Surface Modification of CdSe(ZnS) quantum dots for biomedical applications

Ann Winzell

Performed at
University of Washington
Department of Bioengineering

2010-02-15
LITH-IFM-A-EX-10/2223-SE
Surface Modification of CdSe(ZnS) quantum dots for biomedical applications

Ann Winzel

2010-02-15
LITH-IFM-A-EX-10/2223-SE

Supervisor
Dr. Buddy D. Ratner
Department of Bioengineering
University of Washington
Seattle, USA

Examiner
Dr. Kajsa Uvdal
Department of Physics, Chemistry and Biology
Division of Molecular physics
Linköping University
Linköping, Sweden
Abstract

Quantum dots are inorganic nanocrystals of semiconductor metals that have unique light emitting properties. Due to their tunable and narrow emission profile, broad absorption spectra, resistance to photobleaching and high level of brightness they have emerged as inorganic fluorophores and numerous applicabilities for in vitro, in situ as well as in vivo studies are present. The chemical nature of the quantum dot surface needs to be altered in order to make the inorganic nanoparticles applicable to biological systems. Water soluble and biocompatible particles that limit unspecific binding to proteins can be obtained through functionalization of the surface coating with appropriate molecules.

In this pilot study, two surface modification strategies were performed upon two commercially available quantum dots in order to attach the zwitterionic molecules L-cysteine and thiolated sulfobetaine methacrylate, both shown to create non-fouling and biocompatible surfaces.

A biphasic exchange method was successfully used to perform ligand exchange of Qdot® ITK™ Organic Quantum Dots (QD-Organic) in order to exchange the structurally unknown, native lipophilic coating to one consisting of the amino acid L-cysteine (QD-Cysteine). The quantum dots transferred from the organic to the aqueous phase after the natively hydrophobic coating was changed to the hydrophilic L-cysteine. A characteristic mass fragment of protonated trioctylphosphine oxide (TOPO) was found for QD-Organic, using TOF-SIMS, suggesting TOPO is a part of the native coating. Further, the mentioned mass fragment was no longer present after the exchange. The C (1s) XPS-spectrum showed a new peak for carboxylic carbon, characteristic for L-cysteine, and expected changes in elemental composition were consistent with measured changes for all relevant elements. Large amounts of buffer remained after purification, suggesting the purification protocol needs further evaluation. Traces of the native coating were found in the C (1s) XPS-spectrum for QD-Cysteine, indicating not all ligands were exchange.

Additionally, a strategy for surface functionalization of Qdot® 655 ITK™ amino (PEG) quantum dots (QD-PEG-NH$_2$) with L-cysteine and thiolated sulfobetaine methacrylate was outlined and performed, using Michael addition and the heterobifunctional linker 3-Maleimidobenzoic acid N-hydroxysuccinimide ester. Unfortunately, no indications of successful attachment of the linker to the quantum dot have been found, neither by TOF-SIMS nor XPS, and thus functionalization with L-cysteine and tSBMA was not achieved. In theory, the proposed coupling chemistry used during the pilot study is promising, but further experiments are needed to obtain a successful and optimized protocol for the functionalization.
## Abbreviations and acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHLA</td>
<td>dihydrolipoic acid</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethyl formamide</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width half maximum</td>
</tr>
<tr>
<td>MBA</td>
<td>3-maleimidobenzoic acid</td>
</tr>
<tr>
<td>MBA-NHS</td>
<td>3-maleimidobenzoic acid N-hydroxysuccinimide ester</td>
</tr>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
</tr>
<tr>
<td>PEG</td>
<td>poly (ethylene glycol)</td>
</tr>
<tr>
<td>QD-Cysteine</td>
<td>Qdot® ITK™ Organic Quantum Dots – L-cysteine</td>
</tr>
<tr>
<td>QD-Organic</td>
<td>Qdot® ITK™ Organic Quantum Dots</td>
</tr>
<tr>
<td>QD-PEG-NH₂</td>
<td>Qdot® 655 ITK™ amino (PEG) quantum dots</td>
</tr>
<tr>
<td>QD-PEG-MBA</td>
<td>Qdot® 655 ITK™ amino (PEG) quantum dots-3-maleimidobenzoic acid</td>
</tr>
<tr>
<td>QD-PEG-MBA-Cysteine</td>
<td>Qdot® 655 ITK™ amino (PEG) quantum dots-3-maleimidobenzoic acid – L-cysteine</td>
</tr>
<tr>
<td>QD-PEG-MBA-Sulfobetaine</td>
<td>Qdot® 655 ITK™ amino (PEG) quantum dots-3-maleimidobenzoic acid – Sulfobetaine methacrylate</td>
</tr>
<tr>
<td>SBMA, tSBMA</td>
<td>sulfobetaine methacrylate, thiolated sulfobetaine methacrylate</td>
</tr>
<tr>
<td>TOF-SIMS</td>
<td>time-of-flight secondary ion mass spectrometry</td>
</tr>
<tr>
<td>TOPO</td>
<td>trioctylphosphine oxide</td>
</tr>
<tr>
<td>UV-vis</td>
<td>ultraviolet-visible spectroscopy</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
</tbody>
</table>
## Table of Contents

Abstract ............................................................................................................. i  
Abbreviations and acronyms ........................................................................... ii  
1. Introduction .................................................................................................. 1  
   1.2 Aim ........................................................................................................ 2  
2. Background ................................................................................................ 5  
   2.1 Quantum dots ....................................................................................... 5  
   2.2 Surface modifications .......................................................................... 6  
      2.2.1 Water solubility ........................................................................... 6  
      2.1.2 Biofunctionalization .................................................................. 8  
      2.1.3 Biocompatibility and specificity ................................................. 8  
      2.1.4 Size and charge .......................................................................... 9  
   2.3 CdSe(ZnS) quantum dot ....................................................................... 9  
   2.4 Low fouling surfaces .......................................................................... 11  
      2.4.1 Zwitterionic coating .................................................................. 11  
   2.5 N-hydroxysuccinimide (NHS) - ester crosslinking ............................. 12  
   2.6 Michael addition ................................................................................ 13  
   2.7 Time-of-flight secondary ion mass spectrometry (TOF-SIMS) .......... 13  
   2.8 X-ray Photoelectron Spectroscopy (XPS) ......................................... 14  
   2.9 Dynamic Light Scattering (DLS) .......................................................... 15  
   2.10 Ultraviolet-visible spectroscopy (UV-vis) ....................................... 16  
3. Material ...................................................................................................... 17  
4. Experimental setup ..................................................................................... 18  
   4.1 Surface functionalization of Qdot® 655 ITK™ amino (PEG) quantum dots 18  
      4.1.1 Attachment of linker to QD-PEG-NH₂ ...................................... 18  
      4.1.2 Attachment of L-cysteine to QD-PEG-MBA ............................. 19  
      4.1.3 Attachment of thiolated sulfobetaine methacrylate to QD-PEG-MBA 20  
      4.1.4 Purification .................................................................................. 20  
   4.2 Ligand exchange of Qdot® ITK™ Organic Quantum Dots ................. 21  
      4.2.1 Ligand exchange ........................................................................ 21  
      4.2.2 Purification .................................................................................. 22  
4.3 Analysis .................................................................................................... 23  
   4.3.1 Sample preparation ....................................................................... 23
1. Introduction

During the past decade, the field of nanotechnology has been in the focus of many groups in the research community. In the biomedical field, luminescent quantum dots have emerged as inorganic fluorophores due to their unique light emitting properties, such as tunable and narrow emission profile, broad absorption spectra, resistance to photobleaching and high level of brightness (Xing and Rao 2008).

Quantum dots have numerous applicabilities for in vitro, in situ as well as in vivo studies. Intracellular targets, such as the marker for breast cancer, Her2, and specific intracellular proteins have been labeled both in fixed and live cells using antibody linked quantum dots (Medintz, et al. 2005). Quantum dots conjugated to oligonucleotides have been used as probes in fluorescent in situ hybridization assays for detection of chromosome mutations and abnormalities (Pathak, et al. 2001). Further, quantum dots are highly applicable for imaging purposes, where large photostability, enabling long recording times, and high level of brightness is important for in vivo cellular and tissue targeting. Cellular behavior of Dictyostelium cells, early and late in their development, has been compared and the fate of different colonies could be monitored due to the possibility of long-term imaging and multicolor targeting (Jaiswal and Simon 2004). Multiplexed profiling, simultaneous study of multiple targets such as biomarkers for cancer diagnosis, is an important feature. It is possible due to the size tunable and narrow emission profile and broad absorption spectra of the quantum dots, enabling simultaneous emittance of different wavelengths at the same excitation wavelength (Xing and Rao 2008).

A promising application of quantum dots is fluorescent imaging of diseases, such as tumors, in human objects. The demands and requirements of the particles are high for both such in vivo labeling, and for in vitro diagnostics. Firstly, great specificity is required, since the number of background biomolecules that may create false positive results is high. Thus, eliminating unspecific binding to cells and proteins is of great importance. Secondly, biocompatibility is crucial since non-toxicity is required for biological applications. Further, the harsh cellular environment requires the quantum dots to be stable in water for different pH values and resistant to oxidative degradation (Xing and Rao 2008) (Jaiswal and Simon 2004).

Quantum dots are inorganic nanocrystals of semiconductor metals such as cadmium and selenium, and the chemical nature of the outer surface needs to be altered in order to fulfill the requirements mentioned above and make them applicable to biological systems. Surface modifications are performed to create the appropriate surface coating, functioning as a chemical and a physical barrier to the surrounding and providing anchors for functionalization with biomolecules (Hezinger, Tebmar and Göpferich 2008). Although plenty of research on surface modifications of quantum dots has been conducted, yet more study is required in obtaining a low fouling, pH stable and water soluble coating containing functional groups that does not interfere with the physical properties of the particle such as the quantum yield.
The knowledge of the low fouling character of dually charged surfaces has led to the idea of using the zwitterionic molecules L-cysteine and thiolated sulfobetaine methacrylate to create a “stealth surface”. Both molecules have shown great non-fouling and biocompatible characteristics (Ladd, et al. 2008) (Choi, et al. 2007). Also, the molecules are short and contain a thiol group, enabling the size of the functionalized quantum dot to be kept small and providing means for attachment to the inorganic core-shell using different conjugation chemistries.

During this project, different functionalization strategies have been investigated to attach the two molecules to the surface of semiconductor CdSe(ZnS) quantum dots.

1.2 Aim

The aim of this project was to use different modification strategies to create a non-fouling, “stealth” surface that would render water soluble and biocompatible quantum dots that were non-toxic and contained functional groups for further tagging to biomolecules. Two surface modification strategies were designed to two different commercially available quantum dots, Qdot® 655 ITK™ amino (PEG) quantum dots (QD-PEG-NH₂) and Qdot® ITK™ Organic quantum dots (QD-Organic). Both particles have a core of cadmium and selenium and a shell of zinc sulfide. The former having an inner amphiphilic coating conjugated with amine terminated poly(ethylene glycol)- (PEG) chains, making them natively water soluble, and the latter having a lipophilic coating, making them natively soluble in organic solvents.

A protocol for surface functionalization of QD-PEG-NH₂ was outlined, using the heterobifunctional linker 3-Maleimidobenzoic acid N-hydroxysuccinimide ester (MBA-NHS). MBA contains both an amine reactive group, N-hydroxysuccinimide (NHS), and a maleimide group which is reactive to thiols. The goal was to attach two different molecules, L-cysteine and thiolated sulfobetaine methacrylate (tSBMA). Firstly, MBA would be attached to the amine group of the PEG chain via NHS-ester crosslinking, through an amide bond formation, see schematic drawing in Figure 1

![Figure 1 - Schematic drawing of the attachment of MBA to the amine groups of QD-PEG-NH₂ using NHS-ester crosslinking.](image)
Secondly, the thiol group of L-cysteine or tSBMA would be attached to the maleimide group of MBA using Michael addition, see schematic drawing in Figure 2.

![Figure 2 - Schematic drawing of the attachment of the thiol group of cysteine to the maleimide group of MBA via the thiol group using Michael addition. The same reaction principle was used for attachment of tSBMA.](image)

In the proposed protocol, ligand exchange would be performed on QD-Organic using a biphasic exchange method, in order to exchange the original lipophilic coating to one consisting of the amino acid L-cysteine, as presented in Figure 3.

