Advances in Colloid and Interface Science 215 (2015) 28–45

Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

journal homepage: www.elsevier.com/locate/cis

Surface functionalization of quantum dots for biological applications

Ajay Singh Karakoti [a,*], Ritesh Shukla [b], Rishi Shanker [b], Sanjay Singh [b,**]

[a] Battelle Science and Technology India Pvt. Ltd., 302, Panchshil Technology Park, Hinjewadi, Pune 411057, India
[b] Institute of Life Sciences, School of Science and Technology, Ahmedabad University, University Road, Ahmedabad 380009, Gujarat, India

ARTICLE INFO

Available online 15 November 2014

Keywords:
Quantum dots
Surface modifications
Imaging
Covalent coupling
Silanization

ABSTRACT

Quantum dots are a group of inorganic nanomaterials exhibiting exceptional optical and electronic properties which impart distinct advantages over traditional fluorescent organic dyes in terms of tunable broad excitation and narrow emission spectra, signal brightness, high quantum yield and photo-stability. Aqueous solubility and surface functionalization are the most common problems for QDs employed in biological research. This review addresses the recent research progress made to improve aqueous solubility, functionalization of biomolecules to QD surface and the poorly understood chemistry involved in the steps of bio-functionalization of such nanoparticles.

Contents

1. Introduction . . . 29
2. Comparison of optical properties of QDs with conventional fluorophores for biological applications . . . 29
3. Need for surface modification . . . 30
   3.1. Ligand exchange processes . . . 32
   3.2. Surface silanization . . . 32
      3.2.1. Silanization process . . . 34
   3.3. Amphiphilic combination . . . 35
      3.3.1. Poly(acrylic acid)-based polymer with hydrophobic side chain . . . 36
      3.3.2. Poly(maleic anhydride) copolymers . . . 37
      3.3.3. Block copolymers . . . 37
   3.4. Coating strategies used for cross-linked shell . . . 37
      3.4.1. BIEE 1,2 bis-(2-iodoethoxy) ethane . . . 37
      3.4.2. Glutaraldehyde . . . 37
      3.4.3. Disulfide based cross linkers . . . 37
      3.4.4. Micellar phospholipid coating . . . 37
      3.4.5. Polyacrylate coating . . . 38
4. Functionalization chemistry for attaching biomolecules (DNA, peptides, antibodies, proteins, PEG) . . . 38
   4.1. Adsorption of functional molecules . . . 38
   4.2. Covalent coupling of biomolecules . . . 39
      4.2.1. Amide coupling . . . 39
      4.2.2. Thiol binding . . . 40
      4.2.3. Click chemistry . . . 41
5. Other coating methods . . . 41
   5.1. PEG-based coating . . . 41
   5.2. Aldehyde based methods . . . 41
   5.3. Carbohydrate based methods . . . 41
   5.4. Use of carbonyl diimidazole (CDI) . . . 41

* Corresponding author. Tel.: +91 20 40211000; fax: +91 20 40211002.
** Corresponding author. Tel.: +91 79 26302414; fax: +91 79 26302419.
E-mail addresses: ajay.karakoti@battelle-india.com (A.S. Karakoti), sanjay.singh@ahduni.edu.in (S. Singh).

http://dx.doi.org/10.1016/j.cis.2014.11.004
0001-8686/

6. Limitations and summary . . . 41
Acknowledgments . . . 42
References . . . 42

1. Introduction

Semiconductor quantum dots (QDs) are inorganic compounds composed of groups II–VI or III–V elements with unique optical properties [1–3]. The origin of these properties arises from the confinement of the states of charge carriers by physical reduction in the size of the nanoparticles. In essence, when the size of the particles is decreased below the exciton Bohr's radius of the bulk material it causes the energy levels to possess atom like properties and become discrete as compared to the continuum energy levels observed in bulk materials [3,4]. The properties of such nanoparticles can be described by a classical particle in a box quantum theory where the states of material become quantized. Semiconductor nanoparticles that demonstrate such properties are called as quantum dots. The distance between the discrete energy levels depend on the size of the quantum dots and increases with decrease in the size of the QDs. The dependence of electronic structure on the size of the nanoparticles thus provides a tunable handle to the optical properties of nanoparticles such as the optical absorption and emission characteristics. These characteristics have been discussed at length in several other review articles that address the issues of fluorescence, quantum confinement, Stoke's shift, quantum yield, blinking and resonance observed in QDs [5,6]. However, in current article, a short description of these properties and their limitations will be discussed in context of the biological applications. This review will emphasize on the aqueous solubility and attachment of biologically relevant ligands to QD surface to develop a basic understanding of various functionalization strategies for different biological applications of QDs.

Amongst all the disciplines of research in science and technology, biology being directly connected to human life shares complex economic, social and ethical challenges. The increase in expectancy of life has led to extensive research in early stage diagnosis and detection of medical problems. The medical treatment of diseases now requires continuous monitoring and localized application of drugs in addition to the early prediction of the effectiveness of treatment regime. The whole body exposure of drug or radiation is discouraged for the treatment of localized problem at a specific site of an organ. Thus the new age treatment of diseases requires precise control over the local delivery and action of the drug. In addition, to understand the complexity of several diseases and the efficacy of treatment of diseases against any drug it is necessary to understand the complex spatio-temporal interplay of biological molecules in various cellular processes [7]. Nanomaterials, such as gold, iron oxide (Fe_3O_4), carbon nanotubes and cerium oxide, have been recently explored for intrinsic enzyme mimetic properties, and hold great promise in colorimetric detection and monitoring systems used for simple and rapid disease diagnosis and imaging [8–15].

For decades, the intra and extra cellular imaging and *in-vitro* assay detection have been achieved through fluorescent labeling by dye molecules. However, the physico-chemical properties of the organic/bio-organic fluorophores and dye molecules are limited by their fixed and narrow absorption and broad emission profiles [3,4,16]. In addition, the restricted photo-stability of these fluorophores does not provide long term monitoring, imaging and multiplexing capacity without the requirement of complex instrumentation or post processing and analysis. Inorganic semiconductors are therefore a better option for replacement or conjugation of organic fluorophores with QDs [16,17]. Over the last two decades, the use of QDs have expanded for various biological applications including but not limited to:

a) *In-vitro* and *in-vivo* imaging probes
b) Pathogens and toxin detections
c) Gene technology/profiling
d) Fourier resonance energy transfer (FRET)

Apart from many other unique properties the size dependent fluorescence makes semiconductor QDs a versatile tool for various imaging applications in *in-vitro* and *in-vivo* imaging as compared to the conventional organic fluorescent tags [18,19].

2. Comparison of optical properties of QDs with conventional fluorophores for biological applications

The quantum confinement of QDs gives rise to unique size dependent properties such as the tunable absorption and emission from same type of QDs with size [20,21] The most important properties of conventional fluorophores that allow their use in biological applications such as *in-vitro* and *in-vivo* imaging are:

a) Position of excitation and emission spectrum
b) Width of the excitation and emission spectrum
c) The Stoke's shift
d) Photo stability of dyes on long term exposure to light
e) The decay lifetime of excitons

Conventional dyes and fluorophores generally show maximum absorption and emission at a fixed wavelength. The control over change in the absorption and emission wavelength would require a design of new organic compounds and complicated synthesis protocols [22]. Conventional fluorophore molecules also suffer from narrow absorption spectra and thus require excitation by light of specific wavelength being different for every organic molecule [3,4,16]. In addition, conventional dyes possess broad emission spectra and narrow Stoke's shift that causes the overlap of spectra of different dyes limiting the use of multiple dyes to tag different biomolecules or sections of cells [22–25]. In contrast, quantum dots demonstrate broad absorption and narrow emission spectra with large effective Stoke's shift. Fig. 1a and b depicts a direct comparison of absorption and emission spectra of QDs and Rhodamine 6G highlighting the narrower emission and broad absorption spectra that makes these ideal candidate for multiple imaging [3,4]. The broad absorption arises from the fact that in single crystal QDs the absorption of a photon above the semiconductor band gap creates an electron hole pair that has an increasing probability of occurrence as the energy of the photon is increased. The electronic structure of QDs can be varied easily by controlling the size of QDs and this allows tuning absorption and emission wavelength [26]. In fact, QDs can be excited at any single wavelength below their characteristic absorbance to obtain varying emission by just changing the size of the quantum dots viz. CdSe QDs (Fig. 2a). A very large Stoke's shift can also be observed that allows labeling of multiple biomolecules and cell compartments to be visualized under one single excitation (Fig. 2a). This is particularly useful to develop techniques such as multicolor imaging of cells under continuous illumination (Fig. 2b). Although to achieve such high level of complexity in *in-vitro* or *in-vivo* cellular imaging; a site selective targeting of QDs is required which emphasizes the need for controlled surface functionalization of quantum dots.

The radiative recombination of electrons and holes is also characterized by longer lifetime allowing QDs to demonstrate long fluorescence lifetimes as opposed to shorter lifetime of conventional fluorophores that is helpful in time gated detection of QDs to separate their signal from the background auto-fluorescence of cellular matter [29]. To obtain and retain such high fluorescence properties and quantum

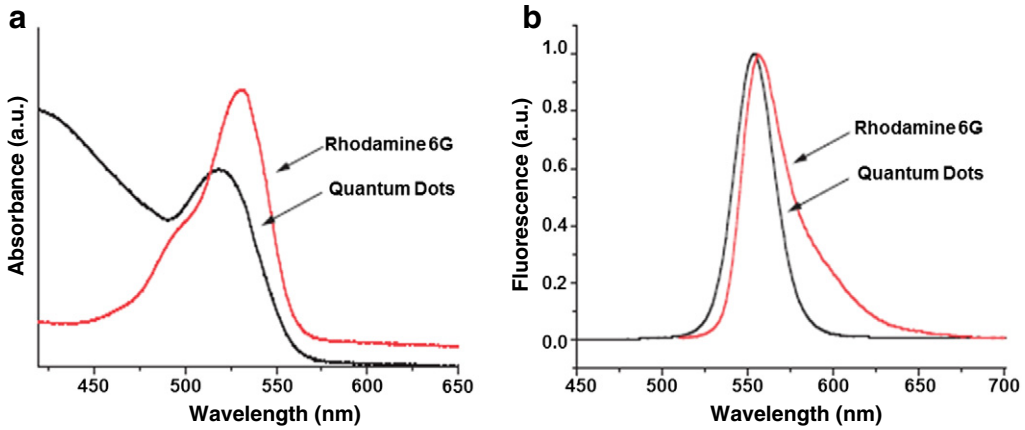

Fig. 1. Comparison of the absorption a) and emission b) behavior of quantum dots with Rhodamine 6G dye.
Reprinted from Ref [3], Copyright (2002) with permission from Elsevier.

yield special efforts are required in the design of functional QDs [30]. As the size of particles such as QDs is decreased, it increases the number of atoms representing the surface of QDs as compared to the bulk. Exposure of a large number of surface atoms increases the overall surface energy as more number of atoms with unsatisfied co-ordination (or with dangling bonds) are present on the surface of the QDs [31]. To compensate for this high energy, surface atoms usually undergo a redistribution and reconstruction resulting in phase changes [32] or creation of surface defects such as vacancies. These surface defects in the crystal structure can act as temporary traps for the electrons (or holes) and prevents their radiative recombination with the holes (or electrons) [33]. Such trapping events can decrease the overall quantum yield and also causes intermittent fluorescence (or blinking) and can limit the use of QDs for fluorescence [34] applications. Surface passivation of QDs with suitable shells can overcome this problem and protects the surface atoms of core quantum dots from undergoing oxidation or other chemical reactions with the environment [35,36]. Thus, intelligent techniques are required to passivate the surface of QDs and increase their quantum yield and photo stability especially in the presence of aggressive oxidizing environment.

High resistance to photo bleaching is another desired property for designing fluorescence tags and QDs display exceptional stability against photo bleaching on continuous excitation [25,38–40]. As compared to the organic fluorophores that bleach after only few minutes of exposure to the external radiation, QDs can cycle through repeated excitation and emission cycles for several hours. Quantum dots show high stability against a number of organic dyes such as Alexa 488 and Rhodamine 6G [4,5,41,42]. Fig. 3a shows the comparison of photo stability of Alexa 488 with streptavidin modified red QDs in labeling of 3T3 cells. While Alexa 488 photo bleaches within 60 s the fluorescence from red QDs retains bright intensity. QDs have been reported to exhibit stable emission over several hours (Fig. 3b) when compared to dye molecules that bleach within 10 min of exposure to light [37].

In addition, the unique electronic structure and availability of high surface area for adsorption allow the resonance transfer of electrons across the molecules make them ideal candidate for sensing applications in Fluorescence Resonance Energy Transfer (FRET) quenching assays for detection of biomolecules.

3. Need for surface modification

Recent developments show a promising future for biological applications of QDs. Even though QDs possess a very high photo stability, tunable fluorescence under single wavelength excitation and longer lifetime as compared to conventional fluorophores; bare uncoated QDs cannot be used directly for biological applications [43]. The need for surface passivation can be broadly categorized in three areas:

A) Surface properties of QDs (Fluorescence/emission characteristics) — As stated earlier, high surface energy associated with crystalline nanoparticles can form surface defects that can quench the fluorescence properties of bare QDs [4,44,45]. In

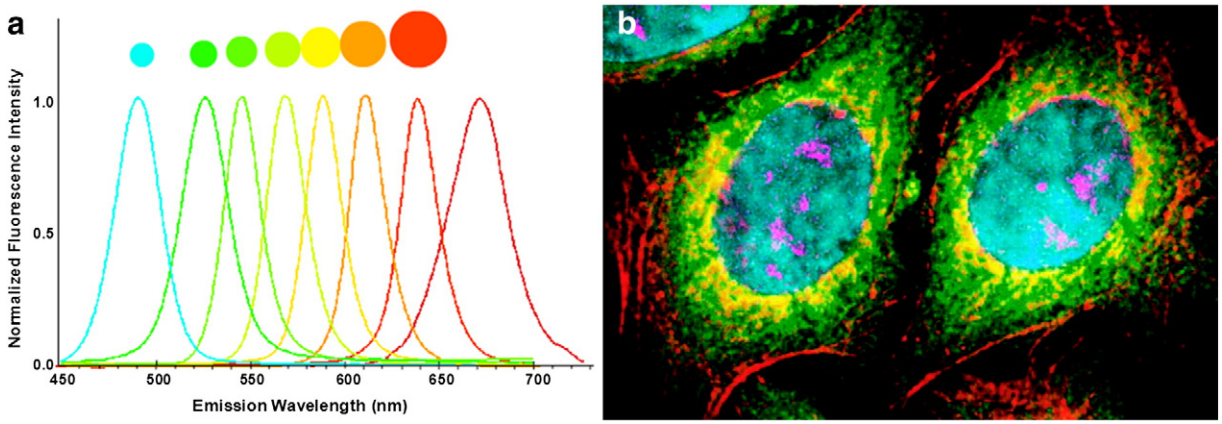

Fig. 2. a) Normalized photoemission of CdSe quantum dots under single wavelength excitation achieved by selectively varying the size of the quantum dots. b) Pseudo colored image of human epithelial cells by staining with five different sizes of QDs. Actin filaments — Red (705 nm), Nucleus — Cyan (655 nm), Ki67 protein — Magenta (605 nm), Microtubules — Green (565 nm), Mitochondria — Orange (525 nm).
Reprinted from Refs [3,27,28], Copyright (2002 & 2005) with permission from Elsevier and Macmillan Publishers Ltd.

