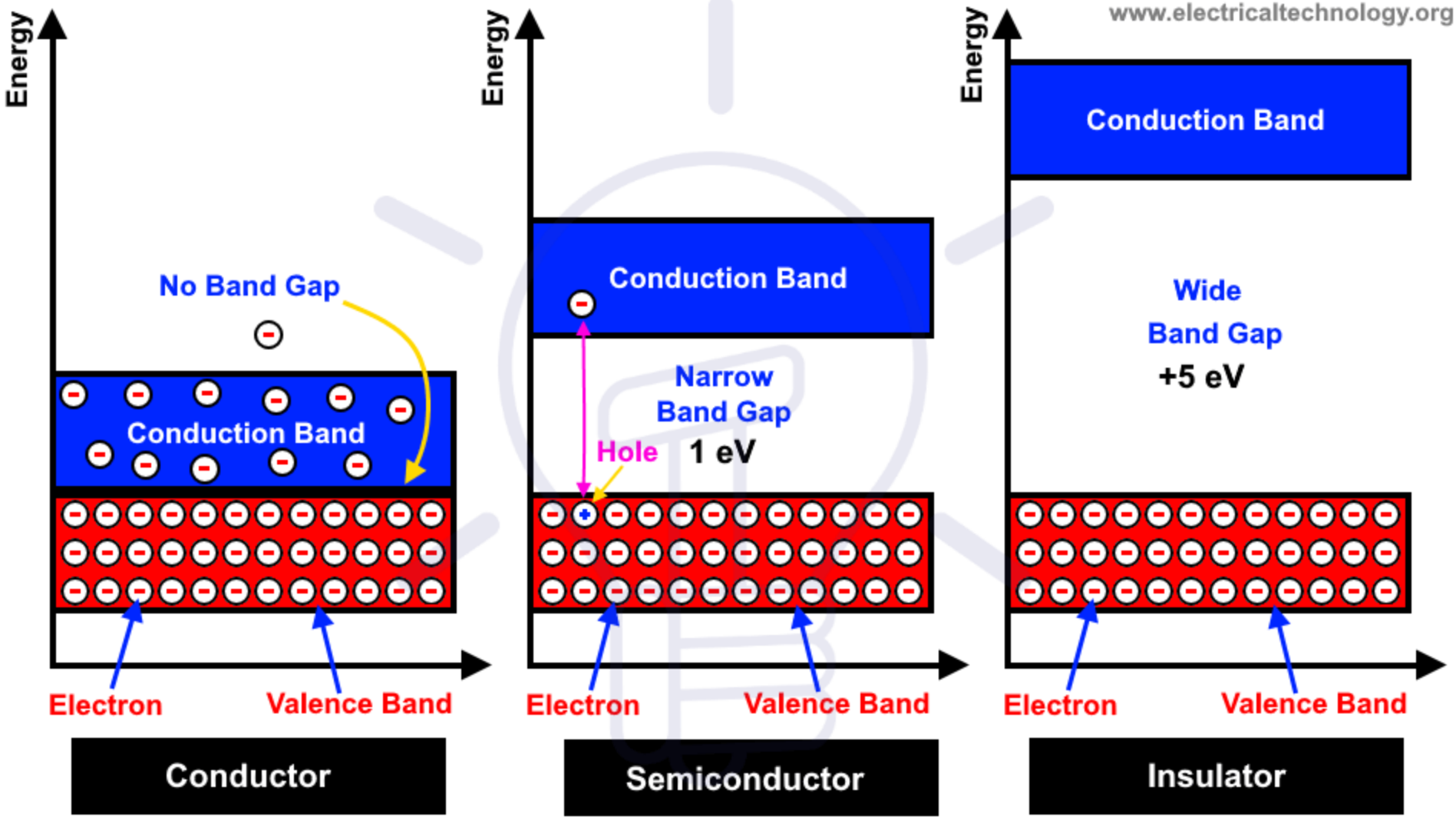
EMERGING MATERIALS AND TECHNIQUES FOR DEVEOPING BIOSENSORS

Quantum Dots (QDs)