![Figure 3 - Schematic drawing of the ligand exchange of QD-Organic, yielding QD-Cysteine.](image)
X-ray photo electron spectroscopy (XPS), time of flight secondary ion mass spectrometry (TOF-Sims) and dynamic light scattering (DLS) are used to evaluate the results of the modifications.


2 Background

2.1 Quantum dots

Quantum dots are atom clusters of semiconducting materials, composed of $10^2$ to $10^5$ atoms from group II to VI of the periodic table. Electrostatically, these nanocrystals are regarded as intermediates between small molecules and bulk materials. The energy levels are discrete and the crystals can thus be regarded as atomic elements, but as more atoms interact the highest occupied atomic levels of the species form a valence band of a bulk material. The same applies for the lowest unoccupied atomic level, which upon atomic interaction forms the conduction band of the quantum dot. The space between the valence and the conduction band forms the band gap. The band width depends on the number of interacting atoms and is thus size dependent (Schmid 2004).

Upon energy transfer to a semiconductor, by light or heat, the mobility of electrons increase. As an electron moves, a hole with a positive charge is created, which also is mobile. The mobility of these electron-hole pairs in the material are called excitons. By solving the Schrodinger equation, one finds that the energy of the excitons increase when the size of the quantum dot decrease. This can be visually seen for CdSe quantum dots, where the exciton is created by energy from visual light. Different sizes of the dot appear in different colors depending on which wavelength is absorbed. In analogy with the creation of excitons upon absorption of specific wavelengths, the recombination of the hole-pair leads to specific wavelengths being emitted. The larger the nanocrystals, the smaller the energy gap and thereby longer wavelengths are emitted upon recombination of the electron-hole pair. By controlling the growth of the particle, thus limiting the size, one can create luminescent particles with different emission wavelengths for the same excitation wavelength, illustrated in Figure 4 (Atkins and De Paula 2006).

![Figure 4](image-url)

*Figure 4- Different sized quantum dots emit light of different wavelengths upon excitation with the same long wavelength UV-lamp. Redrawn from (Invitrogen 2009)*
There are several techniques for production of quantum dots. They can be lithographically defined, expatially self assembled or colloidal. The latter differs from the first two since it is based on chemical synthesis using wet chemistry. Also, the colloidal quantum dots are “free” nanoparticles dispersed in solution, making them applicable for biomedical applications, whilst the others are attached to a solid support (Schmid 2004).

For biomedical purposes, particle stability is of utmost importance, since leaking of toxic ions could be harmful to the cells in the organism that might be studied (Kirchner, et al. 2005). By coating the core with a shell of another semiconductor, making the colloidal crystals heterostructured, the particle stability is increased. The shell layer removes surface defects and passivates the surface, thereby preventing non-radiative electron-hole recombination, leading to increased quantum yield and stronger photo-luminescence (Guyot-Sionnest 2008).

Besides the inorganic core and shell, colloidal quantum dots usually comprise a native hydrophobic coating layer, which is formed during particle synthesis (Hezinger, Tebmar and Göpferich 2008).

2.2 Surface modifications

For biological applications, such as in vitro and in vivo studies, the quantum dots must be water soluble and biocompatible, meaning having low unspecific binding of proteins, being non-toxic and stable against oxidation and degradation for different pH values. This while keeping its physiochemical properties, such as quantum yield, photostability and emission wavelength. The particles also have to be kept small while having functional groups for further tagging to biomolecules. Since the nanocrystals’ properties are determined by the nature of the surface coating, the need for surface modifications with a careful ligand design, depending on the application, is crucial.

2.2.1 Water solubility

Firstly, the prepared hydrophobic quantum dots, that are only solvable in non-polar solvents, need to be made soluble in aqueous solutions. There are two main strategies to achieve water solubility: Surface ligand exchange, where the hydrophobic coordinating ligands from the synthesis are exchanged, and ligand capping, where the ligands from the synthesis remain but are capped with appropriate amphiphilic polymers. Both strategies have positive and negative aspects and the best suited method depends on the application. When performing complete surface exchange, the quantum dot diameter is kept small, but at the risk of having an incomplete surface coverage, causing poor quantum yield and low particle stability. When capping the existing ligands on the other hand, the particle size is significantly increased but the photophysical parameters are more likely undisturbed and aggregation is less of an issue due to greater stability in the surface coating (Hezinger, Tebmar and Göpferich 2008).
When performing surface ligand exchange, illustrated in Figure 5, the coordinating ligands of the hydrophobic coating are exchanged by heterobifunctional ligands or silica derivatives, in a mass driven process. Using bifunctional ligands, the hydrophobic end exchanges the coordinating ligands while the hydrophilic end is extended into solution. Most common is using thiolated polymers, where the thiol group links to the quantum dot and the hydrophilic group is extended in to solution, enabling water solubility (Medintz, et al. 2005). Commonly used are thiolated poly(ethylene glycol)-PEG polymers, yielding good water solubility. Monothiolated molecules such as mercaptoacetic acid and cysteine residues as well as dithiolated polymers, like dihydrolipoic acid (DHLA), have successfully been attached. The downside of using thiolated molecules is the sensitivity to oxidation of the thiol group, upon which the surface molecule detaches from the quantum dot and causes particle aggregation over time.

![Figure 5-Schematic drawing of the ligand exchange reaction where the hydrophobic surface ligands are exchanged by bifunctional ligands. Redrawn from (Walling, Novak and Shepard 2009)](image)

Other exchange methods use grafting dendrons or dendrimers, such as poly(amidoamine), leading to a dense surface coating, containing a large amount of functionalization groups (Hezinger, Tebmar and Göpferich 2008). Displacement with silane derivatives is a more complicated process, than using thiolated compounds, but yields a more stable coating. Extensive cross-linking between silane molecules enables water solubility even if thiol groups detach from the quantum dot surface due to oxidation (Walling, Novak and Shepard 2009).

Ligand capping, illustrated in Figure 6 is performed by using amphiphilic polymers, where the lipophilic end of the polymer interacts with the hydrophobic surface ligands of the quantum dot and the hydrophilic end, usually containing carboxyl groups, is extended into the polar aqueous solution. The modification can be done by using different di-, or tri-block copolymers, consisting of a hydrophilic backbone and hydrophobic side-chains (Hezinger, Tebmar and Göpferich 2008). Solubilization can also be performed through encapsulation of the hydrophobic nanocrystal in the core of phospholipid micelles (Dubertret, et al. 2002), in the hydrophobic pockets of cyclodextrin (Palaniappan, Hackneyb and Liu 2004) or by the use of calixarenes (Takashi, et al. 2005).
2.1.2 Biofunctionalization

Secondly, usage of quantum dots for biomedical purposes requires functionalization with biomolecules. Different methods for conjugating the quantum dot to biomolecules, such as proteins, can be used, through covalent coupling, specific interactions, physical adsorption and electrostatic interactions. Incorporating functional groups, such as COOH, NH₂, SH or OH, in the surface coating provides means for specific and covalent attachment, either directly to the biomolecule or via small and low-molecular bifunctional linkers containing anchor groups for further reactions. Carbodiimide coupling, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and NHS is a common way for direct linkage between COOH and NH₂ groups. Another way is attaching streptavidin to the coating of the quantum dot, for further linkage to biotinylated biomolecules. For non-covalent linkage, electrostatic interactions can be used. When the hydrophilic coating of the particles terminates in negatively charged groups, as is the case with COOH under biological conditions, positively charged proteins can interact with the coating. Functionalization can also be performed by chemisorption of thiolated biomolecules direct to the quantum dot (Xing and Rao 2008).

2.1.3 Biocompatibility and specificity

An important aspect of the surface coating for biomedical purposes is biocompatibility. In this case, that means limiting toxicity, such as interrupting normal cellular behavior, in the biological system to which it is introduced. Leaching of Cd²⁺ ions from the core have shown to induce cytotoxicity, which is enhanced upon oxidation of the core material but repressed by encapsulating with ZnS shell and organic layers (Kirchner, et al. 2005). Cytotoxicity decreases with increasing number of surface layers, as long as the coatings have complete surface coverage, are stable and remain intact (Walling, Novak and Shepard 2009) (Smith, et al. 2006).

The coating is also responsible for reducing non-specific protein adsorption as well as non-specific binding to cell membranes. Wanted is a coating that enables strong interaction with the intended molecule at the same time as no unspecific binding exists. Using Poly(ethylene glycol) (PEG) as part of the surface coating is today the most applied functionalization for increased specificity and reduction of unspecific binding (Liu, Howarth, et al. 2008).
2.1.4 Size and charge
When designing the surface coating of quantum dots for biomedical purposes, both in vitro and in vivo, size is an important consideration. The final size is greatly dependent on the charge of the coating, since it effects both the adsorption of serum proteins and the hydrodynamic diameter, which is the apparent size of the hydrated particle in solution (Atkins and De Paula 2006). For in vitro applications such as cellular imaging, small particle sizes are required in order to prevent steric hindrance and possible interference with the function of labeled cells, proteins, receptors or synapses. In vivo, the particle size can affect the biodistribution and pharmacokinetics. The optimal size differs depending on application. Large particles, with a hydrodynamic diameter of 19 nm, have been successful in mapping sentinel lymph nodes. More stringent size limits were found in an in vivo study concerning total body clearance through the renal system, where a hydrodynamic diameter of less than 6 nm was required (Liu, et al. 2007).

The Bawendi group investigated how the charge of the coating effected the final particle size in serum. Purely anionic- (DHLA) or cationic- (cysteamine) coatings adsorbed serum proteins, increasing the hydrodynamic diameter more than 15 nm, while a neutral coating (DHLA-PEG) showed no protein adsorption, but could not be soluble when synthesized smaller than 10 nm. A zwitterionic coating (L-cysteine) showed no protein adsorption while having a hydrodynamic diameter of less than 5 nm. Further, the zwitterionic quantum dots were subjected to an in vivo study, and could be excreted from rodents through the renal system (Choi, et al. 2007).

As stated earlier, ligand capping with amphiphilic polymers yields more stable but larger particles, on the order of 20-30 nm for a core-shell size of 4-6 nm, while ligand exchange enables smaller sizes at the expense of possibly lower surface coverage and thus instability (Hezinger, Tebmar and Göpferich 2008).

2.3 CdSe(ZnS) quantum dot
The company Invitrogen sell differently modified Qdot® nanocrystals, such as Qdot® 655 ITK™ amino (PEG) quantum dots (QD-PEG-NH₂) and Qdot® ITK™ Organic Quantum Dots CdSe(ZnS) quantum dots (QD-Organic), which were used during this project. Both comprise a core of a mixture of the semiconductors cadmium and selenium (CdSe) and are coated with a shell of zinc sulfide (ZnS). The core-shell is encapsulated in a polymeric coating, which differs for the two used quantum dots, to which biomolecules can be conjugated. A Schematic drawing of a conjugated Qdot® nanocrystal is presented in Figure 7.
Qdot® 655 ITK™ amino (PEG) quantum dots have an inner amphiphilic polymer coating, making the particles water soluble. Information concerning the molecular structure of this coating was not provided by the supplier. Additionally, an outer coating of functionalized PEG-chains has been covalently attached to reduce non specific binding. The PEG chains are functionalized with a terminating amino group, for further tagging to biomolecules, presented in Figure 8.

Qdot® ITK™ Organic Quantum Dots CdSe(ZnS) quantum dots, illustrated in Figure 9 have a lipophilic coating, making them soluble in organic solvents. The molecular structure of the lipophilic coating was not provided by the supplier.
2.4 Low fouling surfaces

Non-specific protein adsorption to surfaces is a major concern for biomedical applications. For implants and in vivo applications the adsorption of serum proteins and cell lysate can induce an inflammatory response leading to thrombosis and implant encapsulation. For in vitro studies, unspecific protein adsorption from biological media to the surface limits the accuracy and specificity and can create false positive and negative results. The need for a surface that minimizes the unspecific protein adsorption, a “non-fouling” or “super-low fouling” surface is apparent (Ladd, et al. 2008). Today, the most commonly used non-fouling materials are surfaces modified with PEG or oligo (ethylene glycol), and derivatives thereof (Hana Vaisocherova’, et al. 2008).

