DNA Circuits Based on Isothermal Constrained Loop Extension DNA Amplification

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Abstract. In this paper, we first describe the isothermal constrained loop extension DNA amplification (ICLEDA), which is a new variant of amplification combining the advantages of rolling circle amplification (RCA) and of strand displacement amplification (SDA). Then, we formalize this process in terms of the theory of formal languages and show, on the basis of this formulation, how to manage OR and AND gates. We then explain how to introduce negation, which allows us to prove that, in principle, it is possible to implement the computation of any boolean function on DNA strands using ICLEDA.

1 Introduction

The first attempt to use DNA for solving computational problems was done by Adleman [1]. Since that time several models of computation using DNA have been proposed, we refer to [2] for an overview. Boolean circuits play an important role in this research. Their structure allows us to implement them in a simple way on DNA support. There are several designs simulating bounded fan-in circuits [10] and semi-unbounded fan-in circuits [9] (in both cases AND and OR gates are used). Another approach can be found in [3] where circuits with NAND gates are simulated or in [13] where the construction is based on the operation of hybridization of molecular beacons.

In this article we use an approach similar to [9][2]. More precisely, we use only true values and the true value of any gate will be encoded by a specific DNA sequence. However, we don’t use hybridization to assembly a resulting (answer) molecule like in these papers. Instead we use a special type of amplification to express the presence of signals (true values for some gates) which can further trigger the amplification of new signals following the circuit. Such an approach does not require temperature cycles and can be executed autonomously.
The most common in-vitro DNA strand replication method (also called “DNA amplification”) is based on PCR. This method is based on a series of primer extension cycles with changing temperature conditions to allow for strand separation at the beginning of each cycle.

Rolling-circle amplification (RCA) is another method of strand replication based on circular DNA molecules [5] and is inspired from the natural replication mechanisms of some viruses [6]. The important observation is that this method does not require changing the conditions of the test tube for DNA amplification and produces long single stranded DNA molecules including multiple complementary copies of the circular template DNA fragment. This procedure was used in DNA computing as a basis for the simulation of the resolution refutation in [7].

The strand displacement amplification (SDA) is based on the ability of a restriction enzyme to nick a modified recognition site and the ability of a polymerase to initiate synthesis at the nick and displace a downstream DNA strand during replication [11]. Both above methods allow us to produce DNA strands in isothermal conditions. There are methods using both RCA and SDA, for example ramification-extension method (RAM) [12].

In this article we consider a new isothermal DNA replication method, called ICLEDA for Isothermal Constrained Loop Extension DNA Amplification, described in [8]. It makes it possible to produce short linear and single stranded DNA strands in isothermal conditions. Importantly in the perspective of a practical application, the amplification is also possible when the template molecules are immobilized on a support. We formalize the amplification process in terms of formal languages. Such a formal system is constructed from a number of elements, which we call amplification loop complexes (or simply loop complexes) that can be in two states: blocked or unblocked. A loop complex in unblocked state produces infinitely the corresponding DNA strand (signal). The transition from a blocked to an unblocked state is done by annealing and primer extension. As a result we can simulate a signaling cascade whose nodes correspond to AND and OR gates. The result is collected in one of the two output nodes, corresponding to the true or false value of the corresponding boolean function.

We also consider a more general framework concerning double-stranded DNA molecules that are partially hybridized and that can be dissociated by annealing with other single stranded DNA and/or by primer extension. We give a description of the corresponding objects and operations in terms of the formal language theory. This notation gives us a simple way to describe the simulation of logical gates and the construction of the circuit.

2 The mechanism

In this section, we first describe the ICLEDA amplification process defined in [8] on which the whole work is based. Then, we show how this mechanism allows us to devise a configuration, which we call the loop complex, which will later on allow us to implement logical gates in this context. Note that the word loop
refers to the shape of the biophysical complex we consider rather than the computational device which is usually understood by this term, this is why the term complex is attached to loop in this denomination. The bio-physical description of the amplification process and of the loop complex is the content of Subsection 2.1.

In a second subsection, we propose a formalization of the process described in Subsection 2.1, see Subsection 2.2. Later, we shall switch to a bit more abstract formalism which will be more suited for computation purposes.

2.1 The amplification and the loop

The ICLEDA amplification method designed in patent [8] is to some extent a combination of RCA and SDA amplification. We refer to this patent for more technical information.

