BIOCOMPATIBILITY OF CADMIUM-SELENIDE QUANTUM DOT

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Abstract

Quantum dots are a group of semiconducting nanomaterials with unique optophysical properties.QDs with superior optical properties are a promising alternative to organic dyes for fluorescence biomedical applications. They are inorganic fluorophores that have size-tunable emission, strong light absorbance, and very high levels of brightness and photostability.The most popular type of QDs is CdSe, cadmium selenide have become one family of the most extensively studied fluorescent semiconductor nanocrystals due to their suitable and tunable bandgap throughout the visible spectrum. Hidrophobic CdSe QDs are insoluble in aqueous solution and can´t directly employed in biomedical applications.They are necessarily made water soluble by surface modifyng 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 molecule.

Key words: core/shell/ligand structure, hidrophobic QD, biocompatibility, hidrophilic QD, functionalization

Introduction

In the life sciences, fluorescence is widely used as a significant technique for people to study and understand the biological structure of organism, the cell-cell interaction and the interplay of biomolecules. In this technique, kinds of fluorophores are developed to label, detect and image the bio-targets. These fluorophores are small molecules, proteins or quantum dots (QDs) (Funct, J. 2011). QDs are semiconductor nanoparticles with the three dimensions confined to 2–8 nm length scale composed of groups II-VI or III-V elements. This particles are defined as particles whit physical dimensions smaller than the exciton Bohr radius[1]. Because of their reduces size, QDs behave differently from bulk solids due to the quantum – confinement effects that are responsible for their remarkably attractive properties intermediate between compounds and single molecules, namely intensive photoluminescence (Chomoucka, J et al, 2012). QDs play an important role in the imaging and as highly fluorescent probes for biomedical sensing that have good sensitivity, long stability, good biocompatibility, and minimum invasiveness.The most popular types of quantum dots include CdSe, CdTe, and ZnSe. The most commonly studied and used quantum dots is cadmium selenide.

Properties of quantum dots

Quantum dots have proven their potential in biomedical fields due to their excelent optical properties. At such small sizes quantum dot behaves differently to other forms of semiconductor.The electron and hole can move around in the bulk material, but there is the Coulomb attraction that keeps them together, and the electron orbits the hole at some average distance as in the Bohr model of the atom. As an example, in the semiconductor CdSe the radius of the electron orbit in the bulk material is ~56 Å. So, if you chemically synthesize a CdSe nanocrystal that has a radius smaller than 56 Å, you begin to "squeeze" the electron and hole, and there is confinement energy. The quantum mechanical confinement causes the energy states to shift to higher levels, or blue shift. The smaller you make the nanocrystal, the more you squeeze the electron and the hole, and the higher the energy levels go. So, large CdSe nanocrystals absorb and emit in the red, small nanocrystals absorb and emit in the blue, and the wavelength of emission can be tuned by size (Figure 1) (Rosenthal, S. J., 2011)

[1] The natural separation distance between the positive and negative charges in the excited state of a material

