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On-chip multiplexed solid-phase nucleic acid hybridization assay using spatial profiles of immobilized quantum dots and fluorescence resonance energy transfer

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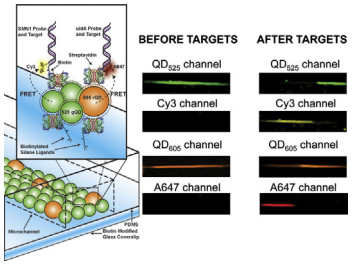
HIGHLIGHTS

- Solid-phase multiplexed QD-FRET nucleic acid assay in electrokinetic fluidic chip.
- Concurrent detection of two oligonucleotides based on channel length coverage.
- Selection of "turn-on" and "turn-off" signals from two acceptor dyes and two colors of immobilized QDs, respectively.
- No loss in assay sensitivity when implementing multiplexed assay format.

GRAPHICAL ABSTRACT



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ABSTRACT

A microfluidic based solid-phase assay for the multiplexed detection of nucleic acid hybridization using quantum dot (QD) mediated fluorescence resonance energy transfer (FRET) is described herein. The glass surface of hybrid glass-polydimethylsiloxane (PDMS) microfluidic channels was chemically modified to assemble the biorecognition interface. Multiplexing was demonstrated using a detection system that was comprised of two colors of immobilized semi-conductor QDs and two different oligonucleotide probe sequences. Green-emitting and red-emitting QDs were paired with Cy3 and Alexa Fluor 647 (A647) labeled oligonucleotides, respectively. The QDs served as energy donors for the transduction of dye labeled oligonucleotide targets. The in-channel assembly of the biorecognition interface and the subsequent introduction of oligonucleotide targets was accomplished within minutes using a combination of electroosmotic flow and electrophoretic force. The concurrent quantification of femtomole quantities of two target sequences was possible by measuring the spatial coverage of FRET sensitized emission along the length of the channel. In previous reports, multiplexed QD-FRET hybridization assays that employed a ratiometric method for quantification had challenges associated with lower analytical sensitivity arising from both donor and acceptor dilution that resulted in reduced energy transfer pathways as compared to single-color hybridization assays. Herein, a spatial method for quantification that is based on in-channel QD-FRET profiles provided higher analytical sensitivity in the multiplexed assay format as compared to single-color hybridization assays. The selectivity of the multiplexed hybridization assays was demonstrated by discrimination between a fully-complementary sequence and a 3 base pair sequence at a contrast ratio of 8 to 1.

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1. Introduction

The impact of nanomaterials on the development of bioassays and biosensing strategies continues to grow, offering numerous possibilities to achieve advantageous analytical performance [1]. One class of nanomaterials that has attracted the attention of many researchers is that of the colloidal semi-conductor nanocrystals or quantum dots (QDs). Unique optical properties of QDs include greater photostability and brightness than organic fluorophores; broad absorption spectra where a single excitation source can excite multiple colors of QDs; narrow, symmetric, size-tunable emission spectra and long photoluminescence (PL) lifetimes (>20 ns) [2]. Overall, these properties render QDs suitable for optical multiplexing when used as donors in fluorescence resonance energy transfer (FRET) to construct detection methods [3]. The use of QDs for biosensing applications has typically focused on two formats: (1) as labels, and (2) as active components of a transduction element [4]. For the latter format, FRET is a commonly used transduction method, where a ratiometric approach for analysis enables minimal variations from heterogeneity introduced by sample preparation and fluctuations in the instrumental response [5–7]. Solution-phase assays for the detection of a wide variety of target analytes ranging from metal ions [8], small molecules [9], nucleic acids [10,11] and proteases [12,13] that use QDs as energy donors in FRET have been reported. While much success has been realized through solution-phase assays, solid-phase assays have proved advantageous from the standpoint of achieving a combination of high sensitivity [14], reusability and regeneration [15].

A number of solid-phase platforms for the immobilization of selective chemistry for assay development, including microsphere beads [16,17], glass beads [15], optical fibers [18–22], paper [23,24], microtitre plates [25] and microfluidics [14,26], that use QDs as either labels or as donors in FRET have been reported. Multiplexing has also been achieved in these solid-phase assays using either spatial registration [27], spectral barcoding [16,17] or spectral discrimination [18,28,29]. Examples of solid-phase multiplexed assays include protease assays using QDs as donors and gold nanoparticles as acceptors on the surface of a glass slide [30]; multiplexed detection of nucleic acid hybridization using a mixed film of immobilized multicolor QDs (donors) and oligonucleotide probes on the surface of an optical fiber [18] and a microtitre plate [25]; and multiplexed toxin analysis in single wells of a microtiter plate using QDs as labels [29].

