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Bioconjugated Quantum Dots for *In Vivo* Molecular and Cellular Imaging

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Abstract

Semiconductor quantum dots (QDs) are tiny light-emitting particles on the nanometer scale, and are emerging as a new class of fluorescent labels for biology and medicine. In comparison with organic dyes and fluorescent proteins, they have unique optical and electronic properties, with size-tunable light emission, superior signal brightness, resistance to photobleaching, and broad absorption spectra for simultaneous excitation of multiple fluorescence colors. QDs also provide a versatile nanoscale scaffold for designing multifunctional nanoparticles with both imaging and therapeutic functions. When linked with targeting ligands such as antibodies, peptides or small molecules, QDs can be used to target tumor biomarkers as well as tumor vasculatures with high affinity and specificity. Here we discuss the synthesis and development of state-of-the-art QD probes and their use for molecular and cellular imaging. We also examine key issues for *in vivo* imaging and therapy, such as nanoparticle biodistribution, pharmacokinetics, and toxicology.

Keywords

Quantum dots; nanocrystals; nanoparticles; nanotechnology; fluorescence; molecular imaging; cellular imaging; drug delivery; cancer; biomarkers; toxicology

1. Introduction

The development of biocompatible nanoparticles for molecular imaging and targeted therapy is an area of considerable current interest [1–9]. The basic rationale is that nanometer-sized particles have functional and structural properties that are not available from either discrete molecules or bulk materials [1–3]. When conjugated with biomolecular affinity ligands, such as antibodies, peptides or small molecules, these nanoparticles can be used to target malignant tumors with high specificity [10–13]. Structurally, nanoparticles also have large surface areas for the attachment of multiple diagnostic (e.g., optical, radioisotopic, or magnetic) and therapeutic (e.g., anticancer) agents. Recent advances have led to the development of biodegradable nanostructures for drug delivery [14–18], iron oxide nanocrystals for magnetic resonance imaging (MRI) [19,20], luminescent quantum dots (QDs) for multiplexed molecular diagnosis and *in vivo* imaging [21–25], as well as nanoscale carriers for siRNA delivery [26, 27].

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Due to their novel optical and electronic properties, semiconductor QDs are being intensely studied as a new class of nanoparticle probe for molecular, cellular, and *in vivo* imaging [10–24]. Over the past decade, researchers have generated highly monodispersed QDs encapsulated in stable polymers with versatile surface chemistries. These nanocrystals are brightly fluorescent, enabling their use as imaging probes both *in vitro* and *in vivo*. In this article, we discuss recent developments in the synthesis and modification of QD nanocrystals, and their use as imaging probes for living cells and animals. We also discuss the use of QDs as a nanoscale carrier to develop multifunctional nanoparticles for integrated imaging and therapy. In addition, we describe QD biodistribution, pharmacokinetics, toxicology, as well as the challenges and opportunities in developing nanoparticle agents for *in vivo* imaging and therapy.

2. QD Chemistry and Probe Development

QDs are nearly spherical semiconductor particles with diameters on the order of 2–10 nanometers, containing roughly 200–10,000 atoms. The semiconducting nature and the size-dependent fluorescence of these nanocrystals have made them very attractive for use in optoelectronic devices, biological detection, and also as fundamental prototypes for the study of colloids and the size-dependent properties of nanomaterials [28]. Bulk semiconductors are characterized by a composition-dependent bandgap energy, which is the minimum energy required to excite an electron to an energy level above its ground state, commonly through the absorption of a photon of energy greater than the bandgap energy. Relaxation of the excited electron back to its ground state may be accompanied by the fluorescent emission of a photon. Small nanocrystals of semiconductors are characterized by a bandgap energy that is dependent on the particle size, allowing the optical characteristics of a QD to be tuned by adjusting its size. Figure 1 shows the optical properties of CdSe QDs at four different sizes (2.2 nm, 2.9 nm, 4.1 nm, and 7.3 nm). In comparison with organic dyes and fluorescent proteins, QDs are about 10–100 times brighter, mainly due to their large absorption cross sections, 100–1000 times more stable against photobleaching, and show narrower and more symmetric emission spectra. In addition, a single light source can be used to excite QDs with different emission wavelengths, which can be tuned from the ultraviolet [29], throughout the visible and near-infrared spectra [30–33], and even into the mid-infrared [34]. However QDs are macromolecules that are an order of magnitude larger than organic dyes, which may limit their use in applications in which the size of the fluorescent label must be minimized. Yet, this macromolecular structure allows the QD surface chemistry and biological functionality to be modified independently from its optical properties.

2.1. QD Synthesis

QD synthesis was first described in 1982 by Efros and Ekimov [35,36], who grew nanocrystals and microcrystals of semiconductors in glass matrices. Since this work, a wide variety of synthetic methods have been devised for the preparation of QDs in different media, including aqueous solution, high-temperature organic solvents, and solid substrates [28,37,38]. Colloidal suspensions of QDs are commonly synthesized through the introduction of semiconductor precursors under conditions that thermodynamically favor crystal growth, in the presence of semiconductor-binding agents, which function to kinetically control crystal growth and maintain their size within the quantum-confinement size regime.

The size-dependent optical properties of QDs can only be harnessed if the nanoparticles are prepared with narrow size distributions. Major progress toward this goal was made in 1993 by Bawendi and coworkers [39], with the introduction of a synthetic method for monodisperse QDs made from cadmium sulfide (CdS), cadmium selenide (CdSe), or cadmium telluride (CdTe). Following this report, the synthetic chemistry of CdSe QDs quickly advanced, generating brightly fluorescent QDs that can span the visible spectrum. As a result, CdSe has become the most common chemical composition for QD synthesis, especially for biological

applications. Many techniques have been implemented to post-synthetically modify QDs for various purposes, such as coating with a protective inorganic shell [40,41], surface modification to render colloidal stability [42,43], and direct linkage to biologically active molecules [44,45]. QD production has now become an elaborate molecular engineering process, best exemplified in the synthesis of polymer-encapsulated (CdSe)ZnS (core)shell QDs. In this method, CdSe cores are prepared in a nonpolar solvent, and a shell of zinc sulfide (ZnS) is grown on their surfaces. The QDs are then transferred to aqueous solution through encapsulation with an amphiphilic polymer, which can then be cross-linked to biomolecules to yield targeted molecular imaging agents.

In the design of a QD imaging probe, the selection of a QD core composition is determined by the desired wavelength of emission. For example, CdSe QDs may be size-tuned to emit in the 450–650 nm range, whereas CdTe can emit in the 500–750 nm range. QDs of this composition are then grown to the appropriate wavelength-dependent size. In a typical synthesis of CdSe, a room-temperature selenium precursor (commonly trioctylphosphine-selenide or tributylphosphine-selenide) is swiftly injected into a hot (~300°C) solution containing both a cadmium precursor (dimethylcadmium or cadmium oleate) and a coordinating ligand (trioctylphosphine oxide or hexadecylamine) under inert conditions (nitrogen or argon atmosphere). The cadmium and selenium precursors react quickly at this high temperature, forming CdSe nanocrystal nuclei. The coordinating ligands bind to metal atoms on the surfaces of the growing nanocrystals, stabilizing them colloiddally in solution, and controlling their rate of growth. This injection of a cool solution quickly reduces the temperature of the reaction mixture, causing nucleation to cease. The remaining cadmium and selenium precursors then can grow on the existing nuclei at a slower rate at lower temperature (240–270°C). Once the QDs have reached the desired size and emission wavelength, the reaction mixture may be cooled to room temperature to arrest growth. The resulting QDs are coated in aliphatic coordinating ligands and are highly hydrophobic, allowing them to be purified through liquid-liquid extractions or via precipitation from a polar solvent.

Because QDs have high surface area to volume ratios, a large fraction of the constituent atoms are exposed to the surface, and therefore have atomic or molecular orbitals that are not completely bonded. These “dangling” orbitals serve as defect sites that quench QD fluorescence. For this reason, it is advantageous to grow a shell of another semiconductor with a wider bandgap on the core surface after synthesis to provide electronic insulation. The growth of a shell of ZnS on the surface of CdSe cores has been found to dramatically enhance photoluminescence efficiency [40,41]. ZnS is also less prone to oxidation than CdSe, increasing the chemical stability of the QDs, and greatly decreasing their rate of oxidative photobleaching [46]. As well, the Zn^{2+} atoms on the surface of the QD bind more strongly than Cd^{2+} to most basic ligands, such as alkyl phosphines and alkylamines, increasing the colloidal stability of the nanoparticles [47]. In a typical shell growth of ZnS on CdSe, the purified cores are again mixed with coordinating ligands, and heated to an elevated temperature (140–240°C). Molecular precursors of the shell, usually diethylzinc and hexamethyldisilathiane dissolved in TOP, are then slowly added [40]. The (CdSe)ZnS nanocrystals may then be purified just like the cores.

More recently, it has become possible to widely engineer the fluorescence of QDs by changing the material composition while maintaining the same size. The technological advances that made this possible were the development of alloyed QDs [29,30] and type-II heterostructures [32]. For example, homogeneously alloying the semiconductors CdTe and CdSe in different ratios allows one to prepare QDs of 5 nm diameter with emission wavelengths of 620 nm for CdSe, 700 nm for CdTe, and 800 nm for the $\text{CdSe}_{0.34}\text{Te}_{0.67}$ alloy [30]. Alternatively, type-II QDs allow one to physically separate the charge carriers (the electron and its cationic counterpart, known as the hole) into different regions of a QD by growing an appropriately

chosen material on the QD as a shell [32]. For example, both the valence and conduction band energy levels of CdSe are lower in energy than those of CdTe. This means that in a heterostructure composed of CdTe and CdSe domains, electrons will segregate to the CdSe region to the lowest energy of the conduction band, whereas the hole will segregate to the CdTe region, where the valence band is highest in energy. This will effectively decrease the bandgap due to the smaller energy separating the two charge carriers, and emission will occur at a longer wavelength. By using different sizes of the core and different shell thicknesses, one can engineer QDs with the same size but different wavelengths of emission.

2.2. Surface Modification

QDs produced in nonpolar solutions using aliphatic coordinating ligands are only soluble in nonpolar organic solvents, making phase transfer an essential and nontrivial step for the QDs to be useful as biological reporters. Alternatively, QD syntheses have been performed directly in aqueous solution, generating QDs ready to use in biological environments [48], but these protocols rarely achieve the level of monodispersity, crystallinity, stability, and fluorescent efficiency as the QDs produced in high-temperature coordinating solvents. Two general strategies have been developed to render hydrophobic QDs soluble in aqueous solution: ligand exchange, and encapsulation by an amphiphilic polymer. For ligand exchange, a suspension of TOPO-coated QDs are mixed with a solution containing an excess of a heterobifunctional ligand, which has one functional group that binds to the QD surface, and another functional group that is hydrophilic. Thereby, hydrophobic TOPO ligands are displaced from the QD through mass action, as the new bifunctional ligand adsorbs to render water solubility. Using this method, (CdSe)/ZnS QDs have been coated with mercaptoacetic acid and (3-mercaptopropyl) trimethoxysilane, both of which contain basic thiol groups to bind to the QD surface atoms, yielding QDs displaying carboxylic acids or silane monomers, respectively [44,45]. These methods generate QDs that are useful for biological assays, but ligand exchange is commonly associated with decreased fluorescence efficiency and a propensity to aggregate and precipitate in biological buffers. More recently it has been shown that these problems can be alleviated by retaining the native coordinating ligands on the surface, and covering the hydrophobic QDs with amphiphilic polymers [10,23,49]. This encapsulation method yields QDs that can be dispersed in aqueous solution and remain stable for long periods of time due to a protective hydrophobic bilayer surrounding each QD through hydrophobic interactions. No matter what method is used to suspend the QDs in aqueous buffers, they should be purified from residual ligands and excess amphiphiles before use in biological assays, using ultracentrifugation, dialysis, or filtration. Also, when choosing a water solubilization method, it should be noted that many biological and physical properties of the QDs may be affected by the surface coating, and the overall physical dimensions of the QDs are dependent on the coating thickness. Typically the QDs are much larger when coated with amphiphiles, compared to those coated with a monolayer of ligand.

