Topics in Current Chemistry (2020) 378:35
https://doi.org/10.1007/s41061-020-0296-6

REVIEW

Quantum Dot Bioconjugates for Diagnostic Applications

María Díaz-González[1] · Alfredo de la Escosura-Muñiz[1] · Maria Teresa Fernandez-Argüelles[2] · Francisco Javier García Alonso[3] · Jose Manuel Costa-Fernandez[2]

Received: 30 December 2019 / Accepted: 29 February 2020 / Published online: 26 March 2020
© Springer Nature Switzerland AG 2020

Abstract

Quantum dots (QDs) are a special type of engineered nanomaterials with outstanding optoelectronic properties that make them as a very promising alternative to conventional luminescent dyes in biomedical applications, including biomolecule (BM) targeting, luminescence imaging and drug delivery. A key parameter to ensure successful biomedical applications of QDs is the appropriate surface modification, i.e. the surface of the nanomaterials should be modified with the appropriate functional groups to ensure stability in aqueous solutions and it should be conjugated with recognition elements capable of ensuring an efficient tagging of the BMs of interest. In this review we summarize the most relevant strategies used for surface modification of QDs and for their conjugation to BMs in preparation of their application in nanoplatforms for luminescent BM sensing and imaging-guided targeting. The applications of conjugations of photoluminescent QDs with different BMs in both in vitro and in vivo chemical sensing, immunoassays or luminescence imaging are reviewed. Recent progress in the application of functionalized QDs in ultrasensitive detection in bioanalysis, diagnostics and imaging strategies are reported. Finally, some key future research goals in the progress of bioconjugation of QDs for diagnosis are identified, including novel synthetic approaches, the need for exhaustive characterization of bioconjugates and the design of signal amplification schemes.

Keywords Biosensing · Diagnostics · Luminescence · Nanoparticles · Quantum dots · Surface functionalization

This article is part of the Topical Collection "Surface-modified Nanobiomaterials for Electrochemical and Biomedicine Applications"; edited by "Alain R. Puente-Santiago, Daily Rodríguez-Padrón".

✉ Jose Manuel Costa-Fernandez
jcostafe@uniovi.es

Extended author information available on the last page of the article

1 Introduction

Nanotechnology encompasses the fabrication, characterization, manipulation and application of materials that have at least one dimension within the range 1–100 nm. When the size of the material is below this threshold, the material behaves differently from the same material with macroscopic dimensions due to the quantum confinement of the electrons when the dimensions are smaller than the Bohr radius, giving rise to unique and extraordinary physicochemical properties [1].

Nanomaterials can be classified according to different criteria, with the most frequent criterion based on the dimensions of the nanomaterial. Three-dimensional (3-D) nanomaterials are those with three dimensions larger than the nanometer scale, but which are composed of individual building blocks that are within the nanometer scale range, such as nanocomposites. Two-dimensional (2-D) nanomaterials are those that present one dimension in the nanometer scale and include, for example, thin films or nanocoatings. One-dimensional (1-D) nanomaterials possess two dimensions within the range of 1–100 nm and only one dimension larger than the nanometer scale; these nanomaterials include, among others, nanotubes, nanorods and nanowires. Finally, zero-dimensional (0-D) nanomaterials are those with three dimensions within the range 1–100 nm, including nanoparticles (NPs) such as metal NPs, semiconductor quantum dots (QDs), and carbon-based QDs (CQDs) [2].

The development of novel nanomaterials has gained increasing interest in recent decades due to their fascinating physicochemical properties, which have a a great potential for application in different research areas and industries, such as (bio)analytical chemistry [3, 4], water treatment systems [5], catalysis, electrocatalysis [6–9], cancer treatment [10], energy storage devices [11], among others. Although little information is currently available on the production of QDs, it was possible to estimate worldwide and Europe-wide production and use of ten different nanomaterials, including QDs, from a 2012 survey sent to companies producing and using engineered nanomaterials, with the results indicating that the estimated production of QDs was about 10 t/year or lower [12].

Inorganic semiconductor nanocrystals, or QDs, have demonstrated a range of unique optoelectronic properties and represent novel, attractive options in many biomedical applications [13–16]. For example, QDs have been widely employed for fluorescence sensing and bioimaging due to their exceptional photoluminescent characteristics, including the capability to tune the emission wavelength just by controlling the size of the NP. Although conventional organic fluorescent molecules are widely used in bioimaging applications, QDs are superior luminescence tags in terms of their photophysical properties, namely, QDs have broad excitation spectra and narrow and sharp emission spectra and large Stokes shifts (>100 nm). Such optoelectronic properties are of great value in multiplexed applications [17, 18] as by using a single light source it is possible to simultaneously excite multiple QDs of different sizes (multiple emission peaks). Additionally, QDs have very high molar adsorption coefficients, as well as higher

quantum yields than organic fluorophores. Consequently, fluorescent NPs are nearly 20-fold brighter and many thousand-fold more stable against photobleaching than conventional organic dyes [19, 20]. Such exceptional optical behavior justifies the rapid emergence of QDs as valuable photoluminescent probes in many analytical applications.

In particular, the use of NPs for diagnostic purposes is increasing exponentially due to their highly valuable optoelectronic properties and small size. Additionally, recent advances in such areas as surface modification and functionalization have given rise to the improved colloidal stability of NPs in complex media and biological buffers and biocompatibility, while allowing the NPs to bond to recognition elements [21]. In this context, especially relevant are the developments in the synthesis of NPs with interesting optical properties that overcome the limitations of traditional organic dyes, including the synthesis of gold NPs (AuNPs), semiconductor QDs, gold nanoclusters, silver nanoclusters, rare-earth-based NPs, carbon dots and dye-loaded NPs [22].

However, despite the exceptional properties demonstrated by QDs developed to date, their applications in clinical analysis, especially during the first decades of development, can still be considered to be somehow limited, in part due to their low targeting efficiency and eventual high toxicity, both of which could hinder their application in in vivo imaging. These limitations underlie the many research efforts to develop QDs exhibiting low biological toxicity (e.g. those based on an Ag_2S core) [23]. Additionally, in the absence of any molecular moiety being attached to the NP surface, QDs generally show nonselective distribution acros different organisms, thus failing to satisfy the minimum requirements for appropriate molecular imaging. Clearly, the development of QD-based nanoprobes requires a previous surface functionalization of the NPs to facilitate the various approaches used in targeting-guided imaging techniques. In addition, to ensure the required biocompatibility of the nanoprobes to be used in in vivo imaging or sensing, an appropriate surface functionalization of the NPs is required.

The aim of this review is to highlight advances in the use of QDs in diagnostic applications. In the following sections, we first introduce and briefly describe the main types of QDs used in bioanalysis. This is followed by a section that focuses on the strategies of solubilization and stabilization of QDs in aqueous solutions under physiological conditions and then by a section in which approaches used for the functionalization of QDs with biological molecules are summarized. The functionalization of QDs is a key aspect of their use and a requirement before they can be employed for the detection of analytes in biological matrices. Thereafter, we describe some of the most relevant applications of QD bioconjugates in the optical imaging of biomarkers, including a review of the in vitro applications of QDs in medicine in which different detection schemes based on the bioconjugation of QDs to antibodies, aptamers, peptides or other types of recognition elements. In this context, we provide an overview of recent advances in the development of low-cost, portable and easy-to-use QD-based biosensing devices for clinical applications (point-of-care). The final section discusses future prospects with the intention to indicate the direction that research on the use of QDs in diagnostic applications is heading.

2 QDs: Nature and Types

Despite the many problems encountered in the initial attempts to synthesize colloidal fluorescent semiconductor NPs, such as lack of reproducibility and reduced optical quality, important advances have been achieved in this field. In pioneer studies on the routine preparation of colloidal QDs, the core of the QDs was usually capped with an organic layer that coordinates with core-metal sites and stabilizes the QD surface, thereby preventing an irreversible flocculation (aggregation) of the nanocrystals [24]. Unfortunately, these protective ligands are also hydrophobic, and thus nanocrystals capped with such coatings are not compatible with bioanalytical assay conditions. Consequently, the QD surface should be further modified by attaching the appropriate hydrophilic functional groups to allow dispersion of the QDs in aqueous solutions while maintaining their high photoluminescence quantum yield.

Although fluorescence emission is the most exploited property of QDs, doping the core of the nanocrystals with transition elements has been a strategy adopted to provide the QDs with new improved multimodal characteristics for biomedical applications. Additionally, during recent years, many research groups have tried to overcome the problem of eventual cytotoxicity of the more conventional heavy-metal based QDs. Approaches based on carbon-based nanomaterials are one of the most promising strategies. In this section, we briefly review the nature and characteristics of QDs typically used in clinical and biomedical applications.

2.1 Semiconductor QDs

Quantum dots are spherical semiconductor photoluminescent NPs with a diameter ranging between 2 and 10 nm. Since the dimensions of the NP are smaller than the Bohr radius, the energy levels are quantized. As a consequence, the optoelectronic properties of QDs depend on their size due to quantum confinement effects and differ from the properties observed for the same bulk material [25]. In fact, due to the quantized energy levels, QDs generate an intense emission of photoluminescence: when the semiconductor QD is irradiated with a light source, the absorption causes an electron to move from the lower energy valence band to a higher energy conduction band, following which an electron–hole pair is generated, and its recombination gives rise to the emission of intense photoluminescence. Other optical features that make QDs very appealing for use in analytical applications include broad absorption spectra, narrow and symmetric emission bands that can be tuned by changing the composition and size of the NP (see Fig. 1), large Stoke shifts and high photostability [26, 27].

The energy band gap decreases with increases in QD diameter, and as a result the emission wavelength shifts to longer wavelengths. Hence, for QDs with the same composition, the emission can be tuned by just modifying the size of the NP (referred to as size-dependent emission). In addition, the QD can be synthesized with different semiconductors, such as CdS, CdSe, CdTe, ZnS, ZnSe,

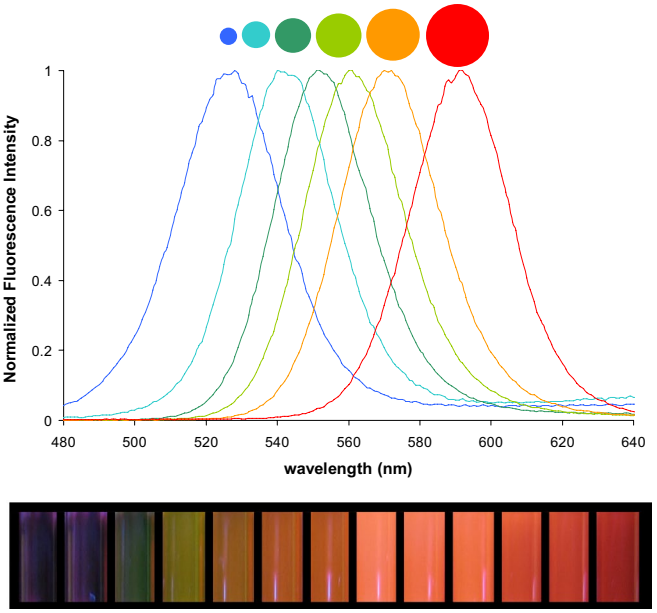

Fig. 1 Size-tuneable fluorescence spectra of CdSe quantum dots (QDs) of different diameter sizes. Bottom bar shows images of the colloidal suspensions of the differently sized QDs under UV light. Reprinted from Fernandez-Argüelles et al. [28], copyright 2010, with permission from Wiley

PbS, InP, among others, all of which also affect their spectral properties. Such features of QDs are very attractive because emission in a wide range of spectra, from the ultraviolet (UV) up to the near-infrared (NIR), can be obtained by simpley changing the composition and size of the QD. Additionally, all QDs generate broad absorption bands, meaning that a single excitation source can be used to efficiently excite QDs with different emission wavelengths, a characteristic which, in addition to their high luminescent quantum yields and long photostability (e.g.: CdS/ZnS QDs are nearly 20-fold brighter and 100-fold more stable than the widely used Rhodamine 6G [19]), is of great interest in multianalyte detection [16, 29].

The synthesis of semiconductor QDs can be performed in both organic hydrophobic solvent and aqueous media, although the synthetic routes that generate QDs with the best optoelectronic properties are those carried out in nonpolar solvents and using hydrophobic ligands. As a result, while the QDs obtained through these routes present outstanding photoluminescent properties, they tend to aggregate and precipitate in aqueous solutions. This renders it necessary to modify their surface with molecules that present hydrophilic groups oriented towards the medium, a configuration which bestows the QDs with good colloidal stability in aqueous media. The most common strategies to transfer QDs from hydrophobic organic media to aqueous solution are summarized in section Stabilization Strategies for QDs in Aqueous Media.

2.2 Metal-Doped QDs

In recent years, advances made in the rational design of nanomaterials have contributed to the development of hybrid NPs that combine the interesting size- and shape-dependent properties of semiconductor QDs with a long-lived phosphorescence-type emission. In this context, the incorporation of suitable atoms or ions into host lattices has generated a novel type of QDs that are very promising for use in bioanalytical applications [30]. ZnS, ZnO, ZnSe, CdS and CdSe QDs can be used as host lattices into which other transition-metal and lanthanide ions, including Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Ag^{+}, Pb^{2+}, Cr^{3+}, Eu^{3+}, Tb^{3+}, Sm^{3+} and Er^{3+}, are incorporated as dopant agents, giving rise to luminescent QDs with novel properties [31]. Consequently, host lattices such as as ZnS and ZnO are being widely studied because, first, they do not contain toxic metals and thus potentially have a lower toxicity and, second, they are characterized by a larger energy band gap, which allows the incorporation of more doping agents, which is very attractive in terms of developing dual-doped QDs [32, 33]. The introduction of the dopant typically increases the photoluminescence lifetime of the QD (see Fig. 2), producing a phosphorescence-like emission that overcomes the limitations of fluorescent NPs or dyes due to the removal of the fluorescence background commonly found in biosensing and bioimaging applications [14].

Most of the synthetic routes described to incorporate the doping agent into the host structure are based on wet chemistry procedures carried out in organic media, typically under high temperatures, or in aqueous media, through precipitation or microemulsion methods in order to control the size and shape of the nanocrystal as well as obtain a homogeneous distribution of the dopant in the host matrix.

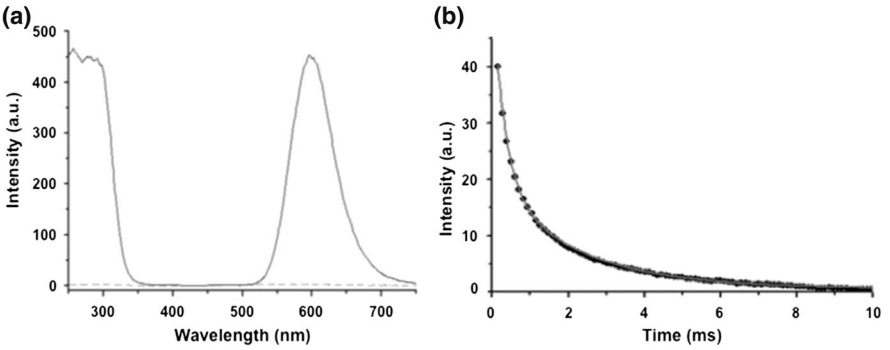

Fig. 2 **a** Excitation and emission spectra of colloidal Mn:ZnS QDs (solid line) and of colloidal ZnS QDs (broken line), **b** decay curve of luminescence emission of colloidal Mn:ZnS QDs with a first lifetime component in the range of 0.3 ms and a second longer lifetime component of around 2.1 ms Reprinted from [34], copyright 2012, with permission from Elsevier

2.3 Carbon-Based QDs (C-dots)

Inorganic semiconductor QDs have been intensely evaluated as luminescent nanomaterials for bioanalytical applications, but carbon-based NPs, such as CQDs and graphene QDs (GQDs), have also drawn attention as attractive alternatives due to their high photoluminescence quantum yields, low photobleaching effects, high biocompatibility and low toxicity, while avoiding the use of heavy metals commonly found in semiconductor QDs [35]. Additionally, carbon-based NPs possess an exceptional colloidal stability in aqueous media as a consequence of their small size, since Brownian motion provides sufficient energy to inhibit aggregation between them [36, 37].

The most remarkable property of CQDs is most likely their excitation wavelength-dependent fluorescence emission (see Fig. 3), which makes them excellent alternative NPs for optical imaging applications [38]. The principle of such characteristic emission is not fully understood, and the origin of the fluorescence emission of CQDs remains a topic of heated discussion. In this context, CQDs obtained through different synthetic routes or using different precursors present different optical behaviors, suggesting that CQDs are quite complex. In fact, the scientific community has yet to agree on an explanation of the optical properties of CQDs, which have been variously attributed to surface state emission, intrinsic band emission, triple ground state emission, dipole emission involving electron–phonon coupling, transition from surface electrons to valence holes, self-trapped excitons and even to the presence of small organic molecules [39, 40].

The synthesis of CQDs has typically employed graphite as the carbon source, and a surface passivation of the CQDs is frequently necessary in order to obtain better fluorescence properties. However, green methods for the synthesis of CQDs based on the use of natural precursors are gaining in importance, as in addition to these synthesis methods being cost effective and environmentally friendly, the CQDs obtained do not require any surface modification, present high photoluminescence and have excellent stability in aqueous media [41].