2.4.1 Zwitterionic coating

Zwitterions are electrostatically neutral molecules that carry both negatively and positively charged groups. Inspired by the fact that the cell membrane, consisting mainly of zwitterionic phospholipids, is believed to be non-thrombogenic, research has been made on zwitterionic materials as low-fouling surfaces (Chang, et al. 2006). Results have shown that materials containing both cationic and anionic groups on the same monomer residue are low fouling and biocompatible. The low protein adsorption is attributed to hydrogen bonding as well as the formation of a hydration repulsive force. The later is formed by a hydration layer, created upon interactions between the hydration shells formed around the dually charged groups. Not only is the presence of dual functional groups important, the surface density and the distance between the two charged groups also affect the low-fouling behavior (Ladd, et al. 2008).

Sulfobetaine methacrylate (SBMA) is a zwitterionic monomer, carrying both an anionic $\text{SO}_3^-$ group and a cationic $\text{N}^+$ -group, as illustrated in Figure 10. Sulfobetaine polymers, Poly(SBMA), belong to the family of poly(betaine) polymers and it has been shown that surfaces coated with poly(SBMA) highly resist protein adsorption and can be regarded as low-fouling (Zhang, et al. 2006). Attachment of a thiol group to the molecule enables functionalization and attachment using different coupling chemistries.

![Figure 10 - Schematic drawing of tSBMA](image-url)
A naturally occurring zwitterionic thiol is L-cysteine, presented in Figure 11, formed upon reduction of the amino acid cystine. Cystine is a dimer comprising two cysteine monomers, coupled through the formation of a disulfide bridge upon oxidation of the thiol groups (Berg, Tymoczko and Stryer 2002). The monomer is short, approximately 0.6nm, and electrostatically neutral, containing the dual functional groups NH$_3^+$ and COO$^-$. The small size and zwitterionic structure makes L-cysteine coatings promising for creating low-fouling surfaces. Nanocrystals modified with a cysteine coating have shown to be biocompatible, water soluble, easily functionalized and compact due to the small size (Liu, Choi, et al. 2007).

**2.5 N-hydroxysuccinimide (NHS) - ester crosslinking**

NHS-esters are readily used crosslinking molecules due to their reactivity towards primary amines. A covalent amide bond is formed between the primary amine and the ester group, and the NHS-group is released (Hermanson 2008). During this project, the heterobifunctional linker MBA-NHS was used, which contain the amine reactive group in one end, enabling covalent attachment of MBA to the primary amine of QD-PEG-NH$_2$, as presented in Figure 12.
2.6 Michael addition

Michael addition is a conjugate addition reaction, where a Michael acceptor reacts with a nucleophile. A Michael acceptor is an α, β-unsaturated compound, such as derivatives of quinone, maleimides, maleic acids or electron deficient alkenes. Strictly, the term Michael addition refers to a reaction where the nucleophile is a carbanion, but the same reactions will take place when the nucleophile is a thiol, which is the case for this project. Further, the Michael acceptor used, the maleimide group of MBA-NHS-ester, is especially suited since it reacts gently and quickly with thiols in room temperature (Davison, et al. 1999).

2.7 Time-of-flight secondary ion mass spectrometry (TOF-SIMS)

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a surface analysis technique that provides information of the molecular structure of the outer 10-20 Å of materials.

A surface is sputtered with highly energetic primary ions, which collides with the sample on the surface and sets the atoms in motion. The energy of the primary ions is high enough to break bonds near the collision site, leading to extensive fragmentation of the sample and emission of atomic particles. The collisions are less energetic further away from the collision site, which leads to less bond breaking and thereby emission of molecular fragments. Particles of the outer 2-3 monolayers have sufficient energy to leave the surface and are ejected as secondary ions, electrons or neutral atoms and molecules.

The generated secondary ions are separated according to mass to charge ratio (m/z) by a Time-of-flight analyzer. The ions are accelerated in an extraction field to the same energy E, before entering a flight tube of length L, which contains no field. Depending on the mass of the ion, the time it takes to pass through the tube differs. Smaller ions travel with higher velocity and thereby reach the detector earlier than larger ions. The mass of the ions is determined through measurements of the flight time and by applying the formula presented in Equation 1.

\[
E = \frac{mv^2}{2} = \frac{mL^2}{2zt}
\]

Equation 1 – The relationship between energy-E, flight time-t, mass to charge ratio (m/z) and length of the flight tube-L

Positive or negative ion mass spectra are acquired from TOF-SIMS, showing the number of detected ions for each (m/z). The peaks found in the spectrum can be identified by evaluating the mass of the signals and comparing them to the mass of ions of the molecules and components present in the sample, or fragments of those ions (Belu, Graham and Castner 2003).
2.8 X-ray Photoelectron Spectroscopy (XPS)

The analysis technique of X-ray Photoelectron Spectroscopy (XPS), also called Electron Spectroscopy for Chemical Analysis is a surface analysis technique used to determine qualitative and semi-quantitative atomic composition and chemistry. The technique enables non-destructive analysis, revealing the elements present in approximately the outer 100 Å of a surface (Ratner and Castner 1997).

By emitting a beam of X-ray photons upon a surface, photons with specific energies hit and excite the electrons in the sample on the surface. Core electrons, as well as valence electrons, can be excited to leave the atom as photo electrons, as presented in Figure 13.

![Figure 13- Schematic drawing of the XPS process showing the excitation of a 1s core level electron. Redrawn from (Barnes 1998)](image)

Measuring the kinetic energy of the emitted electrons, while having knowledge of the photon energy and the work function of the spectrometer, makes it possible to calculate the binding energy by using the principle of energy conservation, presented in Equation 2.

\[
E_{kin} = h \nu - (E_\beta + \phi)
\]

**Equation 2- The principle of energy conservation;** $E_{kin}$ = Kinetic energy of the electron, $h \nu$ = Energy of photon, $E_\beta$=Binding energy and $\phi$=work function of the spectrometer.
All elements have characteristic core electron binding energies, and thus the element from which the electron came, can be identified. The binding energy of an electron is the strength of the interaction between it and the nucleus, and depends on the core level (orbital) in which it originates. The binding energy of a certain core level increases with increasing nucleus charge and thus atomic number (Petoral 2005).

Data from XPS analysis are presented in a spectrum, showing the number of electrons detected as a function of their binding energies. Qualitatively, the chemical composition of a sample can be revealed by examining and associating the binding energy lines in the spectrum with specific elements, except for hydrogen and helium. Semi-quantitative analysis enables determination of the relative amount of the elements present. The intensity of the binding energy lines in a spectrum not only depend on the amount of the element present, but also on factors such as the photoemission cross-section. It is the probability for photoemission from an element at a given core level and determines how easily detected an element’s presence is. Elements such as zinc and cadmium have a large photoemission cross section, resulting in distinct lines whilst selenium has a very low cross section and thus larger amounts of the element is needed to distinguish it in a survey scan. Also instrumental factors, like the inelastic mean free path of the emitted electron, the escape depth and the efficiency of the spectrometer, affect the intensity. Measuring the area under the peaks and correcting them for the above mentioned factors enables determination of the percentages of the present elements (Wagner, Briggs and Seah 1990) (Ratner and Castner 1997).

When an element form bonds with other elements, the binding energies between the nucleus and the core electrons are changed. An atom in a higher oxidation state has higher binding energy, due to greater columbic attraction, than when in a lower oxidation state. The size of this chemical shift depends on the chemical environment, and the more electronegative the bonded element is, the greater the shift. By looking at the chemical shift, one can characterize a material and receive information about existing functional groups and chemical environments (Barnes 1998).

### 2.9 Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) is a technique used to determine the size of small particles in solution by measuring the Brownian motion. Brownian motion is the random movement of particles in a solution caused by the solvent molecules, and the velocity of the motion is defined as the translational diffusion coefficient, D. The hydro-dynamic diameter of a sphere, having the same translational diffusion coefficient as the one measured, can be calculated by using the Stokes-Einstein equation. Diffusion patterns are not only dependent on the core size, but are also effected by the shape of the measured particles, the conformation of the surface structures and the ion content of the medium.

The sample is illuminated by a laser source and when light hits the moving particles, it is scattered in all directions. Constructive and destructive interference of the scattered light
causes intensity fluctuations that are optically detected. The fluctuations as functions of time are used by the correlator in the instrument to create a correlation function. The size distribution, a plot showing the relative intensity of scattered light by particles in different sizes, is obtained by applying various algorithms to the function. The intensity size distribution yields information of the particle sizes present in the sample (Malvern Instruments 2009).

2.10 Ultraviolet-visible spectroscopy (UV-vis)

Ultraviolet-visible spectroscopy (UV-vis) is a technique used for quantitative measurements such as determining the concentration of absorbing species in a solution. Photons with wavelengths in the ultra violet or visible region are passed through a sample and the absorption of the sample is retrieved by measuring the decrease in light intensity (Harris 2007). Absorbance is proportional to concentration and the concentration of absorbing species can be calculated using the Beer-Lambert law, presented in Equation 3.

\[ A = \varepsilon bc \]

Equation 3 - Beer-Lambert law; A=absorbance, b=path length, c=concentration and \(\varepsilon\)=molar extinction coefficient.
# 3 Material

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical formula</th>
<th>Distributor</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Maleimidobenzoic acid N-hydroxysuccinimide ester</td>
<td>C_{15}H_{10}N_{2}O_{6}</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>C_{3}H_{6}O</td>
<td>EMD</td>
<td>HPLC Grade</td>
</tr>
<tr>
<td>Chloroform</td>
<td>CHCl_{3}</td>
<td>EMD</td>
<td>&gt; 99.9%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>C_{2}H_{6}OH</td>
<td>Decon Labs, Inc</td>
<td>USP specified</td>
</tr>
<tr>
<td>Iso-Propanol</td>
<td>C_{3}H_{6}OH</td>
<td>Sigma Aldrich</td>
<td>&gt;99.9%</td>
</tr>
<tr>
<td>L-Cystiene</td>
<td>C_{3}H_{7}NO_{6}S</td>
<td>Aldrich</td>
<td>97%</td>
</tr>
<tr>
<td>Methyl alcohol anhydrous</td>
<td>CH_{3}</td>
<td>Mallinckrodt</td>
<td>&gt; 99.9%</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>CH_{2}Cl_{2}</td>
<td>EMD</td>
<td>HPLC Grade</td>
</tr>
<tr>
<td>Millipore water</td>
<td>H_{2}O</td>
<td>Barnstead Nanopure</td>
<td>Diamond 18.2 M-Ohm w/ 0.2um filter</td>
</tr>
<tr>
<td>N,N dimethyl formamide</td>
<td>C_{3}H_{7}NO</td>
<td>Sigma-Aldrich</td>
<td>&gt; 99.9%</td>
</tr>
<tr>
<td>Phosphate buffered saline, pH 7.4</td>
<td></td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>Qdot® 655 ITK™ amino (PEG) quantum dots</td>
<td></td>
<td>Invitrogen</td>
<td></td>
</tr>
<tr>
<td>Qdot® ITK™ Organic Quantum Dots, 1 µM in decane</td>
<td></td>
<td>Invitrogen</td>
<td></td>
</tr>
<tr>
<td>Sodium Carbonate anhydrous</td>
<td>Na_{2}CO_{3}</td>
<td>Fisher</td>
<td>&gt;99.5%</td>
</tr>
<tr>
<td>Thiolated sulfobetaine methacrylate</td>
<td>C_{10}H_{21}NO_{5}S_{2}</td>
<td>Synthesized by Jeanette Stein at the University of Washington</td>
<td></td>
</tr>
</tbody>
</table>
4 Experimental setup

Described in this section are the experiments that were performed and the analyses yielding the results presented in chapter 5.

4.1 Surface functionalization of Qdot® 655 ITK™ amino (PEG) quantum dots

QD-PEG-NH₂ (Invitrogen) were intended to be functionalized with the zwitterionic molecules L-cysteine and tSBMA, by the use of the heterobifunctional linker MBA (Sigma).

4.1.1 Attachment of linker to QD-PEG-NH₂

Establishment of the protocol for the attachment was inspired by the book Bioconjugate techniques (Hermanson 2008) and consultations with Dr. Shai Garty and Dr. Anna Galperin. NHS-ester crosslinking was used to form an amide bond between the amine group of QD-PEG-NH₂ and the carboxylic group of MBA-NHS, as presented in Figure 14.

The molar ratio used between QD-PEG-NH₂ and MBA-NHS was (QD: MBA-NHS) = (1:1000). MBA was dissolved in the organic solvent N,N dimethyl formamide (DMF) [C₃H₇NO] to a concentration of 0.25 mM. QD-PEG-NH₂, 8 µM in borate buffer pH 8.3 [Na₂B₄O₇·H₂O, H₃BO], was added to the solution and the mixture was put on a shaking table for 30 minutes in room temperature.

