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

MILAN N. STOJANOVIĆ[#, a], DRAGAN B. NIKIĆ[a] and DARKO STEFANOVIĆ[b]

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

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 $(i_1 \wedge i_5) \vee (i_2 \wedge i_6)$. 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 componenet 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 gates[1] 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 beacons[4] and deoxyribozymes (DNA-based nucleic acid catalysts.)[5] The concordance of inputs and outputs of these gates allows tiling of gates in solution[1] 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 $(i_1 \wedge i_2) \vee (i_3 \wedge i_4)$. 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.

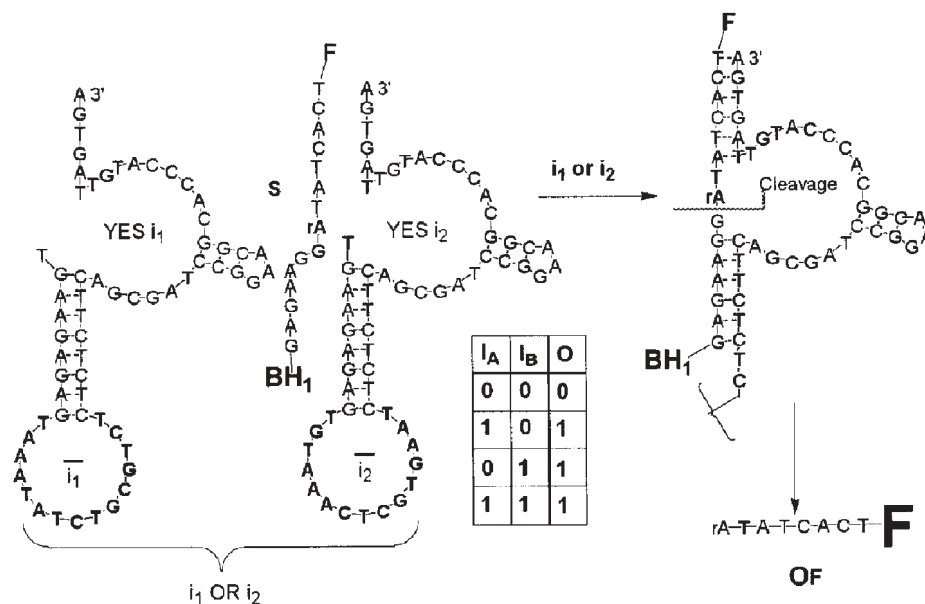[#]   Correspondence: mns18@columbia.edu.

Fig. 1. Two YES gates (YES i_1 and YES i_2) 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, YESi_1 and YESi_2), initially reported as catalytic molecular bea-cons,[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 cleav-age 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 sub-strate a fluorophore (fluorescein, **F**, $\lambda_{em} = 520$ nm, $\lambda_{ex} = 480$ nm) is efficiently quenched by a "dark" quencher without any emission of its own (Black Hole 1, BH$_1$). Cleavage of
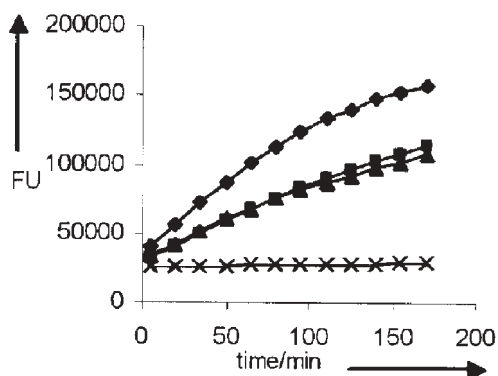