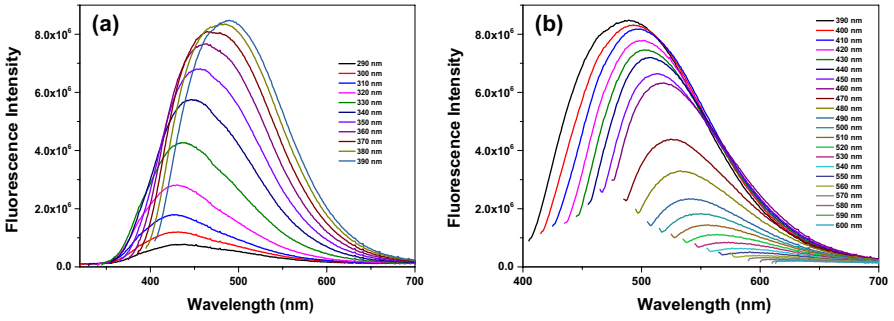

Fig. 3 Emission spectra of black pepper carbon QDs (CQDs) under different excitation wavelengths. **a** excitation wavelength from 290 to 390 nm, **b** excitation wavelength from 390 to 600 nm. Reprinted with permission from Vasimalai et al. [39], copyright 2018 Beilstein-Institut

The potential of CQDs for use in diagnostic applications has increased recently with the metal doping of NPs with N and lanthanides (e.g. Gd and Yb), resulting in co-doped nanomaterials exhibiting not only strong fluorescence but also high constrast capabilities in magnetic resonance imaging (MRI) and computed tomography (CT) [42]. This simple approach has enabled the design of multimodal QDs for bioimaging applications. However, studies on the surface modification of these co-doped NPs with appropriate recognition biomolecules (BMs) for targeted bioimaging are still needed.

3 Stabilization Strategies for QDs in Aqueous Media

As mentioned in a previous section, conventional high-quality fluorescent QDs are commonly synthesized in organic solvents at high temperature. However, the QDs must be made water-compatible (stable in aqueous and biological media so that they maintain their optoelectronic properties) if the intenstion is to use them in bioanalytical applications. To this end, surface modification of the QDs after synthesis is a must; as well, QDs should have functional groups available on their surface for further bioconjugation to BMs.

The appropriate QD surface passivation also can solve some of the problems typically affecting these NPs. First, crystalline NPs can easily form surface defects that quench the fluorescence properties of naked QDs [43]. Second, naked QDs can suffer from surface oxidation, photochemical degradation and/or the leaching of metal ions from the NP core after exposure to ionic or biological media, which affects their optoelectronic properties and produces undesirable cytotoxicity [44]. Thus, modification of the QD surface with the appropriate ligands is essential not only to stabilize the NPs in physiological media (particularly important if they are going to be used in clinical applications) but also to reduce nanocrystal surface defects, thereby minimizing QD reactivity and toxicity. Moreover, despite the significant progress achieved in the synthesis of QDs, biological uses of QDs require that such NPs be modified into biocompatible probes. In this context, the availability of robust and versatile NP surface chemistries are invaluable strategies to achieve stabilization of the QDs in biological buffers while preserving their original photophysical properties and providing adequate reactive groups for further bioconjugations. The three main strategies employed for hydrophilization of QDs (based on the attachment of polar functional groups to the surface of the QD) are summarized in Fig. 4.

As shown in Fig. 4, a universal and simple approach is based on ligand exchange of the original hydrophobic coating of the QDs (e.g. trioctylphosphine oxide [TOPO] chains). In this method, the original coating is removed and replaced with bifunctional molecules that often bind to the QD surface (e.g. through a thiol end) and which have a hydrophilic functional group on the other end (such as carboxyl or sulfonic acids) that provides the required NP solubility in aqueous and polar media and is also available for further bioconjugation [19]. Bidentate ligands, such as dithiothreitol (DTT) or dihydrolipoic acid (DHLA), as well as oligomeric phosphines, peptides and crosslinked dendrons are widely used to obtain QDs that are stable in an aqueous environment [45].

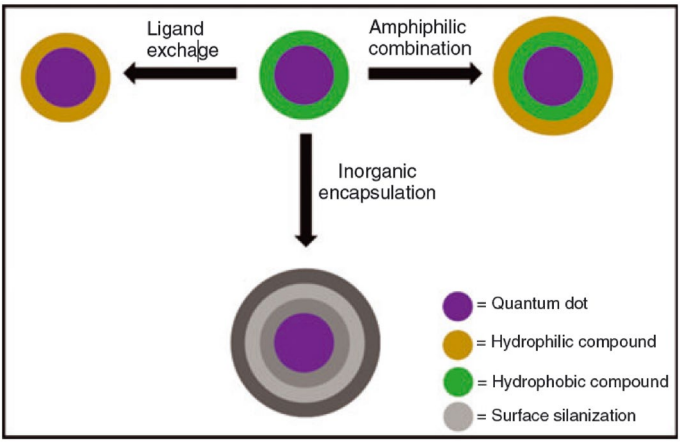

Fig. 4 The three QD phase-transfer approaches commonly used for aqueous stabilization of bare nanoparticles (NPs): the ligand or cap exchange process; bonding of amphiphilic polymers and phospholipids to hydrophobic groups on the surface of the QDs; and surface silanization of the core NP. Reprinted from Karakoti et al. [46], copyright 2015, with permission from Elsevier

Another method commonly used to achieve QD stability in an aqueous environment is based on the well-known silica chemistry used for inorganic encapsulation of the hydrophilic structures (e.g. surface silanization) of the QDs through the generation of a silica shell around the NP surface [46]. This is a very attractive approach to achieve water stabilization of QDs as the silica surface is non-toxic, chemically inert and optically transparent. In this method, typically a precursor molecule, such as mercaptopropyltrimethoxysilane (MPTMOS), is added to the QDs (which replaces the hydrophobic surface chains of the QD). The thiol groups of the MPTMOS react with the inorganic surface of the QD, and the methoxysilane groups polymerize through the formation of siloxane bonds, thus generating a highly crosslinked protective shell around the QD. An additional advantage of this approach is that silica exhibits a high degree of biocompatibility, and it is a simple process to functionalize its surface with appropriate (bio)analyte recognition BMs. Consequently, QDs encapsulated in a silica layer are highly suitable for further bioanalytical applications [47].

The third common approach to achieve water stabilization of QDs is to transfer the nonpolar QDs into an aqueous media combined with the use of amphiphilic polymers. Here, the hydrophobic shell of QDs (e.g. TOP [trioctylphosphine]/TOPO) interacts with the hydrophobic alkyl chains of the amphiphilic polymeric structures through hydrophobic or electronic interactions. The hydrophilic groups of the amphiphilic polymer used will then remain oriented to the external part of the QD surface, thereby providing the required water stability [48]. A large number of amphiphilic copolymers are available for use in this approach, such as polymaleic anhydride [49]) and polyelectrolytes (poly-acrylamide [50], or biopolymers such as DNA [51]). Application of a polyethylene glycol (PEG)-based coating to QDs is another alternative often used to provide stability and biocompatibility to the NPs,

although QDs coated with PEG spacers have reduced nonspecific protein binding, which may often limit the applicability of these NPs in bioanalytical methodologies. For such uses, PEG molecules should be previously activated with appropriate functional groups (e.g. amine, thiols or carboxyls) to provide hydrophilic bridges between the QD surface and the PEG chains [52].

A variant of this third stabilization approach consists of the encapsulation of the hydrophobic QDs in appropriate hydrophilic vehicles, such as liposomes [53]. The hollow spherical structure of liposomes and the high loading capacity makes them attractive carriers for hydrophobic QDs. Moreover, the surface of liposomes can be easily modified with the appropriate functional groups so as to allow a simple further bioconjugation with proteins, thereby minimizing nonspecific interactions of water-soluble and water-insoluble QDs with surface material and amplifying the analytical signal due to the possibility to incorporate several QDs in a single nanoliposome. In this context, a signal amplification platform based on the measurement of fluorescence from QDs encapsulated in liposomes has been recently proposed for the highly sensitive detection of human telomerase activity [54]. In the approach described, similar to a typical hybridization bioassay, biotinylated liposomes containing the QDs were recognized by a capture probe conjugated with streptavidin. In a final step, the QD-encapsulated liposomes were disrupted by the controlled addition of Triton X-100, and the fluorescence intensity of the released QDs was measured to detect telomerase activity [54]. Liposomes containing hydrophobic QDs have also been employed for tumor imaging applications through the specific recognition of aptamers conjugated to the surface of the liposomes (see Fig. 5) [55].

However, a significant drawback of liposomes is their low stability when entering in vivo media. Additionally, QDs stabilized by this approach have a substantially increased hydrodynamic diameter, which could limit their application in bioimaging and targeting procedures.

It must also be taken into account that the procedure selected for hydrophilization of the QDs likely affects their suitability in subsequent bioconjugation and future bioanalytical applications. For example, some approaches can significantly increase the hydrodynamic ratios of the NPs, which in turn can lead to non-specific binding or reduced accessibility to some targets. In this context, ligand exchange provides QDs with a small hydrodynamic diameter but also with lower photoluminescence quantum yields, while encapsulation results in larger nanoprobes sizes with higher quantum yields [56].

4 QD Bioconjugation Strategies

One of the main challenge to the use of QDs in biomedical applications can be considered to be the generation of robust bonding between the appropriate target recognition molecule and the surface of the NP, as this bonding will be a key parameter affecting the direct application of the QDs in biological media. In this section, we summarize some of the most relevant physicochemical processes used to attach BMs to the QD surface, a process referred to as bioconjugation.

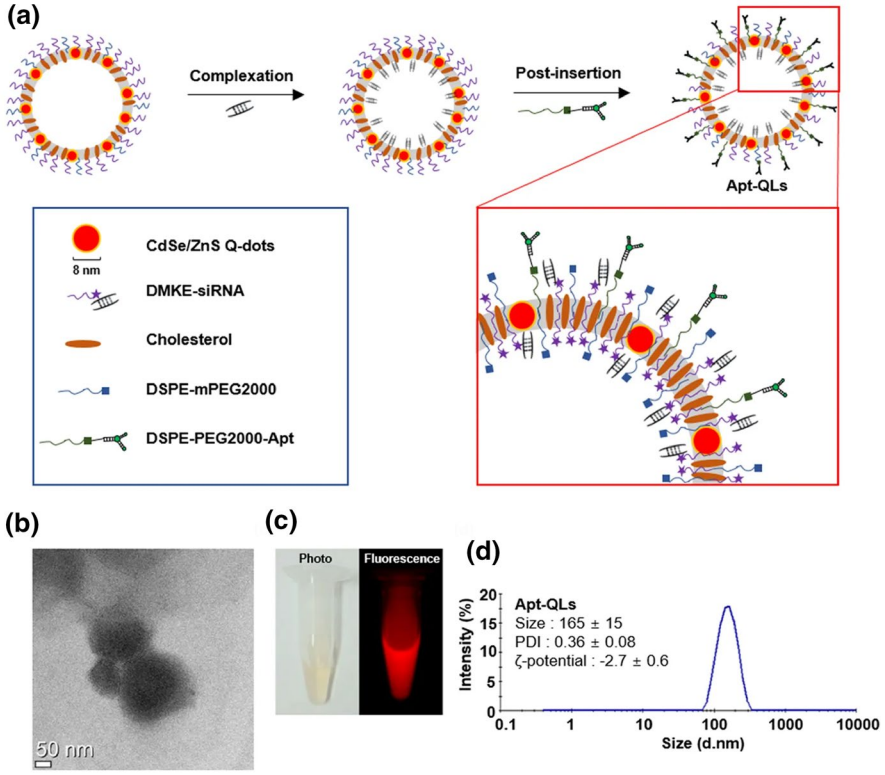

Fig. 5 **a** Schematic illustration of the synthesis of aptamer-conjugated liposomes containing QDs and an aptamer (*Apt-QLs*) against the epidermal growth factor receptor. **b** Liposomes containing QDs were visualized by transmission electron microscopy. **c** Fluorescence emission was verified (excitation wavelength [λ_{ex}] 550 nm, emission wavelength [λ_{em}] 620 nm). **d** Dynamic light scattering revealed generation of 165-nm-diameter liposomes. Reprinted from Kim et al. [55]

All NPs have a very high surface-to-volume ratio; consequently, the role of the NP surface is of paramount importance [57]. The properties of the NP surface are determined not only by their own chemical nature, but also by the layer of capping molecules, referred to as ligands because they bind to the QD surface metals in a similar way as ligands do to the central atom in the metal complexes. Thus, ligands "stop particles aggregating, resist nonspecific adsorption of surrounding molecules, and provide a conjugation point for functional biomolecules" [58]. The synthetic method utilized to prepare QDs determines not only the size, shape and chemical nature of the QDs, but also the ligands that cap their surface. In fact, these ligands are chosen mainly because they have to control the size, shape and polydispersity of the QDs during the synthesis and to maintain their homogeneous dispersion in the solvent post synthesis. Ligand exchange reactions extend the versatility of QD material.

The main elements of the bioconjugation process requiring attention have been described in a comprehensive article written by Sapsford et al. [56] and comprise:

(1) Control over the BM/QD ratio. The desirable ratio varies with the type of the application. It should be noted that QDs are usually larger than BMs, with the possible exception of some large proteins. It should also be noted that the reactions are interfacial in nature and that such interfaces inherently polydisperse across a population of QDs
(2) Control over the orientation of the BM on the QD. Optimal activity of both the QD and the BM should be maintained.
(3) Control over the separation between QD and BM. This point is crucial if the platform QD–BM is to be used in a Förster resonance energy transfer (FRET) experiment [59]
(4) Control over the strength of the QD–BM bond. Most clinical or in vivo experiments require permanent and, therefore, strong linkage.

Many different approaches can be used to immobilize BMs onto the QD surface, with the simplest method to link a BM to a QD surface being adsorption. However, in adsorption, the attachment of a BM is rather tenuous and maintained by weak interactions only, such as hydrogen bonding, London dispersion and Coulombic forces and lone-pair electrons [60]. As an example, the proteins present in the human body tend to bind nonspecifically onto the surface of QDs, a process which is to be avoided. A closely related approach is based on pure electrostatic interactions between the BM and the QD [61]. However, while this method of functionalization is generally straightforward and fast, electrostatic interaction, similar to simple adsorption, suffers from serious disadvantages, including instability, lack of orientation control on the BM and on the BM/QD ratio [62, 63]. Therefore, the most common routes to achieve bioconjugation consist of the four shown in Fig. 6 [56].

4.1 Direct Union of BMs

BMs can be joined to a semiconductor QD surface through the direct covalent union of the BMs to the surface of the QD semiconductor (route 1 in Fig. 6). Thus, proteins, peptides and nucleic acids can be bonded to the QD surface metal atoms (especially Zn) through their (cysteine) thiol and (histidine) imidazole groups [56]. Thiol and histidine motifs could eventually be added to the "natural" BMs and, occasionally, thiol groups are created, reducing peripheral S–S bonds. Occasionally, BMs are bonded directly to the QD in the synthesis processes (biological templating) [56].

4.2 BMs Bonded to a Ligand

A more general method to join BMs to a QD surface is to bind covalently the BM to a ligand that has been previously attached to the QD surface (route 2 in Fig. 6). It should be pointed out that GQDs usually contain carboxyl, hydroxyl, carbonyl and epoxide external groups [64] and that carbon-based QDs (CQDs) can be synthesized

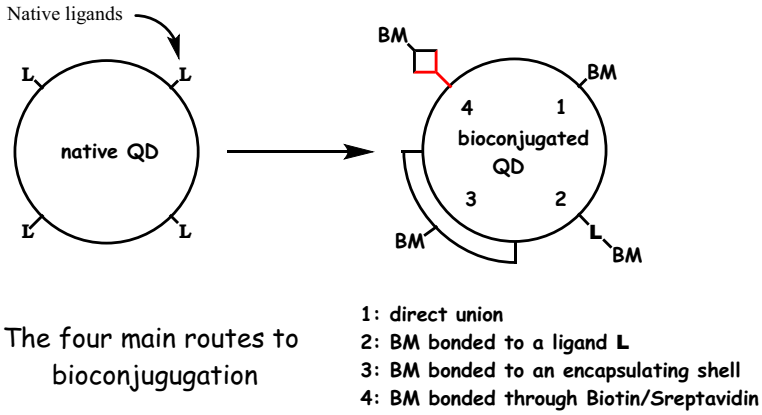

Fig. 6 The four main routes to join a biomolecule (*BM*) to a semiconductor QD surface. (1) Through a direct covalent bond, (2) through a covalent bond between the BM and a ligand (*L*) that was previously anchored onto the QD surface, (3) covalent binding of the BM to a terminal functional group integrated in an encapsulating shell; (4) taking advantage of the specific union biotin/streptavidin

to have selectively carboxylic, amine or other groups in their surface [65]. On the contrary, native semiconductor QDs, with some exceptions being those prepared in water [66, 67], do not have the appropriate ligands able to be robustly bonded to a BM. However, this is not a problem because, as seen in the preceding section, the original ligands can be replaced by others. The new ligands could be monodentate or bidentate simple molecules, such as 3-mercaptopropionic acid (MPA) or dihydrolipoic acid (DHLA), respectively [68], that are attachable to the QD surface through the thiol group(s) and which possess a free terminal carboxylic group to join the BM. However, the new ligands are usually complex molecules with an anchoring group(s) (e.g. polythiol), a spacer chain (frequently a hydrophilic segment) and a terminal functional group (carboxylic or amine groups among others) [69]. Very often, the spacer chain is PEG, that is the whole ligand is a bifunctional PEG molecule [67] because PEG is biocompatible and highly soluble in water and stabilizes QDs against aggregation.

4.3 BMs Bonded to an Encapsulating Shell

The native ligand exchange method is associated with a number of problems, such as a relatively weaker bond between the thiol group and the metal of the QD surface, reduced photoluminescent quantum yield, among others [61, 63]. An alternative is the encapsulation of the QDs (route 3 in Fig. 6), either with a layer of amorphous silica or with a copolymer.

The formation of a silica outer sphere (silanization) increases the solubility and stability of the QDs and retains most of the emission properties [63]. A variety of silanization processes have been described [63, 67, 70, 71]. In general, all are laborious and require several steps [63], including bonding of the silica layer onto the QD surface through an anchoring group. The most commonly used anchoring groups are

thiol or amino groups (A in Fig. 7a). The silica layer which also possess functional groups at the periphery, such as thiol and amino, among others (F in Fig. 7a).

