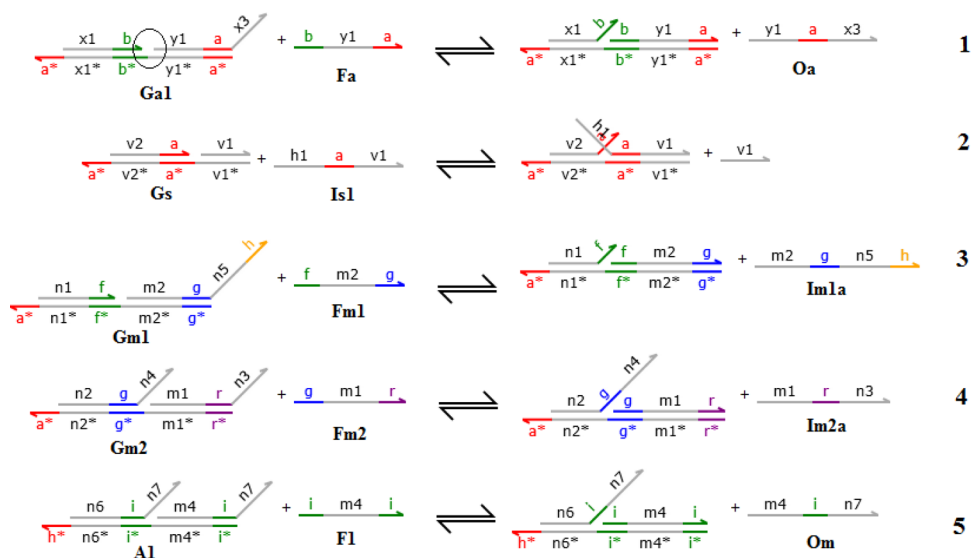


Figure 17. Main leak reactions in our DNA gates: reaction 1 in the addition gate; reaction 2 in the subtraction gate; reactions 3, 4, and 5 in the multiplication gate.

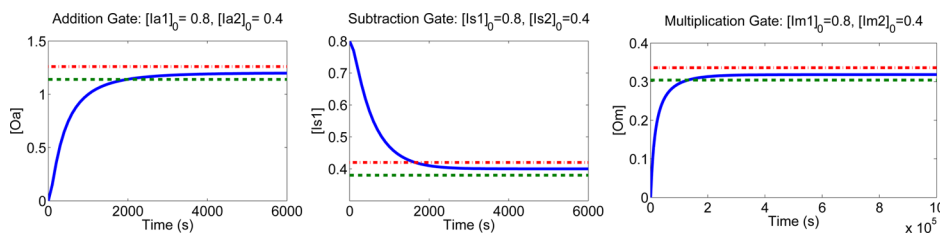


Figure 18. Examples to show the execution of our gates when the input range is (0,1). The vertical axes represent the concentrations of output DNA strands. The ranges between the red and green dotted lines are the valid output ranges. We do not show the curves for the whole simulated period (7.2×10^5 seconds) for the convenience to see the shapes of the curves at the early stage.

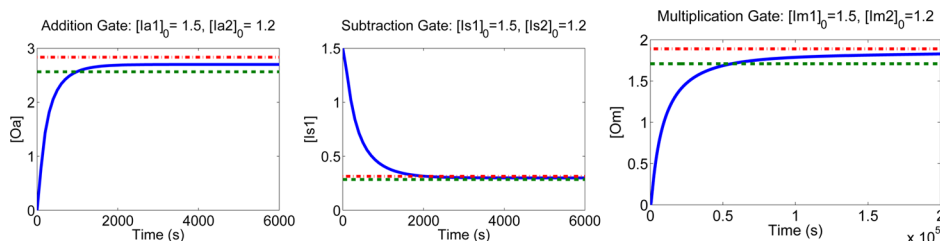


Figure 19. Examples to show the execution of our gates when the input range is (0,2). The vertical axes represent the concentrations of output DNA strands. The ranges between the red and green dotted lines are the valid output ranges. We do not show the curves for the whole simulated period (7.2×10^5 seconds) for the convenience to see the shapes of the curves at the early stage.