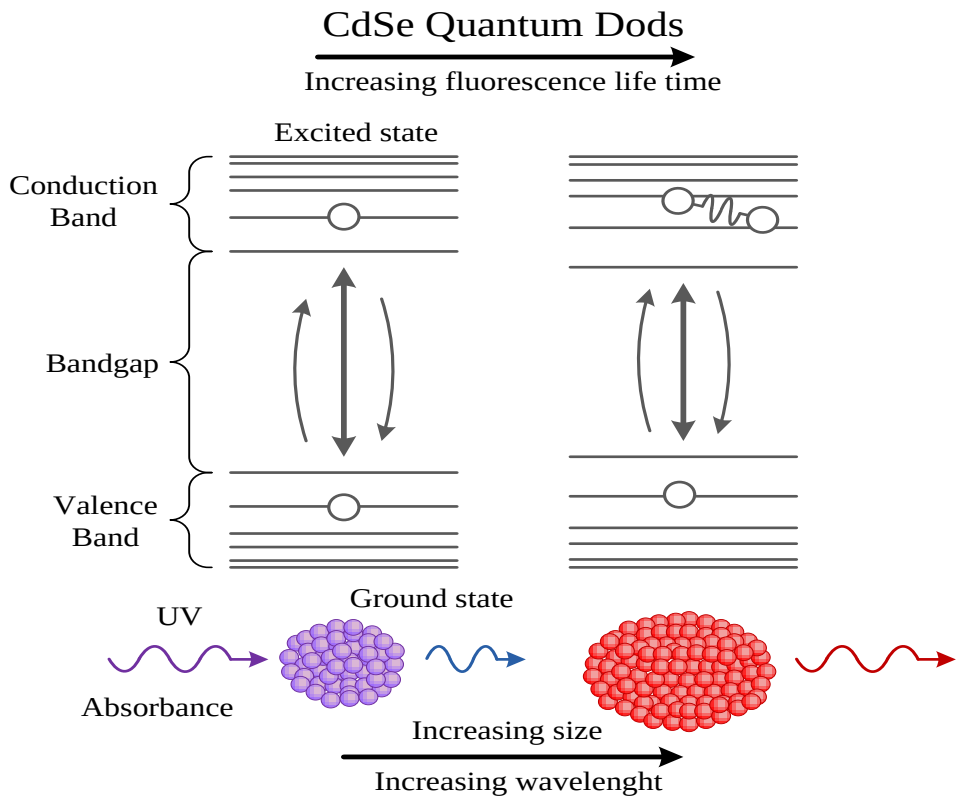

Figure 1. Bandgap energy and size dependent wavelenght of CdSe quantum dot

In a bulk material the electrons will occupy multiple energy levels. These energy levels are so close that they are considered continous. However, there some energy levels that the electrons cannot occupy collectivelly known as the bandgap. Quantum dots exibith size-dependent discrete energy levels. Most electrons occupy energy levels below this bandgap in an area known as the valence band . If an external stimulus is applied, an electron may move from the valence band to the conduction band (Figure 1). The electron in the conductance band and the hole it has left in the valence band are collectively known as an exciton. When the electron returns to a lower energy level, narrow, symmetric emission energy spectrum occurs.The energy bandgap increases with decrease in the size of the QD, thus yielding a size-dependent rainbow of colors (Chan et al, 2002).