The encapsulation of a semiconductor QD with an amphiphilic copolymer is possible due to the ability of the latter's long hydrophobic tails to interact and interdigitate with the pristine QD ligands (such as TOP, TOPO, hexadecylamine, stearic acid, etc.) [67], thereby leaving the hydrophilic and functionalized segments in contact with the water molecules of the solvent [61, 63, 70]. Although the copolymers of maleic anhydride or acrylic acid are the most popular [61, 63, 70, 72, 73], there are many others (e.g. phospholipid–PEG copolymers, which possess a terminal functional group [74]) (see Fig. 7b).

Some authors consider the envelopment of several QDs inside a polyethylenimine coat [75] or inside the bilayer of a liposome [76, 77], as specific cases of QD encapsulation. Although these examples are not strictly comparable to those described in Fig. 7, in which every QD is singularly encapsulated, there is certainly some similarity.

4.4 BMs Bonded Through Biotin/Streptavidin

Another method used very frequently to join BMs to the QD surface is based on the strong interaction between biotin and avidin, with a dissociation constant of 10^{-15} M [78]. Avidin, a protein found in egg white, contains four identical subunits, each with a single biotin-binding site. Biotin is vitamin H. In this method (route 4 in Fig. 6), it is considered advantageous to substitute avidin with deglycosylated avidin derivatives, such as streptavidin or neutravidin [56, 62]. There are a variety of biotin

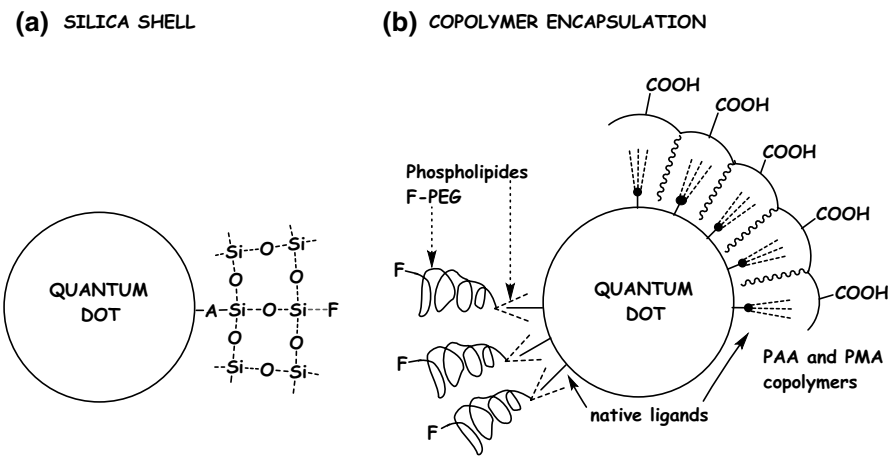

Fig. 7 Encapsulation of a semiconductor QD with silica shell (**a**) or with copolymers (**b**). The silica layer binds to the QD surface through an anchoring group (*A*) and possesses functional groups (*F*) at the periphery. The copolymers most frequently used for encapsulation are polymaleic anhydride (*PMA*)- or poly(acrylic acid) (*PAA*)-based copolymers, but end-functionalized polyethylene glycol (*F-PEG*) phospholipids are also used

derivatives that make the biotinylation of QDs and BMs a rather straightforward process [56, 62]. QDs and BMs may be also functionalized with avidin, although it should be noted that the attachment of avidin to the QD during initial modification will probably obscure one or more available biotin binding sites [56]; at a later moment, however, avidin-functionalized QDs could be attached to biotinylated BMs. Conversely, biotin-functionalized QDs can be used to join avidin-functionalized BMs. Even biotin-labeled QDs can be coupled to biotin-labeled BMs through an intermediary avidin linker [56, 61] although unexpected results may be obtained since the avidin can bind up to four biotin moieties [56, 61].

There are many more types of specific non-covalent affinity between pairs of molecules other than avidin/biotin interactions that are useful in bioconjugation; these include histidine–nickel nitrilotriacetic acid interaction, barnase–barstar interaction and antibody–ligand interaction [62].

4.5 Covalent Coupling Strategies

The focus in this section is on covalent bonding between ligands anchored onto the QD surface (directly or attached to an encapsulating shell) and the incoming BMs. In this regard, we consider biotin and avidin (or straptividin or neutravidin) to be BMs.

The number of functional groups useful for bioconjugation reactions is rather limited in natural BMs [56] (see Fig. 8). Carboxylic and amino groups are present in peptides and proteins not only as terminal groups but also as side groups in peptides such as aspartic or glutamic acids or lysine, respectively. Less common in peptides and proteins are the thiol, phenol and imidazole groups that can be found in the amino acids cysteine, tyrosine and histidine, respectively. Other functional groups

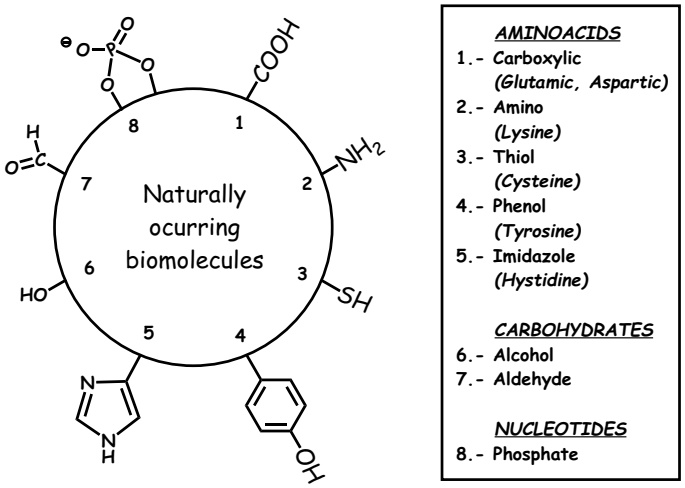

Fig. 8 The most important functional groups found in naturally occurring BMs. *1–5* Functional groups present in amino acids: carboxylic, amino, thiol, phenol, imidazole. *6*, *7* Functional groups from carbohydrates and derivatives: alcohol and aldehyde. *8* Functional group derived from nucleotides: phosphate

that are available in even less common amino acids, such as tryptophan, will not be considered in this review. Carbohydrates and their derivatives provide alcohol and aldehyde as reactive groups, the latter are obtained by oxidation of the former. Nucleic acids possess sugars, phosphates and some bases (the bases are not included in Fig. 8 because they are not usually modified).

Although the number of available functional groups in BMs for bioconjugation reactions would appear to be low, the number of possible reactions by which BMs can be joined covalently to the ligands attached to the QDs described in the literature and used in the commercial sources is rather numerous [56, 62, 63, 71, 76, 79–81]. However, only a small portion of these seem to have been actually used when QDs were involved [62, 63, 82].

One of the most well-studied and easy-to-perform bioconjugation reactions is between a terminal carboxylic acid and a peripheral amine group, conducted under mild conditions, to yield an amide group, with the help of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) and sulfo-NHS (*N*-Hydroxysulfosuccinimide sodium salt). Despite all the disadvantages that this method presents, it is still widely used [62, 63, 82]. An alternative is to use carbonyldiimidazol (CDI) instead of the EDC/sulfo-NHS pair [63, 83].

Other common routes are the reaction of amines with carbonyl groups to yield imine groups, which are usually subsequently reduced with sodium cyanoborohydride [62, 63], and the Michael addition of a terminal thiol to a maleimido group [63, 80]. Another popular coupling method is the utilization of heterobifunctional molecules, such as sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo–SMCC). The NHS ester end of sulfo-SMCC can react with primary amine groups, and the other terminal maleimido function can add a thiol [62, 63]. Similar crosslinker molecules are described in the literature [56, 62, 80].

4.6 Bioorhogonality

The traditional coupling methods described in this review thus far have a number of limitations, among which the most important is undesirable side reactions [56]. The solution to this and other problems is bioorthogonal chemistry. Bioorthogonal chemical reactions involve only the target functions (in QDs and BMs) and do not affect the other functional groups present in either the affected QD and BM or in the biological environment [84, 85].

Although numerous biorthogonal chemical reactions are described in the literature [84–86], only some of these seem to have been carried out when QDs are involved (see those described in Fig. 9). The latter include the copper-catalyzed alkyne-azide cycloaddition (click chemistry); the cycloaddition between tetrazine and strained double bonds (tetrazine ligation); and hydrazone formation by reacting hydrazine and carbonyl groups (hydrazine ligation). It should be noted that not one of the functional groups shown in Fig. 8, i.e those present in "natural" BMs, is involved in these bioorthogonal reactions. This is, of course, the most important advantage of bioorthogonal chemical reactions: the reactions, as described in Fig. 9, do not affect normal molecules present in the biological milieu. Some effort would

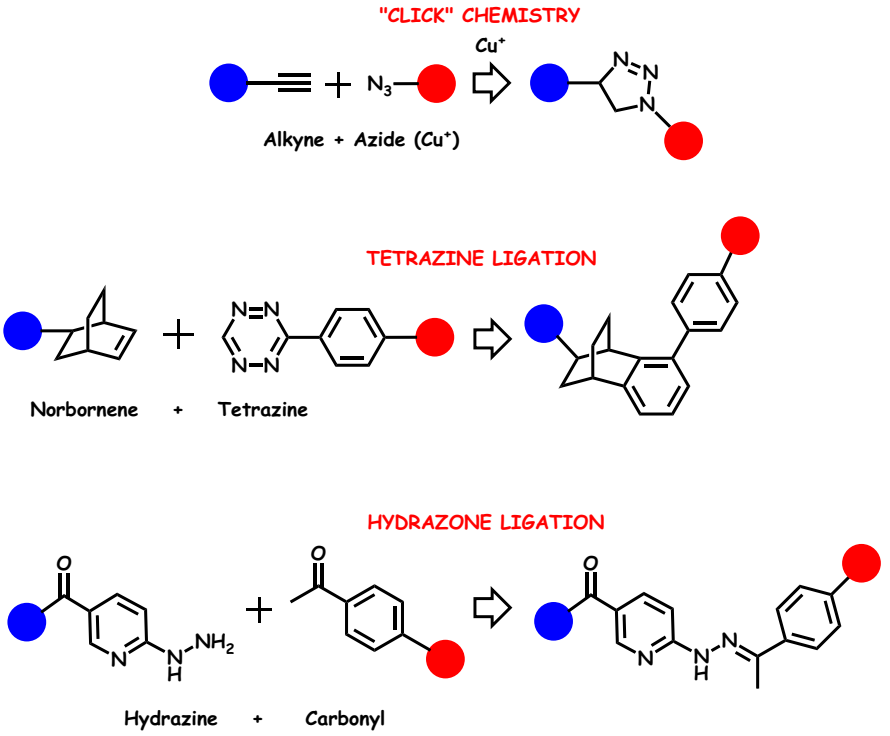

Fig. 9 The main bio-orthogonal reactions carried out utilizing QDs. Top: copper-catalyzed alkyne-azide cycloaddition. Middle: cycloaddition between tetrazine and strained double bonds. Bottom: hydrazone formation by reacting hydrazine and carbonyl groups

need to be made to design procedures for attaching these "new" functional groups to the natural BMs, however no difficulties are foreseeable to bind these to the QDs. In this regard, it has been determined that virtually any functional group can be site-specifically introduced into peptides and nucleotides as needed during the initial synthesis or through subsequent modification [56].

5 QD Biosensing Applications: Point-of-Care Diagnostics

Quantum dot–BM hybrids (i.e. those bound to antibodies, DNA, aptamers, etc.) have been used as sensing probes in a wide variety of in vitro bioassays and biosensors for the detection of different clinical relevant BMs. QDs can be used as labels in a wide spectrum of detection methods (Fig. 10). Most of the reported QD-based bioassays and biosensors have been developed by using QDs as fluorescence labels (Fig. 10a) in fluorescence quenching-based (turn-off), fluorescence enhancement-based (turn-on) and—especially—FRET assays [87–92]. FRET is a very sensitive technology for studying BM interactions that involves the transfer of energy from

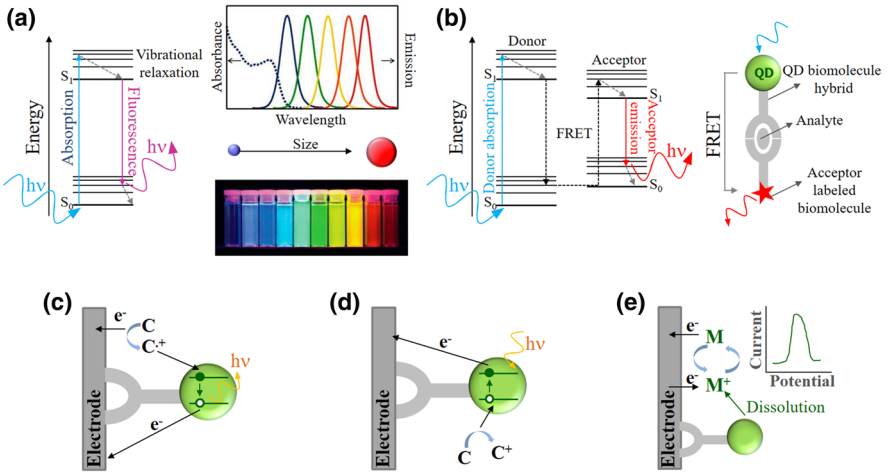

Fig. 10 Detection methods for QD-based biosensing. **a** Fluorescence. Jablonski diagram explaining the effect of fluorescence (left), and a photograph and emission spectra illustrating size-controlled fluorescence of QDs. Adapted from Wen et al. [87], with permission. **b** Förster resonance energy transfer (*FRET*): Jablonski diagram explaining the effect of FRET (left) and schematic illustration of a FRET-based sandwich bioassay (right). **c** Electrochemiluminescence (ECL): schematic illustration explaining anodic ECL. A hole is created by the electrode in the valence band of QDs with the concomitant injection of an electron from a previously oxidized coreactant (*C*). The recombination of electron and hole lead to an anodic ECL emission. **d** Photoelectrochemical (PEC) reaction: schematic illustration explaining anodic PEC reaction. An electron–hole pair is created on QDs after their photoexcitation. The electron transferred from the valence band to the conduction band of the photoexcited QDs is then ejected to the electrode with the concomitant transfer of electrons from an electron donor (*C*), generating an anodic photocurrent. **e** Electrochemical (EC) reaction: schematic illustration explaining the electrochemical detection of QDs by anodic stripping voltammetry. After the dissolution of QDs, metallic species are deposited (reduced) on the electrode and re-oxidized again to be detected Adapted from from Wen et al. [87], copyright 2017, with permission

an excited state donor (usually a fluorophore) to a proximal (< 10 nm), ground-state acceptor (Fig. 10b).

Semiconductor QDs (QDs) have been by far the most reported type of QDs applied in this field [93]. The main advantages of QDs over the organic dyes typically used as fluorescence labels include long fluorescence lifetime, broad absorption spectra, very narrow emission spectra and stability against photobleaching [94]. Another interesting advantage of QDs is their size-controlled luminescence (Fig. 10a). This property allows for the simultaneous determination of different BMs by using a single excitation wavelength [95, 96]. However, the multiplexing capability of QDs as optical labels is quite limited (5–6 BMs). Greater multiplexing capabilities have been obtained by embedding different-sized QDs with different intensity levels into polymeric particles at precisely controlled ratios [97, 98]. This strategy provides a QD barcode technology that in theory is able to create more than million codes (Fig. 11). The main limitations of QDs are their low long-term stability, intrinsic blinking, complex synthesis and complexation and high cost of production.

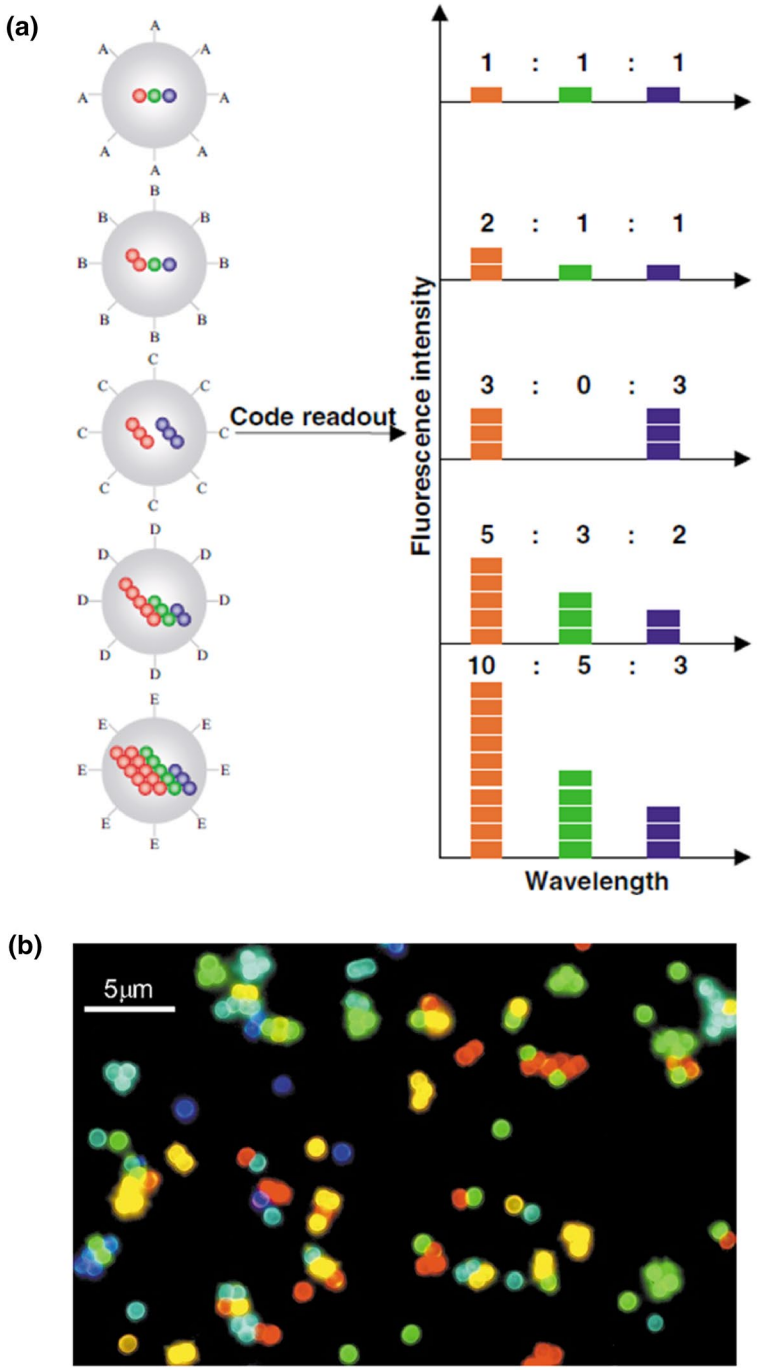

Fig. 11 Quantum dot barcode technology. **a** Schematic illustration showing optical encoding scheme using wavelength and intensity via multiple QDs embedded within microparticles. **b** Color micrograph showing microbeads. Reprinted from Han et al. [97], copyright 2001, with permission from Nature Springer

In addition to fluorescence assays, electrochemiluminescence (anodic/cathodic) (Fig. 10c), photoelectrochemical (anodic/catodic) (Fig. 10d) and especially electrochemical (Fig. 10e) QD-based biosensing systems have also been reported [64, 99, 100]. Electrochemical devices have emerged as the main alternative to fluorescence assays due to their high sensitivity and simple and inexpensive instrumentation [64, 100]. Similar to their optical counterparts, most of the reported electrochemical QD-based bioassays and biosensors rely on the use of QDs as electroactive labels. QDs have been exploited for electrochemical detection on the basis of their elemental compositions. Stripping voltammetry has been the electroanalytical technique most widely employed to quantify the metal ions released upon QD solubilization [101–104]. Based on the different chemical composition, QDs have been used as electroactive labels for multiplexed analysis [105].

