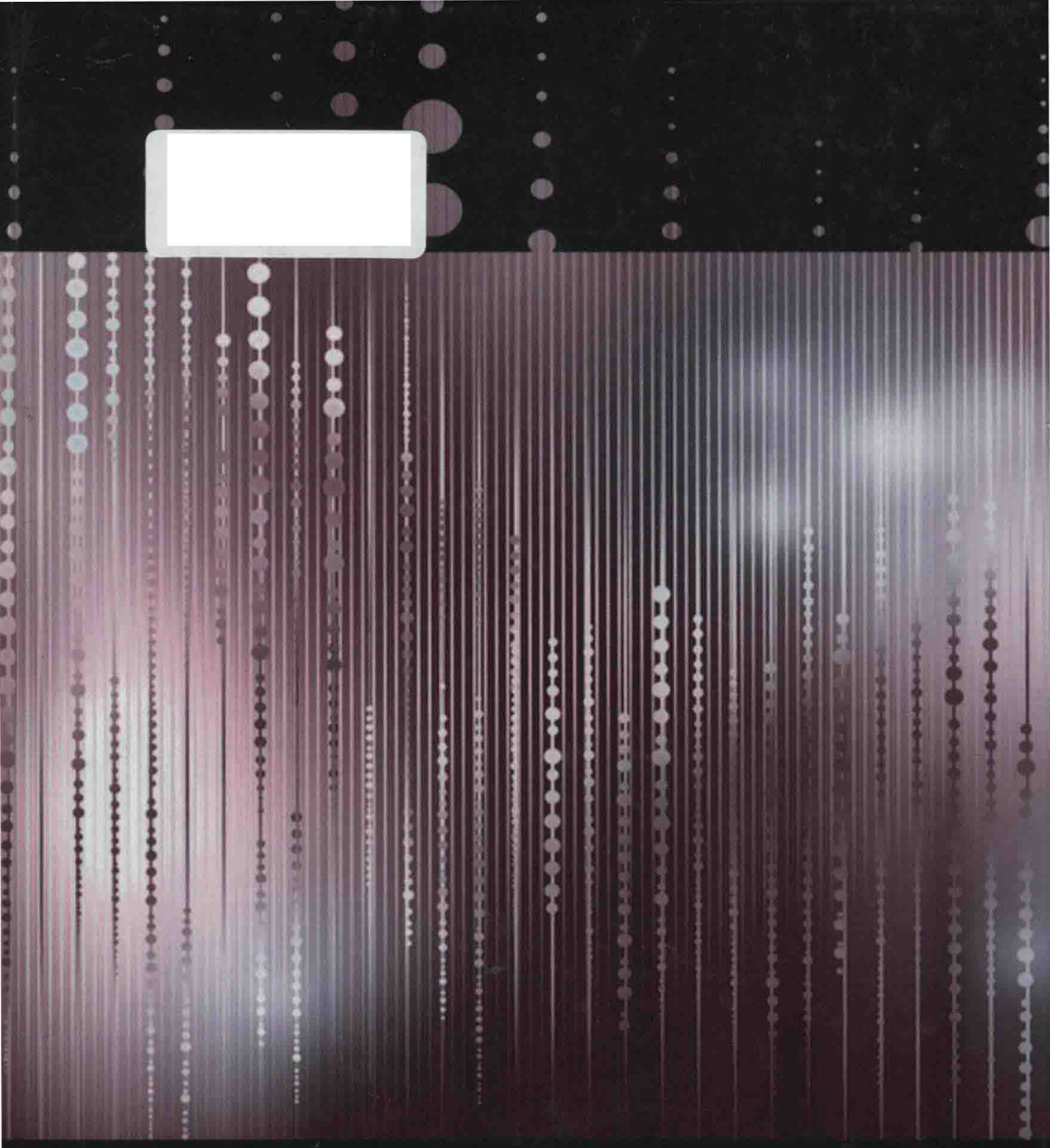

Quantum Dot System Fabrications

Advanced Concepts and Applications

Eva Murphy

Quantum Dot System Fabrications: Advanced Concepts and Applications

Edited by **Eva Murphy**

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Preface

The advanced concepts and applications in quantum dot system fabrications are elucidated in this all-inclusive book. It focuses on some quantum dot system (QDS) production techniques which take into consideration the dependence of structure, dimension and composition on growth processes and conditions such as temperature, strain and deposition rates. This book is a comprehensive compilation of fundamental studies conducted in a similar way as the ones conducted in Physics, Chemistry, and Material Science, with detailed account of various researches and latest information on developments related to QDS systems.

Various studies have approached the subject by analyzing it with a single perspective, but the present book provides diverse methodologies and techniques to address this field. This book contains theories and applications needed for understanding the subject from different perspectives. The aim is to keep the readers informed about the progress in the field; therefore, the contributions were carefully examined to compile novel researches by specialists from across the globe.

Indeed, the job of the editor is the most crucial and challenging in compiling all chapters into a single book. In the end, I would extend my sincere thanks to the chapter authors for their profound work. I am also thankful for the support provided by my family and colleagues during the compilation of this book.

Editor

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Synthesis of Glutathione Coated Quantum Dots

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1. Introduction

QDs play an important role mainly in the imaging and as highly fluorescent probes for biological sensing that have better sensitivity, longer stability, good biocompatibility, and minimum invasiveness. The fluorescent properties of QDs arise from the fact, that their excitation states/band gaps are spatially confined, which results in physical and optical properties intermediate between compounds and single molecules. Depending on chemical composition and the size of the core which determines the quantum confinement, the emission peak can vary from UV to NIR wavelengths (400–1350 nm). In other words, the physical size of the band gap determines the photon's emission wavelength: larger QDs having smaller band gaps emit red light, while smaller QDs emit blue light of higher energy (Byers & Hitchman 2011). The long lifetime in the order of 10–40 ns increases the probability of absorption at shorter wavelengths and produces a broad absorption spectrum (Drummen 2010).