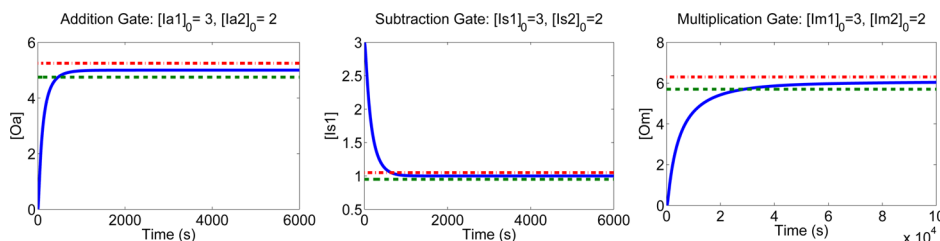


Figure 20. Examples to show the execution of our gates when the input range is (0,4). The vertical axes represent the concentrations of output DNA strands. The ranges between the red and green dotted lines are the valid output ranges. We do not show the curves for the whole simulated period (7.2×10^5 seconds) for the convenience to see the shapes of the curves at the early stage.

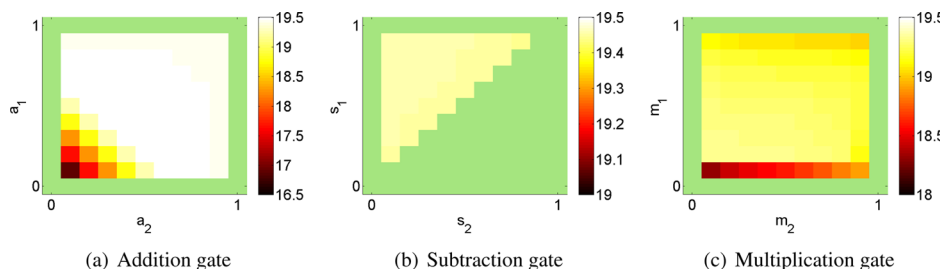


Figure 21. Performance of the gates when the input range is (0,1). The color represents $\log_2(t)$ where t is the time (seconds) that the output stays within the valid output range. $\log_2(t)$ is used instead of t simply for convenience in plotting.

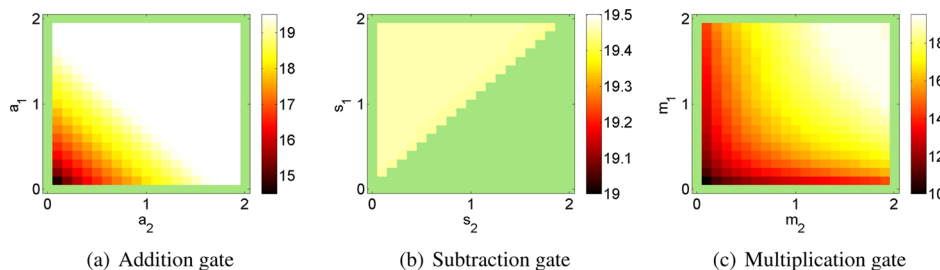


Figure 22. Performance of the gates when the input range is (0,2). The color represents $\log_2(t)$ where t is the time (seconds) that the output stays within the valid output range. $\log_2(t)$ is used instead of t simply for convenience in plotting.

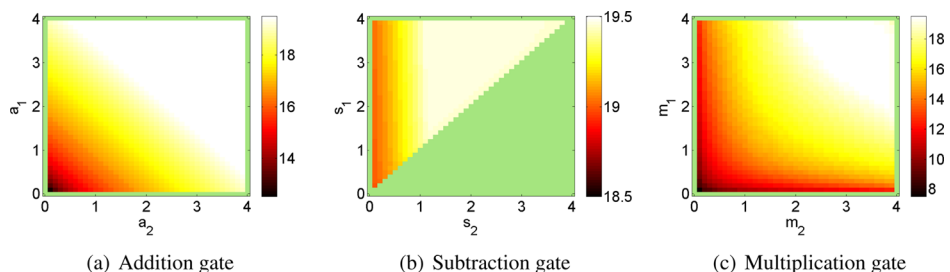


Figure 23. Performance of the gates when the input range is (0,4). The color represents $\log_2(t)$ where t is the time (seconds) that the output stays within the valid output range. $\log_2(t)$ is used instead of t simply for convenience in plotting.

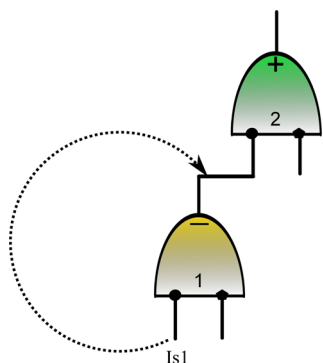


Figure 24. $Is1$ can skip gate 1 and go to gate 2.

