Published in final edited form as:

Proc SPIE. 2009 March 3; 7189: 718904–.

'Giant' multishell CdSe nanocrystal quantum dots with suppressed blinking: Novel fluorescent probes for real-time detection of single-molecule events

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Abstract

We reported for the first time that key nanocrystal quantum dot (NQD) optical properties—quantum yield, photobleaching and blinking—can be rendered independent of NQD surface chemistry and environment by growth of a very thick, defect-free inorganic shell (Chen, et al. J. Am. Chem. Soc. 2008). Here, we show the precise shell-thickness dependence of these effects. We demonstrate that 'giant-shell' NQDs can be largely non-blinking for observation times as long as 54 minutes and that on-time fractions are independent of experimental time-resolution from 1–200 ms. These effects are primarily demonstrated on (CdSe)CdS (core)shell NQDs, but we also show that alloyed shells comprising $\text{Cd}_x\text{Zn}_{1-x}\text{S}$ and terminated with a non-cytotoxic ZnS layer exhibit similar properties. The mechanism for suppressed blinking and dramatically enhanced stability is attributed to both effective isolation of the NQD core excitonic wavefunction from the NQD surface, as well as a quasi-Type II electronic structure. The unusual electronic structure provides for effective spatial separation of the electron and hole into the shell and core, respectively, and, thereby, for reduced efficiencies in non-radiative Auger recombination.

Keywords

nanocrystal quantum dot; suppressed blinking; non-blinking; core-shell

1. INTRODUCTION

Semiconductor nanocrystal quantum dots (NQDs) are considered near-ideal fluorophores for bio-labeling and molecular probe applications based on their unique particle-size-tunable optical properties—efficient broadband absorption and narrow-band emission. Compared to alternative fluorophores, such as organic dyes, NQDs are characterized by significantly enhanced photostability.¹ Despite these enabling characteristics, NQD optical properties are frustratingly sensitive to their surface chemistry and chemical environment. The coordinating organic ligands used to passivate the NQD surface during growth are retained following preparation and are strong contributors to such bulk NQD optical properties as quantum yields (QYs) in emission. Unfortunately, ligands are labile and can become uncoordinated from the NQD surface and, as organic molecules, they can be damaged by exposure to the light sources used for NQD photoexcitation. Ligand loss through physical separation or photochemistry results in uncontrolled changes in QYs and, in the case of irreversible and complete loss, in permanent "darkening" or photobleaching.

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In addition, NQDs are characterized by significant “blinking” (fluorescence intermittency) at the single NQD level.² While a precise mechanism has yet to be universally accepted, blinking is generally considered to arise from an NQD charging process in which an electron (or a hole) is temporarily lost to the surrounding matrix or captured to surface-related trap states.^{2,3,4} The charged NQD is susceptible to efficient non-radiative Auger recombination processes. Thus, the NQD emission turns “off” when the NQD is charged and turns “on” again when NQD charge neutrality is regained. Blinking reduces the overall ‘brightness’ of the NQD emitter and interferes with applications requiring real-time monitoring of single biomolecules.

We recently reported for the first time that these key optical properties—QY, photobleaching and blinking—can be rendered independent of NQD surface chemistry and chemical environment by growth of a very thick inorganic shell.⁵ Due to the unusually thick shell, we called these NQDs ‘giant’ NQDs (g-NQDs). It is known that addition of an inorganic shell of a higher bandgap semiconductor material (e.g., ZnS onto CdSe) can generally enhance QYs and improve stability.^{6,7,8} However, the optical properties of conventional (core)shell and (core)multishell NQDs remain susceptible to ligand loss, changes in ligand identity through ligand-exchange reactions, and/or ligand concentration.^{9,10} In contrast, it has been reported that so-called ‘antiblinking reagents’¹¹ can be used to suppress blinking,^{12,13,14} where such organic reagents likely serve as charge mediators or charge compensators.^{9,11,13,14}

