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Instant Detection of Pathogens Using Programmable RNA Aptamer Networks

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We have developed a novel technology that overcomes limitations of methods currently used for pathogen detection. This technology uses RNA aptamer networks, which can be programmed for detection of multiple pathogens and stored at ambient temperature. RNA aptamers can be quickly and cost-effectively synthesized *in vitro* which represents a major advantage of this approach because the need to use animals or cultured cells for aptamer production is eliminated.

In our detection strategy, we use three to five protein-specific aptamers (denoted as PR in Fig. 1) and incorporate them into an RNA-based detection network (denoted as DN in Fig. 1). Such a network acts like a "molecular octopus" and binds simultaneously to all targeted proteins. The interaction between networked aptamers and pathogen is extremely strong because it is driven by avidity rather than affinity. To visualize pathogen detection, we developed a separate fluorescent network (denoted as FN in Fig. 1). It is equipped with several copies of RNA aptamer (denoted as GF in Fig. 1) that binds 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI). Upon binding of DFHBI, our RNA aptamer network emits bright green fluorescence. Moreover, we equipped both DN and FN networks with several copies of a cross-linking module (denoted as XL-aptamer in Fig. 1) that can bind to itself. Interactions between XL-aptamers stimulate the binding of the FN network to the DN network. The fluorescence of DN:FN complexes is so strong that it can be readily observed by the naked eye under visible blue light as provided by "Dark reader", an inexpensive ($400) hand-held lamp.

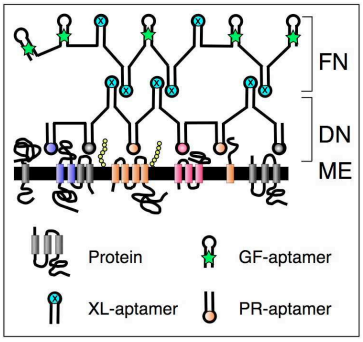

Fig.1. Schematic representation of programmable RNA-aptamer networks. Fluorescent (FN) and detection (DN) networks are composed of green fluorescent (GF), protein recognizing (PR) and cross-linking (XL) aptamers. ME denotes a bacterial membrane intercalated with proteins.

The simplest way to detect pathogens on the surface of an infected object is to spray this object first with the solution containing DN networks and then with the solution containing FN networks. After washing off unbound DN:FN complexes, bacteria can be observed with the naked eye under the Dark Reader lamp (Fig. 2). The above-described approach is simple and effective, but on some occasions it produces false positives due to unspecific binding of DN networks to the contaminated object. To avoid this drawback and to use RNA aptamer networks more economically, we will integrate RNA aptamer networks into a robust microfluidic chip developed earlier by one of us, Dr. Jong W. Hong. This chip will sequentially process nanoliter volumes of liquids to isolate bacterial cells, bind to them DN and FN networks and then wash off unbound DN:FN complexes.

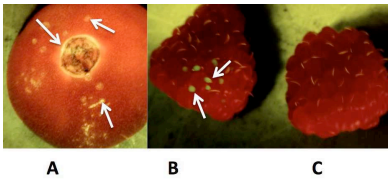

Fig. 2. Naked eye visualization of Salmonella cells on the intentionally infected (A) tomato and (B) raspberry. (C) Uninfected raspberry. Arrows indicate fluorescent DN:FN complexes. The presence of the pathogen in the spots that are marked by white arrows was confirmed by a PCR-based analysis

Auburn University has a patent pending application for the programmable RNA aptamer network technology.

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