DNA Logic Gates

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Molecular Logic Gates Connected through DNA Four-Way Junctions**

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Microprocessor systems based on semiconductor logic gates employ electronic input and output signals. Each type of system of electronic gates has a specific input-output signal correlation, which is described by a truth table.[1] A critical feature that contributes to the success of modern electronic circuits is input-output signal homogeneity: that the same voltage value emerging as the output of one gate can be admitted as the input to another gate. This facilitates the building of large arrays of interconnected logic gates that can perform selected functions of varying complexity. The development of even-more-powerful microprocessors depends on the progress in downsizing their components. It has been suggested that building molecular circuits in a bottom-up manner is a promising alternative to the miniaturization of semiconductor microprocessors.[2] Substantial efforts have been undertaken to create synthetic molecules that are capable of performing logic operations.[3] One of the biggest shortcomings of existing approaches is the lack of universal connectivity. For example, some molecular logic gates use chemicals and optical input signals and produce fluorescence as an output.[4] The fluorescent signal can be conveniently detected, but has limited functional value as an input for the downstream molecules. Therefore, only the small-scale integration of such gates have been achieved.[3a] Herein, we report a new design of molecular logic gates that are made of deoxyribooligonucleotides, which promises to solve this connectivity problem.

DNA is considered to be an excellent building block for molecular logic gates.[5,6] However, in most of the reported designs, input/output homogeneity was not preserved:[5] the gates controlled by oligonucleotide inputs generated enzymatic activity,[5a,b] hole transfer,[5d] or fluorescence as output signals.[5c,e,f] One approach for gate communication used DNAzyme-assisted oligonucleotide output ligation[5i] or release.[5j] However, the enzyme-assisted communication suffers from slow response, owing, in part, to low catalytic efficiency of the DNAzymes. At the same time, catalytic action is not required for gate communication. Indeed, enzyme-free DNA logic gates can be connected using strand displacement hybridization:[6] an output oligonucleotide that is displaced from the DNA duplex by an input oligonucleotide serves as an input for a downstream gate. However, such systems produce a response after several hours, even in the case of simple model networks, because several relatively slow strand displacement hybridization events must occur consecutively for signal transduction. Herein, we suggest an alternative approach that uses the association of strands of DNA when the signal is high and their dissociation when the signal is low.

Recently, we introduced a three-component probe for nucleic acids analysis;[7] the probe consists of two triethylene-glycol-modified DNA strands, α and β, a molecular beacon (MB) probe, a fluorophore, and a quencher-conjugated DNA hairpin (Figure 1). These three oligonucleotides co-existed in solution in a dissociated state when a nucleic acid analyte was absent; the MB adopted a stem-loop conformation and the fluorescent signal was low (Figure 1, left). The addition of a DNA analyte led to a cooperative hybridization of the two strands to the analyte and to MB, thus resulting in the formation of a DNA four-way junction (4J) like structure (Figure 1, right). In this complex, the fluorophore and the quencher were remote from each other, which resulted in a high level of fluorescence.[7] The oligoethylene glycol linkers were required to fix a particular conformation of the 4J structure, in which the MB probe adopts an elongated form and thus generates the high fluorescence signal.[7] On the one hand, the probe is a tool for DNA/RNA analysis; on the other hand, it functions as a YES logic unit (diode logic). As a logic unit, it recognizes an oligonucleotide input (DNA analyte) and generates an oligonucleotide output of another sequence, which is composed of the two signal-transmitting arm sequences. This output can be conveniently detected by hybridization with a MB probe or, alternatively, it can be recognized by downstream logic units as an input. In our proof-of-concept study, we used the 4J-based design to create NOT and AND logic units and we demonstrate the feasibility

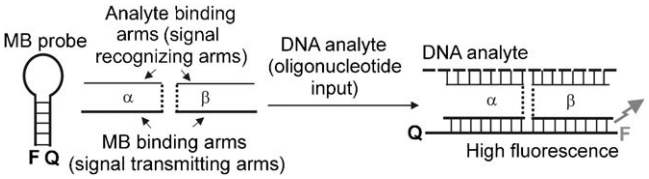

Figure 1. Binary DNA probe for nucleic acid analysis. Triethylene glycol linkers are depicted as dashed lines.

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of an integration between the two gates to produce the ANDNOT logic gate.

