Review Article

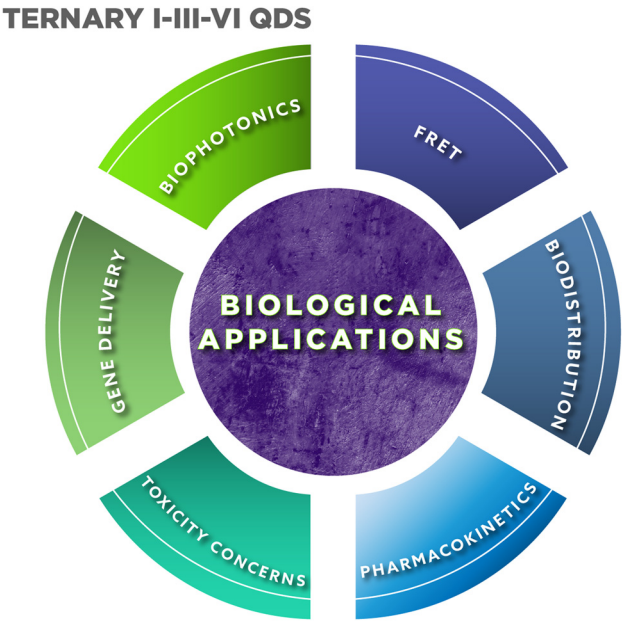
Olanrewaju A. Aladesuyi, Thabang C. Lebepe, Rodney Maluleke, and Oluwatobi S. Oluwafemi*

Biological applications of ternary quantum dots: A review

https://doi.org/10.1515/ntrev-2022-0136
received March 24, 2022; accepted May 15, 2022

Abstract: Semiconductor nanomaterials, also known as quantum dots (QDs), have gained significant interest due to their outstanding optical properties with potential biological and biomedical applications. However, the presence of heavy toxic metals such as Cd, Pb, and Hg in conventional QDs have been a major challenge in their applications. Therefore, it is imperative to seek a viable alternative that will be non-toxic and have similar optical properties as the conventional QDs. Ternary I–III–VI QDs have been found to be suitable alternatives. Their optical properties are tunable and have emissions in the near-infrared region. These properties make them useful in a wide range of biological applications. Hence, this review focuses on the recent progress in the use of ternary QDs in Forster resonance energy transfer (FRET), nanomedical applications such as drug and gene delivery. It also discusses the biophotonic application of ternary I–III–VI QDs in optical imaging, biosensing, and multimodal imaging. Furthermore, we looked at the pharmacokinetics and biodistribution of these QDs, and their toxicity concerns. Finally, we looked at the current status, challenges, and future directions in the application of these ternary QDs.

Keywords: quantum dots, imaging, biophotonics, FRET, biodistribution, gene delivery



Graphical abstract

1 Introduction

Luminescent semiconductor nanoparticles known as Quantum dots (QDs) has been the pivot of most research endeavour in recent times [1,2]. QDs remain one of the important emerging functional materials in biomedical research due to their excellent optical, electronic, and physical properties, which make them preferred over conventional fluorophores [3–6]. By virtue of the quantum confinement effect, they exhibit size influenced fluorescent emission. Therefore, wide emission spectra within the range of 450–1,500 nm can be achieved by adjusting their size. The change in bandgap that accompanies the size increase allows the emission of photons by the QDs from the visible to near infrared region [7–9]. The size-induced optical properties is of much interest in biological applications such as bioimaging, biosensing, drug delivery *etc.*, because fluorescence is one of the most important phenomena in biological related research.

However, recently ternary I–III–VI QDs have been preferred to the conventional II–VI binary QD due to

* **Corresponding author: Oluwatobi S. Oluwafemi,** Department of Chemical Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, Johannesburg, South Africa; Centre for Nanomaterials Science Research, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, Johannesburg, South Africa, e-mail: oluwafemi.oluwatobi@gmail.com
Olanrewaju A. Aladesuyi, Thabang C. Lebepe, Rodney Maluleke: Department of Chemical Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, Johannesburg, South Africa; Centre for Nanomaterials Science Research, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, Johannesburg, South Africa

the absence of toxic heavy metals such as Cd and Pb. This has made them less toxic and environmentally friendly. Besides the low toxicity, ternary QDs also possess excellent optical and electronic properties such as tunable absorption band to the near-infrared (NIR) region, wide Stokes shift, large photofluorescence lifetime, *etc.* [10,11]. These properties are very important for various therapeutic applications. The tunable absorption characteristic ability of ternary QDs influences their fluorescence in animal cells and tissues. Consequently, ternary QDs have been investigated for bioimaging, *in vivo* and *in vitro* applications. These sets of QDs have also been used as drug conjugates for effective and safe delivery of the drug to the target point. A lot of studies have been carried out on these ternary QD/drug conjugates, especially in relation to photodynamic therapy (PDT), a treatment for diverse forms of cancers, infections, and inflammations. It is an alternative to surgery and chemotherapy. In recent years, technology involving the use of QD-based bioimaging and biosensing have been on the increase [12]. This will definitely lead to new frontiers in nanochemistry.

Although reviews on the application of non-toxic ternary QDs in biological and biomedical fields have been reported, in this review, we discussed extensively the application of these ternary QDs in FRET, pharmacokinetics, and biodistribution. The synthesis strategies, core/shell structure, functionalisation, and bioconjugation of ternary QDs have been extensively discussed in our previous work [13]. Thus, this particular review highlights the recent development on the use of ternary QD drug conjugates for therapeutic and diagnostic purposes. We begin by discussing the tunable optical properties of ternary I–III–VI QDs, followed by their applications in FRET systems, therapeutics, biophotonics, and pharmacokinetics. Finally, their toxicity-related issues and future outlook are also discussed.

2 Tunable optical properties of ternary I–III–VI QDs

Ternary I–III–VI QDs (where I = Cu or Ag, III = Ga or In, and VI = S or Se) are made up of less toxic elements, thereby making them preferred to the conventional binary QDs. Quantum confinement experienced by these sets of QDs allows for their tunable optical properties. The distortion in size and bandgap experienced when their composition is altered causes the emission of photons from the visible to the NIR region and allows the tuning of absorption spectra [14–17]. The size-dependent optical properties and emission of photons in the NIR region are some of the reasons for their applications in biological research [18,19]. Their coefficient of absorption, photostability, high quantum yield, good luminescence decay time, and large Stokes shift also contribute to their usefulness in biological research [20,21]. The band gaps displayed by some of these QDs include 1.05 eV (CISe), 1.5 eV (CIS), 1.87 eV (AIS), and 1.2 eV (AISe) to mention a few [22,23].

In biomedical applications of ternary QDs, tuning of emission spectra is paramount, as fluorescence is a vital parameter in biomedical research. The tuning of these optical properties is largely achieved through the variation in atomic compositions of the QDs and the introduction of other elements into their composition [24–26]. Kang *et al.* [27] reported a 12 and 34.7% increase in the tuning of emission wavelength for CISe/ZnS QDs and AgInSe/ZS QDs, respectively. This was observed when the composition of In was increased for both Cu/In ratio and Ag/In ratio in both QDs (Figure 1a and b). Also, appropriate shelling, doping, and functionalization have been found to increase the photoluminescence (PL) intensity, enhance stability, remove surface defects, and facilitate emission in the NIR of ternary QDs [28–30]. This is beneficial to biological applications. For effective charge carriers, shelling or coating material should possess a wider bandgap than the core. Also, the crystal and lattice parameters must be similar to ensure unhindered epitaxial-like growth of the shell [31]. In addition to the safety advantage, ternary QDs display longer fluorescence lifetimes and long excited state lifetimes when compared to other forms of QD, making them good fluorophores favourable in biological applications.

3 FRET of ternary I–III–VI QDs

Energy transfer through a non-radiative process from a fluorescent donor to a lower energy acceptor through dipole-dipole interaction is referred as FRET [32]. Consequently, the fluorescence intensities of the donor are reduced, while that of the acceptor molecule are increased. This energy transfer occurs within a critical radius known as Forster radius. This analytical method is sensitive, reliable, and widely used in biological experiments [20,33]. FRET technique has also been found to be more sensitive to nanoscale changes in distance between acceptor and donor moieties [34]. Recently, ternary QDs have been employed as energy donors and acceptors in FRET systems in order to improve efficiency and analytical effectiveness [35]. Figure 2a describes the mechanism of the FRET process.

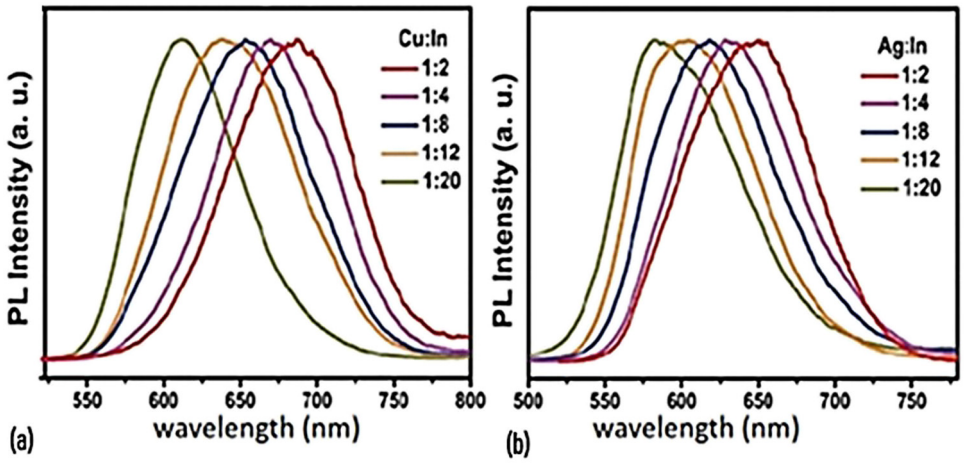


Figure 1: (a) PL spectra of CISe/Zns, (b) AISe/Zns core shell/QD at various Cu/In and Ag/In precursor ratios; with permission from ref. [27]; Green Chemistry.

It involves the absorption of photon by a parent QD leading to an energy transfer from its excited state to the ground state of the nearest accepting chromophore through non-radiative means [36].