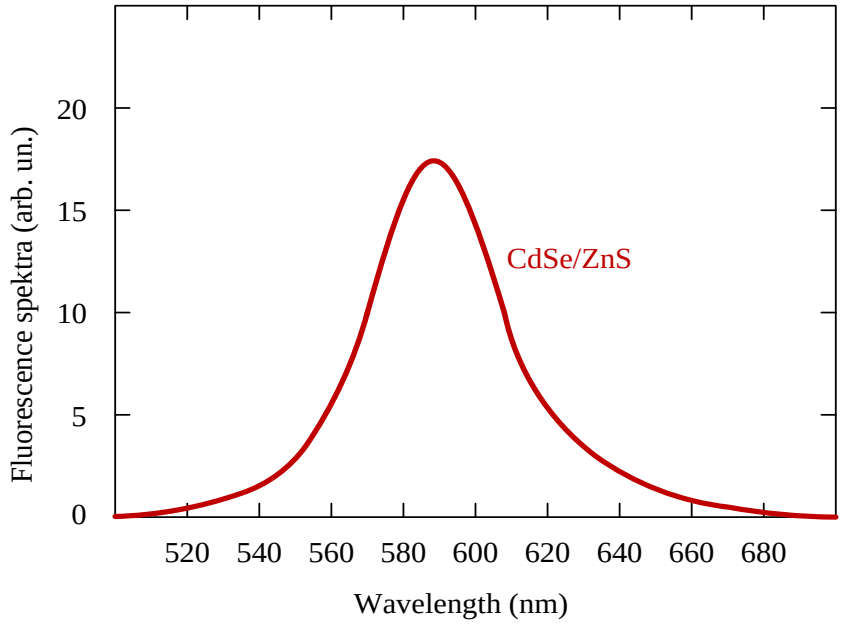

Figure 2. CdSe/ZnS fluorescence spectra

Adventures quantum dots compared to convenctional fluorofores

Since the first mention in the literature, in 1998, QDs have been studied extensively and the applications of these fluorescent nanocrystals range from imaging fixed and live cells all the way to fluoroimmunoassays rendering innovative diagnostic methodologies (Bruchez Jr. *et al*., 1998; Chan & Nie, 1998). 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 (Smith M, 2006). One additional feature of QDs is that they can emit in the infrared and near-infrared regions. 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. Quantum dots exhibit excellent photostability and broad absorption spectra, narrow emission spectra, and higher luminescence and quantum yield than conventional fluorophores under appopriate conditions (Table 1).

Core/shell structure CdSe/ZnS quantum dot. The classic and most commonly used quantum dots is cadmium selenide. Among various semiconductor materials, CdSe has been most extensively studied because its size-dependent photoluminescence is tunable across the visible spectrum. The emission wavelength of CdSe/ZnS quantum dots lies in the range of 470-655 nm (Figure 2.). Quantum dots are used as bare core or as core/shell structures. Although these “core” quantum dots determine the optical properties of the conjugate, they are by themselves unsuitable for biological probes owing to their poor stability and quantum yield (Malik, P. 2013). In fact, the quantum yield of quantum dot cores has been reported to be very sensitive to the presence of particular ions in solution (Chen Y,2002). A bare nanocrystal core is highly reactive and toxic, resulting in a very unstable structure that is prone to photochemical degradation. Also, the core crystalline structure has surface

Table 1. The comparasion of CdSe quantum dots and organic dye

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Brightness emission	(10-100) times than the best organic fluorophores
Fluorescent life times	(10-40) ns unlike conventional organic dyes~few ns
Molar extinction coefficient	10-100 fold higher than most organic dyes
Stokes shift	Large separation between the excitation and emission wavelenght maximum 300-400 nm
Autofluorescente contribution	Quantum dots permit reduce autofluorescente contribution
Light source	Single QD light source
Emission spectra	QDs have narow and symmetrical spectra 13nm-full width at half maximum
Photostability	Inorganic nature of QDs contribute their photostability
Photobleaching	QDs have 100 times stable
Quantum yield	To 90% with ZnS shell

irregularities that lead to emission irregularities like blinking. Capping the core with a semiconductor material of a higher bandgap, not only increases the stability and quantum yield, but also passivates the toxicity of the core by shielding reactive Cd^{2+} ions from being exposed to photo-oxidative environments, e.g. exposure to UV and air (Figure 3) . In the case of cadmium selenide cores (2-7nm in diameter), zinc sulfide ZnS (approx 1.5 nm thick) or cadmium sulfide CdS is generally used (Alivisatos, 2005) (Figure 4). "Core-shell" quantum dots are much brighter, and more stable in various chemical environments (Alivisatos, 2005). Fluorescence properties are determined by the core materials and the shell layer removes surface defects and prevents nonradiative decey leading to a significant improvement in the particle stability and fluorescence quantum yields. These core-shell quantum dots are hydrophobic and only organic soluble as prepared. The outmost surface of these nanoparticle is passivated with a organic ligand.

The ligands, usually long chain molecules ensure that the quantum dot can be printed in a liquid during manufacturing, as well as play an important role in the electronic properties. These organic capping ligands are always nonpolar, which means that another step in the synthesis of biologically useful nanocrystals is to make them soluble in a buffer. An original approach to this problem was to exchange the nonpolar ligands on the surface of the nanocrystal with polar ligands, usually using a thiol functional group for attachment to the surface of the nanocrystal (Chan and Nie, 1998; Pathak et al., 2001).