Integration of microfluidics for the development of assays offers advantages that include relatively low cost [31], fast analysis times [32], small sample volumes, portability and improved sensitivity [33]. Only a limited number of studies have reported the integration of QD-based multiplexed solid-phase assays within microfluidic systems [27,34]. Typically QDs have been used as labels, and the fluidic network offers containment of sample solution to achieve proximity with selective chemistry that is immobilized on the surface of microsphere beads. For example, Jokerst et al. [27] reported on the implementation of sandwich-based multiplexed immunoassay within a microfluidic chip using multicolor QDs as labels for the detection of three cancer markers. The selective chemistry was immobilized on agarose beads and captured within a chip using microstructures, where the spatial registration of the agarose beads provided multiplexing capability. In another example, Hu et al. [34] used microfluidics for surface patterning of capture antibodies and QD-labeled secondary antibodies for selective and sensitive multiplexed detection of femtomolar concentrations of two cancer biomarkers in serum. Multiplexed detection of nucleic acid hybridization using QDs as labels and integration with on-chip detection has also been demonstrated using spectral barcoding, where microbeads were infused with multicolor QDs at different ratios to yield a distinctive spectral signature for each analyte [17].

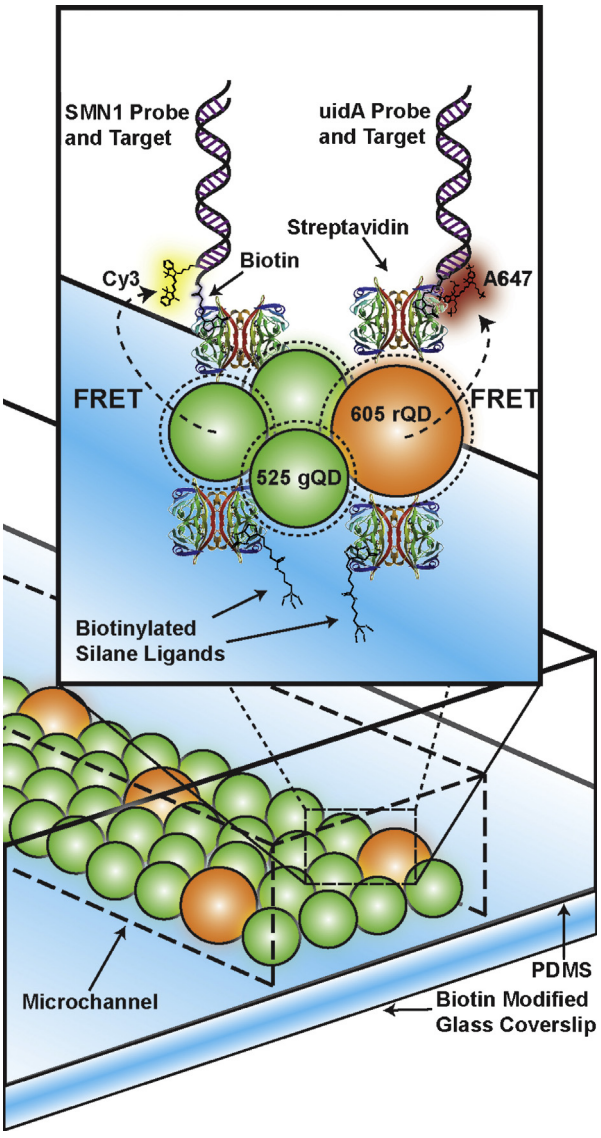

Fig. 1. Representation of a cross-sectional area of a hybrid glass/PDMS based microfluidic channel showing the design of the solid-phase multiplexed nucleic acid hybridization assay using immobilized multicolor QDs as donors in a FRET based transduction scheme. Streptavidin conjugated gQDs and rQDs were immobilized on a biotin modified glass surface, and subsequently conjugated with two types (SMN1 and uidA) of biotinylated probe oligonucleotides. Hybridization with Cy3 labeled SMN1 and A647 labeled uidA target oligonucleotides provided the proximity for FRET sensitized emission from Cy3 and A647 dyes upon excitation with a 402 nm diode laser.

The utility of QDs in microfluidics has been limited to only labels for solid-phase assays and no examples of multiplexed solid-phase QD-FRET assays integrated within microfluidics have been described.

Herein, we build on our earlier study using a single QD-FRET system to transduce oligonucleotide targets [14], and investigate a solid-phase assay for the multiplexed interrogation of nucleic acid hybridization within an electrokinetically operated microfluidic chip. The design of the transduction interface is depicted in Fig. 1. The glass surface of a hybrid glass/polydimethylsiloxane (PDMS) microfluidic chip was chemically modified with a biotin functionality to immobilize streptavidin-coated green-emitting CdSe/ZnS QDs (gQDs) and red-emitting CdSe/ZnS QDs (rQDs). The in-channel delivery of the two colors of QDs was done using electroosmotic flow (EOF). The surface of the QDs were subsequently bioconjugated with two types of biotin terminated oligonucleotide probe

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