

Chapter 2

Quantum Dots

Abstract In the past 30 years, quantum dots (QDs) have developed a lot from their kinds to the various application areas. Traditional nanocrystals are usually composed of elements from groups III–V, II–VI, or IV–VI of the periodic table, such as CdS, CdSe, CdTe, CdS@ZnS, CdSe@ZnS, CdSeTe@ZnS. These QDs own excellent fluorescence properties and have been widely used in biosensing and intracellular or in vivo imaging. However, the leaked cadmium ions are culprits for the observed cytotoxicity of cadmium-based QDs, which hampers their further practical applications. Later, with the demand for more biocompatible QDs as the signal reporter, cadmium-free quantum dots (CFQDs) were introduced, such as silicon QDs (Si QDs), carbon dots (C-dots), and graphene QDs (GQDs). In this chapter, the kinds of these traditional quantum dots and new emerging quantum dots as well as their preparation and functionalization are discussed in detail. Additionally, as a viable alternative to QDs, the metal nanoclusters also displayed great potentials as luminescent labels for fluorescent biosensing and bioimaging. Thus, the relevant description of metal nanoclusters is also included in this chapter.

Keywords Quantum dots • Metal nanoclusters • Preparation and functionalization • Bioconjugation

2.1 Traditional Quantum Dots

History of QDs begins with their first discovery in glass crystals in 1980 by Russian physicist Ekimov [1]. Systematic advancement in the science and technology of QDs was driven after 1984, when Luis Brus derived a relation between size and bandgap for semiconductor nanoparticles by applying a particle in a sphere model approximation to the wave function for bulk semiconductors [2, 3]. However, it took nearly a decade for a new promotion in QD research until the

successful synthesis of colloidal CdX ($X = \text{S}, \text{Se}, \text{Te}$) QDs with size-tunable band-edge absorption and emissions by Murray et al. [4]. So far, CdX is the most investigated QDs due to their excellent optical and electrochemical properties. However, with the further application in biological area, the toxicity of cadmium ion in CdX was paid more and more attention. In order to improve the biocompatibility as well as the PL quantum yield and stability of these core nanocrystals, a layer of a few atoms with a higher bandgap semiconductor was introduced to encapsulate the core nanocrystals to form core-shell nanocrystals. The luminescence efficiency is significantly improved when the nanocrystals are passivated on their surface by a shell of a larger bandgap semiconductor and the leaching of metal ions from the core is blocked well by this structure [5, 6]. At the beginning, CdSe/ZnS and CdSe/CdS are the most intensively studied [5, 7]. Later, more and more other “core-shell” QDs were developed, such as CdSe/ZnSe [8], CdTe/CdS [9], CdTe/ZnS [10], and even CdTe/CdS/ZnS “core/shell/shell” QDs [11]. Reiss et al. proposed a simple synthetic route for the preparation of CdSe/ZnSe core/shell nanocrystals applying zinc stearate as a zinc source. Based on the literature, they firstly synthesized CdSe core nanocrystals in a mixed TOPO/HAD solvent with a molar ratio of 60–80 % HAD and using CdO, complexed by dodecylphosphonic acid, as cadmium precursor. Then, ZnO was complexed with dodecylphosphonic acid and slowly injected together with TOPSe into a mixture of HAD-/TOPO-containing CdSe core nanocrystals. After the formation of CdSe/ZnSe QDs, mercaptocarboxylic acids were introduced to make them water soluble and the photoluminescence efficiencies in organic solvents as well as in water after functionalization with mercaptoundecanoic acid could reach 60–85 % [8]. In our group, CdSeTe@ZnS-SiO₂ QDs were prepared with ZnS-like clusters filled into the SiO₂ shell via a microwave-assisted approach (shown in Fig. 2.1). The mercaptopropionic acid (MPA)-capped green-emitting CdSeTe alloy quantum dots were firstly prepared and purified. After that, the CdSeTe QDs were coated with a silica layer at room temperature in the presence of Zn²⁺ and glutathione (GSH). Lastly, the silica-coated QDs were refluxed under microwave irradiation. With the increase in reaction time, the fluorescence gradually changed from dim green to bright orange under a 365-nm excitation and quantum yield was enhanced from 11.9 to 56.9 % with rhodamine 6G as standard before and after the ZnS-SiO₂ coating [12].

