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Applicability of Quantum Dots in Biomedical Science

Slavica Brkić

Abstract

Quantum dots (QDs) are novel class of inorganic fluorophore with superior photophysical properties. Superior optical properties are a promising alternative to organic dyes for fluorescence biomedical applications. These nanoparticles have size-tunable emission, strong light absorbance, and very high levels of brightness and photostability. Highly luminescent QDs are prepared by coating the core with another material, resulting in core-shell quantum dots that are more stable in various chemical environments. These core-shell QDs are hydrophobic and only organic soluble as prepared. Hydrophobic QDs are insoluble in aqueous solution and cannot be directly employed in biomedical applications. They are necessarily made water soluble by surface modifying them with various bifunctional surface ligands or caps to promote aqueous solubility and enhancing biocompatibility. To make them useful for biomedical applications, QDs need to be conjugated to biological molecules without disturbing the biological function of these molecules. Most of the current studies were designed to ask questions concerning the physicochemical properties of novel QD products, not QD toxicity *per se*. The potential toxicity of the QDs is a cause for concern because they are made of heavy metals. The limitation of heavy metal–containing QDs stimulates extensive research interests in exploring alternative strategies for the design of fluorescent nanocrystals with high biocompatibility.

Keywords: quantum dot, band gap, core/shell/ligand structure, hydrophobic QD, biocompatibility, hydrophilic QD, functionalization, toxicity

1. Introduction

Quantum dots (QDs) are semiconductor nanoparticles that are restricted in three dimensions, typically with a diameter of 2–8 nm [1]. These particles are defined as particles with physical dimensions smaller than the exciton Bohr radius [2]. QDs are a bridge between

bulk materials and atomic or molecular structures and characterized by composition-dependent band gap energy. These particles are light-emitting nanocrystals with novel optical and electrical properties. Properties of QDs have attracted great interest in biology and medicine in the recent years [3]. Compared with organic dyes and fluorescent proteins, semiconductor QDs offer several unique advantages. However, the major concerns about potential toxicity of QDs have cast doubts on their practical use in biology and medicine [3].

2. The band gap energy

The small size of QDs lead to what is known as "quantum confinement" [4]. The quantum confinement effects occur when size of nanoparticle smaller than exciton Bohr radius. An exciton Bohr radius is the distance in an electron-hole pair in a bulk semiconductor. QDs are defined as particles with physical dimensions smaller than the exciton Bohr radius. The quantum confinement means that the energy levels that the electrons inhabit become discrete, with a finite separation between them. However, there are some energy levels that the electrons cannot occupy, which are collectively known as band gap [5]. Most electrons occupy energy levels below this band gap in the area known as the valence band, indeed most energy levels in the valence band are occupied. If, however, an external stimulus is applied, an electron may move from the valence band to the conduction band, i.e., those energy levels above the band gap. When the QD is hit by incident light, it absorbs a photon with a higher energy than that of the band gap of the composing semiconductor. When the electron returns to a lower energy level, a narrow, symmetric energy band emission occurs [6]. The wavelength of photon emissions depends not only on the material from which the dot is made but also its size; the smaller the size of the QDs, the larger the band gap energy and QDs emit blue light, larger QDs having smaller band gaps emit the larger wavelength. Recombination occurs when an electron from a higher energy level relaxes to a lower energy level and recombines with an electron hole. This process is accompanied by the emission of radiation, which can be measured to give the band gap size of a semiconductor. The energy of the emitted photon in a recombination process of a QD can be modeled as the sum of the band gap energy, the confinement energies of the excited electron and the electron hole, and the bound energy of the exciton [1]:

$$E = E_{bandgap} + E_{confinement} + E_{exciton} \qquad (1)$$

The confinement energy can be modeled as a simple particle in an one-dimensional box problem and the energy levels of the exciton can be represented as the solutions to the equation at the ground level ($n = 1$) with the mass replaced by the reduced mass. The magnitude of this confinement energy:

$$E_{confinement} = \frac{\hbar^2 \pi^2}{2 d^2}\left(\frac{1}{m_e} + \frac{1}{m_h}\right) = \frac{\hbar^2 \pi^2}{2\mu d^2}, \qquad (2)$$

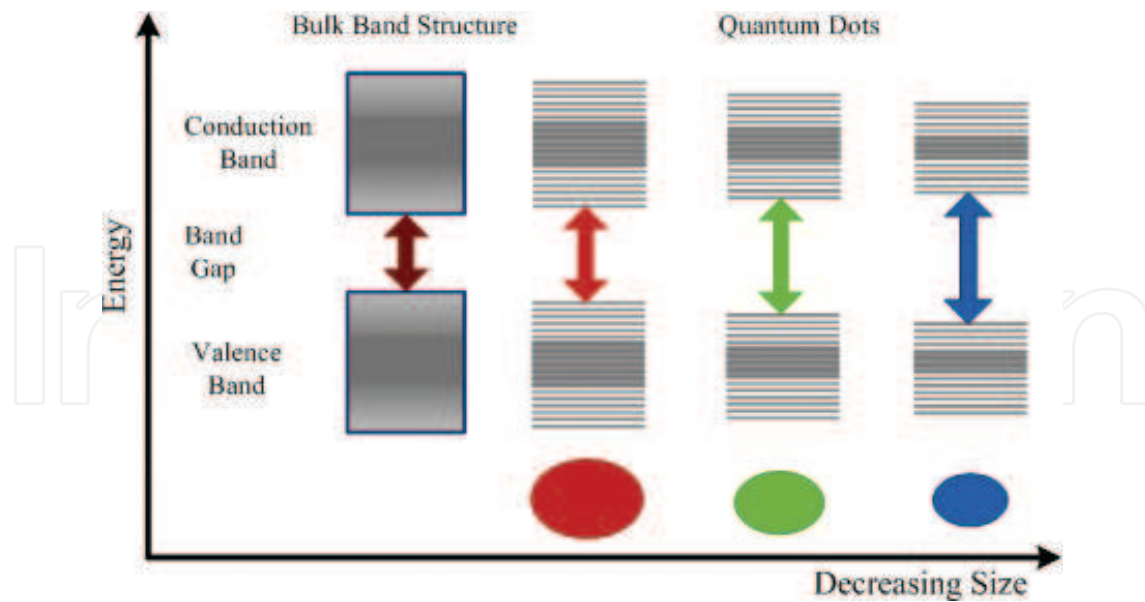**Figure 1.** Size-dependent band gap energy [2].

