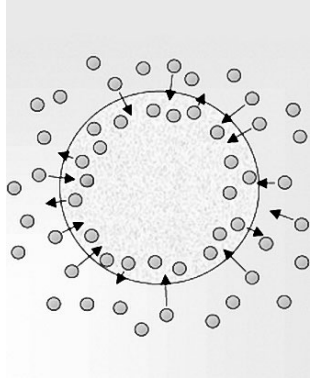
DOI: 10.1002/adma.200500786

Quantum Dots in Biological and Biomedical Research: Recent Progress and Present Challenges

By *Jesse M. Klostranec* and *Warren C. W. Chan**



The marriage of nanomaterials with biology has produced a new generation of technologies that can profoundly impact biological and biomedical research. Quantum dots (Qdots) are an archetype for this hybrid research area and have gained popularity and interest from diverse research communities because of their unique and tunable optical properties. In this Review, we will describe their history and development, optical and electronic properties, and applications in biology and medicine. A critical evaluation of barriers impacting current Qdot technologies will be discussed and insights into the future outlook of the field will be explored.

1. Introduction

Recent advances in the integration of colloidal nanostructures with biology and medicine have created tremendous excitement.[1–4] Figure 1 shows an exponential increase in the number of published reports on the use of nanostructures in biology during the last ten years; data was obtained using Medline. One major reason for this trend is the rapid progress

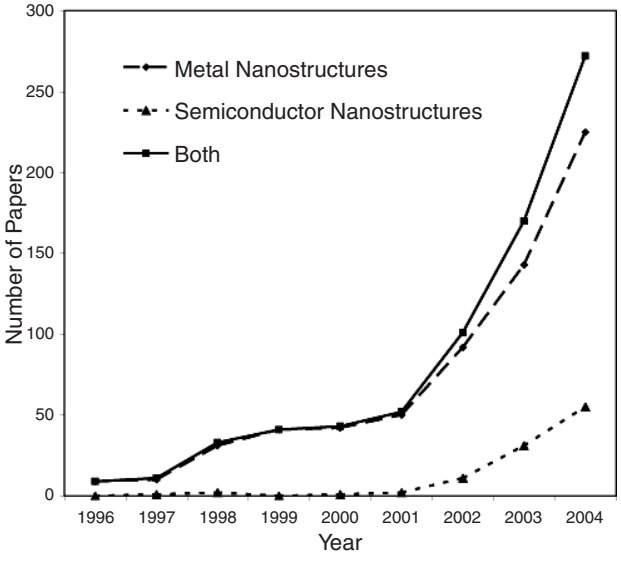


Figure 1. Number of published manuscripts betweeen 1996 and 2004 that report the use of nanostructures (specifically, Qdots and metallic) in biological applications. The data was compiled using the Medline database. In all searches, a manuscript had to contain the nanostructure or material term: semiconductor, quantum dots, metallic, magnetic, iron oxide, gold, gold colloids, silver or metal colloids; and the biology or biomedical term: biology, biological application, diagnostics, therapy, cell, DNA, or protein. A research manuscript was only counted if it met the following criteria: i) use of semiconductor or metallic nanoparticles, and ii) report or mention of biological or medical applications.

[*] Dr. W. C. W. Chan, J. M. Klostranec
Institute of Biomaterials and Biomedical Engineering
University of Toronto, Rosebrugh Building
164 College Street, M5S 3G9 (Canada)
E-mail: warren.chan@utoronto.ca
Dr. W. C. W. Chan, J. M. Klostranec
The Terrence Donnelly Centre for Cellular and Biomolecular Research
University of Toronto
160 College St., M5S 3E1 (Canada)
Dr. W. C. W. Chan
Department of Materials Science and Engineering
University of Toronto, Mining Building
170 College St., M5S 3E1 (Canada)

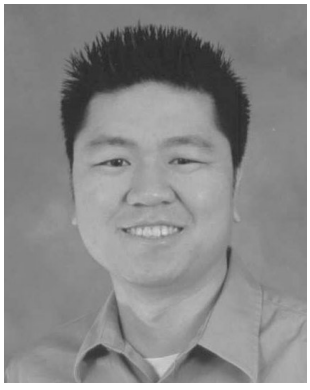
[**] W.C.W.C. acknowledges CIHR (Regenerative Medicine and Nanomedicine and Novel Tools Grant), CFI (New Opportunities and Infrastructure Operating Fund), OIT (New Opportunities), NSERC (Discovery Grant), Connaught Foundation, and University of Toronto (Start-up Fund) for research support. J.M.K. would like to acknowledge the NSERC for a graduate student fellowship.

made by physical scientists in the development of synthetic protocols for manipulating and characterizing nanostructure size, shape, and composition, and another is the development of chemistry to interface these nanostructures with biology.[5–17] Nanostructures have now been exploited in biosensing, cell labeling, animal imaging, and therapy. This hybrid field has now been termed bionanotechnology, nanobiotechnology, or nanomedicine.

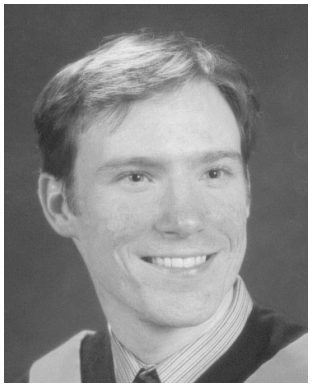
In this Review, we describe the advancements in quantum dots (Qdots) for biological research. Qdots are defined as semiconductor structures with physical dimensions that are smaller than the exciton Bohr radius (an exciton is an electron–hole pair).[17–19] Usually, this occurs when the size of the semiconductor is below 100 nm; within this size range, these nanometer-sized structures have tunable properties. This gives biomedical researchers a large set of precursors for building tools to address important questions and to diagnose and treat diseases. The story of Qdots—from development in chemistry and physics laboratories to applications in biology—will be described. This story provides an excellent glimpse of how and why nanostructures are important tools for biomedical research. Specifically, this Review describes the recent progress in Qdot design and applications, but we also detail the major barriers that currently prevent Qdots from mainstream biomedical use. We refer the interested reader to several excellent review articles on Qdots for biology, which go into greater detail than this Review in certain aspects of this technology.[3, 20–24]

2. Quantum-Dot Synthesis and Surface Modification for Biomedical Research

In the 1970s and early 1980s, understanding the photophysical properties of semiconductor structures was important for a broad range of computer and electronic applications. It was theorized that the physical properties of structures in an intermediate size range (between single atoms and bulk) could be tuned by alteration of size and shape.[25–28] Since those inaugural manuscripts, research in the 1980s focused on the synthesis of semiconductor nanostructures, which are now called quantum dots (Qdots). These Qdots are zero-dimensional electron systems with properties that are dependent on the spatial confinement of the electrons. For electronics and computer applications, such a system allows an engineer to synthesize a large set of nanometer-sized building blocks for constructing faster and smaller computer chips or more efficient light-emitting devices. The first methods for synthesizing colloidal Qdots were conducted by Henglein[27] and Rossetti et al.;[28] CdS Qdots were formed by mixing cadmium and sulfide salts in an aqueous buffer. Researchers in the late 1980s and early 1990s attempted to improve the overall optical qualities of these Qdots (e.g., improving particle monodispersity and size tunability) by investigating the effect of reaction conditions (e.g., solvents, salts, pH, temperature) on their morphology and optical properties.[29–31] These early fundamental research findings provided the guide for the current state of the art in Qdot



Warren C. W. Chan received his B.S. degree in 1996 from the University of Illinois (Urbana-Champaign, IL, USA) and Ph.D. degree in 2001 from Indiana University (Bloomington, IN, USA). He conducted post-doctoral training at the University of California (San Diego, CA, USA). Currently, he is an Assistant Professor at the University of Toronto in the Institute of Biomaterials and Biomedical Engineering. He is also affiliated with the Department of Materials Science and Engineering and the Terrence Donnelly Center for Cellular and Biomolecular Research. His research interest is in the development of nano- and microtechnology for cancer and infectious-disease diagnosis. In his short career, his publications have been cited over 1000 times. He has received the BF Goodrich Young Inventors Award and Lord Rank Prize Fund award in Optoelectronics.



Jesse M. Klostranec received his B.Eng. degree in Aerospace Engineering from Carleton University (Ottawa, ON, Canada) in 2004, specializing in aerodynamics. He is presently a Ph.D. candidate in Professor Chan's lab in the Institute of Biomaterials and Biomedical Engineering at the University of Toronto and is affiliated with the Terrence Donnelly Center for Cellular and Biomolecular Research. He is currently on a Natural Sciences and Engineering Research Council (NSERC) fellowship. The emphasis of his research is integrating micro- and nanotechnologies for medical diagnostics.

synthesis, an organometallic synthetic procedure developed by Murray et al.[6] However, the "Greener" method, which uses metallic salts and organic stabilizing agents, is starting to gain popularity.[32–34] Currently, these are the only two synthetic procedures (or minor variations of these two methods) to produce Qdots with the desired optical properties (i.e., high quantum yield, narrow fluorescence emission, broad absorption profile, and stability against photobleaching) for biomedical research and applications.

