Aqueous phase synthesis and functionalization of semiconductor quantum dots for biomedical applications

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2015

Wang, Y. (2015). Aqueous phase synthesis and functionalization of semiconductor quantum dots for biomedical applications. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/65458>

<https://doi.org/10.32657/10356/65458>

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**AQUEOUS PHASE SYNTHESIS AND
FUNCTIONALIZATION OF SEMICONDUCTOR
QUANTUM DOTS FOR BIOMEDICAL APPLICATIONS**

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A thesis submitted to the Nanyang Technological University
in fulfilment of the requirement for the degree of
Doctor of Philosophy

2015

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deep gratitude to my supervisor, Professor Yong Ken-Tye, who has provided me with valuable guidance throughout the 4 years' PhD study. His constant encouragement and lightening instruction broadened my knowledge, my mind, and even my life.

Special thanks should be given to my group mates and the staffs in Photonics Lab I and II, for their generous support though out the entire project.

Finally, I wish to thank my loving friends and family for everything.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	III
ABSTRACT.....	1
Chapter 1 Introduction	2
1.1 General Introduction.....	2
1.2 Concept of This Thesis	4
Chapter 2 Literature Review	6
2.1 Introduction	6
2.2 Physical Properties of QDs.....	7
2.2.1 Structure and General Properties.....	7
2.2.2 Type I and Type II QDs.....	9
2.2.3 Doped QDs	10
2.3 Chemistry of Quantum Dots.....	11
2.3.1 Organic Phase Synthesis of QDs	11
2.3.2 Phase transfer of QDs.....	13
2.3.3 Aqueous Phase Synthesis of QDs.....	16
2.3.4 Biofunctionalization of QDs.....	18
2.4 Biological Applications of Quantum Dots	20
2.4.1 Functionalized QDs for Biosensing.....	20
2.4.2 Bioconjugated QDs for <i>In Vitro</i> Cell maging.....	25
2.4.3 Bioconjugated QDs for <i>in vivo</i> applications.....	28
2.4.4 Multimodal Imaging with QDs	35
2.4.5 Quantum Dot Toxicity	36
Chapter 3 Aqueous Phase Synthesis of CdTe Quantum Dots using Mixed-ligands System.....	40
3.1 Introduction	40
3.2 Experimental Section.....	42
3.2.1 Chemicals	42
3.2.2 Synthesis Method	42
3.2.3 Characterization.....	44
3.2.4 Cell Culture and Cell Viability Test.....	44
3.3 Results and Discussion	45
3.3.1 Using L-Cysteine and MPA Individually to Prepare CdTe QDs	45

TABLE OF CONTENTS

3.3.2 Synthesis of CdTe QDs Using Mixed Stabilizing Agents.....	54
3.3.3 Cytotoxicity of CdTe QDs.....	57
3.4 Conclusion.....	58
Chapter 4 Manganese-doped ZnSe QDs for MR/optical Dual Modal Imaging	60
4.1 Introduction.....	60
4.2 Experiment	64
4.2.1 Chemicals	64
4.2.2 Characterization.....	64
4.2.3 Synthesis of Mn Doped ZnSe Core	64
4.2.4 Epitaxial Growth of Mn-doped ZnS Shell.....	65
4.3 Results and Discussion	65
4.3.1 Preparation of Mn:ZnSe/ZnS QDs	65
4.3.2 Preparation of SQDs as MR/optical dual mode probes	73
4.4 Conclusion.....	78
Chapter 5 Quantum Dot Based Theranostic Agents for Pancreatic Cancer Therapy	80
5.1 Introduction	80
5.2 Experiments and Results	83
5.2.1 Materials.....	83
5.2.2 Preparation of SiRNA Bector Based on QD/polymer Nanocomplexes	83
5.2.3 Preparation of SiRNA Vector Based on SQD-liposome Hybrids	84
5.2.4 Preparation of CPT Drug Carrier and Gene/drug Co-delivery System Based on QD-liposome Hybrids	86
5.2.5 Characterizations	86
5.2.6 SiRNA Transfection and Gene Expression Analysis Study	87
5.2.7 Cell Viability Evaluation.....	88
5.3 Results and Discussion	89
5.3.1 QD/polymer nanoplex for SiRNA delivery.....	89
5.3.2 SQD Loaded Liposome for Co-delivery of SiRNA and Hydrophobic Drug	105
5.4 Conclusions	115
Chapter 6 Conclusions and Future Work	117
6.1 Conclusions	117
6.2 Ongoing and Future work.....	119
6.2.1 Synthesis of Cd-free QDs With Tunable or NIR Emission.....	119
6.2.2 <i>In vivo</i> Applications.....	121
REFERENCES.....	122

TABLE OF CONTENTS

LIST OF FIGURES	141
SUMMARY OF ABBREVIATIONS.....	145
PUBLICATION LIST	147

ABSTRACT

In this thesis, efforts have been made to investigate and optimize the aqueous synthesis route in order to prepare high quality QDs. Reaction for high quality CdTe QD preparation was firstly investigated. It was found that, species and concentration of surfactants are important factors which greatly influence the growth dynamics, optical properties and stability of the QDs. A mix-ligands system composed of mercaptopropionic acid (MPA) and Cysteine (Cys) was then optimized to generate high quality CdTe QDs.

To avoid the heavy metal related toxicity issues, high quality Mn-doped ZnSe QDs (d-dots) were synthesized as an alternative to Cd-based QDs. The d-dots are characterized with large Stokes shift and long PL lifetime, and found to be biocompatible for biological applications. A sandwiched core/shell QD (SQD, Mn:ZnSe/ZnS/ZnMnS) was also formulated as high quality contrast agent for both optical fluorescence imaging and magnetic resonance imaging (MRI).

After proper surface modification and bio-functionalization, the d-dots were developed as theranostic agents for *in vitro* cancer diagnosis and therapy. Two types of nanocarriers, namely *d-dot*/polymer nanoplex and *d-dot*/liposome hybrid, were developed as optically traceable nanocarrier for cancer cell targeted gene delivery. The therapeutic effect was confirmed by the significantly suppressed expression of the targeted gene sequence (mutant K-Ras) at mRNA level. The SQD/liposome hybrid was also developed as a multifunctional platform for MR/optical dual mode imaging and gene/drug co-delivery.

Chapter 1 Introduction

1.1 General Introduction

Quantum dots (QDs) are semiconductor nanocrystals whose electrons and holes are quantum-confined in all three spatial dimensions. They have attracted much attention during the past few decades due to their unique physical properties as distinguished from the corresponding bulk materials and have shown great potential in the fabrication of next generation optoelectronic devices[1-3]. Since their first demonstration as optical probes for cell labeling in the late 90s[4, 5], QDs have emerged as a new class of fluorescent contrast agents for biomedical applications. In contrast with conventional organic dyes or fluorescent proteins, QDs possess unique optical properties. They have broad absorption band, narrow emission band, composition- or size-tunable emission from visible to near infrared (NIR) range, superior brightness, long fluorescent lifetime and more importantly, they are highly resistant to photo-bleaching[6] and have a relatively large surface area ready for functionalization[7]. Based on these advantages, lots of novel applications have been developed for biosensing and bioimaging, such as cell tracking, multi-channel and multi-modal imaging.

The solution phase synthesis method has revolutionized the QD fabrication technology for its capability of molar scale production. Colloidal QDs prepared using the organometallic methods have been widely reported with high quantum yield, narrow emission bandwidth and extraordinary optical and colloidal stability in solvent dispersions. To date, these high qualities have been successfully achieved on compound QDs based on II-IV (e.g. CdSe, CdTe, CdS, PbS and HgS) or III-V elements (e.g. InP, InAs). However, in the organometallic method, hazardous

precursors and high temperature reaction are normally involved, while complicated post-synthesis phase transfer steps have to be carried out to make the QDs dispersible in aqueous environment before using them for biomedical applications. Recently, aqueous route for synthesizing high quality QDs has attracted considerable research attention, as it is more economic, environment-friendly and simple to operate. More importantly, the QDs synthesized in aqueous phase are initially dispersible in biological environment and ready for biofunctionalizations, therefore show great advantage over the traditional organometallic method in biomedical applications.

For most of the biosensing and bioimaging applications, biocompatibility of the QD is another fundamental issue of concern. Although the potential toxicity of the particles is to some extent complicated by the surface modification, size and shape effect, delivery site/route and dosage, the dominant issue is thought to be the toxicity of its intrinsic compositions. So far, cadmium-based QDs had caught most of the attention and subsequently dominated the research field for quite a few years. It was found that, oxidation or degradation of the QDs can take place in biological environments, and the heavy metal ions released from the particle surface were found to cause acute toxic effects. Although lots of efforts have been directed to improve the compatibility of QDs in biomedical applications through proper surface modification (e.g. organic or inorganic capping), their potential toxicity still remains a major debating and unsettle issue. For this reason, preparation of high quality QDs that do not contain any toxic heavy metal components is extremely desirable for biomedical applications.

1.2 Concept of This Thesis

The overall goal of this work is to investigate the aqueous route to synthesize high quality, biocompatible and multifunctional colloidal quantum dots (QDs) and explore their applications in biomedical field.

In Chapter 2, a brief review is given to introduce the basic concepts of quantum dots, as well as the research background for their biomedical applications. In particular, the physical properties, chemical methods for synthesis and surface modification of QDs are described. Then, the biomedical applications of the QDs are introduced in terms of biosensing, *in vitro* and *in vivo* imaging, while novel applications for multimodal imaging are also highlighted. Finally, problems and research barriers related to toxic effects are briefly described.

Chapter 3 describes how CdTe QDs are synthesized via an aqueous method and the reaction conditions were optimized to achieve high qualities. In this CdTe QD reaction model, the growth dynamic of colloidal QD and the influence of the surfactants (i.e. concentration and species) in an aqueous synthesis reaction were investigated. A mixed-surfactant system consisting of mercaptopropionic acid (MPA) and cysteine (Cys) ligands was then investigated to control the QD's colloidal synthesis reaction and to fabricate QDs with tunable properties.

Chapter 4 explores the aqueous phase synthesis of high quality Mn-doped ZnSe QDs for replacing the commonly used heavy-metal based nanocrystals in bioimaging applications. Because the doped QDs do not contain any toxic heavy metal components, they should be more favourable for biomedical applications. In this chapter, a sandwiched core/shell QD (Mn:ZnSe/ZnS/ZnMnS, SQD) was also constructed as a high quality dual-mode probe for MR/optical imaging.

Chapter 5 demonstrates the applications of aqueous phase synthesized manganese doped zinc selenide QDs (which will henceforth be abbreviated as Mn:ZnSe *d*-dots, or simply *d*-dots) for *in vitro* gene delivery and therapy of pancreatic cancer cells. For this purpose, two types of nanocarriers, *d*-dot/polymer nanoplex and *d*-dot/liposome hybrid, were developed as theranostic agents for optically traceable siRNA delivery. The siRNA transfection caused RNA interference (RNAi) and significant silencing of the target mutant K-Ras gene. The *d*-dot/liposome hybrid was also demonstrated as a flexible nanoplatform for combined gene/chemotherapy of pancreatic cancer cells. Because the *d*-dots are free of heavy metal ions, they are promising candidates to develop safe and applicable contrast agents for theranostic applications.

The conclusions and suggestions for future work are presented in Chapter 6.

Chapter 2 Literature Review

2.1 Introduction

Quantum dots (QDs) are semiconductor nanocrystals with electrons and holes quantum-confined in all three spatial dimensions. Due to their unique physical/optical properties as distinguished from the corresponding bulk materials, QDs have attracted great attention during the past few decades. Since the first demonstration of using QDs as optical probes for cell imaging in the late 90s, QDs have been intensively employed as fluorescent contrast agents for biological applications. In contrast with conventional organic dyes and fluorescent proteins, QDs possess broad absorption band, size-tunable emission covering from visible to near infrared (NIR) range, superior brightness, long fluorescence lifetime and more importantly, they are highly resistant to photo-bleaching. Based on these advantages, numerous novel imaging techniques have been developed in the field of biophotonics, including deep tissue imaging and single molecule monitoring with multi-channel capabilities. Although the fabrication of highly luminescent colloidal QDs with narrow emission bandwidth has been realized by organic solution phase synthesis method, the QDs capped with hydrophobic ligands cannot be applied in biological systems directly and tedious steps are needed to make them water-dispersible. On the contrary, QDs prepared by using aqueous phase synthesis are highly appreciated since they can be directly used for bioconjugation and bioimaging without the need of using complex reaction parameters such as toxic precursors and high temperatures.

2.2 Physical Properties of QDs

2.2.1 Structure and General Properties

It is well known that for semiconductor materials, scaling down the physical size to nanometer range (1~100 nm), at least in one spatial dimension, can induce a set of new electronic and optical properties different from their bulk counterpart without changing the composition and lattice

Fig.2.1 (A) Schematic illustration of the density of states in metal and semiconductor clusters. (B) Density of states in one band of a semiconductor as a function of dimension[10].

structures. In nano-sized materials, a narrow potential well, with a dimension comparable with the de Broglie wavelength of the electron wave function, can confine the motion of electron/hole strongly and therefore results in quantized energy states. For this reason, this size-dependent electronic band structure modulation is widely known as the "quantum confinement effect"[8, 9]. Quantum dots are semiconductor nanocrystals spatially confined in all three dimensions (Fig.2.1). Due to the quantum confinement effect, the original quasi-continuous states of the material turn into discrete levels, corresponding to hybridization of finite number of atomic orbitals. The energy gap between the bottom of the conduction band and the top of the valance band in QDs expands as their size decreases, and therefore, greater energy is involved

in the process of exciton generation/recombination, which may cause a blue-shift of the absorption/photoluminescence (PL) peaks. For this three-dimensionally confined semiconductor materials, a "particle-in-a-sphere model" has been developed to describe the energy states[9]. Because of the atomic-like discrete structure in optical and electrical characteristics, QDs are sometimes referred as artificial atoms[9], and the strongly size-tunable properties make them unique nanomaterials for applications ranging from optoelectrical device to bioimaging.

Additionally, single QDs tend to show a blinking behavior (intermittent emission), which is thought to be a barrier for continuous imaging and tracking at single molecule level. It was reported that, this blinking behavior is related to surface traps and Auger ionization of QDs[11]. Auger recombination of exciton may eject carriers trapped in surface states into surrounding matrix. The emission will restore if only the ejected carriers return and neutralize the QDs. To minimize the Auger recombination effect, wide bandgap materials (e.g. ZnS, CdS) are typically used to overcoat the QD core to form a Type I core-shell structure. The wide bandgap shell can remove the surface trap states and also act as barrier to QD ionization[11]. For the same reason, some thiol-containing compounds (e.g. β -mercaptoethanol, BME) are also used for surface passivation to suppress the QD blinking and improve the emission efficiency[12].

To better understand other properties of QDs, such as size-dependent Stokes shift, polarized photoluminescence and long lifetime of band-edge luminescence and magnetic field induced lifetime shortening, other factors should be taken into consideration, including the shape, lattice asymmetry, electron-hole exchange interaction, impurities, defects and external field. These factors have great effects on

the fine structure of energy states and spectrum, especially in strong confinement region (size smaller than the exciton Bohr radius)[8, 13].

2.2.2 Type I and Type II QDs

Examples of Type I structure include CdSe/CdS, CdSe/ZnS and InAs/CdSe, while some other configurations such as CdTe/CdSe, CdS/ZnSe are usually categorized as Type II QDs. Fig.2.2 illustrates the energy band alignment of the two types. In Type I dots, excitons (electron and hole pairs) are effectively confined in the core, since both conduction and valence band edges of the core are located within the shell bandgap. In case of Type II dots, the band edges of the core are higher or lower than those of the shell. As a result, a significantly red-shifted emission can be observed due to the shrinking offset between electron and hole-occupied states[14]. Additionally, the spatially separated carriers at the heterostructures interface produce a strong local electric field, which induce a great transient Stark shift of the absorption spectrum with respect to the emission. As a result, Type II dots have a advantage to be applied as laser gain media to reduce absorption losses due to Auger

Fig.2.2 Schematic illustration of the energy band alignment of Type I and Type II core/shell QDs.

Fig.2.3 Strain-tuning of the band alignment and optical properties of CdTe/ZnSe heterostructures[15].

process and lower the lasing threshold[1]. However, recent research works have also revealed that, the lattice mismatch induced strain may alter the band alignment of the core/shell structure and, therefore, influence the optical and electronic properties[15]. For example, Fig.2.3 illustrates band energy changes in CdTe/ZnSe core/shell QDs induced by lattice strain that may cause conversion of the standard Type-I heterojunctions to Type-II band alignment when the core is 'squeezed' and the shell is 'stretched'.

2.2.3 Doped QDs

Similar with bulk semiconductor materials, introducing dopants ions is another way to tailor the optical and electronic properties of the QDs. By doping the wide band gap host with various transition (Mn^{2+} , Cu^{2+} , Co^{2+} and Ni^{2+}) or lanthanide ions (Cr^{3+} , Eu^{3+} , Tb^{3+} , and Er^{3+}), doped QDs (*d*-dots) can be prepared with tunable emission colors[16]. So far, Mn- and Cu-doped ZnSe/S QDs are the most frequently reported *d*-dots. Mn dopant typically introduce orange-red emission peaks at 575~610nm, and PL of Cu-

doped ZnSe can be tunable between 470~550 nm. As for Mn-doped QDs, excitons generated in host will transfer energy to the Mn dopant states, where recombination known as *d-d* transition (commonly 4T_1 - 6A_1 transition) occur causing Mn dopant emission and a significant Stokes shift. Unlike the band edge emission, the PL color of the *d-d* transition is not size-tunable, while a longer lifetime, on the order of milliseconds, is normally characterized, since the transition is spin forbidden[17]. As for Cu-doped QD, the recombination is between the host conduction band and the *d*-orbital of a copper ion, therefore the emission wavelength is tunable with the host size[18]. In comparison to conventional QDs, the enhanced ensemble Stokes shift in *d*-dots is desired for many applications including opto-electronic devices and biomedical imaging, as it eliminates self-quenching due to Förster resonance energy transfer or re-absorption[19, 20]. Additionally, with further coating layer of ZnS shell, emission of Mn *d*-dots are reported to be less sensitive to chemical, photochemical and even thermal disturbances, because the inner core atomic-like dopant states do not couple to the lattice phonon strongly[19].

2.3 Chemistry of Quantum Dots

2.3.1 Organic Phase Synthesis of QDs

Fabrication of high quality QDs with high crystallinity, well-passivated surface and monodispersed size was first presented by Murray and coworkers[21, 22]. Synthesis of metal chalcogenide (II-VI) QDs is completed using pyrolysis of the organometallic precursors at high temperature, where metal alkyls (dimethylcadmium, diethylcadmium, dibenzylmercury) and organophosphine chalcogenide (selenium-organophosphine) are employed as metal and group VI sources, respectively. The reaction is carried out in a solution of trioctylphosphine oxide (TOPO) or trioctylphosphate (TOP) with the metal precursor. The TOPO or TOP acts as steric

barriers on QD surface to modulate the growth rate and prevent aggregation due to van der Waals force. The chalcogenide precursor is then injected rapidly to raise the precursor concentration above the threshold of nucleation, leading to the formation of crystal nucleus with a large quantity. After the precursor concentration falls below the threshold, the growth of QDs continues and samples with a series of different colors can be obtained by quenching the reaction at different time points. As illustrated in Fig.2.4, the growth rate of the QDs is related to the monomer concentration and particle size. These two parameters have generated a concept of “focusing” and “defocusing” modes for the growth process of QDs[23]. Since increasing the radius of large crystals requires incorporation of more atoms, at high monomer concentration, smaller particles show faster growth rate than that of the larger ones and monodispersed particle sizes can be obtained. In contrast, a low concentration of precursor leads to a slow growth rate and broad size-distribution due to the Ostwald ripening process, in which QDs smaller than critical size dissolve and the “released materials” support further growth of larger ones with lower surface energy. In order to

Fig.2.4 ‘Focusing’ and ‘defocusing’ growth modes of QDs, and growth rate determined by particle size and concentration of the monomer present[23].

solve this problem, an improvement of the synthesis method has been made by Peng and coworkers[24], in which additional precursor with calculated dosage was injected after the nucleation stage to accelerate the growth process and maintain a narrow size distribution of the resulting QDs.

Furthermore, the development of the core/shell structure is another additional major improvement required to obtain high quality QDs. For instance, coating a CdSe core with a mono or multilayer of wide band gap materials (ZnS or CdS) will provide high quantum yield (QY), less toxicity, and high resistance to photooxidation[25-27]. The synthesis method of the shell structure is similar to that of the core, except that the reaction temperature as well as the addition rate of the shell precursors should be adjusted carefully to prevent Ostwald ripening of the QD cores and homogeneous nucleation[26]. Additionally, a size-selective precipitation process can also be introduced to produce mono-sized QDs. For example, titration of nonsolvent[21] or preferential evaporation of solvent[28] can induce aggregation and precipitation of QDs with larger sizes due to van der Waals forces.

2.3.2 Phase transfer of QDs

Although high quality QDs can be produced in organic phase as mentioned above, the dots capped with hydrophobic surfactants such as TOPO, TOP or tertiarybutylphosphine (TBP), are not water-soluble and therefore, cannot be directly utilized in bioimaging or biosensing applications, which usually have strict requirements of water solubility, biocompatibility, targeting ability, etc. For this reason, subsequent careful engineering of the QD surface is necessary, and to some extent more important than the fabrication of QDs themselves. In order to integrate biological functions, functional groups on QD surface, such as carboxyl (-COOH) or amino (-NH₂) groups, are also required as a bridge to crosslink functional molecules,

such as antibody, DNA segment, peptide, etc. For these reasons, complex post-processing procedures are generally required to make the hydrophobic QDs water-dispersible. Typical schemes for phase transfer include: 1) ligands exchange with bifunctional thiolated molecules or oligomeric phosphines[4, 5], 2) encapsulation in amphiphilic polymer or phospholipids[29, 30], and 3) coating with a hydrophilic silica shell[31, 32]. Since different capping methods are developed for specific uses, their general applicability is limited[6].

The first method, ligand exchange, is a conventional, easy-to-perform method in which aliphatic TOPO or TOP surfactants on the QD surface are replaced with bifunctional ligands by mass action. For binding with QDs the ligands should exhibit at least one anchoring moiety reactive to QD surface metal atoms. To render QDs water-soluble, functional groups on the other end should be hydrophilic, which would also cause Coulomb repulsion between the QDs to avoid aggregation or flocculation in biological medium[4, 33]. For example, thiolated molecules -such as mercapto acid, cysteine, cysteine containing peptides and oligomeric phosphines- are frequently employed for phase transfer[4, 5, 34-39].

However, although this strategy is easy to perform, mono-mercapto ligands cannot stay on the QD surface permanently due to the dynamic nature of the binding between thiol groups and QD surface atoms, and this consequently results in unstable colloidal dispersions[40]. This situation can be improved by using dihydrolipoic acid or oligomeric phosphine, which are reported to keep the QD dispersion stable for more than one year[33, 37, 40]. Furthermore, the ligand exchange process replacing the original coating with new capping ligands, may cause formation of surface defects and deteriorate the chemical stability and fluorescence efficiency[6].

Encapsulation of the dots with a layer of amphiphilic polymers is the second strategy to achieve water solubility. Different from ligand exchange, in this method, the original protective TOPO/TOP layer is not removed but kept in place. Driven by hydrophobic attraction, the hydrophobic parts of the amphiphilic polymers interleave the TOPO/TOP coating to form a bilayer structure while the hydrophilic parts stretch out into the aqueous environment mediating the solubility[29, 41, 42]. In this case, di- or tri-block copolymers and phospholipids are commonly used, while the hydrophilic ends can be functionalized and serve as anchor points for further conjugation with possible biomolecules to integrate biological functions[29, 30, 41-45]. Beside copolymers and phospholipids, there are also reports on QDs encapsulated in a polysaccharide coating[43]. Because the native hydrophobic protective layer is sustained, the electron and hole trapping states on QD surface are effectively passivated and therefore, higher fluorescence efficiency could be obtained as compared with the ligand exchange methods. This bilayer structure may also contribute to an improved chemical stability[7, 46]. However, multiple QD co-encapsulation is normally observed for this method and consequently results in a relatively large particle size[6, 47], which may hinder their uses in sensitive sensing and imaging applications[6].

Using a silica shell functionalized with hydrophilic groups is the third choice for surface modification. Owing to the versatile silane chemistry, QDs can be functionalized with various bio-functional groups[5, 31]. However, similar with the polymer encapsulation, the silica shell could dramatically raise the overall particle size.

2.3.3 Aqueous Phase Synthesis of QDs

In comparison to conventional phase transferring method, water-soluble QDs directly prepared via an aqueous synthesis method exhibit better biocompatibility, colloidal stability[48] and smaller hydrodynamic sizes. More importantly, the aqueous phase strategy does not require high temperature conditions, use of highly toxic chemical precursors or complicated reaction steps. In view of these considerations, the preparation of high-quality QDs directly in aqueous phase is highly preferable, especially for biological applications.

Similar to the organometallic method, the preparation of QDs in aqueous phase requires a stabilizing agent to mediate the growth kinetics of QDs and prevent them from aggregation. Typically, short chain thiolated molecules are employed as surfactants in the reaction process. The thiolated molecules, which contain a carbon-bonded sulfhydryl, are generally terminated with either carboxyl or amino groups at the other end of the molecules. With thiol groups (or referred as sulfhydryl group, -SH) acting as anchor moieties, the thiolated molecules bind to the QD surfaces and generally exhibit either negatively charged carboxyl or positively charged amino groups, which induce formation of an electrical double layer (EDL) structure (diffusion layer and adsorption layer) around the dots and keep them dispersible in the polar solvent due to electrostatic repulsions [49, 50]. Taking CdTe QDs as an example, cadmium precursor (Cd-ligands complexes) with proper reactivity can be achieved through complexation of Cd^{2+} ions and thiolated ligands in water solution at suitable pH and thermal environment. After that, addition of TeH_2 gas or oxygen free NaHTe solution as Te^{2-} source will result in the formation of CdTe nuclei, which will grow to QDs with the desired size at the expense of thermal treatment[51]. Similar to QDs in organic phase, growth of QDs in aqueous phase initially proceeds via a cluster–

molecule aggregation mechanism and is then governed by Ostwald ripening (OR) at the depleted precursor concentration. Despite the precursor concentration and reactivity, the properties and growth kinetics of the QDs are also affected by the EDL structure, because the growth of the QDs requires diffusion of monomers to the QDs surface, while small Cd containing species are thought to passivate the surface defects more effectively without disturbing the existing coordination. However, the EDL structure is greatly influenced by solution surroundings, including precursor concentrations/molar ratios, pH value and, most importantly, the species of the precursor and stabilizing agent.

To date, there remain a few challenges in synthesizing QDs in aqueous phase. For example, the process is time-consuming and the QDs have a broad size distribution. Standard protocols are required to overcome these drawbacks. In recent works[52], a high degree of precursor supersaturation was applied to cause accelerated nucleation rate and decelerated growth at the same time, which means a better separation between nucleation and growth process. As a result, particles with monodispersed sizes can be achieved. On the other hand, because the size defocusing is more pronounced during a long time reaction for larger particle growth, reaction conditions should be optimized to obtain a fast growth rate. For example, microwave irradiation and hydrothermal processes were employed for thermal reactions[53, 54]. Accelerated growth rates at high temperature (180°C) generate QDs with narrow FWHM spectrum and bright luminescence, because the elevated temperature favors a high crystallinity and reduces the surface trap states. Additionally, the addition of optimized stabilizing agents may also contribute to a controllable synthesis process and improved properties of the QDs. In comparison to the more hydrophobic long chain length mercapto acids (such as MHA and MUA), which cause compact packing layer and

slow down monomer diffusion[55, 56], shorter chain thiols (such as MPA or TGA) enable the dots growth at faster rates and therefore, are widely employed as stabilizing agents. On the other hand, hydrolysis of unstable ligands (GSH and Cys) during high temperature reactions may cause drastic deterioration of the PL emission and colloidal stability, because the surfactants become inadequate to passivate QD surface states and prevent aggregations[57]. Nevertheless, it has been argued that, excessive stabilizers in the reaction mixture could distort the surface and cause generation of new nonradiative defects[58].

2.3.4 Biofunctionalization of QDs

A bioconjugation process usually couples QDs with biomolecules, such as peptide, proteins, DNA segments, which extend the applications of QDs in medical and life science. In comparison with traditional organic dyes and fluorescence proteins, the large surface area of a QD allows conjugations with multiple biomolecules for multiple functions[6], such as recognition moieties for targeted imaging and sensing, drugs for therapy, enzymes to catalyze bioluminescent reactions, chemical transfectants to assist membrane-crossing and cell-internalization, electron or energy acceptors (e.g. organic dyes) for fluorescence energy transfer (FRET) based immunoassay and photodynamic therapy (PDT). The strategies for bioconjugation are flexible and vary with diverse QDs surface conditions and also the functional entities to link with.

QDs with capping layer exhibiting $-\text{COOH}$ or $-\text{NH}_2$ can be conjugated with biomolecules through covalent crosslink. For instance, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)-based condensation reaction can be employed to crosslink carboxylated QDs to the molecules exhibiting primary amine groups[29, 38, 59]. This method has been widely used for QD-streptavidin preparation[60, 61].

Similarly, QDs capped with amine groups could be crosslinked to thiolated molecules or molecules containing active ester moieties[5, 62]. As an example, the standard scheme for preparing QD-antibody conjugates was achieved by maleimide-catalyzed condensation between amine-modified QD and sulfhydryl groups on reduced antibody[63, 64]. It should be noted that, these crosslinking methods may cause problems, such as poorly controlled orientation or number of the conjugated molecules on a QD surface, which may reduce their bioactivity and weaken the performance as biofunctional materials.

To date, optimization of the molecule immobilization on nanoparticle surface is still an active research area[46, 65, 66]. It is worth mentioning that protein or peptide bearing functional groups with high binding affinity to QD surface atoms (e.g. thiolate residues) could be directly conjugated to the dots by replacing the original capping ligands. This direct binding strategy makes it possible to achieve surface protection, water-phase solubility and biofunctionalization of the dots in a simple one-step surfactant exchange process[67-69]. Furthermore, attributed to the development of peptide toolkit, modulation of the QD surface properties can be realized by customization of the peptides, while different functions can be integrated[59, 70-72].

Electrostatic binding is another choice to prepare QD-bioconjugates. Because of Coulombian interaction, molecules or proteins engineered with charged domains could be self-assembled onto a QD surface bearing opposite charges[37, 73, 74]. In addition to these methods, more complex schemes such as noncovalent biotin–avidin binding[61, 62], nickel-based histidine tagging[75] and using of adaptor proteins as bridging ligands[73] have been reported for flexible and versatile QD surface modification. Although multiple bioconjugation methods have been established and showed great value in applications, problems related to potential immunogenicity[76]

and impairment of the molecule's bio-functions still need to be evaluated[30, 65, 66, 77].

2.4 Biological Applications of Quantum Dots

2.4.1 Functionalized QDs for Biosensing

In comparison with organic dyes and protein fluorophores, QDs exhibit unique photophysical and photochemical properties and thus enjoy great advantages as fluorescence probes in sensitive optical biosensing applications. Developments in surface engineering and bioconjugation strategies will keep on benefiting the specificity and versatility of QD-based in vitro sensing methods.

The immunoassay is a useful tool for clinical tests, disease diagnostics and other biomedical applications. Developments in QD surface engineering have enabled them to be used as novel fluorescent probes for sensing and analyzing of a wide variety of chemical and biological analysts. For example, a competitive fluoroimmunoassay based on QD-antibody conjugates has been developed for detection of diverse targets, from small molecules to proteins, virus and bacteria[78-81]. For example, on ultrasensitive Western blot analysis based on QD-monoclonal antibody conjugates was carried out with electrophoresis to screen protein expression in cell or tissue with ultrahigh specificity and throughput[79, 82, 83]. It is worth noting that, in order to obtain ultrahigh sensitivity or rapid screening capability, novel readout methods, such as electrochemiluminescence (ECL) assay, barcode, micro fluidics technique and other readout strategies are widely applied[84-88]. In addition, multichannel detection of toxins, drug chemical residues or cancer biomarkers, has been demonstrated with QD-based sandwich immunoassays employing different multicolored antibody-QD conjugates[87, 89, 90]. Although multichannel detection can be easily achieved by introducing different color QDs with narrow emission spectra, efforts are still required

Fig.2.5 Schematic representation of intracellular c-Myc mRNA detection based on the wireless ECL biosensor. CdSe@ZnS QD-DNA conjugates provided an intracellular hybridization process that correlated with the relative levels of intracellular c-Myc mRNA[91].

to address the possible cross-reaction between molecule probes and other non-specific issues, which may limit the number of analyte species in a simultaneous detection.

DNA or RNA segments, acting as recognition moieties, can also be conjugated on QD surface to form fluorescent probes for genetic targets analysis. The high specificity of hybridization between QD-DNA probes and the target strand with complementary sequence essentially benefits the accuracy and number of multiplexed detections which can be achieved. For example, in a barcode configuration, polymeric microbeads functionalized with different oligonucleotide probes are optically coded with distinguishable fluorescence colors or intensity levels by loading with different QD populations[92-95]. Presence of target analytes pre-labeled with organic fluorophores lead to colocalization of the target signal and the coding signal from microbeads. In a recent study, up to nine different gene fragments from pathogens

such as syphilis, HIV, malaria, hepatitis B and C, were simultaneously detected with high fidelity by using nine barcodes[92]. This result suggests that QD barcodes may be powerful toolkits for rapid gene mapping and infectious disease detection.