Unlike the organic-ligand or organic-shell approach, however, the all-inorganic shell approach reported by us⁵ and others¹⁵ allows for effective isolation of the NQD core excitonic wavefunction from the NQD surface and surface environment. In this way, the ‘giant-shell’ NQD is structurally similar to physically grown epitaxial QDs, for which optical properties are stable and blinking is not observed.¹⁶ Interestingly, it was reported in the case of (CdSe)ZnS (core)shell NQDs that shell thickness (up to ~7 monolayers) did not impact NQD blinking properties.¹⁷ It is not clear whether materials (e.g., improperly applied shells) or systems issues led to the conclusions reported for the (CdSe)ZnS NQD system. In the case of the (CdSe)CdS (core)shell system, the thick-shell structural regime is also characterized by an unusual electronic structure—Quasi Type II—for which non-radiative Auger processes are suppressed. Together, the novel physical and electronic structures enable important new NQD optical properties both at the ensemble and single-NQD level.

2. METHODOLOGY

Recently, (core)shell growth techniques have been refined to allow for precise control over shell thickness and shell monolayer additions. Specifically, a technique developed originally for the deposition of thin-films onto solid substrates—known as successive ion layer adsorption and reaction (SILAR)—was adapted for NQD shell growth.^{10,18} Here, homogenous nucleation of the shell composition is largely avoided and higher shell-growth temperatures are tolerated because the cationic and anionic species do not coexist in the growth solution. This method has allowed for growth of thick shells, comprising many shell monolayers, without loss of NQD size monodispersity and with superior shell crystalline quality. Originally demonstrated for a single-composition shell (CdS over CdSe) up to five monolayers thick,¹⁸ the approach has been extended to multishell architectures,¹⁰ and now to thick (~7–9 shell monolayers)¹⁵ and ‘ultra-thick’ shell systems (>10 monolayers).⁵ The resulting nanocrystals are highly crystalline, uniform in shape, and electronically well passivated.^{5,10,18}

The specific synthetic methods followed for the preparation and characterization of ‘giant’ (CdSe)CdS and (CdSe)alloyed-shell NQDs with suppressed blinking and remarkably enhanced stability are as follows:

2.1 Synthesis of CdSe-based giant-shell NQDs

Materials—Cadmium oxide, oleic acid (90%), 1-octadecene (ODE, 90%), dioctylamine (95%), octadecylamine, zinc oxide, sulfur, selenium pellet, and trioctyl phosphine (TOP) were purchased from Aldrich and used without further purification. Trioctyl phosphine oxide (TOPO) (90%) was purchased from Strem and used without further purification.

The synthesis of giant CdSe/multishell NQDs was based on a SILAR approach with minor modification.^{10,18} The CdSe core was prepared by injection of 1 ml 1.5 M Se-TOP solution into a hot solution containing 1.5 g octadecylamine, 0.5 g TOPO, 5 g octadecene, and 0.2 mmol Cd-oleate under standard air-free condition. After injection of Se-TOP at 290 °C, the temperature was set at 250 °C for CdSe NQD growth. After ten minutes, the solution was cooled down to room temperature, and CdSe NQDs were collected by precipitation with acetone and centrifugation. CdSe core NQDs were re-dispersed in hexane.

About 1.5×10^{-7} mol CdSe core NQDs in hexane were put into a 100 mL flask with 3 g ODE and 3 g dioctylamine. Instead of the primary amine used in the literature procedures, a secondary amine was chosen as the ligand to prevent the reaction between Cd-oleate and the amine ligands. 0.2 M elemental sulfur dissolved in ODE, 0.2 M Cd-oleate in ODE and 0.2 M $\text{Zn}_x\text{Cd}_{1-x}$ -oleate ($x = 0.13, 0.49, 0.78$, respectively) were used as precursors for shell growth. The quantity of precursors for each monolayer of shell was calculated according to the volume increment of each monolayer shell, considering the changing total NQD size with each successive monolayer grown. The reaction temperature was set at 240 °C. Growth times were 1 h for sulfur and 3 h for the cation precursors. CdSe-based g-NQDs of different shell compositions were synthesized. For example, CdSe cores with varying monolayers of CdS shell from 7 to 19, as well as CdSe cores with 11 monolayers of CdS shell plus 6 monolayers of $\text{Zn}_x\text{Cd}_{1-x}\text{S}$ alloy shell (successively richer in Zn from nominally 0.13 to 0.80 atomic-% Zn) and 2 monolayers of ZnS shell, were prepared.

