Fluorescence

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Single Nonblinking CdTe Quantum Dots Synthesized in Aqueous Thiopropionic Acid**

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Colloidal semiconductor nanocrystals, also known as quantum dots (QDs), have received considerable attention in recent years because of their unique optical properties including photostability, brightness, narrow but tunable emission spectra, and a broad spectral excitation cross section. These properties make them promising candidates for optoelectronic and biological applications.[1,2] At the single-particle level, however, QDs exhibit severe fluorescence intermittence or blinking (i.e., bright "on" intervals with fluorescence alternate with almost dark "off" intervals without fluorescence or with fluorescence of reduced intensity under continuous laser excitation) on a wide time scale from milliseconds to minutes, and in most cases, on/off events follow an inverse power-law probability distribution.[3] This phenomenon has been ubiquitously observed for luminescent nanocrystal samples of different compositions and structures[4] and represents an intrinsic limitation for certain practical uses, especially as a single-photon light source for quantum information processing and continuous monitoring of single labeled biomolecules. It is very important to develop new approaches for the synthesis of nonblinking QDs or to suppress blinking.

The optical properties of luminescent QDs, such as quantum yield, photostability, and blinking statistics, are highly dependent on their surface chemistry and synthesis conditions.[5,6] At present, most semiconductor QDs are prepared almost to completion in an organic phase by using solvents with high boiling points, such as trioctylphosphane (TOP) or trioctylphosphane oxide (TOPO) or a mixture of TOP and TOPO, and the QDs have to be modified by using surface-chemistry methods to make the surfaces hydrophilic before they are coupled to biomolecules. To date, it is accepted wisdom that all QDs synthesized in organic phases exhibit blinking that is difficult to overcome. In recent years, methods of preparing fluorescent QDs in aqueous phases

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have been significantly improved.[7] Aqueous syntheses provide certain advantages, compared with those of organic phases, such as low toxicity, inexpensiveness, and especially that the products have high quantum yields (40–67%), excellent water-solubility, and biological compatibility such that some have been used in fixed- and live-cell imaging.[8] Aqueous syntheses of luminescent QDs are receiving more and more attention.[9] However, the optical properties of single QDs synthesized in the aqueous phase remain unclear. It is a question of nature as to whether single QDs synthesized in the aqueous phase blink, as do those prepared in the organic phase. Herein, we study the luminescent properties of individual CdTe QDs, synthesized in aqueous solutions, by using total internal reflection fluorescence microscopy (TIRFM). Surprisingly, we found that individual CdTe QDs synthesized in thiopropionic acid (TPA) aqueous solutions did not blink, which is in contrast to those synthesized in organic phases.

CdTe QDs in this study were synthesized in aqueous phases by using the reaction between Cd^{2+} ions and NaHTe in solution in the presence of TPA as a stabilizer; quantum yields of the products were 40–50%. Figure 1 shows a 500 ms exposure–time fluorescence image of single CdTe QDs, embedded in a poly(ethylene oxide) (PEO) matrix, at room temperature. In the fluorescence images, individual QDs are shown to be distributed homogeneously in the PEO matrix and no aggregation is observed. The non-uniform spatial distribution of spot intensities is a consequence of the exponential decay of the evanescent field generated upon total internal reflection at the coverslip–PEO interface and brightness variations of different QDs, and these do not influence the time dependence of the fluorescent behavior of each spot, which was also observed and discussed previously.[10] Water-soluble PEO was used to disperse QDs onto coverslips to avoid aggregation of CdTe QDs during sample preparation. We found that PEO is a good matrix to disperse water-soluble CdTe QDs on coverslips for obtaining high-quality fluorescence images of individual QDs (Figure 1). Furthermore, fluorescence spectroscopy also confirms that PEO, unlike polyvinylpyrrolidone (PVP), does not quench the fluorescence of CdTe QDs (data not shown). A typical fluorescence spectrum of single CdTe QDs is given in the Supporting Information). Figure 2 shows the fluorescence intensity trajectories, together with background intensity, of five random individual CdTe QDs with a time resolution of 200 ms for durations of 500 s under continuous wave excitation. Continuous fluorescence emission of individual CdTe QDs is clearly visible. Indeed, all individual CdTe QDs in the field of view, which could be traced simultaneously by TIRFM, did not blink (see Supporting Information for the corresponding video). To rule out possible influence from the integration time scale, the fluorescence intensity trajectory with a time resolution of 50 ms was also recorded, which showed a similar continuous fluorescence emission (see Supporting Information). For comparison, we also imaged individual CdSe/ZnS QDs prepared in an organic phase (Jiayuan Quantun Dots Co.). Fluorescence blinking for these CdSe/ZnS QDs was observed, which is consistent with previous reports.[3] The intensity trajectories and the corresponding videos are available in the Supporting Information. These data demonstrate that our TIRFM system is a very powerful tool for the characterization of blinking of QDs. Why do CdTe QDs synthesized in the aqueous phase not blink? To date, the blinking mechanism of QDs is not clear but thought to arise from electron (or hole) ejection from an Auger process, pure electron tunneling into the surrounding matrix, or electron capture in trap states of the surface of the particle.[3a,c,11] It was shown that blinking may exhibit unique kinetics by different degrees of surface passivation or by altering synthesis conditions and that the associated mechanism is related to defect sites or unsaturated dangling bonds located at the surface of the QDs.[6a,b] CdTe QDs used in our study were synthesized in aqueous phases in the presence of TPA as a stabilizer and their surfaces were well coated with TPA. We speculate that TPA has a critical effect on the optical properties of CdTe QDs synthesized in aqueous solution. The thio moiety in TPA is an excellent electron donor and donates electrons to the surface traps, thus rendering them incapable of accepting electrons from the carrier or serving as channels