Besides those analytical applications, QDs-based optical probes have also been developed to study cellular and subcellular gene expression[34, 96, 97]. Fluorescence *in situ* hybridization (FISH) assays performed with hydroxylated QDs-oligonucleotide probes was firstly introduced by Pathak and coworkers for the labeling of Y chromosome in human sperm cells[34]. After that, multiplex FISH assay for mRNA subcellular localization has also been reported by Chan and coworkers, and sensitive determination of housekeeping genes with QD-DNA conjugates was demonstrated by Han and Song[96, 97]. Most recently, an on-chip ECL protocol has been developed for detection of mRNA targets in tumor cells, which exhibits ultrahigh sensitivity compared to FISH method[91]. As shown in Fig.2.5, QD was employed as an optical traceable transfection carrier to mediate the cellular internalization of reporter DNA probes. After intracellular hybridization process, expression of target mRNA in tumor cell can be indirectly evaluated by the ECL detection of reporter DNA liberated from cell lysates.

Förster (or Fluorescence) resonance energy transfer (FRET) describes a mechanism of energy transfer from donor excited states to a proximal acceptor chromophore molecule through nonradiative dipole-dipole coupling. The energy transfer efficiency depends on the donor-acceptor distance, relative orientation, and the overlap between donor emission and acceptor absorption spectra[98]. Classical FRET sensing strategies based on fluorescent proteins have been developed as tools for fundamental biochemical research, such as analyte detection and molecular level interaction monitoring (e.g. molecule binding or conformation change)[99, 100]. In

comparison to those conventional fluorophores, QDs are considered as spectacular FRET donor candidates for bio-sensing applications. Because of the broad absorption spectrum and narrow emission profile, QDs with different colors can be simultaneously excited using a single excitation source to provide multichannel detecting ability. More importantly, excitation can be selected wisely to avoid any direct excitation and photobleaching of the acceptor dyes[101]. Also, because of the size-tunable photoluminescence (PL) of QDs, the energy transfer efficiency can be optimized by tuning the overlap between donor emission and acceptor absorption profiles[102]. Furthermore, due to the large two-photon absorption cross-sections of QDs, NIR two-photon excitation can be applied to drive intracellular FRET detection with higher signal-to-background ratio[103]. Fig.2.6a illustrates an example of typical

Fig.2.6 TNT titration of the QD-TNB2-45 nanosensor assembly. A) Schematic of the assay. Addition of TNT displaces the quencher TNB-BHQ10 and thus the QD recovers its emission. The data resulting from the increase in QD PL are plotted as the difference signal versus concentration, both in B) linear and C) logarithmic scales[104].

FRET design[104]. A sandwich structure is formed between quencher-labeled target-analogs and QDs functionalized with recognition moieties, such as nucleic acid, peptides or antibodies. Target analytes present in solution will compete with the analog-quencher complex and restore the photoluminescence of the initially quenched QDs. In this case, QD-anti-TNT antibody (TNB2-45) conjugates was developed for TNT (2,4,6-trinitrotoluene) detection. QDs exposure to soluble TNT induced a systematic and concentration-dependent QD PL recovery, which demonstrated a high sensitivity of the detection scheme (see Fig.2.6b&c).

Although QDs have been extensively used for FRET sensing, several limitations should be mentioned. For instance, using QDs with larger size or inappropriate capping strategy may lead to large center-to-center distance between the QD and the attached acceptor molecules, which substantially cause a relatively low energy transfer efficiency and degrade the sensitivity[105]. To improve the situation, a two-step FRET is developed. By introducing a ‘mediator fluorophore’ between donor and acceptor, a long distance step-wise energy transfer was demonstrated with high efficiency[105]. This limitation can also be circumvented by increasing the number of acceptor linked to QD[106] or by use of multichromophore protein as acceptor[107]. It is worth highlighting that, in addition to organic dyes or fluorescent proteins, Au nanoparticles (AuNPs) can also act as a quencher being paired with the QD to form an energy transfer structure[108, 109]. The size-tunable absorption of the AuNP is favorable towards a more controllable donor quenching rate[102]. On the other hand, because of the broad absorption band and long lifetime, QDs are normally not ideal FRET acceptors[110]. However, these properties benefit QDs being used as energy acceptors in developing self-illuminating optical probes for *in vivo* imaging. In this case, bioluminescence resonance energy transfer (BRET) or chemical energy transfer

(CEF) can be activated without external light excitation, and the energy transferred to QDs is generated by chemical reactions (e.g. luciferase mediated oxidation of substrates)[111-113]. Due to the absence of back ground noise caused by excitation illumination, these self-illuminating probes enjoy high sensitivity for molecule detection and could be promising contrast agents for *in vivo* imaging, where the tissue autofluorescence is a major impediment towards high contrast and deep tissue imaging[102].

2.4.2 Bioconjugated QDs for *In Vitro* Cell maging

Since the first demonstrations using QDs for cell labeling in 1998[4, 5], a variety of QD probes have been developed. After conjugation with recognition moieties, such as ligands or antibodies, QDs can be highly specific to label the corresponding ligand receptors and antigens on cell membrane, which enables us to visualize and optically monitor the movement of single membrane protein/lipids[61, 65, 114-120]. For example, QDs coupled with antibodies against Her2 receptor, which is over-expressed on many kinds of breast cancer cells, were prepared for specific targeting of Her2-positive cancer cells[42, 121]. QDs conjugated with EGF (epidermal growth factor) were reported to exhibit specificity to EGFR membrane receptors over-expressed on various cancer cells[122-124]. Other ligands such as folic acid, RGD peptide, and aptamers have been demonstrated for labeling of folate receptor, $\alpha_v\beta_3$ integrin, PSMA and other membrane proteins[115, 125, 126].

In addition to cell labeling and membrane protein tracking, numerous cell organelles and components have been labeled and monitored by QDs, such as intracellular molecules, nuclear antigens, microtubules, actin filaments, etc.[42, 127-133]. For example, in a work by Wu, specific labeling of subcellular structures by QDs was achieved, with the QD-based probes showing substantial advantages over

organic dyes in multiplexed target detection[42]. More recently, monitoring of intracellular molecules motion, such as EB1 (microtubule-associated protein) and single myosin V (actin-associated protein), has also been demonstrated with high spatial and temporal resolution[127, 130]. As shown in Fig.2.7, after conjugation with QDs, continuous movements of individual EB1 protein along the microtubule structure were monitored optically, while the mean velocity was also evaluated[127]. This result suggests the ability of QD for monitoring dynamic behavior of biomolecule over long time, and the potential to study the complex biological structures or functions based on single-molecule events.

Several methods can be used to deliver the nanoparticles into the cells through the lipid bilayer cell membrane. In addition to the native low-efficient nonspecific endocytosis[43, 134], targeted and enhanced cellular uptake can be achieved by receptor-mediated process when the dots are immobilized by membrane receptors[124, 135]. In order to facilitate escaping of the particles from the endosomes, cationic lipid/peptides, such as lipofectamine and nona-arginine penetrating peptide (CCP), was also introduced for QDs surface monification[136, 137]. For example, Duan et al. have developed cell-penetrating QDs employing hyperbranched copolymer PEI-g-PEG (PEG-grafted-polyethylenimine) as surface coating[138]. In this method, the cationic coating will induce a proton sponge effect, which increases the osmotic pressure, swells and ruptures the endosomes, and in turn release the QDs into cytoplasm. Mechanical delivery by microinjection and electroporation are alternative methods to deliver homogeneously dispersed QDs into cytoplasm[133, 139]. However, these methods are either infeasible for large sample sizes or associated with cell death and aggregations^{165, 170}.

Fig.2.7 Tracking of QD-ND/EB1 on spindle structures following incubation of the QDs ($\lambda=545\text{nm}$) with cell extracts. (a) Rhodamine-labeled spindle structure (red channel) with QD-ND/EB1 (green channel) and the overlay of the two channels (shown in yellow). (b) Temporal image sequence (5s/frame) of a single QD-ND/EB1 moving on a microtubule (see arrow). (c) A switch between a fast and slow movement. (d) Mean velocity histogram extracted from a collection of individual QD-ND/EB1 moving on the spindle structures (3 independent experiments, 3 structures)[127].

2.4.3 Bioconjugated QDs for *in vivo* applications

In vivo imaging is another important application area of QDs. Comparing with conventional imaging approaches in clinical diagnostics, such as MRI (magnetic resonance imaging), PET (positron emission tomography), X-Ray CT (X-Ray computed tomography) and ultrasonography, *in vivo* optical imaging provides a more cost-effective way and potentially offers a high resolution. To date, numerous *in vivo* imaging applications using functionalized QDs have been demonstrated, such as *in vivo* cell tracking[41, 136, 140, 141], vasculature imaging[142-154], tumor imaging and targeted therapy[41, 59, 63, 119, 121, 135, 142, 145, 148, 155-159]. Most importantly, these are in most cases non-invasive and enable real time imaging of cell activity monitoring.

Because of the outstanding chemical and optical stability, QDs labeling allows researchers to visualize *in vivo* activities in real time even for a relatively extended period of time, on the order of months. In the first demonstration performed on single frog (*Xenopus*) embryo, which was microinjected with QDs, development of the embryo and QD distribution were monitored in real-time under continuous excitation[29]. Additionally, QDs have also been introduced directly into the blood stream for vasculature imaging. In the work by Larson et al, green-emitting QDs has been intravenously administered to mice and dynamically visualized in capillaries hundreds of micrometers deep in the skin[148]. In comparison with conventional contrast agents for vasculature imaging (e.g. FITC-dextran), QDs provide better contrast between vessels and the surrounding matrix, require much lower concentration[150, 156], or even provide a chance to image single molecule targets in vessel[157]. Imaging of sentinel lymph nodes (SLNs) with QDs has caught great attention in the past decades since they play an important role in the immune system

and cancer metastasis[143, 147, 149, 151, 153, 158, 160]. Kim et al have reported the use of NIR QDs for labeling the SLNs in cancer surgery on animal models (mouse and pig), which provided the surgeon with direct visual guidance throughout the SLN mapping and resection procedure[145] (Fig.2.8). It should be noted that the size and surface coatings of QDs greatly affect their migration in the lymphatic system. In comparison with larger ones (>10 nm), QDs with smaller hydrodynamic size can drain to a long distance[151], thereby are more suitable for lymphatic drainage mapping or image-guided resection.

Fig.2.8 Images of the surgical field in a pig injected intradermally with 400 pmol of NIR QDs in the right groin. Top to bottom: before injection (autofluorescence), 30 s after injection, 4 min after injection and during image-guided resection. Color video, NIR fluorescence and color-NIR merge images are shown from left to right[145].

Tumor imaging has been the most important *in vivo* application of QDs during the past decade, for which numerous configurations have been employed to improve the targeting efficiency. The first demonstration was carried out by Akerman and coworkers in 2002, in which visible QDs conjugated with three different peptides were intravenously injected into nude mice bearing breast cancer[59]. After several minutes circulation, fluorescence of histological sections suggested specific distributions of QDs in tumor vasculature and organs. The first whole animal scale targeted imaging was reported by Gao et al in 2004[41]. In this work, antibody against PSMA was coupled with the QD for cancer targeting due to its high binding affinity to cancer-specific cell surface biomarkers. After intravenous injection and circulation, fluorescence image of the mice suggested an efficient accumulation of QDs in the subcutaneously implanted prostate tumor. In addition to these active targeting approaches, where specific ligands against cancer biomarkers are employed[63, 135, 154, 161, 162], targeted tumor imaging can also be achieved via a passive mode mediated by EPR effect (enhanced permeability and retention)[163-165]. As a result of porous blood vessels formed in tumor tissue and also non-effective lymphatic drainage, QDs without bioaffinity can passively accumulate in tumor microenvironment and fluorescently label the tumor. Furthermore, attribute to their optical property and tumor targeting ability, QDs have been incorporated with drug formulations, by either surface linkage or loading in polymer particles, to form a traceable drug delivery system[159, 166, 167]. These studies suggest that QDs are nano-platforms with great potential for *in vivo* imaging and future cancer diagnostics/therapy.

Because of the tissue scattering and absorption, the penetration depth of the light is wavelength dependent. It was found that two spectral windows are preferred for in

vivo imaging, namely 700-900 nm and 1200-1600 nm, where the tissues have minimal absorption, autofluorescence and decreased Rayleigh scattering[7, 160, 168] (Fig.2.9). For this reason, recent *in vivo* imaging studies were mostly carried out using NIR QDs, while multi-photon excitation of QDs using low intensity NIR light can further suppress the tissue autofluorescence[148, 158, 169-171]. Additionally, polyethylene glycol (PEG), a bioinert polymer, has become a popular QD coating to reduce non-specific interaction, immune response and accumulation in the reticuloendothelial system (RES). Reduced QD trapping in liver, spleen, bone marrow and lymphatic system has been reported for PEG coated QDs, as well as enhanced targeting specificity due to longer circulation lifetime in the blood[154, 158, 164, 172]. Detailed studies also showed that molecular weight, chain length and coverage degree of the PEG polymer greatly affect the *in vivo* behavior of QDs[45, 174, 175]. In addition to surface chemistry, biodistribution and clearance behavior are also associated with the overall hydrodynamic diameters of the nanoparticles. For example,

Fig.2.9 The NIR window is ideally suited for *in vivo* imaging because of minimal light absorption[168].

Fig.2.10 Blood half-life (hours) of GNPs/mPEG as a function of particle size (nm) and mPEG molecular weight (kDa). Half-life generally improved as particle diameter decreased and mPEG molecular weight increased[164].

Fig.2.11 Left: Schematic depiction of a multistage nanoparticle drug delivery system. The initial 100-nm multistage nanoparticle delivery system accumulates preferentially around leaky vessels in tumor tissue. By cleaving away the gelatin scaffold with MMP-2, 100-nm QDGelNPs change size to 10-nm QD NPs, which can deeply penetrate the dense collagen matrix of the interstitial space. Right: *In vivo* images of QDGelNPs and silica QDs (diameter = 105 nm) after intratumoral coinjection into the HT-1080 tumor. (Scale bar: 100 μ m)[173].

size-dependent circulation lifetime of PEG-QD has been presented by Chan's group (Fig.2.10). Since nanoparticles with large and small sizes have distinct preferences for tumor tissue accumulation and penetration respectively, the author provided design parameters of nanoparticles for either optimized tumor diagnosis or drug delivery[164]. More recently, a multistage delivery system was demonstrated by Wong et al, where size-changing nanoparticle facilitate delivery of QDs to tumor tissue with both sufficient quantity and deep penetration[173]. In this case, after efficient accumulation of large particles in tumor vascular and the following extravasation, a size 'shrinking' of the QD gelatin nanoparticle (~100 nm) was triggered by proteases (MMP-2), which is highly expressed in tumor microenvironment (Fig.2.11, left). The released QDs with smaller sizes (~10 nm) exhibit better diffusion in dense collagen matrix of the interstitial space (Fig.2.11, right).

Accumulation of QDs in the organs due to lack of excretion and the consequent toxic risks may be the main reasons why successful examples of *in vivo* clinic applications are absent. Although renal clearance can be helpful to avoid the accumulation effects, the process is highly complicated by particles' size and surface conditions, while a renal clearance threshold of 5~6 nm has been reported, which means QDs with larger size may not be excreted effectively[176]. Recent encouraging results have been published, where nanoparticles with suitable size and surface coating exhibited both good biocompatibility and tumor targeting ability, and excretion of QDs via kidney could be confirmed by ICP-MS analysis or even fluorescence of the urine sample(Fig.2.12)[154, 172].

Fig.2.12 Renal clearance of QD710-Dendron. (A) Fluorescence imaging of mice ventral before and after tail-vein injection of QD710-Dendron (200 pmol) or PBS at 30, 60, and 120 min, respectively. (B) The fluorescence imaging of urine samples collected after 90 min and the urine from the mice injected with QD710-Dendron had a strong fluorescent signal. (C) UV-vis absorption and (D) Fluorescence emission spectra of the urine samples show the characteristic spectra of QD710-Dendron[154].

2.4.4 Multimodal Imaging with QDs

As important tools for medical diagnosis and analysis, imaging techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT) and optical imaging (OI) are widely employed in clinic applications. Because each single imaging modality may have its own specific advantages and limitations for particular applications, the design and fabrication of multimodal imaging probes has recently become an active research topic, where probes of complementary modalities could be united to compensate the deficiencies of the single modes. Among them, fusion of OI with MRI is one of the most active research fields for multimodal imaging. Offering high *in vivo* spatial resolution and deep tissue penetration, MRI is one of the most popular tools for noninvasive anatomy analysis and pre-surgical diagnosis in human body[177, 178]. Typical clinical contrast comes from tissue-specific differences in the proton density, while Type- T_1 and T_2 contrast agents based on paramagnetic chelates (e.g. Gd and Mn-chelate) and superparamagnetic nanoparticles (MNP, e.g. FePt and iron oxide (SPIO)), respectively, are usually administered orally or intravenously to receive enhanced visibility[179-182]. On the other hand, optical imaging is cost-effective and more powerful to offer a high resolution, especially at subcellular levels. Since QDs have lately been emerging as a new class of optical contrast agents, the preparation and application of semiconductor QDs have received considerable research attention. In contrast with conventional fluorophores used for bioimaging, such as organic dyes or fluorescent proteins, QDs exhibit unique advantages including broad absorption bands, tunable emission colour, high resistance against photobleaching and relatively large surface area for biofunctionalization[183]. Consequently, they have been developed as novel optical probes in applications such as long term cellular imaging, and

multiplexed molecule sensing[5, 183, 184]. More recently, magnetic QDs have become attractive nanoscaffolds in developing MRI/optical dual-mode imaging probes.

So far, several strategies have been reported for magnetic QDs fabrication, including: 1) Co-encapsulating QDs and MNP (e.g. SPIOs) within silica or polymer carrier materials to create QD/MNP composites[185-190]; 2) Fusing QDs and MNP directly as a core/shell or hetero-dimer structure[191-194]; and 3) Conjugating the dots with paramagnetic chelates (e.g Gd, Dy or Mn chelates)[195-197]. In view of the tunability of NPs integration (different types and payload ratios), the first strategy is clearly the most versatile one for MRI/optical probe preparation. However, the relatively large size (>50 nm) of the hybrids likely imposes limits on their *in vivo* and clinic applications. Although, much smaller single nanoparticle based probes (~10 nm) can be achieved via the other two approaches, direct combination of QD and MNP generally causes quenching of QD PL, and paramagnetic chelates adherence has limited payload[179], of which the relaxivity is also complicated by the surface chemistry (e.g. number of Gd coordination sites on chelants, tumbling rate on surface, the water-exchange rate and water coordination)[195, 198]. Doping QDs with paramagnetic transition metal ions is another straightforward method to incorporate magnetic properties into single QDs, which will be discussed in Chapter 4.

2.4.5 Quantum Dot Toxicity

The toxicity issue has been raised and received more and more attention not long after the QDs have been introduced into biomedical research. Since toxicity assessment of QDs is critically important for their practical application in biomedical fields, lots of studies have been carried towards a better understanding of their adverse effects. However, due to the discrepancy in the available reports of QDs toxicity assessments,

it is difficult to draw an unambiguous conclusion on the exact mechanism of the toxic effects. For example, QDs with different surface coating and sizes may experience different local conditions due to the discrepancy in cellular uptake, intracellular interaction and localization, and in turn show different toxic effects[199]. Sometimes, the toxicity may also come from the surface-covering molecules themselves[200]. Moreover, besides the inconsistent results from the many parameters used to evaluate QDs toxicity, such as proliferation, apoptosis, genetic variations, cellular morphology or metabolic activity, toxicity assessment may also be complicated by the cell lines for modeling, which may exhibit varying tolerances to QD-induced toxicity[201].

Although there are still several ongoing debates in this area and more systematic studies are still required, the release of heavy metal ions was frequently mentioned to explain the toxic effects, according to most reported results. For QDs containing heavy metals, such as cadmium, lead and mercury, the risk from the degradation is quite obvious. Although the heavy metal core material can be encapsulated in an organic coating to enhance its chemical stability, degradation and heavy metal release from these particles due to surface oxidation were found when exposure to photolytic and intracellular oxidative conditions[27, 202-204].

The particle size is another concern when evaluating the QDs toxicity. Since particle size affects the surface-volume ratio and the risk of ion release, there is a claim that the dosing parameter should be described in terms of surface area, rather than simply in mass or concentration basis[200, 205]. In some recent work, toxicity assessments of QDs and soluble Cd salt at similar Cd concentrations are compared to confirm the Cd ion induced cytotoxicity. The distinctly different effects observed between free Cd and QD suggest that, toxicity of the dots is further complicated by

effects of bioaccumulation, abnormal local concentration and nanoscale effects of the particles[201, 206-209].

In addition to chemical degradation, free radical generation is another concern for QD toxicity. Photosensitive QD transfers energy or electrons to molecular oxygen and cause the formation of singlet oxygen, which in turn reacts with water or other molecules and catalyzes production of reactive oxygen/nitrogen species (ROS)/(RNS), such as hydroxyl radical ($\cdot\text{OH}$), superoxide anion ($\cdot\text{O}_2$), hydrogen peroxide (H_2O_2) and peroxynitrite ($\cdot\text{ONOO}$)[210-213]. Generation of these free radicals was reported to cause DNA nicking and breaking[210, 214], cell apoptosis and loss of metabolic functions[215]. However, recent study suggested that oxidative stress is the principle mechanism of QD toxicity only when the concentration is low. With increasing concentration, Cd release becomes the major reason of adverse effects[216]. Also, it is worth mentioning that there are reports taking the advantage of this free radical generation process and using it for photodynamic therapy (PDT) aiming at targeted cancer treatment[217-219] .

Strategies have been explored to reduce the potential toxicity from the degradation and the free radical generation. The ZnS shell coating method is usually introduced, which slows down the oxidation process by limiting the transport of oxygen to the core surface. However, this method along with other coating strategies using small molecules, polymers, proteins or silicon dioxide do not fully solve the problem while ROS generation and degradation of the shells and consequently the core material still occurs[27, 199, 212, 220]. Nevertheless, for those applications where long term toxicity is not a major concern, the coating strategies offer a variety of possibilities to improve the biocompatibility and other performances of the QDs. More recently, progresses have been made in developing cadmium-free QDs, such as III-V (e.g.

Chapter 2 Literature Review

InP)[118, 146, 221, 222], Mn/Cu doped Zinc chalcogenide[126, 223-226], Si[158, 227-229] and others material based QDs (e.g. CuInS₂, CuInSe₂, AgInS₂)[230-232].

These QDs, given the name “new generation QDs”, provide competitive properties and make themselves as promising candidates for a wide range of future applications.

Chapter 3 Aqueous Phase Synthesis of CdTe Quantum Dots using Mixed-ligands System

Quantum dots (QDs) exhibit unique optical properties including size-tunable fluorescence, superior brightness, broad absorption and tiny emission bandwidth. These properties have made QDs promising candidates for bioimaging and biosensing applications. In this work, CdTe QDs were synthesized in aqueous phase using both mercaptopropionic acid (MPA) and cysteine (Cys) as capping agents. Growth kinetics and optical properties of the resulted QDs were found to be dependent on both species and concentration of surfactant molecules. Considering the distinct nature and advantages of MPA and Cys, a mixed-ligands system was proposed as a potential approach to fabricate QDs with specific optical property and functions for *in vitro* and *in vivo* bioimaging applications.

3.1 Introduction

Highly luminescent QDs with narrow emission bandwidths have been realized by organometallic method[21]. However, the reaction is complicated by use of hyper-toxic precursors, a high temperature environment, and more importantly, the QDs capped with hydrophobic TOPO/TOP ligands are not able to be applied in biological systems directly and tedious post-processing procedures are needed to make them water dispersible. These phase transfer steps are always accompanied with either deteriorated quantum yield (QY) or an increase in the overall hydrodynamic size of QDs[6, 233, 234].

On the contrary, QDs prepared using aqueous phase methods with hydrophilic thiols or polyphosphates[48] as stabilizing agents are highly appreciated since they

are ready for bioconjugation and can be directly used in biological system[46]. Stabilizing agents, also known as surfactants, play significant roles in QD fabrication, including growth rate control, surface dangling bonds passivation and stabilizing the particles in polar solvent[7]. Generally, in comparison to long chain length mercapto acids (such as mercaptoundecanoic acid (MUA) or Mercaptohexanoic acid (MHA)) which are hydrophobic, short chain length thiol ligands such as mercaptopropionic acid (MPA) or thioglycolic acid (TGA) are widely employed as stabilizing agents, since they enable high growth rate of the nanoparticles and also provide better solubility in polar solvents[55, 235]. More recently, cysteine (Cys) has been reported as a new surfactant used to mediate QDs growth in an aqueous phase preparation method[236]. In this case, QDs capped with Cys exhibit both carboxyl and amino functional groups on the surface that can be used for bioconjugation[237]. Also, using Cys is a convenient way to fabricate QDs with positively charged surfaces[48] for applications such as DNA imaging and gene delivery. However, results from the literature demonstrated that Cys is unstable and susceptible to hydrolysis and oxidation[57, 238, 239]. Without effective passivation and protection by the capping layer, QDs may lose fluorescence or even degrade in the biological environment and in turn cause toxic effects[200].

In this work, CdTe QDs with emission ranging from 500 to 700 nm were directly synthesized in aqueous phase by using both Cys and MPA as surfactants. We aimed to investigate the effects of these two different thiol ligands on the growth kinetics and optical properties of CdTe QDs in aqueous phase. We began our study by using MPA and Cys individually to prepare QDs and comparison was made between these two different surfactants capped QDs with respect to their growth rate, QY and emission bandwidth. The results suggested that both species and concentration of

surfactant molecules applied in the reaction mixture strongly influence the growth kinetics and optical properties of the QDs. These findings have prompted us to investigate the use of a mixed ligands system containing both MPA and Cys for synthesizing CdTe QDs. Based on our findings, the mixed-ligands system for CdTe QDs synthesis can be optimized by carefully adjusting the concentration ratio between MPA and Cys, which allows one to specifically tailor QDs with desirable emission wavelength, reasonable brightness, narrow bandwidth, enhanced photostability and biocompatibility for bioimaging applications.

3.2 Experimental Section

3.2.1 Chemicals

Cadmium perchlorate hydrate ($\text{Cd}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$), sodium borohydride (NaBH_4), tellurium (Te) powder (99.8%, 200 mesh), L-cysteine (99.5%, Cys), 3-mecaptopropionic acid (MPA) and thiazolyl blue tetrazolium bromide (MTT assay) were purchased from Sigma-Aldrich. All the chemicals are used as received. Ultrapure water with 18.2 M Ω /cm was used for sample preparation in all steps.

3.2.2 Synthesis Method

In our experiment, Te powder and NaBH_4 were used for Te^{2-} precursor preparation [240]. Briefly, 48 mg of tellurium powder and 30 mg of sodium borohydride were mixed with 5 ml nitrogen-saturated DI water. Then, the mixture was stirred for 3~8 hours until all the Te powder dissolved and the solution become either colorless or light pink. The chemical reaction that took place in this step can be expressed as[240]:

Owing to the strong reducing ability of H^{-1} , Te can be reduced to valence of -2 while even H^+ ions in water could be reduced with generation of H_2 . To ensure an efficient reduction of Te, the reaction must be kept away from oxygen. In our strategy, the

water was heated (50~70°C) and then saturated with nitrogen for 40 mins to remove dissolved oxygen, and the vessel for reaction was sealed away from air while an outlet was left open for releasing the H₂ gas generated in the process. On the other hand, Cd(ClO₄)₂•6H₂O was employed as Cd²⁺ source in the reaction, before which, Cys and/or MPA were introduced to prepare the Cd-ligand complex as the Cd precursor. Specifically, a mixture of Cd(ClO₄)₂•6H₂O and surfactants were loaded into a three-necked flask and dissolved in 100 ml of DI water. The pH value of the mixture was adjusted to 12 by dropwise titration with a 2M NaOH solution. Furthermore, in our research, the molar ratio between the Cd²⁺ and the surfactants was investigated as an important factor for QDs growth control. The molar ratio between Cd and Te was fixed at 4.2 throughout all the experiments in this work[237]. In practice, 672 mg of Cd(ClO₄)₂•6H₂O and Cys (MPA) with varied concentrations were used in the parallel reactions. After a quick injection of the fresh NaHTe precursor, the reaction solution was vigorously stirred and heated to 100 °C. The chemical reaction can be expressed as below[241]:

Within hours, a series of QDs emitting with different wavelengths, varying from 500 to 700 nm, can be produced at different time intervals during the reaction (10 min ~ 8 h). In order to remove unreacted precursors, excess surfactants and byproducts, purification of the as-prepared QDs was performed. Basically, an equal volume of ethanol was mixed with the as-prepared QDs solution, leading to precipitation of the hydrophilic QDs. The precipitate was collected by centrifugation (6000 rpm, 10 min) and re-dispersed in DI water for further characterization or *in vitro* cell experiments.

3.2.3 Characterization

UV-visible absorption spectra were obtained from a spectrophotometer (Shimadzu UV-2450). Photoluminescence (PL) spectra were measured at room temperature using a spectrofluorometer (Fluorolog-3 spectrofluorometer) with an excitation at 470 nm. The quantum yield (QY) of the QDs was estimated based on a standard procedure described in literature[242], where Rhodamine 123 (Rh₁₂₃) in ethanol, having a QY of 96%, was selected as the reference. The optical densities (OD) of both the sample and the reference were adjusted to 0.05 ~ 0.06 OD at the excitation wavelength (470 nm). Based on UV-vis absorption and PL emission measurements, QY can be calculated using Eq.3.4 and Eq.3.5. To investigate the photostability of the QDs, a 10 W UV lamp was employed for irradiation.

$$QY_S = QY_R \times \frac{A_S \times F_R \times n_S^2}{A_R \times F_S \times n_R^2} \quad (\text{Eq.3.4}),$$

$$F = 1 - 10^{-D} \quad (\text{Eq.3.5}).$$

The subscripts 'S' and 'R' refer to our QD sample and the reference dye Rh₁₂₃, respectively. *A* is the integrated area under the corrected fluorescence spectrum; *D* is the optical density at the excitation wavelength; *n* is the refractive index of the solvent.

3.2.4 Cell Culture and Cell Viability Test

Human pancreatic cancer cells, Panc-1 (CRL-1469, American Type Culture Collection), were maintained in culture with Dulbecco's Modified Eagle's Medium (DMEM, Hyclone), supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 µg/mL penicillin (Gibco) and 100 µg/mL streptomycin (Gibco). Cells were cultured at 37°C in a humidified atmosphere with 5% CO₂. Cell viability was tested by the MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (Sigma) assays. Briefly, panc-1 cells seeded in a 96-well plate at a density of 5000 cells/well were incubated with different concentrations of CdTe QDs for 24 hours. For each assay, 20

μL of 5 mg/ml MTT in PBS was added followed by incubation for another 4 hours. After removing the culture medium, 150 μL of 100% dimethylsulfoxide (DMSO, Sigma) was added followed by 5 minutes gentle shaking to dissolve the precipitate. Absorbance was then measured with a microplate reader (Bio-Rad) at the wavelength of 490 nm. The cell viability was obtained by normalizing the absorbance of the sample well against that of the control well and expressed as a percentage, assigning the viability of non-treated cells as 100%.

3.3 Results and Discussion

3.3.1 Using L-Cysteine and MPA Individually to Prepare CdTe QDs

Based on the synthesis method described above, L-cysteine (Cys) or 3-mecaptopropionic acid (MPA) capped CdTe QDs were successfully synthesized in aqueous phase, and tunable emission color ranging from green to red can be obtained by manipulating the reaction time and the reaction conditions (Fig.3.1a, photograph of the QDs solution under UV irradiation). Representative absorption and photoluminescence spectra of the QDs obtained at different reaction time are shown in Fig.3.1b&c. As can be observed from the spectra, both the band-edge absorption (Abs) peaks and PL emission (PL) profiles can be detected shortly after injection of Te precursor (5 min after injection), which indicates the formation of the CdTe QDs. As the reaction time increases, the red-shifting peak wavelengths of the Abs and PL profile imply the growth of the QDs in size. It is worth noting that, growth of red emitting QDs (PL peak wavelength > 600 nm) was thought to be mainly supported by an Ostwald Ripening process, which can be evidenced by the broadening of the Abs and PL profiles. In Fig.3.1d, we present the time evolution profiles of the Abs and PL peak wavelengths of the QDs shown in Fig3.1b&c. As the reaction continues, the red-shifts of the Abs and PL peaks followed a similar trend. In addition, a global Stokes

Fig.3.1 (a) Photograph of QDs under UV irradiation. (b) UV-vis absorption spectra and (c) PL emission spectra of the QDs prepared with different reaction time. (d) Time evolution of the Abs and PL peak wavelengths of the QDs.

shifts about 40~50 nm were observed (involve energy offset 25~30 mV), which can be attributed to a combined effect of band-edge fine-structure splitting, phonon-assisted emission and size polydispersity[243].

