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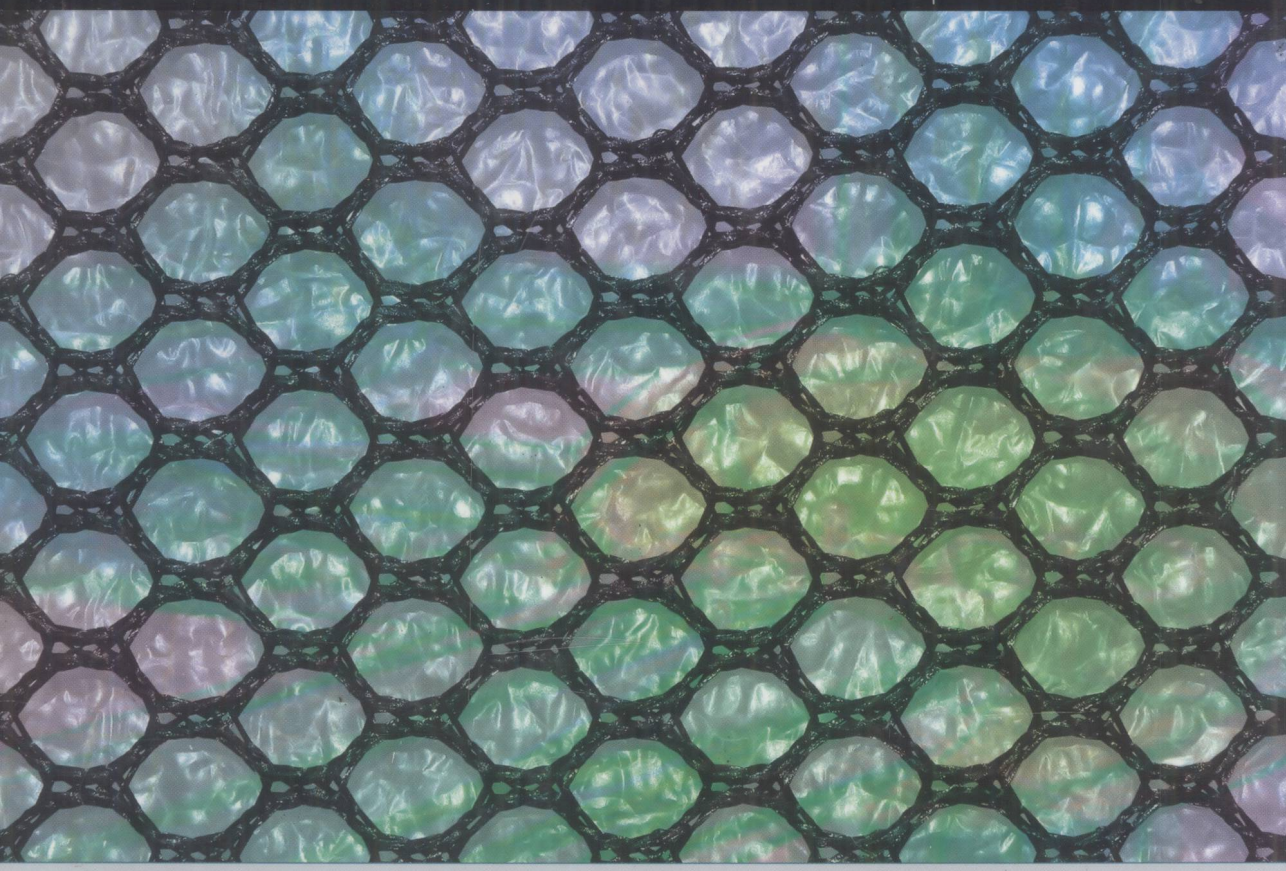

Inorganic Nanoprobes

FOR BIOLOGICAL SENSING AND IMAGING

Inorganic Nanoprobes for Biological Sensing and Imaging

Hedi Mattoussi
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Editors

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Contents

CHAPTER 1

Colloidal Quantum Dots: Synthesis, Photophysical Properties, and Biofunctionalization Strategies