Regardless of the detection method used for QD-based biosensing, most of the reported systems are time-consuming and have to be performed in centralized laboratories by highly skilled personnel; consequently, they are not suitable for "point-of-care" (POC) diagnostics. Modern healthcare systems require diagnostic platforms for the real-time remote monitoring of health biomarkers in their striving towards a more patient-centered approach to care [106]. In this context, the development of simple and cost-effective POC diagnostic systems able to obtain useful information instantly at the sampling site has been the focus of biosensing research in recent years.

Microfluidic systems have become an increasingly attractive alternative to the centralized laboratory assays for POC applications. Microfluidic devices allow conventional assays to be performed using an automated and high-throughput approach, thereby providing advantages such as small reagent consumption, low costs, portability and short analysis time. Examples of on-chip single and multiplexed assays for medical diagnostics based on QDs with optical and electrochemical detection can be found in literature [107–112]. However, the performance of most of these microfluidic devices require the implementation of bulky and energy-consuming off-chip fluidic handling components (pumps, valves) and non-miniaturized detectors, making them unsuitable for POC measurements. This has led to the emergence of paper-based devices as a class of microfluidic devices that can work without the need of any off-chip fluidic handling element, an advantage which has a significant potential for POC applications [113]. In these devices, the liquids are driven by capillary forces and therefore do not require external components. Furthermore, compared to silicon, glass and other polymeric materials used for the fabrication of microfluidic chips, paper is cheap, biodegradable, widely available, flexible and easily modified with BMs. Although the capability of paper-based devices is limited in terms of sensitivity and reproducibility, it has been claimed that these devices are future of point-of-use testing, thanks mainly to their simplicity, low cost and disposability.

In recent years, smartphone devices, which have millions of users worldwide, including third-world regions, have been adapted to tackle limitations in the instrumentation used for POC applications. Owing to the low cost, functionalities, accessibility, small size and ease of use of smartphones, their integration into the development of easy-operable POC devices is very promising [114]. Moreover, the connectivity of smartphones provides the possibility to share data through the cloud

for backup or remote processing of the results, which is highly demanded in modern healthcare systems.

Recent advances in QD-based microfluidic paper devices and QD barcode POC applications are discussed in more detail in subsequent sections.

5.1 QD-Based Microfluidic Paper Devices

Paper-based devices can be classified into dipsticks assays, lateral flow assays (LFAs) and microfluidic paper analytical devices (μPADs). The dipstick assays are the simplest paper-based devices and are based on a paper strip pre-stored with reagents that is dipped into a sample to perform a chemical test (e.g. pH strips). LFAs are the most widely reported form of paper devices (e.g. pregnancy test) [115]. The basic structure of a lateral flow strip comprises four different parts that are fixed in a backing card (Fig. 12a): a sample pad, on which the sample is dropped; a conjugation pad, on which labeled tags conjugated to the biorecognition elements are immobilized; a reaction membrane, which contains test and control lines for reactions; and an absorbent pad, which reserves waste and prevents backflow. Briefly, when a sample is placed onto the sample pad, the sample flows via capillary forces towards the end of the strip. If the analyte is present, the immobilized bioconjugate on the conjugation pad binds to the analyte and continues migrating along the test. As the sample moves along the device, the binding reagents placed on the reaction membrane bind to the analyte at the test line. Bioconjugates free of analyte finally react with specific bioreceptors immobilized on the control line of the membrane (Fig. 12b). μPADs are the most complex but most versatile paper devices [116]. In these devices the formation of hydrophilic channels with hydrophobic barriers enables multidirectional and multidimensional flow that allows for complex bioassays to be performed.

Most of the reported paper devices rely on the use of AuNPs as labels, with qualitative (naked-eye) or semi-quantitative colorimetric detection. The main limitations of colorimetric detection of AuNPs include limited quantitative dynamic ranges and low sensitivity, even with reader systems [117, 118]. In this context, the use of QDs as fluorescence labels is becoming increasingly popular. Fluorescence detection is a good choice to improve both the limit of detection and the dynamic range of colorimetric paper-based devices. However, an important drawback of fluorescence detection in POC applications is the need for complex reader systems to interpret the results.

QDs have been used as signal reporters in the development of different types of paper-based biosensors. Sapountzi et al. [119] designed the first QD-based dipstick for visual detection of nucleic acids and single nucleotide polymorphisms (SNPs) in the human genomic using a common digital camera and a UV lamp for fluorescence imaging. As low as 1.5 fmol levels of double-stranded DNA were clearly detected by naked eye using CdSe/ZnS core–shell QDs as signal reporters. The dipstick performance was accurate and reproducible and was also successfully applied to real sample analysis.

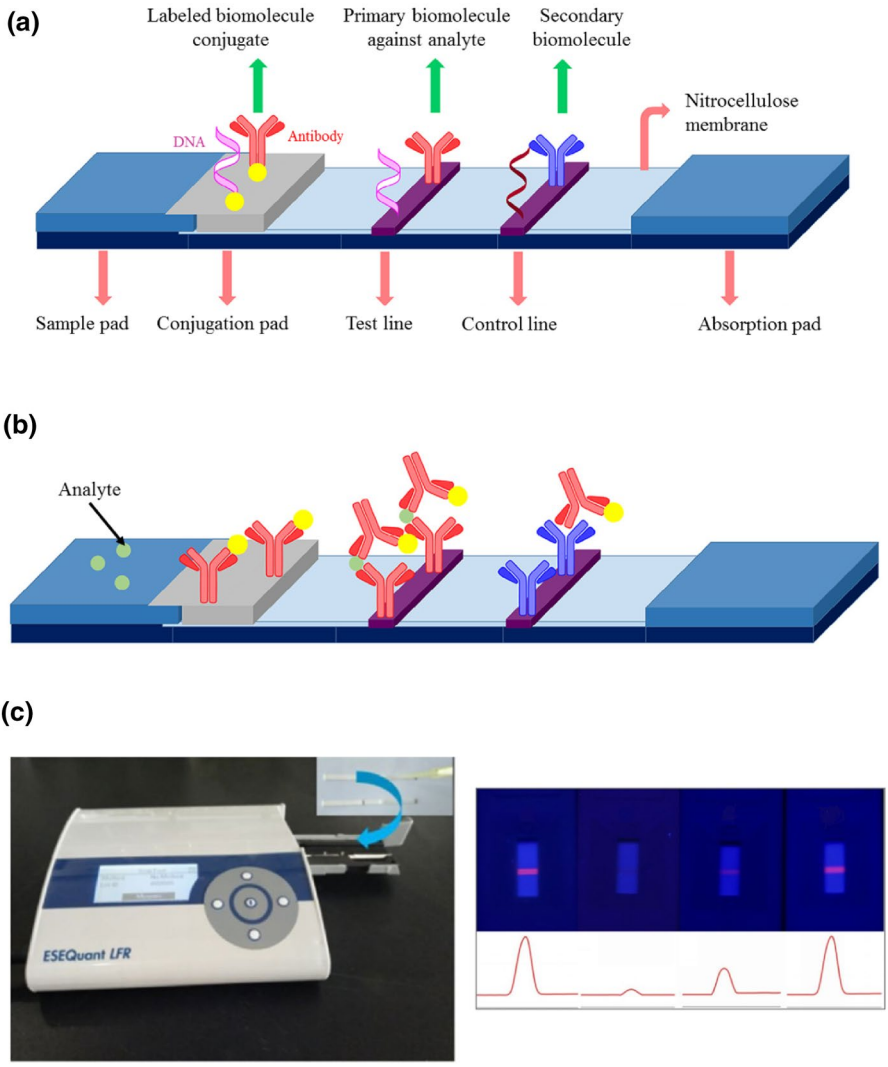

Fig. 12 Quantum dot-based lateral flow assays (LFAs). **a**, **b** Schematic representation of the basic structure of a LFA (**a**) and of a sandwich immunoassay format LFA (**b**). Reprinted from Bahadır and Sezgintürk [115], copyright 2015, with permission from Elsevier. **c** Photograph of a portable fluorescence test strip reader and images of the QD-based fluorescence test strips with different concentrations of the analyte (glutathione) together with the curves obtained from the reader. Reprinted from Liu et al. [123], copyright 2018, with permission from Elsevier

Examples of single and multiplexed fluorescence-based LFAs for a wide variety of clinical relevant BMs using QD-hybrids (conjugated to protein or DNA) as signal reporters can be found in the literature (Table 1) [120–128]. As shown in Table 1, the use of QDs as labels in the development of LFAs has enabled the fast and sensitive determination of different protein biomarkers and DNA. The performance

Table 1 Fluorescence quantum dot-based lateral flow assays

Analyte(s)	Quantum dot	Assay format	Assay time/limit of detection	Real sample	Reference
Heart fatty acid binding protein	CdTe	Sandwich immunoassay	15 min/221 pg mL^{-1}	Human serum	[120]
C-reactive protein (CRP) Interleukin-6 (IL-6)	CdTe	Sandwich immunoassay	30 min/0.3 μg mL^{-1} (CRP) 0.9 pg mL^{-1} (IL-6)	Human serum	[121]
Carcinoembryonic antigen (CEA)	CdSe/ZnS	Sandwich immunoassay	15 min/0.049 ng mL^{-1}	Human serum	[122]
Glutathione	CdSe@ZnS	Displacement assay	10 min/25 nM	HeLa cells extract samples	[123]
Gastric cancer carbohydrate antigen 72-4	CdSe/ZnS	Sandwich immunoassay	10 min/2 IU mL^{-1}	Human serum	[124]
Human immunodeficiency virus-DNA	CdTe	SDA[a]	15 min/0.76 pM	Human serum	[125]
Influenza A nucleoprotein	605-nm QDs	Sandwich immunoassay	1.5 fmol	–	[126]
Simultaneous alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA)	546-nm QDs 620-nm QDs	Sandwich immunoassay	15 min/3 ng mL^{-1} (AFP) 2 ng mL^{-1}(CEA)	Human serum	[127]
Simultaneous influenza A virus subtypes H5 and H9	CdSe/ZnS QDs	Sandwich immunoassay	15 min/0.016 HAU (H5) 0.25 HAU (H9)	Human serum	[128]

HAU Hemagglutinating units, *IU* international units, *QDs* quantum dots

[a]DNA strand displacement amplification

of such QD-based LFAs was found to be similar to that of conventional methods [120–123], and they have been successfully applied for BM determination in real samples.

QD micro/nanospheres have also been employed as fluorescent labels in LFAs. These spheres, prepared by embedding a large amount of QDs into polymeric or silica beads, yield a substantially enhanced fluorescence signal, resulting in an increased sensitivity. Following this strategy, Rong et al. [129] reported a smartphone-based fluorescent LFA for the highly sensitive and selective detection of Zika virus nonstructural protein 1, using CdSe/ZnS QDs encapsulated in polymer microspheres as labels (Fig. 13a). Within only 20 min, the optimized sandwich lateral-flow immunoassay achieved sensitive detection of Zika protein with limits of detection (LODs) of 0.045 and 0.15 ng mL^{-1} in buffer and serum, respectively.

QD nanobeads were used by Li et al. [130] to develop a LFA for prostate specific antigen (PSA). These authors fabricated QD nanobeads (diameter 60 nm) by encapsulating CdSe/CdS/Cd_xZn_{1-x}S/ZnS QDs with modified poly(tert-butyl acrylate-co-ethyl acrylate-co-methacrylic acid). A very sensitive (LOD 0.33 ng/mL) and selective response to PSA was obtained in only 15 min using a portable fluorescence test strip reader. This sandwich lateral-flow immunoassay was also successfully evaluated in clinical serum samples.

As already mentioned, one of the main limitations of fluorescence-based LFAs is the need of expensive and bulky fluorescence readers for quantitative analysis. Most of the fluorescence-based LFAs reported to date use portable strip readers tht are not suitable for POC applications (Fig. 3c). Even when they are portable, such fluorescence readers are still bulky and can only operate for few hours without a power supply. Less reported but remarkable examples of handheld devices [121, 122, 124, 128] and smartphone-based fluoresce readers [126, 129] can also be found in literature.

Smartphone-based platforms have also been used as detectors in QD-based FRET assays on paper substrates. FRET offers the possibility of a ratiometric quantification approach that is able to correct for environmental factors and to self-calibrate. Ratiometric fluorescent intensities can also be easily monitored using a smartphone by simple splitting of the red, green and blue channels in a captured image.

In this context, Petryayeva et al. [131] reported the use of a smartphone detector of FRET-based paper test strips for thrombin activity in serum and whole blood using CdSe/CdS/ZnS as donors and Alexa Fluor 647 (A647) as acceptor (Fig. 13b). Immobilized QDs, conjugated with an A647-labeled peptide substrate, respond to thrombin activity through the loss of FRET between the QD and A647, with recovery of quenched QD photoluminescence. Quantitative results were obtained in less than 30 min with a LOD of 18 NIH units mL^{-1} of activity in whole blood.

Following a similar strategy, FRET-based assays for the quantification of a protein biomarker of epithelial tumors [132] and the DNA diagnostic of spinal muscular atrophy disorder and *Escherichia coli* [133] have been reported (Fig. 13c). CdSe/ZnS donors were used in combination with the Cy3 fluorescent dye acceptor for a sensitive and selective detection of both the epithelial cell adhesion protein and the oligo sequences. As in the μPADs, the hydrophilic areas where QDs were immobilized on these paper-based assays were defined by wax patterning.

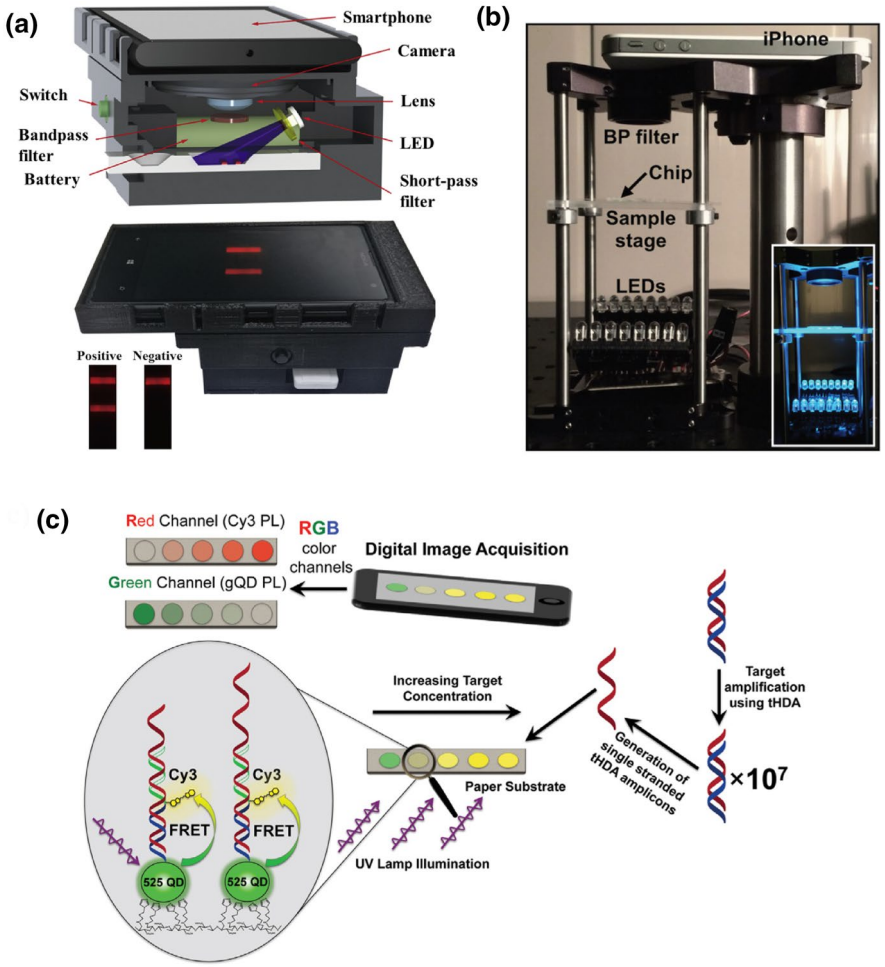

Fig. 13 Smartphone-based QD biosensing systems. **a** Three-dimensional schematic and photograph of the smartphone-based fluorescence LFA reader developed for Zika detection. *LED* Light-emitting diode. Reprinted from Rong et al. [129], copyright 2019, with permission from Elsevier. **b** Photograph of the setup used for smartphone readout of the QD-FRET test strip for thrombin activity. *BP* Band-pass. Reprinted from Petryayeva et al. [131], copyright 2015, with permission from Royal Society of Chemistry. **c** Schematic illustration of the ratiometric QD-FRET assay for DNA diagnostic of spinal muscular atrophy disorder and *Escherichia coli*. *gQD* Graphene QD, *PL* photoluminescence, *tHDA* thermophilic helicase-dependent isothermal amplification. Reprinted with permission from Noor et al. [133], copyright 2015, with permission from Elsevier.

