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Review article

Polymer coating of quantum dots – A powerful tool toward diagnostics and sensorics

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Abstract

The use of quantum dots for biological and biomedical applications is one of the fastest moving fields of nanotechnology today. The unique optical properties of these nanometersized semiconductor crystals make them an exciting fluorescent tool for in-vivo and in-vitro imaging as well as for sensoric applications. To apply them in biological fluids or aqueous environment it is essential to modulate the chemical nature of quantum dot surfaces to alter their solubility and add additional chemical functionalities. By employing different coating technologies they cannot only be rendered water soluble but also functionalized to fulfill different tasks, like receptor targeting or sensing of low molecular weight substances. To achieve this goal different polymeric coatings are applied to provide solubility in water and additional functional groups for attachment. Taken together the versatile modifications described in this review make quantum dots a promising alternative to conventional fluorescent dyes and may offer possibilities for new future developments.

Keywords: Quantum dots; Polymer coating; Biocompatible; Sensorics; Diagnostics; Nanoparticles

1. Introduction

In the last decade, colloidal quantum dots have drawn tremendous attention as a new class of fluorophores for a wide range of diagnostic and sensoric applications. Their unique optical properties lead to major advantages in fluorescence detection and imaging in molecular and cell biology [1]. Linking these inorganic semiconductor nanoparticles to biological molecules like peptides [2], proteins [3–5] and DNA [6,7] was achieved just as well as adapting them for the development of multicolor fluorescent labels for in-vitro and in-vivo imaging [8,9]. Successful sensing applications of these systems were developed for analytes, like small ions and more complex molecules, like sugars or neurotransmitters [10–12]. The most commonly used quantum dots are of the cadmium chalcogenide group due to ease of synthesis and handling. Their inherent optical properties emerge from their semiconductor nature and are namely the bright and stable fluorescence and the broad excitation spectra with high absorption coefficients. These unique properties are the reason why quantum dots have significant advantages over common organic dyes and genetically engineered fluorescent proteins in many biological and biomedical applications. Compared to organic dyes they offer possibilities like multiplexed imaging and long-term investigations, e.g. for cell uptake studies and in-vivo imaging, due to their tunable emission wavelength and an increased photostability up to several months [4,8]. Nevertheless, quantum dot surfaces have to be protected and functionalized to provide biocompatibility, biostability and suitable surface functions for these applications.

A major step toward the applicability of the nanoparticulate systems for sensorics and diagnostics is therefore the design of an adequate coating of their inorganic surface. This coating should provide two functions, a chemical

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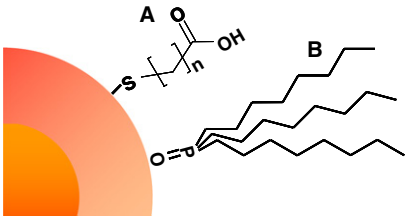


Fig. 1. Schematic drawing of the quantum dot surface with (A) a hydrophilic mercaptoalkane acid applied for water-solubility and (B) a lipophilic trioctylphosphine ligand from synthesis.

and physical stabilization of the quantum dots as well as the ability to modify them for a wide range of applications by attaching certain surface groups. The beginning of this continuous evolution was made with the first water-soluble quantum dots coated with mercaptopropionic acid, already applicable to chemical functionalization utilizing the free carboxylic group (Fig. 1). These quantum dots were further improved by a rapid development of a wide range of polymeric ligands and amphiphilic polymers coordinating on top of the nanocrystal surface. These polymer and ligand coatings are focused on the different facets of the biological applications and even extend into new fields of relevance, like the technique of lifetime imaging or special applications, such as single molecule detection. Consequently, various polymers and ligands have been developed for the differing application areas. Moreover, also two fundamentally different ways of surface coating for the similar applications were adapted, each with its own advantages and disadvantages. This review will provide a summary and comparison of the different polymer based coating strategies and the relevant organic polymers used for the modifications.

2. Quantum dots

Nowadays quantum dot probes are photostable and water-soluble nanoparticulate systems, which display a bright luminescence and offer the possibility to tune their size and emission wavelengths [13]. All these improvements can be ascribed to a series of technological developments providing new functionalities to the inorganic materials. It has started with the first highly crystalline and monodisperse cadmium selenide nanocrystals published by Bawendi et al. [14], synthesized in a hot coordinating solvent. This evolution was followed by improving the photostability and brightness of these quantum dots by coating them with different semiconductor materials thereby passivating the oxidation-sensitive semiconductor surface [15]. In 1998, the first synthetic approaches to water-soluble semiconductor nanocrystals were published [3,4]. Today quantum dots are not only composed of cadmium selenide (CdSe) but of many other semiconducting materials derived from the II and VI elemental groups (e.g. CdTe, CdS, CdHg, ZnS) and III and V elemental groups (e.g. InAs, InP, GaAs) of the periodic table. The emissions of these quantum dots span the whole spectral range from ultraviolet to near-infrared [16–20].

Possessing a size range of 1–10 nm diameter, quantum dots (QDs) are so-called quasi zero-dimensional, single, mostly spherical semiconductor nanocrystals [21,22]. Due to their small dimensions, they exhibit several exciting new optical properties in addition to the classical properties of bulk semiconductors, distinguishing them from common organic fluorescent dyes, e.g. of the cyanin or rhodamine group.

2.1. Optical properties

A classical attribute of quantum dots is their broadband absorption of light with increasing absorption coefficients at higher energies (i.e. smaller wavelengths) [23], which are an order of magnitude larger compared to organic dyes [24]. Furthermore, quantum dots exhibit many non-classical characteristics, as their tunable emission spectra with highly confined Gaussian distributions of the emission wavelengths. Emission peaks of CdSe quantum dots are as narrow as 25 nm full-width at half maximum (fwhm) at room temperature, unlike common organic dyes displaying asymmetric emission peaks with up to several 100 nm width. Additionally, the energetically lowest absorption peak of these dots is located only a few nanometers lower than the maximum emission wavelength. These characteristics allow an excitation of multiple QDs of different emission wavelength with only one excitation wavelength (Fig. 2), making multiplexed imaging of differently modified particles possible. Besides the spectral attributes, the semiconductor nature of quantum dots gives rise to a long photostability, up to several weeks or even months [25]. The slow decay rates of the exited states correspond with a long luminescence lifetime. The general luminescence lifetime of CdSe quantum dots at room temperature is composed of multiexponential decay rates with different lifetimes of 5, 20–30 and 80–500 ns, resulting in a general lifetime of 20–30 ns [26,27]. However, the chemical and physical cause of the multiexponential decay rates still remains vague and has to be further investigated.