In addition, we have systematically investigated the effects of surfactants on QD PL properties. For this purpose, parallel experiments with surfactant/Cd ratios = 1.3, 1.6, 1.9, 2.2 and 2.5 were carried out with a same pH value (~12) and Cd/Te molar ratio (4.2). As shown in Fig.3.2A, for MPA mediated reactions, a rapid red-shift in the QDs emission peak was observed during the first two hours of reaction in case of MPA/Cd=1.3, while NIR-emitting QDs (PL wavelength >700 nm) can be obtained after 8 hours of reaction. However, increasing the MPA/Cd ratio dramatically slowed down the kinetic growth rate of the QDs. For example, when a MPA/Cd ratio of 2.5 was applied, only green and yellow-emitting QDs (PL wavelength < 590 nm) were obtained during the 8 hours reaction. In this case, in order to obtain QDs with emission peak above 630 nm, a reaction with duration more than 20 hours was required. It was reported that upon adjusting the MPA/Cd ratio to be above 1.5, the majority of the Cd precursors exists in the form of Cd-MPA dithiol-complex instead of monothiol-complex[244, 245]. Because the release of the Cd^{2+} from the dithiol-complex is less effective in comparison to that from the monothiol-complex, further increasing the MPA concentration can reduce the QD nucleation activity and slow down the growth rate[246]. However, an opposite trend was observed when Cys was used as the capping agent, i.e., higher growth rates of QDs can be achieved by increasing the Cys surfactant concentration applied in the reaction solution. As shown in Fig.3.2B, when a Cys/Cd ratio of 2.5 was used, the QDs emission peak red-shifted to 600 nm within the first 2 hours of reaction and QDs with NIR emission can be

Fig.3.2 Time evolution profiles of the emission peak wavelengths of MPA-capped (A) and Cys-capped (B) CdTe QDs. Surfactant/Cd ratio = 1.3, 1.6, 1.9, 2.2 or 2.5.

Fig.3.3 Three-dimensional plot of the data in Fig.3.2, showing the emission peak wavelength versus reaction time and surfactant/Cd molar ratio.

obtained after 8 hours of reaction. When the Cys/Cd ratio was reduced from 2.5 to 1.6, a decelerated growth of the nanocrystals was observed.

In Fig.3.3, 3D plots of temporal evolution of the PL peak wavelengths are presented to summarize the growth kinetics of CdTe QDs mediated by MPA or Cys. The data was fitted with the equation, $z = A_0 + A_1x + A_2y + A_3x^2 + A_4xy + A_5y^2$,

which is provided for empirical predictions of the emission peak in various conditions but has no physical meaning. In this expression, the emission peak wavelength is referred as z , while x and y are reaction time and surfactant/Cd ratio, respectively. The best fit was obtained when $A_0=1016$, $A_1=0.6338$, $A_2=-497.6$, $A_3=-0.0004542$, $A_4=-0.1216$ and $A_5=117.8$ for surfactant MPA, and $A_0=461.9$, $A_1=0.1072$, $A_2=52.02$, $A_3=-0.0002978$, $A_4=0.1394$ and $A_5=-7.448$ for Cys-mediated reactions. A more obvious comparison between the impacts of these two surfactants on the growth kinetics of the QDs is shown in Fig.3.4, where the QDs' emission peaks resulted after the 8 hours synthesis are plotted against the surfactant/Cd ratio.

To explain the opposite trend observed in Cys mediated reactions, we propose that both the concentration and species of the surfactants affect the Cd precursor reactivity, which has a strong influence on the nucleation rate and growth kinetics of the nanocrystals. It has been reported that the reactivity of Cd precursors is dramatically affected by the bonding strength as well as the steric effects of the thiol ligands[247, 248]. In our case, when a low Cys/Cd ratio was used (such as 1.3), it is reasonable to

Fig.3.4 Emission peak wavelength obtained for QDs fabricated after 8 hours of reaction versus thiols/Cd molar ratio.

hypothesize that the Cd precursors are mainly in form of Cd-Cys monothiol-complexes. As a result of the weak Cd-thiol bonding, these highly reactive monothiol-complexes can initiate formation of a large population of nuclei during the nucleation stage, while the precursors in the reaction solution could be over-consumed and become inadequate to support the subsequent growth process. On the other hand, the Cd precursors can also be in form of dithiol-complex, when the Cys/Cd ratio was increased to a higher value (e.g. 2.5). In these cases, because of the lower reactivity of the Cd precursors, a smaller number of nuclei would be generated during the nucleation process. As a result, there will be more Cd precursors that can survive the nucleation step to promote the growth process, and therefore cause the rapid shift of the QDs emission peak to longer wavelengths. On the contrary, as for MPA mediated reactions, because of the strong binding between Cd and MPA, when a high MPA/Cd ratio was applied, formation of dithiol-complexes will lead to very low precursor reactivity and consequently slowing down the growth of QDs. In addition, we also speculate that disulfide bonds may form between Cys molecules at high concentration and this will lower the total concentration of “active” Cys molecules to passivate the QD surface. This may allow more monomers to access the QDs surfaces and result in an accelerated growth process at the Cys/Cd ratio of 2.5.

The quantum yield of the samples synthesized in different reactions was also studied. Fig.3.5a shows the evolution of QY of the samples with respect to their emission peak wavelengths. From this Figure, one can suggest that the QY of the aqueous-phase synthesized QDs is greatly affected not only by the types of surfactant species, but also by the concentration of the surfactant used. It is noticeable that the QY of MPA-capped QDs increases as the particles size grow larger, and higher QY can be obtained by lowering the ratio of MPA/Cd. Tuning the ratio from 2.5 to 1.6

significantly enhances the QY two to four-fold. However, further reduction of MPA/Cd to 1.3 did not raise the QY of QDs. On the contrary, poor QY was observed. Although, a molar ratio of 1.3 favors a high growth rate, QDs with longer emission wavelength beyond 640 nm suffer a dramatic decrease in QY, and more importantly, a less stable QDs dispersion was observed. This poor QY and stability of QDs should be attributed to the hydrolysis of the capping ligands at high reaction temperatures, which results in insufficient ligands left to passivate QDs with larger sizes[57]. We noticed that, if enough MPA is present in the reaction, the effect of hydrolysis is negligible and good QY and colloidal stability of QDs can be obtained. However, in

Fig.3.5 Quantum yield versus emission wavelength peak of: a) MPA- and b) Cys-capped CdTe QDs. Surfactant/Cd = 1.3, 1.6, 1.9, 2.2 or 2.5.

comparison with MPA, the effect of hydrolysis is apparent at any given ligands concentrations when L-cysteine is used as stabilizer. Fig.3.5b shows the QY of Cysteine-capped CdTe QDs. Typically, as the particle size grows larger, QY firstly increases and then decreases quickly after reaching a maximum at certain wavelengths. It is also interesting to observe that the peak wavelength at which QDs exhibit the highest QY shifts to larger wavelengths as the concentration of Cys increases in the reaction mixture. For example, a change of the Cys/Cd ratio from 1.3 to 2.5 caused a shift of the brightest emission wavelength from 550 nm to 660 nm. These results suggest that fabrication of QDs with larger peak emission wavelengths and high QY efficiency can be achieved by using a high Cys/Cd molar ratio. It is worth mentioning that further coating the CdTe QDs with high bandgap ZnS or ZnTe shell improves the QY by 30 to 50%. However, in this study, we are focusing on investigating the impact of ligands on the growth kinetics and optical property of the

Fig.3.6 Emission bandwidth of QDs prepared with different surfactant concentrations and species. Generally, using cysteine as stabilizer leads to smaller FWHM (full width at half maximum) compared with MPA.

“base” CdTe QDs. The effect of the coating shell on the CdTe QDs will be reported separately.

Besides the QY analysis, the emission bandwidth is another important factor to assess the quality of QDs. Two types of QDs emission bandwidth broadening have been reported, namely homogeneous and inhomogeneous broadening[249], and the latter one is due to size-distribution defocusing, which should be suppressed in the synthesis process. The emission bandwidths of the CdTe QDs synthesized in different reaction conditions are presented in Fig.3.6. It is obvious that, in our reaction condition (pH=12, Cd precursor concentration=16 mM, Cd/Te molar ratio=4.2 and ligands/Cd=1.3~2.5) Cysteine shows an advantage over MPA as surfactant in fabricating QDs with narrower emission bandwidths. This phenomenon can be ascribed to the suppression of the Ostwald ripening process due to the higher monomer activity of Cd-Cys than that of Cd-MPA.

The results shown in this section proved that the species and concentration of surfactant molecules applied in the reaction have great influences on QDs growth kinetics as well as their final optical properties. Using these surfactants independently, MPA and Cys have their own advantages to obtain desirable QDs with specific optical property. Specifically, good chemical stability against hydrolysis and degradation make MPA a good candidate to synthesize red to near infra-red QDs with good brightness, preferable photo- and colloidal-stability. On the other hand, narrower emission bandwidths can be achieved by using cysteine to mediate the growth of QDs. Also using cysteine as surfactant is a popular way to fabricate QDs with pH-tunable surface charge and they can be used for gene delivery application. To further probe the advantages offer by these two surfactants for making QDs, a mixed-ligands

system consisting of MPA and Cys was developed to prepare QDs dispersion and the results will be discussed in the following section.

3.3.2 Synthesis of CdTe QDs Using Mixed Stabilizing Agents

Similar with the preparations of MPA- or Cys-capped CdTe QDs, QDs with stable fluorescent emission were successfully fabricated using a mixed MPA-Cys ligands system in this study. In order to investigate optimal conditions high quality CdTe QDs preparation, reactions with varied Cys/MPA molar ratio were carried out in parallel experiments. Fig.3.7 shows the time evolution of the emission peak wavelength of the CdTe QDs at different Cys/MPA ratios. For convenience purpose, the ratio between Cys and MPA is expressed in terms of percentage of the total Cys concentration. When the molar ratio of $n_{surfactant}/n_{Cd}$ was adjusted to 2.2, the discrepancy in growth kinetics between pure MPA- and Cys-based reaction can be observed in accordance with previous results. Meanwhile, we noticed that the growth rate can be tailored in our mixed-ligand system by simply varying the ratio between the two thiol ligands. On the contrary, with the condition

Fig.3.7 Time evolution of the peak emission wavelength of QDs when Cys and MPA are employed in combination with different ratios. Surfactant/Cd = 2.2 (A) or 1.6 (B).

Fig.3.8 (a) Quantum yield of QDs versus emission peak wavelength. Surfactant/Cd = 1.6. (b) Bandwidth of QDs emission versus emission peak wavelengths. Surfactant/Cd = 2.2.

of $n_{thiol}/n_{Cd} = 1.6$, variations in the Cys/MPA ratio exhibited no obvious influence on the growth kinetics and this is because of the similar growth rates of MPA- and Cys-based reactions at $n_{surfactant}/n_{Cd} = 1.6$ (see Fig.3.4).

Although Tian et al have been reported that QY can be enhanced when the dots are capped by both glutathione and thioglycolic acid[250], a similar result was not observed in our experiments. Instead, a 1:1 mixture of the two surfactants resulted in moderate brightness and bright range of QDs (Fig.3.8a). On the other hand, although MPA is more stable against hydrolysis and therefore can withstand longer reaction time to support growth of red-emitting QDs, the growth of the QDs was accompanied with significant bandwidth broadening, which could be due to the Ostwald ripening process. However, in our MPA-Cys co-mediated reactions, broadening of the bandwidth can be slowed by introducing more Cys ligands, while the bright PL emission can also be maintained due to the presence of stable MPA ligands (Fig.3.8b). Based on these observations, it is expectable that the reaction parameters of the

mixed-ligands system can be tuned to tailor the brightness and color pureness of the QDs for specific applications.

To evaluate the effects of the surfactants on QD photostability, diluted samples ($OD = 0.05$) in cuvettes were continuously illuminated by UV light and the PL intensity was monitored over 45 mins. As shown in Fig.3.9, the fluorescence of QDs capped with Cys dropped quickly to almost 0 within 15 mins of irradiation, while QDs capped with mixed Cys-MPA showed excellent photostability for 45 mins. The most stable PL emission was obtained when the QDs were capped with mixed MPA-Cys (1:1).

When compared to the branched Cys ligands, MPA has higher chemical stability, stronger binding with the surface Cd atoms, and more compact capping layer due to its linear structure. For these reasons, it can passivate the surface defects and protect the QD from photo-oxidation more effectively. On the other hand, photochemical

Fig.3.9 Photostability measurement of QDs capped with mixed stabilizing ligands. Surfactant/Cd=1.6. The samples are irradiated by UV light (350nm) with power of 10W. Photoluminescent (PL) intensity of the samples is measured at different time points.

degradation of the vulnerable Cys molecules on the QD surface may cause incorporation of sulfur atoms to form a wide band gap CdS shell over the CdTe core, which can enhance the PL intensity[48]. However, excessive degradation of the surfactant protection layer will lead to oxidation or even degradation of the QD. The excellent photostability observed from the mixed-ligands capped QDs implies that the photochemical degradation process can be controlled or optimized via mixed-ligands capping, so as to obtain high resistance against photobleaching.

3.3.3 Cytotoxicity of CdTe QDs

The cytotoxicity of the QDs was evaluated with a Panc-cell viability assay using MTT reagent. The CdTe QDs synthesized with emission peak at 560 and 585 nm (that will henceforth be denoted as QD560 and QD585) were tested in our experiment. As shown in Fig.3.10, the toxicity of the QDs is not only dosage-dependent, but also affected by particle size and surface properties. QDs-Cys25% was observed to be less

Fig.3.10 Cell viability of Panc-1 cells treated with CdTe QDs with different concentrations for 24 hours.

toxic than QDs-Cys75%. Excluding the toxic effects caused by the stabilizer itself, since Cys is much safer than MPA, chemical degradation and release of the Cd components can be the reason for the toxic effects. Because Cys is unstable and the Cys-Cd binding affinity is less than that for MPA-Cd, a MPA capping layer can provide better protection of the core against the harsh conditions in a biological environment. On the other hand, toxicity of QD560 is remarkably higher than Q585. Because of the quantum confinement effects, QD560 emitting at shorter wavelength should have smaller sizes as compared to QD585. Smaller particles with higher surface-to-volume ratio were thought to have more surface atoms risking oxidation and desorption from the lattice. Also, it might be easier for smaller dots to pass through the cell membrane and give rise to a dosage-dependent effect. For these reasons, toxicity of the QDs should also be size-related. Similar results have been published by Shiohara et al[205].

3.4 Conclusion

Aqueous phase synthesis of CdTe QDs has been conducted in pure- and mixed-stabilizing agents systems. The growth process and properties of as-prepared QDs have been studied. It was found that the species used as surfactants as well as their concentrations are important factors which greatly influence the growth dynamics and the final properties of the resulting QDs. MPA-capped QDs have better stability and higher QY, and using L-cysteine as surfactant determines a narrow bandwidth and flexibility in bioconjugations. Considering the distinct nature and advantages of MPA and Cys, a mixed-ligands system was studied as a potential way to fabricate QDs with desired qualities and functions. The results suggest that the usage of mixed-ligands with a proper synthesis process design can be employed to fabricate QDs with optimized brightness, color pureness and enhanced photo-stability. Cellular studies

indicate that the cytotoxic effect of the QDs was affected by both the capping ligands and the particle size.

Chapter 4 Manganese-doped ZnSe QDs for MR/optical Dual Modal Imaging

The use of cadmium-based QDs in biomedical applications has made substantial progress during the past few years. However, many research groups have revealed the breakdown and degradation of QDs in biological systems[27, 176, 251]. Release of heavy metal ions from the particle surface to the biological environment may induce severe acute toxic effects. Although, several *in vitro* and *in vivo* studies have demonstrated that proper protective inorganic and organic coatings on QDs surface will significantly reduce the nanoparticle toxicity[200, 252], their potential toxicity remain a major debating and unsettled issue for *in vivo* and clinical research. In this chapter, we explore the aqueous phase synthesis of high quality Mn-doped ZnSe QDs for replacing the commonly used heavy-metal based nanocrystals in bioimaging applications. Because the doped QDs do not contain any toxic heavy metal components, they should be more favourable for biomedical applications. In this chapter, a sandwiched core-shell QD (Mn:ZnSe/ZnS/ZnMnS, SQD) was also constructed as a high quality dual-mode probe for MR/optical imaging. Biomedical applications of the prepared Mn:ZnSe QDs will be discussed in Chapter 5.

4.1 Introduction

Mn²⁺ doped II–VI QDs (*d*-dots, principally Zn chalcogenides) have attracted significant research attention because of their unique optical, magnetic and electronic properties induced by exciton-dopant coupling[253, 254]. In comparison to those undoped counterparts, which are normally characterized by band-edge emission, PL of *d*-dots is generally determined by the atomic-like emission states introduced by the

Mn dopants. The fast energy transfer from the host to the dopant states cause quenching of the host PL but lead to a red-shifted emission corresponding to a $^4T_1-^6A_1$ transition. The substantial Stokes shift shows great advantages in LED or bioanalysis applications, as it avoids the issue of self-quenching[20, 223]. Furthermore, owing to the $^4T_1-^6A_1$ transition which is spin-forbidden and local field-dependent, the *d*-dots exhibit a much more stable PL emission against temperature and environment changes[223, 255]. Additionally, this stable PL emission is also much longer-lived, i.e. it has a decay time much longer than that of the scattered light and autofluorescence of the background tissue, which is favourable for time-resolved imaging, as it can eliminate the background interferences in biosensing and bioimaging applications[17, 256].

Mn-doped QDs with superior brightness have been synthesized by using nucleation doping strategies[223] or forming Type I core/shell structures in which the Mn^{2+} ions are mostly located close to the centre of the dots[257]. Recently, these high quality doped dots have been demonstrated as stable and biocompatible optical labels in some bioimaging applications. On the other hand, because of the paramagnetic nature of the Mn ions, Mn doped QDs also attracted research attention in developing new types of contrast agents for magnetic resonance imaging (MRI) or MR/optical dual mode imaging.

Because optical or MR imaging modality has its own specific advantages and limitations, design and fabricate nanoprobes that can combine the two imaging modalities within a single nanosystem enable cross-evaluation of the same nanoparticle through different imaging platforms. When optical imaging has the advantages of low-expense and offering high resolution at subcellular level, MRI is a popular tool for noninvasive anatomy analysis and pre-surgical diagnosis in human

body as it can offer high in vivo spatial resolution and deep tissue penetration. In typical MRI scanning, spins of water protons aligned with the external magnetic field are forced into the transverse plane by an RF pulse excitation at the resonant frequency. After that, longitudinal magnetization (M_z) of the water protons recovers to the equilibrium state ($M_{z,eq}$, lower energy) progressively. As described in Eq.4.1,

$$M_z(t) = M_{z,eq}(1 - e^{-t/T_1}) \quad (\text{Eq.4.1})$$

T_1 which generates the contrast in T_1 -weighted imaging reflects the relaxation time of the protons' spins. Although the imaging contrast typically comes from the tissue-specific differences in proton density, *Type- T_1* agents based on paramagnetic chelates (e.g., Gd and Mn-chelate) are usually applied to achieve enhanced sensitivity, which can speed up the spin-lattice relaxation process significantly.

Although the magnetic properties of core-doping type *d*-dots have been explored in some research papers[258, 259], MRI applications have seldom been reported. Firstly, high quality *d*-dots with bright emission were mostly reported with low Mn doping level (typically ~5 atom. %), while incorporation of large amount of impurities will quench the PL[260, 261]. Secondly, unlike T_2 contrast agents, the T_1 imaging mode requires direct and fast interaction of the paramagnetic Mn with surrounding water protons so as to alter their relaxation time effectively[182]. For these reasons, these luminescent core-doping type *d*-dots may not be ideal T_1 probe applicants to produce a strong MRI contrast. More recently, shell-doping type QDs, in which Mn^{2+} ions were introduced to provide the QDs with paramagnetic properties instead of PL emission, have been established as novel MR/optical dual-mode imaging probes. In these cases, a Mn-doped ZnS shell has been deposited over a highly emissive CdSe or CdTe core[262, 263], and the relaxivity of the dots can be enhanced by increasing the shell doping concentration. Because of the direct interaction between the surface Mn

ions and water protons, these shell doped QDs should be more desirable for T_1 mode MRI applications than the core-doped d -dots. However, significant deterioration of the core emission was observed upon direct deposition of the heavily doped shell, where the Mn^{2+} ions at the surface were thought to serve as quencher states. This is a limiting effect in our study aimed at improving the QDs' performance in both MR and optical imaging modes. Furthermore, the toxicity concerns due to the Cd-based core are additional key issues hampering their translation to clinical applications.

To explore the complementary advantages of the Mn dopant emission (e.g. stability, long lifetime, large stoke shift) and MRI ability, in this work we describe aqueous phase preparation of a MR/optical dual-mode probe based on Mn-doped QD. Firstly, high quality Mn-doped ZnSe QDs were synthesized via a modified nucleation doping strategy, while the optimal reaction conditions were investigated in terms of doping concentration, precursor/surfactant ratios, temperature and reaction time. Based on the d -dots characterized by bright and stable Mn dopant emission, Mn:ZnSe/ZnS/ZnMnS sandwiched QDs (SQDs) were constructed to prepare high quality MR/optical dual-mode probes. In this design, the paramagnetic Mn^{2+} ions fixed at the SQDs surface are able to alter the relaxation time of surrounding water protons efficiently, and, therefore, generate strong T_1 signals in MRI. Relaxivity of the QDs can be enhanced by increasing the shell doping level. More importantly, it was observed that a thick un-doped ZnS transition layer sandwiched between the emissive core and the doped cap-shell was helpful to avoid deterioration of the core emission after the ZnMnS shell capping, and thus eliminating the contradiction in achieving high quality performance in both MR and optical imaging modes.

4.2 Experiment

4.2.1 Chemicals

Zinc acetate ($\text{Zn}(\text{OAc})_2$), manganese acetate tetrahydrate ($\text{Mn}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$), selenium (Se), sodium borohydride (NaBH_4), 3-mercaptopropionic acid (MPA), sodium hydroxide (NaOH) and sodium sulfide nonahydrate ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) were purchased from Sigma-Aldrich. 18.2 M Ω ·cm ultrapure water was used throughout the experiments.

4.2.2 Characterization

The UV-visible absorption spectra were obtained from a spectrophotometer (Shimadzu UV-2450). Photoluminescence (PL) spectra and lifetime were collected using a Fluorolog-3 spectrofluorometer. Quantum yields (QYs) of the Mn:ZnSe QD were determined by comparing the integrated emission of diluted *d*-dots to CdSe QDs with matched absorbance. The QY of the CdSe reference sample was calibrated by rhodamine123. The hydrodynamic size distribution profile and the zeta potential of the nanoparticle formulation were measured by a particle size analyzer system (90Plus, Brookhaven Instruments). Transmission electron microscopy (TEM) images were obtained using a JEOL JEM 2010 microscope.

4.2.3 Synthesis of Mn Doped ZnSe Core

Synthesis of Mn doped ZnSe QD was carried out in aqueous phase, in which short-chain thiol ligand MPA was used as surfactant to mediate the nucleation and growth of the nanoparticles. To prepare the Se^{2+} precursor, Se powder was reduced by NaBH_4 in oxydron-free environment. Briefly, Se and NaBH_4 with a molar ratio of 1:1.5 were mixed in nitrogen-saturated DI water and gently stirred under N_2 atmosphere. After 1~3 hours reaction at room temperature, colorless Se^{2+} solution of 0.02M was obtained and kept at 4°C in dark before use. In a typical procedure for Zn and Mn

precursors preparation, 0.01 mmol $\text{Mn}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ and 0.19 mmol $\text{Zn}(\text{OAc})_2$ were mixed together with 0.4 mmol MPA in 200 mL DI water in three-neck flask at room temperature followed by titration with 2 M NaOH to pH=11. To investigate the effects of Mn dopant concentration in forming high quality $\text{Mn}_x\text{Zn}_{1-x}\text{Se}$ QDs, x was adjusted from 0 to 16%. After a quick injection of the fresh NaHSe precursor (Zn/Se=1:0.9) at 100°C with vigorous stirring, the reaction solution was slowly cooled to 80°C and kept refluxing. Samples collected at different time intervals were purified by ethanol and centrifugation to remove excess surfactants and by products.

4.2.4 Epitaxial Growth of Mn-doped ZnS Shell

In order to construct the core/shell structure, $\text{Zn}(\text{OAc})_2$ and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ were employed to support the growth of the ZnS shell. According to the method described in Reference [264], amount of the shell precursors were calculated so as to achieve desired shell thickness. To avoid homogeneous nucleation, the precursors were added via multiple slow injections. Similarly, a mixture of Mn and Zn precursor was introduced for epitaxial growth of the Mn-doped ZnS shell, while the Mn feeding ratio was varied to adjust shell doping level. Finally, multistep purifications and ligand exchange of the samples were performed to ensure that physical absorbed Mn^{2+} ions on the QD surface can be thoroughly removed before characterization.

4.3 Results and Discussion

4.3.1 Preparation of Mn:ZnSe/ZnS QDs

Water-soluble Mn:ZnSe *d*-dots were directly synthesized in aqueous solutions using MPA as stabilizing agent[19, 257]. As for the previously described doped-QDs synthesis, controllable distribution of the dopants in the host is essential to achieve reproducible and desirable optical properties, because the dopants can act as effective electron-hole recombination centers only if they are incorporated into the host lattice

and isolated from surface[19]. For this reason, a modified nucleation-doping strategy (Fig.4.1a) was adapted to separate nucleation and host growth[265]. Specifically, formation of the *d*-dots commenced with nucleation-doping initialized by NaHSe injection at 100°C. Because Mn^{2+} is less reactive towards Se^{2-} than Zn^{2+} , the high temperature was necessary to activate the incorporation of Mn^{2+} into the host lattice at the nucleation stage. Once nucleation completed, the reaction temperature was cooled down to 80°C, and a small amount of Zn precursor was added to support the host growth. Fig.4.2 shows a typical temporal evolution of UV-vis absorption and PL emission of the *d*-dots. The appearance of the Mn emission peak at 590 nm in the PL spectrum (Fig.4.2b) shortly after the NaHSe injection indicates the successful incorporation of Mn ions into the host lattice. The efficient energy transfer from band

Fig.4.1 Illustration of the preparation process for: (a) Mn-doped ZnSe/ZnS QDs, and (b) sandwiched QDs (SQDs).

Fig.4.2 (a) UV-vis absorption, and (b) PL emission of the *d*-dots obtained at different refluxing time (nucleation temperature = 100°C).

excitons to Mn states quenched the band-edge emission (400 nm) and trap emission (480 nm) of the host (Fig.4.3). The PL decay curve of the Mn emission at 590 nm can be fitted with a single exponential function with an estimated lifetime of $\tau = 0.815$ ms (Fig.4.4). This long luminescent lifetime further confirms that the 590 nm emission is originated from the spin-forbidden 4T_1 - 6A_1 transition.

On the contrary, *d*-dots prepared at lower injection temperature (80°C) exhibit very weak Mn emission but strong band-edge emission (Fig.4.5), implying that the Mn incorporation at a milder nucleation condition was less effective. Unlike the band-edge emission, the peak wavelength of the 4T_1 - 6A_1 transition remained unaltered at 590 nm, which is independent of the reaction time or size change. The intensity of the emission increased steadily and reached the maximum value after 1~2 h refluxing, whereas refluxing beyond 3 h resulted in significant decrease of the Mn emission. This could be due to the Ostwald ripening process, which is evidenced by the broadening of the absorption profile (Fig.4.2a). It was known that, in the absence of

Fig.4.3 Energy band structure of Mn-doped ZnSe QDs and the recombination pathways of the photo-excited excitons.

Fig.4.4 Time-resolved PL decay of 590 nm-emitting *d*-dots. τ is estimated to be 0.815 ms.

Fig.4.5 PL emission of the *d*-dots obtained at a nucleation temperature of 80°C.

sufficient precursors, growths of the nanocrystals are primarily supported by sacrificing the smaller ones. We suppose that the Mn dopants in those shrinking *d*-dots may lose their PL when they come close to the surface trap states. Moreover, for those growing dots, re-deposition of the released Mn^{2+} may also generate surface states serving as deep electron traps or nonradiative recombination centers[266]. These alternative exciton relaxation pathways are in competition with the radiative decay at the band-edge or the isolated Mn^{2+} centers, and therefore cause quenching of

the *d*-dots.

Furthermore, consistent with earlier publications[260, 267], the PL of the *d*-dots was found to be greatly affected by the Mn doping concentration. Although increasing the Mn^{2+} contents allows more efficient energy transfer from the ZnSe host to dopants[268, 269], Mn phosphorescence with high quantum yield (QY, 17%) was only achievable in lightly doped QDs (initial Mn doping ratio $\sim 4\%$), while raising the doping level beyond 6% resulted in significant PL deterioration (Fig.4.6). The explanation for this concentration quenching effect involves the formation of defects due to: 1) Changes of the reaction conditions, 2) Shift of crystalline phase[260], 3)

Fig.4.6 (a) PL spectra and (b) QY of *d*-dots prepared with different Mn doping levels.

Creation of Mn doping-induced quencher states (e.g. Mn^{3+} or Mn^{4+})[267], and 4) A fast resonant energy transfer between Mn^{2+} neighbors (excited Mn^{2+} to unexcited Mn^{2+}) via dipole-dipole interaction. It is worth noting that the multi-step energy migration among the Mn^{2+} ions via dipole-dipole interaction can drastically increase the probability of energy lost at traps or quencher centers located close to the excitation path[270, 271]. Although heavily doped *d*-dots (>10%) could be more desirable for high contrast MR imaging, their optical emission was found to be totally quenched. Based on these results, we deduce that it is unfeasible to prepare high quality MRI/optical dual-mode probes by simply raising the doping level of these core-doping type QDs. Other reaction conditions (i.e. precursor concentrations, MPA/Zn ratio, Zn/Se ratio) were also optimized following the investigation method described in Chapter 3 to obtain high quality *d*-dots (data not shown).

Enhancement of the quantum efficiency was accessible via epitaxial growth of a wide band gap ZnS shell over the Mn-doped core. It is known that, by forming a Type I core/shell structure, charge carrier trapping due to the surface states can be partially eliminated, because the wavefunctions of electron and hole are principally confined in the core region. In this work, the thickness of the ZnS shell was adjusted by supplying different amounts of precursors (Zn^{2+} and S^{2-}) during the shell growth reaction. In order to obtain hetero-epitaxial growth of the shell, the addition of the precursors was divided into multiple low-dose injections to avoid homogeneous nucleation of the shell species. TEM images of the *d*-dot (S_0) and the *d*-dot/ZnS (S_{1-3}) with varying shell thickness are presented in Fig.4.7a, from which the proposed ZnS epilayer growth can be evidenced by the remarkable size increase of the nanocrystals (7.0, 7.5, 8.1 and 8.7 nm for $\text{S}_0, 1, 2, 3$). Based on an approximation that one monolayer (ML) of ZnS has the average thickness of 0.31nm (i.e. the (111) interplanar spacing for zinc

Fig.4.7 (a) TEM images of the *d*-dot (S₀) and the *d*-dot/ZnS (S₁₋₃) with varying shell thickness. (b) Electron diffraction patterns of S₀ and S₃. (c) Size histograms of the sample S₀₋₃ shown in (a).

blende ZnS)[264], the ZnS shells in S_{1-3} were estimated to be about 0.8, 1.8 and 2.7 MLs, respectively. The resulted d -dot/ZnS QDs were characterized by a single set of diffraction rings with a zinc blend lattice (Fig.4.7b), which further confirm the epitaxial growth of the core/shell structures instead of homogeneous ZnS nucleation.

Fig.4.8a shows the UV-vis and PL spectra of the d -dots prepared with different shell thickness. As expected, PL intensity of the d -dots was greatly enhanced after shell capping, and the QY of Mn emission reached a maximum value of 27% when 1.8 MLs of ZnS was deposited (Fig.4.8b). Although surface trap states can be well-passivated by the wide band gap ZnS, further growth of the shell was found to

Fig.4.8 (a) UV-vis and PL spectra of the d -dots prepared with different shell thickness ($S_0=0$ nm, $S_1=0.25$ nm, $S_2=0.55$ nm, $S_3=0.85$ nm, $S_4=1.14$ nm); (b) Revolution of QY against the shell thickness.

remarkably lower the QY. This observation is in agreement with former studies[234, 272]. It is believed that the lattice mismatch (5% for ZnSe/ZnS) can induce interactive straining in a heteroepitaxial core/shell structure. Because of the poor elastic nature of ZnS shell ($B_u=77.1$ GPa) and also the low compressibility of the large size core (7 nm)[15], relaxation of the stretched shell as it grows in thickness will cause the formation of lattice defects at the core-shell interface. Additionally, the growth of the ZnS shell was accompanied with gradual shifts of the absorption and band-edge emission peaks to longer wavelengths. These slight red-shifts can be attributed to the partial leakage of the excitons into the shell region. It is also worth noting that, because of the localized nature of the Mn states, which are only sensitive to the changes in local ligand field[273], the peak wavelength of the Mn emission remained at 590 nm and was not influenced by the core/shell structure. Moreover, the reduction in wavefunction overlap between the less confined excitons and the core Mn^{2+} dopants should also be responsible for the Mn emission decrease, since the energy transfer rate is determined by the exciton-dopant electronic coupling efficiency[273].