The most popular types of QDs are composed of semiconductors of periodic group II-VI (CdTe, CdSe, CdS, ZnSe, ZnS, PbS, PbSe, PbTe, SnTe), however also other semiconductor elements from III-V group such as In, Ga, and many others can be used for QDs fabrication (e.g. InP) (Wang & Chen 2011). Particularly, much interest in nanocrystals is focused on the core/shell structure rather than on the core structure (Gill et al. 2008). Majority of sensing techniques employing QDs in biological systems are applied in solution (colloidal form). Up to present days, the most frequently used approaches have been reported on the preparation of colloidal QDs: hydrophobic with subsequent solubilisation step, direct aqueous synthesis or two-phase synthesis. Compared with hydrophobic or two-phase approaches, aqueous synthesis is reagent-effective, less toxic and more reproducible. Furthermore, the products often show improved water-stability and biological compatibility. The current issue solved in the area of QDs synthesis is to find highly luminescent semiconducting nanocrystals, which are easy to prepare, biocompatible, stable and soluble in aqueous solutions. Thus, the semiconductor core material must be protected from degradation and oxidation to optimize QDs performance. Shell growth and surface modification enhance the stability and increase the photoluminescence of the core.

The key step in QDs preparation ensuring the achievement of above mentioned required properties is based on QDs functionalization. Most of these approaches are based on bioconjugation with some biomolecule (Cai et al. 2007). Many biocompatible molecules can be used for this purpose; however glutathione (GSH) tripeptide possessing the surface amino and carboxyl functional groups gained special attention, since it is considered to be the most powerful, most versatile, and most important of the body's self-generated antioxidants. GSH coated QDS can be further modified, for example with biotin giving biotinylated-GSH QDs which can be employed in specific labelling strategies (Ryvolova et al. 2011). Namely, these biotin functionalized GSH coated QDs have high specific affinity to avidin (respectively streptavidin and neutravidin) (Chomoucka et al. 2010).