2.3. Bioconjugation

Water-soluble QDs may be cross-linked to biomolecules such antibodies, oligonucleotides, or small molecule ligands to render them specific to biological targets. This may be accomplished using standard bioconjugation protocols, such as the coupling of maleimide-activated QDs to the thiols of reduced antibodies [22]. The reactivities of many types of biomolecules have been found to remain after conjugation to nanoparticles surfaces, although possibly at a decreased binding strength. The optimization of surface immobilization of biomolecules is currently an active area of research [50,51]. The surfaces of QDs may also be modified with bio-inert, hydrophilic molecules such as polyethylene glycol, to eliminate possible nonspecific binding, or to decrease the rate of clearance from the bloodstream following intravenous injection. QDs have also emerged as a new class of sensor, mediated by energy transfer to organic dyes (fluorescence resonance energy transfer, FRET) [52–54]. It has also recently been reported

that QDs can emit fluorescence without an external source of excitation when conjugated to enzymes that catalyze bioluminescent reactions, due to bioluminescence resonance energy transfer (BRET) [55].

Figure 2 depicts the most commonly used and technologically advanced QD probes. Biologically nonfunctional QDs may be prepared by using a variety of methods. As shown from left to right (top), QDs coated with a monolayer of hydrophilic thiols (e.g. mercaptoacetic acid) are generally stabilized ionically in solution [45]; QDs coated with a cross-linked silica shell can be readily modified with a variety of organic functionalities using well developed silane chemistry [44]; QDs encapsulated in amphiphilic polymers form highly stable, micelle-like structures [23,49]; and any of these QDs may be modified to contain polyethylene glycol (PEG) to decrease surface charge and increase colloidal stability [56]. Also, water-soluble QDs may be covalently or electrostatically bound to a wide range of biologically active molecules to render specificity to a biological target. As shown in Figure 2 (middle), QDs conjugated to streptavidin may be readily bound to many biotinylated molecules of interest with high affinity [23]; QDs conjugated to antibodies can yield specificity for a variety of antigens, and are often prepared through the reaction between reduced antibody fragments with maleimide-PEG-activated QDs [22,57]; QDs cross-linked to small molecule ligands, inhibitors, peptides, or aptamers can bind with high specificity to many different cellular receptors and targets [58, 59]; and QDs conjugated to cationic peptides, such as the HIV Tat peptide, can quickly associate with cells and become internalized via endocytosis [60]. Further, QDs have been used to detect the presence of biomolecules using intricate probe designs incorporating energy donors or acceptors. For example, QDs can be adapted to sense the presence of the sugar maltose by conjugating the maltose binding protein to the nanocrystal surface (Figure 2, bottom) [53]. By initially incubating the QDs with an energy-accepting dye that is conjugated to a sugar recognized by the receptor, excitation of the QD (blue) yields little fluorescence, as the energy is nonradiatively transferred (grey) to the dye. Upon addition of maltose, the quencher-sugar conjugate is displaced, restoring fluorescence (green) in a concentration-dependent manner. QDs can also be sensors for specific DNA sequences [52]. By mixing the ssDNA to be detected with (a) an acceptor fluorophores conjugated to a DNA fragment complementary to one end of the target DNA and (b) a biotinylated DNA fragment complementary to the opposite end of the target DNA, these nucleotides hybridize to yield a biotin-DNA-fluorophore conjugate. Upon mixing this conjugate with QDs, QD fluorescence (green) is quenched via nonradiative energy transfer (grey) to the fluorophore conjugate. This dye acceptor then becomes fluorescent (red), specifically and quantitatively indicating the presence of the target DNA. Finally, QDs conjugated to the luciferase enzyme can nonradiatively accept energy from the enzymatic bioluminescent oxidation of luciferins on the QD surface, exciting the QDs without the need for external illumination [55].

3. Live-Cell Imaging

Researchers have achieved considerable success in using QDs for *in vitro* bioassays [61,62], labeling fixed cells [23] and tissue specimens [63,64], and for imaging membrane proteins on living cells [58,65]. However, only limited progress has been made in developing QD probes for imaging inside living cells. A major problem is the lack of efficient methods for delivering monodispersed (that is, single) QDs into the cytoplasm of living cells. A common observation is that QDs tend to aggregate inside cells, and are often trapped in endocytotic vesicles such as endosomes and lysosomes.

3.1. Imaging and Tracking of Membrane Receptors

QD bioconjugates have been found to be powerful imaging agents for specific recognition and tracking of plasma membrane antigens on living cells. In 2002 Lidke *et al.* coupled red-light emitting (CdSe)ZnS QDs to epidermal growth factor, a small protein with a specific affinity

for the erbB/HER membrane receptor [58]. After addition of these conjugates to cultured human cancer cells, receptor-bound QDs could be identified at the single-molecule level (single QDs may be distinguished from aggregates because the fluorescent intensity from discrete dots is intermittent, or “blinking”). The bright, stable fluorescence emitted from these QDs allowed the continuous observation of protein diffusion on the cellular membrane, and could even be visualized after the proteins were internalized. Dahan *et al.* similarly reported that QDs conjugated to an antibody fragment specific for glycine receptors on the membranes of living neurons allowed tracking of single receptors [65]. These conjugates showed superior photostability, lateral resolution, and sensitivity relative to organic dyes. These applications have inspired the use of QDs for monitoring other plasma membrane proteins such as integrins [50,66], tyrosine kinases [67,68], G-protein coupled receptors [69], and membrane lipids associated with apoptosis [70,71]. As well, detailed procedures for receptor labeling and visualization of receptor dynamics with QDs have recently been published [72,73], and new techniques to label plasma membrane proteins using versatile molecular biology methods have been developed [74,75].

3.2. Intracellular Delivery of QDs

A variety of techniques have been explored to label cells internally with QDs, using passive uptake, receptor-mediated internalization, chemical transfection, and mechanical delivery. QDs have been loaded passively into cells by exploiting the innate capacity of many cell types to uptake their extracellular space through endocytosis [76–78]. It has been found that the efficiency of this process may be dramatically enhanced by coupling the QDs to membrane receptors. This is likely due to the avidity-induced increase in local concentration of QDs at the surface of the cell, as well as an active enhancement caused by receptor-induced internalization [58,77,79]. However, these methods lead to sequestration of aggregated QDs in vesicles, showing strong colocalization with membrane dyes. Although these QDs cannot diffuse to specific intracellular targets, this is a simple way to label cells with QDs, and an easy method to fluorescently image the process of endocytosis. Nonspecific endocytosis was also utilized by Parak *et al.* to fluorescently monitor the motility of cells on a QD-coated substrate [78]. The path traversed by each cell became dark, and the cells increased in fluorescence as they took up more QDs. Chemically-mediated delivery enhances plasma membrane translocation with the use of cationic lipids or peptides, and was originally developed for the intracellular delivery of a wide variety of drugs and biomolecules [60,80–83]. The efficacy of these carriers for the intracellular delivery of QDs is discussed below (Section 3.3 and Section 3.4). Mechanical delivery methods include microinjection of QDs into individual cells, and electroporation of cells in the presence of QDs. Microinjection has been reported to deliver QDs homogeneously into the cytoplasm of cells [49,83], however this method is of low statistical value, as careful manipulation of single cells prevents the use of large sample sizes. Electroporation makes use of the increased permeability of cellular membranes under pulsed electric fields to deliver QDs, but this method was reported to result in aggregation of QDs in the cytoplasm [83], and generally results in widespread cell death.

Despite the current technical challenges, QDs are garnering interest as intracellular probes due to their intense, stable fluorescence, and recent reports have demonstrated that intracellular targeting is not far off. In 2004, Derfus *et al.* demonstrated that QDs conjugated to organelle-targeting peptides could specifically stain either cellular mitochondria or nuclei, following microinjection into fibroblast cytoplasm [83]. Similarly, Chen *et al.* targeted peptide-QD conjugates to cellular nuclei, using electroporation to overcome the plasma membrane barrier [60]. These schemes have resulted in organelle-level resolution of intracellular targets for living cells, yielding fluorescent contrast of vesicles, mitochondria, and nuclei, but not the ability to visualize single molecules. Recently Courty *et al.* demonstrated the capacity to image individual kinesin motors in HeLa cells using QDs delivered into the cytoplasm via osmotic

lysis of pinocytotic vesicles [84]. By incubating the cells in a hypertonic solution containing QDs, water efflux resulted in membrane invagination and pinocytosis, trapping extracellular QDs in endosomal vesicles. Then a brief incubation in hypotonic medium induced intracellular water influx, rupturing the newly formed vesicles, and releasing single QDs into the cytosol. All of the QDs were observed to undergo random Brownian motion in the cytoplasm. However if these QDs were first conjugated to kinesin motor proteins, a significant population of the QDs exhibited directional motion. The velocity of the directed motion and its processivity (average time before cessation of directed motion) were remarkably close to those observed for the motion of these conjugates on purified microtubules *in vitro*. Although this work managed to overcome the plasma membrane diffusion barrier, it highlighted a different problem fundamental to intracellular imaging of living cells, which is the impossibility of removing probes that have not found their target. In this report, the behavior of the QDs was sufficient to distinguish bound QDs from those that were not bound, but this will not be the case for the majority of other protein targets. Without the ability to wash away unbound probes, which is a crucial step for intracellular labeling of fixed, permeabilized cells, the need for activateable probes that are 'off' until they reach their intended target is apparent. However QDs have already found a niche for quantitative monitoring of motor protein transport and for tracking the fate of internalized receptors, allowing the study of downstream signaling pathways in real time with high signal-to-noise and high temporal and spatial resolution [58, 67,68,85,86].

3.3. Tat-QD Conjugates

Cell-penetrating peptides are a class of chemical transfectants that have garnered widespread interest due to the high transfection efficiency of their conjugated cargo, versatility of conjugation, and low toxicity. For this reason, cell-penetrating peptides such as polyarginine and Tat have been investigated for their capacity to deliver QDs into living cells [81,85,87], but the delivery mechanism and the behavior of intracellular QDs are still a matter of debate. Considerable effort has been devoted to understanding the delivery mechanism of these cationic carrier, especially the HIV-1-derived Tat peptide, which has emerged as a widely used cellular delivery vector [88–93]. The delivery process was initially thought to be independent of endocytosis because of its apparent temperature-independence [89–93]. However, later research showed that the earlier work failed to exclude the Tat peptide conjugated cargos bound to plasma membranes, and was largely an artifact caused by cellular fixation. More recent studies based on improved experimental methods indicate that Tat peptide-mediated delivery occurs via macropinocytosis [94], a fluid-phase endocytosis process that is initiated by the binding of Tat-QD to the cell surface [90]. These new results, however, did not shed any light on the downstream events or the intracellular behavior of the internalized cargo. This kind of detailed and mechanistic investigation would be possible with QDs, which are sufficiently bright and photostable for extended imaging and tracking of intracellular events. In addition, most previous studies on Tat peptide-mediated delivery are based on the use of small dye molecules and proteins as cargo [89–93], so it is not clear whether larger nanoparticles would undergo the same processes of cellular uptake and transport. This understanding is needed for the design and development of imaging and therapeutic nanoparticles for biology and medicine.

Ruan *et al.* have recently used Tat peptide-conjugated QDs (Tat-QDs) as a model system to examine the cellular uptake and intracellular transport of nanoparticles in live cells [95]. The authors used a spinning-disk confocal microscope for dynamic fluorescence imaging of quantum dots in living cells at 10 frames per second. The results indicate that the peptide-conjugated QDs are internalized by macropinocytosis, in agreement with the recent work of Dowdy and coworkers [90]. It is interesting, however, that the internalized Tat-QDs are tethered to the inner surface of vesicles, and are trapped in intracellular organelles. An important finding is that the QD-loaded vesicles are actively transported by molecular

machines (such as dyneins) along microtubule tracks to an asymmetric perinuclear region called the microtubule organizing center (MTOC) [96]. Furthermore, it was found that Tat-QDs strongly bind to cellular membrane structures such as filopodia, and that large QD-containing vesicles are able to pinch off from the tips of filopodia. These results not only provide new insight into the mechanisms of Tat peptide-mediated delivery, but also are important for the development of nanoparticle probes for intracellular targeting and imaging.