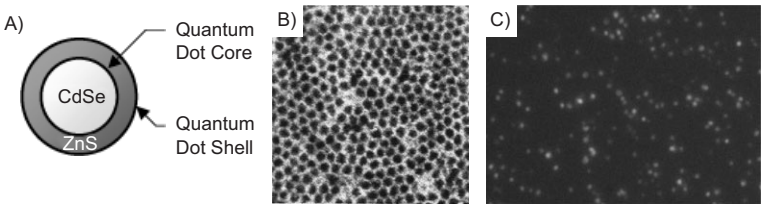


Figure 2. Schematic, transmission electron microscopy, and optical fluorescence microscopy images of the most commonly used Qdot (ZnS-capped CdSe) in biomedical research and technology. A) Schematic depicting a core Qdot (CdSe) coated with a thin inorganic shell (ZnS). B) Transmission electron microscopy image of 4.0 nm core/shell Qdots (reprinted with permission from [8], Copyright 1996, American Chemical Society). C) Fluorescence microscopy image of single Qdots.

For organometallic CdSe Qdot synthesis, dimethylcadmium and selenium are initially dissolved in the organic solvent tri-*n*-octylphosphine at defined ratios (usually at a molar ratio of 1.4:1.0) and injected in a hot coordinating solvent of tri-*n*-octylphosphine oxide (TOPO; 350 °C) under Ar gas. Nucleation and growth of CdSe Qdots are observed after injection and lowering of the temperature from 350 to 300 °C by a change in the color of the solution (from clear to light yellow to orange to red). Since the growth of Qdots is dependent upon the process of Ostwald ripening,[35,36] a specific size of Qdots can be obtained by significant reduction in the reaction temperature. For example, the rapid lowering of the temperature (e.g., from 300 to 200 °C) prevents the Qdots from growing further and allows one to isolate the 4.0 nm Qdots.

To use Qdots in biology, it is extremely important to passivate or cap the CdSe Qdots with a layer of ZnS or CdS. The ZnS or CdS improves the fluorescence quantum yield of the Qdots and protects them against photo-oxidation (which is important for minimizing cytotoxicity and for enhancing photostability).[8,9,37–40] To produce a ZnS capping layer, a solution of dimethylzinc and hexamethyldisilathiane (in tri-*n*-octylphosphine) can be slowly dripped into the reaction vessel after isolating or obtaining CdSe Qdots of a desired size. The low temperature and slow drip rate prevents the nucleation of ZnS Qdots. The thickness of the ZnS shell is mediated by the amount of dimethylzinc and hexamethyldisilathiane injected into the reaction vessel. The ZnS shell has a larger bandgap energy than CdSe, eliminating the core's surface defect states. Also, the ZnS shell has a similar bond length to the CdSe, minimizing crystal-lattice strain and allowing for epitaxial growth. We show a schematic of CdSe/ZnS-capped core/shell Qdots in Figure 2, along with their transmission electron and optical fluorescence microscopy images.

Even with advances in synthesis, obtaining biomedically useful Qdots is still an art; each synthetic batch of Qdots may yield different optical qualities. From one synthesis to the next, a researcher may produce Qdots with different quantum yields and fluorescence spectra. As a result, many research groups are continuing to investigate the effects of organic coordinating agents (e.g., phosopholipids and aminoalkanes) and temperature on improving the reproducibility of Qdot synthesis.[6,37,41–43] Even microfluidic technology has been employed for synthesizing Qdots; it has been suggested that microfluidic technology will permit precise manipulation of coordinating solvents, precursor reagent concentrations, and temperature.[44,45]

So what other improvements in Qdot synthesis are still required? One major Qdot synthetic research goal is to prepare large amounts of Qdots (>1 g) with a high quantum yield (100 %) and a narrow fluorescence full-width at half maximum (<30 nm). Also, an effort toward preparing alloyed Qdots, where the optical properties of the Qdots are tuned by composition rather than size or shape, and doped Qdots, where the Qdots have multiple properties, for example, optical and magnetic, are needed.[46–49]

Qdots synthesized by these methods are nonpolar and insoluble in aqueous solvents, and therefore, they are not compatible with biological systems. Qdots are hydrophobic after synthesis because of the coordinating agent. Hence, a polar Qdot surface has to be created before their use in biology. Several methods have been developed (see Fig. 3) but none of these techniques appear to be ideal. An ideal coating should A) prevent Qdots from flocculating during long-term storage, B) efficiently convert the organic-soluble Qdots to water-soluble, C) maintain the Qdot fluorescence quantum yield, and D) maintain the sub-10 nm Qdot size. The current coating strategies do not fulfill all of these requirements.

Current coating strategies are mediated either by chemical exchange or hydrophobic–hydrophobic interaction. Alivisatos and Nie and their co-workers were the first groups to use chemical exchange for modifying the surface chemistry of Qdots.[14,15] In the chemical-exchange method, a bifunctional molecule, such as mercaptoacetic acid (MAA), competes with TOPO (or another organic stabilizer) for binding to a metal atom on the Qdot surface. With excess bifunctional molecules in solution, the thiol functional groups (from the MAA) outcompete the phosphonic oxides (from the TOPO) for binding onto the metal atoms. If the bifunctional molecules contain a polar functional group that is opposite to the thiol functional group, the Qdots become highly polar and soluble in aqueous solvents. Unfortunately, the disadvantages of this technique are rapid flocculation and decrease in fluorescence quantum yield of the Qdots. Libchaber and Wu and their co-workers were the first groups to use the hydrophobic–hydrophobic interactions to water-solubilize Qdots.[50,51] With this method,

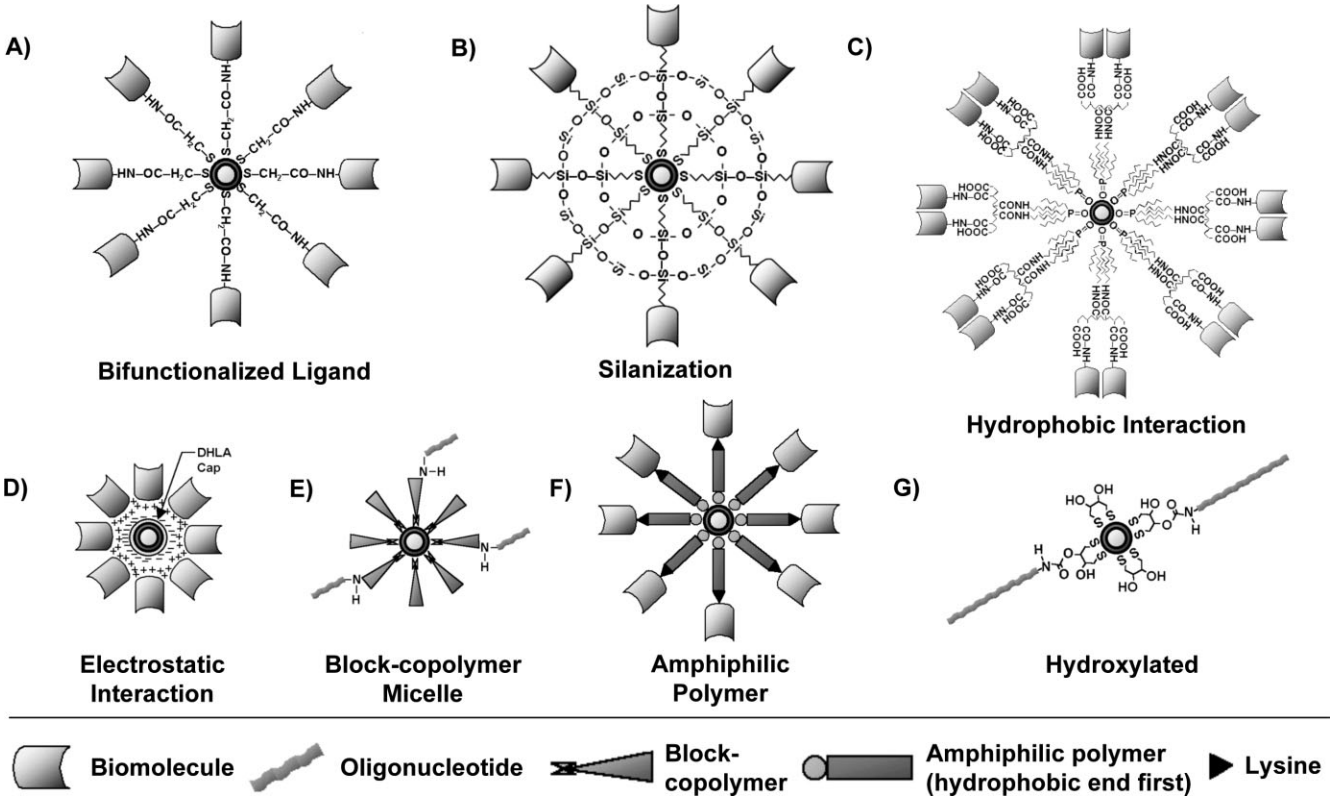


Figure 3. Schematic of current Qdot surface coatings that permit Qdots to interface with biological systems and biorecognition molecules. We call these surface coatings: A) bifunctionalization, B) silanization, C) hydrophobic–hydrophobic interaction, D) electrostatic interaction, E) micelle encapsulation, F) amphiphilic polymer, G) hydroxylation. In the schematic, the thicknesses of the surface coatings with respect to the size of Qdots are not drawn to scale.

an amphiphilic molecule, such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(poly(ethylene glycol))-2000], interacts with a TOPO molecule through hydrophobic–hydrophobic interactions on the Qdot surface. The amphiphilic molecule can then be crosslinked to prevent desorption from the surface. The Qdots become polar because of the protruding polar functional groups. Disadvantages of this technique are the procedural complexity of the method, the high cost of coating reagents, and an increase in the overall size of the Qdots after coating.

