On the ligand shell complexity of strongly emitting, water-soluble semiconductor nanocrystals

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Applications aren’t the only thing in the world.  
It’s interesting in understanding what the world is made of.  
Richard P. Feynman (1918 - 1988)
Abstract

Colloidal semiconductor nanocrystals (NCs) have attracted a great deal of interest as bright and stable chromophores for a variety of applications. Their superior physicochemical properties depend on characteristics of the inorganic core, as well as on the chemical nature and structure of the stabilizing organic ligand shell. To evaluate the promising material, a thorough knowledge of structure-property relationships is still demanded. The present work addresses this challenge to three water-soluble NC systems, namely thiol-capped CdTe, thiol-capped CdHgTe, and DNA-functionalized CdTe NCs with special emphasis on the investigation of structure, modification, and influence of the ligand shell.

Remarkably, CdTe NCs show bright emission in the visible spectral region and can be synthesized in high quality directly in water. It was shown that the aqueous synthesis also facilitates the preparation of strongly near-infrared (NIR) emitting CdHgTe NCs. The current work presents a detailed study on parameters, by which the emission can be tuned, such as the growth time, the initial Cd : Hg ratio, and the choice of ligand. These insights contribute to the knowledge, which is essential for the design of highly emissive and long-term stable NIR emitting NCs. Further variations of the NC/ligand system include the modification of the ligand shell of CdTe NCs with oligonucleotides based on the strong attachment of DNA molecules to the NC. The successful functionalization of NCs with single-stranded DNA molecules is very promising for the precise and programmable assembly of NCs using DNA origami structures as templates.

For both, functionality and optical properties, the surface chemistry of the NCs plays a substantial role and was subject to an extensive investigation. As there is no generally applicable technique to determine the amount of stabilizers and the structure of the ligand shell, the presented study is based on a combination of various methods particularly tailored to the analysis of water-soluble CdTe NCs capped by short-chain thiols. CdTe NCs served as a model system for the described analysis of the ligand shell, since they are thoroughly studied regarding synthesis and features of the core. Aiming for the quantification of thiols, a straightforward colorimetric assay, the Ellman’s test, is for the first time introduced for the analysis of NCs. Accompanied by elemental analysis an approximate number of thiols per NC becomes accessible. Moreover, theoretical calculations were performed to estimate the amount of ligand that would cover the NC in a monolayer of covalently bound molecules. In contrast to these results, the experimental values point to a larger amount of thiols immobilized on the NC. Attempts to remove the ligand indicate the presence of Cd in the ligand shell and thermogravimetric studies show that the ligands are not loosely assembled in the ligand shell. The outstanding conclusion of these findings involves the presence of Cd-thiol complexes in the ligand shell. Further
Results unambiguously show that the amount of Cd-thiol complexes present in the NC solution strongly influences the concentration-dependent emission yield of the NCs. Additional studies dedicated to the considerable influence of the ligand shell highlight a strong effect of pH, NC concentration, type and purity of the solvent, and the number of precipitation steps on the emission of water-soluble semiconductor NCs. These substantial investigations emphasize the need to carefully control the conditions applied for handling, optical measurements, and application of NCs.

In order to gain a deeper insight into the complex structure of the native ligand shell, techniques deliberately chosen for the \textit{in situ} analysis were applied for thioglycolic acid-capped CdTe NCs. Information from dynamic light scattering (DLS) regarding the stability and the shell thickness are consistent with previous results showing a large ligand network on the NC surface and a decreasing stability of the NCs upon dilution. Importantly, nuclear magnetic resonance (NMR) spectroscopy allows for the distinction of bound and free ligands directly in solution and proves the presence of these species for the NCs studied. In particular, the results indicate that the ligands are not strongly bound to the NC core and that both, free and bound ligand species, consist of modified thiol molecules, such as Cd-thiol complexes. These findings support previous assumptions and allow to establish a distinct picture of the ligand shell of water-soluble semiconductor NCs. Further insights were obtained from small-angle X-ray scattering (SAXS), which facilitates the identification and the determination of the composition of NC core as well as ligand shell. Element-specific SAXS yields the final proof of the presence of Cd in the ligand shell. The model developed for the optimal fitting of the experimental scattering curves additionally confirms the findings from the other methods.

In conclusion, the present work contributes to the challenging goal of a comprehensive knowledge of interactions between the NC core and the ligands. The fundamental development of a structural model of water-soluble CdTe NCs including information on stoichiometries is accomplished by the combination of the techniques presented and emphasizes the challenge to assign a clear border between the ligand shell and the Cd-thiol complexes in solution. Altogether, CdTe NCs capped by thioglycolic acid are best described by a crystalline core surrounded by a water-swollen Cd-thiolate shell that considerably affects the optical properties of the system. Notably, the results of the versatile study provide the opportunity to control the overall properties and to evaluate water-soluble semiconductor NCs for particular applications in photonics and optoelectronics.
Contents

Abstract i

Abbreviations v

List of Figures vii

List of Tables x

1 Introduction 1

2 Strongly emitting, water-soluble semiconductor nanocrystals 12
   2.1 State-of-the-art . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 12
   2.2 Colloidal synthesis of CdTe nanocrystals . . . . . . . . . . . . . . . . . . . 19
   2.3 Colloidal synthesis of CdHgTe nanocrystals . . . . . . . . . . . . . . . . . . 23
   2.4 DNA-modified CdTe nanocrystals . . . . . . . . . . . . . . . . . . . . . . . 34
   2.5 Summary . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 37

3 Analysis of the amount of ligands and its influence on optical properties 39
   3.1 Short-chain thiol molecules . . . . . . . . . . . . . . . . . . . . . . . . . . 39
      3.1.1 Analytics . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 39
         3.1.1.1 Ellman’s test . . . . . . . . . . . . . . . . . . . . . . . . . 39
         3.1.1.2 Inductively coupled plasma optical emission spectroscopy . 42
         3.1.1.3 Thermogravimetric analysis . . . . . . . . . . . . . . . . 44
      3.1.2 Modeling . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 46
      3.1.3 Optics . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 47
         3.1.3.1 Reliable determination of photoluminescence quantum yields 47
         3.1.3.2 Dilution studies . . . . . . . . . . . . . . . . . . . . . . . 49
         3.1.3.3 Precipitation studies . . . . . . . . . . . . . . . . . . . . 52
         3.1.3.4 Influence of the solvent . . . . . . . . . . . . . . . . . . 54
   3.2 Modification with DNA molecules . . . . . . . . . . . . . . . . . . . . . . . 56
      3.2.1 Optics . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 56
      3.2.2 Microscopy . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 58
   3.3 Discussion . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 61
## 4 Analysis of the ligand shell structure

- **4.1 Dynamic light scattering**
  - 4.1.1 Theory ........................................... 64
  - 4.1.2 Results and discussion ......................... 67
- **4.2 Nuclear magnetic resonance spectroscopy**
  - 4.2.1 Theory ........................................... 70
  - 4.2.2 Results and discussion ......................... 73
- **4.3 Small-angle X-ray scattering**
  - 4.3.1 Theory ........................................... 79
  - 4.3.2 Results and discussion ......................... 81
- **4.4 Summary and development of a structural model** .......................... 86

