Fluorescence Resonance Energy Transfer in CdSe/ZnS−DNA Conjugates: Probing Hybridization and DNA Cleavage

Ron Gill, Itamar Willner,* Itzhak Shweky, and Uri Banin*

Institute of Chemistry and the Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received: August 29, 2005; In Final Form: September 22, 2005

Nucleic-acid-functionalized CdSe/ZnS quantum dots (QDs) were hybridized with the complementary Texas-Red-functionalized nucleic acid. The hybridization was monitored by following the fluorescence resonance energy transfer from the QDs to the dye units. Treatment of the QD/dye DNA duplex structure with DNase I resulted in the cleavage of the DNA and the recovery of the fluorescence properties of the CdSe/ZnS QDs. The luminescence properties of the QDs were, however, only partially recovered due to the nonspecific adsorption of the dye onto the QDs. Similarly, nucleic-acid-functionalized Au nanoparticles (Au NPs) were hybridized with the complementary Texas-Red-labeled nucleic acid. The hybridization was followed by the fluorescence quenching of the dye by the Au NPs. Treatment of the Au NP/dye DNA duplex with DNase I resulted in the cleavage of the DNA and the partial recovery of the dye fluorescence. The incomplete recovery of the dye fluorescence originated from the nonspecific binding of the dye units to the Au NPs. The nonspecific binding of the dye to the CdSe/ZnS QDs and the Au NPs is attributed to nonprotected surface vacancies in the two systems.

The coupling of biomolecules and semiconductor (SC) nanoparticles (quantum dots) attracts substantial research efforts directed to the development of new imaging, labeling, and sensing systems.[1] This is motivated by the favorable fluorescence properties of semiconductor QDs as compared to conventional organic dyes. The QDs exhibit narrow emission bands that are tunable by their sizes, compositions, and shapes. Due to their continuous absorption, a single laser may be used to excite multiple colored QDs, a significant advantage over the multiple light sources required to excite a series of organic dyes. Furthermore, the stability of QDs toward photodegradation and photobleaching turns the particles to be an attractive material for analytical purposes. These advantageous luminescence properties of SC quantum dots (QDs) were used to develop fluorescence labels for immuno-assays,[2] for the optical coding of mammalian cells,[3] and for the in vivo targeting and imaging of cancer cells.[4]

A quantum dot release in response to a target analyte was demonstrated as a biosensing concept for intracellular QD sequestering and delivery using a CdS nanoparticle entrapped in the pocket of a GroEL chaperonin complex that released its QD cargo upon interaction with ATP.[5] Fluorescence resonance energy transfer (FRET) from SC QDs to dye molecules was used to follow protein−protein interactions,[6] and to develop DNA and telomerase activity biosensors.[7] For example, M13 phage DNA was hybridized with a nucleic acid associated with a CdSe/ZnS core/shell QD. The replication of the duplex assembly in the presence of the nucleotide mixture of dNTPs and Texas Red−dUTP resulted in the incorporation of Texas Red units into the replica and the formation of an active structure for the generation of FRET.[7]

