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COMMUNICATION

CdSe/ZnS core shell quantum dot-based FRET binary oligonucleotide probes for detection of nucleic acids†‡

Yiru Peng,§[a,c] Chunmei Qiu,§[b] Steffen Jockusch,[a] Amy M. Scott,[a] Zengmin Li,[b] Nicholas J. Turro*[a] and Jingyue Ju*[b]

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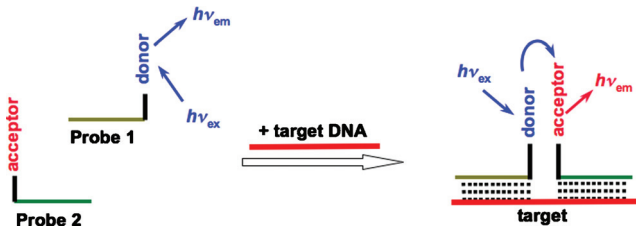
We report the design, synthesis, and characterization of a binary oligonucleotide probe for selective DNA or RNA detection. The probe is based on fluorescence resonance energy transfer (FRET) from quantum dot (CdSe/ZnS core shell) DNA conjugates to organic dye (cyanine-5) DNA conjugates. Selective hybridization of the donor/acceptor DNA conjugates to target DNA enhances FRET and a change in fluorescence signature was observed.

Major advances on nucleic acid sequence detection that combine high quantum yield photoluminescence materials with hybridization techniques have been reported over the last decades. Among the most effective sensors for DNA detection are binary probes (BP).[1,2] BPs consist of donor and acceptor fluorophores that are attached to two different oligonucleotide sequences and serve as a fluorescence resonance energy transfer (FRET) donor–acceptor pair when hybridized to adjacent sites of a target DNA sequence (Scheme 1). In the absence of target DNA, fluorescence from the donor is observed. However, in the presence of target DNA hybridization brings both parts of the BP together and FRET becomes an efficient quenching pathway of the donor fluorescence leading to acceptor fluorescence (Scheme 1). The selective hybridization of the probes to the target produces a unique fluorescence signature that can be used for detecting target DNA or RNA *in vitro* and *in vivo*.

Several different donor–acceptor pairs have been described for BPs. Among the most popular are organic fluorescence dyes such as rhodamine–cyanine systems.[1,2] Disadvantages of organic dyes are their inferior photostability and short fluorescence lifetime (~4 ns). The use of metal complexes with long-lived excited state lifetimes, such as ruthenium complexes as a donor, allowed to generate delayed acceptor luminescence.[3]



Scheme 1 Schematics of a binary probe (BP) for target DNA detection.

Ruthenium(II)-polypyridyl complexes possess long-lived luminescent metal to ligand charge transfer excited states and energy transfer from these excited states to singlet exited states of organic dyes, such as a cyanine dye (Cy5), is spin forbidden. Although spin forbidden energy transfer is slow, the long excited state lifetime of ruthenium complexes (~1 μs) easily compensates, leading to efficient energy transfer. The slower energy transfer rate and long donor excited state lifetime generates delayed acceptor fluorescence of the organic dye. This delayed acceptor fluorescence allows background luminescence suppression by time-gated luminescence detection.[3] However, a disadvantage of these metal complex–organic dye energy transfer systems is the relatively low absorption cross section of the metal complexes.

Quantum dots (QDs) are luminescing semiconductor nanocrystals, such as CdSe, CdS, CdTe, and ZnS. QDs show some superior properties compared to organic dyes and metal complexes, such as large absorption cross section, size-tunable emission wavelength, narrow emission bandwidth, high luminescence quantum yields, long emission lifetimes and excellent photostability.[4] Because of these favorable properties, QDs have attracted great interest for medical diagnostic, drug delivery, DNA-based nanosensors and solar energy conversion.[5–9] Among the large variety of different QDs, CdSe/ZnS core shell nanocrystals[10] appear to be most promising candidates for constructing BPs.