## 5 Conclusion and perspectives .......................... 88

## A Experimental section ................................. 91

- **A.1 Materials** ...................................... 91
- **A.2 Syntheses**
  - A.2.1 CdTe ........................................... 91
  - A.2.2 CdHgTe ......................................... 92
  - A.2.3 CdTe/CdS-DNA .................................. 92
- **A.3 Methods**
  - A.3.1 X-ray diffraction and combustion elemental analysis ............. 93
  - A.3.2 Optical spectroscopy ................................ 94
  - A.3.3 Ellman's test .................................... 94
  - A.3.4 Inductively coupled plasma optical emission spectroscopy ........ 95
  - A.3.5 Thermogravimetric analysis .......................... 95
  - A.3.6 Theoretical calculations ............................ 95
  - A.3.7 Dilution experiments ................................ 96
  - A.3.8 Precipitation experiments ............................ 96
  - A.3.9 Quenching and assembly studies of CdTe/CdS-DNA ................... 96
  - A.3.10 Assembly of CdTe/CdS-DNA on DNA origami structures ............ 97
  - A.3.11 Transmission electron microscopy ........................ 97
  - A.3.12 Dynamic light scattering ............................. 98
  - A.3.13 Nuclear magnetic resonance spectroscopy .......................... 98

## Bibliography ........................................... 101

## Danksagung ............................................. 116

## Erklärung ............................................... 119
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-TG</td>
<td>1-thioglycerol</td>
</tr>
<tr>
<td>2-ME</td>
<td>2-mercaptoethanol</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>APDMMS</td>
<td>aminopropyltrimethoxysilane</td>
</tr>
<tr>
<td>ASAXS</td>
<td>anomalous small-angle X-ray scattering</td>
</tr>
<tr>
<td>AUC</td>
<td>analytical ultracentrifugation</td>
</tr>
<tr>
<td>BBQ</td>
<td>BlackBerry®</td>
</tr>
<tr>
<td>CNT</td>
<td>carbon nanotube</td>
</tr>
<tr>
<td>DDA</td>
<td>dodecylamine</td>
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<tr>
<td>DFT</td>
<td>density functional theory</td>
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<tr>
<td>DHLA</td>
<td>dihydrolipoic acid</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOSY</td>
<td>diffusion-ordered spectroscopy</td>
</tr>
<tr>
<td>DTNB</td>
<td>5,5'-dithiobis(2-nitrobenzoic acid)</td>
</tr>
<tr>
<td>EA</td>
<td>elemental analysis</td>
</tr>
<tr>
<td>EDS</td>
<td>energy-dispersive X-ray spectroscopy</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
</tr>
<tr>
<td>EXAFS</td>
<td>extended X-ray absorption fine structure</td>
</tr>
<tr>
<td>FID</td>
<td>free induction decay</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
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<tr>
<td>HDA</td>
<td>hexadecylamine</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HSQC-INEPT</td>
<td>heteronuclear single quantum coherence-insensitive nuclei enhanced by polarization transfer</td>
</tr>
<tr>
<td>HT</td>
<td>hexane-1-thiol</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>inductively coupled plasma mass spectrometry</td>
</tr>
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<td>ICP-OES</td>
<td>inductively coupled plasma optical emission spectroscopy</td>
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<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>LA</td>
<td>lipoic acid</td>
</tr>
<tr>
<td>MD</td>
<td>molecular dynamics</td>
</tr>
<tr>
<td>MPA</td>
<td>mercaptopropionic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NC</td>
<td>nanocrystal</td>
</tr>
<tr>
<td>NIR</td>
<td>near-infrared</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOESY</td>
<td>nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>NP</td>
<td>nanoparticle</td>
</tr>
<tr>
<td>OA</td>
<td>oleic acid</td>
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<tr>
<td>OAm</td>
<td>octylamine</td>
</tr>
<tr>
<td>ODPA</td>
<td>octadecylphosphonic acid</td>
</tr>
<tr>
<td>PAA</td>
<td>poly(acrylic acid)</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>PEO</td>
<td>poly(ethylene oxide)</td>
</tr>
<tr>
<td>PFG-NMR</td>
<td>pulsed magnetic field gradient nuclear magnetic resonance</td>
</tr>
<tr>
<td>PL</td>
<td>photoluminescence</td>
</tr>
<tr>
<td>PMMA</td>
<td>poly(methyl methacrylate)</td>
</tr>
<tr>
<td>QD</td>
<td>quantum dot</td>
</tr>
<tr>
<td>QY</td>
<td>quantum yield</td>
</tr>
<tr>
<td>R101</td>
<td>Rhodamine 101</td>
</tr>
<tr>
<td>R6G</td>
<td>Rhodamine 6G</td>
</tr>
<tr>
<td>rcf</td>
<td>relative centrifugal force</td>
</tr>
<tr>
<td>RF</td>
<td>radio frequency</td>
</tr>
<tr>
<td>ROESY</td>
<td>rotating frame nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>SANS</td>
<td>small-angle neutron scattering</td>
</tr>
<tr>
<td>SAXS</td>
<td>small-angle X-ray scattering</td>
</tr>
<tr>
<td>SFG</td>
<td>sum frequency generation</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TG</td>
<td>thermogravimetric analysis</td>
</tr>
<tr>
<td>TGA</td>
<td>thioglycolic acid</td>
</tr>
<tr>
<td>TNB</td>
<td>2-nitro-5-thiobenzoic acid</td>
</tr>
<tr>
<td>TOP</td>
<td>trioctylphosphine</td>
</tr>
<tr>
<td>TOPO</td>
<td>trioctylphosphine oxide</td>
</tr>
<tr>
<td>trNOESY</td>
<td>transfer nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
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<tr>
<td>vis</td>
<td>visible</td>
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<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
</tbody>
</table>
List of Figures

1.1 Increase of the ratio of surface atoms with decreasing NC size 3
1.2 General components of a colloidal NP 4
1.3 Overview of widely used classes of ligands to stabilize NCs in organic solvents 5

2.1 Chemical structures of thiols widely used as capping ligands 17
2.2 Scheme of the synthesis of CdTe NCs in aqueous medium 19
2.3 Evolution of absorption and emission maxima of TGA-capped CdTe NCs with growth time 20
2.4 Characterization of 2.6 nm sized CdTe-TGA NCs using TEM, XRD, and EA 21
2.5 Decrease of the relative PL QY with decreasing pH for MPA- and TGA-capped CdTe NCs 21
2.6 Overview of the emission of CdTe and CdHgTe NCs 25
2.7 Absorption and emission spectra of TGA-capped CdHgTe NCs taken after different growth times 26
2.8 Temporal evolution of the absorbance maximum during Ellman’s test indicating the binding strength of thiols to the NC 27
2.9 TEM images of CdHgTe NCs obtained after different growth times 27
2.10 PL QY values for different MPA-capped CdHgTe NCs 29
2.11 Growth kinetics for different initial Cd : Hg ratios and different ligands applied 30
2.12 TEM images of CdHgTe NCs prepared applying different initial Cd : Hg ratios 30
2.13 Change of the PL QY and of the emission band of CdHgTe NCs capped with different ligands upon variation of the pD 33
2.14 Schematic of the used approach to functionalize CdTe NCs with DNA molecules 35
2.15 Absorption and emission spectra of CdTe-TGA NCs, CdTe/CdS-TGA NCs, and T1-capped CdTe/CdS-TGA 36

