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Highly Stable Dextran-Coated Quantum Dots for Biomolecular Detection and Cellular Imaging

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Dextrans have long been used in detection and medicine, because of their low toxicity and excellent biocompatibility. Methods for attaching them to a diverse range of recognition and therapeutic molecules have been developed over many years. These properties make them ideal as coating agents for nanoparticles with applications in detection, imaging, and medicine. In this report, we show that semiconductor quantum dots (QDs) coated with dextrans are highly luminescent, and stable, under a wide range of conditions in the presence of reagents and metabolites such as those which are commonly encountered in biological detection and imaging. Stability is monitored by Förster resonance energy transfer (FRET) titrations with fluorescent avidin, and their use under physiological conditions is demonstrated by tracking their progress in living cells. The surface chemistry is not disrupted by physiological (10 mM) concentrations of glutathione, making them compatible with intracellular environments. Dextran-coated QDs conjugated to streptavidin bind only to the surface of cells that have been biotinylated. These results show that the advantageous properties of dextrans can be extended to QDs and their application to single-cell imaging.

Introduction

Semiconductor quantum dots (QDs) are photoluminescent nanoparticles with dimensions smaller than the Bohr exciton radius of the corresponding bulk material. This quantum confinement gives rise to unusual optical properties, including narrow size-tunable emission spectra and the possibility of exciting multiple colors at a single wavelength far removed (> 100 nm) from the wavelengths at which they emit. Together with their small size, high quantum yields, and resistance to photobleaching, these properties have made QDs attractive alternatives to fluorescent dyes for biological detection and microscopic imaging.[1−6] High-quality QDs are still mainly synthesized from organometallic precursors in hot coordinating organic solvents in which they are stabilized by hydrophobic capping ligands such as trioctylphosphine/trioctylphosphine oxide (TOP/TOPO) or hexadecylamine.[7,8] QDs capped in this way are not soluble in aqueous solution; therefore, further modification is necessary before they are suitable for use in biological applications. There are two main strategies for achieving this. In the first of these, the entire particle (including the original hydrophobic ligands) is encapsulated in a micelle,[9] or amphiphilic polymers.[10−12] QDs encapsulated in this way are usually stable for long periods and have high quantum yields, but their hydrodynamic diameter (HD) is often significantly larger than that of their semiconductor core. This increase in size makes them less useful for Förster resonance energy transfer (FRET) quenching assays and hinders their access to molecular targets for in vivo imaging. These methods are also complicated to perform and expensive, because they involve the synthesis or purchase of specialized chemicals. In the second main strategy, the hydrophobic capping ligands are replaced by ligands terminating in hydrophilic functional groups. The simplest examples of such ligands are monodentate alkanes such as mercaptoacetic acid (MA). These do not produce a significant increase in HD;

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however, luminescence is quenched and stability is poor, because of their subsequent dissociation.[13,14] Stability and luminescence are both improved by capping with bidentate ligands,[15,16] bidentate ligands linked to polymers,[17,18] and multidentate polymers and dendrons;[19−21] however, these methods also require specialized chemistry. In this paper, we report a new method for conjugating recognition molecules to QDs with multidentate mercaptodextrans, and we describe their use in detection and cellular imaging.

Dextrans are water-soluble polymers that are widely used as an interface between inorganic surfaces and biological environments, because of their inertness and resistance to nonspecific binding.[22,23] Their use as coatings for biosensor surfaces and magnetic particles is well-known,[24−27] and, more recently, they have been used to coat gold and silver nanoparticles for use in a diverse range of detection methods.[28−35] Dextrans have also been used to coat QDs. Rosenzweig and colleagues used a layer-by-layer approach to prepare water-soluble clusters of QDs,[36] and, more recently, Earhart and colleagues have conjugated dextrans to QDs,[37] based on a method that was originally developed by Weiss, Alivisatos, and colleagues.[38] The QDs were first encapsulated in a silica/siloxane shell followed by covalent attachment of activated dextran molecules to pendant functional groups. Potential problems with this approach are that silica coating produces a significant increase in size, and the resulting conjugates are only stable for a few weeks, presumably because of the slow dissolution of silica, which occurs even at neutral pH.[39] In the work by Earhart[37] and other reports describing QDs coated with sugar molecules,[40−42] the dextran itself was a participant in subsequent agglutination reactions; however, there is a large body of work showing that dextrans can be functionalized with a variety of other molecules.[43] Because of their size, this includes the possibility of functionalizing the same molecule of dextran with recognition molecules (antibodies, oligonucleotides, haptens, etc.) and anchoring groups for attachment to nanoparticles and other substrates. In previous work, we have shown how such dextrans can be conjugated to gold and silver nanoparticles,[31−35] but QDs are more problematic, because they must be transferred from organic to aqueous solution before they can be conjugated biological molecules that are neither soluble nor stable in the former. Here, we describe how dextrans functionalized with recognition molecules can be conjugated to QDs.