The inverter, or NOT gate, switches from a high to low output signal when an input is applied. In our design, the NOT strand hybridized to MB_{NOT} by signal-transmitting arms (Figure 2a). The MB probe was in a fluorescent opened conformation. Indeed, a high signal was observed when a NOT strand was incubated with MB_{NOT} (Figure 2b, curve 1 and Figure 2c, bar 1). The hybridization of an input (I_{NOT}) destroyed the 4J-like motif and released MB_{NOT} from the complex (Figure 2a, right): the fluorescent signal was low (Figure 2b, curve 2). Notably, only a few minutes of incubation were sufficient to register the high fluorescence output. The ratio of the high and low outputs was stable for at least one hour (Figure S1, Supporting Information). The data obtained after polyacrylamide gel electrophoresis (PAGE) of the samples 0, 1, and 2 confirmed this mechanism for the operation of the NOT gate (Figure S2, Supporting Information).

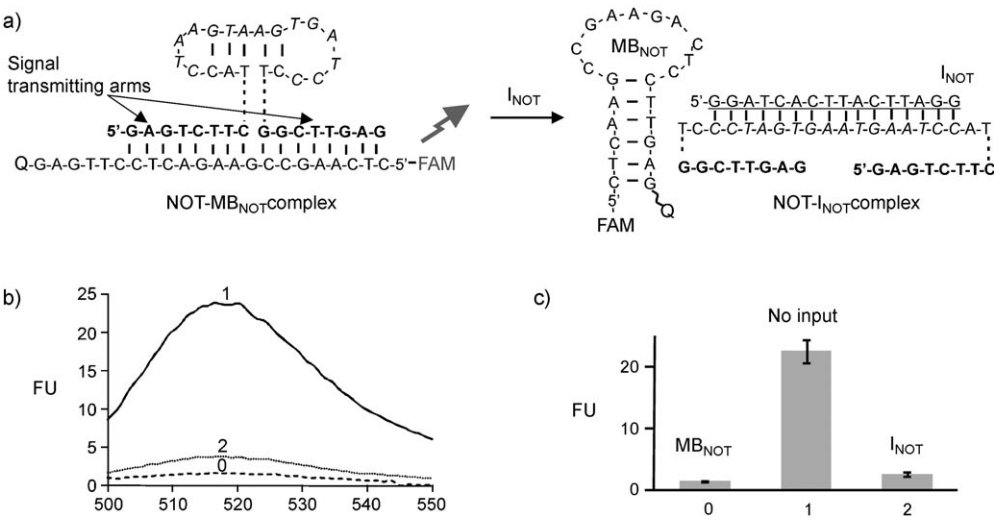

Figure 2. NOT gate. a) Predicted secondary structure of the NOT gate in the absence (left) or presence (right) of the oligonucleotide input I_{NOT}. Triethylene glycol linkers are depicted as dashed lines. FAM and Q are fluorescein and dabcyl groups, respectively. Signal transmitting arms are in bold font. The input recognizing fragment is in italic font. The input sequence is underlined. b) Fluorescence emission spectra of the NOT gate in the absence (1) or presence (2) of the input oligonucleotide; fluorescence background of MB probe (0). c) Fluorescent intensities at 517 nm (average values of three independent experiments) after 15 min of incubation.

The simplest AND logic gate, the two-input AND gate, generates high output only when both inputs are introduced simultaneously (Figure 3b). In our design the AND logic gate consisted of the MB probe, MB_{AND}, and the other three oligonucleotide hairpins, namely AND_a, AND_b, and AND_c (Figure 3a). In the absence of inputs, the hairpins coexisted in solution in the dissociated form. Hybridization of the input oligonucleotides to AND_a and AND_b resulted in opening the communication modules (underlined italic) of these two strands. AND_c hybridized to the communication modules, whilst MB_{AND} was cooperatively bound by the signal-transmitting arms of strands AND_a and AND_b, thus resulting in the formation of a 4J-containing associate, with MB adopting an elongated conformation (Figure 3a, $I1_{AND}$,$I2_{AND}$ complex). The observed fluorescent signal corre-

