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Blink and you'll miss it: a new biosensing strategy with nucleic acids

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Abstract: Fluorescent biosensors typically use energy or electron transfer to modulate the emission from a fluorophore. This requirement often makes it difficult to change the biosensor to make it selective to a difference target. In this research highlight we describe a recently reported strategy that relies, for the first time, on fluorescence blinking from nucleic acid-coupled quantum dots to report the presence of a target molecule. This strategy produces a decoupled biosensor, whose fluorescence output is not directly modulated by interaction with the target. The resulting biosensor can be readily modified to sense any target that can be selectively bound to nucleic acids and is therefore much more widely applicable than the vast majority of fluorescent sensors that have been reported.

Keywords: DNA, quantum dot, fluorescence, blinking, biosensor

In 2016, there will be an estimated 1,685,210 new cancer cases diagnosed and 595,690 cancer deaths in the US. The SEER US Government Statistics Registry shows that the average 5-year survival rate for all cancers for the years 2006-2012 has increased to 69% (up from 50% for 1975–1977). This increase in survival can be attributed to technological advances resulting in better treatment and improvement in early diagnosis. However, the 5-year relative survival rate for certain cancers such as liver, pancreatic, and lung remains very low. This is further exemplified by the significant difference in survival rate between identification of said cancers at stage 1 (6%-16%) to a very low survival rate (1%-3%) at stage IV [1]. Therefore, the early detection of these diseases is paramount in ensuring the increased 5-year survival rates of patients. The development and implementation of newly emerging biological sensor (biosensors) technology could be instrumental in the early detection of relevant oncogenes, which could enhance the early preventive treatment and possible elimination without the need for intensive chemo- or radiotherapy, resulting in improvements in patient quality of life and overall chance of survival [2].

The pursuit of enhanced early detection has led to many developments in the technology behind biosensors [3,4]. One approach utilizes programmable nucleic acids that re-associate in the presence of a target. Recently, we have shown that when these biosensors are conditionally activated and allowed to cross-link commercially available streptavidin-decorated quantum dots (QDs), the output signal can be seen by observing changes in the stochastic fluorescent blinking activity of the particles [5,6]. This unique application to the recognition of biological molecules allows for the expansion of a library of QD-based biosensors with the added value of easily detecting targets via confocal fluorescence spectroscopy.

Quantum dots (QDs) are colloidal, luminescent, semiconductor nanoparticles which have emerged with a significant role as nanomaterials for biosensor development [7]. The use of QDs has increased due to their robust fluorescence (resistance to photo bleaching and oxidation), size-tunable excitation/emission properties, and their amenability to chemical modification [8]. With very few exceptions, fluorescent biosensors report detection events by changing their emission brightness or color after an analyte enables (or disables) an energy/electron transfer pathway in the sensor. This often means that the analyte directly attaches to the part of the sensor that modulates the output fluorescence, e.g. a FRET acceptor that is brought close to a QD donor via an analyte-mediated binding interaction. Consequently, interaction with the analyte directly modifies the sensor's emission processes, i.e. the sensing and output regions of the biosensor are coupled: if you change one you often

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need to change the other, and this means that a biosensor that works well for one analyte often cannot easily be modified to work equally well with another.

Our detection strategy utilizes a target-induced formation of cross-linked QD lattices to detect the presence of a biomolecule. We are able to identify the presence of these lattices via differences in the fluorescence blinking of individual vs. aggregate clusters of QDs [9]. In the presence of a target DNA (i.e. K-ras with codon 12 mutation), isothermal, thermodynamically-driven nucleic acid strand displacement is initiated via ssDNA 'toehold' overhangs to form double-biotinylated double-stranded (ds) DNAs [10]. The addition of QDs to dually biotinylated ds DNAs causes the spontaneous formation of QD-DNA lattices. The assembly of the programmable nucleic acid sensor and these QD lattices were extensively characterized using native polyacrylamide gel electrophoresis (native-PAGE), agarose gel electrophoresis, and confocal fluorescence spectroscopy coupled with time correlated single photon counting.

