INSTITUTE OF PHYSICS PUBLISHING NANOTECHNOLOGY
Nanotechnology **17** (2006) 5875–5881 doi:10.1088/0957-4484/17/23/027

Improvement of the photostability of thiol-capped CdTe quantum dots in aqueous solutions and in living cells by surface treatment

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Received 17 August 2006, in final form 2 October 2006
Published 16 November 2006
Online at stacks.iop.org/Nano/17/5875

Abstract
The surface treatment of thiol-capped CdTe quantum dots (QDs) was carried out with a small amount of sodium borohydride ($NaBH_4$) in aqueous solution at room temperature. The treatment effectively enhanced the photostability of QDs and increased the photoluminescence (PL) efficiency by a factor of two as against the original ones. By measuring the PL trajectories of single QDs with a total internal reflection fluorescence microscope under the same irradiation density of 0.72 μW μm^{-2} at 532 nm, the photostable lifetimes were determined to be 15.2 ± 5.9 s for surface-treated QDs, and 5.8 ± 1.9 s for the original ones, respectively. Both the original and treated QDs could be ingested into human hepatocellular carcinoma cells (QGY) by incubation. The photobleaching of cellular QDs was measured with a confocal microscope. Remarkable enhancement was found for the photostability of the surface-treated QDs in QGY cells, demonstrating its advantage for cellular labelling applications.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Semiconductor quantum dots (QDs), with a radius smaller than the Bohr radius, have strong size-dependent photoluminescence (PL) properties due to the quantum confinement effect of charge carriers [1]. Since Nie and Alivisatos first reported cellular labelling with hydrophilic QDs [2, 3], QDs have received widespread attentions from biological and medical fields [4–9]. Compared with the conventional organic fluorescent probes, QDs have advantages in cellular labelling, such as higher PL quantum efficiency, size tunable PL, wide continuum absorption, narrower luminescent band, and higher photostability [10–12].

For the synthesis of QDs, the commonly used approach is the high-temperature reaction of the semiconductor material in organic solvents in the presence of surfactants. Monodisperse and stable particles (QDs) are formed in the reaction. Because of their small size, QDs have a large surface/volume ratio,

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and many inhomogeneous defects exist on the surface area. These defects cause a non-radiative relaxation resulting in a low PL efficiency. So, a shell of a semiconductor material with higher band gap is usually grown around the core of the QDs to passivate the particle surfaces and increase the PL efficiency [13]. These core/shell QDs still need to be treated again by replacing the hydrophobic surface with ligands such as mercaptoacetic acid to make the QDs become hydrophilic [14, 15]. Though finally water-soluble QDs with PL efficiency around 0.6 can be obtained, the synthetic procedure is complicated.

An alternative way developed recently by Zhang *et al* [16] is the hydrothermal route, in which water-soluble QDs could be prepared directly in aqueous solution at a reaction temperature around 200 °C. This is a simple and efficient way, but it pays the price of harvesting a much lower concentration of QDs with a relatively lower PL efficiency.

Among these water-soluble QDs, no matter what the synthesis method is, the thiol-capped QD is a commonly used type [17]. However, photochemical instability of thiol-capped QDs was reported [18], although they are comparatively more photostable than the organic fluorophores [2, 3]. Recently, we studied the photostability of thiol-capped CdTe QDs in living cells, and found that those QDs were photobleached under the focused light from a microscope objective lens, especially when the intracellular QD concentration was low [19]. Therefore, the improvement of the photostability of QDs is still a challenge for practical applications, especially for cellular imaging.

It was reported that reducing agents such as beta-mercaptoethanol increased the photostability of QDs when they existed in QD solutions [20, 21]. In the present work, $NaBH_4$ was used as a reducing agent to treat the surfaces of thiol-capped CdTe QDs since $NaBH_4$ was found to change the surface property as well as the PL efficiency of some II–VI compound QDs [22]. Our results show that, after $NaBH_4$ treatment, the photostability of QDs was remarkably enhanced in aqueous solutions and in living cells as well.