Advance nanomaterial for developing optical biosensors

Materials may be classified into 3 categories based on level of conductivity

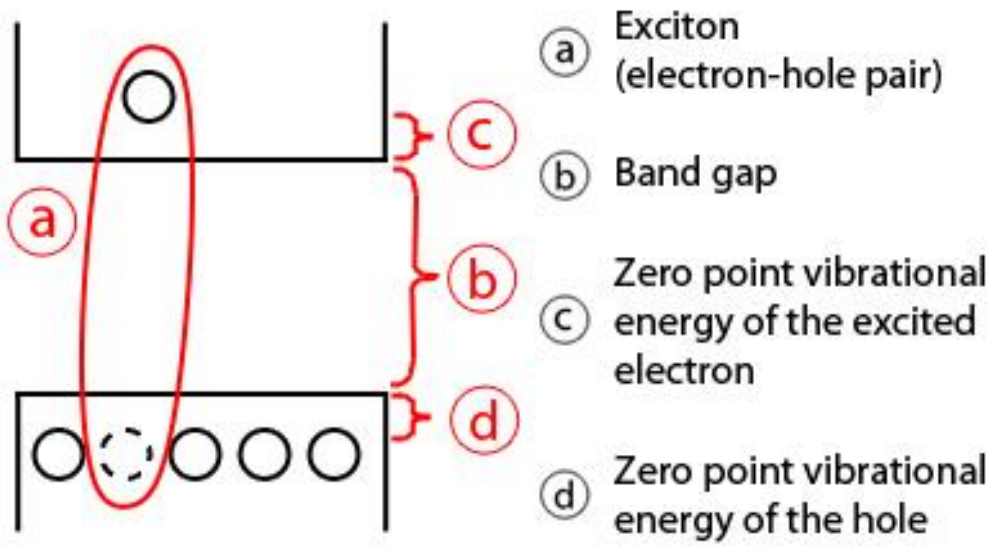


QDs are a special class of semiconductor materials, which are crystals composed of periodic groups of II-VI, III-V, or IV-VI materials.
Example: CdSe (IIb-VI)

The sizes of QDs are ranging from 2-10 nanometers (10-50 atoms) in diameter.

Applying a stimulus such as heat, voltage, or photon flux can induce some electrons to jump the forbidden gap to the conduction band.

The valence location they vacate is referred to as a hole since it leaves a temporary "hole" in the valence band electron structure.

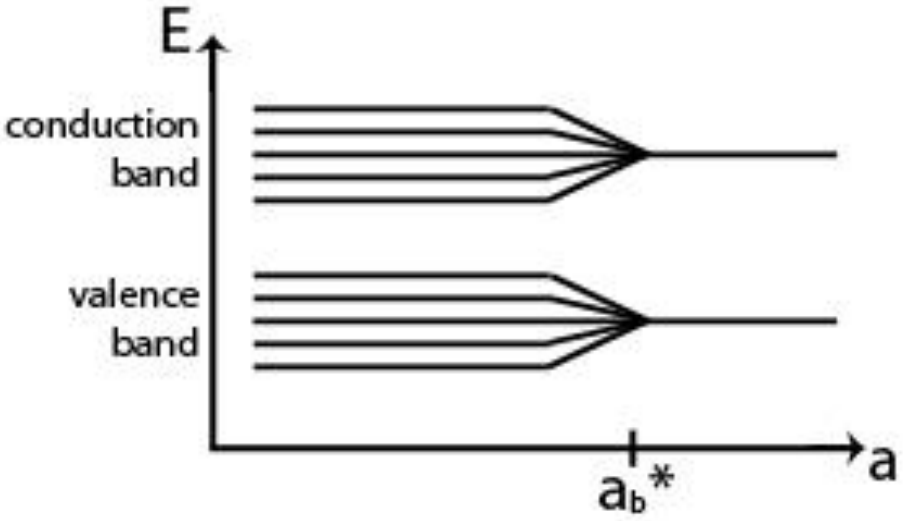


The raised electron and the hole taken as a pair are called an exciton.