Experimental Section

Materials. TOPO-capped CdSe@ZnS core−shell QDs in toluene were obtained from Evident Technologies, Inc. (Troy, NY); according to the supplier's data sheet, QDs emitting at 620 nm had a nominal crystal diameter of 7.2 nm and a molar extinction coefficient of 3.25×10^5 at 598 nm. Paramagnetic polystyrene microspheres coated with streptavidin were obtained from Invitrogen; they had a mean diameter of 2.8 μm and were supplied at a concentration of $\sim 6.7 \times 10^8$ microspheres per milliliter (10 mg/mL). Aminodextran (MW 70 kDa, 16 primary amines per molecule) was obtained from Molecular Probes (Eugene, OR). Mercaptoacetic acid (abbreivated as MA, also known as thioglycolic acid), 5,5′-dithio-bis-(2-nitrobenzoic acid) (also known as Ellman's reagent), water-free polyethyleneimine (abbreviated as PEI; MW $\approx$ 25 kDa) and tetramethylammonium hydroxide (25% solution in methanol; abbreviated as TMA) were obtained from Aldrich. Avidin, biotin, biotin-3-sulfo *N*-hydroxysuccinimidyl ester (biotin-NHS-sulfo), bovine serum albumin (BSA), L-glutathione (reduced), streptavidin, 2-(4-hydroxyphenylazo)benzoic acid (HABA), 2-iminothiolane (also known as Traut's reagent; HCl salt), sodium azide, and Tween 20 were obtained from Sigma. DyLight Fluor 649 sulfo-*N*-hydroxysuccinimidyl ester (DyLight Fluor NHS) was obtained from Thermo Fisher Scientific UK, Ltd.

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Other reagents were of Analar quality (or equivalent grade) or higher. Tissue culture medium (Dulbecco's Modified Eagle's Medium; DMEM) was obtained from GibcoInvitrogen (Invitrogen Ltd., U.K.). Fetal calf serum (FCS) was obtained from Harlan Seralab (U.K.). Hela cells were obtained from the European Collection of Cell Cultures (ECACC).

Equipment. Magnetic precipitation of Dynal microspheres was performed using an MPC-S sample concentrator (Dynal/Invitrogen), and the (slow-tilt) rotation of microspheres was performed on a MX2 sample mixer (Dynal/Invitrogen). UV/vis spectra were recorded on a Hewlett−Packard Model 8452A diode array spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescent Spectrometer. The excitation wavelength was 400 nm, the excitation and emission slits were 10 nm, and the PMT potential was 700 V. Particle sizing was carried out after passing the particles through a 0.1 μm filter (Ultra Free-MC filter unit from Millipore) with a Zeta Plus analyzer supplied by Brookhaven Instruments (Redditch, U.K.); the refractive indices used for size determination were 1.69 (real) and −0.002 (imaginary). Epifluorescence images of microspheres were acquired with a Leica Model DMBL fluorescence microscope equipped with a Model SPOT 2 camera system (SPOT Diagnostic Instruments, Inc., Sterling Heights, MI) and custom filters matched to the emission peaks of the QDs. The excitation wavelength was 400 nm, and the image acquisition time was 250 ms.

Quantum Dots Functionalized with Mercaptoacetic Acid. QDs (100 μL in toluene, as supplied by Evident) in a polypropylene vial were washed and centrifugally precipitated with methanol (4 × 1 mL) at 16 400*g* for 15 min. The final pellet was resuspended in 50 μL of chloroform, to which 25 μL of MA, followed by 25 μL of TMA, were added. After sealing the vial to prevent the release of harmful vapor, the mixture was sonicated for 1 min and then maintained at 60 °C in a water bath for 1 h. At the end of this time, the QDs were centrifugally precipitated for 10 min at 16 400*g*. The supernatant was carefully removed and discarded in a fume hood, and then the pellet was washed with methanol (3 × 1 mL) at 16 400*g* for 15 min. The final pellet was suspended in 0.5 mL of 10 mM sodium bicarbonate solution and stored in darkness.

Quantum Dots Functionalized with Polyethyleneimine. QDs (100 μL in toluene, as supplied by Evident) in a polypropylene vial were washed and centrifugally precipitated with methanol (4 × 1 mL) at 16 400*g* for 15 min. The final pellet was resuspended in 50 μL of chloroform, to which 5 μL of PEI was added. The mixture was sonicated for 1 min and then maintained at 60 °C in a water bath for 1 h. At the end of this time, the QDs were purified in a 100 k centrifugal filter unit (Amicon Ultra-4; Millipore, Bedford, MA) with water as the washing solution. The pH of the retained QDs was adjusted to 7.0 with dilute HCl.

Functionalized Dextrans. Dextrans functionalized with mercapto groups only were prepared by adding 2.5 mg of Traut's reagent to 6.25 mg of aminodextran in 5 mL of bicarbonate solution. After stirring for 2 h, the functionalized dextran was purified via gel exclusion chromatography on Sephadex G25 with water as the eluent. Dextrans functionalized with biotin and mercapto groups were prepared by adding 0.71 μmol (0.275 mg) of biotin-NHS in 100 μL of dry dimethylformamide (DMF) dropwise to 6.25 mg of aminodextran in 5 mL of bicarbonate solution. After stirring for 2 h, 2.5 mg of Traut's reagent was added, and after stirring for an additional 2 h, the functionalized dextran was purified on Sephadex G25 with water as the eluent, or by dialysis against water (3 × 250 mL). The concentration of dextran in the dialyzed solution was estimated by multiplying the original concentration by the ratio of the volumes before and after dialysis. The concentration of biotin in the dialyzed solution was determined as follows: HABA was dissolved in 10 mM NaOH to a final concentration of 2.42 mg/mL. Avidin was dissolved in a 50 mM sodium phosphate buffer (pH 6.0) containing 0.15 M NaCl to a final concentration of 1 mg/mL. A 0.6 mM solution of biotin was prepared in a 50 mM sodium phosphate buffer (pH 6.0). The absorbance at 500 nm of a solution made by mixing 0.5 mL of avidin solution, 0.5 mL of water, and 25 μL of HABA was taken as the zero calibrator. The absorbance then was measured at 2-min intervals after adding 5 μL increments of the biotin solution to the zero calibrator solution. To determine the concentration of biotin in the biotinylated dextran, 0.2 mL of dialyzed solution was mixed with 0.5 mL of avidin solution, 0.3 mL of water, and 25 μL of HABA and then the biotin concentration was determined with reference to the calibration curve. The concentration of −SH groups in the dialyzed solution was determined from the increase in absorbance at 412 nm upon mixing 0.2 mL of the dialyzed solution with 0.75 mL of a 2 mM Ellman's reagent in a 0.1 M sodium bicarbonate solution, using an extinction coefficient of 13 600 M^{-1} cm^{-1}.