3.4. QDs with Endosome-Disrupting Coatings

Duan and Nie [97] developed a new class of cell-penetrating quantum dots (QDs) based on the use of multivalent and endosome-disrupting (endosomolytic) surface coatings (Figure 3). Hyperbranched copolymer ligands such as PEG-grafted polyethylenimine (PEI-g-PEG) were found to encapsulate and stabilize luminescent quantum dots in aqueous solution through direct ligand binding to the QD surface. Due to the cationic charges and a “proton sponge effect” [98–100] associated with multivalent amine groups, these QDs could penetrate cell membranes and disrupt endosomal organelles in living cells. This mechanism arises from the presence of a large number of weak bases (with buffering capabilities at pH 5–6), which lead to proton absorption in acidic organelles, and an osmotic pressure buildup across the organelle membrane [100]. This osmotic pressure causes swelling and/or rupture of the acidic endosomes and a release of the trapped materials into the cytoplasm. PEI and other polycations are known to be cytotoxic, however the grafted PEG segment was found to significantly reduce the toxicity and improve the overall nanoparticle stability and biocompatibility. In comparison with previous QDs encapsulated with amphiphilic polymers, the cell-penetrating QDs were smaller in size and exceedingly stable in acidic environments [56]. Cellular uptake and imaging studies revealed that these dots were rapidly internalized by endocytosis, and the pathways of the QDs inside the cells showed dependence on the number of PEG grafts of the polymer ligands. While higher PEG content led to QD sequestration in vesicles, the QDs coated by PEI-g-PEG with fewer PEG grafts are able to escape from endosomes and release into the cytoplasm.

Lovric *et al.* [101] recently reported that very small QDs (2.2 nm) coated with small molecule ligands (cysteamine) spontaneously translocated to the nuclei of murine microglial cells following cellular uptake through passive endocytosis. In contrast, larger QDs (5.5 nm) and small QDs bound to albumin remained in the cytosol only. This is fascinating because these QDs could not only escape from endocytotic vesicles, but were also subjected to an unknown type of active machinery that attracted the QDs to the nucleus. Nabiev *et al.* [102] studied a similar trend of size-dependent QD segregation in human macrophages, and found that small QDs may target nuclear histones and nucleoli after active transport across the nuclear membrane. They found that the size cut-off for this effect was around 3.0 nm. Larger QDs eventually ended up in vesicles in the MTOC region, although some QDs were found to be free in the cytoplasm. This group proposed that the proton sponge effect was also responsible for endosomal escape, as small carboxyl-coated QDs could buffer in the pH 5–7 range. These insights are important for the design and development of nanoparticle agents for intracellular imaging and therapeutic applications.

4. In Vivo Animal Imaging

Compared to the study of living cells in culture, different challenges arise with the increase in complexity to a multicellular organism, and with the accompanying increase in size. Unlike monolayers of cultured cells and thin tissue sections, tissue thickness becomes a major concern because biological tissue attenuates most signals used for imaging. Optical imaging, especially fluorescence imaging, has been used in living animal models, but it is still limited by the poor transmission of visible light through biological tissue. It has been suggested that there is a near-infrared optical window in most biological tissue that is the key to deep-tissue optical imaging [103]. The rationale is that Rayleigh scattering decreases with increasing wavelength, and that

the major chromophores in mammals, hemoglobin and water, have local minima in absorption in this window. Few organic dyes are available that emit brightly in this spectral region, and they suffer from the same photobleaching problems as their visible counterparts, although this has not prevented their successful use as contrast agents for living organisms [104]. One of the greatest advantages of QDs for imaging in living tissue is that their emission wavelengths can be tuned throughout the near-infrared spectrum by adjusting their composition and size, resulting in photostable fluorophores that are stable in biological buffers [24].

4.1 Biodistribution of QDs

For most *in vivo* imaging applications using QDs and other nanoparticle contrast agents, systemic intravenous delivery into the bloodstream will be the main mode of administration. For this reason, the interaction of the nanoparticles with the components of plasma, the specific and nonspecific adsorption to blood cells and the vascular endothelium, and the eventual biodistribution in various organs are of great interest. Immediately upon exposure to blood, QDs may be quickly adsorbed by opsonins, in turn flagging them for phagocytosis. In addition, platelet coagulation may occur, the complement system may be activated, or the immune system can be stimulated or repressed (Figure 4). Although it is important for each of these potential biological effects to be addressed in detail, so far there are no studies that directly examine blood or immune system biocompatibility of QDs *in vivo* or *ex vivo*. However, a recent review article by Dobrovolskaia and McNeil addresses the immunological properties of polymeric, liposomal, carbon-based, and magnetic nanoparticles [105]. Considering the many factors that may affect systemically administered QDs, such as size, shape, charge, targeting ligands, etc., the two most important parameters that affect biodistribution are likely size and the propensity for serum protein adsorption.

The number of papers published on quantum dot pharmacokinetics and biodistribution is limited, but several common trends can be identified. It has been consistently reported that QDs are taken up nonspecifically by the reticuloendothelial system (RES), including the liver and spleen, and the lymphatic system [106–108]. These findings are not necessarily intrinsic to QDs, but are strictly predicated upon the size of the QDs and their surface coatings. Ballou and coworkers reported that (CdSe)ZnS QDs were rapidly removed from the bloodstream into organs of the RES, and remained there for at least 4 months with detectable fluorescence [107]. TEM of these tissues revealed that these QDs retained their morphology, suggesting that given the proper coating, QDs are stable *in vivo* for very long periods of time without degradation into their potentially toxic elemental components. A complimentary work by Fischer, *et al.* showed that nearly 100% of albumin-coated QDs were removed from circulation and sequestered in the liver within hours after a tail vein injection, much faster than QDs that were not bound to albumin [108]. Within the liver, QDs conjugated to albumin were primarily associated with Kupffer cells (resident macrophages). From a clinical perspective, it may be possible to completely inhibit the accumulation of QDs and avoid potential toxic effects if they are within the size range of renal excretion. Recent publications have focused on this insight. Frangioni and coworkers demonstrated that the renal clearance of quantum dots is closely related to the hydrodynamic diameter of the nanoparticle and the renal filtration threshold (~5–6 nm) [109]. Of equal importance to the QD size, is that the surface does not promote protein adsorption, which could significantly increase QD size above that of the renal threshold, and promote phagocytosis. However, it is unlikely that even small QDs could be entirely eliminated from the kidneys, as it has also been found that small QDs (~9 nm) may directly extravasate out of blood vessels, into interstitial fluid [110].

For targeted imaging, specific modulation of the biodistribution of QD contrast agents is the main goal. One way to increase the probability of bioaffinity ligand-specific distribution is to increase the circulation time of the contrast agent in the bloodstream. QD structure and surface

properties have been found to strongly impact the plasma half-life. It was demonstrated by Ballou *et al.* [107] that the lifetime of anionic, carboxylated QDs in the bloodstream of mice (4.6 minutes half-life) is significantly increased if the QDs are coated with PEG polymer chains (71 minutes half-life). This effect has also been documented for other types of nanoparticles and small molecules, in part due to decreased nonspecific adsorption of the nanoparticles, an increase in size, and decreased antigenicity [111]. In a more recent study using perfused porcine skin *in vitro*, Lee, *et al.* demonstrated that carboxylated QDs were extracted more rapidly from circulation, and had greater tissue deposition than PEG coated QDs [112]. It is important to note that a bioaffinity molecule may also be prone to RES uptake, despite a strong affinity for its intended target. For example, Jayagopal *et al.* reported that QD-antibody conjugates have a significantly longer circulation time if the Fc antibody regions (non-antigen binding domains) are immunologically shielded to reduce nonspecific interactions [113].

4.2. *In Vivo* Vascular Imaging

One of the most immediately successful applications of QDs *in vivo* has been their use as contrast agents for the two major circulatory systems of mammals, the cardiovascular system and the lymphatic system. In 2003, Larson *et al.* demonstrated that green-light emitting QDs remained fluorescent and detectable in capillaries of adipose tissue and skin of a living mouse following intravenous injection [114]. This work was aided by the use of near-infrared two-photon excitation for deeper penetration of excitation light, and by the extremely large two-photon cross-sections of QDs, 100–20,000 times that of organic dyes [115]. In other work, Lim *et al.* used near-infrared QDs to image the coronary vasculature of a rat heart [116], and Smith *et al.* imaged the blood vessels of chicken embryos with a variety of near-infrared and visible QDs [117]. The later report showed that QDs could be detected with higher sensitivity than traditionally used fluorescein-dextran conjugates, and resulted in a higher uniformity in image contrast across vessel lumina. Jayagopal *et al.* [113] recently demonstrated the potential for QDs to serve as molecular imaging agents for vascular imaging. Spectrally distinct QDs were conjugated to three different cell adhesion molecules (CAMs), and intravenously injected in a diabetic rat model. Fluorescence angiography of the retinal vasculature revealed CAM-specific increases in fluorescence, and allowed imaging of the inflammation-specific behavior of individual leukocytes, as they freely floated in the vessels, rolled along the endothelium, and underwent leukostasis. The unique spectral properties of QDs allowed the authors to simultaneously image up to four spectrally distinct QD tags.

For imaging of the lymphatic system, the overall size of the probe is an important parameter for determining biodistribution and clearance. For example, Kim *et al.* [24] intradermally injected ~16–19 nm near-infrared QDs in mice and pigs. QDs translocated to sentinel lymph nodes, likely due to a combination of passive flow in lymphatic vessels, and active migration of dendritic cells that engulfed the nanoparticles. Fluorescence contrast of these nodes could be observed up to 1 cm beneath the skin surface. It was found that if these QDs were formulated to have a smaller overall hydrodynamic size (~9 nm), they could migrate further into the lymphatic system, with up to 5 nodes showing fluorescence [110]. This technique could have great clinical impact due to the quick speed of lymphatic drainage and the ease of identification of lymph nodes, enabling surgeons to fluorescently identify and excise nodes draining from primary metastatic tumors for the staging of cancer. This technique has been used to identify lymph nodes downstream from the lungs [106,118], esophagus [119], and from subcutaneous tumors [120]. Recently the multiplexing capabilities of QDs have been exploited for mapping lymphatic drainage networks. By injection of QDs of different color at different intradermal locations, these QDs could be fluorescently observed to drain to common nodes [121], or up to 5 different nodes in real time [122]. A current problem is that a major fraction of the QDs remain at the site of injection for an unknown length of time [123].

4.3. *In Vivo* Tracking of QD-Loaded Cells

Cells can also be loaded with QDs *in vitro*, and then administered to an organism, providing a means to identify the original cells and their progeny within the organism. This was first demonstrated on a small organism scale by microinjecting QDs into the cytoplasm of single frog embryos [49]. As the embryos grew, the cells divided, and each cell that descended from the original labeled cell retained a portion of the fluorescent cytoplasm, which could be fluorescently imaged in real time under continuous illumination. In reports by Hoshino *et al.* [124] and Voura *et al.* [82], cells loaded with QDs were injected intravenously into mice, and their distributions in the animals were later determined through tissue dissection, followed by fluorescence imaging. Also Gao *et al.* loaded human cancer cells with QDs, and injected these cells subcutaneously in an immune-compromised mouse [10]. The cancer cells divided to form a solid tumor, which could be visualized fluorescently through the skin of the mouse. Rosen *et al.* recently reported that human mesenchymal stem cells loaded with QDs could be implanted into an extracellular matrix patch for use as a regenerative implant for canine hearts with a surgically-induced defect [125]. Eight weeks following implantation, it was found that the QDs remained fluorescent within the cells, and could be used to track the locations and fates of these cells. This group also directly injected QD-labeled stem cells into the canine myocardium, and used the fluorescence signals in cardiac tissue sections to elaborately reconstruct the locations of these cells in the heart. With reports that cells may be labeled with QDs at a high degree of specificity [80,81], it is foreseeable that multiple types of cells may be simultaneously monitored in living organisms, and also identified using their distinct optical codes.

4.4. *In Vivo* Tumor Imaging

Imaging of tumors presents a unique challenge not only because of the urgent need for sensitive and specific imaging agents of cancer, but also because of the unique biological attributes inherent to cancerous tissue. Blood vessels are abnormally formed during tumor-induced angiogenesis, having erratic architectures and wide endothelial pores. These pores are large enough to allow the extravasation of large macromolecules up to ~400 nm in size, which accumulate in the tumor microenvironment due to a lack of effective lymphatic drainage [126–129]. This “enhanced permeability and retention” effect (EPR effect) has inspired the development of a variety of nanotherapeutics and nanoparticulates for the treatment and imaging of cancer (Figure 5). Because cancerous cells are effectively exposed to the constituents of the bloodstream, their surface receptors may also be used as active targets of bioaffinity molecules. In the case of imaging probes, active targeting of cancer antigens (molecular imaging) has become an area of tremendous interest to the field of medicine because of the potential to detect early stage cancers and their metastases. QDs hold great promise for these applications mainly due to their intense fluorescent signals and multiplexing capabilities, which could allow a high degree of sensitivity and selectivity in cancer imaging with multiple antigens.

