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Implementing a two-layer feed-forward catalytic DNA circuit for enzyme-free and colorimetric detection of nucleic acids

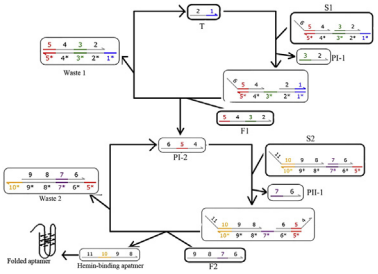
Hadi Ravan

Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran

HIGHLIGHTS

- A catalytic DNA circuit for enzyme-free and colorimetric detection of nucleic acids.
- The circuit can detect 4 pM of the target DNA after a 2-h incubation.
- The circuit can truly discriminate a spurious target with one nucleotide mismatch.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, a highly sensitive and specific bio-sensing platform for enzyme-free and colorimetric detection of nucleic acids has been developed. The biosensor is composed of two DNA nanostructures and two fuel strands that construct the foundation of a feed-forward catalytic DNA circuit. Upon binding the target strand to a specific DNA nanostructure, the circuit is run in order that at the end a hemin-binding aptamer, with the ability to convert a colorless substrate into a colored substance is released. Based on this strategy, 4 pM of the target DNA can be easily detected in serum samples by naked eyes after only a two-hour incubation with the circuit; meanwhile, if the incubation time is extended to 3 h, the biosensor can detect 1 pM of the target DNA. Besides the elevated sensitivity, the circuit can truly discriminate a spurious target containing one nucleotide mismatch with high specificity. Overall, the enzyme-free catalytic DNA circuit can be used as a sensitive alternative method to enzyme-based biosensors for the specific and cost-effective detection of nucleic acids.

1. Introduction

One of the main aspects of living cells is their ability to transduce and amplify a low extracellular signal to a huge intracellular response. The discovery of such phenomena in living organisms has inspired researchers to develop signal amplifiers that are able to detect a low concentration of an input biomolecule for producing a high signal output. In this respect, several signal amplification strategies have been exploited for bio-sensing of nucleic acids, including enzyme-mediated signal amplification [1–3], nanoparticle-based signal enhancement [4,5], DNA template reactions [6], and enzyme-free nucleic acid circuits [7,8]. Among them, enzyme-free DNA circuits due to robustness across diverse conditions [9,10], low cost in terms of dependency to sophisticated reagents such as protein enzymes, and programmability of the reaction networks [11,12] have recently become more popular.

Non-enzymatic nucleic acid circuits can be divided based on the reaction strategy into three major classes, including entropy-driven

E-mail address: ravan@uk.ac.ir.

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catalysis (EDC), hybridization chain reaction (HCR), and catalytic hairpin assembly (CHA) [13]. The functional part of all classes is a single-stranded toehold domain containing between 3 and 8 nucleotides that binds to an input nucleic acid [14–16]. Upon binding of the toehold, a reversible strand displacement reaction occurs between the target and one or more pre-hybridized strands. However, these amplifiers in the basic format have not been sensitive enough to be used in diagnostic applications. To further improve signal amplification, in the present study, an amplification strategy by combining a two-layer catalytic DNA circuit with an HRP-mimicking DNAzyme sequence was developed. The assay provides a highly sensitive, highly specific, enzyme-free and colorimetric detecting platform for nucleic acids.

Fig. 1 represents the analytical framework of the feed-forward catalytic DNA circuit for the detection of nucleic acids. The circuit is composed of two substrate nanostructures, S1 and S2 and two fuel strands, F1 and F2 (each DNA sequence contains several functional domains labeled numerically) (Table S1). The reaction is initiated by hybridization of a target strand T to the toehold domain 1* on the S1 complex. After binding, a strand displacement reaction can occur between domain 2 of the T strand and a pre-hybridized strand, PI-1, on S1 complex. Then, domain 3 of the PI-1 spontaneously dissociates and provides a binding site for domain 3 of the F1 strand. With a new toehold-mediated strand displacement reaction, the other pre-hybridized strand, PI-2, is released and enters the second layer of the circuit. In addition, upon hybridization of the F1 strand, the T strand is also dissociated and enters a new cycle of PI-2 generation. At the second layer, PI-2 serves as the input and triggers the release of a sequestered HRP-mimicking DNAzyme sequence from S2 complex. Here, PI-2 is also released in a manner like the T strand. In this feed-forward circuit (the output of one layer serves as the input for the subsequent layer), a single target can generate many PI-2s and activate many DNAzyme molecules. Based on this strategy, the two-layer DNA circuit can detect a target DNA 150 times more sensitive than the one-layer DNA circuit.