4.3.2 Preparation of SQDs as MR/optical dual mode probes

For MR/optical dual-mode probes preparation, a mono-layer of ZnMnS (doped-shell, d-shell) was deposited over the premade Mn:ZnSe/ZnS QDs, whereby forming a QD with Mn:ZnSe/ZnS/ZnMnS sandwiched structure (SQD). For example, SQD₅ and SQD₃₀ are produced by growing a ZnMnS shell over the sample S₂, while the Mn feeding ratio applied for d-shell growth are 5 and 30%, respectively. Representative TEM images of SQD₅ and SQD₃₀ and the corresponding size histograms are shown in Fig.4.9.

Longitudinal relaxation time (T_1) measurements were performed on a 3.0 T MRI instrument at room temperature. Acting as paramagnetic centers, the Mn ions are

Fig.4.9 TEM images and size histograms of SQD₅(left) and SQD₃₀(right).

expected to shorten the longitudinal relaxation time of surrounding water protons, so as to generate MR signals in T_1 weighted imaging. To investigate the effectiveness of the paramagnetic d -dots or SQDs as T_1 agents, as shown in Fig.4.10, the concentration-independent relaxivity (r_1) was derived from the slope of the longitudinal relaxation rates of water protons ($R_1=1/T_1$) against the molar concentrations of the particles (particle relaxivity, r_{1QD}). r_{1QD} of the Mn:ZnSe core (d -dot), d -dot/ZnS, SQD_{5%} and SQD_{30%} were estimated to be 242.6, 97.2, 532.7 and 1030.6 $\text{mM}^{-1}\text{s}^{-1}$, respectively. When compared to the d -dot core, SQDs capped with ZnMnS shell exhibited a much higher relaxivity, and the value was tuneable by varying the shell doping level. On the other hand, a very low value of r_{1QD} was obtained for d -dots capped with un-doped ZnS shell. These results suggest that, in comparison with those identical ions isolated from the surface which can generate a bright and stable dopant emission, the Mn^{2+} ions present at the surface have a direct

Fig.4.10 Longitudinal relaxation rate (R_1) plotted against QD concentration.

and more efficient interaction with the spin of water protons, and therefore play the dominant role in rI_{QD} enhancement. According to these results, a heavily doped shell should be applied to receive higher relaxivity, although spin-spin interaction between Mn^{2+} ions at high Mn concentration may partially counteract the rI enhancement[263].

Although a heavily Mn-doped shell is favourable for enhanced rI relaxivity and robust signal in T_1 -mode imaging, however, PL deterioration induced by the shell dopants compromise the use of high doping levels and become a major hindrance to develop high quality MR/optical dual-mode probes. In line with the reported shell doped CdSe or CdTe QDs, direct epitaxial growth of a Mn-doped ZnS shell over the d -dot core resulted in a significant reduction of the core-emission. In order to investigate the influences of the surface Mn dopants on the PL property of the QDs, PL spectra of ZnSe QD capped with a $Zn_{95\%}Mn_{5\%}S$ shell were measured in parallel experiments (Fig.4.11). It is worth noting that the shell-located dopants failed to generate the $^4T_1-^6A_1$ transition-related PL emission; instead, they caused deterioration of the ZnSe core emission. As compared to the core-doped Mn^{2+} ions, the electronic

Fig.4.11 PL spectra of ZnSe QD capped with ZnMnS shell.

coupling between the exciton and the surface dopants can be too weak to cause an efficient energy transfer, the strength of which has been reported to depend on the spatial distribution of the wavefunctions of both charge carriers of the exciton and dopant ions[273]. However, the excited Mn states may lose the energy to adjacent non-recombination surface states, and therefore quench the band gap emission without giving the dopant emission. Furthermore, because of the lattice constant difference between Mn-S and Zn-S, non-uniform growth of the ZnMnS may produce plenty of lattice defects at the core-shell interface, and consequently quench the long-lived core emission.

Fortunately, according to our results, this shell doping-induced PL quenching effect can be effectively eliminated via a sandwiched structure. The PL spectra of SQDs prepared with different transition-shell thickness are shown in Fig.4.12. It is noticeable that, as the sandwiched un-doped ZnS shell thickness increases, the emission of the core is less likely to be affected by the shell dopants. Because of the potential barrier introduced by a thick ZnS layer, the photo-generated excitons could be properly confined inside the core region without interacting with the non-

Fig.4.12 PL spectra of SQDs prepared with different transition-shell thickness.

Fig.4.13 Energy structure illustration of the doped Type II core/shell SQD.

recombination states located at the surface (Fig.4.13). More importantly, a thick ZnS sandwich layer may also serve as a buffer zone to shield the emissive core from local lattice distortions and defects that may arise due to non-uniform growth of the heavily doped shell.

Fig.4.14a shows the T_1 -weighted MR image, as well as photograph of the SQDs under UV excitation. Images of *d*-dots QDs and Mn:ZnSe/ZnS core/shell QDs with same molar concentration are also presented for comparison. As compared to Mn:ZnSe/ZnS QDs, enhanced T_1 signals were visible for SQDs, which present more Mn ions at the surface. Increasing the Mn concentration in the SQD shell produces a

Fig.4.14 (a) T_1 -weighted MR images and photograph of *d*-dots, *d*-dot/ZnS, SQD_{5%} and SQD_{30%}. (b) T_1 -weighted MR images and photographs of SQD_{30%} with varying solution concentrations. The photos show the PL emission under UV excitation at 350nm with a power of 10 W.

higher MR intensity, and more importantly, it has little obvious adverse effects on the PL brightness. Since these SQDs possess high relaxivity and bright PL emission at the same time, they should be promising MRI/optical probes for dual mode imaging applications.

4.4 Conclusion

In this work, Mn-doped ZnSe/ZnS sandwiched core/shell QDs (SQDs) were developed as dual-mode imaging probes with high PL efficiency and MRI ability. To obtain a highly efficient PL emission, an aqueous synthesis method was firstly optimized to prepare orange-emitting Mn-doped ZnSe quantum dots (*d*-dots). The Mn doped ZnSe core could be capped with a wide bandgap ZnS shell to obtain enhanced quantum efficiency and improved photo- and chemical stability. Because of the paramagnetic nature of Mn^{2+} ions, a Mn-doped ZnS cap-shell was deposited over the emissive core to enhance the T_1 contrast in magnetic resonance imaging (MRI). Because of the to direct and fast interaction between Mn and the surrounding water protons, the presence of a large quantity of Mn^{2+} ions at the QDs surface determines a

high MR relaxivity. However, a high level Mn shell doping was found to cause severe quenching of the core emission. To overcome the incompatibility in obtaining high quality dual-mode probes with both a desirable high PL efficiency and enhanced MRI contrast, SQDs were constructed with an un-doped ZnS layer sandwiched between the Mn:ZnSe core and the ZnMnS cap-shell. A thick ZnS shell can confine the photo-generated excitons inside the core region and, therefore, suppresses the non-radiative transition pathway in the doped shell. Furthermore, the ZnS shell may also serve as a transition layer to shade the emissive core from the lattice strain of the doped shell, eliminating lattice defects formation at the core surface. *In vitro* cellular experiments, including biocompatibility test and biomedical applications of the *d*-dots, will be discussed in Chapter 5.

Chapter 5 Quantum Dot Based Theranostic Agents for Pancreatic Cancer Therapy

In this chapter, we demonstrate the applications of aqueous phase synthesized manganese doped zinc selenide QDs (Mn:ZnSe *d*-dots) for *in vitro* gene delivery and therapy of pancreatic cancer cells. For this purpose, *d*-dot/polymer nanoplex and *d*-dot/liposome hybrids were developed as theranostic agents for optically traceable siRNA delivery. The siRNA transfection caused RNA interference (RNAi) and significant silencing of the target mutant K-Ras gene. The *d*-dot/liposome hybrid was also demonstrated as a flexible nanoplatform for combined gene/chemotherapy of pancreatic cancer cells. Because the *d*-dots are free of heavy metal ions, they are promising candidates to develop safe and applicable contrast agents for theranostic applications.

5.1 Introduction

Pancreatic cancer is one of the most common cancers with a 5-year survival rate less than 5%[274]. As this disease is extremely difficult to treat with conventional therapies, development of novel therapeutic approaches is urgently needed. Due to the advancement of cancer biology in the past few decades, RNA interference (RNAi), which is a sequence specific post-transcriptional gene silencing mechanism induced by double-stranded RNA (dsRNA), has been developed as a novel platform for gene therapy of disease[275]. Chemically synthesized small-interfering RNAs (siRNAs) can be delivered to tumor cells for initiating specific degradation of target messenger RNA (mRNA) of complementary sequence thereby inhibiting the expression of the corresponding cancer-promoting genes[275, 276]. However, application of naked

siRNA molecules is challenged by limited uptake efficiency and short half-life due to nuclease-mediated degradation[277]. Recently, viral vectors and synthetic materials, such as liposomes, peptide dendrimers, polymers and inorganic nanoparticles (e.g. noble metals, metal oxides, nanocarbons and mesoporous silica), have been investigated as potential carriers for gene delivery[278-287]. Because of the novel optical properties of the semiconductor QDs, such as broad excitation profile, tunable colour and high resistance to photobleaching[6, 29, 67, 148], in the last few decades, extensive research works have been published exploring their potential applications in biomedical field. In some recent research works, serving as diagnostic agents, the QDs have been integrated with therapeutic agents (e.g. drugs and siRNA) to develop multifunctional theranostic (diagnostic therapy) nanomedicines, which have the potential to visualize the delivery process in the long term, therefore enable us to investigate and optimize the delivery dynamics to achieve improved therapeutic efficacy with reduced side effects[166, 288].

However, most of the reported QD-based gene vectors are developed by using cadmium-based QDs synthesized in organic phase, and their potential toxicity remains a major debating and unsettled issue for them to be translated for *in vivo* and clinical research. To overcome this challenge, in this study, we developed manganese doped zinc selenide QDs (*d*-dots) as a biocompatible nanocarrier for *in vitro* gene delivery. Because the Mn:ZnSe *d*-dots do not contain any heavy metal compositions, they are more acceptable for real-life biomedical applications compared to the traditional types of QDs (e.g. CdSe, CdTe, PbS, PbSe and InAs)[289].

For genetic therapy of pancreatic cancers, we designed a siRNA sequence specifically targeting the mutant K-Ras gene with a point mutation at codon 12, which is present in approximately 90% of all types of pancreatic cancers and associated with

increased cell proliferation and resistance to apoptosis[290-292]. Two types of gene carriers were developed based on the Mn:ZnSe QDs, namely *d*-dot/polymer nanoplexes and *d*-dot/liposome hybrids. For *d*-dot/polymer nanoplex preparation, a layer-by-layer (LBL) assembling method was adopted to modify the *d*-dots surface with cationic polymer poly(allylamine hydrochloride) (PAH) or polyethylenimine (PEI) in generating positive surface potential for complexing with K-Ras siRNA molecules. The nanoplexes were colloiddally stable for weeks while the *d*-dots were highly stable against photobleaching. Owing to the unique and stable PL properties of the *d*-dots, siRNA transfection and the subsequent intracellular release profile were monitored by fluorescence imaging. Furthermore, the amine-terminated surface could be further modified to obtain multiple bio-functions, including cancer cell targeting ability. For *d*-dot/liposome preparation, a solvent dispersion method was used. In this strategy, *d*-dot and siRNA molecules are co-encapsulated in spherical vesicles of amphiphilic lipid bi-layers. Flow cytometry studies suggested that cancer cell targeted delivery can be achieved after surface functionalization by folic acid (FA). The expression of the mutant K-Ras mRNA in transfected cells was observed to be significantly suppressed, confirming the therapeutic effects. More importantly, in comparison to the *d*-dot/Polymer nanoplex, the liposome structure is more versatile for theranostic applications, since the therapeutic agents can be either enclosed in the internal aqueous compartment or embedded within the lipid membrane. Tunable combinations of therapeutic agents could benefit the development of personalized nanomedicines. For example, we demonstrated a gene-drug co-delivery system based on the *d*-dot/liposome hybrids, in which the hydrophobic drug camptothecin (CPT) was incorporated as an example for chemotherapy. Cell viability studies showed that both the *d*-dot/PAH nanoplex and *d*-dot/liposome hybrid are biocompatible, and thus

can be promising platforms in developing and optimizing the next generation nanocarriers for targeted gene(chemo) therapy of pancreatic cancer *in vivo*.

5.2 Experiments and Results

5.2.1 Materials

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), Poly(allylamine hydrochloride) (PAH, Mw =15 kDa), Polyethylenimine (PEI, Mw=1.8 kDa), chloroform (CHCl₃), methanol (CH₃OH), cysteamine hydrochloride (2-aminoethanethiol hydrochloride, AET·HCl), folic acid (FA), phosphate buffered saline (PBS, pH = 7.4), and N-Hydroxysuccinimide (NHS) and camptothecin (CPT) were purchased from Sigma-Aldrich. 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-PEG-Methoxy (DSPE-MPEG, MW = 2000) and 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-PEG-Amine (DSPE-PEG-NH₂, MW = 2000) were obtained from Laysan Bio Inc. FAM-labeled small interference RNA (K-Ras siRNA^{FAM}, sense strand: 5'-FAM-GUUGGAGCUG**AUGGCGUAGUU**-3'; Antisense: 5'-CUACGCC**AUCAGCUCCAACUU**-3', italic bold indicates the K-Ras mutation site) were purchased from Shanghai GenePharma (China). 18.2 MΩ·cm ultrapure water was used throughout the experiments.

5.2.2 Preparation of SiRNA Bector Based on QD/polymer Nanocomplexes

The *d*-dots were developed as siRNA vectors through a Layer-by-Layer (LbL) assembly method. Cationic polyelectrolyte PAH was employed to modify the surface potential of the dots. 50 µl of *d*-dots stock solution (1mg mL⁻¹) were washed with ethanol and centrifuged at 10,000 rpm. Next, 250 µl of PAH solution of different concentration (0.05~1 mg mL⁻¹, in DI water) were added to re-disperse the *d*-dots

precipitate, followed by short sonication and vortex for 20 min. After that, the *d*-dot/PAH_{0.05~1} particles were collected by centrifugation at 15,000 rpm for 10 min to remove the non-adsorbed PAH. The resulting *d*-dot/PAH particles were dispersed in 200 μ l of DEPC-treated water while aggregates were removed by centrifugation at 2000 rpm for 1 min. For siRNA loading, 100 μ l of 10 μ M K-Ras siRNA^{FAM} solution were then introduced to the *d*-dot/PAH particles dispersion with gentle vortex and left undisturbed for 40 min. Subsequently, the *d*-dot/PAH/siRNA^{FAM} complex was further incubated with 8 μ l of PAH solution (1 mg mL⁻¹, in DI water) for 1 h to form *d*-dot/PAH/siRNA^{FAM}/PAH nanoplexes. Centrifugation was used to remove the excess PAH from the nanoplexes dispersion. The nanoplexes were redispersed in 100 μ l of DEPC-treated water for cell transfection experiments. In a parallel experiment, PEI, a frequently reported polymeric gene transfecting material, was employed to form *d*-dot/PEI nanoplexes. To serve as a reference for *d*-dot/PAH, the *d*-dot/PEI nanoplexes were prepared following the same preparation method.

Covalent conjugation of the *d*-dot/PAH nanoplexes with folic acid (FA) was conducted using the standard EDC/NHS condensation method. Firstly, 1.5 mg of FA was dissolved in 2 mL PBS (pH 7.4), followed by mixing with 200 μ L EDC (0.1 M in PBS) and 200 μ L of NHS (0.2 M in PBS) for 1 h, allowing the carboxyl groups of FA to be activated. Subsequently, a 250 μ l nanoplexes solution was added and the mixture was gently stirred at room temperature for 2 hours. The FA-conjugated nanoplexes were obtained by centrifugation and washed with PBS for 3 times to remove unreacted chemicals.

5.2.3 Preparation of SiRNA Vector Based on SQD-liposome Hybrids

In this strategy, the MPA surfactant present on QDs surface was firstly replaced by NH₂-terminated AET molecules to obtain a positively charged surface. For this

purpose, the QDs solutions were dialyzed in semi-permeable membranes for 48 hours to remove the MPA molecules in the QD solution and reduce the number of MPA molecules dynamically attached on the QDs surface. When the QDs precipitated, the supernatant was removed, followed by addition of an AET solution (0.5 M, in DI water) to re-disperse the QDs precipitates. After gentle vortex for 30 mins, the QDs were purified by ethanol and centrifugation to remove excess AET molecules.

Formation steps of liposomes incorporating SQD/siRNA clusters are schematically illustrated in Fig.5.14, 9 mg DSPE-mPEG and 1 mg DSPE-PEG-NH₂ were dissolved in a 3:1 (v/v) chloroform/methanol mixture. The organic solvent was evaporated under N₂ flow, and multilamellar vesicles (MLV) were produced after drying in vacuum for 1 hour at room temperature to remove any residual traces of organic solvent. The dried lipid film was sealed and stored at 4°C until use. The dried lipid film was hydrated with a SQD/siRNA suspension (200 µl) for 2 min at 50°C, followed by addition of 800 µl DEPC treated water to make a final volume of 1 ml (final lipid concentration 10 mM). Formation of SQD/siRNA-liposome (L-SQD/siRNA) was achieved by bath sonication of the suspension at 10°C for 5 min. It was visible that the cloudy MLV suspension became optically clear after sonication, signifying the complete transformation of MLV into small unilamellar vesicles (ULV) which shift the Mie-scattering maximum to the UV range. Prior to measurements or surface functionalizations, purification of the *d*-dots-liposome hybrid was repeated twice to remove excess lipids, empty vesicles or un-encapsulated QDs. For this purpose, the suspension was centrifuged at 12000 rpm for 5 min, and the precipitates were then redispersed in 500 µl DI water (final SQD concentration: 1 mg mL⁻¹).

Covalent conjugation of folic acid (FA) to SQD-liposome was conducted using the standard EDC/NHS reaction. Firstly, 1.5 mg of FA were dissolved in 2 mL PBS

(pH 7.4), followed by mixing with 200 μ L EDC (0.1 M in PBS) and 200 μ L of NHS (0.2 M in PBS) for 1 h, allowing the carboxyl groups of FA to be activated. Subsequently, 500 μ l of L-SQD stock solution were added and the mixture was gently stirred at room temperature for 3 hours. The f-L-SQD obtained by centrifugation was washed with PBS for 3 times to remove unreacted chemicals. The purified FA-conjugated SQD-liposome (f-L-SQD) solution was redispersed in 500 μ l DI for further application and characterization.

5.2.4 Preparation of CPT Drug Carrier and Gene/drug Co-delivery System Based on QD-liposome Hybrids

As for drug carrier preparation, SQD-liposomes were constructed similar as the L-SQD siRNA vectors, except that the hydrophobic drug camptothecin (CPT) was incorporated in between the liposomal bilayers, rather than the encapsulated vesicles (Fig.5.14c). Specifically, 9 mg DSPE-mPEG and 1 mg DSPE-PEG-NH₂ were dissolved in a 3:1 chloroform/methanol solution, combined with a 100 μ l CPT chloroform solution. Incorporation of the hydrophobic CPT into MLV was achieved by evaporating the organic solvent by N₂ flow and vacuum. After hydration with QDs water solution, *d*-dots-L-CPT hybrids were produced by bath sonication at 4°C for 5 min. The hybrids were purified by centrifugation at 12000 rpm for 5 min, and then redispersed in 500 μ l DI water.

5.2.5 Characterizations

The UV-visible absorption spectra were obtained from a spectrophotometer (Shimadzu UV-2450). Photoluminescence (PL) spectra and lifetimes were collected using a Fluorolog-3 spectrofluorometer. Quantum yields (QYs) of the Mn:ZnSe QDs were determined by comparing the integrated emission of diluted *d*-dots to CdSe QDs with matched absorbance. The QY of the CdSe reference sample was calibrated by

rhodamine 6G. The hydrodynamic size distribution profile and the zeta potential of the nanoparticle formulation were measured by a particle size analyzer system (90Plus, Brookhaven Instruments). Fourier transform infrared (FT-IR) spectra were measured by a Shimadzu FT-IR spectrometer. All measurements were performed at room temperature. Transmission electron microscopy (TEM) images were obtained using a JEOL model JEM 2010 microscope at an acceleration voltage of 200 kV. The specimens were prepared by drop-casting the sample dispersion onto an amorphous carbon-coated 300 mesh copper grid, which was placed on filter paper to absorb excess solvent.

5.2.6 SiRNA Transfection and Gene Expression Analysis Study

Panc-1 (ATCC® CRL-1469™) cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 $\mu\text{g mL}^{-1}$ penicillin (Gibco) and 100 $\mu\text{g mL}^{-1}$ streptomycin (Gibco). Cells were cultured at 37°C in a humidified atmosphere with 5% CO₂. Panc-1 cells were seeded in 6-well plates to approximately 30% cell confluence, and the culture medium was replaced with DMEM prior to the treatment. Then *d*-dot/PAH (PEI)-siRNA^{FAM} nanoplexes dispersion was added to the cell plates to give a final incubation concentration of the nanoplexes around 10 $\mu\text{g mL}^{-1}$. After 4 h incubation at 37°C in humidified atmosphere with 5% CO₂, the cells were washed with PBS for three times and harvested for transfection efficiency determination. The cellular uptake efficiency was quantitatively evaluated by using a FACS Calibur flow cytometer (Becton Dickinson, Mississauga, CA). For gene expression analysis at 48 h post-transfection, the treated cells were continuously cultured in DMEM with 10% FBS. The cells were harvested and washed by PBS. The total RNA was extracted using TRIzol reagent (Invitrogen) and quantitated by a spectrophotometer (Nano-Drop ND-1000). After

that, RNA was reverse transcribed to cDNA using the reagent kit from Promega according to the vendor's instructions. Real time quantitative RT-PCR was then carried out for quantitative analysis of K-Ras relative mRNA expression by normalizing against the expression of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is one of the most commonly used housekeeping genes adopted for gene expression comparisons. Forward and reverse primers used in RT-PCR were 5'-AGAGTGCCTTGACGATACAGC-3', 5'-ACAAAGAAAGCCCTCCCCAGT-3' for K-Ras mRNA, and 5'-ACCACAGTCCATGCCATCAC-3', 5'-TCCACCACCCTGTTGCTGTA-3' for GAPDH, respectively. In parallel experiments d-dot/PAH complex and free siRNA^{FAM} with the same dosage level were introduced as negative controls while the commercial transfection reagent OligofectamineTM (Invitrogen) coupled siRNA^{FAM} was used as positive control.

5.2.7 Cell Viability Evaluation

Cell viability was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide) assays. The toxic effects of the *d*-dot nanocarriers were tested on different cell lines, including two human pancreatic cancer cell lines (Panc-1 and Miapaca-2), human breast cancer cells (MDA-MB-231) and mouse leukaemic monocyte macrophage cells (RAW 264.7). The cells were seeded in a 96-well plate at a density of 5000 cells/well and incubated with different concentrations of *d*-dot/PAH nanoplexes for 48 h. In parallel experiments, different types of particles (i.e. *d*-dot, CdTe and CdTe/ZnS QDs, *d*-dot/PEI nanoplexes and *d*-dot/PAH-FA) were tested on a RAW 264.7 macrophage cell line and the biocompatibility of these particles was compared at the same dosage addition. In each assay, 20 μ l of 5 mg mL⁻¹ MTT in PBS were added and the cells were incubated for 4 h. Subsequently, 150 μ l of 100% dimethylsulfoxide (DMSO, Sigma) was added to dissolve the precipitate with 5 min

gentle shaking. Absorbance was then measured with a microplate reader (Bio-Rad) at the wavelength of 490 nm. The cell viability was calculated as the ratio of the absorbance of the sample well to that of the control well and expressed as a percentage, assigning the viability of non-treated cells as 100%.

5.3 Results and Discussion

5.3.1 QD/polymer nanoplex for siRNA delivery

The *d*-dots were developed as siRNA vectors through a Layer-by-Layer (LbL) assembly method. Fig.5.1a illustrates the preparation steps, in which the cationic polymer poly(allylamine hydrochloride) (PAH) was employed for complexing with K-Ras siRNA molecules through electrostatic absorption. In comparison with some other polymeric transfecting materials, such as PDDAC, and PAMAM dendrimers, which have been reported with acute *in vitro* toxic effects, the PAH polymer could be more feasible for *in vivo* applications owing to its biocompatibility[280].

It is worth noting that, by varying PAH concentration (Fig.5.2), the *d*-dot/PAH clusters can be prepared with varying hydrodynamic sizes (47~200 nm), and the surface zeta-potential was also tunable over a wide range (+20 to +48 mV). As shown in Fig.5.3, agarose gel electrophoresis was applied to examine the loading of siRNA molecules onto *d*-dot/PAH particles. The bright band A, B and C correspond to the free and the unbound siRNA. Because of the electric field applied, the unbound negatively charged siRNA shifted to bottom (anode) and was stained by the fluorescent ethidium bromide in gel. The results suggest that *d*-dots without PAH coating cannot absorb negatively charged siRNA, while a higher loading efficiency was observed for *d*-dot/PAH clusters with higher zeta potential. In parallel

Fig.5.1 (a) Schematic illustration of the preparation steps of the *d*-dot/polymer nanoplex-based siRNA vectors. (b) TEM images of *d*-dots and *d*-dots/PAH nanoplexes.

Fig.5.2 (a) Hydrodynamic size, and (b) Surface zeta potential of the *d*-dot/PAH and *d*-dot/PEI clusters prepared using polymer solution of different concentrations.

Fig.5.3 Agarose gel electrophoresis examining the binding between *d*-dot/PAH clusters and siRNAs. (I) *d*-dot/PAH_{0.1} (+31 mV) only, (II) free siRNA only, and siRNA mixed with (III) MPA stabilized *d*-dot (-17 mV), (IV) *d*-dot/PAH_{0.05} (+24 mV) or (V) *d*-dot/PAH_{0.1} (+31 mV).

experiments, *d*-dot/PEI nanoplexes prepared under same conditions generally exhibited a higher zeta potential (+35 to +58 mV) than the *d*-dot/PAH. The higher charge density of PEI can be more beneficial for loading of the oppositely charged gene material. After siRNA loading, a second PAH coating layer was applied, generating a surface with positive charges ($\sim +30$ mV). Because of the primary amine groups present by PAH, the capping layer can promote cellular uptake or provide anchor moieties for further modification with functional molecules. In Fig.5.4, we monitored the hydrodynamic size and zeta potential changes of the particles during the LbL assembly. The surface charge reversals clearly indicated deposition of alternating layers of the positively charged PAH and negatively charged siRNAs. It is noticeable that hydrodynamic size of the particles increases dramatically after coating of the first PAH layer. This observation, together with the TEM image (Fig.5.1b), indicates that multiple *d*-dots have been co-encapsulated to form a relatively large *d*-dot/PAH cluster. The hydrodynamic size change of the as-produced nanoplex

formulations was monitored over a period of 2 weeks (Fig.5.5). Result suggests that the nanoplex possesses an excellent colloidal stability.

Fig.5.4 Monitoring the change in particle hydrodynamic size and zeta potential during the LBL construction steps to prepare *d*-dot/PAH_{0.1}/siRNA^{FAM}/PAH nanoplexes.

Fig.5.5 (a) Hydrodynamic size distribution of *d*-dot/PAH_{0.1}/siRNA^{FAM}/PAH nanoplexes measured by DLS. (b) Colloidal stability of *d*-dot/PAH_{0.1}/siRNA^{FAM}/PAH nanoplexes dispersed in DEPC treated water at 25°C. The hydrodynamic size of the complexes was monitored over a period of 2 weeks.

Fig.5.6 Photoluminescence (PL) spectra of *d*-dot/PAH, free siRNA^{FAM} and *d*-dot/PAH/siRNA^{FAM} nanoplexes excited by UV ($\lambda=350$ nm) or blue ($\lambda=450$ nm) light sources. Real image of *d*-dot/PAH/siRNA^{FAM} under different excitations are shown in inset pictures.

Fig.5.7 Photostability comparison between *d*-dots and FAM fluorophores. Panc-1 cells transfected with *d*-dot/PAH-siRNA^{FAM} under continuous irradiation by a mercury light source. Fluorescent images of the cells were taken at different time intervals. Evolution of the relative PL intensity of the *d*-dot and FAM signals were plotted.

As shown in Fig.5.6, we investigated the PL capability of the prepared *d*-dot/PAH-siRNA^{FAM} nanoplexes, while PL spectra of *d*-dot/PAH and free siRNA^{FAM} are also presented for comparison. The clustering of the nanoplexes seems cause a decrease in the *d*-dots and FAM emission efficiency, yet it has little influence on the characteristic peak wavelengths. More importantly, fluorescence resonance energy transfer (FRET) between the *d*-dots and FAM was not observed. This is quite different from the red-emitting CdSe QDs previously reported for gene release monitoring[293]. For multichannel imaging purpose, narrow band-pass filter are normally employed to avoid crosstalk between adjacent channels, while the PL bandwidth of the fluorescent labels are requested to be narrow. Actually, in our case, crosstalk between the FAM labels (520 nm) and *d*-dots (590 nm) signals was fundamentally avoided by employing excitations of different wavelengths (i.e. UV and blue light for *d*-dot and FAM, respectively), because neither of the excitations can activate the two labels simultaneously. More importantly, owing to the large Stokes shift of the *d*-dots, the wavelength of the light source for FAM excitation can be selected in a wide range (theoretically 400 to 500 nm) without activating the *d*-dots. Consequently, the *d*-dots have an advantage over conventional band-edge emitting QDs, as they may simplify the requirements for the optical system and provide a chance to include more optical channels. Additionally, the photostability of the nanoplex was also investigated. Fluorescent images shown in Fig.5.7 suggest that, compared with FAM fluorophores, the inorganic *d*-dots are more suitable probes for long term optical tracing, because they exhibit increased resistance against photobleaching.

The *d*-dot/PAH nanoplexes were applied to deliver siRNAs that target the mutant oncogenic K-Ras gene in pancreatic cancer cells. In Fig.5.8a, the successful delivery

of the siRNAs can be easily identified through the green fluorescence from the FAM label at 4 h post transfection. Similar results were also observed in *d*-dot/PEI-siRNA^{FAM} treated cells and the positive control group using commercially available transfection reagent Oligofectamine™. As a comparison, there is no fluorescent signal in the cells treated with free siRNAs, suggesting that the naked siRNAs are not able to penetrate the cell membrane without the assistance of transfection agents. More interestingly, the overlay of the FAM and the *d*-dots channels shows both co-localization (yellow, a merge of green and red) and delocalization within the *d*-dot/polymer-siRNA^{FAM} treated cells. This indicates that siRNAs were slowly released from the *d*-dots nanoplex surface. By further monitoring cells for 72 h post transfection, a variation in the intracellular distribution of *d*-dots can be observed. Furthermore, unlike Oligofectamine™ formulation, where no FAM signals were detected at 72 h post transfection, the FAM fluorescent signals remained visible in the cells transfected by the *d*-dot/polymer nanoplexes (Fig.5.8b). This suggests that a fraction of siRNA^{FAM} molecules that are tightly bound to the *d*-dot/PAH (PEI) cluster surface have survived from degradation process within cells, and they may need longer time to be released to the cytoplasm. The cationic polymer coating plays an essential role in siRNA loading and release kinetics. Insufficient coating will result in less positively charged nanoplex and relatively weak siRNA surface binding. On the contrary, a higher siRNA loading efficiency can be achieved by using a highly charged polymer coating. However, this approach will cause reduced siRNA release rate since the siRNA will be tightly bound by the strong electrostatic force. This situation will certainly influence the therapeutic activity of the nanoplex formulation. Our results suggest that a zeta potential between +20 mV and +40 mV is an optimum range for the nanoplex to successfully and effectively deliver siRNAs to the cells.

Fig.5.8 (a) Fluorescent image of Panc-1 cells treated with: (i) free siRNA^{FAM}, (ii) Oligo-siRNA^{FAM}, (iii) *d-dot*/PAH-siRNA^{FAM} and (iv) *d-dot*/PEI-siRNA^{FAM} for 4 hours. (b) After 4 hours transfection with (v) Oligo-siRNA^{FAM}, (vi) *d-dot*/PAH-siRNA^{FAM}, and (vii) *d-dot*/PEI-siRNA^{FAM}, the Panc-1 cells were further incubated in DMEM, and the images were taken at 72 hours post-transfection.

Fig.5.9 Transfection efficiency of Panc-1 cells determined by flow cytometry analysis.

(A) Representative pictures, where cells were treated with (i) blank, (ii) free siRNA^{FAM}, (iii) *d-dot*/PAH, (iv) Oligo-siRNA^{FAM} (v) *d-dot*/PAH-siRNA^{FAM} and (vi) *d-dot*/PEI-siRNA^{FAM} for 4 hours. (vii), (viii) and (ix) were collected at 72hours post-transfection, corresponding to (iv), (v) and (vi), respectively. (B) Percentage of cells transfected after 4 h treatment, evaluated from experiments shown in (A). The shown points are average values $\pm$ SD. *, P-value < 0.005 vs blank, siRNA and d-dots. (C) Average FAM fluorescence intensity of the treated cells (4- and 72 hours post-transfection) counted from experiments shown in (A).