Figure 14 - Schematic drawing of the reaction for attachment of the linker MBA-NHS to QD-PEG-NH₂, where the interacting groups of the reactants and the added part of the product are marked in blue and the leaving group in red.
4.1.2 Attachment of L-cysteine to QD-PEG-MBA

Michael addition, described previously, was chosen for attaching L-cysteine (Sigma) to the functionalized QD-PEG-NH₂, through a conjugate addition between the thiol group of L-cysteine and the maleimide group of the attached linker MBA, as presented in Figure 15.

L-cysteine was added to the solution of QD-PEG-MBA in DMF. The amount of L-cysteine added was based on the molar ratio between QD-PEG-NH₂ 8 µM and L-cysteine, and varied between (QD:L-cysteine) = (1:300000) and (1:500000). Upon addition of the crystalline powder, the solution was put on a shaking table for 18 hours in room temperature.
4.1.3 Attachment of thiolated sulfobetaine methacrylate to QD-PEG-MBA

Based on the procedure outlined for attaching L-cysteine to QD-PEG-NH$_2$, the same reaction mechanism was utilized for attaching tSBMA to QD-PEG-NH$_2$ via the linker MBA, as presented in Figure 16.

![Figure 16 - Schematic drawing of the reaction for attachment of tSBMA to QD-PEG-MBA, where the interacting groups of the reactants are marked in red and the added part of the product marked in blue.](image)

The amount of tSBMA added was based on the molar ratio between QD-PEG-NH$_2$ 8 µM and tSBMA, and the ratio used was (QD-PEG-NH$_2$:tSBMA) = (1:350000). tSBMA was dissolved in DMF to a concentration of 56 mM prior to the addition to QD-PEG-MBA in DMF. The solution was put on a shaking table in room temperature for 18 hours.

4.1.4 Purification

The solutions of QD-PEG-MBA-Cysteine and QD-PEG-MBA-Sulfobetaine in DMF were purified, and the solvent was changed from DMF to water, through dialysis against milli-pore water. A membrane of regenerated cellulose with a molecular weight cut-off of 3500 Dalton from Krackeler Scientific Incorporation was used. During dialysis, molecules of lower molecular weight than the cut off value pass through the membrane and out in to the surrounding water, while molecules of larger weight are retained in the dialysis bag (Berg, Tymoczko and Stryer 2002). This diffusion process enables removal of the solvent DMF as well as excess MBA and L-cysteine/tSBMA while the quantum dots are retained. Prior to dialysis, the samples were diluted with milli-pore water (H$_2$O), using the same volume of H$_2$O as the total volume of DMF used in the reactions. This was done in order to prevent possible degradation of the cellulose membrane used upon contact with DMF. Water was changed every three hours during the 4 days of dialysis in order to achieve good purification.
4.2 Ligand exchange of Qdot® ITK™ Organic Quantum Dots

QD-Organic (Invitrogen) were subjected to ligand exchange in order to replace the initial lipophilic coating to one consisting of the zwitterionic L-cysteine.

4.2.1 Ligand exchange

Establishment of the protocol for the surface exchange was inspired by research conducted and published by the Bawendi group at Massachusetts Institute of Technology (Liu, Choi, et al. 2007). In order to perform the ligand exchange, QD-Organic needed to be dispersed in the organic solvent chloroform, requiring replacement of the decane solvent in which the organic quantum dots were received. Solvent exchange was performed through flocculation, according to manual from supplier (Invitrogen 2005). QD-Organic, 1 μM was solved in decane and mixed with methanol and iso-propanol at the volumetric ratio of 1:3:1. The solution was centrifuged for 5-10 minutes at 4000 rpm, upon which the pellet that formed was redispersed in chloroform.

The reaction for the ligand exchange is illustrated in Figure 17.

![Figure 17 - Schematic drawing of the ligand exchange of QD-Organic from the original lipophilic coating to that of L-cysteine. L-cysteine was dissolved in the aqueous buffer of sodium carbonate, pH 9.0 [Na₂CO₃], to a concentration of 6 mM and mixed with QD-Organic 0.5 μM in chloroform at the molar ratio (QD:L-cysteine)=(1:30000). Two drops of ethanol were added in order to break the surface tension, enabling an easier emulsification. The mixture was vigorously stirred, vortexed at high speed, for 2 hours to emulsify the organic and aqueous phase.](image-url)
4.2.2 Purification

Purification of the aqueous phase containing the quantum dots was performed by first separating the two clearly visible phases in the Eppendorf tube. Possible remains of chloroform in the aqueous phase were removed by pouring the solution on to a teflon sheet in the chemical hood and letting the chloroform evaporate. Secondly, the sample was purified from excess L-cysteine and the buffer was changed from sodium carbonate to water by using Millipore-Amicon ultra centrifugal filters. Centrifugation was performed 10 times at 3000rpm. Sodium carbonate pH 9.02 was added after centrifugation cycles 1, 2 and 3, whereas water was added after cycles 4 through 10.
4.3 Analysis
Verification of the protocols consists of a number of steps. First the samples were prepared and then these were subjected to analysis, using UV-vis, XPS, TOF-SIMS and DLS.

Initially, to verify the presence of quantum dots in the samples, UV-vis measurements were performed. In order to evaluate the success of the functionalization of QD-PEG-NH$_2$ as well as the ligand exchange of QD-Organic, surface analysis was performed by the use of XPS, TOF-SIMS and DLS. Each step of the functionalization of QD-PEG-NH$_2$ was investigated, leading to five different samples: QD-PEG-NH$_2$, QD-PEG-MBA, QD-PEG-MBA-Cysteine and QD-PEG-MBA-Sulfobetaine. Evaluation of the ligand exchange of QD-Organic required two samples being analyzed, QD-Organic and QD-Cysteine.

4.3.1 Sample preparation
In order to remove any aggregates formed, the purified samples in water were put in Eppendorf tubes and centrifuged for 5 minutes at 3500 rpm. The pellet containing aggregates was removed and the procedure was repeated two more times on the supernatant.

Prior to XPS and TOF-SIMS, the samples were deposited on silicon wafer (10x10mm) by drip coating. The silicon wafers were cleaned through a series of 5 minute sonications, repeated twice for each solution, firstly in methylene chloride (dichloromethane), secondly in acetone and finally in methanol. The surfaces were blown dry in N$_2$-gas.

Using drip coating, with drops ranging from 2 µl to 4 µl, the samples were added repeatedly to two spots on each surface until a thick layer of deposited sample was visible. A vacuum desiccator was used to dry the applied drops between each addition, enabling faster removal of water from the sample due to reduced pressure. Before analysis, the surfaces were subjected to UV-light to visually validate the presence of a thick surface coating containing the luminescent quantum dots.

4.3.2 UV-vis
Absorbance measurements were performed at the Bioengineering department (University of Washington) on an Agilent 8453 UV-Visible spectrophotometer. Spectra were obtained with the wavelength 350 nm, at which the molar extinction coefficient for the quantum dots is 4 400 000. A rectangular cuvette with a width of 1 cm was used. The concentration of quantum dots was calculated using Lambert-Beers law.

4.3.3 TOF-SIMS
ToF-SIMS experiments were carried out at the National ESCA and Surface Analysis Center for Biomedical problems (NESAC-BIO) (University of Washington, Seattle, USA) using an TOF-SIMS 5-100 from ION-TOF GmbH (Münster, Germany). Five positive and three negative mass spectra were collected for each sample by rastering a 25 keV pulsed Bi$_3^+$ beam over a 100 x 100 µm$^2$ area of the sample surface. The ion dose was limited to 5 x 10$^{11}$ Bi$_3^+$ ions per cm$^2$ to avoid sample damage. The beam hit the target at an incidence angle of 45°.
Spectra were calibrated in mass using the CH$_3^+$, C$_2$H$_3^+$, C$_2$H$_5^+$ and C$_3$H$_5^+$ peaks in positive mode and CH$^-$, OH$^-$, C$_2$H$^-$ peaks in negative mode. Nitrogen containing fragments (e.g., NH$_4^+$, C$_4$H$_{10}$N$^+$, CN$^-$, CNO$^-$) were also added to calibrate the spectra related to nitrogen containing samples.

Multivariate analysis was performed using principal component analysis (PCA) provided by the PLS Toolbox v. 2.0 (Eigenvector Research, Manson, WA) for MATLAB (the MathWorks, Inc., Natick, MA), which is described in detail elsewhere (Wagner and Castner 2001) (Belu, Graham and Castner 2003). The peaks in each spectrum were normalized to the sum of the selected intensities to correct for variations in the total secondary ion yields between different spectra. The data were then mean-centered, and PCA was performed.

All TOF-SIMS experiments as well as analyses and data interpretations have been performed by Jeremy Brison, PhD at NESAC-BIO, University of Washington.

4.3.4 XPS

*Instrumental description*

XPS experiments were carried out at NESAC-BIO, University of Washington (UW) and at Linköping University (LIU).

At LIU, the XPS spectra were taken on a VG Microlab Auger Spectrometer. This instrument has a 310 F detector and uses an unmonochromatic Al Kα X-ray. Narrow scans for C (1s) and S (2p) for QD-PEG-NH$_2$ were acquired, using the pass energy 50eV.

At UW, XPS spectra were taken on a Surface Science Instruments S-probe spectrometer. This instrument has a monochromatized Al Kα X-ray and a low energy electron flood gun for charge neutralization. X-ray spot size for these acquisitions was on the order of 800 mm. Pressure in the analytical chamber during spectral acquisition was less than 5 x 10$^{-9}$ Torr. The pass energy for survey spectra was 150 eV and for narrow scans 50 eV. The take-off angle was 55°, yielding a sampling depth of approximately 50 Å. Survey scans and narrow scans for relevant elements for the samples QD-PEG-MBA, QD-PEG-MBA-Cysteine, QD-PEG-MBA-Sulfobetaine, QD-Organic and QD-Cysteine were performed.

The ESCA 2000 A Analysis Software was used to determine peak areas and to calculate the elemental compositions from peak areas. Additionally, XPSPeak41 was used to analyze the spectra.
**Charge compensation and calibration**

Interpretation of XPS spectra is a complicated matter and there are several crucial factors to take into consideration. Compensation of surface charging and accurate calibration of the binding energy scale are important in order to interpret the acquired data correctly.

When a non-conductive sample is analyzed, the sample area irradiated by X-rays accumulates a positive charge. The positive charge on the surface retards the emitted electrons, thereby reducing the kinetic energy of the photoelectrons which leads to inaccurate binding energies. By using a low energy electron flood gun, the accumulated charge caused by the departing electrons is neutralized. But any small differences in the surface charge across the area have a broadening effect on the shape of the peaks, since the electron flood depresses the potential of the whole surface and not only the parts where charging occurs. The effect of differential charging has been investigated by Bryson (Bryson 1987).

Further, in order to acquire the true binding energies of the photoelectrons, correction due to charging using an internal reference is needed. A common way is by assigning the C (1s) of aliphatic carbons to 285.0 eV. Generally, the entire spectrum has the same energy displacement, but not always. A critical issue is that of varying electrical properties on a surface. When large quantities of sample have been deposited on a surface and thus the samples are inhomogeneous, the surface potential may differ between different layers. This can result in the C1s of aliphatic carbons from one layer, or region, of the surface appearing at a different binding energy than from another layer or region (Metson 1999).

The binding energies were calibrated using an internal reference, the C1s peak of aliphatic carbon at 285.0 eV. According to the stochiometric composition of the outer coating of QD-PEG-NH₂, the most prevalent type of carbon is that of ether carbons from the PEG chain, but in the C1s spectrum the aliphatic carbon peak is much larger. This is not consistent with theory unless additional aliphatic carbons are present in the structurally unknown, inner amphiphilic coating. The length of the PEG chain is unknown, but for all lengths, the ratio between carbon and oxygen is between 2 and 2.5. The observed ratio in the XPS measurement, presented in Table 3, is 3.5, suggesting that indeed additional carbon is present. Further, TOF-SIMS measurements indicate that the inner coating of QD-PEG-NH₂ as well as the coating of QD-Organic contains trioctylphosphine oxide (TOPO). A TOPO molecule, [C₂₄H₅₂PO], consists of 24 aliphatic carbons. This motivates the assignment of the largest peak to aliphatic carbons. Calibration was performed in the same manner for all spectra.