where m_e is the effective mass of the electron, m_h is the effective mass of the hole, μ is the reduced mass of the exciton system, and d is diameter of the confinement [7]. The bound exciton energy can be modeled by using the Coulomb interaction between the electron and the positively charged hole. The negative energy is proportional to Rydberg's energy (R_y = 13.6 eV) and inversely proportional to the square of the size-dependent dielectric constant, ε_r. Energy of exciton:

$$E_{exciton} = -\frac{1}{\varepsilon_r^2}\frac{\mu}{m_e}R_y. \qquad (3)$$

Using these models and spectroscopic measurements of the emitted photon energy E, it is possible to measure the band gap of QDs. Decreasing the size of a QD results in a higher degree of confinement, which produces an exciton of higher energy, thereby increasing the band gap energy as can be seen in **Figure 1**.

3. Biomedical application of quantum dots

Nanotechnology has been heralded as a new field that has the potential to revolutionize medicine, as well as many other seemingly unrelated subjects, such as electronics, textiles, and energy productions. In 1998, the potential of these nanoparticles for applications involving biological labeling was first reported. The first successful application of QDs to medical diagnostics has been demonstrated by immunofluorescent labeling of fixed cells and tissues and immunostaining of membrane proteins on living cells [8]. The fact that several QDs can be excited by the same wavelength of light opens up several multiplexing potentials, including high-throughput screening of biological samples [9]. Size-tunable

absorption and emission property of QDs is an extremely valuable property for biological imaging as they can be tuned all the way from the UV to the near-infrared of the spectrum. For biological and medical applications, it is of importance to study the photophysical properties of QDs.

3.1. Photophysical properties of quantum dots

In the biomedical sciences, fluorescence is used as a powerful tool for labeling, imaging, tracking, detection, and therapy. The fluorescent labeling of biological molecules using organic fluorophore and QDs. QDs are new class of fluorophores that offer several advantages over traditional fluorophores. These particles have broad absorption and narrow emission spectra, high quantum yield, long life-time, high brightness, and stable against photobleaching. A great advantage of QDs compared to the classical fluorophore which emits light in the infrared and near-infrared regions, as the absorption of tissues is minimal in this region. The most popular types of QDs include CdSe, CdTe, and ZnSe. The most commonly studied and used QD is cadmium selenide. The broad absorption spectra of the QDs allows single wavelength excitation of emission from different-sized QDs. Multicolor optical coding for biological assays has been achieved by using different sizes of quantum dots with precisely controlled ratios [10]. In order to apply QDs in biomedical imaging, recent studies have focused on developing near-infrared luminescent QDs which exhibit an emission wavelength ranging from 700 to 900 nm [3]. The use of the near-infrared (NIR) photons is promising for biomedical imaging in living tissue due to longer attenuation distances and a better tissue staining without interfering with autofluorescence, since most of the tissue chromophores weakly absorb light in the infrared range of wavelengths. Hemoglobin and water have lower absorption coefficient and scattering effects in the NIR region (650–900 nm). The QDs emission can be set to a NIR area by adjusting QD size or by incorporating rare-earth activators. The unique robust optical properties of QDs and their surface properties that allow biocompatibility and heteroconjugation make QDs highly promising fluorescent labels for biological applications with significant superiority over classic organic fluorophores [10].

3.1.1. Size-dependent optical properties

Quantum dots exhibit size-dependent discrete energy levels. The energy gap increases with decrease in the size of the nanocrystal, thus yielding a size-dependent rainbow of colors. The wavelength of the emission photon depends not on the material from which the dot is made but on its size. The ability to control the size of QD enables the manufacturer to determine the wavelength of emission, which in turn determines the color of light the human eye perceives. The smaller the dot is closer to the blue end of the spectrum, and the larger the dot is closer to the red (**Figure 2**) [9]. Light wavelengths from ultraviolet to infrared region (400–4000 nm) can be achieved with variation of the size and composition of nanoparticles [11]. The optical properties of QDs will change with proximity of quantum dots to each other.
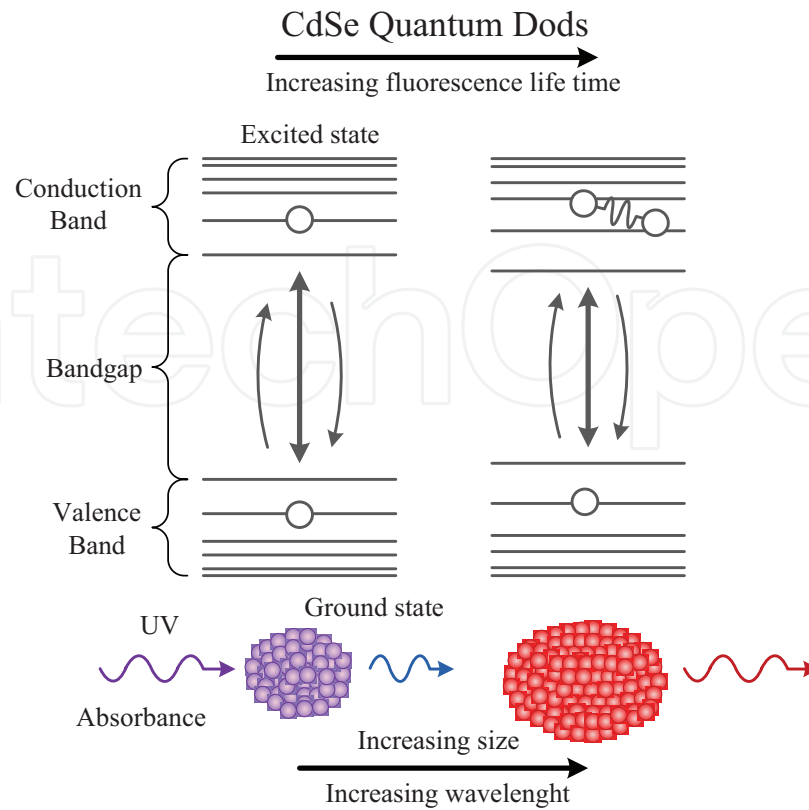
Figure 2. Band gap energy and size dependent wavelength of CdSe quantum dot [12].