The unique optical characteristics of semiconductor nanocrystals are based on an effect called quantum confinement (hence the name quantum dots), caused by the restriction of electrons and holes in all three dimensions [28]. Quantum confinement describes an effect arising in nanocrystals smaller than their so-called Bohr exciton radius. Like classical semiconductors, the nanoparticles possess a valence and a conducting band. However, in quantum dots these bands are quantized with energy values directly related to the nanocrystal size (Fig. 3). This quantization of the energy results in discrete size dependent emission wavelengths of a single quantum dot. The overall energy bandgap between the valence and the conducting band changes its value dependent on the variation of nanocrystal size, and can be described similar to the quantization arising from the 'particle in a box' model [29]. The moment a

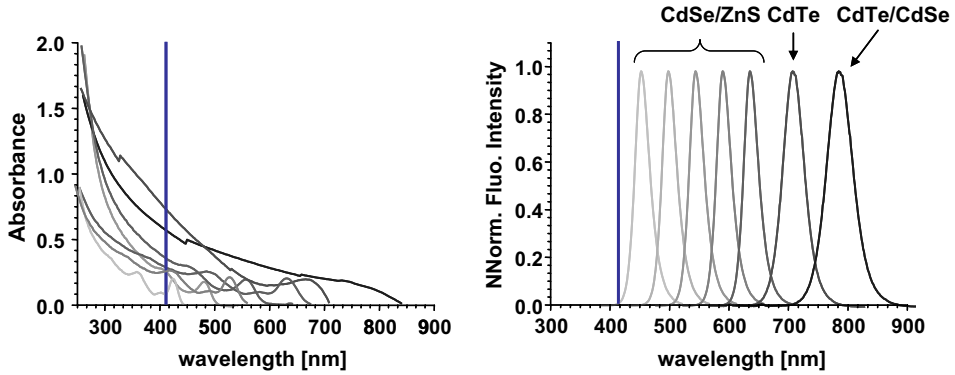


Fig. 2. Absorbance and fluorescence spectra of CdSe/ZnS, CdTe and CdTe/CdSe quantum dots of various sizes with a laser line at 405 nm used for excitation.

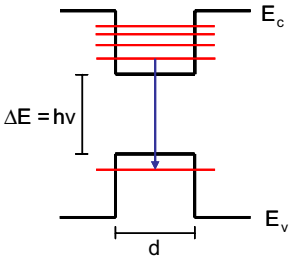


Fig. 3. Schematic drawing of the band structure of a quantum dot with E_c conducting band, E_v valence band, d diameter of the quantum dot and ΔE the energy between the valence and the conducting band, referring to the emission wavelength.

photon enters a quantum dot, a quasi-particle is created. This so-called 'exciton', an electron-hole pair, is formed when the electron is promoted from the valence band to the conducting band by energy absorption. The missing electron in the valence band leaves a hole of opposite electric charge behind. This hole is bound to the promoted electron by the Coulomb force. Upon recombination of the electron with its hole, light of a certain wavelength is emitted, corresponding to the respective bandgap energy.

2.2. Synthesis

Among various synthesis routes leading to cadmium chalcogenide quantum dots, the high temperature synthesis in coordinating solvents is the best-investigated strategy [30]. This synthetic route for nanoparticles of high monodispersity and high crystallinity [31] is performed via the decomposition of metal-organic or organometallic precursors at elevated temperatures. The precursors are composed of an organic part coordinating or binding to the added metal or metalloid. In general, the formation of the quantum dots is carried out at high temperatures between 180 and 310 °C, depending on the selected precursors and solvents. During the reaction, the chosen temperature and the reaction time determine the size of the nanoparticles, since nucleation of seed crystals and deposition of new material on existing crystals take place depending on the respective temperature of the solvent.

The reasons for the development of a broad range of synthetic strategies are the various possibilities of solvents and precursors. The chosen coordinating solvent is capable to "dissolve" the metal precursors and moreover it frequently also acts as ligand or capping agent for the resulting quantum dots (Fig. 1). Essential for these coordinating components are the functional groups (phosphines, phosphineoxides, amines and carboxy groups) suitable to graft on the nanocrystal surface. The attached groups stabilize the quantum dots during their formation, but they are also essential for later solubilization and capping strategies (Fig. 5). Frequently, toxic and pyrophoric components, like mixtures of trioctylphosphine oxide (TOPO), trioctylphosphine (TOP) and hexadecylamine (HDA) [32,33], are used. Nevertheless, nowadays also some other, less toxic coordinating substances, like fatty acids, or also mixtures of coordinating solvents with less pyrophoric non-coordinating solvents, like octadecene (ODE) [34,35], are utilized for nanocrystal synthesis.

However, not only the used solvents but also the used precursors have changed since the first organometallic approaches. The initially applied precursor dimethylcadmium [$Cd(CH_3)_2$] was highly toxic and pyrophoric resulting in difficult conditions for the synthesis. Most recently, Peng and Peng and other workgroups have applied less toxic and easier manageable cadmium precursors, like CdO or $Cd(CH_3COO)_2$, for the formation of high-quality CdX (X = S, Se, Te) quantum dots [31,36,37].

A common procedure to enhance the photoluminescence properties of CdX (X = S, Se, Te) quantum dots is the overgrowth of an additional passivating inorganic shell. This shell is composed of a second semiconducting material with a larger bandgap, e.g. ZnS or ZnSe (Fig. 4). The larger bandgap here provides a protection of the surface against oxidation, and additionally entraps the excitons in the core, resulting in reduced luminescence quenching caused by CdX surface defects. Furthermore, particularly Zn-containing shells exhibit a much greater affinity to thiol groups than the mere core material [38,39], which is of high

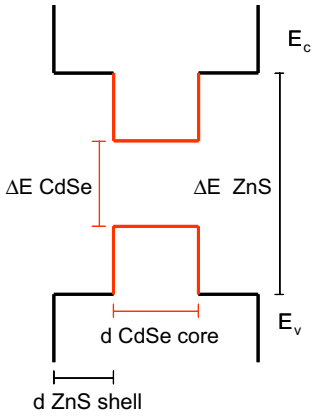


Fig. 4. Schematic drawing of the core–shell system of a CdSe/ZnS quantum dot with E_c conducting band, E_v valence band, d diameter of the quantum dot and ΔE the energy between the valence and the conducting band.

importance for the later applied coating strategies leading to functionalized quantum dots. The general synthesis procedures, as well as the core formations, are all organometallic approaches in contrast to later described organic polymer coating strategies.