To check the stability of g-NQDs with regard to purification, these NQDs were precipitated from growth solution and dispersed in hexane as described above. Further, they were subsequently subjected to multiple “purification” steps in which they were completely precipitated with methanol followed by re-dispersion in hexane. This process was repeated up to seven times and without loss of solubility. QYs in emission were measured (see below) in growth solution, as well as after each precipitation/re-dispersion cycle. As controls, CdSe core NQDs and standard CdSe core/shell and core/multishell NQDs were similarly prepared, purified and measured for QY.

CdSe g-NQDs were transferred into water by stirring purified NQDs ($\sim 5 \times 10^{-9}$ mol) in hexane with 1 mmol mercaptosuccinic acid in 5 mL deionized water overnight. Mercaptosuccinic acid was neutralized by tetramethylammonium hydroxide in water. The pH of the water was ~ 7 . Mercaptosuccinic acid-capped g-NQDs were collected by centrifugation, and were then re-dispersed in a small amount of water and precipitated again using an excess of methanol to remove excess mercaptosuccinic acid. Finally, the purified mercaptosuccinic acid-capped g-NQDs were dispersed in deionized water to form optically clear solutions.

2.2 Ensemble and single-NQD characterization

Ensemble NQD absorption and emission spectra were recorded on a CARY UV-VIS-NIR spectrophotometer and a NanoLog fluorometer, respectively. Quantum yields (QYs) for the g-NQDs and the various NQD control samples in hexane were measured by comparing the NQD emission with that of an organic dye (Rhodamine 590 in methanol). The excitation wavelength was 505 nm and emission was recorded from 520 nm – 750 nm. The QY of Rhodamine 590 was taken to be 95%, and those for the NQD samples were calculated by comparing the emission peak areas of the NQDs with the known dye solution. Specifically, the NQD QYs were calculated using the formula:

$$QY_{NQDs} = Abs_{dye} / Abs_{NQDs} * Peak\ area\ of\ NQDs / peak\ area\ of\ dye * QY_{dye} * (RI_{dye}^2 / RI_{NQDs}^2) \quad (1)$$

$$RI_{dye} - \text{refractive index of dye solution in methanol, } = 1.3284 \quad (2)$$

$$RI_{NQDs} - \text{refractive index of CdSe NQD solution in hexane, } = 1.3749 \quad (3)$$

The absorbance of the dye and the CdSe NQD solutions were controlled between 0.01 and 0.05 optical density. The absorbance and emission for each sample were measured twice at two different concentrations. The reported NQD QYs comprise averages of the two measurements. In an effort to obtain more accurate results, ~five or more measurements were conducted at different concentrations.

For photobleaching and blinking studies, freshly diluted (~0.1–50 pM) g-NQDs in either HPLC-grade toluene or deionized water were dispersed onto a clean quartz slide. Photobleaching behavior was assessed during continuous sample irradiation for several hours at a time over several days using a 532 nm, 180 mW continuous wave (cw) frequency double-YAG laser. Blinking behavior was assessed using two approaches:

“Slow” blinking data (temporal resolution ~200 ms) were obtained using a standard wide-field micro-photoluminescence (micro-PL) experiment, where the data set was collected over an area of 40×40 μm from multiple individual NQDs simultaneously. The excitation source was the 532 nm, 180 mW cw laser focused to ~50 μm diameter spot, and emission was monitored using a liquid-nitrogen-cooled charged-coupled device.