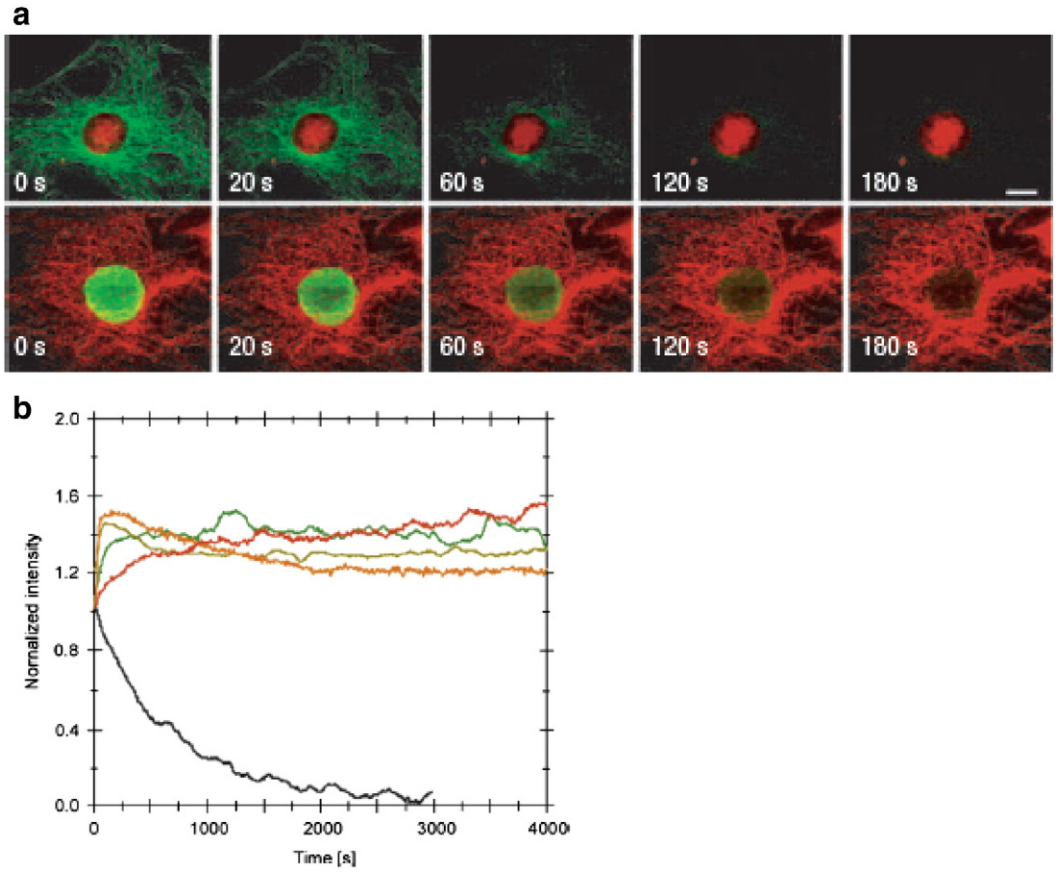

Fig. 3. Photo stability of QDs as compared to conventional fluorophores. a) Simultaneous labeling of 3T3 cells with QD 630-streptavidin (red) for nuclear antigens and Alexa Fluor 488 (green) for microtubules in top row and reversible labeling of Alexa Fluor 488 (green) for nuclear antigens and QD 630-streptavidin (red) for microtubules in bottom row shows the higher photo stability of QDs. b) Time dependent fluorescence intensity of QDs covered with silane shell as compared to Rhodamine 6G excited at 488 nm shows the bleaching of dye within 10 min of exposure as opposed to high stability of QDs over 4 h).
Reprinted from Refs [28,37] Copyright (2005 & 2001) with permission from Macmillan Publishers Ltd. and American Chemical Society.

addition, bare QDs can undergo surface oxidation, photochemical degradation and leaching of metal ions from the QD core can also occur on long term exposure of QDs to ionic media or cellular media (in biomedical applications) resulting in metal ion toxicity [46–48]. Thus, it is essential to cap the surface of QDs with stable compounds to reduce the surface defects and high reactivity. ZnS is often used as a capping agent to increase the stability and performance of QDs and enhance the quantum yield at room temperature [49].

B) Solubility in aqueous and biologically relevant media — While capping of QDs with an outer shell such as ZnS improve the stability and yield of QDs it does not improve the solubility of QDs in aqueous media. Quantum dots (core or core shell) are usually produced by high temperature processes in organic solvents (such as toluene, octane and hexane) stabilized by hydrophobic groups such as amines or phosphines to control their size and prevent further agglomeration. The core QDs can be capped with ZnS shell to give core shell morphology in a single step during the synthesis. However, the intrinsic solubility of QDs stabilized with such hydrophobic ligands in aqueous solution is poor. In order to increase the solubility in the aqueous media the surface of QDs can be modified with hydrophilic ligands [50–53]. There are three main strategies to replace or overcoat the QDs with hydrophilic ligands:

1) Ligand exchange — Cap exchange or ligand exchange is the process of substitution of native hydrophobic ligands such as TOP (trioctylphosphine), TOPO (trioctylphosphine oxide), HDA (hexadecylamine) on the surface of quantum dots with hydrophilic ligands through mass action [16,53]. Each of these substituting ligands generally possesses bifunctional groups such as a) thiols (–SH) to bind to the ZnS shell on the QD surface and b) carboxyls (–COOH), amines ($–NH_2$) or hydroxyls (OH) to improve water solubility and to provide attachment to secondary biomolecules such as proteins, drugs or antibodies [52,53].
2) Surface silanization — It is a process of capping the surface of QDs with a thin continuous layer of silanes that can be crosslinked [54]. The main advantage of silanization process lies in the fact that the ligand molecules are highly cross linked and hence forms a very stable capping agent. The end terminal groups of the silane shell can expose either their thiol, phosphonate or methyl terminal ends for further processing [53].
3) Amphiphilic combination — This method preserves the native TOP/TOPO/HDA layers on the surface and relies on the hydrophobic attraction between the hydrophobic groups of diblock or triblock copolymers with surface hydrophobic groups of QDs [55,56]. The solubility in aqueous solution is then provided through the hydrophilic groups of the block copolymers.

C) Targeted delivery of QDs — Aqueous solubility of QDs is not sufficient for *in-vitro* and *in-vivo* applications of quantum dots [4,57]. While each of the above-mentioned processes for achieving the solubility of QDs has its own advantages and disadvantages, the choice of one method over the other usually depends upon the end application and the ease of availability or familiarity of one method over the other. A common factor of all the modification processes that provide aqueous solubility is the choice of the ligand molecules because these ligands also serve as the anchor points for achieving further functionalization of QDs with biomolecules such as peptides, DNA, proteins and

drugs [58–60]. Several surface capping strategies of QDs with respective choices of ligands and mechanism of interaction between QDs and functional biomolecules have been reported (Table 1). It is evident that several alternative strategies and ligands have been developed over the last decade for specific biological applications. The target specific delivery of quantum dots is essential to minimize their exposure to non-relevant cells, increase greater contrast and localized application of FRET based processes [4,58]. The strategies for bio-functionalization revolve around the two main anchor points — the native thiol bonding between the sulfur groups from ZnS capped QDs and the bi-functional thiol ligands or silanes [28] and like hydrophobic interaction between the hydrophobic ligands from QDs and hydrophobic end from block co-polymers [61]., Phase transfer and particle functionalization, have served as the two most common strategies for QD surface modification with biomolecules, linkers used and the processes involved (Table 2).

A brief description of the strategies to increase the aqueous solubility and attach various biomolecules has been discussed in the subsequent sections.

3.1. Ligand exchange processes

The ligand exchange process can simply be defined as the replacement of an existing nonfunctional ligand with a mono or bi-functional ligand that provides QDs with additional properties such as solubility, mobility and targeting [28,66,76,77]. As stated earlier the surface of QDs is capped with organic long chain ligands such as TOP (trioctylphosphine), TOPO (trioctylphosphine oxide), HDA (hexadecyl amine), OA (oleic acid) and TDPA (tetradecyl phosphonic acid) [78,79]. These ligands prevent the aggregation and growth of QDs during synthesis and passivate surface defects to preserve quantum yield [79–81]. Organic ligands provide excellent solubility and stability to quantum dots in organic non-coordinating solvents and can be exchanged with water soluble ligands through simple mass action [82]. Ligands on the surface of QDs in their native solvent are in a state of dynamic equilibrium with the solvent. This means that the ligands continuously leave the surface of QDs and go to the solvent and free ligands from the solvent attach to the newly available site [83]. Ligand exchange process takes advantage of this dynamic equilibrium process by addition of a new ligand that can compete for the surface of quantum dots. In order to replace the existing ligands on the surface of QDs the concentration of the replacing ligands should be at least equal to the concentration of existing ligands and the replacing ligands should have higher affinity for the surface of QDs [28,49,77,84]. The ligand exchange can still be carried out by increasing the concentration of replacing ligands well above the concentration of the existing ligands if the surface affinity of the replacing ligands is low, thereby increasing the local probability of attachment of replacing ligands [85].

Querner et al. [86] described replacing ligand as an X–Y–Z moiety where X is a functional group with relatively higher affinity for quantum dot surface, Y is the spacer and Z is the terminal functional group that can provide the solubility and additional linking ability to QDs. Thus, for replacing an existing ligand with a desired ligand the most important property is the metal–ligand binding affinity [53,87]. In most biological applications the QDs have a top layer of ZnS shell and thus the metal–ligand bond becomes of considerable importance. Thiols, such as mercaptoacetic acid (MAA), mercaptopropanoic acid (MPA), dihydrolipoic acid (DHLA), dithiothreitol, and carbodithiolates are widely used as the ligand of choice to replace existing ligands on the surface of QDs. While the thiol group in these ligands serve as the binding site to QDs, the carboxyl groups, usually negatively charged at neutral pH (or are present as carboxylate anion at a pH above their pKa (acid dissociation constant)), provides negative charge to the surface of QDs and stabilize QD suspension through electrostatic repulsion. Computational modeling of the attachment of thiols to the surface of quantum dots have confirmed that thiols primarily bond to the zinc metal on the surface through Zn – S bonds along with a weaker sulfur – sulfur bond [88–91]. The affinity of sulfur atoms for the metal drives the exchange process on the surface of QDs however; this affinity is relatively weaker and can be improved by using disulfide linking groups. Ligand exchange reaction to improve the solubility and functionality of quantum dots have two unique advantages: a) the simplicity of the ligand exchange process and b) the diameter of QDs after ligand exchange can be tightly controlled to relatively smaller value as compared to silanization and amphiphilic attachment of ligands. These advantages have resulted in synthesis of a large number of ligands to provide additional stability and functionality for biological applications of quantum dots. The small diameter of QDs is important for the use of QDs in applications such as FRET quenching assays as compact ligands increase the access of QDs to the molecular targets in *in-vivo* imaging [73]. Despite these advantages the ligand exchange process has several disadvantages:

i. The relatively weaker interaction between metal at QD surface and S atoms in ligands results in longer exchange reaction times exceeding well over several hours.
ii. The electrostatic stability of QDs is limited in high salt concentration medium and leads to agglomeration of QDs at a concentration exceeding few hundred millimolar.
iii. The thiol molecules form disulfides over long term storage and detach from the surface of QDs causing their aggregation and oxidation in aqueous media.
iv. The ligand exchange process alters the surface of the QDs and irreversibly decreases the quantum yield of the QDs after ligand exchange process.

Some of the drawbacks of weaker thiol–metal interactions have been addressed by utilization of disulfide linkages that are more stable than the monothiol ligands [85,92]. DHLA based approaches have improved the stability of QDs against oxidation and allowed long term storage of QDs with relatively higher quantum yields. Higher stability of QDs in these ligands arises from the chelating effect of the disulfide linkages with metal atoms on the surface of QDs. However, the DHLA suspensions are stable at relatively alkaline pH and tend to agglomerate under slightly acidic conditions due to the protonation of the carboxylate anion at acidic pH thereby neutralizing the electrostatic stability [85] . Susumu and coworkers have utilized PEG terminated DHLA as an alternative strategy to overcome this limitation of stability in acidic pH. PEG-terminated DHLA capped QDs were further attached to thioctic acid to synthesize a series of amine, carboxyl, hydroxyl and biotin terminated QDs using a scheme of reaction shown in Fig. 5 [93].

While several types of ligands have been developed for enhancing the stability and yield of QDs in aqueous suspension the problem of aggregation and long term stability persists especially when QDs are dialyzed against buffers or water for purification following attachment of biofunctional molecules. Further advancement in the field using phosphine, peptides and cross linked dendrons have been made but these processes adds complexity to the ligand exchange process and increase the size of the QDs thereby compromising the two most unique advantages of the ligand exchange process [95].

3.2. Surface silanization

The process of creating a layer of amorphous silica on the surface of nanoparticles is called silanization. The electrochemical properties of silica make it a suitable material for increasing the solubility of QDs in aqueous media while retaining most of its emission properties. Silica shows anomalous behavior in water as it does not coalesce above its iso-electric point (IEP) like most oxides and shows strong stability at near neutral pH even under high salt concentration. The high stability

Table 1
Strategies and surface functional groups for enhancing the aqueous solubility and attachment of QDs with biomolecules for biological applications [28].
Reprinted from Ref [28], Copyright (2005), Macmillan Publishers Ltd.

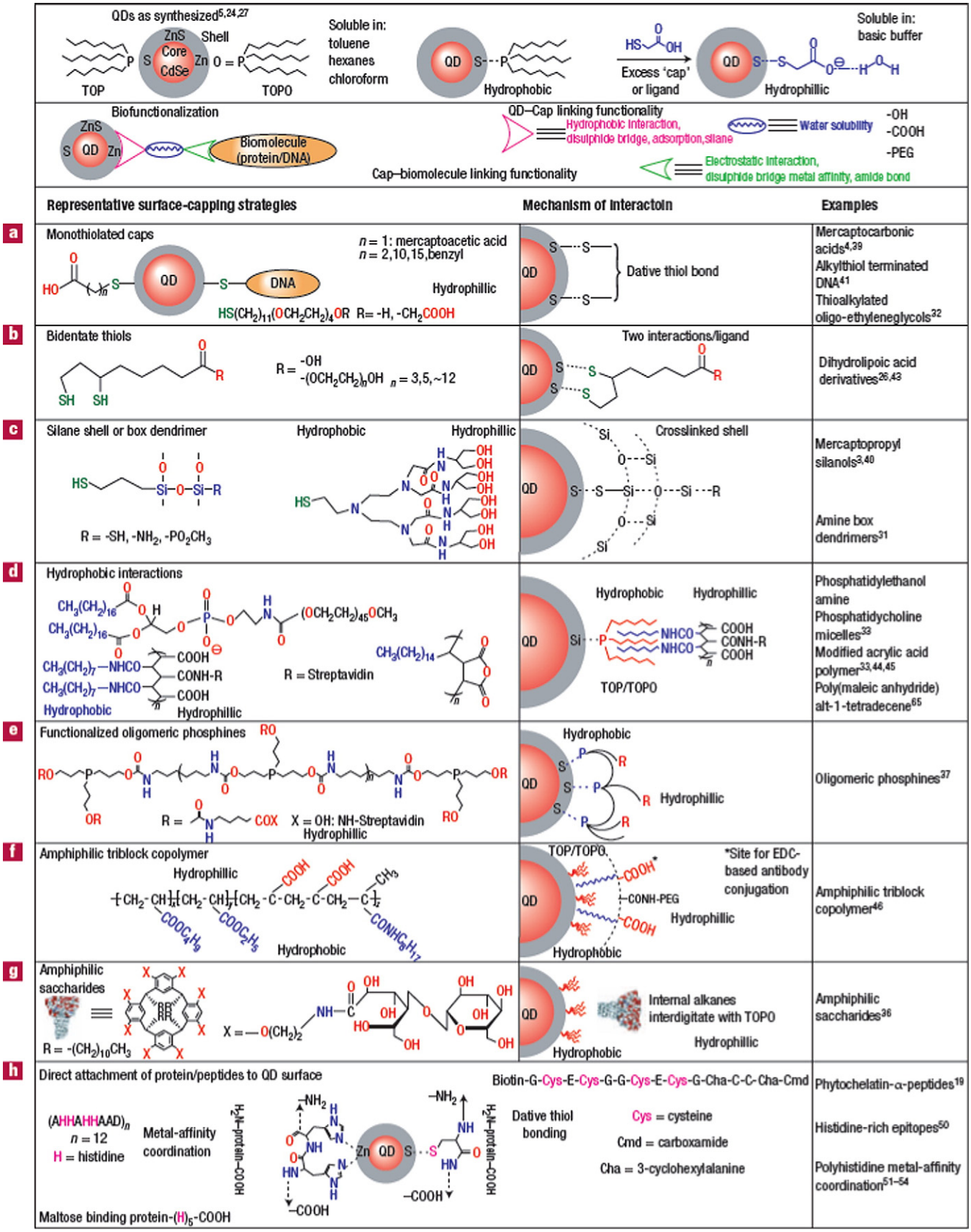

Table 2
Common strategies and processes involved in the surface bio-functionalization of QDs for possible biomedical applications.