3.1 Chemical reaction scheme and calibration of the Ellman’s test for thiol quantification 40
3.2 Thiol molecules tested to give reliable results in quantitative analysis by Ellman’s test 41
3.3 Results of the TG-FTIR analysis of TGA-capped CdTe NCs and proposed mechanisms for decomposition 45
3.4 Results from MD simulations for rotating ligands on a surface for TGA and the obtained values for TGA and MPA 46
3.5 Calculated number of ligands per NC and ligand density for a ligand mono-
layer depending on the NC radius ................................. 47
3.6 Absorption and emission spectra of adjusted dilutions of R6G, R101, and a QD sample for PL QY determination ................................. 48
3.7 Schematic of the dilution principle ................................. 49
3.8 Absorption and emission spectra of 2.6 nm sized CdTe-TGA NCs and change
of the PL QY with decreasing NC concentration ..................... 50
3.9 Comparison of the concentration dependence of the PL QY for MPA- and TGA-capped CdTe NCs and influence of the initial molar ratios on the PL QY ................................. 51
3.10 Comparison of the concentration dependence of the PL QY for TGA-, MPA-
and GSH-capped CdHgTe NCs ................................. 52
3.11 TEM images of 2.6 nm sized CdTe-TGA NCs of as-prepared sample and after
one and two precipitation steps ................................. 53
3.12 Comparison of the relative PL QY and the amount of ligand upon precipitation
and for differently sized CdTe-TGA NCs ................................. 53
3.13 Different PL QY values of CdTe NCs depending on the solvent show the highest values for solutions of D₂O ................................. 54
3.14 Schematic of the quenching experiment ................................. 56
3.15 Schematic of the assembly experiment ................................. 57
3.16 Emission spectra of differently sized DNA-functionalized CdTe/CdS NCs, their
mixture, and their mixture containing the complementary DNA strand ................................. 57
3.17 TEM images of obtained assemblies from differently sized and DNA-func-
tionalized CdTe/CdS NCs ................................. 58
3.18 Complementary binding of DNA-modified NCs to overhangs of the DNA ori-
gami structure allows the precise assembly of NCs ................................. 59
3.19 AFM images and three-dimensional profile of 3.2 nm sized T1-CdTe/CdS-
TGA NCs assembled on six-helix bundles ................................. 59
3.20 AFM images and corresponding height profiles of single six-helix bundles con-
taining 3.2 nm sized T1-CdTe/CdS-TGA NCs ................................. 60
3.21 Chemical structures of possible Cd-thiol complexes ................................. 63
4.1 Principle of the DLS measurement and data processing ................................. 64
4.2 Intensity distribution from DLS measurements and zeta potential of differently
concentrated, 2.5 nm sized CdTe-TGA NCs ................................. 68
4.3 Splitting of the spin energy levels of the ¹H nucleus in a magnetic field ................................. 70
4.4 Basic pulse sequence for a spin echo DOSY experiment ................................. 72
4.5 ¹H-NMR spectrum of 2.6 nm sized CdTe-TGA NCs in D₂O ................................. 73
4.6 ¹H-NMR spectra of pure TGA, a Cd salt/TGA mixture and CdTe-TGA NCs
and for differently sized NCs ................................. 74
4.7 DOSY spectrum of 2.6 nm sized CdTe-TGA NCs in D₂O ................................. 76
4.8 The $^1$H-NMR resonance peak and peak for the diffusion coefficients obtained from DOSY change with decreasing NC concentration ........................................ 77
4.9 Schematic setup of SAXS measurements .......................................................... 79
4.10 Photograph of the setup and sample chamber for ASAXS measurements at BESSY II ............................................................................................................. 81
4.11 Scattering curves obtained from ASAXS measurements on 2.7 nm sized CdTe-TGA NCs at energies below the Cd-K, Cd-L3 and Te-L3 edge ......................... 82
4.12 Fits of ASAXS data for 2.7 nm sized CdTe-TGA NCs based on a core/shell system and a shell exponentially migrating into the solvent .................................. 83
4.13 Results for the core radius and the particle radius including the ligand shell for CdTe-TGA NCs obtained from SAXS ......................................................... 83
4.14 SAXS measurements of differently sized CdTe-TGA NCs and at different NC concentrations ................................................................. 84
A.1 Chemical structure of DNA containing a sequence of phosphorothioate modification of the DNA backbone ................................................................. 93
A.2 Emission spectra of CdTe-TGA NCs in the presence of EDTA and/or Ellman’s reagent ............................................................................................................. 94
A.3 Chemical structure of the used BlackBerry® Quencher 650 ................................ 96
A.4 Fits for ASAXS data for CdTe-TGA NCs based on core/shell system I, II and a shell exponentially migrating into the solvent combined with core/shell system II 99
List of Tables

1.1 Selection of methods for the analysis of the NC ligand shell ........................................... 10
2.1 Relevant properties of bulk CdTe presenting the basis for NCs with size-tunable emission in the visible spectral region ................................................................. 19
2.2 Lattice parameters in zinc blende crystals and the resulting lattice mismatch ....................... 23
2.3 Characteristics of a set of CdHgTe NC samples obtained after different growth times .......................... 28
2.4 Characteristics of different CdHgTe NC samples synthesized applying varied Cd : Hg ratios ......................... 29
2.5 Characteristics of different CdHgTe NC samples capped by TGA, MPA, and GSH .......................... 31
2.6 Sequences of the DNA molecules used for functionalization of NCs ........................................ 35
3.1 Comparison of results from Ellman’s test with ICP-OES and additional structural information about elemental composition ........................................... 42
3.2 Elemental composition obtained from ICP-OES for CdTe-TGA and -MPA after precipitation and freeze drying ......................................................... 44
3.3 Comparison of values for the ligand density on the NC surface from literature and from the current work ......................... 62
4.1 Change of the zeta potential of CdTe-TGA NCs with the number of precipitation steps ......................... 69
A.1 Comparison of ICP-OES results for diluted and dissolved NC samples of the same concentration ......................................................... 95
1 Introduction

Motivation Modern achievements in material science and technology are driven by the miniaturization and the development of materials that perform more complex and efficient tasks. In the microelectronic industry this is depicted by Moore’s Law predicting the doubling of the number of transistors per integrated circuit every two years, which involves the gradual decrease in size of the transistor based on conventional silicon technology. However, as the transistor size will decrease to edge lengths of a few atoms, silicon technology will soon reach its physical limits and novel electronic architectures and components are in demand. Especially inorganic nanoparticles (NPs) present a perfect candidate as building blocks for new functional elements in computing hardware. Also, the biomedical industry is driven by the resolution of biological processes on the molecular level and the mimicry of complex natural mechanisms on the nanoscale, for which NPs gathered considerable attention. A promising approach to new technologies is presented by the development of nanoscaled materials via the so-called ‘Bottom-up’ approach. The synthesis and use of colloidally prepared NPs evolved as a strong research field in the last decades. Especially semiconductor NPs find their first industrial applications in TVs, e-book readers and notebooks.

