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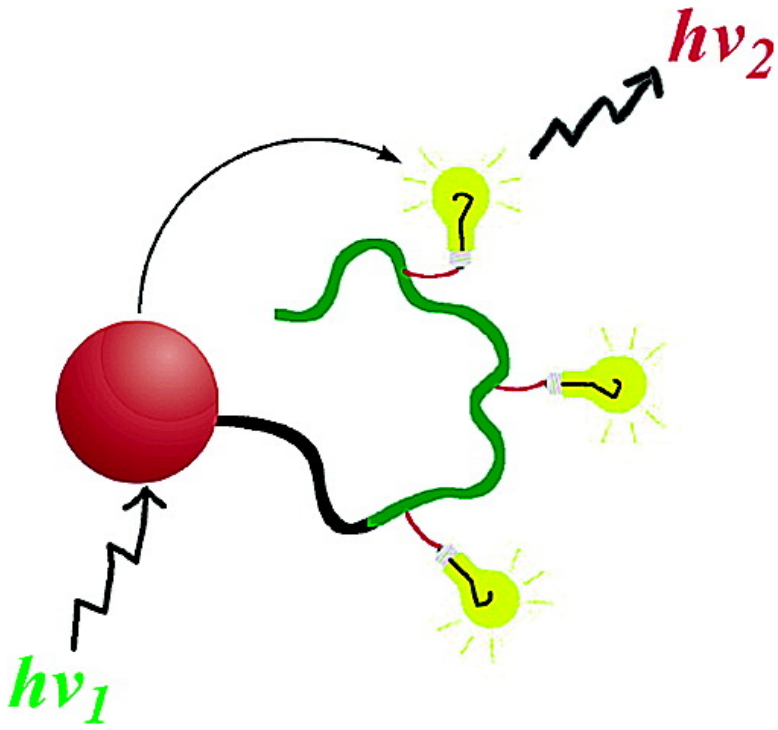

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Lighting-Up the Dynamics of Telomerization and DNA Replication by CdSe−ZnS Quantum Dots

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Hybrid systems consisting of semiconductor quantum dots (QDs) coupled to biomaterials find growing interest in the developing research area of nanobiotechnology.[1,2] Photochemically induced fluorescence resonance energy transfer (FRET) between molecular fluorophores[3−5] or the quenching of excited chromophores by metal nanoparticles[6] was reported to probe DNA hybridization processes and, specifically, the formation and dissociation of hairpin structures. The replication of DNA on bulk surfaces was recently applied to the amplified bioelectronic detection of DNA,[7] and the incorporation of redox-active units into the replicated DNA has enabled the electrochemical probing of the dynamics of replication.[8] The unique photophysical properties of semiconductor quantum dots establish the possibility of applying semiconductor nanoparticles as efficient fluorescence labels[9] or as photoelectrochemical probes.[10] Here, we report on the use of CdSe−ZnS core−shell quantum dots (QDs) as photochemical centers for lighting-up the dynamics of telomerization or of DNA replication occurring on the respective nanoparticles by fluorescence resonance energy transfer (FRET) to dye units incorporated into the new synthesized telomer or DNA replica, Scheme 1. CdSe−ZnS biocompatible QDs (4.2 nm, λ_{em} = 400 nm, 20% luminescence quantum yield) were stabilized by mercaptopropionic acid modified with the thiolated oligonucleotide (**1**) (sequence:5′-HS-$(CH_2)_6$TTTTTTAATCCGTCGAGCAGAGTT-3′). Analysis of the particles indicate that ca. 25 nucleic acid units are associated with each nanoparticle (for the modification of the CdSe−ZnS QDs and their characterization, see Supporting Information). The (**1**)-functionalized semiconductor nanoparticles were incubated with a dNTP mixture (dATP, dCTP, and dGTP, 0.5 mM each) that included Texas-Red 14-dUTP (TR-dUTP) (**2**) (100 μM) in the presence of telomerase (extracted from HeLa cells, 10 000 cells). Figure 1A shows the fluorescence spectra of the system, at 25 °C, upon excitation of the CdSe−ZnS QDs at λ = 400 nm, as a function of telomerization time. The CdSe−ZnS QDs emit at λ = 560 nm, prior to the introduction of telomerase. After the addition of telomerase, and as telomerization proceeds, the fluorescence of the QDs decreases, with the concomitant increase of the characteristic emission of the dye at λ = 610 nm. Control experiments reveal that excitation of the nucleotide mixture that includes the dye-modified dUTP in the absence of the CdSe−ZnS QDs at λ = 400 nm does not lead to an observable emission at λ = 560 nm, but excitation of the system at λ = 550 nm leads to a high fluorescence signal characteristic to the dye. Further control experiments indicate that treatment of the (**1**)-modified CdSe−ZnS QDs with TR-dUTP or with telomerase do not lead to any time-dependent increase in the fluorescence of the dye. These experiments indicate that no nonspecific adsorption of TR-dUTP onto the particles takes place. Also, telomerization in the presence of dNTPs, that includes the unlabeled dUTP, does not yield any quenching of the QDs emission. As the emission of the CdSe−ZnS QDs λ_{max} = 560 nm coincides with the absorbance band of the dye (**2**), the emission observed upon telomerization is attributed to fluorescence resonance energy transfer (FRET) from the nanoparticles to the dye molecules incorporated into the telomeric units by telomerase.