QDs FRET-based probes have been applied in biological systems such as bioimaging [37], photodynamic therapy [38], diagnostic, and biosensing [39,40]. Their unique properties such as size-dependent optical properties, photostability, tunable emission NIR, and resistance to photobleaching makes them a preferred candidate for nanosensors when compared to conventional organic FRET pairs [18,19]. These sets of QDs permit the excitation of different emission wavelengths by a single excitation source. This is possible as a result of their large range of absorption wavelengths and their relatively wide extinction coefficient. These properties make them suitable as bio-sensors in detecting multiplex analysis [41,42]. QDs have been widely utilised as FRET donors for a variety of acceptors. Until now, the widely used QD for FRET-based probes and sensors are either CdSe or CdTe, which hinders biological

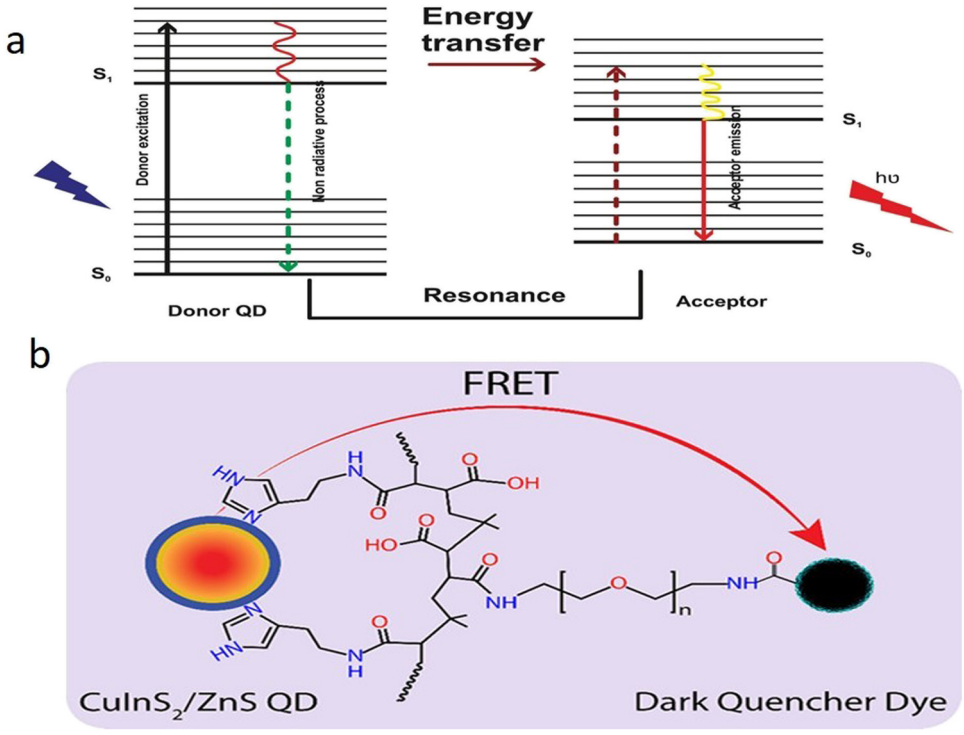


Figure 2: (a) Schematic diagram of energy transfer between a donor and an accepting chromophore; with permission from ref. [36]; Elsevier. (b) Schematic illustration of FRET process between functionalized CIS/ZnS QDs and IR Dye QC-1; with permission from ref. [45]; ACS.

applications due to the toxic nature of Cd ions [43]. Therefore, the use of Cd-free QDs such as ternary I–III–VI QD is vital. Their broad absorption and narrow emission spectra allow single-wavelength excitation of multiple donors and can avoid crosstalk with acceptor fluorophores [20]. Xing *et al.* [44] developed a FRET biosensing system using MnCuInS/ZnS (MCIZ) and urchin-like Au-NPs as a donor-acceptor pair. The FRET pair was developed for the purpose of detecting HER2 protein. In the study, MnCuInS/ZnS QDs were encapsulated in BSA for easy conversion of MnCuInS/ZnS QDs from the organic phase into an aqueous solution. The resulting nanoparticles were applied as the energy donor, and the fluorescence intensity was enhanced to a large extent. As for the energy acceptor, urchin-like Au nanoparticles (Au-NPs) were selected mainly due to its stability and quenching ability for NIR fluorescence. Thus, the NIR MnCuInS/ZnS@BSA and urchin-like Au-NPs were designed as a novel donor–acceptor pair for FRET assay. The proposed FRET-based biosensor produced an enhanced FRET effect for highly sensitive detection of HER2 in human serum samples with a detection range of 2–100 ng mL^{-1} and a low detection limit of 1 ng mL^{-1}. This sensing system reduces the interference of other biomolecules in the NIR region. It also showed the potential of application in *in vitro* and *in vivo* diagnosis.

Xia *et al.* [45] reported a study of FRET between colloidal CIS/ZnS QDs and a dye molecule (IRDye QC-1). The increasing quenching of CIS/ZnS emission and reduction in its photoluminescence decay time following the increasing amount of conjugated dye molecules show that the CIS QDs acted as the energy donor while the dye molecule was the energy acceptor in a donor–acceptor FRET system. Dynamics of the QD-dye FRET pair was simulated using two experimental models, which were based on the multiexponential PL decay of the QDs (Figure 2b). The result showed a donor–acceptor distance (6.5 nm), which is in agreement with the hydrodynamic radius of the amine-functionalised QDs. The study described the potential of using non-toxic ternary QDs-based FRET nanoprobes in wide range biological applications such as biosensing, biomedical imaging, photodynamic therapy *etc.*

4 Therapeutic applications of ternary QD

Conjugated ternary QDs can act as a vehicle for the transmission of a substance of interest to the specific site inside the body or specific organ where it is needed. In addition to the non-toxic nature of these QDs, other properties such as their water solubility, excellent biocompatibility, and controlled release profile of drugs at target sites make them preferred nano-carriers compared to other carriers such as liposomes, chitosan, and polymer nanoparticles [46]. Selected therapeutic applications of drug and gene delivery are discussed here.

4.1 Drug delivery system

Recently, QDs interaction with biological systems has received much interest in pharmacy, pharmacology, and medicine [47]. Ternary QDs permit attachment of various ligands with different functionalities and have been considered as good candidates for drug delivery systems. This is due to their excellent loading capacity of both hydrophobic and hydrophilic therapeutic drugs, regulated release profile, and increased therapeutic ability of the drugs [48]. QDs-loaded drug formulation is beneficial in pharmacokinetics for achieving targeted delivery, improved uptake by cells, and long circulation lifetime [49]. The technology involved in the application of these QDs in drug delivery is evolving by the day. These QDs can be cross-linked to peptides, antibodies, and other biomolecules to target biological systems, making them applicable in diagnosis and drug delivery. For effective QDs drug delivery, factors such as *in vivo* stability, solubility, and biodistribution should be carefully considered. The drug to be administered is usually entrapped in the QDs with the aim of attaching it to a particular surface of the cell. On reaching the target site, the QDs dissociate in order to release the drug. The release of the drug can be triggered by certain parameters such as light or heat [50]. It may also require certain biological reactions, temperature, and pH control of the target sites. Drug entrapped QDs can distort the mechanism of membrane transport and improve to a large extent the absorption of the drug in the affected cells. QDs also enhance properties such as water-solubility and release function, consequently increasing the drug efficacy and reducing the side effect [51,52]. Wu *et al.* showed that AIS/ZnS-methotrexate (anticancer drug) conjugate displayed effective drug delivery and imaging functionalities with minimal toxicity [53]. In another study, Ruzycka-Ayoush *et al.* [54] used AIS/ZnS modified with MUA, L-cysteine, and lipoic as a carrier for doxorubicin (DOX) in treating lung cancer cells. Inhibition of migratory potential of A549 cells was observed for the DOX-QD conjugates at tolerable toxicity levels. The study further revealed the therapeutic efficiency of DOX-loaded AIS/ZnS indicating

their promising role as a novel drug delivery agent to lung cancer cells. DOX loaded with MUCI aptamer – $(CGA)_7$ functionalised CIS QDs was reported to effectively deliver the drug to the targeted prostate cancer cells [55]. The authors also reported a connection between the PL intensity of the CIS QDs and doxorubicin, thereby making it possible to keep track of the cancer drug concentration.

4.2 Gene delivery

Recently, gene therapy has been of much interest as a major therapeutic option in the treatment of various forms of genetic diseases. Several diseases occur as a result of either malfunctioning or the absence of endogenous genes within the body system. The effectiveness of the body's defence system can also be determined and controlled by the activities of specific genes. Vast knowledge and understanding of certain genetic pathways will impact to a large extent on healthcare matters, especially genetically acquired or inherited diseases [56,57]. In addition, genetic therapy can either regulate or reverse certain social disorders such as drug addiction behavioural or mental disorders [58].

One of the ways of achieving gene therapy is through gene augmentation therapy. It involves enhancing or boosting the function of a deficient or malfunctioning gene through the introduction of an appropriate plasmid DNA (pDNA) within which an active genetic component can be incorporated. A lot of pre-clinical studies have shown the use of engineered viruses (viral vectors) to be effective in gene delivery; however, their translation on the human application have been discouraged due to the high risk of mutagenicity and immunogenicity associated with the application [59,60]. Consequently, the emergence of "non-viral" genes are being explored as a viable alternative. These non-viral genes offer several advantages some of which include: ease of preparation, less immunogenicity, lack of recombination potential *etc.*

Among the limitations of successful gene therapy is the inability to deliver sufficient genes to the target tissues and cells and the inability to monitor gene delivery and therapeutic response from the affected tissues and cells [61,62]. Over time, commonly used non-viral vectors such as liposomes and certain polymeric NPs have displayed shortcomings in the area of visualizing and monitoring the DNA delivery process [63]. Recently, the use of QDs as non-viral vectors have been shown to accomplish effective monitoring of gene therapy through fluorescence imaging [64,65]. The use of QDs with appropriate surface modification has been employed as a vehicle for integrating genetic materials such as plasmid DNA and RNA for targeted gene delivery to the required cells and tissues, and this is gaining much attention in pharmacotherapy [58,66]. Ternary QDs show greater potential in gene therapy applications when compared to other highly luminescent QDs due to the absence of toxic elements and its inherent optical properties. $CuInS_2$ ternary QDs functionalised with polyethylenimine (PEI) followed by adsorption into microbubbles (MBs) were used for modal imaging and gene delivery by Yang *et al.* [67]. The functionalised ternary QDs were conjugated with plasmid DNA and were delivered to the targeted tumour site (Figure 3). The result from the study showed that the derived composite MBs@QDS@PEI/pDNA enhances both ultrasound and fluorescent imaging. *In vitro* experiment also confirmed that pDNA could be released from the derived composite and it is a promising material for targeted gene delivery. In another study, Subramaniam *et al.* [68] carried out the removal of oncogene genes in brain tumour cell line (U87) using SiRNA. For effective delivery of the SiRNA, they are conjugated to $AgInS_2$-ZnS (ZAIS) QDs using PEI. The result indicated that the QDs-siRNA were appreciably taken up by the cells and the oncogene genes were knocked off. Also, a green fluorescence decline of 80% was observed, indicating effective translocation of SiRNA into the cells.