“Fast” blinking data (temporal resolution ~down to 1 ms) were obtained using a time-correlated single-photon-counting experiment (note: the time-resolution of the TCSPC is the rep rate of the laser; however, signal-to-noise issues limit the effective time-resolution to >1ms). Using a confocal microscope, a 60X 0.75NA objective was used to collect the image as well as to focus the beam onto a single NQD in a 1 μm spot. A 150 fs frequency-doubled Ti-sapphire pulsed laser (440 nm, 42 pJ/pulse) was used to excite the NQD, and a Si avalanche photodiode was used to detect emitted photons.

A computer program designed to extract the intensity fluctuations of all individual NQDs for these series of images was used to analyze blinking statistics.

Note: dynamic light scattering (DLS) data for the solutions used to make the sub-monolayer films for blinking and photobleaching studies showed no evidence for clustering or aggregation of the g-NQDs, and their DLS-extracted hydrodynamic diameters (HD's) were

consistent with those derived from TEM plus 2 ligand layers.⁵ Additionally, the observed blinking statistics were independent of the concentration of NQDs in each experiment.

3. RESULTS AND DISCUSSION

Here, we report preliminary follow-on results as an extension of our original account,⁵ where a detailed analysis of these results will be reported elsewhere. Originally, we reported that under continuous excitation conditions, significantly suppressed blinking behavior was observed for g-NQDs (single-composition shells and alloyed shells) in contrast with in-house-prepared and commercial conventional-NQD control samples.⁵ The distinctive single-NQD fluorescence behavior and the blinking statistics for a large population (>500) of single g-NQDs are shown in Figure 1. The experimental temporal resolution is ~200 ms, and in all such cases, the total observation time is a remarkable 54 minutes. In the case of classically blinking NQDs (e.g., commercial NQDs and in-house synthesized thinner-shell NQDs), >70% of the NQDs have on-time fractions (fraction of total observation time that a single-NQD is 'on') of <0.2. In contrast, ~50% of the g-NQDs in Figure 1 are non-blinking—have an on-time fraction of >0.99. Previously, only organic surface-ligand approaches had been used successfully to suppress blinking in NQDs, but none compare to the environmental and temporal robustness of the all-inorganic thick-shell approach.

We observe a clear dependence of blinking properties on shell thickness. Specifically, a (CdSe)CdS NQD comprising a 5-monolayer shell behaves wholly unlike (CdSe)CdS NQDs comprising 7 or more monolayer shells (Figure 2). In contrast with the thicker-shell variations, no fraction of the 5-monolayer NQD sample is non-blinking for the duration of the observation period (54 minutes), and, in fact, even at observation times as short as 100 s, the fraction of 5-monolayer NQDs that are 'on' for >99% of the time is zero. On the contrary, the thicker-shell NQDs are characterized by non-blinking NQD fractions that range from >0.10 to ~0.50 for the longest observation time of 54 minutes. This distinct cutoff between conventional-NQD and g-NQD behavior may arise from a threshold shell thickness having been reached that corresponds to the thickness necessary to effectively confine the core excitonic wavefunction from NQD-surface effects and/or to transition from a Type I to a Quasi Type II electronic structure, for which Auger efficiencies are reduced. Interestingly, blinking statistics continue to improve with increasing shell thickness until 16 CdS monolayers (Figure 2), suggesting that there is little advantage to indefinitely increasing the shell thickness. The limit to shell thickness may correspond to a structural regime in which strain effects induce formation of defect sites that result in enhanced non-radiative trap emission. Finally, the decline in blinking statistics is not linear. Non-blinking NQDs become blinking-NQDs at a faster rate in the first 5 minutes of observation. After 5 minutes, a non-blinking NQD is more likely to remain so. Thus, the possibility for long-term stability is apparent in these systems.