Kimihiro Susumu, Igor L. Medintz, and Hedi Mattoussi

1.1 Introduction

Fluorescence tagging of biological molecules is a commonly used approach in biotechnology that has relied on conventional organic fluorophores and fluorescent proteins [1–3]. All available organic fluorophores and fluorescent proteins, however, have some inherent limitations that reduce their effective use to develop biological sensing and imaging. Among these, the most limiting properties are narrow excitation spectral windows, broad photoluminescence (PL) spectra, and low resistance to chemical and photo-degradation [4, 5]. Luminescent semiconductor nanocrystals—often referred to as quantum dots (QDs), such as those made of CdSe and PbSe cores—in comparison offer several unique properties and promise significant advantages in certain bioanalytical and imaging applications [4–8]. Because they have broad absorption envelopes, extending from the ultraviolet (UV) to the band edge, it is possible to simultaneously excite QDs of different emission colors at a single wavelength, making them suitable for multiplexing applications. This promising feature is very difficult to achieve with conventional organic fluorophores. Depending on the materials used, QDs can emit light over a wide range of wavelengths from the visible to near infrared (NIR) regions of the optical spectrum [4, 5, 9–15]. Since the first reports on the use of QD in biology were published, there have been several demonstrations showing that QDs conjugated with biomolecular receptors (including peptides, proteins, and DNA) can be used in a range of biological applications, such as sensing, imaging, and diagnostics. However, successful integration of QDs in biotechnology necessitates a thorough understanding of the nanocrystals, namely their reproducible synthetic routes, surface treatment/functionalization, and biocompatibility.

In this chapter, we provide an overview of the most commonly used synthetic schemes to make colloidal nanocrystals, along with their photophysical and structural characterization. We will then describe some of the most effective schemes reported to date to promote water solubility of these nanocrystals and discuss the simplest conjugation techniques that can be reproducibly applied to colloidal QDs.

1.2 Chemistry and Physics of Semiconductor Quantum Dots

1.2.1 Basic Physical Properties of Semiconductor Quantum Dots

Semiconductor nanocrystals (or QDs) are small crystalline assemblies of a given material consisting of a few hundred to several thousand atoms. Their sizes range anywhere from 10Å to 1,000Å in radius; the exact range depends on the constituent elements used to make the nanocrystals. They can be divided into two main subgroups: (a) Self-assembled QDs are usually grown in vacuum via molecular beam epitaxy or other lithography techniques; these tend to be anisotropic in shape, "pancake like," and overall larger at least within the 2-D plane (see Figure 1.1) [10, 16]. (b) Solution grown nanocrystals are colloidal in nature, and they can be spherical with a radius of 10 ~ 200Å, as well as cubic, rod-like, triangular, and so on (see Figure 1.1) [17]. Their colloidal feature stems from the fact that they are surface-capped with organic ligands that promote their dispersion in the solution environment.

These nanoscale assemblies are neither wholly atomic nor bulk semiconductors. Instead, they exhibit novel electronic properties attributed to what is commonly referred to as quantum confinement effects: this is the spatial confinement of intrinsic electron and hole carriers to the physical dimensions of the nanocrystal material rather than to bulk length scales. Because they somewhat combine properties that are both bulk-like and atomic-like, they have often been described as "artificial atoms." These confinement effects manifest when the nanocrystal size becomes comparable to, or smaller than, the bulk Bohr exciton radius [10, 18, 19]. One of the best-known and -understood confinement effects is the widening of the energy band gap with decrease of nanocrystal size. This manifests itself as a blue shift of the first absorption peak and the photoluminescence maximum with decreasing particle size, along with the appearance of discrete energy states in both the valence and conduction bands (Figure 1.1). However, resolution in the excited state energy levels and separation between the valence and conduction band levels depends on the type of semiconducting materials used.

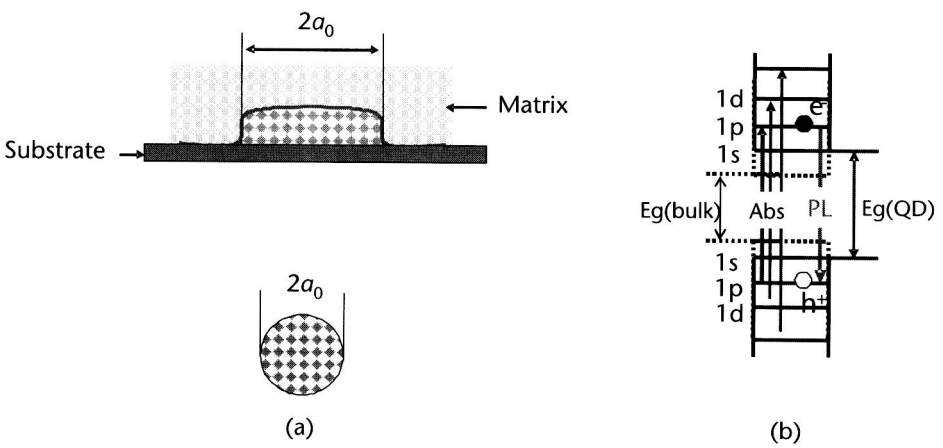

Figure 1.1 (a) Schematic representation of two QDs, a self-assembled "pancake" nanocrystal (top) and a colloidal nanocrystal (bottom). (b) Representation of the discrete energy levels expected and observed for a nanocrystal, due to quantum confinement effects.

