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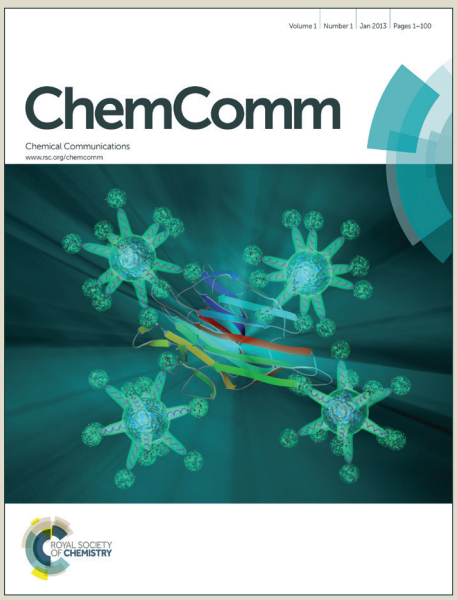

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Nuclease-Containing Media for Resettable Operation of DNA Logic Gates

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We designed and tested a system that allows DNA logic gates to respond multiple times to the addition of oligonucleotide inputs. After producing an output signal, the system spontaneously resets to the background state. This system does not require any operator action to achieve reset of a DNA logic gate, and may become useful for construction of reusable DNA-based computational devices.

Originally introduced by Milan Stojanovic and collegues,[1] DNA logic gates have received ever growing attention.[2] It has been proposed that biocompatible DNA-based computational circuits could be used in biomedical applications.[3] In order to use DNA logic gates for constructing complex biomedical devices, a number of technological challenges must be addressed. These include organization of DNA logic gates in arrays of communicating gates; design of complex DNA circuits; powering the circuits; and achieving resettable operation mode for multiple reuses. Herein we address the last problem by introducing a novel approach to achieve resettable operation of DNA logic gates without the need for external input.

The approach utilizes λ exonuclease (λ exo), an enzyme that hydrolyses 5'phosphorylated strands of DNA duplexes.[4] The enzyme is intensively used in a variety of biomedical assays,[5] and is well suited for our application since, it can remain active for a long time at room temperature. Additionally, λ exo specifically cleaves one strand in a DNA duplex, allowing other DNA components to remain intact during long operation periods. In this proof-of-concept study we optimized λ exo-containing media for the resettable operation of YES and AND logic gates.

The design principle for λ exo-containing media for resettable operation is illustrated in Figure 1 for a YES gate. At the initial state (Fig. 1a), the system consist of λ exo, a molecular beacon probe (**MB1**),[6] DNA strands **YES1** and **YES2** and input complement **I1~~m~~** (input 1 minus). The MB probe is in a stem-loop conformation with low fluorescence, which corresponds to a binary '**0**'. Addition of excess amount of a 5' phosphorylated **I1** input results in concurrent formation of both the signaling YES complex and input-compliment (**I1-I1~~m~~**) duplex (Fig. 1b). The YES complex produces high fluorescence due to the switch of **MB1to the** extended conformation,[7] which corresponds to a binary '**1**'.

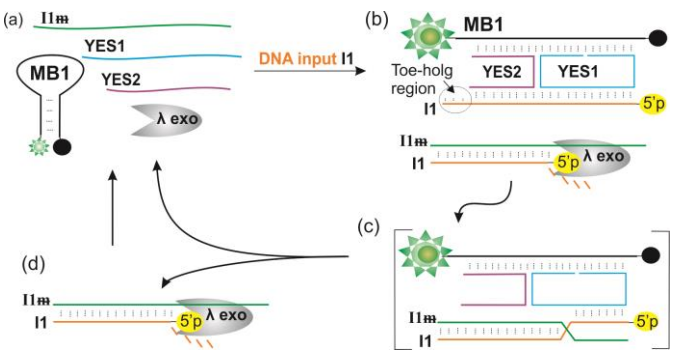

Fig. 1 Reset system for DNA logic gates. (a) Four oligonucleotides: **MB1** probe, **YES1, YES2**, and **I1~~m~~** coexist in solution in the absence of a DNA input. Lambda exonuclease (λ exo) does not cleave the oligonucleotides. (b) Addition of a 5'-end phosphorylated input **I1** leads to its association with other strands in the two complexes: fluorescent quadripartite YES complex and **I1-I1~~m~~** duplex. The DNA input **I1** is cleaved by λ exo in the duplex **I1-I1~~m~~**. The toe-hold region of input **I1** is circled. (c) **I1~~m~~** binds and removes **I1** from the quadripartite complex. (d) **I1** in the **I1-I1~~m~~** complex is cleaved by λ exo, which resets the system back to the original state. The sequences of the DNA strands are shown in Supporting Information (SI).

