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Deoxyribozyme-Based Logic Gates

Milan N. Stojanovic,*,† Tiffany Elizabeth Mitchell,† and Darko Stefanovic‡,§

Contribution from the Division of Clinical Pharmacology and Experimental Therapeutics, Department of Medicine, Columbia University, New York, and Department of Computer Science, University of New Mexico, Albuquerque, New Mexico

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Abstract: We report herein a set of deoxyribozyme-based logic gates capable of generating any Boolean function. We construct basic NOT and AND gates, followed by the more complex XOR gate. These gates were constructed through a modular design that combines molecular beacon stem-loops with hammerhead-type deoxyribozymes. Importantly, as the gates have oligonucleotides as both inputs and output, they open the possibility of communication between various computation elements in solution. The operation of these gates is conveniently connected to a fluorescent readout.

Introduction

We are interested in the development of molecular-scale computational elements[1] as crucial components of multifunctional molecular platforms that can convert specific recognition of multiple molecular disease markers to intervention at the cellular level. Our long-term goal is to construct macromolecular systems able to enter specific cell types and therein sense multiple molecular markers of diseases. Ensuing signals could be analyzed to result in a simple binary output, for example, cell death or cell survival.

We identified oligonucleotides[2,3] as candidates for platform components for the following reasons: (1) various selection and amplification procedures can rapidly generate specific sensitive oligonucleotide-based recognition elements ("aptamers") against protein disease signatures;[4,5] (2) short aptamers can be selected to recognize and, consequently, home-in on cellular surface markers;[6] (3) significant knowledge regarding the stability and intracellular delivery of oligonucleotides has been acquired in development of antisense therapeutics[7] and gene delivery; (4) recognition elements based on oligonucleotides[8] or small molecules[9] can be modularly attached to the catalytic nucleic acids to yield aptazymes or allozymes that act as sensors using product oligonucleotides (modified through cleavage or ligation) as outputs and small molecules[8] or proteins[9,10] as inputs; (5) changes in secondary structures of aptamers can be coupled to recognition[11] of analytes by oligonucleotides with a concurrent potential for triggering drug delivery.[12]

To construct an integrated macromolecular platform, its elements must be able to communicate to each other without macroscopic interfaces. It has been recognized that the primary obstacle to development of practical applications of molecular-

* To whom correspondence should be addressed. Box 84, Department of Medicine, Columbia University, 630W 168th Street, New York, NY 10032. E-mail: mns18@columbia.edu. Telephone: (212) 305-1152. Fax: (212) 305-3475.

§ To whom correspondence should be addressed. Department of Computer Science, Farris Engineering Center #157, University of New Mexico, Albuquerque, NM 87131. E-mail: darko@cs.unm.edu.

‡ University of New Mexico.

† Columbia University.

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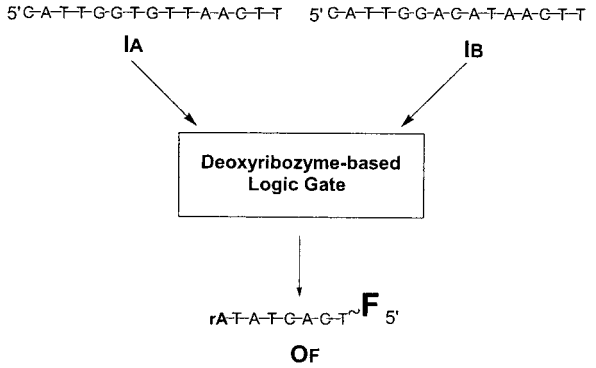

Figure 1. Basic concept: input oligonucleotides $\mathbf{I_A}$ and $\mathbf{I_B}$ result in the presence or absence of output fluorescent product $\mathbf{O_F}$, depending on the interactions with deoxyribozyme-based logic gates

scale computation is the inability to establish communication among the inputs and outputs of individual elements in solution.[1a,13] Our recent demonstration of general allosteric control of deoxyribozymes (DNA-based catalysts[14]), with phosphodiesterase activity by oligonucleotides is important in this context,[15] because the product oligonucleotide (output) of one catalyst could be used as an allosteric effector (input) of another catalyst, thereby allowing communication between various elements of the multifunctional platform without a change in phase. In this report, we expand the principles from our earlier work and describe deoxyribozymes that behave as molecular-scale logic gates,[1a] thus taking the key step toward developing the analytical function of the oligonucleotide-based multifunctional molecular platforms. We define two oligonucleotides $\mathbf{I_A}$ and $\mathbf{I_B}$ as inputs for our logic gates, and a cleaved product oligonucleotide $\mathbf{O_F}$ as an output (Figure 1). Their presence indicates an input/output of 1 and their absence an input/output of 0. Additionally, we connect the catalytic cleavage of substrate **S** to the increase in fluorescence, to facilitate detection of output in homogeneous solution. We present here the basic set of NOT ($\neg$) and AND[16] ($\wedge$) gates, followed by a combination of two deoxyribozymes that behaves as an eXclusive OR[17] ($\underline{\vee}$ or XOR) gate.[18]

