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Photoinduced Enhancement in the Luminescence of Hydrophilic Quantum Dots Coated with Photocleavable Ligands

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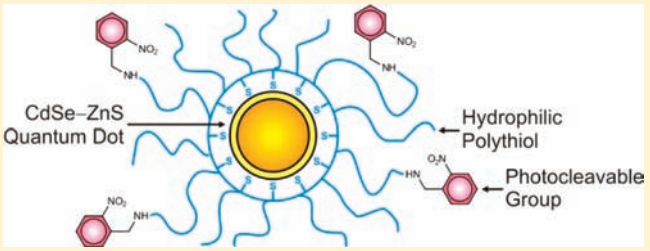
ABSTRACT: In search of strategies to photoactivate the luminescence of semiconductor quantum dots, we devised a synthetic approach to attach photocleavable 2-nitrobenzyl groups to CdSe–ZnS core–shell quantum dots coated with hydrophilic polymeric ligands. The emission intensity of the resulting nanostructured constructs increases by more than 60% with the photolysis of the 2-nitrobenzyl appendages. Indeed, the photoinduced separation of the organic chromophores from the inorganic nanoparticles suppresses an electron-transfer pathway from the latter to the former and is mostly responsible for the luminescence enhancement. However, the thiol groups anchoring the polymeric envelope to the ZnS shell also contribute to the photoinduced emission increase. Presumably, their photooxidation eliminates defects on the nanoparticle surface and promotes the radiative deactivation of the excited quantum dots. This effect is fully reversible but its magnitude is only a fraction of the change caused by the photocleavage of the 2-nitrobenzyl groups. In addition, these particular quantum dots can cross the membrane of model cells and their luminescence increases by ~80% after the intracellular photocleavage of the 2-nitrobenzyl quenchers. Thus, photoswitchable luminescent constructs with biocompatible character can be assembled combining the established photochemistry of the 2-nitrobenzyl photocage with the outstanding photophysical properties of semiconductor quantum dots and the hydrophilic character of appropriate polymeric ligands.



INTRODUCTION

Photocaged fluorophores[1−8] and photoactivatable fluorescent proteins[9−11] switch from a nonemissive to an emissive state upon illumination at an appropriate wavelength and are valuable analytical tools for the investigation of biological samples. Indeed, their introduction into a specimen of interest permits the local activation of fluorescence under optical control. In turn, fluorescence photoactivation offers the opportunity to monitor the diffusion of a labeled target as well as to resolve in time spatially indistinguishable labels. As a result, these photoresponsive probes in combination with fluorescence imaging can be exploited to assess the dynamics of biological processes and visualize the subtleties of biological structures.

Photocaged fluorophores generally rely on the photoinduced cleavage of appropriate functional groups to activate the fluorescence of organic chromophores.[1−8] Similarly, their genetically encoded counterparts activate the fluorescence of organic chromophores, embedded within polypeptidic frameworks, on the basis of photoisomerizations and proton transfer in the excited state.[9−11] The photophysical properties of organic chromophores, however, are inferior to those of certain inorganic nanoparticles. Specifically, semiconductor quantum dots[12−17] have huge one- and two-photon absorption cross sections, long luminescence lifetimes, and excellent photobleaching resistances compared to most organic dyes and fluorescent proteins. In addition, their narrow and symmetric emission bands can be tuned across the visible region with careful adjustments in elemental composition and physical dimensions. Thus, the identification of mechanisms to photoactivate the luminescence of quantum dots can translate into the development of photocaged probes with improved performance.

Recently, we developed biocompatible CdSe–ZnS core–shell quantum dots coated with amphiphilic polymeric ligands.[18] The organic envelope around the inorganic core of these nanostructured constructs preserves their photophysical properties, while ensuring aqueous solubility and biocompatibility. In addition, the polymeric ligands permit the covalent attachment of organic dyes via amide-bond formation. On the basis of these considerations, we envisaged the possibility of attaching photocleavable groups to our hydrophilic quantum dots in order to activate their luminescence under optical control in aqueous environments. Indeed, a recent report[19] demonstrates that the photocleavage of 2-nitrobenzyl groups, adsorbed on the surface of hydrophilic CdTe–CdS InP–ZnS or CdHgTe–ZnS core–shell quantum dots, leads to a

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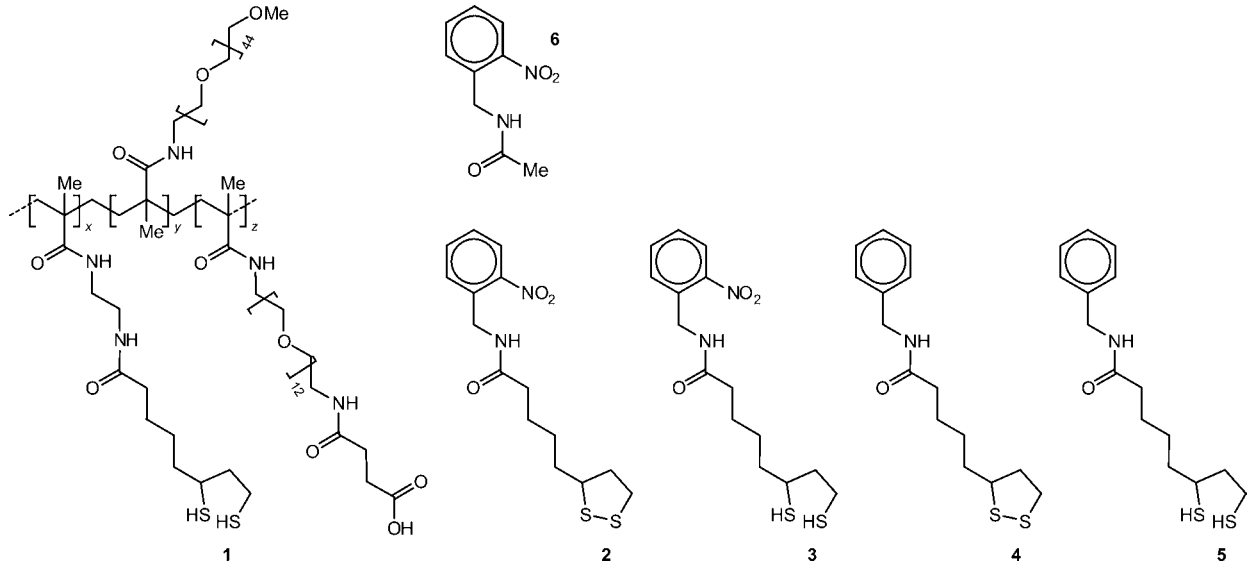