5 Biophotonic applications of ternary QDs

Biophotonics is the science of utilizing generated photons/light for imaging and identifying biological matter [69]. It is an exciting frontier that involves a fusion of photonics and biology. The knowledge and application of biophotonics allow for early detection of diseases and offer possibilities for diagnostic, imaging, and light-assisted and light-guided therapy, to mention a few. Over time, QDs have found usefulness in biophotonic sensing and imaging, and they have been widely used for this purpose [70,71]. Ternary I–III–VI QDs, in particular, are of high stability, low cost, and low toxicity than the conventional cadmium-based QDs, thereby making them an ideal candidate for biophotonic applications. The tuning of optical properties of ternary I–III–VI QDs enables them to be useful for successful imaging and monitoring biological sites and also to understand their molecular interactions. Multimodal imaging of biological species can be achieved

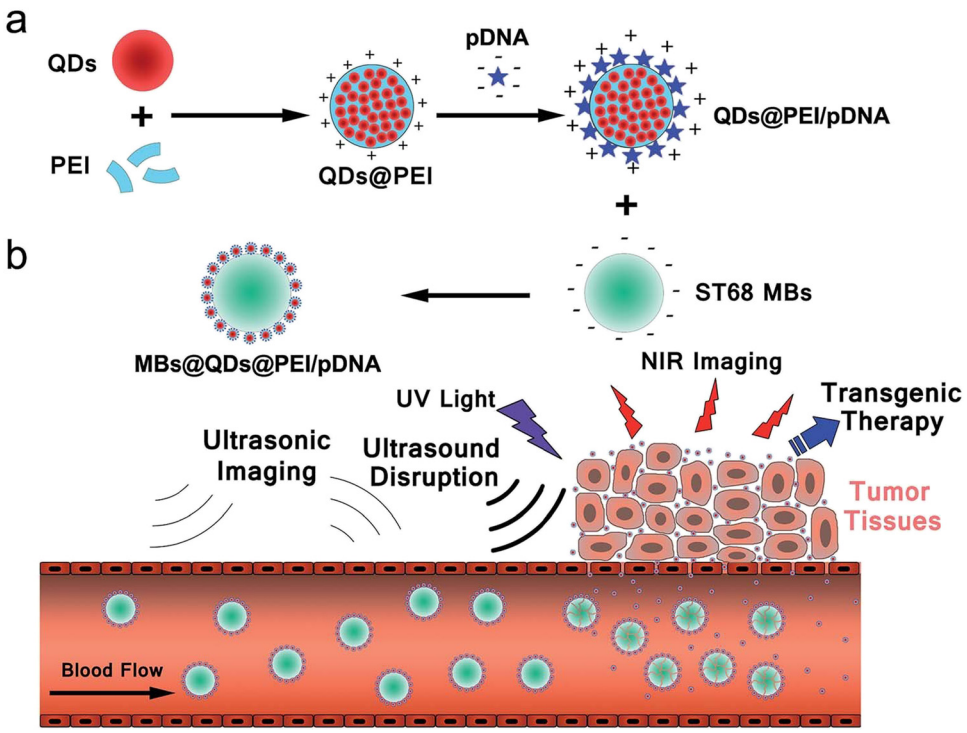


Figure 3: Schematic diagram of: (a) MBs@QDs@PEI/pDNA formation, (b) NIR fluorescence and target delivery by ultrasound-targeted microbubble destruction; with permission from ref. [67]; Royal Society of Chemistry.

when these QDs are combined with contrast agents. This information allows for the early detection of certain diseases as the molecular interaction that occurs prior to these diseases can be monitored [71,72]. Detailed molecular imaging can be achieved when these QDs are integrated into various types of imaging techniques. QD nanoprobes that are sensitive to pH, temperature, or even the activities of biomolecules within a cell or tissue can be employed for detailed molecular imaging [73,74]. In this section, we will discuss briefly the selected biophotonic applications of these QDs. These include; optical imaging, biosensing, photo-therapeutic, and multimodal imaging.

5.1 Optical imaging

In biological and biomedical research, optical image is a very important tool due to its capacity to give *in-vivo* and *in-vitro* information of high resolution [25,48]. This technique has numerous advantages, including high sensitivity, low cost, portability, and potential for multiplex images. Optical imaging is a useful technique in surgery endoscopic procedures. It is applied in early tumour detection and image-guided cancer resection. For surgery to be successful in cancer treatment, the tumour removal has to be maximised, the duration of surgery shortened, and the damage experienced by the neighbouring tissues has to be minimal [75] Optical imaging permits real-time visualisation of the tumours, therefore, allowing image-guided surgery. The use of QDs in tumour imaging and detection for image-guided surgery is gaining attention by the day [76,77] The use of QDs is convenient, non-invasive, offers excellent resistance to photo-bleaching and allows for long term visualisation for intra-operative image-guided surgery [75,78]. Their inherent properties such as tunable emission spectra, large Stokes shift, high photostability *etc*., make them preferred to the conventional traditional organic dyes in many imaging applications [79] Furthermore, unlike the traditional organic dye, they can be easily bioconjugated with biomolecules for targeted imaging due to their large surface area and rich surface chemistry.

In their study, Liu *et al.* [80] synthesised water-soluble luminescent $AgInS_2$ QDs as NIR probes for *in vivo* and *in vitro* targeting and imaging of tumours. The water solubility was achieved by their encapsulation with F127 triblock micelle polymers. The bioconjugated QDs displayed sharp PL spectra and quantum yield (QY) of 35%. With the use of a whole-body animal optical imaging set-up, the conjugated $AgInS_2$ nanocrystals formulation was employed for passive targeted delivery to the

tumour site. The result from the study indicates that the tumour tissue accumulation occurred due to the passive process as a result of the enhanced permeability and retention effect. The small size, high luminescence, and high QY of $AgInS_2$ make it a viable candidate as a biological contrasting agent for sensing and imaging purposes. $CuInS_2$ and $AgInS_2$ QDs are the most commonly used ternary I–III–VI QDs [80]. Among other reasons, the ability to achieve their synthesis under mild conditions (moderate temperature) and the use of non-toxic precursors is a major reason for this [81]. Various reports have described the application of $CuInS_2$ and $AgInS_2$ in optical imaging [82,83]. $CuInS_2$/ZnS QDs encapsulated with chitosan micelle was used for tumour targeting of micelles in cells by Deng *et al.* [84] The study showed the application of biocompatible $CuInS_2$ QDs in multicolour bioimaging applications. In another development, $AgInS_2$ QDs encapsulated with multidentate polymer was used for *in vivo* imaging by Tan *et al.* [85] The results showed that these QDs were suitable for deep tissue imaging as shown by the excellent contrast images observed.

5.2 Ternary QDs-based biosensing

Biosensing is crucial in medical diagnostics. Body fluids such as urine, saliva, sweat *etc.*, when collected from humans, are powerful indicators of any malfunctioning part of the body and can play an important role in disease diagnostics. Recently, the unique properties of QDs have been harnessed in the detection of various chemical and biological interactions [52,85]. The high stability, low cost, and minimal toxicity of ternary QDs make them attractive for biosensing applications. Utilizing poly-(dimethyl diallyl ammonium chloride) (PDAD) conjugated 3-Mercaptopropionic acid capped $CuInS_2$ ternary QD as a probe, Liu *et al.* [86] developed an effective fluorescence detection system for lysozyme. The concentration of lysozyme in the body fluids such as serum and urine suggests the presence of diseases such as leukaemia, meningitis *etc.* Lysozyme is an essential biomarker, and its selective sensing is vital. The study reveals that the $CuInS_2$ QDs show good selectivity for lysozyme over other proteins, suggesting the application of $CuInS_2$ as NIR fluorescence probe for lysozyme assay. As shown in the schematic diagram in Figure 4, the fluorescence of the conjugated $CuInS_2$ was quenched by adding lysozyme aptamer. This was attributed to the electrostatic attraction between the PDAD and the aptamer as confirmed by the previous studies [87]. The authors also envisaged the potential application of this ternary QDs for lysozyme selective detection and quantification in relation to biomedical and biological research. In another study, 3- aminophenyl boronic acid functionalised $CuInS_2$ QDs were applied as PL probes for the detection of dopamine, a very important catecholamine neurotransmitter

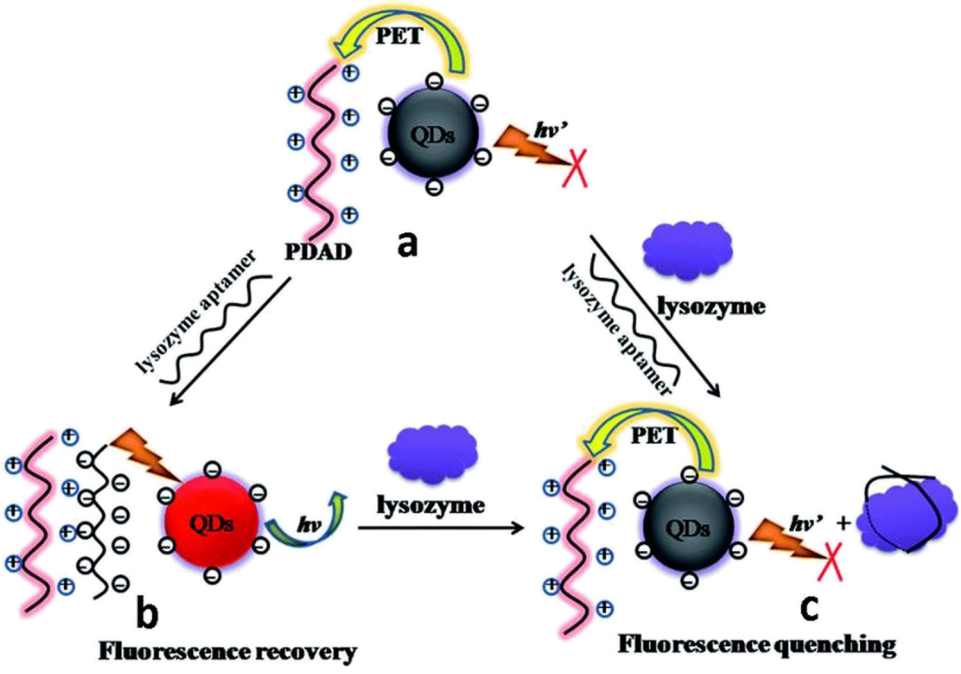


Figure 4: Strategy for lysozyme sensing. (a) Coexistence of the MPA-capped $CuInS_2$ QDs and cationic polyelectrolyte PDAD. (b) Electrostatic bonding of the lysozyme aptamer and PDAD. (c) Bonding of the lysozyme aptamer and lysozyme; with permission from ref. [86]; Royal Society of Chemistry.

that controls a wide variety of human behaviours, such as learning, motor control, arousal enforcement to mention a few [88]. The acid-functionalised QDs were reactive toward vicinal diols to produce cyclic esters in alkaline solutions. This reaction generated quenched fluorescence signal, which was applied as probes for dopamine detection. Based on the fluorescence quenching of the functionalised $CuInS_2$, other diols such as catechol, pyrogallol, and gallate can also be successfully detected.