Results and Discussion

Background and Design. Deoxyribozymes with various catalytic abilities have been developed with the advent of selection and amplification procedures.[8] For the purpose of demonstrating computational elements based on deoxyribozymes, we choose two previously reported deoxyribozymes named **E6**[19] and **8-17**.[20] Both catalysts cleave the phosphodiester backbone of a chimeric substrate **S** at the site of a single ribonucleotide (rA) embedded in a deoxyribonucleotide framework. The single ribonucleotide was used during the selection process to ensure a defined cleavage site. Importantly, the selection process to generate similar deoxyribozymes is well developed; should the need arise, we can isolate multiple additional deoxyribozymes with different substrates within weeks.

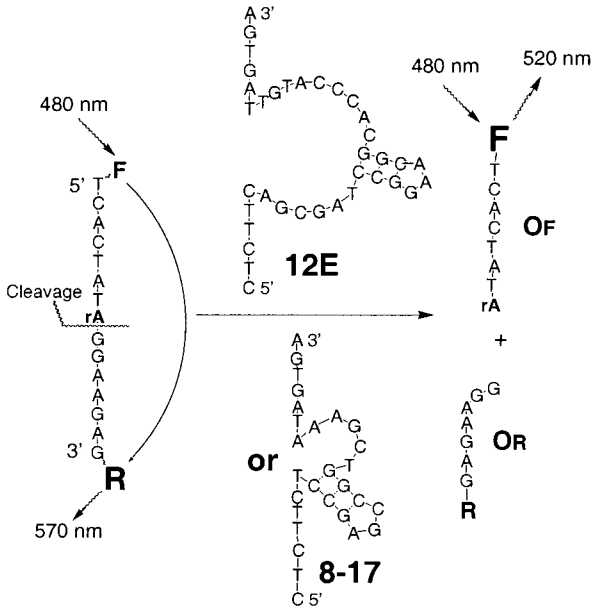

Figure 2. Fluorogenic cleavage of double end-labeled substrate by deoxyribozymes **E6** or **8-17** into products $\mathbf{O_F}$ and $\mathbf{O_R}$. Fluorescein (**F**) emission is quenched by distance-dependent fluorescence resonance energy transfer to tetramethylrhodamine (**R**), and upon cleavage fluorescence increases (larger font **F**).

As demonstrated in previous experiments,[9] when oligonucleotide **S** is double end-labeled with a fluorescein donor (**F**) at the 5′-terminus and a tetramethylrhodamine acceptor (**R**) at the 3′-terminus, cleavage of **S** by deoxyribozymes results in an approximately 10-fold increase[21] in fluorescein emission intensity at 520 nm ($\lambda_{exc} = 480$ nm), as a consequence of separation of donor from the acceptor (Figure 2).

Of the two deoxyribozymes used here, the original **8-17** is more active with a reported turnover of around 1 min^{-1}, in comparison to a 0.04 min^{-1} turnover of the original **E6**.[22] However, the catalytic core of the **8-17** is fixed, and the internal loop (AGC) cannot be replaced with extended sequences. In contrast, the internal loop of **E6** (GAA) can be replaced with an arbitrary sequence.

One of the important characteristics of catalytic oligonucleotides is the ability to design them modularly[8] by combining

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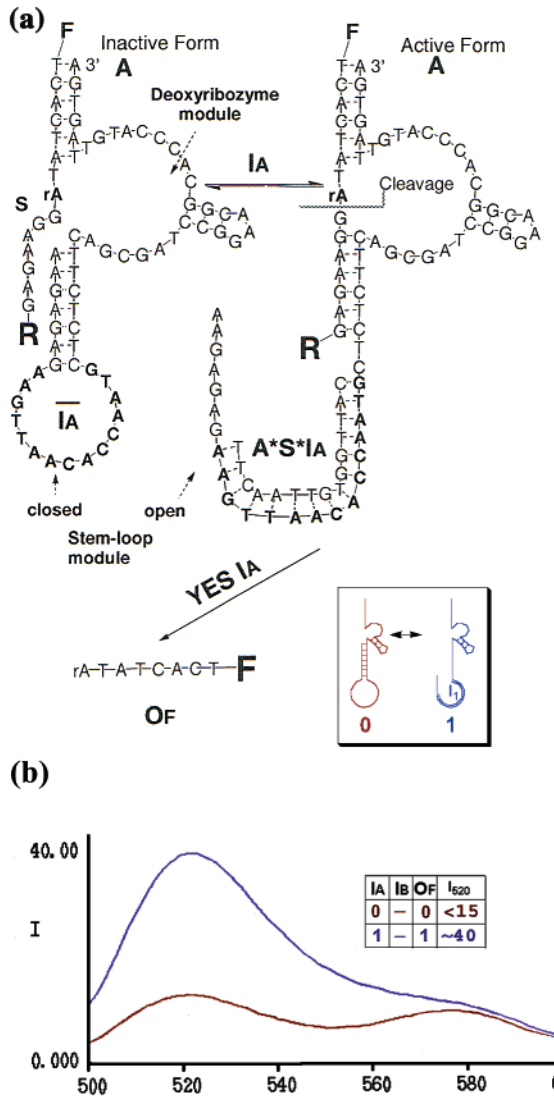