Although optical sensing has been by far the dominant detection method applied in the field of paper microfluidics, examples of electrochemical QD-based paper devices for clinical diagnostics can also be found in literature [134–136]. Electrochemical sensing offers an alternative detection system for quantitative analysis in paper devices due to its simplicity, portability and sensitivity. Small-sized electrodes can be easily integrated into the paper strip (Fig. 14a) or fabricated directly onto the

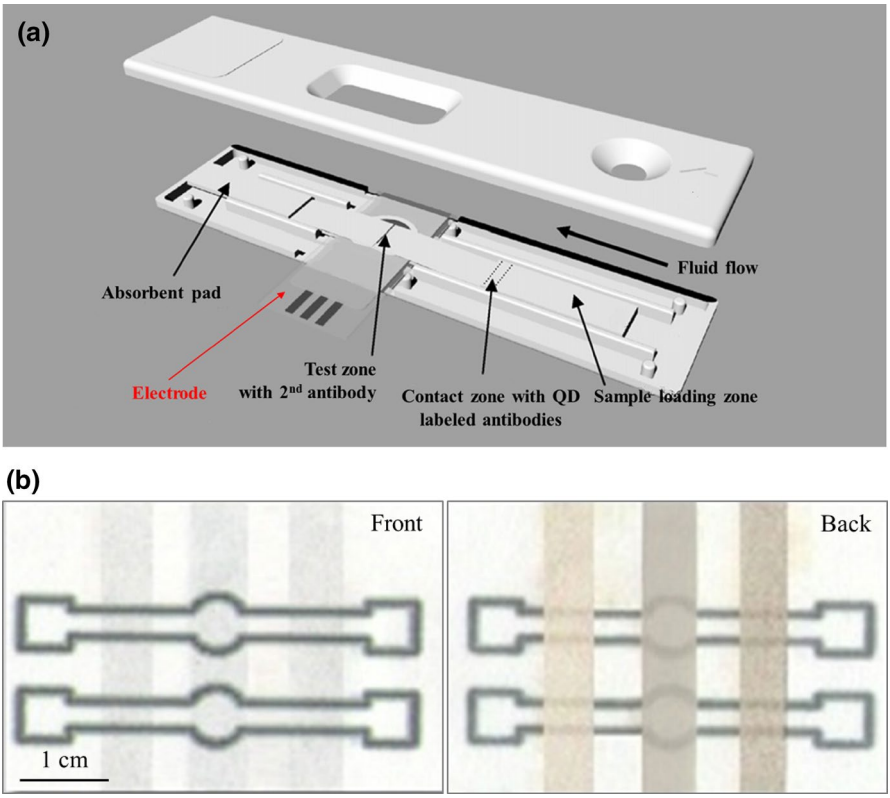

Fig. 14 Electrochemical QD-based paper devices. **a** Schematic diagram of an electrochemical LFA that integrates a screen-printed electrode underneath the test zone of the strip for QD detection. Reprinted from Lin et al. [134], copyright 2008, with permission from Elsevier. **b** Photographs of the front and back of an electrochemical paper device with the electrodes deposited directly onto the paper substrate. Microfluidic pattern is wax-printed on the front face of the paper and the electrodes are deposited by sputtering on the back side of the paper. Reprinted with permission from Kokkinos et al. [135], copyright 2018, with permission from the American Chemical Society

paper substrate (Fig. 14b). These devices, combined with commercially available miniaturized "potentiostats" appear to be a very interesting alternative to fluorescence-based paper devices.

5.2 QD Barcode POC Systems

Barcode assays for POC applications are in high demand by modern healthcare systems [137]. Barcode assays are capable of simultaneously detecting multiple targets from patient samples, thereby increasing the speed of the analysis and improving the precision and accuracy of the diagnosis. As already mentioned, QDs can be used as barcoded probes by embedding them into polymeric particles [97]. In this framework, Gao et al. [138] reported a simple strategy to automate barcode assays in order to make them suitable for POC applications. A microfluidic device was designed to

perform all steps of the assay using barcoded polystyrene microbeads consisting of magnetic NPs (FeO) and QDs (ZnS-capped CdSeS). An on-chip sandwich hybridization assay for the detection of genetic targets for human immunodeficiency virus (HIV), hepatitis B and syphilis was successfully performed in only 20 min, with a LOD of 1.2 nM. The POC application of this microfluidic device is, however, hindered due to the bulky instrumentation required for the fluorescence detection.

A smartphone reader combined with QD barcoding technology was used by Ming et al. [139] in the development of a low-cost chip-based wireless multiplex diagnostic device (Fig. 15). QD barcodes were prepared using different ratios of eight different QDs embedded into a polymeric matrix. These QD barcodes were then arrayed on microfabricated glass slides to create a multiplex chip platform that is simple to use and easy to transport. This handheld device (Fig. 15) was found to be capable of detecting down to 1000 viral genetic copies per milliliter, thereby enabling the diagnosis of patients infected with HIV or hepatitis B in < 1 h.

6 Biomedical Labeling and Imaging

Functionalization of QDs enables them to play a major role in the field of diagnosis and medicine and improves the capabilities of molecular imaging techniques. High-quality functional images (e.g. with high contrast to allow proper differentiation) are required in molecular imaging. Among the different available techniques, fluorescence imaging is a powerful tool to effectively image eventual interactions occurring at the molecular level directly, at real time and with relatively high sensitivity.

However, the conventional fluorescent dyes typically used as contrast agents in imaging techniques are inadequate for optimal performance as they suffer from a poor tissue specificity, low stability (when entering biological media), photobleaching problems and reduced signal penetration. Alternatively, QDs appear to be an

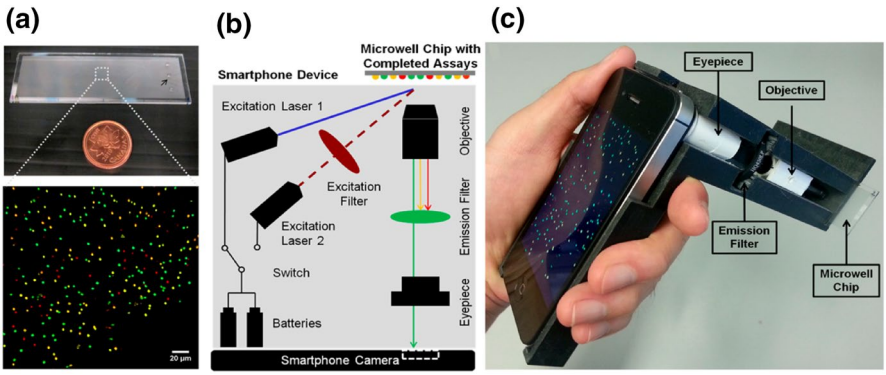

Fig. 15 Overview of the smartphone device utilizing QD barcodes. **a** Photograph of microwell chip containing different barcodes in each well and image of four different QD barcodes arrayed on the surface of the chip captured by a smartphone. **b** Schematic illustration of the detection system performance. **c** Image of the smartphone device. Reprinted from Ming et al. [139], copyright 2015, with permission from the American Chemical Society

exciting class of fluorescent probes for use in photoluminescence imaging due to their tunable optical properties, high stability and ability to be used in guided-targeting based on NP surface functionalization with appropriate recognition elements (e.g. antibodies, aptamers, peptides, etc.). Researchers have explored various methods based on QD functionalization to enhance fluorescence imaging. In this section, we include a brief overview of the emerging applications for bioconjugated QDs in bioimaging for clinical diagnosis.

6.1 Bioconjugated QDs for Biomedical Labeling and Imaging

Researchers are increasingly focused on the luminescence-based imaging of biological specimens for use in biomedical studies and, in particular, in medical diagnostics as developing technological advances point to enhanced biomedical capabilities (e.g. innovative fluorescence-based imaging systems). Typical fluorescent imaging reagents can be endogenous, which often require an enzyme-mediated process inside the organism to stimulate the production of measurable visible light, or exogenous; the latter are currently the more versatile and popular fluorescent imaging agents. NP-based optical contrast agents (including QDs) fall within the group of exogenous reagents that are still dominated by sensitive organic fluorescent probes. However, important advances in the bioconjugation of QDs to selected recognition elements (e.g. antibodies, peptides, genetic material, etc.) constitute a significant trust in the development of novel and improved bioimaging methodologies [140]. The QD–BM bioconjugates thus produced constitute outstanding nanoplatforms for the fluorescence labeling of target BMs. Such fluorescent labels have been successfully employed in fluorescence imaging in studies on the single-molecule dynamics of living cells, monitoring of intracellular protein–protein interactions, disease detection in deeper tissues, detection of tumor cells based on selective binding of the tailored, bioconjugated QDs to known cancer biomarkers, and many more [141].

Pioneer studies on the use of QDs as luminescence tags for imaging exploited the high sensitivity of the luminescence from these NPs to the surface state. Eventual chemical or physical interactions between chemical species present in the media and the surface of the QDs would result in detectable changes in the fluorescence emission. Based on this basic approach, Liu et al. evaluated mercaptoacetic acid (MAA)-capped CdSe/ZnSe/ZnS QDs for the detection of changes in pH within SKOV-3 human ovarian cancer cells [142]. These authors made use of the changes in the intrinsic fluorescence emission of QDs with pH, with more intense fluorescence emission obtained at higher pHs. Therefore, after the QDs were uptaken by endocytosis within the lysosomes in both fixed and living cells, where the pH is rather low, the addition of chloroquine produced an increase on the pH and an enhancement of the photoluminescence intensity of the QDs [142].

However, it must be taken into account that such methods based on the direct interaction of the analytes with the QDs suffer from poor selectivity and so have a rather limited applicability in real-life settings. However, advances in the controlled bioconjugation of QDs to active recognition elements explain the recent emergence of a myriad of applications of QDs for use in target-guided

fluorescence imaging. Selective detection of multiple tumor biomarkers, monitoring of molecular surface dynamics of membrane-associated molecules, measurement of cell motility or quantification of molecular interactions at the cellular and subcellular levels through fluorescent imaging are just some examples of the growing importance of this field [140]. More specifically, the prominent relevance of cancer research in the life sciences has promoted studies on the location and distribution of tumors and tumour cells using fluorescent in vivo imaging. To obtain an insight in the trafficking of cancer cells, such cells are labeled with QDs that have been surface modified with antibodies. As an example, in one study QDs were encapsulated in carboxylated triblock polymeric micelles and conjugated with anti-mesothelin antibodies to allow targeting of the cancerous areas [143]. An in vivo imaging study demonstrated that the QD-loaded nanomicelles, surface modified with the antibodies, targeted the pancreatic tumor site in only 15 min after intravenous injection, thereby illustrating the potential of these structures as promising nanoscale platforms for early human pancreatic cancer detection.

The first challenge when designing imaging contrast agents based on QDs is to successfully deliver the nanoplatform into the cell. Cellular penetration of QDs can be achieved using different approaches based on both active and passive transportation. The major transport pathway of QDs into cells is endocytosis, a process in which eukaryotic cells ingest a part of their plasma membrane to swallow external objects. Passive transduction of QDs into the cell is mostly enabled via electrostatic interaction with the plasma membrane. This is the most likely mechanism in the case of water-stabilized QDs without functionalization with BMs. However, in this latter scenario there is no guarantee of efficient uptake.

It must be mentioned that to date the mechanisms of cellular uptake and cytotoxicity of NPs are still largely understood. Some studies have demonstrated that cellular uptake pathways are strongly dependent on cell type and cell differentiation (e.g. monocytes showed cellular uptake of QDs surface modified with carboxylic acid, while lymphocytes did not) [144]. Other studies have demonstrated that QD coatings highly affect their cellular uptake. As an example, the surface modification of QDs with PEG was found to block non-specific QD delivery into the cells, while QDs coated with carboxyl or amine groups could be internalized quickly and at large amounts by different types of cells [145]. Thus, it is difficult to provide a general statement on the mechanisms for cellular uptake of QDs. The results of a study by Xiao et al. [145] suggest a potential pathway for QD cellular uptake mechanism consisting of three main stages. The first is endocytosis, which may occur through two major mechanisms: phagocytosis and pinocytosis. In the case of small QDs it is envisaged that endocytosis happens via micropinocytosis (a subcategory of pinocytosis that is preferred for the uptake of smaller particles through the formation of endocytic vesicles of different sizes). The second stage is sequestering in early endosomes, and the third stage is translocation to later endosomes or lysosomes. Xiao et al. found that endocytosis was probably assisted by receptors specific to ligands with negative charges [145]. All of these findings are of great relevance to improve the specific targeting of QDs in bioanalytical and medical applications as they are related to reducing non-specific targeting.

Conversely, QDs active transportation is characterized by ligand–receptor-mediated transportation using ligands, such as peptides, proteins or antibodies. In addition, QD surface engineering is critical to minimize undesired nonspecific binding adsorptions of QD probes in biological media. Surface functionalization of QDs with uncharged hydrophilic moieties (e.g. PEG) or with zwitterion molecules produces highly water-stable nanoprobes while efficiently eliminating nonspecific binding (typically brought about by hydrophobic and/or pure electrostatic interactions).

The use of antibody–QD conjugates for guided-target labeling is probably the most commonly used strategy in molecular imaging, although targeting can also be achieved via bioconjugation of the QDs with peptides, DNA, modified proteins, etc. Actually, a single QD (depending on its size) can be simultaneously conjugated with several proteins and peptides [146]. It is well known that peptides are efficient carriers of QDs inside living cells. Therefore, peptide-modified QDs conjugates have been widely used to target cellular BMs, including growth factor receptors, G protein-coupled receptors, integrins and even ion channels [147].

Moreover, small BMs can be also bioconjugated to QDs for in vitro imaging. This is the case of aptamers, which are nucleic acid species that have been engineered to bind to various molecular targets, such as small molecules, proteins, nucleic acids and even cells, tissues and organisms [148]. As an illustration, in one study ZnS/CdSe fluorescent QDs were surface modified with PEG (for aqueous stabilization and biocompatibility) and streptavidine (to further bioconjugate the NP to an appropriate BM receptor) [149]. The streptavidine–PEG–QD was then labeled via streptavidine–biotin with a biotinylated aptamer that can specially bind the epidermal growth factor receptor varient III (EGFRvIII), which is specially distributed on the surface of glioma cells. This labeled aptamer (QD-Apt) nanoprobe was then employed in a fluorescence-guided surgery to allow safe resection of glioma [149]. In this study, the biodistribution of the QD-Apt nanoprobe and the capabilities of targeted imaging of glioma in situ using orthotopic glioma models were evaluated. Different studies were carried out, including an evaluation of the capability of the QD-Apt to image U87-EGFRvIII (a human primary glioblastoma cell line [U87] that overexpresses EGFRvIII) tumor areas. At 4 weeks of tumor development, a whole-body fluorescence imaging examination was performed in different mice after QDs (not bioconjugated) or QD-Apt were injected via the tail vein for 6 h. As can be seen in Fig. 16, the image of the mice harboring the tumor which were administered the QD-Apt (group 1) had strong fluorescence signals in tumor areas, while the group administered QDs (group 2) had no obvious fluorescence signals in the tumor region. In addition, the mice having U87 tumors (groups 3 and 4) had no significant fluorescence in tumor areas, regardless of whether they were administered QD-Apt or QDs. In brief, the developed nanoprobe (QD-Apt) hasa great potential as a novel fluorescence contrast agent for the molecular diagnosis, image-guided surgery, and postoperative examination of gliomas.

Clearly, engineering more compact nanoprobes for fluorescence imaging is currently a research area of great practical interest. A key aspect in this reasearch area is to try to enhance the final uptake of the QD-based nanoprobes in the cellular media. To achieve this goal, much effort is directed towards avoiding (or reducing) eventual QD aggregation or deposition in endosomes or lysosomes. In addition, studies to

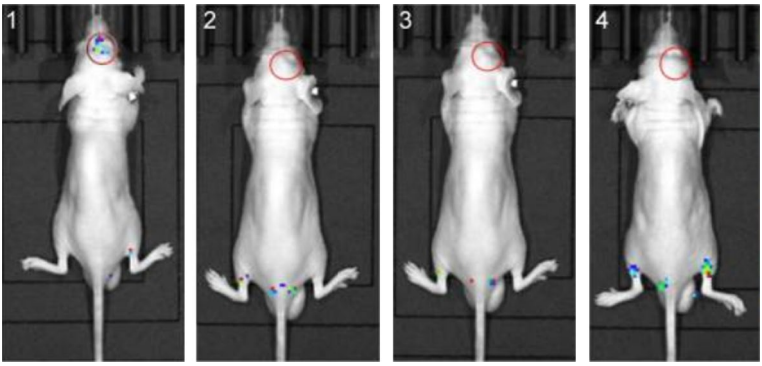

Fig. 16 Effects of a QD-Apt on tumor imaging and body distribution in vivo. Fluorescence images of the mice after injection of QDs or QD-Apt via the tail vein for 6 h. The red circles indicate the tumor regions. The mice of groups 1 and 2 had U87-EGFRvIII (a human primary glioblastoma cell line [U87] line that overexpressed epidermal growth factor receptor varient III [EGFRvIII]) tumors, while the mice of group 3 and group 4 had U87 tumors. In addition, the mice of groups 1 and 3 were administered QD-Apt, while the mice of groups 2 and 4 were administered QDs. Scale bar: 100 μm. Magnification ×200. Reprinted from Tang et al. [149], copyright 2017, with permission from Dove Medical Press Ltd

achieve an efficient QD surface engineering are critical for minimizing undesired nonspecific binding problems of QD nanoprobes when they enter the biological media (a problem that limits the applicability of these nanostructures).