3. Biocompatible quantum dots

Water-solubility, high stability against oxidation and subsequent degradation, small diameters and funtionalizable groups are essential for the application of quantum dots in biological systems. Since unmodified nanocrystals exhibit extremely hydrophobic surface ligands, like trioctylphosphineoxide or hexadecylamine resulting from the organometallic synthesis, they are not suited for biological applications due to their insolubility in aqueous media. Due to this fact, a hydrophilization of their surface is an essential prerequisite for their application in most of the here described experiments.

Since first reports on water-soluble QDs were published, a wide range of coating and capping strategies providing a water-soluble shell arose, having different effects on the properties of the modified particles. The strategies can be divided into two fundamentally different ways solving this problem via functional polymers. One approach completely replaces the surface bound ligands remaining from synthesis; the other only caps the present ligands on the QDs with suitable amphiphilic polymers (Fig. 5). Both approaches have advantages and disadvantages for the obtained water-soluble particles. Replacing the original hydrophobic surface ligands by amphiphilic ones leads to particles with a small final diameter. These composites are often only a few nanometers larger than the core quantum dots. Nevertheless, the exchange of the surface coating often results in poor quantum yields and strongly affects the physicochemical and photophysical stability of QDs in buffered solutions. Instead, surface capping chemistries retain the original surface ligands and therefore preserve

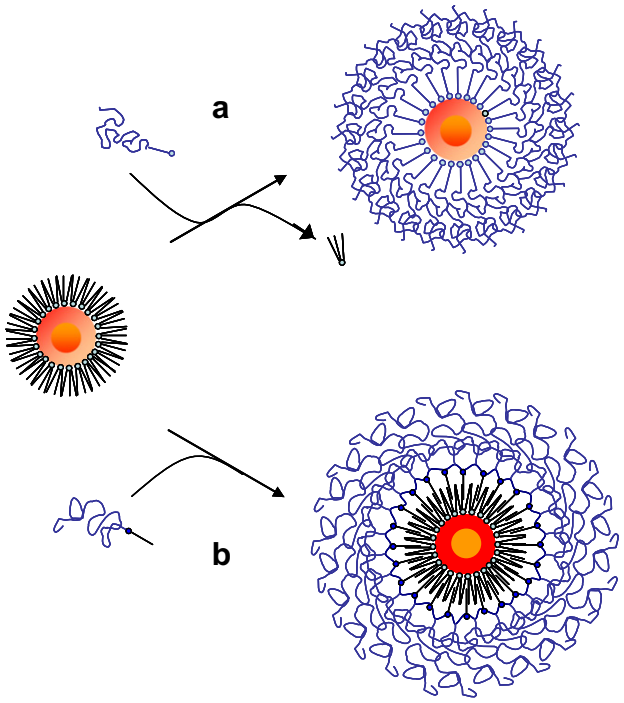


Fig. 5. Scheme of the (a) ligand exchange and (b) the ligand capping strategy.

the photophysical properties of the nanocrystals. However, this approach results in particles with a final size three or four times larger than the original nanocrystal diameter.

The huge variety of different surface modifications results in quantum dots of very different optical and chemical properties. Indeed this diversity is necessary for the multiplicity of applications semiconductor nanocrystals undergo in diagnostics and sensorics. Properties like particle size and charge, as well as application relevant parameters, like chemical and photophysical stability, photoluminescence intensity and cytotoxicity, have to be considered to choose the optimal system for each application.

The focus of the following review will be set on coating strategies with organic substances. For completeness, it has to be mentioned that there are various possibilities for inorganic coating of quantum dots with silica or titania. These coating strategies are based on the same two principles of ligand exchange or ligand capping to anchor the inorganic coating on the nanocrystal surface. This is followed by the formation of another inorganic layer, shielding the quantum dot and rendering it water-soluble [40–51].

3.1. Effects of surface coating

Coatings can change the quantum dot properties with two different aspects, one is the photophysical, the other the physicochemical point of view. The affected photophysical characteristics are the emission wavelength, the quantum yield and the photostability, directly influencing each other due to the physics of the quantum dots.

The natural quantum dot capping, resulting from synthesis, protects the surface against surface defects and oxidation. Too many surface defects result in a decrease of quantum yield, because excitons can emit their energy in a non-radiative way. Additionally, the photostability is largely influenced by the occurring photooxidation of the surface, and the larger the likelihood for an oxidation due to imperfect coating, the worse the observed photostability. Finally, the occurring surface oxidation is also responsible for an effect called 'blueing' of the quantum dots, which is a shift of the emission wavelengths towards blue color [52–55]. In case the surface of a nanocrystal gets oxidized, the remaining emitting semiconductor core gets smaller (Fig. 6). When the core gets smaller the emission wavelength shifts to higher energies and therefore smaller wavelengths [56,57].

Consequently, the exchange of the original capping causes an increased likelihood to suffer damages due to incomplete coverage and imperfect grafting of ligands. Additionally, thiol-containing ligands used in many approaches are susceptible to oxidation of the thiol group, leading to detachment of the coating from the surface. Here again the mere capping of the initial ligands with amphiphilic polymers reduces the likelihood to suffer from surface defects and in most cases provides a much better protection against oxidation due to the much thicker shell on top of the particles.

The physicochemical attributes of the nanocrystals affected by different coating strategies are their size, the charge and the aggregation stability of the particle suspension in biological systems. However, these particle attributes are often the most critical subject for the design of a new coating. The ligand exchange method on the one hand yields particles of a small final diameter, but together with an oxidation sensitivity of the thiol grafting ligands. This then may result in an aggregation of the quantum dots, due to the loss of surface shielding. However, the beneficial small dimensions of the QDs with exchanged ligands retain in some applications the comparable low stability against aggregation. The capping of the ligands on the other hand produces comparatively large polymer-coated particles, which in some cases even have more than one QD inside the coating layer [58]. Nevertheless, these bigger particles provide a good chemical stabilization of the surface and a reliable protection against aggregation.