Strategies	Processes	Linkers	Bio-molecules attached	Type of QDs	Ref
Phase transfer	Ligand exchange	Polyethylene glycol (PEG) grafted polyethylenimine	–	CdSe/CdS/ZnS	[62]
		3-mercaptopropionic acid (3-MPA)	DNA	CdSe/ZnS	[63]
		Lipoic acid	–	CdSe	[64]
	Silanization	Biotin–streptavidin	IgG	CdSe/ZnS	[65]
	Ligand modification	Thiotic acid	PEG	CdSe–ZnS	[66]
	Polymer coating	Ttrioctylphosphine oxide (TOPO) and hexadecylamine	Amphiphilic polymers	CdSe/ZnS	[67]
	Additional coating layer	1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride and N-hydroxysulfosuccinimide (Sulfo-NHS) EDC/NHS)	Anti-CD3	CdTe (CdS and ZnS layers)	[68]
Particle functionalization	Chemical functional groups	Mercaptocarboxylic acids	PEG	CdSe/ZnS	[69]
	Polyethylene glycol	Amine	PEG/folate	CdTe	[70]
	Biomolecules	Mercaptopropionic acid	DNA	CdSe/ZnS	[71]
		Amino-polyethylene-glycol and sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate	Peptides	CdSeTe/ZnS	[72]
		Mercaptopropionic acid	Proteins	$Zn_xHg_{1-x}Se$	[73]
		Carboxylic acid functionalized poly(vinyl alcohol)	Glucose oxidase	CdS	[74]
		Polyethylene glycol and thiol	IgG	$CdTe/SiO_2$	[75]
		Modification of polyacrylic acid with both N-octylamine (OA) and 5-amino-1-pentanol	Rhodamine isothiocyanate (RITC)	CdSe/ZnS	[75]

beyond the IEP of silica arises from the strong short range forces of the order of 4 nm. The hydrogen bonded layers of water on the surface of silica undergoes hydrogen bonding with silica surface that modifies the water network at the surface and causes strong steric repulsion. The steric repulsion is sufficiently strong to reduce agglomeration even at higher volume fractions. Thus, silica as a surface layer is more advantageous than just electrophoretic stabilization of double layers as they can endure large variations in pH and particle concentrations. In addition, to the stability of the dispersion, coating QDs with silica layer does not alter the optical properties of QDs. Even though the particle size increases by silica encapsulation of QDs, the sols display the properties of core QDs and do not display large changes in the exciton absorbance or PL. One of the other key features of silica coating is the reduction of surface interaction with atmospheric oxygen. Thus, the photostability of QDs can be greatly increased by preventing surface oxidation of metals to oxides. Silica encapsulated QDs are claimed to be at least 100 times more stable to photochemical oxidation than bare uncoated QDs. A direct comparison of the photochemical stability of the silica encapsulated QDs with citrate coated CdS was reported (Fig. 6) [96]. Citrate coated CdS show photochemical degradation within 24 h of exposure to daylight while the silica encapsulated CdS were highly stable to photo-oxidation.

The dissolution of CdS occurs through the oxidation step in which S is oxidized by oxygen to SO_4^{2-}. Silica coated prevents the access of oxygen to the CdS surface thereby reducing the dissolution and increasing the photostability of CdS.

3.2.1. Silanization process

The silanization of QDs is a laborious process that goes through multiple steps. The first step involves the activation of QD surface for attaching the first layer of silane molecules (often called as primer layer) to QD surface. This would require an exchange of non-polar ligands such as TOPO and HDA on the surface of QDs to be replaced with more polar ligands that can be dispersed in ethanol or water. Most developments have been made in the surface activation step of QDs for silanization. Earlier Correa-Duarte et al. [95,97] exchanged the citrate coated QDs with silica coating by using (3-sulfanylprosulfanylpropyl) trimethoxysilane (MPS) as the first layer of silica followed by solvent exchange in ethanol. The silica shell was then grown in thickness using the conventional Stober process by addition of tetraethyl orthosilicate (TEOS) to the colloidal solution.

Nann and Mulvaney used the gradual hydrophobic–hydrophilic phase transfer of QDs in solvent by exchanging the TOPO and HDA ligands on the QD surface with MPS in tetrahydrofuran (THF) [98].

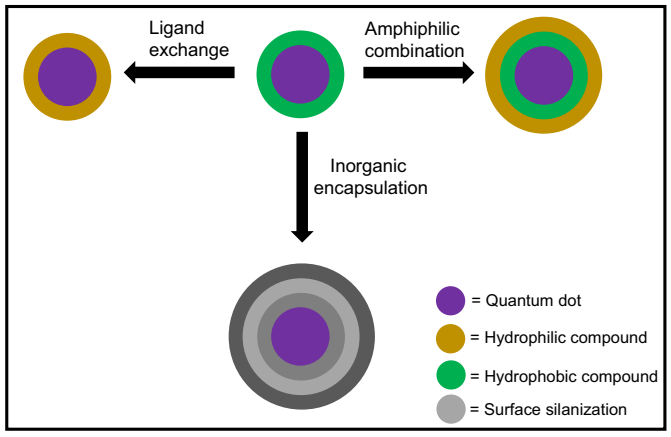

Fig. 4. Strategies for increasing the solubility of CdSe QDs includes ligand exchange or cap exchange process, amphiphilic combination of diblock or triblock copolymers and phospholipids that bond to hydrophobic groups and surface silanization of core QDs.

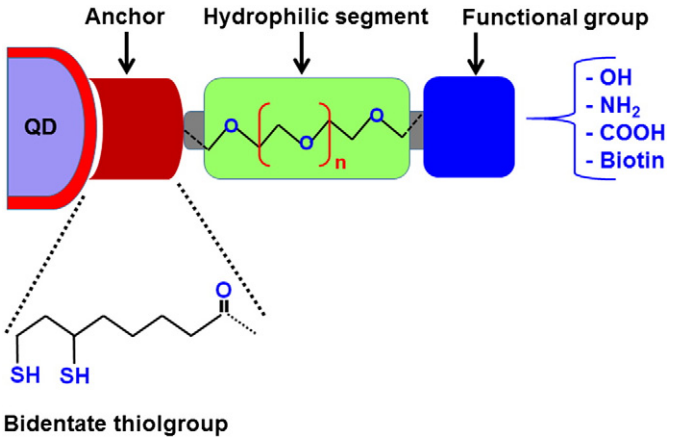

Fig. 5. PEG-terminated DHLA capped QDs and their attachment to secondary functional group for further functionalization of water soluble QDs.
Reprinted from Ref [94], Copyright (2007), American Chemical Society.

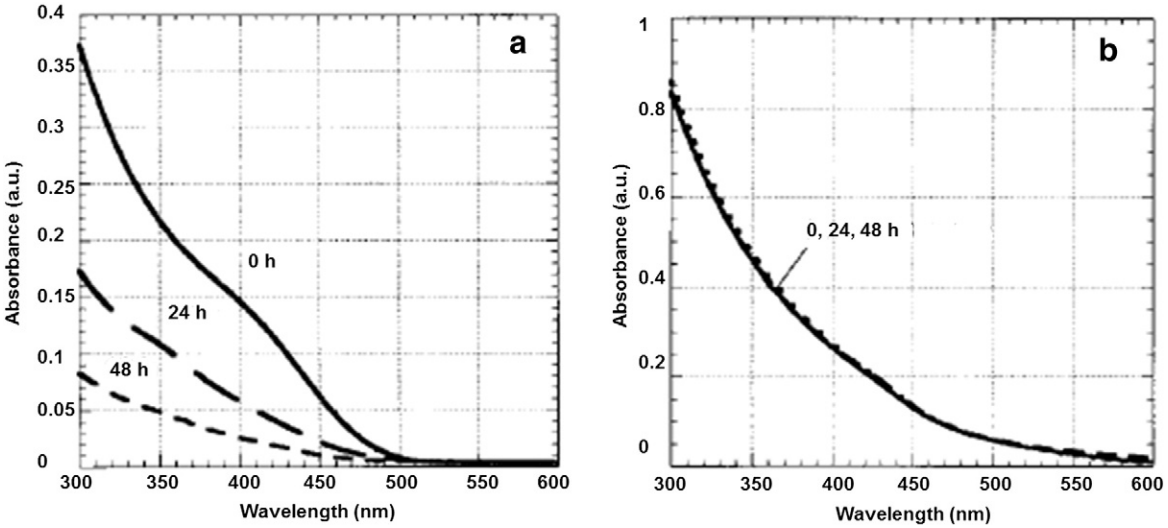

Fig. 6. Absorption spectra of citrate (a) and silica (b) coated CdS quantum dots as a function of exposure to daylight in an air saturated solution. Reprinted from Ref [96], Copyright (1998), Elsevier.

The MPS coated QDs were dissolved in water free polar solvents such as methanol and ethanol and the silica shell thickness was increased by varying the concentration of TEOS, water, ammonia and concentration of MPS capped QDs. Exchanging MPS with 3-aminopropyl(trimethoxysilane) (APS) results in the formation of clusters of QDs trapped in silica matrix. A right balance of water and ammonia is required in creating a uniform and thick shell of silica around the QDs. Water in general catalyzes the hydrolysis of TEOS causing nucleation of silica particles over the QDs [99]. Thus increasing the water content serves two purposes a) increase the rate of nucleation b) provide colloidal stability to the silica encapsulated QDs through ionization of surface silanol ($SiOH \Leftrightarrow SiO^- + H^+$) thereby providing electrostatic stabilization [100,101]. The concentration of MPS coated QDs is very critical for controlling the aggregation and polydispersity in size. Generally, a very low concentration of QDs (50 nM) leads to the formation of one quantum dot per silica while increasing the concentration can cause multiple quantum dots to be encapsulated within the silica matrix along with an increase in size of the silica shell. The effect of QD concentration on the final particle size of the silica encapsulated QDs has been observed (Fig. 7). Nann and Mulvaney [98] proposed that the size of the final particles can be calculated by the following equation:

$$d = 2r = 2^3\sqrt{\frac{3}{4\pi}V_{tot}}$$

where V_{tot} is the total volume of one particle $= V_{QD} + V_{SiO_2}$ and the total amount of silica per particle (V_{SiO2}) can be calculated from $V_{SiO2} = n_{TEOS} / (N_{QD} \times C)$ where n_{TEOS} is the number of moles of TEOS, N_{QD} is the number of QD particles and C is the molar density of silica in mol L^{-1}. The calculated values of the size of silica encapsulated QDs is shown as dashed line that is in agreement with the experimental values (in Fig. 7).

The encapsulation of QDs in silica through the solvent exchange route gives single quantum dots in silica matrix with relatively uniform size and narrow size distribution however, the particles obtained have often low quantum yield (up to 18%) and can be synthesized in very low concentrations. In addition, the exchange is surface ligands do now allow the addition of substances that can stabilize the spectral characteristics of silica over coated QDs. Zhelev et al. [102] reported the synthesis of QDs without exchanging their primary surface coordinating ligands (TOPO, HDA or ODA) in a laborious process involving QD micelle formation.

The configuration of such a molecule and the particle size distribution from the TEM is shown in Fig. 8. The micelle consists of a single QD (such as CdSe, CdS, CdSe/ZnS) with its capping ligand and a hydrophobic chain of silica precursor (n-octyltriethoxysilane OTS). The micelle was then covered by a silica precursor such as triethoxy vinylsilane (TEVS) and finally the surface is covered by a third silica precursor (3-(2-aminoethylamino)-propyl-trimethoxysilane) containing terminal amine groups [104]. The termination of silane in the amine groups allows further conjugation of silica encapsulated QDs with biological and/or dye molecules by classical amide coupling.

3.3. Amphiphilic combination

Several reports have shown the stabilization of QDs by ligand exchange, chemical modification of surface by various functional groups and other covalent modification, however, suffers from several drawbacks. (i) Small ligand having one head group attached to the QDs surface can easily desorb and affect the stabilization, especially in suspension media free of excess unbound ligands. (ii) Although, it has been found that thiol-containing ligands bind relatively strongly to

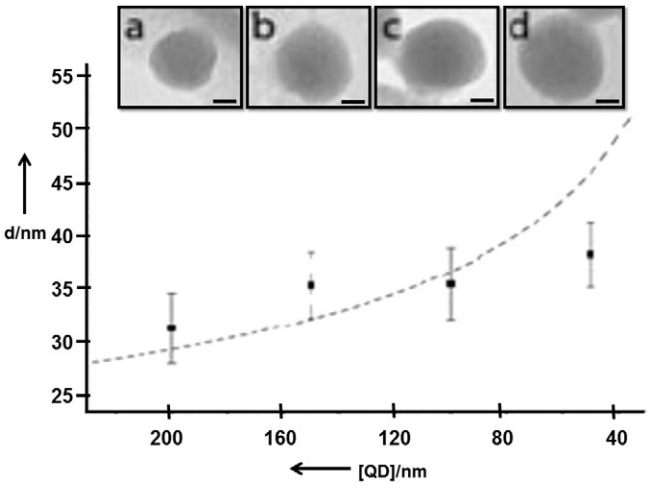

Fig. 7. The effect of increasing the concentration of QDs on the total size of the silica encapsulated QDs. Dotted lines represent the theoretically calculated values and the scale bars on the image represent 10 nm.
Reprinted from Ref [98].

