



Quantum dots can emit visible light of any wavelength, leading to these multi-colored solutions of colloidal quantum dots.

# Quantum Dots

## Illuminating biological applications of quantum dots

By Sarah J. Shareef

If you have ever looked out over a city from an airplane at night, you can easily distinguish the highways because of the street lights that border them. Like street lamps in a city, quantum dots can highlight individual molecules in a vast tissue of thousands of cells. Traditionally, most biological imaging occurs via fluorescent dye molecules that emit limited wavelengths of light and whose luminescence decreases with time. On the other hand, a quantum dot, acting as a single large atom, can maintain emission at any user-specified wavelength in the visible spectrum. Quantum dots are of interest to biologists and physicists alike, as these fluorescent semiconductor nanoparticles have unique physical properties that lend themselves to a vast array of biological applications. In fact, these nano-scaled dots are lighting

up an entire field of interdisciplinary research!

Although foundational research into nano-scaled semiconductors was already underway by 1973 when Leo Esaki won the Nobel Prize in Physics for his work on semiconductor quantum devices, quantum dots were not recognized for their biological applications until the pioneering papers of the Alivisatos lab and the Nie lab in 1998 [1]. In these papers, both Alivisatos and Nie independently demonstrated the use of colloidal quantum dots in biological labeling and suggested that these quantum dots could provide many benefits over traditional fluorescent organic molecules, such as strong luminescence, resistance to photobleaching, narrow emission spectrum, and diameter dependent fluorescence [2, 3]. Since then,

research into the physics of quantum dots has set the groundwork for the recent advances in biological imaging

### How Quantum Dots Work

Ranging from 1 nm to 100 nm in diameter, quantum dots are most often composed of atoms of transition metals, such as cadmium, silver, lead, and gold. Quantum dots fluoresce or emit visible light when energized by electromagnetic radiation ranging from visible, to infrared, to ultraviolet light. The wavelength of emitted light is dependent specifically on the diameter of the quantum dot; large quantum dots emit red light, while smaller quantum dots emit blue light. Due to its resistance to photobleaching, quantum dots continue to emit light long after conventionally used organic fluorescent

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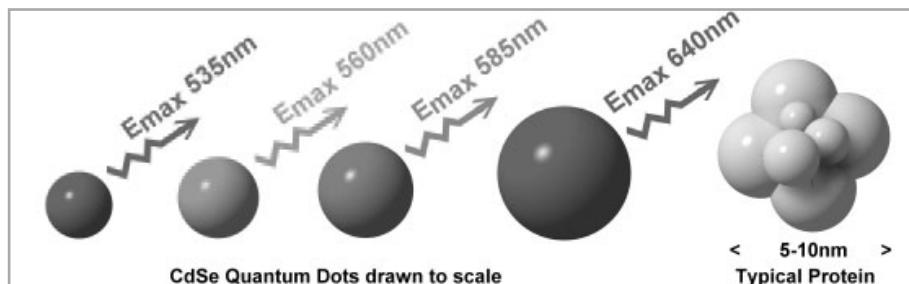
## Focus: Illuminating Science

dyes have faded into the background. The ability to easily and reliably choose a specific emission wavelength and their longer lasting fluorescence make quantum dots ideal for biological imaging, especially for identifying components in whole tissue samples. To understand why quantum dots have these advantageous properties, we must understand the physics of nano-scaled materials.

### Quantum Confinement

The physical properties of quantum dots center on the theory of quantum confinement, which explains how the electrical properties of quantum dots give rise to their fluorescence. Because quantum dots are semiconductors, electrons are not tightly bound to their specific constituent atoms. Just as an atom is surrounded by energy levels occupied by its electrons, a quantum dot can be thought to have its own energy levels that contain these loosely bound electrons. Thus, the quantum dot is nicknamed the artificial atom.

Quantum confinement explains that the electrons in a quantum dot are confined to discrete energy levels when the radius of the quantum dot is less than the Excitation Bohr Radius, a function of the material's properties. When the electrons are excited, they move to a higher energy level, leaving a 'hole' equivalent to positive-charge in its place. The excited electron and positive hole together are known as an exciton. When an electron returns to its original energy level, the energy difference between the excited and original energy levels is released as a photon. Quantum



Emission spectra depends on the quantum dot diameter. Smaller quantum dots have higher energy emission and thus are seen in blue solutions. On the other hand, larger quantum dots emit lower energy red light, which has a wavelength of 640 nm.

confinement also explains that when electron energy levels are discrete the emission wavelength becomes dependent on the size of the particle. Thus, the color of light emitted by a quantum dot can be chosen by adjusting its diameter [4,5].

### Biological Applications

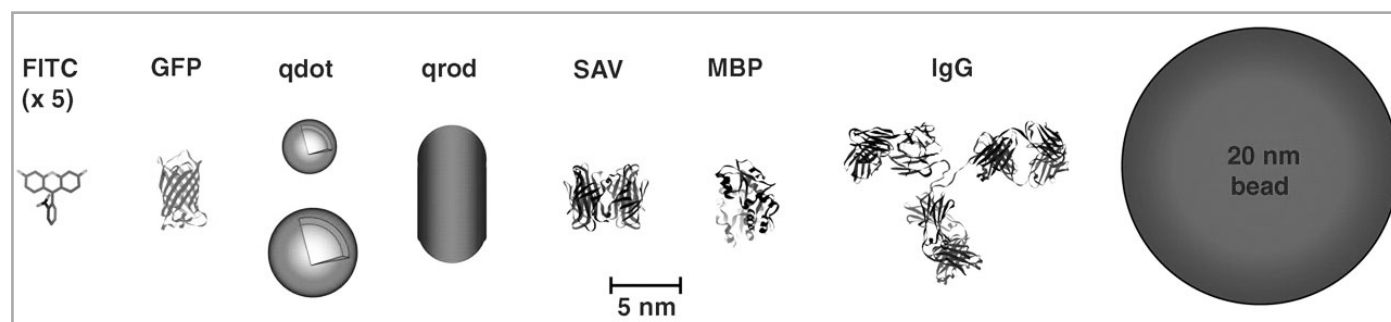
Many of the electronic and physical properties of quantum dots make them ideal for various biological applications. With a fluorescent signal that is 20 times as bright as that of a typical organic dye and 100 times more resistant to photobleaching, quantum dots overcome several of the limitations to biological imaging by providing clear images with a high signal to noise ratio [3]. Quantum dots are also especially advantageous for 3D tissue imaging because they allow for localization of fluorescence at different depths in a 3D section. The resistance to photobleaching allows longer observation of quantum dot labeled tissue samples under confocal microscopy. Also, quantum dots can be used for long-term tracking of individual cells.