Figure 1. Structures of the copolymer **1** and model compounds **2**–**6**.

significant luminescence enhancement.[20−24] These particular nanoparticles were prepared relying on the established ability of dihydrolipoic acid derivatives to anchor organic ligands on the surface of quantum dots.[25] These compounds, however, have only two thiol groups per molecule, while our polymers incorporate multiple anchoring groups along the same macromolecular backbone. In fact, our polydentate ligands can impose long-term stability on the coated quantum dots in aqueous environments, while permitting the covalent attachment of 2-nitrobenzyl photocages. In this work, we report an experimental protocol to couple 2-nitrobenzyl groups to our polymer-coated CdSe−ZnS core−shell quantum dots, together with the synthesis of appropriate model systems and the photochemical and photophysical properties of the resulting photoresponsive nanostructured assemblies.

RESULTS AND DISCUSSION

Design and Synthesis of the Ligands. The copolymer **1** (Figure 1) incorporates anchoring thiol groups, hydrophilic poly(ethylene glycol) chains and connecting carboxylic acids along a common macromolecular backbone. When combined with preformed CdSe−ZnS core−shell quantum dots, coated with tri-*n*-octylphosphine oxide (TOPO), this polymer adsorbs on the surface of the inorganic nanoparticles, displacing the TOPO ligands, to generate water-soluble quantum dots.[18b] The carboxylic acids appended to the polymeric envelope around these nanostructured constructs can then be coupled to chromophores with pendant primary amines, under the assistance of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and the sodium salt of 3-sulfo-*N*-hydroxysuccinimide (sulfo-NHS).[18b] Thus, our hydrophilic quantum dots can be reacted with 2-nitrobenzylamine on the basis of this experimental protocol to generate nanoparticles with photocleavable 2-nitrobenzyl groups on their surface. The investigation of the photochemical and photophysical properties of the resulting conjugates, however, requires also the preparation of appropriate model compounds. In particular, the dithiolane **2**, bisthiol **3** and amide **6** incorporate a 2-nitrobenzyl appendage and permit the investigation of the photolysis of this particular group in solution, its spectroscopic response after adsorption on the surface of model quantum dots and the characterization of its redox behavior respectively. Instead, the bisthiol **5** lacks the nitro group necessary to promote photolysis and, after adsorption on the surface of model quantum dots, permits the investigation of the influence of the anchoring thiol groups on the nanoparticles under irradiation.

We synthesized the dithiolanes **2** and **4** in one step from the corresponding benzylamine and thioctic acid under the assistance of *N*,*N*′-dicyclohexylcarbodiimide (DCC). We then reduced their disulfide linkage with sodium borohydride to generate the bisthiols **3** and **5**, respectively. Similarly, we prepared the amide **6** in one step from 2-nitrobenzylamine and acetic anhydride.

Synthesis and Spectroscopy of the Models. The anchoring thiol groups of **3** and **5** encourage the adsorption of both compounds on the surface of preformed CdSe−ZnS core−shell quantum dots coated with TOPO. Indeed, both compounds displace the native TOPO ligands of the nanoparticles, when stirred with the latter in ethanol for 12 h. In both instances, the modified quantum dots can then be isolated after reiterative centrifugation and filtration steps and the presence of the organic ligands on their surface can be confirmed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Specifically, the ^{1}H NMR spectra of the quantum dots, recorded before and after treatment with the bisthiols, reveal the appearance of resonances for the aromatic rings of the two ligands.

The absorption spectrum (***a*** in Figure 2) of the model compound **2**, recorded in tetrahydrofuran (THF), shows a band for the 2-nitrobenzyl chromophore at 261 nm. Upon ultraviolet illumination, this particular band decays with the concomitant growth of the characteristic absorption of 2-nitrosobenzaldehyde at 286 nm (***b*** in Figure 2). These spectral changes are consistent with the expected photoinduced cleavage of the 2-nitrobenzyl group.[4]

We observed a similar behavior for the ligand **3**, after its adsorption on the surface of preformed CdSe−ZnS core−shell quantum dots. Specifically, the absorption spectrum (***c*** in Figure 2) of the modified quantum dots reveals a band for the 2-nitrobenzyl chromophore[26] at 256 nm and the band gap absorption of the CdSe core at 440 nm. The intensities of the two bands indicate the average number of 2-nitrobenzyl groups

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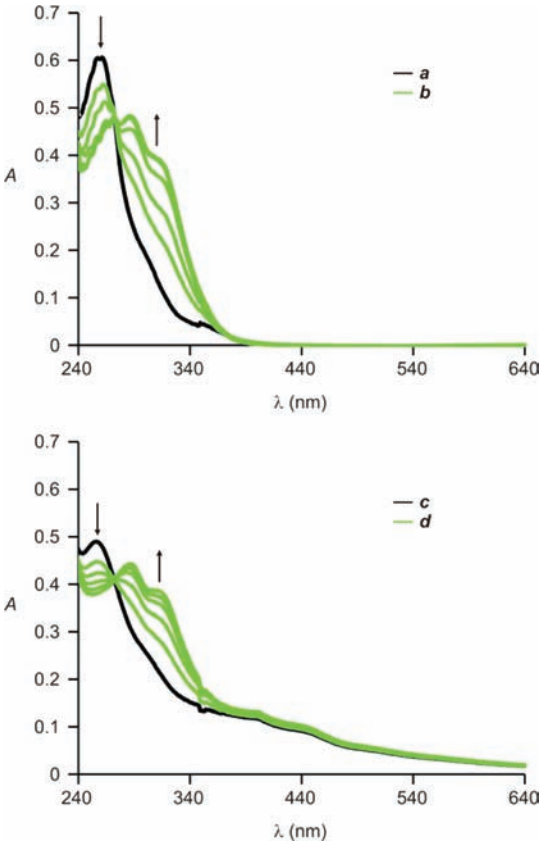

Figure 2. Absorption spectra of **2** (0.2 mM, THF, 25 °C) before (***a***) and after (***b***) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 3, 6, 12, 24, and 35 min). Absorption spectra of CdSe–ZnS core–shell quantum dots (5 μM, THF, 25 °C) coated with **3** before (***c***) and after (***d***) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 5, 10, 20, 35, and 45 min).

