Implicit-OR tiling of deoxyribozymes: Construction of molecular scale OR, NAND, and four-input logic gates*

MILAN N. STOJANOVIĆ¹,², DRAGAN B. NIKIĆ¹ and DARKO STEFANOVIĆ³

¹Division of Experimental Therapeutics and Clinical Pharmacology, Department of Medicine, Columbia University, Box 84, 630 W 168th Street, New York, NY 10032, USA and ²Department of Computer Science, University of New Mexico, Albuquerque, New Mexico 87131, USA

(Received 12 November 2002)

Abstract: We recently reported the first complete set of molecular-scale logic gates based on deoxyribozymes. Here we report how we tile these logic gates and construct new logic elements: OR, NAND, and the first element with four inputs (i₁∧i₂)∨(i₃∧i₄). Tiling of logic gates was achieved through a common substrate used for core deoxyribozyme; degradation of this substrate defines the output. This kind of connection between logic gates is an implicit-OR tiling, because it suffices that one component of the network is active for the whole network to give an output of 1.

Keywords: molecular-scale logic elements, deoxyribozymes, allosteric control.

INTRODUCTION

We recently reported a complete set of molecular scale logic gates¹ based on nucleic acid catalysts.² These gates have oligonucleotides as both inputs and outputs and they were constructed by modular design,³ combining stem-loop controlling elements of molecular beacons⁴ and deoxyribozymes (DNA-based nucleic acid catalysts.)⁵ The concordance of inputs and outputs of these gates allows tiling of gates in solution¹ and potentially performing calculation of arbitraty Boolean formulae. In our initial report we presented an example of tiling two ANDNOT (also known as NOTIF or ONLY) gates into an XOR system. We now present following results: (1) tiling of two detector (YES) gates into an OR system; (2) tiling of two NOT gates into a NAND system; (3) tiling of two AND gates into the first ever reported four-input system (i₁∧i₂)∨(i₃∧i₄). The tiling is accomplished around a common substrate, i.e., the gates operate in parallel, preserving single-layer disjunctive normal form. This type of tiling is called implicit OR tiling, because activation of either constituent gate is sufficient to render the system active. Furthermore, with regard to activity of individual gates as inputs, a truth table describing the relationship between inputs and output corresponds to the truth table of an OR gate.

* Dedicated to Professor Miroslav J. Gašić on the occasion of his 70th birthday.
# Correspondence: mns18@columbia.edu.
A YES gate (Fig. 1, YESi1 and YESi2), initially reported as catalytic molecular beacons,4 consists of a single stem-loop which inhibits the catalytic cleavage of substrates. Binding of the complementary input oligonucleotide to the loop region opens the stem and releases the substrate recognition region for binding with substrate and initiates the cleavage reaction. Thus, YES gates behave as sensors for the presence or the absence of oligonucleotides, with cleavage products as outputs. For convenient read-out of the output we introduced a fluorogenic substrate to follow deoxyribozyme reactions.4 In this substrate a fluorophore (fluorescein, $\lambda_{em} = 520$ nm, $\lambda_{ex} = 480$ nm) is efficiently quenched by a “dark” quencher without any emission of its own (Black Hole 1, BH1). Cleavage of

Fig. 1. Two YES gates (YESi1 and YESi2) tiled around common substrate (S) to yield OR system. Input oligonucleotides are complementary to loops (bold fonts). Presence of input oligonucleotides opens up the stem loop and allows substrate recognition process to complete. Consequent cleavage of substrate results in the increase of fluorescence (larger bold F).

A YES gate (Fig. 1, YESi1 and YESi2), initially reported as catalytic molecular beacons,4 consists of a single stem-loop which inhibits the catalytic cleavage of substrates. Binding of the complementary input oligonucleotide to the loop region opens the stem and releases the substrate recognition region for binding with substrate and initiates the cleavage reaction. Thus, YES gates behave as sensors for the presence or the absence of oligonucleotides, with cleavage products as outputs. For convenient read-out of the output we introduced a fluorogenic substrate S to follow deoxyribozyme reactions.4 In this substrate a fluorophore (fluorescein, $\lambda_{em} = 520$ nm, $\lambda_{ex} = 480$ nm) is efficiently quenched by a “dark” quencher without any emission of its own (Black Hole 1, BH1). Cleavage of

Fig. 2. Fluorescence changes (in relative units FU) over time for i1 OR i2 (250 nM total concentrations of both components) gate in the presence of (from bottom to top): no inputs, i1, i2, both inputs.
substrate separates the fluorophore from the quencher, resulting in several-fold increase in fluorescence.

In this work, we tiled two YES gates with different inputs around a common substrate. This arrangement will produce a system that yields an output (cleavage product) if either of two YES gates is activated. Such a system would represent a quintessential implicit OR tiling, and it performs an OR function with either of two inputs producing an active-form deoxyribozyme, triggering the cleavage of the substrate and formation of the cleavage product. Figure 1 shows the structures of two gates tiled around the common substrate, and a schematic representation of the OR truth tables. The first gate YES i1 is activated by the input oligonucleotide i1 and does not sense the presence of second input oligonucleotide i2. The second gate YES i2 has exactly the opposite be-

Fig. 3. Two NOT gates (NOTi3 and NOTi4) tiled around common substrate (S) to yield i3ORi4 system. Input oligonucleotides are complementary to loops (bold fonts). Presence of input oligonucleotides opens up the stem loop and destroys the catalytic core. Consequent cleavage of substrate results in the increase of fluorescence (larger F).

Fig. 4. Fluorescence changes (in relative units) over time for i3NAND i4 (250 nM total concentrations of both components) gate in the presence of (from top to bottom): no inputs, i3, i4, both inputs.