Figure 3. (a) Single input sensor gate (**A**) is activated by the input oligonucleotide $\mathbf{I_A}$. For design principles, please see ref 15a. The other input oligonucleotide $\mathbf{I_B}$ does not activate deoxyribozyme. Insert schematically represents inactive gate with closed loop (output 0, brown) and active gate with open loop (output 1, blue). **(b)** Fluorescence spectra (relative intensity vs emission wavelength, $\lambda_{exc} = 480$ nm, $t = 6$ h) of the solution containing gate, **S**, and either (from top to bottom) $\mathbf{I_A}$ (output 1, blue) or no input oligonucleotide (brown); insert: truth table for YES gate.

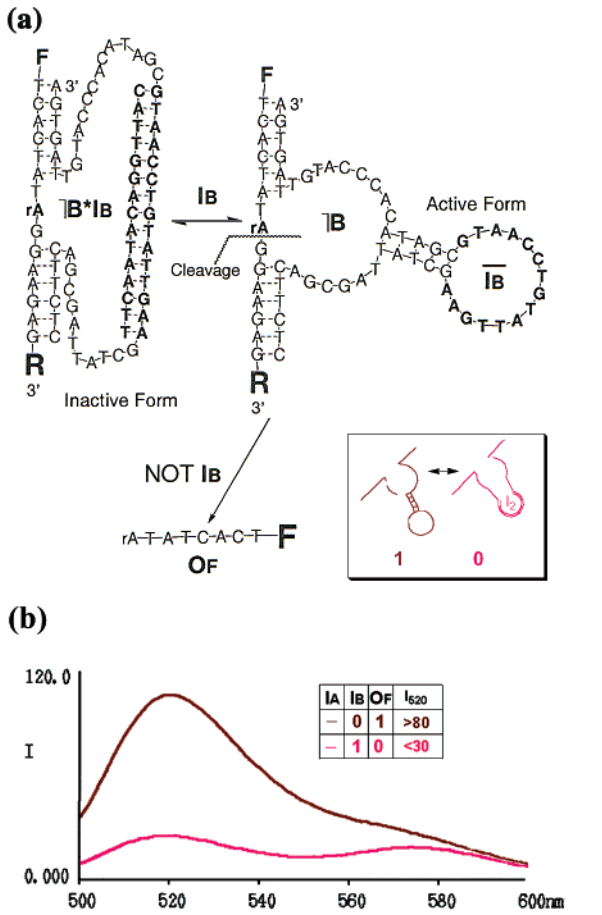

Figure 4. (a) Single-input NOT gate (**¬B**) is constructed through substitution of a nonconserved loop in the deoxyribozyme with beacon stem loop complementary to the input. The deoxyribozyme is inactive in a complex with $\mathbf{I_B}$, while $\mathbf{I_A}$ has only minimal inhibitory influence; insert schematically represents active gate with closed loop (output 1, brown) and inactive gate with open loop (output 0, magenta). **(b)** Fluorescence spectra (relative intensity vs emission wavelength, $\lambda_{exc} = 480$ nm, $t = 12$ h) of the solution containing gate, **S**, and either (from top to bottom): no input oligonucleotides (output 1, brown line) or $\mathbf{I_B}$ (output 0, magenta). (Insert) Truth table for NOT gate.

controlling elements and catalytic regions. Indeed, by applying modular design, we had previously used stem-loop controlling elements (inspired by molecular beacons[23]) to construct deoxyribozymes allosterically promoted by oligonucleotides (i.e., catalytic molecular beacons,[15a] Figure 3). Such stem-loops are closed (self-hybridized) in the absence of oligonucleotide input complementary to the loop region; however, in the presence of input complementary to the loop they undergo stem opening. To build molecular-scale computation elements making use of this design, we again combined stem-loop controlling elements with substrate recognition arms, but we also decided to target the nonconserved loop of **E6** and possibly achieve negative allosteric regulation by oligonucleotides. The attachments of single stem-loops to deoxyribozymes would produce single-input sensor gates, like YES and NOT, while attachments of more than one stem-loop would lead to the dual-input computation elements, like AND and XOR.