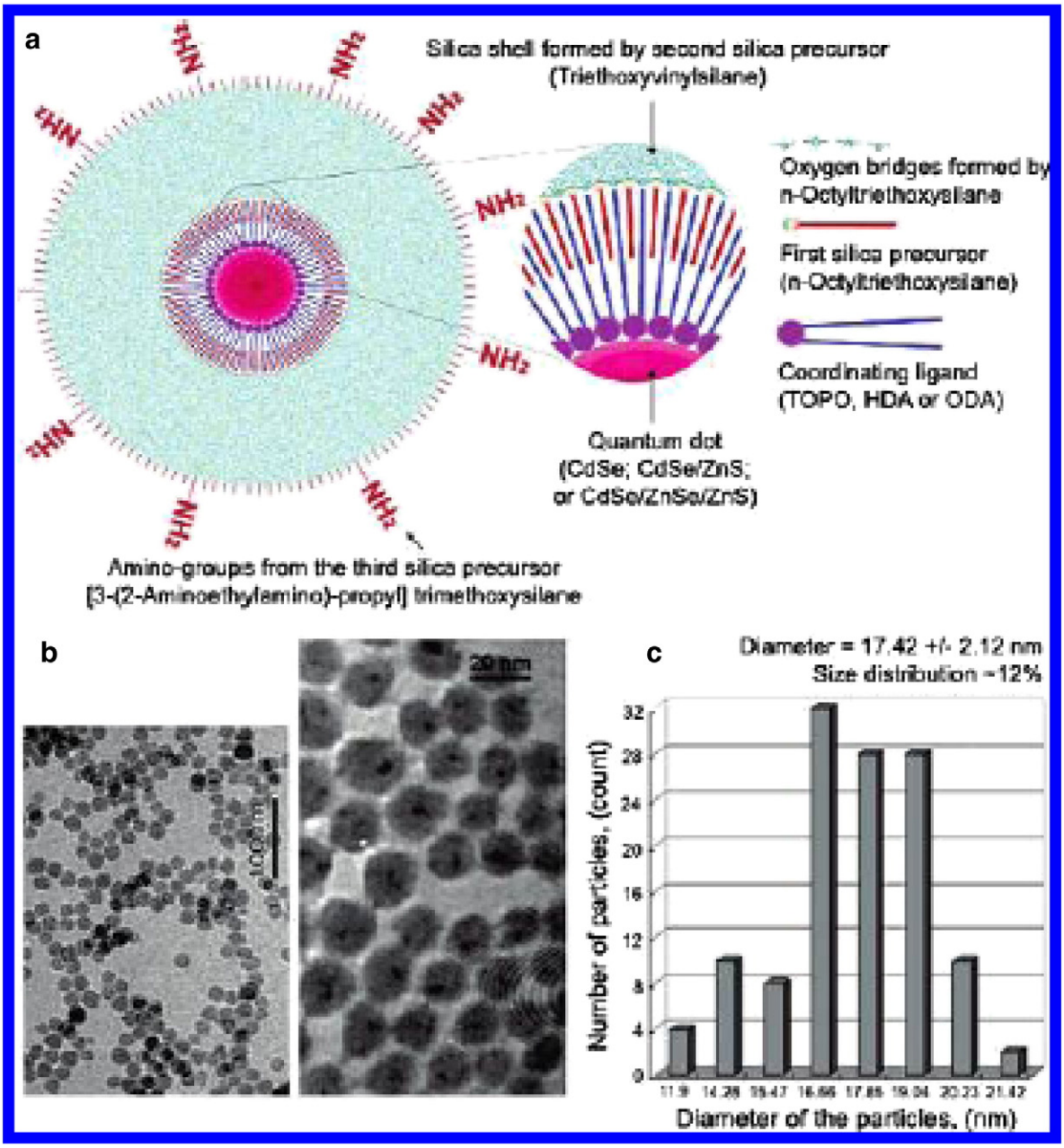

Fig. 8. a) Model structure of silica encapsulated single QDs prepared using micelle based encapsulation b) representative TEM images show the monodisperse core shell nanoparticles and c) the particle size histogram shows the narrow size distribution of the matrix.
Reprinted from Ref [103], Copyright (2006), American Chemical Society.

QDs, in general the ligand molecule has to be carefully chosen to the given core material [59]. Further, multifunctional ligand molecule for surface coating has always shown additional advantages such as enhanced stabilization, chemical modification, which has been well established in a variety of reported protocols [59,94]. Interestingly, using amphiphilic molecules for coating QDs prevents facile desorption of the polymer molecule, due to many contact points, from the particle surface e.g. by thermal fluctuations. For example, the amphiphilic coating bind the amphiphilic molecule with hydrophobic ligand molecules of QDs by hydrophobic interaction and does not depend on either material composition or exact type of ligand molecule. Such observations are mainly based on hydrophobic interaction of hydrocarbon chains and van der Waals forces between the molecules. Finally, the amphiphilic molecules coated QDs exhibit the same physical and chemical surface properties independent of core material [59]. Out of several amphiphilic combination molecules reported in literature, few are discussed below:

3.3.1. Poly(acrylic acid)-based polymer with hydrophobic side chain

Poly(acrylic acid), PAA, is a highly charged linear polyelectrolyte, containing carboxylic acid groups which could be modified with aliphatic amines by amide bonds [105]. Wu et al. [41] have shown that PAA modified with octylamine can be used for phase transferring QDs from organic to aqueous suspension media and is probably used for commercial water soluble QDs. For phase transfer applications, amphiphilic polymers follow simple methods, wherein they dissolve in organic solvent with QDs, such as QDs with TOP/TOPO ligands. After evaporation of organic solvent, the obtained solid can be dissolved in an aqueous suspension. Wu et al. [41] have shown that the stability of

such QDs can be enhanced by further crosslinking lysine by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) chemistry [106]. It was also demonstrated that PAA backbone can be modified with a mixture of octylamine and isopropylamine for efficient QDs coating and stabilization. Kairdolf et al. have produced a CdTe/CdSe QDs in presence of PAA modified (40%) with dodecylamine, yielding amphiphilic QDs soluble in both organic and aqueous solvents [107].

3.3.2. Poly(maleic anhydride) copolymers

Poly(maleic anhydride) (PMA) copolymers are another class of amphiphilic polymers, that can be synthesized by copolymerization of maleic anhydride with olefins making it an alternating copolymer [59]. In PMA copolymers, unlike PAA, the hydrophobic chains are not randomly grafted rather aligned in alternating layers with a higher density of carboxylic acid functional groups. During phase transfer reaction the maleic anhydride rings hydrolyze upon contact with water and open to form two carboxylic acid groups [108]. Several commercial PMA derivatives are available and have been shown to modify QDs successfully leading to stable aqueous suspension, such as poly(maleic anhydride alt-1-tetradecene), poly(maleic anhydride alt-1-octadecene) etc [109]. The self-reacting property of maleic anhydride with primary amines and alcohols can be exploited for modification of polymer before it is used for QDs surface modification [110]. The reaction between maleic anhydride ring with amine gives carboxylic acid functional group and it has been seen that in the case of PEG resulting in increased stability in biological environment [111]. Other modifications have also been reported where hydrophobic side chains, consisting of dodecylamine, are modified by fluorescent dyes, biotin, sugars or PEG etc. functional molecules by covalent modification, leaving a part of the anhydride rings intact [112].

3.3.3. Block copolymers

Block-copolymers are another class of amphiphiles, being used for nanomaterials surface modification including QDs, consists of a hydrophobic and a hydrophilic part [113,114]. These polymers readily form micellar structure with either hydrophilic or hydrophobic part aligned inside, in contrast to their suspension solvent. Such structures have been used for nanomaterial synthesis and capping and even phase transfer from one solvent to another has been demonstrated [115,116]. Certain block copolymers have also been reported to laterally cross link [117–119] and the thickness of the polymer coat over nanoparticle surface can be controlled by choice of appropriate polymer with suitable block lengths. [120,121] In a recent study by Jia et al. [122], QDs were incorporated within micelles to form Pluronic-QD micelles thus making a novel micro reactor. Enzymes such as glucose oxidase (GOX) and horseradish peroxidase (HRP) were respectively labeled with fluorescent dyes present in close vicinity of QDs, thus resulting FRET occurs between the QDs and dyes. Such study demonstrates a versatile platform for multienzyme colocalization and an effective strategy to characterize multienzyme immobilization and colocalization, which can be applicable to many other systems. Similarly, location control and separation of the quantum dots (QDs) within the microspheres was achieved by a supramolecular assembly of block copolymer micelles to control the Förster resonance energy transfer efficiency between the different-colored QDs [123].

3.4. Coating strategies used for cross-linked shell

3.4.1. BIEE 1,2 bis-(2-iodoethoxy) ethane

BIEE has been used as a bifunctional cross-linker which provides tunable hydrophilicity depending on the solution pH in micelles. Shell cross-linked (SCL) micelles with pH receptive core have been reported by Luo et al. [124]. Limited reports are available regarding the use of BIEE in stabilizing and creating cross-linked shells in metal NPs, metal-oxide NPs and quantum dots. Luo et al. [124] reported core/shell type AuNPs stabilized with a monolayer of double hydrophobic block copolymer, poly(2-dimethylamino)ethyl methacrylate)-b-poly(ethylene oxide) (PDMA-b-PEO) having thiol group at the chain end. Further, the thiolated PDMA-b-PEO was exchanged with citrate groups present on Au NPs surface and BIEE was used to selectively cross-link the PDMA residues in the inner shell. Interestingly, it was observed that while there was no change in exhibiting reversible pH responsiveness in both cross-linked and uncross-linked Au NPs, the shell cross-linked Au NPs showed a robust core-shell nanostructure with high colloidal stability. Similarly, a dendritic-linear block copolymer modified superparamagnetic iron oxide nanoparticles (SPIONs) were synthesized by Wu et al. [125]. SPIONs consist of a Fe_3O_4 magnetic nanoparticle core and a dendritic-linear block copolymer, the focal point polyamidoamine-type dendron-b-poly(2-dimethylaminoethyl methacrylate)-b-poly(N-isopropylacrylamide) (PAMAM-b-PDMAEMA-b-PNIPAM) shell can be synthesized by two-step atom transfer radical polymerization (ATRP). Here to reverse the aggregation of SPIONs, a crosslinking reaction between PDMAEMA block and 1,2-bis(2-iodoethoxy)ethane (BIEE) was used (Fig. 4).

3.4.2. Glutaraldehyde

Glutaraldehyde reacts with amine, thiol, phenol, or imidazole functional groups present in biomolecules where the most reactive amino acid side-chains are nucleophiles [126]. These nucleophiles attack the carbonyl group of glutaraldehyde and form an imine. Using this strategy, Santos and co-workers reported the conjugation of $CdS/Cd(OH)_2$ QDs functionalized with concanavalin-A (Con-A) lectin using glutaraldehyde coated $CdS/Cd(OH)_2$ QDs. Con-A lectin is a protein which binds specifically to glucose/mannose residues expressed in the plasma membrane. In another attempt, the same group synthesized $CdS/Cd(OH)_2$ QDs functionalized with glutaraldehyde as efficient fluorescent labels for imaging of living human red blood cells. This study was aimed to determine the antigen-A expression in subgroups of group A erythrocytes.

3.4.3. Disulfide based cross linkers

The conjugation of antibodies to QDs through cross linking of QDs amine with the sulfhydryl group has been shown as a very successful approach. QDs coated with polyamidoamine (PAMAM) and polyisoprene have shown great potential for applications in targeted delivery for cancer and bioimaging [127–129]. Although many biomedical applications of QDs have been shown by using disulfide based cross linkers, this approach can often result in alteration of physical and chemical states of QDs which leads to decrease in quantum efficiency. Jin et al. [130] have reported that surface modification of CdSe/Te CdS QDs with GSH in a tetrahydrofuran—water solution results in only 22% quantum yield. Further, the stability of QDs under *in vivo* experimental conditions are not well understood. The disulfide bond between thiol group and QDs can break due to oxidation which can cause aggregation of QDs.

3.4.4. Micellar phospholipid coating

QDs encapsulated within phospholipid micelles and liposomes are attractive biomedical agents for several reasons: first, the encapsulation process does not alter the surface of QDs, second, the optical properties are preserved, and third, the dense surface phospholipid layer avoids the nonspecific adsorption, which is one of the major hurdles in the *in vivo* success of drug delivery. One of the pioneering reports from Dubertret et al. [131] showed that CdSe/ZnS QDs encapsulated in phospholipid block copolymer micelle can be used for *in vitro* and *in vivo* imaging. Further, its DNA conjugate acted as *in vitro* fluorescent probe and hybridized with the specific complimentary sequences. Overall, this system provided better colloidal stability and reduced photobleaching in various biological environments when compared with other similar systems. PEG-grafted phospholipid micelles have also been reported to encapsulate $CdTe_{1-x}Se_x/CdS$ QDs [132]. Micelle-encapsulated QDs were conjugated with a cyclic arginine–glycine–aspartic acid (cRGD)

peptide which targeted the $\alpha_v\beta_3$ integrins, overexpressed in angiogenic tumor vasculatures. Erogbogbo et al. [133] reported silicon QDs and iron oxide nanoparticles coencapsulated in phospholipid-polyethylene glycol (DSPE-PEG) micelles and showed their luminescence stability in a prostate cancer microenvironment under *in vivo* condition. Lipid-coated QDs have also been synthesized by many groups and have been used in cellular and *in vivo* imaging [134–136].

3.4.5. Polyacrylate coating

Polymeric coating over QDs are effective on providing steric stabilization and moieties to further functionalize with biomolecules. Polyacrylate provides stabilization to colloidal nanoparticles by electrostatic repulsion. Additionally, each polymer chain provides multiple sites for adsorption and the coated QDs would have several free carboxyl group presented for further functionalization. Celebi et al. [137] reported the synthesis of poly(acrylic acid) stabilized cadmium sulfide QDs in aqueous solution. They found that the maximum quantum yield was QD size and polyacrylate's molecular weight dependent. The synthesized QDs showed a quantum yield of 17% and were stable for more than 8 months. Wei et al. [138] synthesized TAT-functionalized quantum dots using polyacrylate TAT-QD(polyacrylate) studied for localization of TAT-QD(polyacrylate) in cells. They found that these QDs were localized in both the perinuclear regions and the lysosomes. They also observed that TAT-QD(polyacrylate) were comparatively more stable and less cytotoxic than TAT-QDs synthesized by other methods. The best available methods for high quality QDs synthesis are based on organic ligands such as TOPO which results in non-aqueous dispersed QDs. This limits the utilization of these QDs in biological applications as most biological interactions take place in aqueous media. In this important aspect, polyacrylic acids have also been used to prepare water soluble QDs via a ligand exchange process between organic soluble QD-TOPO and polyacrylic acid [139].

