Cascaded FRET in Conjugated Polymer/Quantum Dot/Dye-Labeled DNA Complexes for DNA Hybridization Detection

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As the human genome project has uncovered the full sequence of human genomes and the postgenome technologies have been rapidly developed, DNA hybridization detection has become increasingly important.1 A variety of DNA detection formats are now available, including both heterogeneous assays with surface-bound array-based probes and homogeneous solution-based detection schemes.2 A number of those rely on fluorescence signal and, in particular, on fluorescence (or Förster) resonant energy transfer (FRET).3 Both conjugated polymers and semiconductor nanocrystals (colloidal quantum dots, QDs) have been suggested for use as fluorescent entities in DNA detection assays.4–12 Assays based on water-soluble cationic conjugated polymers allow one to utilize a simple binding strategy to DNA through electrostatic interactions with ionic side groups and benefit from the light-harvesting properties along the polymer backbone.4–8 QDs have been increasingly used as donors in FRET-related studies, including optical DNA diagnostics.9–12

QDs are photostable, highly efficient fluorophores with a strong band gap luminescence tunable by size as a result of the quantum confinement effect.13 FRET in QD/DNA/organic dye conjugates has been used to study DNA hybridization,14,15 cleavage,16 and replication.17 Most of the reported approaches required covalent conjugation of DNA to the QD surface (e.g., by EDC/NHS coupling reaction), which often decreases the colloidal stability and/or the emission intensity of QDs.18,19 This limitation can be overcome by the use of a sensing platform relying on the electrostatic interaction of dye-labeled DNA with the oppositely charged QD surface in a similar fashion as in conducting polymer-based detection assays.4–8

In this paper, we propose a simultaneous use of conjugated polymers and semiconductor QDs for DNA hybridization detection, which can potentially combine advantages of both light-harvesting and DNA-binding properties of water-soluble polymers with photostability, light-harvesting ability, and FRET donor property of QDs. A blue-emitting conjugated polymer with positively charged side chains electrostatically self-assembles on the negatively charged surface of red-emitting CdTe QDs. The so-formed hybrid complex has a net positive charge and thus attracts negatively charged dye-labeled DNA molecules (Scheme 1). The water-soluble, cationic, conjugated polymer, poly[9,9-bis(3′-((N,N-dimethyl)-N-ethylammonium)propyl)-2,7-fluorene-alt-1,4-phenylene]dibromide (PDFD), and water-soluble CdTe quantum dots (QDs) are designed to provide a cascaded FRET for DNA hybridization detection. PDFD has two functions in the detection scheme: as a light-harvesting antenna, it enhances the emission of QDs by the first level FRET and inverts the sign of the surface charge of QDs, thus providing a positively charged surface to allow negatively charged dye-labeled DNA to interact with the resulting complex. This interaction causes the second level FRET to infrared-emitting dye labeled on the probe DNA, providing a reliable signal-on sensing platform discriminating between complementary and non-complementary DNA. A detailed spectroscopic study offers a clear description of photophysical processes in the designed polymer/QD/DNA complex, providing ample potential for further sensitivity and selectivity improvements.

KEYWORDS: homogeneous assay · DNA hybridization · semiconductor nanocrystals · conjugated polymers · FRET

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dimethyl-N-ethylammonium)propyl)-2,7-fluorene-alt-1,4-phenylene]dibromide (PDFD), is synthesized by a previously reported procedure with slight modifications.21 CdTe QDs (3.2 nm diameter) are synthesized directly in water as previously reported using thioglycolic acid (TGA) molecules as surface ligands (Scheme 1).22 TGA-capped CdTe QDs are strongly emitting room temperature quantum yield of the sample used here is 43%, stable in water and common buffers,23 as the complexes reported here are, and negatively charged at slightly basic pH.24 Fluorescent labeled DNA is used to monitor the interaction of the PDFD/QD complex and DNA through a double FRET process (Scheme 1): FRET 1 from PDFD to QDs and FRET 2 from QDs to the dye on the DNA. The infrared dye IRD700 is selected as the acceptor dye because its absorbance has no spectral overlap with the emission of PDFD but a sufficient overlap with the emission of QDs (Figure 1a). A detailed spectroscopic study reported here provides a clear description of photophysical processes in the designed polymer/QD/DNA complex.

RESULTS AND DISCUSSION

Upon titration of PDFD solution with CdTe QDs, emission of PDFD is quenched with a Stern–Volmer quenching constant of $1.2 \times 10^8$ M$^{-1}$ and almost disappears at the ratio of five PDFD molecules per QD. The Stern–Volmer constant provides a measure for PL quenching efficiency and points out the formation of the PDFD/QD complex.6 At the same time, the QD photoluminescence (PL) is enhanced due to the FRET 1. The binding of PDFD and QDs is confirmed by the inversion of electrophoretic mobility of the colloidal solution from $3.89 \times 10^{-8}$ to $1.57 \times 10^{-8}$ m$^2$/Vs upon titration of PDFD with QDs. The PDFD and QD concentrations are fixed at $2.5 \times 10^{-7}$ and $5 \times 10^{-8}$ M, respectively, for further measurements.