Stokes shift

One of the most common features of QDs is the photoluminescence redshift relative to absorption, also called Stokes shift. It was named after Irish physicist George G. Stokes. Stokes shift is the difference between QD's peak excitation and the peak emission wavelengths (**Figure 3**) [5]. The energy associated with emission is typically lower than the excitation light. The redshift of emission peaks, with respect to absorption spectra, is size dependent. Stokes shift is commonly observed in semiconductor QDs, and is one of the most important quantities that determine the optical properties of QDs. The large separation between the excitation and emission spectra of the QDs improves the detection sensitivity, as the entire emission spectra of QDs can be detected. The Stokes shift of semiconductor QDs can be as large as 300–400 nm, depending on the wavelength of the excitation light. As the radius of quantum dot increases, the redshift decreases and disappears beyond a certain radius. Each QD has a unique emission maximum; changing the dot size leads to the emission maximum shift. In addition, QDs have very broad absorption spectra, and can be excited over the entire visual wavelength range as well as far into ultraviolet [13]. Because of their exceptionally large Stokes shifts of up to 400 nm, QDs can be used for the multicolor detection with a single wavelength excitation source (**Figure 4**) [14].
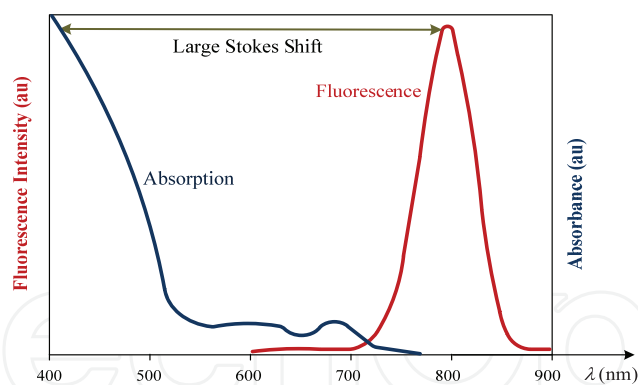
Figure 3. Broad absorption and narrow emission spectrum [15].

Fluorescence intensity and lifetime

The fluorescence of QDs is generated when the excited electron emits photon and returns to the ground-state. The lifetime is a delay between the moment of absorbtion a photon from the light sorce and moment of emitted light (**Figure 5**). The lifetime of 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 [10]. Long lifetime provides difference of QD fluorescence signal from background fluorescence. For example, see [17]. The life time of QDs and fluorophores is shown in **Figure 6**.

In order to extend the lifetime of the QDs, rare earths can be incorporated into the QDs which create local quantum states.
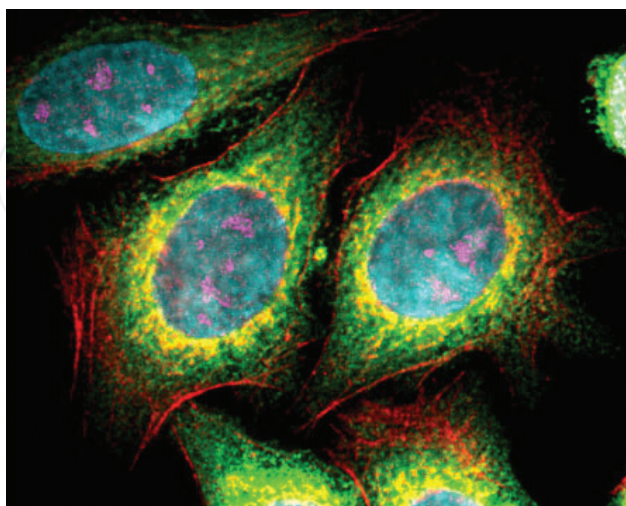

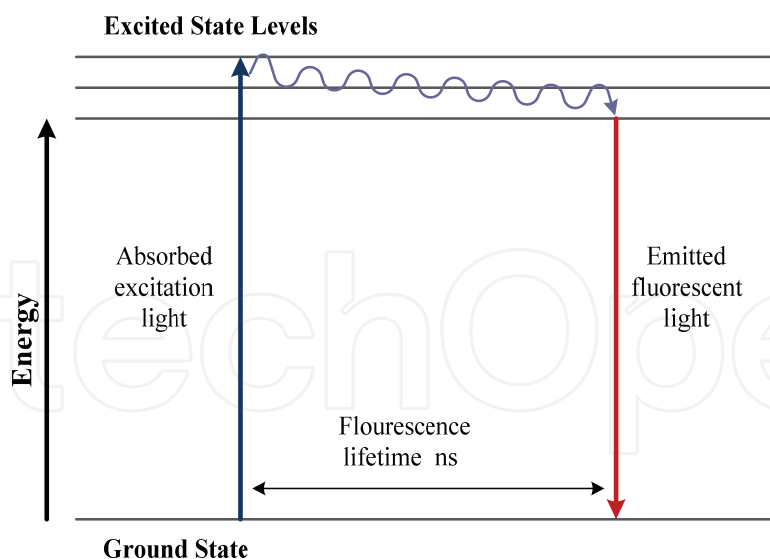**Figure 4.** Color of light depends on size quantum dot.