6.2 NIR Optical Imaging

Fluorescent QDs with emission within the visible light spectral range are mostly limited to in vitro bioimaging applications because tissues have strong absorbance in the visible light region. Additionally, endogenous autofluorescence in the visible light spectrum from biological components present in living tissues may significantly interfere with signals from QD-labeled BMs. An alternative approach to overcoming such limitations which has recently attracted high interest is the preparation of nanoprobes made of QDs with emission in the NIR spectral region. NIR light is able to pass more efficiently through biological tissues, suffering from much lower absorption and minimum biological autofluorescence, making QDs with emission in the NIR range highly attractive in terms of high imaging resolution even when the aim is to label deeper tissues in vivo [150]. As a result, NIR QDs are more attractive than visible light-emitting QDs for in vivo mapping and imaging applications as the use of NIR emission substantially increases contrast, sensitivity and penetration depth while avoiding optical damage to the body [151].

As an example, Bawendi and colleagues bioconjugated NIR InAs(ZnCdS) QDs with a polymeric imidazole ligand via a ligand exchange strategy, obtaining nanoprobes with bright and stable emission in the NIR (700–900 nm) region [152]. These authors then evaluated the potential of such nanoprobes to image tumor vasculature in vivo. As can be seen in Fig. 17, they demonstrated that the NIR-emitting QDs offered superior depth and contrast when compared to green QDs.

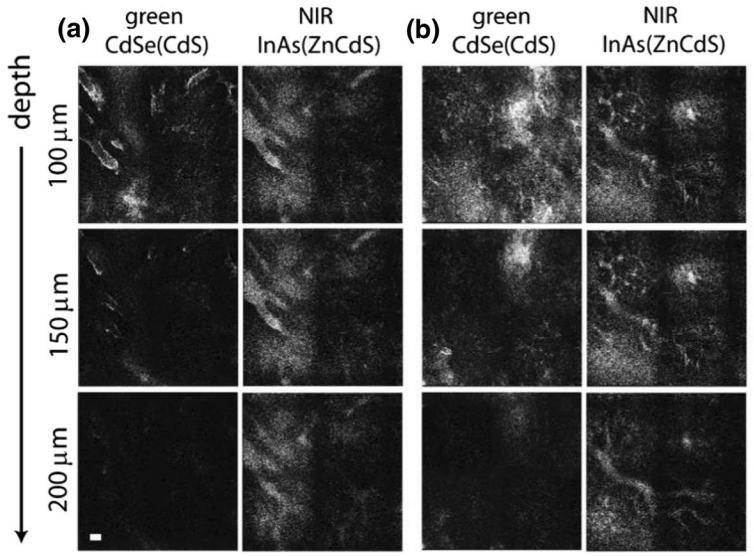

Fig. 17 In vivo grayscale Multiphoton microscopy images with 850 nm excitation (**a**, **b**) of the vasculature in a mammary tumor in a mouse with CdSe(CdS) (green channel) and InAs(ZnCdS) (near-infrared channel [*NIR*]) poly(PEG12)-PIL QDs injected intravenously and imaged simultaneously. Scale bar: 100 µm. Reprinted with permission from Allen et al. [152], copyright 2010, with permission from the American Chemical Society

The NIR QDs clearly imaged the deep vasculature of a mammary cell with an excellent resolution up to 200 µm, while the visible light-emitting QDs produced an image with much lower contrast and emission intensity [152].

Two of the most robust NIR-emitting QDs are the ternary $CuInS_2$ and $CuInSe_2$ core–shell systems. Quite recently, $CuInSe_2$/ZnS QDs with a NIR emission (709 nm) were synthesized and surface conjugated with the tumor targeting peptide Cys-Gly-Lys-Arg-Lys (CGKRK) through a PEG linker [153]. The authors observed that QDs functionalized with the peptide increased NP uptake by the tumor by more than twofold as compared to QDs functionalized with PEG alone. The bioconjugated QDs allowed real-time imaging of the tumor homing process in live mice for a long period of time, and the functionalized QDs showed high photostability and were cleared fairly slowly (half-life of approx. 7 h).

Imaging experiments carried out in vivo demonstrated an improvement in the overall signal-to-noise ratio of > 100-fold when NIR QDs emitting at 1320 nm (NIR-II region) were employed as contrast agents rather than QDs emitting at 850 nm (NIR-I region), with reduced autofluorescence and a superior tissue penetration observed at the longer wavelengths. Thus, more recently there have been some efforts to try to develop QDs that emit in the NIR-II window, based on NP cores of different metallic elements, such as PbSe, PbS or hybrid CdHgTe. More recently, NIR-II QDs have also been synthesized that avoid the use of toxic elements: a mirage of reports propose Ag_2S QDs as NIR-II emitting agents [154].

To date, there have been only a few reports on successful specific targeting employing Ag_2S QDs [23]. In all such reportes, Ag_2S NPs were surface functionalized with appropriate recognition elements for in vivo imaging following different bioconjugation approaches. In a very recent study, Ag_2S NPs were surface functionalized with plerixafor, a small molecule drug used for the inhibition of CXC chemokine receptor 4 (CXCR4). The bioconjugate was used for in vivo imaging of metastatic breast cancer cells based on the selective linkage of the functionalized Ag_2S NPs to highly metastatic breast cancer cells (4T1 tumor model) via their CXCR4 receptor [155]. Moreover, the use of the surface-decorated Ag_2S NPs together with their photothermal properties resulted in a unique and tumor-specific theranostic element.

6.3 Multimodal Imaging

There is currently an increasing interest among researchers on the development of new nanomaterials for multimodal imaging applications in biology and medicine. In this context, multimodal fluorescent-magnetic based nanomaterials deserve particular attention as they can be used both as diagnostic and drug delivery tools, which could facilitate the diagnosis and treatment of many diseases.

The frequently investigated QD-based hybrid-NPs with multiple capabilities are probably the magnetic-QDs. As an example, differently sized infrared-emitting QDs have been incorporated, together with variable amounts of Fe-based magnetic NPs, into poly(styrene/acrylamide) copolymer nanospheres for the preparation of fluorescent-magnetic nanocomposites [156]. The dual-encoded nanobioprobes developed as such exhibited different luminescent behavior (due to the different sizes of the NPs) and magnetic susceptibility. They were proven to be capable of simultaneously recognizing and separating multiple biocomponents from complex samples when three kinds of lectins were used as the targets.

Multimodal imaging can integrate structural/functional information from several imaging modalities, thus promising more accurate diagnosis than any single imaging modality. One important advantageous feature of liposome encapsulation is the possibility to co-immobilize several NPs exhibiting different properties to develop multimodal imaging platforms. In a very recent article, Xu et al. reported the integration of a theranostic liposome (QSC-Lip) with superparamagnetic iron oxide NPs (SPIONs) and QDs and cilengitide (CGT) into one platform, with the aim to target glioma in magnetic targeting (MT) for guiding the surgical resection of glioma [157]. In vivo dual-imaging studies show that QSC-Lip not only produces an obvious negative-contrast enhancement effect on glioma by MRI but also makes tumor emitting fluorescence under MT.

Among the different techniques widely used for molecular imaging, MRI is currently one of the main in vivo imaging techniques used routinely in diagnosis, while fluorescence imaging is nowadays most widely used for in vitro studies; thus these two imaging techniques are complementary. Clearly, there is much research interest directed towards preparing fluorescent imaging/MRI imaging dual-modality nanoprobes to be used in many diagnostic and biomedical applications, such

as cell labeling, enzyme activity measurements, tumor diagnosis and therapy and anatomical localization and real-time assessment during surgery [158]. Nanostructures based on fluorescent QDs can be synthesized to provide magnetic properties to the nanomaterial, thereby creating opportunities for multi-modality biomedical imaging. Fluorescent QDs exhibiting magnetic susceptibility can be synthesized following four different methodologies: metal doping, covalent conjugation, isocrystal growth and co-encapsulation or electrostatic assembly. In particular, a large number of fluorescent imaging/MRI dual-modality imaging nanoprobes combine Gd^{3+} or Mn^{2+} ions with QDs. As an example, a dual contrast nanoreagent was developed by doping Gd ions into $CuInS_2$/ZnS QDs (Fig. 18) [159]. The resulting NPs exhibited NIR fluorescence emission and MRI contrast capabilities with a high longitudinal relaxivity (r1), which was 2.5-fold higher than that of clinically approved Gd agents. In addition, the in vivo imaging experiments showed that the Gd-doped NPs could enhance both NIR fluorescence and T1-weighted MRI of tumor tissue through passive targeting accumulation.

7 Conclusions and Perspectives

In general, photoluminescent QD probes are widespread and used in countless biomedical applications. For biosensing, a great potential of QD-conjugates also lies in multiplexing as well as in vitro and in vivo fluorescent imaging. There has been major progress in the development of in vivo drug delivery systems, and interest remains high in this area. It is important to note that all of these applications are possible because of the advances in QD stabilization in biological media by appropriate surface functionalization and their bioconjugation to suitable BMs.

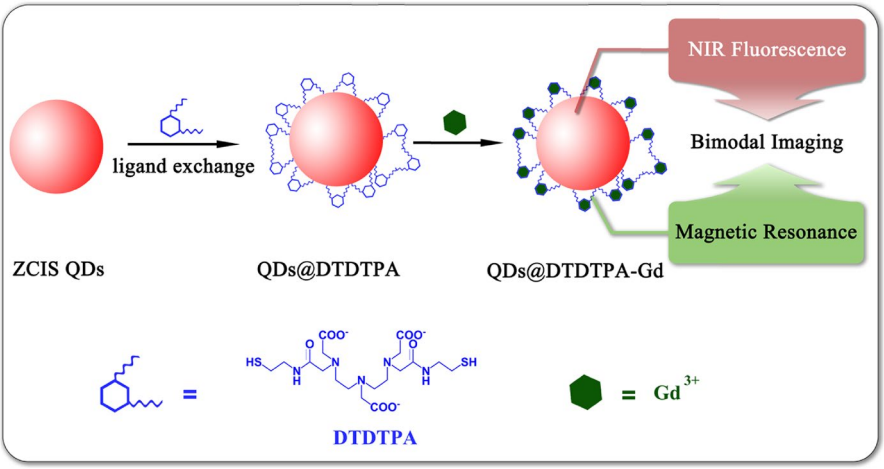

Fig. 18 Fabrication procedure and functional description of the Gd-doped QDs with dual-mode imaging capabilities. Reprinted from Yang et al. [159], copyright 2017, with permission from the American Chemical Society

All of the recent advances in bioconjugation chemistry has made it possible to attach almost any BM of interest to the surface of a QD. However, work is till needed to further enhance the development and applications of QD bioconjugates. In this context, we have identified the following issues.

The orientation of the BMs is a key issue that still needs to be addressed. Many often random orientations are sufficient when the QDs are used in conventional hybridization applications. However, controlled orientations may be needed for the assembly of functionalized 3D structures.

The functionalization of QDs with different BMs (e.g. antibodies, peptides, nucleic acids or aptamers) offer a wide range of opportunities for applying the nanoassemblies in clinical diagnosis, including ultrasensitive detection of disease biomarkers, in vivo targeted imaging or drug delivery applications. The multiplexing capabilities of these QDs open new avenues for the use of differently sized QDs for the simultaneous detection of multiple biomarkers (a key aspect in efficient clinical diagnosis). Moreover, encapsulation of multiple QDs in high-volume nanocarriers (e.g. nanosomes or PLGA NPs) may enable the construction of a panel of multifunctional systems for targeted drug delivery and molecular imaging.

The development of new multimodal imaging nanoprobes is a focus of many researchers. The use of NPs as imaging probes offers several advantages over conventional molecular-scale contrast agents; these include high loading capacity, where the concentration of the imaging agents can be controlled within each NP during the synthesis process; tunable surface that can potentially extend the circulation time of the contrast agents in the blood or target them to specific locations in the body; or provision of multimodal imaging capacities because NPs can combine two or more contrast properties, which can be used in multiple imaging techniques simultaneously [160]. Recent advances in nanotechnology has enabled the development of multifunctional QDs by doping the nanocrystals with appropriate metals, thus integrating two or more imaging contrast agents and thereby enabling their detection by different imaging techniques [42].

One of the major challenges when developing novel bioassay methods for clinical applications is the requirement for high sensitivity in the detection because of the ultralow concentrations of the biomarkers to be detected. Here, the use of QDs as tags in immunoassays could be a powerful approach to achieve the desired ultrasensitivity. As an example, ultrahigh sensitivity for BMs could be easily achieved through metal deposition on the surface of the NP tags acting as catalytic seeds, thus effectively amplifying the size of the metallic NPs after the immunoassay [161].

Obviously, QDs cannot be safely used as tags for in vivo applications until the problem of their toxicity is solved. Despite very extensive studies of toxicity of QDs in different cellular and animal models, the in vivo toxicological effect of QDs remains controversial [37]. The possible release of toxic heavy metals from the core of the QDs as a result of intensive UV illumination has to be taken into account [44]. The preparation of heavy metal-free QDs is being addressed as a promising avenue to overcome such toxicity problems. Additionally, it must be considered that an ideal solubilization strategy should reduce QD toxicity and undesirable nonspecific QD uptake by living tissues, thus reducing cytotoxic effects.

Paper-based microfluidic systems have been revealed as the most suitable platform for POC analysis, with the use of QDs as labels becoming increasingly popular in the development of this type of systems. Although fluorescence QD-based POC systems using hand-held readers or even smartphone-based detectors have been successfully reported, they suffer from an important limitation related to the need for bulky and complex detectors for quantification. However, it is expected that the use of alternative detection methods (e.g. electrochemical) and the rapid development of portable devices and mobile phone technology will allow the miniaturization of the detection systems for POC devices in the near future. Miniaturized signal-recording devices also require a merging of QD barcode technology and POC testing. In the sensing field, there is also a great expectation for recently developed GQDs and CQDs.

To summarize, even though there is still a long road to go before bioconjugated QDs are considered to be routine in in vitro and especially in vivo diagnosis, overall we firmly believe that the rapid development of new bioconjugated nanomaterials will move bioconjugated QDs forward to real-life diagnostic applications in modern biology and medicine.

Acknowledgements Financial support from the FC-GRUPIN-ID/2018/000166 project (Asturias Regional Government, Spain) and the CTQ2017–86994-R and CTQ2016–79412-P projects (MINECO, Spain) is gratefully acknowledged. A. de la Escosura-Muñiz acknowledges the MICINN (Spain) for the "Ramón y Cajal" Research Fellow (RyC-2016-20299).