4. Functionalization chemistry for attaching biomolecules (DNA, peptides, antibodies, proteins, PEG)

In order to utilize water soluble QDs for applications in bioimaging, detection and other applications including drug delivery, the QDs have to be attached to functional biomolecules such as proteins, enzymes, antibodies and nucleic acids [140]. All these biologically relevant molecules have enough binding sites available that can be attached to QDs to achieve cell targeting, attachment of selective toxins, conjugation to FRET coupling molecules or simply increasing the total uptake of QDs in cells [141] (Fig. 9). For example proteins, antibodies and peptides contain several amine and carboxyl groups that can be coupled to the surface modified quantum dots through simple amide bonding [59]. Similarly amine modified DNA can be immobilized on the surface of QDs through amine coupling. DNA by itself has several coupling sites such as phosphate, amines and hydroxyl and it can also bind non-specifically with QDs and other molecules present in the system [142]. This nonspecific binding is found to be entropically feasible. DNA functionalized quantum dots are exploited in colorimetric detection assays for gene detection taking the advantage of specific and complimentary binding of the DNA molecules [18]. Coupling of PEG molecules have been shown to increase the cellular uptake as well increase the residence time of nanoparticles in the body [143]. PEGylated QDs can thus provide the biocompatibility as well as increase the retention of QDs so that smaller doses of nanoparticles can reach the targets by avoiding uptake by the reticuloendothelial system (RES) of the body [93]. However, PEG molecules need to be activated with functional groups such as amine, thiols or carboxyls to achieve covalent ligation with QDs. High molecular weight PEG molecules can adsorb on the surface of QDs thereby providing another form of non-specific binding however, such a weak adsorption can be lost in cellular environment exposing the QDs to RES system. Similarly, carbohydrates such as dextran have been used to provide necessary biocompatibility and solubility to the QDs [53]. Dextran coated quantum dots can tolerate a wide range of pH and cellular conditions without affecting their fluorescence and FRET properties [144]. Thus depending upon the final application of QDs their surface is decorated with molecules that can provide the required functionality to the quantum dots. In essence, the surface of QDs needs to be engineered specific to each selective application and has been a focus of current research. Such hybridized quantum dots and biomolecules then acts as a single unit that complements each other for unique biological applications. For example, in bio-imaging applications the quantum dots serve as the imaging tag while the attached antibody may serve as the unique targeting agents through the specific antigen binding action [140]. Extensive research in this field have led to the development of several approaches for attachment of various types of biomolecules to the surface of QDs and covering each strategy is outside the scope of this review. The common strategies for attachment of biomolecules can be classified as covalent attachment and non-specific adsorption (Fig. 9). The intention of this review is not to provide exhaustive analysis of various strategies for covalent coupling of QDs but to serve as a useful guide to source detailed published protocols for attachment of biomolecules.

4.1. Adsorption of functional molecules

Adsorption of biomolecules over the surface of nanoparticles is a common strategy for attaching biological molecules to the nanoparticles [59,145]. The nanoparticles possess a large surface area that can be utilized for non-specific adsorption of large molecules such as polymers, long chain organic molecules, proteins, enzymes and nucleic acids. This strategy results in non-selective binding of molecules and does not depend upon any functional attachment. The adsorption strategy is mostly dependent upon electrostatic interaction of charged biomolecules such as proteins with the oppositely charged terminal ends of the ligand coated QDs. Relevant protein molecules can be engineered to express positively charged domains on their surface that can self-assemble electrostatically over the surface of negatively charged carboxyl terminated QDs [49,146]. Electrostatic approaches have been exploited to attach a variety of engineered proteins such as MBP and Protein G to the surface of QDs [25]. Another strategy involves a weak chemical interaction such as hydrogen bonding between the biomolecules and the ligands. Such an adsorption strategy is also dependent upon the type of biomolecules being adsorbed on the surface, for example, the adsorption of oligonucleotides on the surface of MAA coated CdSe/ZnS quantum dots and its dependence on the type of solvent, pH of medium, ionic strength, presence of denaturants and type of oligonucleotide was reported by Algar and Krull [147]. It was found that the pH plays an important role in adsorption of oligonucleotide and that the adsorption was higher in acidic pH conditions when the carboxyl groups of the ligands QDs were protonated. The adsorption reduced significantly in alkaline or near neutral pH and suggests hydrogen bonding as a primary mechanism of adsorption of oligonucleotides. Further, evidence of the hydrogen bonding was gathered by addition of formamide to the buffered solution of QDs. Formamide molecule is known to disrupt the hydrogen bonding interaction of nucleic acids. It was observed that the adsorption of oligonucleotides on the surface of MAA coated QDs decreases with an increasing concentration of formamide and confirmed that the adsorption process is primarily driven by hydrogen bonding for attachment of oligonucleotides [148,149]. Even though adsorption strategies are simple in the sense that it does not involve another chemical step for conjugation of molecules to the surface of quantum dots, it has several drawbacks such as a) the non-specific interaction is difficult to quantify and thus the QDs to protein ratio cannot be controlled b) the orientation of attached biomolecules cannot be controlled being non-specific c) the interaction is usually weaker especially when present in cellular environment where a host of other ligands can compete for the surface of quantum dots. The orientation of biomolecules such as antibodies is absolutely critical for their attachment to the intended target

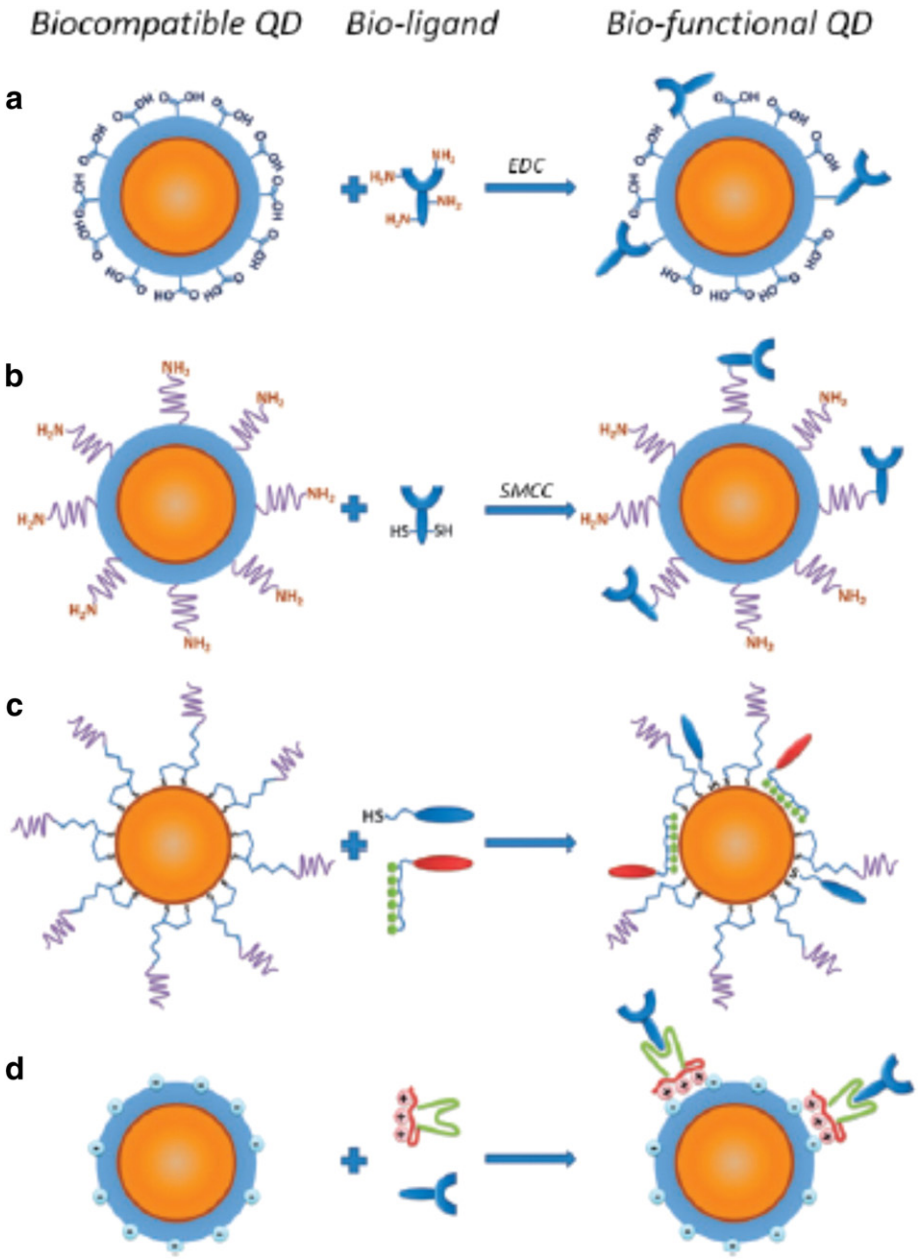

Fig. 9. Common strategies for surface functionalization of quantum dots with selected biomolecules. A and B depict the surface engineering of QDs with biomolecules using covalent conjugation strategies such as EDC coupling or sulfhydryl coupling while C represents the non-covalent coordination of thiol groups or polyhistidine tags with the surface metal atoms of QDs. A versatile strategy for electrostatic adsorption of charged biomolecules on the ligand coated QDs is depicted in D.
Reprinted from Ref [2], Copyright (2010), Royal Society of Chemistry.

and difficult to control and predict in the case of non-specific interaction causing low avidity rendering them non-functional. Thus covalent attachment strategies are more popular to achieve a strong bonding and correct orientation of the biomolecules conjugated at the surface of QDs.

4.2. Covalent coupling of biomolecules

4.2.1. Amide coupling

It is possible to solubilize QDs in water by attachment of various ligands on their surface (Section 3). The choice of ligands is made in a way to provide a terminal functional group (usually $-NH_2$, –COOH, –OH or –SH) for further attachment to biomolecules. It is quite common to use carboxyl terminated functional groups on the surface of QDs as these can be conjugated to free amines on proteins, peptides or antibodies through the formation of simple amide bonds [150]. The advantage of forming amide bonds is the simple water soluble process that usually occurs in alkaline buffered conditions which preserves structure and property of proteins [59]. In addition, the amide coupling does not require the use of lengthy spacers thereby preserving the hydrodynamic size of the QDs. The only increase in hydrodynamic size is directly related to the size of the functional molecule attached to the surface [151]. Carbodiimide coupling is quite frequently used for coupling carboxyl functionalized QDs with amine terminated biomolecules. 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) is the most commonly used coupling agent for the formation of amide bonds using the scheme shown in Fig. 10 [152]. Usually amide coupling proceeds with high efficiency and the yield can be increased by stabilizing the intermediate. However, EDC alone is not very efficient as a crosslinking agent as it cannot react very quickly with the amine. This

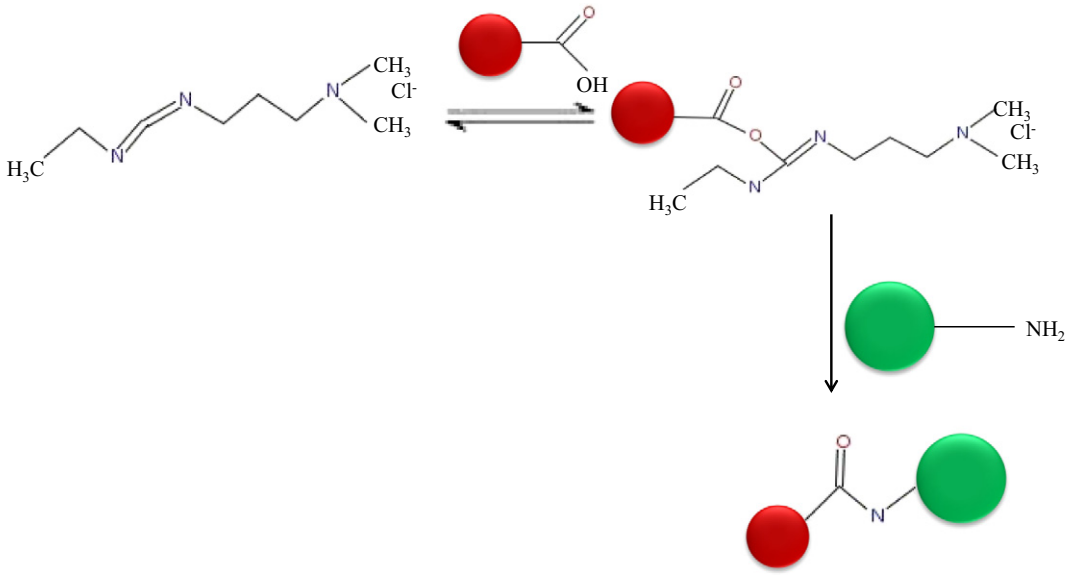

Fig. 10. The schematic EDC coupling of a carboxyl terminated QD to an amine terminated biomolecules and the use of NHS ester for increasing the total yield of the reaction.

allows the O-acyl isourea intermediate to undergo hydrolysis resulting in the regeneration of carboxylic acid.

The efficiency of the reaction can be increased by stabilizing the O-acyl isourea intermediate and pushing the reaction in forward direction [153,154]. The intermediate can be stabilized by using N-hydroxy succinamide (NHS) ester as a stabilizing agent to form an amine reactive intermediate that increases the efficiency of the reaction [155]. The charged analog of NHS also known as sulfo-NHS can be used as an efficient alternative to NHS and provides sufficient stability to allow a 2 step reaction for the formation of amide [156]. The sulfo-NHS ester stabilized EDC coupling reaction has been shown to proceed with retention of as high as 50–80% enzymatic activity depending on the enzyme [157]. For silica encapsulated quantum dots that bear hydroxyl groups on the surface of the silica the EDC coupling can be used to form ester linkage between the carboxyl groups of the biomolecules with the hydroxylated silica surface.

4.2.2. Thiol binding

Disulfide binding of QDs with peptides binding specifically to thiols directly yields functionalized and soluble quantum dots and have been explored as an alternative to the two step solubilization and functionalization procedure [2]. The advantage of using peptides over other compounds lies in the ability to customize various types of peptides than can act as anchoring or targeting agents [158]. Similar procedure in which the thiol terminated functional group on the surface of QDs or silica shell can be attached to sulfur containing amino acid such as cysteine has been tried in the past [75]. Disulfide bonds are covalent in nature and can be used to form zero length bonds between the QDs and the target biomolecules [159]. In addition, maleimide coupling can be used to conjugate biomolecules on the surface of QDs through a sulfo SMCC mediated linker reaction [53,160–163].

The terminal amine/thiol group on the surface of QDs can be attached to the terminal thiol/amine group of proteins, DNA and peptides. The sulfo SMCC acts as the spacer as well as linker molecule and increases the overall size of the QDs (Fig. 11). The stability of such a bond in cellular media is higher than the disulfide bonds as it can undergo disulfide exchange reaction in cellular media with a variety of competing thiol groups. Such a scission can be used strategically to deliver drug loaded nanoparticles/QDs to the specific target where the QDs are released with the cleavage of the thiol bonds by selective enzymes present at the cellular site. Derfus et al. [164] reported the use of Sulfo-SMCC strategy to target the QDs for siRNA and siRNA and tumor-homing peptide delivery to tumor cells. In an attempt by Schumacher et al. [165] a synthetic peptide and R-phycoerythrin dye were conjugated with QDs for detecting *Bacillus anthracis* spores. Similarly, Tiwari et al. [166] has also used Sulfo-SMCC method to conjugate anti-HER2 antibody conjugated CdSe/CdZnS QDs for fluorescence imaging of breast cancer cells. They synthesized the HER2Ab coated QDs by three independent methods, EDC/sulfo-NHS (3-sulfo-N-hydroxysuccinimide sodium salt), iminothiolane/sulfo-

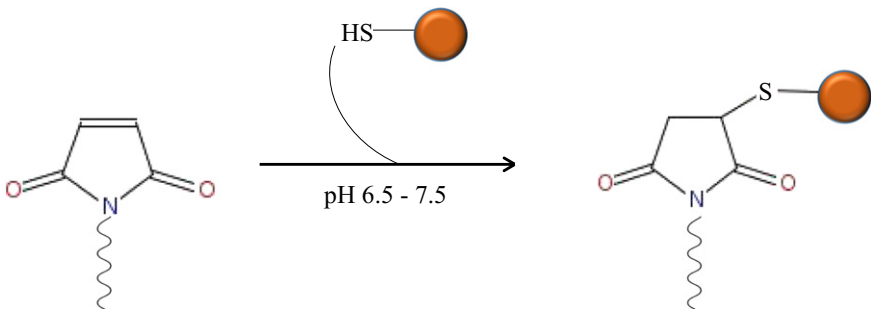

Fig. 11. Maleimide coupling strategy for functionalization of amine terminated QDs with thiol terminated biomolecules.