In more detail, colloidal semiconductor nanocrystals (NCs), also referred to as quantum dots (QDs) possess size-tunable absorption and emission bands, high emission yields, and superior thermal and photochemical stability compared to those of organic dyes. NCs have attracted a great deal of interest for recent technological developments, such as optoelectronics and electronics, fabrication of light-emitting diodes and composites, color conversion, energy scavenging, optical sensing, and biological imaging, to just name a few. For applications involving near-infrared (NIR) emitting chromophores, semiconductor NCs are ideal systems due to their superior emission yields and became valuable materials for a variety of applications ranging from solar cells, security barcodes, and active materials for telecommunication. NIR emitting NCs are also of interest as fluorescent reporters for bioimaging and as biological sensors due to improved tissue penetration, lower background interference, and reduced photochemical damage.

However, until now, the lack of the complete understanding of parameters influencing their photophysics and its control hampers the routine application of these promising materials. Colloidal semiconductor NCs consist of an inorganic core and an organic shell of stabilizing molecules. On the one hand the nature of colloidal NCs as inorganic-organic hybrid materials offers great flexibility for engineering of properties and the development of novel features from synergistic effects. On the other hand this causes the need for the exact understanding of structure-property relationships, which drives basic research to a detailed understanding of

1. INTRODUCTION
the used materials on the molecular level and the prediction of their behavior in application-relevant environments. In more detail, the development of applications must necessarily follow the understanding of the stabilizer-NC interactions as well as the influence and finally the control of the stabilizer/NC ratio on the properties of the NC. This is similarly true for stability and toxicity tests. Especially the eventually desired use of QDs in e.g. routine assays or the reliable evaluation of their toxicity require the definition of application-relevant NC properties to be always characterized in a standardized way. By the gain of profound knowledge about the structure and the influence of the ligand shell, it will be possible to evaluate semiconductor NCs as new materials for various applications and compare their performance with different alternatives.

The present work contributes to this goal for water-soluble semiconductor NCs. An overview of the topic and already published knowledge about the surface chemistry of semiconductor NCs is given in the subsequent part of the introduction to place the present work into the context of contemporary research. Chapter 2 introduces the three model systems used. CdTe as the best studied water-soluble semiconductor NC material serves as model system for the development of suitable techniques to study the ligand shell. CdHgTe is representative for more complex alloy NCs emitting in the near-infrared optical region. As an example for highly functional systems, deoxyribonucleic acid (DNA)-functionalized CdTe NCs were studied in addition. The following study aims for the investigation of the behavior of the ligand shell upon application-relevant changes in the environment, such as pH, concentration, solvent as well as type and amount of ligand. Along with this, the influence of the ligand shell on stability and optical properties of the NCs is studied. Suitable methods to analyze the amount of ligands and possible binding types as well as to monitor the changes of optical properties are presented in chapter 3. Furthermore, the modification of the ligand shell with single DNA molecules is investigated. In this context, techniques for the characterization of the NC surface chemistry are developed to evidence the achievement and to estimate the degree of biofunctionalization. In chapter 4 advanced methods are presented to clarify the overall structure of the ligand shell leading finally to a structural model for thiol-capped CdTe NCs. These studies provide the basis for a better understanding of the chemical nature of the NC-ligand interface and for the design of highly fluorescent, functional and stable materials.

**The role of the surface chemistry** The strong need to study the surface of semiconductor NCs is further emphasized by the size effects that occur due to the nanoscale dimensions of the inorganic crystals. There are two reasons why nanomaterials show properties different to the bulk, namely quantum size effects and surface effects. Decreasing the size of a NC to sizes smaller than the bulk exciton Bohr radius results in the appearance of the quantum confinement effect, introduced by Ekimov, Efros, and Brus. The confinement of the exciton leads to the evolution of new features in the electronic structure of the semiconductor, which become size- and shape-dependent, such as discrete energy levels instead of energy bands and a size-dependent energy gap. Additionally, surface effects come into play, which are caused
by the fact that atoms at the surface have fewer neighbors than in the bulk, resulting in lower coordination number and unsatisfied bonds. Surface atoms are therefore less stabilized than in the bulk and lead to different reactivities depending on the amount of dangling bonds. The smaller the size of the NPs, the larger is the fraction of atoms at the surface, as depicted in figure 1.1. The graph illustrates that the surface-to-volume ratio scales with the inverse size of the NP. Considering a spherical NP with the radius $r$, the surface area scales with $r^2$. The volume as well as the total number of atoms $N$ is proportional to $r^3$. The fractions of the atoms at the surface is then proportional to the surface area divided by the volume, thus to the inverse radius $r^{-1}$ or considering the number of atoms to $N^{-1/3}$. This scaling effect causes a high percentage of atoms that are located at the surface of small NPs, as shown for cubic and spherical metal NPs. Figure 1.1 shows the scaling law for spherical CdTe NCs with a zinc blende crystal structure and points out that already for NPs with a diameter smaller than 3.3 nm more than one third of all atoms are located at the surface. Surface related properties follow the same scaling law or vice versa all properties that show a linear dependence on $r^{-1}$ or $N^{-1/3}$ are related to the surface effect. Examples are melting points and charging energies of NCs that change proportionally to the reciprocal value of their radii to a first approximation.

The significant contribution of the surface to the total free energy due to the large surface-to-volume ratio makes interactions between the surface atoms and surfactant molecules crucially relevant. As the surface atoms show different reactivities due to different coordination numbers, edge atoms, corner atoms, and atoms of different facets bind foreign atoms or molecules with varying strength. This is also important for catalytic use, as corner atoms show the highest affinity to adsorbates. Finally, the mentioned surface effects on the nanoscale strongly emphasize the significant contribution of the surface to the overall properties and therefore the need to understand the exact influence of the NC’s surface.

\[\text{Figure 1.1: The ratio of surface atoms strongly increases with decreasing size of the NC, which underlines the tremendous effect of the surface chemistry on the overall properties of the material. The data points were obtained from calculation for spherical cutouts of the cubic crystal lattice of CdTe. The lines show the reciprocal fit.}\]
1. INTRODUCTION

Figure 1.2: In general, a colloidal NP consists of an inorganic core and an organic ligand shell. Nature and properties of both parts have substantial influence on the characteristics of the final material.