With the use of a similar approach, the same research group reported the use of $CuInS_2$ QDs fluorescent probe for the determination of dicyandiamide. This approach was used in differentiating dicyandiamide from other amino acid and nitrogen pollutants such as melamine in milk samples. Highly luminescent $AgInS_2$/ZnS QDs with a long PL lifetime of 424.5 ns and QY of 40% have been used as fluorescent probes for detecting intracellular Cu^{2+} ions in HeLa cells [89].

5.3 Multimodal molecular imaging

Molecular imaging is one of the fastest growing areas of science. It is the ability to view the inner body system of humans to better understand the biological complexities and achieve the treatment of diseases. It entails the non-invasive study of the *in vivo* biological process both at a cellular and molecular level. Multimodal imaging using nanoparticles in synergy with integrated functional entities have attracted a lot of interest, leading to a wide range of imaging tools for biomedical applications [90–92]. Multimodal imaging technique can be described as the combination of two or more imaging techniques. This can be achieved through the use of multi-modal probes and contrast agents that permit improved visualisation of biological materials and much-improved reliability of accumulated data [93]. As no molecular imaging technique is perfect, no one imaging modality can provide all the necessary information; therefore, it is important to come up with a synergistic approach of combining the advantage of one technique with another and also minimizing the disadvantage of such technique over the other. For example, MRI technique have been widely used as an imaging tool in cancer diagnosis due to certain exemplary features such as non-invasiveness and strong contrast in soft tissues. However, the technique still has shortcomings, such as inadequate imaging speed and low sensitivity, whereas Positron Emission Tomography is highly sensitive but has weak resolution. Recently, multimodal imaging approach has received widespread attention from researchers [94]. Guo *et al.* [95], in their work, successfully synthesised Gd-doped Zn-CIS ternary QDs for the purpose of magnetic resonance and fluorescence dual imaging purposes. The incorporation of Gd into the ZCIS QD is to ensure greater MRI enhancement without interfering with the fluorescence properties of the ZCIS/QDs. MRI and fluorescence imaging studies were successfully carried out for both *in vivo* and *in vitro* studies. The result from the study revealed that the Gd-doped ternary QDs have the ability to function as a dual-modal contrasting agent and can simultaneously produce MRI contrast enhancement and fluorescence emission for *in vivo* imaging. In addition, the synthesis of NIR emitting Zn-Cu-In-Se/Zn1-xMnxS core/shell QDs was carried out by Sitbon *et al.* [96]. Under optimised conditions, synthesised Mn-doped ternary QDs were found to be suitable as multimodal *in vivo* probes when used as MR/NIR fluorescence imaging of regional lymph nodes in mice. Cheng *et al.* [97] reported the synthesis of MR/Fluorescence nanoprobe using covalently conjugated Gd-diethylenetriaminepentaacetic acid (DTPA) and $CuInS_2$/ZnS. The as-synthesised dual modal probe was functionalised with folic acid to achieve active tumour targeting. Results from the study indicate that the dual nanoprobe is an effective T1 contrast agent with longitudinal relaxivity value of 3.72 $mM^{-1}S^{-1}$ and selectivity towards HeLa, HepG2, and MCF-7 cells. The use of ternary QDs base probes in various multimodal imaging applications has also been reported in various studies. They include silica nanohybrids embedded $CuInS_2$/ZnS as tri-modality MR/fluorescence imaging probes [98], $CuInS_2$/ZnS Mn as bi-modal nanoprobes for MRI and PL in *in vivo* imaging applications [99], and $AgInS_2$–ZnS combined with $MnFe_2O_4$ for PL/MR dual-modal imaging [100]. Jiang *et al.* [101] used $CuFeSe_2$ as a nanotheranostic image for the multimodal imaging photothermal therapy of cancer (Figure 5), Yang *et al.* [102] used DTPA-Gd modified $CuInS_2$/ZnS for bimodal MR/NIR imaging,

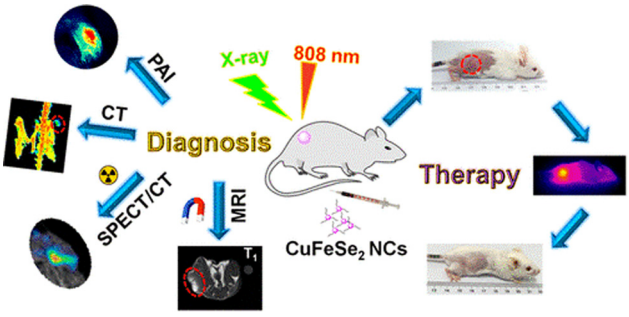


Figure 5: Illustration on the use of $CuFeSe_2$ ternary quantum dot (TQD) for multimodal imaging photothermal therapy of cancer; with permission from ref. [101]; ACS.

while Zhang *et al.* [103] used $CuInS_2$/ZnS and DTPA coupled imaging for MR/fluorescent imaging.

6 Pharmacokinetics and bio-distribution of ternary I–III–VI QDs

The study of the absorption, distribution, metabolism, and excretion of drugs from the body is known as pharmacokinetics [104,105]. Pharmacokinetics addresses the biodistribution of drugs from the vascular space into organs and tissues of the body system and their removal from the body through metabolism and excretion over a period of time. The mode of distribution in the cells, tissues, and organs of the body system is an important characteristic of any drug formulation. Bio-distribution measurement provides key information about the presence or absence of a drug at a particular targeted site where the accumulation is desirable. In pharmacokinetic studies, the drug concentration in the plasma and all other connecting tissues is being analysed over time. This is to ascertain the extent of metabolism and excretion of the drug. This is achieved by serial collection and analysis of the blood samples. Additional labelling has to be employed for non-fluorescent drugs so that they can be detected in the blood. Radioactive labelling is the most widely used for this purpose [106]. However, PL-based detection of non-fluorescent drugs can be achieved by labelling with non-toxic QDs [107]. The use of QDs for drug labelling can, to a large extent, influence drug fate and ensure favourable pharmacokinetics. The size and surface characteristics of these QDs influence their performance as drug carriers; therefore, encapsulation of the hydrophobic drug in the QD-drug formulation may be necessary to improve the absorption and availability [108,109]. In addition, the rate of liver metabolism is reduced with the use of QDs thereby leading to prolonged retention, increased half-life, and reduced opsonisation [110].

The level of success or failure of any targeted therapy procedure can be ascertained if the biodistribution of the drug-QDs formulations is fully understood [111]. QDs-based labelling is preferred to radioactive labelling due to the following; (i) it provides a more reliable biodistribution profile, (ii) it permits monitoring of biodistribution in live animals, thereby reducing the number of animals required for analysis and also provide a more reliable data because the issue of animal to animal variation between time point would not arise, and (iii) side effect is minimised significantly [112–114]. In separate studies, the biodistribution of CIS/ZnS QDs/folic acid conjugated systems in major body organs over a period of time was carried out by Deng *et al.* [115] and Yong *et al.* (2010) [116]. The QDs-drug conjugate in both cases was intravenously injected into nude tumour-bearing mice. Deng *et al.* [115] observed a 4 h post-injection clearance at the liver and tumour sites for the nude mice bearing A549 tumours. Yong *et al.* [116] observed a 30 min clearance of the QDs conjugate at the liver and the spleen. In another work, Li *et al.* [117] studied the biodistribution of CIS/ZnS QDs in healthy mice after intravenous injection. The results (Figure 6) showed that most of the QDs are accumulated in the lung.

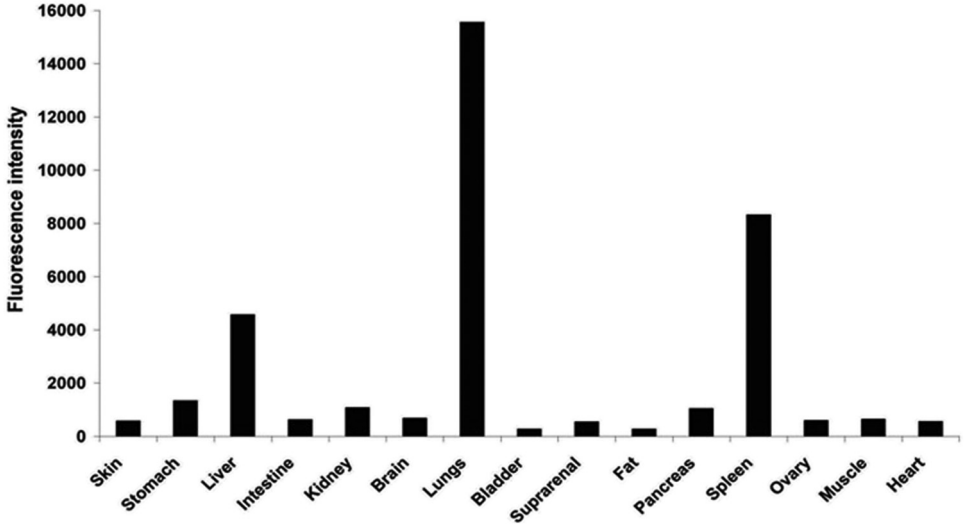


Figure 6: Biodistribution of CIS/ZnS QDs in major organs of a mouse after 24 h of administration; with permission from ref. [117]; ACS.

7 Toxicity concerns of ternary QDs

The toxicity of QDs remains a major obstacle for these set of nanoparticles to be used in clinical research. The toxic or non-toxic nature of QDs goes a long way in determining their intrinsic behaviour and characteristics. The main source of QDs toxicity has been ascribed to the release of heavy metal ions such as Cd^{2+}, Pb^{2+}, and Hg^{2+} which are present in their composition [56,118]. Other factors determining the toxicity or non-toxicity of QDs include size, colour, absence or presence of protective shells, behaviour of capping agents, chirality of capping agents, nature of the core, to mention a few [119,120]. This is of great concern to biomedical and biological researchers and has prompted the development of heavy metal ions-free QDs such as ternary I–III–VI QDs. Several studies have described ternary QDs as "non-toxic" [121–125]. Using histological imaging, Chen *et al.* [126] carried out the *in vitro* and *in vivo* immunotoxicity studies of $CuInS_2$ (PEGylated) using Dc 2.4 cell line and BALB/c mice, respectively. Their findings showed that PEGylated $CuInS_2$ QDs altered the function of Dc 2.4 *in vitro* but showed little or no toxicity to the immune system *in vivo*. This indicates that this set of PEGylated ternary QDs is biocompatible and has the potentials for bioapplications. In another recent development, our group evaluated the cytotoxicity of bare CIS/ZnS QDs and mTHPP porphyrin conjugated CIS/ZnS QDs on BHK 21 (normal fibroblast cell line) and THP-1(leukaemia cancer cell line) [127]. The study showed that both the bare and mTHPP porphyrin conjugated QDs have excellent biocompatibility towards BHK 21, while dosage-dependent toxicity was observed for the cancer cell lines (Figure 7).