Quantum Dots Coated with Functionalized Dextran. Twice the minimum amount of functionalized dextran required to prevent any acid-induced precipitation (see the Supporting Information) in 0.5 mL of water was mixed, in a 1:1 ratio, with 0.5 mL of MA functionalized QDs in a 10 mM bicarbonate solution and then dialyzed against deionized water (4 × 1 L) for a total of 48 h. The concentration of QDs in the dialyzed solution (based on the supplier's extinction coefficient) was 3.6 μM. Following dialysis, the QDs were purified by centrifugal precipitation for 4 h at 16 400*g* and resuspended in water three times. Centrifugal precipitation under these conditions leads to the formation of a firm pellet of QDs from which the supernatant can be efficiently removed. Three cycles of precipitation and resuspension in water leads to QDs that are free from unbound dextran.

Fluorescent Avidin and FRET Titrations. A quantity (0.1 mg) of DyLight Fluor NHS (MW = 1008) was dissolved, with rapid vortexing, with 1.3 mg of avidin (MW = 68 kDa) in PBS (15 mM sodium phosphate (pH 7.4), containing 0.15 M NaCl). After being rotated for 2 h, the solution was dialyzed against PBS (3 × 250 mL)

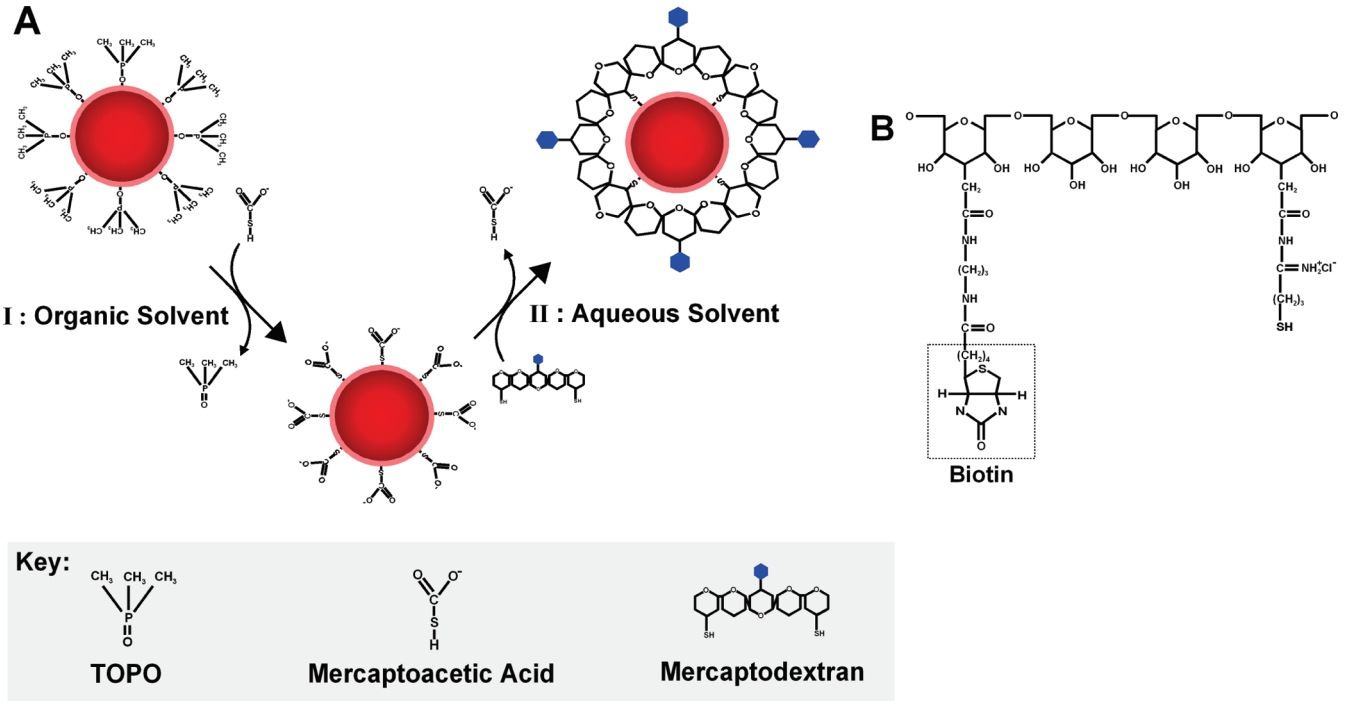

Figure 1. (A) Scheme for preparing water-soluble QDs coated with mercaptodextrans. In step I, hydrophobic QDs are rendered water-soluble with mercaptoactetic acid (MA), and in step II, MA is replaced with multidentate dextrans. (Note: The key does not show full structural formulas.) (B) Structures of biotinylated mercaptodextran; the ratio of biotins to −SH groups was ~1:15.