2.2 New Emerging Quantum Dots

For traditional QDs, cadmium is the main element for their composition. However, it is well known that leaked cadmium ions are culprits for the observed cytotoxicity of cadmium-based QDs, which hampers their further applications to cellular or in vivo study. With the demand for more biocompatible QDs as the signal reporter, the emphasis has shift toward the fabrication of cadmium-free quantum dots (CFQDs) for applications in biology, such as silicon QDs (Si QDs), carbon dots (C-dots), graphene QDs (GQDs), Ag₂Se, Ag₂S, InP, CuInS₂/ZnS. Strictly, some of them are

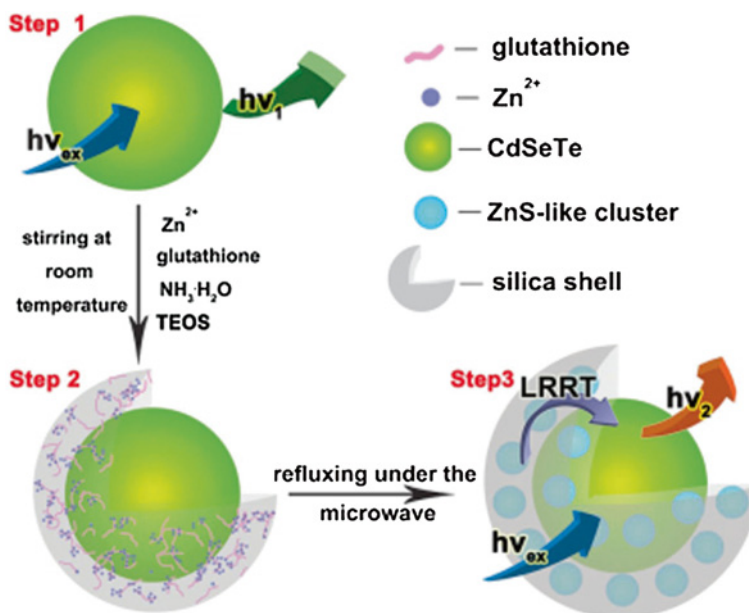


Fig. 2.1 Synthetic pathway for the preparation of CdSeTe@ZnS-SiO₂ QDs. Reproduced with permission from Ref. [12]. Copyright 2012, Royal Society of Chemistry

not new emerging member, such as silicon QDs, which appeared even as early as 1990. But due to their good biocompatibility, we summarized Si QDs in this section and metal nanoclusters are also included because of their excellent properties and wide applications as luminescent probes for biosensing and bioimaging.

2.2.1 Silicon Dots

Silicon has been known to be an indirect bandgap semiconductor with poor optical properties for a long time. It was until the 1990s when efficient light emission from silicon was reported by Canham [13] and quantum confinement to explain features of porous silicon absorption spectra was proposed by Lehman [14] that silicon nanocrystals attracted more and more interests of researchers. There are three distinct photoluminescence bands for Si QDs, one in the infrared, one in the red, and one in the blue light range. The strongest advantage of Si QDs as optical reporter lies in their good biocompatibility. Si QDs were claimed to be at least 10 times safer than Cd-based QDs under UV irradiation [15], and Canham even proposed nanoscale Si as a food additive [16]. Until now, numerous methods have been reported to produce colloiddally and optically stable, water-dispersible Si QDs, incorporating a range of bottom-up and top-down approaches [17]. However, a key obstacle for their applications in bioimaging results from their oxidative

degradation in the biological environment. For a solution, surface modification is necessary. Erogbogbo et al. prepared Si QDs through a nanoparticle synthesis, surface functionalization, PEGylated micelle encapsulation, and bioconjugation process. The obtained Si QDs could be used in multiple cancer-related in vivo applications, including tumor vasculature targeting, sentinel lymph node mapping, and multicolor NIR imaging in live mice, which showed great potentials of Si QDs as biocompatible fluorescent probes for both in vitro and in vivo imaging [18].