For most biomedical applications, a mechanism to attach biorecognition molecules (e.g., oligonucleotides, antibodies, or peptides) onto the Qdot surface is needed. The additional purpose of the coating molecule on the Qdots surface (besides improving the polarity) is to provide organic functional groups (–COOH, NH_3) for conjugation to biorecognition molecules. These groups permit the linkage of biorecognition molecules with Qdots via the popular EDC (1-ethyl-3-(3-dimethylamino propyl)carbodiimide) assisted crosslinking method.[15,51] In this reaction, the carboxylic acid functional groups on the surface of the Qdots can covalently couple to primary amino groups on proteins or oligonucleotides. An amide bond joins these two entities. A drawback of this technique is that EDC-mediated conjugation can lead to aggregation of Qdots if the Qdot-to-protein-to-EDC concentrations are not optimized. As a result, several groups have developed other strategies to coat Qdots with biorecognition molecules. Akerman et al. used a chemical exchange method, where MAA on Qdot surfaces is exchanged with thiolated peptides via chemical equilibria.[52] Mattoussi et al. used adaptor amino acid sequences (via electrostatic interactions) to link proteins to the Qdot surfaces.[53,54] Furthermore, Qdot companies sell streptavidin-coated Qdots, where a streptavidin–biotin interaction mediates the linkage between Qdots and biorecognition molecules. The optical properties and size should be monitored at each step, from the modification of the Qdot surface coating to the coupling of biorecognition molecules onto the Qdots. The inability to characterize these two factors can have a significant impact on their use in biomedical experiments and their results.

3. Optical Properties of Qdots

The optical properties of Qdots can be described by conventional semiconductor physics and quantum mechanics. In semiconductor systems, energy bands called valence and conduction bands exist; the energy difference between the two

bands is called the bandgap energy. When a semiconductor is optically or electrically excited, static electrons (electrons located in the valence band) become mobile (electrons located in the conduction band) within the semiconductor matrix. When it becomes mobile, an electron leaves behind a hole and after a certain period of time (called the lifetime, ≈20 ns), the electrons and holes recombine. In some cases, the electron–hole recombination causes the Qdots to emit photons at a rate of 8×10^6 photons per second (assuming a quantum yield of 30 %). The amount of energy required to induce the electrons to become mobile (and in effect, produce Qdot fluorescence emission) is dependent upon the bandgap energy; bandgap energy is related to the Qdot's size, shape, and composition. This dependence is due to the quantum confinement effect and will only occur when the size of the nanostructure is on the order of the exciton Bohr radius.[55,56] Figure 4 shows several vials of different-sized Qdots and their fluorescence emission upon excitation with a hand-held UV lamp. The tunable emission is attractive to many biomedical researchers since fluorescence is commonly used in cell, tissue, and animal experiments.

Other biomedically attractive properties of Qdots include their continuous absorption profile (this allows different Qdot emissions to be excited simultaneously using a single wavelength), 20 ns fluorescence lifetime (this permits Qdots to be used in time-resolved fluorescence bioimaging[57]), stability against photobleaching[58] (this permits Qdots to be used in monitoring biological events, such as protein tracking), large Stokes shift (this prevents spectral cross talk, enhancing the detection signal), and their inherent brightness (this permits a single Qdot to be imaged). Table 1 compares the optical properties of Qdots with organic fluorophores. Since there are already several excellent review articles that provide full descriptions of these optical properties, we refer the interested reader to these articles.[17–19,59–62]

Alivisatos, Nie, and their co-workers convincingly described many optical advantages of Qdots over organic fluorophores for biomedical research.[14,15,63] At the time, the impact of biological environments and coating molecules on Qdot optical properties was not described or even perceived. Hines and Guyot-Sionnest reported that environment did not appear to impact ZnS-capped CdSe Qdot fluorescence.[8] However, biological environments are far more complicated than organic solvents; hence, some of the complications of using Qdots in biology is the limited understanding of how surface-coating molecules, biorecognition molecules, salt concentrations, pH, and temperature affect the overall optical and electronic properties of Qdots. These conditions can impact the fluorescence signal of the Qdots. For example, we have observed Qdots becoming brighter after surface modification in standard room conditions.[64] Silver and Ou reported the fluorescence of Qdots in endocytic cellular vesicles became markedly brighter after photoactivation, followed by slow photobleaching.[65] For many biomedical experiments, the ability to predict changes in the optical property of a probe in different biological conditions is important. The optical emissions of probes are used for detection of biological events or for quantitive analysis, and unexplained fluctuations (that cannot be standardized) can lead to false interpretation of results.

Finally, we want to mention the intermittent blinking behavior of Qdots.[66–68] It has been suggested that the blinking behavior of Qdots may impair their use in biodetection and single-molecule tracking studies because the camera will not detect the Qdot signal when they are in a dark state (or off state). The off signal of Qdots can range from 1 ms to 1 s. For bulk measurements, this may not be a problem since camera acquisition times could be longer than the Qdot off time. There might be a problem for single-molecule studies if the biological event occurs at a faster rate than the blinking off time. However, many biological events (e.g., receptor–ligand complexation) are longer than 1 s.[69] Dahan et al. did not report any

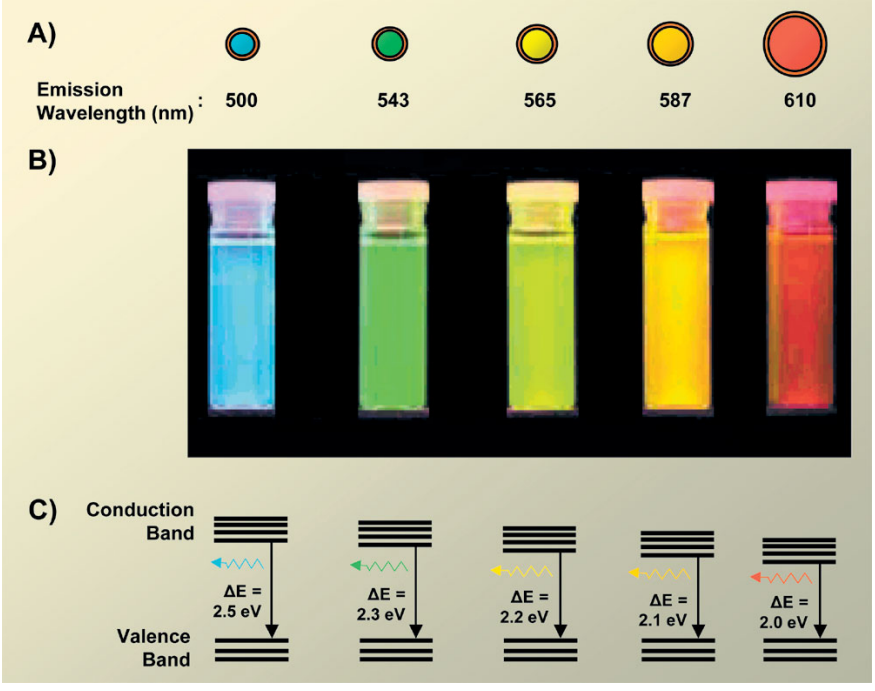


Figure 4. Tunable optical properties of Qdots. A) Schematic of the size-dependent optical properties of Qdots (not drawn to scale). As the Qdot becomes larger, the optical emission shifts from blue to red. The size range of the Qdots is 2–10 nm. B) Corresponding to (A), real-color emission of vials filled with different-sized Qdots suspended in chloroform, excited by a hand-held UV lamp (adapted from [80] with permission, copyright 2001, Nature Publishing Group). C) The corresponding bandgap energies for the Qdots shown in (A) and (B).