The increase in toxicity for the QDs-porphyrin conjugate against the THP-1 cancer cell lines at higher dosage concentrations was attributed to the synergistic effect of the QDs and porphyrin.

Adequate surface passivation is required to improve quantum yield, ensure stability, and avoid surface defects when synthesizing QDs. The use of ZnS as shelling/passivation material has been widely accepted among diverse QD systems due to its large bandgap and ease of synthesis. Furthermore, studies have shown that ZnS shells reduce the core QDs toxicity in *in vitro* and *in vivo* application through the following mechanism; prevention of particle degradation, trapping of toxic ions and avoiding the formation of reactive oxygen stress (ROS) [128,129].

Using the Murine model, Kays *et al.* [130] carried out the *in vivo* biodistribution and cytotoxicity evaluation of bare CIS QDs, Zn-doped CIS (ZCIS) QDs, and ZnS passivated CIS (CIS/ZnS) QDs over a period of 1, 7, and 28 days. The result showed a quick clearance with about 25% of the CIS QDs remaining in the major organs (liver, kidney, and lungs) after 28 days. Organ index (organ weight divided by total body weight), a very sensitive method of determining toxicity, was used in measuring the organ-based toxicity of these QDs. The analysis showed an appreciable increase in the organ index of the liver and spleen for both bare CIS QDs and ZCIS QDs, while no significant increase was observed for CIS/ZnS QDs. The organ index for the kidney was not consistent: however, an appreciable difference in mass was observed at only one-time point each for CIS/ZnS (day 7) and CIS (day 28) QDs.

To have a clearer understanding of the organ-based toxicity of these QDs, the histopathology of the mouse was carried out. The organs' micrograph compared to the control is shown in Figure 8. Geographic necrosis was observed in the liver of the CIS-QDs-dosed mice, mild inflammation was noted in the liver of the ZCIS-

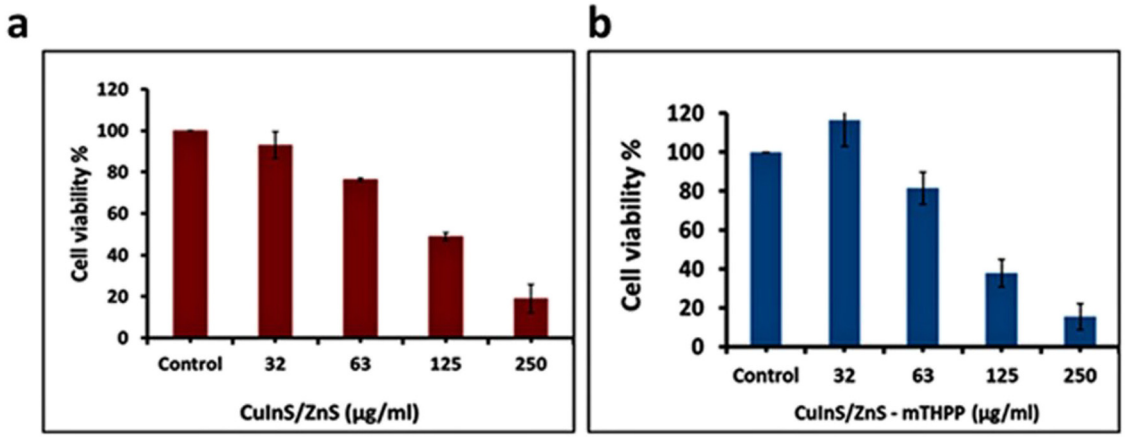


Figure 7: Cytotoxicity effect of: (a) CIS/ZnS QD and (b) CIS/ZnS–mTHPP conjugate against THP-1 cancer cells; with permission from ref. [127]; Springer Nature.

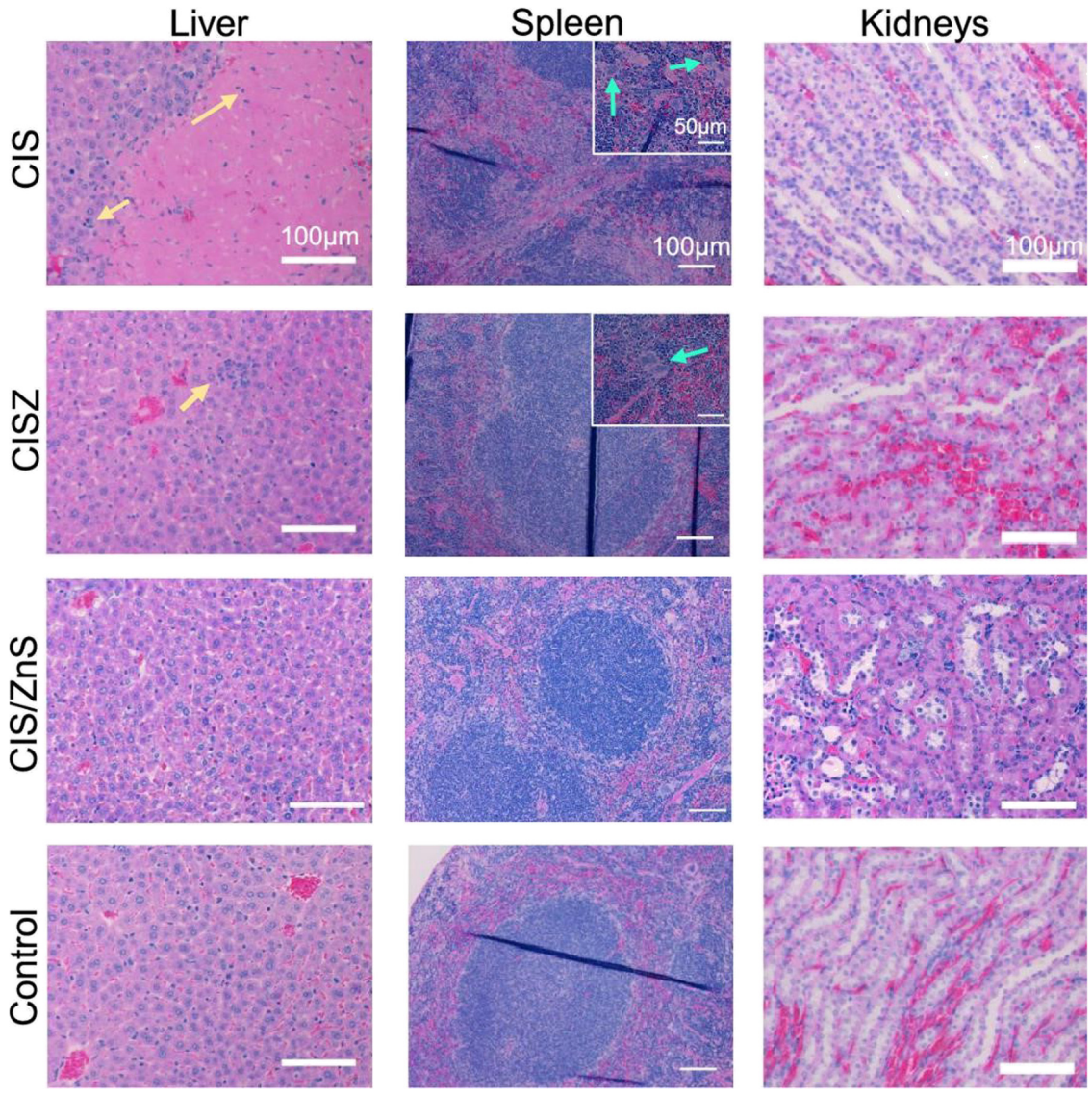


Figure 8: Micrograph of the organs (liver, spleen, and kidney) in the histopathological studies of QDs-dosed mice. The yellow arrow shows the inflammatory cells, while the blue arrow shows the multinucleated cells; with permission from ref. [129]; ACS.

QDs-dosed mice, whereas no inflammation was seen in the micrographs of the CIS/ZnSQDs-dosed mice and that of the control. In the CIS QDs and ZCIS QDs spleen, multinucleated giant cells were observed. This is an indication of an inflammatory response. Disruption of the tissue architecture was also noted in the CIS QDS spleens together with shrinkage of the pulps. No notable histological changes were observed in the kidneys for all the tested QDs. These results suggest that the non-toxic nature of ternary QDs is not only due to the absence of heavy toxic metals such as Cd and Pb but passivating with ZnS minimises the level of degradation, and subsequently the toxicity of the QDs.

A solid conclusion cannot be made regarding the toxicity of these TQDs because the toxicity is associated with many factors such as surface charge and modifications of the capping agents, as mentioned earlier. Therefore, more investigations are still required concerning the toxicity of these materials.

8 Conclusion and future outlook

The use of ternary QDs as an alternative to the Cd, Hg, and Pb-based QD due to their non-toxic nature have gained much attention. The ease of tuning their PL toward the NIR by altering their compositions has given it much attention among researchers. These desirable properties encourage their use in diverse biological and

biomedical applications such as biosensing, bioimaging, and cancer treatment procedures such as PDT and PTT. In this study, we discussed the recent progress in the use of ternary QDs in various biological and biomedical applications. Recent development relating to ternary QDs therapeutic and diagnostic applications is described. The use of these sets of QDs in energy transfer systems such as FRET, biophotonic applications such as biosensing and bioimaging, and their nanomedical applications is also discussed in this review.