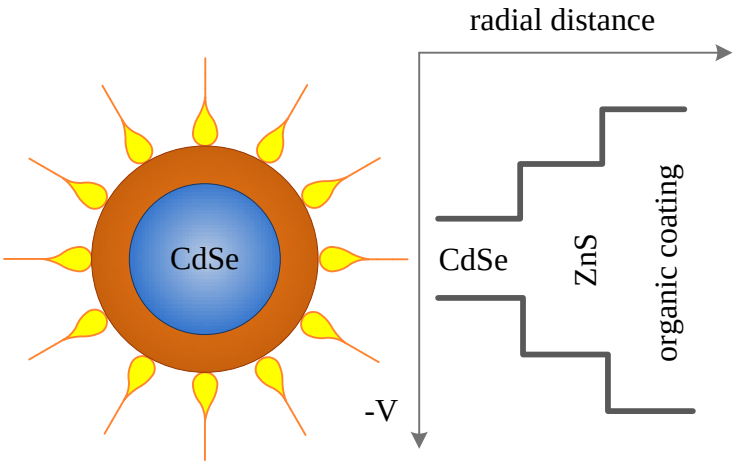

Figure 3. Bandgap energy core/shell/ligand structure

The three important features of quantum dots used in the display industry, called core-shell quantum dots, are the core, shell, and ligand[2] (Figure 4).