Table 1. Comparison of the typical optical properties of green-emitting CdSe/ZnS Qdots, the organic fluorophore rhodamine 6G, and the green fluorescent protein (S65T mutant). Data was compiled from the following references: [6, 15, 34, 37, 57, 119–129].

Properties	Green-emitting Qdots	Rhodamine 6G organic fluorophore	Green fluorescent protein
Emission wavelength [nm]	550	555	509
Full-width at half maximum [nm]	20–40	40–55	35–45
Absorbance profile [nm]	<520	530	487
Quantum yield [%]	20–65	50–90	68
Molar absorptivity [$M^{-1}cm^{-1}$]	10 000–100 000	80 000	39 200
Fluorescence lifetime [ns]	15–20	1–5	3
Other properties	Tuneable emission Photostable Large surface area Continuous absorbance profile	Small Spectral tail	Tuneable emission Genetic incorporation Spectral tail

problems with imaging the kinetic activities of single glycine receptors (labelled with Qdots) in the membrane of live neurons.[70]

4. In Vitro Applications of Qdots

We will now describe some of the challenges that must be overcome before Qdots advance toward mainstream biological applications. Qdots were initially considered as potential optical probes to replace organic fluorophores for biological applications because of their advantageous properties. However, due to some limitations (e.g., size of particles after coating, difficulty in working with them), many researchers in the field would conclude that after seven years, Qdots are more likely to complement existing organic fluorophores in many applications rather than replace them. Nevertheless, a diverse range of reported biological applications of Qdots have been demonstrated. Some of them are depicted in Figure 5. Thus far, researchers have demonstrated the use of Qdots for cell labelling,[51,58,71–73] tracking cell migration,[74,75] flow cytometry,[76–78] fluorescence in situ hybridization,[74,79] whole-animal contrast agents,[1,80,81] pathogen detection,[82,83] genomic and proteomic detection,[84,85] fluorescence resonance energy transfer (FRET) sensors,[86,87] and high-throughput screening of bio-

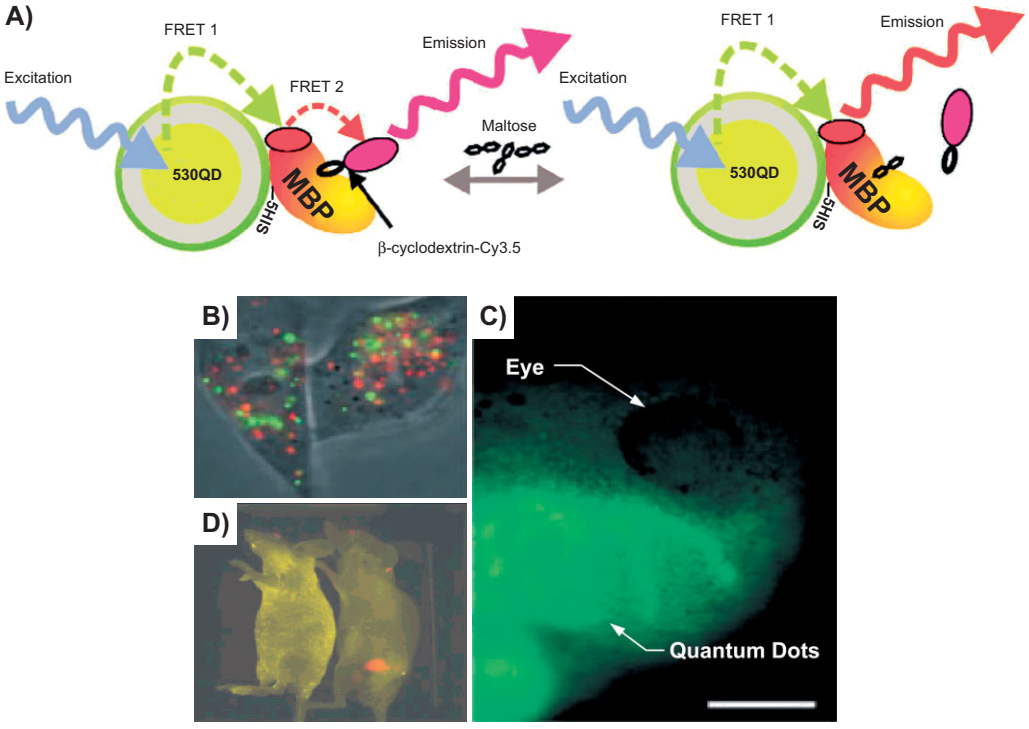


Figure 5. Examples of in vitro and in vivo applications of Qdot probes. A) Example of the design of a fluorescence resonance energy transfer (FRET) sensor. Shown on the left is a 530 Qdot-MBP-Cy3-β-CD-Cy3.5 maltose sensor. Addition of more maltose releases many *β*-CD-Cy3.5 from the complex, increasing Cy3 emission. Abbreviations: MBP: maltose binding protein; β-CD: β-cyclodextrin (reproduced from [86] with permission, copyright 2003, Nature Publishing Group). B) Multicolor staining of HeLa cell with red-light-emitting EGF-Qdots and green-light-emitting Qdots (reproduced from [72]). C) In vivo labeling of a *Xenopus* embryo with green-micelle-coated Qdots, showing the migration pattern of neural crest cells; scale bar is 0.5 mm (reproduced from [50] with permission, copyright 2002, American Association for the Advancement of Science). D) Image of Qdots targeting prostate cancer in vivo in a mouse bearing a xenograft tumor. The tumor was targeted using orange-red-emitting Qdot probes. A control (no tumor) mouse is shown on the left (reproduced from [81] with permission, copyright 2004, Nature Publishing Group).

molecules.[84,88] These demonstrated applications show the broad utility of Qdots.

In the near future, we envision Qdots will have a major impact in systems biology research. An important emphasis in biology is the elucidation and mapping of biomolecule networks that dictate the function and viability of a cell.[89–93] This will impact many research fields, from molecular diagnostics to tissue engineering to molecular biology. There has been a lack of available tools for analyzing biomolecules in real time and at the single-molecule level. Qdots and other nanostructures are expected to play an immense role in these studies; the main reason for this is Qdots are similar in size to proteins and have tunable emission. In living cells, Qdots can be designed to color-code the biomolecules of interest, and, because of their brightness and photostability, they can be imaged and monitored for long periods of time. The patterns of movement of Qdots can indicate biomolecule activity. Image analysis can show 1) families of molecules interacting with one another, 2) the duration of binding interactions, and 3) rates of molecular assemblies.

Combining Qdots with discoveries from systems biology research can also lead to improvements in molecular diagnostics. The detection of multiple molecules (markers) in serum, cells, or tissues by Qdot color emission can improve diagnostic efficiency since interpretation of a diagnosis will be based on multiple targets. The current state of the art in molecular diagnostics relies only on the detection of a single protein or gene, which can lead to false positive diagnosis.[94,95] However, for cell- and tissue-imaging applications, only three-to-four Qdot colors for protein labeling of cells have been demonstrated in peer-reviewed publications. In contrast, up to nine organic fluorophores have been used for labelling cells (although this requires a special optical setup for analysis).[96] Why?

What are the major challenges of using Qdots for cell biology? There are several limitations associated with using Qdots. These include 1) lack of availability of Qdots (only recently have more than five colors of Qdots been made commercially available), 2) variability in surface chemistry that has prevented effective coating with biorecognition molecules, 3) lack of fundamental research on the optical properties of Qdots in biological environments, 4) nonspecific binding that may reduce the detection sensitivity of Qdots, and 5) instrumentation limitation (detectors have optimum detection at specific wavelength regions).

The first issue has already been addressed; multiple companies are currently selling Qdots. As of June 2006, at least eight different colored, biocompatible Qdots are commercially available. However, the fluorescence full-width at half maximum and quantum yields appear to vary from one color emission to another (and this may be a limitation for multicolor applications of Qdots). Many researchers are addressing the second issue. The current coatings are far from ideal for using Qdots for cellular imaging. Current coatings are large (can add >10 nm), adding bulk to the Qdot; this may interfere with the binding of Qdots to biomolecules and may also impact the overall detection signal (in the event of labeling proteins being in close proximity to each other). The third issue has already been described in Section 2. The fourth issue is a major drawback because nonspecific binding of Qdots to surfaces can limit detection sensitivity and, more importantly, lead to false signals. The degree of nonspecific binding appears to vary from surface coating to surface coating and cell type to cell type. Currently, the answer to preventing Qdot nonspecific binding is to coat poly(ethylene glycol) (PEG) onto the Qdots surface;[52] PEG has been a popular polymer in the pharmaceutical industry for reducing nonspecific uptake of drugs and drug-delivery systems.[97] Since PEG can degrade under long-term storage, it may not be the final answer to address all of the nonspecific binding requirements, and therefore, further research efforts will be needed. The fifth and final issue is also another important problem. With the advancement of Qdot probes, the design of specialized instrumentation for optimal Qdot detection has been lacking. The variable quantum efficiency of charge-coupled device (CCD) cameras in a broad emission range does not provide optimal optical-signal sensitivity for Qdots with different emission colors (i.e., the signal may be higher for green-emitting in comparison to red-emitting Qdots because of the quantum efficiency of the camera, even if the concentrations of green- and red-emitting Qdots are the same on or in a cell). Another issue may be the optical excitation; if Qdots of different emission are excited with the same wavelength, the molar-absorptivity value is different between the two different colors (and therefore, this will affect the overall fluorescence emission intensity). Lastly, we want to mention that cytotoxicity does not appear to be a major issue for using Qdots for long-term cell-tracking studies. Derfus et al.[39] and Kirchner et al.[40] showed that coating chemistry could prevent cytotoxicity in culture. Jaiswal et al. and Derfus et al. have shown the use of Qdots for long-term tracking of cells in culture.[58,72] For in vivo applications, toxicity will be more important; we will discuss this further in section 5. If Qdots are to fulfill the promise of multicolor labelling at the single-molecule level to study biomolecular events in living cells and enhance diagnosis of diseases, these issues must be addressed.