In this report, we describe the design, synthesis and *in vitro* testing of a BP with a QD-based donor and an organic dye based acceptor (cyanine dye, Cy5) (Scheme 2). The existing methods for the construction of QD-DNA conjugates include streptavidin-biotin interaction,[11,12] glycosidic bonding,[13] electrostatic interaction,[14] metal-thiol bonding,[15] and carbodiimide reaction.[16,17] Because of the large size of QDs in addition to the necessary protective coating of QDs, long spacers for linking are

[a]*Department of Chemistry, Columbia University, New York, NY, 10027, USA*
[b]*Department of Chemical Engineering, Columbia University, New York, NY, 10027, USA*
[c]*College of Chemistry & Material Sciences, Fujian Normal University, Fuzhou, 350007, China*

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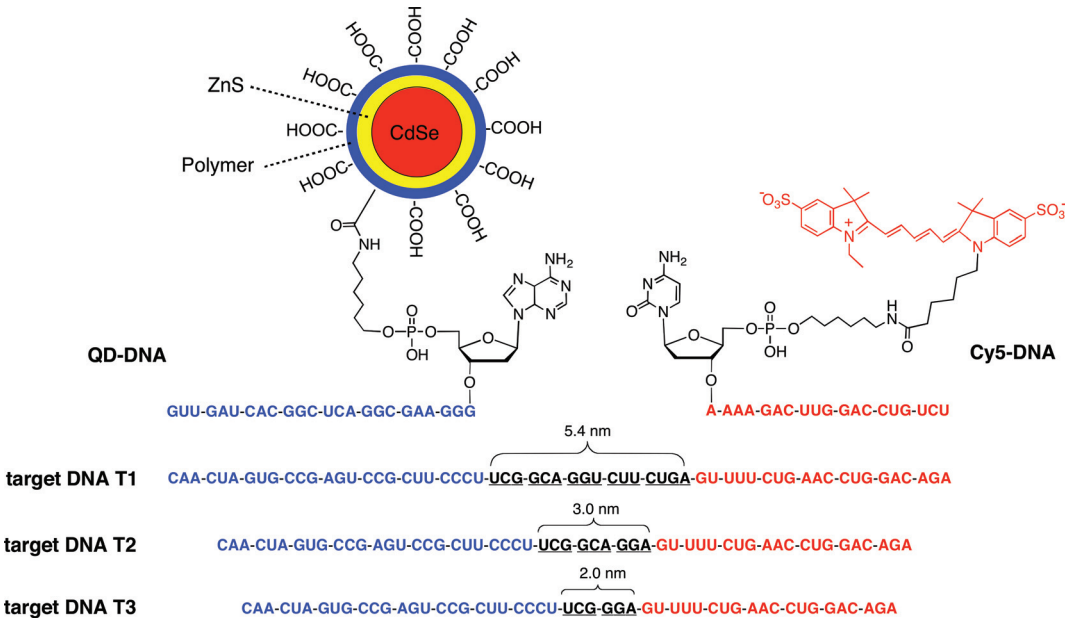
§ Equal contribution.



Scheme 2 Structures of binary probes and target DNA T1–T3.

undesirable, because of reduced energy transfer efficiencies. Therefore, we selected a short linking strategy through carbodiimide reaction.[18] Commercially available CdSe/ZnS core shell nanocystals with a polymer coating and carboxylic acid external surface (Qdot605, Invitrogen) were covalently linked to the 3′ end of amino-modified oligonucleotides through carbodiimide coupling chemistry (see ESI for details‡). The QD-DNA conjugates were assessed by gel electrophoresis. Compared with free QD bands in the chromatograms, a new band which exhibited decreased mobility corresponding to the QD-DNA conjugate was clearly resolved (Fig. S1, ESI‡). As acceptor, the organic dye Cy5 was selected because of its good transparency at the QD excitation wavelength (460 nm) and good overlap of Cy5 absorption with Qdot605 emission, which is required for good FRET efficiencies. The Förster distance, the donor–acceptor distance with 50% energy transfer efficiency, was calculated from the spectral overlap integral of donor (QD) fluorescence and acceptor (Cy5) absorbance according to established procedures (see ESI for details‡).[19,20] The estimated Förster distance in our FRET system is 6.9 nm and is in the typical range of other BP systems involving organic dye donor/acceptor pairs. The Cy5-DNA conjugates were synthesized as described in ESI.‡

Steady-state fluorescence analysis was performed to assess the performance of the BP for target DNA analysis. Fig. 1 shows the fluorescence spectrum of an aqueous Tris buffer solution[21] of QD-DNA and Cy5-DNA with an excitation wavelength of 460 nm. In the absence of target DNA (spectrum a) the fluorescence spectrum is dominated by the QD luminescence at 605 nm. The very weak fluorescence at 667 nm originates from Cy5 and is caused by weak direct absorption of excitation light (460 nm) by Cy5. In the presence of target DNA the fluorescence spectrum changes significantly (Fig. 1b). The luminescence intensity of QD decreased to approximately 1/5 of its original intensity in the absence of target and strong fluorescence of Cy5 was observed at 667 nm. The inset of Fig. 1 shows the