Figure 5. Fluorescence lifetime [2].

Brightness and photostability

Minimizing overlap between total excitation and emission bands enhances the clarity and brightness of the fluorescing QD by avoiding re-absorption of emitted light into nearby quantum dots—characteristics that display manufacturers and end-users find highly desirable. Broad absorption spectra make it possible to excite all QDs simultaneously with a single light source and minimize sample autofluorescence by choosing an appropriate excitation wavelength. QDs have very large molar excitation coefficient in the order of $0.5–5 × 10^6$ M^{-1}, about 10–50 times larger than that of organic dyes. QDs are able to absorb 10–50 times more photons than organic
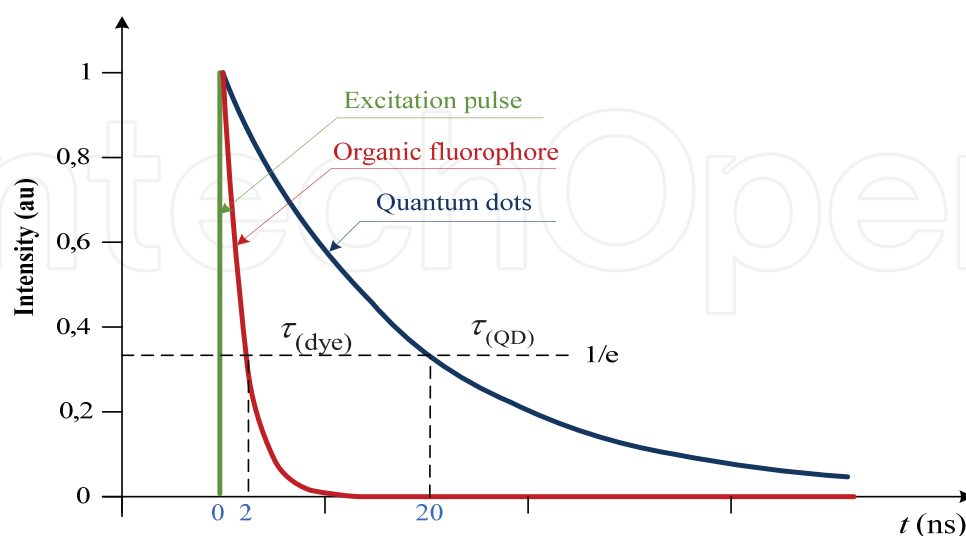

Figure 6. Fluorescence lifetime quantum dots and organic fluorophore [19].

dyes at the same excitation photon flux, leading to a significant improvement in the probe brightness. Owing to their inorganic nature, QDs have minimal interaction with the surrounding environment which contributes to their photostability. Inorganic QDs are more photostable under ultraviolet excitation than organic molecules, and their fluorescence is more saturated [18].

Quantum yield

Quantum yield is a measure of the "brightness" of a fluorophore and is defined as the ratio of the number of photons emitted to the number of photons absorbed. Some organic dyes have quantum yields approaching 100%, but conjugates (from biological affinity molecules) made from these generally have a significantly lower quantum yield. QDs are relatively efficient with regards to conversion of the excitation light into emission, where the quantum yield is generally over 50%. QDs retain their high quantum yield even after conjugation to biological affinity molecules [14]. The fluorescence quantum yield gives the efficiency of the fluorescence process.

Photobleaching

Photobleaching (fading) is the photochemical alteration of a dye or a fluorophore molecule, such that it is permanently unable to fluoresce. This is caused by cleaving of covalent bonds or non-specific reactions between the fluorophore and surrounding molecules. QDs are several thousand times more stable against photobleaching than organic dye molecules, and are thus ideal probes for fluorescent spectroscopy and bioimaging applications [20].

4. Core/shell structure quantum dot

Quantum dots are used as bare core or as core/shell structures. Although these "core" QDs determine the optical properties of the conjugate, they are by themselves unsuitable for biological probes owing to their poor stability and quantum yield [21]. In fact, the quantum yield of QD cores has been reported to be very sensitive to the presence of particular ions in solution [22]. A bare nanocrystal core is highly reactive and toxic, resulting in a very unstable structure that is prone to photochemical degradation. The core is highly reactive due to their large surface area/volume ratio, resulting in a very unstable structure which is particularly prone to photochemical degradation. Capping the core with a semiconductor material of a higher band gap not only increases the stability and quantum yield, but also passivates the toxicity of the core by shielding reactive ions from being exposed to photo-oxidative environments, e.g., exposure to UV and air (**Figure 7**). Traditionally, the typical QDs consist of a II–IV, IV–VI, or III–V semiconductor core (CdTe, CdSe, Pb, Se, GaAs, GaN, InP, and InAs), which is surrounded by a covering of wide band gap semiconductor shell as zinc sulfide ZnS or cadmium sulfide CdS is generally used (approx. 1.5 nm thick) [23]. Capping core nanocrystals with ZnS has shown to increase stability and performance, producing QDs with improved photophysical and chemical properties at room temperature. However, ZnS capping alone is not sufficient to stabilize the core, particularly in biological solutions [24]. Quantum dots do not exhibit aqueous solubility as they are generally synthesized in organic solution. The
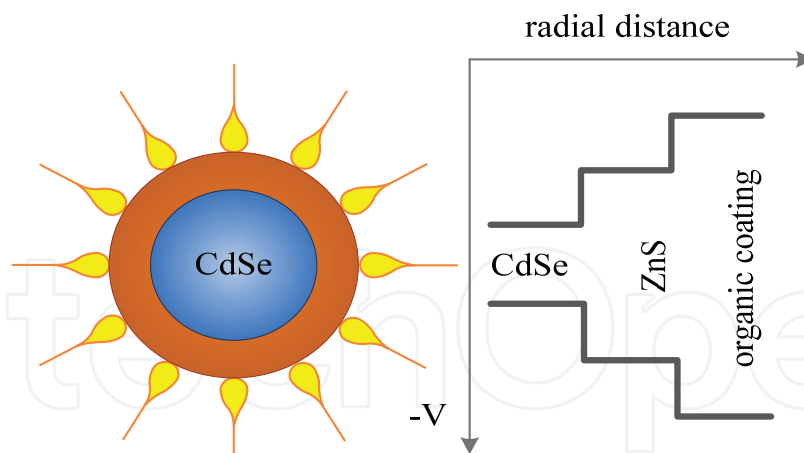
Figure 7. Band gap energy core/shell/ligand structure [12].

outmost surface of these nanoparticles is passivated with an organic ligand.[1] QDs are made water soluble by surface modifying them with various caps to ensure aqueous solubility and enhancing biocompatibility. The three important features of QDs used in the display industry, called core-shell QDs, are the core, shell, and ligand (**Figure 8**).