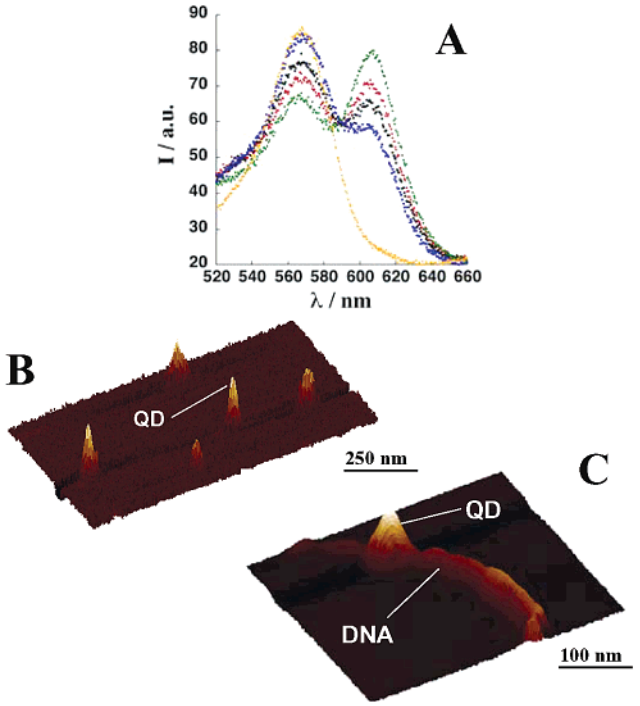

Figure 1. (A) Emission spectra upon the time-dependent telomerization on the CdSe−ZnS QDs: blue, before addition of telomerase; black, red, and green, after 10, 30, and 60 min of telomerization, respectively; orange, in the presence of telomerase before the addition of dNTPs and TR-dUTP. (B) AFM image of QDs before telomerase treatment. (C) AFM image of a CdSe−ZnS QD after 60 min of telomerization (images recorded on mica surfaces activated with 5 mM $MgCl_2$). z-bar: 5 nm.

Atomic force miscroscopy (AFM) images of the CdSe−ZnS QDs prior to the telomerization, Figure 1B, and the image of a nanoparticle after telomerization, Figure 1C, were recorded on freshly cleaved mica surfaces, under ambient conditions. While the height of the nanoparticle is ca. 4 nm, its lateral dimensions are distorted due to the tip dimensions. The nanoparticle, after incubation in the presence of telomerase, reveals beautifully the synthesized hinged DNA strands. The height of the telomeric DNA chains is ca. 1 nm, whereas their length is ca. 300 nm. This corresponds to a telomerase-induced elongation of ca. 1000 base units in the telomeric DNA chain.