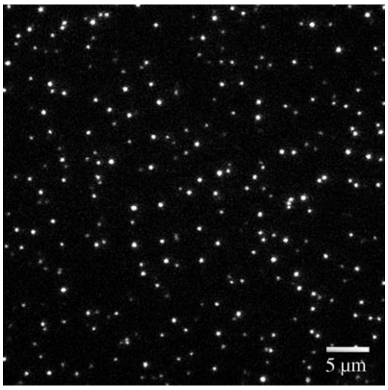

Figure 1. TIRFM image (45×45 µm^2) of single CdTe QDs embedded in a PEO matrix at room temperature. Emission peak at 689 nm; exposure time: 500 ms.

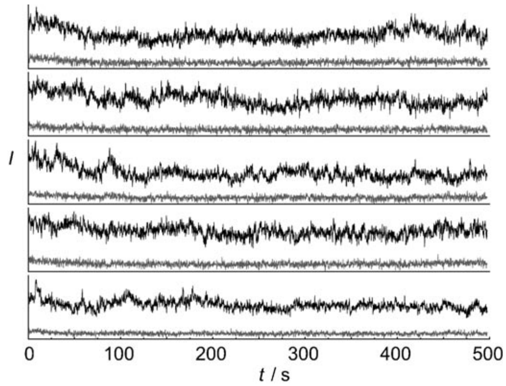

Figure 2. Fluorescence-intensity trajectories of five random individual CdTe QDs (emission peak at 689 nm) with a temporal resolution of 200 ms for durations of 500 s. The local background (gray) was extracted from the average intensity around each spot.

for efficient charge tunneling from the interior of the QDs to the surrounding matrix. Recently, it was reported that the blinking of QDs can be suppressed by antiblinking agents, such as β-thioethanol (BTE) and thioethylamine (TEA), or when the surfaces of QDs were coordinated with organic ligands, such as oligo(phenylene vinylene) (OPV).[6c–e] To confirm the role of TPA on the surface of the QDs used in our study, TPA on the surfaces of CdTe QDs was exchanged with citrate, and the fluorescence-intensity trajectories of citrate-coated QDs were recorded (Figure 3); surprisingly, blinking was observed. In addition, the fluorescence of citrate-coated QDs decreases significantly and photobleaches rapidly under continuous laser excitation. This result is attributed to ligand exchange or surface damage of the CdTe QDs. Furthermore, we investigated the fluorescent emission of individual CdTe QDs synthesized in an aqueous phase in the presence of glutathione, another stabilizer that contains a thio group.[12] The results show that glutathione-coated CdTe QDs also do not blink (data not shown). Our data illustrate that thio-based compounds, such as TPA and glutathione, play a key role in suppressing the blinking of CdTe QDs and improving their photostability. So far, the synthesis of QDs such as CdTe, CdSe, ZnSe, and HgTe in aqueous solutions has involved the use of thio-containing compounds as stabilizers, which coat the surface of these QDs.

