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Optical applications of quantum dots in biological system

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This review presents a simple introduction on the unique properties and general synthesis of quantum dots (QDs) in which we lay emphasis on the optical applications in the biological system. The detection of biological molecules such as DNA, protein and enzyme, the cell-based analysis and *in vivo* animal imaging are mainly discussed.

quantum dots, optical application, biological system

1 Introduction

Quantum dots (QDs), or semiconductor nanocrystals, are a kind of nanoscale inorganic material composed of groups II–VI or III–V elements, defined as particles with physical dimension smaller than the exciton Bohr radius [1, 2] (Figure 1). They have been widely studied due to their unique optical properties including high quantum yield, low photobleaching, high photochemical stability, size-tunable emission and simultaneous excitation of multiple fluorescence colors [3–5]. These optical properties enable quantum dots to exceed conventional organic dyes as fluorophores and seek applications in the biological system.

Since the first description of QD synthesis in 1982 by Efros and Ekimov [6, 7], a wide variety of preparation methods have been put up, employing different media such as aqueous solution, high-temperature organic solvents, and solid substrates [8–10]. In 1998, Alivisatos's and Nie's groups simultaneously demonstrated that the water-soluble and biocompatible QDs could be prepared by appropriate surface modification and such QDs are potential as highly sensitive fluorescent biomarkers [11]. Till now, CdSe/ZnS core/shell QDs remain best available for almost all biological applications [12]. CdSe QDs can be synthesized through

Figure 1 Ten distinguishable emission colors of ZnS-capped CdSe QDs excited with a near-UV lamp. From left to right (blue to red), the emission maxima are located at 443, 473, 481,500, 518, 543, 565, 587, 610, and 655 nm [1].

TOP/TOPO method and the coating can be composed by a layer of wider bandgap semiconductor such as ZnS and a layer of other functional molecules [13, 14]. Thanks to these preparation and surface modification, QDs are playing important roles in biological applications.

QDs have been combined with the technologies of fluorescence, reversible optics, electrochemiluminescence (ECL) [15–18], cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV). In optical applications, they have been widely studied in the detection of biomolecules including DNA, protein and enzyme, cell-based applications and *in vivo* animal imaging. On one hand, QDs replace conventional organic dyes due to their better photostability in application of DNA microar-

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rays [19], fluorescent *in situ* hybridization (FISH) [20, 21], and optical immunoassay for protein detection [22–24]. On the other hand, QDs have been combined with technology of Förster Resonance Energy Transfer (FRET) as excellent donors and they have played essential roles in application of molecular beacons (MB) [25], single quantum-dot-based DNA nanosensors [26] and protein detection [27–29]. Gold nanoparticles have also contributed as efficient quenchers with QD-FRET in the detection of DNA [30, 31], protein [32, 33] and enzyme [34–36]. Besides these two major aspects, we have also introduced interesting QD-embedded mi- crobeads for the multiplexed detection of DNA [37]. As for cell-based applications, we mainly discuss cellular labeling [38-43] and related technologies such as cell injection [44] and chemically-mediated delivery [45]. We also include QDs in *in vivo* animal imaging in visual [46] and NIR regions [47–52] and present their application in vasculature imaging [53–58].

2 Detection of biomolecules

2.1 Application of quantum dots in DNA detection

Specific nucleic acid detection has shown great importance in rapid disease diagnosis, gene sequencing, food and environment analysis [59–61]. Based on the hybridization of targets to the probe DNA immobilized on a solid support [59–61], quantum dots have been widely used in the detection of DNA.

2.1.1 QDs as fluorophores in colorimetric detection of DNA

QDs have singular advantage over conventional fluophores in photostability, which is essential in most fluorescence application [62]. They maintain a high level of brightness and photobleaching threshold after repeated cycles of excitation and fluorescence for hours, higher than organic fluorophores which bleach only a few minutes on exposure to light [63, 64]. The photostable property of QDs allows them to exceed traditional fluorescence markers in routine biological experiments, such as DNA microarray [19] and FISH [20, 21].