Fig. 2. Fluorescence changes (in relative units FU) over time for i_1 OR i_2 (250 nM total concentrations of both components) gate in the presence of (from bottom to top): no inputs, i_1, i_2, both inputs.
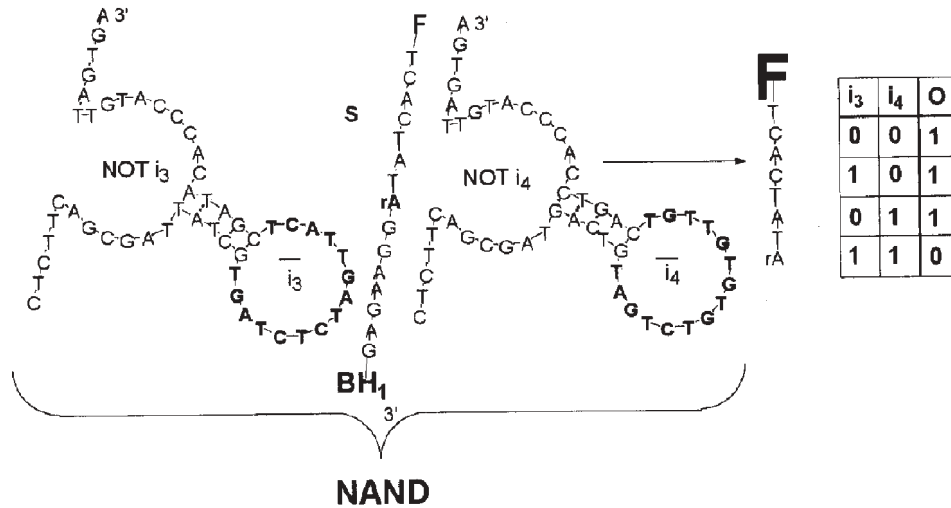
Fig. 3. Two NOT gates (NOTi$_3$ and NOTi$_4$) tiled around common substrate (S) to yield i$_3$ORi$_4$ system. Input oligonucleotides are complementary to loops (bold fonts). Presence of input oligonucleotides opens up the stem loop and destroys the catalytic core. Consequent cleavage of substrate results in the increase of fluorescence (larger F).

substrate separates the fluorophore from the quencher, resulting in several-fold[6] 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 i$_1$ is activated by the input oligonucleotide i$_1$ and does not sense the presence of second input oligonucleotide i$_2$. The second gate YES i$_2$ has exactly the opposite be-
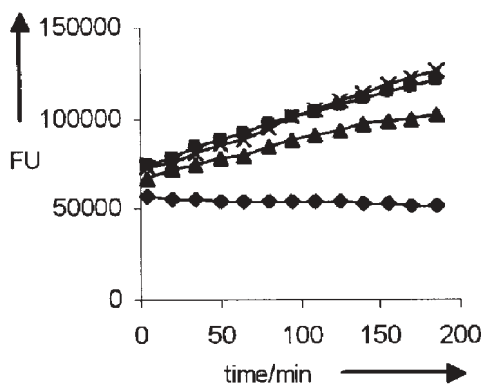

Fig. 4. Fluorescence changes (in relative units) over time for i$_3$NAND i$_4$ (250 nM total concentrations of both components) gate in the presence of (from top to bottom): no inputs, i$_3$, i$_4$, both inputs.
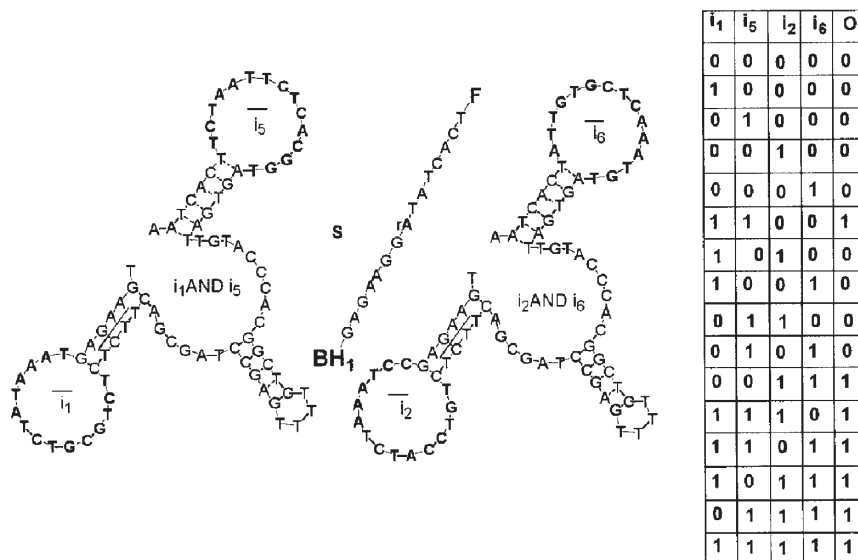
324 STOJANOVIĆ, NIKIĆ and STEFANOVIĆ

i₁	i₅	i₂	i₆	O
0	0	0	0	0
1	0	0	0	0
0	1	0	0	0
0	0	1	0	0
0	0	0	1	0
1	1	0	0	1
1	0	1	0	0
1	0	0	1	0
0	1	1	0	0
0	1	0	1	0
0	0	1	1	1
1	1	1	0	1
1	1	0	1	1
1	0	1	1	1
0	1	1	1	1
1	1	1	1	1