at 4 °C for a total of 36 h. The concentration of DyLight Fluor in the dialyzed solution was determined from the absorbance at 649 nm, and the concentration of avidin was determined from the absorbance at 280 nm, after subtracting the absorbance of DyLight Fluor at this wavelength. Monitoring of stability in PBS-Tween (PBS containing 1 mg/mL BSA, 0.05% Tween-20, and 10 mg/L sodium azide) and DMEM was performed by incubating different ratios of fluorescent avidin with 1.5 pmol of biotin-QDs for 15 min at room temperature. Determination of stability in the presence of glutathione was performed by incubating 1.5 pmol of biotin-QDs with different concentrations of glutathione for 1 h in 0.2 M phosphate buffer (pH 7.4), containing 0.1 M NaCl at room temperature. At the end of this time, PBS-Tween containing one molar equivalent of fluorescent avidin was added. Emission spectra were obtained after incubating for an additional 15 min.

Streptavidin-Coated Quantum Dots. Biotin QDs were incubated for 1 h with a 5-fold molar excess of streptavidin in 1 mL of PBS, and then centrifugally precipitated for 4 h at 16 400*g* and resuspended in 1 mL of PBS three times.

Cell Culture, Cell Imaging, and Biotinylation. Hela cells were grown in DMEM supplemented with 10% FCS (v/v) and 1% nonessential amino acids (v/v), at 37 °C and 5% CO_2. They were plated in 35-mm glass coverslip culture dishes (Iwaki) at 5×10^4 cells/mL. Dextran- and PEI-coated QDs, and MA-capped QDs, were added directly to the culture dish and incubated on the microscope stage at 37 °C and 5% CO_2. They were imaged by confocal microscopy using a Zeiss Model LSM510 with a Plan-apochromat 63× 1.3 NA oil-immersion objective. Excitation of QDs was performed using a 561-nm diode laser. Data capture was carried out with LSM510 version 3 software (Zeiss, Germany). For short- duration time-lapse experiments, images were acquired every 500 ms for 1 min, and for long- duration time-lapse experiments that were aimed at monitoring cell survival upon repetitive QD illumination, images were acquired every 7 min for 24 h. Cells were biotinylated by replacing the DMEM medium with PBS containing 1 mg/mL biotin-NHS-sulfo. After incubating at room temperature for 30 min, the PBS-containing biotin was replaced (after three washes with PBS) with complete medium containing streptavidin-QDs and the cells were imaged as described previously.

Results and Discussion

The method that we have developed to coat QDs with functionalized dextrans is shown in Figure 1A. In step I, the CdSe@ZnS QDs are rendered water-soluble with MA and suspended in 10 mM $NaHCO_3$; in step II, the MA-capped QDs are mixed with the minimum amount of functionalized dextran required to prevent any acid-induced precipitation. The functionalized dextrans used in this work had the structure shown in Figure 1B, and the amount added was determined via titration, as described in the Supporting Information. In the absence of added dextran, the QDs precipitate, as previously reported for QDs capped with monodentate alkanes.[1] Fourier transform infrared (FTIR) spectra (see Figure E1 in the Supporting Information) show that bands observed at 1724, 1586, and 1381 cm^{-1} that are present in the spectrum of MA-capped QDs[44] are either very weak or absent

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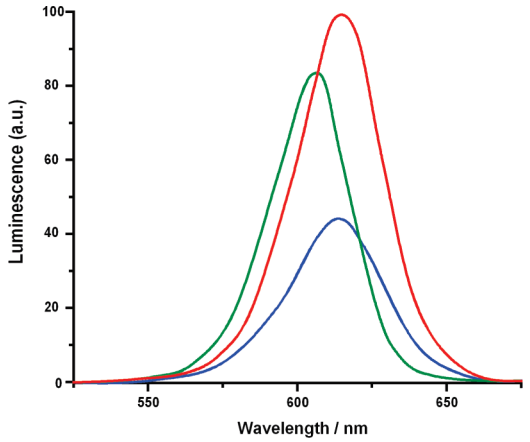

Figure 2. Emission spectra of dextran-capped QDs in PBS-Tween (red line), TOPO-capped QDs in toluene (green line), and MA-capped QDs in 10 mM sodium bicarbonate solution (blue line). The QD concentrations were the same in all solutions.

from the spectrum of dialyzed QDs, indicating that most or all of the MA has dissociated. Figure 2 compares the emission spectra of dextran-coated QDs with TOPO- and MA-capped QDs at the same concentration. The quantum yield of the quantum dots was 57% (according to personal communication from Evident Technologies); therefore, these results indicate that quantum yields after capping with MA and dextran were ~30% and ~70%, respectively. The increase in quantum yield when replacing MA with dextran is consistent with results obtained by others, showing that luminescence is quenched by thiolated ligands, especially when, similar to MA, these are charged.[45] The emission peaks of both the MA- and dextran-coated QDs were red-shifted by 12 nm, compared with the TOPO-capped QDs, as has been previously been reported for QDs capped with mercapto ligands in aqueous solution.[45,46] The full width at half-maximum (fwhm) of the MA- and dextran-coated QDs was 3 nm broader than the TOPO-capped QDs. TEM images (see Figure 3) showed that the QDs were not aggregated, and particle size measurements (see Figure E8 in the Supporting Information) indicated that dextran-coated QDs had a mean diameter of 26.6 nm, compared to 7.7 nm for MA-capped QDs.