The spectral shifts made to calibrate the photoelectron binding energies can be found in Appendix - XPS.
**Analysis of spectra**

Survey scans were performed for binding energies 0 – 1100 eV. The surface elemental composition was determined by associating the electron lines in the survey scan with specific core levels of elements. Narrow scans were conducted for binding energies of each relevant element. The obtained experimental spectra of sufficient resolution and quality were analyzed. The peak positions corresponding to different oxidation states of the elements identified to some extent. Corresponding peak areas were investigated by normal peak fitting procedures (Ratner and Castner 1997).

Assignment of the peaks was difficult since not all information of the structure of the coating of the starting material, the unmodified quantum dots, was available. With that information, interpretation of data acquired by XPS would have been simplified. The molecules analyzed were large, making the assignment complicated, since each atom effects the binding energy of the surrounding atoms.

**4.3.5 DLS**

Dynamic light scattering was performed at the Bioengineering department (University of Washington) using a Malvern Nanosizer ZS. The dispersant used was water with refractive index of 1.330 and viscosity of 0.8872 cP. The refractive index of the material was 2.42 and the material absorption was 0.10. Analysis was performed in a low volume glass cuvette at the temperature of 25° C. Three consecutive measurements were performed, each with duration of 60 seconds or 70 seconds.

The mean and standard deviation of the hydrodynamic diameter for each sample was calculated from the three curves obtained from the size distribution by number.
5 Results and discussion

Presented are results from the experiments outlined in chapter 4. Observations made during the modifications as well as data from TOF-SIMS, XPS and DLS are presented and discussed.

5.1 Surface functionalization of Qdot® 655 ITK™ amino (PEG) quantum dots

In this section, all analysis steps performed to investigate the outcome of the proposed protocol will be reported, even though results from some of the used techniques were inconclusive.

5.1.1 Observations

The unmodified quantum dots were of an intense red color. Upon functionalization of QD-PEG-NH₂ with MBA, the sample still showed a reddish color, although paler and not as intense. The pale red color was maintained after further functionalization with L-cysteine and tSBMA. The samples were diluted with water before purification in order to lower the concentration of the solvent DMF because of the dialysis membrane not being verified as stable for usage of the solvent. Decreased concentrations of quantum dots after the functionalizations were measured through absorbance measurements using UV-vis, and are presented in Table 1. The concentration of the unmodified quantum dots, stated by the supplier, was 8 µM. The measured concentration of QD-PEG-MBA was as expected, whilst the concentrations of QD-PEG-MBA-Cys and QD-PEG-MBA-Sulfobetaine both were half of what was expected when taking the dilution factor in consideration. A small amount of quantum dots must have been lost, either in the Eppendorf tubes used or in the dialysis membrane. Since the volumes worked with were very small, in the 100 µl range, the loss of one drop has a great impact. The observed decreased intensity in color is thus partly a result of the lower concentration of quantum dots upon dilution, but also due to loss of sample. Even though the concentrations were low, the results indicate that the particles were present and luminescent after the modifications.

<table>
<thead>
<tr>
<th></th>
<th>Concentration of quantum dots [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD-PEG-MBA</td>
<td>238</td>
</tr>
<tr>
<td>QD-PEG-MBA-Cysteine</td>
<td>44</td>
</tr>
<tr>
<td>QD-PEG-MBA-Sulfobetaine</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 1 - Concentration of quantum dots after each functionalization.
5.1.2 TOF-SIMS

The mass spectrum of QD-PEG-NH₂ is presented in Figure 19 and of QD-Organic in Figure 18. A strong characteristic peak was found in the QD-Organic sample at m/z -387.35. This peak has the same mass as a protonated TOPO molecule. When looking at the spectrum for QD-PEG-NH₂, a peak of the same m/z (387.35) was detected. The chemical structure of the inner amphiphilic coating, to which the amine terminated PEG chain is attached, is not known. However, the result presented in the mass spectrum suggests that TOPO is a part of this inner coating. This was useful and helpful information for interpretation of the XPS data of the functionalizations that were performed to QD-PEG-NH₂.

Figure 18 - Mass spectrum of QD-Organic.

Figure 19 - Mass spectrum of QD-PEG-NH₂.
The linker MBA contains a maleimide group, which was intended for attachment of L-cysteine and tSBMA. The mass fragment $C_4H_2NO_2^-$ is a characteristic fragment from unreacted maleimide groups. The negative mass spectrum for the m/z of this fragment is presented in Figure 20.

The m/z where the peak for this mass fragment should be is marked by the black arrow. Unfortunately, no peak was detected in the green curve, of QD-PEG-MBA, or in the black curve, of QD-PEG-MBA-Sulfobetaine. The peak in red, of QD-PEG-NH$_2$, is probably an interference and comes from another fragment or substance, since no maleimide is present in this sample. The results indicate that MBA has not been attached to QD-PEG-NH$_2$ and that there is no unreacted MBA present in the QD-PEG-MBA-Sulfobetaine sample.

![Figure 20 - Negative mass spectra for m/z of the fragment C$_4$H$_2$NO$_2^-$ for QD-PEG-NH$_2$ (red curve), QD-PEG-MBA (green curve) and QD-PEG-MBA-Sulfobetaine (black curve).](image)

The mass fragment $C_4H_2NO_2S^-$ is a characteristic mass fragment from reacted maleimide. The negative mass spectrum for the m/z of this fragment is presented in Figure 21. The m/z where the peak for this mass fragment should be is marked by the black arrow. There were not any peaks detected in the red curve or the green curve, of QD-PEG-NH$_2$ and QD-PEG-MBA, respectively. Unfortunately, no peak was present in the black curve of QD-PEG-MBA-Sulfobetaine either. The results indicate that there is no reacted MBA present in the QD-PEG-MBA sample or in the QD-PEG-MBA-sulfobetaine sample. Thus, no indications of sulfobetaine attachment to QD-PEG-MBA have been found.
Figure 21 - Negative mass spectra for m/z of the fragment $\text{C}_4\text{H}_2\text{NO}_2\text{S}^-$ for QD-PEG-NH$_2$ (red curve), QD-PEG-MBA (green curve) and QD-PEG-MBA-Sulfobetaine (black curve).
5.1.3 XPS
The success of the functionalizations was investigated by examining changes in elemental composition as well as by analyzing the chemical shifts detected in the peak fitted C (1s) spectra of the functionalizations. Additionally survey spectra for all samples and the peak fitted S (2p) XPS- spectrum for QD-PEG-NH$_2$ are presented. Tables of the peak assignment for carbon and sulphur, as well as the O (1s) spectra for the samples, can be found in Appendix A - XPS.

The chemical structures of the substances involved in the modification of QD-PEG-NH$_2$ are illustrated in Figure 22. The quantum dot, as supplied, has an inner amphiphilic coating to which the amine terminated PEG-chain has been covalently attached. No information regarding the chemical structure of the inner coating has been provided by the supplier and has not been included in the drawings in order to simplify the illustrations. The molecules attached in the modifications consist of the elements carbon, nitrogen, sulfur and oxygen. All these elements are also a part of the unmodified PEG-coated quantum dot and therefore focus was put on following changes in relative ratios for these elements upon modification.

![Figure 22 - Schematic drawings of the structures analyzed by XPS. Upper: QD-PEG-NH$_2$; Second: QD-PEG-MBA; Third: QD-PEG-MBA-Cysteine; Bottom: QD-PEG-MBA-Sulfobetaine.](image-url)
The survey scan of the unmodified QD-PEG-NH₂, presented in Figure 23, showed characteristic binding energy lines for the core-shell elements cadmium, zinc and sulfur. The intensity of the binding energy lines depends not only on the amount of element present, as previously stated. This is seen in the survey scan of QD-PEG-NH₂, where the lines for cadmium and zinc are easily distinguishable while the lines for the selenium core is of much less intensity and hard to detect in the wide scan, even though selenium is present. Energy lines characteristic for carbon, oxygen and nitrogen are also seen in the survey spectrum.

![Survey scan for the unmodified QD-PEG-NH₂.](image)

The survey scans for QD-PEG-MBA, QD-PEG-MBA-Cysteine and QD-PEG-MBA-Sulfobetaine, are presented in Figure 24, Figure 25 and Figure 26, respectively. All show the core-shell elements of the quantum dot, Cd, Se, Zn and S. Further, the elements C, N and O were detected in all samples. For QD-PEG-MBA, the sulfur originates from the shell structure since sulfur is not present in MBA. Sodium contamination was present for all samples. Since no sodium was added during the functionalizations, it must originate from the borate buffer, containing sodium borate, in which QD-PEG-NH₂ is supplied and stored. QD-PEG-MBA-Cysteine also showed calcium contamination and the element has not been involved in the functionalization and must be a contamination from the lab where the reaction was performed or during surface application.
Figure 24 - Survey scan for QD-PEG-MBA.

Figure 25 - Survey scan for QD-PEG-MBA-Cysteine.
Surface elemental composition was constructed from the survey scan of each species and is presented in Table 2.

<table>
<thead>
<tr>
<th>Element</th>
<th>QD-PEG-NH$_2$ [%]</th>
<th>QD-PEG-MBA [%]</th>
<th>QD-PEG-MBA-CYS [%]</th>
<th>QD-PEG-MBA-SB [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>72.2</td>
<td>74.4</td>
<td>66.7</td>
<td>75.5</td>
</tr>
<tr>
<td>Oxygen</td>
<td>20.8</td>
<td>16.7</td>
<td>23.8</td>
<td>17.8</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.4</td>
<td>3.4</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Sulfur</td>
<td>2.3</td>
<td>1.3</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.4</td>
<td>1.9</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.4</td>
<td>0.6</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Selenium</td>
<td>-</td>
<td>0.6</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.54</td>
<td>1.1</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>-</td>
<td>-</td>
<td>3.3</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2 - Atomic composition of QD-PEG-NH$_2$, QD-PEG-MBA, QD-PEG-MBA-Cysteine and QD-PEG-MBA-Sulfobetaine.
The observed ratio between carbon and oxygen, calculated from the elemental composition, for the samples is presented in Table 3. According to the stochiometric composition of the outer coating of QD-PEG-NH₂, the most prevalent type of carbon is that of ether carbons from the PEG chain. The length of the PEG chain is unknown, but for all lengths, the ratio between carbon and oxygen is between 2 and 2.5. The observed ratio in the XPS measurement is 3.5, suggesting that additional carbon must be present in the inner amphiphilic coating. Further, TOF-SIMS measurements indicate that the inner coating of QD-PEG-NH₂ contains trioctylphosphine oxide (TOPO). TOPO, with the molecular formula \([C_{24}H_{52}PO]\), contains a lot of aliphatic carbon and could thus explain the large amount of carbon detected by XPS. Additional molecules, not identified in this study, are likely a part of the inner coating.

<table>
<thead>
<tr>
<th></th>
<th>C/O ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD-PEG-NH₂</td>
<td>3.5</td>
</tr>
<tr>
<td>QD-PEG-MBA</td>
<td>4.5</td>
</tr>
<tr>
<td>QD-PEG-MBA-Cysteine</td>
<td>2.8</td>
</tr>
<tr>
<td>QD-PEG-MBA-Sulfobetaine</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Table 3 – The carbon to oxygen ratio for QD-PEG-NH₂, QD-PEG-MBA, QD-PEG-MBA-Cysteine and QD-PEG-MBA-Sulfobetaine.

The stochiometric composition of the attached molecules, presented in Table 4, was calculated in order to further evaluate changes in atomic composition after each step in the modifications.

<table>
<thead>
<tr>
<th></th>
<th>MBA [%]</th>
<th>L-cysteine [%]</th>
<th>Thiolated sulfobetaine methacrylate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>73.3</td>
<td>42.9</td>
<td>55.6</td>
</tr>
<tr>
<td>Oxygen</td>
<td>20.0</td>
<td>28.6</td>
<td>27.8</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>6.7</td>
<td>14.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Sulfur</td>
<td>-</td>
<td>14.3</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Table 4 - Calculated atomic composition of the attached molecules in the functionalization of QD-PEG-NH₂.

In order to detect changes for the elements relevant to the surface coating, independent of contaminants and core elements of the quantum dot, the compositions presented in Table 2 were normalized. This was done by dividing the percentage of each of the four relevant substances carbon, nitrogen, sulfur and oxygen with the sum of their percentages. The theoretical composition of the attached molecules was compared to the normalized XPS data obtained before the modification, to give information regarding the expectation of the relative amount of each element to increase, decrease or be unchanged upon successful attachment.