The transfection efficiency of the siRNA into Panc-1 cells was quantitatively evaluated by flow cytometry analysis. Fig.5.9a shows the representative plots of the FAM intensity in cells treated with different formulations. After 4 hours incubation, strong FAM signals were detected in cells transfected by Oligo agent, *d*-dot/PAH and *d*-dot/PEI nanoplexes. These results are consistent with the fluorescent imaging analysis, indicating considerable accumulation of siRNAs inside the cells. The fractions of cells detected with strong FAM signals were counted to be 73.7%, 72.3% and 71.3%, respectively (Fig.5.9b). On the contrary, no evident FAM signal was detected for the cases of free siRNA^{FAM}, *d*-dot nanoplexes only and blank cell groups. Fig.5.9c shows the average fluorescence intensity per cell count recorded in the flow cytometry evaluation. Because the average FAM intensity is two times higher than that of Oligo-siRNA^{FAM}, we consider that siRNA transfection by *d*-dot/PAH nanoplexes is more effective. Comparatively, even higher transfection efficiency was achieved by using PEI as the polymer coating. The enhanced siRNA accumulation could be attributed to the high surface charge density of *d*-dot/PEI nanoplex. It is also noticeable that, after 72 hours of incubation, the fluorescence signals almost disappeared in Oligo transfected cells (Fig.5.9a and c). In contrast, around 56% and 77% of intensity still remained for *d*-dot/PAH and *d*-dot/PEI transfected cells. These observations are in accordance with our imaging results (Fig.5.8b), suggesting the protection and the partial release of siRNAs inside the cells.

Regulated expression of the targeted mRNA, regarded as the direct proof of successful RNAi process, was carried out to evaluate the therapeutic capability of the nanoplex formulation. Fig.5.10 shows the relative expression levels of mutant K-Ras mRNA in Panc-1 cells treated with different formulations after 72 hours of transfection. No evident differences in the expression levels were observed in the cells

treated with *d*-dot/polymer complexes only or free siRNAs. In contrast, the expression level of the mutant K-Ras mRNA in cells transfected by Oligo-siRNA and *d*-dot/PAH-siRNA was found to be significantly suppressed to 34% and 43%, respectively. In comparison with *d*-dot/PAH, the silencing effect was not further promoted by using *d*-dot/PEI (46%), although it induced higher transfection efficiency. These results, together with the residual FAM intensity detected by flow cytometry at 72 h post-transfection, imply that the *d*-dot/PAH complex with lower zeta potential could be more favourable than *d*-dot/PEI for gene release. Since the zeta potential of the *d*-dot/polymer complex is essential for siRNA binding and its value is tunable over a wide range, the release kinetics of siRNA should be optimized in the future to obtain improved knockdown efficiency or sustained release for long term gene treatment.

Fig.5.10 K-Ras mRNA relative expression levels in Panc-1 cells detected by real-time RT-PCR: (a) Blank, (b) Free siRNA, (c) *d*-dots, (d) Oligo-siRNA, (e) *d*-dot/PAH-siRNA and (f) *d*-dot/PEI-siRNA complexes. Data are presented as average values $\pm$ SD.

*, P-value < 0.005 vs blank, siRNA and *d*-dots.

MTT assays were conducted to evaluate the cytotoxicity of *d*-dots and *d*-dot/polymer complexes. As shown in Fig.5.11a, in addition to the *d*-dots, cytotoxicity evaluation for MPA-stabilized CdTe and CdTe/ZnS core/shell QDs, which have similar physical and optical properties, were also performed in this study as positive

Fig.5.11 (a) Cytotoxicity tests of four types of nanoparticles, i.e. *d*-dots, PAH-coated *d*-dots, CdTe QDs and CdTe/ZnS core/shell QDs. RAW 264.7 cells were treated with different concentrations of the particles for 48 hours. The particles were MPA-stabilized except for *d*-dot/PAH complexes. (b) Cytotoxicity tests of the *d*-dot/polymer nanocomplexes and d-dot/PAH-FA. Data for *d*-dot/MPA was presented as reference.

controls. The test was firstly conducted using macrophage cell line (RAW 264.7). Because macrophage cells internalized foreign particles nonspecifically through the phagocytosis process, the cytotoxicity evaluation could be less sensitive towards the discrepancy in internalization/accumulation rate of the particles with different surface modifications. At 48 h post treatment, CdTe QDs without ZnS shell capping exhibit severe toxicity. The 50% cell viability (IC₅₀, half maximal inhibitory concentration) was determined to be less than 10 $\mu\text{g mL}^{-1}$. Comparatively, the ZnS-capped CdTe QDs were found to be less toxic. The IC₅₀ was greatly improved to above 40 $\mu\text{g mL}^{-1}$ although the influence of the particles on cell viability was still pronounced, especially at high dosage concentrations. Thus, the ZnS capping layer may protect the Cd-core from fast degradation, however, it cannot prevent the release of heavy metal ions to the biological environment, especially when the QDs are exposed to intracellular oxidative conditions.

Fig.5.12 Cytotoxicity tests of the *d*-dot/PAH nanoplex on four different cell lines, i.e. RAW 264.7, Panc-1, Miapaca-2 and MDA-MB-231. The cells were treated with different concentrations of *d*-dot/PAH for 48 hours.

In stark contrast, after 48 h of incubation with *d*-dots, the viability of the treated cells were maintained over 90% for concentrations as high as $160\ \mu\text{g mL}^{-1}$. These results indicate that the *d*-dots are highly biocompatible. Furthermore, the viabilities of the RAW 264.7 cells treated with *d*-dot/polymer nanoplexes are shown in Fig.5.11b clearly indicate that the toxicity of the *d*-dot nanoplexes was mainly determined by the polymer coating, rather than the inorganic *d*-dots. In comparison with the *d*-dot/PEI reference group, higher viabilities were evaluated for cells treated with an equal amount of *d*-dot/PAH nanoplexes. To further confirm the biocompatibility of the *d*-dot/PAH, we investigated the toxic effects of the *d*-dot/PAH complexes on different cell lines, including human breast cancer cells (MDA-MB-231) and two human pancreatic cancer cell lines (Panc-1 and Mipaca-2). Fig.5.12 shows that, at 48 hours after exposure, all the four cell lines maintained over 80% viability across a wide range of dosages up to $160\ \mu\text{g mL}^{-1}$. These results suggested that the as-prepared *d*-dot/PAH complexes (zeta potential of $\sim +30\ \text{mV}$) are highly biocompatible. Given that our *d*-dots formulations are free of heavy metals substance, they will be a promising optical contrast agent for biomedical applications ranging from imaging to drug delivery.

Because the PAH polymer exhibits plenty of primary amino groups which can serve as anchor moieties to conjugate with different functional molecules, the *d*-dot complexes capped with a second PAH layer could be further modified to receive multiple bio-functions. For example, we conjugated folic acid (FA) on the *d*-dot/PAH to obtain ability for cancer cell targeted gene delivery. Fig.5.13a schematically illustrates the preparation of folic acid conjugated nanoplexes, where EDC/NHS condensation method was applied for crosslinking. According to our results, after conjugation with FA, the zeta potential of the nanoplex changed from $+30\ \text{mV}$ to a

negative value (-18 mV). Compared with positive charges, a negatively charged surface helps to reduce non-specific internalization of the particle by cells[294]. The MTT results shown in Fig.5.11b confirm the biocompatibility of these *d*-dot/PAH-FA formulations. Surface characterization of the FA conjugated *d*-dot/PAH were studied by using FT-IR spectroscopy. For comparison, spectra of PAH, *d*-dot/PAH, FA and *d*-dot/PAH-FA are shown in Fig.5.13b. The *d*-dot/PAH complexes exhibit the characteristic peaks of the amine group in PAH polymer, including the N-H stretching mode at 3433 cm^{-1} and the NH_2 deformation vibration at 1627 cm^{-1} . The two bands at 2924 and 2862 cm^{-1} correspond to asymmetric and symmetric stretching vibration of $-\text{CH}_2$, respectively. Compared with the PAH polymer in hydrochloride form, the NH_3^+ deformation vibration at 1512 cm^{-1} disappeared in *d*-dot/PAH after forming the complexes, accompanying with presence of a new band at 1095 cm^{-1} which is assigned to the C-N stretching mode. Conjugation of folic acid onto *d*-dot/PAH was substantiated by the emergence of both the amide I band at 1635 cm^{-1} and the amide II band at 1512 cm^{-1} , which are attributed to C=O stretch and NH deformation in the secondary amides, respectively. Meanwhile, a series of characteristic IR absorption peaks of FA located at 1697 cm^{-1} (carboxyl), 1605 cm^{-1} (benzene), and 1481 cm^{-1} (hetero-ring) were also visible in the spectra of *d*-dot/PAH-FA. All these results substantiated that the folic acid molecules were successfully conjugated on *d*-dot/PAH surfaces.

To demonstrate the ability of the *d*-dot/PAH-FA for cancer cell targeted siRNA delivery, fluorescent images of Panc-1 cells treated with non-conjugated and FA-conjugated complexes are compared in Fig.5.13c. Prior to transfections, Panc-1 cells in (ii) and (iv) were pre-saturated with free folic acid to block FA receptors (FAR) available on cell surface. As shown in the fluorescent images, Panc-1 cells were

Fig.5.13 (a) Schematic illustration of the method to prepare FA conjugated *d*-dot/PAH nanoplexes. (b) FT-IR spectra of PAH, *d*-dot/PAH, folic acid and *d*-dot/PAH-FA nanocomposites. (c) Fluorescence image of Panc-1 cells treated with unconjugated (i, ii) and FA-conjugated (iii, iv) nanoplexes. The cells in (ii) and (iv) were pre-saturated with free FA before incubation with the nanocomposites.

stained with FA conjugated nanoplexes after 2 hours incubation (iii), whereas minimal signal was observed from cells pre-saturated with FA (iv). These results suggested that the uptake of the particles involves the interaction between FA and FAR. In contrast, accumulations of the particles were observed in both unsaturated and FAR-blocked cells for unconjugated nanoplexes, suggesting a non-specific nature of their uptake due to their positive surface charges. Considering that the FAR is overexpressed on many human cancer cell lines, these results indicate that the particles can be functionalized with folic acid for cancer cell targeted gene delivery. More importantly, these results exemplified that the amine groups present on the second PAH layer could be employed to link with molecules to obtain multiple bio-functions. For example, to explore the applications for *in vivo* gene delivery and tumor therapy, PEG should be conjugated for improved stability in serum and prolonged circulation time of the particles after intravascular administration[295].

5.3.2 SQD Loaded Liposome for Co-delivery of SiRNA and Hydrophobic Drug

A solvent dispersion method was adapted to prepare the liposome-SQD (L-SQDs) assemblies (Fig.5.14a)[296]. To form the liposome structure, a mixture of phospholipid and organic solvent (chloroform/methanol=3:1) was firstly evaporated in vacuum until a lipid thin film was obtained. The formation of SQD-encapsulated multilamellar vesicles (MLV) was accomplished by hydration of the dry lipid film by aqueous solution of the SQDs at 50°C. After that, the MLV was disrupted by sonic energy to produce small unilamellar vesicles (SUV), which typically have much smaller size (20~100 nm). The SQD-loaded SUVs were purified and recollected by centrifugation. The hydrodynamic size of the L-SQDs was estimated to be around 57 nm via DLS measurement (Fig.5.15a). The successful encapsulation of multiple QDs

Fig.5.14 Schematic illustration of preparation steps of the L-QDs based siRNA (drug) carriers.

in one SUV liposome was verified by TEM image shown in Fig.5.15b, which also reveals the uniform size distribution of the L-SQDs (40 ~ 50 nm). The phospholipid layer consists of PEG-functionalized DSPE (DSPE-mPEG, 90%) and PEG-DSPE modified with -NH₂ (DSPE-PEG-NH₂, 10%), and the molar ratio was optimized to receive proper surface charge (Fig.5.15c). The negative zeta-potential (~ -30 mV) and the PEG present at the surface may provide a good colloidal stability in biological environment or a prolonged circulation lifetime after intravenous administrations, as they help to suppress non-specific molecule absorption on the surface and reduce non-specific cellular uptake. Additionally, the primary amine groups introduced by the NH₂-functionalized lipids at the surface can be further modified, allowing for biofunctionalization of the L-SQDs assemblies. As illustrated in Fig.5.14, a standard

Fig.5.15 (a) Hydrodynamic size distribution of the L-SQDs measured by DLS; (b) TEM image of the L-SQDs. The SUV lipid membrane fragments can be visualized in HRTEM image (inset); (c) Zeta-potential of L-SQD is tunable by varying the molar ratio between DSPE-mPEG and DSPE-PEG-NH₂.

EDC/NHS coupling method was adapted to conjugate L-SQDs with folic acid (FA). Because folate receptors (FRs) are over-expressed on the surface of many tumor cell lines but shows limited expression on normal cells[297, 298], the FA-functionalized L-SQDs (f-L-SQDs) was prepared to achieve cancer cell targeting affinity.

Labeling of pancreatic cancer cells (Panc-1) with the f-L-SQDs was demonstrated in Fig.5.16. After 4 hours incubation, the cellular uptake of the f-L-SQDs was clearly evidenced by the robust PL emission from the SQDs, while a minimal signal was observed from cells treated with unconjugated L-SQDs and cells pre-saturated with FA before the incubation with f-L-SQDs. These results demonstrate the specific nature of the cellular uptake, which involves the interaction between FA and FR, and therefore indicate the cancer cell targeting affinity of the f-L-SQDs. Furthermore, owing to the dual-mode ability of the SQD, accumulations of the L-SQDs inside the cells were also detectable via T_1 -weighted MR imaging of the cell lysates (Fig.5.16,

Fig.5.16 Fluorescent images of Panc-1 cells. (a) Blank cells, (b) Cells treated with L-SQD, (c) Cells treated with FA functionalized L-SQD and (d) Cells presaturated with excess FA before incubation with f-L-SQD. T_1 -weighted MR images of the cell lysis in centrifuge tubes are presented in the insets.

Fig.5.17 Cytotoxicity tests of the SQR and f-L-SQR hybrids upon Panc-1 cells after 48 h incubation.

insets). In accordance with the fluorescence images, stronger MR signals were observed from the f-L-SQDs treated group, indicating the enhanced cellular uptake after FA-functionalization.

The biocompatibilities of the SQDs and FA-L-SQD hybrids were verified using a MTT cell viability assay. In comparison with the conventional high quality QDs (e.g. CdSe, CdTe, PbS), which have the concerns of toxicity due to heavy metal ions release, SQDs are free of toxic heavy metal components and therefore should be more suitable for biomedical applications. As can be observed in Fig.5.17, SQDs and f-L-SQDs with a concentration up to $160\ \mu\text{g mL}^{-1}$ exhibited no noticeable influence on the viability of the Panc-1 cells after 48 h of treatment. These preliminary results suggest that the f-L-SQD hybrids can be non-toxic and biocompatible materials for biological applications, although long-term and *in vivo* investigations are still required to exclude any other potential risks before moving forward to clinic research.

The biocompatible L-SQD assemblies were then developed as versatile theranostic tools for traceable gene- and chemotherapy of pancreatic cancer cells. As a model therapeutic gene material, small-interfering RNAs (siRNAs) targeting the mutant K-Ras gene with a point mutation at codon 12 was selected for genetic therapy of Panc-1 cells, which is associated with increased cell proliferation and resistance to apoptosis. Fig.5.14b illustrates the preparation steps of the L-SQD based gene vehicles. Prior to siRNA loading, the MPA molecules on QDs surface were replaced by $-\text{NH}_2$ terminated cysteamine (AET) via a simple ligand replacement process. Because of the dynamic nature of the binding between MPA and the QD surface Zn atom, the addition of excess AET will replace the MPA ligand via a mass action. However, the direct addition of AET will cause aggregation (flocculation) of the QD dispersion and block reaction, because there is a transition stage when the MPA are

insufficiently replaced. In this stage, the neutral surface charges cannot keep the QDs well dispersed, because the Coulomb force is too weak. For this reason, in our procedure, a dialysis treatment was conducted to remove the MPA molecules in the QD solution and reduce the number of MPA molecules dynamically attached on the QDs surface. When the QDs precipitated due to the lack of surfactants, excess AET was added to re-disperse the QDs, which dramatically raise the zeta-potential, skipping the transition stage. With this method, the zeta potential of the SQD was changed from -22 mV to a positive value (+23 mV), and its water dispersion is stable for months. More importantly, the bright PL emission of SQDs was maintained after the ligands replacement (Fig.5.14), while a similar reaction process was found to cause significant PL deterioration in both un-doped ZnSe/ZnS QDs and Mn:ZnSe QDs without ZnS capping. We tend to attribute the stable PL emission of the SQD to a collective effect of ZnS shell protection and the localized nature of Mn emission, which is less sensitive towards environment changes [273].

The results of the chemical characterization of the particles' surface by FT-IR measurement is shown in Fig.5.18. The new bands at 3200 cm^{-1} and 1085 cm^{-1} are assigned to the ν_{NH} and ν_{C-N} stretching modes of the primary amine group, respectively. Although δ_{NH} (NH_2 deformation) and ν_{COO^-} (antisymmetric stretch) around 1556 cm^{-1} cannot be distinguished, the weakening of ν_{COO^-} (symmetric stretch, 1413 cm^{-1}), $\nu_{C=O}$ (1648 cm^{-1}) and ν_{OH} (3433 cm^{-1}) is still observable after the replacement. These results confirm that the carboxyl-terminated MPA ligands were effectively replaced by the primary amine-terminated AET.

Because the backbone of nucleic acids is negatively charged, the positive zeta

Fig.5.18 FT-IR spectra of SQD/MPA and SQD/AET.

Fig.5.19 Fluorescent images of Panc-1 cells treated with f-L-SQD/siRNA^{FAM}. (a) Channel of FAM; (b) Channel of SQD; (c) The overlay of (a) and (b); (d) The overlay of (a), (b) and bright field image.

potential facilitates the binding of siRNA molecules onto SQD surfaces, which was confirmed by electrophoresis (Fig.5.14). After siRNA attachment, the zeta potential of the QDs decreased to a negative value (~ -20 mV). The SQD/siRNA^{FAM} compositions were then encapsulated by phospholipid bilayer membrane followed by FA functionalization to generate f-L-SQD/siRNA assemblies. It is worth noting that, using the positively charged SQDs as gene 'sorbents', almost all the siRNA^{FAM} molecules can be encapsulated and recollected, while limited loading efficiency was observed by PL measurement of the supernatant for free siRNA^{FAM} encapsulation.

The results of siRNA transfection of Panc-1 cells using f-L-SQD as delivery agent are illustrated in Fig.5.19, where 3'-Fluorescently labeled siRNA (siRNA^{FAM}) was used for visualization. Because of the robust PL signals from SQDs (red channel) and FAM labels (green channel), the effective accumulation of SQDs and siRNA^{FAM} inside the cells can be visualized via fluorescence imaging. At the same time, no fluorescent signal could be visualized in the cells treated with free siRNAs, showing that the naked siRNAs cannot penetrate the cell membrane without transfection agents (Fig.5.8a, i).

The siRNA transfection efficiency was further quantitatively evaluated by flow cytometry analysis. The obtained results, shown in Fig.5.20, are consistent with the fluorescent imaging results, and no evident FAM signals were detected in blank cells, cells treated with free siRNA or L-SQDs without siRNA loading. In contrast, 9.2% of the cells were transfected when L-SQDs were used as gene carriers, and the transfection efficiency was enhanced to 70% after FA conjugation. Additionally, for cells pre-saturated with excess amount of FA, transfection efficiency was found to be greatly suppressed to 16.9%. These results suggest that the f-L-SQD assemblies can be developed as traceable and multifunctional vehicles to facilitate cancer cell

Fig.5.20 Transfection efficiency of Panc-1 cells determined by flow cytometry analysis, expressed as percentage of cells transfected after 4 h treatment.

Fig.5.21 K-Ras mRNA relative expression levels in Panc-1 cells were detected by real-time RT-PCR. (a) Blank, (b) Free siRNA, (c) L-SQD, (d) f-L-SQD/siRNA.

targeted siRNA delivery. As a direct proof of sequence specific post-transcriptional gene silencing induced by the siRNA, regulated expression of the targeted mRNA was monitored. The relative expression levels of mutant K-Ras mRNA in treated Panc-1 cells are shown in Fig.5.21. The mRNA expression level in f-L-SQD/siRNA

transfected cells was significantly suppressed to 46%, which demonstrate the therapeutic effect through RNA interfering (RNAi).

With respect to drug delivery for cancer chemotherapy, camptothecin (CPT), which showed remarkable anticancer activity by inducing DNA damage and apoptosis, was selected as a model drug. As shown in Fig.5.14c, CPT-loaded SUV was obtained via solvent evaporation followed by hydration and probe sonication. Because of the CPT's hydrophobic nature, the evaporation of the organic solvent causes the formation of dry lipid-CPT films with CPT molecules embedded in the hydrophobic leaflets of phospholipid membranes. Hydration of the lipid-CPT films by SQD solutions, followed by probe sonication, finally produce the drug-QD co-encapsulated liposomes. It is worth noting that the liposome encapsulation is necessary for CPT delivery, since direct administration of any hydrophobic drug is limited due to its low solubility in aqueous medium. The liposomes were then conjugated with FA to receive receptor-mediated cancer cell uptake. Finally, co-delivery of drug and gene materials were explored by forming a SQD-gene-drug co-encapsulated system.

Figure 5.22 Cell viability of Panc-1 cells after a 48 hrs treatment with f-L-SQD, f-L-SQD /siRNA, f-L-SQD/CPT and f-L-SQD/siRNA-CPT.

MTT cell viability assays were conducted to evaluate therapeutic effects of the L-SQD based gene and drug carriers. Fig.5.22 presents the viabilities of cells exposed to different formulations with same incubation duration and concentration. In contrast to SQD and f-L-SQDs, significant reductions in cell viability were observed in f-L-SQD/siRNA and f-L-SQD/CPT treated groups. The siRNA molecules delivered by f-L-SQD induce sequence-specific silencing of the mutated K-ras gene, therefore hinder the proliferation of the cells and result in lower cell viability as compared to the untreated group. Free siRNA treatment failed to trigger RNAi and therapeutic effects because of the poor transfection efficiency. On the other hand, after loading with CPT, the f-L-SQD/CPT hybrids were found to cause considerable cytotoxic effects. More importantly, even lower cell viability was observed in cells treated with siRNA-CPT co-encapsulated together with f-L-SQD, which suggests the feasibility of using the f-L-SQD hybrid as a drug-gene co-delivery system for chemo-gene therapy.

5.4 Conclusions

In this Chapter, in order to explore biomedical applications of the QDs, the Mn-doped ZnSe QDs were developed to be used as theranostic agent for pancreatic cancer cell imaging and therapy. Specifically, two types of gene carriers were developed based on the *d*-dots, namely *d*-dot/polymer nanoplexes and *d*-dot/liposome hybrids. Owing to the unique and stable PL properties of the *d*-dots, siRNA transfection was optically traceable. Cancer cell-targeted siRNA delivery was achieved by functionalizing the nanocarriers with cancer targeting ligands (FA). The therapeutic effect was confirmed by the significantly suppressed expression of the targeted gene sequence (mutant K-Ras) at mRNA level. More importantly, the *d*-dot/liposome hybrid based on the SQDs was demonstrated to be a promising multifunctional platform for MR/optical dual mode imaging and gene/drug co-delivery. Owing to the flexibility of the *d*-

dot/liposome hybrid, applications could be extended to *in vivo* tumour diagnosis and targeted gene-chemo therapy, while tunable combinations of therapeutic agents could benefit the development of personalized nanomedicines.

Chapter 6 Conclusions and Future Work

6.1 Conclusions

In summary, this thesis investigates a few aqueous phase synthesis methods to develop high quality and multifunctional colloidal quantum dots (QDs) and explore their applications as theranostic agents for pancreatic cancer diagnosis and therapy.

In comparison with the traditional organometallic method, the preparation of colloidal QDs directly in aqueous phase has been attracting increased attention, since the procedure is more economic, more environmental-friendly and easy to perform. More importantly, because the QDs are initially water dispersible and ready for bioconjugation, the aqueous synthesis methods are highly desirable for biological applications. In this thesis, efforts have been made to investigate and optimize the aqueous synthesis route in order to prepare high quality QDs, which should have bright and stable PL emission with narrow bandwidths. Specifically, the reaction for synthesizing high quality CdTe QDs was firstly investigated. It was found that the used surfactant species and their concentration are important factors which greatly influence the growth dynamics, optical properties and stability of the resulting QDs. A mixed-ligands system composed of mercaptopropionic acid (MPA) and cysteine (Cys) was then optimized to generate high quality CdTe QDs.

Although high quality CdTe QDs with size-tunable color can be synthesized using an aqueous method, these cadmium-based QDs are associated with Cd^{2+} ion release in the biological environment and were found to cause serious cytotoxic effects according to our *in vitro* cell viability test. To avoid the heavy metal related toxicity issues, high quality Mn-doped ZnSe QDs (*d*-dots) were synthesized as an alternative to Cd-based QDs. The *d*-dots are characterized by a large Stokes shift and long PL

lifetime, which are unique optical properties desirable for bioimaging applications. Most importantly, the *d*-dots were found to be biocompatible, and thus, should be promising candidates for biological applications. Furthermore, in order to develop multifunctional probes for multi-modal imaging, a sandwiched core/shell QD (SQD, Mn:ZnSe/ZnS/ZnMnS) was formulated as high quality contrast agent for both optical fluorescence imaging and magnetic resonance imaging (MRI). The paramagnetic Mn²⁺ ions dopants present in core and shell produce a ⁴T₁-⁶A₁ PL transition and at the same time also enhanced *T*₁ MRI signals, respectively. Quenching of PL due to heavy doping of shell was observed and resolved by employing a thick ZnS sandwiched transition shell layer.

To explore potential biomedical applications of the biocompatible QDs, after proper surface modification and bio-functionalization, the *d*-dots were developed as theranostic agents for in vitro cancer diagnosis and therapy. Specifically, two types of nanocarriers, namely *d*-dot/polymer nanoplex and *d*-dot/liposome hybrid, were developed as theranostic agents for small interfering RNA delivery into pancreatic cancer cells. Because of the unique optical properties of the QDs, the gene transfection process is optically traceable. Cancer cell targeted gene delivery was achieved by functionalizing the nanocarriers with cancer targeting ligands (FA). The therapeutic effect was confirmed by the significantly suppressed expression of the targeted gene sequence (mutant K-Ras) at mRNA level due to the RNAi process. More importantly, the *d*-dot/liposome hybrid based on the SQDs was demonstrated as a promising multifunctional platform for MR/optical dual mode imaging and gene/drug co-delivery. In this liposome structure, hydrophobic anti-cancer drug can be co-encapsulated with siRNA molecules and the dual-mode SQDs. Due to their flexibility, the applications of the *d*-dot/liposome hybrid could be extended to *in vivo*

tumour diagnosis and targeted gene-chemo therapy, while tunable combinations of therapeutic agents could benefit the development of personalized nanomedicines.

6.2 Ongoing and Future work

6.2.1 Synthesis of Cd-free QDs With Tunable or NIR Emission

As described in previous chapters, we have successfully prepared high quality Mn:ZnSe QDs as an alternative to replace the Cd-based QDs used in biomedical applications. However, the Mn-doped QDs normally requires UV light for excitation, while the emission color is not tunable to some extent. These intrinsic properties will limit its performance in some applications, such as deep tissue imaging and multiplexed sensing. For this reason, Cd-free QDs with tunable emission color and or NIR emission are considerably needed. To solve these problems, our ongoing projects aim to synthesis high quality and biocompatible Cu-doped ZnS QDs, CuInS₂ QDs and Ag₂S QDs as alternatives to Cd-based QDs, which have the size-tunable emission color covering the visible-NIR range.

Fig.6.1 Photograph of Cu:ZnS QDs, Mn:ZnSe QDs and CuInS₂ QDs excited by UV light.

Fig.6.2 Representative UV-vis and PL spectra of Cu:ZnS QDs and CuInS₂ QDs.

Some preliminary results are presented in Fig.6.1 and Fig.6.2. We have synthesized a series of Cd-free QDs based on Cu-doped ZnS and ternary alloyed CuInS₂. The emission color of these QDs ranges from blue (420 nm) to NIR (720 nm), which has already covered the emission range of the conventional CdTe QDs. As compared to Mn-doped QDs, Cu-doped QDs synthesized via growth doping method exhibit size-tunable emission color, because the radiative recombination involves the host conduction band and the *d*-orbital of a copper ion. More importantly, the CuInS₂ QD exhibits tuneable absorption/emission in visible-NIR range, thus can be promising candidates for *in vivo* and deep tissue imaging. So far, aqueous synthesis of high quality Cu-doped, CuInS₂ and Ag₂S QDs are seldom reported. In our future work,

based on these preliminary results, efforts will be made to optimize the synthesis chemistry to achieve improved optical properties.

6.2.2 *In vivo* Applications

In Chapter 4 and 5, we have prepared high quality Mn-doped ZnSe QDs based theranostic agents for *in vitro* cancer cell diagnosis and therapy. Specifically, the *d*-dot-lioposome hybrids are highly biocompatible and are flexible platforms to integrate diagnostic agents and therapeutic materials. Furthermore, because of the MRI ability of the encapsulated SQDs and the PEG functional groups present on the phospholipid bi-layers, which can prolong the circulation time of the liposome in blood stream, the SQD-liposome hybrids are promising for *in vivo* applications. In our future work plans, FA- or antibody-functionalized liposome formulations will be investigated as gene or drug carriers for targeted tumor therapy *in vivo*. Since the SQDs can be visualized in both MR and optical modes, the accumulation of the carriers is supposed to be traceable via both real-time MRI and fluorescent image of tissue sectioning. Biodistribution and metabolism of the nanocarriers can also be investigated, while optimization of the nanocarrier design can also be carried out to improve the specificity of the tumor targeted drug delivery.

REFERENCES

- [1] V. I. Klimov, S. A. Ivanov, J. Nanda, M. Achermann, I. Bezel, J. A. McGuire, *et al.*, "Single-exciton optical gain in semiconductor nanocrystals," *Nature*, vol. 447, pp. 441-446, 2007.
- [2] M. C. Schlamp, X. G. Peng, and A. P. Alivisatos, "Improved efficiencies in light emitting diodes made with CdSe(CdS) core/shell type nanocrystals and a semiconducting polymer," *Journal of Applied Physics*, vol. 82, pp. 5837-5842, Dec 1997.
- [3] H. Mattoussi, L. H. Radzilowski, B. O. Dabbousi, E. L. Thomas, M. G. Bawendi, and M. F. Rubner, "Electroluminescence from heterostructures of poly(phenylene vinylene) and inorganic CdSe nanocrystals," *Journal of Applied Physics*, vol. 83, pp. 7965-7974, Jun 1998.
- [4] W. C. W. Chan and S. M. Nie, "Quantum dot bioconjugates for ultrasensitive nonisotopic detection," *Science*, vol. 281, pp. 2016-2018, Sep 1998.
- [5] M. Bruchez, M. Moronne, P. Gin, S. Weiss, and A. P. Alivisatos, "Semiconductor nanocrystals as fluorescent biological labels," *Science*, vol. 281, pp. 2013-2016, Sep 1998.
- [6] I. L. Medintz, H. T. Uyeda, E. R. Goldman, and H. Mattoussi, "Quantum dot bioconjugates for imaging, labelling and sensing," *Nature Materials*, vol. 4, pp. 435-446, Jun 2005.
- [7] W. B. Cai, A. R. Hsu, Z. B. Li, and X. Y. Chen, "Are quantum dots ready for in vivo imaging in human subjects?," *Nanoscale Research Letters*, vol. 2, pp. 265-281, Jun 2007.
- [8] A. L. Efros, M. Rosen, M. Kuno, M. Nirmal, D. J. Norris, and M. Bawendi, "Band-edge exciton in quantum dots of semiconductors with a degenerate valence band: Dark and bright exciton states," *Physical Review B*, vol. 54, pp. 4843-4856, 1996.
- [9] V. I. Klimov, *Nanocrystal Quantum Dots 2nd Edition*. New York, 2009.
- [10] A. P. Alivisatos, "Semiconductor Clusters, Nanocrystals, and Quantum Dots," *Science*, vol. 271, pp. 933-937, February 16, 1996.
- [11] M. Nirmal, B. O. Dabbousi, M. G. Bawendi, J. J. Macklin, J. K. Trautman, T. D. Harris, *et al.*, "Fluorescence intermittency in single cadmium selenide nanocrystals," *Nature*, vol. 383, pp. 802-804, Oct 1996.
- [12] S. Hohng and T. Ha, "Near-complete suppression of quantum dot blinking in ambient conditions," *Journal of the American Chemical Society*, vol. 126, pp. 1324-1325, Feb 2004.
- [13] C. de Mello Donegá, M. Bode, and A. Meijerink, "Size- and temperature-dependence of exciton lifetimes in CdSe quantum dots," *Physical Review B*, vol. 74, p. 085320, 2006.
- [14] P. Reiss, M. Protiere, and L. Li, "Core/Shell Semiconductor Nanocrystals," *Small*, vol. 5, pp. 154-168, Jan 2009.
- [15] A. M. Smith, A. M. Mohs, and S. Nie, "Tuning the optical and electronic properties of colloidal nanocrystals by lattice strain," *Nature Nanotechnology*, vol. 4, pp. 56-63, Jan 2009.
- [16] P. Wu and X.-P. Yan, "Doped quantum dots for chemo/biosensing and bioimaging," *Chemical Society Reviews*, vol. 42, pp. 5489-5521, 2013.
- [17] C. Gan, Y. Zhang, D. Battaglia, X. Peng, and M. Xiao, "Fluorescence lifetime of Mn-doped ZnSe quantum dots with size dependence," *Applied Physics Letters*, vol. 92, pp. -, 2008.

