

Quantum Dot-Based Molecular Beacons for Quantitative Detection of Nucleic Acids with CRISPR/Cas(N) Nucleases

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ABSTRACT: Strategies utilizing the CRISPR/Cas nucleases Cas13 and Cas12 have shown great promise in the development of highly sensitive and rapid diagnostic assays for the detection of pathogenic nucleic acids. The most common approaches utilizing fluorophore-quencher molecular beacons require strand amplification strategies or highly sensitive optical setups to overcome the limitations of the readout. Here, we demonstrate a flexible strategy for assembling highly luminescent and colorimetric quantum dot–nucleic acid hairpin (QD-HP) molecular beacons for use in CRISPR/Cas diagnostics. This strategy utilizes a chimeric peptide–peptide nucleic acid (peptide-PNA) to conjugate fluorescently labeled DNA or RNA hairpins to ZnS-coated QDs. QDs are particularly promising alternatives for molecular beacons due to their greater brightness, strong UV absorbance with large emission offset, exceptional photostability, and potential for multiplexing due to their sharp emission peaks. Using Förster resonance energy transfer (FRET), we have developed ratiometric reporters capable of pM target detection (without nucleotide amplification) for both target DNA and RNA, and we further demonstrated their capabilities for multiplexing and camera-phone detection. The flexibility of this system is imparted by the dual functionality of the QD as both a FRET donor and a central nanoscaffold for arranging nucleic acids and fluorescent acceptors on its surface. This method also provides a generalized approach that could be applied for use in other CRISPR/Cas nuclease systems.

KEYWORDS: CRISPR/Cas, Molecular beacons, Quantum dots, Ratiometric, Förster resonance energy transfer, Peptide nucleic acids

INTRODUCTION

The CRISPR/Cas family of proteins gained prominence in 2010 as RNA-guided endonucleases that serve as a bacterial defense mechanism against viral infection.¹ It was recognized that bacteria incorporated bits of viral DNA into their genomes between motifs called clustered regularly interspaced short palindromic repeats (CRISPR) and, upon transcription, CRISPR-associated (Cas) proteins would bind the CRISPR RNA (crRNA) guided by a trans-activating crRNA (tracrRNA).² This duplex then served as a guide to specifically target and degrade any viral DNA matching the CRISPR-encoded spacer. The combination of crRNA and tracrRNA in a single guide RNA (sgRNA or gRNA) reduced the complexity of this system and enabled rapid advances in applications of CRISPR/Cas toward programmable gene editing, knockdown, and diagnostics, earning the Nobel Prize in Chemistry in 2020.³



The implications of CRISPR/Cas for pathogenic nucleic acid detection were quickly realized with the Cas13 (type VI, RNA-targeting) and Cas12 (type V, DNA-targeting) CRISPR/Cas systems upon the discovery of their propensity to undergo nonspecific trans-cleavage of nucleic acids only when bound to target nucleic acids.^{4–6} Unlike traditional nucleases, the Cas13 and Cas12 proteins are effectively inert prior to being assembled with a gRNA into a Cas/gRNA ribonucleoprotein (RNP). The gRNA itself is composed of two domains: a RNA loop motif with sequence and secondary structure that is

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Development Of Molecular Beacons For Nucleic Acid Detection

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