

DNA biosensors that reason

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ABSTRACT

Despite the many designs of devices operating with the DNA strand displacement, surprisingly none is explicitly devoted to the implementation of logical deductions. The present article introduces a new model of biosensor device that uses nucleic acid strands to encode simple rules such as “IF DNA_strand_1 is present THEN $disease_A$ ” or “IF DNA_strand_1 AND DNA_strand_2 are present THEN $disease_B$ ”. Taking advantage of the strand displacement operation, our model makes these simple rules interact with input signals (either DNA or any type of RNA) to generate an output signal (in the form of nucleotide strands). This output signal represents a diagnosis, which either can be measured using FRET techniques, cascaded as the input of another logical deduction with different rules, or even be a drug that is administered in response to a set of symptoms. The encoding introduces an implicit error cancellation mechanism, which increases the system scalability enabling longer inference cascades with a bounded and controllable signal–noise relation. It also allows the same rule to be used in forward inference or backward inference, providing the option of validly outputting negated propositions (e.g. “diagnosis A excluded”). The models presented in this paper can be used to implement smart logical DNA devices that perform genetic diagnosis in vitro.

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1. Introduction

Competitive hybridization between DNA strands is the foundation of a process called strand displacement. This method is used in biomolecular computation to implement computing operations (Reif and Sahu, 2009; Takahashi et al., 2006; Rinaudo et al., 2007; Macdonald et al., 2008; Smolke, 2009; Soloveichik et al., 2008; Cardelli, 2009; Cockroft and Ghadiri, 2007; Wasiewicz et al., 2001; Zhang et al., 2007; Seelig et al., 2006). In short, it can be defined as follows: a strand A displaces another strand B from a complex $A'B$, due to the higher affinity between A and A' and the greater stability of the duplex AA' .

Despite the many designs of DNA devices operating with strand displacement, surprisingly none is explicitly devoted to the implementation of logical deductions, which, in mathematics and logic, is called inference: given a set of facts (e.g. “ DNA_strand_1 AND DNA_strand_2 are present”) and a set of implication rules (e.g. “IF DNA_strand_1 AND DNA_strand_2 are present THEN $disease_A$ ”), the inference process derives new facts as conclusions of the original set of implications and facts (the conclusion is “ $disease_A$ ”).

Our interest in performing inference with DNA strands is mainly inspired by the previous work of Ran et al. (2009). They developed an autonomous molecular system that, encoding facts and rules as

DNA strands and using restriction enzymes as an inference engine, was able to perform simple logical deductions. Our goal is the same, but using different biological hardware. Instead of using restriction enzymes, we take the DNA strand displacement as the main biological operation. With this motivation, we already presented an initial version (Rodríguez-Patón et al., 2010) implementing logical inference with DNA strands using strand displacement.

2. Materials and methods

2.1. Previous work

Biomolecular computation is a discipline that merges computer science and biotechnology, using biomolecules as information processing substrate. Leonard Adleman's seminal work (Adleman, 1994) emerged as the proof of concept of this new discipline. Adleman was the first to solve difficult computing problems, such as the Hamiltonian path problem (finding a path in a graph that visits each vertex exactly once), taking advantage of the parallel processing capabilities of recombinant DNA. Subsequently Richard Lipton was equally successful at tackling another difficult mathematical problem (Lipton, 1995), using the same set of basic DNA operations: synthesis, amplification, append, extraction, detection and polymerization. By applying this set of operations on a population of DNA strands, encoding all the potential solutions of the problem, they were able to filter out only the right ones.

During the early years of this discipline, the problems approached were mainly combinatorial, but, in 2006, there

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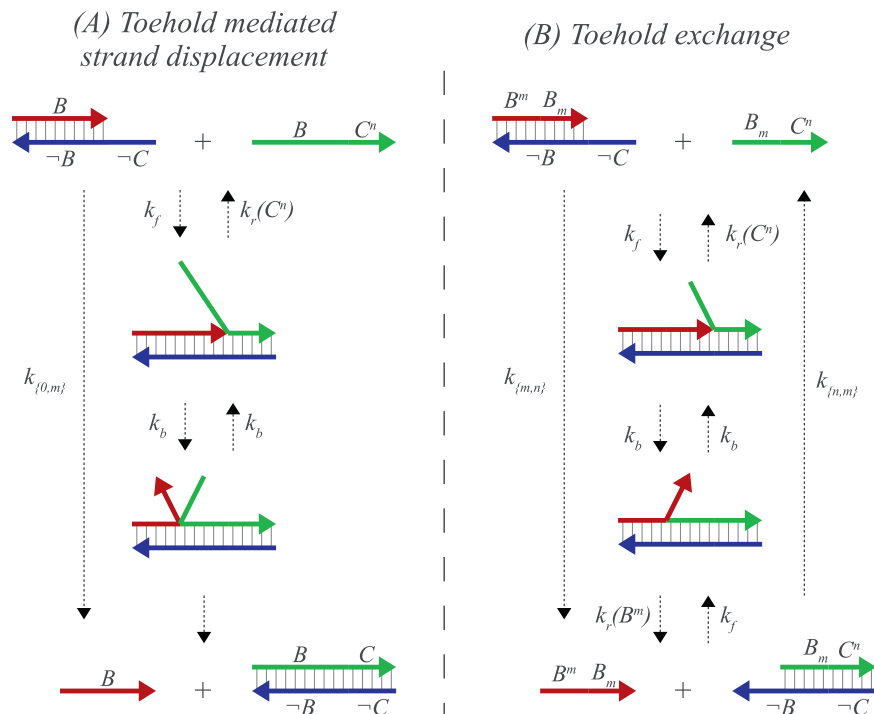


