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Abstract

Ultrasensitive Detection and Discrimination of Cancer-Related Single Nucleotide Polymorphisms Using Poly-Enzyme Polymer Bead Amplification †

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We report the development of a new ultrasensitive approach for label-free DNA detection using magnetic nanoparticle (MNP)-assisted rapid target capture/separation in combination with signal amplification using poly-enzyme tagged polymer nanobead. The sensor uses a MNP linked capture DNA and a biotin modified signal DNA to sandwich bind the target followed by ligation to provide high single-nucleotide polymorphism discrimination. Only the presence of a perfect match target DNA yields a covalent linkage between the capture and signal DNAs for subsequent binding to a neutravidin-modified horseradish peroxidase (HRP) enzyme via the strong biotin–neutravidin interaction. This converts each captured full match DNA target into a HRP which can convert millions of copies of a non-fluorescent substrate (amplex red) to a highly fluorescent product (resorufin) for great signal amplification. The use of polymer nanobead each tagged with thousands of copies of HRPs as the signal amplifier greatly improves the signal amplification power, leading to greatly improved sensitivity. This biosensing approach can specifically detect an unlabelled DNA target down to 10 aM with a wide dynamic range of 5 orders of magnitude (from 0.01 fM to 1000 fM). Furthermore, our approach has a high discrimination between a perfectly matched gene and its cancer-related single-base mismatch targets (SNPs): it can positively detect the perfect match DNA target even in the presence of 100-fold excess of co-existing SNPs. This sensing approach also works robustly in clinical relevant media (e.g., 10% human serum) and gives almost the same SNP discrimination ratio as that in clean buffers. Therefore, this ultrasensitive SNP biosensor appears to be well-suited for potential diagnostic applications of genetic diseases.