2. Experimental details

2.1. Synthesis of CdTe QDs

2.1.1. Synthesis of thiol-capped CdTe QDs. Thiol-capped CdTe QDs were prepared in our laboratories by a modified hydrothermal route. The details of the synthesis procedure can be found in our previous work [17]. Briefly, with a molar ratio of 2:1, sodium borohydride was used to react with tellurium in water to prepare sodium hydrogen telluride (NaHTe, 1 mM). A fresh NaHTe solution was added to a nitrogen-saturated $CdCl_2$ solution in the presence of thioglycolic acid (TGA) at pH 9.0 in an ice–water bath. The molar ratio was fixed at 1:1.5:0.5 ($[Cd^{2+}]$:[TGA]:$[Te^{2-}]$). Then, the CdTe precursor solution (40 ml) was put into a Teflon-lined stainless steel autoclave with a volume of 50 ml. The autoclave was maintained at the desired temperatures for certain times. After hydro-cooling to room temperature, thiol-capped CdTe QDs dispersed in water were obtained.

2.1.2. Surface treatment. The as-prepared CdTe QD solution (1 mg ml^{-1}) was added with a reducing agent, $NaBH_4$ (1 wt% of the QD amount), for surface treatment. The mixture was stirred for 24 h at room temperature. After treatment, the dispersed QDs were precipitated from the solution using excess ethanol. Then, the solution was centrifuged to harvest the QDs. The QD powders obtained were dried in vacuum and stored in a nitrogen-filled container for subsequent use. To prepare the surface-treated QD solution, a certain amount of powder was dissolved into water to give a desired concentration.

2.2. Cell culture

2.2.1. QGY cells. The QGY cells (human hepatocellular carcinomacell line 7701) were obtained from the cell bank of Shanghai Science Academy [23]. The cells were seeded into culture dishes containing RPMI1640 medium with 10% calf serum, 100 units ml^{-1} penicillin, 100 $\mu g\ ml^{-1}$ streptomycin and 100 $\mu g\ ml^{-1}$ neomycin, and incubated in a fully humidified incubator at 37 °C with 5% of CO_2.

When the cells reached 80% confluence with normal morphology, the solution of original QDs or $NaBH_4$-treated QDs was added into the cell dishes with a final concentration of 100 $\mu g\ ml^{-1}$. These cell dishes were then incubated for 3–4 h. Under such an incubation concentration of QDs, no detectable damage to cells was observed. After incubation, these QD-loaded cells were washed with phosphate-buffered saline (PBS) three times to remove the unbound QDs, and fixed with 10% formol (pH 7.4) for 10 min at room temperature. Finally, the fixed cells were washed with PBS and made ready for PL imaging measurements.

2.3. Experimental measurements

2.3.1. Cellular images. Cellular images were acquired with a confocal laser scanning microscope (Olympus, FV-300, IX71). A water immersion objective (60×) and a matched pinhole were used in measurements. Using the z-scan mode, the images in different layers can be recorded to obtain the three-dimensional distribution of intracellular QDs and the relative PL intensities.

2.3.2. Photobleaching measurements in living cells and in solutions. The photobleaching of cellular QDs was measured with the point-stay mode of the confocal microscope with an appropriate filter set. In this mode, the focused laser beam was fixed at the selected spot of the cell and the time-dependent PL intensities were recorded by a photomultiplier tube (PMT). The spot size of the focused laser beam was about 1 μm in diameter. The irradiation power density was then calculated by measuring the laser power with a power meter (Coherent, Lasercheck).

The photobleaching of QDs in aqueous solution was investigated in a small cuvette under a continuous laser irradiation at 532 nm (Coherent, Nd:YVO_4). The decay of the PL signal was recorded by a PMT with a band-pass filter (585–640 nm).