Some of these issues are also pertinent to using Qdots in biosensor applications. Some of the first-reported biosensor systems involved FRET,[86,87] where Qdots acted as donors and organic fluorophores acted as acceptors (see Fig. 5). In FRET, biomolecular sensing or detection occurs when the Qdot-donor emission signal decreases while the organic-fluorophore emission signal enhances. These FRET experiments have only been demonstrated in solution. For these homogeneous assays, understanding how different surface molecules affect their fluorescence emission is vital to their use. Signal variations (that indicates detection) must be derived from detecting the molecule of interest and not from other factors.

Biologists commonly analyze complex samples of protein, DNA, and carbohydrates using heterogeneous or surface-based assays. Examples include dot-blotting and enzyme-linked immunoassays. Multiplexed analysis, where multiple molecules are detected simultaneously, is extremely important

since it can provide rapid detection and analysis. An example is DNA and protein microarrays, where thousands of biomolecules are detected within one day of analysis. For Qdots, exploitation of the multicolor emission may one day permit their use in the same fashion as the microarrays. Surface-based multicolor detection using different emitting Qdots has been demonstrated for detection of toxins. Goldman et al. used five different-colored Qdots to detect five different toxins simultaneously.[85] Design of Qdot optical barcodes have also been demonstrated for multiplex analysis. Nie and co-workers placed different colored Qdots inside polystyrene beads to make barcodes for rapid detection (having similar analytical capability to the microarray).[84] A depiction of such microbeads is given in Figure 6. However, with surface-based techniques, nonspecific-binding effects can be a major problem since they can influence the noise of the detection scheme, decreasing the signal-to-noise (SNR) ratio.

5. In Vivo Applications of QDots

A new and exciting avenue of research for Qdots is their application as a contrast agent for in vivo imaging. Organic fluorophores and chemiluminescence probes are currently the most commonly used optical probes for animal imaging.[91,98,99] However, a limitation of optical contrast agents is the lack of available probes that emit in the near-IR (NIR) emission range (>650 nm). The NIR emitting window is appealing for biological optical imaging because of the low tissue absorption and scattering effects in this emission range.[100,101] The bounds of the NIR optical window for animal imaging are typically set at 650–900 nm (Fig. 7). Recently, Frangioni and co-workers showed other NIR emission windows (1025–1150 nm, 1225–1370 nm, and 1610–1710 nm) for maximum in vivo optical imaging.[102] A limited amount of organic fluorophores emitting in the NIR are commercially

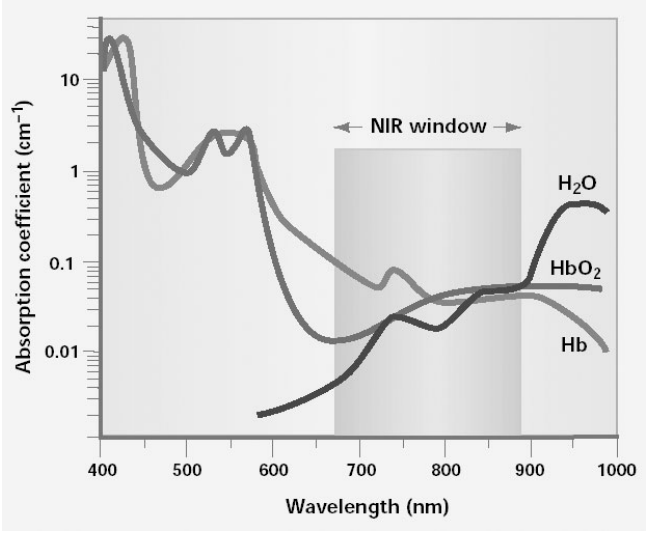


Figure 7. Near-IR (NIR) in vivo imaging window. For in vivo imaging, the ideal optical probe will require in vivo excitation and emission. In the NIR region, the absorbance spectra of interfering biological molecules (e.g., water and hemoglobin) are minimal compared to the visible region. Design of high-quality NIR-emitting Qdots may yield novel contrast agents for in vivo imaging and detection (adapted from [100] with permission, copyright 2001, Nature Publishing Group).

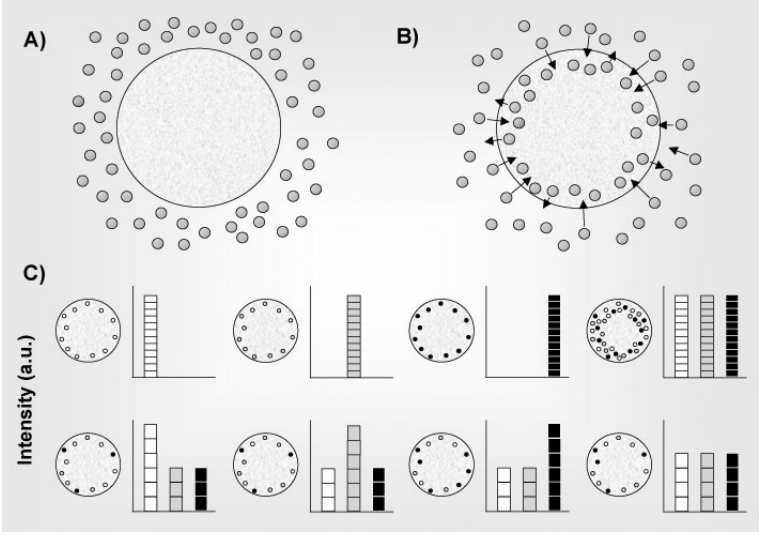


Figure 6. The incorporation of Qdots in high-throughput molecular screening. Qdot spectral signatures are encoded into microbeads for the design of optical molecular barcodes. A) A blank polystyrene bead is placed in solution with Qdots. B) In an equilibrating process, Qdots diffuse into the beads and are stabilized inside the beads by hydrophobic–hydrophobic interactions. C) By varying the number and colors of Qdots encoding the microbeads, a set of spectrally distinct probes can be produced that can identify surface-recognition molecules.

available, the most popular ones being the cyanine-dye series. Since the optical properties of Qdots can be tuned by size and composition, it should be possible to prepare a series of NIR-emitting Qdots for animal imaging. CdTe, CdTeSe, InPAs, PbS, and PbSe have been successfully synthesized with NIR emission.[46,47,80,103] Only the CdTe, CdTeSe, and InPAs Qdots have been demonstrated for animal imaging.

Thus far, there are only a handful of manuscripts demonstrating Qdots as in vivo contrast agents. Akerman et al.[52] conjugated Qdots to peptides that can target tumors in tumor-bearing mice and they observed tumor-tissue sections stained with Qdots under epifluorescence microscopy. Gao et al.[81] demonstrated whole-animal imaging of prostate cancer in mice using Qdots conjugated to antibodies. Kim et al.[80] demonstrated the use of Qdots for sentinel-lymph-node mapping in pigs, which helps guide surgeons in the removal of tumor cells. Voura et al. tracked Qdot-labelled tumor cells as they extravasated into lung tissue.[104] Finally, Rao et al. showed the use of bioluminescence resonance energy transfer (similar to FRET)

to excite Qdots for in vivo imaging without the use of a laser excitation source.[105]