The mechanism is represented on Fig. 1(a). The loop complex is a circular molecule composed of two parts: the amplifiable fragment (2) and the loop link (1). The arrow represents the 3’ end of the amplifiable fragment. We represent this molecule schematically as on Fig. 1(b). For the sake of commodity we split the amplifiable fragment in 3 parts (3,4,5 on the picture) corresponding to the 3’ end, middle and 5’ end of the amplifiable fragment.

The amplification mix contains primers (101) which hybridize to the 3’ part of the amplifiable fragment (3). They can be further extended by DNA polymerase (102) present in the mix, see Fig. 2(a). The loop link (1) length is small compared to the length of the DNA fragment: typically 1 to 5 nm. It can be a simple chemical link joining the extremities of the DNA fragment, or a biochemical link between biotin moieties attached to the extremities of the DNA fragment via a streptavidin protein. The DNA fragment is also short in regards to the stiffness of double stranded DNA. In conditions where the biochemical replication reactions can take place, double stranded DNA molecules shorter
than 300 – 500 nucleotides are too stiff for their extremities to come into close proximity. In other words, a circular DNA molecule shorter than 300 nucleotides cannot exist in full double stranded form, but is found as stretches of double stranded portions separated by single stranded portions. This is true also for the loop complexes used in ICLEDA. At some point the complex will be composed from a single stranded DNA having \( n \) nucleotides from the 5' end of the amplifiable fragment, a double-stranded DNA corresponding to the 3' part of the amplifiable fragment, the extended primer and the linking loop of special length.

![Image of DNA molecules](image)

**Fig. 2.** The amplification process: primer extension (a); maximum stretch of amplifiable fragment and the opening of the 5' end of the double strand (b); a second amplification started (c). Notation: link loop (1), amplifiable fragment (3,4,5), DNA polymerase (102), (extended) primer (101), single stranded fragment (104).

Since the two extremities of the amplifiable fragment are linked to each other this gives a geometric constraint for the loop. In order to continue the reaction either the single strand part should be extended to the maximum or the double stranded part should open at the opposite extremity. At some level of tension the energetic preference will be to continue the extension of the primer by DNA polymerase, while the opposite end will detach by Brownian motion. So, at the same time the double stranded fragment will be opened at 5' part and one nucleotide will be added by DNA polymerase. However it should be noted that the number of nucleotides on the double stranded part remains unchanged, due to the geometric constraints of the loop.

Since no more nucleotides are bound at the 3' end of the amplifiable fragment (3) at some point it becomes accessible for a hybridization with a new primer, see Fig. 2(b). The extension is blocked when it reaches the end on the amplifiable fragment (105) because of the presence of non-natural nucleotides in the link, see Fig. 2(c).

Fig. 3 shows a loop complex (1) which has three attached primers being in different stages of the duplication. The first primer (101a) is paired to the 3' part of the amplifiable fragment (3) and is ready to be extended. The second primer (101b) is in the process of the extension and it is paired by its 3' end to the central part (4) of the amplifiable fragment where its extension continues,
while its 5’ part is a single stranded DNA. The third primer (101c) reached the end and is not extended anymore, but it is still paired by its 3’ end to the 5’ end (5) of the amplifiable fragment. The progression of the extension is blocked by the loop link (1), while its 5’ part is progressively detached from the amplifiable fragment by the progression of the extension of the second primer. Besides its role in the amplification process, the link also enables the possibility of attaching the loop complex to a surface without hindering the replication mechanism.

![Fig. 3](image-url) The amplification process: three molecules in different stages of duplication. Notation: link loop (1), amplifiable fragment (3,4,5), DNA polymerase (102), (extended) primers (101a,b,c), single stranded fragments (104b,c).

Now we remark that if in the mix a fragment of a single stranded DNA that matches the 3’ part of the amplifiable fragment is present, then it can stick to the amplifiable fragment as shown on Fig. 4(a). We call such a strand a trigger. When a trigger is attached to the loop complex, no amplification can be done. A trigger can be detached from the loop complex by an activator that matches by its 3’ end a part of the trigger strand as shown on Fig. 4(b). Once bound to the trigger the activator can be extended by DNA polymerase and this will release the trigger, so the loop complex will be able to start the amplification process.