The first steps toward this goal were undertaken in 2002 by Akerman *et al.*, who conjugated QDs to peptides with affinity for various tumor cells and their vasculatures [130]. After intravenous injection of these probes into tumor-bearing mice, microscopic fluorescence imaging of tissue sections demonstrated that the QDs specifically homed to the tumor vasculature. In 2004 Gao *et al.* demonstrated that tumor targeting with QDs could generate tumor contrast on the scale of whole-animal imaging [10]. QDs were conjugated to an antibody against the prostate-specific membrane antigen (PSMA), and intravenously injected into mice bearing subcutaneous human prostate cancers. Tumor fluorescence was significantly greater for the actively targeted conjugates compared to nonconjugated QDs, which also accumulated passively through the EPR effect. Using similar methods, Yu *et al.* were able to actively target and image mouse models of human liver cancer with QDs conjugated to an antibody against

alpha-fetoprotein [131], and Cai *et al.* showed that labeling QDs with RGD peptide significantly increased their uptake in human glioblastoma tumors [132].

The development of clinically relevant QD contrast agents for *in vivo* imaging is certain to encounter many roadblocks in the near future (see Section 5), however QDs can currently be used as powerful imaging agents for the study of the complex anatomy and pathophysiology of cancer in animal models. Stroh *et al.* [133] demonstrated that QDs greatly enhance current intravital microscopy techniques for the imaging of tumor microenvironment. The authors used QDs as fluorescent contrast agents for blood vessels using two-photon excitation, and simultaneously captured images of extracellular matrix from autofluorescent collagen, and perivascular cell contrast from fluorescent protein expression. The use of QDs allowed stark contrast between the tumor constituents due to their intense brightness, tunable wavelengths, and reduced propensity to extravasate into the tumor, compared to organic dye conjugates. In this work, the authors also used QD-tagged beads with variable sizes to model the size-dependent distribution of various nanotherapeutics in tumors. As well, the authors demonstrated that bone marrow lineage-negative cells, which are thought to be progenitors for neovascular endothelium, were labeled *ex vivo* with QDs and imaged *in vivo* as they flowed and adhered to tumor blood vessels following intravenous administration. More recently, Tada *et al.* used QDs to study the biological processes involved in active targeting of nanoparticles. The authors used QDs labeled with an antibody against human epidermal growth factor receptor 2 (HER2) to target human breast cancer in a mouse model [134]. Through intravital fluorescence microscopy of the tumor following systemic QD administration, the authors could distinctly observe individual QDs as they circulated in the bloodstream, extravasated into the tumor, diffused in extracellular matrix, bound to their receptors on tumor cells, and then translocated into the perinuclear region of the cells. The combination of sensitive QD probes with powerful techniques like intravital microscopy and *in vivo* animal imaging could soon lead to major breakthroughs in the current understanding of tumor biology, improve early detection schemes, and guide new therapeutic designs.

5. Nanoparticle Toxicity

Great concern has been raised over the use of quantum dots in living cells and animals due to their chemical composition of toxic heavy metal atoms (e.g. Cd, Hg, Pb, As, Pb). Presently the most commonly used QDs contain divalent cadmium, a nephrotoxin in its ionic form. Although this element is incorporated into a nanocrystalline core, surrounded by biologically inert zinc sulfide, and encapsulated within a stable polymer, it is still unclear if these toxic ions will impact the use of QDs as clinical contrast agents. It may be of greater concern that QDs, and many other types of nanoparticles, have been found to aggregate, bind nonspecifically to cellular membranes and intracellular proteins, and induce the formation of reactive oxygen species. As previously stated, QDs larger than the renal filtration threshold quickly accumulate in the reticuloendothelial system following intravenous administration. The eventual fate of these nanoparticles is of vital importance, but so far has yet to be elucidated.

5.1. Cadmium Toxicity

In the only long-term, quantitative study on QD biodistribution to date, Yang, *et al.* showed that after intravenous administration of cadmium-based QDs, the concentration of cadmium in the liver and kidneys gradually increased over the course of 28 days, as determined via ICP-MS [135]. The cadmium levels in the kidneys eventually reached nearly 10% of the injected dose, compared to 40% in the liver. Although it was not apparent if the cadmium was in the form of a free ion, or remained in the nanocrystalline form, fluorescence microscopy revealed the presence of intact QDs in both the liver and kidneys. However the redistribution of the cadmium over time may signify the degradation of QDs *in vivo*, since the natural accumulation sites of Cd²⁺ ions are the liver and kidneys [79,136,137]. In acute exposures, free cadmium

also may be redistributed to the kidneys via hepatic production of metallothionein [138]. Whether or not this is the specific mechanism observed in this report should be the focus of detailed *in vivo* validation studies. Nevertheless, these findings stress that (a) QD size and nonspecific protein interaction should be minimized to allow renal filtration, or else QDs will inevitably accumulate in organs and tissues of the RES, lung, and kidney, and (b) the potential release of the elements of the QD and their distribution in specific organs, tissues, cell types, and subcellular locations must be well understood.

In general, most *in vitro* studies on the exposure of cells to QDs have attempted to relate cytotoxic events to the release of potentially toxic elements and/or to the size, shape, surface, and cellular uptake of QDs. Because the toxicity of Cd^{2+} ions is well documented, a significant body of work has focused on the intracellular release of free cadmium from the QDs. Cd^{2+} ions can be released through oxidative degradation of the QD, and may then bind to sulfhydryl groups on a variety of intracellular proteins, causing decreased functionality in many subcellular organelles [139]. Several groups have investigated methods to quantify the amount of free Cd^{2+} ions released from QDs, either intracellularly or into culture media, by ICP-MS or fluorometric assays, leading to the conclusion that Cd^{2+} release correlates with cytotoxic manifestations [79,140,141]. Derfus, *et al.* facilitated oxidative release of cadmium ions from the surface of CdSe QDs by exposure to air or ultraviolet irradiation [79]. Under these conditions, CdSe QD cores coated with small thiolate ligands were toxic. Capping these QDs with ZnS shells or coating with BSA rendered the QD cores less susceptible to oxidative degradation and less toxic to primary rat hepatocytes, implicating the potential role of cadmium in QDs cytotoxicity. The decrease in QD cytotoxicity of CdSe QDs with the overgrowth of a ZnS shell has since been verified in several reports [139,142]. If it is revealed in the future that Cd^{2+} release is a major hindrance for the use of QDs in cells and in animals, several new types of QDs that have no heavy metals atoms may be useful for advancing this field [143,144].

5.2. Toxicity Induced by Colloidal Instability

Presently it is nearly impossible to drawing firm conclusions about the toxicity of QDs in cultured cells due to (a) the immense variety of QDs and variations of surface coatings used by different labs and (b) a technical disparity in experimental conditions, such as the duration of the nanoparticle exposure, use of relevant cell lines, media choice (e.g. with or without serum), and even the units of concentration (e.g. mg/ml versus nM). Nonetheless, the cytotoxicity of QDs reported in the literature has strongly correlated with the stability and surface coatings of these nanoparticles, which can be separated into three categories. (1) Core CdTe QDs that are synthesized in aqueous solution and stabilized by small thiolate ligands (e.g. mercaptopropionic acid or mercaptoacetic acid). These QDs have been widely used due to their ease of synthesis, low cost, and immediate utility in biological buffers. However, because these QDs are protected only by a weakly bound ligand, they are highly prone to degradation and aggregation, and their cytotoxicity toward cells in culture has been widely reported [140,145]. (2) Core/shell CdSe/ZnS QDs synthesized in nonpolar solvents and transferred to water using thiolate ligands. CdSe is less prone to oxidation than CdTe, and ZnS is even more inert, and therefore these QDs are much more chemically stable. With direct comparison to CdTe QDs, these nanocrystals are significantly less cytotoxic, although high concentrations have been found to illicit toxic responses from cells [140]. Because these QDs are coated with a ZnS shell, the origin of this cytotoxicity is still unclear, whether it is from degradation of the shell, leading to cadmium release, or if it is caused by other effects. When coated with small ligands, these QDs have similar surface chemistries compared to aqueous CdTe QDs, burdened by significant dissociation of ligands from the QDs, rendering the nanoparticles colloiddally unstable [146]. This propensity for aggregation may contribute to their cytotoxicity, even if free cadmium is not released. Importantly for the comparison between CdSe/ZnS QDs and their cadmium-only counterparts (CdSe or CdTe core QDs), thiolate

ligands bind more strongly to zinc than to cadmium, which may contribute colloidal stability. (3) Core/shell CdSe/ZnS QDs synthesized in nonpolar solvents and transferred to water via encapsulation in amphiphilic polymers or cross-linked silica. These QDs have been found to be significantly more stable colloiddally, chemically, and optically when compared to their counterparts coated in small ligands [56]. For this reason, they have been found to be nearly biologically inert in both living cells and living animals [10,24,49,60,79,107,114,147]. Only when exposed to extreme conditions or when directly injected into cells at immensely high concentrations have these QDs been found to elicit toxic or inflammatory responses [49,142].

It is feasible that a significant amount of toxicological data obtained for QDs thus far has been considerably influenced by the colloidal nature of these nanoparticles. The tendency for nanoparticles to aggregate, precipitate on cells in culture, nonspecifically adsorb to biomolecules, and catalyze the formation of reactive oxygen species (ROS) may be just as important as heavy metal toxicity contributions to toxicity. For example, Kircher et al. found that CdSe/ZnS QDs coated with an amphiphilic polymer shell induced the detachment of human breast cancer cells from their cell culture substrate [139]. This effect was found to also occur for biologically inert gold nanoparticles coated with the same polymer, thus ruling out the possibility of heavy metal atom poisoning. Microscopic examination of the cells revealed that the nanoparticles precipitated on the cells, causing physical harm. Indeed, carbon nanotubes, which are entirely composed of harmless carbon, have been found to be capable of impaling cells and causing major problems in the lungs of mammals [148]. Nonspecific adsorption to intracellular proteins may also impair cellular function, especially for very small QDs (3 nm and below), which can invade the cellular nucleus [101], binding to histones and nucleosomes [102], and damage DNA *in vitro* [149,150]. QDs are also known to catalyze the formation of ROS [145,151], especially when exposed to ultraviolet radiation. In fact, Cho *et al.* exposed cells to CdTe QDs in cell culture and determined that their cytotoxicity could only be accounted for with the effects of ROS generation, as there was no dose-dependent relationship with intracellular Cd²⁺ release, as determined with a cadmium-reactive dye [140]. However, protection of the surface of QDs with a thick ZnS shell may greatly reduce ROS production [152,153]. Despite a significant surge of interest in the cytotoxicity of nanoparticles, there is still much to learn about the cytological and physiological mediators of nanoparticle toxicology. If it is determined that heavy metal composition plays a negligible role in QD toxicity, QDs will have as good of a chance as any other nanoparticle at being used as clinical contrast agents.

6. Dual-Modality QDs for Imaging and Therapy

In comparison with small organic fluorophores, QDs have large surfaces that can be modified through versatile chemistry. This makes QDs convenient scaffolds to accommodate multiple imaging (e.g., radionuclide-based or paramagnetic probes) and therapeutic agents (e.g. anticancer drugs), through chemical linkage or by simple physical immobilization. This may enable the development of a nearly limitless library of multifunctional nanostructures for multimodality imaging, as well as for integrated imaging and therapy.

