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Near-Complete Suppression of Quantum Dot Blinking in Ambient Conditions

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Colloidal semiconductor quantum dots are attractive fluorophores for biological imaging because of broad absorption and narrow emission spectra, and they are brighter and far more photostable than organic dyes.[1] Surface passivation by a semiconductor layer with higher band gap or polymers was reported to improve optical properties of quantum dots such as quantum yield and photobleaching.[2] However, severe intermittence in emission (also known as blinking) has been universally observed from single dots[3] and has been considered an intrinsic limitation difficult to overcome. This is unfortunate because growing applications in spectroscopy of single biological molecules[4] and quantum information processing using single-photon sources[5] could greatly benefit from long-lasting and nonblinking single-molecule emitters. For instance, in a recent application of single-dot imaging, the tracking of membrane receptors was interrupted frequently due to the stroboscopic nature of recording.[6] Blinking can also reduce the brightness in ensemble imaging via signal saturation. Here we show that passivation of the quantum dot surfaces with thiol moieties suppresses blinking with the emission duty cycle approaching 100% while maintaining biocompatibility.

For single-molecule experiments, a narrow channel was made between a cleaned quartz microscope slide (Finkenbeiner) and a coverslip using double-sided adhesive tape. The surface was treated with 40 μL of 1 mg/mL biotinylated BSA (Sigma) in 10 mM Tris-HCl, pH 7.4, 50 mM NaCl (TN buffer). After 10 min of incubation, and washing out with TN buffer, 40 μL of 20−100 pM water-soluble, streptavidin-coated CdSe/ZnS quantum dots[7] (Qdot 655 nm or Qdot 585 nm, Quantum Dot Corp.) were injected into the channel. The concentration of the quantum dot solution was adjusted to give good surface density for single-molecule experiments. After checking that fluorescent spots were well separated from one another, we injected 60 μL of TN buffer with the test chemicals given in the text. The wide-field microscope is based on an inverted microscope (Olympus IX70) with a 60× water immersion objective with numerical aperture of 1.2 (Olympus, Melville, NY) and an intensified CCD camera (Intensified Pentamax, Roper Scientific, Trenton, NJ) that can record the intensities from several hundred single molecules simultaneously. Solid-state 532 nm lasers (Crystalaser) were used to excite the molecules. Data were acquired using software written in Visual C++ (Microsoft).

In TN buffer, individual dots (Qdot 655 nm) excited at 532 nm showed severe blinking behavior (upper trace in Figure 1a), 100 ms bin time). However, for most of quantum dots, the blinking behavior all but disappeared in the presence of 140 mM of β-mercaptoethanol (BME) (lower trace in Figure 1a). The effect of BME is immediate since the blinking was suppressed as soon as the BME buffer was delivered via a flow system (upper trace in Figure 1b). There seems to be no permanent change to the quantum dot properties because the blinking behavior reappeared immediately after flowing in the TN buffer for the most of quantum dots (lower trace in Figure 1b). Measured at the ensemble level, BME greatly reduces emission saturation at high excitation intensities, consistent

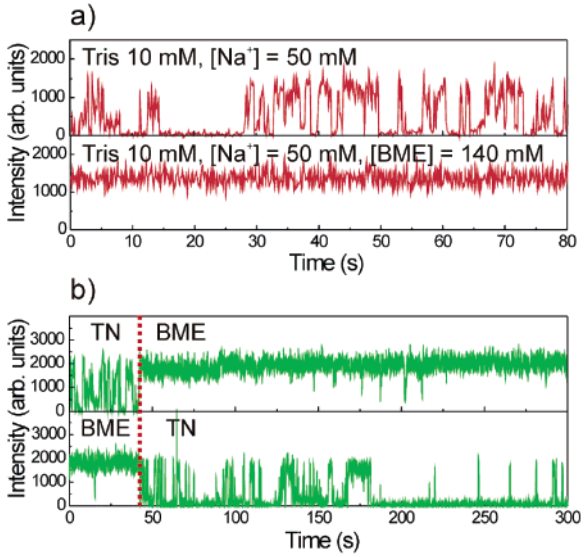

Figure 1. (a) Typical intensity time traces of CdSe single-molecule quantum dots with emission peak at 655 nm in TN buffer (upper panel) and in TN buffer with 140 mM BME (lower panel). (b) Upper panel shows a single dot (emission peak at 585 nm) intensity trace as TN buffer with 140 mM BME was injected at ∼40 s (red dotted lines) into the sample, displacing TN buffer from the sample. Lower panel shows a time trace of a reverse case where BME buffer was washed away using TN buffer at ∼40 s.

with blinking suppression and increased emission duty cycle (see Supporting Information).