2.9.1. Simulation of the Circuit To Compute $f(x)$. The rate constants used are the same as those for evaluating the gates (see section 4). The simulation is done for all $x \in \{0.01, 0.02, \dots, 0.99\}$. The benchmark to quantify the performance of the circuit is the time that the output stays within the valid output range (between $0.95f(x)$ and $1.05f(x)$) during the 7.2×10^5 seconds that we simulated. Figure 26a shows an example of how our circuit executes when $x = 0.7$. The range between the red dotted line and green dotted line is the valid output range. The quick growth of the output at the beginning is due to gate 3 (addition gate) which operates faster than the multiplication gates. The slow growth later is mainly from the multiplication gates which operate slower. A summary of our simulation data is shown in Figure 26b. It is observed that the output for larger inputs stays in the valid output range for a longer time because the influence of leak reactions is relatively smaller for larger inputs.

2.10. An Analog DNA Circuit To Compute $g(x,y) = 2y - y^2x$ for $x,y \in (0,2)$ and $g(x,y) > 0$. Figure 27 shows an analog DNA circuit to compute $g(x,y) = 2y - y^2x$ for $x,y \in (0,2)$ and $g(x,y) > 0$. The simulation is done for all combinations of x and y , where $x,y \in \{0.1, 0.2, \dots, 1.9\}$ and $g(x,y) > 0$. The benchmark to quantify the performance of the circuit is the time that the output stays within the valid output range (between $0.95g(x,y)$ and $1.05g(x,y)$) during the 7.2×10^5 seconds that we simulated. Figure 28a shows how the circuit executes when $x = 0.5$ and $y = 1$. The output went up first because it was produced by a subtraction gate (gate 1) and the input strands $Is1$ of gate 1 came sooner than the input strands $Is2$, because $Is1$ was produced by an addition gate (gate 2) but $Is2$ was produced by a subcircuit composed of two multiplication gates (gate 3 and gate 4). The output eventually went down because of the cancellation reactions in gate 1. The time that the output stayed in the valid output

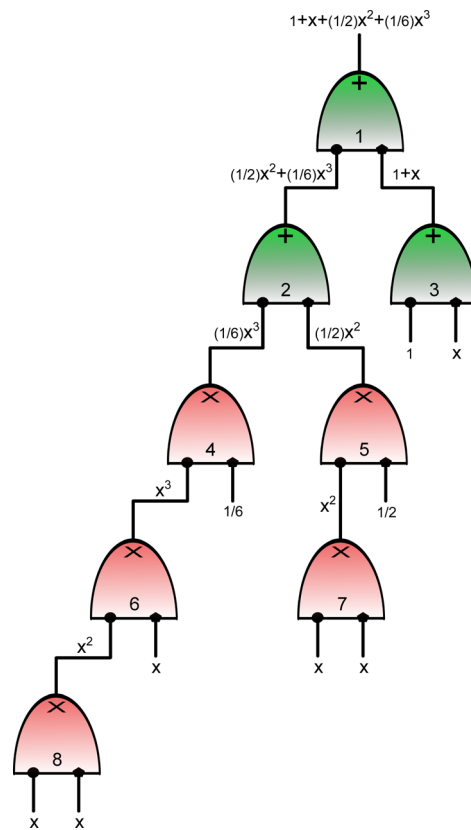


Figure 25. A circuit to compute $f(x) = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!}$ for $0 < x < 1$. Each wire is assigned a formula which describes an input of the circuit or what the subcircuit under the wire computes. Each gate is assigned a number for the convenience to describe the circuit design. The input ranges of gate 2, gate 4, gate 5, gate 6, gate 7, and gate 8 are (0,1). The input range of gate 1 is (0,4). The input range of gate 3 is (0,2). The input ranges are chosen according to the upper bound of the inputs of a gate may encounter in the circuit.

range during its growth did not count to the benchmark. A summary of our simulation data is shown in Figure 28b.