Sensor and NOT Gates. We have previously[15a] reported single-input sensor gates (sometimes referred to as YES gates in chemical literature) that directly transduce oligonucleotide input into output (i.e., 1 → 1, 0 → 0). For example, we combined in the gate **A** (Figure 3a) **E6** with a stem-loop (anti-$\mathbf{I_A}$ or $\overline{\mathbf{I_A}}$) complementary to $\mathbf{I_A}$. As detailed elsewhere,[15a] the stem-loop inhibits the catalytic module through overlap of the stem with the 5′-substrate recognition domain of the deoxyribozyme. Hybridization of $\mathbf{I_A}$ to the complementary loop opens the stem, reverses intramolecular competitive inhibition to allow binding of substrate to proceed. A solution containing two sensor gates with different inputs, but the same output oligonucleotide would behave as an implicit OR gate (not shown), which is active when at least one of the two inputs is present.

Single-input NOT gates invert any input data (i.e., 0 → 1, 1 → 0). To perform this function, we introduce herein the deoxyribozyme **¬B** that is inhibited by a specific oligonucleotide input, $\mathbf{I_B}$ (Figure 4a). The NOT gate is constructed by replacing the nonconserved loop of the **E6** catalytic core with a stem-

(23) For traditional molecular beacons, see: Tyagi, S.; Bratu, D. P.; Kramer, F. R. *Nat. Biotechnol.* **1998**, *16*, 49−53 and references therein.

loop sequence complementary to $\mathbf{I_B}$. Hybridization of $\mathbf{I_B}$ with the anti-$\mathbf{I_B}$ opens the required stem structure of the core, distorting its shape and inhibiting its function. Unlike the behavior of YES gate **A**, where a complementary input causes a promoting effect based on the reversal of intramolecular inhibition, an input to $\neg\mathbf{B}$ causes intermolecular inhibition by creating a ternary complex ($\neg\mathbf{B}^*\mathbf{S}^*\mathbf{I_B}$) unable to cleave the substrate.

As observed through changes in fluorescence (Figure 4b) the presence of $\mathbf{I_B}$ is translated into the absence of $\mathbf{O_F}$ and, vice versa, the absence of $\mathbf{I_B}$ yields the presence of $\mathbf{O_F}$. NOT gates are less discriminatory in their interactions with mismatched oligonucleotides, and there is some mild inhibition by a triple mutant $\mathbf{I_A}$[24] (Supporting Information).

Importantly, two NOT gates with different input oligonucleotides and the same output oligonucleotide operating in parallel behave as an implicit NAND gate (not shown), based on DeMorgan's laws: $\neg\mathbf{A} \vee \neg\mathbf{B} = \neg(\mathbf{A}\wedge\mathbf{B})$.

AND Gates. Our next goal was to create an AND gate that independently recognizes two inputs and provides output product only in the presence of both. We relied on the fully modular nature of catalytic molecular beacons, which we previously firmly established,[15a] and attached a controlling element to each end of a single catalyst (**8-17**) to obtain $\mathbf{A}\wedge\mathbf{B}$. In this design, in the absence of its proper input either of the attached stem-loop structures would independently inhibit output formation. As shown in Figure 5a, in the absence of $\mathbf{I_A}$ the 5′-substrate-recognition arm is blocked through an intramolecular hybridization that forms the stem of the anti-$\mathbf{I_A}$ loop; analogously, in the absence of $\mathbf{I_B}$ the 3′-substrate-recognition arm is blocked through intermolecular hybridization with the stem of the anti-$\mathbf{I_B}$ loop. Only upon hybridization of both loops to complements (inputs) will both stems be opened, allowing recognition of **S** and its catalytic cleavage.

In Figure 5b we present fluorescence of a solution of $\mathbf{A}\wedge\mathbf{B}$ and **S** with different combinations of oligonucleotide inputs. Fluorescence emission at 520 nm remains near background level (substrate only) when only $\mathbf{I_A}$ or $\mathbf{I_B}$ is present, increasing only when both inputs are present. Therefore, $\mathbf{A}\wedge\mathbf{B}$ behaves as an AND gate, using oligonucleotides $\mathbf{I_A}$ and $\mathbf{I_B}$ as inputs and providing oligonucleotide $\mathbf{O_F}$ as an output.

XOR Systems. As described above, an implicit OR gate could be constructed from two sensor gates with different inputs, but the same output oligonucleotide and this gate are active when at least one of the two inputs is present. A catalytic XOR (eXclusive OR) gate, however, must be active only when one (and only one) input is present. This is perhaps the most difficult dual-input gate to construct, because under one set of circumstances an input must trigger an output, while under another set of circumstances the very same input must inhibit the same output. To solve this problem, we designed XOR as a two-component system. Two groups of gates would operate in an implicit OR fashion, each group having identical substrates; however, deoxyribozymes of each group would be active in the presence of one input, but inactive upon addition of a second input. Importantly, the same input, which activated one group of deoxyribozymes, would be the deactivating input of the second group, and vice verse.

