Aptamer-Based Microfluidic Biosensor for Rapid Detection of RNA Degrading Agents in Challenging Environments

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Ribonucleic acid (RNA) molecules fold into complex tertiary structures that display various catalytic activities and interact specifically with both small molecules and macromolecules. This structural and functional diversity provides a platform for identifying viable building blocks for novel RNA nanostructures that could be used for the development of RNA-based biosensors for detecting pathogens and chemical agents that could threaten our food safety and security. As RNA is readily degraded by ubiquitous ribonucleases (RNases) and metal ions, manufacturing RNA-based nanodevices is challenging. In particular, tracing the source of RNA degrading activities is a time-consuming and costly endeavor.

We used RNA aptamer technology to design and build a microfluidic biosensor for rapid detection of RNase and lead (Pb) contamination. The sensing strategy is illustrated in Figure 1A. In our assay, 3,5-difluoro-4-hydroxybenzylidene imidazoline (DFHBI) binds to its RNA aptamer and emits strong fluorescence. Figure 1B shows the structure of the BFHBI-binding aptamer. The two-stranded design increases sensitivity of our biosensor. Our preliminary studies indicate that a single cleavage of the two-stranded RNA aptamer extinguishes its fluorescence. Figure 1C shows detection of RNase A, RNase T1 and Pb(Ac)2 using a microfluidic chip. It contains 60 compartments for fluorescent double-stranded RNA aptamer and RNase or metal ion contaminated solutions. Traces of both RNA degrading agents are detected in 0.4 nL samples under strictly controlled conditions.

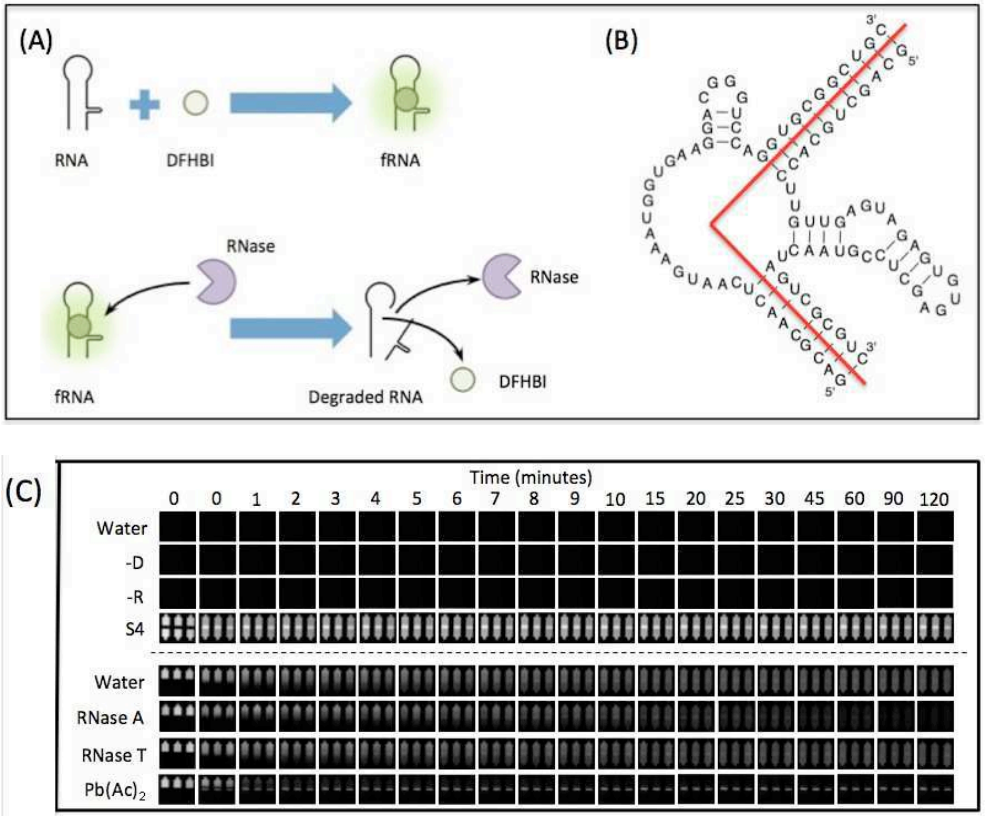

Fig. 1. Detection of RNA-degrading agents. (A) Principle of detection; (B) 2D structure of a BFHBI-binding aptamer; (C) Analysis of 20 (in triplicate) samples contaminated by RNases and $Pb(Ac)_2$.

Auburn University has a patent pending application for the above described technology.

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