2.2.2 Carbon Dots

C-dots are a new class of carbon nanomaterials with sizes below 10 nm, which were first obtained during purification of single-walled carbon nanotubes through preparative electrophoresis in 2004 [19]. Since the discovery of their excellent optical property, C-dots have attracted wide attentions and displayed great potentials in biological applications. A special optical property of C-dots is that besides normal or down-converted photoluminescence, they were shown to possess excellent up-converted PL (UCPL), which enables the design of high-performance, complex catalyst systems based on C-dots for efficient utilization of the full spectrum of sunlight [20–23]. Additionally, C-dots can exhibit PL emission in the near-infrared (NIR) spectral region under NIR light excitation, which is particularly significant and useful for in vivo bionanotechnology because of the low autofluorescence and high tissue transparency in the NIR region [24, 25]. Except strong fluorescence, C-dots also own other properties such as electrochemical luminescence [26–28], photoinduced electron transfer property [29, 30], photocatalysis [22], optoelectronics [31, 32], which all extend their applications in various areas.

As a type of C-dots, the GQDs have also attracted a lot of interest from researchers over the past few decades because of their fascinating optical and electronic properties. As graphene is a zero-bandgap material, in principle, the bandgap of graphene can be tuned from 0 eV to that of benzene by varying their sizes [33, 34]. The 1D graphene sheets could be converted into 0D GQDs, which assume numerous novel chemical and physical properties due to the pronounced quantum confinement and edge effects [35, 36]. Although GQDs are considered as a member of C-dot family, there are still some differences between them [37]. The C-dots are either amorphous or crystalline, while GQDs possess graphene lattices inside the dots, regardless of the dot sizes [38]. Additionally, luminescent C-dots comprise discrete, quasi-spherical carbon nanoparticles with sizes below 10 nm, while GQDs are always defined as the graphene sheets with lateral dimensions than 100 nm in single, double, and few (3 to <10) layers [31, 34]. In general, the average sizes of GQDs are mostly below 10 nm, and up to now, the largest diameter of GQDs reported is 60 nm, which is dependent on the preparation methods [39]. Similar to C-dots, the properties of photoluminescence, good electron mobility and chemical stability, electrochemical luminescence, and photocatalyst of GQDs have been widely employed in the fabrication of numerous sensors and bioimaging

[37, 40]. Except Si QDs, C-dots, or GQDs, other kinds of cadmium-free QDs have also been well developed due to their good biocompatibility and excellent optical properties, such as InP [41], InP/ZnS [42], CuInS₂/ZnS [43, 44], Ag₂Se [45], Ag₂S [46]. They all showed promising potentials in biological imaging applications.

2.2.3 Metal Nanoclusters

As a viable alternative to QDs, fluorescent metal nanoclusters, known as ultra-small size, good biocompatibility, and excellent photostability, have become a new class of fluorescent labels for biological applications. Among them, Au and Ag nanoclusters attract much more attentions. Actually, at the beginning of the observation of photoluminescence from the noble metals, little attention was paid due to the extremely low quantum yield (QY) of 10^{-10} and much more interests have been attracted by researchers until the much enhanced QY reached to the range of 10^{-3} to 10^{-1} [47]. Until now, a lot of Au and Ag NCs stabilized with different scaffolds (protein, peptide, and oligonucleotide) have been developed and applied for the detection of thiol compounds [48], metal ions [49, 50], protein [51, 52], DNA [53], RNA [54] as well as intracellular and in vivo bioimaging. Dickson and coworkers successfully transferred poly (acrylic acid)-stabilized Ag NCs (PA-SCs) to anti-actin Ab/C12 and anti- α -tubulin/C12 conjugates to obtain fluorogenic silver cluster biolabels for cell surface labeling [55]. Wang et al. reported fluorescent Au NCs could be spontaneously biosynthesized by cancerous cell incubated with micromolar chloroauric acid solutions, a biocompatible molecular Au (III) species, which could not occur in noncancerous cells. They further realized in vivo self-bioimaging of tumors by subcutaneous injections of millimolar chloroauric acid solution near xenograft tumors of the nude mouse model of hepatocellular carcinoma or chronic myeloid leukemia. This opens up promising opportunities of fluorescent metal nanoclusters for in vivo bioimaging [56]. Specially, DNA-stabilized Ag NCs possess obvious advantage in DNA biosensing because of the easy assembly of DNA sequence. Werner and colleagues designed a nanocluster beacon to detect a DNA sequence related to the human *Braf* oncogene based on an interesting phenomenon that the red fluorescence of DNA-stabilized Ag NCs could be enhanced 500-fold when placed in proximity to guanine-rich DNA sequences [57].