Microarray combined with QD-conjugated labels provided simultaneous detection of two multiple genetic markers [19]. Microarray analysis has offered high throughput, convenience, capability and opportunities for genetic detection [65]. As most biochemical assays require secondary detection of a label, the label detection is a determinant of sensitivity [19]. Sequenced oligonucleotide or DNA are used as probes to find the target DNA with hybridization to ensure the binding specificity. Semiconductor nanoparticles such as CdSe/ZnS (core/shell) structure are attractive for biolabeling due to their narrow and tunable emission [66, 67], negligible photobleaching [68], etc. They also surpass conventional organic fluorescent dyes as they require neither high incubation temperature nor overnight incubation. Therefore, the DNA-QD conjugates have emerged as promising labels in the detection of DNA array [69].

FISH offers a cytogenetic technique to detect the presence or absence of DNA on chromosomes. In routine research and clinical uses, it is used in gene mapping, quantitation of gene copy number in tumors with gene amplification, and quantitation of the density of telomere repeats at the ends of human and mammalian chromosomes [21]. While these experiments are often obviated by the photo-bleaching of conventional organic dyes, QD labels offer a more stable and quantitative mode of FISH in research and clinical applications [21]. QDs used in FISH are also required to be soluble and stable in physiological buffer condition, with its surface efficiently derivatized with oligonucleotides [20]. Modification of QDs with the hydroxylated surface enables them to have similar solubility properties but less nonspecific absorptions than those with carboxylic surfaces [70, 71].

2.1.2 QD-FRET in DNA detection

Förster Resonance Energy Transfer (FRET) is a widely used optical method in hybridization detection. When an excited fluorescent dye molecule comes close to a dye of lower excitation energy in a few nanometer ranges, the energy is transferred to the latter one, which show fluorescence while the fluorescence of the originally excited molecule is quenched [72]. QDs function as donors in FRET and enable longer imaging both *in vitro* and *in vivo* applications due to their better photostability than conventional dyes. The QD-FRET application in molecular beacons (MBs), combination with gold nanoparticles, and single-quantum-dot-based DNA nanosensors was reported [25].

A molecular beacon is usually composed of an oligonucleotide, a 5′ end fluorophore and a 3′ end quencher. In the absence of the target DNA sequence, MBs form hairpin structure by the hybridization of complementary sequences of the stem section [25]. In this structure, the fluorophore and the quencher are close，so the energy is transferred to the quencher and no fluorescent signal is observed [25]. In the presence of target DNA, MBs undergo conformational change due to its strong hybridization with the target DNA, and thus the fluorophore and the quencher are separated and a fluorescent signal is observed [25]. MBs do not require washing step and offer high specificity. The photostability of QDs suggests the application of MBs *in vivo* and the broad adsorption spectra of QDs indicate the potential in multicolor MBs detection [25].

Gold nanoparticles (AuNPs) have drawn much attention because of their high extinction coefficient and a broad absorption spectrum in a visible light that is overlapped with the emission wavelength of usual energy donors [73]. Therefore AuNPs are also attractive FRET quenchers for quantum dots. Several FRET methods using QDs and AuNPs respectively as donors and quenchers are reported in

ref. [73]. It has been studied that effective close contact with gold nanoparticles can be achieved by hybridization of an oligonucleotide with a 5′-end functionalized with a QD and a complementary oligonucleotide with a 3′-end functionalized with a gold nanoparticle [30]. Gold nanorods (GNRs) have also been utilized as an optimal quencher for QD-emission quenching [31]. ss-DNA conjugated GNRs were hybridized with QD-ssDNA for sensitive DNA detection [31]. GNRs have the strong optical absorption which enhances the quenching effect, and their large surface area and higher surface energy also increase the quenching site and improve further the quenching efficiency. Therefore, GNRs have brought some advantages in a quenching system compared to gold nanospheres [31].

An ultrasensitive nanosensor based on single QD-FRET has been developed to detect low concentration of DNA in a separation-free format [26]. In this system, QDs are linked to DNA probes to capture DNA targets, which bind to dye-labelled reporter strands and thus a FRET donor acceptor ensemble is formed [26]. The QD also functions as a concentrator that amplifies the target signal by confining several targets in a nanoscale domain [26]. Unbound nanosensors produce near-zero background fluorescence, but on binding to even a small amount of target DNA, they generate a very distinct FRET signal [26].

