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Metal, Metal Oxides and Metal Sulphides for Biomedical Applications

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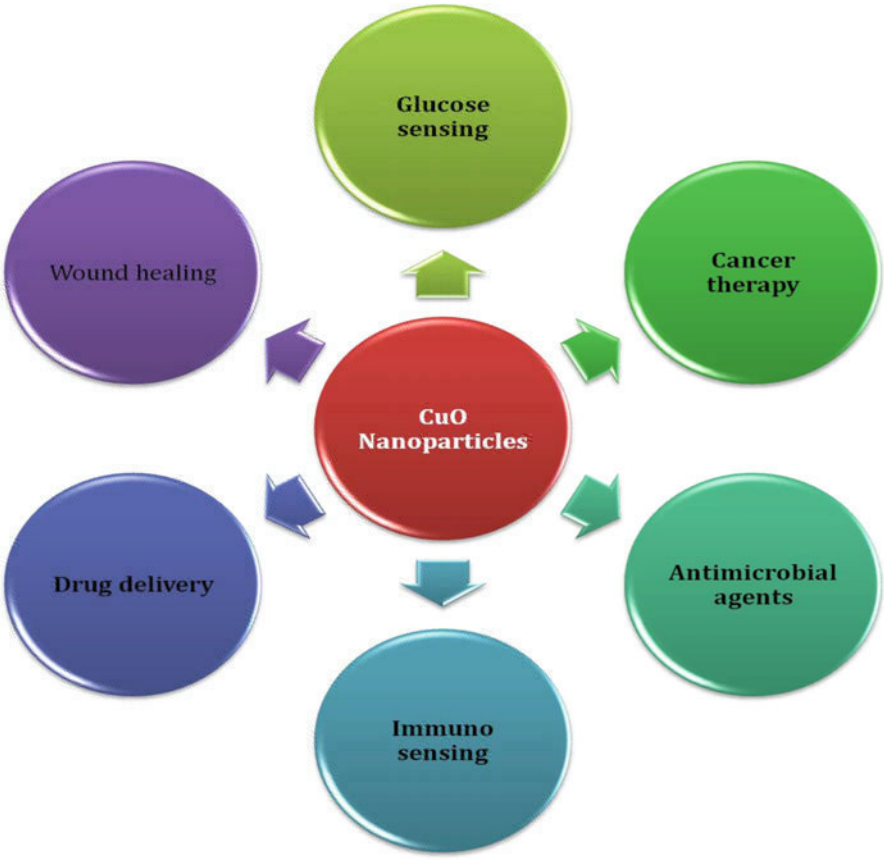
Preface

In the history of science, we often find that the study of some natural phenomenon has been the starting point in the development of a new branch of knowledge

Sir. C. V. Raman

The design of new materials for improved healthcare has always been at the core of medicinal research. The recent discovery of nanomaterials of unprecedented properties due to their nanosize opened a new vista for inventing new approaches for bioengineering, diagnosis, and therapy of many diseases. This book reviews the synthesis, toxicity, biosensing, and therapeutic use of metals, metal oxides, and metal sulphides. The chapters detail four main areas: functional nanoparticles for therapies, nanomaterials for bioimaging, biosensors for monitoring diseases, and green synthesis of advanced materials.

Chapter 1 by Joicy and Thangadurai reviews the applications of sulfide nanomaterials for bioimaging and biosensing, with focus on fluorescence, magnetic resonance, and acoustics. Here, sulfide-based chalcogenide materials appear non-toxic and highly performing in terms of optical properties. Biomedical applications of Ti alloys, for example, in dentistry, are presented by Rokaya et al. in Chap. 2, with emphasis on surface, mechanical, corrosion, and biological properties. Medicinal applications of metal complexes are discussed in Chap. 3 by Prasad et al. Chapter 4 by Parsanathan and Jagadeeshan explains the links between various metals, for example, Zn, Cr, Cu, Mn, Mg, and Fe, and the metabolic syndrome causing diabetes, obesity, and cardiovascular diseases. Antimicrobials based on metal and metal oxide–based nanomaterials are reviewed in Chap. 5 by Nas et al.



Application of CuO nanoparticles in the fields of medicine and biology (Chap. 9)

Cancer diagnosis and treatment benefits from new nanoparticles made of metals, metal oxides, and metal sulphides are reviewed by Pachaiappan* and Kovendhan Manavalan in Chap. 6. Chapter 7 by Kalambate and Huang discusses the use of electrochemical biosensors for the quantification of disease biomarkers and drug molecules by voltammetry, impedimetry, and potentiometry. Therapy and bioimaging have recently improved with the design of novel inorganic nanoparticles, as presented by Prabha et al. in Chap. 8. The eco-friendly applications of metals and metal oxides in drug delivery and bio-imaging are reviewed by Prasad et al. in Chap. 9. Chapter 10 by Jagadeeshan and Parsanathan describes the use of nanomaterials made of metal oxides to treat cancer. Varier et al. and Jayamurali

et al. discuss the neurotoxicity, cardiotoxicity, hepatotoxicity, and developmental and reproductive toxicity of metals such as Hg, Pb, Al, Fe, Cd, and As in Chaps. 11 and 12. We thank very much the contributing authors for their valuable contributions.

Arica, Chile — Saravanan Rajendran
Riyadh, Saudi Arabia — Mu. Naushad
Chennai, Tamil Nadu, India — D. Durgalakshmi
Aix-en-Provence, France — Eric Lichtfouse

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Dr. Mu. Naushad extends his appreciation to the Deanship of Scientific Research at King Saud University for the support.

