A new DNA computing model for the NAND gate based on induced hairpin formation

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Received 2 April 2003; received in revised form 22 April 2004; accepted 22 April 2004

Abstract

Hairpin structure of DNA molecules has been widely employed in a variety of biosensors and in nanoscale molecular assembly applications. For example, the well known molecular beacons can report the presence of specific nucleic acids in homogeneous solutions with high accuracy. Recently, Smith et al. proposed the induction of hairpin formation through sequence-specific binding of a small-molecule ligand to a G–G mismatch. Not only did this method offer great flexibility in controlling hairpin formation, more importantly the induced hairpin maintains high degree of sensitivity toward specific hybridization. In this contribution, we present a theoretical model for the logical NAND gate based on induced hairpin formation.

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Keywords: DNA computing; Induced hairpin formation; Logical NAND gates

1. Introduction

Since Adleman’s seminal publication (Adleman, 1994), the new emerging field of DNA computing has attracted ever-growing attention from researchers. In an effort to seek potential applications where DNA computers can outperform silicon-based counterparts, Ogihara and Ray (1999) first proposed to simulate Boolean circuits by DNA molecules. The authors claimed the time of the real-time simulation of the class NC is proportional to the depth of the circuit. Amos et al. (1998) described an improved simulation of Boolean circuits that runs in time proportional to the depth of the circuit. Furthermore, they have also described how transitive closure of an n × n matrix could be computed using network of logical gates based on DNA molecules (Dunne et al., 1998). Mulawka et al. (1999) proposed an implementation of the NAND gate through the restriction nuclease Fok I. The major drawback of these methods is that the logical gate will be destroyed by the digestion of enzymes at the end of the computation process. This resulted in lack of reusability for the next process of computation, and thus greatly limited their applications in future DNA computers. In addition, Perez (2002) proposed a logical system based on the deoxyribozyme-based gates. Its implementation seems to be rather complicated and thus needs further experimental verification for large scale computation. In this contribution, we present a theoretical model for the logical NAND gate based on induced hairpin formation.

We will briefly review the Boolean circuit in Section 2, followed by, in Section 3, the introduction...
of hairpin structure and Smith’s idea for the induced hairpin formation through the G-G mismatching. We then describe the construction of the NAND gates in Section 4, and finally we will conclude in Section 5 with a brief discussion and the application of our DNA-based NAND gate.

2. Boolean circuits

Boolean circuits, which represent the Boolean functions, are an important Turing-equivalent model of parallel computation (Harrison, 1965; Dunne, 1998). An n-input bounded fan-in Boolean circuit may be viewed as a directed, acyclic graph, $G$, with two types of node: n-input nodes with in-degree (i.e., input lines) zero, and gate nodes with maximum in-degree two. The directed edge shows the flow of information. Each input node is associated with a unique Boolean variable $x_i$ from the input set $X_n = (x_1, x_2, \ldots, x_n)$. Each gate node, $g_i$, is associated with some Boolean function $f_i \in \Omega$ ($\Omega$ refers to a circuit basis). A complete basis, for example, $\Omega = \{\lor, \land\}$, is the minimal set of functions that are able to express all possible Boolean functions. Traditionally, a normal Boolean circuit is a leveled structure where the logical gates in a level are all same. Any Boolean circuit can be mathematically transferred into the normal form through polynomial steps (Dunne, 1998). A small instance Boolean circuit with four input variables and three logical gates is shown in Fig. 1.

In simulating Boolean circuits, the first step is to select an appropriate complete basis that can be easily implemented by the specific configuration of DNA molecules. Because the NAND function itself provides a complete basis (Amos et al., 1998), the realization of the NAND function yields a far less complicated simulation than using other complete bases. For example, we can implement the AND ($\land$) gate and the OR ($\lor$) gate through NAND gates as follows:

$$x_1 \land x_2 = \text{NAND}(\text{NAND}(x_1, x_1), \text{NAND}(x_2, x_2))$$

$$x_1 \lor x_2 = \text{NAND}(\text{NAND}(x_1, x_1), \text{NAND}(x_2, x_2))$$

The truth table of the NAND gate is shown in Table 1.