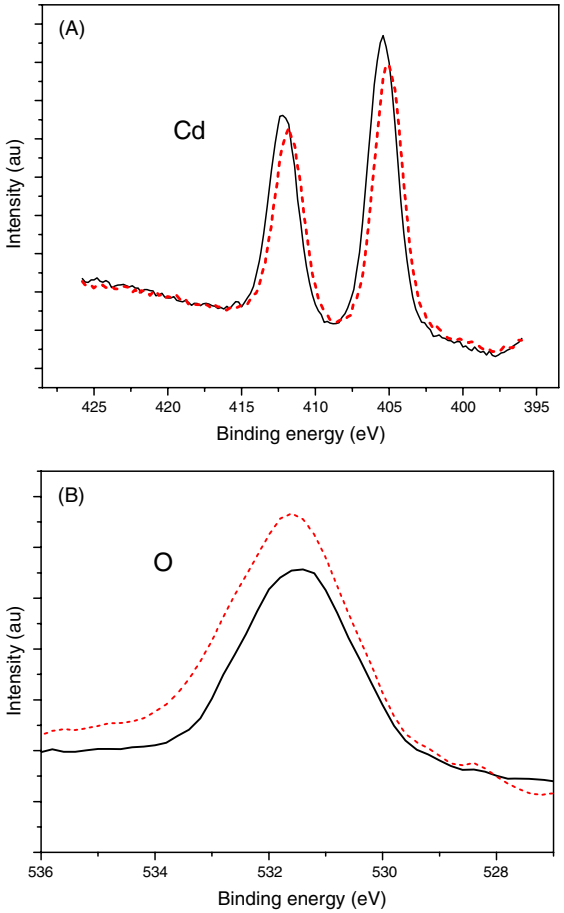

Figure 1. Cd 3d (A) and O 1s (B) XPS spectra of the original (solid lines) and surface-treated (dotted lines) CdTe QDs.

2.3.3. Photobleaching measurement of single QDs. To quantitatively compare the photostability of the original and surface-treated QDs, the photostable lifetimes of these two kinds of single QDs were measured with a total-internal-reflection fluorescence microscope (TIRFM). The TIRFM setup was similar to that in the literature [24], built around an inverted microscope (Olympus IX71). In this method, the evanescence wave from the laser beam was totally reflected from the bottom surface of the prism, which only excited the QDs that were very close to the prism bottom surface. The QD aqueous solution at a very low concentration of 7 μg ml^{-1} was sealed in a pair of cover-slips, which was coupled via immersion oil to the bottom plane of the prism. A polarized 532 nm laser beam (Photop SUWTECH, DPGL-2100F) was incident through the prism onto the bottom interface at 69° and was totally reflected internally. The PL signals were imaged using a 60× NA 1.4 objective (oil-coupled to the cover-slip) onto an intensified CCD camera (DVC-Intensicam 1312M-LV-1B) mounted at one of the microscope exit ports with a band-pass filter. The camera format was 1300 × 1030. At the operating gain of 60 k, about 20 photons per pixel were required to produce a signal-to-dark noise ratio of 3. The PL images of a set of single QDs were acquired continuously with an exposure time of 43 ms. The excitation power density was 0.72 μW μm^{-2} in all measurements. According to the obtained trajectories of the PL intensities, the photostable lifetime of each QD was determined.

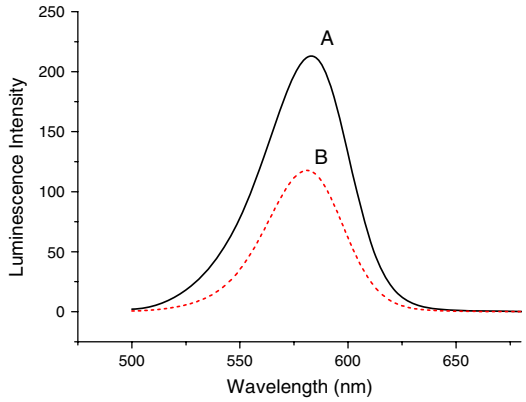

Figure 2. PL spectra of the treated (A) and original (B) CdTe QDs in aqueous solutions excited at 400 nm.

2.3.4. X-ray photoelectron spectroscopy measurements. X-ray photoelectron spectroscopy (XPS) spectra were measured with a PHI-5000C ESCA system (Perkin Elmer) with Al Kα radiation (1486.6 eV). The sample powders were directly pressed onto a self-supported disc (10 mm × 10 mm) mounted on a sample holder and then transferred into the analyser chamber. The wide-scanning spectrum (0–1200 eV) and the high-resolution spectrum were recorded simultaneously. Binding energies were calibrated by using the containment carbon peak (C 1s = 284.6 eV).