If QD conjugates are to be useful for detection, they must be stable during storage and in the presence of reagents that are commonly present in biological buffers. QDs were stored at a concentration of 1.5 μM (~9×10^{14} particles per milliliter) at 4 °C in sterile water, and the stability was monitored by FRET titration with fluorescent avidin. FRET (Förster resonance energy transfer) is the name given to nonradiative energy transfer from an electronic excited state to an acceptor. It is dependent on the inverse sixth power of the distance between the donor and the acceptor; therefore, it can be used to distinguish

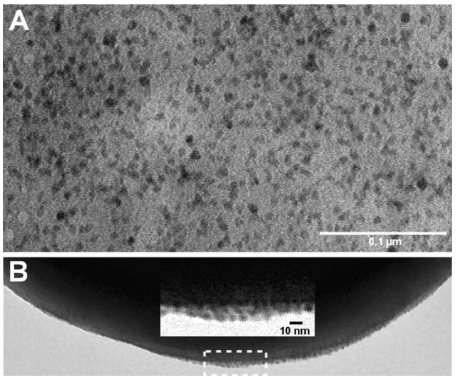

Figure 3. (A) TEM image of dextran-coated QDs. (B) TEM images of biotin-QDs bound to streptavidin-coated paramagnetic beads. In the low-magnification image, QDs are visible as spheres located at the edge of the bead; in the higher-magnification image (inset), they are visible as individual spheres ~10 nm in diameter. An epifluorescence image of the beads used to obtain these images is shown in Figure E5 in the Supporting Information.

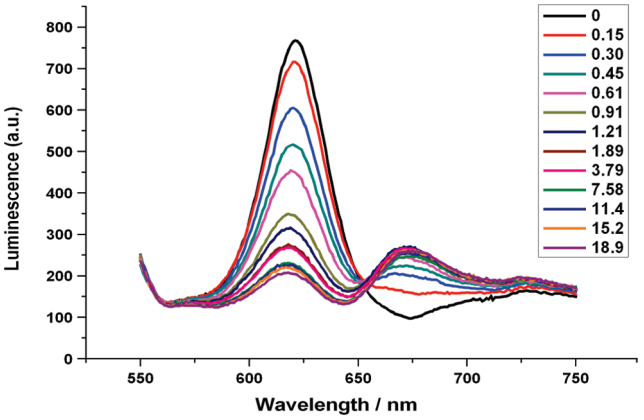

Figure 4. Emission spectra of 15 nM biotin-QDs with different molar ratios of fluorescent avidin; the legend (shown as an inset) presents the molar ratios.

between fluorescent molecules bound to QDs and unbound molecules in the same solution.[47−49] FRET titrations were conducted with avidin that had been labeled with DyLight Fluor 649, as described in the Supporting Information; DyLight Fluor 649 has an emission peak at 670 nm, but no absorbance at 400 nm (which is the wavelength used to excite the QDs). QDs coated with biotin-functionalized dextran (biotin-QDs) were incubated with different ratios of fluorescent avidin for 1 h at room temperature in phosphate buffered saline (PBS) that also contained 1 mg/mL BSA and 0.05% Tween-20 (PBS-Tween). The molar concentration of the QDs in these titrations was known from the extinction coefficient ($\varepsilon = 3.25 \times 10^5$ cm^{-1} at 598 nm) and the molar concentrations of fluorescent avidin were known from the UV/vis spectrum, as described in the Supporting Information. As the molar ratio of avidin to QDs increased, the emission intensity of the QDs decreased, as shown in Figure 4. Figure 5 shows how the emission intensities at 612 nm (QDs) and 670 nm (DyLight Fluor) are dependent

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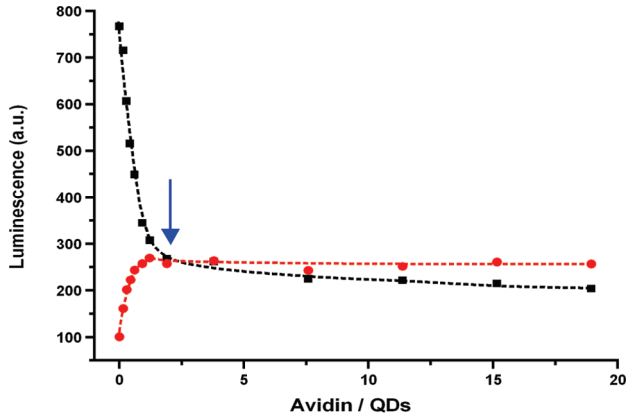

Figure 5. Plots of luminescence intensity at 620 nm (black squares) and 670 nm (red circles). The blue arrow indicates the ratio at which no more avidin molecules can be accommodated in close proximity to the QDs.

on the molar ratio of avidin to QDs. In both cases, the intensities reach a plateau where the biotin-QDs are unable to bind any more avidin molecules. The inflection points (blue arrow in Figure 5) indicate that this point is reached when each QD is bound to a mean of ~2 avidin molecules. No decrease in the number of avidin molecules per QD was detected when similar titrations were performed at intervals over a period of three months (see Figure E9 in the Supporting Information). FRET titrations were also used to investigate the stability of biotin-QDs in a cell growth medium (DMEM) containing 10% FCS. This contains a mixture of metabolites and proteins, including millimolar concentrations of amino acids such as cystine and lysine that might be expected to displace thiolated ligands; however, results have shown that biotin remained conjugated to the QDs. We also investigated the effect of pH and ionic strength on dextran-coated QDs. There was a slight decrease in emission at low pH, but even at pH 3, the QDs were still bright, while NaCl had no effect, even when it was present at saturating concentrations such as those shown in Figure E6 in the Supporting Information.