3. Induced hairpin formation

A hairpin-loop is a secondary structural motif frequently observed in both single stranded DNA and RNA where a fragment of the sequence is self-complementary. It is generally composed of two parts (shown in Fig. 2): a single stranded loop and a double stranded stem. Its loop may be destroyed by two ways: (1) the stem hybrid can be separated by heating the buffer above its melting temperature or by the addition of urea; (2) if there exists a loop hybrid whose rigidity and length could preclude the simultaneous existence of the stem hybrid, then the
Hairpin-loop will undergo a spontaneous conformational reorganization that forces the stem apart. The second case can be accomplished through elaborately designing the sequences of the loop and stem. Traditionally, the loop part is designed longer than the stem as the GC content in the stem part is higher than that in the hairpin loop. As the hairpin structure presents a higher specific hybridization than a linear probe, it has been widely used in a variety of detection applications, such as sensitive monitoring of polymerase chain reactions, real-time detection of DNA/RNA hybridization in living cells, and in DNA mutation analysis. Experimental results have shown that it can discriminate even a single-base mismatch (Tan et al., 2000).

In normal circumstances, base A always bonds with T and G with C in DNA molecules. But previous researchers (Nakatani et al., 2001a,b,c) have demonstrated that a naphthyridine dimer can specifically bind to a G–G mismatch in double stranded DNA (dsDNA). The binding of naphthyridine dimer to a short dsDNA sequence containing a G–G mismatch lowers its free energy of hybridization and raises its melting temperature. Smith et al. (2002) have successfully induced the formation of hairpin structure by utilizing this property. The DNA monolayer was immobilized on a gold surface, and the stem part contained two –GGG– mismatching segments as follows:

$$5'$$-AAAGGGTTTGGGT-$$3'$$

$$3'$$-TTTGGGAAAGGGA-$$5'$$

Hairpin formation was observed when the surface was exposed to naphthyridine dimer, and its destruction occurred in the absence of the dimmer because of the six G–G mismatches in the stem part. Because the naphthyridine dimer can be added or removed easily over many cycles, this provides a convenient means to control the formation of the hairpin structure. In this contribution, we propose to simulate the NAND gate through induction of hairpin formation.

### 4. Theoretical model of the NAND gate

The simulation process mainly takes place in three phases: First, the logical gate evaluates its unique inputs and presents a specific configuration that indicates its computing result. Specifically, when the output is “1”, the DNA strands representing the gate take on a hairpin structure and otherwise keep linear. Second, the output strands are added only at the end of those strands whose value is “1”. Finally, under the action of endonuclease, only those strands whose value is “1” can output the corresponding output strands added at their 3’ end.

#### 4.1. Encoding requirements on the NAND gate

The strands representing the NAND gate mainly consist of the following: (1) two subsequences $$x_1$$ and $$x_2$$ of length $$l$$ which correspond to the two input variables; (2) two partially complementary sequences of length $$l$$ which contain some G–G mismatches as in (Smith et al., 2002). They will hybrid to form the stem part in the presence of the naphthyridine dimer. Because the gate strands are to be immobilized on a chemically modified surface, a spacer sequence is needed to separate them from the surface (shown in Fig. 3). Additionally, the 3’ end of the gate strands ends with a subsequence $$5'$$-CAA$$3'$$.

The output variable $$z$$ is encoded by a unique sequence of length $$l$$ and its 5’end begins with a subsequence $$5'$$-TTG$$3'$$. This makes it possible to form a recognition site, $$5'$$-CAA$$3'$$ TTG$$3'$$, as the output sequences are added at the 3’ end of the gate strands. Endonuclease Hpa I can be used to cut this recognition site in the middle position as it is double stranded. Finally, parameters of $$l$$ and $$m$$ should make sure that the induced hairpin formation can overcome any loop hybrid of length $$l$$ but fail to prevent the whole loop hybrid of length $$2l$$. As for the design of the loop and the stem, it can be accomplished easily by readily available software.
4.2. Steps to simulate the NAND gate

(1) Immobilizing the 5′ end of the gate strands on a chemically modified surface as shown in Fig. 3. As there are four different input cases for a standard NAND gate, we present four oligonucleotides to represent them in the following Figs. 3–7.