2.3 Preparation and Functionalization

2.3.1 Cadmium-Based Quantum Dots

A lot of synthetic methods have been developed for QD preparation, which can be divided into two classifications: physical approach and chemical approach. Physical approach, which was mainly referred to epitaxial growth and/or

nanoscale patterning, has been widely used to provide QDs predominantly by the combination of high-resolution electron beam lithography and subsequent etching. However, the production of defection formation, size nonuniformity, poor interface quality, and even damage to the bulk of the crystal itself became the main disadvantages of this method. In contrast, the size and composition of QDs prepared by chemical approach are easily controlled and the average size distribution varies within 5–10 %. The basic process of this method relies on the pyrolysis of organometallic and chalcogen precursors, where rapid nucleation followed by slower and steady growth is desired [58]. A typical protocol involved the following steps: Firstly, heat tri-*n*-octylphosphine oxide (TOPO) to a high temperature under argon or nitrogen atmosphere and then inject a hot solution containing the precursors to initiate rapid homogeneous nucleation. After removing the heat to lower the temperature of the reaction mixture quickly, the crystal growth continues for some time at a lower temperature [59]. Take the synthesis of cadmium selenide (CdSe) QDs, for example. Me_2Cd was chosen as the Cd source, and TOPSe was selected as chalcogen source due to their ease of preparation and good stability. The precursors were rapidly injected into the hot TOPO solution at 300 °C with vigorous stirring to produce a deep yellow/orange solution. Such rapid injection was accompanied by a sudden decrease in temperature to ~180 °C, and the nucleation was stopped. After restoring to heat the reaction flask to 230–260 °C, the growth of QDs continued and their sizes were further controlled by the reaction time [4]. However, Me_2Cd as the raw material was toxic, combustible, expensive, and unstable at room temperature. Meanwhile, the formed insoluble metallic precipitate after the injection of Me_2Cd into the hot TOPO also limited the wide use of this method. As a safer alternative, cadmium oxide (CdO) was chosen as an effective cadmium precursor first proposed by Peng et al. CdO, TOPO, and HPA/TDPA were loaded in a three-neck flask. At about 300 °C, reddish CdO powder was dissolved and generated a colorless homogenous solution. Then, the introduction of tellurium, selenium, and sulfur stock solutions yields high-quality quantum rods and dots of CdTe, CdSe, and CdS. This one-pot synthetic scheme brought a major step toward a green chemistry approach for synthesizing high-quality semiconductor nanocrystals [60, 61].

However, the applications of QDs in biological systems required they are water soluble. Therefore, numerous methods have been developed for creating hydrophilic QDs. One effective route is to exchange the hydrophobic layer of organic solvent with hydrophilic ligands such as thiol-containing molecules [62–66] and peptides [67], which was designated as “cap exchange.” Another route is native surface modification, for example encapsulation by a layer of amphiphilic diblock [68, 69], triblock copolymers [70, 71], silica coating [72, 73], or phospholipid micelles [74, 75]. This two-step method for the hydrophilic QDs preparation broadened their biological applications greatly. However, direct synthesis of hydrophilic QDs in aqueous solution might be more favorable because of its simplicity, high reproducibility, and lower toxicity [76]. To achieve this aim, 3-mercaptopropionic acid (MPA) [77–79], 2-mercaptoethylamine acid (MA), thioglycolic acid (TGA) [80], and L-cysteine [81–83] are used as the stabilizers for one-step

synthesis of hydrophilic QDs. Gao et al. prepared water-soluble CdTe QDs through the reaction between Cd^{2+} and NaHTe, with TGA as the stabilizer. They controlled the ratio of Cd^{2+} , NaHTe, and TGA and adjusted the pH value to 4.5–5.0. The combination between thiol group of TGA and Cd^{2+} promoted not only the hydrophilic stability but also the photoluminescent quantum yield [84, 85]. Besides, microwave-assisted green synthesis has been popular for the preparation of QDs. In comparison with conventional thermal techniques, microwave dielectric heating has a few merits, such as fast heating and 1–2 orders of magnitude increase in the kinetics of the reaction rate. Specifically, microwave dielectric heating could realize the rapid and homogeneous growth of nanocrystals, which is extraordinarily beneficial for preparing high-quality NCs. With the help of microwave irradiation, CdTe, CdTe/ZnS, and CdTe/CdS/ZnS nanocrystals with high photoluminescent quantum yield and excellent photostability were synthesized successfully by He et al. [86–88].