Glutathione is a physiological antioxidant that is present in cells at concentrations of up to 10 mM (2 μM in serum,[50] 2 mM in red blood cells,[51] and 10 mM in liver cells[52]). It is well-known that surface chemistries based on bonded S atoms can be displaced by physiological concentrations of glutathione, and several reports have described how this can be harnessed to trigger the release of molecules from nanoparticle delivery vehicles.[53−56] Studies demonstrating stable surfaces chemistries in the presence of glutathione are less well-documented:

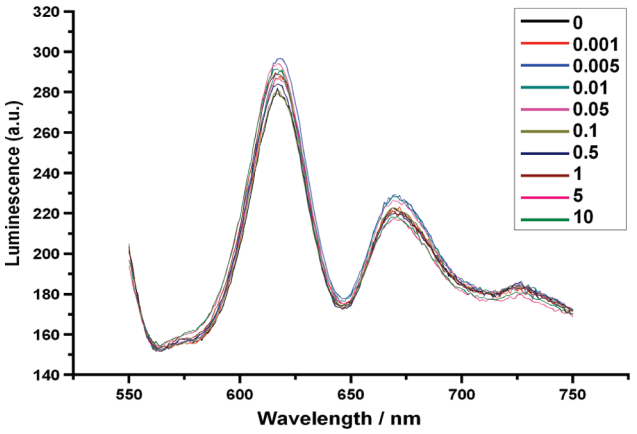

Figure 6. Emission spectra of biotin-QDs after incubation with different concentrations of glutathione, followed by a molar equivalent of fluorescent avidin (numbers shown in the inset indicate glutathione concentrations (in mM)).

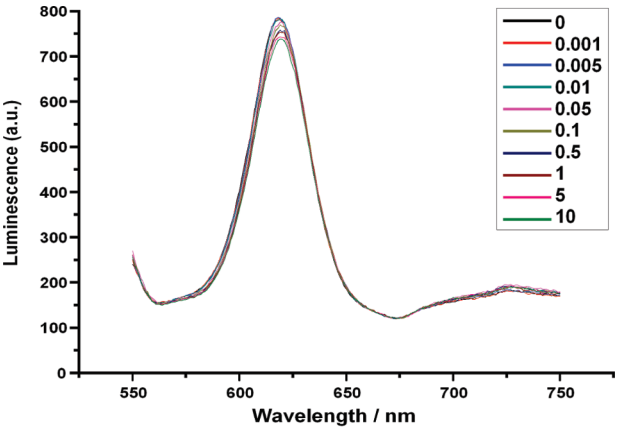

Figure 7. Emission spectra of biotin-QDs after incubation with different concentrations of glutathione, followed by a molar equivalent of fluorescent avidin in the presence of 1 mM biotin (numbers shown in the inset indicate glutathione concentrations (in mM)).

Li-Shishisdo and colleagues found that 10 mM glutathione more than doubled the luminescence intensity of commercial polymer-coated QDs,[57] and Yu and colleagues found that glutathione decreased the luminescence of lactose-capped QDs, but they did not investigate if the capping agent had been displaced.[58] Figure 6 shows the effect of incubating biotin-QDs with glutathione in a 0.2 M phosphate buffer for 1 h, followed by incubation with a molar equivalent of fluorescent avidin. Energy transfer from the QDs to fluorescent avidin occurs at all concentrations of glutathione, but emission at 670 nm is abolished if the QDs are incubated with fluorescent avidin and 1 mM biotin, as shown in Figure 7. This is an important result, because it shows that the dextran surface chemistry is stable in the presence of physiological concentrations of glutathione.

Among the most important applications of QDs are those in which signals are tracked over extended periods

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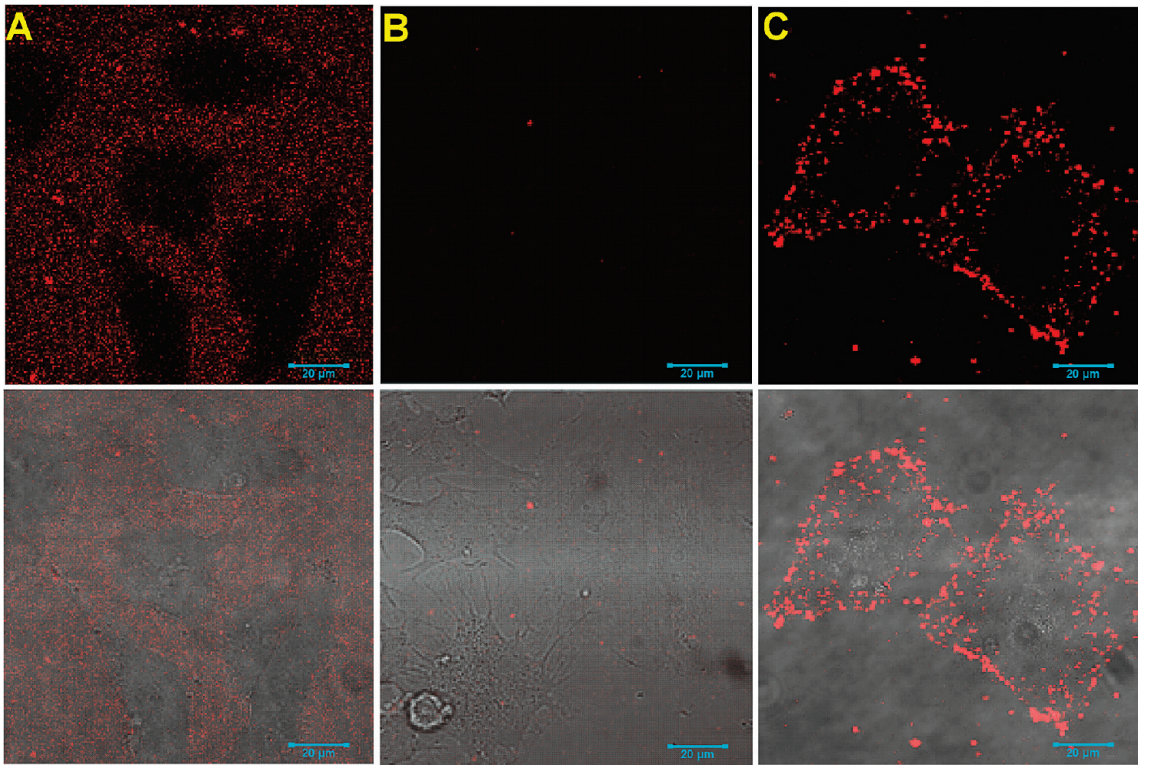