Fig. 5. Two AND gates (i_1ANDi_5 and i_2ANDi_6) tiled around common substrate (**S**) to yield $(i_1 \wedge i_5) \vee (i_2 \wedge i_6)$ 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.

havior, *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 $a \vee b$ 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.*, NOTi_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 (NOTi_3 and NOTi_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 $\neg(a \wedge b)$ 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.*, $\neg a \vee \neg b$) into NAND gate (*i.e.*, $\neg(a \wedge b)$) 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.*,
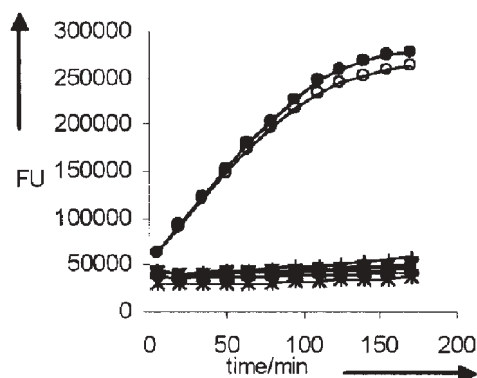
Fig. 6. Fluorescent changes of $(i_1{\wedge}i_5){\vee}(i_2{\wedge}i_6)$ system in the presence of inputs (from top to bottom): (i_1,i_5), (i_2,i_6), (i_1,i_6), $(i_1,i_2),i_1,i_5,i_2,i_6$. Increase is seen only when two matched inputs are present that activate any one of the constituent gates.

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_1ANDi_5 and i_2ANDi_6 (Fig. 5), which we tile in the $(i_1{\wedge}i_5){\vee}(i_2{\wedge}i_6)$ 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: $(i_1{\wedge}i_2){\vee}(i_1{\wedge}i_3){\vee}(i_1{\wedge}i_4){\vee}(i_2{\wedge}i_3){\vee}(i_2{\wedge}i_4){\vee}(i_3{\wedge}i_4)$.

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.

И З В О Д

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

МИЛАН Н. СТОЈАНОВИЋ[a], ДРАГАН Б. НИКИЋ[a] и ДАРКО СТЕФАНОВИЋ[б]

[a]*Division of Experimental Therapeutics and Clinical Pharmacology, Department of Medicine, Columbia University, Box 84, 630W 168th Street, New York, NY 10032, USA u* [б]*Department of Computer Science, University of New Mexico, Albuquerque, New Mexico 87131, USA*

Наша група је недавнао конструисала први комплетан скуп логичких кола од део-ксинуклеотидних ензима (деоксирибозима). У овом раду ми комбинујемо (слажемо) ова логичка кола и конструишемо нове елементе: OR, NAND и први елемент са четири улаза $(i_1 \wedge i_5) \vee (i_2 \wedge i_6)$. Комбиновање логичких кола смо постигли тиме што појединачни ензими деле супстрат, чија деградација дефинише излаз кола. Овакво слагање ензима у раствору називамо имплицитно OR слагање, јер је довољно да макар један конститутиван ензим буде активан, па да цело коло да излаз 1.

(Примљено 12. новембра 2002)

REFERENCES

1. P. Ball, *Nature* **406** (2000) 118
2. M. N. Stojanović, T. E. Mitchell, D. Stefanović, *J. Am. Chem. Soc.* **124** (2002) 3555
3. G. M. Emilsson, R. R. Breaker, *Cell. Mol. Life. Sci.* **59** (2002) 596
4. M. N. Stojanović, P. de Prada, D. W. Landry, *ChemBioChem.* **2** (2001) 411
5. R. R. Breaker, G. F. Joyce, *Chem. Biol.* **2** (1995) 655
6. This number varies with the structure of substrate from 2–30.