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Abstract

Complex DNA Nanomachines for the Ultrasensitive Detection of Ribonucleic Acids †

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1. Background

DNA nanomachines were developed as highly accurate alternatives to costly and unreliable methods used for measuring gene expression levels. However, despite their potential, DNA nanomachines also come with several limitations. One major drawback is their tendency to interact with various biological molecules, which can compromise their functionality. Additionally, background noise often hampers their performance, leading to reduced sensitivity and less precise results.

2. Goal

The goal of our work was to create DNA nanomachines for the highly sensitive detection of RNA extracted from cell culture that can be used for assessing mRNA concentrations without amplification.

3. Methods

DNA nanomachines were partially preassembled to add more auxiliary structures. Additional quenchers were added to the structure to decrease the background noise and increase the dynamic range and limit of detection. The pre-assembly was performed with the gradual cooling of samples after a short boiling period. The resulted pre-assembled structures were confirmed via PAGE gel. Samples were incubated at 55 °C and different times of exposure were analyzed. The DNA nanomachines were characterized via limits of detection and their effectivity was assessed on total RNA extracted from cell culture.

4. Results

The results of the optimization experiments showed that incorporating a specific amount of DNA nanomachine with quenchers effectively reduced the background noise and non-specific fluorescence, therefore leading to more accurate evaluation of the signal. The limit of detection experiments revealed that the designed structures were capable of detecting their target at concentrations as small as picomolar. Notably, the machines equipped with quenchers exhibited even greater sensitivity, further enhancing their performance.

5. Conclusions

The optimization experiments conducted on DNA nanomachines have unequivocally demonstrated that their sensitivity can be significantly enhanced through the incorporation of new designs and additional elements. This breakthrough paves the way for the potential utilization of these advanced constructions as highly efficient point-of-care diagnostics in the future.

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