Occurring in both modification methods is the aqueous solubilization with additional charged groups on the surface. The mostly occurring chemical moieties are carboxyl and amino groups which offer the additional possibility for further functionalization with specific biomolecules. However, the use of these systems raises the risk of aggregation in biological environments caused by ionic interactions. The destabilization of anionic shells happens, for example, caused by increased ionic strengths of the aqueous solutions, increased temperature or complex salt mixtures. Therefore, another frequently used technique altering the physicochemical attributes of the particles is the PEGylation of an existing polymer shell, yielding uncharged colloids. This modification furthermore results in a reduced unspecific uptake in cells, and moreover, prevents adsorption of proteins on the polymer shells. Also the risk of agglomeration in biological fluids is remarkably reduced [59].

3.2. Ligand exchange strategies

Among the different strategies of ligand exchange (Table 1 and Fig. 5), various thiol ligands, including dithiols and thiol dendrimers, have been studied extensively. One of the easiest ways to obtain water-solubility is the attachment of thiolated poly(ethylene glycol) polymers [60–64]. Main advantages of the thiolated PEGs are the easy synthesis, ease of handling and the versatile applications. Due to these facts, the PEG ligands are widely used for solubilization, besides other suitable ligands such as thioglycolic acid, mercaptopropionic acid or dihydrolipoic acid [65]. The often introduced second functional group of the ligand, e.g. amino or carboxy groups, provides the possibility of further functionalization steps. However, an extra benefit of poly(ethylene glycol) coatings is the reduced unspecific cellular uptake of the modified uncharged particles [59] as mentioned earlier. Depending on the strived goal, varying polymer chain lengths and number of binding dentates are used. The two mainly applied types are the mono [63,64] and the bidentate [62] thiols. The latter ligand obviously grafts more effectively on the nanocrystal surface and therefore provides a much better stabilization of the

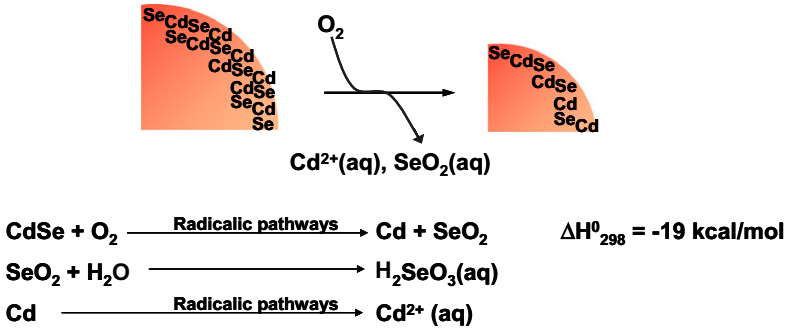
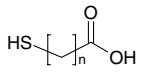
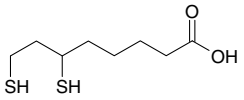
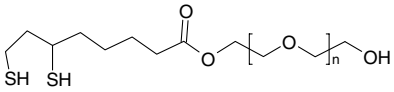
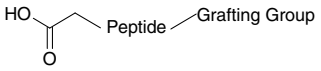
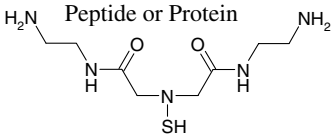
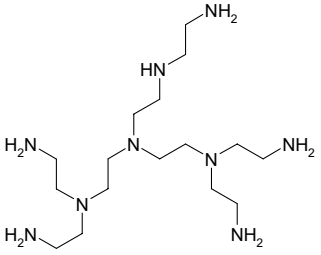
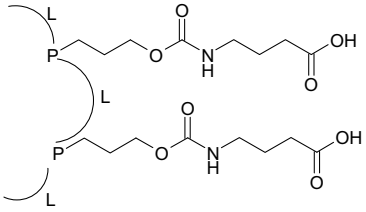
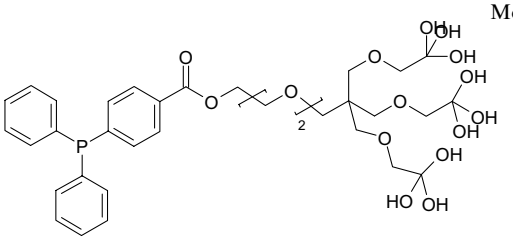


Fig. 6. Schematic drawing and reaction scheme of the photooxidation on the nanocrystal surface of CdSe [54].

Table 1
Examples of ligands and polymers used for ligand exchange with their mechanism of interaction with the semiconductor and the intended application

Ligands	Mechanisms of interaction	Applications	References
 Mercaptoalkane acid	Monodentate thiol bond	Metal ion sensing	[92–94]
 Dihydrolipoic acid	Bidentate thiol bond	FRET experiments, ion sensing	[11,12]
 PEGylated dihydrolipoic acid	Bidentate thiol bond	Cancer marker detection, live cell labelling, organelle tracking	[61,63,64]
 Peptide or Protein	Monodentate thiol group, leucine zipper, cysteine domain, histidine tag	Tumor vascular imaging, intracellular targeting	[68,69]
 Generation Poly (amidoamine) dendrimer	Monodentate thiol bond	Transfection agent	[73]
 branched poly(ethyleneimine)	Multidentate amine bond	Transfection agent	[74]
 multidentate phosphine polymer	Multidentate phosphine bond	Lymph node mapping	[77]
 Poly(ether) dendron	Monodentate phosphine bond	–	[78]

nanocrystals in aqueous solution. Nevertheless, the remaining disadvantages of these simple coating agents are the reduced photoluminescence intensity and the lack of long-term chemical stability of the thiol groups.