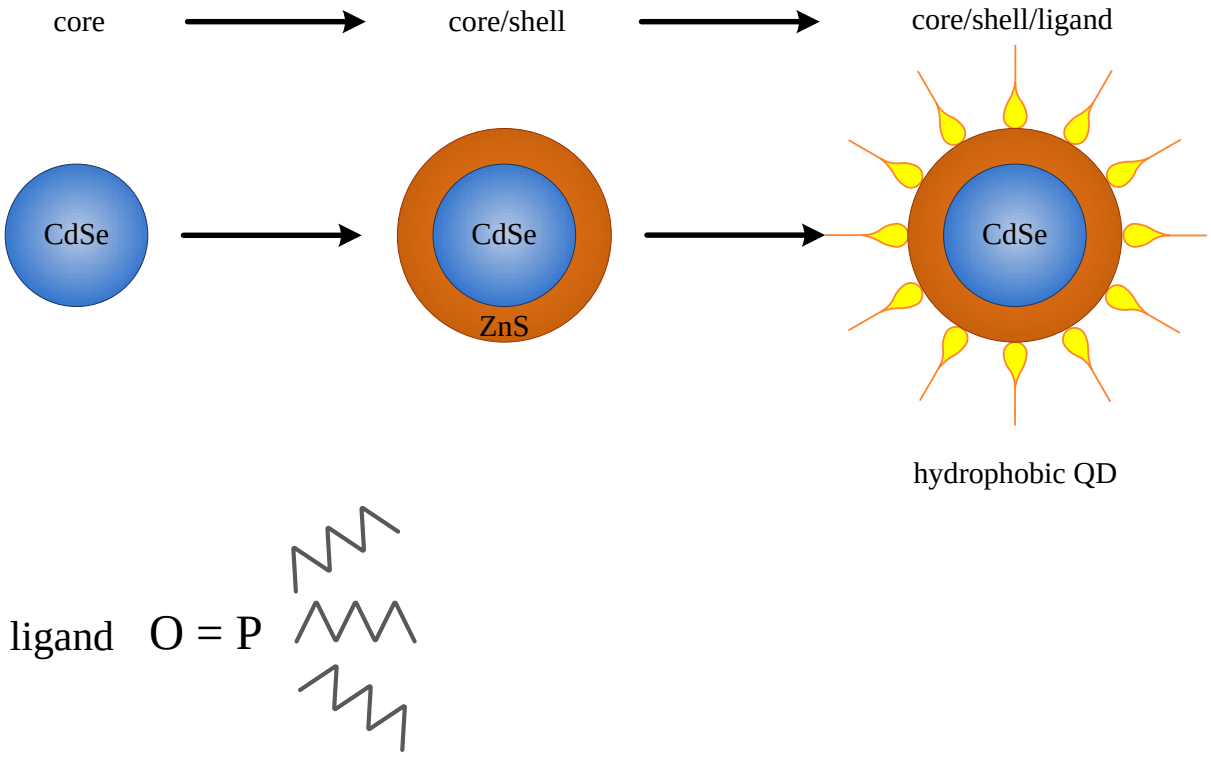

Figure 4. Core/shell/ligand structure of hidrophobic CdSe quantum dot

The ligands, usually long chain molecules ensure that the quantum dot can be printed in a liquid during manufacturing, as well as play an important role in the electronic properties. These organic capping ligands are always nonpolar, which means that another step in the synthesis of biologically useful nanocrystals is to make them soluble in a buffer. An original approach to this problem was to exchange the nonpolar ligands on the surface of the

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

nanocrystal with polar ligands, usually using a thiol functional group for attachment to the surface of the nanocrystal (Chan and Nie, 1998; Pathak et al., 2001).
When these three features of quantum dots 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. Quantum dots do not exhibit aqueous solubility as they are generaly synthesized in organic solution and are surface –stabilized with hydrofobic organic ligands. Thus, they are necessarily made water soluble by surface modifying them with various bifunctional surface ligands or caps to promote aqueous solubility and enhancing biocomptibility.Quantum dots must be conjugated with molecules which have the capabilities of recognizing the target. These surface modifications can olso help prevent aggregation, reduce the nonspecific binding, and are critical to achieving specific target imaging in biological studies.

Surface coatings and water-solubility

The key to develop QDs as a tool in biological systems is to achieve water solubility, biocompatibility and photostability, and importantly, to provide flexible quantum dot surface chemistry/functionality that will enable efficient coupling of these fluorescent inorganic probes to reagents capable of targeting and/or sensing ongoing biological processes (Hongyou, F et al., 2005). Quantum dots, single or core/shell structures, do not exhibit aqueous solubility. After synthesis, the core/shell CdSe/ZnS quantum dot must be covered with an organic layer or incorporated within the organic shell to make them water-soluble and biocompatible (Weal M et al.,2010).
Some of the tehniques used to achive solubilisation include ligand exchange, surface silanization and phase transfer methods..

The ligand exchange method. Native cap exchange is a strategy in which the native hydrophobic layer (TOPO/TOP)[3] cap is replaced with a bifunctional moiety that can bind QDs from one side while exposing hydrophilic groups on the surface to achieve optimal dispersion. The exchange of the hydrophobic surfactant molecules with bifunctional molecules, which are hydrophyllic on one side and hydrophobic on the other, to bind to the ZnS shell on the QD (Alivisatos et al., 2005). The ligand exchange procedure entails replacing the as-grown ligand e.g., phosphine oxide introduced during the QDs synthesis with new biocompatible polymers (Figure 5). These biocompatible polymers usually have functional anchor groups, such as thiol, amine and carboxyl, which can passivate QDs more strongly than the original ligand.Most often thiols (-SH) as functional groups are used to bind to the ZnS and carboxyl (-COOH) groups are used as hydrophyllic ends. The resulting QDs are soluble in both aqueous and polar solvents. 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.