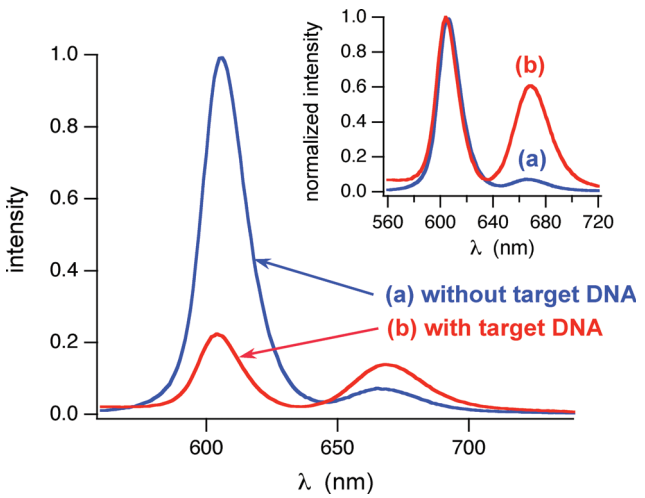


Fig. 1 Fluorescence spectra of aqueous buffer solutions of QD-DNA (0.2 μM) and Cy5-DNA (5 μM) in the absence (a) and presence (b) of target DNA T2 (5 μM). Excitation wavelength 460 nm. The inset shows normalized spectra.

two fluorescence spectra normalized to the QD fluorescence to demonstrate the significant change in fluorescence signature upon addition of target DNA. The FRET efficiency between the QD donor and Cy5 acceptor and the efficiency of target detection was evaluated by the contrast ratio (signal to background ratio; S/B) using eqn (1):

$$\mathrm{S/B} = (I_{667}\ \text{with target}/I_{667}\ \text{without target})/(I_{605}\ \text{with target}/I_{605}\ \text{without target}) \quad (1)$$

In eqn (1) I_{667} and I_{605} are the fluorescence intensities of Cy5 and QD, respectively, in the presence and absence of target.

Table 1 Fluorescence lifetimes (τ) and corresponding fluorescence contribution (%) of binary probes in absence and presence of target DNA

QD-DNA[a] + Cy5-DNA[b]	τ/ns[cf] [λ_{ex} 460 nm, λ_{em} 605 nm] (contribution, %)	τ/ns[df] [λ_{ex} 460 nm, λ_{em} 667 nm] (contribution, %)	τ/ns[ef] [λ_{ex} 659 nm, λ_{em} 667 nm] (contribution, %)
No target	11 (84) 3.2 (16)	1.4 (87) 5.1 (13)	1.5
+T3	10 (87) 1.9 (13)	1.4 (77) 3.4 (23)	1.7
+T2	8.2 (80) 0.6 (20)	1.4 (80) 3.5 (35)	1.7
+T1	7.3 (76) 0.8 (23)	1.4 (87) 4.4 (13)	1.6

[a] 0.2 μM. [b] 5 μM. [c] QD excitation/QD emission. [d] QD excitation/Cy5 emission. [e] Cy5 excitation/Cy5 emission. [f] Error limit 10%.

A contrast ratio of S/B = 8 was observed, which is similar to BPs using organic dyes.

The hybridization kinetics was followed by steady-state fluorescence spectroscopy in order to determine the optimum incubation time for hybridization of BP with the target DNA (Fig. S3, ESI‡). A complete hybridization process required about 5 h. The hybridization time is relatively long and may be due to the high density on the QD surface which might slow down the hybridization process.

FRET efficiency is known to depend strongly on the distance between the donor (QD) and acceptor (Cy5). This distance dependence was studied by varying the number of DNA bases in the target DNAs T1, T2 and T3 between the donor and acceptor (Scheme 2). Steady-state fluorescence spectra for three different DNA targets with varying spacing, T1 (16 bases), T2 (9 bases), and T3 (6 bases), were recorded after hybridization with BPs for 5 h. The S/B ratio increased as the number of bases between the QD donor and Cy5 acceptor increased, with T1 having a S/B ratio of 9 and the longest distance of 5.4 nm (16 bases) and with T3 having a S/B ratio of 5.5 (shortest distance, 2.0 nm, 6 bases). Because of the large size of QD (~16 nm) and high density of DNA on the QD surface, probably a larger distance is favorable for QD hybridization with Cy5 due to the lowest steric hindrance. In addition, the largest spacer length (5.4 nm in T1) is still well below the Förster distance of the QD/Cy5 pair (6.9 nm).