An alternative method for a solubilization is the surface absorption of small thiol-containing organic molecules, functionalized with additional anionic or cationic moieties. These solubilized QDs subsequently are covered using an oppositely charged polymer, e.g. with derivatives of poly(acrylamide) causing a stabilization of the ligand shell [66]. However, not only synthetic polymers can attach onto this charged layer, also proteins readily adsorb on it [5]. What could be a problem, some work groups turn into a benefit by attaching proteins on the nanocrystal surfaces. Furthermore, the application of engineered peptides and proteins for functional coatings of quantum dots is a fast growing field in nanocrystal modification. For these custom-designed proteins biologically relevant domains, like targeting sequences, are fused with attachment domains for the quantum dots, like thiol-containing cysteine domains, cationic histidine tags [67] or the leucine zipper peptides [5]. Nevertheless, also simply thiolated proteins are utilized for direct attachment on nanocrystal surfaces providing modified nanoparticles [68]. A further improvement is the coattachment of thiolated PEGs and engineered peptides on one particle surface [69]. This method provides specific binding on the one hand, but on the other hand also reduces the adsorption of different other proteins on the quantum dots and enhances their overall biocompatibility.

Another possibility to exchange existing surface ligands is the application of grafting dendrons or dendrimers, which are three-dimensional, highly branched and almost monodisperse macromolecules [70]. Dendrons and dendrimers itself are core–shell nanostructures consisting of a core, starting point for the polymerization, interior branch cells and an exponentially increasing number of functional groups on the surface. The mostly used dendrimers for nanocrystal capping are for example functionalized poly(amidoamine) (PAMAM) polymers [71–73]. An important attribute of these PAMAM polymers is their ability to effectively penetrate cell walls, making them also useful as commercial transfection agents. The PAMAM polymers possess a large number of primary and tertiary amine groups at the surface and in the interior branches of the molecule, which are known to allow for DNA complexation and which can also graft to quantum dot surfaces, while additionally improving the fluorescent properties of the modified semiconductor nanoparticles [32]. Nevertheless, they only possess poor affinities to nanocrystal surfaces and do not offer stabilization against particle aggregation because of their charged groups. Due to this fact, the utilized PAMAM dendrimers have to be further modified with additional thiol groups, known to graft better on the surface of quantum dots. Hence, a significant improvement of their affinity to nanocrystal surfaces is achieved. Surprisingly, these dendrimer coated quantum dots seem to transfect better than higher generation dendrons alone in first cell studies [73], which can be explained with altered particles sizes compared to the free polymers. Thus, the composites may be a promising innovation for the transfection of cells.

An alternative is the attachment of polymers to nanocrystals via the use of amines. As mentioned above, this chemical moiety is known for only a weak binding to semiconductor surfaces. However, some work groups succeeded in functionalizing quantum dots with mere amine-containing polymers, like poly(ethyleneimine) (PEI) [74], also being an effective transfection agent. The PEI coated quantum dots exhibited an effective phase-transfer and a good solubility in polar solvents. However, the coatings with PEI polymers unfortunately seemed to enhance the photooxidation of the quantum dot and therefore increased the darkening of the coated nanocrystals. Other applied amine-containing polymers are poly(*N*,*N*-dimethylaminoethyl methacrylate)s [75]. It was shown that these polymers do not only effectively passivate the surface of the nanocrystals, but also provide robust colloidal stabilization in various biological environments. Additionally, the polymer-coated particles exhibit an increase in quantum yields compared to the uncoated ones, which can be ascribed to the photoluminescence enhancing effect of the amines.

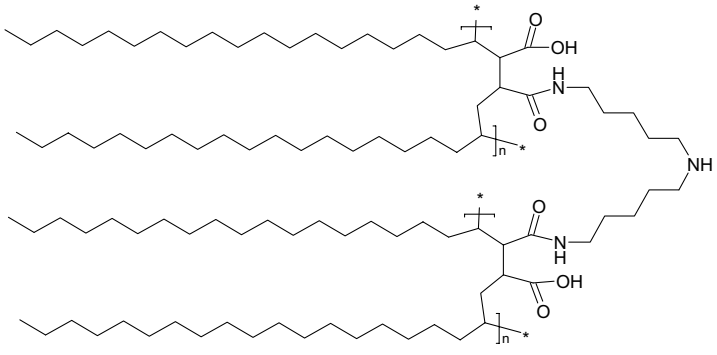
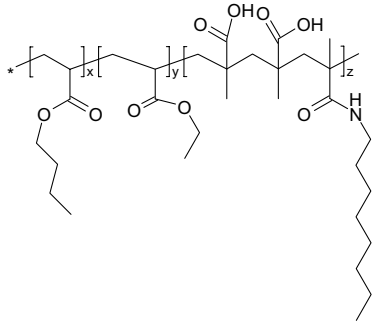
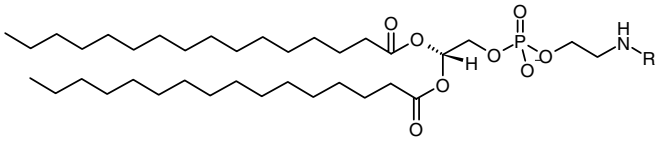
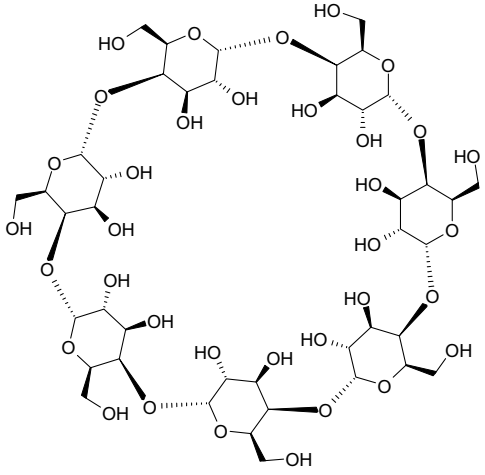
To overcome the drawback of reduced photoluminescence intensity with all the previously described grafting groups, several phosphine-containing polymers were developed, which show more similarities with the original ligands. In 2003, Bawendi et al. synthesized multidentate phosphine oxide polymers [76,77] composed of three sublayers enclosing the nanocrystal, an inner phosphine layer, a linking layer between the phosphine group and an outer functionalized layer. The attachment of phosphines here provides quantum yields up to 40%, while the oligomeric outer layer can be easily modified with functional moieties, for example PEG chains [77]. Taken together, these multidentate ligands provide chemically stable and highly fluorescent quantum dots. A potential application for these particles is, for example, the lymph node mapping due to their exceptionally small hydrodynamic radii of 15–20 nm, allowing successful penetration through tissues.

