DNA ORIGAMI BASED NANOANTENNA FOR ENHANCED FLUORESCENCE DETECTION OF microRNA

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Liquid biopsies provide valuable clinical information through the analysis of biomarkers present in body fluids, such as peripheral blood samples [1]. This approach offers significant potential for diagnosis, clinical decision-making, and prognosis assessment. Among the various classes of biomarkers, microRNAs (miRNAs) which are short, non-coding RNA molecules (19–25 nucleotides in length), play key regulatory roles in cancer progression [2]. Numerous miRNAs associated with different types of cancer have been identified, and some show strong promise as targets for liquid biopsy-based diagnostics. However, the detection of miRNAs remains challenging due to the need for high sensitivity and specificity. Moreover, quantitative analysis is essential to monitor changes in miRNA concentrations. A high degree of multiplexing capability is also crucial, as the clinical relevance of miRNAs depends on the ability to simultaneously profile multiple miRNAs and identify specific disease expression patterns [3]. Finally, the direct detection of low-abundance analytes can be further hindered by low signal-to-background ratios. In this work, we propose plasmonic nanoantennas (NAs) designed to enhance the fluorescence-based detection of miRNAs. Plasmonic NAs are structures constituted of plasmonic metallic nanoparticles (NPs) that can intensify and localize electromagnetic fields enhancing the excitation and emission rate of a fluorophore placed in the proximity [4]. The construction of the proposed NA involved the synthesis and functionalization of metallic NPs and the precise attachment of these NPs to DNA origami to form a dimer NA structure. The NPs employed in this work are silver-coated gold nanorods (Au@Ag NRs), synthesized from gold nanorods (AuNRs). The silver shell was formed through a controlled overgrowth process, achieved by adding silver nitrate and ascorbic acid to a suspension of AuNRs (53 × 18 nm) stabilized with the surfactant BDAC, maintained at 65 °C. The synthesized Au@Ag NRs (58 × 29 nm) were functionalized with thiol-modified T18 ss DNA oligonucleotides. DNA origami NAs were then assembled via hybridization between the T18-functionalized Au@Ag NRs and complementary A18 ssDNA strands protruding from the long-shaped DNA origami structure. The resulting structures were characterized using transmission electron microscopy (TEM) and dark-field microscopy. To assess fluorescence enhancement, the DNA origami was modified with a fixed ATTO680 dye, and fluorescence signals were recorded using wide-field microscopy. Single-molecule fluorescence intensities were first measured from DNA origami structures without Au@Ag NRs to establish a reference. The same measurements were then performed on the NAs. Enhancement values were quantified by comparing fluorescence intensities from spots corresponding to the NAs against the average reference intensity. The results revealed that the dimer NAs achieved fluorescence enhancement factors up to 1000-fold, with an average value of 285±251. The strong fluorescence enhancement achieved by the plasmonic dimer NAs demonstrates their potential for highly sensitive miRNA detection. By modification of the DNA origami, the designed NAs can be further applied to the detection of miRNAs. The bioassay consists of the binding of the unlabeled analyte to capture strands placed on the DNA origami in the center of the gap, and the detection of the analyte by binding of a second strand labeled with the dye, as shown in Figure 1. This approach provides a promising platform for the development of liquid biopsy assays and improvement of cancer diagnostics.

References

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Figures

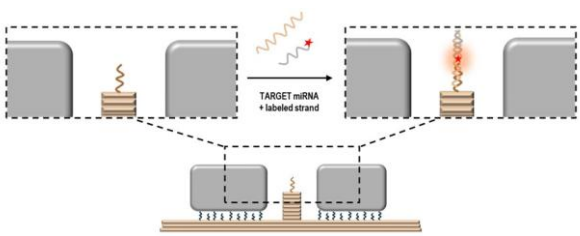

Figure 1. DNA origami-based nanoantenna for miRNA detection.