SMCC and sulfo-SMCC coupling methods and found that SMCC coupling with partially reduced antibody was the most effective for the detection of HER2 expression in breast cancer cells.

4.2.3. Click chemistry

Click chemistry is a powerful technique for combinatorial synthesis of new compounds through highly selective and rapid synthesis [167–169]. Copper catalyzed 1–3 dipolar cycloaddition of azides with alkynes is one of the most popular examples of click chemistry [168, 170]. Click chemistry can be used as a technique that not only improves the ligation reaction of QDs but also to attach a library of biomolecules and coupling agents. Click reactions proceed with the formation of a carbon-heteroatom bond that is thermodynamically stable and proceeds usually at room temperature with high yield however, the only limitation lies in the synthesis of terminal alkyne or azide groups on the clicking QD surface and biomolecules which proceeds with low yield [171,172]. The stability of terminal alkynes and azides makes the reactants stable and can be introduced easily in a variety of biomolecules and linking groups [172–175]. Additionally, through careful control of the stoichiometry, multiple functionalities can be attached to the same quantum dot possessing an alkyne or azide termination.

5. Other coating methods

5.1. PEG-based coating

Polyethylene-glycol (PEG) compounds with discrete chain lengths are known to provide biocompatibility to nano and microparticles [176]. QDs coated with PEG spacers reduce nonspecific protein binding and escape from reticuloendothelial system (RES) which provides longer circulation time in the blood. Currently several PEG reagents are available with carboxylate or thiol or lipoamide terminal group as a monofunctional, bifunctional or heterogenous bifunctional terminal groups [177]. These reagents are effective for producing hydrophilic bridges between an adsorptive surface and an affinity ligand. Several efforts have been devoted for the phase transfer of QDs from organic to aqueous media using PEG. Yu et al. [111] reported the synthesis of amphiphilic polymer poly(maleic anhydride-alt-1-octadecene) (PMAO)-PEG through the reaction between PMAO and primary amine-terminated PEG methyl ethers. This reagent was simply mixed with hydrophobic QDs and stirred overnight. Interestingly, these PMAO-PEG-coated QDs showed the same optical properties and quantum yield as hydrophobic QDs. In order to explore the biological application, these QDs were modified with antibodies to recognize the Her2 receptor from cancer cells. In another report by Lv et al. [178] PEGylated chitosan derivatives (N-octyl-N-mPEG-chitosan, mPEG = poly(ethylene glycol) monomethyl ether; OPEGC) were synthesized vis Schiff base reduction reaction between chitosan and mPEG-aldehyde, where chitosan acts as the backbone of the grafted copolymers, and mPEG-aldehyde providing the hydrophilic chain. The resulting QDs showed narrow size distribution, high quantum yield, good water dispersibility and low cytotoxicity.

5.2. Aldehyde based methods

A reliable and popular method for attaching antibodies or other proteins to QDs involves reductive amination where amines on the protein/antibody conjugate with aldehyde groups of the QDs. This reaction involves the formation of an initial Schiff base between the aldehyde and amine groups, which is then reduced to a secondary amine by a mild reducing agent. Iyer et al. [179] have developed a bis-aryl hydrazone linkage strategy for the coupling of protein pre-coated QDs to antibodies. This strategy involves Schiff base mediated conjugation between aromatic aldehydes and aromatic hydrazines. Another advantage of this method is that the functional linkers do not cross-react with other side groups present in the side chains of natural amino acids. These QDs were also used for the specific targeting of endogenous epidermal growth factor receptors in breast cancer cells. The same approach was further extended for optical mapping of RNA polymerases bound to combed genomic DNA *in vitro*.

5.3. Carbohydrate based methods

The biocompatibility and specific receptor recognition ability make carbohydrates as potential targeting ligands to modify quantum dots for site-specific bio applications. Carbohydrates not only facilitate specific targeting but they also confer water solubility and biocompatibility as well to the QDs, which is necessary for their safe use in bio-based applications. Carbohydrate functionalization to QDs happens in one of two ways, either the carbohydrate ligand is synthesized at first and later functionalized to the surface; or the carbohydrate is attached to the surface through some bioconjugation method. Tamura and co-workers synthesized a mannose displaying trioctylphosphine-derivative and synthesized CdSe–ZnS QDs via the ligands surface addition instead of TOPO [180]. Sun et al. [181] reported the synthesis of branched glycopolymer, displaying galactose–glucose disaccharides, with a biotinylated end group attached to streptavidin-coated CdSe–ZnS QDs and successfully showed the capture and detection of nanomolar lectin.

5.4. Use of carbonyl diimidazole (CDI)

Another method for addition of carboxylic group to on the QDs surface is the use of CDI as catalyst. CDI activates the hydroxyl group to form reactive imidazole carbamates. Further, this reactive group, in an aqueous coupling buffer, reacts with primary amine-containing ligands via removal of imidazole groups and formation of carbamate linkages. The coupling process is slow and occurs in alkaline conditions (pH of ~10). Using this strategy, several reports have been published for surface modification of QDs with antibodies or other biomolecules [182]. Jin et al. [182] used CDI as catalyst to develop QD surfaces with sulfonates or quaternary ammoniums, which endowed QDs excellent colloidal stability independent of the pH and ionic strength and achieved stable and flexible bioconjugations. Scholl et al. [183] reported the alteration of QDs surface using CDI and showed that the modification of traditional Western immunoblotting using a technique to count quantum-dot-tagged proteins on optically transparent PVDF membranes can be utilized for ultrasensitive detection of proteins up to 0.2 pg.

6. Limitations and summary

QDs have shown tremendous promise in the biological applications such as gene labeling, FRET sensing as well as selective fluorescent labeling of cellular matrix. However, for successful application of these strategies, the native core QDs have to be tailored to specific environment and biological application. Thus, the success of QDs for any relevant biological application depends on the successful ligation of the QDs that should not interfere with its unique properties as compared to chemical fluorophore molecules. This has opened new avenues for the scientific community as it mandated the collaboration between material scientists and biologists with chemists to develop specific strategies for functionalization and solubilization of QDs. The functionalization of quantum dots to increase their solubility and reduce toxicity results in larger size and lower yield than the pure QDs. This limits the application of large sized QDs as compared to the conventional organic fluorophores in accessing targeted biomolecules and in FRET applications. In addition, the multiple functionalization steps involved in the synthesis of a useful biomolecules-QDs composite structure makes the purification of final compound extremely difficult especially considering the large surface area of small QDs that can adsorb a variety of impurities on the surface. Multiple purification steps required for removing the excessive ligands and toxic chemicals from the QDs suspension

make the procedure lengthy and process expensive for commercial use. These multiple steps also reduce the yield of the final product and also decrease the total quantum yield of the QDs. The surrounding environment such as pH, salt concentration and oxidation of QDs by surrounding chemical media reduce the quantum yield further. Further, the stability of colloidal suspension for a long period in cellular media is compensated due to agglomeration of QDs.

The toxicity of quantum dots due to leaching of Cd or Se ions from QDs is one of the major concerns for biomedical applications of QDs. Several reports suggests the cytotoxicity and alteration of cellular function of the pure and functionalized QDs. Pure QDs have been demonstrated as toxic while there are mixed reports on the toxicity of biocompatible ligand coated QDs and it is difficult to formulate a concrete theory whether the toxicity is inherited from leaching of ions or from adsorbed impurities from several chemical reactions during functionalization. Thus, before the full potential of QDs is realized, complete toxicity profiles of functionalized quantum dots with well-defined protocols and purification procedures needs to be established. It is clear that the physical, chemical and fluorescent properties of QDs is unparalleled by any other currently existing inorganic or organic molecules. In addition the quantum confinement effect gives them tunable fluorescent properties by just varying the size of the QDs that has resulted in numerous applications such as FRET sensors, labeling of cellular proteins, cell tracking, pathogen and toxin detection and *in vivo* animal imaging. In addition to this, selective functionalization of QDs is currently being explored for gene profiling and optical bar coding for high throughput gene expression monitoring and monitoring drug discover assays. There is a lot of interest in the synthesis of magnetic and fluorescent composites of QDs as an ideal agent for complimentary magnetic and fluorescent tracking and deep tissue imaging. A relatively unexplored area of QD blinking may be harnessed in the form of biological sensors by tuning the blinking process with functionalized agents. While endless possibilities for biomedical applications of QDs exist, the prime requirement will be the design of functional biomolecules and their conjugation strategies to make them multifunctional and low or non-toxic.

Acknowledgments

The financial assistance for the Centre for Nanotechnology Research and Applications (CENTRA) by The Gujarat Institute for Chemical Technology (GICT) is gratefully acknowledged. The manuscript number is ILS #027.

References

[1] Zrazhevskiy P, True LD, Gao X. Multicolor multicycle molecular profiling with quantum dots for single-cell analysis. Nat Protoc Oct 2013;8(10):1852–69.
[2] Zrazhevskiy P, Sena M, Gao X. Designing multifunctional quantum dots for bioimaging, detection, and drug delivery. Chem Soc Rev Nov 2010;39(11):4326–54.
[3] Chan WC, Maxwell DJ, Gao X, Bailey RE, Han M, Nie S. Luminescent quantum dots for multiplexed biological detection and imaging. Curr Opin Biotechnol Feb 2002;13(1):40–6.
[4] Jamieson T, Bakhshi R, Petrova D, Pocock R, Imani M, Seifalian AM. Biological applications of quantum dots. Biomaterials Nov 2007;28(31):4717–32.
[5] Chan WC, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science Sep. 25 1998;281(5385):2016–8.
[6] Cheng X, Lowe SB, Reece PJ, Gooding JJ. Colloidal silicon quantum dots: from preparation to the modification of self-assembled monolayers (SAMs) for bioapplications. Chem Soc Rev Apr 21 2014;43(8):2680–700.
[7] Patra CR, Bhattacharya R, Patra S, Basu S, Mukherjee P, Mukhopadhyay D. Lanthanide phosphate nanorods as inorganic fluorescent labels in cell biology research. Clin Chem Nov 2007;53(11):2029–31.
[8] Ambrosi A, Airo F, Merkoci A. Enhanced gold nanoparticle based ELISA for a breast cancer biomarker. Anal Chem Feb 1 2010;82(3):1151–6.
[9] Gao L, Zhuang J, Nie L, Zhang J, Zhang Y, Gu N, et al. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. Nat Nanotechnol Sep 2007;2(9):577–83.
[10] Cui R, Han Z, Zhu JJ. Helical carbon nanotubes: intrinsic peroxidase catalytic activity and its application for biocatalysis and biosensing. Chemistry Aug 16 2011;17(34):9377–84.
[11] Singh S, Dosani T, Karakoti AS, Kumar A, Seal S, Self WT. A phosphate-dependent shift in redox state of cerium oxide nanoparticles and its effects on catalytic properties. Biomaterials Oct 2011;32(28):6745–53.
[12] Karakoti A, Singh S, Dowding JM, Seal S, Self WT. Redox-active radical scavenging nanomaterials. Chem Soc Rev Nov 2010;39(11):4422–32.
[13] Pirmohamed T, Dowding JM, Singh S, Wasserman B, Heckert E, Karakoti AS, et al. Nanoceria exhibit redox state-dependent catalase mimetic activity. Chem Commun (Camb) Apr 28 2010;46(16):2736–8.
[14] Karakoti AS, Singh S, Kumar A, Malinska M, Kuchibhatla SV, Wozniak K, et al. PEGylated nanoceria as radical scavenger with tunable redox chemistry. J Am Chem Soc Oct 14 2009;131(40):14144–5.
[15] Asati A, Santra S, Kaittanis C, Nath S, Perez JM. Oxidase-like activity of polymer-coated cerium oxide nanoparticles. Angew Chem Int Ed Engl 2009;48(13):2308–12.
[16] Wang Q, Kuo Y, Wang Y, Shin G, Ruengruglikit C, Huang Q. Luminescent properties of water-soluble denatured bovine serum albumin-coated CdTe quantum dots. J Phys Chem B Aug 31 2006;110(34):16860–6.
[17] Jaiswal JK, Simon SM. Potentials and pitfalls of fluorescent quantum dots for biological imaging. Trends Cell Biol Sep 2004;14(9):497–504.
[18] Zhang Y, Wang TH. Quantum dot enabled molecular sensing and diagnostics. Theranostics 2012;2(7):631–54.
[19] Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R, Nann T. Quantum dots versus organic dyes as fluorescent labels. Nat Methods Sep 2008;5(9):763–75.
[20] Drummen GP. Quantum dots-from synthesis to applications in biomedicine and life sciences. Int J Mol Sci 2010;11(1):154–63.
[21] Tang H, Xu G, Weng L, Pan L, Wang L. Luminescence and photophysical properties of colloidal ZnS nanoparticles. Acta Mater 2004;52(6):1489–94.
[22] Haugland RP. The handbook—a guide to fluorescent probes and labeling technologies. Tenth ed. San Diego: Invitrogen Corporation; 2005.
[23] Hagan AK, Zuchner T. Lanthanide-based time-resolved luminescence immunoassays. Anal Bioanal Chem Jul 2011;400(9):2847–64.
[24] Lakowicz JR, Gryczynski II, Gryczynski Z. High throughput screening with multiphoton excitation. J Biomol Screen 1999;4(6):355–62.
[25] Sapsford KE, Pons T, Medintz IL, Mattoussi H. Biosensing with luminescent semiconductor quantum dots. Sensors 2006;6:925–53.
[26] Dohnalova K, Alexander N, Poddubny A, Alexei A, Prokofiev AA, de Boer WD, et al. Surface brightens up Si quantum dots: direct bandgap-like size-tunable emission. Light Sci Appl 2013;2.
[27] Smith AM, Nie S. Chemical analysis and cellular imaging with quantum dots. Analyst Aug 2004;129(8):672–7.
[28] Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater Jun 2005;4(6):435–46.
[29] Rossetti R, Brus L. Electron-hole recombination emission as a probe of surface chemistry in aqueous cadmium sulfide colloids. J Phys Chem 1982 11/01 2014/07/03;86(23):4470-4472.
[30] Rasmussen TE, Jauffred L, Brewer J, Vogel S, Torbensen ER, Lagerholm BC, et al. Single molecule applications of quantum dots. J Mod Phys 2013;4:27–42.
[31] Behari J. Principles of nanoscience: an overview; 2010.
[32] Karakoti AS, Sanghavi S, Nachimuthu P, Yang P, Thevuthasan S. Probing the Size- and Environment-Induced Phase Transformation in CdSe Quantum Dots. J Phys Chem Lett 2011 11/03 2014/07/07;2(22):2925-2929.
[33] Wang X, Qu L, Zhang J, Peng X, Xiao M. Surface-Related Emission in Highly Luminescent CdSe Quantum Dots. Nano Lett 2003 07/02 2014/07/03;3(8):1103-1106.
[34] Dahan M, Levi S, Luccardini C, Rostaing P, Riveau B, Triller A. Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. Science Oct 17 2003;302(5644):442–5.
[35] Xie R, Kolb U, Li J, BaschÃ© T, Mews A. Synthesis and Characterization of Highly Luminescent CdSeâ˜Core CdS/Zn0.5Cd0.5S/ZnS Multishell Nanocrystals. J Am Chem Soc 2005 04/29 2014/07/03;127(20):7480-7488.
[36] Hohng S, Ha T. Near-Complete Suppression of Quantum Dot Blinking in Ambient Conditions. J Am Chem Soc 2004 01/20 2014/07/03;126(5):1324-1325.
[37] Gerion D, Pinaud F, Williams SC, Parak WJ, Zanchet D, Weiss S, et al. Synthesis and Properties of Biocompatible Water-Soluble Silica-Coated CdSe/ZnS Semiconductor Quantum Dotsâ€ J Phys Chem B 2001 06/05 2014/07/13;105(37):8861-8871.
[38] Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, et al. Quantum dots for live cells, in vivo imaging, and diagnostics. Science Jan 28 2005;307(5709):538–44.
[39] Ozkan M. Quantum dots and other nanoparticles: what can they offer to drug discovery? Drug Discov Today Dec 15 2004;9(24):1065–71.
[40] Dabbousi BO, Rodriguez-Viejo J, Mikulec FV, Heine JR, Mattoussi H, Ober R, et al. (CdSe) ZnS core-shell quantum dots: synthesis and optical and structural characterization of a size series of highly luminescent materials. J Phys Chem B 1997;101:9463–75.
[41] Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, et al. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol Jan 2003;21(1):41–6.
[42] Panchuk-Voloshina N, Haugland RP, Bishop-Stewart J, Bhalgat MK, Millard PJ, Mao F, et al. Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates. J Histochem Cytochem Sep 1999;47(9):1179–88.
[43] Cinteza LO. Quantum dots in biomedical applications: advances and challenges. J Nanophotonics 2010;4(1).
[44] Manna L, Scher EC, Li LS, Alivisatos AP. Epitaxial growth and photochemical annealing of graded CdS/ZnS shells on colloidal CdSe nanorods. J Am Chem Soc Jun 19 2002;124(24):7136–45.
[45] Hess BC, Okhrimenko IG, Davis RC, Stevens BC, Schulzke QA, Wright KC, et al. Surface transformation and photoinduced recovery in CdSe nanocrystals. Phys Rev Lett Apr 2 2001;86(14):3132–5.

