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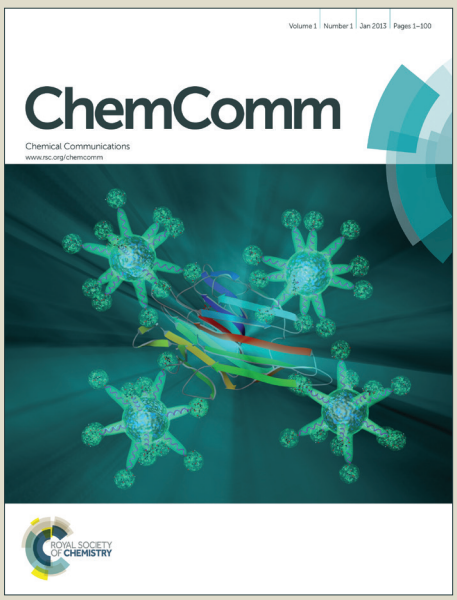

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Divide and Control: Split Design of Multi-Input DNA Logic Gates

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Logic gates made of DNA have received significant attention as biocompatible building blocks for molecular circuits. The majority of DNA logic gates, however, are controlled by the minimum number of inputs: one, two or three. Here we report a strategy to design a multi-input logic gate by splitting a DNA construct.

It is believed that DNA logic circuits capable of processing oligonucleotide inputs can be used as building blocks for biocompatible computational cuircuts.[1] One possible application of such computational nano-devices is the analysis of RNA markers (in blood or tissue) to detect cancerous processes and signal the abnormalities or possibly correct them. Numerous designs of DNA logic gates have been introduced,[2] including recently published examples of gold nanoparticle-associated gates and their delivery into cells,[3] toehold-mediated DNA logic gates based on host-guest DNA-GNPs,[4a] DNA-hydrolysing deoxyribozyme[4b] and four-way junction-driven DNA strand displacement.[5] However, most of the reported designs are limited to logic gates that process one, two or maximum three inputs.[6] At the same time, some practical applications require multi-input logic gates. For example, a 4-bit arithmetic logic unit uses 4- and 5-input AND logic gates.[7a] Moreover, concurrent analysis of multiple biomarkers is required for accurate diagnosis of genetic and infectious diseases.[7b] Here, we report a design principle that enables conversion of a DNA construct responding to 2 inputs into a multi-input logic device.

Figure 1 illustrates the design of one of the first DNA logic gates,[2a] a 2-input AND gate (**2iAND**) based on an RNA-cleaving deoxyribozyme (Dz). The gate represents an inactivated Dz sequence. The inactivation is achieved due to the presence of two stem-loop structures serving as input-recognition modules. The stems block the substrate-binding arms and a part of the Dz catalytic core. Hybridization of input oligonucleotides **I1** and **I2** to the loop fragments of the input-recognition modules of the Dz **2iAND** gate destabilizes the stem structures, thus releasing the substrate-binding arms for binding to a fluorophore- and a quencher–labelled reporter substrate (**F substrate**). The activated Dz cleaves the substrate and separates the fluorophore from the quencher, thus producing high fluorescence output signal. The design of the Dz **2iAND,** as well as other related Dz-based logic gates, was used to build the most advanced and sophisticated systems in molecular computation explored so far. For example, they were used to design tic-tac-toe game by the coordinated action of 23,[8] or 128 gates.[9] Most recently, the gates were used for the design of multi-layer computational cascades[10] and a molecular calculator with a 7-segment digital display.[11] However, no more than 3-input Dz gates have been reported so far, to the best of our knowledge.[6a]

