Abstract
A simple proof-of-principle type of simulation of chemically implemented logic gate is proposed in this paper using the structural switching of single stranded DNA strand from i-motif to hairpin and vice versa which is triggered by regulating H+ and copper (II) ions. The advantage of such chemically induced gates lies in its fast response time, controllability and reusability feature. Later on this simulation process is expressed in the form of algorithm to evaluate AND-OR Boolean circuit.

Key words: DNA, AND gate, OR gate, Boolean Circuit.

Introduction
Watson and Crick claimed novel prize in 1953 for the discovery of the double helical structure of DNA. For ages DNA is known as primary genetic material responsible for transmitting genetic information from one generation to another until Adleman(1), for the first time recognized the information processing property of DNA and demonstrated the first ever DNA synthetic computer by solving an instance of HPP. With time, enormous numbers of research are reported to employ DNA computing approach for solve several diverse fields of problems. DNA Boolean circuit simulation draws most of the interest and efforts. Ogihara and Ray (2,3) proposed the first DNA system that can simulate bounded Boolean circuit with complexity proportional to size of circuit. Since then several theoretical and experimental models and algorithms have been proposed. Analogous to silicon logic gate, molecular logic gate is expected to act as fundamental for biological or molecular computers (4)-(14). However the efficiency of most of such model is limited by involving too many error prone and time consuming biochemical processes. Recently secondary structure of DNA such as G-quadruplex and i-motif structure finds their way into several gate simulation models due to their unique properties such as highly specific binding properties, polymorphic versatility, and self assembly (15-19). There are several models reported where i-Motif structure coexisted with G-quadruplex but such models experienced the set back in terms of its complex maintenance and high cost (20,21). In few models G-quadruplex structure is solely used (22) whereas in some other models i-motif structure is solely used in construction of gate designing (23,24). Yunhua et al. (24) demonstrated DNA logic gates using only i-motif structural induction in response to the presence of H+, Ag+, and I- as inputs. This simple technology has potential to simulate the functionality of OR and INHIBIT gate.

Henry Albert Day et al. (25) published the switchability of pH induced i-motif structure to hairpin and vice-versa. During the experiment they initially induced i-motif structure in human telomeric sequence at pH 5.5(folding time of 100 milli seconds) which was altered to hairpin structure by adding Cu²⁺ at room temperature without changing the pH. The folding time of i-motif structure to hairpin on adding Cu²⁺ was estimated to be $44 \pm 2$ seconds. Further they
successfully reverse the hairpin structure to i-motif structure by adding EDTA. The total time span of a complete cycle of folding or unfolding is 100 seconds only.

In this application based paper, it is proposed that AND-OR gate can be chemically induced by using the switching property proposed by H.A Day and his group. The author proposed an algorithm to simulate logic gates and evaluation of Boolean circuit using secondary structural switching of C-rich chain of DNA. Instead of encoding the gate strand and the input strands in the form of DNA sequence, in this paper the ions and pH are programmed and controlled. It is expected that this model have several advantages such as cost effectiveness, fast response time, reusability and easy implementation.

Preliminaries

DNA i-motif: For decades it's been believed that DNA remain in only B-form double helical structure as proposed by Watson and Crick but after extensive studies in following years it is well established that DNA can be found to be in several different secondary structures such as in A and Z forms, triplexes, three and four way junctions and quadruplexes. i-motif or i-tetraplex structure is one of such secondary structures of DNA which recently gains lot of interests. It is a four stranded structure formed by intercalating two pair duplexes of anti parallel orientation which are held together via hydrogen bond between cytosine-cytosine base pairs. There are several ways to enhance the formation of i-motif such as acidic pH of the solution or at neutral pH with the presence of certain ions like Ag⁺(27) etc. In human genome i-motif sequence present as complementary sequence of G-quadruplex forming sequence such as human telomeric i-motif sequence (hTelC)(26). Several research works are reported expecting to use i-motif structures for anticancer drug development and gene regulation (28,29) and several other applications such as construction of biosensors(pH sensors), logic circuits, nanomachines and functional materials like proton-fuelled i-motif nanomotor, DNA “bipedal walkers”.

Fig. 1: Switching of C-rich sequence to i-motif structure

DNA hairpin: DNA hairpin structure is obtained when two self complementary segment in a single stranded DNA fold itself to self-hybridize. The resulting structure consists of two parts: stem part and the loop part. Stem part are the two self complementary sequences and the loop portion is the sequence which doesn’t take part in the hybridization. The stem-loop structure owes its stability to the length, involvement of G-C pairs, and number of mismatches in the stem. Hairpin structure finds its application in several biomedical and nano-technology field.

Fig. 2. Single stranded to hairpin structure.

Logic Gate and Boolean circuit simulation: In a Boolean circuit, computational units which are responsible for carrying out information processing are called gates, connected to each other by a network of inputs and outputs. The output processing of gates are based on the present inputs available at that moment and don’t have any memory about the past. The size and the depth of the circuit are the standards to measure the complexity of any circuit. Boolean circuit can be categorized in three types depending on the inputs and outputs associated:

- Unbounded fan-in Boolean circuit: No limitation to the numbers of inputs to both AND-OR gates.
Semi-unbounded fan-in Boolean circuit: AND gate is limited to maximum of two inputs and no limitation to the numbers of inputs to OR gate.