2. Glutathione as promising QDs capping agent

GSH is linear tripeptide synthesized in the body from 3 amino acids: L-glutamate, L-cysteine, and glycine (Y.F. Liu & J.S. Yu 2009) (Fig. 1.). These functional groups provide the possibility of being coupled and further cross-linked to form a polymerized structure (Zheng et al. 2008). Thiol group of cysteine is very critical in detoxification and it is the active part of the molecule which serves as a reducing agent to prevent oxidation of tissues (J.P. Yuan et al. 2009). Besides its thiol group acting as capping agent, each GSH molecule also contains one amine and two carboxylate groups (Chomoucka et al. 2009).

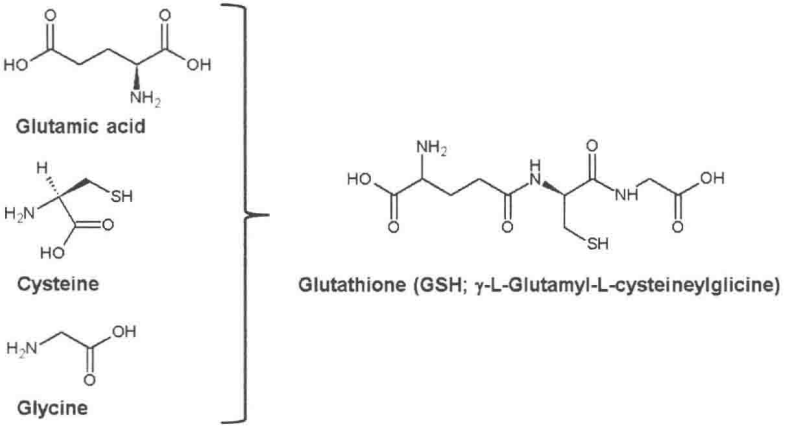

Fig. 1. Structure of glutathione

GSH is presented in almost all living cells, where it maintains the cellular redox potential. The liver, spleen, kidneys, pancreas, lens, cornea, erythrocytes, and leukocytes, have the highest concentrations in the body, ordinarily in the range from 0.1 to 10 mM. It belongs to powerful anti-viral agents and antioxidants for the protection of proteins, which neutralize free radicals and prevent their formation (Helmut 1999). Moreover, it is considered to be one of the strongest anti-cancer agents manufactured by the body. GSH´s important role is also in the liver for detoxification of many toxins including formaldehyde, acetaminophen, benzpyrene and many other compounds and heavy metals such as mercury, lead, arsenic and especially cadmium, which will be discussed later concerning the toxicity level of Cd-based QDs. GSH is involved in nucleic acid synthesis and helps in DNA repairing (Milne et

al. 1993). It slows the aging processes; however its concentration decreases with age. GSH must be in its reduced form to work properly. Reduced GSH is the smallest intracellular thiol (-SH) molecule. Its high electron-donating capacity (high negative redox potential) combined with high intracellular concentration (milimolar levels) generate great reducing power. This characteristic underlies its potent antioxidant action and enzyme cofactor properties, and supports a complex thiol-exchange system, which hierarchically regulates cell activity.

3. Synthesis of hydrophobic QDs

The synthesis of the most frequently used semiconducting colloidal QDs, consisted of metal chalcogenides (sulphides, selenides and tellurides), is based either on the usage of organometallic precursors (e.g. dimethylcadmium, diethylzinc), metallic oxide (e.g. CdO, ZnO) or metallic salts of inorganic and organic acids (e.g. zinc stearate, cadmium acetate, cadmium nitrate (Bae et al. 2009)). The sources of chalcogenide anion are usually pure chalcogen elements (e.g. S, Se, Te). Whatever precursor is used, the resulted QDs are hydrophobic, but their quantum yields (QY) are higher (in the range of 20–60 %) compared to the QDs prepared by aqueous synthesis route (below 30 %). However, the trend is to avoid the usage of organometallic precursors, because they are less environmentally benign compared to other ones, which are more preferable (Mekis et al. 2003).