The CdSe−ZnS quantum dots enabled also the detection of a viral DNA by following the replication process by FRET. In this system, the primer (**3**) (sequence: 5′-HS-$(CH_2)_6$-CCCCCACGT-TGTAAAACGACGGCCAGT-3′) complementary to M13ϕ DNA

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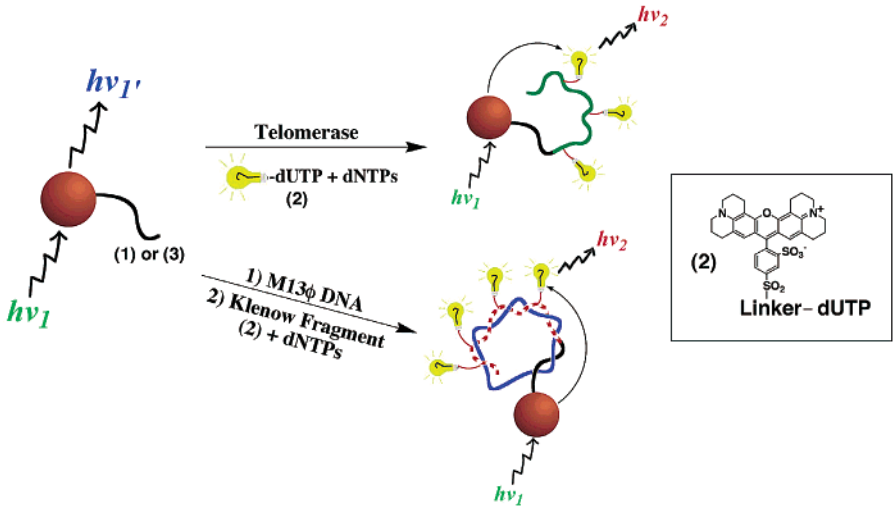
Scheme 1. Telomerization and Replication on Nucleic-Acid-Functionalized CdSe−ZnS QDs with the Incorporation of Texas Red-Labeled dUTP



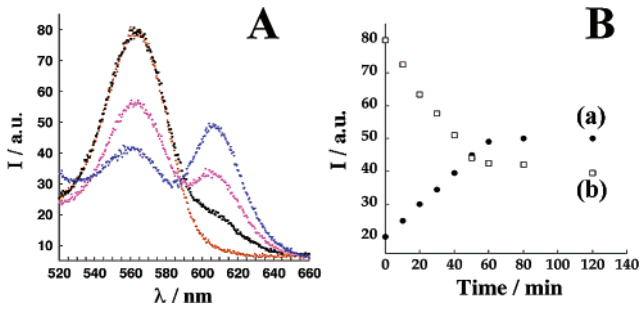


Figure 2. (A) Emission spectra upon the time-dependent DNA replication on the CdSe−ZnS QDs: orange, before addition of TR-dUTP; black, purple, and blue, after 1, 30, and 60 min of replication. (B) Time-dependent decrease of the CdSe−ZnS emission (a) and the dye fluorescence increase (b), upon replication on the QDs.

was assembled on the CdSe−ZnS QDs. Hybridization of the (**3**)-functionalized QDs with M13ϕ DNA (0.1 pmol), followed by replication in the presence of polymerase (klenow fragment, 10 units) and a mixture of dNTPs containing the TR-dUTP, yield the dye-labeled DNA replica. The DNA replication could be followed by FRET from the QDs to the incorporated dye unit, Figure 2A. The time-dependent decrease in the emission of the CdSe−ZnS QDs and the fluorescence increase of the dye, upon polymerization on the QDs, is depicted in Figure 2B. The two processes follow similar kinetics as expected and are saturated after ca. 60 min of reaction. A set of control expriments identical to those described for the telomerization process was performed to elucidate that the enhancement of the dye emission originates from a specific FRET process to the dye-labeled DNA replica, rather than from nonspecific adsorption of the dye (for more details, see Supporting Information).

Besides the significance of the systems in probing enzymatic processes on DNA, the results may be applied for the fast and sensitive detection of cancer cells and as an amplification route for analyzing DNA on chip arrays. Furthermore, recent activities in nucleic acid research use hybridization processes as logic gates.[11] The polymerase-induced replication of DNA on the surface of luminescent QDs represents an "AND" gate where FRET readout occurs only if hybridization and replication proceed.

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Supporting Information Available: The synthesis and characterization of the functionalized QDs, experimental protocols for the enzymatic processes, and detailed control experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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