Bounded fan-in Boolean circuit: Both AND and OR gates can have maximum of two inputs. In this paper a Bounded fan-in Boolean circuit is proposed.

i. **AND gate simulation**: AND gate evaluates 1 if all of its inputs are true otherwise it gives output as 0. The functionality of AND gate can be considered as serial connection. In this paper theoretic realization of AND gate is demonstrated at molecular level by regulating H⁺ and Cu²⁺ (CuCl₂) content. The entire process can be represented in the form of an algorithm (illustrated in Fig 3):

```
AND_operator (main_strand, T₁)
{
  T₁•! T₁ U main_strand;
  if (X₁ = 0)
    no titration of H⁺ to T₁; // no change in the structure of main_strand
    if (X₂ = 0 || X₂ = 1)
      No Cu²⁺ is added; // no change in the i-motif structure
      Output = 0;
  else
    if (X₁ = 1)
      Titrate H⁺ to T₁; // main_strand change to i-motif structure
      if (X₂ = 1)
        add Cu²⁺ to T₁; // i-motif structure change to hairpin structure;
        Output = 1;
      else
        don’t add Cu²⁺ to T₁; // No change in the i-motif structure;
        Output = 0;
    }
}
```

Fig. 3. Algorithm for AND gate

In the above algorithm the main_strand is the DNA strand (5’-TAA-CCC-TAA-CCC-TAA-CCC-3’) upon which all structural changes are observed, depending on the inputs (either H⁺ or Cu²⁺ or both). T₁ is the test tube where the reactions are carried out. The output is read as 1 when the structure of main_strand attain the hairpin shape which happens only when both the inputs are present and in any other case the structure of main_strand remains in either linear strand or in i-motif structure which is read as output 0 (illustrated in Fig 4.).

![Fig. 4. Demonstration of AND gate.](image-url)
Table 1: Two input AND gate

<table>
<thead>
<tr>
<th>X1</th>
<th>H⁺</th>
<th>0</th>
<th>0</th>
<th>1</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>X2</td>
<td>Cu²⁺</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Output</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**OR gate simulation**: OR gate evaluates to 1 if any of its inputs are true otherwise as 0. The functionality of OR gate is like parallel connection. Unlike AND gate where H⁺ and Cu²⁺ acts as inputs, OR gate simulation is realized regulating only H⁺ in the test tube. The entire process can be represented in the form of an algorithm (illustrated in Fig 5):

```
ORgate_operation (main_strand, T₁)
{
    T₁ = T₁ ∨ main_strand;
    if (X₁==1 || X₂==1 || X₁==1&& X₂==1)
        Titrate H⁺ to T₁;//main_strand change to i-motif structure
        Output=1;
    Else
        No H⁺ is titrated to T₁;// no change to main_strand
        Output=0;
}
```

**Fig 5. Algorithm for OR gate**

Main_strand is a C-rich strand having potential to attain i-motif structure (5'-TAA-CCC-TAA-CCC-TAA-CCC-TAA-CCC-3'). When H⁺ is added to a test tube containing main_strand the pH of the solution changed to slight acidic and hence i-motif structure is obtained. The output is read as 1 in case of this molecular OR gate when the structure of main_strand attains the i-motif structure, which happens when either of the input is present (illustrated in Fig 6.).

Table 2: Two input OR gate

<table>
<thead>
<tr>
<th>X1</th>
<th>H⁺</th>
<th>0</th>
<th>0</th>
<th>1</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₂</td>
<td>H⁺</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Output</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**iii. Boolean Circuit Evaluation**: Fig 7. illustrated a diagram of a three leveled Boolean circuit with level 0 consists of inputs, level 1 consists of intermediate gates (AND and OR) and level 2 has AND gate. Any Boolean circuit can be visualized as a directed acyclic graph with all the gates as nodes connected to each other in such a way that the output of one level serves as input to the higher level. The algorithm to emulate a Boolean circuit is shown in Fig 8.

**Fig 6. Demonstration of OR gate.**

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Boolean_operation (main_strand,T,i)
for j=1 to j=level_max
  for k=1 to k=gate_max
    if (gk==AND)
      AND_operation(main_strand,T,i);
    if (gk==OR)
      ORgate_operation(main_strand,T,i);
  END for
END for

Fig 7. Instance of a AND-OR Boolean circuit.

As the time incurred for each cycle of switching is only a few 100 seconds [25], it can be concluded that the proposed logic gate has fast response time. Similarly the model is reusable as several cycles of switching are possible for same strand of DNA as the linear strand can be restored every time by controlling the amount and concentration of pH, Cu^2+ or EDTA in the solution.

The model exploits the potential of three different structural outputs (linear, i-motif, hairpin) on the basis of pH condition and cationic concentration in the solution which provide a new insight to design DNA logic gate and Boolean circuits.

Conclusion
In this paper a new gate design strategy is proposed to simulate AND and OR gate individually as well as to evaluate any AND-OR Boolean circuit by controlling H^+ and/or Cu^2+. The conformational structural change of C-rich DNA strand to i-motif structure and hairpin structure is utilized throughout the operations. The advantage of such chemically induced gates lies in its fast response time, controllability and reusability feature. Also the model offers a great potential in designing several nano machines as three structural outputs are possible using same strand of DNA. However this model lacks the feature of full automation and parallelism.

References