References

- [18] S. K. Panda, S. G. Hickey, H. V. Demir, and A. Eychmüller, "Bright White-Light Emitting Manganese and Copper Co-Doped ZnSe Quantum Dots," *Angewandte Chemie International Edition*, vol. 50, pp. 4432-4436, 2011.
- [19] N. Pradhan and X. Peng, "Efficient and color-tunable Mn-doped ZnSe nanocrystal emitters: Control of optical performance via greener synthetic chemistry," *Journal of the American Chemical Society*, vol. 129, pp. 3339-3347, Mar 21 2007.
- [20] J. H. Yu, S.-H. Kwon, Z. Petrasek, O. K. Park, S. W. Jun, K. Shin, *et al.*, "High-resolution three-photon biomedical imaging using doped ZnS nanocrystals," *Nature Materials*, vol. 12, pp. 359-366, Apr 2013.
- [21] C. B. Murray, D. J. Norris, and M. G. Bawendi, "SYNTHESIS AND CHARACTERIZATION OF NEARLY MONODISPERSE CDE (E = S, SE, TE) SEMICONDUCTOR NANOCRYSTALLITES," *Journal of the American Chemical Society*, vol. 115, pp. 8706-8715, Sep 1993.
- [22] C. B. Murray, C. R. Kagan, and M. G. Bawendi, "Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies," *Annual Review of Materials Science*, vol. 30, pp. 545-610, 2000.
- [23] Y. Yin and A. P. Alivisatos, "Colloidal nanocrystal synthesis and the organic-inorganic interface," *Nature*, vol. 437, pp. 664-670, Sep 2005.
- [24] X. G. Peng, J. Wickham, and A. P. Alivisatos, "Kinetics of II-VI and III-V colloidal semiconductor nanocrystal growth: "Focusing" of size distributions," *Journal of the American Chemical Society*, vol. 120, pp. 5343-5344, Jun 1998.
- [25] M. A. Hines and P. Guyot-Sionnest, "Synthesis and characterization of strongly luminescing ZnS-Capped CdSe nanocrystals," *Journal of Physical Chemistry*, vol. 100, pp. 468-471, Jan 1996.
- [26] B. O. Dabbousi, J. RodriguezViejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, *et al.*, "(CdSe)ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites," *Journal of Physical Chemistry B*, vol. 101, pp. 9463-9475, Nov 1997.
- [27] A. M. Derfus, W. C. W. Chan, and S. N. Bhatia, "Probing the cytotoxicity of semiconductor quantum dots," *Nano Letters*, vol. 4, pp. 11-18, Jan 2004.
- [28] A. Chemseddine and H. Weller, "HIGHLY MONODISPERSE QUANTUM SIZED CDS PARTICLES BY SIZE-SELECTIVE PRECIPITATION," *Berichte Der Bunsen-Gesellschaft-Physical Chemistry Chemical Physics*, vol. 97, pp. 636-637, Apr 1993.
- [29] B. Dubertret, P. Skourides, D. J. Norris, V. Noireaux, A. H. Brivanlou, and A. Libchaber, "In vivo imaging of quantum dots encapsulated in phospholipid micelles," *Science*, vol. 298, pp. 1759-1762, Nov 2002.
- [30] T. Pellegrino, L. Manna, S. Kudera, T. Liedl, D. Koktysh, A. L. Rogach, *et al.*, "Hydrophobic nanocrystals coated with an amphiphilic polymer shell: A general route to water soluble nanocrystals," *Nano Letters*, vol. 4, pp. 703-707, Apr 2004.
- [31] D. Gerion, F. Pinaud, S. C. Williams, W. J. Parak, D. Zanchet, S. Weiss, *et al.*, "Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots," *Journal of Physical Chemistry B*, vol. 105, pp. 8861-8871, Sep 2001.
- [32] T. Nann and P. Mulvaney, "Single quantum dots in spherical silica particles," *Angewandte Chemie-International Edition*, vol. 43, pp. 5393-5396, 2004.
- [33] H. T. Uyeda, I. L. Medintz, J. K. Jaiswal, S. M. Simon, and H. Mattoussi, "Synthesis of compact multidentate ligands to prepare stable hydrophilic quantum dot fluorophores," *Journal of the American Chemical Society*, vol. 127, pp. 3870-3878, Mar 2005.

References

- [34] S. Pathak, S. K. Choi, N. Arnheim, and M. E. Thompson, "Hydroxylated quantum dots as luminescent probes for in situ hybridization," *Journal of the American Chemical Society*, vol. 123, pp. 4103-4104, May 2001.
- [35] S. Kim and M. G. Bawendi, "Oligomeric Ligands for luminescent and stable nanocrystal quantum dots," *Journal of the American Chemical Society*, vol. 125, pp. 14652-14653, Dec 2003.
- [36] W. Z. Guo, J. J. Li, Y. A. Wang, and X. G. Peng, "Conjugation chemistry and bioapplications of semiconductor box nanocrystals prepared via dendrimer bridging," *Chemistry of Materials*, vol. 15, pp. 3125-3133, Aug 2003.
- [37] H. Mattoussi, J. M. Mauro, E. R. Goldman, G. P. Anderson, V. C. Sundar, F. V. Mikulec, *et al.*, "Self-assembly of CdSe-ZnS quantum dot bioconjugates using an engineered recombinant protein," *Journal of the American Chemical Society*, vol. 122, pp. 12142-12150, Dec 2000.
- [38] G. P. Mitchell, C. A. Mirkin, and R. L. Letsinger, "Programmed assembly of DNA functionalized quantum dots," *Journal of the American Chemical Society*, vol. 121, pp. 8122-8123, Sep 1999.
- [39] E. R. Goldman, E. D. Balighian, H. Mattoussi, M. K. Kuno, J. M. Mauro, P. T. Tran, *et al.*, "Avidin: A natural bridge for quantum dot-antibody conjugates," *Journal of the American Chemical Society*, vol. 124, pp. 6378-6382, Jun 2002.
- [40] W. J. Parak, D. Gerion, T. Pellegrino, D. Zanchet, C. Micheel, S. C. Williams, *et al.*, "Biological applications of colloidal nanocrystals," *Nanotechnology*, vol. 14, pp. R15-R27, Jul 2003.
- [41] X. H. Gao, Y. Y. Cui, R. M. Levenson, L. W. K. Chung, and S. M. Nie, "In vivo cancer targeting and imaging with semiconductor quantum dots," *Nature Biotechnology*, vol. 22, pp. 969-976, Aug 2004.
- [42] X. Y. Wu, H. J. Liu, J. Q. Liu, K. N. Haley, J. A. Treadway, J. P. Larson, *et al.*, "Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots," *Nature Biotechnology*, vol. 21, pp. 41-46, Jan 2003.
- [43] F. Osaki, T. Kanamori, S. Sando, T. Sera, and Y. Aoyama, "A quantum dot conjugated sugar ball and its cellular uptake on the size effects of endocytosis in the subviral region," *Journal of the American Chemical Society*, vol. 126, pp. 6520-6521, Jun 2004.
- [44] L. C. Mattheakis, J. M. Dias, Y. J. Choi, J. Gong, M. P. Bruchez, J. Q. Liu, *et al.*, "Optical coding of mammalian cells using semiconductor quantum dots," *Analytical Biochemistry*, vol. 327, pp. 200-208, Apr 2004.
- [45] B. Ballou, B. C. Lagerholm, L. A. Ernst, M. P. Bruchez, and A. S. Waggoner, "Noninvasive imaging of quantum dots in mice," *Bioconjugate Chemistry*, vol. 15, pp. 79-86, Jan-Feb 2004.
- [46] A. M. Smith, H. W. Duan, A. M. Mohs, and S. M. Nie, "Bioconjugated quantum dots for in vivo molecular and cellular imaging," *Advanced Drug Delivery Reviews*, vol. 60, pp. 1226-1240, Aug 2008.
- [47] F. Erogbogbo, C.-W. Chang, J. May, P. N. Prasad, and M. T. Swihart, "Energy transfer from a dye donor to enhance the luminescence of silicon quantum dots," *Nanoscale*, vol. 4, pp. 5163-5168, 2012.
- [48] N. Gaponik, D. V. Talapin, A. L. Rogach, K. Hoppe, E. V. Shevchenko, A. Kornowski, *et al.*, "Thiol-capping of CdTe nanocrystals: An alternative to organometallic synthetic routes," *Journal of Physical Chemistry B*, vol. 106, pp. 7177-7185, Jul 2002.
- [49] H. Zhang, Y. Liu, C. L. Wang, J. H. Zhang, H. Z. Sun, M. J. Li, *et al.*, "Directing the growth of semiconductor nanocrystals in aqueous solution: Role of electrostatics," *Chemphyschem*, vol. 9, pp. 1309-1316, Jun 2008.

References

- [50] Y. S. Park, Y. Okamoto, N. Kaji, M. Tokeshi, and Y. Baba, "Aqueous phase-synthesized small CdSe quantum dots: adsorption layer structure and strong band-edge and surface trap emission," *Journal of Nanoparticle Research*, vol. 13, pp. 5781-5798, Nov 2011.
- [51] S. B. Brichkin and E. V. Chernykh, "Hydrophilic semiconductor quantum dots," *High Energy Chemistry*, vol. 45, pp. 1-12, Jan 2011.
- [52] A. Priyam, S. Ghosh, S. C. Bhattacharya, and A. Saha, "Supersaturation driven tailoring of photoluminescence efficiency and size distribution: A simplified aqueous approach for producing high-quality, biocompatible quantum dots," *Journal of Colloid and Interface Science*, vol. 333, pp. 195-201, May 2009.
- [53] H. Zhang, L. P. Wang, H. M. Xiong, L. H. Hu, B. Yang, and W. Li, "Hydrothermal synthesis for high-quality CdTe nanocrystals," *Advanced Materials*, vol. 15, pp. 1712-+, Oct 2003.
- [54] Y. He, H. T. Lu, L. M. Sai, W. Y. Lai, Q. L. Fan, L. H. Wang, *et al.*, "Microwave-assisted growth and characterization of water-dispersed CdTe/CdS core-shell nanocrystals with high photoluminescence," *Journal of Physical Chemistry B*, vol. 110, pp. 13370-13374, Jul 2006.
- [55] A. S. Blum, M. H. Moore, and B. R. Ratna, "Quantum dot fluorescence as a function of alkyl chain length in aqueous environments," *Langmuir*, vol. 24, pp. 9194-9197, Sep 2008.
- [56] T. Fang, K. Ma, L. Ma, J. Bai, X. Li, H. Song, *et al.*, "3-Mercaptobutyric Acid as an Effective Capping Agent for Highly Luminescent CdTe Quantum Dots: New Insight into the Selection of Mercapto Acids," *The Journal of Physical Chemistry C*, vol. 116, pp. 12346-12352, 2012/06/07 2012.
- [57] W. Y. Mao, J. Guo, W. L. Yang, C. C. Wang, J. He, and J. Y. Chen, "Synthesis of high-quality near-infrared-emitting CdTeS alloyed quantum dots via the hydrothermal method," *Nanotechnology*, vol. 18, Dec 2007.
- [58] H. Borchert, D. V. Talapin, N. Gaponik, C. McGinley, S. Adam, A. Lobo, *et al.*, "Relations between the Photoluminescence Efficiency of CdTe Nanocrystals and Their Surface Properties Revealed by Synchrotron XPS," *The Journal of Physical Chemistry B*, vol. 107, pp. 9662-9668, 2003/09/01 2003.
- [59] M. E. Akerman, W. C. W. Chan, P. Laakkonen, S. N. Bhatia, and E. Ruoslahti, "Nanocrystal targeting in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, pp. 12617-12621, Oct 2002.
- [60] R. Edgar, M. McKinstry, J. Hwang, A. B. Oppenheim, R. A. Fekete, G. Giulian, *et al.*, "High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, pp. 4841-4845, Mar 2006.
- [61] M. Dahan, S. Levi, C. Luccardini, P. Rostaing, B. Riveau, and A. Triller, "Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking," *Science*, vol. 302, pp. 442-445, Oct 2003.
- [62] F. Pinaud, D. King, H. P. Moore, and S. Weiss, "Bioactivation and cell targeting of semiconductor CdSe/ZnS nanocrystals with phytochelatin-related peptides," *Journal of the American Chemical Society*, vol. 126, pp. 6115-6123, May 2004.
- [63] W. B. Cai, D. W. Shin, K. Chen, O. Gheysens, Q. Z. Cao, S. X. Wang, *et al.*, "Peptide-labeled near-infrared quantum dots for imaging tumor vasculature in living subjects," *Nano Letters*, vol. 6, pp. 669-676, Apr 2006.
- [64] Y. Xing, Q. Chaudry, C. Shen, K. Y. Kong, H. E. Zhau, L. Wchung, *et al.*, "Bioconjugated quantum dots for multiplexed and quantitative immunohistochemistry," *Nature Protocols*, vol. 2, pp. 1152-1165, 2007.

References

- [65] O. Lieleg, M. Lopez-Garcia, C. Semmrich, J. Auernheimer, H. Kessler, and A. R. Bausch, "Specific integrin Labeling in living Cells using functionalized nanocrystals," *Small*, vol. 3, pp. 1560-1565, Sep 2007.
- [66] S. Pathak, M. C. Davidson, and G. A. Silva, "Characterization of the functional binding properties of antibody conjugated quantum dots," *Nano Letters*, vol. 7, pp. 1839-1845, Jul 2007.
- [67] X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, *et al.*, "Quantum dots for live cells, in vivo imaging, and diagnostics," *Science*, vol. 307, pp. 538-544, Jan 2005.
- [68] S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara, and A. M. Belcher, "Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly," *Nature*, vol. 405, pp. 665-668, Jun 2000.
- [69] C. T. Dameron, R. N. Reese, R. K. Mehra, A. R. Kortan, P. J. Carroll, M. L. Steigerwald, *et al.*, "BIOSYNTHESIS OF CADMIUM-SULFIDE QUANTUM SEMICONDUCTOR CRYSTALLITES," *Nature*, vol. 338, pp. 596-597, Apr 1989.
- [70] F. Q. Chen and D. Gerion, "Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells," *Nano Letters*, vol. 4, pp. 1827-1832, Oct 2004.
- [71] B. C. Lagerholm, M. M. Wang, L. A. Ernst, D. H. Ly, H. J. Liu, M. P. Bruchez, *et al.*, "Multicolor coding of cells with cationic peptide coated quantum dots," *Nano Letters*, vol. 4, pp. 2019-2022, Oct 2004.
- [72] J. B. Delehanty, I. L. Medintz, T. Pons, F. M. Brunel, P. E. Dawson, and H. Mattoussi, "Self-assembled quantum dot-peptide bioconjugates for selective intracellular delivery," *Bioconjugate Chemistry*, vol. 17, pp. 920-927, Jul 2006.
- [73] E. R. Goldman, G. P. Anderson, P. T. Tran, H. Mattoussi, P. T. Charles, and J. M. Mauro, "Conjugation of luminescent quantum dots with antibodies using an engineered adaptor protein to provide new reagents for fluoroimmunoassays," *Analytical Chemistry*, vol. 74, pp. 841-847, Feb 2002.
- [74] H. Mattoussi, J. M. Mauro, E. R. Goldman, T. M. Green, G. P. Anderson, V. C. Sundar, *et al.*, "Bioconjugation of highly luminescent colloidal CdSe-ZnS quantum dots with an engineered two-domain recombinant protein," *Physica Status Solidi B-Basic Research*, vol. 224, pp. 277-283, Mar 2001.
- [75] J. M. Slocik, J. T. Moore, and D. W. Wright, "Monoclonal antibody recognition of histidine-rich peptide encapsulated nanoclusters," *Nano Letters*, vol. 2, pp. 169-173, Mar 2002.
- [76] D. Marshall, R. B. Pedley, J. A. Boden, R. Boden, R. G. Melton, and R. H. J. Begent, "Polyethylene glycol modification of a galactosylated streptavidin clearing agent: Effects on immunogenicity and clearance of a biotinylated anti-tumour antibody," *British Journal of Cancer*, vol. 73, pp. 565-572, Mar 1996.
- [77] L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. Reynolds, R. L. Letsinger, R. Elghanian, *et al.*, "A fluorescence-based method for determining the surface coverage and hybridization efficiency of thiol-capped oligonucleotides bound to gold thin films and nanoparticles," *Analytical Chemistry*, vol. 72, pp. 5535-5541, Nov 2000.
- [78] A. C. Vinayaka, S. Basheer, and M. S. Thakur, "Bioconjugation of CdTe quantum dot for the detection of 2,4-dichlorophenoxyacetic acid by competitive fluoroimmunoassay based biosensor," *Biosensors & Bioelectronics*, vol. 24, pp. 1615-1620, Feb 2009.
- [79] S. Kale, A. Kale, H. Gholap, A. Rana, R. Desai, A. Banpurkar, *et al.*, "Quantum dot bio-conjugate: as a western blot probe for highly sensitive detection of cellular proteins," *Journal of Nanoparticle Research*, vol. 14, Mar 2012.

References

- [80] L. J. Yang and Y. B. Li, "Simultaneous detection of Escherichia coli O157 : H7 and Salmonella Typhimurium using quantum dots as fluorescence labels," *Analyst*, vol. 131, pp. 394-401, 2006.
- [81] W. T. Liu, L. Zhu, Q. W. Qin, Q. Zhang, H. H. Feng, and S. Ang, "Microfluidic device as a new platform for immunofluorescent detection of viruses," *Lab on a Chip*, vol. 5, pp. 1327-1330, 2005.
- [82] B. Scholl, H. Y. Liu, B. R. Long, O. J. T. McCarty, T. O'Hare, B. J. Druker, *et al.*, "Single Particle Quantum Dot Imaging Achieves Ultrasensitive Detection Capabilities for Western Immunoblot Analysis," *Acs Nano*, vol. 3, pp. 1318-1328, Jun 2009.
- [83] R. Bakalova, Z. Zhelev, H. Ohba, and Y. Baba, "Quantum dot-based western blot technology for ultrasensitive detection of tracer proteins," *Journal of the American Chemical Society*, vol. 127, pp. 9328-9329, Jul 2005.
- [84] R. Cui, H. C. Pan, J. J. Zhu, and H. Y. Chen, "Versatile immunosensor using CdTe quantum dots as electrochemical and fluorescent labels," *Analytical Chemistry*, vol. 79, pp. 8494-8501, Nov 2007.
- [85] L. Y. Chen, C. L. Chen, R. N. Li, Y. Li, and S. Q. Liu, "CdTe quantum dot functionalized silica nanosphere labels for ultrasensitive detection of biomarker," *Chemical Communications*, pp. 2670-2672, 2009.
- [86] J. M. Klostianec, Q. Xiang, G. A. Farcas, J. A. Lee, A. Rhee, E. I. Lafferty, *et al.*, "Convergence of quantum dot barcodes with microfluidics and signal processing for multiplexed high-throughput infectious disease diagnostics," *Nano Letters*, vol. 7, pp. 2812-2818, Sep 2007.
- [87] M. H. Yang, A. Javadi, and S. Q. Gong, "Sensitive electrochemical immunosensor for the detection of cancer biomarker using quantum dot functionalized graphene sheets as labels," *Sensors and Actuators B-Chemical*, vol. 155, pp. 357-360, Jul 2011.
- [88] J. A. A. Ho, Y. C. Lin, L. S. Wang, K. C. Hwang, and P. T. Chou, "Carbon Nanoparticle Enhanced Immuno-electrochemical Detection for Protein Tumor Marker with Cadmium Sulfide Biotracers," *Analytical Chemistry*, vol. 81, pp. 1340-1346, Feb 2009.
- [89] E. R. Goldman, A. R. Clapp, G. P. Anderson, H. T. Uyeda, J. M. Mauro, I. L. Medintz, *et al.*, "Multiplexed toxin analysis using four colors of quantum dot fluororeagents," *Analytical Chemistry*, vol. 76, pp. 684-688, Feb 2004.
- [90] C. F. Peng, Z. K. Li, Y. Y. Zhu, W. Chen, Y. Yuan, L. Q. Liu, *et al.*, "Simultaneous and sensitive determination of multiplex chemical residues based on multicolor quantum dot probes," *Biosensors & Bioelectronics*, vol. 24, pp. 3657-3662, Aug 2009.
- [91] M. S. Wu, G. S. Qian, J. J. Xu, and H. Y. Chen, "Sensitive Electrochemiluminescence Detection of c-Myc mRNA in Breast Cancer Cells on a Wireless Bipolar Electrode," *Analytical Chemistry*, vol. 84, pp. 5407-5414, Jun 2012.
- [92] S. Giri, E. A. Sykes, T. L. Jennings, and W. C. W. Chan, "Rapid Screening of Genetic Biomarkers of Infectious Agents Using Quantum Dot Barcodes," *Acs Nano*, vol. 5, pp. 1580-1587, Mar 2011.
- [93] M. Y. Han, X. H. Gao, J. Z. Su, and S. Nie, "Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules," *Nature Biotechnology*, vol. 19, pp. 631-635, Jul 2001.
- [94] H. X. Xu, M. Y. Sha, E. Y. Wong, J. Uphoff, Y. H. Xu, J. A. Treadway, *et al.*, "Multiplexed SNP genotyping using the Qbead (TM) system: a quantum dot-encoded microsphere-based assay," *Nucleic Acids Research*, vol. 31, Apr 2003.
- [95] J. R. E. Shepard, "Polychromatic microarrays: Simultaneous multicolor array hybridization of eight samples," *Analytical Chemistry*, vol. 78, pp. 2478-2486, Apr 2006.

References

- [96] P. M. Chan, T. Yuen, F. Ruf, J. Gonzalez-Maeso, and S. C. Sealton, "Method for multiplex cellular detection of mRNAs using quantum dot fluorescent in situ hybridization," *Nucleic Acids Research*, vol. 33, 2005.
- [97] Y. Choi, H. P. Kim, S. M. Hong, J. Y. Ryu, S. J. Han, and R. Song, "In situ Visualization of Gene Expression Using Polymer-Coated Quantum-Dot-DNA Conjugates," *Small*, vol. 5, pp. 2085-2091, Sep 2009.
- [98] J.R.Lakowicz, Ed., *Principles of Fluorescence Spectroscopy*. New York, 2006, p.^pp. Pages.
- [99] E. A. Jares-Erijman and T. M. Jovin, "FRET imaging," *Nature Biotechnology*, vol. 21, pp. 1387-1395, Nov 2003.
- [100] A. Miyawaki, "Visualization of the spatial and temporal dynamics of intracellular signaling," *Developmental Cell*, vol. 4, pp. 295-305, Mar 2003.
- [101] K. E. Sapsford, L. Berti, and I. L. Medintz, "Materials for fluorescence resonance energy transfer analysis: Beyond traditional donor-acceptor combinations," *Angewandte Chemie-International Edition*, vol. 45, pp. 4562-4588, 2006.
- [102] I. L. Medintz and H. Mattoussi, "Quantum dot-based resonance energy transfer and its growing application in biology," *Physical Chemistry Chemical Physics*, vol. 11, pp. 17-45, 2009.
- [103] A. R. Clapp, T. Pons, I. L. Medintz, J. B. Delehanty, J. S. Melinger, T. Tiefenbrunn, *et al.*, "Two-photon excitation of quantum-dot-based fluorescence resonance energy transfer and its applications," *Advanced Materials*, vol. 19, pp. 1921-+, Aug 2007.
- [104] E. R. Goldman, I. L. Medintz, J. L. Whitley, A. Hayhurst, A. R. Clapp, H. T. Uyeda, *et al.*, "A hybrid quantum dot-antibody fragment fluorescence resonance energy transfer-based TNT sensor," *Journal of the American Chemical Society*, vol. 127, pp. 6744-6751, May 11 2005.
- [105] I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman, B. Fisher, and J. M. Mauro, "Self-assembled nanoscale biosensors based on quantum dot FRET donors," *Nature Materials*, vol. 2, pp. 630-638, Sep 2003.
- [106] C. Y. Zhang, H. C. Yeh, M. T. Kuroki, and T. H. Wang, "Single-quantum-dot-based DNA nanosensor," *Nature Materials*, vol. 4, pp. 826-831, Nov 2005.
- [107] I. L. Medintz, T. Pons, K. Susumu, K. Boeneman, A. M. Dennis, D. Farrell, *et al.*, "Resonance Energy Transfer Between Luminescent Quantum Dots and Diverse Fluorescent Protein Acceptors," *Journal of Physical Chemistry C*, vol. 113, pp. 18552-18561, Oct 2009.
- [108] A. D. Quach, G. Crivat, M. A. Tarr, and Z. Rosenzweig, "Gold Nanoparticle-Quantum Dot-Polystyrene Microspheres as Fluorescence Resonance Energy Transfer Probes for Bioassays," *Journal of the American Chemical Society*, vol. 133, pp. 2028-2030, Feb 2011.
- [109] X. Li, J. Qian, L. Jiang, and S. L. He, "Fluorescence quenching of quantum dots by gold nanorods and its application to DNA detection," *Applied Physics Letters*, vol. 94, Feb 2009.
- [110] A. R. Clapp, I. L. Medintz, B. R. Fisher, G. P. Anderson, and H. Mattoussi, "Can luminescent quantum dots be efficient energy acceptors with organic dye donors?," *Journal of the American Chemical Society*, vol. 127, pp. 1242-1250, Feb 2005.
- [111] M. K. So, C. J. Xu, A. M. Loening, S. S. Gambhir, and J. H. Rao, "Self-illuminating quantum dot conjugates for in vivo imaging," *Nature Biotechnology*, vol. 24, pp. 339-343, Mar 2006.
- [112] Z. Y. Xia and J. H. Rao, "Biosensing and imaging based on bioluminescence resonance energy transfer," *Current Opinion in Biotechnology*, vol. 20, pp. 37-44, Feb 2009.
- [113] G. B. Kim and Y. P. Kim, "Analysis of Protease Activity Using Quantum Dots and Resonance Energy Transfer," *Theranostics*, vol. 2, pp. 127-138, 2012.

References

- [114] J. Lee, Y. Choi, K. Kim, S. Hong, H. Y. Park, T. Lee, *et al.*, "Characterization and Cancer Cell Specific Binding Properties of Anti-EGFR Antibody Conjugated Quantum Dots," *Bioconjugate Chemistry*, vol. 21, pp. 940-946, May 2010.
- [115] M. H. Ko, S. Kim, W. J. Kang, J. H. Lee, H. Kang, S. H. Moon, *et al.*, "In vitro Derby Imaging of Cancer Biomarkers Using Quantum Dots," *Small*, vol. 5, pp. 1207-1212, May 2009.
- [116] V. Roullier, S. Clarke, C. You, F. Pinaud, G. Gouzer, D. Schaible, *et al.*, "High-Affinity Labeling and Tracking of Individual Histidine-Tagged Proteins in Live Cells Using Ni²⁺-Tris-nitrilotriacetic Acid Quantum Dot Conjugates," *Nano Letters*, vol. 9, pp. 1228-1234, Mar 2009.
- [117] H. Bannai, S. Levi, C. Schweizer, M. Dahan, and A. Triller, "Imaging the lateral diffusion of membrane molecules with quantum dots," *Nature Protocols*, vol. 1, pp. 2628-2634, 2006.
- [118] K. T. Yong, H. Ding, I. Roy, W. C. Law, E. J. Bergey, A. Maitra, *et al.*, "Imaging Pancreatic Cancer Using Bioconjugated InP Quantum Dots," *Acs Nano*, vol. 3, pp. 502-510, Mar 2009.
- [119] K. C. Weng, C. O. Noble, B. Papahadjopoulos-Sternberg, F. F. Chen, D. C. Drummond, D. B. Kirpotin, *et al.*, "Targeted tumor cell internalization and imaging of multifunctional quantum dot-conjugated immunoliposomes in vitro and in vivo," *Nano Letters*, vol. 8, pp. 2851-2857, Sep 2008.
- [120] F. Koepfel, J. K. Jaiswal, and S. M. Simon, "Quantum dot-based sensor for improved detection of apoptotic cells," *Nanomedicine*, vol. 2, pp. 71-78, Feb 2007.
- [121] H. Tada, H. Higuchi, T. M. Wanatabe, and N. Ohuchi, "In vivo real-time tracking of single quantum dots conjugated with monoclonal anti-HER2 antibody in tumors of mice," *Cancer Research*, vol. 67, pp. 1138-1144, Feb 2007.
- [122] N. Kawashima, K. Nakayama, K. Itoh, T. Itoh, M. Ishikawa, and V. Biju, "Reversible Dimerization of EGFR Revealed by Single-Molecule Fluorescence Imaging Using Quantum Dots," *Chemistry-a European Journal*, vol. 16, pp. 1186-1192, 2010.
- [123] V. Sigot, D. J. Arndt-Jovin, and T. M. Jovin, "Targeted Cellular Delivery of Quantum Dots Loaded on and in Biotinylated Liposomes," *Bioconjugate Chemistry*, vol. 21, pp. 1465-1472, Aug 2010.
- [124] D. S. Lidke, P. Nagy, R. Heintzmann, D. J. Arndt-Jovin, J. N. Post, H. E. Grecco, *et al.*, "Quantum dot ligands provide new insights into erbB/HER receptor-mediated signal transduction," *Nature Biotechnology*, vol. 22, pp. 198-203, Feb 2004.
- [125] V. Bagalkot, L. Zhang, E. Levy-Nissenbaum, S. Jon, P. W. Kantoff, R. Langer, *et al.*, "Quantum dot - Aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on Bi-fluorescence resonance energy transfer," *Nano Letters*, vol. 7, pp. 3065-3070, Oct 2007.
- [126] M. Geszke, M. Murias, L. Balan, G. Medjandi, J. Korczynski, M. Moritz, *et al.*, "Folic acid-conjugated core/shell ZnS:Mn/ZnS quantum dots as targeted probes for two photon fluorescence imaging of cancer cells," *Acta Biomaterialia*, vol. 7, pp. 1327-1338, Mar 2011.
- [127] A. Dif, F. Boulmedais, M. Pinot, V. Roullier, M. Baudy-Floc'h, F. M. Coquelle, *et al.*, "Small and Stable Peptidic PEGylated Quantum Dots to Target Polyhistidine-Tagged Proteins with Controlled Stoichiometry," *Journal of the American Chemical Society*, vol. 131, pp. 14738-14746, Oct 2009.
- [128] J. A. Liu, S. K. Lau, V. A. Varma, B. A. Kairdolf, and S. M. Nie, "Multiplexed Detection and Characterization of Rare Tumor Cells in Hodgkin's Lymphoma with Multicolor Quantum Dots," *Analytical Chemistry*, vol. 82, pp. 6237-6243, Jul 2010.
- [129] J. B. Delehanty, C. E. Bradburne, K. Susumu, K. Boeneman, B. C. Mei, D. Farrell, *et al.*, "Spatiotemporal Multicolor Labeling of Individual Cells Using Peptide-Functionalized

References

- Quantum Dots and Mixed Delivery Techniques," *Journal of the American Chemical Society*, vol. 133, pp. 10482-10489, Jul 2011.
- [130] P. Pierobon, S. Achouri, S. Courty, A. R. Dunn, J. A. Spudich, M. Dahan, *et al.*, "Velocity, Processivity, and Individual Steps of Single Myosin V Molecules in Live Cells," *Biophysical Journal*, vol. 96, pp. 4268-4275, May 2009.
- [131] S. R. Nelson, M. Y. Ali, K. M. Trybus, and D. M. Warshaw, "Random Walk of Processive, Quantum Dot-Labeled Myosin Va Molecules within the Actin Cortex of COS-7 Cells," *Biophysical Journal*, vol. 97, pp. 509-518, Jul 2009.
- [132] S. Clarke, F. Pinaud, O. Beutel, C. J. You, J. Piehler, and M. Dahan, "Covalent Monofunctionalization of Peptide-Coated Quantum Dots for Single-Molecule Assays," *Nano Letters*, vol. 10, pp. 2147-2154, Jun 2010.
- [133] A. M. Derfus, W. C. W. Chan, and S. N. Bhatia, "Intracellular delivery of quantum dots for live cell labeling and organelle tracking," *Advanced Materials*, vol. 16, pp. 961-+, Jun 2004.
- [134] L. W. Zhang and N. A. Monteiro-Riviere, "Mechanisms of Quantum Dot Nanoparticle Cellular Uptake," *Toxicological Sciences*, vol. 110, pp. 138-155, Jul 2009.
- [135] H. Ding, K. T. Yong, W. C. Law, I. Roy, R. Hu, F. Wu, *et al.*, "Non-invasive tumor detection in small animals using novel functional Pluronic nanomicelles conjugated with anti-mesothelin antibody," *Nanoscale*, vol. 3, pp. 1813-1822, 2011.
- [136] E. B. Voura, J. K. Jaiswal, H. Mattoussi, and S. M. Simon, "Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy," *Nature Medicine*, vol. 10, pp. 993-998, Sep 2004.
- [137] B. R. Liu, Y. W. Huang, J. G. Winiarz, H. J. Chiang, and H. J. Lee, "Intracellular delivery of quantum dots mediated by a histidine- and arginine-rich HR9 cell-penetrating peptide through the direct membrane translocation mechanism," *Biomaterials*, vol. 32, pp. 3520-3537, May 2011.
- [138] H. W. Duan and S. M. Nie, "Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings," *Journal of the American Chemical Society*, vol. 129, pp. 3333-3338, Mar 2007.
- [139] I. L. Medintz, T. Pons, J. B. Delehanty, K. Susumu, F. M. Brunel, P. E. Dawson, *et al.*, "Intracellular delivery of quantum dot-protein cargos mediated by cell penetrating peptides," *Bioconjugate Chemistry*, vol. 19, pp. 1785-1795, Sep 2008.
- [140] A. B. Rosen, D. J. Kelly, A. J. T. Schuldt, J. Lu, I. A. Potapova, S. V. Doronin, *et al.*, "Finding fluorescent needles in the cardiac haystack: Tracking human mesenchymal stem cells labeled with quantum dots for quantitative in vivo three-dimensional fluorescence analysis," *Stem Cells*, vol. 25, pp. 2128-2138, Aug 2007.
- [141] B. H. Jun, D. W. Hwang, H. S. Jung, J. Jang, H. Kim, H. Kang, *et al.*, "Ultrasensitive, Biocompatible, Quantum-Dot-Embedded Silica Nanoparticles for Bioimaging," *Advanced Functional Materials*, vol. 22, pp. 1843-1849, May 2012.
- [142] B. Ballou, L. A. Ernst, S. Andreko, T. Harper, J. A. J. Fitzpatrick, A. S. Waggoner, *et al.*, "Sentinel lymph node imaging using quantum dots in mouse tumor models," *Bioconjugate Chemistry*, vol. 18, pp. 389-396, Mar-Apr 2007.
- [143] Y. Hama, Y. Koyama, Y. Urano, P. L. Choyke, and H. Kobayashi, "Simultaneous two-color spectral fluorescence lymphangiography with near infrared quantum dots to map two lymphatic flows from the breast and the upper extremity," *Breast Cancer Research and Treatment*, vol. 103, pp. 23-28, May 2007.
- [144] A. Jayagopal, P. K. Russ, and F. R. Haselton, "Surface engineering of quantum dots for in vivo vascular imaging," *Bioconjugate Chemistry*, vol. 18, pp. 1424-1433, Sep-Oct 2007.