When these three features of QDs are tuned to how we need them, we develop exciting new applications. In order to utilize in a biological environment, they need to be made hydrophilic. Thus, they are necessarily made water soluble by surface modifying them with various bifunctional surface ligands or caps to promote aqueous solubility and enhancing biocompatibility [25]. QDs must be conjugated with molecules which have the capabilities of recognizing the target. These surface modifications can also help prevent aggregation, reduce the nonspecific binding, and are critical in achieving specific target imaging in biological studies [26].

4.1. Surface coatings and water-solubility

The key to develop QDs as a tool in biological systems is to achieve water solubility, biocompatibility, and photostability [27]. QDs, single or core/shell structures, do not exhibit aqueous solubility. After synthesis, hydrophobic QD must be covered with an organic layer or incorporated within the organic shell to make them water-soluble and biocompatible [28]. Some of the techniques used to achieve solubilization include ligand exchange, surface silanization, and phase transfer method [29].

4.1.1. The ligand exchange method

Native cap exchange is a strategy in which the native hydrophobic layer (TOPO/TOP)[2] cap is replaced with a bifunctional moiety that can bind QDs from one side while exposing hydrophilic

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[1]As organic ligands, molecules and ions, containing O, S, N, and P, are bonded so that their electronic pair can produce a covalent bond with the central atom.
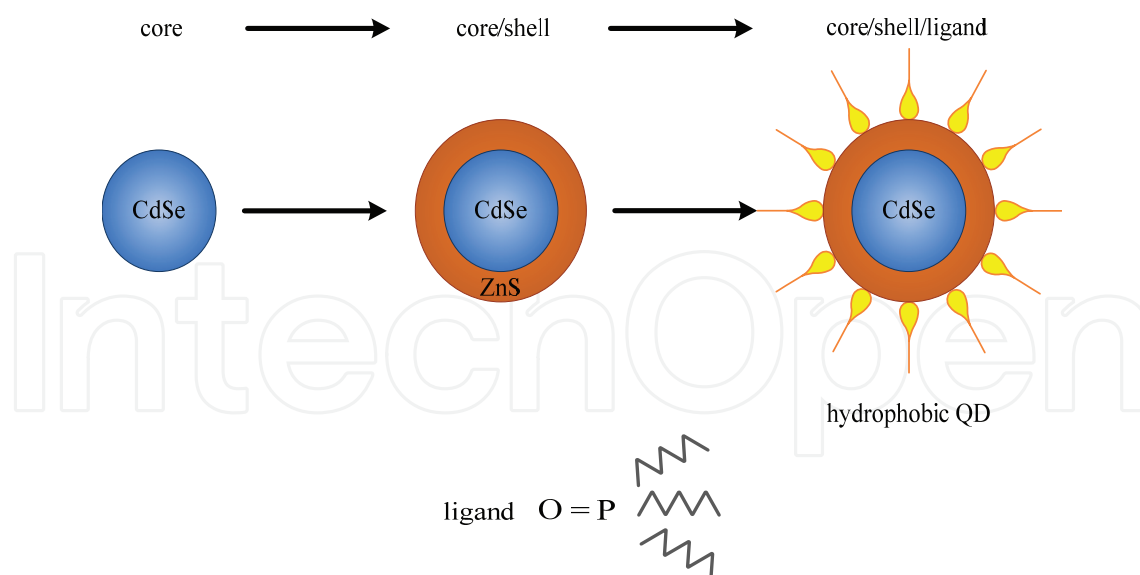[2]TOPO/TOP: Typically trioctylphosphine oxide/trioctylphosphine.

Figure 8. Core/shell/ligand structure of hydrophobic quantum dot [12].

groups on the surface to achieve optimal dispersion [30]. The ligand exchange method includes the exchange of the hydrophobic surfactant molecules with bifunctional molecules, which are hydrophilic on one side and hydrophobic on the other, to bind to the ZnS shell on the QD [23] (**Figure 9**). These biocompatible polymers usually have functional anchor groups, such as thiol, amine, and carboxyl, which can passivate QDs more strongly than the original ligand [31]. Most often thiols (−SH) as functional groups are used to bind to the ZnS and carboxyl (−COOH) groups are used as hydrophilic ends. The resulting QDs are soluble in both aqueous and polar solvents [29]. Biomolecules, such as proteins, peptides, and DNA, were also conjugated to the free carboxyl groups by crosslinking to the reactive amine. This process did not affect the optical characters of the QDs compared with the original QDs.

4.1.2. Surface silanization

Surface silanization involves the growth of a silica shell around the nanocrystal. As silica shells are highly cross-linked, they are very stable [32]. Silica coating enhances the mechanical stability of colloidal QDs and protects them against oxidation and agglomeration. The adventure of silica encapsulation is QDs chemical stability over a much broader pH range compared to carboxy-terminated ligands [29]. The chemistry of glass surfaces can be readily extended to silanized QDs, providing more flexibility for bioconjugation (**Figure 9**).