The combination of ternary QDs with contrast agents such as radioactive labels for multi-imaging and multimodal purposes will be helpful in understanding the complex nature of tumours and other diseases, therefore, enhancing their treatment and management. The possibility of developing new multi-modal probes through the integration of ternary QDs with suitable contrasting agents is a research area that can be fully exploited in the near future. The biodistribution and toxicity should be fully understood with respect to the size, shape, surface properties, and manner of administration in the respective animal models. The retention time of these QDs in relation to the presence and absence of targeting ligands should also be fully understood. Even though the ternary QDs are devoid of toxic metals such as Cd, Pb, Hg *etc*., their other constituents *i.e*. In and Se are not totally benign in living systems [112]. Critical assessment of their genotoxicity, immunotoxicity, and pharmacokinetics still need to be carried out before they can be employed in humans. Furthermore, comparative studies of their toxicity with other forms of toxic metals-free QDs such as graphene and silicon need to be extensively carried out. Their toxicological effect in various biological model is very important and need to be urgently developed. Also, the multiple methods used in evaluating the toxicity profiles of QDs make the comparison of the results challenging. The existence of a uniform and standard method of determining the toxicity of these QDs will be appreciated in biological and biomedical research. However, surface modifiers such as peptides, biocompatible polymers, DNA/RNA can be employed to further reduce their toxicity. Researchers also need to have a full understanding of the mechanism of these QDs in living systems so that their clinical applications will be achieved soon. In addition, the proper understanding of the mechanism will ensure the control of their optical and electronic properties such as PL, QY *etc*., which will give rise to more biological applications. For emission dependent applications such as bioimaging and sensing, a reduction in photoluminescence line width will be advantageous. In the near future, attention should be given to developing ternary QDs-based therapeutic and imaging methods for the treatment of malignant cancer cells.

Acknowledgments: The authors would like to thank the National Research Foundation (N.R.F) under its Competitive Programme for Rated Researchers (CPRR), Grants No. 129290, the University of Johannesburg (URC) and the Faculty of Science (FRC) for financial support. OAA also thanks Covenant University, Ota, Ogun State, Nigeria, for allowing him to take up a post-doctoral fellowship at the Department of Chemical Sciences, NANOBEW research group, University of Johannesburg under the supervision of Prof. S.O. Oluwafemi.

Funding information: The authors would like to thank the National Research Foundation (N.R.F) under its Competitive Programme for Rated Researchers (CPRR), Grants No. 129290, the University of Johannesburg (URC) and the Faculty of Science (FRC) for financial support.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

References

[1] Soheyli E, Azad D, Sahraei R, Hatmnia AA, Alinazari M. Synthesis and optimization of emission characteristics of water-dispersible Ag-In-S quantum dots and their bactericidal activity. Colloids Surf B Biointerfaces. 2019;182:110389.

[2] Zhou J, Liu Y, Tang J, Tang W. Surface ligands engineering of semiconductor quantum dots for chemosensory and biological applications. Mater Today. 2017;20:360–76.

[3] Maluleke R, Sakho EHM, Oluwafemi OS. Aqueous synthesis of glutathione-capped $CuInS_2$/ZnS quantum dots-graphene oxide nanocomposite as fluorescence "switch OFF" for explosive detection. Mater Lett. 2020;269:127669. doi: 10.1016/j.matlet.2020.127669.

[4] Jawhar NN, Soheyli E, Yazici AF, Mutlugun E, Sahraei R. Preparation of highly emissive and reproducible Cu–In–S/ZnS core/shell quantum dots with a mid-gap emission character. J Alloy Compd. 2020;824:153906.

[5] Mohamed W, Abd El-Gawad H, Mekkey S, Galal H, Handal H, Mousa H, et al. Quantum dots synthetization and future prospect applications. Nanotechnol Rev. 2021;10(1):1926–40.

[6] Bai X, Purcell-Milton F, Gun'ko YK. Optical properties, synthesis, and potential applications of Cu-based ternary or

quaternary anisotropic quantum dots, polytypic nanocrystals, and core/shell heterostructures. Nanomaterials (Basel). 2019;9(1):85. doi: 10.3390/nano9010085.
[7] Gil HM, Price TW, Chelani K, Bouillard JG, Calaminus SDJ, Stasiuk GJ. NIR-quantum dots in biomedical imaging and their future. iScience. 2021;24:102189.
[8] Toufanian R, Piryatinski A, Mahler AH, Iyer R, Hollingsworth JA, Dennis AM. Bandgap engineering of indium phosphide-based core/shell heterostructures through shell composition and thickness. Front Chem. 2018;6:567.
[9] Geng B, Yang D, Pan D, Wang L, Zheng F, Shen W, et al. NIR-responsive carbon dots for efficient photothermal cancer therapy at low power densities. Carbon. 2018;134:153–62.
[10] Raievska O, Stroyuk O, Dzhagan V, Solonenko D, Zahn RTD. Ultra-small aqueous glutathione-capped Ag–In–Se quantum dots: luminescence and vibrational properties. RSC Adv. 2020;10:42178–93.
[11] Tsolekile N, Nelena S, Oluwafemi OS. Porphyrin as diagnostic and therapeutic agent. Molecules. 2019;24:2669.
[12] Hsu JC, Huang CC, Ou KL, Lu N, Mai FD, Chen JK, et al. Silica nanohybrids integrated with $CuInS_2$/ZnS quantum dots and magnetite nanocrystals: multifunctional agents for dual-modality imaging and drug delivery. J Mater Chem. 2011;21:19257–66.
[13] Aladesuyi OA, Oluwafemi OS. Synthesis strategies and application of ternary quantum dots-in cancer therapy. Nano-Structures Nano-Objects. 2020;24:100568.
[14] Smith M, Nie S. Chemical analysis and cellular imaging with quantum dots. Analyst. 2004;129:672–7. doi: http://dx.doi.org/10.1039/B404498N.
[15] Girma WM, Dehvari K, Ling YC, Chang JY. Albumin-functionalized $CuFeS_2$/photosensitizer nanohybrid for single-laser-induced folate receptor-targeted photothermal and photodynamic therapy. Mater Sci Eng C Mater Biol Appl. 2019;101:179–89.
[16] Dai M, Ogawa S, Kameyama T, Okazaki K, Kudo A, Kuwabata S, et al. Tunable Photoluminiscence from the visible to near infra-red wavelength region of non-stoichiometric $AgInS_2$ nanoparticles. J Mater Chem. 2012;22:12851–8.
[17] Gawande MB, Goswami A, Felpin FX, Asefa T, Huang X, Silva R, et al. Cu and Cu based nanoparticles: synthesis and application in catalysis. Chem Rev. 2016;116:3722–811.
[18] Bharti S, Kaur G, Jain S, Gupta S, Tripathi SK. Characteristics and mechanism associated with drug conjugated inorganic nanoparticles. J Drug Target. 2019;27:813–29.
[19] Yue L, Li H, Liu Q, Guo D, Chen J, Sun Q, et al. Manganese-doped carbon quantum dots for fluorometric and magnetic resonance (dual mode) bioimaging and biosensing. Microchim Acta. 2019;186(5):315.
[20] Chen B, Zhong H, Zhang W, Tan Z, Li Y, Yu C, et al. Highly Emissive and Color-Tunable $CuInS_2$-Based Colloidal Semiconductor Nanocrystals: Off-Stoichiometry Effects and Improved Electroluminescence Performance. Adv Funct Mater. 2012;22:2081–8.
[21] Mohan CN, Renuga V, Manikandan A. Influence of silver precursor concentration on structural, optical and morphological properties of $Cu_{1-x}Ag_xInS_2$ semiconductor nanocrystals. J Alloy Compd. 2017;729:407–17.
[22] Algar WR, Stewart MH, Scott AM, Moon WJ, Medintz IL. Quantum dots as platforms for charge transfer-based biosensing: challenges and opportunities. J Mater Chem B. 2014;2:7816–27.
[23] Chuang PH, Lin CC, Liu RS. Emission-tunable $CuInS_2$/ZnS quantum dots: structure, optical properties, and application in white light-emitting diodes with high color rendering index. ACS Appl Mater Interfaces. 2014;6:15379–87.
[24] Varghese JR, Oluwafemi OS. The photoluminescence and biocompatibility of $CuInS_2$-based ternary quantum dots and their biological applications. Chemosensors. 2020;8:101.
[25] Jain S, Bharti S, Bhullar GK, Tripathi SK. I–III–VI core/shell QDs: synthesis, characterizations and applications. J Lumin. 2020;219:116912.
[26] Van der Stam W, Berends AC, de Mello Donega C. Prospects of colloidal copper chalcogenide nanocrystals. ChemPhysChem. 2016;17:559–81.
[27] Kang X, Yang Y, Huang L, Tao Y, Wang L, Pan D. Large-scale synthesis of water-soluble $CuInSe_2$/ZnS and $AgInSe_2$/ZnS core/shell quantum dots. Green Chem. 2015;17:4482–8.
[28] Chen Y, Li S, Huang L, Pan D. Green and facile synthesis of water-soluble Cu–In–S/ZnS core/shell quantum dots. Inorg Chem. 2013;52:7819–21.
[29] Mir IA, Das K, Akhter T, Ranjan R, Patel R, Bohidar HB. Eco-friendly synthesis of $CuInS_2$ and $CuInS_2$@ZnS quantum dots and their effect on enzyme activity of lysozyme. RSC Adv. 2018;8:30589–99.
[30] Xia C, Winckelmans N, Prins PT, Bals S, Gerritsen HC, De Mello Donegá C. Near-infrared-emitting $CuInS_2$/ZnS dot-in-rod colloidal heteronanorods by seeded growth. J Am Chem Soc. 2018;140(17):5755–63.
[31] Girma WM, Fahmi MZ, Permadi A, Abate MA, Chang JY. Synthetic strategies and biomedical applications of I–III–VI ternary quantum dots. J Mater Chem B. 2017;5:6193–216.
[32] Lakowicz JR. Principles of fluorescence spectroscopy. 3rd edn. New York, NY, USA: Springer; 2006. p. 1–954.
[33] Shrestha D, Jenei A, Nagy P, Vereb G, Szöllősi J. Understanding FRET as a research tool for cellular studies. Int J Mol Sci. 2015;16(4):6718–56.
[34] Hillisch A, Lorenz M, Diekmann S. Recent advances in FRET: distance determination in protein—DNA complexes. Curr Opin Struct Biol. 2001;11:201–7.
[35] Muñoz R, Santos EM, Galan-Vidal CA, Miranda JM, Lopez-Santamarina A, Rodriguez JA. Ternary quantum dots in chemical analysis. Synthesis and detection mechanisms. Molecules. 2021;26(9):2764.
[36] Schiffman JD, Balakrishna RG. Quantum dots as fluorescent probes: synthesis, surface chemistry, energy transfer mechanisms, and applications. Sens Actuators B Chem. 2018;258:1191–214.
[37] Chou K, Dennis A. Förster resonance energy transfer between quantum dot donors and quantum dot acceptors. Sensors. 2015;15(6):13288–325.
[38] Lucky SS, Soo KC, Zhang Y. Nanoparticles in photodynamic therapy. Chem Rev. 2015;115:1990–2042.
[39] Long F, Gu C, Gu AZ, Shi H. Quantum Dot/Carrier–Protein/Haptens conjugate as a detection nanobioprobe for FRET-based immunoassay of small analytes with all-fiber microfluidic biosensing platform. Anal Chem. 2012;84:3646–53.