Accordingly, we combined YES and NOT gates in a single molecule to construct $\mathbf{A}\wedge\neg\mathbf{B}$ (A AND NOT B, Figure 6a). We

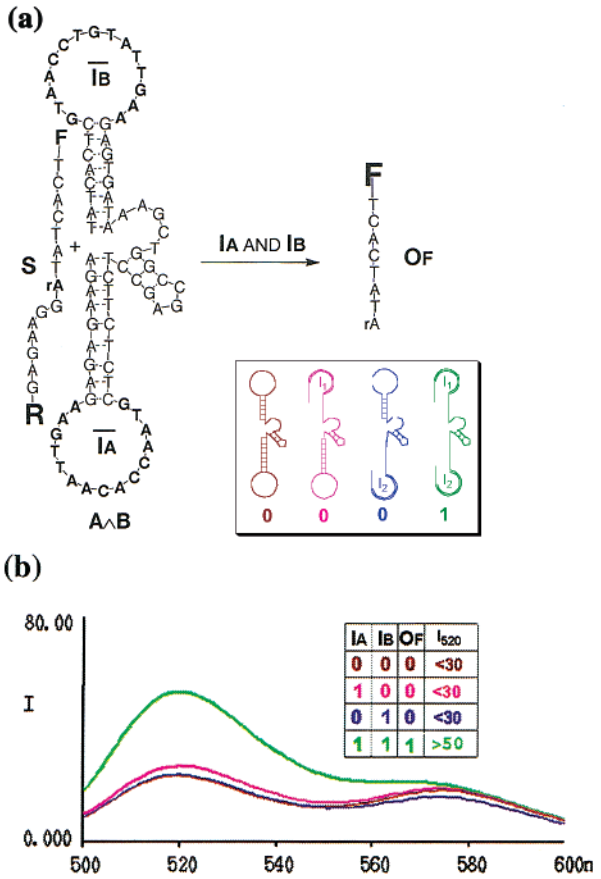

Figure 5. (a) AND gate ($\mathbf{A}\wedge\mathbf{B}$) is constructed through attachment of two loops complementary to input oligonucleotides to the 5′- and 3′-ends of the deoxyribozyme; deoxyribozyme is active only if both inputs are present; insert schematically presents inactive gate (output 0) with closed one (magenta and blue) or both loops (brown) and active gate with both loops open (output 1, green). (b) Fluorescence spectra (relative intensity vs emission wavelength, $\lambda_{exc} = 480$ nm, $t = 4$ h) of the solution containing $\mathbf{A}\wedge\mathbf{B}$, **S**, and (from top to bottom): $\mathbf{I_A}$ and $\mathbf{I_B}$ (output 1, green line), only $\mathbf{I_B}$, only $\mathbf{I_A}$, or no input oligonucleotides. (Insert) Truth table for AND gate.

attached a stem-loop recognizing $\mathbf{I_A}$ to a position at the 5′-end (where it inhibits the catalysis, as in an YES gate) and a stem-loop recognizing $\mathbf{I_B}$ to the internal position of the **E6** catalytic motif (where it does not influence catalysis without an input, as in a NOT gate). Thus, $\mathbf{A}\wedge\neg\mathbf{B}$ is inhibited by the 5′-stem-loop when $\mathbf{I_A}$ is absent, but is also inhibited by an open internal stem in the presence of $\mathbf{I_B}$. This gate is active only in the presence of $\mathbf{I_A}$ and in the absence of $\mathbf{I_B}$, as can be seen in the Figure 6B. We also constructed deoxyribozyme $\mathbf{B}\wedge\neg\mathbf{A}$ (B AND NOT A, not shown separately, please see Figure 7a) in an analogous manner. A stem-loop recognizing $\mathbf{I_B}$ was attached to the 5′-end where it inhibits catalysis, and we placed a stem-loop complementary to $\mathbf{I_A}$ in an internal position of the **E6** catalytic motif. Thus, $\mathbf{B}\wedge\neg\mathbf{A}$ behaves in the opposite manner of $\mathbf{A}\wedge\neg\mathbf{B}$: it is active only when $\mathbf{I_B}$ is present and $\mathbf{I_A}$ is absent.

Present together in solution in an implicit OR arrangement $\mathbf{A}\wedge\neg\mathbf{B}$ and $\mathbf{B}\wedge\neg\mathbf{A}$ behave as a single XOR gate, $\mathbf{A}\underline{\vee}\mathbf{B}$, that uses $\mathbf{I_A}$ and $\mathbf{I_B}$ as inputs and $\mathbf{O_F}$ as an output. As seen in Figure 7b, $\mathbf{A}\underline{\vee}\mathbf{B}$ shows no increase in fluorescence in the absence or in the presence of both inputs, while the presence of only $\mathbf{I_A}$ or $\mathbf{I_B}$ yields an increase in fluorescence.