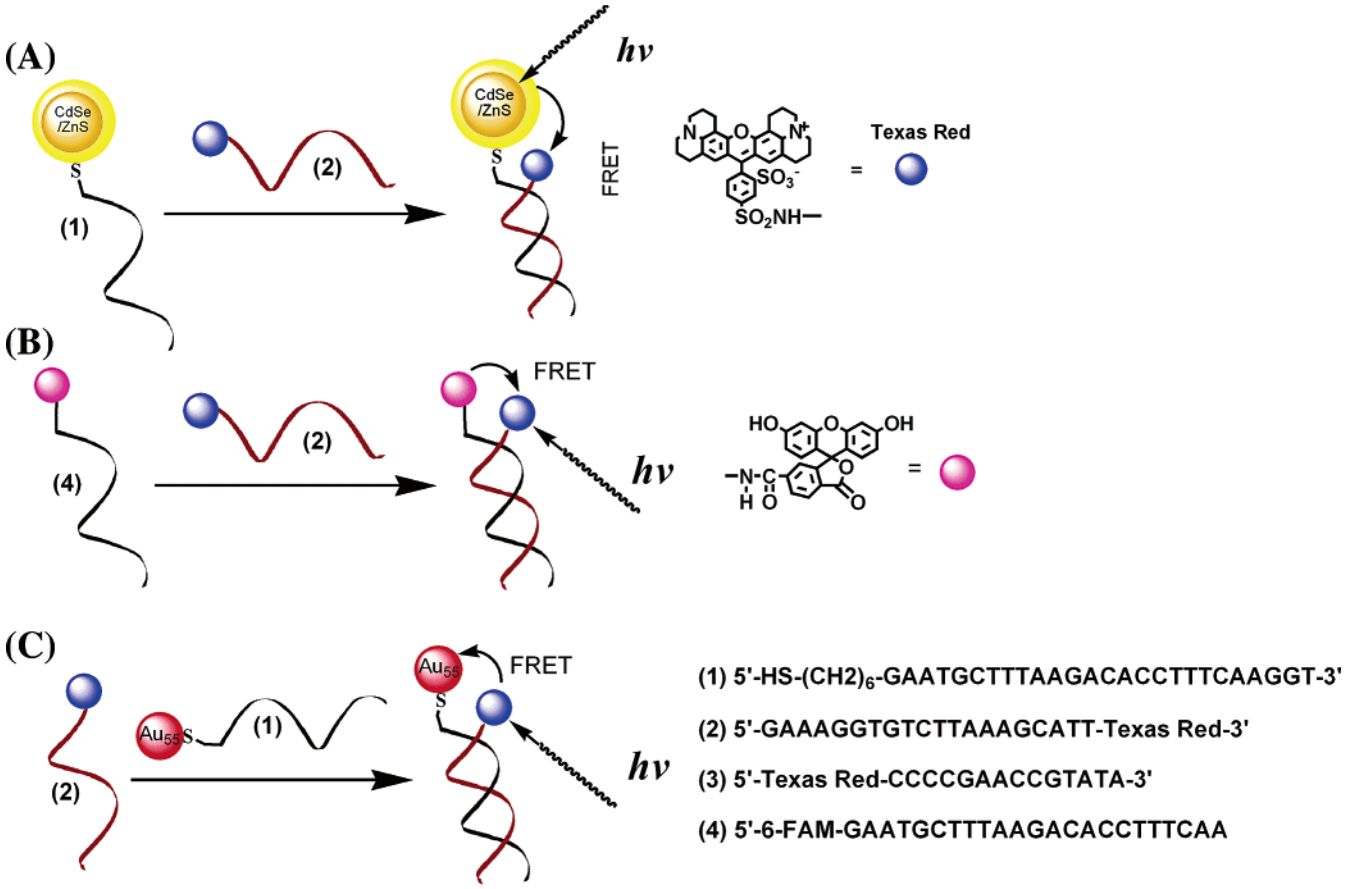
Recently, nucleic acid−CdSe hybrids were used as probes to identify small-interfering RNA (si-RNA).[8] The hybridization of the CdSe−nucleic acid probes with the dye-labled si-RNA results in a FRET process, which was used to detect the synthesis of si-RNA. The FRET efficiency in this system is, however, low due to the long spaces separating the CdSe QDs from the dye units. Semiconductor QDs were also employed as active components for the generation of photocurrents that probe enzyme reactions, enzyme inhibition,[9] and DNA hybridization.[10] Recently, the formation of double-stranded DNA and the separation of the duplexes by an excess of the complementary DNA strand were studied by the quenching of the luminescence of the quantum dots in CdSe QD/Au nanoparticle conjugates.[11] Here, we report on the FRET occurring in different duplex DNA configurations consisting of CdSe/ZnS QD/dye and Au nanoparticle (Au NP)/dye assemblies. We apply the process to follow the DNase I cleavage of the DNA and reveal the nonspecific adsorption of dyes on the nanoparticles. The nonspecific adsorption of the dye on the QDs might introduce some future drawbacks in the application of the QDs as bioanalytical labels. The restoration of the luminescence properties of the QDs upon the DNase I scission of the dye-functionalized duplex DNA provides, however, an efficient tool to discriminate between the specific hybridization of the dye-functionalized DNA with the complementary nucleic acid, linked to the QDs, and the nonspecific association of the dye-modified nucleic acid with the QDs. The results might have further broad implications in developing QD-based multiplexed sensing arrays, and particularly for the high-resolution-based FRET microscopy of ultradense arrays.[12]