The most common approach to the synthesis of the colloidal hydrophobic QDs is the controlled nucleation and growth of particles in a solution of organometallic/chalcogen precursors containing the metal and the anion sources. The method lies in rapid injection of a solution of chemical reagents into a hot and vigorously stirred coordinating organic solvent (typically trioctylphosphine oxide (TOPO) or trioctylphosphine (TOP)) that can coordinate with the surface of the precipitated QDs particles (Talapin et al. 2010). Consequently, a large number of nucleation centres are initially formed at about 300 °C. The coordinating ligands in the hot solvents prevent or limit subsequent crystal growth (aggregation) via Ostwald ripening process (small crystals, which are more soluble than the large ones, dissolve and reprecipitate onto larger particles), which typically occurs at temperatures in the range of 250–300 °C (Merkoci 2009). Further improvement of the resulting size distribution of the QDs particles can be achieved through selective preparation (Mićić & Nozik 2002). Because these QDs are insoluble in aqueous solution and soluble in nonpolar solvents only, further functionalization is required to achieve their solubilization. However, this inconveniency is compensated with higher QY of these QDs as mentioned previously.

3.1 Solubilization of hydrophobic QDs

Solubilization of QDs is essential for many biological and biomedical applications and presents a significant challenge in this field. Transformation process is complicated and involves multiple steps. Different QDs solubilization strategies have been discovered over the past few years. Non-water soluble QDs can be grown easily in hydrophobic organic solvents, but the solubilization requires sophisticated surface chemistry alteration. Current methods for solubilization without affecting key properties are mostly based on exchange of the original hydrophobic surfactant layer (TOP/TOPO) capping the QDs with hydrophilic one or the addition of a second layer (Jamieson et al. 2007). However, in most cases, the surface exchange results in not only broadening of the size distribution but also

in reductions of QY from 80% in the organic phase to about 40% in aqueous solution (Tian et al. 2009).

The first technique involves ligand exchange (sometimes called cap exchange). The native hydrophobic ligands are replaced by bifunctional ligands of surface anchoring thiol-containing molecules (see Fig. 2.) (usually a thiol, e.g. sodium thioglycolate) or more sophisticated ones (based on e.g. carboxylic or amino groups) such as oligomeric phosphines, dendrons and peptides to bind to the QDs surface and hydrophilic end groups (e.g. hydroxyl and carboxyl) to render water solubility. The second strategy employs polymerized silica shells functionalized with polar groups using a silica precursor during the polycondensation to insulate the hydrophobic QDs. While nearly all carboxy-terminated ligands limit QDs dispersion to basic pH, silica shell encapsulation provides stability over much broader pH range. The third method maintains native ligands on the QDs and uses variants of amphiphilic diblock and triblock copolymers and phospholipids to tightly interleave the alkylphosphine ligands through hydrophobic interactions (Michalet et al. 2005; Xing et al. 2009). Aside from rendering water solubility, these surface ligands play a critical role in insulating, passivating and protecting the QD surface from deterioration in biological media (Cai et al. 2007).