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Saravanan Rajendran received his Ph.D. in Physics–Material Science in 2013 from the Department of Nuclear Physics at the University of Madras, Chennai, India. He was awarded the University Research Fellowship (URF) during the years 2009–2011 by the University of Madras. After working as an Assistant Professor at Dhanalakshmi College of Engineering, Chennai, India, during the years 2013–2014, he was awarded SERC and CONICYT-FONDECYT Postdoctoral Fellowship, University of Chile, Santiago, in the years of 2014–2017. Dr. Rajendran has worked (2017–2018) in the research group of Professor John Irvine, School of Chemistry, University of St Andrews, UK, as a Postdoctoral Research Fellow within the framework of a EPSRC-Global Challenges Research Fund for the removal of Blue-Green Algae and their toxins. He is currently working as a Research Scientist in the Faculty of Engineering, Department of Mechanical Engineering, University of Tarapacá, Arica, Chile. Addition to this, he is also working as a Research Associate in SERC, Santiago, Chile. Dr. Rajendran is the Associate Editor for *International Journal of Environmental Science and Technology* (Springer). His research interests focus in the area of nanostructured functional materials, photophysics, surface chemistry, and nanocatalysts for renewable energy and wastewater purification. Dr. Rajendran has published in several international peer-reviewed journals, eight book chapters, and seven books published by renowned international publishers.

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Chapter 1
Metal Sulfide Nanostructures for Bioimaging and Biosensing Applications

S. Joicy and P. Thangadurai

Contents

Abstract Medical field has been growing enormously in the recent years in terms of diagnosis as well as treatment. People are interested to do diagnosis quickly and more importantly with high precision. In this aspect, many researches have been carried out to develop and improve diagnostic tools. In particular, imaging and sensing are the two major tools that are highly useful for medical diagnostics. There are two aspects in developing these tools. One is the technology aspect, and the second is the development of materials that are used in these tools. More precisely, the imaging as well as sensing devices work on the type of materials, and their performance is highly depending on the quality of the materials. These materials are mostly used as probes, based on their active property. The probe can be optical, electric, magnetic and electronic depending on the requirement. Thus, the importance of the materials in medical diagnostics has been improving, especially with the development of nanomaterials. Materials are highly researched for their

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applications in bioimaging and biosensing. In this aspect, the present chapter has been written on the application of sulfide nanomaterials for bioimaging and biosensing. Out of many types of materials, the sulfide-based chalcogenide materials are nontoxic and highly performing especially in terms of their optical properties and can be exploited for these applications. Many imaging techniques based on fluorescence, magnetic resonance and acoustic are discussed in this chapter. As far as the biosensing is concerned, fluorescent sensors, calorimetric sensor and electrochemical sensors are discussed.

Keywords Metal sulfides · Bioimaging · Image-guided therapy · Biosensing

Abbreviations

CDT	Chemodynamic therapy
CT	X-ray computed tomography
DNA	Deoxyribonucleic acid
FLI	Fluorescence imaging
FRET	Fluorescence (or Förster) resonance energy transfer
GCE	Glassy carbon electrode
IRT	Infrared thermal
MOST	Multispectral optical tomography
MRI	Magnetic resonance imaging
PAI	Photoacoustic imaging
PAT	Photoacoustic tomography
PDT	Photodynamic therapy
PET	Positron emission tomography
PTT	Photothermal therapy
RT	Radio therapy
ssDNA	Single-stranded deoxyribonucleic acid
TRT	Thermoradiotherapy

1.1 Introduction

Currently, biomedical research is one of the vast-growing research areas with advanced technologies due to its direct impact on human health. Bioimaging and biosensing are two different significant aspects of biomedical research. The main focus of those fields lies in the development of probes for selective detection and specific treatment of infectious diseases. These two directions of biomedical research field are individually classified based on their functionality. Bioimaging is an important method that visualizes and monitors the physical and molecular events in living cells. Bioimaging utilizes photons, X-rays, magnetic resonance, ultrasounds and positrons as imaging sources. By utilizing those imaging sources, wide range of

imaging techniques such as optical imaging, magnetic resonance imaging, photoacoustic imaging and positron emission tomography have been developed for understanding basic molecular aspects of living organisms and earlier detection of diseases. Bioimaging studies are typically achieved in two kinds of environments, which are, in vitro and in vivo, in which the efficiency of the developed imaging agents could be determined. On the other hand, biosensing mainly deals with the quantitative and qualitative detection of biological analytes by characterizing absorption, emission and electrocatalytic behavior of the sensor system. The biological analytes include nucleic acids, proteins, vitamins, virus and glucose. On the basis of signal transduction, biosensors can be classified as optical, electrochemical and thermal biosensors.

Nanotechnology plays a major role in scientific development of biomedical research. Unique physical and chemical features of nanomaterials differ from their bulk counterparts due to their quantum size effect. Nanomaterials are materials with morphological features whose sizes are smaller than 100 nm, at least in one dimension. With the development of nanotechnologies, various nanomaterials with unique properties are being introduced into bioimaging and biosensing applications. Those nanomaterials include metal oxides (Andreescu et al. 2012), metal sulfides (Martynenko et al. 2017; Yadav et al. 2019), carbon materials (Wen et al. 2015; Hong et al. 2015) and graphene (Shen et al. 2012; Hai et al. 2018). Nanomaterials offer more advantages such as biomedical agents due to their smaller size, large surface-to-volume ratio and ease of surface modification. Among the most fascinating nanomaterials, metal cations with sulfide-based nanomaterials have been employed intensively to serve as bioimaging and biosensing nanoagents for various biological applications because of their unique optical, magnetic, electronic and catalytic properties. These unique properties of metal sulfides can be tunable by altering size, shapes, composition and doping, which facilitate opportunities to enhance the bio-performance (Yong et al. 2010; Geszke-Moritz et al. 2013a; Gao et al. 2015; Zhao et al. 2017; Yu et al. 2017). Metal sulfides made in various nanostructured forms such as quantum dots, nanoparticles, nanorods, nanotubes, nanosheets and nanocubes have been prepared and used for biomedical applications.

The performance of bioimaging and biosensing nanoagents based on metal sulfides depends significantly on their size, dimension, morphology and surface activity. For instance, hollow and porous nanostructures are more favorable for high drug loading capacity because those nanostructures provide more space to load drugs (Li et al. 2016; Wang et al. 2016; Zhang et al. 2019). Next, biosensor based on one-dimensional nanostructures such as nanorods and nanotubes is attractive for detection of biological analytes due to their confined electron transportation along the one-dimensional direction (Zhang et al. 2008; Guan et al. 2015). On the other hand, high density of active surface sites over a large area of two-dimensional metal sulfides makes them ideal for biosensing application (Vilian et al. 2019).