CdSe/ZnS core/shell QDs, diameter 3.8 ± 0.8 nm (core diameter 2.9 nm), were prepared by modification of the literature procedure for the synthesis of core/shell QDs.[13] These QDs were further transformed into water-soluble QDs by ligand exchange with 3-mercapto-propionic acid (MPA)[14] (Supporting Information). The resulting QDs were functionalized with the thiolated nucleic acid (**1**). The average surface coverage of the nucleic

* Authors to whom correspondence should be addressed. Phone: 972-2-6585272. Fax: 972-2-6527715. E-mail: willnea@vms.huji.ac.il.

10.1021/jp054874p CCC: $30.25
Published on Web 11/19/2005

SCHEME 1[a]



[a] (A) FRET within a CdSe/ZnS QD/Texas-Red-functionalized duplex DNA. (B) FRET process in the DNA **3**/**2** duplex structure. (C) FRET process in the DNA duplex structure consisting of the **1**-functionalized Au NP and **2**.

acid was estimated to be ~6 per particle (measured by determining the amount of noncoordinated DNA spectroscopically; see Supporting Information). The quantum yield of the QDs in chloroform was 70%, and the quantum yield of the MPA-functionalized QDs in water was 25%. After modification with the nucleic acid, a further decrease in the quantum yield, to a value of 7%, was observed. The decrease in the quantum yield upon the association of the nucleic acids may be attributed to the further extensive processing necessary for binding, which may create additional surface defects. The Texas-Red-modified nucleic acid (**2**) was hybridized with the **1**-modified CdSe QD, Scheme 1A. Texas Red was selected as the acceptor dye since its absorbance has a high degree of overlap with the emission of the QDs, yet its absorbance at the excitation wavelength of the CdSe particles, $\lambda = 400$ nm, is negligible. Figure 1 shows the absorbance and emission features of the CdSe/ZnS QDs and of Texas Red, respectively. Figure 2, curves a−g, shows the time-dependent fluorescence spectra of the system upon the hybridization. The CdSe/ZnS QD exhibit a fluorescence band at $\lambda = 580$ nm. As hybridization proceeds, the emission from the QDs decreases, and the new fluorescence band at $\lambda = 615$ nm, characteristic to Texas Red, appears and increases in intensity. The system reaches ca. 60% quenching of the initial luminescence of the CdSe QDs. Figure 2, curve h, shows the fluorescence spectrum of the Texas-Red-modified nucleic acid (**2**) solution in the absence of the CdSe QDs, upon excitation at $\lambda = 400$ nm (the wavelength corresponding to the QD excitation). A minute fluorescence intensity originating from the direct excitation of the dye is observed, consistent with the fact that the dye does not absorb at the excitation wavelength

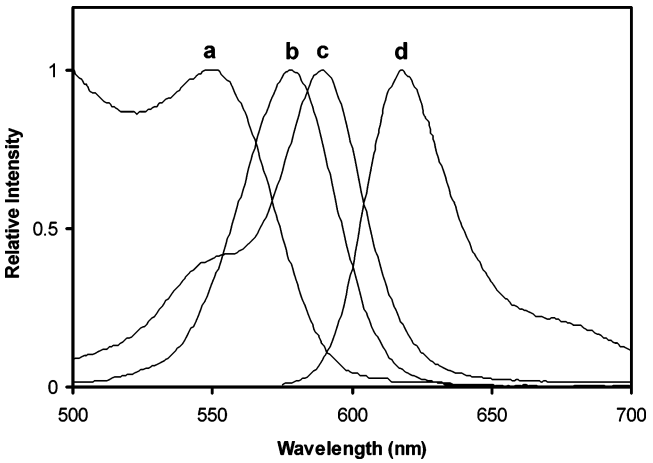


Figure 1. Normalized absorption and emission spectra corresponding to (a) absorption of the CdSe−ZnS QDs, (b) emission spectrum of the CdSe−ZnS QDs, (c) absorption of the Texas Red dye, and (d) emission of the Texas Red dye.