6.1. Dual-Modality Imaging

The applications of QDs described above for *in vivo* imaging are limited by tissue penetration depth, quantification problems, and a lack of anatomic resolution and spatial information. To address these limitations, several research groups have led efforts to couple QD-based optical imaging with other imaging modalities that are not limited by penetration depth, such as MRI, positron emission tomography (PET) and single photon emission computed tomography (SPECT) [154–158]. For example, Mulder *et al.* [154] developed a dual-modality imaging probe for both optical imaging and MRI by chemically incorporating paramagnetic gadolinium complexes in the lipid coating layer of QDs [154,155]. *In vitro* experiments showed that

labeling of cultured cells with these QDs led to significant T1 contrast enhancement with a brightening effect in MRI, as well as an easily detectable fluorescence signal from QDs. However, the *in vivo* imaging potential of this specific dual-modality contrast agent is uncertain due to the unstable nature of the lipid coating that was used. More recently, Chen and coworkers used a similar approach to attach the PET-detectable radionuclide ^{64}Cu to the polymeric coating of QDs through a covalently bound chelation compound [158]. The use of this probe for targeted *in vivo* imaging of a subcutaneous mouse tumor model was achieved by also attaching $\alpha_v\beta_3$ integrin-binding RGD peptides on the QD surface. The quantification ability and ultrahigh sensitivity of PET imaging enabled the quantitative analysis of the biodistribution and targeting efficacy of this dual-modality imaging probe. However, the full potential of *in vivo* dual-modality imaging was not realized in this study, as fluorescence was only used as an *ex vivo* imaging tool to validate the *in vivo* results of PET imaging, primarily due to the lower sensitivity of optical imaging in comparison with PET. This imbalance in sensitivity is fundamental to the differences in the physics of these imaging modalities, and points to an inherent difficulty in designing useful multimodal imaging probes. The majority of these probes are still at an early stage of development. The clinical relevance of these nanoplateforms still needs further improvement in sensitivity and better integration of different imaging modalities, as well as validation of their biocompatibility and safety.

It is also noteworthy that recent advances in the synthesis of QDs containing paramagnetic dopants, such as manganese, have led to a new class of QDs that are intrinsically fluorescent and magnetic [159,160]. However the utility of these new probes for bioimaging application is unclear because they are currently limited to the ultraviolet and visible emission windows, and their stability (e.g., photochemical and colloidal) and biocompatibility have yet to be systematically investigated [144]. As well, inorganic heterodimers of QDs and magnetic nanoparticles have generated dual-functional nanoparticles [161,162]. Although these new materials are of great interest, they are still in development and have only recently shown applicability in cell culture, but not yet in living animals [160,163].

6.2. Integration of Imaging and Therapy

Drug-containing nanoparticles have shown great promise for treating tumors in animal models and even in clinical trials [157]. Both passive and active targeting of nanotherapeutics have been used to increase the local concentration of chemotherapeutics in the tumor. Due to the size and structural similarities between imaging and therapeutic nanoparticles, it is possible that their functions can be integrated to directly monitor therapeutic biodistribution, to improve treatment specificity, and to reduce side effects. This synergy has become the principle foundation for the development of multi-functional nanoparticles for integrated imaging and cancer treatment. Most studies are still at a proof-of-concept stage using cultured cancer cells, and are not immediately relevant to *in vivo* imaging and treatment of solid tumors. However, these studies will guide the future design and optimization of multifunctional nanoparticle agents for *in vivo* imaging and therapy [164–167].

In one example, Farokhzad *et al.* reported a ternary system composed of a QD, an aptamer, and the small molecular anticancer drug doxorubicin (Dox) for *in vitro* targeted imaging, therapy and sensing of drug release [165]. As illustrated in Figure 6, aptamers were conjugated to QDs to serve as targeting units, and Dox was attached to the stem region of the aptamers, taking advantage of the nucleic acid binding ability of doxorubicin. Two donor-quencher pairs of fluorescence resonance energy transfer occurred in this construct, as the QD fluorescence were quenched by Dox, and Dox was quenched by the double-stranded RNA aptamers. As a result, gradual release of Dox from the conjugate was found to “turn on” the fluorescence of both QDs and Dox, providing a means to sense the release of the drug. However it is clear that

the current design of this conjugate will not be sufficient for *in vivo* use unless the drug loading capacity can be greatly increased (currently 7–8 Dox molecules per QD).

6.3. QDs for siRNA Delivery and Imaging

QDs also provide a versatile nanoscale scaffold to develop multifunctional nanoparticles for siRNA delivery and imaging. RNA interference (RNAi) is a powerful technology for sequence-specific suppression of genes, and has broad applications ranging from functional gene analysis to targeted therapy [168–172]. However, these applications are limited by the same delivery problems that hinder intracellular imaging with QDs (Section 3.2), namely intracellular delivery and endosomal escape, in addition to dissociation from the delivery vehicle (i.e. unpacking), and coupling with cellular machines (such as the RNA-induced silencing complex or RISC). For cellular and *in vivo* siRNA delivery, a number of approaches have been developed (see ref. [168] for a review), but these methods have various shortcomings and do not allow a balanced optimization of gene silencing efficacy and toxicity. For example, previous work has used QDs and iron oxide nanoparticles for siRNA delivery and imaging [27,166,167,173], but the QD probes were either mixed with conventional siRNA delivery agents [166] or an exogenous compound, such as the antimalaria drug chloroquine, was needed for endosomal rupture and gene silencing activity [173].

Gao *et al.* have recently fine-tuned the colloidal and chemical properties of QDs for use as delivery vehicles for siRNA, resulting in highly effective and safe RNA interference, as well as fluorescence contrast [174]. The authors balanced the proton-absorbing capacity of the QD surface in order to induce endosomal release of the siRNA through the proton sponge effect (see Section 3.4). A major finding is that this effect can be precisely controlled by partially converting the carboxylic acid groups on a QD into tertiary amines. When both are linked to the surface of nanometer-sized particles, these two functional groups provide steric and electrostatic interactions that are highly responsive to the acidic organelles, and are also well suited for siRNA binding and cellular entry. As a result, these conjugates can improve gene silencing activity by 10–20 fold, and reduce cellular toxicity by 5–6 fold, compared with current siRNA delivery agents (lipofectamine, JetPEI, and TransIT). In addition, QDs are inherently dual-modality optical and electron microscopy probes, allowing real-time tracking and ultrastructural localization of QDs during transfection.

7. Concluding Remarks

Quantum dots have been received as technological marvels with characteristics that could greatly improve biological imaging and detection. In the near future, there are a number of areas of research that are particularly promising but will require concerted effort for success:

(1) Design and development of nanoparticles with multiple functions

For cancer and other medical applications, important functions include imaging (single or dual-modality), therapy (single drug or combination of two or more drugs), and targeting (one or more ligands). With each added function, nanoparticles could be designed to have novel properties and applications. For example, binary nanoparticles with two functions could be developed for molecular imaging, targeted therapy, or for simultaneous imaging and therapy. Ternary nanoparticles with three functions could be designed for simultaneous imaging and therapy with targeting, targeted dual-modality imaging, or for targeted dual-drug therapy. Quaternary nanoparticles with four functions can be conceptualized in the future to have the capabilities of tumor targeting, dual-drug therapy and imaging.

(2) Use of multiplexed QD bioconjugates for analyzing a panel of biomarkers and for correlation with disease behavior, clinical outcome, and treatment response

This application should begin with retrospective studies of archived specimens in which the patient outcome is already known. A key hypothesis to be tested is that the analysis of a panel of tumor markers will allow more accurate correlations than single tumor markers. As well, the analysis of the relationship between gene expression from cancer cells and the host stroma may help to define important cancer subclasses, identify aggressive phenotypes of cancer, and determine the response of early stage disease to treatment (chemotherapy, radiation, or surgery).

(3) Design and development of biocompatible nanoparticles to overcome nonspecific organ uptake and RES scavenging

There is an urgent need to develop nanoparticles that are capable of escaping RES uptake, and able to target tumors by active binding mechanisms. This *in vivo* biodistribution barrier might be mitigated or overcome by systematically optimizing the size, shape, and surface chemistry of imaging and therapeutic nanoparticles.

(4) Penetration of imaging and therapeutic nanoparticles into solid tumors beyond the vascular endothelium

This task will likely require active pumping mechanisms such as caveolin transcytosis and receptor-mediated endocytosis, or cell-based strategies such as nanoparticle-loaded macrophages.

(5) Release of drug payloads inside targeted cells or organs

This task will likely require the development of biodegradable nanoparticle carriers that are responsive to pH, temperature, or enzymatic reactions.

(6) Nanotoxicology studies including nanoparticle distribution, excretion, metabolism, pharmacokinetics, and pharmacodynamics in animal models in vivo

These investigations will be vital for the development of nanoparticles beyond their current use as research tools, toward clinical applications in cancer imaging and therapy.

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References

1. Alivisatos AP. The use of nanocrystals in biological detection. *Nat. Biotechnol* 2004;22:47–52. [PubMed: 14704706]
2. Ferrari M. Cancer nanotechnology: Opportunities and challenges. *Nat. Rev. Cancer* 2005;5:161–171. [PubMed: 15738981]
3. Niemeyer CM. Nanoparticles, proteins, and nucleic acids: Biotechnology meets materials science. *Angew. Chem., Int. Ed* 2001;40:4128–4158.
4. Cao YWC, Jin RC, Mirkin CA. Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. *Science* 2002;297:1536–1540. [PubMed: 12202825]
5. Gao XH, Yang LL, Petros JA, Marshal FF, Simons JW, Nie SM. In vivo molecular and cellular imaging with quantum dots. *Curr. Opin. Biotechnol* 2005;16:63–72. [PubMed: 15722017]

6. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science* 2005;307:538–544. [PubMed: 15681376]
7. Nie SM, Xing Y, Kim GJ, Simons JW. Nanotechnology applications in cancer. *Annu. Rev. Biomed. Eng* 2007;9:257–288. [PubMed: 17439359]
8. Rosi NL, Mirkin CA. Nanostructures in biodiagnostics. *Chem. Rev* 2005;105:1547–1562. [PubMed: 15826019]
9. Yezhelyev MV, Gao X, Xing Y, Al-Hajj A, Nie SM, O'Regan RM. Emerging use of nanoparticles in diagnosis and treatment of breast cancer. *Lancet Oncol* 2006;7:657–667. [PubMed: 16887483]
10. Gao XH, Cui YY, Levenson RM, Chung LWK, Nie SM. In vivo cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol* 2004;22:969–976. [PubMed: 15258594]
11. Liu Z, Cai WB, He LN, Nakayama N, Chen K, Sun XM, Chen XY, Dai HJ. In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. *Nat. Nanotech* 2007;2:47–52.
12. Weissleder R, Kelly K, Sun EY, Shtatland T, Josephson L. Cell-specific targeting of nanoparticles by multivalent attachment of small molecules. *Nat. Biotechnol* 2005;23:1418–1423. [PubMed: 16244656]
13. Lee ES, Na K, Bae YH. Polymeric micelle for tumor pH and folate-mediated targeting. *J. Control. Release* 2003;91:103–113. [PubMed: 12932642]
14. Hood JD, Bednarski M, Frausto R, Guccione S, Reisfeld RA, Xiang R, Cheres DA. Tumor regression by targeted gene delivery to the neovasculature. *Science* 2002;296:2404–2407. [PubMed: 12089446]
15. Duncan R. Polymer conjugates as anticancer nanomedicines. *Nat. Rev. Cancer* 2006;6:688–701. [PubMed: 16900224]
16. Couvreur P, Vauthier C. Nanotechnology: Intelligent design to treat complex disease. *Pharm. Res* 2006;23:1417–1450. [PubMed: 16779701]
17. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: Theory to practice. *Pharmacol. Rev* 2001;53:283–318. [PubMed: 11356986]
18. Torchilin VP. Micellar nanocarriers: Pharmaceutical perspectives. *Pharm. Res* 2007;24:1–16. [PubMed: 17109211]
19. McCarthy JR, Kelly KA, Sun EY, Weissleder R. Targeted delivery of multifunctional magnetic nanoparticles. *Nanomedicine* 2007;2:153–167. [PubMed: 17716118]
20. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, de la Rosette J, Weissleder R. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N. Engl. J. Med* 2003;348:2491–2499. [PubMed: 12815134]
21. Rhyner MN, Smith AM, Gao XH, Mao H, Yang L, Nie SM. Quantum dots and multifunctional nanoparticles: new contrast agents for tumor imaging. *Nanomedicine* 2006;1:209–217. [PubMed: 17716110]
22. Xing Y, Chaudry Q, Shen C, Kong KY, Zhau HE, Chung LW, Petros JA, O'Regan RM, Yezhelyev MV, Simons JW, Wang MD, Nie SM. Bioconjugated quantum dots for multiplexed and quantitative immunohistochemistry. *Nat. Protoc* 2007;2:1152–1165. [PubMed: 17546006]
23. Wu XY, Liu HJ, Liu JQ, Haley KN, Treadway JA, Larson JP, Ge NF, Peale F, Bruchez MP. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nat. Biotechnol* 2003;21:41–46. [PubMed: 12459735]
24. Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat. Biotechnol* 2004;22:93–97. [PubMed: 14661026]
25. Yezhelyev MV, Al-Hajj A, Morris C, Marcus AI, Liu T, Lewis M, Cohen C, Zrazhevskiy P, Simons JW, Rogatko A, Nie S, Gao X, O'Regan RM. In situ molecular profiling of breast cancer biomarkers with multicolor quantum dots. *Adv. Mater* 2007;19:3146–3151.
26. Woodle MC, Lu PY. Nanoparticles deliver RNAi therapy. *NanoToday* 2005;8:34–41.
27. Medarova Z, Pham W, Farrar C, Petkova V, Moore A. In vivo imaging of siRNA delivery and silencing in tumors. *Nat. Med* 2007;13:372–377. [PubMed: 17322898]
28. Alivisatos AP. Semiconductor clusters, nanocrystals, and quantum dots. *Science* 1996;271:933–937.