3. Results and discussion

Figure 1 shows the XPS spectra of the CdTe QDs before and after surface treatment. The typical peaks of Cd $3d_{5/2}$ at 405 eV and Cd $3d_{3/2}$ at 411 eV appeared for the original CdTe QDs. After surface treatment, a 0.2 eV shift toward the lower binding energy was found for both peaks (figure 1(A)), suggesting that the oxidized Cd state (CdO) was formed, according to the previous report [25]. The O 1s peak at 531.7 eV (figure 1(B)) was found for original QDs, which should come from the carboxyl oxygen of thioglycolic ligands in these thiol-capped CdTe QDs, and this was enhanced after the treatment, reflecting that more oxygen atoms bound to the QD surface via the treatment, supporting the suggestion of CdO formation [26]. The XPS data are in good agreement with the previous report [22], in which the hydrophobic CdSe and CdTe QDs were treated with $NaBH_4$ in organic solution to form the CdO layer. Our results demonstrate that the $NaBH_4$ treatment can be extended to water-soluble QDs because $NaBH_4$ is also water-soluble.

The PL spectra of QDs in aqueous solutions were measured at room temperature using a fluorescence spectrophotometer (Hitachi, F-2500) with an excitation wavelength of 400 nm. As depicted in figure 2, the PL spectra of the original and treated QDs have the same peak wavelength (585 nm) and bandwidth (40 nm), but the PL intensity of the treated QDs was doubled. Since the CdO has a valence band binding energy of

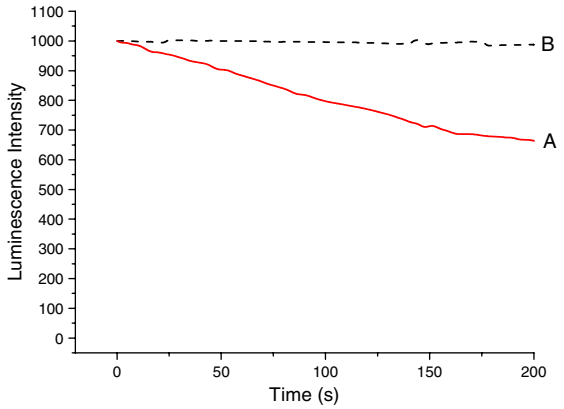

Figure 3. Photobleaching curves of the original (A) and surface-treated (B) QDs in aqueous solutions (0.1 mg ml^{-1}). The irradiation wavelength and power density were 532 nm and 80 mW mm^{-2}, respectively.

5.4 eV [27], which is higher than the CdTe band gap, the passivation effect of CdO is somewhat like that of the core–shell QDs. Thus, the increment of PL efficiency after the $NaBH_4$ treatment can be understood.

We found in our previous work [19] that the thiol-capped CdTe QDs were photobleached under laser irradiation, and the mechanism was mainly due to photooxidation. In this work, the CdO layer formed on the QD core surface was expected to shield the oxygen molecules from interacting with the QD core and to inhibit the photooxidation effectively.

The improvement of the photostability by surface treatment was studied in an aqueous solution (0.1 mg ml^{-1}) under continuous laser irradiation. As shown in figure 3, at the irradiation power density of 80 mW mm^{-2}, the original QDs were notably photobleached, while $NaBH_4$-treated QDs were much more photostable.