For advancing Qdots toward clinical in vivo applications, there are challenges ahead. Although these studies demonstrate the use of Qdots in vivo, several fundamental questions must be addressed: 1) What are the proper doses of Qdots for optimal in vivo imaging? 2) What are the kinetics of Qdots in vivo? 3) How to prevent uptake of Qdots by the reticuloendothelial cells? 4) Are the Qdots toxic to the animal? Dosing requirements have not been properly addressed. The reported Qdot doses have ranged from 1 nmol to upwards of 300 nmol. The dosing concentration will affect the overall signal of the Qdots fluorescing from the tumor. Nie and co-workers showed that a higher concentration of Qdots is required for detection of tumors when the mechanism of tissue accumulation is passive versus active diffusion.[81] Kinetic studies, the study of Qdot distribution in vivo with respect to Qdot accumulation in tumors, have not been thoroughly investigated. Thus far, images and videos of Qdots traversing the blood stream and accumulating in organs have been shown,[106] but there have been very few reports of quantitative measurements. Quantitative studies will permit one to determine the degree of nonspecific binding in vivo, the degree of uptake in diseased sites, the degree of metabolism and clearance of Qdots, and provide information that can assist in the identification of the proper dose for optimum detection. All of these parameters are important in advancing Qdots toward clinical applications. A study by Fischer et al. monitored CdSe/ZnS in vivo pharmacokinetics in Sprague-Dawley rats.[107] The experiments showed mercaptoundecanoic acid-coated and lysine-crosslinked Qdots and bovine serum albumin (BSA)-conjugated Qdots were both cleared from plasma and predominantly sequestered by the liver, with notable uptake also occurring in the spleen, kidneys, and bone marrow (organs that are part of the reticuloendothelial system, RES). In both cases, however, negligible clearance in the urine and feces was observed. Finally, to maximize the use of Qdots as contrast agents, it is important to design Qdots that can escape the cells of the reticuloendothelial system (RES). RES is a defensive mechanism of the body that uptakes nanostructures in vivo and most likely degrades them. If Qdots are trapped in the RES, they will not be able to reach their target site(s), and therefore, their utility as a contrast agent may be compromised. Akerman et al.[52] and Ballou et al.[106] demonstrated that coating Qdot surfaces with PEG could alleviate some of these problems but not completely. A deeper qualitative and quantitative understanding of Qdot surface chemistry, and their relationship to in vivo kinetics and accumulation, will be necessary.

The extent of cytotoxicity of Qdot probes in vivo still needs to be determined and is currently an open-ended question. Heavy metals have known toxicity and Qdots used for biological applications are currently composed of Cd, Zn, Hg, or Pb. Toxicity studies to date have only been done with in vitro culture models. Derfus et al.[39] showed that CdSe Qdots are toxic to primary hepatocyte cells, the main cells in the liver involved in metal detoxification, when illuminated with UV light or exposed to excess hydrogen peroxide. However, when the CdSe Qdots were coated with ZnS, which reduces Qdot photo-oxidation, they observed minimal cytotoxicity. It was concluded that UV light or hydrogen peroxide cause Qdots to photo-oxidize and to release metal ions into cells. The metal ions, in effect, cause cell death. Kirchner et al. extended this study and showed that a different coating chemistry can impact the degree of cytotoxic effects.[40] For example, conjugation of Qdots with the polymer PEG can increase the critical concentration of Qdots before cytotoxicity. Hence, many factors, such as surface chemistry, passivation coatings, water-solubilization coatings, and cell type, determine the cytotoxic effects of Qdots. Although these conclusions came from in vitro experiments, they may be important factors to consider when evaluating the in vivo whole-animal Qdot kinetics and their toxicity. Ballou et al.[106] qualitatively showed the Qdot kinetics are dependent upon surface coatings, but their data do not clearly indicate in vivo Qdot metabolism or clearance. A strong understanding of these mechanisms (in cells and in animals) will be required long before Qdots become useful for in vivo clinical applications.

6. Future Outlook

In the last ten years, Qdots have been extremely important to the field of bionanotechnology. Because of the unique and beautiful emission of Qdots, researchers had envisioned many applications of Qdots that are not possible using organic fluorophores. Along with Qdots, other nanostructures, such as metallic and carbon-based nanoparticles, were added to the mix as precursors that can accelerate both fundamental and applied biological research. As with any new field, problems arise, such as the fluctuations of the Qdots emission in different solvents, cytotoxicity, etc., that were not initially considered when demonstrating the application principle. In order to advance bionanotechnology, attention to studying the fundamental properties of nanostructures in biological systems is essential so that the results obtained from their use in biology are predictive and quantitative.

As Qdots begin to mature, there will be an increasing trend on converging Qdots with other nano- and biotechnologies. Qdots will only be one part of the bionanotechnology story. Novel properties of metallic nanostructures have been discovered (e.g., the fluorescence emission of gold nanoparticles when they are encapsulated in dendrimers,[108] the surface-enhanced Raman effect of molecules adsorbed on metallic surfaces,[109] their size-dependent uptake by cells,[110] and cooperative affinity for target biomolecules with increased packing density of targeting molecules on the metallic nanoparticle surface[111]). There is research emphasis on integration of these nanostructures to construct functional units that can, for example, detect and treat diseases.[112,113] The new "buzz" word for these structures is multifunctional nanostructures. Some recent reports have described how to assemble nanostructures and characterize the effect of integrating nanostructures on the individual nanostructure's optical and electronic

properties.[4,114–116] Eventually, the goal is to be able to assemble and program functions into a structure with dimensions similar to a standard virus (< 150 nm).

Other converging areas of research will be to incorporate nanostructures into analytical devices. Such devices would ideally have multiplexing capabilities (i.e., screening of large amounts of proteins and genes simultaneously) with single-molecule detection capabilities (the ultimate level of detection). Additionally, multimodal imaging probes and detection systems should be areas of increased interest. Already Qdots have been used as part of bimodal imaging probes, pairing their light microscopy capabilities with magnetic resonance imaging (MRI)[117] and electron microscopy (EM)[118] modalities. Areas of applications can be broad—from clinical detection of HIV, to screening of agricultural diseases, to tracking pharmaceutical agents in vivo.

7. Concluding Remarks

Based on recent activities in the field of bionanotechnology, there appears to be a general paradigm underlying bionanotechnology research. In the paradigm, researchers develop and characterize nanostructures, understand and manipulate their surface chemistry for biological applications, and demonstrate their ability to solve biological problems or integrate them into systems or devices for clinical diagnostics. Figure 8 shows a schematic of this sequence. The development and maturity of Qdots in the last twenty years is a good example of this paradigm. In this Review, we have summarized the recent progress of Qdots in biology and offered perspective on the needs of the Qdot field for it to advance toward everyday biological research and applications.

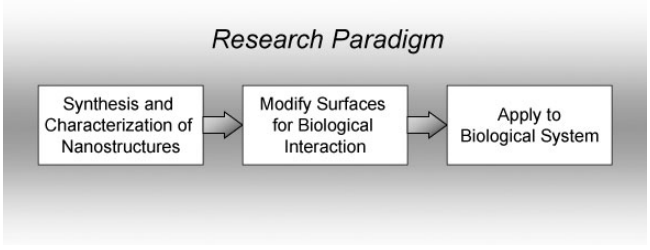


Figure 8. Research paradigm in bionanotechnology. The recipe for research projects in bionanotechnology is 1) the design and characterization of the nanostructures, 2) the integration of the nanostructures with biological systems, and 3) the use of the nanostructures for studying and applying to a specific biological or biomedical problem.