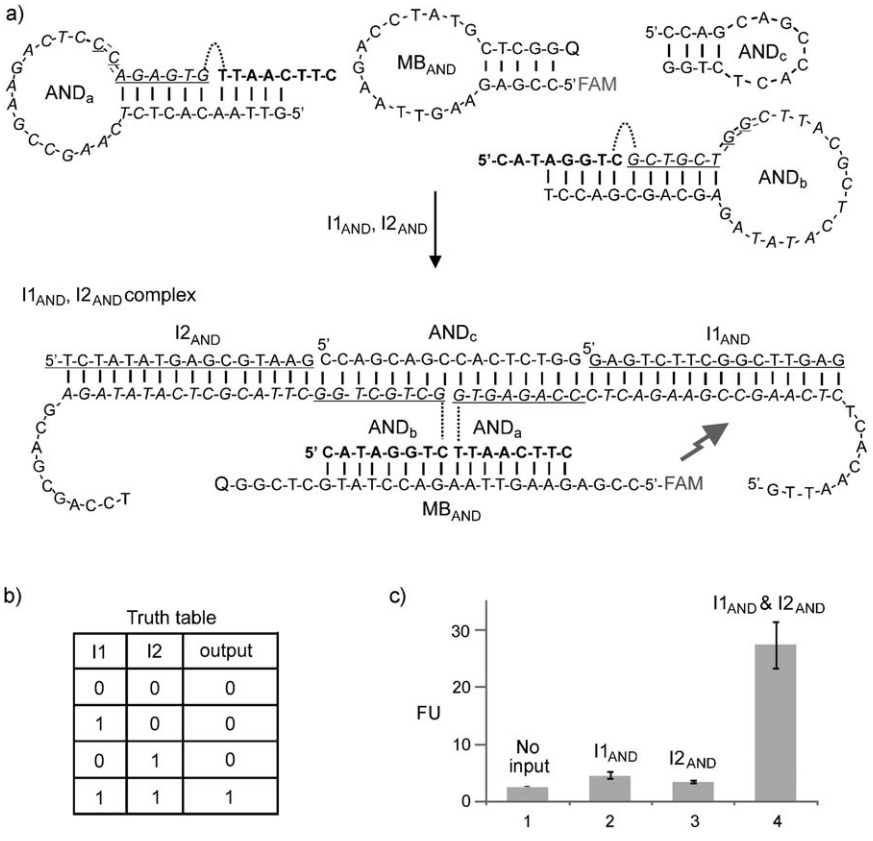

Figure 3. AND gate. a) Predicted secondary structure of the two-input AND gate in the absence (top) or presence (bottom) of both inputs. The signal transmitting arms are in bold font. The input recognizing fragment of AND_a and AND_b strands are in italic font. The input sequences are underlined. The communication fragments of the AND_a and AND_b strands are underlined and in italic font. b) The truth table for the AND gate. c) Relative fluorescence intensities (517 nm) of the AND gate in the presence of various input combinations.

sponded to AND logic: a high fluorescence was detected only when both $I1_{AND}$ and $I2_{AND}$ were added (Figure 3c, sample 4). The formation of a high molecular weight complex in the presence of both inputs was confirmed by native PAGE (Figure S3, Supporting Information).

To demonstrate gate connectivity, we designed an ANDNOT gate by connecting the NOT gate to the AND gate (Figure 4a). The ANDNOT logic gate, an important component of half and full adders, only generates a high output signal in the presence of one of the two inputs, according to the truth table shown in Figure 4b. In our design, the output fragment of the NOT gate was complementary to the input recognition loop of the AND_a strand (Figure 4a). Hybridization of $I2_{AND}$ to AND_b strand triggered cooperative assembling of all seven DNA strands in the $I2_{AND}$ANDNOT complex (Figure 4a). A reporter MB_{AND} was in an open conformation in this DNA associate and the fluorescent signal was high (Figure 4c, bar 3). In the presence of I_{NOT}, which was complementary to the input-recognition region of the NOT gate, the input-bound NOT strand dissociated from the complex, thus causing collapse of the whole structure, which was accompanied by the release of MB_{AND} in solution. PAGE analysis supports this mechanism of ANDNOT gate operation (Figure S4, Supporting Information).