[46] Kloepfer JA, Mielke RE, Nadeau JL. Uptake of CdSe and CdSe/ZnS quantum dots into bacteria via purine-dependent mechanisms. Appl Environ Microbiol May 2005; 71(5):2548–57.
[47] Katari JEB, Colvin VL, Alivisatos AP. X-ray photoelectron spectroscopy of CdSe nanocrystals with applications to studies of the nanocrystal surface. J Phys Chem 1994;98(15):4109–17.
[48] Singh S, Sharma A, Robertson GP. Realizing the clinical potential of cancer nanotechnology by minimizing toxicologic and targeted delivery concerns. Cancer Res Nov 15 2012;72(22):5663–8.
[49] Clapp AR, Medintz IL, Mattoussi H. Forster resonance energy transfer investigations using quantum-dot fluorophores. Chemphyschem Jan 16 2006;7(1):47–57.
[50] Graf C, Dembski S, Hofmann A, Ruhl E. A general method for the controlled embedding of nanoparticles in silica colloids. Langmuir Jun 20 2006;22(13):5604–10.
[51] Gao X, Cui Y, Levenson RM, Chung LW, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol Aug 2004;22(8):969–76.
[52] Kairdolf BA, Mancini MC, Smith AM, Nie S. Minimizing nonspecific cellular binding of quantum dots with hydroxyl-derivatized surface coatings. Anal Chem Apr 15 2008;80(8):3029–34.
[53] Thanha NTK, Green LAW. Functionalisation of nanoparticles for biomedical applications. Nano Today 2010;5:213–30.
[54] Li H, Shih WY, Shih WH. Highly photoluminescent and stable aqueous ZnS quantum dots. Ind Eng Chem Res 2010;49(2):579–82.
[55] Lukehart CM, Scott RA. Nanomaterial inorganic and bioinorganic perspective; 2013 512–3.
[56] Li J, Wu N. Biosensors based on nanomaterials and nanodevices; 2013.
[57] Walling MA, Novak JA, Shepard JR. Quantum dots for live cell and in vivo imaging. Int J Mol Sci Feb 2009;10(2):441–91.
[58] Ho YP, Leong KW. Quantum dot-based theranostics. Nanoscale Jan 2009;2(1):60–8.
[59] Sperling RA, Parak WJ. Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. Philos Transact A Math Phys Eng Sci Mar 28 2010;368(1915):1333–83.
[60] Biju V. Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy. Chem Soc Rev Feb 7 2014;43(3):744–64.
[61] Wang T, Sridhar R, Korotcov A, Ting AH, Francis K, Mitchell J, et al. Synthesis of amphiphilic triblock copolymers as multidentate ligands for biocompatible coating of quantum dots. Colloids Surf A Physicochem Eng Asp Feb 5 2011;375(1–3):147–55.
[62] Duan H, Nie S. Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings. J Am Chem Soc Mar 21 2007;129(11): 3333–8.
[63] Sun D, Gang O. DNA-functionalized quantum dots: fabrication, structural, and physicochemical properties. Langmuir Jun 11 2013;29(23):7038–46.
[64] Palui G, Avellini T, Zhan N, Pan F, Gray D, Alabugin I, et al. Photoinduced phase transfer of luminescent quantum dots to polar and aqueous media. J Am Chem Soc Oct 3 2012;134(39):16370–8.
[65] Yang P, Zhou G. Phase transfer of hydrophobic QDs for water-soluble and biocompatible nature through silanization. Mater Res Bull 2011;46(12):2367–72.
[66] Uyeda HT, Medintz IL, Jaiswal JK, Simon SM, Mattoussi H. Synthesis of compact multidentate ligands to prepare stable hydrophilic quantum dot fluorophores. J Am Chem Soc Mar 23 2005;127(11):3870–8.
[67] Janczewski D, Tomczak N, Han MY, Vancso GJ. Synthesis of functionalized amphiphilic polymers for coating quantum dots. Nat Protoc Oct 2011;6(10):1546–53.
[68] Lo YC, Edidin MA, Powell JD. Selective activation of antigen-experienced T cells by anti-CD3 constrained on nanoparticles. J Immunol Nov 15 2013;191(10):5107–14.
[69] Yildiz I, Deniz E, McCaughan B, Cruickshank SF, Callan JF, Raymo FM. Hydrophilic CdSe–ZnS core-shell quantum dots with reactive functional groups on their surface. Langmuir Jul 6 2010;26(13):11503–11.
[70] Lu M, Zhang W, Gai Y, Yang T, Ye P, Yang G, et al. Folate-PEG functionalized silica CdTe quantum dots as fluorescent probes for cancer cell imaging. New J Chem 2014;38(9):4519–26.
[71] Hushiarian R, Yusof NA, Abdullah AH, Ahmad SA, Dutse SW. A novel DNA nanosensor based on CdSe/ZnS quantum dots and synthesized Fe_3O_4 magnetic nanoparticles. Molecules 2014;19(4):4355–68.
[72] Chen Y, Molnar M, Li L, Friberg P, Gan LM, Brismar H, et al. Characterization of VCAM-1-binding peptide-functionalized quantum dots for molecular imaging of inflamed endothelium. PLoS One 2013;8(12):e83805.
[73] He X, Gao L, Ma N. One-step instant synthesis of protein-conjugated quantum dots at room temperature. Sci Rep 2013;3:2825.
[74] Mansur A, Mansur H, Gonzalez J. Enzyme-polymers conjugated to quantum-dots for sensing applications. Sensors 2011;11(10):9951–72.
[75] Wolcott A, Gerion D, Visconte M, Sun J, Schwartzberg A, Chen S, et al. Silica-coated CdTe quantum dots functionalized with thiols for bioconjugation to IgG proteins. J Phys Chem B Mar 23 2006;110(11):5779–89.
[76] Parak WJ, Boudreau R, Le Gros M, Gerion D, Zanchet D, CM M, et al. Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks. Adv Mater 2002;14(12):882–5.
[77] Mattoussi H, Mauro J, Goldman ER, Anderson GP, Sundar VC, Mikulec FV, et al. Self-assembly of CdSe–ZnS quantum dot bioconjugates using an engineered recombinant protein. J Am Chem Soc 2000;122(49):12142–50.
[78] Ong WL, Rupich SM, Talapin DV, McGaughey AJ, Malen JA. Surface chemistry mediates thermal transport in three-dimensional nanocrystal arrays. Nat Mater May 2013;12(5):410–5.
[79] Bera D, Lei Qian L, Tseng TK, Holloway PH. Quantum dots and their multimodal applications: a review. Mater 2010;3:2260–345.
[80] Talapin DV, Haubold S, Rogach AL, Kornowski A, Haase M, Weller H. A novel organometallic synthesis of highly luminescent CdTe nanocrystals. J Phys Chem B 2001; 105:2260–3.
[81] Hammer NI, Emrick T, Barnes MD. Quantum dots coordinated with conjugated organic ligands: new nanomaterials with novel photophysics. Nanoscale Res Lett 2007;2:282–90.
[82] Mei BC, Susumu K, Medintz IL, Delehanty JB, Mountziaris TJ, Mattoussi H. Modular poly(ethylene glycol) ligands for biocompatible semiconductor and gold nanocrystals with extended pH and ionic stability. J Mater Chem 2008;18: 4949–58.
[83] Olsson C. Synthesis of bifunctional poly(ethylene oxides) and their use as ligands to nanoparticles; 2009.
[84] Kim S, Bawendi MG. Oligomeric ligands for luminescent and stable nanocrystal quantum dots. J Am Chem Soc Dec 3 2003;125(48):14652–3.
[85] Zhang Y, Clapp A. Overview of stabilizing ligands for biocompatible quantum dot nanocrystals. Sensors 2011;11(12):11036–55.
[86] Querner C, Reiss P, Bleuse J, Pron A. Chelating ligands for nanocrystals' surface functionalization. J Am Chem Soc Sep 22 2004;126(37):11574–82.
[87] Alivisatos AP. Semiconductor clusters, nanocrystals, and quantum dots. Science 1996;271:933–7.
[88] Pejchal R, Ludwig ML. Cobalamin-independent methionine synthase (MetE): a face-to-face double barrel that evolved by gene duplication. PLoS Biol Feb 2005; 3(2):e31.
[89] Grapperhaus CA, Tuntulani T, Reibenspies JH, Darensbourg MY. Methylation of tethered thiolates in [(bme-daco)Zn](2) and [(bme-daco)Cd](2) as a model of zinc sulfur-methylation proteins. Inorg Chem Aug 10 1998;37(16):4052–8.
[90] Hammes BS, Carrano CJ. Methylation of (2-methylethanethiol-bis-3,5-dimethylpyrazolyl)methane zinc complexes and coordination of the resulting thioether: relevance to zinc-containing alkyl transfer enzymes. Inorg Chem Feb 26 2001;40(5):919–27.
[91] Chiou SJ, Innocent J, Riordan CG, Lam KC, Liable-Sands L, Rheingold AL. Synthetic models for the zinc sites in the methionine synthases. Inorg Chem Sep 18 2000; 39(19):4347–53.
[92] Parak FG. Proteins in action: the physics of structural fluctuations and conformational changes. Curr Opin Struct Biol Oct 2003;13(5):552–7.
[93] Susumu K, Mei BC, Mattoussi H. Multifunctional ligands based on dihydrolipoic acid and polyethylene glycol to promote biocompatibility of quantum dots. Nat Protoc 2009;4(3):424–36.
[94] Susumu K, Uyeda HT, Medintz IL, Pons T, Delehanty JB, Mattoussi H. Enhancing the stability and biological functionalities of quantum dots via compact multifunctional ligands. J Am Chem Soc Nov 14 2007;129(45):13987–96.
[95] Xu L, Chen C. Physiological behavior of quantum dots. Wiley Interdiscip Rev Nanomed Nanobiotechnol Nov-Dec 2012;4(6):620–37.
[96] Correa-Duarte MA, Giersig M, Liz-MarzÃ¡n LM. Stabilization of CdS semiconductor nanoparticles against photodegradation by a silica coating procedure. Chem Phys Lett 1998;286(5â€“6):497–501.
[97] Correa-Duarte MA, Kobayashi Y, Caruso RA, Liz-Marzan LM. Photodegradation of SiO_2-coated CdS nanoparticles within silica gels. J Nanosci Nanotechnol Mar 2001;1(1):95–9.
[98] Nann T, Riegler J, Nick P, Mulvaney P. Quantum dots with silica shells. Proc SPIE 5705, Nanobiophotonics and Biomedical Applications II; 2005. p. 77.
[99] Serrano IC, Ma Q, Palomares E. QD-"Onion"-Multicode silica nanospheres with remarkable stability as pH sensors. J Mater Chem 2011;21:17673–9.
[100] Yang DZ, Xu SK, Chen QF. Applications of quantum dots to biological probes. Guang Pu Xue Yu Guang Pu Fen Xi Sep 2007;27(9):1807–10.
[101] Arriagada FJ, Osseo-Asare K. Synthesis of nanosize silica in a nonionic water-in-oil microemulsion: effects of the water/surfactant molar ratio and ammonia concentration. J Colloid Interface Sci Mar 15 1999;211(2):210–20.
[102] Zhelev Z, Bakalova R, Ohba H, Jose R, Imai Y, Baba Y. Uncoated, broad fluorescent, and size-homogeneous CdSe quantum dots for bioanalyses. Anal Chem Jan 1 2006;78(1):321–30.
[103] Zhelev Z, Ohba H, Bakalova R. Single quantum dot-micelles coated with silica shell as potentially non-cytotoxic fluorescent cell tracers. J Am Chem Soc May 17 2006; 128(19):6324–5.
[104] Louie A. Multimodality imaging probes: design and challenges. Chem Rev May 12 2010;110(5):3146–95.
[105] Wang KT, Iliopoulos I, Audebert R. Viscometric behaviour of hydrophobically modified poly(sodium acrylate). Polym Bull 1988;20(6):577–82.
[106] Luccardini C, Tribet C, Vial F, Marchi-Artzner V, Dahan M. Size, charge, and interactions with giant lipid vesicles of quantum dots coated with an amphiphilic macromolecule. Langmuir Feb 28 2006;22(5):2304–10.
[107] Kairdolf BA, Smith AM, Nie S. One-pot synthesis, encapsulation, and solubilization of size-tuned quantum dots with amphiphilic multidentate ligands. J Am Chem Soc Oct 1 2008;130(39):12866–7.
[108] Pellegrino T, Parak WJ, Boudreau R, Le Gros MA, Gerion D, Alivisatos AP, et al. Quantum dot-based cell motility assay. Differentiation Dec 2003;71(9–10):542–8.
[109] Di Corato R, Quarta A, Piacenza P, Ragusa A, Figuerola A, Buonsanti R, et al. Water solubilization of hydrophobic nanocrystals by means of poly(maleic anhydride-alt-1-octadecene). J Mater Chem 2008;18(17):1991–6.
[110] Lin C-AJ, Sperling RA, Li JK, Yang T-Y, Li P-Y, Zanella M, et al. Design of an amphiphilic polymer for nanoparticle coating and functionalization. Small 2008;4(3): 334–41.
[111] Yu WW, Chang E, Falkner JC, Zhang J, Al-Somali AM, Sayes CM, et al. Forming biocompatible and nonaggregated nanocrystals in water using amphiphilic polymers. J Am Chem Soc Mar 14 2007;129(10):2871–9.
[112] Lin W, Fritz K, Guerin G, Bardajee GR, Hinds S, Sukhovatkin V, et al. Highly Luminescent Lead Sulfide Nanocrystals in Organic Solvents and Water through Ligand Exchange with Poly(acrylic acid). Langmuir 2008 07/03 2014/07/09;24(15): 8215-8219.