To pin down which moiety takes part in blinking reduction, we tested various chemicals. Only thiol-containing chemicals whose chain lengths are relatively short showed strong effects on blinking reduction, and their efficiency seems to be independent of the number of thiol groups per molecule. The fraction of nearly blinking-free time traces (defined as those that showed two or less blinking events in 80 s) was over 80% at or above 10 mM BME or dithiothreitol, and decreased at lower concentrations (Figure 2). At 1 mM BME 60% of dots were still nearly blinking-free. We could not observe any blinking reduction when we added large thiol-containing chemicals such as glutathione. Therefore, the reduction of blinking seems to occur when the thiol groups bind to ZnS surfaces rather than to the polymer overcoat; we assume that the polymer layer has small holes and that the size of molecule is critical for the penetration to quantum dot surfaces. We also tested the effect of oxygen molecules on blinking because it was reported that photooxidation causes quantum dot degradation.[2] We enzymatically removed oxygen molecules dissolved in TN buffer using glucose oxidase and glucose, but no blinking suppression was observed.

To gain insights into the mechanism for this striking suppression of blinking, we obtained the on- and off-time statistics for various BME concentrations. Histograms of dwell times of on- and off-states were generated using data acquired from approximately 1000

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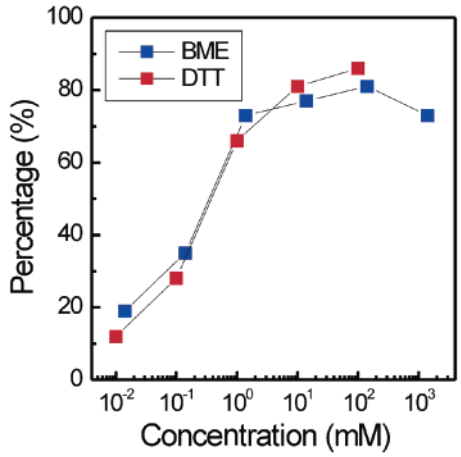

Figure 2. The fraction of traces of quantum dots with 585-nm emission peak which show two or less blinking events for 80 s at several BME (blue squares) and DTT (red squares) concentrations.

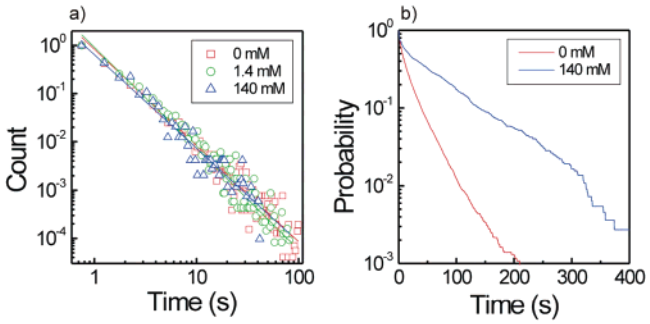

Figure 3. Statistics of on- and off dwell times. (a) Normalized off-time histogram ($P(t)$) at various BME concentrations. Each data set is fitted to the power law ($P(t) \propto t^{-\alpha}$) with similar exponents (α =2.00, 2.10, and 1.94 for 0 mM (red squares), 1.4 mM (green circles), and 140 mM (blue triangles) of BME, respectively) (b) On-time probability distribution vs time, defined as the probability a Qdot remains "on" after indicated time elapses, without (red lines) and with 140 mM of BME (blue lines).

molecules. If the suppression is due to the shortening of the off-time, the off-times would have strong dependence on BME concentration. However, the off-time distributions displayed identical power-law dependence[8] with the similar exponents at all BME concentrations (Figure 3a), while a substantial lengthening of the on-time was observed at 140 mM BME compared with data without BME (Figure 3b). This is in contrast to the observations made near metallic surfaces at cryogenic temperatures[9] in which on−off blinking statistics were maintained but the quantum dots in the normally "off" state were rendered "on" via the surface-enhanced radiative recombination. Therefore, the mechanism of blinking suppression here appears to be novel.

It has been proposed that the off-state is due to a charged dot which lost an electron to a surface trap and that the existence of many such surface traps of various depths is responsible for the power-law dependence.[8] On the basis of our data and previous studies on blinking mechanism, we propose the following as a likely mechanism for blinking suppression. The thiol moiety, a potent electron donor, donates electrons to the surface electron traps, rendering them incapable of accepting electrons from the dot, hence reducing the frequency of blinking. Quenching of the surface traps by the thiol moiety may not be complete, for instance, due to some traps that are not readily accessible, accounting for the remaining blinking events.

Blinking suppression was still efficient at 1 mM BME, a concentration that is much lower than what has been used in many single-molecule experiments in vitro (70−140 mM). This effect, therefore, should allow the *continuous* observation of movements of Qdot-labeled motor proteins (kinesin, myosin, helicase, etc.) and nucleic acids molecules. Yet for certain applications such as live cell imaging or molecules with disulfide bonds, even 1 mM BME may not be compatible. For these applications, it will be desirable to develop new approaches of modifying Qdots based on a better understanding of the blinking suppression mechanism.

In summary, we have shown that the blinking of colloidal semiconductor quantum dots can be suppressed in ambient and biologically relevant conditions. Surface passivation of quantum dot surfaces by the binding of the thiol group seems to be the critical mechanism. This unexpected observation should make quantum dots ideal as single-molecular light sources for a variety of applications.

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Supporting Information Available: Figure showing reduction of emission saturation by BME (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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