In order to quantitatively evaluate the improvement of the photostability, the photostable lifetimes of the original and $NaBH_4$-treated single QDs were measured with TIRFM. In this way, the affections on fluorescence intensity measurements, such as the occasional aggregation of QDs and the concentration fluctuations in the solutions, can be ruled out. In the measurements, only the QDs that adhered to the cover-slip surface were clearly imaged, because the excitation depth of the evanescence wave is very limited. With a very low QD concentration of 7 μg ml^{-1}, the PL images showed that most of the QDs existed as single QDs. Under the excitation of 0.72 μW μm^{-2}, the PL intensity trajectories of QDs were recorded with the continuous image-acquiring mode (43 ms exposure time for each image). From these obtained trajectories, the time evolution (resolution of 43 ms) of the PL intensity of each single QD can be picked out for analysis. Figure 4 shows the typical PL time evolutions from an original single QD and a treated single QD, respectively. Luminescence intermittency (blinking), which is the representative signal of a single QD, was found in figures 4(B) and (D), confirming that

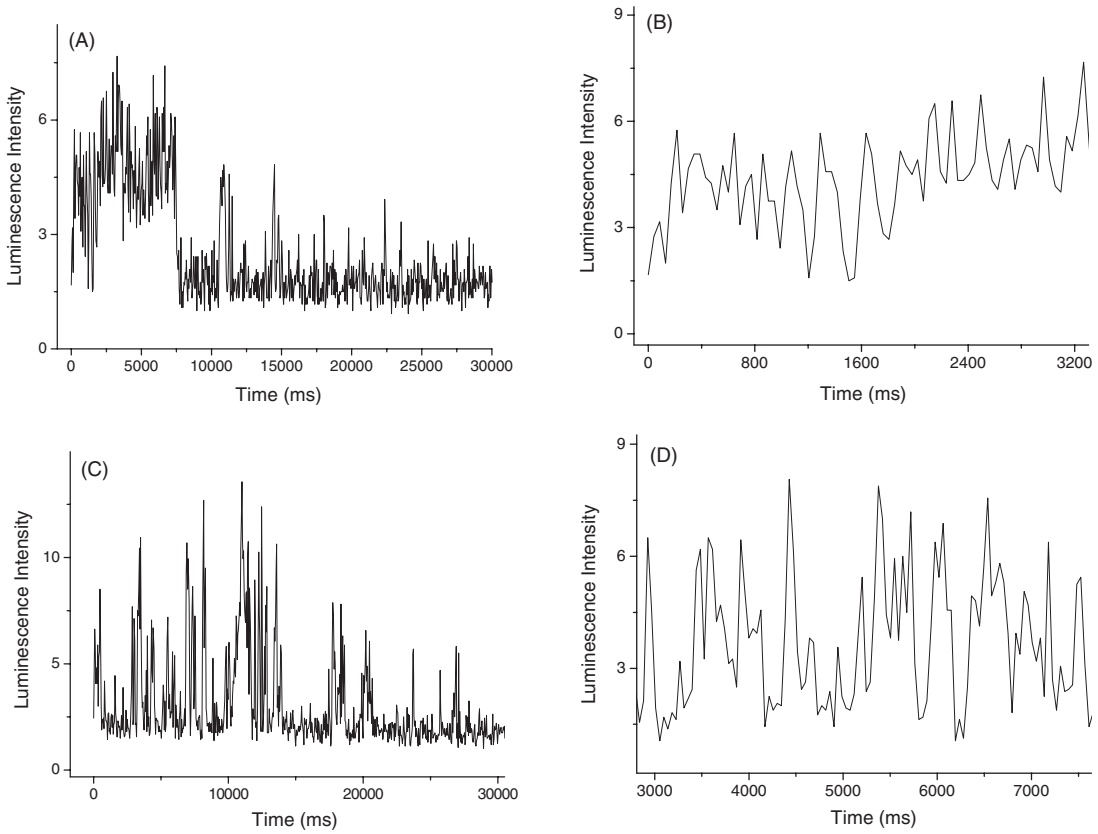

Figure 4. PL intensity trajectories for an original (A) and a treated (C) single CdTe QD with 43 ms binning. (B) and (D) are the zoomed-in trajectories of (A) and (C), respectively, to show the details of the blinking. All trajectories were recorded under the excitation at 532 nm with a power density of 0.72 μW μm^{-2}.