2.1.3 Novel QD-embedded microbeads for multiplexed detection

Compared to conventional dyes, QDs contain broad absorption spectra, which allow excitation by a wide range of wavelength, indicating that the QDs with multiple different colors could be excited by a single wavelength [62]. Unlike organic dyes, QDs also have narrow emission spectra, which can be controlled in a relatively simple manner by the variation of core size and composition, and through the variation of surface coating [62]. Therefore, QDs are well suited to multiplexed imaging due to the narrow emission and abroad absorption spectra [62]. Here, the novel QD-embedded microbeads for multiplexed detection were introduced.

Different size quantum dots (zinc sulfide-capped cadmium selenide nanocrystals) are embedded into polymeric microbeads at precisely controlled ratios for multicolor-optical coding [37]. Because of QDs optical properties such as size-tunable emission and simultaneous excitation, they are ideal for wavelength-and-intensity multiplexing. Theoretically 10 intensity levels and 6 colors could code one million nucleic acid sequences. The embedded QDs have been observed to be spatially separated from each other without undergoing fluorescence resonance energy transfer. Therefore the QD-tagged beads are highly uniform and reproducible. At the same time, the surface of a polymer bead could be conjugated to biomolecular probes such as oligonucleotides, to hybridize with the target DNA. In this way, the smart microstructure has not only molecular recognition abilities but also built-in codes for rapid detection of oligonucleotides (Figure 2). By integrating molecular recognition and optical coding, such beads should find broad application in gene expression studies, high-speed screening, and medical diagnostics [37].

2.2 Detection of protein and enzyme

The detection of biomarkers is extremely important for clinical diagnosis, basic discovery, and a variety of other biomedical applications [22]. Enzyme catalyzes chemical reactions in the biological system and thus the detection of enzymes is usually related to these reactions. QDs are widely used in the detection of proteins and enzymes due to their unique optical properties.

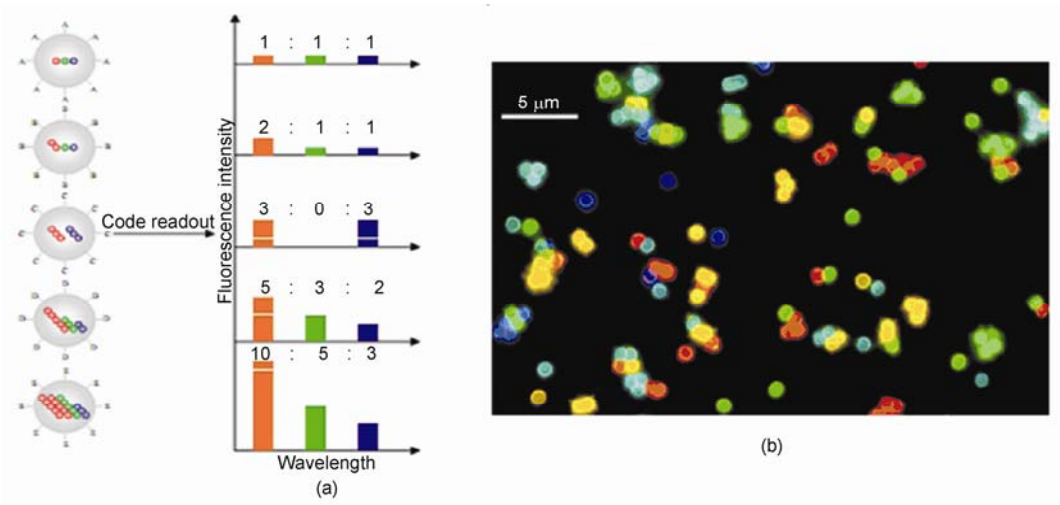

Figure 2 (a) Schematic illustration of optical coding based on wavelength and intensity multiplexing; (b) fluorescence micrograph of a mixture of CdSe/ZnS QD-tagged beads emitting single-color signals at 484, 508, 547, 575, and 611 nm [37].