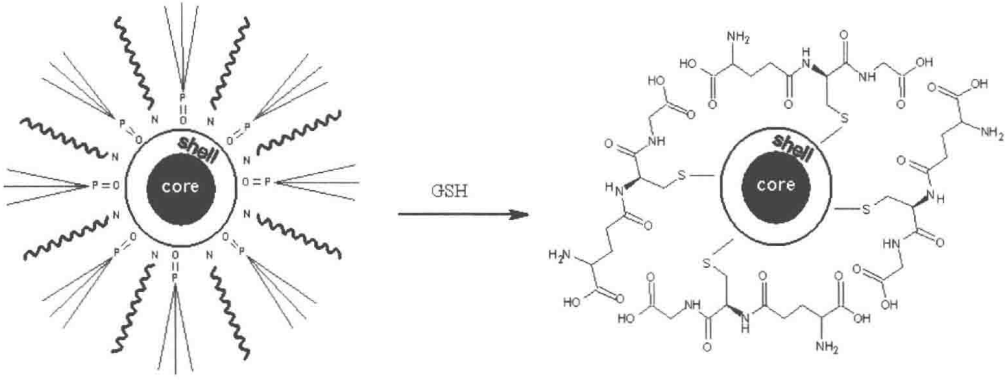

Fig. 2. Schematic representation of water soluble GSH-QDs preparation

An interesting work dealing with synthesis of hydrophobic QDs using chalcogen and metal oxide precursors and their following solubilisation with GSH was recently published by Jin et al. (Jin et al. 2008). The authors prepared highly fluorescent, water-soluble GSH-coated CdSeTe/CdS QDs emitting in near-infrared region (maximum emission at 800 nm) and tested them as optical contrast agents for *in vivo* fluorescence imaging. NIR emitting QDs are very suitable for *in vivo* imaging mainly due to low scattering and the absorption of NIR light in tissues. The preparation is based on surface modification of hydrophobic CdSeTe/CdS (core/shell) QDs with GSH in tetrahydrofuran-water solution. GSH is added in relatively high concentration of 30 mg for 1 ml of solution and its excess is finally removed by dialysis. The resulting GSH-QDs were stocked in PBS (pH = 7.4) and exhibited the QY of 22%.

Similarly, highly luminescent CdSe/ZnS QDs were synthesized by Gill and colleagues, who used GSH-capped QDs, which were further functionalized with fluorescein

isothiocyanate-modified avidin (Gill et al. 2008). The resulting avidin-capped QDs were used in all ratiometric analyses of H_2O_2 and their fluorescence QY was about 20 %.

Tortiglione et al. prepared GSH-capped CdSe/ZnS QDs in three steps (Tortiglione et al. 2007). At first, they synthesized TOP/TOPO-capped CdSe/ZnS core/shell QDs via the pyrolysis of precursors, trioctylphosphine selenide and organometallic dimethylcadmium, in a coordinating solvent. Diethylzinc and hexamethyldisilathiane were used as Zn and S precursors, respectively in the formation of ZnS shell around CdSe core. Due to their hydrophobic properties, CdSe/ZnS QDs were subsequently transferred into aqueous solution by standard procedure of wrapping up them in an amphiphilic polymer shell (diamino-PEG 897). Finally, the PEG-QDs were modified with GSH via formation of an amide bond with free amino groups of the diamino-PEG. These functionalized fluorescent probes can be used for staining fresh water invertebrates (e.g. *Hydra vulgaris*).. GSH is known to promote Hydra feeding response by inducing mouth opening.

4. Aqueous synthesis of GSH coated QDs

The second and more utilized way is the aqueous synthesis, producing QDs with excellent water solubility, biological compatibility, and stability (usually more than two months). Compared with organic phase synthesis, aqueous synthesis exhibits good reproducibility, low toxicity, and it is inexpensive. Basically, the fabrication process of water-soluble QDs takes place in reflux condenser (usually in a three-necked flask equipped with this reflux condenser). Nevertheless, this procedure in water phase needs a very long reaction time ranging from several hours to several days. Recently, new strategies employing microwave-assisted (MW) synthesis, which seems to be faster compared to the reflux one, were published as well (see below).