2.3.2 Cadmium-Free Quantum Dots

Compared with cadmium-based QDs, available protocols for the synthesis of Si QDs are limited. As a whole, strategies for the preparation of Si QDs are generally composed of solution-phase-based methods [89–91], microemulsion synthesis [92], thermally induced disproportionation of solid hydrogen silsesquioxane in a reducing atmosphere, and so on [93]. Swihart's group successfully prepared water-dispersible Si QDs with blue, green, and yellow photoluminescence by the functionalization with acrylic acid in the presence of HF. However, as illustrated, these Si QDs still cannot satisfy the colloidal and spectral stability in biological environments [94]. Then, they further proposed a method for the preparation of water-dispersible and biocompatible Si QDs using phospholipid micelles. They were prepared by laser-driven pyrolysis of silane, followed by HF- HNO_3 etching, and the obtained Si QDs were dispersible in chloroform because of the surface functionalization of styrene, octadecene, or ethyl undecylenate. For water solubility, phospholipid micelles were then introduced and a hydrophilic shell with PEG groups was formed on the surface of Si QDs. Such micelle-encapsulated Si QDs displayed good application as biological luminescent probe in in vitro cell labeling [95]. Kauzlarich proposed a microwave-assisted reaction to produce hydrogen-terminated Si QDs. Two different methods were developed for the water-soluble Si QDs: hydrosilylation produced 3-aminopropenyl-terminated Si QDs, and a modified Stober process produced silica-encapsulated Si QDs. Both of them exhibited a maximum emission at 414 nm with intrinsic fluorescence quantum yield efficiencies of 15 and 23 %, respectively [96].

Synthetic methods for C-dots and GQDs are generally classified into two categories: top-down method and bottom-up method. Top-down methods commonly make use of laser ablation and electrochemical oxidation, where C-dots and GQDs are formed or “broken off” from a larger carbon structure and larger

graphene sheets, respectively. Kang et al. developed an alkali-assisted electrochemical method to prepare C-dots with graphite rods as both anode and cathode and NaOH/EtOH as electrolyte. The judicious cutting of a graphite honeycomb layer into ultrasmall particles leads to tiny fragments of graphite, producing C-dots (Fig. 2.2a) [22]. Pan et al. prepared GQDs with bright blue photoluminescence by hydrothermal cutting of oxidized graphene sheets. The starting material, micrometer-sized ripped graphene sheets obtained by thermal reduction of graphene oxide sheets, was first oxidized in concentrated H_2SO_4 and HNO_3 . After that, the hydrothermal treatment of the oxidized graphene sheets at 200°C was performed, resulting in the dramatical decrease in the size of graphene sheets. Ultrafine GQDs were isolated by a dialysis process (Fig. 2.2b) [36]. Bottom-up approaches typically refer to solution chemistry methods during which C-dots and GQDs are formed from molecular precursors. For example, Li et al. reported a solution-chemistry-based approach to large, stable colloidal GQDs with uniform size and shape based on oxidative condensation reactions. The oxidation of polyphenylene dendritic precursors led to fused graphene moieties, which were then stabilized by multiple 2',4',6'-trialkyl phenyl groups. The crowdedness on the edges of the graphene cores twists the substituted phenyl groups from the plane of the core, leading to alkyl chains closing the latter in all three dimensions and reducing face-to-face interaction between the graphenes to produce large, stable GQDs [97]. Puvvada et al. [98] prepared water-soluble C-dots through microwave-assisted pyrolysis of an aqueous solution of dextrin in the presence of sulfuric

