Multiway study of Fluorescence Resonance Energy Transfer in nanoscale semiconductor labeled DNA

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Abstract
This study is application of bulk measurement in simple, rapid and sensitive detection of DNA, which is critical in diagnosing genetic diseases. Most DNA detection systems (microarrays, for example) regardless of their need for target amplification require separation of unhybridized DNA strands from hybridized strands immobilized on a solid substrate. Here, we demonstrate a hybridization detection method using oligonucleotide functionalized quantum dots as nanoprobes and second order spectrofluorimetric Excitation-Emission data (2D-PLE). In this study, two nucleic-acid functionalized CdSe/ZnS quantum dots (QDs), each with discernible emission wavelengths, at 605 and 705 nm, were designed to hybridize in juxtaposition with the complementary oligonucleotide target capC which was one of the three anthrax-related genes to form a sandwiched nanoassembly. In this system there was no need to functionalize the target DNA. The resulting assembly brings the QD acceptor and the QD donor in to proximity, leading to fluorescence emission from the high energy QD (donor) by means of Fluorescence Resonance Energy Transfer (FRET) on illumination of the low energy QD (acceptor). So, hybridization was monitored by following the fluorescence resonance energy transfer. As a result, detection of sandwiched nanoassembly as a third component indicated the presence of target. Using highly informative 2D-PLE data and applying PARAFAC method to deal with such data an estimate of concentration and spectral profiles were obtained. In the next step using model based chemometric methods, complex formation constants of both double and ternary complex forms were calculated. This is the first report on determination of complex formation constants of this sandwiched nanoassemblies.

Experimental

Part A

Figure 1. QD nanoprobes prepared by surface-functionalizing QDs with target-specific oligonucleotide probes. Two target-specific QD nanoprobes with different emission wavelengths sandwich a target, forming a QD probe target nanoassembly. The nanoassembly is detected as changes in emission of two probes due to the Fluorescence Resonance Energy Transfer (FRET) of the both QD nanoprobes.

Figure 2a. 2D-PLE of probe QD605 conjugated with DNA
Figure 2b. 2D-PLE of probe conjugated with DNA
Figure 2c. 2D-PLE of nanosynthesis of two probes and target DNA.

Data Recording

Part A

Two QD-DNA conjugate nanoprobes and an unmodified target DNA brought the two QD probes within a distance on the order of the forster radius of the QD-QD FRET pair. As a consequence, FRET-sensitized QD fluorescence was observed.

Part B: Titration Data

To consider this FRET process precisely, the hybridization-FRET-sensitized fluorescence of sandwich nanoassembly followed as a function of target concentration.

Reference