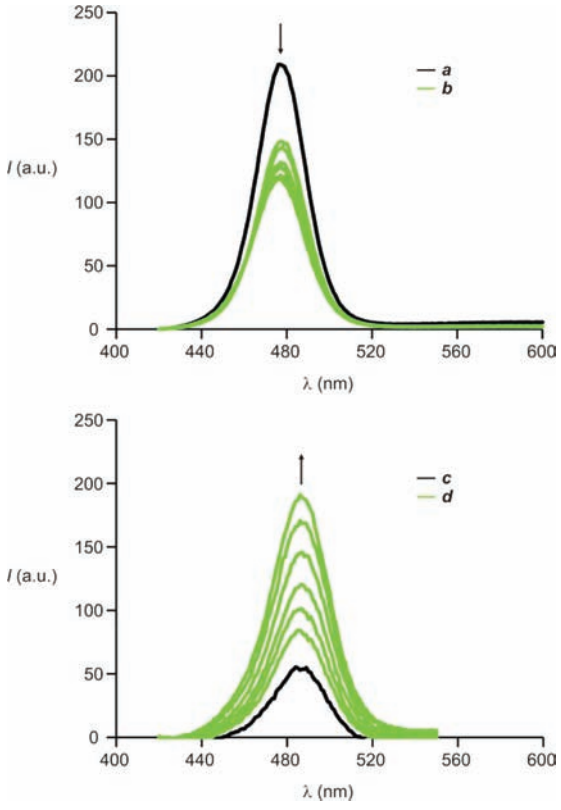

Figure 3. Emission spectra of CdSe–ZnS core–shell quantum dots (1.5 μM, THF, 25 °C, λ_{Ex} = 380 nm) coated with TOPO before (***a***) and after (***b***) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 5, 10, 15, 20, 25, and 30 min). Emission spectra of CdSe–ZnS core–shell quantum dots (5 μM, THF, 25 °C, λ_{Ex} = 400 nm) coated with **3** before (***c***) and after (***d***) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 5, 10, 20, 35, 45, and 55 min).

per quantum dot to be ~18. Upon ultraviolet irradiation, the absorbance at 256 nm decreases and a band for the photogenerated 2-nitrosobenzaldehyde grows at 288 nm (***d*** in Figure 2). Thus, the 2-nitrobenzyl ligands can be photocleaved even when they are adsorbed on the inorganic nanoparticles.

The emission spectra of CdSe–ZnS core–shell quantum dots recorded before and after (***a*** and ***c*** in Figure 3) the adsorption of **3** on their surface reveal a negligible change in emission wavelength, but a significant decrease in emission intensity. Indeed, the emission band of the quantum dots is centered at ~480 nm in both instances, but the luminescence quantum yield drops from 0.32 to 0.09 after the adsorption of **3**. The pronounced decrease in quantum efficiency is, presumably, a result of photoinduced electron transfer from the luminescent inorganic core to the organic ligands. In fact, the oxidation potential of the quantum dots and the reduction potential of the 2-nitrobenzyl group suggest this process to be exergonic with a free energy change of ~−0.4 eV.[27]

Upon ultraviolet irradiation, the emission intensity of the quantum dots coated with **3** increases (***d*** in Figure 3) and, eventually, approaches that recorded before ligand adsorption (***a*** in Figure 3). These observations suggest that the photolysis of the organic ligands removes the quenchers from the surface of the quantum dots and restores their luminescence in full. However, the photoinduced conversion of the 2-nitrobenzyl groups into 2-nitrosobenzaldehyde is irreversible, while the change in luminescence appears to be reversible. Specifically, the emission intensity of the irradiated quantum dots fades over the course of 24 h upon storage in the dark.[28] This apparent contradiction indicates that the photolysis of the 2-nitrobenzyl groups cannot be the sole process responsible for the changes in luminescence.

In order to gain further understanding on these effects, we examined the influence of ultraviolet irradiation on the very same batch of CdSe–ZnS core–shell quantum dots before and after adsorption of the model compound **5**. The benzyl terminus of this particular ligand lacks the nitro group of **3** and cannot be photocleaved. Consistently, the absorption spectra of both sets of quantum dots do not change upon ultraviolet illumination. However, their emission spectra vary significantly with irradiation. Specifically, the luminescence of the native quantum dots decreases irreversibly by 44% (***a*** and ***b*** in Figure 3), whereas that of the nanoparticles coated with **5** increases by 43% (***a*** and ***b*** in Figure 4) under otherwise identical irradiation conditions. Furthermore, the photoinduced luminescence increase observed in the case of **5** is reversible. In fact, the emission intensity decreases upon storage in the dark and can be switched for multiple cycles simply by alternating irradiation and storage steps (***c*** in Figure 4). However, the magnitude of the luminescence changes decreases significantly with the number of switching cycles, presumably, as a result of the gradual degradation of the sample. Furthermore, the luminescence change observed for the quantum dots coated

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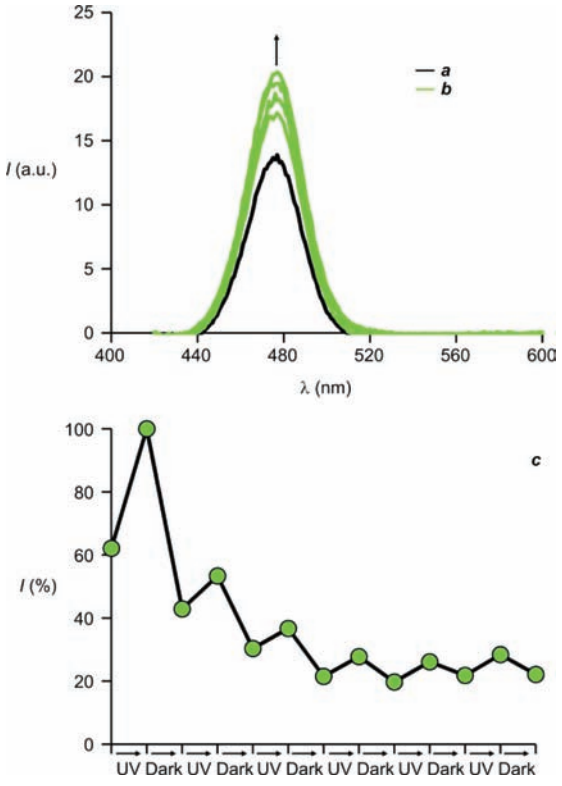