[40] Sapsford KE, Berti L, Medintz IL. Materials for FRET analysis: beyond traditional donor–acceptor combinations. Angew Chem, Int Ed. 2006;45:4562–89.
[41] Abbasi AZ, Amin F, Niebling T, Friede S, Ochs M, Carregal-Romero S, et al. How colloidal nanoparticles could facilitate multiplexed measurements of different analytes with analyte-sensitive organic fluorophores. ACS Nano. 2011;5:21–5.
[42] Kuznetsova V, Tkach A, Cherevkov S, Sokolova A, Gromova Y, Osipova V, et al. Spectral-time multiplexing in FRET complexes of $AgInS_2$/ZnS quantum dot and organic dyes. Nanomaterials. 2020;10(8):1569.
[43] Wegner KD, Hildebrandt N. Quantum dots: bright and versatile *in vitro* and *in vivo* fluorescence imaging biosensors. Chem Soc Rev. 2015;44:4792–834.
[44] Xing H, Wei T, Lin X, Dai Z. Near-infrared MnCuInS/ZnS@BSA and urchin-like Au nanoparticle as a novel donor-acceptor pair for enhanced FRET biosensing. Anal Chim Acta. 2018;1042:71–8.
[45] Xia C, Wang W, Du L, Rabouw FT, van den Heuvel DJ, Gerritsen HC, et al. Forster Resonance Energy Transfer between colloidal $CuInS_2$/ZnS quantum dots and dark quenchers. J Phys Chem C. 2020;124(2):1717–31.
[46] Oluwafemi SO, Sakho EHM, Parani S, Lebepe TC. Chapter 7 in ternary quantum dots - synthesis, properties, and applications. Bioimaging and therapeutic applications of ternary quantum dots. Cambridge: Woodhead publishing; 2021. p. 155–206.
[47] Yao J, Li P, Lin L, Yang M. Biochemistry and biomedicine of quantum dots: from biodetection to bioimaging, drug discovery, diagnostics, and therapy. Acta Biomater. 2018;74:36–55.
[48] Tsolekile N, Parani S, Matoetoe MC, Songca SP, Oluwafemi OS. Evolution of ternary I–III–VI QDs: synthesis, characterization and application. Nano-Struct Nano-Objects. 2017;12:46–56.
[49] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol. 2015;33:941–51.
[50] Zhao MX, Zhu B. The research and applications of quantum dots as nano-carriers for targeted drug delivery and cancer therapy. Nanoscale Res Lett. 2016;11:8–19.
[51] Zhao C, Bai Z, Liu X, Zhang Y, Zou B, Zhong H. Small GSH-Capped $CuInS_2$ quantum dots: MPA-assisted aqueous phase transfer and bioimaging applications. ACS Appl Mater Inter. 2015;7:17623–9.
[52] Bai XL, Wang SG, Xu SY, Wang LY. Luminescent nanocarriers for simultaneous drug or gene delivery and imaging tracking. TrAC-Trends. Anal Chem. 2015;73:54–63.
[53] Wu PJ, Ou KL, Chen JK, Fang HP, Tzing SH, Lin WX, et al. Methotrexate-conjugated $AgInS_2$/ZnS quantum dots for optical imaging and drug delivery. Mater Lett. 2014;128:412–6.
[54] Ruzycka-Ayoush M, Kowalik P, Kowalczyk A, Bujak P, Nowicka AM, Wojewodzka, et al. Quantum dots as targeted doxorubicin drug delivery nanosystems in human lung cancer cells. Cancer Nano. 2021;12:8.
[55] Lin Z, Ma Q, Fei X, Zhang H, Su X. A novel aptamer functionalized $CuInS_2$ quantum dots probe for daunorubicin sensing and near infrared imaging of prostate cancer cells. Anal Chem Acta. 2014;818:54–60.
[56] Lin WJ, Chien WH. Peptide-conjugated micelles as a targeting nanocarrier for gene delivery. J Nanopart Res. 2015;17:349–9.
[57] Mintzer MA, Simanek EE. Nonviral vectors for gene delivery. Chem Rev. 2009;109(2):259–302.
[58] Chen G, Roy I, Yang C, Prasad PN. Nanochemistry and nanomedicine for nanoparticle-based diagnostics and therapy. Chem Rev. 2016;116:2826–85.
[59] Prasad PN. Introduction to nanomedicine and nanobioengineering. Hoboken, N.J: John Wiley & Sons; 2012. Print.
[60] Hyde SC, Gill DR, Higgins CF, Trezise AE, Macvinish LJ, Cuthbert AW, et al. Correction of the ion-transport defect in cystic-fibrosis transgenic mice by gene-therapy. Nature. 1993;362:250–5.
[61] Liang Y, Liu Z, Shuai X, Wang W, Liu J, Bi W, et al. Delivery of cationic polymer-siRNA nanoparticles for gene therapies in neural regeneration. Biochem Biophys Res Commun. 2012;421:690–5.
[62] Wei H, Volpatti LR, Sellers DL, Maris DO, Andrews IW, Hemphill AS, et al. Dual responsive, stabilized nanoparticles for efficient *in vivo* plasmid delivery. Angew Chem. 2013;52(20):5377–81.
[63] Zhang Y, Wang TH. Quantum dot enabled molecular sensing and diagnostics. Theranostics. 2012;2:631–54.
[64] Sanpui P, Pandey SB, Chattopadhyay A, Ghosh SS. Incorporation of gene therapy vector in chitosan stabilized Mn^{2+}-doped ZnS quantum dot. Mater Lett. 2010;64:2534–7.
[65] Zhao J, Qiu X, Wang Z, Pan J, Chen J, Han J. Application of quantum dots as vectors in targeted Survivin Gene siRNA Delivery. OncoTargets Ther. 2013;6:303309.
[66] Yezhelyev MV, Qi L, O'Regan RM, Nie S, Gao X. Proton-sponge coated quantum dots for siRNA delivery and intracellular imaging. J Am Chem Soc. 2008;130(28):9006–12.
[67] Yang Y, Wang J, Li X, Lin X, Yue X. A near infrared fluorescent/ultrasonic bimodal contrast agent for imaging guided pDNA delivery via ultrasound targeted microbubble destruction. RSC Adv. 2015;5(11):8404–14.
[68] Subramaniam P, Lee SJ, Shah S, Patel S, Starovoytov V, Lee KB. Generation of a library of non-toxic quantum dots for cellular imaging and siRNA delivery. Adv Mater. 2012;24(29):4014–9.
[69] Prasad PN. Introduction to biophotonics. Hoboken, NJ: Wiley-Interscience; 2003.
[70] Yong KT. Quantum dots for biophotonics. Theranostics. 2012;2(7):629–30.
[71] Vu TQ, Lam WY, Hatch EW, Lidke DS. Quantum dots for quantitative imaging: from single molecules to tissue. Cell Tissue Res. 2015;360:71–86.
[72] Chen H, Zhang M, Li B, Chen D, Dong X, Wang Y, et al. Versatile antimicrobial peptide-based ZnO quantum dots for *in vivo* bacteria diagnosis and treatment with high specificity. Biomaterials. 2015;53:532–44.
[73] Wang F, Gu Z, Lei W, Wang W, Xia X, Hao Q. Graphene quantum dots as a fluorescent sensing platform for highly efficient detection of Copper (II) ions sensor. Actuat B-Chem. 2014;190:516–22.
[74] Benítez-Martínez S, Valcárcel M. Graphene quantum dots in analytical science. TracTrend Anal Chem. 2015;72:93–113.
[75] Li Y, Li Z, Wang X, Liu F, Cheng Y, Zhang B, et al. *In vivo* cancer targeting and imaging-guided surgery with near infrared-