2.2.1 QDs as fluorophores in colorimetric detection of protein

As mentioned in 2.1.1, QDs' extraordinary photostability enables them to play important roles in immunoassay for protein detection [22, 23]. In these reactions, one antibody is conjugated with QDs, while another antibody is immobilized on a solid face. When the antigen comes, the "sandwich" structure of antibody-antigen-antibody is formed and the quantity of antigen can be determined according to the fluorescence of QDs. Our group reported the CdTe QD label for protein detection, which offers electrochemical and optical signals at the same time [23] (Figure 3). Li *et al.* generated a test strip based on quantum dots and lateral flow, providing rapid and sensitive detection of biomarkers. Ma *et al.* have developed multiplexed fluoroimmunoassays based on two kinds of biofunctional multicolor microspheres through layer-by-layer technology with two different antibodies, anti-human IgG and anti-rabbit IgG, to detect human IgG and rabbit IgG simultaneously [24]. They have also applied the multiplexed fluoroimmunoassays to microfluidic chips [24]. Goldman *et al*. used sandwich immunoassays for the simultaneous detection of four toxins: cholera toxin, ricin, shiga-like toxin 1 and staphylococcal enterotoxin B (SEB), in a single microtiter well [74].

2.2.2 QD-FRET in protein and enzyme detection

The application of the FRET based on QDs opens new opportunities for sensitive detection of biorecognition events. For example, specific binding of different proteins was observed via measurements of FRET between a QD donor attached to one of the proteins, and some organic acceptor dyes attached to the other protein under study. Nagasaki *et al.* developed a FRET system where CdS QDs covered with biotin-PEG/PAMA layer interacted with Taxas red-labeled streptavidin, with the energy transfer in proportion of the concentration of streptavidin [27]. This report showed the potential of QD-FRET system in protein detection and Willard *et al.* described a similar system using biontin-streptavidin interaction [28]. Wang *et al*. used an antigen-antibody FRET system where the emission of antigen (BSA, bovine serum antigen) conjugated red emitting CdTe QDs quenched the luminescence of BSA-antibody conjugated green CdTe QDs [29].

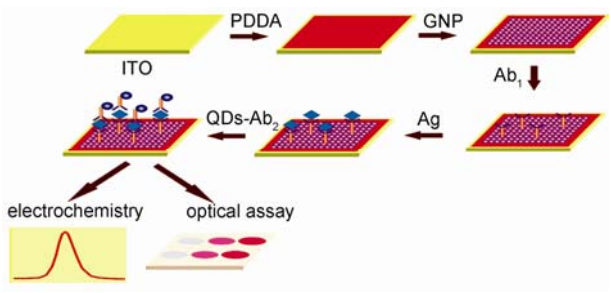

Figure 3 Procedure of electrochemical and optical immunoassays based on CdTe QDs label [23].

In addition, AuNPs have been introduced into the QD-FRET system due to their high extinction coefficient and a broad absorption spectrum in a visible light that is overlapped with the emission wavelength of usual energy donors [32]. Oh *et al.* reported an inhibition assay based on the modulation in FRET efficiency between streptavidin conjugated QDs and biotinlated AuNPs [32]. The same concept was also used to detect glycoproteins [33].

Chang *et al.* have developed protease-activated QD probes by linking AuNP-labeled peptide structure to QDs for the detection of protease activities and modulation [34]. As the peptide contained appropriate cleavage sequences to the protease, the FRET signal of the system could alter when specific cleavage took place [34]. Caspase enzymes, essential in apoptosis, were also detected through cleavage in a FRET system [35]. Choi *et al.* developed aptamer-capped near-infrared PbS quatum dots to selectively detect label-free thrombins [36]. The water-soluble QDs were synthesized with the thrombin-binding aptamer retaining the secondary structure necessary for binding and quenching of the FRET system which was attributed to charge transfer from functional groups on protein to the QD itself [36].

3 QDs for cell-based applications

Biological molecules display different properties and functions in purification and in living multicellular organisms [75]. Therefore, it is desirable to study living cells and organism in real time [75]. The best type of microscopy combining simplicity, resolution, sensitivity and robustness has been fluorescence microscopy [45]. Due to their interesting optical properties, QDs have been introduced as fluorescence labels for biological staining experiments for several years [76, 77]. Compared to organic fluorophores, they offer the following advantages: Because of their narrow emission spectra, more structures can be labeled in multi-color staining and it is possible to excite all colors of fluorescence with one single excitation wavelength; due to the reduced photobleaching it is also easier to obtain 3-dimensional (stacked) images; another advantage lies in the enhanced fluorescence decay time as by making time-gated images the background can be reduced [78].