### 2.2 Formalization

As the main process described in the previous subsection deals with molecules, we shall represent them as words over the four-letter alphabet. However, we shall not represent individual nucleotides. We shall rather consider the places where reactions may occur. Consequently, we shall divide the words in several places, the sensitive ones and the neutral ones. If $A$ is a molecule or a part of it, we denote by $A'$ its complement in the Watson-Crick complementarity rule. Note that $A'$ is also written in the opposite order of its letters with respect to $A$. Molecules are oriented and we denote by the symbol $\diamond$ the head (3’ end) of the molecule, which implies the reading order from left to right for $\diamond A$ and from right to left for $A'\diamond$. 
We consider that if $A$ and $A'$ are both present and if the configurations of the DNA strings to which they belong allow it, they bind each other. Consider that $A$ occurs in a molecule $M$. We write this $M = uAv$ with $u$ or $v$ possibly not present: we then say that $u$ or $v$ is empty. Consider that $A'$ occurs in a molecule $M'$, We similarly denote this by $M' = xA'y$. We assume that $A$ no more occurs neither in $u$ nor in $v$ and that, similarly $A'$ no more occurs neither in $x$ nor in $y$. We also assume that $A$ and $A'$ do not interact with neither of the molecules $u$, $v$, $x$ and $y$ and that these molecules also do no interact with each other. We express this by saying that $u$, $v$, $x$ and $y$ are neutral parts of the molecules to which they belong. This allows us to focus only on $A$ and $A'$ which are called the sensitive parts of the molecules $M$ and $M'$. We shall mark this difference between sensitive and neutral parts of molecules in the notation: sensitive parts will be denoted by capital letters and neutral parts will be denoted by lower case ones.

When the molecules $\circ M$ and $\circ N$ are both present, assuming that $\circ M$ contains the active part $A$ and that $\circ N$ contains $A'$, we denote this by an additive notation: $\circ M \oplus \circ N$. Now, if we replace $\circ M$ and $\circ N$ by their expressions in terms of $A$ and $A'$, we get $\circ uAv \oplus yA'x\circ$ and we now know that as a result we obtain a complex as $A$ and $A'$ get bound to each other. We write the complex as $\circ uAv \otimes yA'x\circ$. Hence the corresponding rule can be written as follows:

$$ \circ uAv \oplus yA'x\circ \vdash \circ uAv \otimes yA'x\circ $$  

As an example, we cannot write

$$ \circ uAv \oplus yBx\circ \vdash \circ uAv \otimes yBx\circ $$  

unless $A = wB't$ or $B = rA's$, we remember the reader that lower case letters denote neutral parts. To avoid unneeded repetition of rules we shall always use the rule in its explicit form 1, considering that in 2, we have neither $A = wB't$, nor $B = rA's$. 

**Fig. 4.** The loop complex blocked by a trigger (a) and the hybridization with further extension used to remove the trigger (b). Notation: link loop (1), amplifiable fragment (3,4,5), trigger (11), DNA strand used to release the trigger (12)
We assume that the operation $\oplus$ is commutative and associative, which corresponds to the fact that $\oplus$ models a situation in which the components are independent and may freely combine or not and in all possible combinations.

The loop complex can be formalized as follows: $\nabla F'uR'$ where $u$ is the neutral part and $F'$ with $R'$ are the sensitive ones, corresponding to the parts 4,3,5 on Fig. 1(b). A trigger can be formalized as $\otimes wX'Fz$, where $w$ and $z$ are the neutral parts and $X'$, $F$ are the sensitive ones.

The working of the loop complex can be formalized as:

$$\nabla F'uR' \vdash F'uR'$$ (3)

Now, if there is a trigger, we have:

$$\nabla F'uR' \oplus \otimes A'Fw \vdash \nabla F'uR' \otimes \otimes A'Fw.$$ (4)

where $u$, $t$ and $w$ are neutral. From subsection 2.1, the result of (4) blocks the application of (3).

We also have two rules for the trigger which occurs in formula (4):

$$\nabla F'uR' \otimes \otimes A'Fw \oplus zF'Ax\otimes \vdash \nabla F'uR' \oplus \otimes A'Fw \otimes zF'Ax\otimes$$ (5)

$$\nabla F'uR' \otimes \otimes A'Fw \oplus Ax\otimes \vdash \nabla F'uR' \oplus \otimes A'Fw \otimes F'Ax\otimes$$ (6)

In these formulas, as $A$ binds with $A'$ which, as a result, detaches the trigger from the loop complex, see Fig. 1. And now, we can see that (3) applies. Note that both formulas (5) and (6) produce almost the same result as the molecule $F'Ax\otimes$ is present in the new complex in both cases. The formula (6) translates the property indicated in Sub-section 2.1 to detach the trigger attached to the complex in the left-hand side of the formulas, it is enough to present the beginning of the active molecules, the process of detachment will produce the continuation of the active parts.