Figure 4. Emission spectra of CdSe–ZnS core–shell quantum dots (56 nM, THF, 25 °C, λ_{Ex} = 380 nm) coated with **5** before (*a*) and after (*b*) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 5, 10, 15, and 20 min). Change in the relative emission intensity (*c*) of CdSe–ZnS core–shell quantum dots (56 nM, THF, 25 °C, λ_{Ex} = 380 nm, λ_{Em} = 476 nm) coated with **5** upon ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 10 min) and storage in the dark (24 h).

with **5** is only a fraction of that detected for those coated with the photocleavable ligand **3**. Indeed, the emission intensity of the nanoparticles coated with **3** increases by 70% upon ultraviolet irradiation.

The behavior of the quantum dots coated with **5** prompted us to assess the response of identical nanoparticles coated with *n*-decanethiol to ultraviolet irradiation. Once again, the absorption spectrum does not change upon illumination, while the emission spectrum reveals an increase in luminescence of 32% (*a* and *b* in Figure 5). As observed for **5**, the photoinduced process is reversible and the luminescence returns to the original value after storing the irradiated nanoparticles in the dark (*c* in Figure 5). These observations suggest that the thiol anchoring groups of **3**, **5** and *n*-decanethiol are responsible for the photoinduced increase in emission intensity. Indeed, literature precedents[29–32] also indicate that thiol ligands lead to significant luminescence enhancements upon prolonged illumination of CdSe quantum dots with and without protective inorganic shells. These noticeable changes are, presumably, a result of the photo-induced oxidation of the thiol groups.[29] In fact, the cyclic voltammogram of **5** shows that the bisthiol appendage can be oxidized to the corresponding disulfide at +0.45 V vs Ag/AgCl. This value in combination with the reduction potential of similar CdSe–ZnS core–shell quantum dots suggests that the photooxidation of the thiol ligands is exergonic with a free energy change of ~−1.5 eV.[27] Presumably, this process eliminates a fraction of the defects on the surface of the

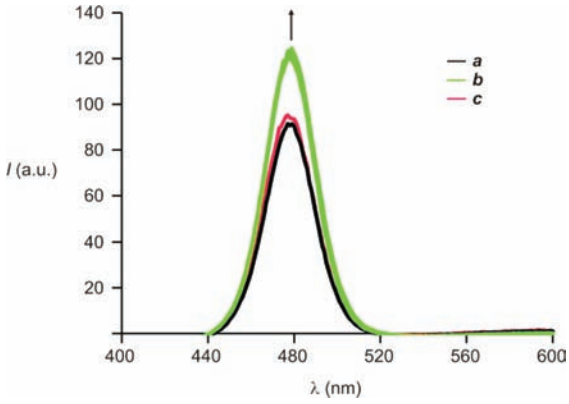

Figure 5. Emission spectra of CdSe–ZnS core–shell quantum dots (1.5 μM, THF, 25 °C, λ_{Ex} = 380 nm) coated with *n*-decanethiol before (*a*) and after (*b*) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 5, 10, 15, 20, 25, and 30 min) and subsequent storage in the dark for 48 h (*c*).

nanoparticles and, therefore, causes an enhancement in luminescence.[33] The thermal back reduction of the ligands can then restore gradually the surface defects and lower the emission intensity back to the original value, leading to the observed luminescence fading upon storage in the dark of an irradiated sample.

Synthesis and Spectroscopy of the Hydrophilic Quantum Dots. The CdSe–ZnS core–shell quantum dots coated with the copolymer **1** can be dispersed in organic solvents as well as in aqueous environments.[18b] The corresponding absorption spectra (*a* in Figures 6 and 7) show the band gap absorption of the nanoparticles to be centered at 450 nm in both THF and neutral phosphate buffer saline (PBS). Furthermore, the spectra do not change upon ultraviolet irradiation in both instances. Instead, the emission band of the quantum dots shifts from 466 nm in THF to 487 nm in PBS with a concomitant decrease in quantum yield from 0.46 to 0.30. In both instances, the emission intensity increases significantly with ultraviolet irradiation up to a stationary value (*b* and *c* in Figures 6 and 7) and then decreases after storage in the dark (*d* in Figures 6 and 7). These changes in luminescence parallel those observed for quantum dots coated with the model ligands **3**, **5** or *n*-decanethiol and, thus, appear to the related to the presence of anchoring thiol groups in the polymeric envelope around the inorganic core. Furthermore, essentially the same behavior can be reproduced in degassed solutions under an atmosphere of argon, indicating that molecular oxygen does not participate in these processes. By contrast, illumination of the very same samples with visible light, rather ultraviolet radiations, does not cause any change in their emission intensity.