The calculated ratios between carbon and oxygen presented in Table 3, as well as the comparison between expected and detected change were used to evaluate the outcome of the modifications. The normalized XPS data, excepted change and detected change for each step in the functionalization are presented in Table 5, 6 and 7.
Table 5 shows the first step in the functionalization, attachment of MBA to QD-PEG-NH$_2$. Carbon and oxygen were expected to be unchanged but an increase and decrease, respectively, was detected. Thus, the carbon relative oxygen ratio increased from 3.5 to 4.5. The stochiometric C/O ratio for MBA is 3.7 and for the solvent DMF, used to solve MBA, the C/O ratio is 3. Unfortunately, no indications of MBA presence, neither attached nor unbound have been found in the C (1s) XPS spectrum or by TOF-SIMS. The increased C/O ratio is therefore believed to be due to insufficient purification from the solvent DMF. The amount of nitrogen in MBA is 6.7 % and the detected amount for QD-PEG-NH$_2$ was 2.4 %, thus it was expected to increase. The data after modification showed an increase to 3.5 %, which theoretically could indicate that MBA has been attached to some extent. Although, as discussed above, it is likely the presence of DMF causing the increase. The sulfur detected for QD-PEG-NH$_2$ as well as QD-PEG-MBA is that of the ZnS shell.

<table>
<thead>
<tr>
<th>Atom</th>
<th>XPS data QD-PEG-NH$_2$ [%]</th>
<th>XPS data QD-PEG-MBA [%]</th>
<th>Expected change QD-PEG-NH$_2$ $\rightarrow$ QD-PEG-MBA</th>
<th>Measured change QD-PEG-NH$_2$ $\rightarrow$ QD-PEG-MBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>73.9</td>
<td>77.7</td>
<td>No Change</td>
<td>Increase</td>
</tr>
<tr>
<td>Oxygen</td>
<td>21.3</td>
<td>17.4</td>
<td>No change</td>
<td>Decrease</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.4</td>
<td>3.5</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Sulfur</td>
<td>2.4</td>
<td>1.4</td>
<td>No change</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

Table 5 - Comparison of atomic composition between QD-PEG-NH$_2$ and QD-PEG-MBA.

Atomic composition, of QD-PEG-MBA and QD-PEG-MBA-Cysteine is presented in Table 6. A decrease in carbon was detected, consistent with expected change since the percentage of carbon in L-cysteine is 42.9 % and the detected carbon amount of QD-PEG-MBA was 77.7 %. The detected amount of oxygen increased significantly, also according to expectation. The C/O ratio has decreased from 4.5 to 2.8. Unfortunately, no indications of either MBA or L-cysteine, attached or unbound, have been found in the C (1s) XPS-spectra and the changes are therefore not attributed to attachment, even though detected and expected change are concurring. The amount of sodium detected for QD-PEG-MBA-Cysteine was twice that found in QD-PEG-MBA and indicates a lot of borate buffer is present in the sample. Borate buffer contains plenty of oxygen and is therefore likely contributing to the large increase of the element. Also, the calcium contamination detected in the survey scan combined with a carbon peak found at high binding energies in the C (1s) XPS-spectrum, suggests that CaCO$_3$ is present in the QD-PEG-MBA-Cysteine sample, which further contributes to the increase in oxygen. The detected decrease in nitrogen is likely due to less DMF present in the sample relative the QD-PEG-MBA sample. The change in sulfur content was too low to be significant.
Comparison of the compositional differences between QD-PEG-MBA-Sulfobetaine and QD-PEG-MBA is presented in Table 7. tSBMA contains a lot of oxygen, 27.8% and thus the content was expected to increase. An increase was detected, but it was quite small. The decrease in the C/O ratio from 4.5 to 4.3 cannot be regarded as significant. Neither reacted nor unreacted maleimide was detected for either sample by TOF-SIMS. It is therefore unlikely that either MBA or tSBMA has been attached to QD-PEG-NH₂. The decrease in nitrogen indicates that less DMF is present in the QD-PEG-MBA-Sulfobetaine sample than in the QD-PEG-MBA sample. The increase in sulfur is too small to be significant.

Table 6 - Comparison of atomic composition of QD-PEG-MBA and QD-PEG-MBA-Cysteine.

<table>
<thead>
<tr>
<th></th>
<th>XPS QD-PEG-MBA [%]</th>
<th>XPS data QD-PEG-MBA-Cysteine [%]</th>
<th>Expected change QD-PEG-MBA → QD-PEG-MBA-Cysteine</th>
<th>Measured change QD-PEG-MBA → QD-PEG-MBA-Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>77.7</td>
<td>71.7</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Oxygen</td>
<td>17.4</td>
<td>25.5</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>3.5</td>
<td>1.6</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.4</td>
<td>1.2</td>
<td>Increase</td>
<td>No significant change</td>
</tr>
</tbody>
</table>

Table 7 - Comparison of atomic composition of QD-PEG-MBA and QD-PEG-MBA-Sulfobetaine.

<table>
<thead>
<tr>
<th></th>
<th>XPS QD-PEG-MBA [%]</th>
<th>XPS data QD-PEG-MBA-Sulfobetaine [%]</th>
<th>Expected change QD-PEG-MBA → QD-PEG-MBA-Sulfobetaine</th>
<th>Measured change QD-PEG-MBA → QD-PEG-MBA-Sulfobetaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>77.7</td>
<td>77.8</td>
<td>Decrease</td>
<td>No change</td>
</tr>
<tr>
<td>Oxygen</td>
<td>17.4</td>
<td>18.4</td>
<td>Increase</td>
<td>Small increase</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>3.5</td>
<td>2.1</td>
<td>Increase</td>
<td>Small decrease</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.4</td>
<td>1.7</td>
<td>Increase</td>
<td>No significant change</td>
</tr>
</tbody>
</table>
The C (1s) XPS spectra for QD-PEG-NH$_2$, QD-PEG-MBA and QD-PEG-MBA-Cysteine are presented in Figure 27. The spectrum for the unmodified quantum dot was acquired at LIU whilst the other two spectra were acquired at UW, and thus two different XPS-instruments were used.

The three C (1s) peaks denoted C1, C2 and C3, respectively are present in all three of the C (1s) XPS-spectra. The Full Width Half Maximum (FWHM) of the peaks is 2 in the QD-PEG-NH$_2$ spectrum and 1.4 in the QD-PEG-MBA and QD-PEG-MBA-Cysteine spectra. The C1 peak is assigned to aliphatic hydrocarbons (C-C and C-H), (Petoral 2005), present in TOPO which is believed to be a part of the inner amphiphilic coating of the quantum dot, as indicated by TOF-SIMS. The peak C2, shifted 1.5 eV from the hydrocarbon peak, is attributed to carbon of the ether groups (C-O-C) in the PEG chain, in good agreement with reported chemical shifts for PEG (Sharma, Johnson and Desai 2004). The remaining peak, C3, is assigned to amide carbon (N-C=O) of 288.0eV (Cheng, et al. 2007)(Xiao, Brunner and Wieland 2004). Amide carbons are present in the native QD-PEG-NH$_2$, where an amide bond was used for attachment of the PEG-chain to the inner amphiphilic coating of the quantum dot.
Comparing the QD-PEG-NH$_2$ and the QD-PEG-MBA spectra it is observed that the C1 peak has increased relative the C2- and the C3 peaks. If the increase in the C1 peak was due to additional hydrocarbons from successful attachment of MBA, an increase in the C3 peak would also have been noticed, since amide carbons are present in the maleimide group of MBA as well as in the amide bond that would have formed upon the NHS-ester crosslinking of MBA to QD-PEG-NH$_2$. As discussed previously, the increase in carbon is likely due to the solvent DMF still present after purification. DMF contains two aliphatic carbons for each amide carbon, and could therefore cause the relative increase of the C1 peak. Since the same peaks are present in all three spectra, there are no indications of successful attachment of MBA.

Small differences between the QD-PEG-MBA and the QD-PEG-MBA-Cysteine spectra are noticed, but no indication of successful attachment of L-cysteine has been found. The intensity of the C1 peak relative the C2 peak has increased. Successful attachment of L-cysteine to the linker as well as presence of unbound L-cysteine associated to QD-PEG-MBA would also cause the hydrocarbon peak to increase in relation to the ether peak. But if that had been the case, an intensity increase would also have been detected at 288.5 eV, which is the characteristic binding energy for carboxylic carbons in L-cysteine (Uvdal, Bodö and Liedberg 1992). Since no such spectral difference was noticed it is unlikely that the increase in hydrocarbons is due to presence of L-cysteine. Further, the C4 peak at 289.5 eV is believed to be a contamination from the lab. The binding energy is typical for carbonate carbons -CO$_3^{2-}$. It is likely that the sample is contaminated with CaCO$_3$, since calcium was detected in the elemental composition and the binding energy of the peak is consistent with reported binding energies for the mentioned substance (Han, et al. 2003).
The C (1s) spectra of QD-PEG-NH₂, QD-PEG-MBA and QD-PEG-MBA-Sulfobetaine are presented in Figure 28. The spectrum for the unmodified quantum dot was acquired at LIU, whilst the other two spectra were acquired at UW, and thus two different XPS-instruments were used.

![C (1s) XPS spectra of QD-PEG-NH₂ (LIU), QD-PEG-MBA (UW) and QD-PEG-MBA-Sulfobetaine (UW).](image)

**Figure 28** - The C (1s) XPS spectra of QD-PEG-NH₂ (LIU), QD-PEG-MBA (UW) and QD-PEG-MBA-Sulfobetaine (UW). The QD-PEG-NH₂ peaks were fit using three C (1s) singlets of FWHM 2. The QD-PEG-MBA and QD-PEG-MBA-Sulfobetaine peaks were fit using three and four C (1s) singlets, respectively, of FWHM 1.4.

The upper two C (1s) spectra, of QD-PEG-NH₂ and QD-PEG-MBA, are identical to the ones presented in Figure 27. The FWHM of the peaks is 2 in the QD-PEG-NH₂ spectrum and 1.4 in the QD-PEG-MBA and QD-PEG-MBA-Sulfobetaine spectra. The peak assignment is the same as previously discussed, with the C1 peak corresponding to aliphatic hydrocarbon, the C2 peak to ether carbon and the C4 peak to amide carbon. The C (1s) spectrum of QD-PEG-MBA-Sulfobetaine is almost identical to the QD-PEG-MBA spectrum, except for the additional and very small C4 peak located at 289.4 eV. As in the QD-PEG-MBA-Cysteine spectrum, this peak is likely due to a CaCO₃ contamination. No indications of attachment or presence of tSBMA were found.
The S (2p) and Se (3p) XPS-spectrum of QD-PEG-NH$_2$, acquired at LIU, is presented in Figure 29.

![Graph showing S (2p) spectrum with peaks S1 and Se3p](image)

Figure 29 - The S (2p) and Se (3p) XPS-spectrum of QD-PEG-NH$_2$ (LIU). The peaks were fit using one S (2p) doublet of FWHM 1.5 and one Se (3p) doublet of FWHM 1.9.

The S (2p) and Se (3p) core level XPS-spectrum for the unmodified quantum dot, QD-PEG-NH$_2$, is presented in Figure 29. Both selenium and sulfur originate from the CdSe(ZnS) core-shell structure of the particle. The spectrum consists of a S (2p) spin-orbit doublet, S1, and a Se (3p) spin-orbit doublet. The S 2p$_{3/2}$ and the S2p$_{1/2}$ peaks, have a FWHM of 1.5 and are separated by 1.2 eV, at the intensity ratio 2:1. The Se3p$_{3/2}$ and the Se3p$_{1/2}$ peaks have a FWHM of 1.9 and are separated by 5.8 eV, at the intensity ratio 2:1.

The shift in binding energy between the S1 peak, assigned to sulfur of the ZnS shell, and the Se 3p peaks is 1.3 eV. The shift correspond well to the energy difference of 1.2 eV, between the same two peaks, reported in XPS studies of the core-shell structure of CdSe(ZnS) quantum dots (Huang, et al. 2004).