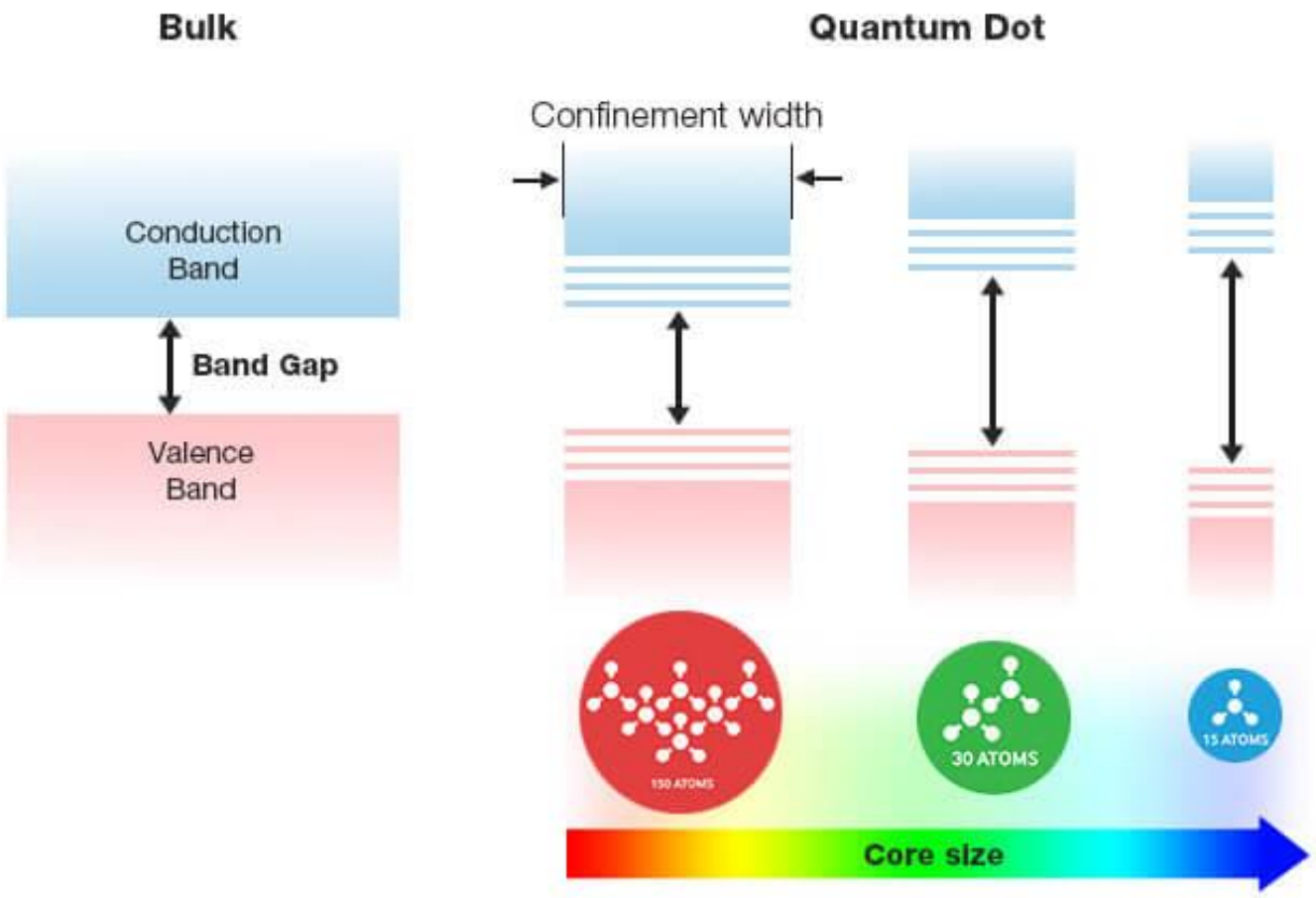
Excitons have an average physical separation between the electron and hole, referred to as the **Exciton Bohr Radius** this physical distance is different for each material.

What is the difference between QD semiconductor and Bulk semiconductor materials?

If the size of a semiconductor crystal becomes small enough that it approaches the size of the material's Exciton Bohr Radius, then the electron energy levels can no longer be treated as continuous - they must be treated as discrete, meaning that there is a finite separation between energy levels.