It can be noted that the formalism allows to explain why we obtain this rule. Indeed, it might be argued that as $F'$ and $F$ should also bind together, we could have the backward reaction:

$$\nabla F'uR' \oplus \otimes A'Fw \otimes zF'Ax\otimes \vdash \nabla F'uR' \oplus \otimes A'Fw \oplus zF'Ax\otimes$$

The reason why we have not this reaction is that as $A'$ is more visible for $A$ that $F'$ is for $F$, $A$ attaches to the trigger which, as a result, lead to its freeing from the complex.

In what follows, to simplify the notations, we introduce the following convention: we represent the triggers $Ax\otimes$ and $zF'Ax\otimes$ as $A$. This allows us to melt the formulas (5) and (6) in a single one, namely:

$$\nabla F'uR' \otimes \otimes A'Fw \oplus A \vdash \nabla F'uR' \oplus \otimes A'Fw \otimes A$$ (7)

If we have to explicit the form of $A$ as $Ax\otimes$ or $zF'Ax\otimes$, we shall speak of a realization of $A$. 
We can see this higher priority of \( A \) in the formalism. Remember that if we wish to read the words in the order given by a run other the molecule from its head to its tail, we have to read the word from the diamond to the opposite end. Define the **apartness** of a sensitive molecule \( X \) with respect to a molecule \( M \) as the number of sensitive parts of \( M \) between the diamond of \( M \) and the position of \( X \). Denote it by \( \text{apart}(X, M) \). We can now define the **apartness** of \( X \) with respect to \( M \) and \( N \), denoted by \( \text{apart}(X, M, N) \), where \( X \) is contained in \( M \) and \( X' \) is contained in \( N \), as the expression \( \text{apart}(X, M) + \text{apart}(X', N) \). Now, we can see that \( \text{apart}(A, \nabla F'uR' \otimes \odot tA'Fw, A) = 0 \) whatever the realization of \( A \): as \( Ax\circ \) or \( zF'Ax\circ \). Now we have that \( \text{apart}(F, \nabla F'uR' \otimes \odot tA'Fw, A) = 1 \), also whatever the realization of \( A \). As the apartness of \( A \) is lower than that of \( F \), the reaction with \( A \) has a higher priority and so it takes place while the reaction with \( F \) does not.

### 3 Implementing boolean functions

In this section we consider the amplification loop \( \nabla F'uR' \). We assume that if a loop \( \nabla F'uR' \) is unblocked, then there will be an **unbounded** number of copies of \( Fu'R \). This assumption results from the observation that once started, the amplification could produce a large enough number of resulting molecules, even if the loop is blocked again afterwards.

This implies that we can consider that initially all loops are blocked, otherwise we substitute them by a large number of DNA molecules corresponding to their result. So the computation in such a system consists in unblocking some loops in some order. This corresponds in a direct manner to boolean circuits where the electrical impulses are propagated in the circuit. The signals we use are always identified by the part at the beginning of the molecule, *i.e.* a signal \( A \) will be given by the string \( \odot Aw \).

It is known that any boolean function can be computed by a boolean circuit with AND, OR and NOT gates. It is possible to omit the NOT gate if signals corresponding to false values of variables are used. We remark that in this case only one type of signal (corresponding to **true**) exists in the circuit and the result is obtained by positional information, *i.e.* there are 2 possible output wires corresponding to the true and the false values of the formula.

From the properties stated in the previous section, we can devise the construction of the **OR**- and the **AND**-gates. First, we start with the **OR**-gate described in Subsection 3.1. In Subsection 3.2, we describe an **AND** gate which is close to the construction of Subsection 5.1. In Subsection 3.3, we describe another variant which is closer to that suggested by [8].

#### 3.1 The **OR**-gate

Considering rule [8], we decide to interpret the production of the molecule \( R \) as the emission of the boolean signal **true**.
In this condition, if the trigger $tA'Fw$ is initially attached to a loop complex $\nabla F'uR'$, forming complex $\nabla F'uR' \otimes oA'Fw$, rule (7) tells us that introducing the molecule $A$ either as $Ax$ or as $zF'Ax$, we obtain $\nabla F'uR' \oplus oA'Fw \otimes A$. As $\oplus$ is commutative, this corresponds to the fact that now rule (3) applies to $\nabla F'uR'$; accordingly, we get again the signal true.