References

1. Prado M, Espiña B, Fernández-Argüelles MT, Diéguez L, Fuciños P, Vial S, Oliveira JM, Reis RL, Boehme K (2016) Detection of foodborne pathogens using nanoparticles. Advantages and trends. In: Barros-Velázquez J (ed) Antimicrobial food packaging. Elsevier, Amsterdam, pp 183–201
2. Trapiella-Alfonso L, Llano-Suárez P, Sanz-Medel A, Costa-Fernández JM, Fernández-Argüelles MT (2017) Analytical nanoscience and nanotechnology. In: Meyers RA (ed) Encyclopedia of analytical chemistry. Wiley, Hoboken, pp 1–24
3. Lopez-Lorente A, Valcarcel M (2016) The third way in analytical nanoscience and nanotechnology: involvement of nanotools and nanoanalytes in the same analytical process. Trends Anal Chem 75:1–9
4. Blanco-López MC, Rivas M (2019) Nanoparticles for bioanalysis. Anal Bioanal Chem 411:1789–1790
5. Ahsan MA, Jabbari V, Imam MA, Castro E, Kim H, Curry ML, Valles-Rosales DJ, Noveron JC (2020) Nanoscale nickel metal organic framework decorated over graphene oxide and carbon nanotubes for water remediation. Sci Total Environ 698:134214
6. Cova CM, Zuliani A, Santiago ARP, Caballero A, Muñoz-Batista MJ, Luque R (2018) Microwave-assisted preparation of Ag/Ag_2S carbon hybrid structures from pig bristles as efficient HER catalysts. J Mater Chem A 6:21516–21523
7. Ahsan MA, Deemer E, Fernandez-Delgado O, Wang H, Curry ML, El-Gendy AA, Noveron JC (2019) Fe nanoparticles encapsulated in MOF-derived carbon for the reduction of 4-nitrophenol and methyl orange in water. Catal Commun 130:105753
8. Ahsan MA, Jabbari V, El-Gendy AA, Curry ML, Noveron JC (2019) Ultrafast catalytic reduction of environmental pollutants in water via MOF-derived magnetic Ni and Cu nanoparticles encapsulated in porous carbon. App Surf Sci 497:143608
9. Ahsan MA, Fernandez-Delgado O, Deemer E, Wang H, El-Gendy AA, Curry ML, Noveron JC (2019) Carbonization of Co-BDC MOF results in magnetic C@Co nanoparticles that catalyze the reduction of methyl orange and 4-nitrophenol in water. J Mol Liq 290:111059

10. Sanad MF, Shalan AE, Bazid SM, Serea ESA, Hashem EM, Nabiha S, Ahsan MA (2019) A graphene gold nanocomposite-based 5-FU drug and the enhancement of the MCF-7 cell line treatment. RSC Advances 9:31021–31029
11. Dominguez N, Torres B, Barrera LA, Rincon JE, Lin Y, Chianelli RR, Ahsan MA, Noveron JC (2018) Bimetallic CoMoS composite anchored to biocarbon fibers as a high-capacity anode for Li-ion batteries. ACS Omega 3:10243–10249
12. Piccinno F, Gottschalk F, Seeger S, Nowack B (2012) Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J Nanopart Res 14:1109
13. Snee PT (2020) Semiconductor quantum dot FRET: untangling energy transfer mechanisms in bioanalytical assays. Trends Anal Chem 123:115750
14. Llano Suarez P, García-Cortés M, Fernández-Argüelles MT, Ruiz Encinar J, Valledor M, Ferrero FJ, Campo JC, Costa-Fernandez JM (2019) Functionalized phosphorescent nanoparticles in (bio) chemical sensing and imaging—a review. Anal Chim Acta 1046:16–31
15. Granada-Ramírez DA, Arias-Cerón JS, Rodriguez-Fragoso P, Vázquez-Hernández F, Luna-Arias JP, Herrera-Perez JL, Mendoza-Álvarez JG (2017) Quantum dots for biomedical applications. In: Nanobiomaterials: nanostructured materials for biomedical applications. Unidad Profesional Interdisciplinaria de Ingeniería y Tecnologías Avanzadas (UPIITA), pp 411–436. https://doi.org/10.1016/B978-0-08-100716-7.00016-7
16. Coto-García AM, Sotelo-González E, Fernández-Argüelles MT, Pereiro R, Costa-Fernández JM, Sanz-Medel A (2011) Nanoparticles as fluorescent labels for optical imaging and sensing in genomics and proteomics. Anal Bioanal Chem 399:29–42
17. Geißler D, Charbonnière LJ, Ziessel RF, Butlin NG, Löhmannsröben H-G, Hildebrandt N (2010) Quantum dot biosensors for ultrasensitive multiplexed diagnostics. Angew Chem 49:1396–1401
18. Hildebrandt N (2011) Biofunctional quantum dots: controlled conjugation for multiplexed biosensors. ACS Nano 5:5286–5290
19. Chan WC, Nie S (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281:2016–2018
20. Sukhanova A, Devy J, Venteo L, Kaplan H, Artemyev M, Oleinikov V, Klinov D, Pluot M, Cohen JH, Nabiev I (2004) Biocompatible fluorescent nanocrystals for immunolabeling of membrane proteins and cells. Anal Biochem 324:60–67
21. Gill R, Zayats M, Willner I (2008) Semiconductor quantum dots for bioanalysis. Angew Chem Int Ed 47:7602–7625
22. Bear J, Charron G, Fernandez-Arguelles MT, Massadeh S, McNaughter P, Nann T (2011) In vivo applications of inorganic nanoparticles. In: Booss-Bavnbek B, Klosgen B, Larsen J, Pociot F, Renstrom E (eds) BetaSys. Systems biology, vol 2. Springer, New York, pp 185–220
23. Lu C, Chen G, Yu B, Cong H (2018) Recent advances of low biological toxicity Ag_2S QDs for biomedical application. Adv Eng Mater 20:1700940
24. Rabani E (2001) Structure and electrostatic properties of passivated CdSe nanocrystals. J Chem Phys 115:1493
25. Jin Z, Hildebrandt N (2012) Semiconductor quantum dots for in vitro diagnostics and cellular imaging. Trends Biotechnol 30:394–403
26. Alivisatos AP (1996) Perspectives on the physical chemistry of semiconductor nanocrystals. J Phys Chem 100:13226–13239
27. Coto-Garcia AM, Fernandez-Arguelles MT, Costa-Fernandez JM, Sanz-Medel A, Valledor M, Campo JC, Ferrero FJ (2013) The influence of surface coating on the properties of water-soluble CdSe and CdSe/ZnS quantum dots. J Nanopart Res 15:1330
28. Fernandez-Arguelles MT, Costa-Fernandez JM, Pereiro R, Sanz-Medel A (2010) Organically modified quantum dots in chemical and biochemical analysis. In: Martinez-Manez R, Rurack K (eds) The supramolecular chemistry of organic–inorganic hybrid materials. Wiley, Hoboken, pp 377–403
29. Wagner MK, Li F, Li J, Li X, Le XC (2010) Use of quantum dots in the development of assays for cancer biomarkers. Anal Bioanal Chem 397:3213–3224
30. Garcia-Cortes M, Sotelo E, Fernandez-Arguelles MT, Encinar JR, Costa-Fernandez JM, Sanz-Medel A (2017) Capping of Mn-doped ZnS quantum dots with DHLA for their stabilization in aqueous media: determination of the nanoparticle number concentration and surface ligand density. Langmuir 33:6333–6341
31. Wu P, Yan XP (2013) Doped quantum dots for chemo/biosensing and bioimaging. Chem Soc Rev 42:5489–5521

32. Panda SK, Hickey SG, Demir HV, Eychmüller A (2011) Bright white-light emitting manganese and copper co-doped ZnSe quantum dots. Angew Chem Int Ed 50:4432–4436
33. Ehlert O, Osvet A, Batentschuk M, Winnacker A, Nann T (2006) Synthesis and spectroscopic investigations of Cu- and Pb-doped colloidal ZnS nanocrystals. J Phys Chem B 110:23175–23178
34. Sotelo-Gonzalez E, Fernandez-Arguelles MT, Costa-Fernandez JM, Sanz-Medel A (2012) Mn-doped ZnS quantum dots for the determination of acetone by phosphorescence attenuation. Anal Chim Acta 712:120–126
35. Mohapatra S, Rout SR, Das RK, Nayak S, Ghosh SK (2016) Highly hydrophilic luminescent magnetic mesoporous carbon nanospheres for controlled release of anticancer drug and multimodal imaging. Langmuir 32:1611–1620
36. Wang J, Zhang Z, Zha S, Zhu Y, Wu P, Ehrenberg B, Chen JY (2014) Carbon nanodots featuring efficient FRET for two-photon photodynamic cancer therapy with a low fs laser power density. Biomaterials 35:9372–9381
37. Chong Y, Ma Y, Shen H, Tu X, Zhou X, Xu J, Dai J, Fan S, Zhang Z (2014) The in vitro and in vivo toxicity of graphene quantum dots. Biomaterials 35:5041–5048
38. Wang J, Zhang P, Huang C, Liu G, Leung KCF, Wáng YXJ (2015) High performance photoluminescent carbon dots for in vitro and in vivo bioimaging: effect of nitrogen doping ratios. Langmuir 31:8063–8073
39. Vasimalai N, Vilas-Boas V, Gallo J, Cerqueira MF, Menéndez-Miranda M, Costa-Fernández JM, Diéguez L, Espiña B, Fernández-Argüelles MT (2018) Green synthesis of fluorescent carbon dots from spices for in vitro imaging and tumour cell growth inhibition. Beilstein J Nanotechnol 9:530–544
40. Righetto M, Privitera A, Fortunati I, Mosconi D, Zerbetto M, Curri ML, Corricelli M, Moretto A, Agnoli S, Franco L, Bozio R, Ferrante C (2017) Spectroscopic insights into carbon dot systems. J Phys Chem Lett 8:2236–2242
41. Dong Y, Wang R, Li G, Chen C, Chi Y, Chen G (2012) Polyamine-functionalized carbon quantum dots as fluorescent probes for selective and sensitive detection of copper ions. Anal Chem 84:6220–6224
42. Bouzas-Ramos D, Cigales-Canga J, Mayo JC, Sainz RM, Ruiz Encinar J, Costa-Fernandez JM (2019) Carbon quantum dots codoped with nitrogen and lanthanides for multimodal imaging. Adv Funct Mater 29:1903884
43. Manna L, Scher EC, Li LS, Alivisatos AP (2002) Epitaxial growth and photochemical annealing of graded CdS/ZnS shells on colloidal CdSe nanorods. J Am Chem Soc 124:7136–7145
44. Coto-Garcia AM, Valledor M, Campo JC, Ferrero FJ, Fernandez-Argüelles MT, Costa-Fernandez JM, Sanz-Medel A (2011) Dynamic analysis of the photoenhancement process of colloidal quantum dots with different surface modifications. Nanotechnology 22:9
45. Wu ZY, Zhao YL, Qiu FP, Li YP, Wang SW, Yang BH, Chen L, Sun JH, Wang JY (2009) Forming water-soluble CdSe/ZnS QDs using amphiphilic polymers, stearyl methacrylate/methylacrylate copolymers with different hydrophobic moiety ratios and their optical properties and stability. Colloid Surface A 350:121–129
46. Karakoti AS, Shukla R, Shanker R, Singh S (2015) Surface functionalization of quantum dots for biological applications. Adv Colloid Interface Sci 215:28–45
47. Aubert T, Soenen SJ, Wassmuth D, Cirillo M, Van Deun R, Braeckmans K, Hens Z (2014) Bright and stable CdSe/CdS@SiO(2) nanoparticles suitable for long-term cell labelling. ACS Appl Mater Interfaces 6:11714–11723
48. Palui G, Aldeek F, Wang WT, Mattoussi H (2015) Strategies for interfacing inorganic nanocrystals with biological systems based on polymer-coating. Chem Soc Rev 44:193–227
49. Fernandez-Arguelles MT, Costa-Fernandez JM, Pereiro R, Sanz-Medel A (2008) Simple bio-conjugation of polymer-coated quantum dots with antibodies for fluorescence-based immunoassays. Analyst 133:444–447
50. Potapova I, Mruk R, Prehl S, Zentel R, Basche T, Mews A (2003) Semiconductor nanocrystals with multifunctional polymer ligands. J Am Chem Soc 125:320–321
51. Mattoussi H, Mauro JM, Goldman ER, Green TM, Anderson GP, Sundar VC, Bawendi MG (2001) Bioconjugation of highly luminescent colloidal CdSe-ZnS quantum dots with an engineered two-domain recombinant protein. Phys Status Solidi B 224:277–283
52. Du Y, Zhong Y, Donga J, Qiana C, Sunc S, Gaod L, Yang D (2019) The effect of PEG functionalization on the in vivo behavior and toxicity of CdTe quantum dots. RSC Advances 9:12218–12225

53. Beloglazova NV, Shmelin PS, Speranskaya ES, Lucas B, Helmbrecht C, Knopp D, Niessner R, De Saeger S, Goryacheva IY (2013) Quantum dot loaded liposomes as fluorescent labels for immunoassay. Anal Chem 85:7197–7204
54. Zavari-Nematabad A, Alizadeh-Ghodsi M, Hamishehkar H, Alipour E, Pilehvar-Soltanahmadi Y, Zarghami N (2017) Development of quantum-dot-encapsulated liposome-based optical nanobiosensor for detection of telomerase activity without target amplification. Anal Bioanal Chem 409:1301–1310
55. Kim MW, Jeong HY, Kang SJ, Choi MJ, You YM, Im CS, Lee TS, Song IH, Lee CG, Rhee KJ, Lee YK, Park YS (2017) Cancer-targeted nucleic acid delivery and quantum dot imaging using EGF receptor aptamer-conjugated lipid nanoparticles. Sci Rep 7: 9474. https://doi.org/10.1038/s41598-017-09555-w
56. Sapsford KE, Algar WR, Berti L, Gemmill KB, Casey BJ, Oh E, Stewart MH, Medintz IL (2013) Functionalizing nanoparticles with biological molecules: developing chemistries that facilitate nanotechnology. Chem Rev 113:1904–2074
57. Boles MA, Ling D, Hyeon T, Talapin DV (2016) The surface science of nanocrystals. Nat Mater 15:141–153
58. Howes PD, Chandrawati R, Stevens MM (2014) Colloidal nanoparticles as advanced biological sensors. Science 346:1247390
59. Hildebrandt N, Spillmann CM, Algar WR, Pons T, Stewart MH, Oh E, Susumu K, Díaz SA, Delehanty JB, Medintz IL (2017) Energy transfer with semiconductor quantum dot bioconjugates: a versatile platform for biosensing, energy harvesting, and other developing applications. Chem Rev 117:536–711
60. Xia X-R, Monteiro-Riviere NA, Riviere JE (2010) An index for characterization of nanomaterials in biological systems. Nat Nanotechnol 5:671–675
61. Zhang F, Lees E, Amin F, Rivera-Gil P, Yang F (2011) Polymer-coated nanoparticles: a universal tool for biolabelling experiments. Small 7:3113–3127
62. Foubert A, Beloglazova NV, Rajkovic A, Sas B, Madder A, Goryacheva IY, De Saeger S (2016) Bioconjugation of quantum dots: review and impact on future application". Trends Anal Chem 83:31–48
63. Karakoti AS, Shukla R, Shanker R, Singh S (2015) Surface functionalization of quantum dots for biological applications. Adv Colloid Interface 215:28–45
64. Pedrero M, Campuzano S, Pingarrón JM (2017) Electrochemical (bio)sensing of clinical markers using quantum dots. Electroanalysis 29:24–37
65. Liu W, Li Ch, Ren Y, Sun X, Pan W, Li Y, Wang J, Wang W (2016) Carbon dots: surface engineering and applications. J Mater Chem B 4:5772–5788
66. Zhou D, Lin M, Chen Z, Sun H, Zhang H, Sun H, Yang B (2011) Simple synthesis of highly luminescent water-soluble CdTe quantum dots with controllable surface functionality. Chem Mater 23:4857–4862
67. Heuer-Jungemann A, Feliu N, Bakaimi I, Hamaly M, Alkilany A, Chakraborty I, Masood A, Casula MF, Kostopoulou A, Oh E, Susumu K, Stewart MH, Medintz IL, Stratakis E, Parak WJ, Kanaras AG (2019) The role of ligands in the chemical synthesis and applications of inorganic nanoparticles. Chem Rev 119:4819–4880
68. Sperling RA, Parak WJ (2010) Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. Philos Trans R Soc A 368:1333–1383
69. Susumu K, Uyeda HT, Medintz IL, Pons T, Delehanty JB, Mattoussi H (2007) Enhancing the stability and biological functionalities of quantum dots via compact multifunctional ligands. J Am Chem Soc 129:13987–13996
70. Zhou J, Liu Y, Tang J, Tang W (2017) Surface ligands engineering of semiconductor quantum dots for chemosensory and biological applications. Mater Today 20:360–376
71. Erathodiyil N, Ying JY (2011) Functionalization of inorganic nanoparticles for bioimaging applications. Acc Chem Res 44:925–935
72. Yu WW, Chang E, Falkner JC, Zhang J, Al-Somali AM, Sayes CM, Johns J, Drezek R, Colvin VL (2007) Forming biocompatible and nonaggregated nanocrystals in water using amphiphilic polymers. J Am Chem Soc 129:2871–2879
73. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Peale F, Bruchez MP (2003) Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol 21:41–46

74. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A (2002) In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science 298:1759–1762
75. Park J, Lee J, Kwag J, Baek Y, Kim B, Yoon CJ, Bok S, Cho S-H, Kim KH, Ahn GO, Kim S (2015) Quantum dots in an amphiphilic polyethyleneimine derivative platform for cellular labeling, targeting, gene delivery, and ratiometric oxygen sensing. ACS Nano 9:6511–6521
76. Zhou J, Yang Y, Zhang C-Y (2015) Toward biocompatible semiconductor quantum dots: from biosynthesis and bioconjugation to biomedical application. Chem Rev 115:11669–11717
77. Qu W, Zuo W, Li N, Hou Y, Song Z, Gou G, Yang J (2017) Design of multifunctional liposome-quantum dot hybrid nanocarriers and their biomedical application. J Drug Target 25:661–672
78. Green NM (1963) The use of [^{14}C]biotin for kinetic studies and for assay. Biochem J 89:585–591
79. Biju V (2014) Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy. Chem Soc Rev 43:737–962
80. Russ Algar W (2017) A brief introduction to traditional bioconjugate chemistry, Chap. 1. In: Algar WR, Dawson PE, Medintz IL (eds) Chemoselective and bioorthogonal ligation reactions: concepts and applications, vol 1. Wiley-VCH Verlag GmbH. & Co. KGaA. In the other chapters of volume 1 the reactions mentioned in chapter 1 are dealt in detail
81. Bioconjugation technical handbook. Reagents for crosslinking, immobilization, modification, biotinylation, and fluorescent labeling of proteins and peptides. Thermo Fisher Scientific. https://www.thermofisher.com/es/es/home/global/forms/life-science/bioconjugation-technical-handbook-download.html. Accessed 11 Mar 2020.
82. Wagner AM, Knipe JM, Orive G, Peppas NA (2019) Quantum dots in biomedical applications. Acta Biomater 94:44–63
83. Lišková M, Voráčová I, Klepárník K, Hezinová V, Přikryl J, Foret F (2011) Conjugation reactions in the preparations of quantum dot-based immunoluminescent probes for analysis of proteins by capillary electrophoresis. Anal Bioanal Chem 400:369–379
84. Algar WR, Prasuhn DE, Stewart MH, Jennings TL, Blanco-Canosa JB, Dawson PE, Medintz IL (2011) The controlled display of biomolecules on nanoparticles: a challenge suited to bioorthogonal chemistry. Bioconjugate Chem 22:825–858
85. Massey M, Algar WR (2017) Nanoparticle bioconjugates: materials that benefit from chemoselective and bioorthogonal ligation chemistries. In: Algar WR, Dawson PE, Medintz IL (eds) Chemoselective and bioorthogonal ligation reactions: concepts and applications, vol 1. Wiley-VCH Verlag GmbH & Co. KGaA, Wienheim, pp 568–586
86. Row RD, Prescher JA (2018) Constructing new bioorthogonal reagents and reactions. Acc Chem Res 51:1073–1081
87. Wen L, Qiu L, Wu Y, Hu X, Zhang X (2017) Aptamer-modified semiconductor quantum dots for biosensing applications. Sensors 17:1736
88. Zhang C, Ding C, Zhou G, Xue Q, Xian Y (2017) One-step synthesis of DNA functionalized cadmium-free quantum dots and its application in FRET-based protein sensing. Anal Chim Acta 957:63–69
89. Long Y, Zhang LF, Zhang Y, Zhang CY (2012) Single quantum dot based nanosensor for renin assay. Anal Chem 84:8846–8852
90. Sanvicens N, Pascual N, Fernández-Argüelles MT, Adrián J, Costa-Fernández JM, Sánchez-Baeza F, Sanz-Medel A, Marco MP (2011) Quantum dot-based array for sensitive detection of *Escherichia coli*. Anal Bioanal Chem 399:2755–2762
91. Wang Y, Gao D, Zhang P, Gong P, Chen C, Gao G, Cai L (2014) A near infrared fluorescence resonance energy transfer based aptamer biosensor for insulin detection in human plasma. Chem Commun 50:811–813
92. Chen L, Tse WH, Chen Y, McDonald MW, Melling J, Zhang J (2017) Nanostructured biosensor for detecting glucose in tear by applying fluorescence resonance energy transfer quenching mechanism. Biosens Bioelectron 91:393–399
93. Cayuela A, Soriano ML, Carrillo-Carrión C, Valcárcel M (2016) Semiconductor and carbon-based fluorescent nanodots: the need for consistency. Chem Commun 52:1311–1326
94. Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R, Nann T (2008) Quantum dots versus organic dyes as fluorescent labels. Nat Methods 5:763–775
95. Wu S, Liu L, Li G, Jing F, Mao H, Jin Q, Zhai W, Zhang H, Zhao J, Jia C (2016) Multiplexed detection of lung cancer biomarkers based on quantum dots and microbeads. Talanta 156–157:48–54