4.1.3. Polymer coating (phase transfer)

Polymer coating (phase transfer) method uses amphiphilic polymers to coat the surface [33, 34]. The hydrophobic alkyl chains of the polymer interdigitate with the alkyl groups on the QDs surface, while the hydrophilic groups orientate outwards to attain water solubility. However, coating with a polymer may increase the overall diameter of the QDs, and this
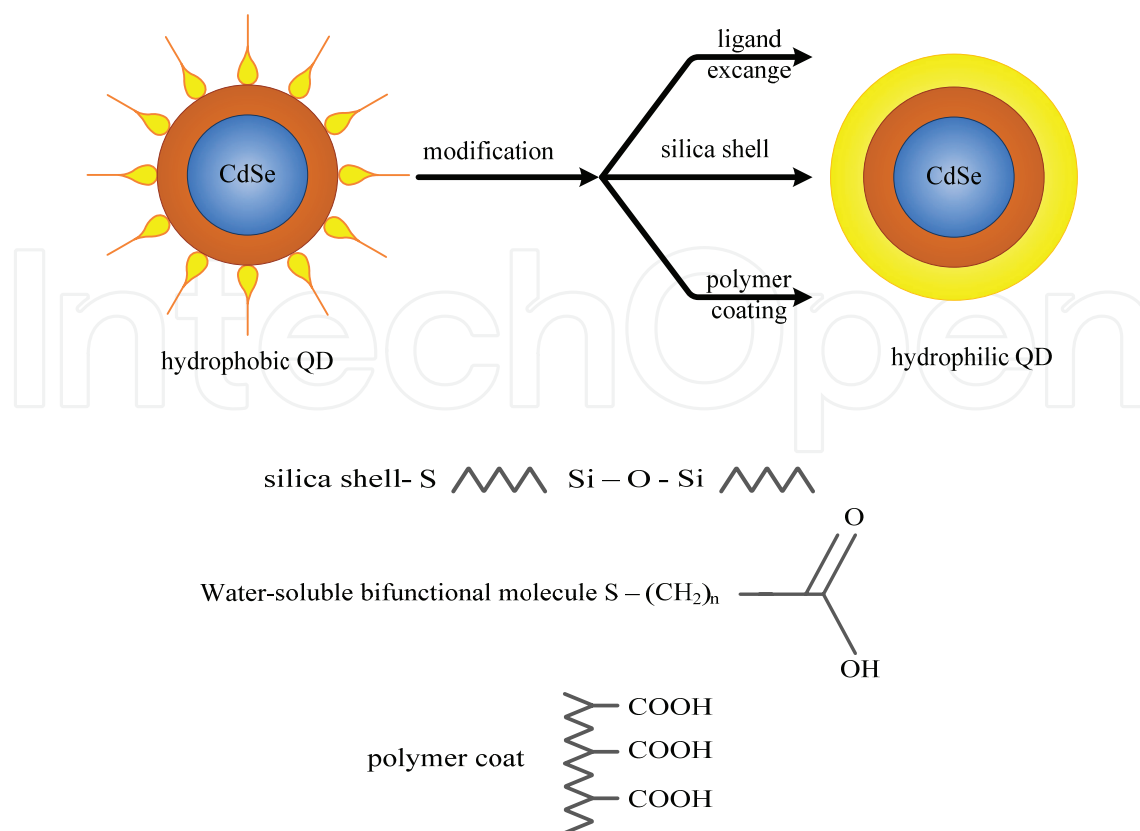
Figure 9. Modification from hydrophobic to hydrophilic quantum dots [12].

may reduce emission and limits their use biological applications [35]. The aqueous coating can then be tagged with various biomolecules of interest. Biomolecules, such as proteins, peptides, and antibodies, were conjugated to the free functional groups by crosslinking to the reactive amine. This process did not affect the optical characters of the QDs compared with the original hydrophobic surfactant layer.

5. Functionalization of quantum dots

Once solubilization has been achieved, QDs can be functionalized by conjugation to a number of biological molecules. QDs are adapted to the desired biological application by conjugation to biological molecules without disturbing the function of these molecules. QDs must be conjugated with molecules which have the capabilities of recognizing the target [36]. Biomolecules include antibodies, peptides, avidin, biotin, oligonucleotides, albumin, or by coating with streptavidin (**Figure 10**) [6]. Owing to large surface area-to-volume ratio, several biomolecules of varying types can be attached to a single QD. Each of these biomolecules provides a desired function which affords multi-functionality [37]. Various surface modification techniques were developed to ensure the specific bioconjugation: covalent linkage, electrostatic attraction, adsorption, and mercapto (−SH) exchange. The choice depends on the features of the biomolecule of interest
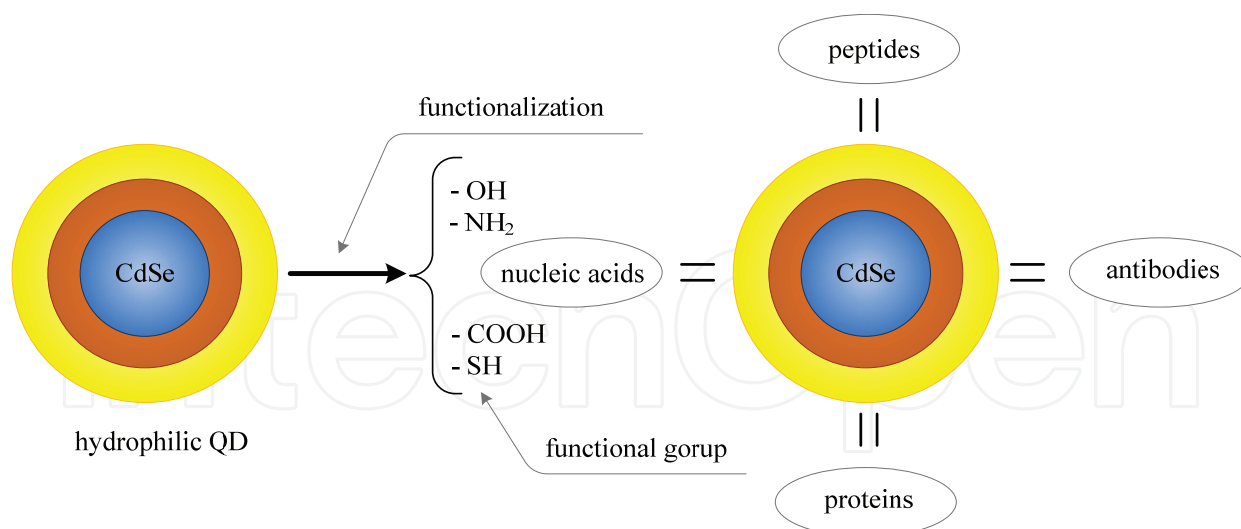
Figure 10. Functionalization of hydrophilic quantum dots [16].