References

- [145] S. Kim, Y. T. Lim, E. G. Soltesz, A. M. De Grand, J. Lee, A. Nakayama, *et al.*, "Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping," *Nature Biotechnology*, vol. 22, pp. 93-97, Jan 2004.
- [146] S. W. Kim, J. P. Zimmer, S. Ohnishi, J. B. Tracy, J. V. Frangioni, and M. G. Bawendi, "Engineering InAsP_{1-x}/InP/ZnSe III-V alloyed core/shell quantum dots for the near-infrared," *Journal of the American Chemical Society*, vol. 127, pp. 10526-10532, Aug 2005.
- [147] H. Kobayashi, Y. Hama, Y. Koyama, T. Barrett, C. A. S. Regino, Y. Urano, *et al.*, "Simultaneous multicolor imaging of five different lymphatic basins using quantum dots," *Nano Letters*, vol. 7, pp. 1711-1716, Jun 2007.
- [148] D. R. Larson, W. R. Zipfel, R. M. Williams, S. W. Clark, M. P. Bruchez, F. W. Wise, *et al.*, "Water-soluble quantum dots for multiphoton fluorescence imaging in vivo," *Science*, vol. 300, pp. 1434-1436, May 2003.
- [149] C. P. Parungo, D. I. Soybel, Y. L. Colson, S. W. Kim, S. Ohnishi, A. M. DeGrand, *et al.*, "Lymphatic drainage of the peritoneal space: A pattern dependent on bowel lymphatics," *Annals of Surgical Oncology*, vol. 14, pp. 286-298, Feb 2007.
- [150] J. D. Smith, G. W. Fisher, A. S. Waggoner, and P. G. Campbell, "The use of quantum dots for analysis of chick CAM vasculature," *Microvascular Research*, vol. 73, pp. 75-83, Mar 2007.
- [151] J. P. Zimmer, S. W. Kim, S. Ohnishi, E. Tanaka, J. V. Frangioni, and M. G. Bawendi, "Size series of small indium arsenide-zinc selenide core-shell nanocrystals and their application to in vivo imaging," *Journal of the American Chemical Society*, vol. 128, pp. 2526-2527, Mar 2006.
- [152] M. Roy, C. J. Niu, Y. H. Chen, P. Z. McVeigh, A. J. Shuhendler, M. K. Leung, *et al.*, "Estimation of Minimum Doses for Optimized Quantum Dot Contrast-Enhanced Vascular Imaging In Vivo," *Small*, vol. 8, pp. 1780-1792, Jun 2012.
- [153] S. H. Bhang, N. Won, T. J. Lee, H. Jin, J. Nam, J. Park, *et al.*, "Hyaluronic Acid-Quantum Dot Conjugates for In Vivo Lymphatic Vessel Imaging," *Acs Nano*, vol. 3, pp. 1389-1398, Jun 2009.
- [154] J. H. Gao, K. Chen, R. Luong, D. M. Bouley, H. Mao, T. C. Qiao, *et al.*, "A Novel Clinically Translatable Fluorescent Nanoparticle for Targeted Molecular Imaging of Tumors in Living Subjects," *Nano Letters*, vol. 12, pp. 281-286, Jan 2012.
- [155] R. Weissleder, C. H. Tung, U. Mahmood, and A. Bogdanov, "In vivo imaging of tumors with protease-activated near-infrared fluorescent probes," *Nature Biotechnology*, vol. 17, pp. 375-378, Apr 1999.
- [156] M. Stroh, J. P. Zimmer, D. G. Duda, T. S. Levchenko, K. S. Cohen, E. B. Brown, *et al.*, "Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo," *Nature Medicine*, vol. 11, pp. 678-682, Jun 2005.
- [157] X. F. Yu, L. D. Chen, K. Y. Li, Y. Li, S. Xiao, X. Luo, *et al.*, "Immunofluorescence detection with quantum dot bioconjugates for hepatoma in vivo," *Journal of Biomedical Optics*, vol. 12, Jan-Feb 2007.
- [158] F. Erogbogbo, K. T. Yong, I. Roy, R. Hu, W. C. Law, W. W. Zhao, *et al.*, "In Vivo Targeted Cancer Imaging, Sentinel Lymph Node Mapping and Multi-Channel Imaging with Biocompatible Silicon Nanocrystals," *Acs Nano*, vol. 5, pp. 413-423, Jan 2011.
- [159] M. Nurunnabi, K. J. Cho, J. S. Choi, K. M. Huh, and Y. H. Lee, "Targeted near-IR QDs-loaded micelles for cancer therapy and imaging," *Biomaterials*, vol. 31, pp. 5436-5444, Jul 2010.
- [160] J. V. Frangioni, "In vivo near-infrared fluorescence imaging," *Current Opinion in Chemical Biology*, vol. 7, pp. 626-634, Oct 2003.

References

- [161] K. T. Yong, R. Hu, I. Roy, H. Ding, L. A. Vathy, E. J. Bergey, *et al.*, "Tumor Targeting and Imaging in Live Animals with Functionalized Semiconductor Quantum Rods," *Acs Applied Materials & Interfaces*, vol. 1, pp. 710-719, Mar 2009.
- [162] K. T. Yong, I. Roy, W. C. Law, and R. Hu, "Synthesis of cRGD-peptide conjugated near-infrared CdTe/ZnSe core-shell quantum dots for in vivo cancer targeting and imaging," *Chemical Communications*, vol. 46, pp. 7136-7138, 2010.
- [163] T. Tanaka, S. Shiramoto, M. Miyashita, Y. Fujishima, and Y. Kaneo, "Tumor targeting based on the effect of enhanced permeability and retention (EPR) and the mechanism of receptor-mediated endocytosis (RME)," *International Journal of Pharmaceutics*, vol. 277, pp. 39-61, Jun 2004.
- [164] S. D. Perrault, C. Walkey, T. Jennings, H. C. Fischer, and W. C. W. Chan, "Mediating Tumor Targeting Efficiency of Nanoparticles Through Design," *Nano Letters*, vol. 9, pp. 1909-1915, May 2009.
- [165] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, and K. Hori, "Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review," *Journal of Controlled Release*, vol. 65, pp. 271-284, Mar 2000.
- [166] K. T. Yong, Y. C. Wang, I. Roy, H. Rui, M. T. Swihart, W. C. Law, *et al.*, "Preparation of Quantum Dot/Drug Nanoparticle Formulations for Traceable Targeted Delivery and Therapy," *Theranostics*, vol. 2, pp. 681-694, 2012.
- [167] H. S. Cho, Z. Y. Dong, G. M. Pauletti, J. M. Zhang, H. Xu, H. C. Gu, *et al.*, "Fluorescent, Superparamagnetic Nanospheres for Drug Storage, Targeting, and Imaging: A Multifunctional Nanocarrier System for Cancer Diagnosis and Treatment," *Acs Nano*, vol. 4, pp. 5398-5404, Sep 2010.
- [168] R. Weissleder, "A clearer vision for in vivo imaging," *Nature Biotechnology*, vol. 19, pp. 316-317, Apr 2001.
- [169] K. T. Yong, I. Roy, H. Ding, E. J. Bergey, and P. N. Prasad, "Biocompatible Near-Infrared Quantum Dots as Ultrasensitive Probes for Long-Term in vivo Imaging Applications," *Small*, vol. 5, pp. 1997-2004, Sep 2009.
- [170] K. T. Yong, "Mn-doped near-infrared quantum dots as multimodal targeted probes for pancreatic cancer imaging," *Nanotechnology*, vol. 20, Jan 2009.
- [171] Z. Khatun, M. Nurunnabi, K. J. Cho, and Y. K. Lee, "Oral Delivery of Near-Infrared Quantum Dot Loaded Micelles for Noninvasive Biomedical Imaging," *Acs Applied Materials & Interfaces*, vol. 4, pp. 3880-3887, Aug 2012.
- [172] M. L. Schipper, G. Iyer, A. L. Koh, Z. Cheng, Y. Ebenstein, A. Aharoni, *et al.*, "Particle Size, Surface Coating, and PEGylation Influence the Biodistribution of Quantum Dots in Living Mice," *Small*, vol. 5, pp. 126-134, Jan 2009.
- [173] C. Wong, T. Stylianopoulos, J. A. Cui, J. Martin, V. P. Chauhan, W. Jiang, *et al.*, "Multistage nanoparticle delivery system for deep penetration into tumor tissue," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, pp. 2426-2431, Feb 2011.
- [174] H. A. Lee, M. Imran, N. A. Monteiro-Riviere, V. L. Colvin, W. W. Yu, and J. E. Riviere, "Biodistribution of quantum dot nanoparticles in perfused skin: Evidence of coating dependency and periodicity in arterial extraction," *Nano Letters*, vol. 7, pp. 2865-2870, Sep 2007.
- [175] T. J. Daou, L. Li, P. Reiss, V. Jossierand, and I. Texier, "Effect of Poly(ethylene glycol) Length on the in Vivo Behavior of Coated Quantum Dots," *Langmuir*, vol. 25, pp. 3040-3044, Mar 2009.
- [176] H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, *et al.*, "Renal clearance of quantum dots," *Nature Biotechnology*, vol. 25, pp. 1165-1170, Oct 2007.
- [177] S. R. Dave and X. Gao, "Monodisperse magnetic nanoparticles for biodetection, imaging, and drug delivery: a versatile and evolving technology," *Wiley*

- Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology*, vol. 1, pp. 583-609, Nov-Dec 2009.
- [178] R. Damadian, "TUMOR DETECTION BY NUCLEAR MAGNETIC RESONANCE," *Science*, vol. 171, pp. 1151-8, 1971 1971.
 - [179] R. Koole, W. J. M. Mulder, M. M. van Schooneveld, G. J. Strijkers, A. Meijerink, and K. Nicolay, "Magnetic quantum dots for multimodal imaging," *Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology*, vol. 1, pp. 475-491, Sep-Oct 2009.
 - [180] A. Louie, "Multimodality Imaging Probes: Design and Challenges," *Chemical Reviews*, vol. 110, pp. 3146-3195, 2010/05/12 2010.
 - [181] S. R. Dave and X. Gao, "Monodisperse magnetic nanoparticles for biodetection, imaging, and drug delivery: a versatile and evolving technology," *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, vol. 1, pp. 583-609, 2009.
 - [182] Z. Zhen and J. Xie, "Development of Manganese-Based Nanoparticles as Contrast Probes for Magnetic Resonance Imaging," *Theranostics*, vol. 2, pp. 45-54, 2012 2012.
 - [183] Y. Wang, R. Hu, G. Lin, I. Roy, and K.-T. Yong, "Functionalized Quantum Dots for Biosensing and Bioimaging and Concerns on Toxicity," *Acs Applied Materials & Interfaces*, vol. 5, pp. 2786-2799, Apr 24 2013.
 - [184] I. L. Medintz, H. T. Uyeda, E. R. Goldman, and H. Mattoussi, "Quantum dot bioconjugates for imaging, labelling and sensing," *Nat Mater*, vol. 4, pp. 435-446, 06//print 2005.
 - [185] D. K. Yi, S. T. Selvan, S. S. Lee, G. C. Papaefthymiou, D. Kundaliya, and J. Y. Ying, "Silica-coated nanocomposites of magnetic nanoparticles and quantum dots," *Journal of the American Chemical Society*, vol. 127, pp. 4990-4991, Apr 13 2005.
 - [186] X. Hong, J. Li, M. J. Wang, J. J. Xu, W. Guo, J. H. Li, *et al.*, "Fabrication of magnetic luminescent nanocomposites by a layer-by-layer self-assembly approach," *Chemistry of Materials*, vol. 16, pp. 4022-4027, Oct 19 2004.
 - [187] N. Gaponik, I. L. Radtchenko, G. B. Sukhorukov, and A. L. Rogach, "Luminescent polymer microcapsules addressable by a magnetic field," *Langmuir*, vol. 20, pp. 1449-1452, Feb 17 2004.
 - [188] J. Kim, J. E. Lee, J. Lee, J. H. Yu, B. C. Kim, K. An, *et al.*, "Magnetic fluorescent delivery vehicle using uniform mesoporous silica spheres embedded with monodisperse magnetic and semiconductor nanocrystals," *Journal of the American Chemical Society*, vol. 128, pp. 688-689, Jan 25 2006.
 - [189] T. R. Sathe, A. Agrawal, and S. Nie, "Mesoporous silica beads embedded with semiconductor quantum dots and iron oxide nanocrystals: Dual-function microcarriers for optical encoding and magnetic separation," *Analytical Chemistry*, vol. 78, pp. 5627-5632, Aug 15 2006.
 - [190] H. Y. Xie, C. Zuo, Y. Liu, Z. L. Zhang, D. W. Pang, X. L. Li, *et al.*, "Cell-targeting multifunctional nanospheres with both fluorescence and magnetism," *Small*, vol. 1, pp. 506-509, May 2005.
 - [191] K. W. Kwon and M. Shim, "gamma-Fe₂O₃/II-VI sulfide nanocrystal heterojunctions," *Journal of the American Chemical Society*, vol. 127, pp. 10269-10275, Jul 27 2005.
 - [192] J. Gao, B. Zhang, Y. Gao, Y. Pan, X. Zhang, and B. Xu, "Fluorescent magnetic nanocrystals by sequential addition of reagents in a one-pot reaction: A simple preparation for multifunctional nanostructures," *Journal of the American Chemical Society*, vol. 129, pp. 11928-11935, Oct 3 2007.
 - [193] H. Kim, M. Achermann, L. P. Balet, J. A. Hollingsworth, and V. I. Klimov, "Synthesis and characterization of Co/CdSe core/shell nanocomposites: Bifunctional magnetic-optical nanocrystals," *Journal of the American Chemical Society*, vol. 127, pp. 544-546, Jan 19 2005.

References

- [194] H. W. Gu, R. K. Zheng, X. X. Zhang, and B. Xu, "Facile one-pot synthesis of bifunctional heterodimers of nanoparticles: A conjugate of quantum dot and magnetic nanoparticles," *Journal of the American Chemical Society*, vol. 126, pp. 5664-5665, May 12 2004.
- [195] H. Yang, S. Santra, G. A. Walter, and P. H. Holloway, "Gd-III-functionalized fluorescent quantum dots as multimodal imaging probes," *Advanced Materials*, vol. 18, pp. 2890-+, Nov 3 2006.
- [196] L. Prinzen, R.-J. J. H. M. Miserus, A. Dirksen, T. M. Hackeng, N. Deckers, N. J. Bitsch, *et al.*, "Optical and magnetic resonance imaging of cell death and platelet activation using annexin A5-functionalized quantum dots," *Nano Letters*, vol. 7, pp. 93-100, Jan 2007.
- [197] J. T. Rosenberg, J. M. Kogot, D. D. Lovingood, G. F. Strouse, and S. C. Grant, "Intracellular Bimodal Nanoparticles Based on Quantum Dots for High-Field MRI at 21.1 T," *Magnetic Resonance in Medicine*, vol. 64, pp. 871-882, Sep 2010.
- [198] V. C. Pierre, M. Botta, and K. N. Raymond, "Dendrimeric gadolinium chelate with fast water exchange and high relaxivity at high magnetic field strength," *Journal of the American Chemical Society*, vol. 127, pp. 504-505, Jan 19 2005.
- [199] J. Lovric, H. S. Bazzi, Y. Cuie, G. R. A. Fortin, F. M. Winnik, and D. Maysinger, "Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots," *Journal of Molecular Medicine-Jmm*, vol. 83, pp. 377-385, May 2005.
- [200] C. Kirchner, T. Liedl, S. Kudera, T. Pellegrino, A. M. Javier, H. E. Gaub, *et al.*, "Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles," *Nano Letters*, vol. 5, pp. 331-338, Feb 2005.
- [201] N. Chen, Y. He, Y. Y. Su, X. M. Li, Q. Huang, H. F. Wang, *et al.*, "The cytotoxicity of cadmium-based quantum dots," *Biomaterials*, vol. 33, pp. 1238-1244, Feb 2012.
- [202] A. Hoshino, K. Hanaki, K. Suzuki, and K. Yamamoto, "Applications of T-lymphoma labeled with fluorescent quantum dots to cell tracing markers in mouse body," *Biochemical and Biophysical Research Communications*, vol. 314, pp. 46-53, Jan 2004.
- [203] A. Hoshino, K. Fujioka, T. Oku, M. Suga, Y. F. Sasaki, T. Ohta, *et al.*, "Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification," *Nano Letters*, vol. 4, pp. 2163-2169, Nov 2004.
- [204] R. Schneider, C. Wolpert, H. Guilloteau, L. Balan, J. Lambert, and C. Merlin, "The exposure of bacteria to CdTe-core quantum dots: the importance of surface chemistry on cytotoxicity," *Nanotechnology*, vol. 20, Jun 2009.
- [205] A. Shiohara, A. Hoshino, K. Hanaki, K. Suzuki, and K. Yamamoto, "On the cyto-toxicity caused by quantum dots," *Microbiology and Immunology*, vol. 48, pp. 669-675, 2004.
- [206] R. F. Domingos, D. F. Simon, C. Hauser, and K. J. Wilkinson, "Bioaccumulation and Effects of CdTe/CdS Quantum Dots on Chlamydomonas reinhardtii - Nanoparticles or the Free Ions?," *Environmental Science & Technology*, vol. 45, pp. 7664-7669, Sep 2011.
- [207] A. Ambrosone, L. Mattera, V. Marchesano, A. Quarta, A. S. Sussha, A. Tino, *et al.*, "Mechanisms underlying toxicity induced by CdTe quantum dots determined in an invertebrate model organism," *Biomaterials*, vol. 33, pp. 1991-2000, Mar 2012.
- [208] T. C. King-Heiden, P. N. Wicinski, A. N. Mangham, K. M. Metz, D. Nesbit, J. A. Pedersen, *et al.*, "Quantum Dot Nanotoxicity Assessment Using the Zebrafish Embryo," *Environmental Science & Technology*, vol. 43, pp. 1605-1611, Mar 2009.
- [209] Y. Y. Su, M. Hu, C. H. Fan, Y. He, Q. N. Li, W. X. Li, *et al.*, "The cytotoxicity of CdTe quantum dots and the relative contributions from released cadmium ions and nanoparticle properties," *Biomaterials*, vol. 31, pp. 4829-4834, Jun 2010.

References

- [210] M. Green and E. Howman, "Semiconductor quantum dots and free radical induced DNA nicking," *Chemical Communications*, pp. 121-123, 2005.
- [211] B. I. Ipe, M. Lehnig, and C. M. Niemeyer, "On the generation of free radical species from quantum dots," *Small*, vol. 1, pp. 706-709, Jul 2005.
- [212] J. Lovric, S. J. Cho, F. M. Winnik, and D. Maysinger, "Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death," *Chemistry & Biology*, vol. 12, pp. 1227-1234, Nov 2005.
- [213] P. Juzenas, R. Generalov, A. Juzeniene, and J. Moan, "Generation of Nitrogen Oxide and Oxygen Radicals by Quantum Dots," *Journal of Biomedical Nanotechnology*, vol. 4, pp. 450-456, Dec 2008.
- [214] A. Anas, H. Akita, H. Harashima, T. Itoh, M. Ishikawa, and V. Biju, "Photosensitized breakage and damage of DNA by CdSe-ZnS quantum dots," *Journal of Physical Chemistry B*, vol. 112, pp. 10005-10011, Aug 2008.
- [215] S. J. Cho, D. Maysinger, M. Jain, B. Roder, S. Hackbarth, and F. M. Winnik, "Long-term exposure to CdTe quantum dots causes functional impairments in live cells," *Langmuir*, vol. 23, pp. 1974-1980, Feb 2007.
- [216] K. G. Li, J. T. Chen, S. S. Bai, X. Wen, S. Y. Song, Q. Yu, *et al.*, "Intracellular oxidative stress and cadmium ions release induce cytotoxicity of unmodified cadmium sulfide quantum dots," *Toxicology in Vitro*, vol. 23, pp. 1007-1013, Sep 2009.
- [217] P. Juzenas, W. Chen, Y. P. Sun, M. A. N. Coelho, R. Generalov, N. Generalova, *et al.*, "Quantum dots and nanoparticles for photodynamic and radiation therapies of cancer," *Advanced Drug Delivery Reviews*, vol. 60, pp. 1600-1614, Dec 2008.
- [218] A. C. S. Samia, X. B. Chen, and C. Burda, "Semiconductor quantum dots for photodynamic therapy," *Journal of the American Chemical Society*, vol. 125, pp. 15736-15737, Dec 2003.
- [219] R. Bakalova, H. Ohba, Z. Zhelev, M. Ishikawa, and Y. Baba, "Quantum dots as photosensitizers?," *Nature Biotechnology*, vol. 22, pp. 1360-1361, Nov 2004.
- [220] T. T. Zhang, J. L. Stilwell, D. Gerion, L. H. Ding, O. Elboudwarej, P. A. Cooke, *et al.*, "Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics measurements," *Nano Letters*, vol. 6, pp. 800-808, Apr 2006.
- [221] D. J. Bharali, D. W. Lucey, H. Jayakumar, H. E. Pudavar, and P. N. Prasad, "Folate-receptor-mediated delivery of InP quantum dots for bioimaging using confocal and two-photon microscopy," *Journal of the American Chemical Society*, vol. 127, pp. 11364-11371, Aug 2005.
- [222] G. J. Stasiuk, S. Tamang, D. Imbert, C. Poillot, M. Giardiello, C. Tisseyre, *et al.*, "Cell-Permeable Ln(III) Chelate-Functionalized InP Quantum Dots As Multimodal Imaging Agents," *Acs Nano*, vol. 5, pp. 8193-8201, Oct 2011.
- [223] N. Pradhan, D. Goorskey, J. Thessing, and X. G. Peng, "An alternative of CdSe nanocrystal emitters: Pure and tunable impurity emissions in ZnSe nanocrystals," *Journal of the American Chemical Society*, vol. 127, pp. 17586-17587, Dec 21 2005.
- [224] N. Pradhan, D. M. Battaglia, Y. C. Liu, and X. G. Peng, "Efficient, stable, small, and water-soluble doped ZnSe nanocrystal emitters as non-cadmium biomedical labels," *Nano Letters*, vol. 7, pp. 312-317, Feb 2007.
- [225] M. E. Mathew, J. C. Mohan, K. Manzoor, S. V. Nair, H. Tamura, and R. Jayakumar, "Folate conjugated carboxymethyl chitosan-manganese doped zinc sulphide nanoparticles for targeted drug delivery and imaging of cancer cells," *Carbohydrate Polymers*, vol. 80, pp. 442-448, Apr 2010.

References

- [226] K. Manzoor, S. Johny, D. Thomas, S. Setua, D. Menon, and S. Nair, "Bio-conjugated luminescent quantum dots of doped ZnS: a cyto-friendly system for targeted cancer imaging," *Nanotechnology*, vol. 20, Feb 2009.
- [227] Z. F. Li and E. Ruckenstein, "Water-soluble poly(acrylic acid) grafted luminescent silicon nanoparticles and their use as fluorescent biological staining labels," *Nano Letters*, vol. 4, pp. 1463-1467, Aug 2004.
- [228] F. Erogbogbo, K. T. Yong, I. Roy, G. X. Xu, P. N. Prasad, and M. T. Swihart, "Biocompatible luminescent silicon quantum dots for imaging of cancer cells," *Acs Nano*, vol. 2, pp. 873-878, May 2008.
- [229] F. Erogbogbo, K. T. Yong, R. Hu, W. C. Law, H. Ding, C. W. Chang, *et al.*, "Biocompatible Magnetofluorescent Probes: Luminescent Silicon Quantum Dots Coupled with Superparamagnetic Iron(III) Oxide," *Acs Nano*, vol. 4, pp. 5131-5138, Sep 2010.
- [230] M. Helle, E. Cassette, L. Bezdetnaya, T. Pons, A. Leroux, F. Plenat, *et al.*, "Visualisation of Sentinel Lymph Node with Indium-Based near Infrared Emitting Quantum Dots in a Murine Metastatic Breast Cancer Model," *Plos One*, vol. 7, Aug 2012.
- [231] K. C. Cheng, W. C. Law, K. T. Yong, J. S. Nevins, D. F. Watson, H. P. Ho, *et al.*, "Synthesis of near-infrared silver-indium-sulfide (AgInS₂) quantum dots as heavy-metal free photosensitizer for solar cell applications," *Chemical Physics Letters*, vol. 515, pp. 254-257, Oct 2011.
- [232] E. Cassette, T. Pons, C. Bouet, M. Helle, L. Bezdetnaya, F. Marchal, *et al.*, "Synthesis and Characterization of Near-Infrared Cu-In-Se/ZnS Core/Shell Quantum Dots for In vivo Imaging," *Chemistry of Materials*, vol. 22, pp. 6117-6124, Nov 2010.
- [233] Y. G. Zheng, S. J. Gao, and J. Y. Ying, "Synthesis and cell-imaging applications of glutathione-capped CdTe quantum dots," *Advanced Materials*, vol. 19, pp. 376-+, Feb 2007.
- [234] R. G. Xie, U. Kolb, J. X. Li, T. Basche, and A. Mews, "Synthesis and characterization of highly luminescent CdSe-Core CdS/Zn_{0.5}Cd_{0.5}S/ZnS multishell nanocrystals," *Journal of the American Chemical Society*, vol. 127, pp. 7480-7488, May 25 2005.
- [235] T. Fang, K. G. Ma, L. L. Ma, J. Y. Bai, X. Li, H. H. Song, *et al.*, "3-Mercaptobutyric Acid as an Effective Capping Agent for Highly Luminescent CdTe Quantum Dots: New Insight into the Selection of Mercapto Acids," *Journal of Physical Chemistry C*, vol. 116, pp. 12346-12352, Jun 2012.
- [236] N. N. Mamedova, N. A. Kotov, A. L. Rogach, and J. Studer, "Albumin-CdTe nanoparticle bioconjugates: Preparation, structure, and interunit energy transfer with antenna effect," *Nano Letters*, vol. 1, pp. 281-286, Jun 2001.
- [237] W. C. Law, K. T. Yong, I. Roy, H. Ding, R. Hu, W. W. Zhao, *et al.*, "Aqueous-Phase Synthesis of Highly Luminescent CdTe/ZnTe Core/Shell Quantum Dots Optimized for Targeted Bioimaging," *Small*, vol. 5, pp. 1302-1310, Jun 2009.
- [238] H. Zhang, P. Sun, C. Liu, H. Gao, L. Xu, J. Fang, *et al.*, "L-Cysteine capped CdTe-CdS core-shell quantum dots: preparation, characterization and immuno-labeling of HeLa cells," *Luminescence*, vol. 26, pp. 86-92, Mar-Apr 2011.
- [239] X. D. Cao, C. M. Li, H. F. Bao, Q. L. Bao, and H. Dong, "Fabrication of strongly fluorescent quantum dot-polymer composite in aqueous solution," *Chemistry of Materials*, vol. 19, pp. 3773-3779, Jul 2007.
- [240] U. K. Gautam and C. N. R. Rao, "Controlled synthesis of crystalline tellurium nanorods, nanowires, nanobelts and related structures by a self-seeding solution process," *Journal of Materials Chemistry*, vol. 14, pp. 2530-2535, 2004.

References

- [241] A. L. Rogach, T. Franzl, T. A. Klar, J. Feldmann, N. Gaponik, V. Lesnyak, *et al.*, "Aqueous synthesis of thiol-capped CdTe nanocrystals: State-of-the-art," *Journal of Physical Chemistry C*, vol. 111, pp. 14628-14637, Oct 2007.
- [242] M. Grabolle, P. Kapusta, T. Nann, X. Shu, J. Ziegler, and U. Resch-Genger, "Fluorescence Lifetime Multiplexing with Nanocrystals and Organic Labels," *Analytical Chemistry*, vol. 81, pp. 7807-7813, Sep 2009.
- [243] F. Meinardi, A. Colombo, K. A. Velizhanin, R. Simonutti, M. Lorenzon, L. Beverina, *et al.*, "Large-area luminescent solar concentrators based on /^{Stokes-shift-engineered}/ nanocrystals in a mass-polymerized PMMA matrix," *Nat Photon*, vol. 8, pp. 392-399, 05//print 2014.
- [244] M. A. Vairavamurthy, W. S. Goldenberg, O. Y. Shi, and S. Khalid, "The interaction of hydrophilic thiols with cadmium: investigation with a simple model, 3-mercaptopropionic acid," *Marine Chemistry*, vol. 70, pp. 181-189, May 2000.
- [245] V. Swayambunathan, D. Hayes, K. H. Schmidt, Y. X. Liao, and D. Meisel, "THIOL SURFACE COMPLEXATION ON GROWING CDS CLUSTERS," *Journal of the American Chemical Society*, vol. 112, pp. 3831-3837, May 1990.
- [246] L. Zou, Z. Y. Gu, N. Zhang, Y. L. Zhang, Z. Fang, W. H. Zhu, *et al.*, "Ultrafast synthesis of highly luminescent green- to near infrared-emitting CdTe nanocrystals in aqueous phase," *Journal of Materials Chemistry*, vol. 18, pp. 2807-2815, 2008.
- [247] W. W. Yu, Y. A. Wang, and X. Peng, "Formation and Stability of Size-, Shape-, and Structure-Controlled CdTe Nanocrystals: Ligand Effects on Monomers and Nanocrystals," *Chemistry of Materials*, vol. 15, pp. 4300-4308, 2003/11/01 2003.
- [248] D. Battaglia and X. G. Peng, "Formation of high quality InP and InAs nanocrystals in a noncoordinating solvent," *Nano Letters*, vol. 2, pp. 1027-1030, Sep 2002.
- [249] A. P. Alivisatos, A. L. Harris, N. J. Levinos, M. L. Steigerwald, and L. E. Brus, "ELECTRONIC STATES OF SEMICONDUCTOR CLUSTERS - HOMOGENEOUS AND INHOMOGENEOUS BROADENING OF THE OPTICAL-SPECTRUM," *Journal of Chemical Physics*, vol. 89, pp. 4001-4011, Oct 1988.
- [250] J. N. Tian, R. J. Liu, Y. C. Zhao, Q. Xu, and S. L. Zhao, "Controllable synthesis and cell-imaging studies on CdTe quantum dots together capped by glutathione and thioglycolic acid," *Journal of Colloid and Interface Science*, vol. 336, pp. 504-509, Aug 2009.
- [251] R. Hardman, "A toxicologic review of quantum dots: Toxicity depends on physicochemical and environmental factors," *Environmental Health Perspectives*, vol. 114, pp. 165-172, Feb 2006.
- [252] L. Ye, K.-T. Yong, L. Liu, I. Roy, R. Hu, J. Zhu, *et al.*, "A pilot study in non-human primates shows no adverse response to intravenous injection of quantum dots," *Nat Nano*, vol. advance online publication, 2012.
- [253] R. N. Bhargava, D. Gallagher, X. Hong, and A. Nurmikko, "OPTICAL-PROPERTIES OF MANGANESE-DOPED NANOCRYSTALS OF ZNS," *Physical Review Letters*, vol. 72, pp. 416-419, Jan 17 1994.
- [254] D. J. Norris, N. Yao, F. T. Charnock, and T. A. Kennedy, "High-quality manganese-doped ZnSe nanocrystals," *Nano Letters*, vol. 1, pp. 3-7, Jan 2001.
- [255] X. Yuan, J. Zheng, R. Zeng, P. Jing, W. Ji, J. Zhao, *et al.*, "Thermal stability of Mn²⁺ ion luminescence in Mn-doped core-shell quantum dots," *Nanoscale*, vol. 6, pp. 300-307, 2014 2014.
- [256] D. Zhu, Y. Chen, L. Jiang, J. Geng, J. Zhang, and J.-J. Zhu, "Manganese-Doped ZnSe Quantum Dots as a Probe for Time-Resolved Fluorescence Detection of 5-Fluorouracil," *Analytical Chemistry*, vol. 83, pp. 9076-9081, 2011/12/01 2011.