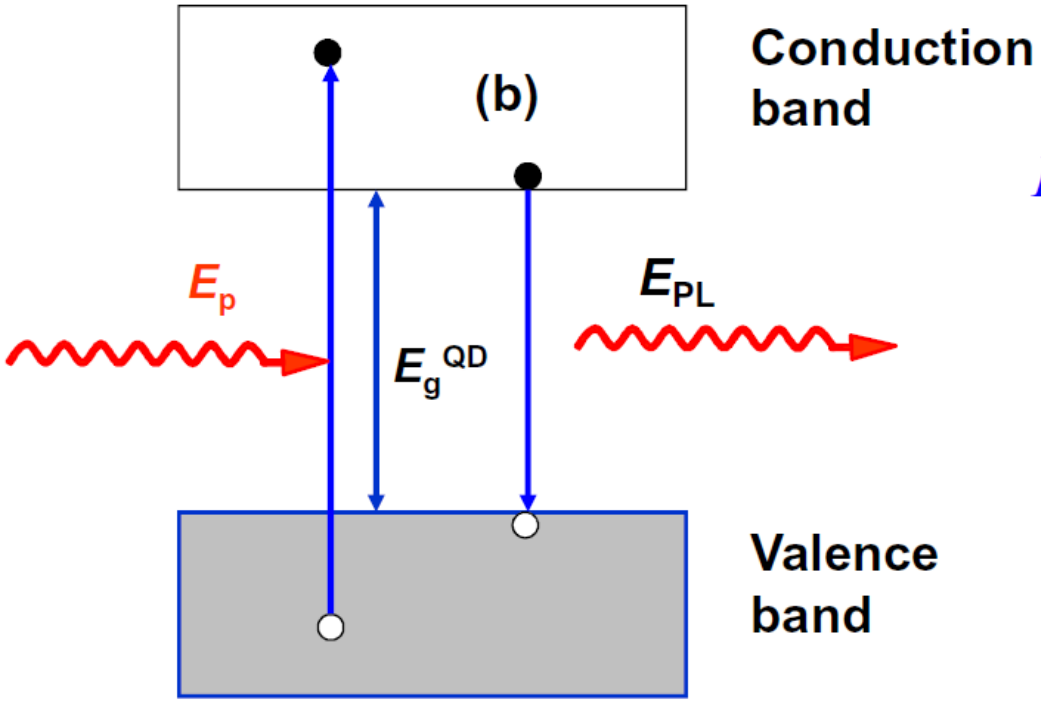
Splitting of energy levels for small quantum dots due to the quantum confinement effect. The horizontal axis is the radius, or the size, of the quantum dots and a_b* is the Exciton Bohr radius.



This situation of discrete energy levels is called **quantum confinement**, and under these conditions, the semiconductor material ceases to resemble bulk, and instead can be called a ***quantum dot***.

Because quantum dots' electron energy levels are discrete rather than continuous, t**he addition or subtraction of just a few atoms to the quantum dot has the effect of altering the boundaries of the bandgap**

Changing the geometry of the surface of the quantum dot also changes the bandgap energy, owing again to the small size of the dot, and the effects of quantum confinement.



$$E_g^{QD} = E_g^{bulk} + \frac{\hbar^2 \pi^2}{2R^2}\left(\frac{1}{m_e} + \frac{1}{m_h}\right)$$

Notice that the smaller the radius of a quantum dot, the larger the bandgap. This means you can control the color of the light emitted by the dots by changing their size!

E_g= Band gap energy, R is the radius, m_e is the free electron mass, m_h is the hole mass, h is the planks constant.