In order to explore the influence of photocleavable 2-nitrobenzyl groups on the luminescence of our hydrophilic nanoparticles, we adapted a literature procedure[34] to prepare two batches of CdSe–ZnS core–shell quantum dots with sufficiently different core diameters to resolve their emission bands across the visible region. Then, we coated each set with the copolymer **1** and illuminated the resulting constructs with ultraviolet radiations for 30 min. This treatment results in an increase in luminescence up to a photostationary value (*c* in Figure 7), because of the photooxidation of the thiol anchoring groups in the polymeric envelope. At this point, we reacted a portion of each set of illuminated quantum dots with

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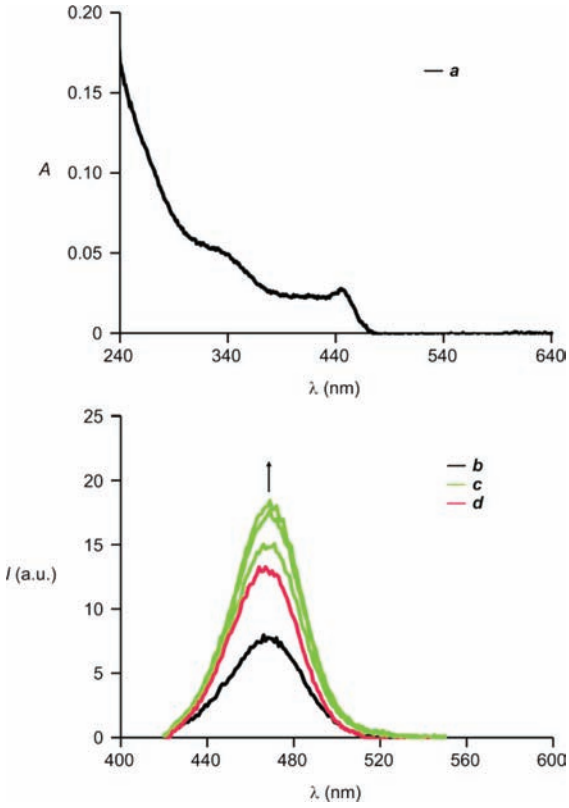

Figure 6. Absorption spectra of CdSe–ZnS core–shell quantum dots (1 μM, THF, 25 °C) coated with **1** (***a***). Emission spectra of CdSe–ZnS core–shell quantum dots (1 μM, THF, 25 °C, λ_{Ex} = 380 nm) coated with **1** before (***b***) and after (***c***) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 10, 20, and 30 min) and subsequent storage in the dark for 12 h (***d***).

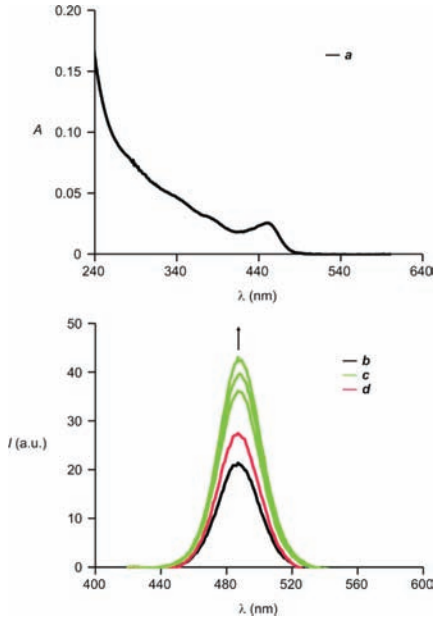

Figure 7. Absorption spectra of CdSe–ZnS core–shell quantum dots (4 μM, PBS, pH = 7.4, 25 °C) coated with **1** (***a***). Emission spectra of CdSe–ZnS core–shell quantum dots (4 μM, PBS, pH = 7.4, 25 °C, λ_{Ex} = 380 nm) coated with **1** before (***b***) and after (***c***) ultraviolet irradiation (365 nm, 0.4 mW cm^{-2}, 10, 20, and 35 min) and subsequent storage in the dark for 12 h (***d***).

2-nitrobenzylamine in PBS, under the assistance of EDC and sulfo-NHS, purified the final conjugates by size-exclusion chromatography and confirmed the presence of 2-nitrobenzyl groups on the nanoparticle surface by ^{1}H NMR spectroscopy. Finally, we combined irradiated and conjugated quantum dots of one dimension with irradiated, but unconjugated, nanoparticles of the other and vice versa. The emission spectra (***a*** and ***c*** in Figure 8) of the resulting dispersions reveal bands

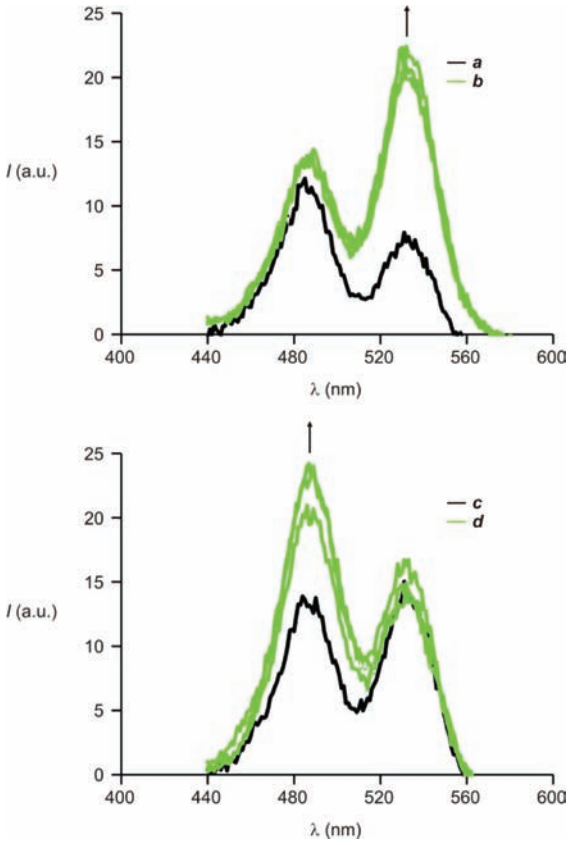

Figure 8. Emission spectra of mixtures (PBS, pH = 7.4, 25 °C, λ_{Ex} = 420 nm) of two sets of CdSe–ZnS core–shell quantum dots, both coated with **1** but differing in core diameter, recorded after ultraviolet irradiation for 30 min (365 nm, 0.4 mW cm^{-2}) and conjugation of 2-nitrobenzylamine to the set emitting at long (***a***) or short (***c***) wavelengths and after further ultraviolet irradiation for 5, 10, and 15 min (***b*** and ***d***). The concentrations of the quantum dots emitting at short wavelengths are 0.1 (***a*** and ***b***) and 1.8 μM (***c*** and ***d***) and those of the ones at emitting long wavelengths are 0.8 (***a*** and ***b***) and 5.6 μM (***c*** and ***d***).