The nucleic acid-driven biosensor (Figure 1A) is comprised of an assembled single biotinylated DNA duplex (1+2) with a 12-nucleotide toehold, along with a complementary single biotinylated ssDNA (3). The difference in Gibb's free energy (-12 kcal/mol) prevents the formation of the double-biotinylated ds DNA in the ((1+2)+3) assembly in the absence of target DNA. Upon the 'sensing' of the target DNA (4), a stabilization of -85 kcal/mol favors formation of double-biotinylated ds DNA (2+3). Finally, the addition of the QDs causes spontaneous lattice formation.

Fluorescence blinking, which is exhibited by lone quantum dots is contrasted with the quasi-continuous fluorescence of aggregated QDs, which allows for an easily distinguishable fluorescent signal when QD lattices are formed (Figure 1B). This approach is reference-free and has the potential for picomolar analyte detection limits. Furthermore, the sensing part of the biosensor is decoupled from the output part (no FRET or electron transfer) so we predict that changing the analyte to which these decoupled biosensors are selective will be much more convenient than conventional fluorescent

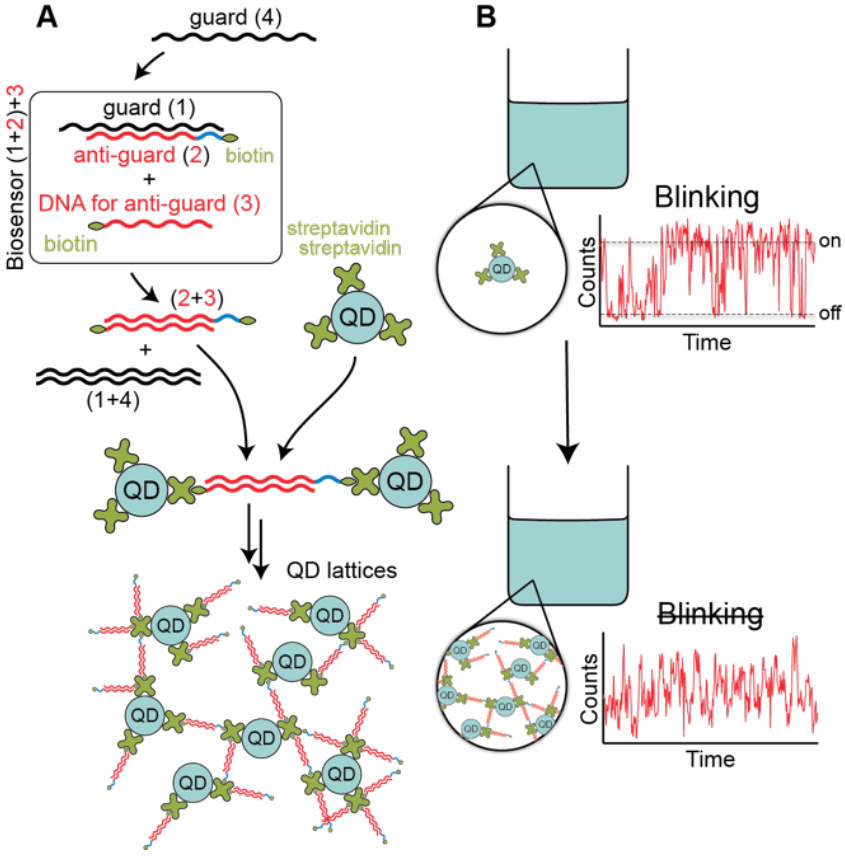

Figure 1. Explaining the concept of biosensing with fluorescent blinking. Thermodynamically driven strand displacement in the presence of target sequence results in formation of double biotinylated duplexes (in red) that, upon addition of free quantum dots (QD) promotes formation of QD lattices. Blue strand sections are required to stabilize biosensor formation.

sensors, because only the sensing region would need to be modified. Hence, this type of biosensor should be readily adaptable to a wide range of biomolecules.

Our strategy offers a robust methodology for the detection of oncogenes and, as the first reported biosensing strategy to utilize QD blinking, it opens possibilities for far more versatile, non-binary systems of detection. While utilizing the same fundamental blinking behavior change, this methodology could quantify much more complicated systems and be used in myriad ways while adding a dependable new detection strategy as a tool in the discovery of further concepts in related fields.

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