[3] TOPO/TOP: Typically trioctylphosphine oxide/trioctylphosphine

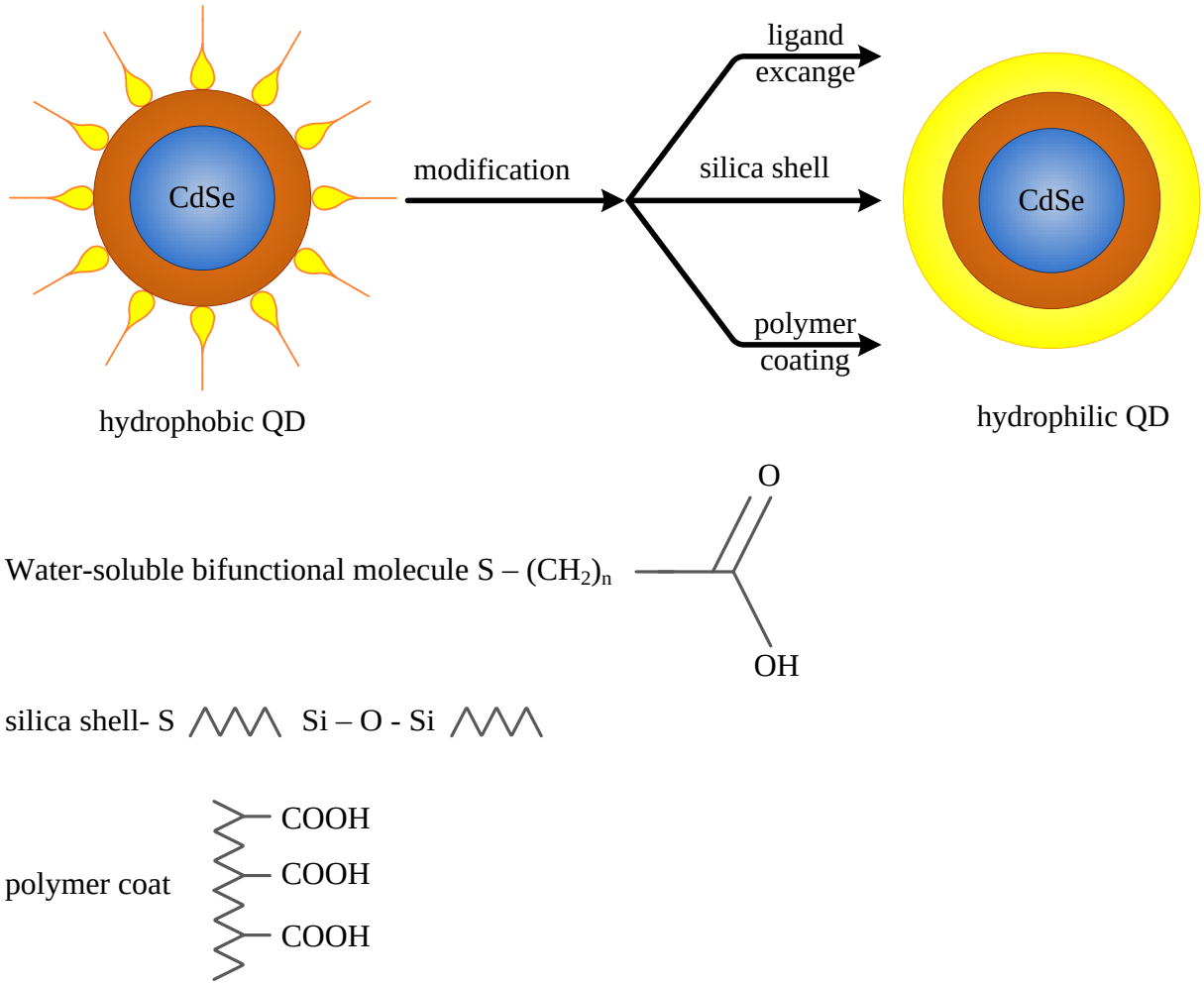

Figure 5. Modification from hidrophobic to hidrophilic quantum dots

Surface silanization involves the growth of a silica shell around the nanocrystal. As silica shells are highly cross-linked they are very stable (Alivisatos, 2004). Silanization offers several advantages over use of thiolated compounds for QD capping. First, the outermost siloxane shell in which the QDs are encapsulated is extensively crosslinked compared with one or a few bonds in the case of thiolated compounds. Second,silica coating enhances the mechanical stability of colloidal quantum dots and protects them against oxidation and agglomeration.The adventure of silica encapsulation is QDs chemical stability over a much broader pH range compare to carboxy-terminated ligands (Chomoucka,J 2012). Finally, the chemistry of glass surfaces can be readily extended to silanized QDs, providing more flexibility for bioconjugation (Figure 5.).

