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Naturally amplified player for biosensing: tmRNA to the rescue

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Abstract

We present a *trans*-translation mediating tmRNA molecule from bacteria that can be used as a marker in microbial diagnostics and/or taxonomical studies. Different bacteria can be detected and specifically identified using surface bound complementary DNA oligonucleotide probes on different sensor platforms. Several tmRNA hybridization techniques were studied on microarray and in real-time on SOI microring resonator biosensor platforms. Microarray based detection technology enabled detection of tmRNA molecules from less than 1 cell equivalent of pathogenic bacteria.

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Keywords:tmRNA, molecular diagnostics, NASBA, microarray, SOI microring resonators

1. Introduction

One of the key moments in developing new biosensing methods, is the selection of proper marker molecules that are most suitable for the needed cause. A new emerging player in bacterial detection is a *trans*-translation mediating molecule called tmRNA (Figure 1), present in all known bacteria with around 1000 copies per cell. In addition to its natural role, tmRNA can be successfully used as a diagnostical marker molecule to detect and distinguish different bacterial species or other taxonomical units.

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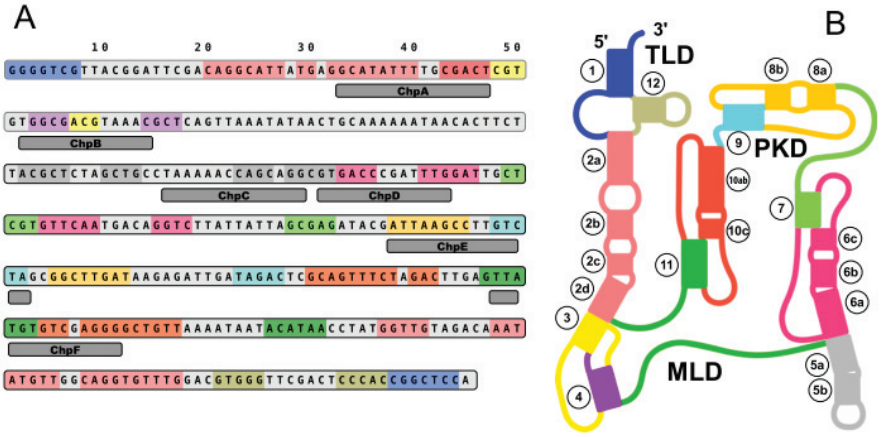

Fig. 1. tmRNA structure and chaperone positions [1]. (A) *S.pneumoniae* R6 tmRNA sequence (NC003098.1) with predicted helices highlighted in color and chaperone structures marked. Prediction and coloring according to tmRNA website [2]. (B) *E.coli* tmRNA structure [3] with corresponding highlighted helices

2. tmRNA hybridization detection

2.1. Chaperone aided tmRNA hybridization

Hybridization based detection of long RNA molecules like tmRNA can be enhanced by introducing complementary helper oligonucleotides to reduce the secondary structure formation of RNA molecule (Figure 1). Such helper "chaperone" molecules contribute strongly to the specificity and the sensitivity, especially on hybridization experiments on lower temperatures where secondary structure formation is more problematic (Figure 2) [1].

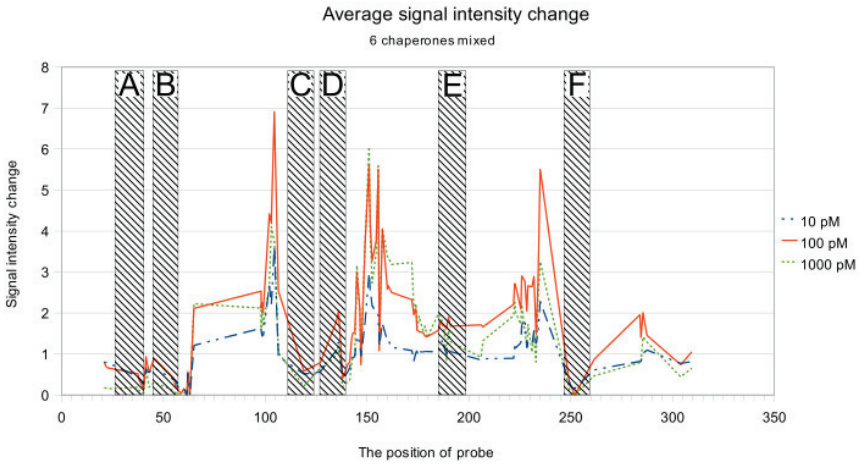

Fig.2. The average relative signal intensity of microarray probes at different chaperone concentrations [1]. The signal intensities are arranged according to probe midpoint position on tmRNA. The chaperone corresponding complementary regons are marked by shaded rectangles. Signal intensity normalized value 1 stands for hybridization signal without chaperones.