2.11. Analog DNA Circuits for Nonpolynomial Functions. Here we discuss how to build analog DNA circuits to compute nonpolynomial functions. The first strategy is using Taylor Series to approximate nonpolynomial functions by polynomial functions. For example, by Taylor Series, we have

$$\exp(x) \approx f(x) = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!}$$

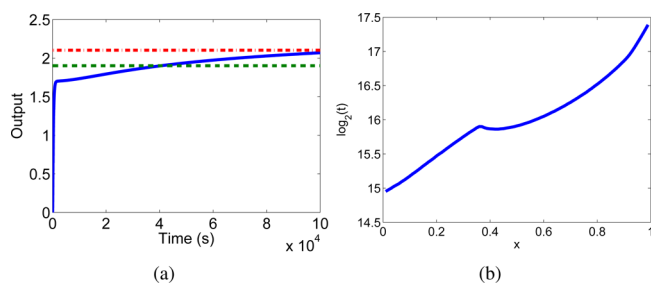


Figure 26. (a) Execution of the circuit to compute $f(x)$ when $x = 0.7$. We do not show the curve for the whole simulated period (7.2×10^5 seconds) for the convenience to see the shape of the curve at the early stage. (b) Performance of the circuit to compute $f(x)$, where t is the time (seconds) that the output stays in the valid output range. $\log_2(t)$ is used for the vertical axis instead of t simply for convenience in plotting.

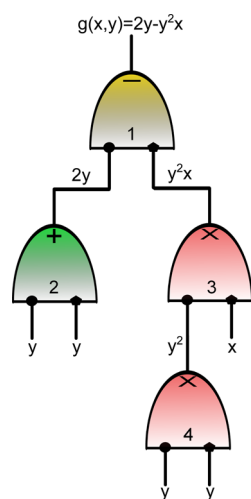


Figure 27. A circuit to compute $g(x, y) = 2y - y^2x$ for $x, y \in (0,2)$ and $g(x, y) > 0$. Each wire is assigned a formula which describes an input of the circuit or what the subcircuit under the wire computes. Each gate is assigned a number for the convenience to describe the circuit design. The input range of gate 1 and gate 3 is $(0,4)$. The input range of gate 2 and gate 4 is $(0,2)$. The input ranges are chosen according to the upper bound of the inputs of a gate may encounter in the circuit.

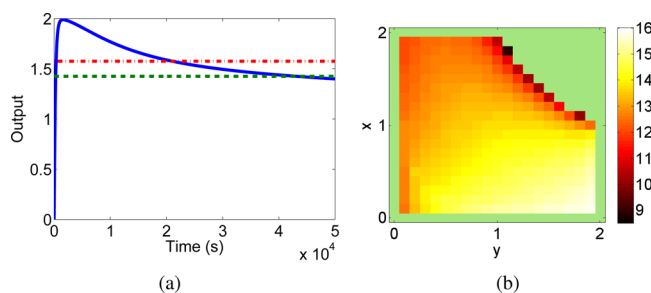


Figure 28. (a) Execution of the circuit to compute $g(x,y)$ when $x = 0.5, y = 1$. We do not show the curve for the whole simulated period (7.2×10^5 seconds) for the convenience to see the shape of the curve at the early stage. (b) Performance of the circuit to compute $g(x,y)$. The color represents $\log_2(t)$ where t is the time (seconds) that the output stays within the valid output range. $\log_2(t)$ is used instead of t simply for convenience in plotting. The unplotted part at the upper-right corner is where $g(x,y) > 0$ is not satisfied.

and the error is small when $0 < x < 1$. Therefore, we can use the circuit in section 2.9 to compute $\exp(x)$ with good approximation when $0 < x < 1$.

The second strategy is using the Newton Iteration. For example, a circuit that computes $r(x) = \frac{1}{x}$ for $0.5 < x < 1$: The Newton Iteration formula to compute $r(x) = \frac{1}{x}$ is $Y_{n+1} = 2Y_n - Y_n^2x$, where $\lim_{n \rightarrow \infty} Y_n = \frac{1}{x}$. The circuit for one iteration is the same as that to compute $g(x,y) = 2y - y^2x$, for $x, y \in (0,2)$ and $g(x,y) > 0$. Each iteration can work well according to the simulation results of the circuit to compute $g(x,y)$. The only issue is to let the iterations happen sequentially. This can be done by adding the circuit for each iteration sequentially as shown in Figure 29. Note that the sequence designs for different iterations are different to prevent the crosstalk among iterations.