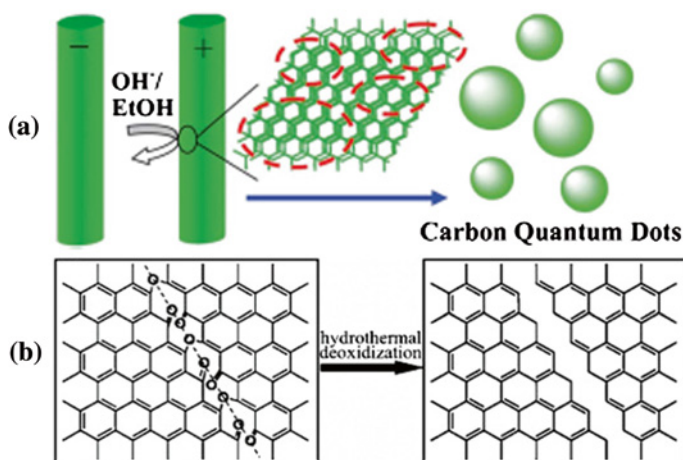


Fig. 2.2 **a** Schematic diagram of electrochemical fabrication of C-dots. Reproduced with permission from Ref. [22]. Copyright 2012, Royal Society of Chemistry. **b** Mechanism for the hydrothermal cutting of oxidized graphene sheets into GQDs. A mixed epoxy chain composed of epoxy and carbonyl pair groups (*left*) is converted into a complete cut (*right*) under the hydrothermal treatment. Reproduced with permission from Ref. [36]. Copyright 2010, Wiley

acid. It should be noted that the cage-opening of fullerene is another bottom-up approach for the synthesis of GQDs. Loh et al. successfully obtained GQDs by metal-catalyzed cage-opening of C_{60} [99].

2.3.3 Metal Nanoclusters

The metal nanoclusters are formed by the reduction of metal ions. However, such reduction of metal ions in aqueous solution always results in large nanoparticles rather than small NCs due to the tendency of NCs to aggregate [100]. Therefore, choosing suitable stabilizer to protect clusters from aggregating and enhancing their fluorescence is the key to obtain small and highly fluorescent metal NCs. Depending on the protection group, Au/Ag NCs are prepared mainly in six scaffolds: thiol-containing small molecules, dendrimers, polymers, DNA oligonucleotides, peptides, and proteins. Each kind of template has different roles in the synthesis of Au or Ag NCs. DNA oligonucleotides are widely employed in the preparation of fluorescent Ag NCs as good stabilizers because silver ions possess a high affinity to cytosine bases on single-stranded DNA. A series of Ag NCs

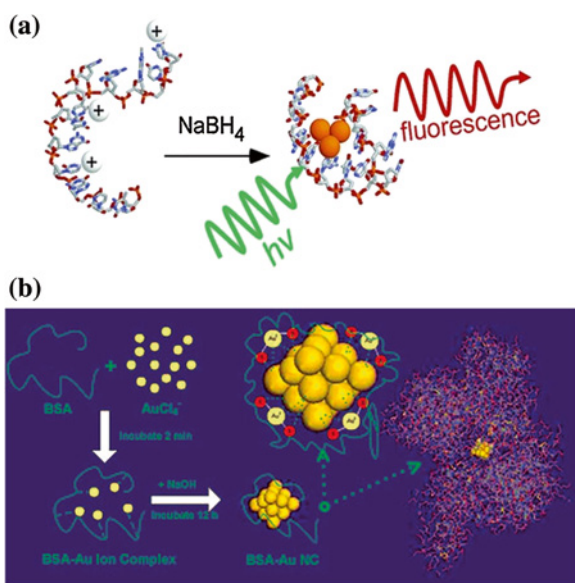


Fig. 2.3 **a** Schematic of the synthesis of DNA-stabilized Ag NCs. Reproduced with permission from Ref. [111]. Copyright 2004, American Chemical Society. **b** Schematic of the formation of Au NCs with BSA as the scaffold. Upon the addition of $HAuCl_4$ to the aqueous BSA solution under vigorous stirring, the protein molecules sequestered Au ions and entrapped them. After adjusting the pH to about 12 with NaOH, the Au ions were reduced by BSA to form Au NCs with red emissions in situ. Reproduced with permission from Ref. [103]. Copyright 2009, American Chemical Society