The current chapter focuses on bioimaging and biosensing applications of most widely used metal sulfides and their nanocomposites. Various imaging techniques where metal sulfides employed as imaging agent have been deliberated in the first part of the chapter. Also, imaging-guided therapy system based on the metal sulfide

nanostructures was also explained with various examples. The second part of the chapter has focused on the biosensing applications based on the metal sulfide nanostructures.

1.2 Metal Sulfide Nanostructures for Bioimaging and Therapy

In medical field, imaging method has become a crucial tool in clinical trials, medical practice and cancer research. At present, numerous technologies such as imaging and therapeutic technologies have been developed and applied for medical imaging and therapy to appropriately diagnose and treat the diseased tissues. Those technologies include photoluminescence imaging (PLI), magnetic resonance imaging (MRI), photoacoustic imaging (PAI), photothermal therapy (PTT), photodynamic therapy (PDT) and chemotherapy. Nanomaterials with transition metal sulfide nanostructures have attracted great attention in the field of biomedical therapy and imaging due to their optical (such as near-infrared absorption and photoluminescence) and magnetic properties. These properties made them potential probes in biomedical field such PLI, PAI, MRI, PDT and PTT. This section presents very successful results of functionalized/conjugated transition metal sulfide nanostructures applied for imaging and imaging-guided therapy.

1.2.1 Fluorescence Imaging

Optical imaging using luminescent probes has greatly attracted fast-growing research interest due to its high specificity and sensitivity and has potential to offer high-resolution images. To date, there have been a number of studies on using transition metal sulfide nanomaterials as optical probes for photoluminescence imaging of cells and tissues, from in vitro and in vivo imaging to specific tissue targeted imaging.

Metal sulfides based on group II–VI semiconductors (ZnS, CdS and HgS) have been attracted and used for fluorescence imaging due to their attractive photophysical properties, improved processability and good host material for luminescent activators (i.e., dopants). Of them, narrow bandgap semiconductor CdS quantum dots have been extensively studied due to its size-tunable absorption and emission properties (Fig. 1.1a) and have been implemented in biomedical applications (Shen et al. 2010; Huang et al. 2013; Shivaji et al. 2018). Conjugated CdS quantum dots were used as fluorescent probes for targeted imaging of various cancerous cell lines. For example, human transferrin protein- conjugated CdS quantum dots with quantum yield of 74% were used for targeted fluorescence imaging of human breast adenocarcinoma cell (MCF-7 cells) (Pedram et al. 2016).

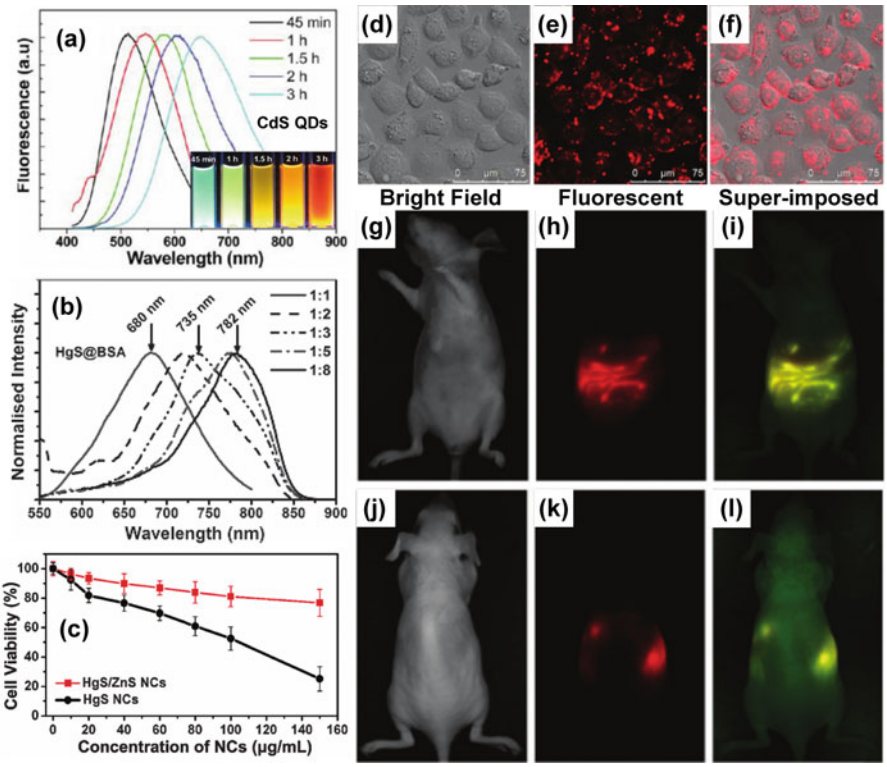

Fig. 1.1 (**a**) Tunable photoluminescence of CdS quantum dots (QDs) by varying size based on the reaction time. (Modified after Aboulaich et al. (2012). Copyright 2012 American Chemical Society). (**b**) Normalized luminescence spectra of HgS@BSA (*BSA – bovine serum albumin*) quantum dots with different Hg/S molar ratios. (Reprinted with permission from Goswami et al. (2012). Copyright 2012 John Wiley and Sons). (**c**) MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) calorimetric assay on Hela cells exposed to HgS–ZnS nanocrystals (NCs) and bare HgS core nanocrystals at different concentrations from 0 to 150μg mL^{-1} for 24 h, respectively. Bright-field (**d**) and confocal fluorescence (**e**) images of Hela cells incubated with HgS–ZnS nanocrystals emitting at 785 nm for 3 h at a concentration of 100μg mL^{-1}, and overlay images of (**d**) and (**e**) are (**f**). The images were obtained by excitation at 633 nm. (**g** to **l**) In vivo NIR fluorescence imaging of a nude mouse with HgS–ZnS nanocrystals emitting at 785 nm. (**g** and **j**) Bright-field images of the nude mouse on the abdominal side and on the backside; (**h** and **k**) unmixed images of the HgS–ZnS nanocrystals fluorescence signal from the abdominal cavity and the backside of the nude mouse with HgS–ZnS nanocrystals injected into the abdominal cavity; (**i** and **l**) superimposed images of autofluorescence of nude mouse and fluorescence of the nanocrystals. (Reprinted with permission from Yang et al. (2015a). Copyright 2015 Royal Society of Chemistry)

In another work, mouse liver cancer cells (CBRH-7919) were imaged by the fluorescent CdS-MAA-PEI quantum dots conjugated with folic acid as targeting ligand through receptor (folate receptor)-mediated endocytosis (Wei et al. 2012).