A broad absorption spectrum cou-

pled with a narrow emission spectrum means that quantum dots have well-defined colors that allow several different molecules to be viewed simultaneously within a confined area. The ability to easily control the fluorescence color of quantum dots by modulating their diameter allows reliable production of quantum dots of any color. With no documented negative effects on cell viability, quantum dots appear to be biocompatible and are even used to label molecules in living animals [6]. Furthermore, because of their small size, quantum dots can be endocytosed into a cell and thus can be used to introduce factors directly into the cell body. Therefore, the physical properties of quantum dots make them useful not only for biological imaging but also to several biotechnologies.

### Quantum Dot Biotechnologies

Advances in biological research incorporating nanotechnology have expanded the applications of quantum dots to Forster resonance energy transfer (FRET) systems and internally-excited fluorescent systems.



Relative sizes of common proteins and quantum dots. Because quantum dots can be about the same size as common proteins, they can be incorporated into biological imaging techniques.

credit: <http://nanocluster.mit.edu/research.php>; <http://www.sciencemag.org/ezp-prod1.hul.harvard.edu/cgi/content/abstract/307/7570/7538/index.html>





Quantum dots can be used as a component of a FRET system, which is used to detect close interaction between two fluorescently labeled molecules. One of the molecules, called the FRET donor, fluoresces when activated by an external signal. The FRET acceptor will be activated by the emission of the FRET donor if it is in close enough proximity. Thus, if the emission of the FRET donor is observed the molecules are not in close contact, but if the emission of the FRET acceptor is observed the molecules are interacting. Boeneman and colleagues constructed a sensitive detection system for caspase-3 protease activity based on FRET using quantum dots. A quantum dot acts as the FRET donor and activates a fluorophore, mCherry, attached to the quantum dot via a protein containing a caspase-3 cleavage site. When the link between the quantum dot and fluorophore is cleaved by caspase-3, the fluorophore is no longer detected and instead only the emission of the quantum dot is observed thereby highlighting the locations of caspase-3 protease activity. Thus, caspase-3 protease activity can be detected and/or quantified from the ratio of FRET donor versus acceptor fluorescence [7].

Another branch of current biotechnology research is designing quantum dots that do not require activation by an external light source.

Removing external excitation would decrease background fluorescence and would enable more specific imaging. Conjugation of the fluorescent enzyme luciferase to the surface of quantum dots has been successful in activating quantum dots. When luciferase binds biotin, it emits energy that activates quantum dots and leads to the release of a photon. Thus, the conjugation of luciferase to quantum dots would enable the use of quantum dots in

biological samples sensitive to external excitation [8].

### Detecting Cancer with Quantum Dots

Quantum dot conjugated-antibodies are being used more frequently to select certain molecules in a tissue. In a proof-of-principle paper, scientists from the Dana Farber Cancer Institute and Harvard Medical School showed that quantum dot-conjugated antibodies could be used to identify sub populations of cancer cells using multiple markers in a single tissue. Because quantum dots have specific emission spectra, antibodies for multiple molecules could be conjugated to different colored quantum dots [9].

Quantum dots conjugated with peptide toxins can also be used to identify disease state cells. Scientists from the Vanderbilt School of Medicine and Department of Physics conjugated quantum dots with two well-characterized neurotoxins, chlorotoxin (CTX) and dendrotoxin (DTX). Chlorotoxin binds to an extracellular matrix enzyme MMP-2 which is important for cell motility function and enables metastasis, while dendrotoxin binds to a specific potassium channel Kv1.1 (Shaker family).

Orndorff and Rosenthal found that glioma cells expressed MMP-2 and Kv1.1 at levels 4 times greater than did non-cancer cells. Therefore, quantum dots conjugated with neurotoxins chlorotoxin and dendrotoxin labeled glioma cells more frequently than non-cancer cells. Because neurotoxins exhibit high affinity for their molecular targets, the neurotoxin-conjugated quantum dots provide a sensitive and long lasting probe that can be used for long term cancer cell tracking even in conditions of low molecular target concentration. The scientists also conducted a multiplexing experiment that demonstrated that two molecular targets could be

identified simultaneously when excited with the same light source by taking advantage of the characteristic broad absorption spectra of quantum dots. These experiments broadened the biological applications of quantum dots [10].

Quantum dots have several inherent physical properties that make them especially relevant to biological applications. While their long-lasting fluorescence immediately allows them to contribute to biological imaging, quantum dots are also being used in other types of biological experiments. For example, quantum dots can also detect cancer cells in vivo. Given the potential of quantum dots to advance biological experimentation, it is not surprising that the incorporation of quantum dots into a vast array of biological applications has occurred so rapidly. It will be of great interest to see the future advances in biology that are due to these nano-sized spheres of light. **H**

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