29. Zhong XH, Feng YY, Knoll W, Han MY. Alloyed $\text{Zn}_x\text{Cd}_{1-x}\text{S}$ nanocrystals with highly narrow luminescence spectral width. *J. Am. Chem. Soc* 2003;125:13559–13563. [PubMed: 14583053]
30. Bailey RE, Nie SM. Alloyed semiconductor quantum dots: Tuning the optical properties without changing the particle size. *J. Am. Chem. Soc* 2003;125:7100–7106. [PubMed: 12783563]
31. Hines MA, Scholes GD. Colloidal PbS nanocrystals with size-tunable near-infrared emission: Observation of post-synthesis self-narrowing of the particle size distribution. *Adv. Mater* 2003;15:1844–1849.
32. Kim S, Fisher B, Eisler HJ, Bawendi M. Type-II quantum dots: CdTe/CdSe(core/shell) and CdSe/ZnTe(core/shell) heterostructures. *J. Am. Chem. Soc* 2003;125:11466–11467. [PubMed: 13129327]
33. Qu LH, Peng XG. Control of photoluminescence properties of CdSe nanocrystals in growth. *J. Am. Chem. Soc* 2002;124:2049–2055. [PubMed: 11866620]
34. Pietryga J, Schaller R, Werder D, Stewart M, Klimov V, Hollingsworth J. Pushing the band gap envelope: Mid-infrared emitting colloidal PbSe quantum dots. *J. Am. Chem. Soc* 2004;126:11752–11753. [PubMed: 15382884]
35. Efros AL, Efros AL. Interband absorption of light in a semiconductor sphere. *Sov. Phys. Semicond* 1982;16:772–775.
36. Ekimov AI, Onushchenko AA. Quantum size effect in the optical-spectra of semiconductor microcrystals. *Sov. Phys. Semicond* 1982;16:775–778.
37. Crouch D, Norager S, O'Brien P, Park JH, Pickett N. New synthetic routes for quantum dots, *Philos. Trans. R. Soc. London, A* 2003;361:297–310.
38. Bhattacharya P, Ghosh S, Stiff-Roberts AD. Quantum dot opto-electronic devices. *Annu. Rev. Mater. Res* 2004;34:1–40.
39. Murray CB, Norris DJ, Bawendi MG. Synthesis and characterization of nearly monodisperse CdE (E = S, Se, Te) semiconductor nanocrystallites. *J. Am. Chem. Soc* 1993;115:8706–8715.
40. Dabbousi BO, Rodriguez-Viejo J, Mikulec FV, Heine JR, Mattoussi H, Ober R, Jensen KF, Bawendi MG. (CdSe)ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. *J. Phys. Chem. B* 1997;110:9463–9475.
41. Hines MA, Guyot-Sionnest P. Synthesis and characterization of strongly luminescing ZnS-capped CdSe nanocrystals. *J. Phys. Chem* 1996;100:468–471.
42. Gerion D, Pinaud F, Williams SC, Parak WJ, Zanchet D, Weiss S, Alivisatos AP. Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots. *J. Phys. Chem* 2001;105:8861–8871.
43. Guo W, Li JJ, Wang YA, Peng XG. Luminescent CdSe/CdS core/shell nanocrystals in dendron boxes: Superior chemical, photochemical and thermal stability. *J. Am. Chem. Soc* 2003;125:3901–3909. [PubMed: 12656625]
44. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. *Science* 1998;281:2013–2016. [PubMed: 9748157]
45. Chan WCW, Nie SM. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 1998;281:2016–2018. [PubMed: 9748158]
46. Talapin DV, Mekis I, Gotzinger S, Kornowski A, Benson O, Weller H. CdSe/CdS/ZnS and CdSe/ZnSe/ZnS core-shell-shell nanocrystals. *J. Phys. Chem. B* 2004;108:18826–18831.
47. Xie RG, Kolb U, Li JX, Basche T. A. Mews, Synthesis and characterization of highly luminescent CdSe-Core CdS/Zn_{0.5}Cd_{0.5}S/ZnS multishell nanocrystals. *J. Am. Chem. Soc* 2005;127:7480–7488. [PubMed: 15898798]
48. Gaponik N, Talapin DV, Rogach AL, Hoppe K, Shevchenko EV, Kornowski A, Eychmuller A, Weller H. Thiol-capping of CdTe nanocrystals: An alternative to organometallic synthetic routes. *J. Phys. Chem. B* 2002;106:7177–7185.
49. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. In vivo imaging of quantum dots encapsulated in phospholipid micelles. *Science* 2002;298:1759–1762. [PubMed: 12459582]
50. Lieleg O, Lopez-Garcia M, Semmrich C, Auernheimer J, Kessler H, Bausch AR. Specific integrin Labeling in living Celts using functionalized nanocrystals. *Small* 2007;3:1560–1565. [PubMed: 17705315]

51. Pathak S, Davidson MC, Silva GA. Characterization of the functional binding properties of antibody conjugated quantum dots. *Nano Lett* 2007;7:1839–1845. [PubMed: 17536868]
52. Zhang CY, Yeh HC, Kuroki MT, Wang TH. Single-quantum-dot-based DNA nanosensor. *Nat. Mater* 2005;4:826–831. [PubMed: 16379073]
53. Medintz IL, Clapp AR, Mattoussi H, Goldman ER, Fisher B, Mauro JM. Self-assembled nanoscale biosensors based on quantum dot FRET donors. *Nat. Mater* 2003;2:630–638. [PubMed: 12942071]
54. Tran PT, Goldman ER, Anderson GP, Mauro JM, Mattoussi H. Use of luminescent CdSe-ZnS nanocrystal bioconjugates in quantum dot-based nanosensors. *Phys. Status Solidi B* 2002;229:427–432.
55. So MK, Xu CJ, Loening AM, Gambhir SS, Rao JH. Self-illuminating quantum dot conjugates for in vivo imaging. *Nat. Biotechnol* 2006;24:339–343. [PubMed: 16501578]
56. Smith AM, Duan HW, Rhyner MN, Ruan G, Nie SM. A systematic examination of surface coatings on the optical and chemical properties of semiconductor quantum dots. *Phys. Chem. Chem. Phys* 2006;8:3895–3903.
57. Xing Y, Smith AM, Agrawal A, Ruan G, Nie SM. Molecular profiling of single cancer cells and clinical tissue specimens with semiconductor quantum dots. *Int. J. Nanomedicine* 2006;1:473–481. [PubMed: 17722280]
58. Lidke DS, Nagy P, Heintzmann R, Arndt-Jovin DJ, Post JN, Grecco HE, Jares-Erijman EA, Jovin TM. Quantum dot ligands provide new insights into erbB/HER receptor-mediated signal transduction. *Nat. Biotechnol* 2004;22:198–203. [PubMed: 14704683]
59. Bagwe RP, Zhao XJ, Tan WH. Bioconjugated luminescent nanoparticles for biological applications. *J. Dispersion Sci. Technol* 2003;24:453–464.
60. Chen F, Gerion D. Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. *Nano Lett* 2004;4:1827–1832.
61. Smith AM, Nie SM. Chemical analysis and cellular imaging with quantum dots. *Analyst* 2004;129:672–677. [PubMed: 15344262]
62. Smith AM, Dave S, Nie SM, True L, Gao XH. Multicolor quantum dots for molecular diagnostics of cancer. *Expert Rev. Mol. Diagn* 2006;6:231–244. [PubMed: 16512782]
63. Fountaine TJ, Wincovitch SM, Geho DH, Garfield SH, Pittaluga S. Multispectral imaging of clinically relevant cellular targets in tonsil and lymphoid tissue using semiconductor quantum dots. *Mod. Pathol* 2006;19:1181–1191. [PubMed: 16778828]
64. Ferrara DE, Weiss D, Carnell PH, Vito RP, Vega D, Gao XH, Nie SM, Taylor WR. Quantitative 3D fluorescence technique for the analysis of en face preparations of arterial walls using quantum dot nanocrystals and two-photon excitation laser scanning microscopy. *Am. J. Physiol.-Reg. I* 2006;290:R114–R123.
65. Dahan M, Levi S, Luccardini C, Rostaing P, Riveau B, Triller A. Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. *Science* 2003;302:442–445. [PubMed: 14564008]
66. Chen HF, Titushkin I, Strosio M, Cho M. Altered membrane dynamics of quantum dot-conjugated integrins during osteogenic differentiation of human bone marrow derived progenitor cells. *Biophys. J* 2007;92:1399–1408. [PubMed: 17114225]
67. Echarte MM, Bruno L, Arndt-Jovin DJ, Jovin TM, Pietrasanta LI. Quantitative single particle tracking of NGF-receptor complexes: Transport is bidirectional but biased by longer retrograde run lengths. *FEBS Lett* 2007;581:2905–2913. [PubMed: 17543952]
68. Rajan SS, Vu TQ. Quantum dots monitor TrkA receptor dynamics in the interior of neural PC12 cells. *Nano Lett* 2006;6:2049–2059. [PubMed: 16968024]
69. Young SH, Rozengurt E. Qdot Nanocrystal Conjugates conjugated to bombesin or ANG II label the cognate G protein-coupled receptor in living cells. *Am. J. Physiol.-Cell Ph* 2006;290:C728–C732.
70. Le Gac S, Vermes I, van den Berg A. Quantum dots based probes conjugated to annexin V for photostable apoptosis detection and imaging. *Nano Lett* 2006;6:1863–1869. [PubMed: 16967992]
71. Koeppel F, Jaiswal JK, Simon SM. Quantum dot-based sensor for improved detection of apoptotic cells. *Nanomedicine* 2007;2:71–78. [PubMed: 17716192]
72. Jaiswal JK, Goldman ER, Mattoussi H, Simon SM. Use of quantum dots for live cell imaging. *Nat. Methods* 2004;1:73–78. [PubMed: 16138413]