Mercury sulfide (HgS) is another type of II-VI narrow bandgap semiconductor whose emission wavelength can also be tailored in NIR-I window by varying their size (Fig. 1.1b) (Yang et al. 2012; Goswami et al. 2012). The NIR photons with longer wavelength in biomedical application provide less tissue scattering and absorption, which leads to deep tissue penetration and minimized background autofluorescence. However, apart from the consideration of optical bioimaging window, toxicity of heavy metals Cd^{2+} and Hg^{2+} is another major blockade in the practical use of Cd and Hg-sulfides, especially for in vivo imaging applications (Tchounwou et al. 2012; L'Azou et al. 2014). Therefore, approaches to nontoxic semiconductor quantum dots have been focused on the preparation of core–shell architectures with an exterior layer of nontoxic material (i.e., ZnS as shell). Also, core–shell structures based on CdS and HgS greatly increase their stability and quantum yields.

Liu et al. (Liu et al. 2014) reported that the CdS–ZnS core–shell quantum dots with QY of 42% can be used as an efficient photoluminescent probe for bioimaging application. After stabilized with pluronic block copolymer micelles, CdS–ZnS quantum dots exhibited a bright emission with QY of ~ 65% and high colloidal photostability. Subsequently, these quantum dots were used for in vitro and in vivo optical imaging studies. Zhang and group (Zhang et al. 2013) have fabricated folic acid-assembled PEI-coated CdS–ZnS quantum dots as a turn-on fluorescent probe for targeted imaging of folate receptor overexpressed cancer cells (HeLa, HepG2 and A549). In another report (Yang et al. 2015a), the biocompatible HgS–ZnS core–shell quantum dots with tunable emission in wide NIR window (785–1060 nm) and higher QY of 43.8% were obtained by aqueous route using glutathione (GSH) as stabilizing agent and used for imaging study. The results of this study showed that the GSH-stabilized HgS–ZnS core–shell quantum dots exhibited significantly lower cytotoxicity (above 80% viability) compared to the GSH-HgS core nanocrystals (Fig. 1.1c). This implies that the ZnS shell proficiently inhibited the release of free-Hg^{2+} ions from the HgS core material and thus reduced cytotoxicity. Then, the resultant low-toxic GSH-stabilized HgS–ZnS core–shell nanocrystals have been proved as an effective NIR fluorescent label in both in vitro (Fig. 1.1d–f) and in vivo (Fig. 1.1g–l) imaging. The penetration depth of NIR emitting HgS–ZnS core–shell nanocrystals in nude mouse reached to 2 cm with a distinct separation of fluorescence of nanocrystals from the background autofluorescence.

On the other hand, a non-toxic zinc sulfide (ZnS) semiconductor is one of the most important versatile and fluorescent materials with a wide bandgap of ~3.6 eV. Particularly, transition metal ions-doped ZnS luminescent probe have received much attention recently as a class of luminescent material, where dopant ions act as a recombination centers for the electron–hole pairs which results in strong and characteristic radiative emission. The potential of various metal ions-doped ZnS nanocrystals (single or co-doped form) in biomedical application has been reported by different research groups (Xu et al. 2011; Bhowal et al. 2018; Selvaraj et al. 2019). In particular, manganese ion (Mn^{2+})-doped ZnS phosphors have been extensively studied for their longer excited state life time and larger Stokes shift. The large Stokes shift is a crucial benefit for bioimaging application in terms of minimalizing

self-reabsorption and distinguishing the photon emission from background autofluorescence. Labeling of cancer cells by folic acid-conjugated Mn:ZnS quantum dots as fluorescent probes was studied by various researchers (Geszke et al. 2011; Geszke-Moritz et al. 2013b; Mohammad et al. 2016) and proved this quantum dots are very specific towards their targets. Another research group (Yu et al. 2013) had developed three photon-excited photoluminescence characteristics of Mn^{2+}-doped ZnS quantum dots for high resolution in vitro and in vivo tumor targeted imaging.

Another interesting longer wavelength (NIR-II, 1000–1400 nm) photon-emitting metal sulfide is silver sulfide, Ag_2S that belongs to I-VI group semiconductor. As NIR-II emitting substance without any heavy metals, the Ag_2S is an ideal candidate for the study of multicolor in vivo imaging and multiple nanodiagnostics with high spatial and temporal resolution with high signal-to-background ratio. Both in vitro and in vivo NIR-II imaging studies by Ag_2S quantum dots have been reported by various research groups. For example, PEGylated Ag_2S quantum dots (particle size ~5.4 nm) with NIR-II emission at 1200 nm (Fig. 1.2a) have been developed as imaging contrast agent for in vivo NIR fluorescence imaging of xenograft 4T1 tumors-bearing mouse (Hong et al. 2012). The experimental results showed that PEG-Ag_2S quantum dots were able to detect the xenograft tumors with high tumor-to-background signal contrast through passive targeting based on enhanced permeability and retention (EPR) effect (Fig. 1.2b, c). Another group (Tang et al. 2015) had synthesized water-dispersible 3-Mercaptopropionic acid (MPA)-capped Ag_2S quantum dots with distinct tunable emission from 500 to 820 nm. In their subsequent work, a cyclic peptide, arginine-glycine-aspartic acid-(D)phenylalanine-lysine (cRGDfk) was conjugated to MPA-Ag_2S quantum dots using EDC-NHS chemistry for fluorescence imaging of different mouse models of cancer. The results of in vivo imaging study (Fig. 1.2d) showed that cRGDfk- conjugated Ag_2S quantum dots were mainly accumulated in the tumor sites with minimal background fluorescence at 24 h postinjection of quantum dot-cRGDfk conjugates through tail vein of bilateral tumor-bearing mouse. The observed result has been further supported with ex vivo biodistribution (Fig. 1.2e) and relative fluorescence intensity analysis of different organs and tissues.