of the QDs. Thus, the fluorescence at $\lambda = 615$ nm, that is intensified upon the hybridization of **2** with the **1**-modified CdSe QDs originates from a FRET process between the QDs and the dye. It should be noted that the molar ratio of CdSe/ZnS QD to **2** in the experiment depicted in Figure 1 is 1:2. Increasing the content of the complementary nucleic acid **2** enhances the quenching efficiency. For example, upon alteration of the CdSe/ZnS QDs to **2** ratio from 1:1 to 1:4, the quenching efficiency increases from 16% to 72%.

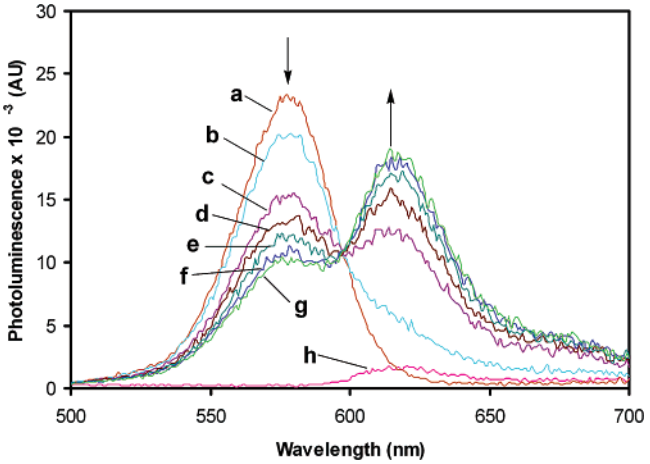


Figure 2. Fluorescence spectra corresponding to the **1**-functionalized QDs 0.25×10^{-6} M at different time intervals of hybridization with **2**, 0.5×10^{-6} M in 10 mM Tris HCl buffer, pH = 7.4, with 2.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$: (a) before addition of **2**, (b) 2 min, (c) 10 min, (d) 22 min, (e) 38 min, (f) 62 min, (g) 90 min after addition of **2**, and (h) fluorescence spectrum of only **2**, 0.5×10^{-6} M, $\lambda_{ex} = 400$ nm.

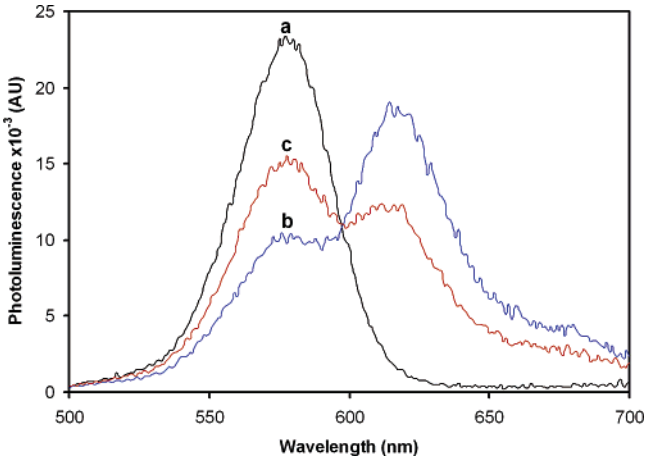


Figure 3. Fluorescence spectra corresponding to (a) the **1**-functionalized QDs, (b) after hybridization of the QDs with **2** for 90 min, and (c) after reacting the **1**-functionalized QD/**2** duplex with DNase I, 2000 U, in a reaction volume of 200 μL, for 10 min.

Interestingly, comparison of the integrated light intensity emitted by the Texas Red dye is higher than the value calculated from the decrease in the integrated light emitted by the CdSe/ZnS QD. For example, after 22 and 62 min of hybridization, the total integrated light intensity emitted by the system is 15% and 20%, respectively, higher than the integrated luminescence immediately after hybridization. This suggests that in the presence of the hybridized dye-functionalized DNA (**2**) a part of the excited QDs, which normally decay rapidly by nonradiative pathways, participate in the FRET process to the dye, resulting in the observed enhanced luminescence.