Figure 8. (A) Confocal fluorescence image (top) and fluorescence image overlaid on a bright-field image (bottom) of Hela cells after 10 min of incubation with mercaptoacetic-capped QDs. (B) Confocal fluorescence image (top) and fluorescence image overlaid on a bright-field image (bottom) of Hela cells after 3 h of incubation with mercaptodextran-coated QDs. (C) Confocal fluorescence image (top) and fluorescence image overlaid on a bright-field image (bottom) of Hela cells after 10 min of incubation with polyethyleneimine (PEI)-coated QDs.

of time. Currently, most cellular imaging is still performed with fluorescent dyes, but these can be bleached by prolonged exposure to excitatory light. For QDs to be useful as alternatives to fluorescent dyes, they must only bind to cellular targets when they are conjugated to the corresponding probe molecule. To investigate nonspecific binding, we incubated Hela cells with mercaptodextran-coated QDs and imaged their localization using time-lapse laser scanning confocal microscopy. For comparison, we also incubated cells with QDs capped with MA, and QDs coated with polyethyleneimine (PEI). MA-capped QDs rapidly became immobilized on the surface of the cells and the surrounding substrate, but they did not interact with them, as shown in Figure 8A, and PEI-coated QDs displayed a typical endosomal localization (spotty fluorescence around the nucleus), as shown in Figure 8C; similar localization is also displayed by peptide-capped gold nanoparticles.[59] Endocytosis of PEI coated-QDs is not surprising, because many transport events in living cells are mediated by anionic cell surface polysaccharides that interact with the peptide transduction domains; however, this is not particularly specific. By contrast, mercaptodextran-coated QDs (see Figure 8B) did not bind to the cells, even when incubated with them overnight. To demonstrate binding to target molecules, we conjugated QDs to streptavidin (streptavidin-QDs) and labeled cell surface proteins with biotin using biotin-NHS-sulfo. The latter reacts with exposed primary amines on the surface of the cells, linking them to biotin by an amide bond and an intervening six-carbon spacer. After unbound biotin was washed away, the cells were incubated with streptavidin-QDs. In contrast to QDs coated only with dextran, streptavidin-QDs (see Figure 9) began to bind to the surface of biotinylated cells within 10 min (see Figure 9 A), and after 3 h (see Figure 9B), some QDs were clearly localized in the intracellular compartment (see z-stack imaging in Figures 9C and 9D), penetrating as far as the nuclear membrane, which they were unable to cross (confirmed by staining with Hoechst 33342 (see Figure 9E)). In control experiments in which the cells were not biotinylated, the streptavidin-QDs did not bind to the cells. Following internalization of streptavidin-QDs, there was no sign of cytoxicity over a period of 24 h (see the movie provided in the Supporting Information), during which cells continued to divide normally. At the end of this period, cells stained with Hoechst 33342 showed no sign of chromatin condensation or fragmentation, which would have indicated cell death (also see Figure E10 in the Supporting Information).

Conclusions

In summary, we have shown how quantum dots (QDs) can be rendered water-soluble and conjugated to recognition molecules with mutidentate mercaptodextrans. In previous work, similar dextrans have been grafted onto gold and silver nanoparticles,[31−35] but QDs are more