Recently, research community have concentrated on multinary I-III-VI_2 and I_2-II-IV-VI_4 group alloyed semiconductor nanomaterials of Cu/Ag-based metal sulfides, such as $CuInS_2$, $AgInS_2$, Cu_2ZnInS_4 and Ag_2ZnInS_4. Because these nanomaterials contain no highly toxic elements and exhibit composition tunable optical and electronic properties, high photoluminescence quantum yield (PLQY) and resistance to photobleaching. Multinary I-III-VI_2 and I_2-II-IV-VI_4 quantum dots with these exclusive features can be applied to bioimaging application. Peng group (Xie et al. 2009) had proposed a greener method to synthesize ZnS coated CuInS quantum dots with high QY of ~30% in non-coordinating solvent. Figure 1.2f shows the emission wavelength of CuInS–ZnS core–shell quantum dots which covers a broad emission window from 500 (visible) to 950 (NIR-I) nm. The CuInS–ZnS quantum dots exhibit good optical characteristics, and are highly hopeful for NIR fluorescent imaging and multicolor imaging. In one of the earlier studies, oil

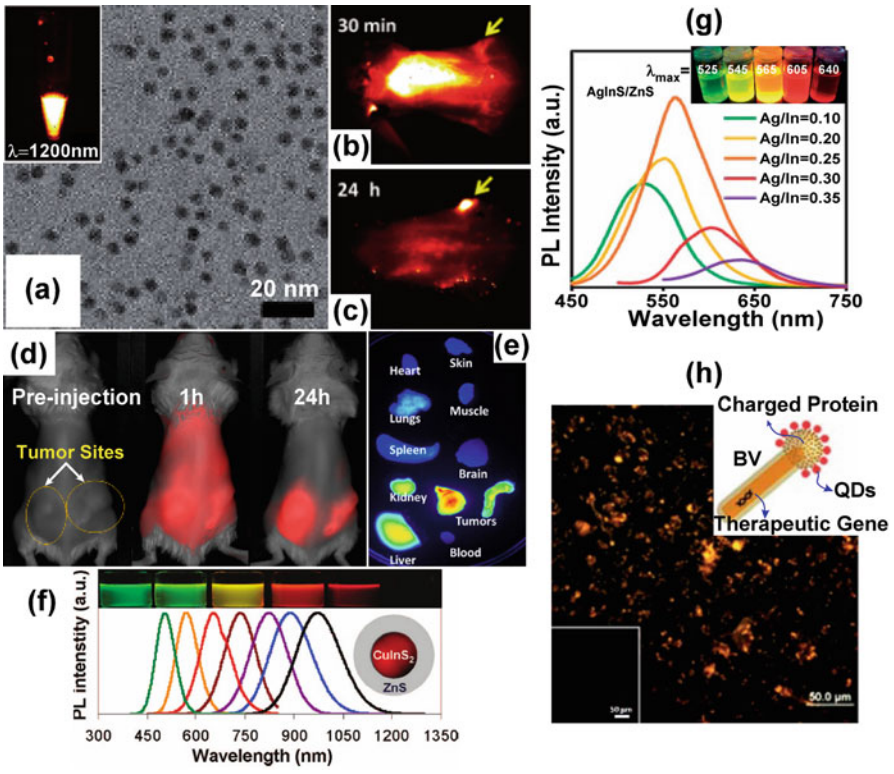

Fig. 1.2 (**a**) A TEM image of 6PEG-Ag_2S quantum dots. Insert image denotes the photoluminescence image of 6PEG-Ag_2S quantum dots suspended in PBS at a concentration of 1.34 mg mL^{-1}. (**b** and **c**) NIR-II fluorescence imaging of a xenograft 4T1 tumor with high uptake of 6PEG-Ag_2S quantum dots. (Modified after Hong et al. (2012). Copyright 2012 John Wiley and Sons). (**d**) Representative in vivo fluorescence imaging of cRGDfK-Ag_2S in 4T1luc tumor-bearing Balb/c mouse at different time points after intravenous administration. (**e**) Representative ex vivo fluorescence image of organ tissues from 4T1luc bilateral tumor bearing mice 24 h postinjection. (Reprinted with permission from Tang et al. (2015). Copyright 2015 American Chemical Society). (**f**) Photoluminescence properties of the $CuInS_2$–ZnS core–shell nanocrystals. (Reprinted with permission from Xie et al. (2009). Copyright 2009 American Chemical Society). (**g**) Photoluminescence spectra of water dispersible AgInS–ZnS quantum dots with varying Ag:In ratios. (Insert: the photograph was taken under ultraviolet light). (**h**) Fluorescence image of HeLa cells incubated overnight with baculoviral vectors (BVs) that are labeled with orange-emitting polyacrylic acid and mercaptoacetic acid ligand-capped AgInS–ZnS (PM-AgInS–ZnS) quantum dots. Insert image (left bottom) shows the fluorescence image of HeLa cells incubated solely with PM-AgInS–ZnS quantum dots. Insert graphical representation denotes the BV labeled with negatively charged PM-AgInS–ZnS quantum dots. (Modified after Regulacio et al. (2013). Copyright 2013 Royal Society of Chemistry)

dispersible $CuInS_2$–ZnS core–shell quantum dots (Yong et al. 2010) were encapsulated within functionalized PEGylated phospholipid micelles, and further conjugated with folic acid for in vivo tumor targeting and imaging. Strong fluorescence signal