Received: April 18, 2005
Final version: May 24, 2006

[1] R. K. Jain, M. Stroh, *Nat. Biotechnol.* **2004**, *22*, 959.
[2] G. M. Whitesides, *Nat. Biotechnol.* **2003**, *21*, 1161.
[3] X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, S. Weiss, *Science* **2005**, *307*, 538.
[4] C. M. Niemeyer, *Angew. Chem. Int. Ed.* **2001**, *40*, 4128.
[5] J. W. G. Wildoer, L. C. Venema, A. G. Rinzler, R. E. Smalley, C. Dekker, *Nature* **1998**, *391*, 59.
[6] C. B. Murray, D. J. Norris, M. G. Bawendi, *J. Am. Chem. Soc.* **1993**, *115*, 8706.
[7] L. R. Becerra, C. B. Murray, R. G. Griffin, M. G. Bawendi, *J. Chem. Phys.* **1994**, *100*, 3297.
[8] M. A. Hines, P. Guyot-Sionnest, *J. Phys. Chem.* **1996**, *100*, 468.
[9] X. Peng, M. C. Schlamp, A. V. Kadavanich, A. P. Alivisatos, *J. Am. Chem. Soc.* **1997**, *119*, 7019.
[10] J. E. Bowen Katari, V. L. Colvin, A. P. Alivisatos, *J. Phys. Chem.* **1994**, *98*, 4109.
[11] D. A. Tomalia, M. Hall, D. Hedstrand, *J. Am. Chem. Soc.* **1987**, *109*, 1601.
[12] S. Iijima, *Nature* **1991**, *354*, 56.
[13] R. D. Averitt, D. Sarkar, N. J. Halas, *Phys. Rev. Lett.* **1997**, *78*, 4217.
[14] M. Bruchez, M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, *Science* **1998**, *281*, 2013.
[15] W. C. W. Chan, S. Nie, *Science* **1998**, *281*, 2016.
[16] R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078.
[17] A. Henglein, *Chem. Rev.* **1989**, *89*, 1861.
[18] A. P. Alivisatos, *Science* **1996**, *271*, 933.
[19] M. Nirmal, L. Brus, *Acc. Chem. Res.* **1999**, *32*, 407.
[20] A. P. Alivisatos, *Nat. Biotechnol.* **2004**, *22*, 47.
[21] J. K. Jaiswal, S. M. Simon, *Trends Cell Biol.* **2004**, *14*, 497.
[22] W. J. Parak, T. Pellegrino, C. Plank, *Nanotechnology* **2005**, *16*, R9.
[23] I. L. Medintz, H. T. Uyeda, E. R. Goldman, H. Mattoussi, *Nat. Mater.* **2005**, *4*, 435.
[24] A. J. Sutherland, *Curr. Opin. Solid State Mater. Sci.* **2002**, *6*, 365.
[25] A. I. Ekimov, A. A. Onushchenko, *JETP Lett.* **1981**, *34*, 345.
[26] A. L. Efros, A. L. Efros, *Sov. Phys.—Semicond.* **1982**, *16*, 772.
[27] A. Henglein, *J. Phys. Chem.* **1982**, *86*, 2291.
[28] R. Rossetti, S. Nakahara, L. E. Brus, *J. Chem. Phys.* **1983**, *79*, 1086.
[29] L. Spanhel, M. Haase, H. Weller, A. Henglein, *J. Am. Chem. Soc.* **1987**, *109*, 5649.
[30] A. R. Kortan, R. Hull, R. L. Opila, M. G. Bawendi, M. L. Steigerwald, P. J. Carroll, L. E. Brus, *J. Am. Chem. Soc.* **1990**, *112*, 1327.
[31] M. L. Steigerwald, A. P. Alivisatos, J. M. Gibson, T. D. Harris, R. Kortan, A. J. Muller, A. M. Thayer, T. M. Duncan, D. C. Douglass, L. E. Brus, *J. Am. Chem. Soc.* **1988**, *110*, 3046.
[32] Z. A. Peng, X. Peng, *J. Am. Chem. Soc.* **2001**, *123*, 183.
[33] I. Mekis, D. V. Talapin, A. Kornowski, M. Haase, H. Weller, *J. Phys. Chem B* **2003**, *107*, 7454.
[34] D. V. Talapin, A. L. Rogach, A. Kornowski, M. Haase, H. Weller, *Nano Lett.* **2001**, *1*, 207.
[35] P. W. Voorhees, *J. Stat. Phys.* **1985**, *38*, 231.
[36] D. V. Talapin, A. L. Rogach, M. Haase, H. Weller, *J. Phys. Chem. B* **2001**, *105*, 12 278.
[37] B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen, M. G. Bawendi, *J. Phys. Chem. B* **1997**, *101*, 9463.
[38] W. G. J. H. M. van Sark, P. L. T. M. Frederix, D. J. Van den Heuvel, H. C. Gerritsen, A. A. Bol, J. N. J. van Lingen, C. de Mello Donega, A. Meijerink, *J. Phys. Chem. B* **2001**, *105*, 8281.
[39] A. M. Derfus, W. C. W. Chan, S. N. Bhatia, *Nano Lett.* **2004**, *4*, 11.
[40] C. Kirchner, T. Liedl, S. Kudera, T. Pellegrino, A. M. Javier, H. E. Gaub, S. Stolzle, N. Fertig, W. J. Parak, *Nano Lett.* **2005**, *5*, 331.
[41] X. Peng, J. Wickham, A. P. Alivisatos, *J. Am. Chem. Soc.* **1998**, *120*, 5343.
[42] M. Green, P. O'Brien, *Chem. Mater.* **2001**, *13*, 4500.
[43] T. Trindade, P. O'Brien, *Adv. Mater.* **1996**, *8*, 161.
[44] E. M. Chan, R. A. Mathies, A. P. Alivisatos, *Nano Lett.* **2003**, *3*, 199.
[45] B. K. H. Yen, A. Gunther, M. A. Schmidt, K. F. Jensen, M. G. Bawendi, *Angew. Chem. Int. Ed.* **2005**, *44*, 5447.
[46] R. E. Bailey, S. Nie, *J. Am. Chem. Soc.* **2003**, *125*, 7100.

