Functional nanoparticle-bioconjugates for biosensing and biocatalysis

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Introduction

Nanoparticles have unique, size-dependent optical and electronic properties that are distinct and unavailable from the bulk, which in combination with the high target binding affinities and specificities of functional biomolecules (*e.g.* protein, DNA and antibody etc.), make them extremely well-suited for sensing and biomedical applications.

Results

Over the past few years, we have developed two types of nanoparticle-bioconjugates based sensors in an attempt to satisfy the different bioassay/biodetection needs. First, we have developed a simple gold nanoparticle (GNP)-aptamer based colorimetric sensor that exploits the unique, aggregation induced colour changes of GNPs (where isolated GNPs are red while aggregated ones are blue/purple) as well as the distinctly different adsorption behaviors of single-stranded (ss) DNAs/aptamers on citrate stabilised GNPs: unstructured, random coiled ssDNAs adsorb strongly onto GNPs to provide effective protection against salt-induced aggregation, whereas target-bound aptamer complexes cannot offer such protection. As a result, a ssDNA aptamer-GNP solution remains red after salt addition without target but turns into blue/purple with target (Figure 1A). Such colour changes are directly visible by the naked eye, making it well-suited for simple colorimetric sensing. We found that this simple sensor can detect specific low nM protein and ~100 nM small molecule targets in seconds by eye without using any instruments. Moreover, it can detect maximum residual limit (MRL) level of illegal additives and harmful residues (*e.g.* aminoglycosides antibiotics) in milk prescribed by EU law in seconds with the naked eye. Therefore, this simple sensor appears well-suited for rapid, on-site detection of relatively high abundant targets, such as illegal additives and harmful residues in food. It should be noted however that this sensor can only work in relatively clean solutions, the presence of large amount non-target proteins and other matrix materials can interfere with the specific target detection.

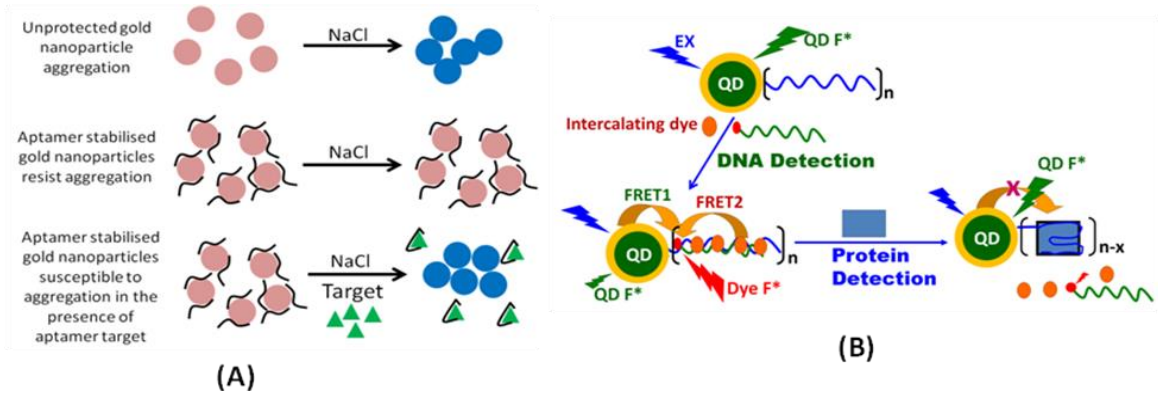

Figure 1: (**a**) Principle of the GNP-aptamer colorimetric sensor. (**b**) Schematic principle of the QD-intercalating dye dual-donor FRET sensor for detection of DNA and unlabelled protein targets.

Second, we have developed a novel intercalating dye (ethidinum bromide, EB)-quantum dot (QD) dual-donor FRET based sensor (Figure 1B). All previous QD-FRET based sensors have based on using the QD as the sole energy donor, whose sensitivity and specificity are limited by challenges associated with the ability of preparating functional QD-bioconjugates that are both copmpact (for high sensitivity) and effectively resisting non-specific adsorption (for high specificity and robustness) because these requirements are incompatible. The dual-donor

FRET system can overcome such limitations because of the enhanced FRET efficiency *via* both the QD and intercalated EBs donors. Moreover, the spatial closeness of the intercalated EBs to the acceptor in the dual-donor FRET system allows for efficient FRET even with relatively bulky QD-bioconjugates (and/or a QD-dye FRET system with relatively small spectral overlap), bypassing the strict requirement of compact QD-conjugates for high FRET in traditional single-donor FRET systems. The dual-donor FRET system has been used for sensitive detection of labelled DNA probes, where hybridization of the DNA probe to the QD-DNA conjugate leads to simultaneous intercalation of EBs, leading to significantly enhanced FRET for each target binding and hence improved sensitivity. This sensor can detect sub-nM level labelled DNA probes with high specificity: it can discriminate between perfect-match and single-base mismatch probes, which is equivalent to a SNP (single nucleotide polymorphysm) discrimination. Moreover, the sensor has been turned into a sensitive, label-free protein sensor *via* the incorporated anti-thrombin DNA aptamer sequence where target protein binding induced complementary DNA displacement and simultaneous removal of both QD and EB FRET signals. The dual-donor FRET sensor can quantitate thrombin down to 35 pM level, 2-4 orders of magnitude more sensitive than previously reported single-donor QD-FRET sensors, suggesting it has strong potential for disease diagnosis. Despite this, the current sensor however cannot work in complex media, *ca.* human serum, and therefore further optimizition of the QD surface and bioconjugation chemistries are still needed to make it suitable for real clinical detection and diagnostic applications.

Publications

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Collaborators

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