Colloidal NCs present inorganic core-shell or core only architectures covered with organic surface ligands that are bound to surface atoms either coordinatively or electrostatically or interact with them via van der Waals forces. Figure 1.2 depicts the two components of a colloidal NC. The inorganic core mainly determines the optical properties, such as the spectral position and bandwidth of the first excitonic absorption maximum, the emission band, the fluorescence quantum yield, and fluorescence dynamics. These properties strongly depend on the semiconductor material, actual core size, shape, and dispersity. The combination of different elements in one nanocrystalline material in the form of core/shell, alloyed or doped NCs is an additional way to alter certain optical features. Furthermore, the amount of defects in a NC strongly affects its properties. For various materials, these relations have been extensively studied. However, the surface chemistry also plays an important role, but is often neglected. The organic shell consisting of ligands, also known as capping molecules, stabilizers or surfactants, is defined by the type and amount of the molecule present on the surface of the NC, the way of binding to the NC and the binding strength, as well as the exact structure of the ligand shell. Primarily, the stabilizers determine solubility, stability of the NCs and allow further functionalization and processing. Therefore, the choice of ligand is mainly considered as possibility to control design parameters like the solubility in different matrices and routes to subsequent (bio)functionalization of NCs. In addition, the ligands provide control of the nucleation and growth kinetics, passivate the NC surface by electronic interaction with surface sites and influence the electronic structure and consequently the optical properties. Controlling the NC surface is very challenging, even more than size and dispersity, because it is not that thoroughly studied so far. However, the control of the surface chemistry is at least of the same importance as the control of the particle size, because of the strong influence of the surface-bound ligands. The different ways, how they can influence the overall properties, will be described below.

An overview of frequently used classes of organic molecules for the stabilization of semicon-
1. INTRODUCTION

Figure 1.3: Overview of widely used classes of ligands to stabilize NCs in organic solvents and corresponding popular examples. In most cases, the ligands consist of a polar head group and a non-polar tail enabling binding to the NC and providing solubility, respectively.

ductor NCs in organic solvents including popular examples is depicted in figure 1.3. A detailed description of the basic ligands and their properties can be found in a review by M. Green. In general, these ligands consist of a polar head group that coordinates to metal atoms in solution and at the NC surface and a non-polar hydrocarbon tail, which enables interaction with the surrounding medium, ideally nonpolar solvents. If water is the solvent of choice, short-chain thiols of different valency with various end groups are among the most frequently used ligands. However, the situation in aqueous solution becomes more complex, as it is difficult to distinguish between the part of the ligand responsible for the coordination to the NC and the part enabling the solubility in the polar medium. Additionally, the ligands often possess charged groups, which introduce pH dependencies of the shell structure. For the use of NCs in biological media, water-compatible, short-chain ligands are favorable to minimize the hydrodynamic diameter of the NCs and enable further (bio)functionalization. Especially for Cd-based NCs, thiols are frequently used, as they can passivate electron trap states and thereby enhance the emission. Further details about thiols as stabilizers for aqueous-based NCs are presented in section 2.1.

To point out the relevance of the stabilizing molecule more clearly, the following part will account for the exact role of the ligand shell for the colloidal synthesis, for application-relevant properties and additional surface related features that influence the overall properties of the material. The main and indispensable feature is to provide solubility. The coordinating molecules render particles stable in various solvents by introducing repulsive interactions between the single NCs. These interactions can be classified into van der Waals interactions (steric...
1. INTRODUCTION

stabilization) and electrostatic interactions (charge stabilization). Thereby, the NCs become soluble in different solvents allowing for easy fabrication and processing, at which variations in the surface capping may lead to changes in stability. By their kinetic- and size-limiting functions the ligands influence the nucleation and growth kinetics during the NC synthesis as well as control reaction pathways. The key for a controlled synthesis is the fact that the stabilizing molecule is dynamically adsorbed to the surface of the growing NC. On the one hand the ligand must be mobile enough on the surface to allow the growth of the NC, on the other hand the binding of the stabilizer must be stable enough to provide colloidal stability. A compromise of both allows the ligand to control the NC size, shape and size distribution. The amount of ligand present during synthesis can influence the shape of the resulting NC, as predicted by calculations for oleic acid-capped PbSe. Chiral ligands can induce defects to the inorganic surface layer of the NC resulting in chiral NCs. There are also reports showing the potential influence of the amount of ligand on recrystallization processes and thereby influencing the crystal structure of the inorganic core.

The strong influence of the exact composition of the ligand shell on various properties that are crucial for the applicability of NCs has been reported. A recent review by Hines and Kamat sums up the effects of QD surface chemistry on optical and electronic properties. The fact that ligand exchange can lead to quenching of the emission underlines the strong dependence of the physicochemical properties of the NCs on the ligand structure. The removal of ligands has shown to be responsible for the formation of surface states and thereby quenching of band gap emission. Moreover, the presence of thiols can inhibit as well as favor hole trapping, emphasizing the crucial importance of the position of the energy levels/redox potentials of the thiols and their interaction with charge carriers. Generally, surface atoms, ligand functional groups, and solvent influence nonradiative and radiative relaxation rates. More precisely, the optical properties can be influenced by (i) the organic-inorganic interface, i.e. the nature and stoichiometry of surface atoms at the NC core. It is reported that the surface coverage of NCs by anions or cations affects the emission properties and highly luminescent CdTe NCs possess much fewer Te atoms at the surface than NCs with low luminescence. In addition, (ii) the organic ligand shell considerably affects the optical properties. The electronic structure of the surfactant molecule contributes to the overall electronic and optical profile of the NC affecting the emission yields. It has been shown that the latter strongly depends on the nature as well as on the amount of the surfactant. It can be assumed that changes of the photoluminescence as well as the emission decay behavior directly reflect changes of the surface coverage of such NCs. It was shown that excessive purification leads to the loss of ligands from the surface and consequently to the decrease of the emission yield. Not only the final amount of ligands per NC, but also the initial ratio of stabilizers present during the NC formation can influence the final quality of the material, as shown for the emission yield. Additionally, ligands influence dynamics of the excitonic decay by mediating charge trapping or by participating in charge transfer, which depends strongly on QD size, length of the ligand and number of ligands. Hence, control of the surface chemistry is important for the
design of NCs with bright and stable emission. Also, the surrounding medium can influence the optical properties of NCs, as the solvent can directly affect the extent of ligand ad- and desorption. Impurities in the solvent can cause quenching, and added molecules to the medium may alter the emission intensity depending on their interactions with the ligand. The ligand molecules further enable subsequent functionalization of the NCs, which is in general possible by electrostatic or van der Waals interactions or covalent linking. Attractive aims include the functionalization with e.g., target-specific biomolecules or functionalities to allow the integration into hybrid materials. Also, the identification of valuable ligands for conductive superstructures of NCs came into focus of recent research, as the ligand influences the carrier mobility in NC solids. Additionally, the surface functionalization plays a key role in NC toxicity, which is of great importance for the evaluation of the material. Summing up, the overall quality of the material is influenced by features such as the nature of the ligand including its electronic structure, the amount of the ligand present in the final NC solution and during synthesis, and the degree of surface functionality. Besides the already mentioned aspects, the influence of varying parameters that lead to changes of the ligand shell structure are emphasized below. These include temperature, pH, and ionic strength of the surrounding medium and the concentration of both surfactant and NC in the final solution. Wuister et al. observed the quenching of the emission of water-soluble CdTe NCs upon decreasing the temperature to values below 270 K and assigned the reversibility to solvent induced changes at the surface. Various studies show the influence of pH on NC luminescence and stability of water-soluble cadmium chalcogenide NCs. It is assumed that the influence of the pH is caused by structural changes of the ligand shell, interactions between charged ligands and the electronic structure of the core, dissociation of ligands from the surface due to protonation, counter ion effects, the additional coordination of Cd to carboxyl groups and is most likely the sum of different contributions. A more detailed investigation of the influence of the pH on the properties of different capped CdTe and CdHgTe NCs will be given in chapter. Furthermore, the surfactant layer is very dynamic. This is on the one hand important for successful ligand exchange and NC growth, but on the other hand hampers the prediction of the material’s performance under relevant conditions. There have been quantitative studies on the dynamic nature of surface ligands. These dynamics can result in changes of the ligand shell during storage and the desorption of ligands from the NC surface upon dilution of the colloidal solution. This emphasizes that depending on the binding strength of the ligand to the NC surface atoms, the NC concentration is crucial for the final properties. As dilution can result in ligand desorption, the loss of emission intensity and decomposition of particles can be the consequence. In particular for biological applications, fluorescent reporters are required that are bright and stable in strongly diluted solutions. Moreover, ligand desorption-related changes of the emission yield can correlate with a reduced stability of the NC, possibly resulting in the release of toxic metal ions or anions. Therefore, a better understanding and control of the stability of the ligand shell are of great importance.
1. INTRODUCTION