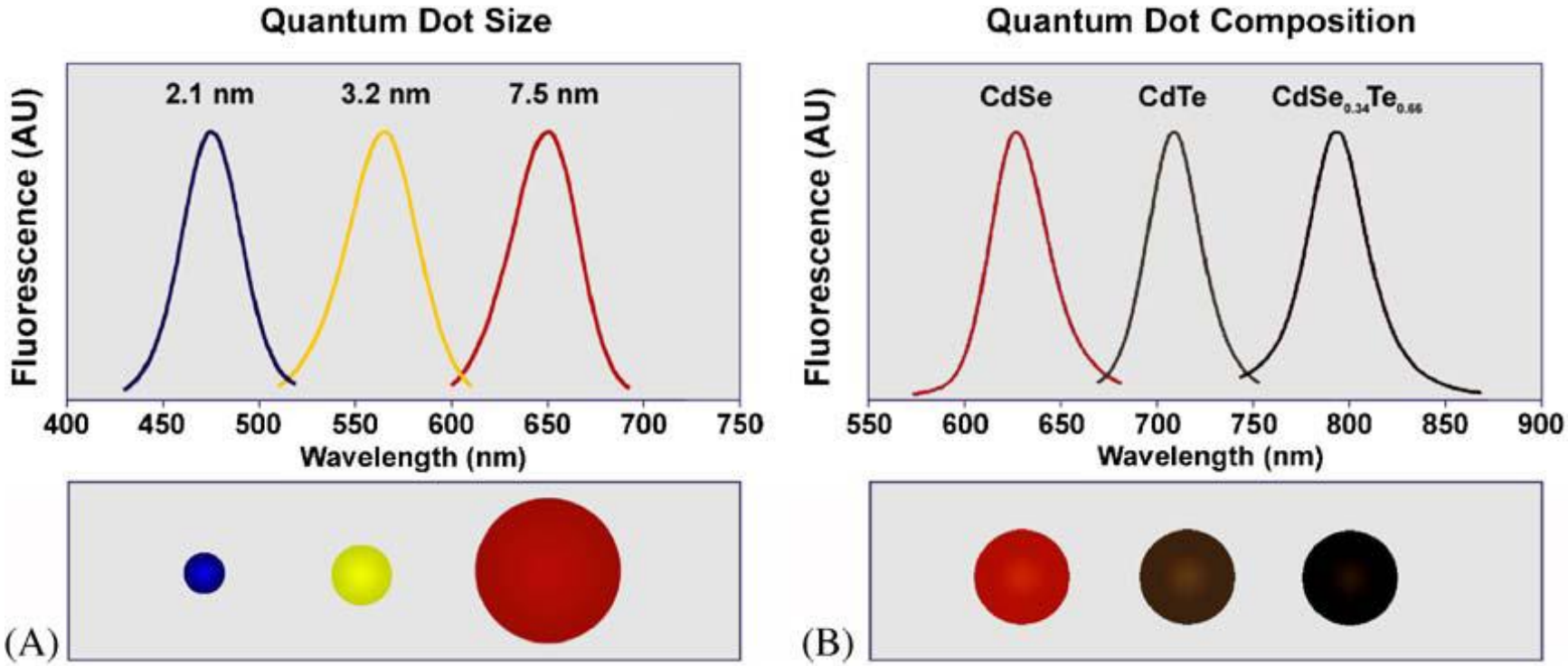
The bandgap in a quantum dot always energetically larger than bulk; therefore, the radiation from quantum dots is "blue shifted"

The size of the bandgap could be controlled by adjusting the size of the dot.

Because the emission frequency of a dot is dependent on the bandgap, it is therefore possible to control the output wavelength of a dot with extreme precision and therefore specify its "color" output depending on the needs of the customer.

Thus quantum dots of the same material, but with different sizes, can emit light of different colors. The physical reason is the quantum confinement effect.

The bandgap energy that determines the energy (and hence color) of the fluorescent light is inversely proportional to the square of the size of the quantum dot.



Tuning the QD emission wavelength by changing the nanoparticle size or composition

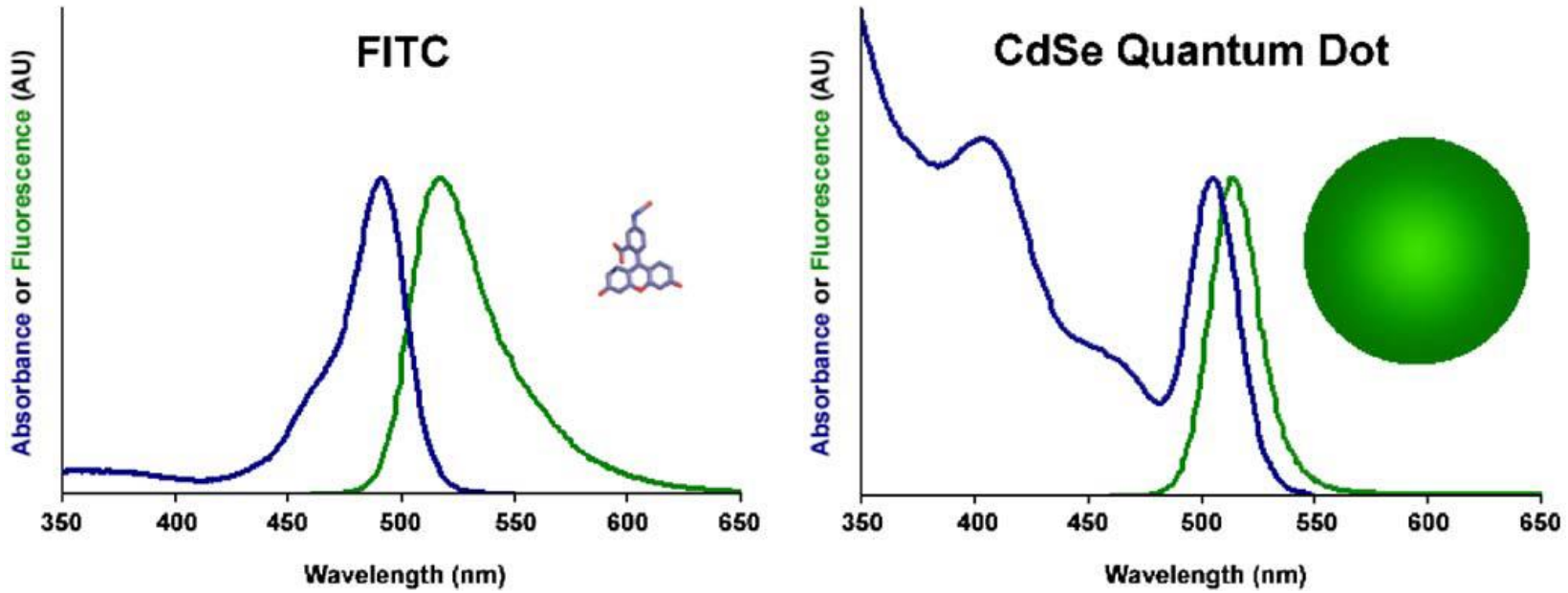
The lifetime of fluorescence is determined by the size of the quantum dot.