was observed from the tumor area of mouse treated with folic acid- conjugated $CuInS_2$–ZnS quantum dots, whereas weak signal was attained from the tumor sites for unconjugated quantum dots. In addition to this, in vivo multiplex luminescence imaging was achieved by administering two different color-emitting $CuInS_2$–ZnS quantum dot micelles (quantum dot 640 and quantum dot 710) subcutaneously. As shown in Fig. 12 of work done by Yong et al. (Yong et al. 2010), the fluorescence signals from two different quantum dots are well distinguished. The results confirm that the efficiency of $CuInS_2$–ZnS core–shell quantum dots bioconjugates as an optical contrast agent for targeted and multiplexed optical bioimaging. In another study, a bright and stable $CuInS_2$–ZnS quantum dots embedded in silica nanobeads were fabricated and further conjugated with holo-Transferrin (TF) protein for targeted cancer cell imaging (Foda et al. 2014). And, in vitro bioimaging study had suggested that the quantum dots@SiO_2@TF conjugates were capable of targeting cancer cells. Yang et al. employed alpha-fetoprotein (AFP) antibody as a tumor targeting ligand (Yang et al. 2016a). Intense fluorescent signal was observed in the cytoplasm of HepG2 cells treated with CuInS–ZnS-AFP probe, while no signal was observed from the control one, bare CuInS–ZnS quantum dots.

On other hand, there are a few research papers demonstrating $AgInS_2$ ternary and $AgZnInS_2$ quaternary quantum dots in the form of bare or core–shell architecture efficiently used for optical in vitro and in vivo imaging (Chang et al. 2012; Shamirian et al. 2015; Tan et al. 2015b; Song et al. 2016a; Mansur et al. 2017). For example, Regulacio and co-workers had demonstrated that $AgInS_2$–ZnS quantum dots coated on baculoviral vectors act as efficient vectors for co-delivery of therapeutic genes and quantum dot luminescent probe to the cells (Regulacio et al. 2013). In this study, air stable $AgInS_2$–ZnS quantum dots with tunable emission from 525 to 640 nm (Fig. 1.2g) and the PLQYs of up to 20% were synthesized by varying Ag:In ratios in aqueous phase in the presence of polycarboxylate (polyacrylic acid, PAA) and thiol-containing ligand (mercaptoacetic acid, MAA). PM-coated $AgInS_2$–ZnS quantum dots display very low cytotoxicity (9.5% for 200μg mL^{-1}, 48 h of incubation) and colloidal stability in the physiological pH range. The negatively charged PM-$AgInS_2$–ZnS-565 nm quantum dots were electrostatically bind with positively charged end of baculoviral vectors (insert graphical representation of Fig. 1.2h). In vitro study on HeLa cells (Fig. 1.2h) suggests that baculoviral vectors attached with PM-$AgInS_2$–ZnS quantum dots can efficiently enter and label the cells. In another study on multinary Ag chalcogenides, Song et al. demonstrated a method for synthesizing ternary $AgInS_2$, quaternary AgZnInS, $AgInS_2$–ZnS and AgZnInS–ZnS quantum dots based on cation exchange and diffusion process (Song et al. 2016b). L-glutathione (GSH) was used to make water-dispersible alloyed core–shell quantum dots, and alpha-fetoprotein antibodies (AFP, a specific indicator in diagnosing hepatocellular carcinoma) were used to conjugate with GSHcapped quantum dots for targeted fluorescent labeling. For in vitro imaging, Hep-G2 cells were treated with GSH-quantum dots@AFP antibody conjugates. Optical images showed that GSH-quantum dots@AFP conjugates bound to the antigen receptors in the cytoplasm of Hep-G2 cells via antigen–antibody reaction. On the contrary, quantum dots without conjugating AFP could

not label the cytoplasm of Hep-G2 cells. According to the above studies, targeted labeling efficiency of multinary Ag-based metal sulfides has been realized.

1.2.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a broadly used diagnostic imaging tool in the clinical procedures and is based on the magnetization properties of atomic nuclei. As an effective imaging technique, MR contrast imaging has advantages such as high spatial resolution (~1 mm), deep tissue penetration and three-dimensional anatomical information. Moreover, MR contrast agents are often used to further improve their imaging sensitivity by interacting with water molecule protons in surrounding tissues, leading to shorten the longitudinal (spin-lattice) and transverse (spin–spin) relaxation times, thereby enhancing image contrast. Paramagnetic and superparamagnetic metal ions are used as contrast agents for MRI. Paramagnetic materials boost the longitudinal relaxation processes (known as T_1 relaxation processes), making brighter MR signal, whereas superparamagnetic and ferromagnetic materials accelerate the transverse relaxation processes (known as T_2 relaxation processes), which has resulted in hypointense MR signal.

Lanthanide metal ion gadolinium (III) has been confirmed to exhibit superior paramagnetic contrast efficiency due to its unique magnetic characteristics. Therefore, incorporation of Gd(III) ions into the host nanomaterials is one of the ways to achieve enhanced MRI signal intensity by serving as paramagnetic module. Metal sulfide nanomaterials embedded with Gd^{3+} dopant or decorated with Gd chelates have been developed as efficient positive (T_1) contrast agents for MR imaging. For instance, Chang et al. synthesized Gd^{3+} ion-doped $CuInS_2$–ZnS core–shell quantum dots in the presence of dual stabilizers glutathione and citric acid trisodium salt by microwave irradiation technique as shown in Fig. 1.3a (Chang et al. 2016). This T_1 contrast agent exhibits a significantly high longitudinal relaxivity ($r_1 = 55.90\ mM^{-1}\ s^{-1}$) and low r_2/r_1 ratio of 1.42 (Fig. 1.3b, c). In a work by Anbazhagan et al., paramagnetic MoS_2-Gd chelate core–shell nanoparticle was synthesized by a multistep process as shown in Fig. 1.3d (Anbazhagan et al. 2016). In this MoS_2-Gd chelate core–shell structured nanoparticles, a conjugated gadolinium-chelate shell significantly improves the magnetic property and longitudinal relaxation rate ($9.4\ mM^{-1}\ s^{-1}$) to the MoS_2 core. In vivo MR imaging was also performed, which displayed hyperintense signal in the kidney, heart and bladder (Fig. 1.3e, f). Transition element manganese(II)-doped nanomaterials are the another category of efficient MRI contrast agents. The potential of 1.5–2 nm-sized Mn^{2+}-doped ZnS quantum dots with different dopant concentrations as MRI-positive contrast agents has been studied by evaluating their relaxivities (Jahanbin et al. 2015). According to MRI studies, Mn^{2+}:ZnS quantum dots generate strong T_1 contrast enhancement. And Mn:ZnS quantum dots exhibit high longitudinal (r_1) relaxivity varying between 20.34 and $75.5\ mM^{-1}\ s^{-1}$ with concentration of Mn^{2+} increasing from 10% to 30%.