Fig. 1. Toehold mediated strand displacement and toehold exchange. Capital letters represent nucleotide domains, and the negation symbol $\neg$ denotes the Watson–Crick complement of the domain (if P is a generic nucleotide domain, $\neg P$ represents its Watson–Crick complement). The use of superscripts and subscripts within a domain P denotes the 5' and 3' portions of the domain, respectively. A superscript value of m indicates the 5'-most m bases of the full domain, whereas a subscript value of m indicates all but the 5'-most m bases of the domain. Thus the concatenation of P^m and P_m form the full domain P for all values of m . (A) *Toehold mediated strand displacement*. Two molecules are illustrated at the top of the figure: (1) a double stranded complex, with the domain B as the upper strand and the concatenation of domains $\neg B$ and $\neg C$ as the lower strand, and (2) a single strand concatenating the domains B and C . As the domains C and $\neg C$ are complementary, they bind to each other with a forward hybridization rate k_f ; the length of the toehold C , n , is short enough to consider this reaction can be reversed with a dissociation rate $k_r(C^n)$, n being the length of domain C . After C and $\neg C$ are bound, the resulting molecule starts a process called *branch migration*, where the two domains with nucleotide sequence B compete to bind to domain $\neg B$ in the lower strand, moving the border between both upper strands left and right. After some point, the strand B will be released from the complex, letting BC and $\neg B - \neg C$ form a double stranded complex whose length is long enough to consider the reaction irreversible. (B) *Toehold exchange*. This method is similar to toehold mediated strand displacement in that a single strand binds by a toehold to a double strand to initiate a branch migration. However, they differ in the length of the initial single strand (invading strand), which is shorter than its counterpart on side A of this figure. In consequence, after the toeholds C and $\neg C$ bind to each other reversibly, the branch migration movements are not enough to achieve a complete displacement of strand B : the incumbent toehold, B^m , is still bound to the lower strand of the complex. The process will finish when B^m spontaneously dissociates from the complex at rate $k_r(B^m)$. Contrary to (A), this last step is reversible since the new double stranded complex has a new toehold, which can bind again to B^m with a hybridization rate k_f . The *bimolecular reaction model (BM)* (Zhang et al., 2007) of toehold exchange approximates the constant rate of the overall reactions depicted by the long arrows at the sides in (B), obviating the need to calculate all the intermediate reaction rates (k_f , $k_r(B^m)$, $k_r(C^n)$, ...). It is denoted as $k_{[m,n]}$, meaning that the lengths of invading toehold (m) and incumbent toehold (n) are all that is needed for its calculation. It can also be applied in toehold mediated strand displacement reactions, assuming an incumbent toehold of length zero (see side A of the figure).

emerged another research line in DNA computing based on the competitive hybridization of DNA: the DNA strand displacement operation. Since then, several contributions have been made in this direction, like, for example, the design of logic gates (Seelig et al., 2006; Cockroft and Ghadiri, 2007), general purpose catalytic gates (Qian and Winfree, 2009, 2011a,b; Qian et al., 2011), DNA automata (Takahashi et al., 2006), as well as theoretical models (Cardelli, 2009).

The strand displacement operation can be used with any type of nucleic acid, like, for example, the different RNA molecules involved in the RNA interference process. Research presented in Xie et al. (2010) approached the usage of synthetic RNA-based biosensors that, by means of the strand displacement operation, transduce input mRNA levels into small interfering RNA (siRNA) that suppress the translation of specific target RNA molecules.

There are some precedents on the use of DNA molecules to perform logical inference, such as the use of self-assembly and polymerase chain reaction (PCR) to build boolean functions (Wasiewicz et al., 2001) and perform inference (Wasiewicz et al., 2000), the molecular representation of formulas and querying using DNA hairpins (Hagiya et al., 1997; Rose et al., 2006), as well as the implementation of deduction with simple rules (Kobayashi, 1999).

The work presented in Ran et al. (2009) reawakened the interest and potential of applying the logic programming paradigm in

biomolecular computation. Based on the DNA automaton concept developed in previous research (Benenson et al., 2004, 2003, 2001), they built a system capable of performing simple logical deductions with DNA molecules. In that work, propositions and implication rules were encoded using double stranded DNA molecules with a free sticky end; when a proposition and an implication had complementary sticky ends, both molecules merged into one; then the enzyme Fok I would cleave the resultant molecule into two new pieces, different from the original fact and implication molecules; one of these new pieces, merged with an auxiliary DNA strand, would represent the conclusion inferred from fact and implication, which could either be cascaded into another inference process or be read as output using FRET techniques (see Fig. 2).

Motivated by Ran et al. (2009), our group presented a system with similar functional power elsewhere (Rodríguez-Patón et al., 2010). It differed as follows:

- Instead of restriction enzymes, we used the DNA strand displacement operation as a biological engine.
- Whereas Shapiro's model represented the logical value "False" as the absence of DNA strands, our model encoded it using the Watson–Crick complementary strand of the corresponding

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