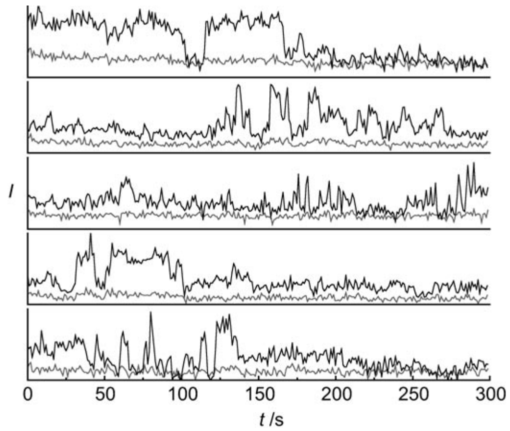

Figure 3. Fluorescence-intensity trajectories of five random individual citrate-coated CdTe QDs with a temporal resolution of 1000 ms for durations of 500 s. The local background (gray) was extracted from the average intensity around each spot.

In conclusion, we have used TIRFM to observe individual CdTe QDs synthesized in aqueous solution and have investigated their fluorescence-emission behavior. We have found that individual CdTe QDs synthesized in TPA solution do not blink. Our experiments have confirmed that the TPA coating on the CdTe QDs plays a key role in suppressing blinking. We believe that these CdTe QDs are suitable for use in single-molecule imaging and single-molecule detection as fluorescent probes as they are water-soluble, biocompatible, bright, and do not blink. However, CdTe QDs, especially after purification, photobleach more rapidly than CdSe/ZnS QDs prepared in an organic phase (see Supporting Information). For individual purified CdTe QDs, fluorescence emission was beyond detection after about 15 min under continuous wave excitation. We speculate that this deterioration is due to the damage of TPA or photooxidation of CdTe. We are working to improve the photostability of the CdTe QDs synthesized in an aqueous phase.

Experimental Section

CdTe QDs were prepared in the presence of TPA (Fluka) as described previously.[7d,e] Briefly, Cd precursor solutions were prepared by mixing a solution of $CdCl_2$ (1.25×10^{-3} M) with a solution of TPA and this mixture was adjusted to a pH of 8–9 with NaOH (1M). The solution was purged with N_2 then vigorously stirred while oxygen-free NaHTe (or NaHSe) solution was injected; the molar ratio of Cd/Te/TPA was 2.5:1:6. The resulting mixture was heated to reflux for different periods of time to control the size of QDs. UV/Vis absorption spectra of TPA-stabilized QDs were obtained by using a Lambda 20 UV/Vis spectrophotometer (Perkin-Elmer). Photoluminescence spectra were recorded on a Varian Cary Fluorescence Spectrophotometer. CdTe QDs with emission peaks at 689 nm or 625 nm were used. Citrate-coated CdTe QDs were prepared by adding purified QDs to a citrate solution (10 mM; pH 8.3) to induce ligand exchange. Glutathione-coated CdTe QDs were synthesized in glutathione solution as described previously.[12]

Coverslips ($24\times32\times0.17$ mm^3) were thoroughly cleaned prior to sample preparation by sonication in chromic acid mixture, NaOH solution (0.1M), ethanol, and ultrapure water (Millipore), each for 15 min, then dried in a jet of N_2. An aqueous solution of CdTe QDs (about 1.0×10^{-8} mol L^{-1}, estimated by the fluorescence correlation spectroscopy) and an aqueous solution of PEO (1%; MW 100000, Sigma) were mixed in a 1:1 ratio. CdSe/ZnS QDs (emission peak at 625 nm, Wuhan Jiayuan Quantum dots Co. Ltd., China) were dispersed in a chloroform solution of PMMA (poly(methyl methacrylate); 2%, Sigma). A small drop of this mixture was carefully coated onto the coverslips by rolling with a clean cylindrical glass rod.

CdTe QDs were imaged by a total internal reflection fluorescence microscope based on an Olympus IX 71 inverted fluorescence microscope (Olympus Optical Co.). A schematic diagram of the TIRFM setup is given in the Supporting Information. Samples were excited with a He–Ne laser operating at 543.5 nm (5 mW, Coherent) and the laser power monitored in front of the microscopy objective (numerical aperture (NA) = 1.45, 60×, Olympus) was measured to be 0.65 mW. Fluorescence from the sample was collected by the same objective, separated from the excitation light by a suitable dichroic mirror (570 nm) and emission filters (long-pass filter, 590 nm), and then focused into a cooled-CCD (charged coupled device) camera with a frame-transfer device (Cascade 650, Photometrics). The frame-transfer device has an imaging array of 653×492 with 7.4×7.4 μm^2/pixel. As the image can be quickly transferred from the sensor area to the frame transfer area, there is no need for a mechanical shutter. When operated in overlap mode, the camera is extremely useful in applications requiring continuous imaging (100% duty cycle) and can capture all the light emitted from the specimen. Image acquisition and processing were performed using the MetaMorph program (Universal Imaging). The local background was extracted from the average intensity around each spot. All measurements were performed at room temperature.

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