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'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 $Cd_xZn_{1-x}S$ 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

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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 photostabilility.[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.

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

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 similiarly 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 mercatosuccinic 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:

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

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

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

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 40x40 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.

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-levelfluctuations 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.

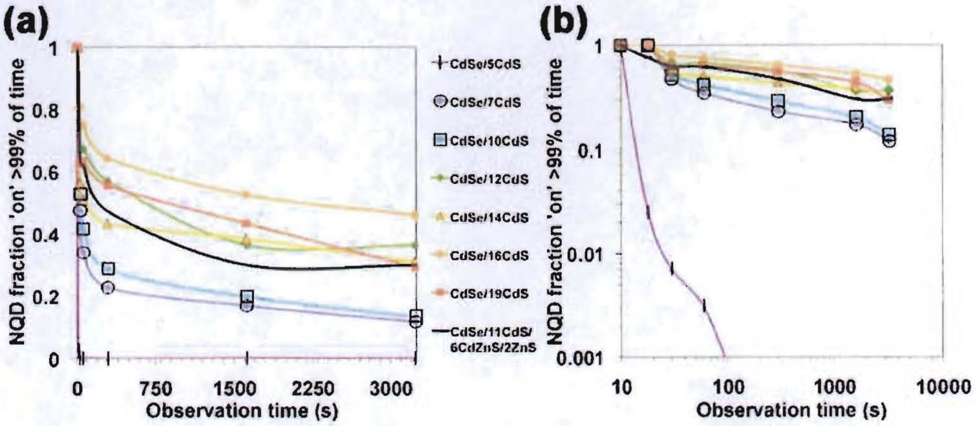

Fig. 2. Shell-thickness dependence of blinking behavior. Fraction of NQDs that are 'non-blinking', defined as 'on' for >99% of the specific observation time versus observation time. (a) Linear plot. (b) Log plot.

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.

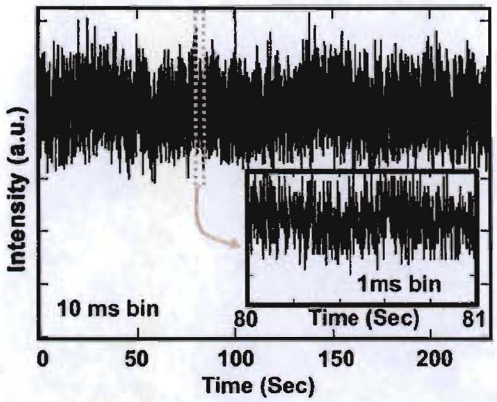

Fig. 3. Blinking data obtained using a time-correlated-single-photon-counting technique showing blinking behavior at timescales down to 1 ms. For non-blinking giant-shell NQDs, no blinking was observed at these faster timescales for the complete observation time of almost 4 minutes.

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