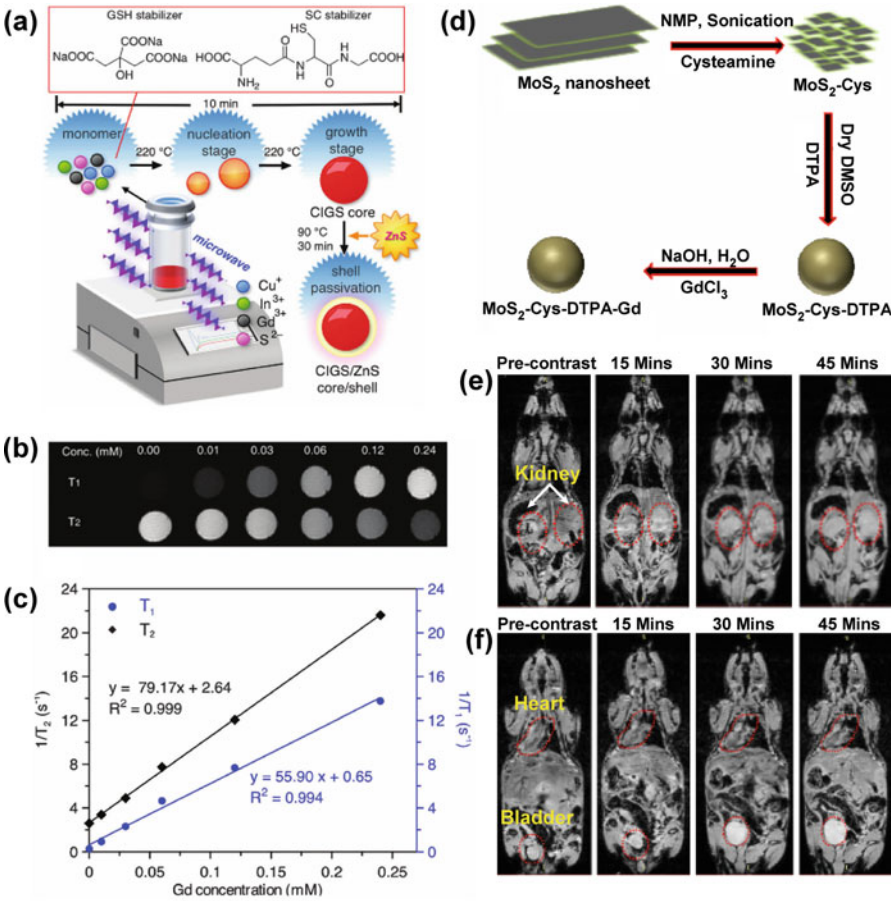

Fig. 1.3 (**a**) Formation of the Gd:$CuInS_2$ core and Gd:$CuInS_2$–ZnS core–shell quantum dots under microwave irradiation. (**b**) T_1- and T_2-weighted images of phantoms containing various Gd concentrations. (**c**) Plots of inverse relaxation times ($1/T_1$ and $1/T_2$) versus Gd concentration of the as-synthesized Gd:$CuInS_2$–ZnS quantum dots. (Reprinted with permission from Chang et al. (2016). Copyright 2016 Royal Society of Chemistry). (**d**) Synthetic approach used to prepare the core–shell gadolinium (Gd)-chelate functionalized MoS_2 nanomaterials. (**e**) The T_1-weighted MR images of the mice injected with MoS_2-Cys-DTPA-Gd at image layer with the kidney. (**f**) T_1-weighted MR images of the mice injected with MoS_2-Cys-DTPA-Gd at image layer with the heart and bladder. (Reprinted with permission from Anbazhagan et al. (2016). Copyright 2016 American Chemical Society)

1.2.3 Photoacoustic Imaging

The photoacoustic imaging (PAI) is otherwise termed as optoacoustic imaging. The PAI has been emerged as promising deep tissue imaging modality that is capable of mapping the optical absorption coefficient of biological tissues via photoacoustic

effect. In PAI, the nanosecond laser pulses absorbed by the biomolecules (endogenous photo-absorbers) undergo time-varying thermal expansion–relaxation process which results in generation of acoustic waves in the tissue. Finally, images are formed by reconstructing the generated photoacoustic signals acquired at different positions around the tissue surface. Detection sensitivity and specific tissue target abilities of PAI modality can be potentially extended by employing exogenous contrast agent. The agents that strongly absorb NIR radiation are highly suitable for high-contrast PAI with enhanced detection sensitivity at depths.