The 5'-phosphorylated input bound to the complement is digested by λ exo, freeing **I1~~m~~** compliment to bind another molecule

of **I1** in solution. Upon complete digestion of the **I1** strands unbound to the YES complex, **I1~~m~~** displaces **I1** from the YES complex (Fig. 1c), causing **MB1** to reform a hairpin thus abrogating the fluorescent output and resetting the system to binary '**0**' state. The **I1** strand in **I1~~m~~-I1** duplex is again digested by λ exo. In this study we applied this approach for YES and AND logic gates and demonstrated 10 and 5 rounds of operation, respectively.

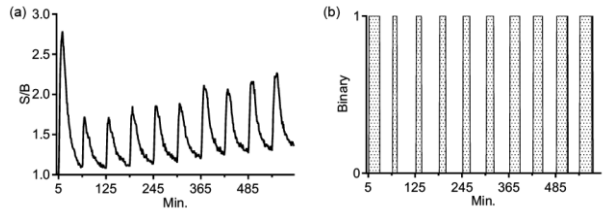

Fig. 2 Multiple response of the YES DNA logic gate. (a) Fluorescent response of the YES logic gate. Signal to background ratio (S/B) calculated from the control sample lacking the input. After 5 min incubation, the input was added every 60 min. (5, 65, 125, 185, 245, 305, 365, 425, 485, and 545 min). DNA strands **MB1** probe, **YES1**, **YES2**, and **I1~~m~~** were incubated in the buffer (50 mM HEPES pH 7.4, 50 mM $MgCl_2$, 20 mM KCl,120 mM NaCl,0.03% Triton X-100, 1% DMSO) containing 75 units of λ exo at 27°C. (b) Digital response of YES logic gate. S/B data was transformed to digital response using the IF function in Microsoft Excel. If the S/B ratio was greater 1.5, the binary value of 1 was assigned; if the S/B ratio was less than 1.5, the binary value was 0. The data represent one experiment, for replicate trials see SI.

In our resettable system, the YES gate had a cycle time of 60 min, and we measured the gate operation for up to ten cycles (Fig. 2a). Upon addition of input, the fluorescence increased immediately and reached its maximum in 5 min, followed by spontaneous decrease within next 60 min. The response of the YES gate was transformed to binary '**0**' and '**1**' by considering any signal above the threshold of 1.5 corresponding to binary '**1**', while signal below the threshold corresponding to binary '**0**' (Fig. 2b). The performance of the YES gate was reproducible (Fig. S2). The formation of YES complex and its degradation during the operational round were confirmed by analysis of the YES gate samples using polyacrylamide gel electrophoresis (Fig. S3).

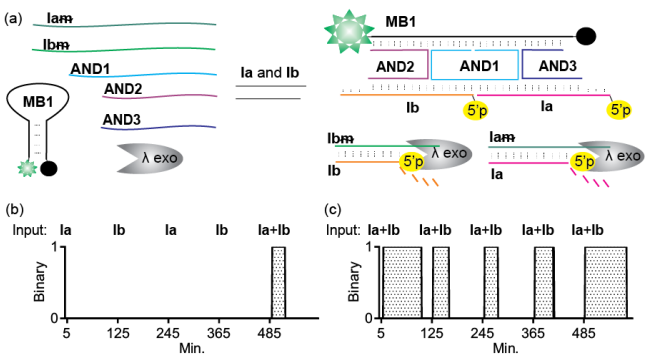

Fig. 3 Resettable AND gate. (a) Design of the AND gate (see Fig. S5 for more details). (b) Digital response of the AND gate in the presence or one or both inputs. (c) Five operational rounds of the AND gate upon addition of both inputs. S/B data was transformed to the digital response using the IF function in Microsoft Excel. If the S/B ratio was greater than 2, the value of 1 was assigned. However, if the S/B ratio was less than 2, the value was 0. (see Fig. S6 for original fluorescent results and multiple repeats).