It is not difficult to obtain a similar sequence of deductions with $\nabla G'vR'$ and the trigger $rB'Gs$. Introducing the molecule $B$, either as $By$ or as $zG'Bx$, we shall also get $R$ by applying the rules. The final construction for the gate is shown on Fig. 5.

Fig. 5. The simulation of the OR gate.

It is plain that if we introduce one of $A$ or $B$ we get also $R$: it is enough to introduce initially in the soup with both the loop complexes $\nabla F'uR'$ and $\nabla G'vR'$ as well as both triggers $oA'Fw$ and $oB'Gs$, so that we may assume that, after a certain time, rule (4) applies giving us:

$$\nabla F'uR' \otimes oA'Fw \oplus \nabla G'vR' \otimes oB'Gs.$$  

¿From rule (7) and the commutativity and associativity, we obtain that if $A$ is poured into the soup, $R$ will be produced, as the complex $\nabla F'uR' \otimes oA'Fw$ will be decomposed into $\nabla F'uR' \oplus oA'Fw \otimes A$. If $B$ is poured, we get a similar result. If both $A$ and $B$ are introduced, we get a similar result as both kinds of complexes are present. In all these three cases, the molecule $R$ is produced.

Accordingly, we obtain that a soup which contains both the loop complexes $\nabla F'uR' \otimes oA'Fw$ and $\nabla G'vR' \otimes oB'Gs$, it behaves like an OR-gate with respect to the occurrence or absence of the molecules $A$ and $B$: the required signal $R$ occurs if at least one of them is present and only in this condition.

We remark that the construction above can be extended to an $n$-ary OR gate. This gives the possibility to simulate semi-unbounded fan-in circuits.

3.2 The AND-gate

We can simulate an AND gate by considering two active regions on the loop, i.e. loops of form $\nabla F'_A uF'_B vR'$, where $F'_A$ and $F'_B$ are active zones for triggers having $A$ and $B$. Then the loop is blocked by two molecules as follows
Now if both triggers $\diamond At'$ and $\diamond Bq'$ are present, then the loop complex can be unblocked:

$$
\nabla F'_a u F'_b v R' \otimes t A' F_A w \otimes q B' F_B s \bigoplus \diamond At' \bigoplus \diamond Bq' \\
\downarrow

\nabla F'_a u F'_b v R' \otimes t A' F_A w \bigoplus \diamond At' \bigoplus q B' F_B s \otimes \diamond Bq' \\
\downarrow

\nabla F'_a u F'_b v R' \bigoplus t A' F_A w \otimes \diamond At' \bigoplus q B' F_B s \otimes \diamond Bq'$$ (8)

In the above derivation the unblocking can start by the signal $At'$, but following the commutativity of $\bigoplus$ it yields the same result.

It is clear that if only one of the triggers $At'$ or $Bq'$ is present, then the loop complex is only partially unblocked (either $\nabla F'_a u F'_b v R' \otimes t A' F_A w$ or $\nabla F'_a u F'_b v R' \otimes q B' F_B s$) and cannot produce the resulting signal.

### 3.3 The initial AND-gate

Another variant of the AND gate is described in [8]. Like in the previous case it is also a complex of 3 molecules, however the loop complex is bound in only one place. Its construction is done in two stages: the loop complex $\nabla F'_a w R'$ is blocked by the trigger $\diamond F_A A'$. After that the molecule $AB'\diamond$ is added into the solution and it will stick to the $A'$ site. Hence, the complex $\nabla F'_a w R' \otimes A' F_A \otimes AB'\diamond$ will be formed, see Fig. 6(b). We remark that since this complex is formed during the preparation stage, we can insure that no molecules $\diamond Au, (u \neq B)$ or $\diamond t F_A Av$ are present in the solution.

Now during the computation the loop complex can be unblocked as follows:

$$
\nabla F'_a w R' \otimes A' F_A \otimes AB'\diamond \bigoplus \diamond Bu \bigoplus \diamond Av \\
\downarrow

\nabla F'_a w R' \otimes A' F_A \bigoplus AB'\diamond \otimes \diamond A' Bu \bigoplus \diamond Av \\
\downarrow

\nabla F'_a w R' \bigoplus AB'\diamond \otimes \diamond A' Bu \bigoplus A' F_A \otimes F_A Av$$ (9)

We remark that unlike the previous case this construction is not symmetric, i.e. first the signal $\diamond Bu$ is removing the molecule $AB'\diamond$ from the complex, freeing the site $A'$, which can be bound after that by the signal $\diamond Au$ that finally unblocks the loop.