The other disadvantages of QDs synthesized through aqueous route are the wider FWHM (the full width at half maximum) and lower QY which can attribute to defects and traps on the surface of nanocrystals (Y.-F. Liu & J.-S. Yu 2009). These defects can be eliminated by the selection of capping agents. The process of functionalization involves ligand exchange with thioalkyl acids such as thioglycolic acid (TGA) (Xu et al. 2008), mercaptoacetic acid (MAA) (Abd El-sadek et al. 2011), mercaptopropionic acid (MPA) (Cui et al. 2007), mercaptoundecanoic acid (MUA) (Aldeek et al. 2008), mercaptosuccinic acid (MSA) (Huang et al. 2007) or reduced GSH.

From these ligands, GSH seems to be a very perspective molecule, since it provides an additional functionality to the QDs due to its key function in detoxification of heavy metals (cadmium, lead) in organism (Ali et al. 2007).

GSH is not only an important water-phase antioxidant and essential cofactor for antioxidant enzymes, but it also plays roles in catalysis, metabolism, signal transduction, and gene expression. Thus, GSH QDs as biological probe should be more biocompatible than other thiol-capping ligands. Concerning the application, GSH QDs can be used for easy determination of heavy metals regarding the fact, that the fluorescence is considerably quenched at the presence of heavy metals. Similarly, GSH QDs exhibit high sensitivity to H_2O_2 produced from the glucose oxidase catalysing oxidation of glucose and therefore glucose can

be sensitively detected by the quenching of the GSH QDs florescence (Saran et al. 2011; J. Yuan et al. 2009).

4.1 QDs synthesis in reflux condenser

This synthesis route usually consists in reaction of heavy metal (Zn, Cd, ...) precursor with chalcogen precursors. Ordinarily used precursors of heavy metals easily dissolving in water are acetates, nitrates or chlorides. The chalcogen precursors can be either commercial solid powders (e.g. Na_2TeO_3 in the case of CdTe QDs) or freshly prepared before using in reaction procedure, e.g. H_2Te (preparation by adding sulphuric acid dropwise to the aluminium telluride (Al_2Te_3) (Zheng et al. 2007a)) or NaHTe (forming by reaction of sodium borohydride ($NaBH_4$) with Te powder (He et al. 2006; Zhang et al. 2003)) in the case of CdTe QDs. However, NaHTe and H_2Te are unstable compounds under ambient conditions; therefore the synthesis of CdTe QDs generally has to be performed in inert reaction systems (see Fig. 3.). Since Na_2TeO_3 is air-stable, all of operations can be performed in the air, avoiding the need for an inert atmosphere. The synthetic pathway is thus free of complicated vacuum manipulations and environmentally friendly.

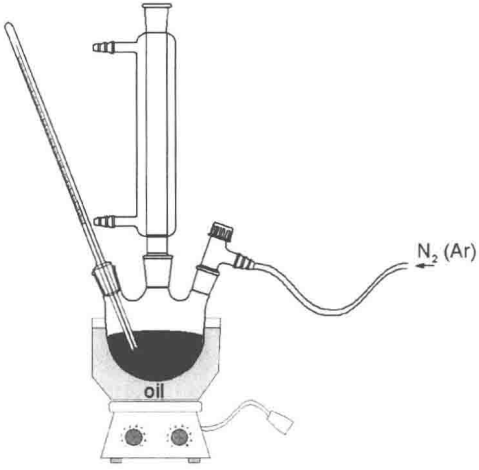

Fig. 3. Schema of apparatus for water soluble QDs preparation in reflux condenser

4.1.1 CdTe QDs capped with GSH

Xue et al. synthesized GSH-capped CdTe QDs by mixing the solutions of cadmium acetate and GSH and following injection of NaHTe solution under argon atmosphere and heating (Xue et al. 2011). After refluxing, QDs were precipitated with an equivalent amount of 2-propanol, followed by resuspension in a minimal amount of ultrapure water. Excess salts were removed by repeating this procedure three times, and the purified QDs were dried overnight at room temperature in vacuum. These GSH-QDs showed excellent photostability and possessed high QY (42 %) without any post-treatment. The authors conjugated the QDs with folic acid and studied how these labelled QDs can specifically target folic acid receptor on the surface of human hepatoma and human ovarian cancer cell to demonstrate their potentially application as biolabels.