Fig. 4. Fluorescence changes (in relative units) over time for i3NAND i4 (250 nM total concentrations of both components) gate in the presence of (from top to bottom): no inputs, i3, i4, both inputs.
behavior, i.e., it is inert in the presence of $i_1$ and reports the presence of $i_2$. Combined in solution these two gates show real-time fluorescent changes (Fig. 2) consistent with performing molecular-scale $a \lor b$ calculation: fluorescence increases rapidly in the presence of one or both inputs, while fluorescence is unchanged without inputs. Finally, the increase in fluorescence is fastest in the presence of both inputs, as the concentration of active deoxyribozyme species is the highest.

NOT gates (e.g., NOT$i_3$ in Fig. 3) have a stem-loop attached to the catalytic core. Recognition of oligonucleotide input complementary to the stem opens up a loop, distorting the catalytic core and rendering the deoxyribozyme inactive.$^1$ Two NOT gates (NOT$i_3$ and NOT$i_4$ in Fig. 3) can be tiled to share a common substrate, analogously to two YES gates. This implicit OR tiling leads to an active gate unless both inhibitory substrates are present. Presence of only one inhibitory oligonucleotide inhibits only one of the constituent gates, leaving the other one active. The two gates acting in unison perform a molecular-scale $\neg(a \land b)$ Boolean calculation and the whole system behaves as a NAND gate, with a truth table given in Fig. 3. Interestingly, our ability to tile two NOT gate in implicit OR fashion (i.e., $\neg a \lor \neg b$) into NAND gate (i.e., $\neg(a \land b)$) is a remarkable demonstration of the validity of DeMorgan’s laws on the molecular scale. In Figure 4b we present the actual experiment, in which changes in the presence of all combinations of inputs support NAND behavior.

AND gates require presence of two input oligonucleotides to be fully active. They are constructed by adding one inhibitory stem-loop at the 5’ end and a second inhibitory stem-loop at the 3’ end of the deoxyribozyme. The length of stem-loops can be adjusted to reduce the background cleavage reaction that leads to imperfect digital behavior, i.e.,

---

Fig. 5. Two AND gates ($i_1$AND$i_5$ and $i_2$AND$i_6$) tiled around common substrate ($S$) to yield $(i_1 \land i_5) \lor (i_2 \land i_6)$ system. Input oligonucleotides are complementary to loops (bold fonts). This system is active only when one or both of the constituent gates senses both input oligonucleotides.
cleavage in the presence of only one input oligonucleotide. Two AND gates could be tiled together around a common substrate to achieve the first-ever reported molecular element with four inputs. We provide here an example of structure-optimized gates $i_1\text{AND}i_5$ and $i_2\text{AND}i_6$ (Fig. 5), which we tile in the $(i_1\land i_5)\lor(i_2\land i_6)$ system that is active if any of the constituent AND gates is active, i.e., matched inputs $(i_1, i_5)$ or $(i_1, i_6)$ must be present pairwise (Fig. 6). Using the same principles, we could now construct alternative systems, in which any combination of two inputs would active fluorogenic cleavage (not shown). For example, systems which would be active if any two or more out of four oligonucleotides is present could be as easily defined through the implicit-OR connection of six AND gates into: $(i_1\land i_2)\lor(i_1\land i_3)\lor(i_1\land i_4)\lor(i_2\land i_3)\lor(i_2\land i_4)\lor(i_3\land i_4)$.

In conclusion, we demonstrated that implicit-OR tiling of individual gates around a common substrate is a valuable tool in constructing systems that perform Boolean calculations in solution. Some of our Boolean formulae are of unprecedented complexity in molecular-scale computations. We are now addressing remaining issues in our approach to perform Boolean calculation of arbitrary complexity with molecular-scale logic gates in solution, including intergate communication.

**EXPERIMENTAL**

All oligonucleotides were synthesized and PAGE purified by IDT DNA (Iowa, USA) and were used as received. Fluorescence measurements were performed on Perkin-Elmer Victor 2 plate reader and each well contained a solution of gates producing at total concentrations of 250 nM, fluorogenic substrate at 2.5 μM concentrations and 20 mM Mg$^{2+}$ ions in HEPES buffer (pH 7.4, 1 M NaCl). Corresponding inputs (or buffer for blanks) were added at a concentration of 2.5 μM to each well and measurement was started immediately.

**Acknowledgments:** We gratefully acknowledge the support of our research efforts by the NSF (EIA-0218262 to MNS and DS), NASA (NAS2-02039 to MNS) and NIH (NIBIB, RO1 EB000675-1 to MNS).
ИЗВОД

ИМПЛИЦИТНО-ОР СЛАГАЊЕ ДЕОКСИРИБОЗИМА: КОНСТРУКЦИЈА МОЛЕКУЛСКИХ ЛОГИЧКИХ КОЛА OR, NAND И ЕЛЕМЕНТА СА ЧЕТИРИ УЛАЗА

МИЛАН Н. СТОЈАНОВИЋ, ДРАГАН Б. НИКИЋ И ДАРКО СТЕФАНОВИЋ

Na grupa je nedavno konstruisala prvi kompletan skup logičkih kola od deoksiribozima (deoksiribozima). U ovom radu mi kombinujemo (slagamo) ova logička kola i konstruisemo nove elemente: OR, NAND i prvi element sa četiri ulaza (i1 ∨ i2)∧ (i3 ∨ i4). Kombinovanje logičkih kola smo postigli time što pojedinačni enzimi dele supstrat, čija degradacija definiše izlaz kola. Ovakvo slagaњe enzima u rastvoru nazivamo implicitno OR slagaњe, jer je dovoljno da makar jedan konstitutivni enzim budu aktivni, pa da celo kolu da izlaz 1.

(Примљено 12. новембра 2002)

REFERENCES

6. This number varies with the structure of substrate from 2–30.