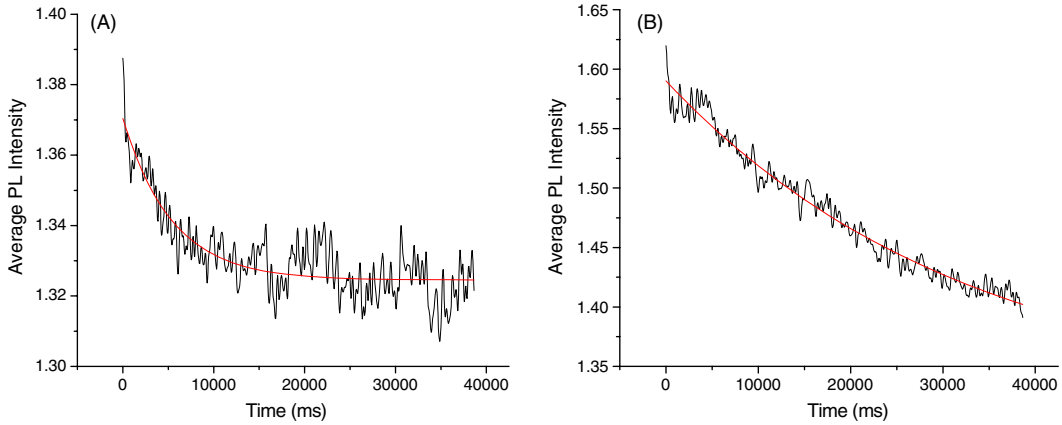

Figure 5. Summed up PL intensities from the continuous QD images measured by total internal reflection. (A) Original CdTe QDs; (B) treated CdTe QDs.

the imaged QDs really existed as single QDs [28]. Comparing the PL intensities between figures 4(A) and (C), the PL from a treated QD was about two times brighter than that from an original QD, providing evidence of the PL enhancement. As was reported previously [29], the photobleaching behaviour of a single molecule differed from that of a bulk system. Similarly, the PL intensity of a single QD did not decrease gradually but kept constant on average in a certain period until losing its brightness completely with a sudden drop, which was the symbolic character of a single QD in its photobleaching process. Such a character is shown in figures 4(A) and (C), indicating that each single QD has its own photostable lifetime. The lifetimes of 7.7 s for an original QD (figure 4(A)) and 14.1 s for a treated QD (figure 4(C)) are illustrative examples. To statistically determine the average photostable lifetime of QDs, about 50 single QDs were picked out one by one for measurements. The average photostable lifetimes of single QDs were determined to be 15.2 $\pm$ 5.9 s for treated QDs and 5.8 $\pm$ 1.9 s for original QDs, respectively. A three-fold increment of the photostability was obtained by $NaBH_4$ treatment.

Each obtained CCD image contained a lot of luminescent QDs like the bright stars in a dark sky. The summed up PL intensity of all the QDs in one image decayed with irradiation time as shown in figure 5. As a statistical result, it is the group behaviour of the photobleaching. The PL intensities showed exponential decays instead of a sudden drop as in the case of a single QD. The fitted exponential curves agree well with the measured ones. The fitted decay times are 5.4 and 32.7 s for original and treated QDs, respectively, providing further evidence for the enhancement of the photostability of QDs by surface treatment.

Although the photostability was improved by $NaBH_4$ treatment, photobleaching still happened. Is photooxidation still the main reason for the photobleaching of those $NaBH_4$-treated QDs? To answer this question, aqueous solutions of surface-treated QDs were bubbled with nitrogen gas, oxygen gas or air, and the photobleaching courses were then studied for comparison as shown in figure 6. When oxygen was purged away from the solution (curve (A)), the QDs were extremely photostable. The more oxygen existed in the solution, the worse the photostability was (curves (B) and (C)),

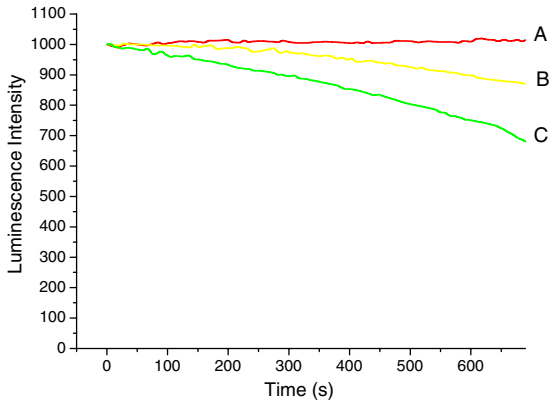

Figure 6. Photobleaching curves of the surface-treated QDs in aqueous solutions (0.07 mg ml^{-1}) bubbled with nitrogen gas to purge away oxygen (A), in air (B) and bubbled with oxygen gas (C).