(2) If an input variable $x_i$ ($i = 1, 2$) is “1”, then its complementary strands are added to the surface and they will anneal with the correspondent subsequence of the gate strands. Next the DNA ligase is added so that the nick between the complementary strands of $x_1$ and $x_2$ can be sealed in case both the two input variables are “1” (the first case in Fig. 4).

(3) Adding naphthyridine dimer to the surface. This will induce the hairpin formation for some of the gate strands. As shown in Fig. 5, if the gate’s output is “1” then it will form a hairpin structure, otherwise it remains linear strand.

(4) Washing the surface with distilled water so that the naphthyridine dimer is completely removed. After this the gate strands in hairpin structure will become single stranded while the double strands remain unchanged (shown in Fig. 6).

(5) Adding the output strands $z$ and the complementary strands of $5′$-$x_2$-$z$-$3′$ ($s$ represents the stem sequence at the 3′ end of gate strands) to the surface. Then ligase is added to seal the nick between...
Fig. 8. Exporting the output strands.

the gate strand and the output strand. Now we have added the output strand \( z \) at the end of these gate strands whose output is “1”, while those whose output is “0” remain unchanged because they are doubly stranded (shown in Fig. 7).

(6) Clearing the surface, and adding enzyme \( Hpa \) to the surface so that the output strands \( z \) are cut off from the gate strands (shown in Figure 8). Collecting these strands \( z \) and purifying them prepared for the input of the next gates. Finally, the surface is regenerated by heating and clearing for the next cycle of calculation.

It seems that steps 4–6 are unnecessary for the simulation of the NAND gate. This is indeed true because in step 3 we have gotten the gate’s output by its configuration that can be easily detected by surface plasmon resonance (SPR) imaging measurement. But the point is that we hope to construct Boolean circuit network with such kind of NAND gates. Because there are so many gates at each level, it is impractical to add their outputs one by one, for otherwise the circuit’s parallelism would be greatly reduced.

5. Conclusion

In this paper, we presented a theoretical model of the NAND gate through the induced hairpin formation. As shown above, only six steps were needed to complete the computational process of the NAND gate. Thus the time consumption will increase linearly with the level number of the network, and has nothing to do with the total number of logical gates. As the NAND gate is encoded by just two input variables and the output variable is added only if its output is “1”, this allows the NAND gate to be reusable for repeated cycles of computation. Further more, our model may be expected to perform the computation with higher reliability as the hairpin formation has high specific hybridization ability. However, this speculation needs further experimental verification. Finally, our model can be easily incorporated with the next generation of DNA chip to form a combinatorial computing network. Thus one can speculate the prospects of a real molecular computer constructed by such NAND gates.

However, as the strands representing a specific gate consist of hundreds of the same oligonucleotides at one small spot on the surface, the inter-strand interactions between them may pose serious problem to affect the computing process. In order to minimize the possibility of such interactions, one effective measure is to control the surface density of the attached oligonucleotides. As we know the average length of the four bases of DNA molecules is 0.35 nm per base, it is estimated that the surface density should be approximately 1000 nm\(^2\) per molecule for a length of 45 bp. The surface density can be measured through the surface plasma resonance (SPR) technology.

Acknowledgements

This project is supported by the National Science Foundation of China under the following Grants Nos.: 60373089, 30370356, and 60274026. The authors wish to acknowledge the anonymous referees’ suggestions to improve this paper. Our appreciation extends as well to E.A. Smith for her help in elucidating properties of the induced hairpin formation.

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