The occurrence of energy transfer between the PDFD and QDs in the PDFD/QD complex (FRET 1) as well as between the PDFD/QD complex and IRD700 dye (FRET 2) is confirmed by PL excitation (PLE) spectroscopy (Figure 1b). First, by comparing the behavior of bare QDs and QDs in the PDFD/QD complex, it can be clearly seen how the excitation of QDs increases when the absorption of PDFD sets at wavelengths below 425 nm, demonstrating the presence of the FRET 1 process. Second, in the PDFD/QD/DNA complex, the shape of the PLE spectra of the IRD700-labeled double-stranded (ds) DNA perfectly matches the one of QDs, revealing the existence of the FRET 2 process. The energy transfer from the QDs to the dye-labeled DNA is evidenced by the faster decay of the QD emission as the concentration of dye-labeled DNA increases (Figure 2a). At the same time, the emission of the IRD700 dye becomes slower due to the delayed excitation provided by feeding from QDs through FRET 2 (Figure 2b).

In order to quantify efficiency of the FRET 1 from PDFD to QDs, steady-state absorption and PL measurements have been performed on bare QDs and on a PDFD/QD complex. Considering the PL increase of QDs upon addition of PDFD, the FRET 1 efficiency can be calculated using the following relation:

$$\Phi_{\text{FRET1}} = \frac{A_{\text{QD}}(\lambda_{\text{exc}})}{A_{\text{PDFD/QD}}(\lambda_{\text{exc}})} = \frac{I_{\text{QD}}(\lambda_{\text{QD}})}{I_{\text{QD}}(\lambda_{\text{QD}}) - 1}$$

where $A_{\text{QD}}(\lambda_{\text{exc}})$ and $A_{\text{PDFD/QD}}(\lambda_{\text{exc}})$ are absorption of the acceptor (bare QDs) and of the donor–acceptor pair (PDFD/QD composite), respectively, at the wavelength of excitation $\lambda_{\text{exc}}$. $I_{\text{QD}}$ and $I_{\text{QD}}(\lambda_{\text{QD}})$ are PL intensities of the QDs in absence and presence of the PDFD, respectively. Applying eq 1 to the absorption and PL data, $\Phi_{\text{FRET1}}$ equals 72%. Time-resolved PL measurements considered in de-
tail in ref 25 have confirmed that the FRET 1 from the PDFD to the QDs is a very fast (\( \sim 50 \) ps) process. The efficiency of the FRET 2 from QDs to infrared dye was estimated by monitoring the reduction of the PL intensity of QDs caused by energy transfer to dye molecules using the following relation:

\[
\Phi_{\text{FRET2}} = 1 - \frac{I_{\text{QD}}}{I_{\text{QD0}}} \tag{2}
\]

where \( I_{\text{QD0}} \) and \( I_{\text{QD}} \) are the PL intensities of QDs in absence and presence of 5 nM of a complementary ds-DNA, respectively. Applying eq 2 to the measured absorption and PL spectra, we found \( \Phi_{\text{FRET2}} \) to be 42%. Assuming random orientations of dipole moments of donors and acceptors, we estimated a Förster radius of 5.8 nm for the PDFD/QD pair and 5.1 nm for the QD/dye pair.

To demonstrate the ability of PDFD/QD complex for DNA hybridization detection, the former is titrated with (i) probe single-stranded (ss) DNA labeled with IRD700 dye, (ii) probe DNA incubated with a non-complementary DNA, and (iii) probe DNA incubated with a complementary DNA to produce ds-DNA. Figure 3a shows PL spectra of the PDFD/QD complex upon incubation with an increasing amount of IRD700-labeled complementary ds-DNA, which displays a progressive loss in QD emission alongside with an increase of the emission intensity of IRD700 dye, being consistent with FRET 2 from PDFD/QD complex to the dye. The ratio of the emission intensities of IRD700 dye and QD (Figure 3b) is proportional to the concentration of DNA, thus allowing a quantitative assay. Importantly, the proportionality constant for complementary DNA is 2.5 times larger than for ss-DNA, allowing us to unambiguously detect the DNA hybridization event. This is a consequence of the higher local charge density of ds-DNA that leads to a stronger electrostatic binding to the PDFD/QD complex. The detection limit of our system as demonstrated here (\(<5\) nM) favorably compares with previously reported values for QD-based DNA hybridization assays.\(^{15,26}\) The dual use of both conjugated polymer and QD extinction offers a potential to lower this limit to picomolar range.\(^{4,5,8}\)

**CONCLUSION**

In summary, a facile approach has been demonstrated for the detection of DNA hybridization, which is based on a cascaded double FRET from a complex of conjugated polymer and CdTe QDs to the dye-labeled DNA. The conjugated polymer provides a dual advantage of (i) a light-harvesting antenna enhancing the emission of QDs and thus potentially offering improved assay sensitivity and (ii) inverting the sign of originally negatively charged QDs and thus providing a positively charged counterpart for negatively charged DNA molecules to electrostatically adsorb on polymer/QD complexes. The difference in electrostatic interaction strength for single-stranded and double-stranded DNA results in different slopes of a linearly increasing ratio of dye-to-QD emission intensities, providing a reliable signal-on sensing platform for the detection of hy-
Our detailed spectroscopic analysis offers a clear description of FRET processes taking place in the polymer/QD/DNA complex and offers ample potential for future sensitivity improvements of the detection platform proposed. It can also be useful for the development of an alternative strategy with an improved selectivity of DNA detection, with cascaded FRET processes taking place to intercalated dyes, thus amplifying optical response from ethidium bromide (EB)-based DNA protocols.

REFERENCES AND NOTES