indicating that photooxidation was still the main reason for photobleaching of these surface-treated QDs. The CdO monolayer was supposed to be formed during the $NaBH_4$ treatment, but it was likely not a compact layer and could not absolutely isolate the QD core from diffusing oxygen molecules. However, the CdO layer already effectively shielded the QD cores, resulting in an enhanced photostability. To minimize the disturbance from the diffusing effect of QDs in aqueous solutions, an unfocused laser beam with a diameter of 4 mm was used in photobleaching measurements. At such a low power density (40 mW mm^{-2}), the photobleaching looks linear because only the initial part of the decay curve is demonstrated.

The goal of using QDs as fluorescent probes is to label the targets in biological systems. Reduced photobleaching of QDs is of particular importance for experiments with long-term imaging, such as fluorescence labelling of transport processes in cells, or tracking the path of some typical molecules [30]. Therefore, the good photostability of intracellular QDs is an elementary requirement for their applications.

Figure 7 shows the PL images of both original ((A)–(C)) and treated ((D)–(F)) QDs in QGY cells under the same excitation and measuring conditions. All the cells were

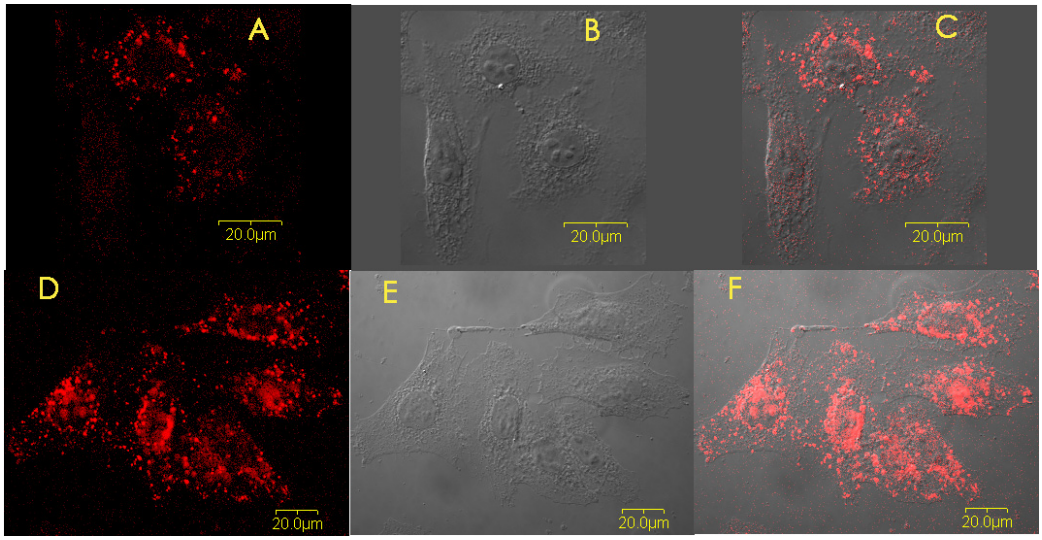

Figure 7. Images of QD-labeled QGY cells. (A) PL image of the original cellular QDs; (B) DIC image of the cells in (A); (C) merged image of (A) and (B). (D)–(F) are the same as (A)–(C) but for surface-treated QDs.

incubated with the same QD concentration (100 μg ml^{-1}) for 4 h and then washed. The PL images in figures 7(A) and (D) were recorded with a 585–630 nm band-pass filter. Figures 7(B) and (E) are the differential interference contrast (DIC) images to exhibit the cell morphology. Figures 7(C) and (F) are the merged images. Both kinds of QDs (treated and untreated) were found to distribute diffusely inside the cells with similar concentrations, but the PL from the treated QDs (figure 7(D)) looked much brighter than that from the original QDs (figure 7(A)) because the treated QDs had a higher PL quantum efficiency.