References

- [257] N. Pradhan, D. M. Battaglia, Y. Liu, and X. Peng, "Efficient, stable, small, and water-soluble doped ZnSe nanocrystal emitters as non-cadmium biomedical labels," *Nano Letters*, vol. 7, pp. 312-317, Feb 2007.
- [258] K.-T. Yong, "Mn-doped near-infrared quantum dots as multimodal targeted probes for pancreatic cancer imaging," *Nanotechnology*, vol. 20, Jan 7 2009.
- [259] S. Santra, H. S. Yang, P. H. Holloway, J. T. Stanley, and R. A. Mericle, "Synthesis of water-dispersible fluorescent, radio-opaque, and paramagnetic CdS : Mn/ZnS quantum dots: A multifunctional probe for bioimaging," *Journal of the American Chemical Society*, vol. 127, pp. 1656-1657, Feb 16 2005.
- [260] E. Sotelo-Gonzalez, L. Rocas, S. Garcia-Granda, M. T. Fernandez-Arguelles, J. M. Costa-Fernandez, and A. Sanz-Medel, "Influence of Mn²⁺ concentration on Mn²⁺-doped ZnS quantum dot synthesis: evaluation of the structural and photoluminescent properties," *Nanoscale*, vol. 5, pp. 9156-9161, 2013 2013.
- [261] A. Nag, S. Chakraborty, and D. D. Sarma, "To dope Mn²⁺ in a semiconducting nanocrystal," *Journal of the American Chemical Society*, vol. 130, pp. 10605-10611, Aug 13 2008.
- [262] S. Wang, B. R. Jarrett, S. M. Kauzlarich, and A. Y. Louie, "Core/shell quantum dots with high relaxivity and photoluminescence for multimodality imaging," *Journal of the American Chemical Society*, vol. 129, pp. 3848-3856, Apr 4 2007.
- [263] L. Jing, K. Ding, S. Kalytchuk, Y. Wang, R. Qiao, S. V. Kershaw, *et al.*, "Aqueous Manganese-Doped Core/Shell CdTe/ZnS Quantum Dots with Strong Fluorescence and High Relaxivity," *Journal of Physical Chemistry C*, vol. 117, pp. 18752-18761, Sep 12 2013.
- [264] B. Dong, L. Cao, G. Su, and W. Liu, "Facile synthesis of highly luminescent UV-blue emitting ZnSe/ZnS core/shell quantum dots by a two-step method," *Chemical Communications*, vol. 46, pp. 7331-7333, 2010 2010.
- [265] P. Shao, Q. Zhang, Y. Li, and H. Wang, "Aqueous synthesis of color-tunable and stable Mn²⁺-doped ZnSe quantum dots," *Journal of Materials Chemistry*, vol. 21, pp. 151-156, 2011 2011.
- [266] P. K. Jain, B. J. Beberwyck, L.-K. Fong, M. J. Polking, and A. P. Alivisatos, "Highly Luminescent Nanocrystals From Removal of Impurity Atoms Residual From Ion-Exchange Synthesis," *Angewandte Chemie-International Edition*, vol. 51, pp. 2387-2390, 2012 2012.
- [267] A. A. Bol and A. Meijerink, "Luminescence quantum efficiency of nanocrystalline ZnS : Mn²⁺. 1. Surface passivation and Mn²⁺ concentration," *Journal of Physical Chemistry B*, vol. 105, pp. 10197-10202, Oct 25 2001.
- [268] H.-Y. Chen, S. Maiti, and D. H. Son, "Doping Location-Dependent Energy Transfer Dynamics in Mn-Doped CdS/ZnS Nanocrystals," *Acs Nano*, vol. 6, pp. 583-591, Jan 2012.
- [269] H.-Y. Chen, T.-Y. Chen, and D. H. Son, "Measurement of Energy Transfer Time in Colloidal Mn-Doped Semiconductor Nanocrystals," *Journal of Physical Chemistry C*, vol. 114, pp. 4418-4423, Mar 18 2010.
- [270] M. Katiyar and A. H. Kitai, "LUMINESCENCE CONCENTRATION QUENCHING DUE TO ENERGY MIGRATION IN ZNS-MN WITH FIXED TRAP DENSITY," *Journal of Luminescence*, vol. 46, pp. 227-234, May 1990.
- [271] B. C. G. G. Blasse, "Luminescent Materials," *Springer-Verlag: Berlin*, 1994.
- [272] F. Aldeek, L. Balan, G. Medjahdi, T. Roques-Carmes, J.-P. Malval, C. Mustin, *et al.*, "Enhanced Optical Properties of Core/Shell/Shell CdTe/CdS/ZnO Quantum Dots Prepared in Aqueous Solution," *Journal of Physical Chemistry C*, vol. 113, pp. 19458-19467, Nov 12 2009.

References

- [273] H.-Y. Chen and D. H. Son, "Energy and Charge Transfer Dynamics in Doped Semiconductor Nanocrystals," *Israel Journal of Chemistry*, vol. 52, pp. 1016-1026, Dec 2012.
- [274] A. Vincent, J. Herman, R. Schulick, R. H. Hruban, and M. Goggins, "Pancreatic cancer," *Lancet*, vol. 378, pp. 607-620, Aug 2011.
- [275] S. M. Elbashir, J. Harborth, W. Lendeckel, A. Yalcin, K. Weber, and T. Tuschl, "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells," *Nature*, vol. 411, pp. 494-498, May 2001.
- [276] A. de Fougères, H. P. Vornlocher, J. Maraganore, and J. Lieberman, "Interfering with disease: a progress report on siRNA-based therapeutics," *Nature Reviews Drug Discovery*, vol. 6, pp. 443-453, Jun 2007.
- [277] K. A. Whitehead, R. Langer, and D. G. Anderson, "Knocking down barriers: advances in siRNA delivery," *Nature Reviews Drug Discovery*, vol. 8, pp. 129-138, Feb 2009.
- [278] R. L. Juliano, "Peptide-oligonucleotide conjugates for the delivery of antisense and siRNA," *Current Opinion in Molecular Therapeutics*, vol. 7, pp. 132-136, Apr 2005.
- [279] W. J. Song, J. Z. Du, T. M. Sun, P. Z. Zhang, and J. Wang, "Gold Nanoparticles Capped with Polyethyleneimine for Enhanced siRNA Delivery," *Small*, vol. 6, pp. 239-246, Jan 2010.
- [280] E. Y. Zhao, Z. X. Zhao, J. C. Wang, C. H. Yang, C. J. Chen, L. Y. Gao, *et al.*, "Surface engineering of gold nanoparticles for in vitro siRNA delivery," *Nanoscale*, vol. 4, pp. 5102-5109, 2012.
- [281] W. J. Li and F. C. Szoka, "Lipid-based nanoparticles for nucleic acid delivery," *Pharmaceutical Research*, vol. 24, pp. 438-449, Mar 2007.
- [282] V. P. Torchilin, "Recent approaches to intracellular delivery of drugs and DNA and organelle targeting," in *Annual Review of Biomedical Engineering*, vol. 8, ed, 2006, pp. 343-375.
- [283] J. M. Lee, T. J. Yoon, and Y. S. Cho, "Recent Developments in Nanoparticle-Based siRNA Delivery for Cancer Therapy," *Biomed Research International*, 2013.
- [284] A. Kwok, G. A. Eggimann, J. L. Reymond, T. Darbre, and F. Hollfelder, "Peptide Dendrimer/Lipid Hybrid Systems Are Efficient DNA Transfection Reagents: Structure-Activity Relationships Highlight the Role of Charge Distribution Across Dendrimer Generations," *Acs Nano*, vol. 7, pp. 4668-4682, May 2013.
- [285] B. Pan, D. Cui, P. Xu, C. Ozkan, G. Feng, M. Ozkan, *et al.*, "Synthesis and characterization of polyamidoamine dendrimer-coated multi-walled carbon nanotubes and their application in gene delivery systems," *Nanotechnology*, vol. 20, Mar 25 2009.
- [286] T. Kim and T. Hyeon, "Applications of inorganic nanoparticles as therapeutic agents," *Nanotechnology*, vol. 25, Jan 10 2014.
- [287] S. B. Hartono, M. Yu, W. Gu, J. Yang, E. Strounina, X. Wang, *et al.*, "Synthesis of multi-functional large pore mesoporous silica nanoparticles as gene carriers," *Nanotechnology*, vol. 25, Feb 7 2014.
- [288] M. S. Muthu, D. T. Leong, L. Mei, and S.-S. Feng, "Nanotheranostics - Application and Further Development of Nanomedicine Strategies for Advanced Theranostics," *Theranostics*, vol. 4, pp. 660-677, 2014 2014.
- [289] D. Zhu, X. Jiang, C. Zhao, X. Sun, J. Zhang, and J.-J. Zhu, "Green synthesis and potential application of low-toxic Mn : ZnSe/ZnS core/shell luminescent nanocrystals," *Chemical Communications*, vol. 46, pp. 5226-5228, 2010.
- [290] S. Guerrero, I. Casanova, L. Farre, A. Mazo, G. Capella, and R. Mangués, "K-ras codon 12 mutation induces higher level of resistance to apoptosis and predisposition to anchorage-independent growth than codon 13 mutation or proto-oncogene overexpression," *Cancer Research*, vol. 60, pp. 6750-6756, Dec 2000.

References

- [291] J. B. Fleming, G. L. Shen, S. E. Holloway, M. Davis, and R. A. Brekken, "Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: Justification for K-ras-directed therapy," *Molecular Cancer Research*, vol. 3, pp. 413-423, Jul 2005.
- [292] T. R. Brummelkamp, R. Bernards, and R. Agami, "Stable suppression of tumorigenicity by virus-mediated RNA interference," *Cancer Cell*, vol. 2, pp. 243-247, Sep 2002.
- [293] J. M. Li, M. X. Zhao, H. Su, Y. Y. Wang, C. P. Tan, L. N. Ji, *et al.*, "Multifunctional quantum-dot-based siRNA delivery for HPV18 E6 gene silence and intracellular imaging," *Biomaterials*, vol. 32, pp. 7978-7987, Nov 2011.
- [294] Z.-G. Yue, W. Wei, P.-P. Lv, H. Yue, L.-Y. Wang, Z.-G. Su, *et al.*, "Surface Charge Affects Cellular Uptake and Intracellular Trafficking of Chitosan-Based Nanoparticles," *Biomacromolecules*, vol. 12, pp. 2440-2446, Jul 2011.
- [295] H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin, and D. G. Anderson, "Non-viral vectors for gene-based therapy," *Nat Rev Genet*, vol. 15, pp. 541-555, 08//print 2014.
- [296] S. Ip, C. M. MacLaughlin, N. Gunari, and G. C. Walker, "Phospholipid Membrane Encapsulation of Nanoparticles for Surface-Enhanced Raman Scattering," *Langmuir*, vol. 27, pp. 7024-7033, Jun 7 2011.
- [297] H. Elnakat and M. Ratnam, "Distribution, functionality and gene regulation of folate receptor isoforms: implications in targeted therapy," *Advanced Drug Delivery Reviews*, vol. 56, pp. 1067-1084, 4/29/ 2004.
- [298] K. Watanabe, M. Kaneko, and Y. Maitani, "Functional coating of liposomes using a folate-polymer conjugate to target folate receptors," *International Journal of Nanomedicine*, vol. 7, pp. 3679-3688, 2012.

LIST OF FIGURES

Fig.2.1 (A) Schematic illustration of the density of states in metal and semiconductor clusters. (B) Density of states in one band of a semiconductor as a function of dimension[10].	7
Fig.2.2 Schematic illustration of the energy band alignment of Type I and Type II core/shell QDs.	9
Fig.2.3 Strain-tuning of the band alignment and optical properties of CdTe/ZnSe heterostructures[15].	10
Fig.2.4 ‘Focusing’ and ‘defocusing’ growth modes of QDs, and growth rate determined by particle size and concentration of the monomer present[23].	12
Fig.2.5 Schematic representation of intracellular c-Myc mRNA detection based on the wireless ECL biosensor. CdSe@ZnS QD-DNA conjugates provided an intracellular hybridization process that correlated with the relative levels of intracellular c-Myc mRNA[91].	21
Fig.2.6 TNT titration of the QD-TNB2-45 nanosensor assembly. A) Schematic of the assay. Addition of TNT displaces the quencher TNB-BHQ10 and thus the QD recovers its emission. The data resulting from the increase in QD PL are plotted as the difference signal versus concentration, both in B) linear and C) logarithmic scales[104].	23
Fig.2.7 Tracking of QD-ND/EB1 on spindle structures following incubation of the QDs ($\lambda=545\text{nm}$) with cell extracts. (a) Rhodamine-labeled spindle structure (red channel) with QD-ND/EB1 (green channel) and the overlay of the two channels (shown in yellow). (b) Temporal image sequence (5s/frame) of a single QD-ND/EB1 moving on a microtubule (see arrow). (c) A switch between a fast and slow movement. (d) Mean velocity histogram extracted from a collection of individual QD-ND/EB1 moving on the spindle structures (3 independent experiments, 3 structures)[127].	27
Fig.2.8 Images of the surgical field in a pig injected intradermally with 400 pmol of NIR QDs in the right groin. Top to bottom: before injection (autofluorescence), 30 s after injection, 4 min after injection and during image-guided resection. Color video, NIR fluorescence and color-NIR merge images are shown from left to right[145].	29
Fig.2.9 The NIR window is ideally suited for in vivo imaging because of minimal light absorption[168].	31
Fig.2.10 Blood half-life (hours) of GNPs/mPEG as a function of particle size (nm) and mPEG molecular weight (kDa). Half-life generally improved as particle diameter decreased and mPEG molecular weight increased[164].	32
Fig.2.11 Left: Schematic depiction of a multistage nanoparticle drug delivery system. The initial 100-nm multistage nanoparticle delivery system accumulates preferentially around leaky vessels in tumor tissue. By cleaving away the gelatin scaffold with MMP-2, 100-nm QDGeINPs change size to 10-nm QD NPs, which can deeply penetrate the dense collagen matrix of the interstitial space. Right: <i>In vivo</i> images of QDGeINPs and silica QDs (diameter = 105 nm) after intratumoral coinjection into the HT-1080 tumor. (Scale bar: 100 μm)[173].	32
Fig.2.12 Renal clearance of QD710-Dendron. (A) Fluorescence imaging of mice ventral before and after tail-vein injection of QD710-Dendron (200 pmol) or PBS at 30, 60, and 120 min, respectively. (B) The fluorescence imaging of urine samples collected after 90 min and the urine from the mice injected with QD710-Dendron had a strong fluorescent signal. (C) UV-vis absorption and (D) Fluorescence emission spectra of the urine samples show the characteristic spectra of QD710-Dendron[154].	34
Fig.3.1 (a) Photograph of QDs under UV irradiation. (b) UV-vis absorption spectra and (c) PL emission spectra of the QDs prepared with different reaction time. (d) Time evolution of the Abs and PL peak wavelengths of the QDs.	46

List of Figures

Fig.3.2 Time evolution profiles of the emission peak wavelengths of MPA-capped (A) and Cys-capped (B) CdTe QDs. Surfactant/Cd ratio = 1.3, 1.6, 1.9, 2.2 or 2.5	48
Fig.3.3 Three-dimensional plot of the data in Fig.3.2, showing the emission peak wavelength versus reaction time and surfactant/Cd molar ratio.	48
Fig.3.4 Emission peak wavelength obtained for QDs fabricated after 8 hours of reaction versus thiols/Cd molar ratio.....	49
Fig.3.5 Quantum yield versus emission wavelength peak of: a) MPA- and b) Cys-capped CdTe QDs. Surfactant/Cd = 1.3, 1.6, 1.9, 2.2 or 2.5.	51
Fig.3.6 Emission bandwidth of QDs prepared with different surfactant concentrations and species. Generally, using cysteine as stabilizer leads to smaller FWHM (full width at half maximum) compared with MPA.	52
Fig.3.7 Time evolution of the peak emission wavelength of QDs when Cys and MPA are employed in combination with different ratios. Surfactant/Cd = 2.2 (A) or 1.6 (B).	54
Fig.3.8 (a) Quantum yield of QDs versus emission peak wavelength. Surfactant/Cd = 1.6. (b) Bandwidth of QDs emission versus emission peak wavelengths. Surfactant/Cd = 2.2.	55
Fig.3.9 Photostability measurement of QDs capped with mixed stabilizing ligands. Surfactant/Cd=1.6. The samples are irradiated by UV light (350nm) with power of 10W. Photoluminescent (PL) intensity of the samples is measured at different time points.	56
Fig.3.10 Cell viability of Panc-1 cells treated with CdTe QDs with different concentrations for 24 hours.	57
Fig.4.1 Illustration of the preparation process for: (a) Mn-doped ZnSe/ZnS QDs, and (b) sandwiched QDs (SQDs).....	66
Fig.4.2 (a) UV-vis absorption, and (b) PL emission of the <i>d</i> -dots obtained at different refluxing time (nucleation temperature = 100°C).....	66
Fig.4.3 Energy band structure of Mn-doped ZnSe QDs and the recombination pathways of the photo-excited excitons.	67
Fig.4.4 Time-resolved PL decay of 590 nm-emitting <i>d</i> -dots. τ is estimated to be 0.815 ms. .	68
Fig.4.5 PL emission of the <i>d</i> -dots obtained at a nucleation temperature of 80°C.	68
Fig.4.6 (a) PL spectra and (b) QY of <i>d</i> -dots prepared with different Mn doping levels.	69
Fig.4.7 (a) TEM images of the <i>d</i> -dot (S_0) and the <i>d</i> -dot/ZnS (S_{1-3}) with varying shell thickness. (b) Electron diffraction patterns of S_0 and S_3 . (c) Size histograms of the sample S_{0-3} shown in (a).	71
Fig.4.8 (a) UV-vis and PL spectra of the <i>d</i> -dots prepared with different shell thickness ($S_0=0$ nm, $S_1=0.25$ nm, $S_2=0.55$ nm, $S_3=0.85$ nm, $S_4=1.14$ nm); (b) Revolution of QY against the shell thickness.....	72
Fig.4.9 TEM images and size histograms of SQD5(left) and SQD30(right).	74
Fig.4.10 Longitudinal relaxation rate (R_l) plotted against QD concentration.	75
Fig.4.11 PL spectra of ZnSe QD capped with ZnMnS shell.	76
Fig.4.12 PL spectra of SQDs prepared with different transition-shell thickness.	77
Fig.4.13 Energy structure illustration of the doped Type II core/shell SQD.	77
Fig.4.14 (a) T_l -weighted MR images and photograph of <i>d</i> -dots, <i>d</i> -dot/ZnS, SQD _{5%} and SQD _{30%} . (b) T_l -weighted MR images and photographs of SQD _{30%} with varying solution concentrations. The photos show the PL emission under UV excitation at 350nm with a power of 10 W.....	78
Fig.5.1 (a) Schematic illustration of the preparation steps of the <i>d</i> -dot/polymer nanoplex-based siRNA vectors. (b) TEM images of <i>d</i> -dots and <i>d</i> -dots/PAH nanoplexes.	90
Fig.5.2 (a) Hydrodynamic size, and (b) Surface zeta potential of the <i>d</i> -dot/PAH and <i>d</i> -dot/PEI clusters prepared using polymer solution of different concentrations.....	90
Fig.5.3 Agarose gel electrophoresis examining the binding between <i>d</i> -dot/PAH clusters and siRNAs. (I) <i>d</i> -dot/PAH _{0.1} (+31 mV) only, (II) free siRNA only, and siRNA mixed with (III)	

MPA stabilized <i>d</i> -dot (-17 mV), (IV) <i>d</i> -dot/PAH _{0.05} (+24 mV) or (V) <i>d</i> -dot/PAH _{0.1} (+31 mV).	91
Fig.5.4 Monitoring the change in particle hydrodynamic size and zeta potential during the LBL construction steps to prepare <i>d</i> -dot/PAH _{0.1} /siRNA ^{FAM} /PAH nanoplexes.	92
Fig.5.5 (a) Hydrodynamic size distribution of <i>d</i> -dot/PAH _{0.1} /siRNA ^{FAM} /PAH nanoplexes measured by DLS. (b) Colloidal stability of <i>d</i> -dot/PAH _{0.1} /siRNA ^{FAM} /PAH nanoplexes dispersed in DEPC treated water at 25°C. The hydrodynamic size of the complexes was monitored over a period of 2 weeks.	92
Fig.5.6 Photoluminescence (PL) spectra of <i>d</i> -dot/PAH, free siRNA ^{FAM} and <i>d</i> -dot/PAH/siRNA ^{FAM} nanoplexes excited by UV ($\lambda=350$ nm) or blue ($\lambda=450$ nm) light sources. Real image of <i>d</i> -dot/PAH/siRNA ^{FAM} under different excitations are shown in inset pictures.	93
Fig.5.7 Photostability comparison between <i>d</i> -dots and FAM fluorophores. Panc-1 cells transfected with <i>d</i> -dot/PAH-siRNA ^{FAM} under continuous irradiation by a mercury light source. Fluorescent images of the cells were taken at different time intervals. Evolution of the relative PL intensity of the <i>d</i> -dot and FAM signals were plotted.	93
Fig.5.8 (a) Fluorescent image of Panc-1 cells treated with: (i) free siRNA ^{FAM} , (ii) Oligo-siRNA ^{FAM} , (iii) <i>d</i> -dot/PAH-siRNA ^{FAM} and (iv) <i>d</i> -dot/PEI-siRNA ^{FAM} for 4 hours. (b) After 4 hours transfection with (v) Oligo-siRNA ^{FAM} , (vi) <i>d</i> -dot/PAH-siRNA ^{FAM} , and (vii) <i>d</i> -dot/PEI-siRNA ^{FAM} , the Panc-1 cells were further incubated in DMEM, and the images were taken at 72 hours post-transfection.	96
Fig.5.9 Transfection efficiency of Panc-1 cells determined by flow cytometry analysis. (A) Representative pictures, where cells were treated with (i) blank, (ii) free siRNA ^{FAM} , (iii) <i>d</i> -dot/PAH, (iv) Oligo-siRNA ^{FAM} (v) <i>d</i> -dot/PAH-siRNA ^{FAM} and (vi) <i>d</i> -dot/PEI-siRNA ^{FAM} for 4 hours. (vii), (viii) and (ix) were collected at 72hours post-transfection, corresponding to (iv), (v) and (vi), respectively. (B) Percentage of cells transfected after 4 h treatment, evaluated from experiments shown in (A). The shown points are average values $\pm$ SD. *, P-value < 0.005 vs blank, siRNA and d-dots. (C) Average FAM fluorescence intensity of the treated cells (4- and 72 hours post-transfection) counted from experiments shown in (A).	97
Fig.5.10 K-Ras mRNA relative expression levels in Panc-1 cells detected by real-time RT-PCR: (a) Blank, (b) Free siRNA, (c) <i>d</i> -dots, (d) Oligo-siRNA, (e) <i>d</i> -dot/PAH-siRNA and (f) <i>d</i> -dot/PEI-siRNA complexes. Data are presented as average values $\pm$ SD. *, P-value < 0.005 vs blank, siRNA and <i>d</i> -dots.	99
Fig.5.11 (a) Cytotoxicity tests of four types of nanoparticles, i.e. <i>d</i> -dots, PAH-coated <i>d</i> -dots, CdTe QDs and CdTe/ZnS core/shell QDs. RAW 264.7 cells were treated with different concentrations of the particles for 48 hours. The particles were MPA-stabilized except for <i>d</i> -dot/PAH complexes. (b) Cytotoxicity tests of the <i>d</i> -dot/polymer nanocomplexes and <i>d</i> -dot/PAH-FA. Data for <i>d</i> -dot/MPA was presented as reference.	100
Fig.5.12 Cytotoxicity tests of the <i>d</i> -dot/PAH nanoplex on four different cell lines, i.e. RAW 264.7, Panc-1, Miapaca-2 and MDA-MB-231. The cells were treated with different concentrations of <i>d</i> -dot/PAH for 48 hours.	101
Fig.5.13 (a) Schematic illustration of the method to prepare FA conjugated <i>d</i> -dot/PAH nanoplexes. (b) FT-IR spectra of PAH, <i>d</i> -dot/PAH, folic acid and <i>d</i> -dot/PAH-FA nanocomposites. (c) Fluorescence image of Panc-1 cells treated with unconjugated (i, ii) and FA-conjugated (iii, iv) nanoplexes. The cells in (ii) and (iv) were pre-saturated with free FA before incubation with the nanocomposites.	104
Fig.5.14 Schematic illustration of preparation steps of the L-QDs based siRNA (drug) carriers.	106
Fig.5.15 (a) Hydrodynamic size distribution of the L-SQDs measured by DLS; (b) TEM image of the L-SQDs. The SUV lipid membrane fragments can be visualized in HRTEM image (inset); (c) Zeta-potential of L-SQD is tunable by varying the molar ratio between DSPE-mPEG and DSPE-PEG-NH ₂	107
Fig.5.16 Fluorescent images of Panc-1 cells. (a) Blank cells, (b) Cells treated with L-SQD, (c) Cells treated with FA functionalized L-SQD and (d) Cells presaturated with excess FA	

List of Figures

before incubation with f-L-SQD. T_1 -weighted MR images of the cell lysis in centrifuge tubes are presented in the insets.....	108
Fig.5.17 Cytotoxicity tests of the SQD and f-L-SQD hybrids upon Panc-1 cells after 48 h incubation.	108
Fig.5.18 FT-IR spectra of SQD/MPA and SQD/AET.....	111
Fig.5.19 Fluorescent images of Panc-1 cells treated with f-L-SQD/siRNA ^{FAM} . (a) Channel of FAM; (b) Channel of SQD; (c) The overlay of (a) and (b); (d) The overlay of (a), (b) and bright field image.	111
Fig.5.20 Transfection efficiency of Panc-1 cells determined by flow cytometry analysis, expressed as percentage of cells transfected after 4 h treatment.	113
Fig.5.21 K-Ras mRNA relative expression levels in Panc-1 cells were detected by real-time RT-PCR. (a) Blank, (b) Free siRNA, (c) L-SQD, (d) f-L-SQD/siRNA.	113
Figure 5.22 Cell viability of Panc-1 cells after a 48 hrs treatment with f-L-SQD, f-L-SQD /siRNA, f-L-SQD/CPT and f-L-SQD/siRNA-CPT.	114
Fig.6.1 Photograph of Cu:ZnS QDs, Mn:ZnSe QDs and CuInS ₂ QDs excited by UV light.	119
Fig.6.2 Representative UV-vis and PL spectra of Cu:ZnS QDs and CuInS ₂ QDs.....	120

SUMMARY OF ABBREVIATIONS

Abs	Absorption
AET	Aminoethanethiol
BRET	Bioluminescence Resonance Energy Transfer
CPT	Camptothecin
Cys	Cysteine
d-dot	Doped Quantum Dot
DEPC	Diethylpyrocarbonate
DI	Deionized
DLS	Dynamic light scattering
ECL	Electrochemiluminescence
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDL	Electrical Double Layer
EPR	Enhanced Permeability And Retention
FA	Folic Acid
FAM	Fluorescein Amidite
FISH	Fluorescence In Situ Hybridization
FRET	Fluorescence Resonance Energy Transfer
FRs	Folate Receptors
FT-IR	Fourier Transform Infrared Spectroscopy
FWHM	Full Width At Half Maximum
GNP	Gold Nanoparticle
IC50	Half Maximal Inhibitory Concentration
LBL	Layer By Layer
MHA	Mercaptohexanoic Acid
ML	Monolayer
MLV	Multilamellar Vesicle
MNP	Magnetic Nanoparticle
MPA	Mercaptopropionic Acid
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MUA	mercaptoundecanoic acid
mRNA	Messenger RNA
NIR	Near Infrared
NP	Nanoparticle
OD	Optical Density
PAH	Poly(allylamine hydrochloride)
PBS	Phosphate-buffered Saline
PDT	Photodynamic Therapy
PEI	Polyethylenimine
PEG	Polyethylene Glycol
PL	Photoluminescence
QD	Quantum Dot
QY	Quantum Yield

Summary of Abbreviations

RES	Reticuloendothelial System
RNAi	RNA Interference
ROS/RNS	Reactive Oxygen/Nitrogen Species
RT-PCR	Reverse Transcription Polymerase Chain Reaction
siRNA	Small Interfering RNA
SQD	Sandwiched Quantum Dot
TBP	Tertiarybutylphosphine
TGA	Thioglycolic Acid
TOPO	Trioctylphosphate
TOPO	Trioctylphosphine Oxide
TEM	Transmission Electron Microscopy
NHS	N-Hydroxysuccinimide
ULV	Unilamellar Vesicles
UV	Ultraviolet

PUBLICATION LIST

- [1] [Y. Wang](#), M. Liu, C. Yang, B. Wu, B. Zhang, et al., "Sandwiched Mn-doped QDs for Dual-Mode Imaging and Theranostic Applications," *Submitted*.
- [2] [Y. Wang](#), C. Yang, R. Hu, H. T. Toh, X. Liu, G. Lin, et al., "Assembling Mn:ZnSe quantum dots-siRNA nanoplexes for gene silencing in tumor cells," *Biomaterials Science*, 2015.
- [3] S. Hu, S. Zeng, B. Zhang, C. Yang, P. Song, T. J. H. Danny, et al., "Preparation of biofunctionalized quantum dots using microfluidic chips for bioimaging," *Analyst*, vol. 139, pp. 4681-4690, Sep 21 2014.
- [4] B. Zhang, R. Hu, [Y. Wang](#), C. Yang, X. Liu, and K.-T. Yong, "Revisiting the principles of preparing aqueous quantum dots for biological applications: the effects of surface ligands on the physicochemical properties of quantum dots," *Rsc Advances*, vol. 4, pp. 13805-13816, 2014 2014.
- [5] G. Lin, R. Hu, W.-C. Law, C.-K. Chen, [Y. Wang](#), H. L. Chin, et al., "Biodegradable Nanocapsules as siRNA Carriers for Mutant K-Ras Gene Silencing of Human Pancreatic Carcinoma Cells," *Small*, vol. 9, pp. 2757-2763, Aug 26 2013.
- [6] [Y. Wang](#), R. Hu, G. Lin, W.-C. Law, and K.-T. Yong, "Optimizing the aqueous phase synthesis of CdTe quantum dots using mixed-ligands system and their applications for imaging of live cancer cells and tumors in vivo," *Rsc Advances*, vol. 3, pp. 8899-8908, 2013 2013.
- [7] [Y. Wang](#), R. Hu, G. Lin, I. Roy, and K.-T. Yong, "Functionalized Quantum Dots for Biosensing and Bioimaging and Concerns on Toxicity," *Acs Applied Materials & Interfaces*, vol. 5, pp. 2786-2799, Apr 24 2013.
- [8] R. Hu, [Y. Wang](#), X. Liu, G. Lin, C. H. Tan, W.-C. Law, et al., "Rational design of multimodal and multifunctional InP quantum dot nanoprobe for cancer: in vitro and in vivo applications," *Rsc Advances*, vol. 3, pp. 8495-8503, 2013 2013.
- [9] K.-T. Yong, [Y. Wang](#), I. Roy, H. Rui, M. T. Swihart, W.-C. Law, et al., "Preparation of Quantum Dot/Drug Nanoparticle Formulations for Traceable Targeted Delivery and Therapy," *Theranostics*, vol. 2, pp. 681-694, 2012 2012.
- [10] [Y. Wang](#), R. Hu, G. Lin, K.-T. Yong, and Ieee, "Multicolored Cell Imaging with Bioconjugated Fluorescent Quantum Dots," *Proceedings of the 2013 Ieee 5th International Nanoelectronics Conference (Inec)*, pp. 476-479, 2013 2013. (*Conference paper*)

BOOK CHAPTER

- . Zhang, B. †; [Wang, Y.](#) †; Hu, R.; Roy, I; Yong, K.-T., Colloidal cadmium-free quantum dots: the next generation of semiconductor nanocrystals for biological applications. Accepted. Chapter for Handbook of Photonics for Biomedical Engineering, Springer, 2016 Approx

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