The (CdSe)11CdS-6Cd_xZn_yS-2ZnS alloyed shell—total shell thickness of 19 monolayers and terminated with 'bio-friendly'¹⁹ ZnS—is plotted in Figure 2 along with the all-CdS variations. The alloyed g-NQD data fall in between those for the (CdSe)10CdS and (CdSe)12CdS g-NQDs, rendering its behavior more like the 10–12 monolayer variations than the 19-monolayer variation. Thus, it appears that the first 11 shell monolayers—the CdS layers—dominate the properties in this system, and the alloyed shells simply provide for a structurally graded transition from CdS to the terminating ZnS. Long-observation-time (54 minutes) blinking data were obtained using the wide-field micro-PL approach described in **Methodology**. This technique has the advantages of permitting data to be collected from multiple NQDs at the same time and of long observation times. Unfortunately, however, the temporal resolution is limited to ~200 ms – 100 ms acquisition time plus an 80 ms CCD read-out time. Therefore, blinking on timescales shorter than 200 ms could be registered as

high-frequency on-level fluctuations in our experiment, and we would not be able to see this ‘blinking’ given our temporal resolution. An example of high-frequency *fluctuations* (as opposed to ‘off’ events) is evident in the time traces shown in Figure 1. However, we determined that the standard deviation of the high-frequency on-level fluctuations that appear in our time traces is approximately equal to the square root of the signal level. Further, all of the analyzed NQDs—both non-blinking and blinking—exhibit approximately the same noise level. Therefore, although the existence of ‘blinking’ events in the fluctuating ‘on’ level could not be ruled out by our ‘slow-blinking’ data, we believed that the high-frequency fluctuations resulted mainly from the random shot noise of the detector.

To confirm that on-time fractions of our g-NQDs are independent of experimental time-resolution from 1–200 ms, we performed follow-on ‘fast-blinking’ studies using a time-correlated single-photon-counting experiment (**Methodology**). As evidenced in Figure 3 for both a 10 ms and a 1 ms ‘bin’ time (temporal resolution), our non-blinking NQDs do not exhibit ‘off’ behavior even at short collection times.

In addition to intriguing and functional blinking properties, the g-NQDs are also characterized by remarkable photostability. Under continuous illumination (532 nm, 180 mW laser), the g-NQDs exhibit limited tendencies for photobleaching or simply do not photobleach. Similar to blinking results, there appears to be a clear threshold behavior manifested between 5 and 7 shell monolayers. Preliminary shell-thickness dependencies are shown here (Figure 4).

4. CONCLUSION

Thick-shell or g-NQDs possess significantly suppressed blinking and substantially improved stability against photobleaching as a function of inorganic-shell thickness. Together, these characteristics will enable bio-labeling and advanced molecular probe applications that were previously impeded by the un-optimized single-NQD-level optical properties characteristic of conventional NQDs. A mechanistic-level understanding of these results, as well as a more detailed understanding of the effects of core and shell composition and structure will be reported elsewhere.

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Fig. 1. On-time histogram of CdSe/16CdS g-NQD. Inset: Fluorescence time trace of a representative single NQD; temporal resolution is 200 ms.

Fig. 2. Shell-thickness dependence of blinking behavior. Fraction of NQDs that are 'non-blinking', defined as 'on' for >99% of the specific observation time versus observation time. (a) Linear plot. (b) Log plot.

Fig. 3. Blinking data obtained using a time-correlated-single-photon-counting technique showing blinking behavior at timescales down to 1 ms. For non-blinking giant-shell NQDs, no blinking was observed at these faster timescales for the complete observation time of almost 4 minutes.

Fig. 4.

(a) Photobleaching behavior of (CdSe)CdS NQDs possessing different shell thicknesses (' n ' = number of shell monolayers) presented as NQD fraction emitting over time. (b) Fluorescence time-traces of representative single NQDs for each shell thickness.