Polymer coating (phase transfer) method uses amphiphyllic polymers to coat the surface (Nann, 2005, Wang et al., 2004). The hydrophobic alkyl chains of the polymer interdigitate with the alkyl groups on the QDs surface while the hydrophyllic groups orientate outwards to attain water solubility. However, coating with a polymer may increase the overall diameter of the QDs and this may reduce emission and limits their use biological applications(Figure 5) (Uyeda et al., 2005).

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.

Functionalization of quantum dots

Once solubilisation has been achieved, QDs can be functionalised 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. Quantum dots must be conjugated with molecules which have the capabilities of recognizing the target (Jin, S. 2011). Biomolecules include antibodies, peptides, avidin, biotin, oligonucleotides, albumin, or by coating with streptavidin (Figure5) (Azzazy, H.M.E. 2007). Surface modifications can olso help prevent aggregation, reduce the nonspecific binding, and are critical to achieving specific target imaging in biomedical studies.

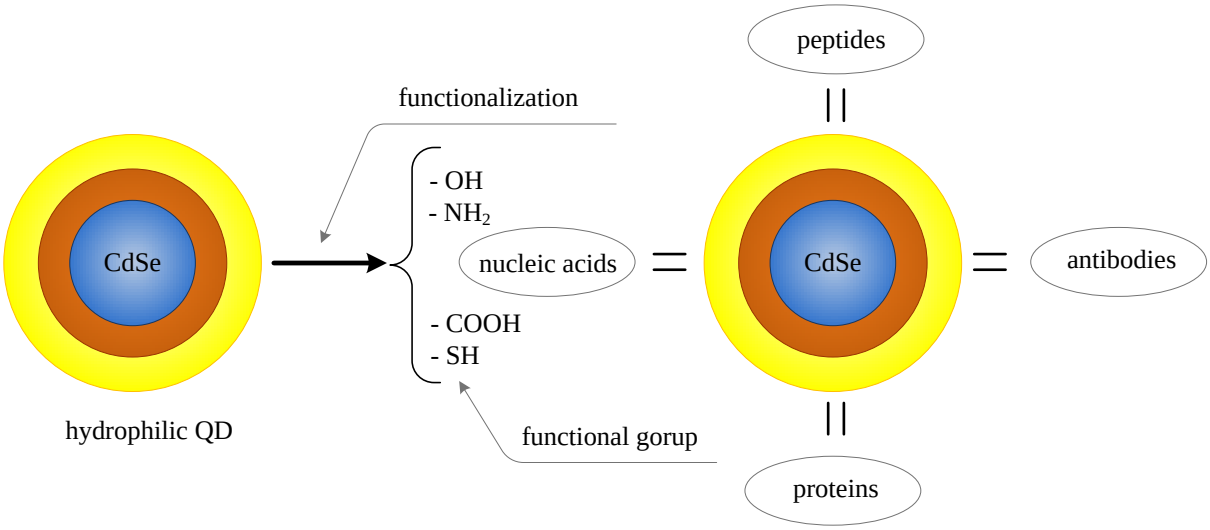

Figure 5. Functionalization of hidrophilic quantum dots

For example by conjugating an antibody to the quantum dot, targeting to specific cells or tissues can be affected *in vivo* or in fluorescent antibody immunoassays. When regular cells transform into cancer cells, their protein-expression profiles change dramatically. Certain proteins, called "cancer cell-specific antigens", are expressed on the surface of cancer cells. For cancer imaging, these antigens might also serve as targets or markers for diagnosis.

A limitation of traditional small-molecule fluorescent dyes is in the labeling of other small molecules, drugs, transporters, and small-molecule probes to cellsurface 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 quantum dots produce conjugates with much greater light outputper binding event, owing to the increased absorbance and emission of the quantum dot. Furthermore, there is the possibility of improved avidity compared o a dye conjugate, owing to the combined effect of many molecules of the binding ligand on the surface of the quantum dot (Charles Z. Hotz, 2007).

In order to suitably functionalize the QDs, there are several methods that have been successfully used for conjugation of QDs to the desired biomolecules. These include electrostatic attraction, covalent linkage, adsorption, and mercapto (-SH) exchange (Azzazy, H.M.E. 2007).