emitting quantum dot bioconjugates. Theranostics. 2012;2:769–76.
[76] Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater. 2005;4:435–46.
[77] Xing Y, Chaudry Q, Shen C, Kong KY, Zhau HE, Chung LW, et al. Bioconjugated quantum dots for multiplexed and quantitative immunohistochemistry. Nat Protoc. 2007;2(5):1152–65.
[78] Tanaka E, Choi HS, Fujii H, Bawendi MG, Frangioni JV. Image-guided oncologic surgery using invisible light: completed pre-clinical development for sentinel lymph node mapping. Ann Surg Oncol. 2006;13(12):1671–81.
[79] Ruan G, Agrawal A, Marcus AI, Nie S. Imaging and tracking of tat peptide-conjugated quantum dots in living cells: new insights into nanoparticle uptake, intracellular transport, and vesicle shedding. J Am Chem Soc. 2007;129:14759–66.
[80] Liu L, Hu R, Roy I, Lin G, Ye L, Reynolds JL, et al. Synthesis of luminescent near-infrared $AgInS_2$ nanocrystals as optical probes for *in vivo* applications. Theranostics. 2013;3(2):109–15.
[81] Nam DE, Song WS, Yang H. Facile, air-insensitive solvothermal synthesis of emission-tunable $CuInS_2$/ZnS quantum dots with high quantum yields. J Mater Chem. 2011;21(45):18220–6.
[82] Loghina L, Chylii M, Kaderavkova A, Slang S, Svec P, Pereira JR, et al. Tunable optical performance in nanosized $AgInS_2$-ZnS solid solution heterostructures due to the precursor's ratio modification. Optical Mater Exp. 2021;11:539–50.
[83] Chinnathambi S, Shirahata N. Recent advances on fluorescent biomarkers of near-infrared quantum dots for *in-vitro* and *in-vivo* imaging. Sci Technol Adv Mater. 2019;20:337–55.
[84] Deng D, Chen Y, Cao J, Tian J, Qian Z, Achilefu S, et al. High-quality $CuInS_2$/ZnS quantum dots for *in vitro* and *in vivo* Bioimaging. Chem Mater. 2012;24:3029–303.
[85] Tan L, Liu S, Li X, Chronakis IS, Shen YA. New strategy for synthesizing $AgInS_2$ quantum dots emitting brightly in near-infrared window for *in vivo* imaging. Colloid Surf B. 2015;125:222–9.
[86] Liu S, Na W, Pang S, Shi F, Su X. A label-free fluorescence detection strategy for lysozyme assay using $CuInS_2$ quantum dots. Analyst. 2014;139(12):3048–54.
[87] Tang D, Liao D, Zhu Q, Wang F, Jiao H, Zhang Y, et al. Fluorescence turn-on detection of a protein through the displaced single-stranded DNA binding protein binding to a molecular beacon. Chem Commun. 2011;47:5485–7.
[88] Olguín HJ, Guzmán DC, García EH, Mejía GB. The role of dopamine and its dysfunction as a consequence of oxidative stress. Oxid Med Cell Longev. 2016;2016:9730467.
[89] Xiong WW, Yang GH, Wu XC, Zhu JJ. Microwave-assisted synthesis of highly luminescent $AgInS_2$/ZnS nanocrystals for dynamic intracellular Cu(II) detection. J Mater Chem B 1. 2013;1(33):4160–5.
[90] Kobayashi H, Longmire MR, Ogawa M, Choyke PL. Rational chemical design of the next generation of molecular imaging probes based on physics and biology: mixing modalities, colors and signals. Chem Soc Rev. 2011;40(9):4626–48.
[91] Louie A. Multimodality imaging probes: design and challenges. Chem Rev. 2010;110(5):3146–95.
[92] Jing L, Ding , KKershaw, Kempson SV, Rogach IM, Gao AL, M. Magnetically engineered semiconductor quantum dots as multimodal imaging probes. Adv Mater. 2014;26(37):6367–86.
[93] Jennings LE, Long NJ. 'Two is better than one' – probes for dual-modality molecular imaging. Chem Commun. 2009;24:3511.
[94] Gao D, Zhang P, Sheng Z, Hu D, Gong P, Chen C, et al. Highly bright and compact alloyed quantum rods with near infrared emitting: a potential multifunctional nanoplatform for multimodal imaging *in vivo*. Adv Funct Mater. 2014;24:3897–905.
[95] Guo W, Yang W, Wang Y, Sun X, Liu Z, Zhang B, et al. Color-tunable Gd-ZnCu-in-S/ZnS quantum dots for dual modality magnetic resonance and fluorescence imaging. Nano Res. 2014;7:1581–91.
[96] Sitbon G, Bouccara S, Tasso M, Francois A, Bezdetnaya L, Marchal, et al. Multimodal Mn-doped I–III–VI quantum dots for near infrared fluorescence and magnetic resonance imaging: from synthesis to *in vivo* application. Nanoscale. 2014;6(15):9264–72.
[97] Cheng CY, Ou KL, Huang WT, Chen JK, Chang JY, Yang CH. Gadolinium-based $CuInS_2$/ZnS nanoprobe for dual-modality magnetic resonance/optical imaging. ACS Appl Mater Interfaces. 2013;5(10):4389–400.
[98] Shen J, Li Y, Zhu Y, Yang X, Yao X, Li J, et al. Multifunctional gadolinium-labeled silica-coated Fe_3O_4 and $CuInS_2$ nanoparticles as a platform for *in vivo* tri-modality magnetic resonance and fluorescence imaging. J Mater Chem B. 2015;3:2873–82.
[99] Ding K, Jing L, Liu C, Hou Y. Magnetically engineered Cd-free quantum dots as dual-modality probes for fluorescence/magnetic resonance imaging of tumors. Biomaterials. 2014;35:1608–161.
[100] Fahmi MZ, Ou KL, Chen JK, Ho MH, Tzing SH, Chang JY. Development of bovine serum albumin-modified hybrid nanoclusters for magnetofluorescence imaging and drug delivery. RSC Adv. 2014;4:32762–72.
[101] Jiang X, Zhang S, Ren F, Chen L, Zeng J, Zhu M, et al. Ultrasmall magnetic $CuFeSe_2$ ternary nanocrystals for multimodal imaging guided photothermal therapy of Cancer. ACS Nano. 2017;11(6):5633–45. doi: 10.1021/acsnano.7b01032.
[102] Yang Y, Lin L, Jing L, Yue X, Dai Z. CuInS2/ZnS quantum dots conjugating Gd (III) chelates for near-infrared fluorescence and magnetic resonance bimodal imaging. ACS Appl Mater Interfaces. 2017;9(28):23450–7.
[103] Zhang J, Hao G, Yao C, Hu S, Hu B, Zhang B. Paramagnetic albumin decorated CuInS2/ZnS QDs for $CD133^+$ glioma bimodal MR/fluorescence targeted imaging. J Mater Chem B. 2016;4(23):4110–8.
[104] Currie GM. Pharmacology, part 2: introduction to pharmacokinetics. J Nucl Med Technol. 2018;46:221–30.
[105] Ratain MJ, Plunkett WK. Principles of pharmakinetics. In: Kufe DW, Pollock RE, Weichselbaum RR, et al. editors. Holland-Frei cancer medicine. 6th edn. Hamilton (ON): BC Decker; 2003.
[106] Gordi T, Baillie R, Vuong le T, Abidi S, Dueker S, Vasquez H, et al. Pharmacokinetic analysis of 14C-ursodiol in newborn infants using accelerator mass spectrometry. J Clin Pharmacol. 2014;54:1031–7.

[107] Zhu C, Chen Z, Gao S, Goh BL, Samsudin IB, Lwe KW, et al. Recent advances in non-toxic quantum dots and their biomedical applications. Prog Nat Sci Mater Int. 2019;29:628–40.
[108] Termsarasab U, Yoon IS, Park JH, Moon HT, Cho HJ, Kim DD. Polyethylene glycol-modified arachidyl chitosan-based nanoparticles for prolonged blood circulation of doxorubicin. Int J Pharm. 2014;464:127–34.
[109] Wang X, Yang C, Wang C, Guo L, Zhang T, Zhang Z, et al. Polymeric micelles with α-glutamyl-terminated PEG shells show low non-specific protein adsorption and a prolonged *in vivo* circulation time. Mat Sci Eng C-Mater. 2016;59:766–72.
[110] Zhang RX, Li J, Zhang T, Amini MA, He C, Lu B, et al. Importance of integrating nanotechnology with pharmacology and physiology for innovative drug delivery and therapy – an illustration with firsthand examples. Acta Pharmacol Sin. 2018;39:825–44.
[111] Yaghini E, Turner HD, LeMarois AM, Suhling K, Naasani I, MacRobert AJ. *In vivo* biodistribution studies and *ex vivo* lymph node imaging using heavy metal-free quantum dots. Biomaterials. 2016;104:182–91.
[112] Muhanna N, MacDonald TD, Chan H, Jin CS, Burgess L, Cui L, et al. Multimodal nanoparticle for primary tumor delineation and lymphatic metastasis mapping in a head-and-neck cancer rabbit model. Adv Healthc Mater. 2015;4:2164–9.
[113] Kim SH, Lee JH, Hyun H, Ashitate Y, Park G, Robichaud K, et al. Near-infrared fluorescence imaging for noninvasive trafficking of scaffold degradation. Sci Rep. 2013;3:1198–8.
[114] Xu G, Zeng S, Zhang B, Swihart MT, Yong K, Prasad PN. New generation cadmium-free quantum dots for biophotonics and nanomedicine. Chem Rev. 2016;116:12234–327.
[115] Deng D, Qu L, Zhang J, Ma Y, Gu Y. Quaternary Zn–Ag–In–Se quantum dots for biomedical optical imaging of RGD-Modified micelles. ACS Appl Mat Interf. 2013;5:10858–65.
[116] Yong KT, Roy I, Hu R, Ding H, Cai H, Zhu J, et al. Synthesis of ternary $CuInS_2$/ZnS quantum dot bioconjugates and their applications for targeted cancer bioimaging. Integr Biol. 2010;2:121–9.
[117] Li L, Daou TJ, Texier I, Kim Chi TT, Liem NQ, Reiss P. Highly luminescent $CuInS_2$/ZnS core/shell nanocrystals: cadmium-free quantum dots for *in vivo* imaging. Chem Mater. 2009;21:2422–9.
[118] Yong KT, Law WC, Hu R, Ye L, Liu L, Swihart MT, et al. Nanotoxicity assessment of quantum dots: from cellular to primate studies. Chem Soc Rev. 2013;42:1236–50.
[119] Maluleke R, Parani S, Oluwafemi OS. Preparation of graphene oxide-$CuInS_2$/ZnS Quantum dots nanocomposite as "Turn-On" Fluorescent probe for the detection of polycyclic aromatic hydrocarbons in aqueous medium. J Fluoresc. 2021;31:1297–302.
[120] Martynenko IV, Baimuratov AS, Weigert F, Soares JX, Dhamo L, Nickl P, et al. Photoluminescence of Ag-In-S/ZnS quantum dots: excitation energy dependence and low-energy electronic structure. Nano Res. 2019;12:1595–603.
[121] Trapiella-Alfonso L, Pons T, Lequeux N, Leleu L, Grimaldi J, Tasso M, et al. Clickable-Zwitterionic copolymer capped-quantum dots for *in vivo* fluorescence tumor imaging. ACS Appl Mater Interfaces. 2018;514:17107–16.
[122] Pons T, Pic E, Lequeux N, Cassette E, Bezdetnaya L, Dubertret B. Cadmium-free 516 CuInS 2/ZnS quantum dots for sentinel lymph node imaging with reduced toxicity. ACS Nano. 2010;5174(5):2531–8.
[123] Helle M, Cassette E, Bezdetnaya L, Pons T, Leroux A, Plénat F, et al. Visualisation of sentinel lymph node with Indium-based near infrared emitting quantum dots in a murine metastatic breast cancer model. PLoS One. 2012;7(8):521.
[124] Long X, Zhang F, He Y, Hou S, Zhang B, Zou G. Promising anodic 529 electrochemiluminescence of nontoxic core/shell CuInS2/ZnS nanocrystals in aqueous 530 medium and its biosensing potential. Anal Chem. 2018;90(5):3563–9.531.
[125] Wang Y, Hu R, Lin G, Roy I, Yong KT. Functionalized quantum dots for biosensing and bioimaging and concerns on toxicity. ACS Appl Mater Interf. 2013;5:2786–99.
[126] Chen T, Li L, Lin X, Yang Z, Zou W, Chen Y, et al. *In vitro* and *in vivo* immunotoxicity of PEGylated Cd-free $CuInS_2$/ZnS quantum dots. Nanotoxicology. 2020;14(3):372–87.
[127] Tsolekile N, Nahle S, Zikalala N, Parani S, Sakho E, Joubert O, et al. Cytotoxicity, fluorescence tagging and gene-expression study of CuInS/ZnS QDS-meso (hydroxyphenyl) porphyrin conjugate against human monocytic leukemia cells. Sci Rep. 2020;10(1):4936.
[128] Tang Y, Han S, Liu H, Chen X, Huang L, Li X, et al. The role of surface 539 chemistry in determining *in vivo* biodistribution and toxicity of CdSe/ZnS core-shell 540 quantum dots. Biomaterials. 2013;34(34):8741–55.
[129] Ye L, Yong KT, Liu L, Roy I, Hu R, Zhu J, et al. A pilot study in non-human primates shows no adverse response to 544 intravenous injection of quantum dots. Nat Nanotechnol. 2012;7(7):453–458. 545.
[130] Kays JC, Saeboe AM, Toufanian R, Kurant DE, Dennis AM. Shell-free copper indium sulfide quantum dots induce toxicity *in vitro* and *in vivo*. Nano Lett. 2020;202020(3):1980–91.