CdSe/ZnS core shell QDs possess significantly longer fluorescence lifetimes (19–65 ns)[10] than organic dyes (1–10 ns). To test if this longer fluorescence lifetime of a QD (donor) can generate delayed fluorescence of Cy5 (acceptor) due to slow FRET, we performed fluorescence lifetime studies using time correlated single photon counting. Fig. 2 shows fluorescence decay traces at 605 nm (QD emission) after excitation with light pulses at 460 nm. The fluorescence of QD-DNA conjugates in buffer solution shows biexponential decays with lifetimes of 3.2 ± 0.3 ns and 10 ± 1 ns. As expected, upon addition of Cy5-DNA, the fluorescence decay of the QD remained nearly the same with a lifetime of 3.2 ± 0.3 ns and 11 ± 1 ns (Fig. 2a). However, in the presence of target DNA, T1, T2, and T3, the fluorescence of the QD decayed significantly faster (Fig. 2b–d). The fastest decay was observed in the presence of T1 (largest distance between donor and acceptor) suggesting most efficient energy transfer to Cy5. This fastest decay is consistent with the highest contrast ratio in steady-state fluorescence measurements (S/B = 9 for T1). Table 1 summarizes the fluorescence lifetimes of the

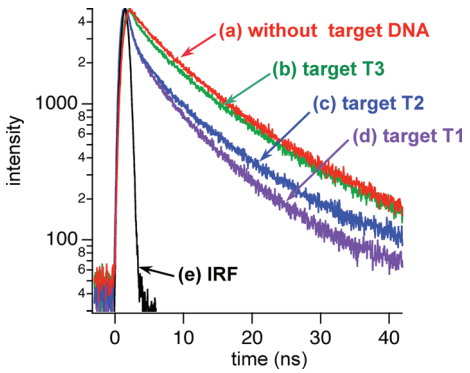


Fig. 2 Decay traces of QD fluorescence (monitored at 605 nm) after excitation with light pulses (460 nm) of aqueous buffer solutions of QD-DNA (0.2 μM) and Cy5-DNA (5 μM) in the absence (a) and presence (b–d) of target DNA (5 μM). IRF: instruments response function (e).

biexponential fits and their corresponding contributions to the fluorescence signal. As can be seen in the second column, the biexponential fits show the shortest lifetime values in the presence of T1 (7.3 ns and 0.8 ns) with the short lifetime component (0.8 ns) having a larger contribution (23%) compared to T2, T3 and in the absence of target. This is indicative of fast energy transfer kinetics. The energy transfer kinetics can be examined from the kinetics of Cy5 fluorescence (monitored at 667 nm) after excitation of QD at 460 nm. The fluorescence decays of Cy5 fitted a biexponential kinetic with lifetimes of 1.4 to 5 ns and showed little variation with different targets (Table 1 and Fig. S5; ESI‡). The fast fluorescence kinetics of Cy5 indicates a rapid energy transfer from QD to Cy5 which generates only a minor delay in Cy5 fluorescence.

In conclusion, QD-DNA conjugates are easily accessible by covalently linking carboxylic acid terminated QDs with DNA through a carbodiimide linkage. We showed that binary hybridization probes for DNA analysis can be constructed from these QD-DNA conjugates (donor) in conjunction with Cy5-DNA (acceptor). Selective hybridization of the donor/acceptor DNA conjugates to target DNA enables fluorescence resonance energy transfer and changes the fluorescence signature significantly. A contrast ratio of S/B = 9 in fluorescence signature was observed under our conditions. This high contrast ratio together with other advantages of QD compared to conventional organic fluorophores should make these QD-based binary probes attractive for DNA and RNA analysis.