Unfortunately, due to poor quality of the S (2p) and Se (3p) XPS-spectra of QD-PEG-MBA, QD-PEG-MBA-Cysteine and QD-PEG-MBA-Sulfobetaine, peak fitting was not applicable.
5.1.4 DLS
Dynamic light scattering was performed to estimate the size of the particles upon each step in the functionalizations. Usage of this technique was a part of the protocol and is therefore presented and discussed, even though no definite conclusions could be drawn from the acquired data.

The hydrodynamic diameter of the particles is determined by the diffusion rate of the particles in solution. Factors such as charge and structure of the surface molecules, and type and concentration of the buffer used affect the diffusion and thus the obtained sizes. QD-PEG-NH₂ was in borate buffer, while QD-PEG-MBA, QD-PEG-Cysteine as well as QD-PEG-MBA-Sulfobetaine were dispersed in water at the time of analysis. The results were used for detecting trends rather than analyzing exact numbers. Also, only three measurements were made for each sample and more repetitions would be needed to make the analysis statistically correct. Results of the measurements can be seen in Table 8.

<table>
<thead>
<tr>
<th></th>
<th>Mean [nm]</th>
<th>Standard deviation [nm]</th>
<th>Measure Difference [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD-PEG-NH₂ no 1</td>
<td>33</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>QD-PEG-NH₂ no 2</td>
<td>36</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>QD-PEG-NH₂ no 3</td>
<td>17</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>QD-PEG-MBA</td>
<td>15</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>QD-PEG-MBA-Cysteine</td>
<td>19</td>
<td>1</td>
<td>2-6</td>
</tr>
<tr>
<td>QD-PEG-MBA-Sulfobetaine</td>
<td>21</td>
<td>1</td>
<td>4-8</td>
</tr>
</tbody>
</table>

Table 8 – Hydrodynamic diameter upon attachment of MBA, L-cysteine and thiolated sulfobetaine methacrylate to QD-PEG-NH₂.

The analysis for unmodified QD-PEG-NH₂ was repeated three times since the measured size the first two times was not in proportion to estimated size. Excepted value was in the range of 15-25 nm, since the supplier estimate the size to be 15-20 nm. The measured mean was between 17 nm and 36 nm and all have large standard deviations. The two large mean values originate from several different sizes present in the sample. Probably both monodisperse quantum dots as well as aggregates were present, making the variation in size large. The sample was centrifuged before the last measurement and the aggregates that sunk to the bottom of the tube were removed. This procedure resulted in a more reasonable mean value of 17 nm, but the standard deviation was still high. The diffusion rate is affected by ions in the media since a double layer of ions can be formed around the particles reducing the speed and thus resulting in a larger apparent hydrodynamic diameter. The quantum dots were in borate buffer, which might have affected the measured size. More accurate results might have been obtained if the media had been changed to water prior to analysis. But due to lack of time and material it was not possible to redo the analysis.
The size of L-cysteine is estimated to approximately 0.6nm, which would lead to an increase of 1.2 nm in core diameter upon successful attachment. A size difference of 2-6 nm was measured between the QD-PEG-MBA and the QD-PEG-MBA-Cysteine samples.

The size of tSBMA is estimated to approximately 2.1 nm, causing an increase in core diameter of about 4.2 nm upon successful modification. A size difference of 4-7 nm was measured between the QD-PEG-MBA and the QD-PEG-MBA-Sulfobetaine samples.

Given no indications that successful attachment of either MBA, L-cysteine and sulfobetaine have been obtained by XPS or TOF-SIMS, the measured differences between the samples only illustrates the difficulties and uncertainties when using DLS. The hydrodynamic diameter depends on many other factors than the actual size of the particles. As noticed for QD-PEG-NH₂, the accuracy in the results is questionable since measurement of the same sample yields such different results. Additionally, many more measurements of each sample would have been needed in order for the measurements to be regarded as statistically correct.
5.2 Ligand exchange of Qdot® ITK™ Organic Quantum Dots

5.2.1 Observations
Initially, the quantum dots were dispersed in chloroform and thus present in the organic phase, while L-cysteine was dissolved in aqueous buffer. Upon mixture, the phases separated with the top layer housing the aqueous phase and the bottom layer the organic phase. As seen in Figure 32, all color and thus the quantum dots were in the bottom layer before the exchange, in consistency with the hydrophobic nature of QD-Organic. After ligand exchange, all color was seen in the top layer as seen in Figure 30. The preference of the aqueous phase indicates that the lipophilic surface ligands have been at least mostly exchanged to hydrophilic L-cysteine and that QD-Cysteine has been formed.

![Figure 32 - QD-Organic; Top layer: cysteine solved in aqueous phase; Bottom layer: QD-Organic solved in chloroform.](image1)

![Figure 30 - QD-Cysteine; Top layer: QD-Cysteine in aqueous phase; Bottom layer: chloroform.](image2)

UV-vis was performed to measure the concentration of QD-Organic prior to ligand exchange and to QD-Cysteine after. The results seen in Table 9 show low concentrations of both native and modified quantum dots, which was expected since very small amounts were used. It showed that the quantum dots were present and luminescent after the ligand exchange.

<table>
<thead>
<tr>
<th></th>
<th>Concentration [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD-Organic</td>
<td>2</td>
</tr>
<tr>
<td>QD-Cysteine</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 9 – Concentration of quantum dots before and after ligand exchange.
5.2.2 TOF-SIMS

The mass spectrum of QD-Organic acquired by TOF-Sims is presented in Figure 33. It shows two strong characteristic mass fragments of masses m/z \(-387.35\) and m/z \(-754.72\). Assignment of the peaks was difficult since the structure of the lipophilic coating was unknown, but an important find was that the mass fragment of m/z \(-387.35\) has the same mass as a protonated TOPO molecule (TOPO + H). This indicates that TOPO is a part of the native coating of QD-Organic. This is supported by the detection of phosphorous in the XPS analysis, to be discussed in the next subsection.

The peaks of the high fragmentation area are due to the ion bombardment and are considered not, or less, characteristic for the sample. Further, a characteristic fragment of Polydimethylsiloxane (PDMS) was detected, which is a common contaminant.

Figure 33 - Mass spectrum of QD-Organic.
The mass spectrum for QD-Cysteine is presented in Figure 34. The above mentioned mass fragment at m/z – 387.35 is not present in the spectrum. This strongly indicates there has been exchange of the native surface ligands.

A large amount of sodium, of m/z=23, was detected, originating from the sodium carbonate buffer which was used to solve L-cysteine before ligand exchange. This could have an impact on the yield of the secondary ions, since sodium ionize well.
5.2.3 XPS

The success of the ligand exchange was investigated by examining changes in elemental composition as well as by analyzing the chemical shifts detected in the peak fitted C (1s) - and S (2p) XPS-spectra for QD-Organic and QD-Cysteine. Tables of the peak assignment for carbon and sulphur, as well as the O (1s) spectra for QD-Organic and QD-Cysteine, can be found in Appendix A - XPS.

The survey scan of the native QD-Organic, presented in Figure 35, showed characteristic binding energy lines for the core-shell elements of the quantum dot, cadmium, selenium, zinc and sulfur. Large amounts of carbon and oxygen were detected which must originate from the structurally unknown lipophilic coating.

![Survey scan of QD-Organic](image)

*Figure 35 – Survey scan of QD-Organic.*

The survey scan of QD-Cysteine, presented in Figure 36, shows the characteristic binding energy lines for the core-shell elements cadmium, zinc and sulfur. As discussed previously, the binding energy lines for selenium are difficult to distinguish in the survey scan, although the element is present. Further, the energy lines for carbon, nitrogen and oxygen are seen in the survey spectrum.
Surface elemental composition of QD-Organic and QD-Cysteine, presented in Table 10, was constructed from the survey scans of the species. The core-shell elements Cd, Se, Zn and S were detected for both quantum dots. The substances relevant for evaluation of the success of ligand exchange of QD-Organic are the ones in L-cysteine, nitrogen, oxygen, sulfur and carbon. They were all detected in both samples and the most abundant elements of the samples were carbon and oxygen. For QD-Organic, silicon was detected, which originates from the silicon surface to which the sample was applied. The detection of phosphorous in the sample supports the indication that the native lipophilic coating contains TOPO, which was found by TOF-SIMS. TOPO is a common ligand used to coat quantum dots and it contains phosphorous. As for QD-Cysteine, large amounts of sodium were detected. L-cysteine was dissolved in sodium carbonate buffer prior to ligand exchange and the results indicate that the purification performed was insufficient and needs to be improved.
In order to detect changes for the elements relevant to the ligand exchange, the compositions presented in Table 10 were normalized in the same manner as describes previously and the normalized atomic composition of QD-Organic and QD-Cysteine can be seen in Table 11. The theoretical composition of L-cysteine, presented in Table 4 was compared to the normalized XPS data obtained before the modification, to give information regarding the expectation of the relative amount of each element to increase, decrease or be unchanged upon successful attachment. The expected change was compared to the detected change to evaluate the outcome. Expected and measured changes are consistent for all four substances and a strong indication of successful exchange.

<table>
<thead>
<tr>
<th>Element</th>
<th>XPS data QD-Organic [%]</th>
<th>XPS data QD-Cysteine [%]</th>
<th>Expected change QD-Organic → QD-Cysteine</th>
<th>Measured change QD-Organic → QD-Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>77.4</td>
<td>56.9</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Oxygen</td>
<td>20.1</td>
<td>38.0</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.5</td>
<td>1.1</td>
<td>Increase</td>
<td>Small Increase</td>
</tr>
<tr>
<td>Sulfur</td>
<td>2.1</td>
<td>4.0</td>
<td>Increase</td>
<td>Increase</td>
</tr>
</tbody>
</table>

Table 11 - Comparison of atomic composition of QD-Organic and QD-Cysteine.
The C (1s) spectrum for QD-Organic and the peak fit C (1s) XPS-spectrum for QD-Cysteine are presented in Figure 37.

Figure 37- The C (1s) XPS- spectra of QD-Organic and QD-Cysteine (UW). The QD-Organic peaks were fit using five C (1s) singlets of FWHM 1.3.

The structure of the native lipophilic coating of QD-Organic was initially unknown, but TOF-SIMS as well as the elemental composition strongly indicates that TOPO is a part of it. But there are likely additional molecules in the coating and given the spectral appearance of QD-Organic, being uniform and consisting of a “bulky” peak, an accurate peak assignment was not possible to make.

The QD-Cysteine C (1s) spectrum however show five C (1s) peaks, denoted C1, C5, C6, C7 and C4, respectively, and the FWHM is 1.3. The C5- and the C7 peaks are both characteristic peaks for L-cysteine. The C5 peak is assigned to the sum of carbons bonded to the thiol group and the amino group of L-cysteine and C7 corresponds to carbon in the carboxylic group, both in good agreement with previous studies of L-cysteine (Uvdal, Bodø and Liedberg 1992). The C1 peak is assigned to aliphatic hydrocarbons (Cheng, et al. 2007), originating from ligands of the initial organic coating still present after the ligand exchange. Further, the C6 peak is likely originating from lipophilic ligands still existing on the surface, since carbon of binding energy 287.2 eV is detected in the QD-Organic spectrum as well. The C4 peak, detected at high binding energy, is assigned to carbonate carbon \(-\text{CO}_3^2-\) (Shchukarev and Korolkov 2004), originating from the sodium carbonate buffer used to dissolve L-cysteine prior to the ligand exchange. A large amount of sodium was detected in the surface elemental composition and it is therefore reasonable that carbonate ions also are present.
The S (2p) XPS-spectrum of QD-Cysteine, acquired at UW, is presented in Figure 38.

The S (2p) and Se (3p) core level XPS-spectrum for QD-Cysteine are presented in Figure 38. Both elements are a part of the CdSe(ZnS) core-shell structure of the particle and additional sulfur is present in L-cysteine, where the thiol group is linked to the (ZnS) coating of the quantum dot.

The spectrum consists of two S (2p) spin-orbit doublets, S1 and S2, and a Se (3p) spin-orbit doublet. The S 2p_{3/2} and the S2p_{1/2} peaks have a FWHM of 1.5 and are separated by 1.2 eV, at the intensity ratio 2:1. The Se3p_{3/2} and the Se3p_{1/2} peaks have a FWHM of 1.1 and are separated by 5.8 eV, at the intensity ratio 2:1.