73. Courty, S.; Bouzigues, C.; Luccardini, C.; Ehrensperger, MV.; Bonneau, S.; Dahan, M. Tracking individual proteins in living cells using single quantum dot imaging. In: Inglese, J., editor. *Methods in Enzymology*. Vol. 414. Amsterdam: Elsevier, Inc.; 2006. p. 211-228.
74. Howarth M, Takao K, Hayashi Y, Ting AY. Targeting quantum dots to surface proteins in living cells with biotin ligase. *Proc. Natl. Acad. Sci. U. S. A* 2005;102:7583–7588. [PubMed: 15897449]
75. Bonasio R, Carman CV, Kim E, Sage PT, Love KR, Mempel TR, Springer TA. U.H. von Andrian, Specific and covalent labeling of a membrane protein with organic fluorochromes and quantum dots. *Proc. Natl. Acad. Sci. U. S. A* 2007;104:14753–14758. [PubMed: 17785425]
76. Hanaki K, Momo A, Oku T, Komoto A, Maenosono S, Yamaguchi Y, Yamamoto K. Semiconductor quantum dot/albumin complex is a long-life and highly photostable endosome marker. *Biochem. Biophys. Res. Commun* 2003;302:496–501. [PubMed: 12615061]
77. Jaiswal JK, Mattoussi H, Mauro JM, Simon SM. Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat. Biotechnol* 2003;21:47–51. [PubMed: 12459736]
78. Parak WJ, Boudreau R, Le Gros M, Gerion D, Zanchet D, Micheel CM, Williams SC, Alivisatos AP, Larabell C. Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks. *Adv. Mater* 2002;14:882–885.
79. Derfus AM, Chan WCW, Bhatia SN. Probing the cytotoxicity of semiconductor quantum dots. *Nano Lett* 2004;4:11–18.
80. Mattheakis L, Dias J, Choi Y, Gong J, Bruchez M, Liu J, Wang E. Optical coding of mammalian cells using semiconductor quantum dots. *Anal. Biochem* 2004;327:200–208. [PubMed: 15051536]
81. Lagerholm B, Wang M, Ernst L, Ly D, Liu H, Bruchez M, Waggoner A. Multicolor coding of cells with cationic peptide coated quantum dots. *Nano Lett* 2004;4:2019–2022.
82. Voura E, Jaiswal J, Mattoussi H, Simon S. Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. *Nat. Med* 2004;10:993–998. [PubMed: 15334072]
83. Derfus AM, Chan WCW, Bhatia SN. Intracellular delivery of quantum dots for live cell labeling and organelle tracking. *Adv. Mater* 2004;16:961–966.
84. Courty S, Luccardini C, Bellaiche Y, Cappello G, Dahan M. Tracking individual kinesin motors in living cells using single quantum-dot imaging. *Nano Lett* 2006;6:1491–1495. [PubMed: 16834436]
85. Nan XL, Sims PA, Chen P, Xie XS. Observation of individual microtubule motor steps in living cells with endocytosed quantum dots. *J. Phys. Chem. B* 2005;109:24220–24224. [PubMed: 16375416]
86. Cambi A, Lidke DS, Arndt-Jovin DJ, Figdor CG, Jovin TM. Ligand-conjugated quantum dots monitor antigen uptake and processing by dendritic cells. *Nano Lett* 2007;7:970–977. [PubMed: 17388641]
87. Delehanty JB, Medintz IL, Pons T, Brunel FM, Dawson PE, Mattoussi H. Self-assembled quantum dot-peptide bioconjugates for selective intracellular delivery. *Bioconjugate Chem* 2006;17:920–927.
88. Langel, U. *Cell-penetrating peptides: processes and applications*. Boca Raton, FL: CRC Press; 2002.
89. Brooks H, Lebleu B, Vives E. Tat peptide-mediated cellular delivery: back to basics. *Adv. Drug Deliver. Rev* 2005;57:559–577.
90. Wadia JS, Dowdy SF. Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer. *Adv. Drug Deliver. Rev* 2005;57:579–596.
91. Gupta B, Levchenko TS, Torchilin VP. Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. *Adv. Drug Deliver. Rev* 2005;57:637–651.
92. Futaki S. Membrane-permeable arginine-rich peptides and the translocation mechanisms. *Adv. Drug Deliver. Rev* 2005;57:547–558.
93. Zorko M, Langel U. Cell-penetrating peptides: mechanism and kinetics of cargo delivery. *Adv. Drug Deliver. Rev* 2005;57:529–545.
94. Conner SD, Schmid SL. Regulated portals of entry into the cell. *Nature* 2003;422:37–44. [PubMed: 12621426]
95. Ruan G, Agrawal A, Marcus AI, Nie SM. Imaging and tracking of Tat peptide-conjugated quantum dots in living cells: new insights into nanoparticle uptake, intracellular transport, and vesicle shedding. *J. Am. Chem. Soc* 2007;129:14759–14766. [PubMed: 17983227]

96. Gonczy P, Pichler S, Kirkham M, Hyman AA. Cytoplasmic dynein is required for distinct aspects of MTOC positioning, including centrosome separation, in the one cell stage *Caenorhabditis elegans* embryo. *J. Cell Biol* 1999;147:135–150. [PubMed: 10508861]
97. Duan HW, Nie SM. Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings. *J. Am. Chem. Soc* 2007;129:3333–3338. [PubMed: 17319667]
98. Neu M, Fischer D, Kissel T. Recent advances in rational gene transfer vector design based on poly (ethylene imine) and its derivatives. *J. Gene Med* 2005;7:992–1009. [PubMed: 15920783]
99. Boussif O, Lezoualch F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP. A Versatile Vector For Gene And Oligonucleotide Transfer Into Cells In Culture And In-Vivo - Polyethylenimine. *Proc. Natl. Acad. Sci. U. S. A* 1995;92:7297–7301. [PubMed: 7638184]
100. Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. *Nat. Rev. Drug Discov* 2005;4:581–593. [PubMed: 16052241]
101. Lovric J, Bazzi HS, Cuie Y, Fortin GRA, Winnik FM, Maysinger D. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *J. Mol. Med.-JMM* 2005;83:377–385.
102. Nabiev I, Mitchell S, Davies A, Williams Y, Kelleher D, Moore R, Gun'ko YK, Byrne S, Rakovich YP, Donegan JF, Sukhanova A, Conroy J, Cottell D, Gaponik N, Rogach A, Volkov Y. Nonfunctionalized nanocrystals can exploit a cell's active transport machinery delivering them to specific nuclear and cytoplasmic compartments. *Nano Lett* 2007;7:3452–3461. [PubMed: 17949046]
103. Weissleder R. A clearer vision for in vivo imaging. *Nat. Biotechnol* 2001;19:316–317. [PubMed: 11283581]
104. Frangioni JV. In vivo near-infrared fluorescence imaging. *Curr. Opin. Chem. Biol* 2003;7:626–634. [PubMed: 14580568]
105. Dobrovolskaia MA, Mcneil SE. Immunological properties of engineered nanomaterials. *Nat. Nanotech* 2007;2:469–478.
106. Soltesz E, Kim S, Laurence R, DeGrand A, Parungo C, Dor D, Cohn L, Bawendi M, Frangioni J, Mihaljevic T. Intraoperative sentinel lymph node mapping of the lung using near-infrared fluorescent quantum dots. *Ann. Thorac. Surg* 2005;79:269–277. [PubMed: 15620956]
107. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. Noninvasive imaging of quantum dots in mice. *Bioconjugate Chem* 2004;15:79–86.
108. Fischer HC, Liu LC, Pang KS, Chan WCW. Pharmacokinetics of nanoscale quantum dots: In vivo distribution, sequestration, and clearance in the rat. *Adv. Funct. Mater* 2006;16:1299–1305.
109. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, Bawendi MG, Frangioni JV. Renal clearance of quantum dots. *Nat. Biotechnol* 2007;25:1165–1170. [PubMed: 17891134]
110. Zimmer JP, Kim SW, Ohnishi S, Tanaka E, Frangioni JV, Bawendi MG. Size series of small indium arsenide-zinc selenide core-shell nanocrystals and their application to in vivo imaging. *J. Am. Chem. Soc* 2006;128:2526–2527. [PubMed: 16492023]
111. Roberts M, Bentley M, Harris J. Chemistry for peptide and protein PEGylation. *Adv. Drug Deliver. Rev* 2002;54:459–476.
112. Lee HA, Imran M, Monteiro-Riviere NA, Colvin VL, Yu WW, Riviere JE. Biodistribution of quantum dot nanoparticles in perfused skin: Evidence of coating dependency and periodicity in arterial extraction. *Nano Lett* 2007;7:2865–2870. [PubMed: 17685663]
113. Jayagopal A, Russ PK, Haselton FR. Surface engineering of quantum dots for in vivo vascular Imaging. *Bioconjugate Chem* 2007;18:1424–1433.
114. Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW, Webb WW. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. *Science* 2003;300:1434–1436. [PubMed: 12775841]
115. Rubart M. Two-photon microscopy of cells and tissue. *Circ. Res* 2004;95:1154–1166. [PubMed: 15591237]
116. Lim YT, Kim S, Nakayama A, Stott NE, Bawendi MG, Frangioni JV. Selection of quantum dot wavelengths for biomedical assays and imaging. *Mol. Imaging* 2003;2:50–64. [PubMed: 12926237]
117. Smith JD, Fisher GW, Waggoner AS, Campbell PG. The use of quantum dots for analysis of chick CAM vasculature. *Microvasc. Res* 2007;73:75–83. [PubMed: 17070560]

118. Parungo C, Colson Y, Kim S, Kim S, Cohn L, Bawendi M, Frangioni J. Sentinel lymph node mapping of the pleural space. *Chest* 2005;127:1799–1804. [PubMed: 15888861]
119. Parungo C, Ohnishi S, Kim S, Kim S, Laurence R, Soltesz E, Chen F, Colson Y, Cohn L, Bawendi M, Frangioni J. Intraoperative identification of esophageal sentinel lymph nodes with near-infrared fluorescence imaging. *J. Thorac. Cardiovasc. Surg* 2005;129:844–850. [PubMed: 15821653]
120. Ballou B, Ernst LA, Andreko S, Harper T, Fitzpatrick JAJ, Waggoner AS, Bruchez MP. Sentinel lymph node imaging using quantum dots in mouse tumor models. *Bioconjugate Chem* 2007;18:389–396.
121. Hama Y, Koyama Y, Urano Y, Choyke PL, Kobayashi H. Simultaneous two-color spectral fluorescence lymphangiography with near infrared quantum dots to map two lymphatic flows from the breast and the upper extremity. *Breast Cancer Res. Treat* 2007;103:23–28. [PubMed: 17028977]
122. Kobayashi H, Hama Y, Koyama Y, Barrett T, Regino CAS, Urano Y, Choyke PL. Simultaneous multicolor imaging of five different lymphatic basins using quantum dots. *Nano Lett* 2007;7:1711–1716. [PubMed: 17530812]
123. Gopee NV, Roberts DW, Webb P, Cozart CR, Siitonen PH, Warbritton AR, Yu WW, Colvin VL, Walker NJ, Howard PC. Migration of intradermally injected quantum dots to sentinel organs in mice. *Toxicol. Sci* 2007;98:249–257. [PubMed: 17404394]
124. Hoshino A, Hanaki K, Suzuki K, Yamamoto K. Applications of T-lymphoma labeled with fluorescent quantum dots to cell tracing markers in mouse body. *Biochem. Biophys. Res. Commun* 2004;314:46–53. [PubMed: 14715244]
125. Rosen AB, Kelly DJ, Schuldt AJT, Lu J, Potapova IA, Doronin SV, Robichaud KJ, Robinson RB, Rosen MR, Brink PR, Gaudette GR, Cohen IS. Finding fluorescent needles in the cardiac haystack: Tracking human mesenchymal stem cells labeled with quantum dots for quantitative in vivo three-dimensional fluorescence analysis. *Stem Cells* 2007;25:2128–2138. [PubMed: 17495112]
126. Jain RK. Delivery of molecular medicine to solid tumors: lessons from in vivo imaging of gene expression and function. *J. Control. Release* 2001;74:7–25. [PubMed: 11489479]
127. Jain RK. Transport of molecules, particles, and cells in solid tumors. *Annu. Rev. Biomed. Eng* 1999;1:241–263. [PubMed: 11701489]
128. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Control. Release* 2000;65:271–284. [PubMed: 10699287]
129. Matsumura Y, Maeda H. A New Concept For Macromolecular Therapeutics In Cancer-Chemotherapy - Mechanism Of Tumorotropic Accumulation Of Proteins And The Antitumor Agent Smancs. *Cancer Res* 1986;46:6387–6392. [PubMed: 2946403]
130. Akerman ME, Chan WCW, Laakkonen P, Bhatia SN, Ruoslahti E. Nanocrystal targeting in vivo. *Proc. Natl. Acad. Sci. U. S. A* 2002;99:12617–12621. [PubMed: 12235356]
131. Yu XF, Chen LD, Li KY, Li Y, Xiao S, Luo X, Liu J, Zhou L, Deng YL, Pang DW, Wang QQ. Immunofluorescence detection with quantum dot bioconjugates for hepatoma in vivo. *J. Biomed. Opt* 2007;12:014008. [PubMed: 17343483]
132. Cai WB, Shin DW, Chen K, Gheysens O, Cao QZ, Wang SX, Gambhir SS, Chen XY. Peptide-labeled near-infrared quantum dots for imaging tumor vasculature in living subjects. *Nano Lett* 2006;6:669–676. [PubMed: 16608262]
133. Stroh M, Zimmer JP, Duda DG, Levchenko TS, Cohen KS, Brown EB, Scadden DT, Torchilin VP, Bawendi MG, Fukumura D, Jain RK. Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo. *Nat. Med* 2005;11:678–682. [PubMed: 15880117]
134. Tada H, Higuchi H, Wanatabe TM, Ohuchi N. In vivo real-time tracking of single quantum dots conjugated with monoclonal anti-HER2 antibody in tumors of mice. *Cancer Res* 2007;67:1138–1144. [PubMed: 17283148]
135. Yang RH, Chang LW, Wu JP, Tsai MH, Wang HJ, Kuo YC, Yeh TK, Yang CS, Lin P. Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. *Environ. Health Persp* 2007;115:1339–1343.
136. Omarova A, Phillips CJC. A meta-analysis of literature data relating to the relationships between cadmium intake and toxicity indicators in humans. *Environ. Res* 2007;103:432–440. [PubMed: 17109840]