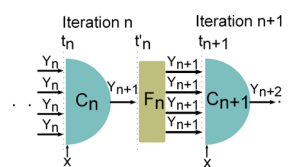


Figure 29. Assume that the circuit for iteration n (C_n) starts to work at time t_n . At t'_n , the output Y_{n+1} is already in the desirable range and we add fanout gate F_n to the reaction tube to prepare the inputs for C_{n+1} . At t_{n+1} , the Y_{n+1} outputs from F_n are ready and we add C_{n+1} with input x . Each time we add fanout gate or circuit for the next iteration to the reaction tube, it should be a tiny volume of concentrated solution to minimize dilution. We want to reduce dilution because it will cause error if we want circuits for all iterations to perform at roughly the same concentration. The time points, e.g., t'_n, t_{n+1} , can be estimated by simulation.

3. DISCUSSION

In this paper, we proposed an architecture for systematic construction of DNA circuits to perform analog arithmetic computation. There are three elementary gates in our architecture. On the basis of these gates, we can build circuits to compute polynomial functions of inputs. Functions beyond the scope of polynomials may also be approximately computed using the techniques that we suggested. Analog computation has certain advantages over digital computation for some applications and this motivated our work.

The prior work on analog computation has explored the computation power of chemical reaction networks (CRNs).^{36–40} For example, Chen et al. showed that semilinear functions can be computed by CRNs. Their work investigates the computation by CRNs in general from a high-level perspective, while we propose concrete DNA systems. These theory works can be very useful in motivating the design of DNA systems. The current work on analog DNA systems has mainly focused on developing DNA circuits to generate continuous dynamics at both theoretical and experimental aspects.^{8,9,11,12} These works are mainly based on some general schemes to implement arbitrary chemical reaction networks (CRNs) with DNA strand displacement circuits.^{10,13} There is still a need of detailed exploration on analog arithmetic computation by DNA circuits.

To improve the performance of our DNA gates, the main work that should be done is leak reduction. The errors caused by the leak reactions keep growing over time until the gates

are used up. This is not particular for our DNA gates. Leak is a common issue for DNA strand displacement circuits. The slow-down mechanism in reaction 1 (forward) (see Figure 11) is important to the performance of our multiplication gate, because it decides the extent that the DNA implementation fulfills the requirement of $k_s \ll k_p, k_1, k_2$ in the high-level chemical reaction network of our multiplication gate.

4. METHODS

We simulated our gates by Language for Biochemical Systems (LBS).²⁷ To speed up the simulation, we used MATLAB (MathWorks) to run the code produced from LBS by Visual GEC.²⁹ We also used MATLAB to visualize our simulation data. The simulation of stochastic model-checking is done by Language for Biochemical Systems (LBS)²⁷ in a beta version of Visual GEC.²⁹ All DNA figures are drawn by Visual DSD.²⁸

Analog gates process continuous signals, and we cannot perform simulations for all the possible inputs. The sampling scheme, using the example of the addition gate, picks all combinations of a_1 and a_2 , where $a_1, a_2 \in \{0.1, 0.2, \dots, r_a - 0.1\}$ where $(0, r_a)$ is the input range. It is the same scheme for the subtraction and multiplication gates. For the subtraction gate, we also require $s_1 > s_2$. The benchmark to quantify the performance of a gate is the time that the output stays within the valid output range.

The rate constant of toehold binding is $2 \times 10^{-3} \text{ nM}^{-1} \text{ s}^{-1}$ for toeholds that are five nucleotides long.³ The rate constant of toehold unbinding is 10 s^{-1} for toeholds that are five nucleotides long.³ The rate constant of branch migration is $8000/x^2 \text{ s}^{-1}$,⁴¹ where x is the length of branch migration domain. Using mismatches, we can tune the speed of DNA strand displacement in a wide range.⁴² We assume that, by modifying the $n1$ domain of Im1 of the multiplication gate such that there are mismatches when Im1 displaces sp17, the rate constant of the branch migration is reduced by 2 orders of magnitude, which is

$$0.01 \times \frac{8000}{|n1|^2} = 0.2 \text{ s}^{-1}$$

where $|n1|$ is the length of domain $n1$. The leak rate constant is $5 \times 10^{-9} \text{ nM}^{-1} \text{ s}^{-1}$.⁸ We choose 5 nM as the unit for concentration in the simulation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.6b00144.

LBS code to simulate the gates and circuits (TXT)

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Author Contributions

T.S. designed the gates and circuits, performed simulation, and wrote the paper. J.R. conceived and supervised the study and wrote the paper. All authors participated in discussing the design and revising the paper and gave final approval to publish this paper.

Notes

The authors declare no competing financial interest.

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