The resulting **1**-functionalized CdSe/ZnS/**2** duplex was then subjected to the hydrolytic enzyme DNase I. Figure 3 shows the fluorescence spectra of the **1**-functionalized CdSe/ZnS QDs before and after hybridization with **2**, curves a and b, respectively, and the fluorescence spectra of the duplex system after treatment with DNase I, curve c. After treatment of the duplex structure with DNase I, the intensity of the FRET band of the dye decreases, and the fluorescence of the CdSe/ZnS QDs increases. These results are consistent with the expectation that DNase I cleaves the duplex DNA, resulting in the removal of the Texas Red units and thus blocking the FRET process and the restoration of the QD luminescence. The elimination of the

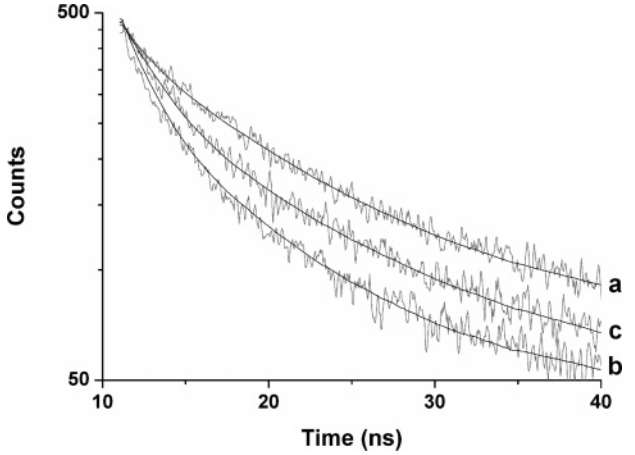


Figure 4. Fluorescence transients corresponding to (a) the **1**-functionalized QDs, (b) after hybridization of the QDs with **2** for 90 min, and (c) after reacting the **1**-functionalized QD/**2** duplex with DNase I, 2000 U, in a reaction volume of 200 μL, for 10 min. All measurements were made at $\lambda = 580$ nm.

FRET process and the recovery of the QD luminescence are, however, incomplete, and the system is stabilized at a state where only 40% of the quenched CdSe/ZnS luminescence is recovered. To understand the origin for the partial recovery of the luminescence of the QDs and the steady-state FRET emission by the dye units, we have further characterized the photophysical properties of the CdSe/ZnS/**2** hybrid system by measuring the fluorescence decay curves, Figure 4. The transients were fit with a biexponential decay convoluted by the system response function. We find that the **1**-functionalized CdSe/ZnS exhibit a lifetime of 10.3 ± 0.4 ns. Upon the hybridization with Texas-Red-modified nucleic acid (**2**), we observe two populations of the CdSe/ZnS QDs. A major population, ca. 82%, reveals a shortened lifetime of 8 ± 0.4 ns, and a population of ca. 18% with a substantially shorter lifetime corresponding to 1.2 ± 0.1 ns. After the treatment of the system with DNase I we find that the long-lived population of the CdSe/ZnS recovers its original lifetime, 10 ± 0.6 ns, yet the DNase I has no effect on the short-lived population (ca. 15% with a lifetime of 1.4 ± 0.14 ns).

A possible explanation for the lifetime features of the QDs after hybridization and DNase I treatment is that the hybridized **2** yields two different populations of QDs; one population of lower content (20%) consists of the dye adsorbed onto the QDs (for example, adsorbed onto defect sites lacking the thiolate) that leads to effective quenching of the QDs and to the short lifetime of these QDs. The second population consists of the hybridized nucleic acid **2**, where the dye is electrostatically repelled by the capping interface of the QDs. This leads to the inefficient quenching of the QDs and to the long-lived particles (ca. 8 ns). Single nanocrystal measurements could assist, in the future, to elucidate the suggested mechanism. The reaction of the QDs with DNase I leads to the cleavage of the associated nucleic acids, but the originally adsorbed dye remains associated with the particles, giving rise to the short-lived population of the QDs. The effective quenching of the luminescence of the CdSe/ZnS QDs by the surface-adsorbed dye units leads to the incomplete restoration of the luminescence of the QDs after treatment with DNase I.

