Complementary RNA-Based Biosensors for Rapid Detection of RNAs Derived From a Single Pathogenic Bacterial Cell

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The detection of pathogenic bacteria is a key to the prevention and identification of problems related to human/animal health and food safety. Advanced techniques such as the polymerase chain reaction (PCR) are primarily used for the amplification of pathogen-specific DNA sequences. Therefore, they will not determine if the bacteria are viable or dead. We developed novel biosensors for the rapid detection of pathogen-specific RNAs. Because RNA is rapidly degraded in dead organisms, only viable cells can be detected. Figure 1 illustrates the principle of cRNA-based detection. First, we amplify the target RNA into its complementary RNA (cRNA) using isothermal RNA amplification (IRA). The cRNA is a synthetic RNA molecule that does not exist in nature and thus, when analyzed, will not suffer from contamination by naturally occurring organisms. As a result, a high level of specificity is achievable. The detection of the cRNAs requires two independent hybridization reactions. In the first reaction, the receptor module captures cRNA molecules. The second reaction involves base pairing of cRNA molecules with various types of enhancers. The requirement for two independent hybridization reactions permits manipulation of the biosensor to optimize the specificity and sensitivity of target RNA detection.

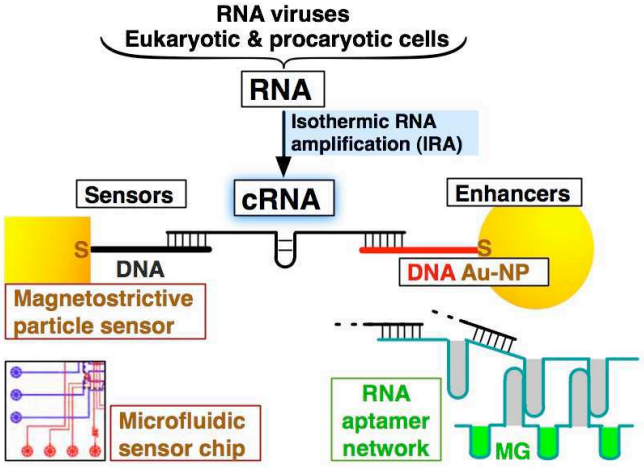

Fig. 1 shows the principal steps for the detection of RNA molecules derived from pathogens. cRNAs are synthetized using IRA. The cRNAs are detected via their hybridization to the receptor and various types of enhancers, either DNA-Au-NPs or RNA aptamer networks.

Our biosensors target tmRNA, an RNA molecule that is present in all bacteria. Because tmRNA is absent in eukaryotic cells, the possibility that RNAs derived from humans, animals and plants will interfere with the detection process is minimal. Our studies demonstrated that 10^{-17} M cRNAs could be detected using DNA-linked gold nanoparticles (DNA-Au-NPs) as both a receptor and an enhancer. Given that each actively dividing *E. coli* cell contains 3,000 tmRNA molecules, and a two-minute-long IRA reaction produces thousands of cRNA copies, the cRNA-based biosensor is able to detect a single cell of *E. coli* grown in a liquid culture. To increase sensitivity of detection, we use RNA networks composed of multiple copies of an aptamer that binds 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI) as an enhancer. When bound to its RNA aptamer, DFHBI emits strong fluorescence that can be readily monitored by minimally trained personnel. Our technologies are expected to have significant impact in food agriculture, medicine, research and national security.

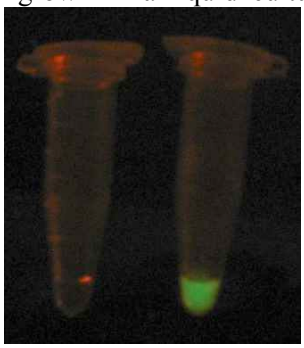

Fig. 2. Rapid detection of the mutant tmRNA molecules using the cRNA-based sensor composed of DNA-Au-NPs (receptors) and DFHBI-binding RNA networks (enhancers). Left tube: Wild-type tmRNAs isolated from the *E. coli* K12 cells. Right tube: Mutant tmRNAs encoding a Histidine-rich peptide.

Auburn University has a patent pending application for the cRNA detection technology.

*We acknowledge the National Science Foundation (Grant 1063536) and the Upchurch Fund for Excellence for funding this project.