Another GSH-functionalized QDS, namely CdTe and CdZnSe, were prepared by Ali et al. (Ali et al. 2007). The first mentioned were synthesized from H_2Te and $CdCl_2$, while in the second case NaHSe, $ZnCl_2$, H_2Se were used. Both types of GSH-capped QDs were coupled with a high-throughput detection system, to provide quick and ultrasensitive Pb^{2+} detection without the need of additional electronic devices. The mechanism is based on selective reduction of GSH-capped QDs in the presence of Pb^{2+} which results in fluorescence quenching that can be attributed to the stronger binding between heavy metal ions and the surface of GSH capping layer.

Also Goncalves and colleagues employed the simple experimental procedure for GSH-capped CdTe QDs fabrication and investigated the fluorescence intensity quenching in the presence of Pb^{2+} ions (Goncalves et al. 2009). Briefly, they mixed $CdCl_2$ and GSH aqueous solutions with freshly prepared NaHTe solution and the mixture was refluxed up to 8 h.

The same reactants for the synthesis of GSH-capped CdTe QDs were used by Cao M. et al. (Cao et al. 2009) and Dong et al. (Dong et al. 2010a). Cao and co-authors studied QDs interactions (fluorescence quenching) with heme-containing proteins and they found their optical fluorescence probes can be used for the selective determination of cytochrome c under optimal pH value. While Dong et al. used their GSH-CdTe QDs as fluorescent labels to link bovine serum albumin (BSA) and rat anti-mouse CD4, which was expressed on mouse T-lymphocyte and mouse spleen tissue. The authors demonstrated that CdTe QDs-based probe exhibited much better photostability and fluorescence intensity than one of the most common fluorophores, fluorescein isothiocyanate (FITC), showing a good application potential in the immuno-labeling of cells and tissues.

Wang and colleagues reported on the preparation of three kinds of water-soluble QDs, MAA-capped CdTe QDs, MAA-capped CdTe/ZnS and GSH-capped CdTe QDs, and compared the change of their fluorescence intensity (quenching) in the presence of As (III)(Wang et al. 2011). Arsenic (III) has a high affinity to reduced GSH to form $As(SG)_3$ thus the fluorescence of GSH coated QDs is reduced significantly in the presence of As (III). MAA-capped CdTe QDs were prepared through reaction of $CdCl_2$ and MAA with subsequent injection of freshly prepared NaHTe solution under vigorous magnetic stirring. Then the precursor solution was heated and refluxed under N_2 protection for 60 min. Finally, cold ethanol was added and MAA-CdTe QDs were precipitated out by centrifugation. A similar procedure was used for GSH-capped CdTe QDs synthesis with only one difference: the precipitation process was repeated for three times in order to eliminate free GSH ligands and salts in the GSH-CdTe QDs colloids. MAA-capped CdTe/ZnS QDs were prepared also similarly. When the CdTe precursor was refluxed for 30 min, $ZnCl_2$ and Na_2S were added slowly and simultaneously to form ZnS shell. After 30 min, the products were separated by the addition of cold ethanol and centrifugation.

Different thiol ligands, including TGA, L-cysteine (L-Cys) and GSH for capping CdTe QDs were also tested by Li Z. et al. (Z. Li et al. 2010). The starting materials were identical as in previous mentioned studies, i.e. NaHTe and $CdCl_2$. The luminescent properties of CdTe QDs with different stabilizing agents were studied by using fluorescence spectra, which showed that CdTe QDs with longer emission wavelength (680 nm) can be synthesized more easily when L-Cys or GSH is chosen as stabilizing agents. Moreover, the authors found that the cytotoxicity of TGA-QDs is higher than that of L-Cys- and GSH-CdTe. Ma et al. also prepared CdTe QDs modified with these three thiol-complex, namely TGA, L-cys and GSH and investigated the interactions of prepared QDs with BSA using spectroscopic methods (UV-VIS, IR and fluorescence spectrometry) (Ma et al. 2010).