It should be noted that the integrated light intensity of the dye-functionalized CdSe QDs, after the scission of the duplex DNA, is approximately 15% higher than the integrated light intensity of the CdSe QDs immediately upon addition of **2**. This is consistent with the fact that cleavage of the duplex DNA

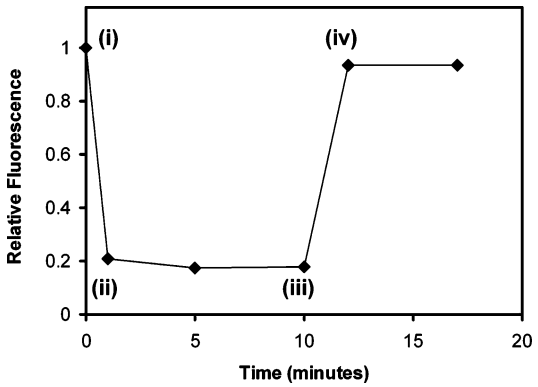


Figure 5. Fluorescence intensities of the FAM dye at $\lambda = 520$ nm upon hybridization of **3**, 1×10^{-6} M, with **2**, 0.1×10^{-6} M, and cleavage of the resulting duplex by DNase I, 2000 U, in 200 μL of reaction medium. (i) Fluorescence of **3** only and addition of **2**. (ii) After hybridization with **2** for 2 min. (iii) Addition of DNase I to the system. (iv) After reacting with DNase I. In all experiments the solution consists of 10 mM Tris-HCl buffer, pH = 7.4, that included 2.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$, $\lambda_{ex} = 480$ nm.

leads to QDs with adsorbed dye units. These dye sites quench excited state species that originally decayed by nonradiative paths and lead to the enhanced emission. Further support that the Texas Red dye adsorbs onto the CdSe QDs was obtained by the reaction of the **1**-functionalized CdSe QDs with the foreign, noncomplementary nucleic acid **3**. Although hybridization does not occur with **3**, a low FRET signal at $\lambda = 615$ nm is observed (ca. 30% of the intensity observed for the FRET signal with the complementary nucleic acid **2**). This low FRET signal was unaffected by the addition of DNase I, implying that it originates from the adsorption of the dye units to the QDs.

To further understand the suitability of QDs to probe the hybridization and cleavage of duplex DNA structures, we examined two additional related systems consisting of dye donor/dye acceptor DNA duplex and dye donor/Au NP acceptor DNA duplex. We studied the hybridization of the 6-carboxyfluorescein (6-FAM)-functionalized nucleic acid **4** with the Texas-Red-modified nucleic acid **2** and the DNase-I-induced cleavage of the duplex structure, using FRET as the readout signal for the reactions, Scheme 1B. The results in the system were compared to a system where the **1**-functionalized Au NPs (1.4 nm) were hybridized with the Texas-Red-modified nucleic acid **2**, and DNase I cleaves the system, Scheme 1C.

Figure 5 shows the normalized fluorescence (at $\lambda = 520$ nm) of the donor-modified nucleic acid **4** in the course of hybridization with **2** and upon cleavage by DNase I. Upon hybridization, 85% of the donor fluorescence is quenched. Upon treatment with DNase I, 95% of the initial fluorescence of the donor is recovered, consistent with the cleavage of the duplex and the dissociation of the acceptor dye from the assembly.