Similarly, on the sector of dendrons and dendrimers, poly(ether)s modified with aryl phosphine focal points were developed [78]. The incorporated phosphine group here provides a strong coordination to the surface without affecting the quantum yield. Moreover, the conic shape of the dendrons seems to be ideal for an adsorption onto the nanoparticles, because the formation of a closely packed polymer shell is possible. These obtained shells then effectively suppress subsequent diffusion of quenching substances like oxygen or other small ions from the solution to the nanocrystal surface.

3.3. Ligand capping strategies

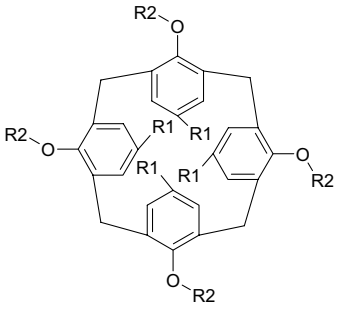
A wide range of amphiphilic polymers for quantum dot surface modification were developed since the first

Table 2
Examples of polymers used for quantum dot capping with their application

Capping polymers	Applications	References
 Polycarboxylic acid crosslinked with diamine	Multiphoton imaging in vivo, labelling of cancer markers and cellular targets	[80,81]
 Triblock copolymer	–	[79]
 Phospholipid	Tracking of plasmid DNA, in-vivo imaging, cell detection	[83, 84,86]
 β-Cyclodextrin	Molecular recognition	[89]

(*continued on next page*)

Table 2 (*continued*)

Capping polymers	Applications	References
 Calix[4]arene	Acetylcholine detection	[91]

publications describing water-solubilization using capping strategies (Table 2 and Fig. 5). Many different ways utilizing di- or triblock copolymers or other amphiphilic polymers (amphipols) were published. The common functionality of all these different polymers is the lipophilic part, intercalating between the aliphatic chains of the surface ligands, and finally covering or encapsulating the whole quantum dot with the original ligands still in place.

One possibility is to use amphipol triblock copolymers of poly(acrylic acid), a polymer that is commercially applied to solubilize membrane proteins in aqueous solutions [79]. A few years ago, related diblock copolymers were developed for the preparation of biocompatible semiconductor nanocrystals in large scale. The used polymer shell here is composed of octylamine-modified poly(acrylic acid) additionally crosslinked with lysine. The modified QDs can further be improved by PEGylation of the carboxylic groups to reduce unspecific binding [80,81].

Another polymer type, which is used for quantum dot functionalization, is the amphiphilic poly(maleic anhydride-alt-1-tetradecene) [82], which can be further crosslinked with a diamine to stabilize the shell. All these amphipols have a hydrophilic backbone with attached hydrophobic side chains, interacting with the aliphatic chains of the ligands present on the nanocrystal surface, bridging between the lipophilic surface ligands and the hydrophilic solution. Solubilization of the nanoparticles in water is mainly funded in the carboxylic groups of acrylic or maleic acid, forming the backbone of the amphipol shell. The shell architecture with the functional group then provides the possibility for further functionalization with antibodies or proteins, suitable to target cancer cells, using standard carbodiimide chemistry [80].

An often-used alternative to coating with amphiphilic polymers is the encapsulation of quantum dots in micelles. The advantage of this method is the applicability of a wide variety of surfactants/lipids with different functionally terminated groups. As micelle building compounds mostly PEG derivatisized phospholipids are applied due to the improved solubilization capability [83,84]. Also many other suitable surfactants, like lipids combined with paramagnetic gadolinium complexes, can be used for nanocrystal encapsulation [85–87], providing the possibility as well for luminescence imaging as for MRI (magnetic resonance imaging). The QD containing micelles preserve the optical properties of the encapsulated quantum dots and additionally offer a high biocompatibility. The drawback of this method is that only nanocrystals of predefined diameters and emission wavelength can be encapsulated by certain micelle building surfactants or lipids. This fact is founded in a given micellar size of a particular surfactant, defining the inner free space available for the quantum dots [83].

A coating method aiming at the sensoric application of quantum dots is the use of different cyclodextrins, where an interaction of the coating with the core is still desirable [88,89]. Here, the hydrophobic pockets of the saccharide oligomers interact with the aliphatic chains of the TOPO present on the nanoparticle surface. Nevertheless, the immobilized cyclodextrins retain their capability of engaging molecular recognition. Due to this fact and the observation of fluorescence changes while analyte binds, this modification method is very promising for possible sensing applications [89]. Another benefit of this capping strategy is the resulting small diameter of the QDs, which is achieved due to the small required space of the cyclodextrins. A related approach to small water-soluble quantum dots without a ligand exchange is the use of calixarenes, similar organic cyclic systems [90,91]. This coating also preserves the emission intensity of the quantum dots and their small diameter. Calixarenes are cyclic oligomers based on a hydroxyalkylation product of phenol and an aldehyde. It was also shown that calixarenes could be functionalized with sugars or peptides to allow biological applications of these systems [90]. However, also derivatization with aliphatic and sulfonato groups was achieved for optical detection of small molecules, like acetylcholine [91].

3.4. Application of surface coatings

The various coating methods, substances and the different characteristics of the resulting nanoparticles open a wide range of application areas, particularly the field of sensorics and diagnostics. For sensorical approaches, it is important to have a surface coating that allows an interaction of the analytes or the reporter molecules with the quantum dot. In contrast the diagnostic applications rely on biocompatibility, especially concerning cytotoxicity and the undesirable adsorption of proteins and possible aggregation.

The basis for sensoric applications of quantum dots is, in most cases, the interaction of the analyte molecule or ion with the nanocrystal surface, leading to a change in the fluorescent properties of the particle. Following this approach, quantum dots were coated with cysteine, thioglycolic acid or related ligands for the detection of several metal ions like Ag^{+} [92], Cu^{2+} [93], Zn^{2+} [94] and also small toxic anions, for example, cyanide [95]. Additionally, coated quantum dots were applied to optical temperature detection [96–98]. The conjugation of selective reagents or reporter molecules to the surface of luminescent nanocrystrals is also utilized for quantum dot probes. Particularly dihydrolipoic acid can be modified with functional moieties for selective K^{+} [11] (Fig. 7a) or even glucose [12] sensing. Nevertheless, these approaches seem to be restricted to a small number of analytes interacting with the surface coatings and the underlying quantum dots. Additionally they possess only a low stability in biological systems and limited applicability in realistic sample arrangements due to possible interactions with similar ions present in the solutions.