### 3.4 Implementing boolean functions

It is known that every boolean function of $n$ variables can be implemented by a boolean circuit using AND, OR and NOT gates. It is possible to eliminate the NOT gate by considering that only true signals can circulate in the circuit. In this case the input of the circuit is not the true or false value for the same
variable $x$, but rather a true value for $x$ or for $\neg x$. The output is also modified: instead of a single output having one of the values true or false, there are two outputs (marked by true and false) and a true value in some of the outputs indicate that the output value of the circuit is true or false.

For a boolean formula $\phi$ such a modified circuit can be constructed by a superposition of two circuits, one computing $\phi$ and the other one computing $\neg \phi$.

If we consider that the two output nodes are combined into a fictive output node then such a circuit is a DAG with the root being the output node and the leaves being the input variables and their negations.

We remark an important similarity between traditional electronic implementation of boolean circuits and our implementation: if a signal (represented by an electric charge in electronics and by DNA molecule in our case) appears at some moment during the computation, then it is sufficiently strong and does not disappear in the consequent steps. This allows us to make a direct analogy between two implementations and use similar construction techniques. This is different from other approaches of simulation of circuits by DNA computing, as we do not need additional amplification phase anymore.

More precisely, let $f : B^n \to B, B = \{0, 1\}$ be a boolean function and let $D = (V, E, F)$ be the circuit implementing this function (where $V = \{1, \ldots, n\}$ is the set of vertices, $E \subseteq V \times V$ is the set of edges and $F : V \to \{x_p, \neg x_p, AND_m, OR_k, NOT_q, out\}, 1 \leq p, q, m, k \leq n$ is the function that labels vertices of the circuit). Then for any inner node $y$ such that $F(y) = OR_m$ (resp. $F(y) = AND_m$) we construct an OR loop (resp. AND loop) as discussed in 3.1 (resp. 3.2). The final gate will send the signal to the output node (the root of the circuit ($F(x) = out$)). A similar construction should be performed for $\neg f$. The final assembly is the union of these two circuits.

In order to compute the result in the initial configuration signals corresponding to $X_k$ (where $X_k$ is either $x_k$ or $\neg x_k$) should be introduced.

We give below an example of such a construction for the following function: $f(x_1, x_2, x_3) = (\neg x_1 \land x_2) \lor (x_1 \land x_2 \land \neg x_3)$. 

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**Fig. 6.** The simulation of the AND gate with two active regions on the loop (a) and with one active region on the loop (b)
The ordinary boolean circuit computing $f$ is given below:

Next we replace the NOT gate by considering that only true values can transit the circuit. This gives the following structure for $f$ (we also numbered the gates):

Now we should construct the circuit (without negation) for $\neg f = (x_1 \land x_3) \lor \neg x_2$:

Now we combine the two constructions. We also add labels to edges going out from the same left node (corresponding to a concrete signal produced by the corresponding gate).
Having in mind that the true value of some node is represented by the presence of the corresponding signal (that labels the edge), it becomes clear that this construction can be directly implemented using loop complexes and corresponding signals by 4 AND gates and 2 OR gates.

The signals $S_1 \ldots S_6$ correspond to the input values and the signals $S_{11}$ and $S_{12}$ to the output. So the computation starts by giving input signals (taking care of not having an input $x$ and $\neg x$ at the same time). Then the gates will act in cascade and one of two output signals ($S_{11}$ if $f$ is true or $S_{12}$ if $f$ is false) will be obtained.

## 4 Conclusions

In this article we present a new method for the simulation of boolean circuits. The use of ICLEDA offers many advantages like a single volume and unchanged reaction conditions. This implies that the corresponding implementation will not need any additional intervention. Moreover, since the loop complexes can be easily attached to the support it is possible to reuse the circuit by washing the tube and by introducing trigger molecules to block the loops. Another advantage of the method is that the signal molecules (corresponding to the true value of some gate) are of a small length; moreover, by introducing compartments it is possible to share some of the signals.

As a further work it remains to experimentally verify the functioning of the method. A partial attempt for this is done in [8] where an evidence of the feasibility of the basic blocs is given. Another interesting question is the further investigation of the framework for the partial hybridization of DNA strings introduced in this article.

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