Tian et al. (Tian et al. 2009) used for the first time GSH and TGA together to enhance stability of water soluble CdTe QDs prepared using NaHTe and $CdCl_2$. The author prepared different-sized CdTe QDs with controllable photoluminescence wavelengths from 500 to 610 nm within 5 h at temperature of 100 °C. When the molar ratio of GSH to TGA is 1:1, QY of the yellow-emitting CdTe (emission maximum at 550 nm) reached 63 % without any post-treatment. The synthesized CdTe QDs possess free carboxyl and amino groups, which were successfully conjugated with insulin for delivery to cells, demonstrating that they can be easily bound bimolecularly and have potentially broad applications as bioprobes.

Yuan et al. replaced NaHTe with more convenient Na_2TeO_3 for preparation of CdTe QDs, namely they used $CdCl_2$ and Na_2TeO_3, which were subsequently mixed with MSA or GSH as capping agent (J. Yuan et al. 2009). The prepared QDs were tested for glucose detection by monitoring QDs photoluminescence quenching as consequence of H_2O_2 presence and acidic changes produced by glucose oxidase catalysing glucose oxidation, respectively. The authors found that the sensitivity of QDs to H_2O_2 depends on QDs size: smaller size presented higher sensitivity. The quenching effect of H_2O_2 on GSH-capped QDs was more than two times more intensive than that on MSA-capped QDs.

4.1.2 CdSe QDs capped with GSH

Compared to CdTe QDs, GSH-capped CdSe QDs are much readily prepared. Jing et al. synthesized TGA-capped CdSe QDs using $CdCl_2$ and Na_2SeO_3, and they used these QDs for hydroxyl radical electrochemiluminescence sensing of the scavengers (Jiang & Ju 2007).

The research group of Dong, mentioned in synthesis of CdTe QDs, also prepared two kinds of highly fluorescent GSH-capped CdSe/CdS core-shell QDs emitting green and orange fluorescence at 350 nm excitation by an aqueous approach (Dong et al. 2010b). The authors used these QDs as fluorescent labels to link mouse anti-human CD3 which was expressed on human T-lymphocyte. Compared to CdSe QDs, they found a remarkable enhancement in the emission intensity and a red shift of emission wavelength for both types of core-shell CdSe/CdS QDs. They demonstrated that the fluorescent CdSe/CdS QDs exhibited much better photostability and brighter fluorescence than FITC.

4.1.3 CdS QDs capped with GSH

Also thiol-capped CdS QDs are less studied in comparison with CdTe QDs. MPA belongs to the most tested thiol ligands for capping these QDS (Huang et al. 2008). Liang et al. synthesized GSH-capped CdS QDs in aqueous solutions from $CdCl_2$ and CH_3CSNH_2 (thioacetamide) at room temperature (Liang et al. 2010). In this synthesis procedure, GSH was added in the final step into previously prepared CdS QDs solution. The obtained GSH coated QDs were tested as fluorescence probes to determine of Hg^{2+} with high sensitivity and selectivity. Under optimal conditions, the quenched fluorescence intensity increased linearly with the concentration of Hg^{2+}.

Merkoci et al. employed another preparation process: GSH and $CdCl_2$ were first dissolved in water with subsequent addition of TMAH (tetramethylammoniumhydroxide) and ethanol. After degassing, HMDST (hexamethyldisilathiane) was quickly added as sulphide precursor, giving a clear (slightly yellow) colloidal solution of water soluble CdS QDs modified with GSH (Merkoci et al. 2007). The authors used these QDs as a model compound