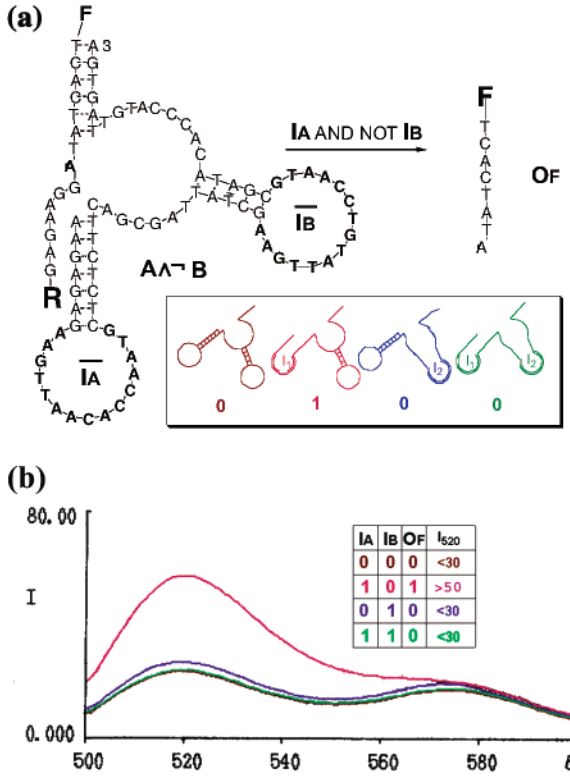

Figure 6. (a) A sensor−inhibitor AND NOT gate (**A**∧¬**B**) is constructed through attachment of two loops complementary to input oligonucleotides, one at the 5′-end, one at the nonconserved loop; catalytic activity in solution is present only if $\mathbf{I_A}$ is present and $\mathbf{I_B}$ is absent; insert schematically represents three inactive states of the gate (outputs 0) with 5′-loop closed (brown) or internal loop open (blue) or both (green) and one active state of the gate (output 1, magenta) with 5′-loop open and internal loop closed. (b) Fluorescence spectra (relative intensity vs emission wavelength, λ_{exc} = 480 nm, t = 12 h) of the solution containing this gate, **S**, and: only $\mathbf{I_A}$ (output 1, magenta), $\mathbf{I_A}$ and $\mathbf{I_B}$ (green), only $\mathbf{I_B}$ (blue), or no input oligonucleotides (brown). (Insert) Truth table for AND NOT gate.

Discussion

Others have reported an AND gate-like operation using nucleic acid catalysts able to sense two small molecules in solution,[25] (or one oligonucleotide and one small molecule[26]). In this work, we demonstrate deoxyribozyme-based logic gates able to analyze input oligonucleotides and operate as NOT, AND, and XOR gates with an oligonucleotide output. Because the set of enzyme-based logic gates described here includes the basis <NOT, AND>, it will suffice to generate any Boolean function, subject only to practical constraints of specific detection and our future ability to serially connect the gates. Consequently, we can now work on implementation of arbitrary binary arithmetic circuits using logic gate representations that are standard in computer engineering.[27] For example, a half-adder takes two bits of input ($\mathbf{I_A}$ and $\mathbf{I_B}$) to produce as outputs a sum digit and a carry digit. Thus a solution containing logic gates described in this report, an XOR gate as the sum digit and an AND gate with a different substrate as the carry digit, would allow the simplest addition (1+1), as has been elegantly described for logic gates based on ion sensors.[28]

The modular design of our gates, demonstrated herein clearly by two deoxyribozymes with switched loops operating in parallel as an XOR gate, points to the generic nature of the constructs; that is, almost any nucleic acid sequences of sufficient length can be now considered for an input. Necessary caveats to such