emission properties, matrix-dependent surface recombination and ligand bonding processes need to be better understood.

Methods used for the analysis of the ligand shell  For the reasons mentioned above, the investigation of the exact surface chemistry of NCs has come in the focus of recent research. The main questions that will be answered by characterizing the ligand shell, concern (i) the identification of constituents of the capping shell, (ii) the amount of ligand molecules bound to the surface, (iii) the exact structure of the ligand shell, and (iv) the binding strength to the surface and the disclosure of possible exchange dynamics. Several reviews tried to answer these questions, such as by Sapsford et al. for surface-functionalized nanomaterials in general. Analytical strategies for the surface characterization of NPs including carbon nanotubes (CNTs) and NCs were reviewed by Zhang et al. A comprehensive study of alkanethiol surface ligands on gold clusters was given by Terrill et al. The review by Morris-Cohen et al. focuses on the analysis of the NC ligand shell and highlights the potential of various spectroscopic techniques, such as nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), optical methods and other analytical techniques. NMR, electron paramagnetic resonance (EPR), FTIR and Raman spectroscopy play an important role in the confirmation of the ligand structure on the NC surface as non-destructive methods. FTIR reveals characteristic resonances of certain functional groups, however, cannot distinguish between free and bound ligands. In contrast, NMR allows the distinction between the two states. In particular, the use of NMR for identification, quantification, and assignment of exchange processes of ligands was intensively studied by Hens and coworkers. Grosso et al. highlight DOSY NMR to probe molecules stabilizing NCs and Hens et al. give a comprehensive overview of the possibilities offered by NMR for the characterization of the NC surface chemistry. A more detailed investigation on these possibilities is given in section 4.2. Unfortunately, these methods are not applicable for every type of ligand and NC, partly demand complex instrumentation, and require high concentrations of materials.