centered at 485 and 531 nm for the two sets of quantum dots present in each mixture. However, the 2-nitrobenzyl groups are conjugated to the nanoparticles emitting at long wavelengths in one instance (***a*** in Figure 8) and to those emitting at short wavelengths in the other (***c*** in Figure 8). The time (<5 h) required to perform all of these experimental steps is relatively short compared to that (>24 h) necessary to revert in full the luminescence enhancement caused by the thiol groups upon ultraviolet illumination. As a result, the further irradiation of the nanoparticles reveals almost exclusively changes in emission intensity caused by the 2-nitrobenzyl groups. In fact, the luminescence of the conjugated quantum dots increases significantly in both mixtures upon illumination (***b*** and ***d*** in Figure 8), as a result of the photoinduced cleavage of their

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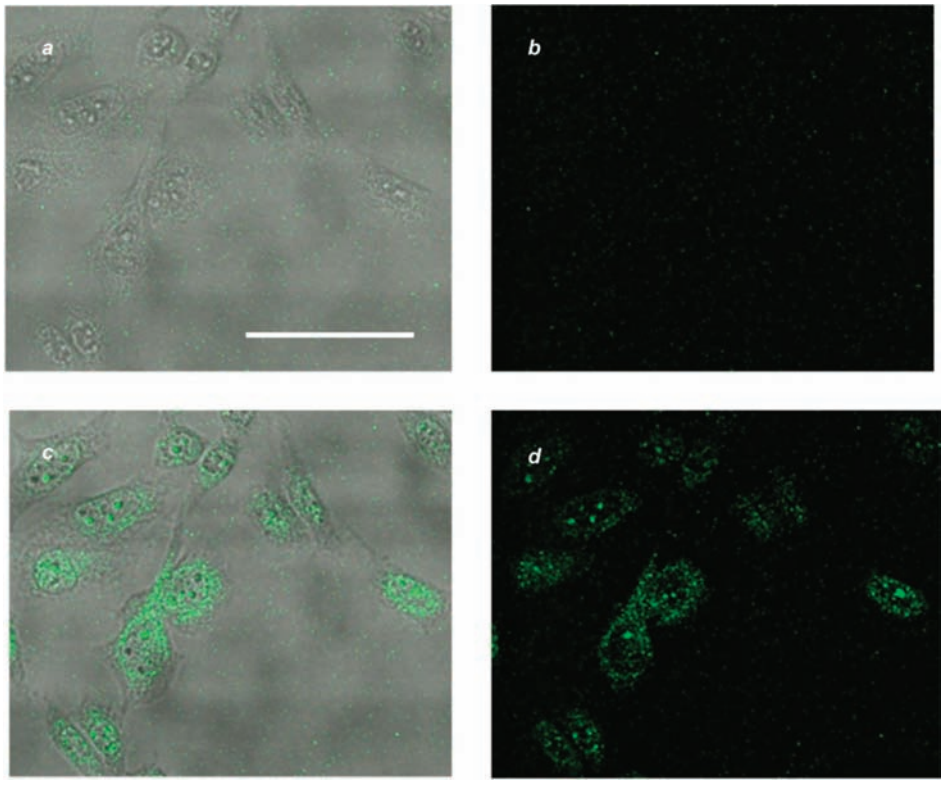

Figure 9. Phase-contrast (***a*** and ***c***) and luminescence (***b*** and ***d***) images (λ_{Ex} = 800 nm, scale bar = 50 μm), recorded before (***a*** and ***b***) and after (***c*** and ***d***) irradiation (365 nm, 0.4 mW cm^{-2}, 30 min), of CHO cells incubated with CdSe–ZnS core–shell quantum dots (30 nM), coated with **1** and conjugated to 2-nitrobenzylamine, for 3 h.

2-nitrobenzyl groups. Instead, the emission intensity of the unconjugated nanoparticles remains essentially unaffected. Thus, these results demonstrate unequivocally that the photolysis of 2-nitrobenzyl quenchers adsorbed on the surface of hydrophilic CdSe–ZnS core–shell quantum dots can indeed be exploited to switch the nanoparticle luminescence under optical control in aqueous environments. Once again, these changes are most likely a consequence of the ability of the 2-nitrobenzyl group to quench the luminescence of the CdSe core on the basis of electron transfer, in agreement with the behavior of analogous quantum dots coated with the model ligand **3**.[27] However, the poly(ethylene glycol) spacers of the polymeric ligand **1**, connecting the quenchers to the inorganic nanoparticles, are significantly longer than the aliphatic chain of the model ligand **3**. Presumably, the relatively flexible polymeric chains fold back to bring the 2-nitrobenzyl groups in close proximity to the ZnS shell and permit the transfer of electrons upon excitation.

Intracellular Luminescence Photoactivation. The photoinduced luminescence enhancement observed in aqueous environment (Figure 8), coupled to the established ability of our polymeric coatings to promote cellular internalization,[18b] encouraged us to investigate the intracellular behavior of nanoparticles bearing the photocleavable quenchers on their surface. Specifically, we incubated Chinese Hamster Ovarian (CHO) cells with CdSe–ZnS core–shell quantum dots, passivated with **1** and conjugated to 2-nitrobenzylamine, for 3 h. We then imaged the resulting specimen with a two-photon fluorescence microscope, operating at an excitation wavelength of 800 nm. The corresponding phase-contrast image (***a*** in Figure 9) clearly reveals the contour of individual cells, which, however, cannot be observed in the luminescence-only counterpart (***b*** in Figure 9). After ultraviolet illumination, the emission intensity of the internalized quantum dots increases significantly and the stained cells become visible in both phase-contrast and luminescence-only images (***c*** and ***d*** in Figure 9). Thus, the photoinduced cleavage of the 2-nitrobenzyl quenchers, with the concomitant enhancement in the nanoparticle luminescence observed in organic and aqueous solutions, occurs also within the intracellular environment. In order to quantify such luminescent enhancement, we measured the emission intensity at intervals of 0.3 μm along lines drawn across individual cells before and after ultraviolet illumination. The resulting averaged intensities indicate a photoinduced luminescence increase of 77%.[35]