The potential of QDs to be used in much more analyte specific FRET (förster resonance energy transfer) based sensors can expand the applicability of semiconductor nanocrystals in sensorics. Here, the tunable wavelengths and the high quantum yields of the nanocrystals theoretically enable efficient energy transfer with a wide number of conventional dyes. Indeed it should be mentioned that FRET efficiencies obtained with QDs as donor species today are still low compared to efficiencies of common dyes, which is mainly founded in the comparatively large size of even thin-coated quantum dots, making it very complicated to bring the acceptor into close proximity to the donor for efficient FRET. Nevertheless, a variety of quantum dot FRET applications were yet developed and strategies arose to improve the energy transfer. Protein-binding sites were studied by FRET investigations, whereas the acceptor dyes are bound to a protein affording FRET when the assembly is adsorbed on the QD surface [99] (Fig. 7b). Not only conventional fluorescent dyes are utilized as acceptors, also several so-called dark quenchers, molecules or nanocrystals only absorbing and not re-emitting the light, were bound to QDs for FRET applications. This consequently results in a detectable decrease of the fluorescence signal upon increasing FRET events. Examples for these systems are the use of functionalized gold nanocrystals for DNA hybridization investigations [100,101] and inhibition assays [102] or alternatively, the application of an organic dark quencher dye for pH sensing [103] and maltose binding assays [104].

The diagnostic approaches of modified quantum dots, on the other hand, are more dependent on impermeable polymer shells and efficient physical and biological shielding of the quantum dot to prevent unspecific adsorption of proteins and fast degradation leading to a fluorescence loss. This can mainly be achieved with densely packed polymer shells and subsequent PEGylation. On the one hand these water soluble and often targeted quantum dots can be used for in-vitro cellular imaging e.g. histology, on the other hand for in-vivo imaging of tumors in small animals. The applied in-vivo imaging here is a non-invasive possibility to detect highly sensitive and in a high contrast deep tissue regions in mice and even larger species without the use of radioactive radiation or larger instrumental setups, like

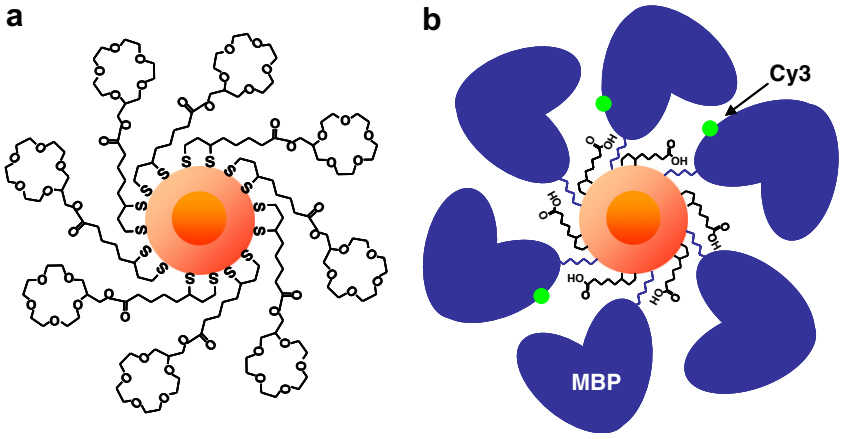


Fig. 7. (a) A K^{+} ion sensitive QD, with a crown ether functionalized dihydrolipoic acid (DHLA) as grafting ligand [82], (b) an example for a QD FRET sensor for protein binding site investigations, with Cy3 as acceptor dye bound to MBP as investigated protein [84].

computed tomography. The intravenous injection of biocompatible quantum dots was performed for blood vessel imaging [81], targeting of tissue specific vascular markers [68], or lymph node mapping [105]. A promising application is also the targeting of tumor cells in vivo using specific antibodies against Her2 markers [80] (Fig. 8). Another improvement in the field of in-vivo imaging are self-illuminating quantum dots, these polymer coated and luciferase modified nanocrystals need no external light for excitation. In this system the chemical energy of the substrate coelenterazine is converted into photon energy by the enzyme luciferase. This photon energy excites the quantum dot through bioluminescence resonance energy transfer (BRET). With this excitation mechanism the autofluorescence is virtually eliminated, however the emitted photons are still absorbed or scattered making sensitive detection necessary [106–108].

In the area of cellular imaging, quantum dot probes are as well used for the tagging of whole cells as for the investigation of single intracellular processes. Many studies here are focused on membrane specific markers due to the easy access from the outside of the cells and the unnecessary passage through the cell membrane. Additionally, also several attempts to internalize quantum dots in live cells have been performed. One approach to internalize the nanoparticles is the use of membrane translocation peptides [110], also attempts at utilizing electroporation or established transfection reagents [111] were performed (Fig. 9). The latter strategy to internalized QDs allows then the targeting of sub-cellular compartments as, for example, mitochondria or the nucleolus using specific targeting peptides allowing for the labelling of the small compartments in living cells for subsequent studies of cellular biology [68,112,113].

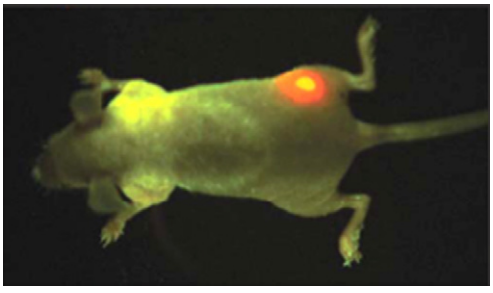


Fig. 8. Red QDs injected into a living mouse marking the location of a tumor [109]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

3.5. Cytotoxicity

Having biological experiments in mind, the cytotoxicity is an important factor to be considered. Quantum dot size, charge and concentration, their outer shell bioactivity and oxidative, photolytic or mechanical stress are all factors that, collectively and individually, can determine their cellular toxicity. For biological applications, it is notable that especially protection of the nanocrystal surface is not only important for the probe stability, but it is also vital to prevent leakage of cytotoxic semiconductor components from the inorganic core. In addition, some coating materials can also have toxic effects on cells on their own, if they are released from the composites.