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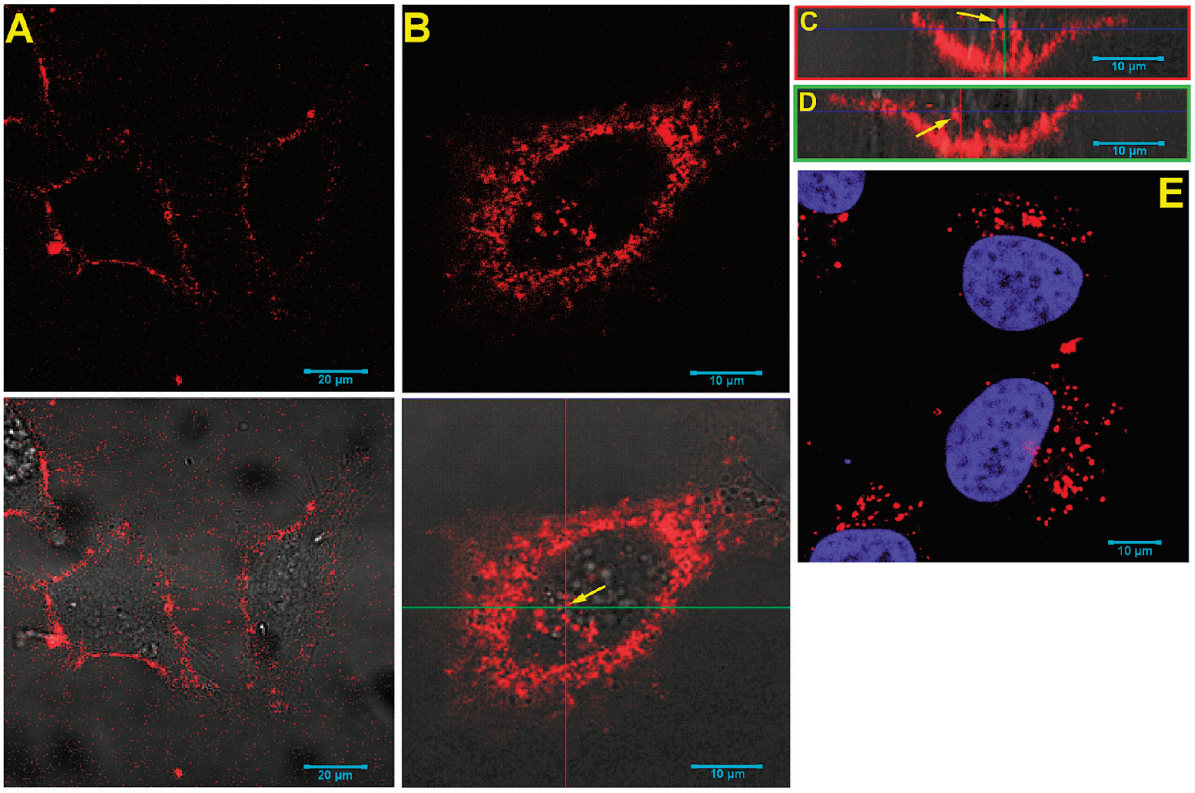

Figure 9. (A) Confocal fluorescence image (top) and fluorescence image overlaid on a bright-field image (bottom) of biotinylated Hela cells after 10 min of incubation with streptavidin-QDs. (B) Confocal fluorescence image (top) and fluorescence image overlaid on a bright-field image (bottom) of biotinylated Hela cells after 3 h of incubation with streptavidin-coated QDs; some QDs (e.g., such as that noted by the yellow arrow) are clearly localized in the intracellular compartment. (C) Projection of z-stack image on red line in Figure 7B, showing QD intracellular localization. (D) Projection of z-stack on green line in Figure 7B, showing intracellular QD localization. (E) Confocal fluorescence image of cells stained with Hoechst 33342 after 20 h of incubation with streptavidin-QDs.

problematic, because the initial step must be performed in an organic solvent in which dextrans and many other biological molecules are not only insoluble, but, in many cases, irreversibly damaged. To overcome this problem, we first rendered the QDs water-soluble with mercaptoacetic acid (MA). The role of the MA is to provide a temporary surface that confers solubility in aqueous solution, in much the same way that gold nanoparticles are stabilized by citrate ions. MA is then exchanged for dextrans functionalized with multiple −SH groups. Because the entire surface is grafted onto the QDs in a single step, the possibility of precipitation or interparticle crosslinking, which sometimes occurs when subsequent chemistry is performed on water-soluble QDs,[15] is avoided. QDs coated in this way are stable over a wide range of pH and in the presence of metabolites and reagents such as those which are commonly encountered in detection and imaging. The diameter of dextran-coated QDs with a semiconductor core diameter of 7.2 nm is 26.6 nm, which is smaller than some commercial streptavidin-QDs,[18,60] but larger than QDs capped with pegylated-DHLA,[18] although we believe this could be decreased by coating with dextrans that have a lower molecular weight. One of the motivations for this work was the observation that the stability of water-soluble QDs is generally enhanced by coating with multidentate ligands such as DHLA; however, even these would be expected to dissociate under acidic conditions when the pH is less than the pK_a value of the thiolate S atom,[61] and in the presence of high concentrations of competing thiols, such as reduced glutathione. In neither case do these conditions disrupt the surface chemistry, and, therefore, we cannot rule out the possibility that bonding forces other than those involving S atoms are involved. QDs coated with dextran do not bind nonspecifically to cells, even when they are incubated with them for 24 h; however, when they are conjugated to appropriate recognition molecules, binding occurs within a matter of minutes. In this report, we have used the interaction between streptavidin and biotin to demonstrate this; however, in previous work, it has been shown that dextrans can be functionalized with a wide variety of different molecules,[62,63] including antibodies,[64,65] oligonucleotides,[34] and drug molecules.[66,67] This suggests that

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the method that we have reported here will be useful for attaching QDs to a broad range of different molecules for use in detection and imaging.

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Supporting Information Available: FTIR spectra, titration of MA capped quantum dots with mercaptodextrans, images of streptavidin magnetic beads coated with biotin-quantum dots, images of quantum dots in different solutions and at different pHs, UV/vis spectrum, and details of fluorescent avidin, particle sizing graphs, FRET titrations of quantum dots at different times during storage, and movie showing the viability of Hela cells 24 h after adding streptavidin-QDs. This information is available free of charge via the Internet at http://pubs.acs.org/.