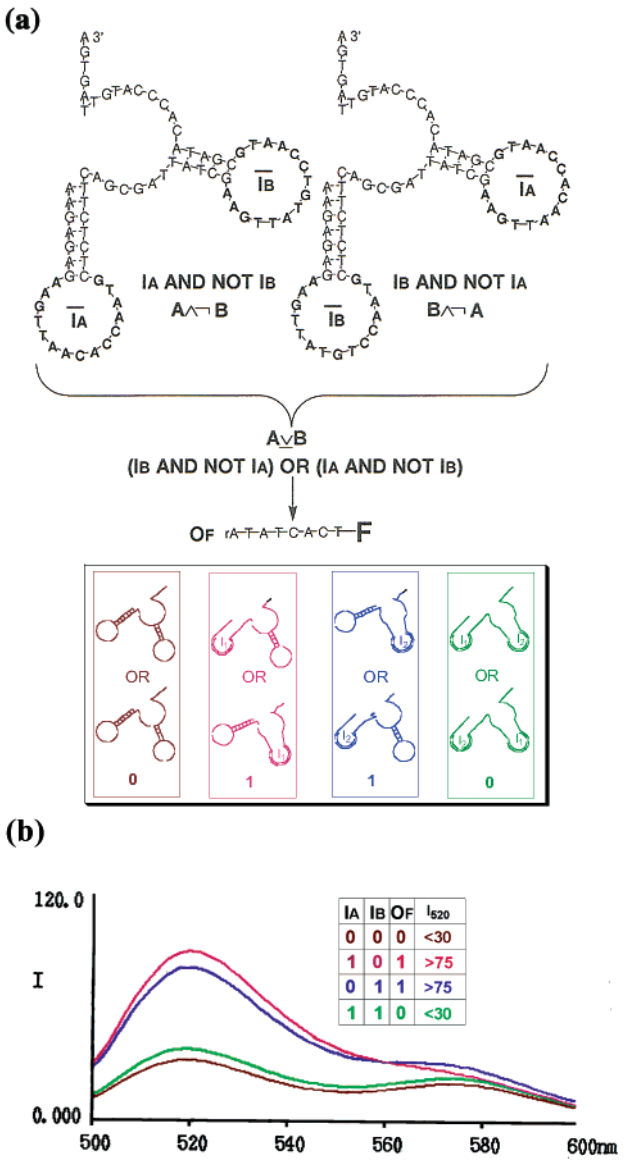

Figure 7. (a) XOR gate (**A**⊻**B**) as a combination of **A**∧¬**B** and **B**∧¬**A** with the same inputs and output; catalytic activity is present in solution if either $\mathbf{I_A}$ or $\mathbf{I_B}$ is present, but not both; insert schematically represents the two active states (outputs 1) of the XOR system, when only one oligonucleotide is present (blue or magenta), and the two inactive states (output 0) with either neither (brown) or both (green) oligonucleotides present. (b) Fluorescence spectra (relative intensity vs emission wavelength, λ_{em} = 520 nm, λ_{exc} = 480 nm, t = 12 h) of the solution containing **A**⊻**B**, **S**, and (from top to bottom): only $\mathbf{I_A}$ (magenta), only $\mathbf{I_B}$ (blue), both (green), or no input oligonucleotides (brown). (Insert) Truth table for XOR gate.

(24) Hamming distance in these oligonucleotide-based computation elements can be defined as number of mismatches that minimizes cross talk between two elements. Thus, at room temperature and high Mg^{2+} concentrations, for 15-mer oligonucleotides the Hamming distance can be realistically set at 3 for YES gates and 4 for NOT gates. For a detailed discussion of Hamming distances in the parallel DNA-based computation, see: (a) Marathe, A.; Condon, A. E.; Corn, R. M. *Dimacs Workshop on DNA Based Computers V*; American Mathematical Society: Providence, RI, June 1999; pp 75−89. (b) Frutos, A. G., Liu, Q., Thiel, A. J.; Sanner, A. M. W.; Condon, A. E.; Smith, L. M.; Corn, R. M. *Nucleic Acids Res.* **1997**, *25*, 4748−4757.

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generality include ensuring that (1) input sequences are not complementary to entities in solution other than their beacon loops, (2) one input oligonucleotide corresponds to a single beacon loop, (3) input oligonucleotides do not form stable secondary structures, and (4) one deoxyribozyme motif cleaves only one substrate motif. Although these conditions limit the maximum number of deoxyribozymes that can operate in parallel in solution, our proposed applications require only a limited number of serial and parallel operations. For example, to streamline the concurrent detection of four molecular disease markers into a single output (e.g., the decision to release a cytotoxic compound) we require only two parallel AND gates (to sense the markers) that are serially connected to a third AND gate. Even for the full adder, we estimate that not more than 20 deoxyribozymes would be needed. In comparison, our preliminary investigations show that tens of thousands of oligonucleotides can be constructed to form a compatible set that satisfies the constraints listed above.[24]

Our gates can be considered fully digital. For example, an **AND** gate in the absence of both activating oligonucleotides is almost indistinguishable by fluorescence spectra from the background cleavage of substrate under our conditions. The interactions of stem-loops (as in molecular beacons) with an excess of complementary oligonucleotides occur rapidly, within one minute;[29] consequently, upon sensing activating inputs, deoxyribozymes that are part of logic gates are immediately ready to proceed with catalytic activity. Despite almost instant activation, in this report we have chosen to perform the analysis of an output after several hours. Such extended incubation periods are the result of our decision to use homogeneous detection and to stress the catalytic nature of the process under multiple substrate turnover conditions.[30] These choices necessitate a large excess of substrate causing a high background fluorescence. Electrophoretic methods of product detection would be able to detect digital activity after several minutes, yet we would loose the simplicity of homogeneous detection. We note that we were able to detect digital behavior in fluorogenic assays after 10 min or less under different conditions.[31]

The multiple turnover conditions are also important since our system is, to the best of our knowledge, the first example of a full set of artificial enzymatic logic gates.[32] The enzymatic nature of our gates ensures that the output fan out will not be the major issue in potential applications where serial connections of deoxyribozymes are needed.