Nanomaterial-based exogenous contrast agents play a crucial role in PAI with high signal-to-noise ratio. Nanostructures with NIR-absorbing metal sulfides such as Ag_2S, MoS_2 and CuS have shown remarkable potential as PAI contrast agents. Due to NIR absorption ability and low toxicity, Ag_2S nanoparticles are promising nanoprobes for PAI. Zhang and co-workers have developed an anti-EGFR affibody (ZEGFR:1907) conjugated Ag_2S quantum dots with average size of 14 nm and exhibited surface plasmon resonance absorption at ~800 nm (Zhang et al. 2018b). Ex vivo and in vivo studies showed good biosafety and excellent efficiency in the NIR-I PAI of tumor cells when illuminated by 808 nm laser (NIR-I window, 100 mW cm^{-2}). In another case, contrast agent of folic acid-modified PF127@Ag_2S quantum dots have been used for photoacoustic imaging of tumor cell targeting (Zhang et al. 2018a). On the other hand, CuS has also received a great attention due to its localized surface plasmonic absorption in NIR window, making CuS as a suitable candidate for PAI. In 2013, Feng et al. had designed and synthesized a smart "off–on" PAI contrast agent of CuS based on the significant change in the NIR absorption that originates from the amorphous–crystalline phase transition in response to the body temperature (Feng et al. 2018). In vivo study proved that the PAI ability of amorphous CuS can be efficiently activated by body temperature. In another study, CuS nanoparticles with absorption tuned to 990 nm as an excellent contrast agent suitable for deep tissue PA tomography (PAT) imaging. Lie's group was first demonstrated in NIR-II PAT using CuS nanoparticles (~11±3 nm) with 1064 nm Nd:YAG laser (Ku et al. 2012). This PA contrast agent allowed visualization of mouse brain after intracranial injection (Fig. 1.4a1) and rat lymph nodes 12 mm below the skin after interstitial injection (Fig. 1.4a2). In addition, agarose gel containing CuS contrast agent embedded in chicken breast muscle could be mapped at a depth of ~5 cm. Recently, nanoprobe of hybrid protein–CuS (CuS@BSA-RGD) nanoparticles possessing high optical absorption at 1064 nm have been fabricated and applied for PAI of orthotopic hepatocellular carcinoma with high signal-to-noise ratio (Yan et al. 2019). Also, the experimental results revealed that this nanoprobe has excellent photoacoustic properties at 1064 nm pulse laser excitation.

As a NIR absorbing material, molybdenum sulfide (MoS_2) nanostructures have also been exploited as contrast agents for enhanced PAI. The MoS_2 is a known material to have layered structure and comparable to graphene for many characteristics. For instance, single-layer MoS_2 nanosheets were employed as efficient probe for highly sensitive PAI of orthotopic brain tumors (Chen et al. 2016). In this study, the MoS_2 nanosheets with different layered nanostructures were prepared by

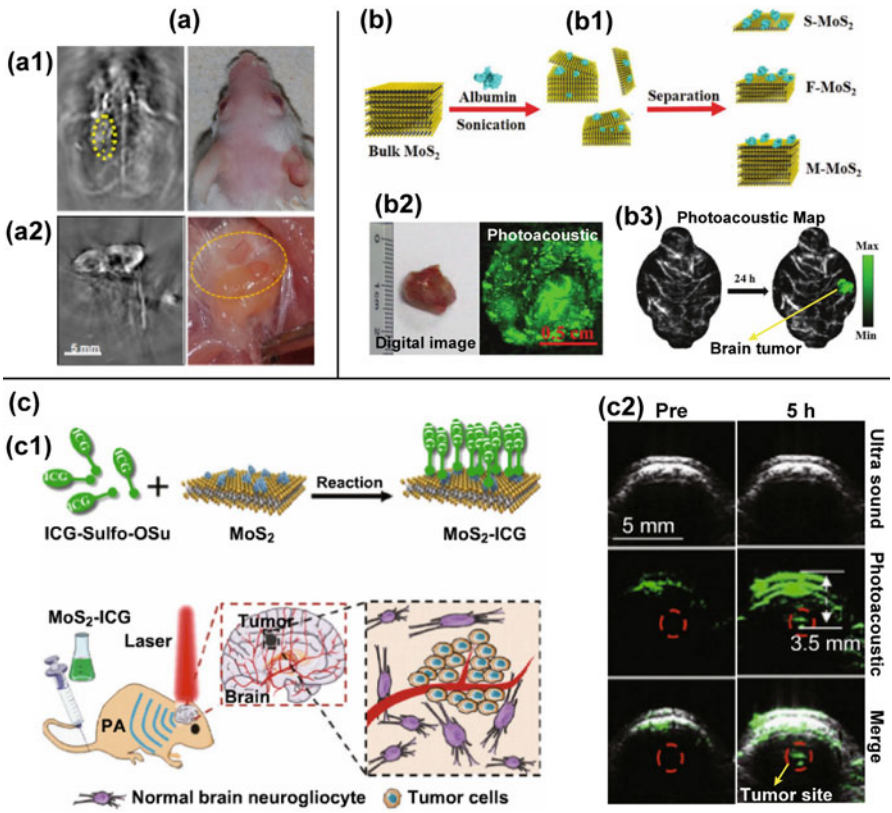

Fig. 1.4 (**a**) Copper sulfide nanoparticles for deep tissue imaging, (a1) representative in vivo PAT images of a mouse brain at 1064 nm 24 h after intracranial injection of 15μL of CuS nanoparticle solution and photograph of the head of the mouse, (a2) PAT image of auxiliary and brachial lymph nodes acquired on the right side of a rat 24 h after interstitial injection of 200μL of CuS nanoparticle solution into the right front paw pad and corresponding photograph of exposed rat under arm after imaging experiment. Yellow circle indicates lymph nodes. (Reprinted with permission from Ku et al. (2012). Copyright 2012 American Chemical Society). (**b**) Single-layer MoS_2 nanosheets with amplified photoacoustic effect for highly sensitive PAI of orthotopic brain tumors, (b1) synthesis procedure of MoS_2 nanosheets with various layered nanostructures, (b2) digital photograph and photoacoustic maximum amplitude projection image of exfoliated tumor at 24 h post i.v. injection of S-MoS_2, (b3) photoacoustic maximum amplitude projection image of brain tumor region before and after 24 h post i.v. injection of S-MoS_2 showing blood vessels (gray) and brain tumor (green color). (Reprinted with permission from Chen et al. (2016). Copyright 2016 John Wiley and Sons). (**c**) Molybdenum disulfide and indocyanine green (MoS_2-ICG) hybrid for in vivo photoacoustic imaging, (c1)MoS_2–ICG hybrid synthesis and its application in photoacoustic imaging of orthotopic brain glioma, (c2) cross-sectional ultrasound, photoacoustic, and their merged images of the brain tumor region before and after 5 h intravenous injection of MoS_2–ICG. (Reprinted with permission from Liu et al. (2018). Copyright 2018 Springer)

S-MoS_2 Single layer MoS_2, *F-MoS_2* few layer MoS_2, *M-MoS_2 multilayer MoS_2*, and *ICG* indocyanine green