In order to demonstrate the applicability of the approach for more complex functional constructs, we designed a two-input AND logic gate (Fig. 3). According to AND logic, two inputs are required to generate high output. In the presence of the two oligonucleotide inputs **Ia** and **Ib,** strands **AND1**, **AND2** and **AND3** hybridize to MB probe and to the input strands and form a DX tile complex.[8] In this complex the MB probe is in an elongated confirmation, thus producing high output signal. Importantly, individual inputs are unable to stabilize the DX complex, producing lower fluorescence. Indeed, the AND gate demonstrated correct digital response producing high fluorescent signal only in the presence of both input **Ia** and **Ib** (Fig. 3b)**.** Upon addition of both inputs the fluorescent signal increased to its maximum value within 10 min (Fig. 3c). The gate was reset to the background in 120 min. The illustrated 5 operational rounds (10 hrs in total) were limited by one working day and can most likely be extended. Remarkably, λ exo maintained its activity through the 5 rounds and did not show any need for increasing the time required for the system resetting. The demonstrated resettable operation of the AND gate demonstrates that multiple toe-hold regions engineered into DNA structures are compatible with the λ exo-containing medium.

Previously reported resettable DNA logic gates utilized DNA strand displacement for resetting the gate to the initial state.[9] In this approach, addition of excess amount of oligonucleotides complementary to the inputs would displace the input strand from the complex with the logic gates, thus resetting DNA logic gates to the original state. While the kinetics of strand displacement is well characterized,[10] this approach has fundamental limitations. For example, it requires an external operation: addition of the input complement to reset logic gates. Moreover, the input-compliment duplex waste product is accumulated, and may interfere in the performance of DNA-based constructs. Indeed, no more than 2-3 operational rounds were reported for this approach.[9,10] Herein we designed and tested a system that uses strand displacement, but eliminates some limitations by using λ exo and input-complement, strands which are permanently present as buffer components. The system allows resettable operation for at least 10 hours without the need for additional action by an operator to reset the system back to its original state. It is possible to use other nucleases in similar approaches. For example, RNase H is a widely used enzyme for related applications[5a,11] which cleaves only RNA strand in RNA-DNA hybrid. However, RNase H would require using RNA-containing input oligonucleotides, which are less stable and more expensive commercial products than phosphorylated DNA oligonucleotides.

Currently, we are able to reset YES and AND gates within minutes at 27°C, which is a reasonable time for many biomedical applications. Furthermore, the kinetics can be modified by the amount of input added and by the concentration of λ exo. One possible approach to shorten the system response is to enable operation of the gates at temperatures closer to 37°C, an optimum for λ exo activity.

Conclusions

We report an approach for resettable operation of DNA logic gates that improves on those reported previously. It eliminates the need for external stimulus to reset the system to the background and avoids accumulation of oligonucleotide waste products. The system utilizes all DNA oligonucleotides and is potentially compatible with DNA logic gates of other designs, which utilize oligonucleotide inputs. Further efforts will be directed toward shortening the operational cycle and applying this system to more complex DNA logic gates and their logic circuits.

Experimental procedure

All oligonucleotides were synthesized by IDT DNA Technologies, Inc. λ exo was purchased from Invitrogen. All experiments were conducted in 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. For YES gate; MB (175 nM), **YES 1** and **YES2** (375 nM), and **I1~~m~~** (150 nM) with 75 units of λ exo were incubated at 27.0° C for 15 min. One unit of λ exo is defined as the amount of enzyme necessary to produce 10 nmol of deoxyribonucleotides from a double strand substrate in 30 min at 37° C. 5'-Phosphorylated input strand (250 nM) was added at 5 min and in 60 min intervals. For AND gate; MB (250 nM), **AND1**, **AND2** and **AND3** strands (500 nM), **Ia** (175 nM), **Ib** (175 nM) strands with 75 units of λ exo were incubated for 30 min at 27.5°C. of **Ia** and **Ib** (both 350 nM) were added at 6 min and then at 2 hr intervals. Fluorescence for both gates was recorded at 517 nm (485 nm excitation) using an Infinite 200 Pro plate reader and Thermo Scientific Nunc black 96 well polystyrene plates.

Notes and references

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Electronic Supplementary Information (ESI) available: Oligonucleotide sequences and the predicted structures of fluorescent complex for YES and AND gates; additional trials of operational rounds for YES and AND gates; polyacrylamide gel electrophoresis (PAGE) analysis of reaction mixture containing YES gate. See DOI: 10.1039/c000000x/

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