Another non-destructive method for indirect characterization of the ligand shell is presented by optical spectroscopy, such as absorption and emission measurements in steady-state or time-dependent. Valuable features could include spectral shifts upon ligand binding or changes in the emission intensity due to quenching processes or loss of ligand. By time-resolved methods it is also possible to monitor ad- and desorption processes. The chemical composition of the NC-ligand system or solely of the molecules bound to the NCs can be determined by destructive methods, such as elemental analysis (EA) and thermogravimetric analysis (TG). EA includes combustion elemental analysis, inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) for quantification of ligand molecules as well as energy-dispersive X-ray spectroscopy (EDS) for the confirmation of the presence of certain elements. These methods possess the drawback of difficult interpretation for NCs consisting of different elements. TG yields the total mass of a ligand and thermodynamic information about the relative binding strength, however upon perturbing
the equilibrium between free and bound ligand. XPS provides information about the elemental composition and the coordination environment. Extended X-ray absorption fine structure (EXAFS) also allows for the development of structural models of the ligand shell, however requires advanced expertise and instrumentation. Analytical ultracentrifugation (AUC), and chromatographic methods like high-performance liquid chromatography (HPLC) or gel electrophoresis allow for the separation of species according to their overall size and charge and therefore the packing density of the ligand. For NCs stabilized with larger ligands like certain amphiphilic polymers or heterobifunctional poly(ethylene glycol) (PEG), also the overall hydrodynamic radius measured by dynamic light scattering (DLS) and the surface charge via zeta potential measurements can help to understand the ligand shell structure. To interpret experimental data, it is also necessary to compare the results to values obtained from calculations and simulations, such as density functional theory (DFT), molecular dynamics (MD) simulations or ab-initio calculations, which are also a prominent way for characterizing the binding of molecules to NC surfaces. More advanced and very specialized techniques like potentiometry and conductometry for the quantification of functional groups, sum frequency generation (SFG) for the identification of the ligand shell structure and small-angle X-ray scattering (SAXS) for monitoring the formation of hybrid materials, play only a minor role for standard characterization of the ligand shell up to now. Table 1.1 gives examples for the mentioned methods already presented in literature for the NC surface study and illustrates the fact that there is no generally applicable technique. There is a variety of methods that have been used depending on core material, type of ligand, system and solvent studied, and the desired information, like amount of ligand, binding strength, surface stoichiometry, dynamics on the surface and more. For this reason, it is also not possible to give a complete overview of all possible methods to study the surface chemistry, as for every system and question a certain set of characterization techniques needs to be chosen. All approaches have the aim in common to solve the main challenges as to find quantifiable analytical signals and separate those signals for free and bound ligands. An equally important goal is to identify the nature and potential amount of binding sites, which might not be a steady value. The table also shows that many studies have been dedicated to cadmium and lead chalcogenides soluble in organic solvents. However, there is still a lack of understanding and control of the surface chemistry of water-soluble semiconductor NCs. Despite the increasing number of publications on the study of the NC surface, challenges still remain for aqueous systems. This includes reliable and simple analytical methods to identify and quantify surface ligands. Methods that ideally enable discrimination between surface-bound ligands and free ligands are important to study parameters that change the ligand density on the NC surface. This will then allow to derive a relation of surface chemistry and properties of the NC system. However, the proposed dynamic nature of aqueous NC surfaces renders studies of their surface chemistry and its influence on their optical properties complicated. The control of their photoluminescence under application-relevant conditions though is one major goal to reach with a detailed study of the surface chemistry, which is presented in the following.
<table>
<thead>
<tr>
<th>Method</th>
<th>Information</th>
<th>Studied System</th>
<th>Conclusions</th>
<th>Examples</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTIR</td>
<td>identification of functional groups</td>
<td>CdSe-, ZnSe-, ZnS-amines</td>
<td>characterization of surface coverage and ligand bonding</td>
<td>determination of adsorption isotherms and binding constants</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdTe-S-donor ligands</td>
<td></td>
<td>reveal absence of -SH group in the ligand shell</td>
<td>87, 88</td>
</tr>
<tr>
<td></td>
<td>binding information</td>
<td>CdTe-TGA, other thiols</td>
<td></td>
<td>resolution of conformational changes of biomolecules upon adsorption</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Raman</td>
<td>CdTe-TGA, MPA, 1-TG</td>
<td>presence of vibrational modes of CdTe core, interface and carboxylate-metal complexes</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>NMR</td>
<td>CdTe-DDA, ZnO-OAm</td>
<td>trNOESY allows distinction of capping from noncapping ligands</td>
<td>113Cd-NMR for assignment of Cd-rich surfaces</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aqueous CdS</td>
<td></td>
<td>ligand-shell morphology of binary thiol mixtures</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>quantification of ligand, structural model</td>
<td>InP-TOPO, PbSe-OA, TOP, PbS-OA, OAm</td>
<td>identification, quantification of free and bound ligand, equilibrium constant for ad-/desorption</td>
<td></td>
<td>76, 77</td>
</tr>
<tr>
<td></td>
<td>binding information</td>
<td>CdSe-TOP/TOPO</td>
<td>TOPO coordinates Cd surface sites, while TOP coordinates Se</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>ligand binding dynamics</td>
<td>CdSe, CdS, PbSe, PbS-carboxylates, Lewis bases</td>
<td>not only ligand but also NC stoichiometry is dynamic</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdSe-OAm</td>
<td>rapid exchange between bound and free state</td>
<td></td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdSe-OA</td>
<td>surface coverage – PL intensity, determination of kinetic constants</td>
<td></td>
<td>73, 74</td>
</tr>
<tr>
<td></td>
<td>exchange process</td>
<td>CdSe-OA, ODPA</td>
<td>OA binds as oleate ions, two-step exchange, ODPA binds strongly</td>
<td></td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdSe-OA, PbSe-OA</td>
<td>short-chain alcohols strip OA ligands under PL reduction</td>
<td></td>
<td>73, 74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdSe-TOPO, HDA</td>
<td>change of shell composition upon purification, PL QY is determined by combination of the presence of tightly and weakly bound ligands</td>
<td></td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>ligand characteristics</td>
<td>zeolite-APDMMS</td>
<td>determination of pKₘ of bound ligands using ³¹H-NMR</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>EPR</td>
<td>identification of capping</td>
<td>TiO₂-thiols, alanine</td>
<td>presence of charge transfer complex</td>
<td></td>
<td>101</td>
</tr>
<tr>
<td>XPS</td>
<td>binding information</td>
<td>CdTe-TGA</td>
<td>weakly emitting NCs show large amount of Te on the surface</td>
<td></td>
<td>46</td>
</tr>
<tr>
<td>Method</td>
<td>Information</td>
<td>Studied System</td>
<td>Conclusions</td>
<td>Examples</td>
<td>Ref.</td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>-------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>structural model</td>
<td>CdTe-TGA, other thiols</td>
<td>confirmation of presence of CdS shell</td>
<td></td>
<td>89,102</td>
</tr>
<tr>
<td></td>
<td>quantification of ligands</td>
<td>CdSe-Cl&lt;sup&gt;-&lt;/sup&gt;</td>
<td>quantification of chlorine ligands</td>
<td></td>
<td>103</td>
</tr>
<tr>
<td>EXAFS</td>
<td>structural model</td>
<td>CdTe-2-ME</td>
<td>separation of structural and dynamic properties of core and Cd-SR shell</td>
<td></td>
<td>104</td>
</tr>
<tr>
<td>EA</td>
<td>overall composition</td>
<td>CdSe-TOPO</td>
<td>stoichiometry of Cd-rich NCs depends on used TOPO concentration</td>
<td></td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Au-thiols</td>
<td></td>
<td>quantification of ligand-chain length-dependent ligand density</td>
<td></td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>CdSe/ZnS-DHLA-proteins, peptides</td>
<td></td>
<td>dye labeling and gel electrophoresis enable determination of NC loading</td>
<td></td>
<td>107</td>
</tr>
<tr>
<td>TG</td>
<td>quantification of ligands</td>
<td>CdSe/CdS/ZnS-PEG</td>
<td>quantification of native ligands and polymer coating</td>
<td></td>
<td>108</td>
</tr>
<tr>
<td>Optics</td>
<td>quantification of ligands</td>
<td>PMMA/PAA NPs</td>
<td>colorimetric method for quantification of surface carboxyl groups</td>
<td></td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>exchange dynamics</td>
<td>CdTe-OA</td>
<td>ligand removal by pyridine evidenced by time-resolved PL spectroscopy</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>ligand binding strength</td>
<td>CdSe-HDA, TOPO, TOP, HT</td>
<td>time-dependent PL studies allow calculation of surface coverage and binding energies</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>CdSe-alkylamines, -thiols</td>
<td></td>
<td>PL depends on both ligand and NC concentration</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>CdS-OA</td>
<td></td>
<td>concentration-dependent number of surface available adsorption sites</td>
<td></td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>CdSe/ZnS-PEO</td>
<td></td>
<td>PL quenching experiments yield picture of ligand shell’s inner structure</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>PMMA/PAA NPs</td>
<td></td>
<td>porous surface shell morphology from labeling experiments</td>
<td></td>
<td>111</td>
</tr>
<tr>
<td>AUC</td>
<td>ligand densities</td>
<td>CdSe/ZnS-DHLA-PEG</td>
<td>sedimentation velocity data allow calculation of ligand packing density</td>
<td></td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Au-PEG</td>
<td></td>
<td>determination of grafting density</td>
<td></td>
<td>113</td>
</tr>
<tr>
<td>Chromatography</td>
<td>resolution of components in ligand-NC distribution</td>
<td>Dendrimer NP-azide, alkyn ligands</td>
<td>resolution and quantification of amounts and ratios of ligand/dendrimer combinations</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Simulations</td>
<td>indication of binding sites</td>
<td>CdSe-various ligands</td>
<td>DFT study, comparison of binding affinities</td>
<td></td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>and coordinating groups</td>
<td>CdSe-various ligands</td>
<td>ab-initio calculations of binding strength of ligands to different facets</td>
<td></td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>surface coverage</td>
<td>Au-alkanethiols</td>
<td>MD simulations of adsorption process and surface coverage</td>
<td></td>
<td>116</td>
</tr>
</tbody>
</table>

†Abbreviations for ligands can be found in figure 1.3 and 2.1. Not yet defined abbreviations include dodecylamine (DDA), hexadecylamine (HDA), aminopropyldimethoxy-silane (APDMMS), poly(ethylene oxide) (PEO), poly(methyl methacrylate) (PMMA), and poly(acrylic acid) (PAA).
2 Strongly emitting, water-soluble semiconductor nanocrystals

In this chapter the focus is set on the most successful examples of water-soluble, thiol-capped NCs, which are promising for a broad range of nano- and biotechnological applications. The first section is dedicated to the overview of the aqueous synthesis of NCs in general, the resulting optical properties and possible surface functionalities based on state-of-the-art research. The simplest systems suited for such studies are thiol-capped CdTe NCs that can be synthesized with high quality in water. Thereby, this method prevents the need for ligand exchange to achieve water solubility as required for other NCs like CdSe which can be prepared in high quality only in organic solvents. Section 2.2 gives insight into the preparation of CdTe NCs and important aspects about NC growth and stability. This is then extended to more complex alloy CdHgTe NCs. Here, the photoluminescence is mainly influenced by the size and the composition of the NC core, i.e. the Cd : Hg ratio and the different stages of mercury penetration into the CdTe crystal. The latter includes the type of ligand and its binding strength to the surface atoms, the ligand density, and the shell composition. In section 2.3 a detailed investigation of growth kinetics and stability of CdHgTe NCs is presented, with special emphasis on the influence of the initial Cd : Hg ratio and of the ligand chosen. The third model system used for the investigation of the ligand shell is presented by CdTe with a modified ligand shell introducing DNA molecules, see section 2.4.