It is clear from our present work that there are two issues that must be addressed before more complex demonstrations of communication between elements and serial connections. First, oligonucleotide products of upstream gates must be sufficiently different from their substrates, to ensure that only products act as inputs for downstream gates. It is relevant in this context that we have successfully constructed stem-loop structures able to act as substrates for deoxyribozymes.[15a] Upon cleavage, these molecules reveal an oligonucleotide stretch previously unavailable for Watson−Crick base pairing. Second, as a consequence of our design, downstream NOT gates would remain active until sufficient inhibitory product has accumulated. This problem, however, also appears in electronic circuits, where synchronization of elements is achieved through clocking function. Similar strategies can be devised for molecular-scale computation elements, and we will address them in our future publications.

We note that our targeted applications do not require reversibility. However, our gates are fully reversible: removal of input oligonucleotides resets them to the initial states. In instances where gates may be attached to surfaces[33] removal of inputs can be achieved by washing; when in solution, input complements could be added, as is standard in state-of-the-art DNA-based machines.[34]

Last, our results provide one possible explanation as to how metabolic control and quorum sensing were organized in early RNA-based organisms,[35] the chemistry of which is postulated to have focused on the production and degradation of various oligonucleotides. For example, networks of AND, NOT, and XOR gates could have been used to monitor the balance of specific oligonucleotide (metabolic) products; accumulation of these above a certain level could have activated or deactivated catabolic pathways.

Conclusions

Conjunction (AND), disjunction (OR), and negation (NOT) are the building blocks of logic; all other operations, no matter how complex, can be obtained by suitable combinations of these. We have successfully constructed a set of molecular-scale logic gates that encompasses these basic functions. The switches are based on deoxyribozymes that use oligonucleotides as both input and output. The design of the control mechanism, based on the conformational changes of stem-loops, can be extended to any nucleic acid catalyst. Almost any group of oligonucleotides can be used to trigger the analytical function of these computation elements and a resultant presence (or absence) of fluorescent oligonucleotide product. We are now formulating communication networks between deoxyribozymes, and integrating recognition and analytical functions with therapeutic effects.

Materials and Methods

Materials. All oligonucleotides were custom-made by Integrated DNA Technologies Inc. (Coralville, IA), and purified by HPLC or PAGE electrophoresis, except 15-mers, $\mathbf{I_A}$ and $\mathbf{I_B}$, which were used crude. Samples were dissolved in RNase- and DNase-free water, separated in aliquots, and frozen at −20 °C until needed. All experiments were performed in autoclaved 50 mM HEPES, 1 M NaCl, pH = 7.5 at room temperature. $MgCl_2$ was obtained from Sigma-Aldrich Co. (St. Louis, MO) and used as 200 mM autoclaved stock solutions in water.

Instrumental. All fluorescent spectra were obtained on a Hitachi Instruments Inc. (San Jose, CA) F-2000 fluorescence spectrophotometer with Hamamatsu xenon lamp. Experiments were performed at excitation wavelength of 480 nm and emission scan at 500−600 nm. Printouts of spectra were scanned and colors manually introduced in Adobe Photoshop 5.5.

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(30) Logic gates have turnover higher than 0.5 per hour (cf. equation in ref 15a).
(31) For an example of $\mathbf{A}\wedge\neg\mathbf{B}$, please see Supporting Information; for other conditions, see refs 15a and 20a.
(32) For an approach to enzymatic logic gates, see: Tuchman, S.; Sideman, S.; Kenig, S.; Lotan, N. *Mol. Electron. Devices* **1994**, *3*, 223−238.
(33) Surface-based approaches to DNA computation are described in our refs 2e,f and 24.
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Procedures. Logic gates (1 μM stock, 10 μL, final concentration 200 nM), oligonucleotides $\mathbf{I_A}$ and $\mathbf{I_B}$ (5 μM stock, 10 μL, final concentration 1 μM), and substrate (30 μM stock, 10 μL, final concentration 6 μM) were mixed in that order. For the "0" input buffer (10 μL) was used instead of $\mathbf{I_A}$ or $\mathbf{I_B}$. Reactions were initiated after 5 min by the addition of Mg^{2+}, (50 mM stock, 10 μL, final concentration 10 mM). After incubation at room-temperature aliquots (5 μL) were diluted to 0.5 mL with HEPES buffer and transferred into a quartz semi-microcuvette for spectrofluorometric analysis.

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Note Added after ASAP: Figures 2, 3a, 4a, 5a, 6a, and 7a were incorrect in the version posted ASAP March 14, 2002; the corrected version was posted April 3. 2002.

Supporting Information Available: 1. Fluorescence spectra for the reaction of $\neg\mathbf{B}$ gate in the presence of $\mathbf{I_A}$, $\mathbf{I_B}$, or both oligonucleotides; 2. R_n values (E_{520}/E_{570}) for the catalytic cleavage by $\mathbf{A}\wedge\neg\mathbf{B}$ in the presence of $\mathbf{I_A}$, $\mathbf{I_B}$, or both oligonucleotides at 10, 30, and 60 min (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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