2. Materials and methods

2.1. Reagents

DNA oligonucleotides designed in this study were synthesized by Bioneer Co. (South Korea), which were purified by the polyacrylamide gel electrophoresis (PAGE) method. Table S1 represents the domain sequences of the circuit. 2, 2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and hemin were purchased from Sigma–Aldrich Chemical Co. (USA). The hemin stock solution was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at −20 °C. All other reagents were of analytical grade.

2.2. Colorimetric measurements

To construct substrate nanostructures, each strand participant in the structure was mixed in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) Buffered Saline (HBS; 10 mM HEPES buffer containing 200 mM NaCl, 20 mM KCl and 1.5 mM $MgCl_2$, pH 7.4). The samples were heated to 95 °C and slowly cooled down to 25 °C at the rate of 1 °C/min. The annealed nanostructures were purified by non-denaturing PAGE based on Qian procedure with some modifications [17]. To obtain better yields in gel purification, the DNA nanostructures were prepared at a high concentration of their components (40 μM of each oligonucleotide). After hybridization, the substrate complexes were loaded on 12% non-denaturing PAGE (19:1 acrylamide: bisacrylamide, 240 μL ammonium persulfate, and 24 μL Tetramethylethylenediamine (TEMED)). The gels were stained with ethidium bromide, and then visualized under UV light. The proper band of the substrates were cut out and eluted in 2 mL of HBS buffer for 2 days. The purified nanostructures

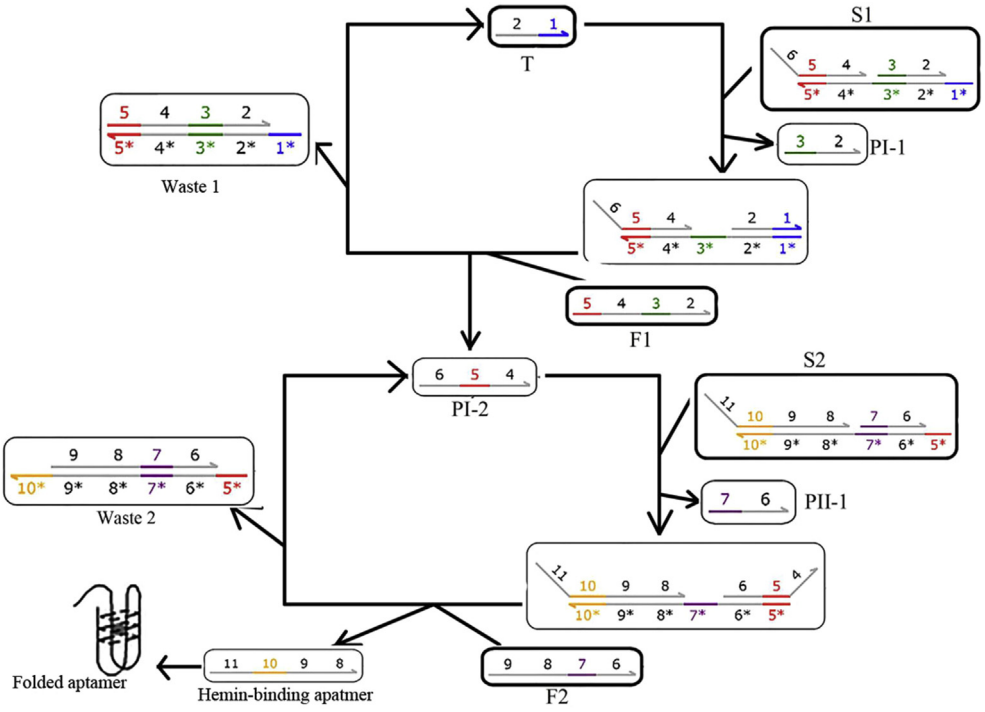

Fig. 1. Schematic illustration of the two-layer feed-forward catalytic DNA circuit. Each DNA sequence contains several functional domains labeled numerically. Upon binding of the target strand to the DNA nanostructure S1, the circuit is run in order that at the end a hemin-binding aptamer, with the ability to convert a colorless substrate into a colored substance, is released. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)