Figure 6A shows the fluorescence spectra of the Texas-Red-functionalized nucleic acid **2** before hybridization, curve a, and after hybridization of the **1**-modified Au NPs, curve b. The dye fluorescence is quenched by the Au NPs (90%). Upon interaction with DNase I, the original dye fluorescence is only partially recovered, to an extent of 65%, curve c. To understand the incomplete recovery of the Texas Red fluorescence upon the treatment with DNase I, we have interacted the Texas-Red-functionalized nucleic acid **3** with the **1**-modified Au NPs. It should be noted that **3** lacks any complementarity with **1**. Figure 6B shows the fluorescence of the **3**-functionalized nucleic acid, before (curve a) and after interaction with the **1**-modified Au NPs, curve b. Although no complementarity exists between **3**

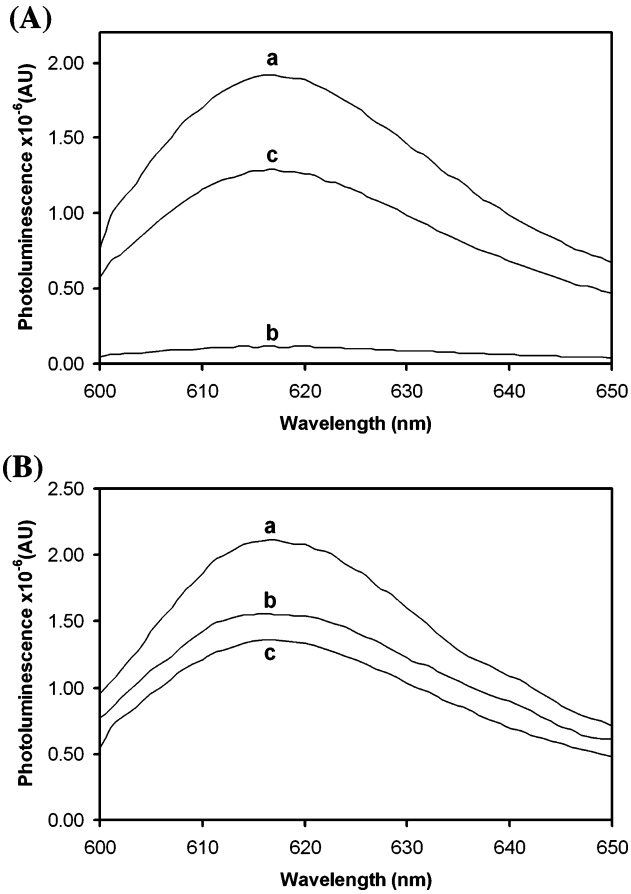


Figure 6. (A) Fluorescence spectra corresponding to (a) **2**, 1×10^{-6} M, (b) after hybridization with the **1**-functionalized Au NPs, 1×10^{-6} M, for 10 min, and (c) after treatment with DNase I, 2000 U, for 10 min in 200 μL of reaction medium, $\lambda_{ex} = 590$ nm. (B) Fluorescence spectra corresponding to (a) **4**, 1×10^{-6} M, (b) after addition of the **1**-functionalized Au NPs, 1×10^{-6} M, for 10 min, and (c) after treatment with DNase I, 2000 U, for 10 min in 200 μL of reaction medium, $\lambda_{ex} = 590$ nm. The composition of the solution is as in Figure 4.

and **1**, the fluorescence of the dye decreases to 75% of the original value. This is attributed to the nonspecific binding of the dye to the Au NPs, leading to local quenching of the dye fluorescence. Upon the addition of DNase I to the system, a further slight decrease of the Texas Red fluorescence to a value of 65% of the original fluorescence is observed. The slight decrease in the luminescence of the system upon the addition of DNase I is attributed to the association of the dye-functionalized nucleic acid with the enzyme that stimulates the quenching of the luminescence of the dye. Remarkably, the degree of quenching of the dye luminescence of **3** by the noncomplementary nucleic-acid-functionalized Au NPs and DNase I (65%) is the same as the restored fluorescence of the dye upon the cleavage of the **1**/**2**−Au NP duplex by DNase. Thus, the incomplete restoration of the Texas Red fluorescence upon scission of the **1**/**2**−Au NP duplex structure is attributed to the nonspecific adsorption of the dye to the Au NP and to the quenching of the dye by DNase I.