Biocompatible quantum dots represent a powerful tool for the direct readout of information down to single molecule level (Weal, M .2010). 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. Quantum dots 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 toumor imaging, tracking of local cancer grown and its distant dissemination, detection and therapy of variors disease, identyfaing of variors types of biomarker, more effective and early diagnosis of cancer, imaging of live tissue etc.

Conclusion

Quantum dots have emerged as a new promising class of fluorescent probes for biomolecular and cellular imaging.In comparasion with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties.The most commonly studied and used QD is CdSe core and shell layer made of ZnS. Quantum dots do not exhibit aqueous solubility as they are generaly synthesized in organic solution and are surface-stabilized with hydrophobic organic ligands. To make them useful for biomedical applications, CdSe/ZnS quantum dots 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 whit living systems. Biocompatible quantum dots represent a powerful tool for the direct readout of information down to single molecule level.

References

J. Funct, 2011.,Biocompatible Polymer/Quantum Dots Hybrid Materials: Current Status and Future Developments Lei Shen Department of Chemistry & Biochemistry, The University of Texas at Austin,

Chomoucka Jet al., Modern Micro and Nanoparticle-Based Imaging Techniques, Sensors (Basel). 2012 Nov 2;12(11):14792-820. doi: 10.3390/s121114792. Review.

Rosenthal, Sandra J. et al., Biocompatible Quantum Dots for Biological Applications, Chem Biol. 2011 Jan 28; 18(1): 10–24.

Chan et al.; Curr., Opin.Biotehnol.2002,13,40

Bruchez M Jr, Moronne M, Gin P et al., (1998) Semiconductor nanocrystals as fluorescent biological labels. Science 281:2013–2016.

Chan WC, Nie S.,(1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection Science: 1998 Sep 25;281(5385):2016-8.

Smith, M.et al, 2006., Engineering luminescent quantum dots for in vivo molecular and cellular imaging. Ann. Biomed. Eng. 34: 3-14.

Chen Y. and Rosenzweig, Z., Luminiscent CdSe quantum dotsas selective ion probes. Anal.Chem.74,5132-5138.

Malik P. et al.,Quantum dots for diagnosis of cancers, Adv. Mat.Lett.2013.4 (11),811-822

Alivisatos P,Gu W, Larabell C., Quantum dots as cellular probes. Annu ReV Biomed Eng 2005;7:77-76

Chan WC, Nie S., Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science. 1998;281:2016–2018. [PubMed]

Pathak, S., et al., Hydroxilated QDs as luminiscent probes for in situ hybridization" J.Am,Chem.Soc. 2001 123(17):4103-4104

Hongyou, F et al., Surfactant-Assisted Synthesis of Water-Soluble and Biocompatible Semiconductor Quantum Dot Micelles, Nano letters 2005

Weal M. et al., Emerging applications of fluorescent nanocrystals quantum dots for MMs detection, 2010.

Alivisatos P., The use nanocrystals in biological detection. Nat.Biotechnol 2004; 22:47-52

Nann, T. Phase transfer of CdSe@ZnS quantum dots using amphiphilic hyperbranched polyethylenimine. *Chem. Commun.* 1735–1736 (2005).

Wang et al., Surface passivation of luminescent colloidal quantum dots with poly(dimethylaminoethyl methacrylate) through a ligand exchange process. J. Am. Chem. Soc. 2004, 126, 7784-7785.

Uyeda, H. et al ,Synthesis of compact multidentate ligands to prepare stable hydrophilic quantum dot fluorophores. J. Am. Chem. Soc. 2005, 127, 3870-3878.

Jin S. et al., Application of Quantum Dots in Biological Imaging, Journal of nanomaterials Volume 2011, article ID 834139,13 pages

Charles Z. Hotz, Applications of Quantum Dots in Biology, Humana Press, 2007

Azzazy H.M.E. et al., From diagnostic to terapy:Prospects of quantum dots, Clinical biochemistry 40 (2007) 917-927