This phenomenon can be understood by a simple extension or adaptation of the expected behavior of a quantum mechanical particle confined in a one-dimensional box of length L to a three-dimensional box (or sphere) of radius a_0, where the potential minimum represents the QD and the barrier to escape originates from the abrupt termination of the semiconducting material at the QD surface (boundary conditions) [10, 18, 19]. In this model/description, a carrier is localized within a potential minimum between two infinite barriers. For a one-dimensional box, effects of carrier confinement manifest in quantization of the carrier energies to discrete values that exhibit inverse square dependence on the length of the box ($E_n \propto n^2/L^2$, with $n = 1,2,3,\ldots$).

For a spherical QD with a radius a_0 treated within the conditions of an infinite potential barrier, the electron and hole energy levels in the particle can be written using the following expression:

$$E_{l,n}^{e,h} = \frac{\hbar^2 \beta_{l,n}^2}{2m_{e,h}a_0^2} \tag{1.1}$$

Here $\beta_{l,n}$ is the nth root of the spherical Bessel function of order l (solution obeying the boundary conditions), $m_{e,h}$ is the effective mass of the electron (e) or hole (h), and a_0 is the radius of the QD. This treatment predicts discrete (quantized) electron-hole (exciton) transitions, along with an increase in the effective band gap energy (or HOMO-LUMO transition) with decreasing particle size, which can also be expressed as:

$$E_g(QD) = E_g(bluk) + \frac{\hbar^2 \pi^2}{2m_{e,h}a_0^2} \tag{1.2}$$

Conversely, (1.1) and (1.2) also predict a decrease in the energy spacing between states with increasing nanocrystal size. It should be noted that the physical behavior of these nanocrystals is also affected by the Coulomb interactions between the confined carriers (these are charged). However, because this contribution/term has a weaker dependence on size (scales as $1/a_0$ compared to $\sim 1/a_0^2$ shown in (1.1)), it is small and is often treated as perturbation to the overall energy values. For the interested readers, there are several excellent review articles on the subject published in the past decade, including those recently assembled by Yoffe [16, 19], Gaponenko [18], Efros [10], and Kippeny [20].

Earlier work probing the optical properties of QDs was primarily focused on understanding the correlation between size and spectral shifts in absorption and luminescence based on confinement effects. Today, the absorption properties of CdSe as well as other QDs are relatively well understood with up to 10 excited states in the absorption assigned and theoretical avoided crossings observed [22]. A representative experimental example of absorption spectra for II–VI colloidal QDs is shown in Figure 1.2.

There is a unique spectroscopic property registered primarily for colloidal QDs, but rarely reported for their self-assembled nanocrystal counterparts. Bawendi and coworkers reported in 1996 that under CW laser excitation, the emission of isolated TOP/TOPO-capped single-particle CdSe and CdSe-ZnS QDs was not continuous.

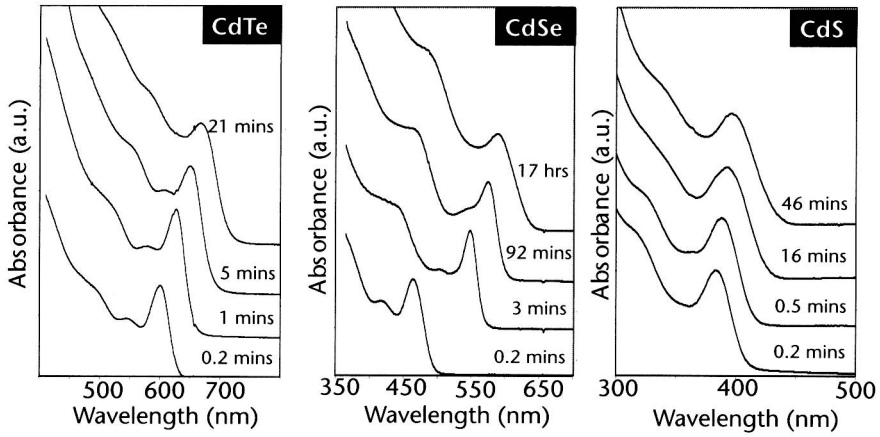

Figure 1.2 Representative absorption spectra for CdS, CdSe, and CdTe QDs prepared using high-temperature solution reaction. The spectra are shown at successive time intervals of nanocrystal growth. The shift in the position of the first absorption peak reflects an increase in the nanocrystal size. Reproduced from [21], with permission from the American Chemical Society.