In summary, the present study has addressed the fluorescence resonance energy transfer processes in dye/nanoparticle DNA conjugates. Our observations reveal that DNase I cleavage of the duplex DNAs associated with the CdSe/ZnS QD/dye or dye/Au NP system restores only partially the luminescence of the QDs or dye, and that the unrecovered fluorescence is due to quenching of the QDs by nonspecifically adsorbed dye (or to nonspecifically adsorbed dye by the Au NPs). We have shown

that the nonspecific adsorption of the dye-functionalized nucleic acid to metal or semiconductor nanoparticles partially results in FRET processes that are independent of the DNA hybridization. The nonspecific binding of dye-labled DNA strands to both SC and Au nanoparticles likely arises from the reactive surfaces of both systems. The MPA ligand is short, and therefore the SC nanoparticle surface is prone for nonspecific binding processes. This nonspecific binding process partially masks the specific hybridization process, yet still the major effect seen in both systems arises from the specific binding. Use of different surface passivation for the nanocrystals may further reduce the problem of nonspecific binding, and therefore improve the prospects of applying FRET with metal and SC nanoparticles for bioanalysis.

Acknowledgment. This research was supported by the German−Israeli Program (DIP).

Supporting Information Available: Methods used for the preparation of the QDs and their functionalization with DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Katz, E.; Willner, I. *Angew. Chem., Int. Ed.* **2004**, *43*, 6042−6108. (b) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4*, 435−446.

(2) (a) Bruched, M. Jr.; Meronne, M.; Gia, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016. (b) Wu, X. Y.; Liu, H. J.; Liu J. Q.; Haley, K. N.; Treadway, J. A.; Larson, J. P.; Ge, N. F.; Peale, F.; Bruchez, M. P. *Nat. Biotechnol.* **2003**, *21*, 41−46.

(3) (a) Mattheakis, L. C.; Dias, J. M.; Choi, Y. J. Gong, J. Bruchez M. P.; Liu, J. Q. Wang E. *Anal. Biochem.* **2004**, *327*, 200−208. (b) Ham, M.; Gao, X.; Su, J. Z.; Nie, S. *Nat. Biotechnol.* **2001**, *19*, 631−635.

(4) Gao, X.; Cui, Y.; Levenson, R. M.; Chung, L. W. K.; Nie, S. *Nat. Biotechnol.* **2004**, *22*, 969−976.

(5) Ishii, D.; Kinbara, K.; Ishida, Y.; Ishii, N.; Okochi, M.; Yohda, M.; Aida, T. *Nature* **2003**, *423*, 628−632.

(6) Willard, D. M.; Carillo, L. L.; Jung, J.; Van Orden, A. *Nano Lett.* **2001**, *1*, 469−474.

(7) Patolsky, F.; Gill, R.; Weizmann, Y.; Mokari T.; Banin, U.; Willner, I. *J. Am. Chem. Soc.* **2003**, *125*, 13918−13919.

(8) Bakalova, R.; Zhelev, Z.; Ohba, H.; Baba, Y. *J. Am. Chem. Soc.* **2005**, *127*, 11328−11335.

(9) Pardo-Yissar, V.; Katz, E.; Wasserman, J.; Willner, I. *J. Am. Chem. Soc.* **2003**, *125*, 622−623.

(10) (a) Willner, I.; Patolsky, F.; Wasserman, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 1861−1864. (b) Gill, R.; Patolsky, F.; Katz, E.; Willner, I. *Angew. Chem., Int. Ed.* **2005**, *44*, 4554−4557.

(11) Dyadyusha, L.; Yin, H.; Jaiswal, S.; Brown, T.; Baumberg, J. J.; Booy, F. P.; Melvin, T. *Chem. Commun.* **2005**, *25*, 3201−3203.

(12) Ebenstein, Y.; Mokari, T.; Banin, U. *J. Phys. Chem. B* **2004**, *108*, 93−99.

(13) Li, J. J.; Wang, Y. A.; Guo, W.; Keay, J. C.; Mishima, T. D.; Johnson, M. B.; Peng, X. *J. Am. Chem. Soc.* **2003**, *125*, 12567−12575.

(14) (a) Wuister, S. F.; Swart, I.; van Driel, F.; Hickey, S. G.; de Mello Donega, C.; *Nano Lett.* **2003**, *3*, 503−507. (b) Xie, R.; Kolb, U.; Li, J.; Basche, T.; Mews, A. *J. Am. Chem. Soc.* **2005**, *127*, 7480−7488.