Various applications of QDs have been found in cellular labeling. Dubertret *et al.* have demonstrated that individual nanocrystals encapsulated in phospholipid block-copolymer micelles could remain stable, nontoxic, and slow to photobleaching when injected to Xenopus embryos [38]. Sukhanova *et al.* have developed NC-Abs conjugates composed of water-soluble CdSe/ZnS core/shell nanocrystals and anti-mouse antibodies, which was successfully used in immunofluorescent detection and three-dimensional confocal analysis of p-glycoprotein overexpressed in the membrane of MCF7r breast adenocarcinoma cells [39]. Kaul *et al.* have applied QDs in mortalin imaging of normal and cancer cells

[40]. Wu *et al*. used immunoglobulin G and streptavidin linked QDs to label the breast cancer marker Her2 on the surface of fixed and live cancer cells [41]. Yezhelyev *et al.* applied quantum dots in multiplexed cell labeling and analysis through conjugating five different primary antibodies, each labeled with a different color quantum dot, against five cancer biomarkers (HER2, EGFR, PR, and mTOR) [42, 43].

A variety of techniques has been employed to label cells internally with QDs, such as passive uptake, receptor-mediated internalization, chemical transfection, and mechanical delivery [79]. Cell injection turns out to be useful for single-cell observation but it costs lots of time when large numbers of cells are to be labeled [44]. Jaswal *et al.* emplyed endocytic uptake of QDs and these live cells labeled were used for long-term multicolor imaging [80]. This is a simple way to label cells although sequestration of aggregated QDs in vesicles might happen [45]. Derfus *et al.* enhanced membrance translocation by using cationic lipids and cationic pepetides in the way of chemically-mediated delivery [45]. Electroporation made use of the increased permeability of cellular membranes under pulsed electric fields to deliver QDs, but this method was reported to result in the aggregation of QDs in the cytoplasm, and generally resulted in widespread cell death. Courty *et al*. demonstrated the capacity to image individual kinesin motors in HeLa cells using QDs delivered into the cytoplasm via osmoticlysis of pinocytotic vesicles [81]. New approaches are in demand to transport the QDs to cytoplasmic cells.

4 Application of QDs in *in vivo* animal imaging

Photostability enables quantum dots to exceed fluorescent dyes or proteins in the application of *in vivo* imaging; they allow images to be recorded over a longer period of time due to their resistance to photo bleaching [42]. Maysinger *et al.* have demonstrated the assessment of QD-induced activation of astrocytes in the brains of transgenic mice through *in vivo* imaging and this method widely applicable for assessing the tissue response to nanoparticles in live animals [46].

Engineering of QDs to fluoresce in the NIR region has been used to increase the signals [47]. Modeling studies have revealed that two spectral windows exist for QD imaging in living subjects, one at 700–900 nm and the other at 1200–1600 nm [48, 49]. Due to its high photon penetration in tissues derivate from low tissue absorption, scattering and auto fluorescence in the NIR region, QDs are suitable for biomedical applications in this region [48]. Lim *et al.* have produced near-infrared QDs optimized for imaging surface vasculature and used them to image the coronary vasculature *in vivo* [49]. Cerussi *et al.* have applied NIR diffused optical spectroscopy into noninvasive diagnostic in breast caners [50]. Jiang *et al.* have realized *in vivo* observations performed on mouse heart by conjugating proteins to quantum dots with an emission at 750 nanometers. In this way they have demonstrated the ability of quantum dots with NIR region emission wavelengths for *in vivo* analysis of deep tissue or non-invasive applications [51]. Zimmer *et al.* conjugated DHLA to NIR emitting InAs/ZnSe core/shell quantum dots and observed within the interstitial fluid in rats [52].

Vasculature imaging is also one of QDs' most important applications as new vasculature establishes a blood supply to a growing tumor [42]. Morgan *et al.* have realized imaging of rat coronary vasculature at a depth of 1.5–2.0 mm through NIR emitting of CdTeMnHg-BSA nanocrystals [53]. Larson *et al.* have developed water-soluble quantum dots for 6 multi-photon fluorescence imaging through intack skin and adipose tissue in mice, which allowed visualization of vasculature at the base of the dermis 900 μm deep [54]. Cai *et al.* have reported *in vivo* targeting and imaging of tumor vasculature using arginine-glycine-aspartic acid peptide-labeled quantum dots, which might aid in cancer detection and management [55] (Figure 4). Smith *et al*. have studied QDs for visualization of blood vessels of the chick chorioallantoic membrane. They have found that QD vascular residence time was tunable through QD surface chemistry modification and the use of QDs with higher emission wavelengths (> 655 nm) virtually eliminated all chick-derived autofluorescence and improved depth-of-field imaging [56]. Stroh *et al.* have combined multiphoton microscopy techniques, transgenic mice and quantum dots, to im-