Instead, they observed that QDs underwent intermittent on/off photoluminescence, now widely identified as the PL "blinking" of single QDs [23]. Blinking of single QD photoluminescence has since been widely reported in a variety of conditions, including extremely dilute dispersions in polymeric films, surface tethered nanocrystals, and more recently cellular media [24]. In particular, there is a nonvanishing probability for a QD to enter a long dark period. The on/off intermittency in QD emission was attributed to Auger ionization of the QD [23, 25, 26]. Despite the remarkable progress made, this phenomenon is still not fully understood.

1.2.2 Synthesis, Characterization, and Capping Strategies

1.2.2.1 Growth of Colloidal Nanocrystals in Doped Glasses

A range of experimental techniques such as e-beam lithography, X-ray lithography, molecular beam epitaxy (MBE), ion implantation, and growth in size-restricted environments has been reported for making small nanocrystallites not only of semiconductors but also of metals; colloidal QDs are a subset of those semiconducting nanocrystals. However, the first reported discovery of QDs by Ekimov and Onuschenko used doped silicate glasses [27–34]. The authors showed that if a supersaturated solution of copper and chlorine in glass was heated at high temperatures, controlled precipitation of CuCl within the glass matrix could take place. They further demonstrated that additional heating and annealing of the glass melt systematically creates collections of nano-scale particulates of crystalline CuCl containing a finite number of atoms, ranging from tens to hundreds of angstroms; the authors initially denoted these structures as *quantum droplets*. Following those remarkable demonstrations, growth of QDs made of II–VI semiconductors (e.g., CdS and CdSe) in glass was achieved by doping the melt with salts of the desired materials/precursors [27, 29, 35]. When the temperature of the glass rapidly decreases, small nuclei of the semiconductor are generated. Following heat treat-

ment over temperatures ranging from 400 to 1,000 degrees C, nucleation and growth culminate in the formation of small spherical crystalline particles of semiconductor dispersed in the amorphous glass matrices. This technique provided highly crystalline nanoparticles in glass host samples that can support very large (a few hundred angstroms) QDs. However, because the QDs remain trapped within a solid glass matrix, these nanocrystals cannot be easily manipulated to alter surface chemistry or improve their size distribution.

1.2.2.2 Synthetic Routes of Dispersible and Highly Luminescent QDs

Solution-phase growth of semiconductor nanoparticles carried out within inverse micelles was demonstrated shortly after the first realization of carrier confinements in semiconductor crystallites using doped silicate glasses by Ekimov and coworkers [28–31, 36–38]. This "wet chemistry" route allows preparation of functionalized, and thus dispersible, nanocrystals, by essentially exploiting the natural geometrical structures created by water-in-oil mixtures upon adding an amphiphilic surfactant such as sodium dioctyl sulfosuccinate (AOT), cetyltrimethylammonium bromide (CTAB), and tetraoctylammonium bromide (TOAB) (see schematics in Figure 1.3). This route was in fact demonstrated for a variety of other inorganic nanoparticles. In this technique, one can vary the water content of the mixture to control the size of the water droplets (nanoscale reaction pools) suspended in the oil phase. Addition of appropriate metal salts to the solution, which naturally migrate to the water pools, initiates nucleation and growth of colloidal nanocrystals. This technique has a few advantages including the fact that the reactions are carried out at room temperature. Furthermore, it provides one the unique ability to perform postsynthesis processing of these materials from solutions. This approach, however, was not able to provide QDs that have good crystalline structure and high photoluminescence quantum yields necessary for potential transition to technological use.