2.1. Real-time detection of tmRNA on SOI microring resonators

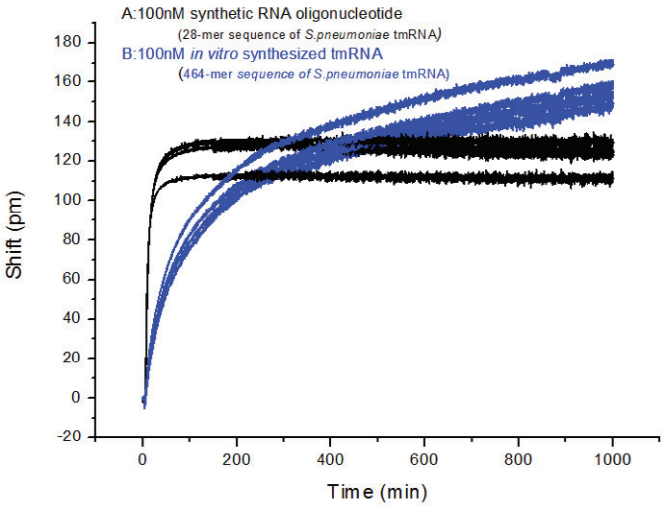

Fig. 3. Hybridization signal of tmRNA molecules on SOI microring resonators. Comparison between hybridization kinetics of short tmRNA molecule segment (28-mer RNA oligonucleotide) and full-length tmRNA (*in* vitro synthesized 464-mer).

Microring resonators are a class of refractive-index-sensitive devices that have recently been applied to monitoring chemical reactions and biomolecular binding events [4,5]. Real-time monitoring and specific identification of different tmRNA molecules (Figure 3) provides bases for developing a multiplexed biosensing device, capable of quick detection of pathogenic bacteria.

2.2. NASBA-microarray technology for tmRNA detection

A complete technological solution for bacterial identification using tmRNA as a marker molecule has been developed [6,7]. Detection of tmRNA molecules from less than 1CFU of pathogen *Streptococcus pneumonia* was demonstrated by combining NASBA amplification of target tmRNA-s with microarray based specific hybridization detection (Figure 4).

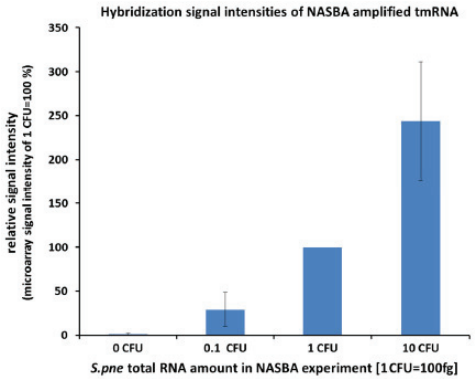

Fig. 4. Microarray signal intensities of NASBA amplified tmRNA from *S.pneumoniae* total RNA dilutions [7].

A robust probe design algorithm for microbial detection on microarray was implemented. The probes were evaluated for specificity and, combined with NASBA (Nucleic Acid Sequence Based Amplification) amplification, for sensitivity. Combining custom designed microarray probes and modified NASBA amplification protocol we were able to detect *S.pneumoniae* tmRNA from a series of total RNA dilutions equivalent to the RNA content of 0.1-10 CFU. The described technological solution and both its separate components web based probe design program SLICSel [7] and NASBA-microarray technology independently [6] are applicative for many different areas of microbial biosensing.

3. Conclusion

We have demonstrated thoroughly the use of tmRNA as a novel marker molecule for bacterial detection and biosensing. Different bacterial detection mechanisms were investigated using DNA/RNA-microarray technology and/or SOI microring resonator biosensing platform.

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