Larger dots have more closely spaced energy levels in which the electron-hole pair can be trapped. Therefore, electron-hole pairs in larger dots live longer causing larger dots to show a longer lifetime.

Quantum dots are particularly significant for optical applications due to their theoretically high quantum yield. One of the most obvious advantage is brightness (owing to the high quantum yield) as well as their stability (allowing much less photobleaching).

quantum dots are 20 times brighter and 100 times more stable than traditional fluorescent reporters.

Due to their bright fluorescence, narrow emission, broad UV excitation and high photostability, QDs have been adopted for *in vitro* bioimaging by many researchers as an alternative to organic based fluorophores



Absorbance (blue) and fluorescence (green) spectra of an organic dye (fluorescein isothiocyanate, FITC, left) and a CdSe QD (right) with identical emission wavelengths.

The emission spectrum of the QD is more narrow and symmetric than that of the dye, and the absorption spectrum extends far into the ultraviolet region.

Example: Single-quantum-dot-based DNA nanosensor

ZHANG et al. Nature materials 4 (2005) page 826

An ultrasensitive nanosensor based on fluorescence resonance energy transfer (FRET) capable of detecting low concentrations of DNA in a **separation-free format** is reported.

This system uses QDs linked to DNA probes to capture DNA targets.

The target strand binds to a dye-labelled reporter strand thus forming a FRET donor–acceptor ensemble.

The resulting assembly brings the fluorophore acceptors and the QD donor into close proximity, leading to fluorescence emission from the acceptors by means of FRET on illumination of the donor.

As a result, detection of acceptor emission indicates the presence of targets

In the absence of targets or in the presence of non-specific targets, fluorescent bursts were detected by the donor detector but not the acceptor detector, suggesting that direct excitation of acceptors or leakage of donor emission into the acceptor detector was minimal. This near-zero background acceptor fluorescence observed in the negative control sample also suggests high detection specificity. The capability of capturing several acceptors by a QD donor helps enhance the overall energy-transfer efficiency.

Here CdSe–ZnS core–shell nanocrystals, 605QD, as donors and Cy5 as acceptors were used. A 488-nm argon laser was used as an excitation light source. Photons emitted from the QDs and Cy5 through individual channels were detected by photodiodes. Single-molecule fluorescence images were taken and analyzed by software.

A nanosensor-based oligonucleotide ligation assay has been demonstrated to successfully detect a point mutation typical of some ovarian tumours in clinical samples.