137. Ikeda M, Watanabe T, Zhang ZW, Moon CS, Shimbo S. The integrity of the liver among people environmentally exposed to cadmium at various levels. *Int. Arch. Occup. Environ. Health* 1997;69:379–385. [PubMed: 9215923]
138. Liu J, Liu YP, Michalska AE, Choo KHA, Klaassen CD. Distribution and retention of cadmium in metallothionein I and II null mice. *Toxicol. Appl. Pharmacol* 1996;136:260–268. [PubMed: 8619234]
139. Kirchner C, Liedl T, Kudera S, Pellegrino T, Javier AM, Gaub HE, Stolzle S, Fertig N, Parak WJ. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. *Nano Lett* 2005;5:331–338. [PubMed: 15794621]
140. Cho SJ, Maysinger D, Jain M, Roder B, Hackbarth S, Winnik FM. Long-term exposure to CdTe quantum dots causes functional impairments in live cells. *Langmuir* 2007;23:1974–1980. [PubMed: 17279683]
141. Chang E, Thekkekk N, Yu WW, Colvin VL, Drezek R. Evaluation of quantum dot cytotoxicity based on intracellular uptake. *Small* 2006;2:1412–1417. [PubMed: 17192996]
142. Maysinger D, Behrendt M, Lalancette-Herbert M, Kriz J. Real-time imaging of astrocyte response to quantum dots: In vivo screening model system for biocompatibility of nanoparticles. *Nano Lett* 2007;7:2513–2520. [PubMed: 17638392]
143. Pradhan N, Battaglia DM, Liu YC, Peng XG. Efficient, stable, small, and water-soluble doped ZnSe nanocrystal emitters as non-cadmium biomedical labels. *Nano Lett* 2007;7:312–317. [PubMed: 17297994]
144. Pradhan N, Peng XG. Efficient and color-tunable Mn-doped ZnSe nanocrystal emitters: Control of optical performance via greener synthetic chemistry. *J. Am. Chem. Soc* 2007;129:3339–3347. [PubMed: 17311383]
145. Lovric J, Cho SJ, Winnik FM, Maysinger D. Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death. *Chem. Biol* 2005;12:1227–1234. [PubMed: 16298302]
146. Aldana J, Wang Y, Peng X. Photochemical instability of CdSe nanocrystals coated by hydrophilic thiols. *J. Am. Chem. Soc* 2001;123:8844–8850. [PubMed: 11535092]
147. Zhang TT, Stilwell JL, Gerion D, Ding LH, Elboudwarej O, Cooke PA, Gray JW, Alivisatos AP, Chen FF. Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics measurements. *Nano Lett* 2006;6:800–808. [PubMed: 16608287]
148. Lam CW, James JT, McCluskey R, Arepalli S, Hunter RL. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit. Rev. Toxicol* 2006;36:189–217. [PubMed: 16686422]
149. Liang JG, He ZK, Zhang SS, Huang S, Ai XP, Yang HX, Han HY. Study on DNA damage induced by CdSe quantum dots using nucleic acid molecular "light switches" as probe. *Talanta* 2007;71:1675–1678. [PubMed: 19071507]
150. Green M, Howman E. Semiconductor quantum dots and free radical induced DNA nicking. *Chem. Commun* 2005:121–123.
151. Zhang Y, He J, Wang PN, Chen JY, Lu ZJ, Lu DR, Guo J, Wang CC, Yang WL. Time-dependent photoluminescence blue shift of the quantum dots in living cells: Effect of oxidation by singlet oxygen. *J. Am. Chem. Soc* 2006;128:13396–13401. [PubMed: 17031951]
152. Ipe BI, Lehnig M, Niemeyer CM. On the generation of free radical species from quantum dots. *Small* 2005;1:706–709. [PubMed: 17193510]
153. Chan WH, Shiao NH, Lu PZ. CdSe quantum dots induce apoptosis in human neuroblastoma cells via mitochondrial-dependent pathways and inhibition of survival signals. *Toxicol. Lett* 2006;167:191–200. [PubMed: 17049762]
154. Mulder WJM, Koole R, Brandwijk RJ, Storm G, Chin PTK, Strijkers GJ, Donega CD, Nicolay K, Griffioen AW. Quantum dots with a paramagnetic coating as a bimodal molecular imaging probe. *Nano Lett* 2006;6:1–6. [PubMed: 16402777]
155. van Tilborg GAF, Mulder WJM, Chin PTK, Storm G, Reutelingsperger CP, Nicolay K, Strijkers GJ. Annexin A5-conjugated quantum dots with a paramagnetic lipidic coating for the multimodal detection of apoptotic cells. *Bioconjugate Chem* 2006;17:865–868.

156. Bakalova R, Zhelev Z, Aoki I, Ohba H, Imai Y, Kanno I. Silica-shelled single quantum dot micelles as imaging probes with dual or multimodality. *Anal. Chem* 2006;78:5925–5932. [PubMed: 16906742]
157. Cai WB, Chen X. Nanoplatfoms for targeted molecular imaging in living subjects. *Small* 2007;3:1840–1854. [PubMed: 17943716]
158. Cai WB, Chen K, Li Z, Gambhir SS, Chen X. Dual-functional probe for PET and near-infrared fluorescence imaging of tumor vasculature. *J. Nucl. Med* 2007;48:1862–1870. [PubMed: 17942800]
159. Santra S, Yang HS, Holloway PH, Stanley JT, Mericle RA. Synthesis of water-dispersible fluorescent, radio-opaque, and paramagnetic CdS : Mn/ZnS quantum dots: A multifunctional probe for bioimaging. *J. Am. Chem. Soc* 2005;127:1656–1657. [PubMed: 15700997]
160. Wang S, Jarrett BR, Kauzlarich SM, Louie AY. Core/shell quantum dots with high relaxivity and photoluminescence for multimodality imaging. *J. Am. Chem. Soc* 2007;129:3848–3856. [PubMed: 17358058]
161. Gu HW, Zheng RK, Zhang XX, Xu B. Facile one-pot synthesis of bifunctional heterodimers of nanoparticles: A conjugate of quantum dot and magnetic nanoparticles. *J. Am. Chem. Soc* 2004;126:5664–5665. [PubMed: 15125648]
162. Gu HW, Yang ZM, Gao JH, Chang CK, Xu B. Heterodimers of nanoparticles: Formation at a liquid-liquid interface and particle-specific surface modification by functional molecules. *J. Am. Chem. Soc* 2005;127:34–35. [PubMed: 15631435]
163. Selvan ST, Patra PK, Ang CY, Ying JY. Synthesis of silica-coated semiconductor and magnetic quantum dots and their use in the imaging of live cells. *Angew. Chem., Int. Ed* 2007;46:2448–2452.
164. Manabe N, Hoshino A, Liang YQ, Goto T, Kato N, Yamamoto K. Quantum dot as a drug tracer in vivo. *IEEE T. Nanobiosci* 2006;5:263–267.
165. Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, Langer R, Farokhzad OC. Quantum dot - aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. *Nano Lett* 2007;7:3065–3070. [PubMed: 17854227]
166. Chen AA, Derfus AM, Khetani SR, Bhatia SN. Quantum dots to monitor RNAi delivery and improve gene silencing. *Nucleic Acids Res* 2005;33:e190. [PubMed: 16352864]
167. Tan WB, Jiang S, Zhang Y. Quantum-dot based nanoparticles for targeted silencing of HER2/neu gene via RNA interference. *Biomaterials* 2007;28:1565–1571. [PubMed: 17161865]
168. Kim DH, Rossi JJ. Strategies for silencing human disease using RNA interference. *Nat. Rev. Genet* 2007;8:173–184. [PubMed: 17304245]
169. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. *Nat. Rev. Mol. Cell Biol* 2007;8:23–36. [PubMed: 17183358]
170. Bumcrot D, Manoharan M, Koteliensky V, Sah DWY. RNAi therapeutics: a potential new class of pharmaceutical drugs. *Nat. Chem. Biol* 2006;2:711–719. [PubMed: 17108989]
171. Meister G, Tuschl T. Mechanisms of gene silencing by double-stranded RNA. *Nature* 2004;431:343–349. [PubMed: 15372041]
172. Dykxhoorn DM, Lieberman J. The silent revolution: RNA interference as basic biology, research tool, and therapeutic. *Annu. Rev. Med* 2005;56:401–423. [PubMed: 15660519]
173. Derfus AM, Chen AA, Min DH, Ruoslahti E, Bhatia SN. Targeted quantum dot conjugates for siRNA delivery. *Bioconjugate Chem* 2007;18:1391–1396.
174. Gao XH, Yezhelyev MV, Qi L, O'Regan RM, Nie SM. Proton-sponge-coated quantum dots for siRNA delivery and imaging. *Nano Lett.* 2008xx-xx

Figure 1.

Size-dependent optical properties of cadmium selenide QDs dispersed in chloroform, illustrating quantum confinement and size tunable fluorescence emission. (a) Fluorescence image of four vials of monodisperse QDs with sizes ranging from 2.2 nm to 7.3 nm in diameter. This image was obtained with ultraviolet illumination. (b) Fluorescence spectra of the same four QD samples. Narrow emission bands (23–26 nm FWHM or full-width at half-maximum) indicate narrow particle size distributions. (c) Absorption spectra of the same four QD samples. Notice that the absorption spectra are very broad, allowing a broad wavelength range for excitation. Both the absorption and emission intensities are plotted in arbitrary units (AU).

Figure 2.
Schematic diagrams of nonfunctionalized and bioconjugated QD probes for imaging and sensing applications. See text for detailed discussion.

Figure 3.

Encapsulation and solubilization of core-shell CdSe/CdS/ZnS quantum dots by using multivalent and hyperbranched copolymer ligands. (a) and (b) Chemical structures of PEI and 19 PEI-g-PEG copolymers consisting of two or four PEG chains per PEI polymer molecule. (c) Schematic diagram showing direct exchange reactions between the monovalent capping ligand octadecylamine and the multivalent copolymer ligands. Adapted with permission from Duan and Nie [97].

Figure 4. Schematic diagram showing QD interactions with blood immune cells and plasma proteins. The probable modes of interactions include (a) QD opsonization and phagocytosis by leucocytes (e.g., monocytes), (b) non-specific QD-cell membrane interactions (electrostatic or hydrophobic), and (c) fluid-phase pinocytosis.

Figure 5. Schematic diagram showing QDs involved in both active and passive tumor targeting. In the passive mode, nanometer-sized particles such as quantum dots accumulate at tumor sites through an enhanced permeability and retention (EPR) effect [126–129]. For active tumor targeting, nanoparticles are conjugated to molecular ligands such as antibodies and peptides to recognize protein targets that are over-expressed on the surface of tumor cells such as the epidermal growth factor receptor (EGFR), the transferrin receptor, or the folate receptor. Courtesy of Dr. Ximei Qian, Emory University, Atlanta, GA 30322, USA.

Figure 6. Schematic illustration of QD-Aptamer-Dox FRET system and its targeted delivery through receptor-mediated endocytosis. (a) QDs-aptamer conjugates (QD-Apt) are fluorescent until they are mixed with the fluorescent drug doxorubicin (Dox), which intercalates with the base pairs of the aptamer and quenches the QD via FRET. (b) Aptamer-specific endocytosis results in cellular internalization of the conjugate, release of cytotoxic Dox, and restoration of fluorescence. Adapted with permission from Bagalkof *et al.* [165].