[47] S. W. Kim, J. P. Zimmer, S. Ohnishi, J. B. Tracy, J. V. Frangioni, M. G. Bawendi, *J. Am. Chem. Soc.* **2005**, *127*, 10 526.
[48] O. E. Raola, G. F. Strouse, *Nano Lett.* **2002**, *2*, 1443.
[49] K. M. Hanif, R. W. Meulenberg, G. F. Strouse, *J. Am. Chem. Soc.* **2002**, *124*, 11 495.
[50] B. Dubertret, P. Skourides, D. J. Norris, V. Noireaux, A. H. Brivanlou, A. Libchaber, *Science* **2002**, *298*, 1759.
[51] X. Wu, H. Liu, J. Liu, K. N. Haley, J. A. Treadway, J. P. Larson, N. Ge, F. Peale, M. P. Bruchez, *Nat. Biotechnol.* **2003**, *21*, 41.
[52] M. E. Akerman, W. C. W. Chan, P. Laakkonen, S. N. Bhatia, E. Ruoslahti, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12 617.
[53] H. Mattoussi, J, M. Mauro, E. R. Goldman, G. P. Anderson, V. C. Sundar, F. V. Mikulec, M. G. Bawendi, *J. Am. Chem. Soc.* **2000**, *122*, 12 142.
[54] H. Mattoussi, J. M. Mauro, E. R. Goldman, T. M. Green, G. P. Anderson, V. C. Sundar, M. G. Bawendi, *Phys. Status Solidi* **2001**, *224*, 277.
[55] L. E. Brus, *J. Chem. Phys.* **1984**, *80*, 4403.
[56] L. E. Brus, *J. Phys. Chem.* **1986**, *90*, 2555.
[57] M. Dahan, T. Laurence, F. Pinaud, D. S. Chemla, A. P. Alivisatos, M. Sauer, S. Weiss, *Opt. Lett.* **2001**, *26*, 825.
[58] J. K. Jaiswal, H. Mattoussi, J. M. Mauro, S. M. Simon, *Nat. Biotechnol.* **2003**, *21*, 47.
[59] A. P. Alivistatos, *J. Phys. Chem.* **1996**, *100*, 13 226.
[60] C. B. Murray, C. R. Kagan, M. G. Bawendi, *Ann. Rev. Mat. Sci.* **2000**, *30*, 545.
[61] H. Weller, *Angew. Chem. Int. Ed.* **1993**, *32*, 41.
[62] H. Weller, *Adv. Mater.* **1993**, *5*, 88.
[63] W. C. W. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, M. Han, S. Nie, *Curr. Opin. Biotechnol.* **2002**, *13*, 40.
[64] W. Jiang, S. Mardyani, H. Fischer, W. C. W. Chan, *Chem. Mater.* **2006**, *18*, 872.
[65] J. Silver, W. Ou, *Nano Lett.* **2005**, *5*, 1445.
[66] M. Nirmal, B. O. Dabbousi, M. G. Bawendi, J. J. Macklin, J. K. Trautman, T. D. Harris, L. E. Brus, *Nature* **1996**, *383*, 802.
[67] S. A. Empedocles, M. G. Bawendi, *Science* **1997**, *278*, 2114.
[68] B. Lounis, H. A. Bechtel, D. Gerion, A. P. Alivisatos, W. E. Moerner, *Chem. Phys. Lett.* **2000**, *329*, 399.
[69] C. Gales, R. V. Rebois, M. Hogue, P. Trieu, A. Breit, T. E. Hebert, M. Bouvier, *Nat. Methods* **2005**, *2*, 177.
[70] M. Dahan, S. Levi, C. Luccardini, P. Rostaing, B. Riveau, A. Triller, *Science* **2003**, *302*, 442.
[71] F. Pinaud, D. King, H. Moore, S. Weiss, *J. Am. Chem. Soc.* **2004**, *126*, 6115.
[72] A. M. Derfus, W. C. W. Chan, S. N. Bhatia, *Adv. Mater.* **2004**, *16*, 961.
[73] M. Howarth, K. Takao, Y. Hayashi, A. Y. Ting, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7583.
[74] S. Pathak, S. Choi, N. Arnheim, M. E. Thompson, *J. Am. Chem. Soc.* **2001**, *123*, 4103.
[75] W. J. Parak, R. Boudreau, M. Le Gros, D. Gerion, D. Zanchet, C. M. Micheel, S. C. Williams, A. P. Alivisatos, C. Larabell, *Adv. Mater.* **2002**, *14*, 882.
[76] J. Zheng, Q. Song, S. Mardyani, W. C. W. Chan, C. W. Wang, *Lab. Hemetol.* in press.
[77] B. C. Lagerhom, M. Wang, L. A. Ernst, D. H. Ly, H. Liu, M. P. Bruchez, A. S. Waggoner, *Nano Lett.* **2004**, *4*, 2019.
[78] X. Gao, S. Nie, *Anal. Chem.* **2004**, *76*, 2406.
[79] Y. Xiao, P. E. Barker, *Nucl. Acids Res.* **2004**, *32*, e28.
[80] S. Kim, Y. T. Lim, E. G. Soltesz, A. M. De Grand, J. Lee, A. Nakayama, J. A. Parker, T. Mihalijevic, R. G. Laurence, D. M. Dor, L. H. Cohn, M. G. Bawendi, J. V. Frangioni, *Nat. Biotechnol.* **2004**, *22*, 93.
[81] X. Gao, Y. Cui, R. M. Levenson, L. W. K. Chung, S. Nie, *Nat. Biotechnol.* **2004**, *22*, 969.
[82] L. Zhu, S. Ang, W. Liu, *Appl. Environ. Microbiol.* **2004**, *70*, 597.
[83] R. Edgar, M. McKinstry, J. Hwang, A. B. Oppenheim, R. A. Fekete, G. Giulian, C. Merril, K. Nagashima, S. Adhya, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4841.
[84] M. Han, X. Gao, J. Z. Su, S. Nie, *Nat. Biotechnol.* **2001**, *19*, 631.
[85] E. R. Goldman, A. R. Clapp, G. P. Anderson, H. T. Uyeda, J. M. Mauro, I. L. Medintz, H. Mattoussi, *Anal. Chem.* **2004**, *76*, 684.
[86] I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman, B. Fisher, J. M. Mauro, *Nat. Mater.* **2003**, *2*, 630.
[87] A. R. Clapp, I. L. Medintz, J. M. Mauro, B. R. Fisher, M. G. Bawendi, H. Mattoussi, *J. Am. Chem. Soc.* **2003**, *126*, 301.
[88] X. Gao, W. C. W. Chan, S. Nie, *J. Biomed. Opt.* **2002**, *7*, 532.
[89] D. Hanahan, R. A. Weinberg, *Cell* **2000**, *100*, 57.
[90] H. Kitano, *Science* **2002**, *295*, 1662.
[91] R. Weissleder, *Nat. Rev. Cancer* **2002**, *2*, 1.
[92] M. Stoll, A. W. Cowley, Jr., P. J. Tonellato, A. S. Greene, M. L. Kaldunski, R. J. Roman, P. Dumas, N. J. Schork, Z. Wang, H. J. Jacob, *Science* **2001**, *294*, 1723.
[93] L. Hood, J. R. Heath, M. E. Phelps, B. Lin, *Science* **2004**, *306*, 640.
[94] S. Pottumarthy, V. C. Wells, A. J. Morris, *J. Clin. Microbiol.* **2000**, *38*, 2227.
[95] Y. Sbihi, A. Rmiqui, M. N. Nieves Rodriguez-Cabezas, A. Orduna, A. Rodriguez-Torres, A. Osuna, *J. Clin. Lab. Anal.* **2001**, *15*, 14.
[96] M. Bigos, N. Baumgarth, G. C. Jager, O. C. Herman, T. Nozaki, R. T. Stovel, D. R. Parks, L. A. Herzenberg, *Cytometry* **1999**, *36*, 36.
[97] R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, R. Langer, *Science* **1994**, *263*, 1600.
[98] R. Weissleder, C. Tung, U. Mahmood, A. Bogdanov, *Nat. Biotechnol.* **1999**, *17*, 375.
[99] C. H. Contag, B. D. Ross, *J. Magn. Reson. Imaging* **2002**, *16*, 378.
[100] R. Weissleder, *Nat. Biotechnol.* **2001**, *19*, 316.
[101] W. Jiang, E. Papa, H. Fischer, S. Mardyani, W. C. W. Chan, *Trends Biotechnol.* **2004**, *22*, 607.
[102] Y. T. Lim, S. Kim, A. Nakayama, N. E. Stott, M. G. Bawendi, J. V. Frangioni, *Mol. Imaging* **2003**, *2*, 50.
[103] L. Bakueva, I. Gorelikov, S. Musikhin, X. S. Zhao, E. H. Sargent, E. Kumacheva, *Adv. Mater.* **2004**, *16*, 926.
[104] E. B. Voura, J. K. Jaiswal, H. Mattoussi, S. M. Simon, *Nat. Med.* **2004**, *10*, 993.
[105] M. So, C. Xu, A. M. Loening, S. S. Gambhir, J. Rao, *Nat. Biotechnol.* **2006**, *24*, 339.
[106] B. Ballou, B. C. Lagerholm, L. A. Ernst, M. P. Bruchez, A. S. Waggoner, *Bioconjugate Chem.* **2004**, *15*, 79.
[107] H. C. Fischer, L. Liu, K. S. Pang, W. C. W. Chan, *Adv. Funct. Mater.* **2006**, *16*, 1299.
[108] J. Zheng, C. Zhang, R. M. Dickson, *Phys. Rev. Lett.* **2004**, *93*, 147 402.
[109] S. Nie, S. R. Emory, *Science* **1997**, *275*, 1102.
[110] B. D. Chithrani, A. A. Ghazani, W. C. W. Chan, *Nano Lett.* **2006**, *6*, 662.
[111] N. L. Rosi, D. A. Giljohann, C. S. Thaxton, A. K. R. Lytton-Jean, M. S. Han, C. A. Mirkin, *Science* **2006**, *312*, 1027.
[112] A. G. Tkachenko, H. Xie, Y. Liu, D. Coleman, J. Ryan, W. R. Glomm, M. K. Shipton, S. Franzen, D. L. Feldheim, *Bioconjugate Chem.* **2004**, *15*, 482.
[113] A. G. Tkachenko, H. Xie, D. Coleman, W. R. Glomm, J. Ryan, M. F. Anderson, S. Franzen, D. L. Feldheim, *J. Am. Chem. Soc.* **2003**, *125*, 4700.
[114] A. Ongaro, F. Griffin, L. Nagle, D. Iacopino, R. Eritja, D. Fitzmaurice, *Adv. Mater.* **2004**, *16*, 1799.
[115] M. G. Grimau, D. Iacopino, A. Avino, B. G. de la Torre, A. Ongaro, D. Fitzmaurice, J. Wessels, R. Eritja, *Helvet. Chim. Acta* **2003**, *86*, 2814.
[116] I. Gryczynski, J. Malicka, W. Jiang, H. Fischer, W. C. W. Chan, Z. Gryczynski, W. Grudzinski, J. R. Lakowicz, *J. Phys. Chem. B* **2005**, *109*, 1088.

[117] W. J. M. Mulder, R. Koole, R. J. Brandwijk, G. Storm, P. T. K. Chin, G. J. Strijkers, C. de Mello Donega, K. Nicolay, A. W. Griffioen, *Nano Lett.* **2006**, *6*, 1.
[118] B. N. G. Giepmans, T. J. Deerinck, B. L. Smarr, Y. Z. Jones, M. H. Ellisman, *Nat. Methods* **2005**, *2*, 743.
[119] M. Unger, E. Kartalov, C.-S. Chiu, H. A. Lester, S. R. Quake, *BioTechniques* **1999**, *27*, 1008.
[120] H. E. Grecco, K. A. Lidke, R. Heintzmann, D. S. Lidke, C. Spagnuolo, O. E. Martinez, E. A. Jares-Erijman, T. M. Jovin, *Microsc. Res. Tech.* **2004**, *65*, 169.
[121] R. Helm, A. B. Cubitt, R. Y. Tsien, *Nature* **1995**, *373*, 663.
[122] Q. S. Hanley, V. Subramaniam, D. J. Arndt-Jovin, T. M. Jovin, *Cytometry* **2001**, *43*, 248.
[123] H. Ditlbacher, N. Felidj, J. R. Krenn, B. Lamprecht, A. Leitner, F. R. Aussenegg, *Appl. Phys. B* **2001**, *73*, 373.
[124] A. M. Michaels, J. Jiang, L. Brus, *J. Phys. Chem. B* **2000**, *104*, 11 965.
[125] M. A. Noginov, M. Vondrova, S. N. Williams, M. Bahoura, V. I. Gavrilenko, S. M. Black, V. P. Drachev, V. M. Shalaev, A. Sykes, *J. Opt. A* **2005**, *7*, S219.
[126] E. T. Knobbe, B. Dunn, P. D. Fuqua, F. Nishida, *Appl. Opt.* **1990**, *29*, 2729.
[127] M. F. Garcia-Parajo, J.-A. Veerman, G. M. J. Segers-Nolten, B. G. de Grooth, J. Greve, N. F. van Hulst, *Cytometry* **1999**, *36*, 239.
[128] M. Ishikawa, E. Sekizuka, S. Sato, N. Yamaguchi, J. Inamasu, H. Bertalanffy, T. Kawase, C. Iadecola, *Stroke* **1999**, *30*, 1679.
[129] R. Y. Tsien, *Annu. Rev. Biochem.* **1998**, *67*, 509.