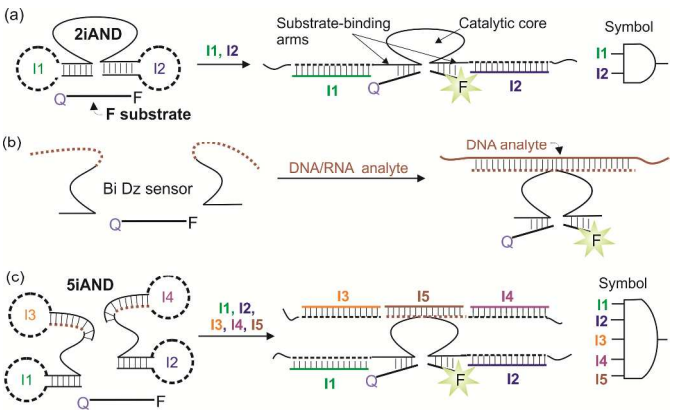

Figure 1: Design of multicomponent AND gates. (a) The two-input deoxyribozyme AND gate (Dz **2iAND**) introduced by Stojanovic and colleagues.[2a] (b) Principle mechanism of binary (split) Dz sensor activated by an oligonucleotide analyte.[13,14] The two parts of Dz sensor bind to the adjacent fragments of a nucleic acid analyte and re-form a catalytic core, which cleaves the reporter substrate. (c) A 5-input AND gate (**5iAND**) designed in this study. Dashed lines indicate input-recognition modules of the gate. Input oligonucleotides **I1**, **I2**, **I3** and **I4** bind to the input-recognition fragments and release the substrate-binding arms (as in panel A) or the **I5**-binding fragment of the split Dz **5iAND**. Input **I5** bridges the two 5iAND strands, thus re-forming the Dz catalytic core (as in panel B).

To increase the number of input-recognition elements in a Dz logic gate we suggest using the binary (split) Dz strategy introduced by us earlier[13] and, independently, by Todd's group,[14] for the detection of RNA and single-stranded DNA analytes (Figure 1b). In this strategy, a Dz core is divided into 2 subunits, and each subunit is then elongated with an analyte-recognition sequence (brown dashed lines in Fig. 1b). Hybridization of a specific DNA or RNA analyte results in re-formation of the catalytic core, which cleaves the substrate followed by fluorescence increase. In this study we used split 10-23 Dz introduced by Mokany et al.[14a]

We combined the Dz gates (Figure 1a) and the binary Dz strategy (Figure 1b) to design a 5-input Dz AND gate (**5iAND**, Figure 1c). For an AND gate, high output is observed only in the presence of all inputs. To design a **5iAND**, five input-recognition domains were needed. Four input-recognition domains were introduced in the loop portions of four stem-loop structures. The Dz substrate-binding arms were caged in the stems of the **I1-** and **I2**-recognition modules (as in the case of **2iAND** gates, Figure 1a). The other two stem-loop structures blocked the binding site for input 5 (**I5**), an oligonucleotide that bridges the two halves of the **5iAND** construct (Figure 1c). Hybridization of inputs **I1** and **I2** to the former two input-recognition modules releases the substrate-binding arms, whereas binding of inputs **I3** and **I4** to the other two input-recognition modules makes the I5-recognition domain available for hybridization with **I5**. Input **I5** bridges the two parts of the **5iAND** and stabilizes the Dz catalytic core similarly to that of a binary Dz sensor (Figure 1b). Therefore, the presence of all five inputs is required to restore the catalytic activity of the Dz construct and ensure high fluorescent signal.

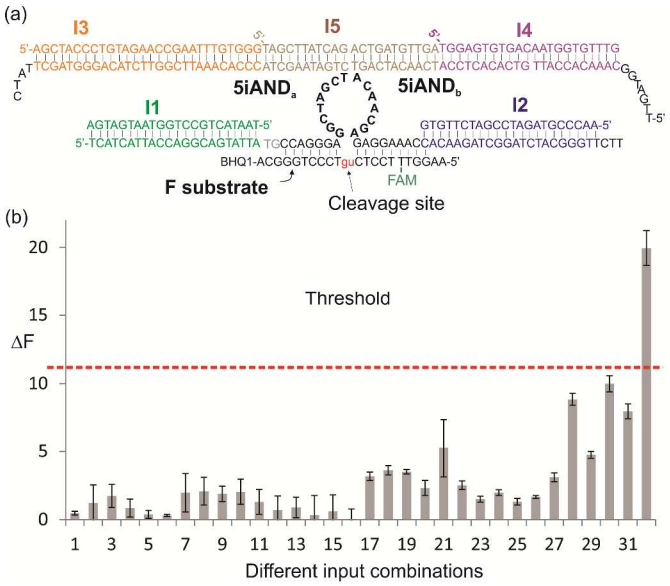

Figure 2: Sequences and performance of the **5iAND** logic gate. (a) Primary and secondary structure of **5iAND** in the presence of all five oligonucleotide inputs (**I1-I5**). (b) Fluorescent response of the gate in the presence of 32 different input combinations after 30 min of incubation. **I1-I5** concentrations were 10 nM. $\Delta F = F - F_0$, where F is the fluorescence of **5iAND** after incubation with different input combinations; F_0 is the fluorescence of F substrate under the same conditions. The average values of three independent experiments with one standard deviation are present. The reaction conditions (temperature, ionic strength, and the concentration of all DNA strands) were optimized.