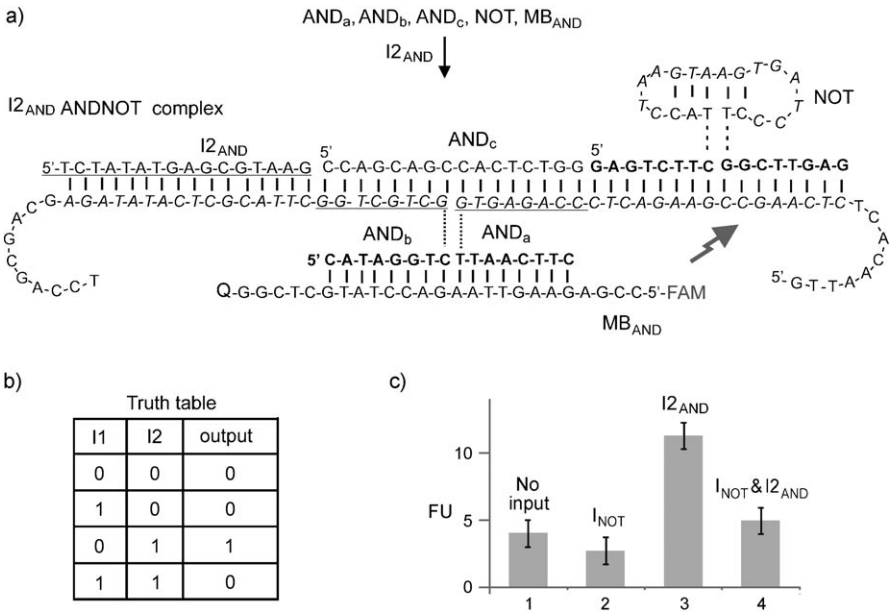

Figure 4. ANDNOT gate. a) Predicted secondary structure of the ANDNOT gate in the presence of $I2_{AND}$ input (high signal output). The signal-transmitting arms are in bold font. The input-recognizing fragment of the AND_a and AND_b strands are in italic font. The input sequences are underlined. The communication fragments of the AND_a and AND_b strands are underlined and in italic font. b) ANDNOT truth table. I1 and I2 in the table correspond to I_{NOT} and $I2_{AND}$ of the designed ANDNOT gate, respectively. c) Fluorescent response of the ANDNOT gate in the presence of various input combinations.

In conclusion, this study introduces a new approach for intermolecular communication between DNA logic gates. Unlike previously studied DNA gates,[5,6] the new design does not require strand displacement hybridization or enzymatic catalysis for output release. Instead, signal transduction is mediated by the association of several DNA strands; this reduces the time for signal transmission. Indeed, the DNA hybridization requires only minutes to be completed.[8] The pivotal elements of this design are the following: 1) Short oligonucleotide fragments function as both inputs and outputs; 2) cooperative action of the two oligonucleotide fragments (signal transmitting arms) is required to communicate a high signal; and 3) transfer of the high signal is mediated by assembling DNA strands in 4J-containing complexes. These are key features that characterize this new approach for intergate communication, the full power of which has yet to be explored.

The chain of the connected logic modules can be easily scaled up in a modular fashion by feeding the output of an upstream gate to the next downstream gate in the chain. Figure 4 demonstrates how the output of the NOT gate (bold) is recognized as an input by the AND gate. The output of the AND gate is reported by the MB probe; however, this output can be recognized by a downstream gate as an input, thus creating a three-level integrated system. The investigation of such multilayer assembling might be the next step in the development of these systems.

Importantly, the DNA four-way junction is a naturally occurring (Holliday junction), well-studied structure.[9] It is used as a building block in a variety of artificial DNA constructs by DNA nanotechnology.[10] One potential advantage of our logic gates is their compatibility with 4J-based constructs that were developed by DNA nanotechnology, such as two-dimensional (2D) DNA lattices made of double-crossover molecules or DNA origami.[10] Incorporation of our logic modules into 2D scaffolds can bring the benefits of higher hybridization rates, a reduced level of undesired background DNA association, and the possibility to transmit a signal over longer distances.

Experimental Section

All oligonucleotides were custom-made by Integrated DNA Technologies, Inc. (Coralville, IA). For the fluorescence assay, oligonucleotides were mixed in a buffer containing 50 mM $MgCl_2$, 10 mM TrisHCl, pH 7.4, at a final concentration of 20 nM for MB probes and 100 nM for all other oligonucleotides. Fluorescent spectra were recorded on a Perkin–Elmer (San Jose, CA) LS-55 Luminescence Spectrometer with a Hamamatsu xenon lamp (excitation at 485 nm; emission 517 nm) after 15 min of incubation at room temperature (22 °C). The data of three independent measurements are presented with an error margin of one standard deviation.

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