96. Zhang W, Hubbard A, Brunhoeber P, Wang Y, Tang L (2013) Automated multiplexing quantum dots in situ hybridization assay for simultaneous detection of ERG and PTEN gene status in prostate cancer. J Mol Diagn 15:754–764
97. Han M, Gao X, Su JZ, Nie S (2001) Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. Nat Biotechnol 19:631–635
98. Kim J, Biondi MJ, Feld JJ, Chan WCW (2016) Clinical validation of quantum dot barcode diagnostic technology. ACS Nano 10:4742–4753
99. Zhao WW, Wang J, Zhu YC, Xu JJ, Chen HY (2015) Quantum dots: electrochemiluminescent and photoelectrochemical bioanalysis. Anal Chem 87:9520–9531
100. Campuzano S, Yáñez-Sedeño P, Pingarrón JM (2019) Nanoparticles for nucleic-acid-based biosensing: opportunities, challenges, and prospects. Anal Bioanal Chem 411:1791–1806
101. Zheng Y, Wang X, He S, Gao Z, Di Y, Lu K, Li K, Wang J (2019) Aptamer-DNA concatamer-quantum dots based electrochemical biosensing strategy for green and ultrasensitive detection of tumor cells via mercury free anodic stripping voltammetry. Biosens Bioelectron 126:261–268
102. Li CC, Hu J, Lu M, Zhang CY (2018) Quantum dot-based electrochemical biosensor for stripping voltammetric detection of telomerase at the single-cell level. Biosens Bioelectron 122:51–57
103. Liu Y, Zhu L, Kong J, Yang P, Liu B (2013) A quantum dots-based electrochemical assay towards the sensitive detection of tumor cells. Electrochem Comm 33:59–62
104. Martín-Yerga D, González-García MB, Costa-García A (2014) Electrochemical immunosensor for anti-tissue transglutaminase antibodies based on the in situ detection of quantum dots. Talanta 130:598–602
105. Kong FY, Xu BY, Xu JJ, Chen HY (2013) Simultaneous electrochemical immunoassay using CdS/DNA and PbS/DNA nanochains as labels. Biosens Bioelectron 39:177–182
106. Christodouleas DC, Kaur B, Chorti P (2018) From point-of-care testing to eHealth diagnostic devices (eDiagnostics). ACS Cent Sci 4:1600–1616
107. Tavares AJ, Noor MO, Vannoy CH, Algar WR, Krull UJ (2012) On-chip transduction of nucleic acid hybridization using spatial profiles of immobilized quantum dots and fluorescence resonance energy transfer. Anal Chem 84:312–319
108. Noor MO, Tavares AJ, Krull UJ (2013) On-chip multiplexed solid-phase nucleic acid hybridization assay using spatial profiles of immobilized quantum dots and fluorescence resonance energy transfer. Anal Chim Acta 788:148–157
109. Kim C, Hoffmann G, Searson PC (2017) Integrated magnetic bead–quantum dot immunoassay for malaria detection. ACS Sens 2:766–772
110. Medina-Sánchez M, Miserere S, Morales-Narváez E, Merkoçi A (2014) On-chip magneto-immunoassay for Alzheimer's biomarker electrochemical detection by using quantum dots as labels. Biosens Bioelectron 54:279–284
111. Krejcova L, Nejdl L, Merlos-Rodrigo MA, Zurek M, Matousek M, Hynek D, Zitka O, Kopel P, Adam V, Kizek R (2014) 3D printed chip for electrochemical detection of influenza virus labeled with CdS quantum dots. Biosens Bioelectron 54:421–427
112. Zhou F, Lu M, Wang W, Bian ZP, Zhang JR, Zhu JJ (2010) Electrochemical immunosensor for simultaneous detection of dual cardiac markers based on a poly(dimethylsiloxane)-gold nanoparticles composite microfluidic chip: a proof of principle. Clin Chem 56:1701–1707
113. Gong MM, Sinton D (2017) Turning the page: advancing paper-based microfluidics for broad diagnostic application. Chem Rev 117:8447–8480
114. Roda A, Michelini E, Zangheri M, Di Fusco M, Calabria D, Simoni P (2016) Smartphone-based biosensors: a critical review and perspectives. Trends Anal Chem 79:317–325
115. Bahadır EB, Sezgintürk MK (2016) Lateral flow assays: principles, designs and labels. Trends Anal Chem 82:286–306
116. Martinez AW, Phillips ST, Butte M, Whitesides GM (2007) Patterned paper as a platform for inexpensive, low-volume, portable bioassays. Angew Chem 119:1340–1342
117. Urusov AE, Zherdev AV, Dzantiev BB (2019) Towards lateral flow quantitative assays: detection approaches. Biosensors 9:89
118. Fu LM, Wang YN (2018) Detection methods and applications of microfluidic paper-based analytical devices. Trends Anal Chem 107:196–211
119. Sapountzi EA, Tragoulias SS, Kalogianni DP, Ioannou PC, Christopoulos TK (2015) Lateral flow devices for nucleic acid analysis exploiting quantum dots as reporters. Anal Chim Acta 864:48–54

120. Savin M, Mihailescu C-M, Matei I, Stan D, Moldovan CA, Ion M, Baciu I (2018) A quantum dot-based lateral flow immunoassay for the sensitive detection of human heart fatty acid binding protein (hFABP) in human serum. Talanta 178:910–915
121. Borse V, Srivastava R (2019) Fluorescence lateral flow immunoassay based point-of-care nanodiagnostics for orthopedic implant-associated infection. Sens Actuators B Chem 280:24–33
122. Qina W, Wang K, Xiao K, Hou Y, Lu W, Xu H, Wo Y, Feng S, Cui D (2017) Carcinoembryonic antigen detection with "Handing"-controlled fluorescence spectroscopy using a color matrix for point-of-care applications. Biosens Bioelectron 90:508–515
123. Liu J, Ji D, Meng H, Zhang L, Wang J, Huang Z, Chen J, Li J, Li Z (2018) A portable fluorescence biosensor for rapid and sensitive glutathione detection by using quantum dots-based lateral flow test strip. Sens Actuators B Chem 262:486–492
124. Yan X, Wang K, Lu W, Qin W, Cui D, He J (2016) CdSe/ZnS quantum dot-labeled lateral flow strips for rapid and quantitative detection of gastric cancer carbohydrate antigen 72-4. Nanoscale Res Lett 11:138
125. Deng X, Wang C, Gao Y, Li J, Wen W, Zhang X, Wang S (2018) Applying strand displacement amplification to quantum dots-based fluorescent lateral flow assay strips for HIV-DNA detection. Biosens Bioelectron 105:211–217
126. Shah KG, Singh V, Kauffman P, Abe K, Yager P (2018) Mobile phone ratiometric imaging enables highly sensitive fluorescence lateral flow immunoassays without external optical filters. Anal Chem 90:6967–6974
127. Wang C, Hou F, Man Y (2015) Simultaneous quantitative detection of multiple tumor markers with a rapid and sensitive multicolor quantum dots based immunochromatographic test strip. Biosens Bioelectron 68:156–162
128. Wu F, Yuan H, Zhou C, Mao M, Liu Q, Shen H, Cen Y, Qin Z, Ma L, Li LS (2016) Multiplexed detection of influenza A virus subtype H5 and H9 via quantum dot-based immunoassay. Biosens Bioelectron 77:464–470
129. Rong Z, Wang Q, Sun N, Jia X, Wang K, Xiao R, Wang S (2019) Smartphone-based fluorescent lateral flow immunoassay platform for highly sensitive point-of-care detection of Zika virus nonstructural protein 1. Anal Chim Acta 1055:140–147
130. Li X, Li W, Yang Q, Gong X, Guo W, Dong C, Liu J, Xuan L, Chang J (2014) Rapid and quantitative detection of prostate specific antigen with a quantum dot nanobeads-based immunochromatography test strip. Appl Mater Interfaces 6:6406–6414
131. Petryayeva E, Algar WR (2015) Single-step bioassays in serum and whole blood with a smartphone, quantum dots and paper in-PDMS chip. Analyst 140:4037–4045
132. Das P, Krull UJ (2017) Detection of a cancer biomarker protein on modified cellulose paper by fluorescence using aptamer-linked quantum dots. Analyst 142:3132–3135
133. Noor MO, Hrovat D, Moazami-Goudarzi M, Espie GS, Krull UJ (2015) Ratiometric fluorescence transduction by hybridization after isothermal amplification for determination of zeptomole quantities of oligonucleotide biomarkers with a paper-based platform and camera-based detection. Anal Chim Acta 885:156–165
134. Lin YY, Wang J, Liu G, Wu H, Wai CM, Lin Y (2008) A nanoparticle label/immunochromatographic electrochemical biosensor for rapid and sensitive detection of prostate-specific antigen. Biosens Bioelectron 23(11):1659–1665
135. Kokkinos CT, Giokas DL, Economou AS, Petrou PS, Kakabakos SE (2018) Paper-based microfluidic device with integrated sputtered electrodes for stripping voltammetric determination of DNA via quantum dot labeling. Anal Chem 90:1092–1097
136. Cincotto FH, Fava EL, Moraes FC, Fatibello-Filho O, Faria RC (2019) A new disposable microfluidic electrochemical paper-based device for the simultaneous determination of clinical biomarkers. Talanta 195:62–68
137. Yang M, Liu Y, Jiang X (2019) Barcoded point-of-care bioassays. Chem Soc Rev 48:850–884
138. Gao Y, Lam AWY, Chan WCW (2013) Automating quantum dot barcode assays using microfluidics and magnetism for the development of a point-of-care device. ACS Appl Mater Interfaces 5:2853–2860
139. Ming K, Kim J, Biondi MJ, Syed A, Chen K, Lam A, Ostrowski M, Rebbapragada A, Feld JJ, Chan WCW (2015) Integrated quantum dot barcode smartphone optical device for wireless multiplexed diagnosis of infected patients. ACS Nano 9:3060–3074
140. Wegner KD, Hildebrandt N (2015) Quantum dots: bright and versatile in vitro and in vivo fluorescence imaging biosensors. Chem Soc Rev 44:4792–4834

141. Zhao M-X, Zeng E-Z (2015) Application of functional quantum dot nanoparticles as fluorescence probes in cell labeling and tumor diagnostic imaging. Nanoscale Res Lett 10:171
142. Liu YS, Sun Y, Vernier PT, Liang CH, Chong SYC, Gundersen MA (2007) pH-sensitive photoluminescence of CdSe/ZnSe/ZnS quantum dots in human ovarian cancer cells. J Phys Chem C 111:2872–2878
143. Ding H, Yong K-T, Law W-C, Roy I, Hu R, Wu F, Zhao W, Huang K, Erogbogbo F, Bergeya EJ, Prasad PN (2011) Non-invasive tumor detection in small animals using novel functional Pluronic nanomicelles conjugated with anti-mesothelin antibody. Nanoscale 3:1813–1822
144. Zhang LW, Bäumer W, Monteiro-Riviere NA (2011) Cellular uptake mechanisms and toxicity of quantum dots in dendritic cells. Nanomedicine 6:777–791
145. Xiao Y, Forry SP, Gao X, Holbrook RD, Telford WG, Tona A (2010) Dynamics and mechanisms of quantum dot nanoparticle cellular uptake. J Nanobiotechnol 8:13
146. Igor L, Medintz HTU, Goldman ER, Mattoussi H (2005) Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater 4:435–446
147. Rosenthal SJ, Chang JC, Kovtun O, McBride JR, Tomlinson ID (2011) Biocompatible quantum dots for biological applications. Chem Biol 18:10–24
148. Ni X, Castanares M, Mukherjee A, Lupold SE (2011) Nucleic acid aptamers: clinical applications and promising new horizons. Curr Med Chem 18:4206–4214
149. Tang J, Huang N, Zhang X, Zhou T, Tan Y, Pi J, Pi L, Cheng S, Zheng H, Cheng Y (2017) Aptamer-conjugated PEGylated quantum dots targeting epidermal growth factor receptor variant III for fluorescence imaging of glioma. Int J Nanomed 12:3899–3911
150. McHugh KJ, Jing L, Behrens AM, Jayawardena S, Tang W, Gao M, Langer R, Jaklenec A (2018) Biocompatible semiconductor quantum dots as cancer imaging agents. Adv Mater 30:e1706356
151. Aswathy RG, Yoshida Y, Maekawa T, Kumar DS (2010) Near-infrared quantum dots for deep tissue imaging. Anal Bioanal Chem 397:1417–1435
152. Allen PM, Liu W, Chauhan VP, Lee J, Ting AY, Fukumura D, Jain RK, Bawendi MG (2010) InAs(ZnCdS) quantum dots optimized for biological imaging in the near-infrared. J Am Chem Soc 132:470–471
153. Liu XY, Braun GB, Zhong HZ, Hall DJ, Han WL, Qin MD, Zhao CZ, Wang MN, She ZG, Cao CB, Sailor MJ, Stallcup WB, Ruoslahti E, Sugahara KN (2016) Tumor-targeted multimodal optical imaging with versatile cadmium-free quantum dots. Adv Funct Mater 26:267–276
154. Shen Y, Lifante J, Ximendes E, Santos HDA, Ruiz D, Juárez BH, Gutiérrez IZ, Vera VT, Retama JR, Rodríguez EM, Ortgies DH (2019) Perspectives for Ag2S NIR-II nanoparticles in biomedicine: from imaging to multifunctionality Nanoscale. Jaque D, Benayas A, del Rosal B 11:19251–19264. https://doi.org/10.1039/C9NR05733A
155. Wang Z, Ma Y, Yu X, Niu Q, Han Z, Wang H, Li T, Fu D, Achilefu S, Qian Z, Gu Y (2018) Targeting CXCR4–CXCL12 axis for visualizing, predicting, and inhibiting breast cancer metastasis with theranostic AMD3100–Ag_2S quantum dot probe. Adv Funct Mater 28:1800732
156. Hu J, Xie M, Wen C-Y, Zhang Z-L, Xie H-Y, Liu A-A, Chen Y-Y, Zhou S-M, Pang DW (2011) A multicomponent recognition and separation system established via fluorescent, magnetic, dualencoded multifunctional bioprobes. Biomaterials 32:1177–1184
157. Xu H-L, Yang JJ, ZhuGe DL, Lin MT, Zhu QY, Jin BH, Tong MQ, Shen B-X, Xiao J, Zhao Y-Z (2018) Glioma-targeted delivery of a theranostic liposome integrated with quantum dots, superparamagnetic iron oxide, and cilengitide for dual-imaging guiding cancer surgery. Adv Healthc Mater 7:1701130
158. Deng Y, Xu A, Yu Y, Fu C, Liang G (2018) Biomedical applications of fluorescent and magnetic resonance imaging dual-modality probe. ChemBioChem 20:499
159. Yang Y, Lin L, Jing L, Yue X, Dai Z (2017) $CuInS_2$/ZnS quantum dots conjugating Gd(III) chelates for near-infrared fluorescence and magnetic resonance bimodal imaging. ACS Appl Mater Interfaces 9:23450–23457
160. Estelrich J, Sánchez-Martín MJ, Busquets MA (2015) Nanoparticles in magnetic resonance imaging: from simple to dual contrast agents. Int J Nanomed 10:1727–1741
161. Garcia-Cortes M, Ruiz Encinar J, Costa-Fernandez JM, Sanz-Medel A (2016) Highly sensitive nanoparticle-based immunoassays with elemental detection: application to prostate-specific antigen quantification. Biosens Bioelectron 85:128–134

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Affiliations

María Díaz-González[1] · Alfredo de la Escosura-Muñiz[1] · Maria Teresa Fernandez-Argüelles[2] · Francisco Javier García Alonso[3] · Jose Manuel Costa-Fernandez[2]

[1] NanoBioAnalysis Group, Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain

[2] GEAB Research Group, Department of Physical and Analytical Chemistry, University of Oviedo, Avda. Julián Clavería 8, 33006 Oviedo, Spain

[3] NanoBioAnalysis Group, Department of Organic and Inorganic Chemistry, University of Oviedo, Avda. Julián Clavería, 8, 33006 Oviedo, Spain