with different fluorescent emission wavelengths have been produced with various DNA sequences as the stabilizers [101]. Contrary to tremendous reports on DNA-templated Ag NCs, studies on the synthesis of luminescent Au NCs with DNA as the capping agents are scarce. Only Chen et al. reported that the atomically monodisperse Au NCs could be obtained by etching gold particles with the help of amino acids, proteins, and DNA under sonication in water [102]. Unlike DNA oligonucleotides, proteins as the scaffolds offer more potentials in fluorescent Au NC formation. The first fluorescent protein-templated Au NCs were reported by Ying and coworkers in 2009. They developed a simple, green synthetic route for the preparation of Au NCs with red emissions based on the capability of bovine serum albumin (BSA) to sequester and reduce Au precursors [103]. Except BSA, other proteins such as lysozyme [104], transferrin [105], and HRP enzyme [106] have also been proved to act as efficient scaffolds for producing fluorescent Au NCs. In 2007, Dickson et al. reported a significant advance in producing fluorescent Ag NCs *in vivo* by ambient temperature photoactivation with nucleolin protein as the scaffold. Inspired by this, they further designed a short peptide incorporating the specific amino acids most prevalent in nucleolin and several cysteine groups to stabilize fluorescent Ag NCs directly in phosphate buffer [107]. Details about thiol-containing molecules, dendrimers, and polymers as the scaffold for the synthesis of Au and Ag NCs can be referred to other reviews [100, 108–110]. The main synthetic approaches for DNA-templated Ag NCs and BSA-stabilized Au NCs are illustrated in Fig. 2.3.

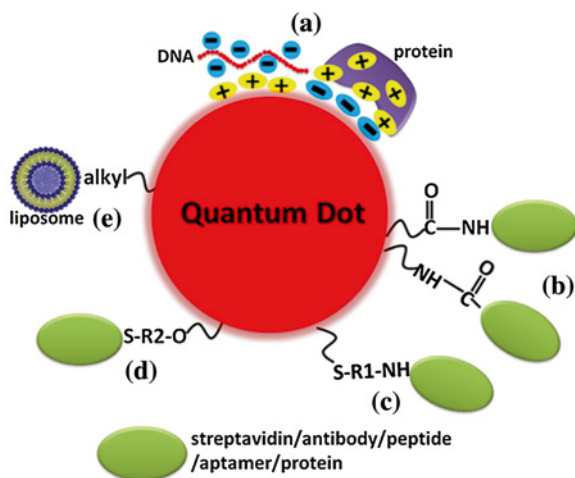


Fig. 2.4 Schematic presentation of various methods for the preparation of QD bioconjugates. **a** Electrostatic interaction between a positively charged protein and a negatively charged QD surface or between a negatively charged oligonucleotide and a positively charged QD surface. **b** Amide bond formation between carboxyl and amino groups by EDC/NHS chemistry. **c** Coupling between amine and thiol groups via the cross-linker SMCC. **d** Conjugation between hydroxyl and thiol groups. **e** Hydrophobic interactions between alkyl on QD surface and lipid or liposome

2.3.4 Quantum Dot Bioconjugation

Labeling of biomolecules, such as oligonucleotides, peptides, and proteins, to the QDs without disturbing the biological function of these molecules is usually required for their applications in biosensing. Commonly used approaches for QD bioconjugation with biological molecules include nonspecific adsorption, multivalent chelation, mercapto (-SH) exchange, and covalent linkage [112]. Several small molecules, such as oligonucleotides and serum albumins, are readily adsorbed to the surface of water-soluble QDs [113, 114]. However, such adsorption is influenced much by the ionic strength, pH, temperature, and the surface charge of the molecule. A more stable linkage is realized by covalently linking biomolecules to the functional groups of QDs using cross-linker molecules. Examples of covalent cross-linking methods include carbodiimide-mediated amide formation, active ester maleimide-mediated amine, and sulfhydryl coupling. Among them, carbodiimide-activated coupling between amine and carboxylic groups displays obvious advantage because most proteins contain primary amine and carboxylic acid groups and no further chemical functionalization is needed for QD conjugation [115, 116]. Figure 2.4 shown below presents a summary of various methods for the preparation of QD bioconjugates.

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Quantum Dots for DNA Biosensing

Zhu, J.-J.; Li, J.-J.; Huang, H.-P.; Cheng, F.-F.

2013, VIII, 91 p. 41 illus., 22 illus. in color., Softcover

ISBN: 978-3-642-44909-3