Acknowledgements

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References

1 A. A. Marti, S. Jockusch, N. Stevens, J. Ju and N. J. Turro, Fluorescent hydrization probes for sensitive and selective DNA and RNA detection, *Acc. Chem. Res.*, 2007, **40**, 402–409.
2 D. M. Kolpashchikov, Binary probes for nucleic acid analysis, *Chem. Rev.*, 2010, **110**, 4709–4723.
3 A. A. Marti, C. A. Puckett, J. Dyer, N. Stevens, S. Jockusch, M. Stevens, J. Ju, J. K. Barton and N. J. Turro, Inorganic–organic hybrid luminescent binary probe for DNA detection based on spin-forbidden resonance energy transfer, *J. Am. Chem. Soc.*, 2007, **129**, 8680–8681.
4 U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke and T. Nann, Quantum dots *versus* organic dyes as fluorescent labels, *Nat. Methods*, 2008, **5**, 763–775.
5 L. Shi, V. D. Paoli, N. Rosenzweig and Z. Rosenzweig, Synthesis and application of quantum dots FRET-based protease sensors, *J. Am. Chem. Soc.*, 2006, **128**, 10378–10379.
6 C.-Y. Zhang, H.-C. Yeh, M. T. Kuroki and T.-H. Wang, Single-quantum-dot-based DNA nanosensor, *Nat. Mater.*, 2005, **4**, 826–831.
7 E. R. Goldman, I. L. Medintz, J. L. Whitley, A. Hayhurst, A. R. Clapp, H. T. Uyeda, J. R. Deschamps, M. E. Lassman and H. Mattoussi, A hybrid quantum dot-antibody fragment fluorescence resonance energy transfer-based TNT sensor, *J. Am. Chem. Soc.*, 2005, **127**, 6744–6751.
8 I. L. Medintz and H. Mattoussi, Quantum dot-based resonance energy transfer and its growing application in biology, *Phys. Chem. Chem. Phys.*, 2009, **11**, 17–45.
9 W. R. Algar and U. J. Krull, Quantum dots as donors in fluorescence resonance energy transfer for the bioanalysis of nucleic acids, proteins, and other biological molecules, *Anal. Bioanal. Chem.*, 2007, **391**, 1609–1618.
10 H.-M. Gong, Z.-K. Zhou, H. Song, Z.-H. Hao, J.-B. Han, Y.-Y. Zhai, S. Xiao and Q.-Q. Wang, The influence of surface trapping and dark states on the fluorescence emission efficiency and lifetime of CdSe and CdSe/ZnS quantum dots, *J. Fluoresc.*, 2007, **17**, 715–720.
11 Y. Xiao and P. E. Barker, Semiconductor nanocrystal probes for human metaphase chromosomes, *Nucleic Acids Res.*, 2004, **32**, 28e.
12 N. C. Cady, A. D. Strickland and C. A. Batt, Optimized linkage and quenching strategies for quantum dot molecular beacons, *Mol. Cell. Probes*, 2007, **21**, 116–124.
13 S. Pathak, S.-K. Choi, N. Arnheim and M. E. Thompson, Hydroxylated quantum dots as luminescent probes for in situ hybridization, *J. Am. Chem. Soc.*, 2001, **123**, 4103–4104.
14 M. Artemyev, D. Kisiel, S. Abmiotko, M. N. Antipina, G. B. Khomutov, V. V. Kislov and A. A. Rakhnyanskaya, Self-organized, highly luminescent CdSe nanorod-DNA Complexes, *J. Am. Chem. Soc.*, 2004, **126**, 10594–10597.
15 I. L. Medintz, L. Berti, T. Pons, A. F. Grimes, D. S. English, A. Alessandrini, P. Facci and H. Mattoussi, A reactive peptidic linker for self-assembling hybrid quantum dot-DNA bioconjugates, *Nano Lett.*, 2007, **7**, 1741–1748.
16 X. Wang, X. Lou, Y. Wang, Q. Guo, Z. Fang, X. Zhong, H. Mao, Q. Jin, L. Wu, H. Zhao and J. Zhao, QDs-DNA nanosensor for the detection of hepatitis B virus DNA and the single-base mutants, *Biosens. Bioelectron.*, 2010, **25**, 1934–1940.
17 D. Zhou, L. Ying, X. Hong, E. A. Hall, C. Abell and D. Klenerman, A compact functional quantum dot-DNA conjugate: preparation, hybridization, and specific label-free DNA detection, *Langmuir*, 2008, **24**, 1659–1664.
18 H. Shen, A. M. Jawaid and P. T. Snee, Poly(ethylene glycol) carbodiimide coupling reagents for the biological and chemical functionalization of water-soluble nanoparticles, *ACS Nano*, 2009, **3**, 915–923.
19 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum Publishers, New York, 1999, 368 pp.
20 N. Stevens, J. Dyer, A. A. Marti, M. Solomon and N. J. Turro, FRET-View: a computer program to simplify the process of obtaining fluorescence energy transfer parameters, *Photochem. Photobiol. Sci.*, 2007, **6**, 909–911.
21 Tris buffer: 10 mM Tris-HCl, 100 mM NaCl, 1 μM EDTA, pH 7.4.

