Target DNA Detection by Strand Displacement and Deoxyribozymogen Amplification

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There is a growing need for sensitive and reliable nucleic acid detection methods that are convenient, inexpensive and portable[1]. Here, we describe a rapid DNA detection system that couples (i) a DNA-based molecular switch (Detector) using toe-hold mediated strand displacement mechanisms[2] with (ii) a cross-catalytic deoxyribozymogen system capable of exponential amplification[3] (Signal Amplifier) and (iii) DNA-functionalized colloidal gold nanoparticles[4], DNA-AuNP (Colorimetric Indicator). The system produces a colorimetric readout exploiting the plasmon resonance effects of aggregated gold nanoparticles (i.e., a red-to-purple color change visible to the naked eye). The system described here can detect 100 nmol of a specific target DNA sequence in a sample within 2 hours under isothermal conditions (room temperature).

The target is an arbitrary single-stranded DNA sequence having the generic sub-sequences a-b-c (read 3′ to 5′). In its inactive form, the Detector is a metastable bulged hairpin sequence with an extended 3′-tail. The bulged sub-sequence is the 10-23 deoxyribozyme[5]. The 10-23 deoxyribozyme is a small, fast (k_{cat}= 0.1 min^{-1}), and efficient ($k_{cat}/k_m > 10^9$) DNA enzyme for cleaving RNA oligonucleotides at purine-pyrimidine junctions. The 10-23 deoxyribozyme is magnesium-dependent and has only 15 nucleotides composing its catalytic core. Short flanking substrate-recognition sequences (7-8 nucleotides) can be programmed to hybridize with, and then cleave, arbitrary oligonucleotide sequences. The 10-23 deoxyribozyme does not cleave DNA linkages and the circularized form of this deoxyribozyme is inactive.

The 3′ end of the Detector has a sub-sequence composition (c*-b*-a* where "*" denotes the reverse-complement sequence) making it susceptible to toe-hold mediated strand displacement by the complementary target DNA. Strand displacement exposes the deoxyribozyme and its two flanking substrate-recognition sequences, a and b. In its active form, the Detector initiates the Signal Amplifier.

The Signal Amplifier employs a mixture of circularized 10-23 deoxyribozymes C1 and C2, each containing two RNA linkages (5'-rArU-) susceptible to cleavage[5]. C2 can hybridize with, and can subsequently be cleaved by a cleaved and linearized C1 (designated L1). Symmetrically, C1 can be cleaved by L2, which is a linearized C2. The products of the activated amplification reaction, L1 and L2, thus accumulate exponentially.

Hence, in the presence of the target DNA, activated Detector molecules initiate the poised, autocatalytic Signal Amplifier reaction by cleaving C2 molecules. The resulting exponential accumulation of L1 and L2 can be readily indicated by a variety of convenient methods. A simple and reliable indicator of the products of the Signal Amplifier reaction is the macroscopic red-to-purple color change sustained when AuNPs are suitably organized. Here, 15 nm AuNP's were functionalized with thiolated single-stranded DNA oligomers having the sequence a* and b*, complementing the a and b sub-sequences of L1. The accumulated product L2 acts as a bridge between AuNPs, causing aggregation, plasmonic coupling and red to purple color change. The relative inability of C1 to serve as the bridging strand is attributed to steric hindrance. The reaction proceeds efficiently when DNA-AuNPs are added after the Signal Amplifier reaction has run for 2 hours.

In order to generalize the system for detection of other DNA targets, the detection and triggering functionality of the Detector was separated into two distinct DNA molecular sequences, i.e., a Transducer sequence and a Trigger sequence. The Transducer, like the original Detector sequence, is a hairpin sequence with an extended 3´-tail. The 3´ end of the Transducer has a sub-sequence composition (z*-y*-x*) making it susceptible to toe-hold mediated strand displacement by the target DNA, x-y-z. The hairpin loop of the Transducer has the sub-sequence a-b-c. When the target hybridizes with the Transducer, the a-b-c sub-sequences are then available for strand displacement of the Trigger sequence, that in turn, initiates the Signal Amplifier in the same manner as the original Detector sequence. In this way, the Trigger, Signal Amplifier and AuNP oligos constitute the core detection system, and when reconfiguring the system for new target sequences, only the Transducer sequence need be modified.

This elaborated detection system was tested using control and biological target DNAs including unique sequence identifiers of *Chlamydia trachomati*[6] (*Ct*) and Human Imunodeficiency Virus[7] (HIV). Combining rationally designed and *in vitro* selected DNA-based technology[8], this detection system demonstrates a rapid, reliable, inexpensive and reconfigurable diagnostic for arbitrary DNA analytes. Coupling this system with nucleic acid sensors for protein, metabolites and metal ions[9], this system could find wide application in medicine, environmental pollution/contamination monitoring[10], electrochemical detection[11], and nanotechnology[12].

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