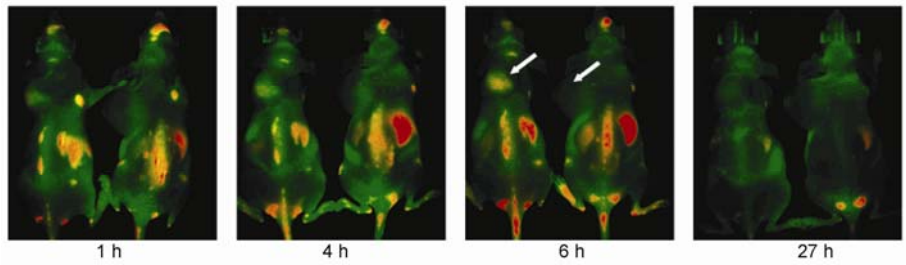

Figure 4 *In vivo* NIR fluorescence imaging of U87MG tumor-bearing mice (left shoulder, pointed by white arrows) injected with 200 pmol of QD705-RGD (left) and QD705 (right), respectively [55].

age and differentiate tumor vessels from both the perivascular cells and matrix [57]. Levene *et al.* have applied quantum dot-labeled serum in microangiographies from deep capillaries and blood vessels [58].

Although QDs exceed traditional organic dyes in use of *in vivo* imaging, there still exist obvious barriers to their further application. QD complexes might be immunogenic and thus cause dangerous immune reactions or become ineffective due to antibody binding; heavy metals in QDs might be toxic to the host; and the size of QD complexes might be difficult to clear and thus lead to aggregation in liver [47]. Various methods have been employed to study the toxicity of QDs *in vivo*, including oxidation and cadmium release [48, 82], surface coating [48, 83] and so on.

5 Conclusion and perspective

QDs have drawn interests due to their unique and advantageous optical properties, which includes broad absorption, narrow size-tunable PL spectra, and superior resistance to photobleaching [84]. They have appeared as a new promising class of fluorescent probes for biomolecular detection, cell-based application and *in vivo* animal imaging. Detections of biomolecules through QD labels and QD-FRET mechanism usually base on a selective binding event or other selective reaction. QDs' size-tunable properties enable them to solve the problems of spectral overlap in conventional organic labels. Moreover, QDs can be immobilized on solid surfaces or embedded in microbeads to realize multiplexed detection. At the same time, QDs optical properties such as photostability and narrow emission spectra also make them robust labels in cell-based applications. Besides these regular advantages, QDs exceed fluorescent dyes in *in vivo* imaging because of their fluorescence in NIR region, which offers low tissue absorption.

However, the use of QDs in biological systems, especially in *in vivo* application, suffers from their toxicity. Due to their chemical composition of toxic metal atoms (e.g. Cd, Hg, Pb, As), hindrance exists when QDs are applied in living cells and animals [85]. Although metal ions like divalent cadmium today are covered with inert zinc sulfide and encapsulated within a stable polymer, they might still be toxic in living bodies. Moreover, QDs could aggregate or bind nonspecifically to cellular membranes and intracellular proteins [85]. It has been reported that concentration of cadmium in th liver and kidneys could gradually increase after intravenous administration of cadmium-based QDs [86]. Former studies have shown that QDs' size, shape, surface coating all could affect their toxicity [85].

Overall, the unique optical properties of QDs and the modulation of those properties have provided researchers versatile toolkit for bioanalysis [87]. Importantly, multiplexed detection is possible as a new type of simple, flexible method for novel diagnostic technologies and intracellular probes [87]. Both quantum efficiency and sensitivity should be increased in the future [88]. As for *in vivo* applications, new types of QDs exempt from heavy metal atoms should be developed [89, 90]. Moreover, nanoparticle distribution, excretion, metabolism, pharmacokinetics, and pharmacodynamics should be included in nanotoxicology studies in animal models *in vivo* [85].

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