[38]. Surface modifications can also help prevent aggregation, reduce the nonspecific binding, and are critical to achieving specific target imaging in biomedical studies [3]. QDs retain excellent stability of optical properties upon conjugation to biomolecules, and can be simultaneously excited by a single light source.

5.1. Covalent linkage

The first bioconjugation method offers a most stable covalent linkage of QDs to biomolecules and is the most commonly used approach for making biofunctionalized QDs for *in vivo* applications. Most water solubilization methods result in QDs covered with carboxylic acid, amino or thiol groups. Under these situations, it is easy to link QDs to biological molecules which also have these functional groups [39]. Using these methods, there have been numerous reports of conjugating QDs with various biological molecules, including proteins, antibodies, peptides, oligonucleotides, and albumin [5].

5.2. Electrostatic interaction

In the second procedure, an electrostatic interaction between QDs and charged adapter molecules or proteins with incorporated charged domains was employed [40, 41]. In this case, the protein of interest can be fused to a positively charged domain that will in turn bind electrostatically to the negatively charged surface of the QDs [6]. In the electrostatic interaction approach, the binding energies are highly dependent on both the chemical environment and the ambient temperature and QDs size can strongly affect the interaction efficiency. Electrostatic interactions generally are not sufficiently specific, however, given the complexity of biological milieu [5]. Therefore, conjugates made by this way are not suitable for *in vivo* or *ex vivo* cell labeling due to the possible interference with positively charged proteins [42].

5.3. Non-specific adsorption

Simple small molecules such as oligonucleotides and various serum albumins were found to readily adsorb to the surface of water-soluble QDs [6]. This adsorption is nonspecific and depends on ionic strength, pH, temperature, and surface charge of the molecule [5].

5.4. Mercapto exchange

Thiol-containing biomolecules can be conjugated to QDs via mercapto exchange. Thiols (SH) bond of mercapto group bind to the surface of the most often used semiconductor materials (CdSe, CdS, CdTe, and ZnS), and therefore QDs can be conjugated to biological molecules bearing mercapto (SH) groups in this way [43]. ZnS-thiol bond is not very strong. The conjugated biomolecules may break off and the QDs may precipitate out of solution [6].

Surface of QDs are decorated with different biomolecules: proteins, peptides, nucleic acids, ligands, or other biomolecules to achieve bioconjugation that mediate specific interactions with living tissue.

6. Toxicity of quantum dots

Most of the current studies were designed to ask questions concerning the physicochemical properties of novel QD products such as fluorescence, detectability, stability, and cell labeling efficacy, and not QD toxicity *per se*. Several studies reveal that the toxicity of QDs depends on many factors which can be summarized as inherent physicochemical properties and environmental conditions. Understanding the potential toxicity of QDs requires a fundamental grasp of QD physicochemical properties. Each QD type will need to be characterized individually to its potential toxicity. Each individual type of QD possesses its own unique physicochemical properties, which in turn determines its potential toxicity or lack thereof. QD sizes, charges, concentrations, outer coating materials and functional groups, oxidation, and mechanical stability all have been implicated as contributing factors to toxicity [44]. Most QDs consist of heavy metal which may be potentially toxic. Possibly the most important aspect of QD toxicity is their stability, both *in vivo* and during synthesis and storage. QDs with an outer ZnS shell, referred to as core-shell QDs, are generally more chemically stable. Degradation of the QD coating may also result in reaction of the QD in undesirable/unanticipated ways *in vivo*. Further, some QD coating materials have themselves been found to be cytotoxic. Cytotoxicity of QDs has been observed in a large number of *in vitro* studies [45], affecting cell growth and viability [22]. Several studies suggest QD cytotoxicity to be due to photolysis or oxidation. Under oxidative and photolytic conditions, QD core-shell coatings have been found to be labile, degrading, and thus exposing potentially toxic "capping" material or intact core metalloid complexes or resulting in dissolution of the core complex to QD core metal components, e.g., Cd and Se [46]. The toxicity is associated with the cadmium, lead, or arsenic containing QDs. For *in vivo* studies, the main concern is the robustness of the surface coating.

An unstable surface coating could expose the core of the QD to UV damage or air oxidation. The toxic heavy metal ions can be easily leaked out into biological systems, if the surfaces are not properly covered by the shells or protected by ligands [44]. The stability of QDs and their resistance to metabolic degradation in live cells would allow long-term imaging studies, and several studies have indicated lack of cytotoxicity for period up to 4 months [47]. Although such QDs should not be acutely toxic as long as their polymer coating is stable enough to restrain the release of cadmium, both short- and long-term safety of QDs will need to be established in toxicological studies in clinically relevant animal models. Studies in cell lines have shown that QDs do not affect cell growth under normal media conditions and short-term administration of QDs into animals seems not to affect the metabolism and behavior of the animals [48]. Xiao and coworkers showed that the cytotoxicity of CdSe quantum was proportional to QDs concentration in the tested range. There was a negligible cytotoxicity at concentration of 20 nM, which is twice of the applied concentration of fluorescent cell imaging test. On the other side, no size-dependent cytotoxicity was observed. For time-dependence, cytotoxicity increased along with prolongation of incubation period, and tended to be stable after 12 h of incubation. Therefore, cytotoxicity can be neglected in a typical fluorescent cell imaging procedure (10 nM/L, 12 h), and the labeling effect to microtubule can be guaranteed satisfactorily [49]. There are also concerns about toxicity related to the molecules used for surface functionalization of the QDs [6]. However, toxicity concerns are still valid for longer periods and *in vivo* applications. Given the highly intrinsic toxicity of cadmium, the biological applications of Cd-QDs have been limited. The limitation of heavy metal containing QDs stimulates extensive research interests in exploring alternative strategies for the design of fluorescent nanocrystals with high biocompatibility. In this case, the practical strategy is to develop highly fluorescent nanoparticles based on nontoxic elements [3].