The shift in binding energy between the S1, corresponding to sulfur of the ZnS shell, and the Se3p peaks is 1.4 eV. The shift is very close to the energy difference between the same two peaks for QD-PEG-NH₂ as previously presented. The two quantum dots are of the same CdSe(ZnS) core-shell material and thus it was expected to find the same peaks. Further, the S2 peak with S2p_{3/2} at 163.4 eV, having one third of the intensity of the S1 peak, is assigned to unbound thiol of L-cysteine (Uvdal, Bodö and Liedberg 1992). Considering purification after the ligand exchange was not sufficient, as previously discussed, the presence of excess and unbound L-cysteine associated to the L-cysteine coated particles is not surprising.

Unfortunately, due to poor quality of the S (2p) XPS-spectrum of QD-Organic, peak fitting was not applicable.
5.2.4 DLS

DLS was performed on QD-Cysteine in water from two different ligand exchanges. The native particles, QD-Organic, are only solvable in organic solvents, which the instrument is not suited for, and could thus not be subjected to the analysis. Only three measurements were done for each sample, making the results statistically uncertain. The results are used as guidance as to whether the results are in the correct range and the absolute values are not important. Obtained sizes can be seen in Table 12.

<table>
<thead>
<tr>
<th></th>
<th>Mean [nm]</th>
<th>Standard deviation [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD-Cysteine no 1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>QD-Cysteine no 2</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 12 - Hydrodynamic diameter of QD-Cysteine, after ligand exchange of QD-Organic.

L-cysteine is a small molecule, approximately 0.6 nm, and thus large measured sizes were not expected. The difference between the values could originate from varieties in the surface coverage and differences in the conformation of the molecule. A low surface coverage means fewer molecules are present and thus less water is attached to the particles, resulting in a faster diffusion rate and yielding in turn a smaller hydrodynamic diameter. If the L-cysteine molecules are extended into the solution the diffusion rate is slower than if they are lying flat on the surface.

Since the hydrodynamic diameter of the native QD-Organic could not be measured, no comparison of before and after could be made. Additionally, too few measurements were performed for the results to be statistically correct. Thus, no conclusion as to the success of the ligand exchange can be drawn from the DLS results.
6 Conclusions
In this pilot study, two surface modification strategies were performed upon two different quantum dots. The success of the modifications was investigated using XPS and TOF-SIMS, but since the structure of the native coating of the commercial particles was unknown, the surface analyses were not straightforward.

Ligand exchange of QD-Organic has successfully been performed, using a biphasic exchange method, in which the original, unknown, lipophilic coating was changed to one consisting of the hydrophilic L-cysteine. Detection of phosphorous in the elemental composition obtained by XPS as well as the characteristic mass fragment for TOPO found by TOF-SIMS for QD-Organic suggests that the native coating at least partly consists of TOPO. The loss of this mass fragment in the mass spectrum for QD-Cysteine strongly indicates that the native coating has been exchanged. Successful exchange of the lipophilic ligands to the hydrophilic L-cysteine is further supported by the visual transfer of the particles from the organic- to the aqueous phase. A new peak, characteristic for the carboxylic group of L-cysteine, was detected in the C (1s) XPS-spectrum for QD-Cysteine, and expected changes in elemental composition concur with measured changes for the elements relevant for L-cysteine- carbon, nitrogen, sulfur and oxygen. Large amounts of sodium carbonate buffer were detected and unbound L-cysteine was found in the S (2p) XPS-spectrum, indicating the purification protocol needs to be further evaluated. Peaks not expected for QD-Cysteine were found in the C (1s) spectra of both QD-Organic and QD-Cysteine, indicating traces of the native lipophilic coating still remain. But the preference of the aqueous phase as well as the loss of the mass fragment of TOPO indicates that most of the ligands were successfully exchanged. Complete surface exchange should be possible to obtain by repeating the experiments of this pilot study, thereby optimizing the reaction conditions and the purification procedure.

Surface functionalization of Qdot® 655 ITK™ amino (PEG) quantum dots with L-cysteine and tSBMA, using the heterobifunctional linker MBA and Michael addition was difficult to achieve. Small amounts of material were available and thus few reactions were performed. No indications of successful attachment of the linker MBA to QD-PEG-NH₂ were found, either by TOF-SIMS or XPS. Since the MBA attachment was unsuccessful, functionalization with L-cysteine and tSBMA was not possible. Large amounts of DMF were still present after purification while neither unbound MBA nor tSBMA nor L-cysteine were detected, suggesting dialysis is not an appropriate purification method for the solvent. Indications that the initially unknown, inner amphiphilic coating of QD-PEG-NH₂, contains TOPO have been found by TOF-SIMS, a useful information when performing surface analysis to the mentioned particles.

It was found that DLS did not contribute to any conclusions regarding the success of the modifications.
7 Improvements and outlook for the future

The surface modifications performed in this pilot study need further evaluation. The ligand exchange of QD-Organic was successful, but in order to completely exchange the lipophilic ligands, the biphasic exchange method should be repeated in order to optimize the reaction conditions and the concentrations of the reactants. During the repeated modifications, improvements to the purification protocol should be made since large amounts of sodium carbonate buffer were detected. A combination of dialysis and filtration vials might be applicable.

For the functionalization of QD-PEG-NH₂, the proposed coupling chemistry is promising in theory, but the protocol needs to be evaluated, optimized and investigated further. At first, focus has to be on achieving successful attachment of MBA to the quantum dot, using NHS-crosslinking. In the next step, the protocol for linkage of the thiol group of L-cysteine and tSBMA to the maleimide group of MBA, using Michael addition, can be evaluated. A different purification method than dialysis should be performed when using DMF as solvent for MBA, since a lot of molecules remained in the samples.

Usage of an additional surface characterization technique, such as IRAS would be helpful. Both for assistance in the evaluation of the outcome of the performed modifications, but also to obtain more information concerning the surface coating of the unmodified, commercially acquired quantum dots. Additional information of the structure of the native lipophilic coating of QD-Organic as well as of the inner amphiphilic coating of QD-PEG-NH₂ is needed. Knowledge of the starting material is crucial in order to fully detect and interpret changes in data brought about by modifications to the surface coating.

Since no conclusions of the success of the modifications could be drawn from the DLS measurements, the technique seems not applicable and should therefore be removed from the protocol.

In the future, applying the biphasic exchange protocol used for ligand exchange of QD-Organic to QD-Cysteine to create a coating of tSBMA or other thiolated molecules on QD-Organic would be interesting since the protocol turned out successful for cysteine. Additionally, evaluation of the quantum yield, pH-stability and biocompatibility of the modified particles, using fluorescent measurements, pH-studies and cell studies would be interesting to perform.
Acknowledgement

I want to start by thanking Kajsa Uvdal at Linköping University for making it possible for me to do my project in Seattle and also for her continuous support. Another important person is Cecilia Vahlberg, who besides from performing XPS analysis and helping me interpret the data have listened, inspired and guided me many times. Additionally, I want to thank all the members of the Division of Molecular Surface Physics and Nanoscience at Linköping University and my opponent Caroline Göransson.

I want to thank all members of the Ratner lab group at the Bioengineering department at the University of Washington and especially Buddy D. Ratner for giving me the opportunity to be a part of his research group. Others that helped me a lot and to whom I am very grateful are Coleen Irwin, Winston Ciridon, Eric Sussman, Anna Galperin and Shai Garty.

I also want to thank the people of NESAC/BIO for performing the surface analyses and letting me prepare my surfaces in their lab. Especially David G. Castner, Laura J Gamble, Liney Arnadottir, Jim Hull, Gilad Zorn, Sirnegeda Techane and importantly Jermey Brison for performing and analyzing TOF-SIMS.

Other people at the University of Washington I want to acknowledge for their help are Matt Mangianillo and Shivang Dave.

Furthermore, a sincere and deep thank you to the Lars Jonsson’s family fund, for financial support in conducting my master’s thesis abroad.

Last but not least I want to thank my family and friends for all the support during my five years of studying. My father for always inspiring me to learn new things and getting me interested in science at an early age, my mother for her continuous enthusiasm and support, my sister for being such a great role model and always supporting me and also my brother for valued comments on my thesis. Finally, thank you Fredrik for keeping me sane and happy by just being yourself.
References


Appendix - XPS

XPS peak assignment

<table>
<thead>
<tr>
<th>Chemical surrounding</th>
<th>QD-PEG-NH₂ (from LIU) [eV]</th>
<th>QD-PEG-MBA (from UW) [eV]</th>
<th>QD-PEG-MBA-SB (from UW) [eV]</th>
<th>QD-PEG-MBA-Cys (from UW) [eV]</th>
<th>QD-Cysteine (from UW) [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>285.0</td>
<td>285.0</td>
<td>285.0</td>
<td>285.0</td>
<td>285.0</td>
</tr>
<tr>
<td>C2</td>
<td>286.4</td>
<td>286.5</td>
<td>286.4</td>
<td>286.4</td>
<td>286.4</td>
</tr>
<tr>
<td>C3</td>
<td>288.0</td>
<td>288.1</td>
<td>288.1</td>
<td>288.1</td>
<td>288.1</td>
</tr>
<tr>
<td>C4</td>
<td>289.4</td>
<td>289.5</td>
<td>289.5</td>
<td>289.5</td>
<td>289.5</td>
</tr>
<tr>
<td>C5</td>
<td>286.5</td>
<td>286.4</td>
<td>286.4</td>
<td>286.4</td>
<td>286.4</td>
</tr>
<tr>
<td>C6</td>
<td></td>
<td></td>
<td>287.2</td>
<td>287.2</td>
<td>287.2</td>
</tr>
<tr>
<td>C7</td>
<td></td>
<td></td>
<td>288.5</td>
<td>288.5</td>
<td>288.5</td>
</tr>
</tbody>
</table>

Table 13 - Peak assignment for C (1s) XPS- spectra.

<table>
<thead>
<tr>
<th>Chemical surrounding</th>
<th>QD-PEG-NH₂ (from LIU) [eV]</th>
<th>QD-Cysteine (from UW) [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>161.7</td>
<td>161.2</td>
</tr>
<tr>
<td>S2</td>
<td>162.9</td>
<td>162.4</td>
</tr>
</tbody>
</table>

Table 14 - Peak assignment for S (2p) XPS- spectra.

Calibration of binding energy scale

<table>
<thead>
<tr>
<th>Sample</th>
<th>Binding energy correction due to charging [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD-PEG-NH₂ (UW)</td>
<td>11.8</td>
</tr>
<tr>
<td>QD-PEG-MBA (UW)</td>
<td>11.7</td>
</tr>
<tr>
<td>QD-PEG-MBA-Cysteine (UW)</td>
<td>12.4</td>
</tr>
<tr>
<td>QD-PEG-MBA-Sulfobetaine (UW)</td>
<td>12.3</td>
</tr>
<tr>
<td>QD-Organic (UW)</td>
<td>12.7</td>
</tr>
<tr>
<td>QD-Cysteine (UW)</td>
<td>12.1</td>
</tr>
<tr>
<td>QD-PEG-NH₂ (LIU)</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

Table 15 – Calibration of the binding energy scale by assignment of the C (1s) peak for aliphatic carbon to 285eV.
Surface functionalization of Qdot® 655 ITK™ amino (PEG) quantum dots
Presented are the O (1s) spectra of QD-PEG-MBA, QD-PEG-MBA-Cysteine and QD-PEG-MBA-Sulfobetaine.

The XPS spectra of O (1s), for QD-PEG-MBA and QD-PEG-MBA-Cysteine, presented in Figure 39, show similar peak appearances. The peaks are centered at 532.5 eV and 531.5 eV, respectively. No conclusions of the success of the modification were drawn.

Figure 39 – The O (1s) XPS-spectra of QD-PEG-MBA and QD-PEG-MBA-Cysteine.
The XPS spectra of O (1s) for QD-PEG-MBA and QD-PEG-MBA-Sulfobetaine, presented in Figure 40, show similar peak appearances, centered at 532.5 eV and 531.8 eV, respectively. No conclusion regarding the success of the modification was made.

Figure 40- The O (1s) XPS- spectra of QD-PEG-MBA and QD-PEG-MBA-sulfobetaine.
**Ligand exchange of Qdot® ITK™ Organic Quantum Dots**

The XPS spectra of O (1s) for QD-Organic and QD-Cysteine, presented in Figure 41, both show a peak centered at 532 eV and no apparent difference between the two can be noticed.

![Graph showing O (1s) XPS spectra of QD-Organic and QD-Cysteine.](image)

Figure 41 - The O (1s) XPS-spectra of QD-Organic and QD-Cysteine.