An oxidation of the surface happens through a variety of chemical pathways, mostly through radical reactions of oxygen combined with UV-irradiation. This leads to the formation of chalcogenoxides (e.g. SO_2, SO_3, SeO_2, TeO_2) and reduced cadmium. These chalcogenoxides can then desorb from the surface and dissolute (e.g. resulting in H_2SO_3, H_2SO_4, H_2SeO_3, $TeO_2(aq)$), the residual reduced cadmium is oxidized back to Cd^{2+}-ions, leading to the subsequent release of free cadmium ions [53–55] (Fig. 6). These soluble Cd^{2+}-ions make the biggest part of the toxic effect that is ascribed to QDs. With increasing impermeability of the surrounding polymer shell the overall cytotoxicity decreases. The toxicity of the used coating material is closely related to the toxicity of the utilized components during their synthesis. However, at the low concentrations needed especially for cellular experiments most reports did not find adverse effects on cell viability, morphology, function or development. Semiconductor nanocrystals are therefore not completely innocuous, but a safe range for their biological application certainly exists [114–116]. This range eventually can be further extended with increasing

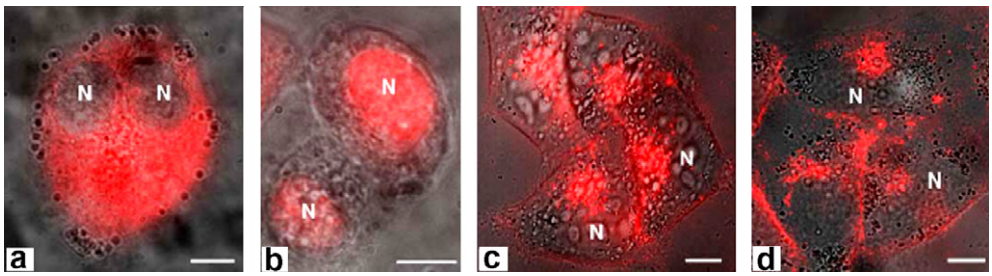


Fig. 9. Various approaches to translocate QDs in cells. (a) QDs microinjected into the cytoplasm (b) into the nucleolus (c) poly(ethylene imine) coated QDs in the cell and (d) Chariot protein functionalized QDs accumulated in the endocytotic vesicles. Pictures are merged fluorescence (red) and brightfield images of HeLa cells. N: nucleolus. Scale bars 5 μm [112]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

quantum yields of the particles and subsequently decreasing detection limits.

4. Conclusion

A great variety of polymeric surface coatings for quantum dots are currently applied for a broad range of different applications. All applications require distinct characteristics of the quantum dots, which are adjustable by the used surface coating polymer. The size and photostability of water-soluble quantum dots here strongly depend on the used capping strategy and the resulting particle architecture.

Ligand exchange on the one hand can produce small particles, but often lacks the long-term stability and photoluminescence intensity. Their resistance against acids or bases and, in some cases, against chemical oxidation is very week. Nevertheless, FRET experiments, for example, urgently require small hydrodynamic radii, and also other sensoric applications depend on the accessibility of the nanocrystal surface, which can only be achieved by attachment of short ligands. For ligand exchange procedures the recent adaptation of phosphine groups is very beneficial due to the improved stabilization of the nanocrystal surface and the additional surface passivation against oxidation, while mere PEGylation mainly provides protection against unspecific protein absorption. For transfection experiments, substitution with cationic polymers, like branched PEI or PAMAM dendrimers, is suggestive.

On the other hand, the ligand capping strategies with their effective shielding of the nanocrystal surface, their subsequently low cytotoxicity and high stability in biological environments are ideal for cellular and in-vivo experiments. These studies rely on a sustained fluorescence in the presence of oxidizing agents and low cytotoxicity. Also in assays conducted in high salt buffers, uncharged and sterically stabilized nanocrystals, like PEG coated QDs, can be of avail. Therefore, protection of the quantum dots with an amphiphilic bilayer, e.g. using phospholipids or amphiphilic polymers, is useful. The amphiphilic capping then can be easily modified with targeting sequences or proteins using carbodiimide chemistry. Subsuming the different strategies, none of the encapsulation methods can be universally optimal for several biological and sensorical applications at once.

5. Future outlook

The different polymeric surface coatings developed in the last decade combining biological materials with inorganic nanocrystals have not only been crucial for the successful use of quantum dots in cell and tissue imaging. Additionally, they have afforded new systems in materials science for the controlled assembly of nanomaterials used in the biological environment. As research continues to produce different nanomaterials with novel unique properties, it will become possible to gather new multimodal imaging agents. Combining QDs for fluorescence imaging with magnetic resonance imaging (MRI) or computed tomography (CT) contrast agents, like Fe_2O_3 [117], FePt [118] or Gd complexes [86], allows deep tissue imaging and fluorescence tracking of one system for sophisticated diagnostic applications. For these innovative applications, new coatings and functional materials are urgently needed to fulfill the difficult tasks lying ahead. In the area of sensorics, QDs can moreover function as effective protein carriers and exciton donors for prototype self-assembled FRET nanosensors for the detection of many relevant signal molecules, like acetylcholine [79] or others. Furthermore, they could even drive biosensors through a two-step FRET mechanism overcoming inherent donor–acceptor distance limitations, already realized with the FRET maltose-binding sensor [96]. To this time, mostly intensity-based measurements with quantum dots have been employed in the fields of sensing and imaging. Indeed, lifetime-based methods will draw more and more attention due to their superior resolution, independence from fluorescence intensity and concentration at the detection point and, finally, the possibility to out-gate the tissue autofluorescence present in all biological systems, like cell cultures or whole animals. For these developments QDs are an especially powerful tool due to their long excited state lifetimes compared to the common organic dyes and the interfering tissue autofluorescence.

However, for all that semiconductor nanocrystals will not overcome the use of conventional organic dyes in biological and sensorical applications. They could complement dye deficiencies in particular approaches such as in-vivo imaging and break up to new applications as long-term imaging and lifetime measurements. Moreover, adapting QD nanoparticles for biological use will teach us important lessons about creating future inorganic–organic hybrids for many other applications.

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