6.1. Nontoxic quantum dots

The last few years of research are focused on QDs that do not contain Cd. QDs made up of III–V semiconductors such as InP. Groups III–V QDs may provide a more stable alternative to groups II–VI (CdSe, Cd, Te, etc.) [50]. QDs due to the presence of a covalent, rather than an ionic bond, and have been reported to have lower cytotoxicity. However these QDs are difficult to prepare on a competitive time scale, and tend to have much lower quantum efficiencies, meaning uptake has been slow [51]. The most highlighted candidates for Cd-free QDs are the InP-based QDs. InP QDs a very attractive candidate for replacing cadmium-based QDs for biological studies. However, till date, there are very little studies reporting the use of InP QDs in bioimaging applications, as they are difficult to prepare because of the sensitivity of precursors and surfactants toward the reaction environment in obtaining good quality InP QDs [52]. Therefore, progresses in the synthesis of InP-based QDs occurred much slowly compared to that of CdSe QDs [53]. InP/ZnS QDs combining the brightness, photostability, and biocompatibility characteristics will serve as a new generation of targeted optical probes for several biomedical applications, including early detection of cancer, replacing the cadmium-based [54]. Silicon nanoparticle is another type of QDs which possess unique properties when their sizes are reduces to below 10 nm. Similar to their predecessors, silicon QDs also have many advantages over traditional fluorescent organic dye. Moreover, owing to silicon's nontoxic

and environment-friendly nature, Si QDs are used as fluorescent probes for bioimaging. Si QDs are produced in nonpolar solvents with hydrophobic ligands on their surface in other to protect the Si core. Therefore, it is a common problem that silicon QDs show poor solubility and unstable photoluminescence in aqueous solutions. Thus, fabricating good water-disperse Si QDs with stable optical characters is vital to their applications in bioimaging studies. In the demand of using biocompatible and nontoxic QDs as nanoprobes, rare-earth (RE) elements are used to fabricate a new type of QDs, such as Gd-doped ZnO QDs [44].

7. Adventures of quantum dots compared to conventional fluorophores

The ability to study molecular and cellular events by using fluorescent probes has broadly impacted many areas in biomedical research. QDs offer several adventures over traditional fluorophores. Traditional fluorophores have certain properties that limit their biomedical applications: narrow absorption and broad emission spectra, low luminescence and quantum yield, rapid photobleaching, and low brightness signal. A limitation of traditional small-molecule fluorescent dyes is in the labeling of other small molecules, drugs, transporters, and small-molecule probes to cell-surface receptors. Conjugates of dyes to these small molecules often lack sensitivity or specificity in the detection of the desired targets. Conjugates of small molecules to QDs produce conjugates with much greater light output per binding event, owing to the increased absorbance and emission of the QD [14]. The fact that several QDs can be excited by the same wavelength of light opens up several multiplexing potentials, including high-throughput screening of biological samples [9]. Size-tunable absorption and emission property of QDs is an extremely valuable property for biological imaging as they can be tuned all the way from the UV to the near-infrared of the spectrum. QDs exhibit better photophysical properties than conventional fluorophores under appropriate conditions. A limitation of traditional small-molecule fluorescent dyes is in the labeling of other small molecules, drugs, transporters, and small-molecule probes to cell-surface receptors. Conjugates of dyes to these small molecules often lack sensitivity or specificity in the detection of the desired targets. Conjugates of small molecules to QDs produce conjugates with much greater light output binding event, owing to the increased absorbance and emission of the QD. Furthermore, there is the possibility of improved avidity compared of a dye conjugate, owing to the combined effect of many molecules of the binding ligand on the surface of the quantum dot [14]. Biocompatible QDs represent a powerful tool for the direct readout of information down to single molecule level [28]. For example, by conjugating an antibody to the QD, targeting to specific cells or tissues can be affected *in vivo* or in fluorescent antibody immunoassays. QDs have shown great potential to provide spatial, temporal, and structural information for biological systems: *in vivo* cell labeling, *in vitro* cell labeling, detection of tumor marker, *in situ* tissue diagnostic, noninvasive tumor imaging, tracking of local cancer grown and its distant dissemination, detection and therapy of various disease, identifying of various types of biomarkers, more effective and early diagnosis of cancer, imaging of live tissue, etc.

8. Conclusion

Quantum dots have emerged as a new promising class of fluorescent probes for biomolecular and cellular imaging. In comparison with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties. QDs do not exhibit aqueous solubility as they are generally synthesized in organic solution and are surface-stabilized with hydrophobic organic ligands. To make them useful for biomedical applications, QDs need to be conjugated to biological molecules without disturbing the biological function of these molecules. Various surface modification techniques were developed to ensure the specific bioconjugation. This is usually achieved by decorating QDs with proteins, peptides, nucleic acids, streptavidin, or other biomolecules that mediate specific interactions with living systems. Biocompatible quantum dots represent a powerful tool for the direct readout of information down to single molecule level. The limiting factor of application QDs *in vivo* is their toxicity. Although QD technology is still not in much use due to their hydrophobicity, toxicity, and many issues need to be solved in order to apply them safely in biomedical applications.

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