Based on these PL images, the photobleachings were further compared between the original and treated cellular QDs. However, the comparison should be made at the same QD cellular concentration. As shown in figure 7, the intracellular distributions of both original and treated QDs are not uniform. The PL intensity in a certain area of the cell can be read out from the recorded image. For the same kind of QDs, the PL intensity was proportional to the local concentration of QDs in that area. The PL quantum efficiency of cellular QDs was supposed to be equal to that of the QDs in aqueous solution since the cytoplasm of cells is the water-dominated solvent. Since the PL quantum efficiency is doubled after surface treatment, the images were screened to find the corresponding areas with PL intensity in figure 7(D) twice that in 7(A). Those two selected areas were supposed to have the same QD local concentration, and the photostabilities were then comparatively measured. The results are shown in figure 8, where the laser irradiation power was 0.3 mW, which is in the power region commonly used for fluorescence measurements with a confocal microscope, and the focused laser spot on the cell was about 1 μm in diameter. Under such an irradiation power density (4×10^{-4} W μm^{-2}), the original QDs were photobleached quickly (curve (A)), losing half of the PL intensity in about 1 s, which would seriously restrict the measurement of cellular imaging. In contrast, the treated QDs endured a much longer photobleaching time of about 5 s

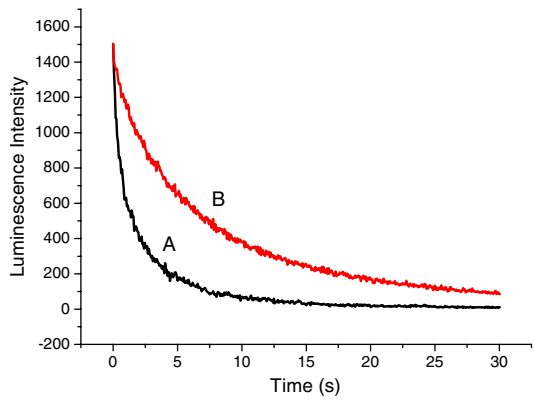

Figure 8. Photobleaching curves of original (A) and treated (B) QDs in QGY cells. The irradiation areas were selected to have the same QD concentration. The irradiation power density was 4×10^{-4} W μm^{-2}.

(curve (B)), indicating that the treated QDs have a much better photostability in living cells as well.

It was reported that the adding of reducing agents, such as beta-mercaptoethanol, to the QD solution could increase the photostability of QDs [20, 21]. However, in this work, the reducing agent $NaBH_4$ was only used to treat the QD surface and it was then filtered out and did not exist in the QD solution any more. Therefore, the mechanism is different. The enhanced QD photostability in this work was supposed to result from the CdO layer formed by surface treatment, which blocked the photooxidation of QDs.

In the $NaBH_4$ treatment, some factors should be noticed. The amount of $NaBH_4$ added into the QD solution and the reaction time are both important. An excess amount of $NaBH_4$ and prolonged reaction time will cause the detachment of thioglycolic ligands from the QD core, resulting in the aggregation and precipitation of QDs. In our experiment, the QDs were properly treated to maintain their good aqueous solubility. No precipitation of the treated QDs was found for

several weeks when the solution was placed in a sealed bottle and kept in the dark.

As described in section 2, after $NaBH_4$ treatment, the QDs were precipitated from the solution using excess ethanol, harvested by centrifugation and dried to become powders. These QD powders were stored in a nitrogen-filled container, and could be used at any time by dissolving them in water. They are very stable and have been used for more than half a year.

4. Conclusion

With $NaBH_4$ treatment, the photostability and PL efficiency of the thiol-capped CdTe QDs in either aqueous solutions or living cells were effectively improved, demonstrating that this treating method is helpful in the applications of QD cellular labelling especially for long-term cellular imaging measurements.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (60578045), the Shanghai Municipal Science and Technology Commission (06ZR14005 and 04DZ05617) and Visiting Scholar Foundation of Key Laboratory in Fudan University.

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