■ CONCLUSIONS

Photocleavable 2-nitrobenzyl groups can be attached covalently to the polymeric coating of hydrophilic CdSe–ZnS core–shell quantum dots via amide-bond formation. Ultraviolet illumination of the resulting constructs in neutral buffer cleaves the 2-nitrobenzyl appendages from the surface of the nanoparticles and leads to a significant luminescence enhancement. Control experiments with model systems suggest that the 2-nitrobenzyl chromophores can accept an electron from the excited quantum dots and quench their luminescence. Therefore, the photoinduced removal of the 2-nitrobenzyl quenchers suppresses this particular electron-transfer pathway and enhances the nanoparticle luminescence. However, the behavior of the model systems also reveals that, in addition to the 2-nitrobenzyl chromophores, the thiol groups responsible for the adsorption of the polymeric coating on the ZnS shell of the quantum dots affect the excitation dynamics of these nanostructured constructs. Even in the absence of the

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photocleavable 2-nitrobenzyl quenchers, ultraviolet illumination of the nanoparticles results in a noticeable luminescence increase. Nonetheless, the magnitude of the emission change caused by the thiol groups is only a fraction of that associated with the cleavage of the 2-nitrobenzyl chromophores. Furthermore, the luminescence enhancement caused by the thiol groups is reversible and is not affected by the presence of molecular oxygen as well as by the nature of the solvent. Electrochemical data suggest that the photooxidation of these particular groups and their thermal back reduction are, presumably, responsible for the reversible change in emission intensity. In addition, the polymeric coating around the inorganic core encourages the internalization of these photoswitchable constructs in model cells. Ultraviolet illumination of stained cells results in the intracellular cleavage of the 2-nitrobenzyl quenchers with a luminescent enhancement approaching 80%. In summary, our results demonstrate that the photolysis of 2-nitrobenzyl groups can be exploited to enhance the luminescence of CdSe–ZnS core–shell quantum dots in organic solvents, aqueous solutions and even inside living cells. However, they also indicate that the thiol groups responsible for ligand adsorption contribute to the photoinduced luminescence enhancement.

EXPERIMENTAL PROCEDURES

Materials and Methods. Chemicals were purchased from commercial sources and used as received with the exception of CH_2Cl_2 and THF, which were distilled over CaH_2 and Na/benzophenone respectively, and H_2O, which was purified with a Barnstead International NANOpure DIamond Analytical system. CdSe–ZnS quantum dots coated with either TOPO or **1** were synthesized according to literature procedures.[18b] All reactions for the synthesis of **2**–**6** were monitored by thin-layer chromatography, using aluminum sheets coated with silica. Electrospray ionization mass spectra (ESIMS) were recorded with a Bruker micrOTO-Q II spectrometer. NMR spectra were recorded with either a Bruker Avance 300 or a Bruker Avance 400 spectrometer. Absorption spectra were recorded with a Varian Cary 100 Bio spectrometer, using quartz cells with a path a length of 0.5 cm. Emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions. Luminescence quantum yields were determined with a fluorescein standard, following a literature protocol.[36] Electrochemical measurements were performed with a CH Instruments 660 electrochemical analyzer under Ar, using a glassy-carbon working electrode (0.3 cm), a Pt counter electrode and a Ag/AgCl (3 M KCl) reference electrode. Samples were irradiated at 365 nm (0.4 mW cm^{-2}) with a Mineralight UVGL-25 lamp or at 562 nm (0.3 mW cm^{-2}) with a Spectral Energy LH150/1 light source and their absorption and emission spectra were recorded immediately after illumination.

Synthesis of 2. A solution of DCC (0.32 g, 1.5 mmol) in CH_2Cl_2 (9 mL) was added dropwise over the course of 30 min to a solution of 2-nitrobenzylamine (0.22 g, 1.4 mmol) and thioctic acid (0.44 g, 2.1 mmol) in CH_2Cl_2 (20 mL) maintained at 0 °C under Ar. The reaction mixture was allowed to warm up to ambient temperature and stirred for 24 h under these conditions. The resulting precipitate was filtered off and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography [$CHCl_3$/MeOH (22:1, v/v)] to afford **2** (0.27 g, 56%) as a brown oil. ESIMS: m/z = 340 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ = 1.37–1.39 (2H, m), 1.58–1.64 (4H, m), 1.79–1.84 (1H, m), 2.15–2.19 (2H, t, 8 Hz), 2.39–2.40 (1H, m), 3.05–3.12 (2H, m), 3.49–3.51 (1H, m), 4.62–4.63 (2H, s), 7.40–7.44 (1H, m), 7.57–7.63 (2H, m), 7.99–8.02 (1H, d, 8 Hz); ^{13}C NMR ($CDCl_3$): δ = 25.3, 27.5, 34.2, 36.3, 38.4, 39.0, 40.2, 56.3, 126.2, 129.7, 131.2, 132.5, 135.9, 146.9, 173.1.

Synthesis of 3. A mixture of **2** (240 mg, 0.7 mmol) and $NaBH_4$ (25 mg, 0.7 mmol) in MeOH (15 mL) was stirred at ambient temperature for 3 h, diluted with aqueous NaCl (1M, 85 mL) and extracted with $CHCl_3$ (3 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent distilled off under reduced pressure to afford **3** (200 mg, 89%) as a light yellow oil. ESIMS: m/z = 342 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ = 1.38–1.40 (2H, m), 1.59–1.65 (6H, m), 1.84–1.87 (1H, m), 2.16–2.19 (2H, t), 2.39–2.41 (1H, m), 3.06–3.13 (2H, m), 3.50–3.52 (1H, m), 4.62–4.64 (2H, s), 7.43–7.45 (1H, m), 7.58–7.64 (2H, m), 8.00–8.03 (1H, m).