A major breakthrough took place in 1993, when Bawendi and coworkers showed that high quality nanocrystals of CdSe QDs with crystalline cores, narrow size distribution (~10 percent), and relatively high quantum yields can be prepared using an *organometallic synthesis* based on the "pyrolysis" of metal-organic precursors [9, 39]. This rationale was confirmed shortly thereafter by other groups including Alivistaos and coworkers [40]. This reaction scheme initially employed

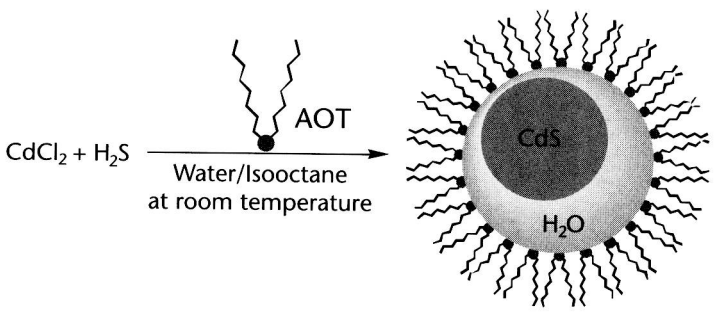

Figure 1.3 Schematic representation of the growth of CdS semiconductor nanocrystals in inverse micelles. Other materials such as CdSe and CdTe have also been prepared using the inverse micelles approach.

dimethylcadmium ($CdMe_2$) and trioctylphosphine selenide (TOP:Se), diluted in trioctylphosphine (TOP), and their rapid injection into a hot (280–300 degrees C) coordinating solution of trioctylphosphine oxide (TOPO) (see schematics in Figure 1.4) [39]. They also demonstrated that size distribution can be further improved by postreaction processing. Following this breakthrough, colloidal QDs could be made with quantum yields (QYs) on the order of 5 to 10 percent at room temperature, making fluorescence-based studies of QDs viable and raising the potential for use in technological applications. The QYs of these nanocrystals can reach near unity at low temperature.

Subsequently, Peng and coworkers further refined the reaction scheme and showed that additional precursors that are less volatile and less pyrophoric than $CdMe_2$ could effectively be employed to prepare high-quality colloidal nanocrystals [21, 43]. In those studies, they and other groups have eventually outlined the importance of impurities—usually acids coordinating to the metal precursors, such as hexylphosphonic acid (HPA) and tetradecylphosphonic acid (TDPA)—in the reaction progress, and showed that these impurities can be externally controlled. They also applied this rationale to making other types of colloidal nanocrystals, including CdTe and CdS as well as Pb-based QDs. In this route, high purity TOPO and controlled amounts of metal coordinating ligands and metal precursors such as CdO, cadmium acetate ($Cd(OAc)_2$), and cadmium acetylacetonate ($Cd(acac)_2$) were used for preparing Cd-based nanocrystals. The high temperature synthetic route was extended to making near-IR emitting QDs by Murray and coworkers (and further confirmed by other groups), using oleic acid and Lead(II) acetate trihydrate or lead oxide for PbSe QDs [14, 44, 45]. In most reported methods the selenium precursor still relies on TOP:Se [14, 21, 43, 46, 47].

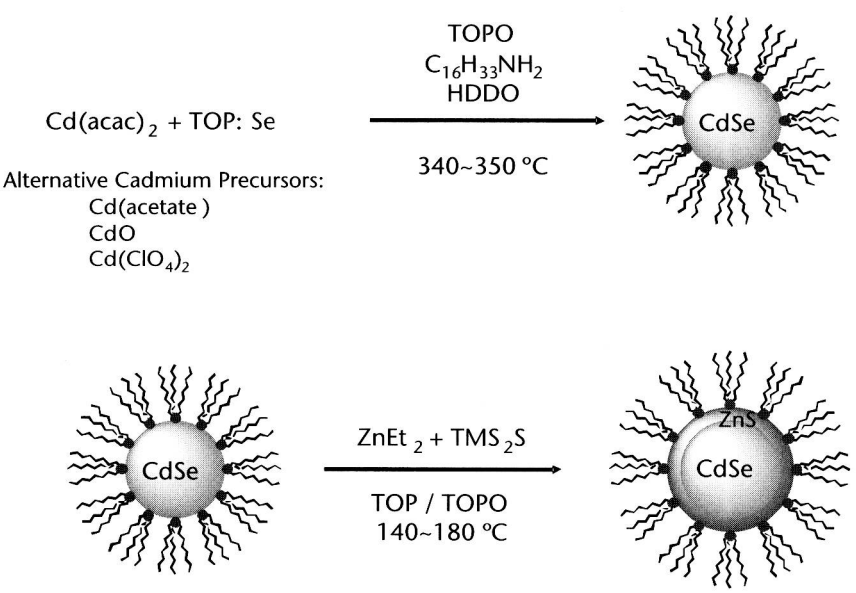

Figure 1.4 (top) Schematic depiction of the high-temperature organometallic reaction and growth method used for colloidal CdSe nanocrystals. (bottom) Reaction scheme for the overcoating CdSe quantum dots with ZnS using the same high-temperature solution route. Additional details can be found in [41, 42].

