Point-of-Contact, DNA-Based Amplifier for Detecting Cancer-Related Micro-RNA in Blood Serum

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Cancer is recognized as a serious global health challenge. Worldwide, there are approximately 1.3 million lung cancer deaths per year [1]. While early detection and diagnosis of cancer leads to decreased mortality rates, current screening methods require significant time and equipment [2]. Recently, increased levels of certain micro-ribonucleic acids (miRNAs) in blood have been linked to the presence of cancer. Micro-RNAs are small, single-stranded, non-coding RNAs that are 21-23 nucleotides in length and regulate genes by suppression of messenger RNAs [3], [4]. Several miRNAs and components of miRNA pathways are amplified in some cancers [5], and hence, can serve as targets for cancer detection. Furthermore, it has recently been shown that miRNAs can be detected in human blood serum [6]. miRNA expression profiling reveals that miRNA signatures can be used for cancer classification and prognosis; for example, miR-25 and miR-223 are highly expressed in the serum of lung cancer patients [6].

Current diagnosis technology requires the isolation of total RNA, production of complementary deoxyribonucleic acid (DNA), and quantitative polymerase chain reaction (PCR) to detect these miRNAs in serum. Developments in DNA computing have shown that it is possible to construct nucleic acid-based chemical networks that accept miRNAs as inputs, perform Boolean logic functions, and generate as an output a large number of DNA strands that can readily be detected. Additionally, recent studies have shown that metastable DNA systems exhibiting autocatalytic or cross-catalytic behavior can be constructed, giving rise to exponential amplification [7]. As miRNAs occur in blood in low abundance, this would allow for amplification without using PCR. In this study, we report a DNA-based cross-catalytic network engineered to amplify specific cancer-related miRNAs. The cross-catalytic DNA system accepts the input of a specific catalyst miRNA and produces, as an output signal, strands that generate an easily detectable fluorescence signal. Sub-components of the DNA network were tested individually and their operation in serum, as well as a mixture of serum with sodium dodecyl sulfate (SDS), is demonstrated. Simulations of the entire cross-catalytic network indicate successful operation and validate the network design.

In order to optimize the design of the cross-catalytic network for operation in human blood serum, rate constants were determined for sub-components of the system. Rate constants for hybridization reactions are of particular relevance to the design of catalytic DNA networks for operation in serum or other body fluids, as are rates of degradation of

nucleic acids due to nuclease activity. As typified in Fig. 1(a), initial experiments using fluorescence resonant energy transfer (FRET) were performed to compare hybridization rates of nucleic acids in phosphate buffered saline (PBS) solution and human blood serum. The use of sodium dodecyl sulfate (SDS) added to serum was explored as a means to suppress nuclease activity without disrupting DNA hybridization, as shown in Fig. 1(b). Hybridization rates in all solutions were within one order of magnitude for both DNA and RNA. With 10% SDS, DNA lifetime in serum is increased. Addition of SDS to serum should allow the cross-catalytic system to amplify cancer specific miRNA. Additionally, operation of the cross-catalytic network was simulated to confirm proper operation. Simulations results indicate that the system remains in a metastable state until a catalyst DNA strand is added to the system. Once the catalyst strand is added, dye-labeled DNA strands are rapidly released into solution, providing a large, easily detectable fluorescence signal.

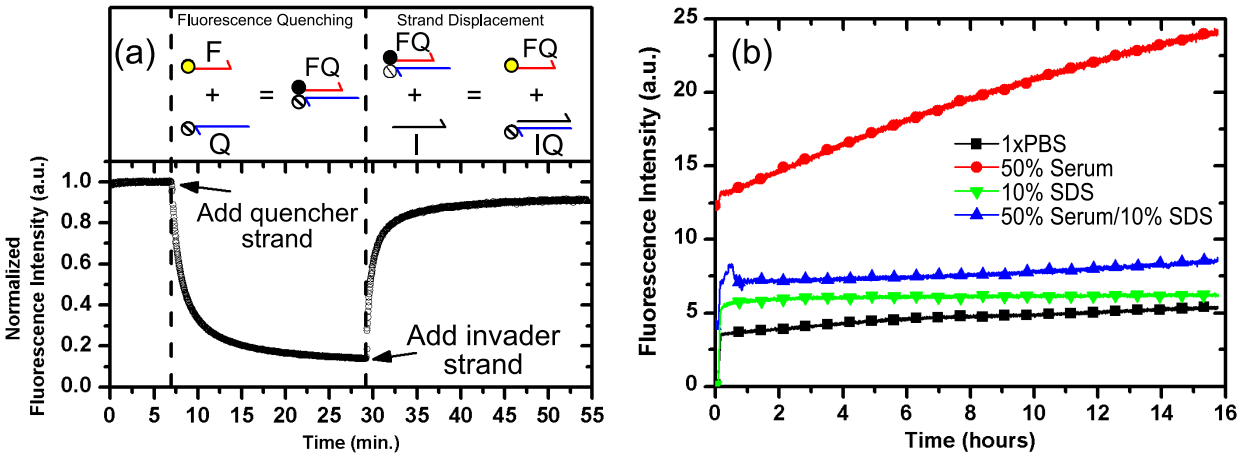

Figure 1. (a) Hybridization and strand invasion kinetics using FRET in 1×PBS. (b) Time dependent dye-quencher pair stability. The addition of 10% SDS to serum greatly increased pair lifetime.

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