[113] Möller M, Spatz JP, Roescher A. Gold nanoparticles in micellar poly(styrene)-b-poly(ethylene oxide) films—size and interparticle distance control in monoparticulate films. Adv Mater 1996;8(4):337–40.
[114] Stevenson JP, Rutnakornpituk M, Vadala M, Esker AR, Charles SW, Wells S, et al. Magnetic cobalt dispersions in poly(dimethylsiloxane) fluids. J Magn Magn Mater 2001;225(1â€"2):47–58.
[115] Nann T. Phase-transfer of CdSe@ZnS quantum dots using amphiphilic hyperbranched polyethylenimine. Chem Commun 2005;13:1735–6.
[116] Dong J, Li J, Zhou J. Interfacial and phase transfer behaviors of polymer brush grafted amphiphilic nanoparticles: a computer simulation study. Langmuir 04/23 2014;30(19):5599–608.
[117] Kim BS, Qiu JM, Wang JP, Taton TA. Magnetomicelles: composite nanostructures from magnetic nanoparticles and cross-linked amphiphilic block copolymers. Nano Lett Oct 2005;5(10):1987–91.
[118] Kim Y, Pyun J, Frechet JM, Hawker CJ, Frank CW. The dramatic effect of architecture on the self-assembly of block copolymers at interfaces. Langmuir Nov 8 2005; 21(23):10444–58.
[119] Cheng C, Wei H, Shi BX, Cheng H, Li C, Gu ZW, et al. Biotinylated thermoresponsive micelle self-assembled from double-hydrophilic block copolymer for drug delivery and tumor target. Biomaterials Feb 2008;29(4):497–505.
[120] Kang Y, Taton TA. Core/shell gold nanoparticles by self-assembly and crosslinking of micellar, block-copolymer shells. Angew Chem Int Ed Engl Jan 7 2005;44(3): 409–12.
[121] Grubbs RB. Nanoparticle assembly: solvent-tuned structures. Nat Mater Aug 2007; 6(8):553–5.
[122] Jia F, Zhang Y, Narasimhan B, Mallapragada SK. Block copolymer–quantum dot micelles for multienzyme colocalization. Langmuir Dec 18 2012;28(50): 17389–95.
[123] Ku KH, Kim MP, Paek K, Shin JM, Chung S, Jang SG, et al. Multicolor emission of hybrid block copolymer–quantum dot microspheres by controlled spatial isolation of quantum dots. Small Aug 26 2013;9(16):2667–72 [2654].
[124] Luo S, Xu J, Zhang Y, Liu S, Wu C. Double hydrophilic block copolymer monolayer protected hybrid gold nanoparticles and their shell cross-linking. J Phys Chem B Dec 1 2005;109(47):22159–66.
[125] Wu X, He X, Zhong L, Lin S, Wang D, Zhu X, et al. Water-soluble dendritic–linear triblock copolymer-modified magnetic nanoparticles: preparation, characterization and drug release properties. J Mater Chem 2011;21(35):13611–20.
[126] Habeeb AJ, Hiramoto R. Reaction of proteins with glutaraldehyde. Arch Biochem Biophys Jul 1968;126(1):16–26.
[127] Akin M, Bongartz R, Walter JG, Demirkol DO, Stahl F, Timur S, et al. PAMAM-functionalized water soluble quantum dots for cancer cell targeting. J Mater Chem 2012;22(23):11529–36.
[128] Geraldo DA, Duran-Lara EF, Aguayo D, Cachau RE, Tapia J, Esparza R, et al. Supramolecular complexes of quantum dots and a polyamidoamine (PAMAM)-folate derivative for molecular imaging of cancer cells. Anal Bioanal Chem Apr 2011;400(2): 483–92.
[129] Poselt E, Schmidtke C, Fischer S, Peldschus K, Salamon J, Kloust H, et al. Tailor-made quantum dot and iron oxide based contrast agents for in vitro and in vivo tumor imaging. ACS Nano Apr 24 2012;6(4):3346–55.
[130] Jin T, Fujii F, Komai Y, Seki J, Seiyama A, Yoshioka Y. Preparation and characterization of highly fluorescent, glutathione-coated near infrared quantum dots for in vivo fluorescence imaging. Int J Mol Sci Oct 2008;9(10):2044–61.
[131] Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science Nov 29 2002;298(5599):1759–62.
[132] Hu R, Yong KT, Roy I, Ding H, Law WC, Cai H, et al. Functionalized near-infrared quantum dots for in vivo tumor vasculature imaging. Nanotechnology Apr 9 2010;21(14):145105.
[133] Erogbogbo F, Yong KT, Hu R, Law WC, Ding H, Chang CW, et al. Biocompatible magnetofluorescent probes: luminescent silicon quantum dots coupled with superparamagnetic iron(III) oxide. ACS Nano Sep 28 2010;4(9):5131–8.
[134] Koole R, van Schooneveld MM, Hilhorst J, Castermans K, Cormode DP, Strijkers GJ, et al. Paramagnetic lipid-coated silica nanoparticles with a fluorescent quantum dot core: a new contrast agent platform for multimodality imaging. Bioconjug Chem Dec 2008;19(12):2471–9.
[135] Carion O, Mahler B, Pons T, Dubertret B. Synthesis, encapsulation, purification and coupling of single quantum dots in phospholipid micelles for their use in cellular and in vivo imaging. Nat Protoc 2007;2(10):2383–90.
[136] Yong KT, Roy I, Law WC, Hu R. Synthesis of cRGD-peptide conjugated near-infrared CdTe/ZnSe core-shell quantum dots for in vivo cancer targeting and imaging. Chem Commun (Camb) Oct 14 2010;46(38):7136–8.
[137] Celebi S, Erdamar AK, Sennaroglu A, Kurt A, Acar HY. Synthesis and characterization of poly(acrylic acid) stabilized cadmium sulfide quantum dots. J Phys Chem B Nov 8 2007;111(44):12668–75.
[138] Wei Y, Jana NR, Tan SJ, Ying JY. Surface coating directed cellular delivery of TAT-functionalized quantum dots. Bioconjug Chem Sep 2009;20(9):1752–8.
[139] Bardajee G, Soleyman R. Surface passivation of CdSe-TOPO quantum dots by poly(acrylic acid): solvent sensitivity and photo-induced emission in water. Iran Polym J 2013;22(12):885–90.
[140] Xing Y, Rao J. Quantum dot bioconjugates for in vitro diagnostics & in vivo imaging. Cancer Biomark 2008;4(6):307–19.
[141] Rosenthal SJ, Chang JC, Kovtun O, McBride JR, Tomlinson ID. Biocompatible quantum dots for biological applications. Chem Biol Jan 28 2011;18(1):10–24.
[142] Murcia MJ, Minner DE, Mustata GM, Ritchie K, Naumann CA. Design of quantum dot-conjugated lipids for long-term, high-speed tracking experiments on cell surfaces. J Am Chem Soc Nov 12 2008;130(45):15054–62.
[143] Oh E, Delehanty JB, Sapsford KE, Susumu K, Goswami R, Blanco-Canosa JB, et al. Cellular uptake and fate of PEGylated gold nanoparticles is dependent on both cell-penetration peptides and particle size. ACS Nano Aug 23 2011;5(8):6434–48.
[144] Wilson R, Spiller DG, Beckett A, Prior IA, Sée V. Highly stable dextran-coated quantum dots for biomolecular detection and cellular imaging. Chem Mater 2010; 22(23):6361–9.
[145] Delong RK, Reynolds CM, Malcolm Y, Schaeffer A, Severs T, Wanekaya A. Functionalized gold nanoparticles for the binding, stabilization, and delivery of therapeutic DNA, RNA, and other biological macromolecules. Nanotechnol Sci Appl 2010;3: 53–63.
[146] Hitchcock AP, Leung BO, Brash JL, Scholl A, Doran A. Soft X-ray spectromicroscopy of protein interactions with phase-segregated polymer surfaces; 2012 731–60.
[147] Algar WR, Krull UJ. Developing mixed films of immobilized oligonucleotides and quantum dots for the multiplexed detection of nucleic acid hybridization using a combination of fluorescence resonance energy transfer and direct excitation of fluorescence. Langmuir Apr 20 2010;26(8):6041–7.
[148] Algar WR, Krull UJ. Interfacial chemistry and the design of solid-phase nucleic acid hybridization assays using immobilized quantum dots as donors in fluorescence resonance energy transfer. Sensors 2011;11(6):6214–36.
[149] Xiao Q, Huang S, Qi ZD, Zhou B, He ZK, Liu Y. Conformation, thermodynamics and stoichiometry of HSA adsorbed to colloidal CdSe/ZnS quantum dots. Biochim Biophys Acta Jul–Aug 2008;1784(7-8):1020–7.
[150] Pereira M, Lai EP. Capillary electrophoresis for the characterization of quantum dots after non-selective or selective bioconjugation with antibodies for immunoassay. J Nanobiotechnol 2008;6:10.
[151] Kairdolf BA, Smith AM, Stokes TH, Wang MD, Young AN, Nie S. Semiconductor quantum dots for bioimaging and biodiagnostic applications. Annu Rev Anal Chem (Palo Alto Calif) 2013;6:143–62.
[152] Donegan J, Rakovich Y. Cadmium telluride quantum dots: advances and applications; 2013 177–8.
[153] Khorana HG. The chemistry of carbodiimides. Chem Rev 1953;53:145–66.
[154] DeTar DF, Silverstein R, Rogers FFJ. Reactions of carbodiimides III. The reactions of carbodiimides with peptide acids. J Am Chem Soc 1966;88:1024–30.
[155] Anderson GW, Zimmerman JE, Callahan FM. The use of esters of N-hydroxysuccinimide in peptide synthesis. J Am Chem Soc 1964;86:1839–42.
[156] Staros JV. N-hydroxysulfosuccinimide active esters: bis(N-hydroxysulfosuccinimide) esters of two dicarboxylic acids are hydrophilic, membrane-impermeant, protein cross-linkers. Biochemistry Aug 17 1982;21(17):3950–5.
[157] Ferguson EL. Chapter 3 synthesis of polymer–trypsin conjugates bioresponsive polymer-phospholipase a2 conjugates as novel anti-cancer agents (Book); 2008 63–5.
[158] Koolpe M, Burgess R, Dail M, Pasquale EB. EphB receptor-binding peptides identified by phage display enable design of an antagonist with ephrin-like affinity. J Biol Chem Apr 29 2005;280(17):17301–11.
[159] Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Peale F, Bruchez MP. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol 2003;21(1):41–6.
[160] Hu R, Law WC, Lin G, Ye L, Liu J, Liu J, Reynolds JL, Yong KT. EGylated Phospholipid Micelle-Encapsulated Near-Infrared PbS Quantum Dots for in vitro and in vivo Bioimaging. Theranostics 2012;2(7):723–33.
[161] Huh S, Chen HT, Wiench JW, Pruski M, Lin VS. Cooperative catalysis by general acid and base bifunctionalized mesoporous silica nanospheres. Angew Chem Int Ed Engl Mar 11 2005;44(12):1826–30.
[162] Bonasio R, Carman CV, Kim E, Sage PT, Love KR, Mempel TR, et al. Specific and covalent labeling of a membrane protein with organic fluorochromes and quantum dots. Proc Natl Acad Sci U S A Sep 11 2007;104(37):14753–8.
[163] Gindy ME, Ji S, Hoye TR, Panagiotopoulos AZ, Prud'homme RK. Preparation of poly(ethylene glycol) protected nanoparticles with variable bioconjugate ligand density. Biomacromolecules Oct 2008;9(10):2705–11.
[164] Derfus AM, Chen AA, Min DH, Ruoslahti E, Bhatia SN. Targeted quantum dot conjugates for siRNA delivery. Bioconjug Chem Sep–Oct 2007;18(5):1391–6.
[165] Schumacher WC, Phipps AJ, Dutta PK. Detection of *Bacillus anthracis* spores: comparison of quantum dot and organic dye labeling agents. Adv Powder Technol 2009;20(5):438–46.
[166] Tiwari DK, Tanaka S, Inouye Y, Yoshizawa K, Watanabe TM, Jin T. Synthesis and characterization of anti-HER2 antibody conjugated CdSe/CdZnS quantum dots for fluorescence imaging of breast cancer cells. Sensors 2009;9(11):9332–64.
[167] Kolb HC, Finn MG, Sharpless KB. Click chemistry: diverse chemical function from a few good reactions. Angew Chem Int Ed Engl Jun 1 2001;40(11):2004–21.
[168] Li Q, Dong T, Liu X, Lei X. A bioorthogonal ligation enabled by click cycloaddition of o-quinolinone quinone methide and vinyl thioether. J Am Chem Soc Apr 3 2013; 135(13):4996–9.
[169] Tornoe CW, Christensen C, Meldal M. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(i)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. J Org Chem May 3 2002;67(9):3057–64.
[170] Sharpless KB, Manetsch R. In situ click chemistry: a powerful means for lead discovery. Expert Opin Drug Discov Nov 2006;1(6):525–38.
[171] Kolb HC, Sharpless KB. The growing impact of click chemistry on drug discovery. Drug Discov Today Dec 15 2003;8(24):1128–37.
[172] Nwe K, Brechbiel MW. Growing applications of "click chemistry" for bioconjugation in contemporary biomedical research. Cancer Biother Radiopharm Jun 2009;24(3): 289–302.
[173] Prescher JA, Bertozzi CR. Chemistry in living systems. Nat Chem Biol Jun 2005;1(1): 13–21.
[174] Speers AE, Cravatt BF. Profiling enzyme activities in vivo using click chemistry methods. Chem Biol Apr 2004;11(4):535–46.

[175] Huisgen R. Kinetics and mechanism of 1,3-dipolar cycloadditions. Angew Chem Int Ed Engl 1963;2(11):633–45.
[176] Karakoti AS, Das S, Thevuthasan S, Seal S. PEGylated inorganic nanoparticles. Angew Chem Int Ed Engl Feb 25 2011;50(9):1980–94.
[177] Bentzen EL, Tomlinson ID, Mason J, Gresch P, Warnement MR, Wright D, et al. Surface modification to reduce nonspecific binding of quantum dots in live cell assays. Bioconjug Chem Nov–Dec 2005;16(6):1488–94.
[178] Lv Y, Li K, Li Y. Surface modification of quantum dots and magnetic nanoparticles with PEG-conjugated chitosan derivatives for biological applications. Chem Pap 2013;67(11):1404–13.
[179] Iyer G, Pinaud F, Xu J, Ebenstein Y, Li J, Chang J, et al. Aromatic aldehyde and hydrazine activated peptide coated quantum dots for easy bioconjugation and live cell imaging. Bioconjug Chem Jun 15 2011;22(6):1006–11.
[180] Tamura J-I, Fukuda M, Tanaka J, Kawa M. Synthesis Of Hydrophilic Ultrafine Nanoparticles Coordinated With Carbohydrate Cluster. J Carbohydr Chem 2002 01/10 2014/10/24;21(5):445-449.
[181] Sun XL, Cui W, Haller C, Chaikof EL. Site-specific multivalent carbohydrate labeling of quantum dots and magnetic beads. Chembiochem Nov 5 2004; 5(11):1593–6.
[182] Jin H, Nam J, Park J, Jung S, Im K, Hur J, et al. Strong polyelectrolyte quantum dot surface for stable bioconjugation and layer-by-layer assembly applications. Chem Commun (Camb) Feb 14 2011;47(6):1758–60.
[183] Scholl B, Liu HY, Long BR, McCarty OJ, O'Hare T, Druker BJ, et al. Single particle quantum dot imaging achieves ultrasensitive detection capabilities for Western immunoblot analysis. ACS Nano Jun 23 2009;3(6):1318–28.