In these high-temperature reaction schemes, it has been demonstrated that applying size selective precipitation using polar solvents such as methanol or ethanol following nanocrystal growth could reduce the particle size distribution/polydispersity of the nanocrystals. In addition to reducing polydispersity, this procedure also removes impurities and precipitated metals from the reaction solution [9, 39]. This cleaning step is crucial for nanocrystals made using less reactive precursors and the various metal-coordinating molecules, since larger amounts of unreacted metals, acids, and amines can be left in the final QD crude samples. Available techniques to characterize nanocrystals include high- and low-resolution transmission electron microscopy (TEM), wide angle X-ray diffraction (XRD), small angle X-ray scattering (SAXS), and absorption and fluorescence spectroscopy, which extract information such as size, distribution width, crystal structure, band edge value and emission energy level (see Figures 1.5 and 1.6 and Table 1.1) [9, 11, 21, 39, 41–43, 46–50]. TEM tends to provide slightly smaller values for the inorganic core than SAXS, for example, because TEM does not take into account the amorphous outermost atomic layer on the nanocrystal surface [51].

Additional details on the synthetic routes, structural characterization, physics of quantum confinements effects, and their implications on the electronic and spectroscopic properties of colloidal QDs can be found in these thorough reviews [17, 52]. A summary of the reported synthetic methods for making colloidal QDs is provided in Table 1.2, with particular emphasis on preparations yielding colloidal QDs

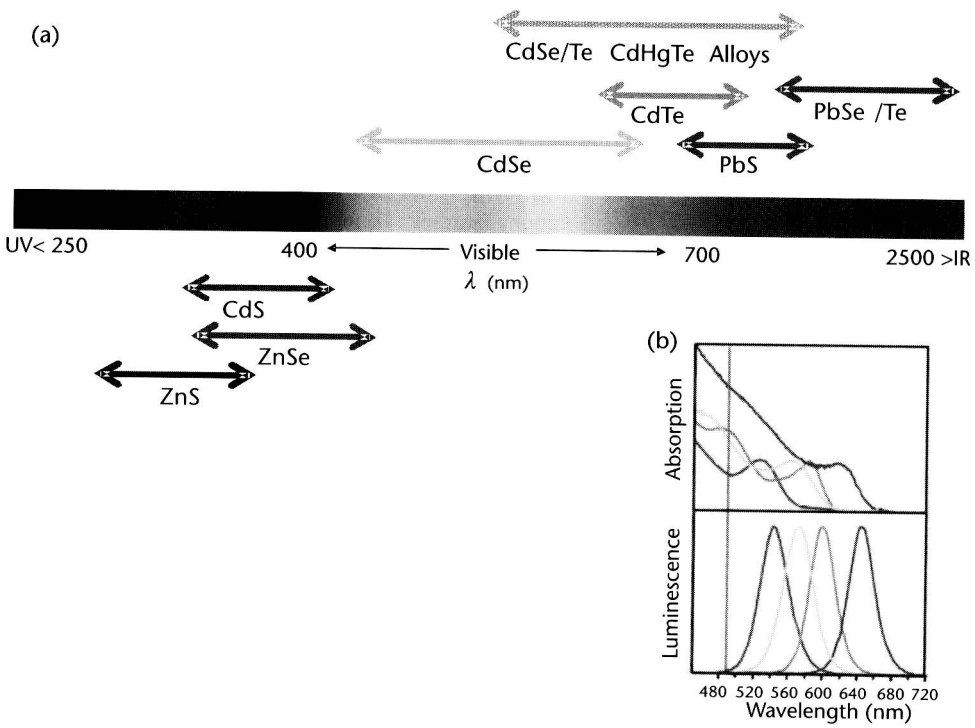

Figure 1.5 (a) Representative set of emission spectral windows for several types of QD materials. Core materials of II–VI, III–V and hybrid "III–VI" are shown. Partially reproduced from [5], with permission from NPG. (b) A representative example of absorption and normalized emission spectra collected for solutions of CdSe-ZnS QDs. Partially reproduced from [4], with permission from AAS. Other materials not represented here include InP and InAs QDs. (See Color Plate 1.)