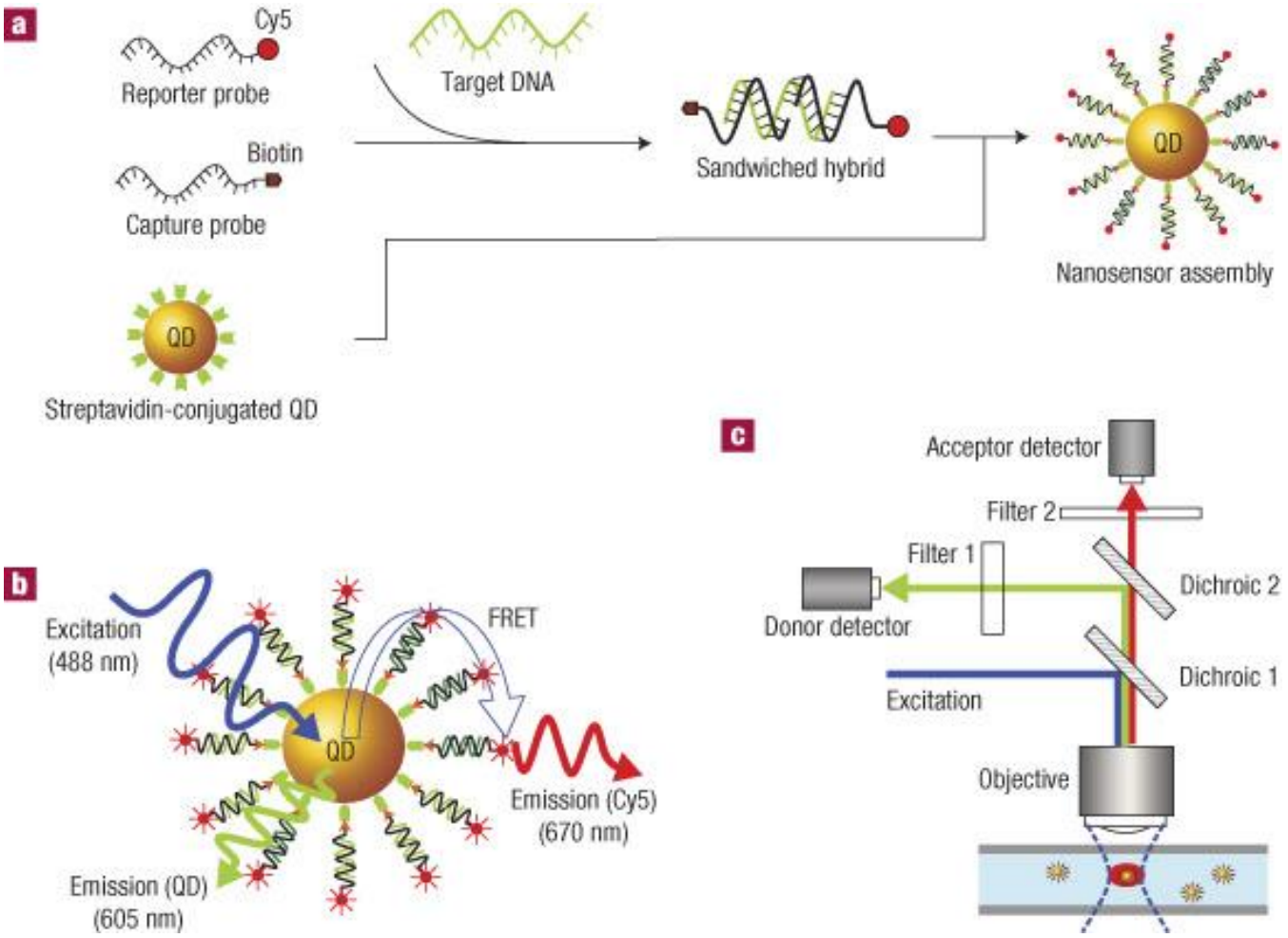


Fig: a. Conceptual scheme showing the formation of a nanosensor assembly in the presence of targets.

b, Fluorescence emission from Cyanine5 (Cy5) on illumination on QD caused by FRET between Cy5 acceptors and a QD donor in a nanosensor assembly.

c, Experimental setup.

Recent advances have shown that nanometer-sized semiconductor particles can be covalently linked with biorecognition molecules such as peptides, antibodies, nucleic acids, or small-molecule ligands for use as biological labels.

High-quality QDs are also well suited for optical encoding and multiplexing applications due to their broad excitation profiles and narrow/symmetric emission spectra.

Quantum dot labels have been successfully used for a variety of bioanalytical purposes, such as DNA hybridization detection, immunoassays, and binding assays using FRET to probe for target events.

Significant advances in the use of QDs as bioanalytical tools for in vitro work have been made in the areas of immunoassays and biosensors.

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