In this proof-of-concept study, we used the sequences of human microRNAs – miR-10b, miR-122, miR-21, miR-200b and miR-99a – for the design of oligonucleotide inputs (Table S1 in Supporting Materials). Changes in expression levels of these microRNAs have been linked to a number of diseases including cancer, cardiovascular and neurodegenerative diseases.[15] Figure 2a demonstrates the complex of the **5iAND** gate with all five inputs. This complex should cleave **F substrate** and produce high fluorescence. Analysis of fluorescence of samples containing **5iAND** and all possible combinations of the 5 inputs revealed highest fluorescence of the sample containing all 5 inputs (Figure 2b), as expected for a correct digital behavior of 5-input AND gates (Figure S1). Indeed, a threshold fluorescence value shown in Figure 2b with a dashed line clearly separates the high output (presence of all five inputs) from the low outputs (all other input combinations). In this proof-of-concept investigation we optimized the reaction temperature, buffer conditions and the concentrations of all oligonucleotides. Importantly, signal-to-noise ratio (S/N) ~2 was observed after 30 min in the presence of low input concentration (10 nM), and the signal above the background was detected within 10 min (data not shown). Analysis of the samples by native gel electrophoresis revealed that significant amount of high molecular weight associate was formed only in the presence of all 5 inputs (Figure S2). This observation supports the hypothesis of catalytic complex formation (shown in Figure 2a) in the presence of all 5 inputs.

Even though molecular logic gates were first suggested more than a decade ago,[2a] and the proof-of-concept studies for such gates are numerous in the latest years, reports on multi-input molecular gates are rare despite their practical significance. The number of possible input states is 2^n, were n is the number of inputs. Increased number of possible input states brings an advantage of more complex gate behaviour. Indeed, modern electronic processors use 4- and 5-input AND logic gates.[7a] At the same time, designing a multi-input biomolecular logic gate may be challenging. Here we demonstrate an approach to design a multi-input DNA logic unit based on a 2-input logic gate: the DNA construct should be divided in portions, followed by controlling each portion by an additional set of oligonucleotide inputs. It is possible to design 3- and 4-input AND gate from the **5iAND** logic gate reported here simply by removing one or two-input recognition modules. Importantly, by using catalytically efficient 10-23 deoxyribozyme core in this study we achieved gate response in the presence of relatively low input concentrations. Indeed, most DNA logic gates process 100-1000 nM oligonucleotide inputs.[2-8,10] The **5iAND** gate reported here responded to the presence of 10 nM inputs within 10 min. This improved sensitivity of **5iAND** reported in this study may be used in the design of sensors for simultaneous analysis of microRNAs in biological samples in a PCR-free format. We hypothesize that logic gates based on 10-23 Dz controlled by even greater number of inputs can be designed by employing additional splitting strategies. For example, Wang and Sen reported an alternative strategy for splitting 10-23 Dz, at its substrate-binding arm.[16] Combining Wang and Sen's strategy with that reported here may enable design of an AND gate that accepts more than 5 inputs.

Conclusions

A 5-input AND gate was designed using the concept of a 2-input AND gate and the split strategy. We hope that the design reported here finds implications and/or inspires construction of DNA devices for practical applications including analysis of multiple biomarkers by DNA logic gates.

Experimental procedure

All oligonucleotides were synthesized by IDT DNA Technologies. All experiments were conducted in a solution containing 50 mM

HEPES, 50 mM $MgCl_2$, 20 mM KCl, 120 mM NaCl, 0.03% Triton X-100, 1% DMSO at pH 7.4, 200 nM F substrate and 10 nM of all other DNA strands, 30 ^{o}C, 30 min. Fluorescence spectra of the samples were recorded on a PerkinElmer (San Jose, CA) LS-55 luminescence spectrometer with a Hamamatsu xenon lamp (excitation at 485 nm; emission 517 nm). More details of the experimental procedure are given in the Supporting Information.

Notes and references

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Electronic Supplementary Information (ESI) available: [details of experimental procedures, sequences of oligonucleotides used in the study, truth table for a 5-input AND gate, and electrophoretic analysis of the 5iANS gate in the presence of different input combinations]. See DOI: 10.1039/c000000x/

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