Synthesis of 4. A solution of DCC (0.37 g, 1.8 mmol) in CH_2Cl_2 (10 mL) was added dropwise over the course of 30 min to a solution of benzylamine (175 μL, 1.6 mmol) and thioctic acid (0.50 g, 2.4 mmol) in CH_2Cl_2 (20 mL) maintained at 0 °C under Ar. The reaction mixture was allowed to warm up to ambient temperature and stirred for 24 h under these conditions. The resulting precipitate was filtered off and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography [$CHCl_3$/MeOH (22:1, v/v)] to afford **4** (0.29 g, 62%) as a yellow oil. ESIMS: m/z = 295 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ = 1.41–1.43 (2H, m), 1.64–1.66 (4H, m), 1.85–1.87 (1H, m), 2.15–2.19 (2H, t, 8 Hz), 2.38–2.42 (1H, m), 3.07–3.14 (2H, m), 3.50–3.53 (1H, m), 4.36–4.38 (2H, s), 7.26–7.28 (5H, m); ^{13}C NMR ($CDCl_3$): δ = 25.9, 29.4, 35.1, 36.8, 38.9, 40.7, 43.9, 56.9, 126.8, 127.9, 128.2, 128.8, 129.1, 138.9, 173.3.

Synthesis of 5. A mixture of **4** (240 mg, 0.8 mmol) and $NaBH_4$ (25 mg, 0.8 mmol) in MeOH (15 mL) was stirred at ambient temperature for 3 h, diluted with aqueous NaCl (1M, 85 mL) and extracted with $CHCl_3$ (3 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent distilled off under reduced pressure to afford **5** (210 mg, 88%) as a colorless oil. ESIMS: m/z = 296 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ = 1.42–1.43 (2H, m), 1.64–1.66 (6H, m), 1.89–1.94 (1H, m), 2.15–2.18 (2H, t, 8 Hz), 2.39–2.43 (1H, m), 3.07–3.15 (2H, m), 3.52–3.55 (1H, m), 4.36–4.39 (2H, s), 7.29–7.32 (5H, m).

Synthesis of 6. A solution of 2-nitrobenzylamine (62 mg, 0.4 mmol) and acetic anhydride (0.39 mL, 0.4 mmol) in CH_2Cl_2 (5 mL) was stirred at ambient temperature for 2 h. The solvent was distilled off under reduced pressure and the residue was crystallized from EtOH to give **6** (48 mg, 61%) as a yellow solid. ESIMS: m/z = 194 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ = 1.97 (3H, s), 4.62–4.64 (2H, s), 7.40–7.46 (1H, m), 7.61–7.69 (2H, m), 8.01–8.04 (1H, d); ^{13}C NMR ($CDCl_3$): δ = 23.5, 31.7, 113.2, 114.7, 116.1, 117.6, 119.9, 120.6, 165.4.

Adsorption of 3 and 5 on the Quantum Dots. A dispersion of CdSe–ZnS core–shell quantum dots coated with TOPO in hexane (0.02 mM, 2 mL) was diluted with EtOH (20 mL) and subjected to centrifugation. The supernatant was discarded and the solid residue was dispersed in $CHCl_3$ (10 mL) and diluted with a solution of either **3** or **4** (300 mg) in $CHCl_3$ (10 mL). The solvent was distilled off under reduced pressure and the residue was dispersed in EtOH (3 mL) and stirred at 70 °C for 12 h in a sealed vial under Ar. After cooling down to ambient temperature, the mixture was diluted with EtOH (5 mL) and transferred to a centrifuge tube. Consecutive aliquots (1 mL) of hexane were added with vigorous shaking until the formation of a precipitate was observed. After centrifugation, the precipitate was separated from the supernatant and dispersed in THF (3 mL) to afford the modified quantum dots.

Conjugation of 2-Nitrobenzylamine to the Quantum Dots. A dispersion of CdSe–ZnS core–shell quantum dots coated with **1** in PBS (3.4 μM, 400 μL, pH = 7.4) was combined with solutions of 2-nitrobenzylamine in DMSO (0.66 mM, 10.3 μL), EDC in PBS (10.4 mM, 25.9 μL, pH = 7.4) and sulfo-NHS in PBS (9.2 mM, 146.8 μL, pH = 7.4). The mixture was stirred at ambient temperature for 4 h and purified by size-exclusion chromatography [GE Healthcare PD-10, PBS (pH = 7.4)] to afford the modified quantum dots.

Intracellular Luminescence Photoactivation. CHO cells were cultured in F-12 nutrient mixture and supplemented with fetal bovine serum (10%, v/v), penicillin (200 U mL^{-1}), streptomycin (200 μg mL^{-1}) and glutamine (2 mM). After reaching confluency, the cells were harvested by trypsinization and seeded at a density of 5 × 10^4 cells mL^{-1} in a six-well plate containing one sterile cover slide (22 mm ×22 mm) per well. The cells were incubated at 37 °C with O_2/CO_2/air (20:5:75, v/v/v) overnight and then in the presence of CdSe–ZnS core–shell quantum dots (30 nM), coated with **1** and conjugated to 2-nitrobenzylamine, for a further 3 h. The coverslips were removed,

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washed with PBS (pH = 7.2, 3 × 1 mL) and fixed onto a glass slide for imaging. The images were recorded on an inverted Leica SP5 confocal/multiphoton microscope, using a two-photon excitation wavelength of 800 nm and collecting the emission between 416 and 540 nm.

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(27) The free energy changes (ΔG°) for the photoinduced electron transfer processes were estimated with equation 1, where e is the elementary charge, E_{Ox} is the oxidation potential of the species donating the electron, E_{Red} is the reduction potential of the species accepting the electron, ΔE_{00} is the optical band gap of the quantum dots, ε_0 is the vacuum permittivity, ε_r is the dielectric constant of the medium, and d is the distance between donor and acceptor (Kavarnos, G. J. *Fundamentals of Photoinduced Electron Transfer*; VCH: New York, 1993). The redox potentials of the quantum dots are +1.35 and −0.91 V vs Ag/AgCl (ref 34). Their optical band gap is 2.82 eV (*c* in Figure 2). The reduction potential of the 2-nitrobenzyl group is −1.10 V vs Ag/AgCl and the oxidation potential the bisthiol anchoring group is +0.45 V vs Ag/AgCl, according to the cyclic voltammograms of the model compounds **6** and **4**, respectively. The distance between the CdSe core and the 2-nitrobenzyl acceptors as well as that between the core and the thiol donors can be estimated to be 3.5 and 2.4 nm, respectively.

$$\Delta G^\circ = eE_{Ox} - eE_{Red} - \Delta E_{00} - \frac{e^2}{4\pi\varepsilon_0\varepsilon_r d} \quad (1)$$

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