2.1 State-of-the-art

Colloidal semiconductor NCs with high quality are usually prepared in organic solvents. The wet-chemical synthesis is based on the reaction of organometallic precursors in organic solvents at high temperatures. The so-called hot-injection method yields NCs with a narrow size distribution, good crystallinity, and bright emission and allows for a variety of inorganic shells grown on the core NC to further improve the emission properties. However, the resulting NCs show restricted compatibility with water and/or air, which may be necessary for assays that require an aqueous environment such as biological investigations. While ligand exchange and transfer to water can be applied, it is time-consuming and usually leads to a strong decrease of the emission intensity, and/or strong increase in size due to thick passivation layers from proteins, SiO$_2$, or polymers, which can also restrict the applicability of the NCs. An alternative approach to the widely used organometallic routes is the aqueous based synthesis. This method shows several advantages, as listed below.
The synthesis of colloidal NCs can be performed by applying mild preparative conditions as it does not require high temperatures (> 100 °C).

The green and biocompatible solvent water is employed, which is environmentally friendly and allows easy fabrication and processing in solution avoiding the use of highly toxic organometals.

The cheap, simple, and reproducible synthetic method can easily be scaled up to industrial requirements, as it enables the production of large quantities in one reaction flask.

The aqueous synthesis allows also the preparation of versatile materials in terms of composition, size, shape, and functionalization of NCs via applying an appropriate capping ligand. There are reports on strongly emitting NCs prepared by the aqueous route, which exhibit photoluminescence yields of up to 80 %.

This method, however, still exhibits some drawbacks, such as the fact that the mild synthetic conditions disable perfect structural localization of atoms in the crystal lattice and prevent efficient control of shape and crystal structure, because the temperature is not high enough to overcome the energy barrier for these changes. For example, CdTe NCs synthesized in water show a zinc blende cubic crystal structure and mainly spherical or quasi-spherical shape, since this is thermodynamically the most stable. However, it is reported that one-dimensional CdTe NCs with wurtzite structure can be prepared by partial removal of the ligand accompanied by a phase transition in form of a recrystallization process.

In general, the colloidal synthesis is based on the use of molecules or complexes introducing atomic species necessary for the NC growth, also referred to as precursors. Following their decomposition to reactive species, so-called monomers, the final nucleation and growth of NCs proceeds from the monomers. CdS was the first material synthesized in water, whereas CdTe is the most successful example. The latter exhibits unique optical properties and is already well studied. A number of reviews of the aqueous synthesis of CdTe NCs exist. The reported method facilitates the preparation of CdTe NCs capped by different thiols with diameters up to 6 nm and a photoluminescence (PL) covering most of the visible up to the NIR spectral range with high PL quantum yields (QYs). Details on the synthesis and properties of CdTe NCs are presented in section 2.2. Based on the synthesis of CdTe, approaches have been developed to obtain alloyed material, such as CdHgTe. It needs to be emphasized that up to now, these are the only NIR and infrared (IR) emissive NCs, which can be synthesized in high quality in water, using a simple one-pot reaction. More details on CdHgTe NCs are given in section 2.3. Subsequently, the main aspects of the growth mechanism will be exemplarily discussed for CdTe. Several investigations have been dedicated to the growth process and rate. It was pointed out that the ligand shell plays a prominent role in the growth process and for the PL QY, because the stabilizing molecule influences the activation energy of the CdTe formation reaction by the participation in the transition-state complex. In general, a quick growth yields NCs with low crystallinity and a large number of defects, whereas a
slow growth promotes a high content of sulfur and a higher probability for NC oxidation. The growth rate is supposed to be influenced by electrostatics on the NC surface and the charge of the Cd-thiol complex, whereas the decomposition of the Cd-thiol complexes rather than diffusion of monomers is discussed as the rate-limiting process. The prominent role of the Cd-thiol complexes is further emphasized in studies by Shavel et al., showing the domination of the uncharged Cd-TGA complex in the NC growth and a clear correlation between the concentration of Cd$^{2+}$ as well as thiol and the PL QY by using numerical calculations. Decreasing the TGA : Cd ratio leads to the increase of PL QY of CdTe NCs due to an increase in relative concentration of the uncharged Cd-thiol complex Cd-SR. A competition of two factors during the synthesis is supposed. (i) The surface quality of the NC improves as a result of an increase in the Cd-SR concentration compared to other complexes by the decrease of the TGA amount. (ii) A sufficient amount of stabilizer is necessary to provide stability and surface passivation achieved by an increasing amount of TGA. Experimentally obtained values for optimal ratios are 1.32, 1.30, and 1.20. As the thiol ligand partly decomposes with prolonged reaction time, the incorporation of sulfur into the NC cannot be prevented. However, a sulfur-enriched shell of CdTe NCs is favorable for stability and PL QY .

The most attractive properties of semiconductor NCs are their optical features. Conventional organic fluorophores typically show extinction coefficients of 10,000 - 100,000 M$^{-1}$cm$^{-1}$, emission decay times of 1 - 10 ns, and photobleaching especially at high illumination intensities. On the contrary, NCs present more attractive systems than dyes because of the unique advantages explained below. NCs exhibit size-tunable absorption and emission bands and very long PL lifetimes. Their emission bands are narrow and symmetrical and do not display the long-wavelength tail common to organic fluorophores. NCs possess a much larger absorption cross-section than organic dyes and molar extinction coefficients of 200,000 - 2,000,000 M$^{-1}$cm$^{-1}$. Furthermore, NCs allow the free choice of excitation wavelength as they absorb at all wavelengths shorter than the onset of absorption. Hence, NCs can be used for spectral multiplexing, *i.e.* many colors can be excited at once and can then be spectrally resolved. Their high photostability and the very low rates of photobleaching are also very important for the use in fluorescence microscopy. This is even more pronounced for NIR emitters. For wavelengths $> 750$ nm, organic dyes are only of very limited use as they possess small QY values in the order of maximum 0.20 in organic solvents and 0.04 in water in the wavelength region of 750 to 950 nm and for wavelengths $> 1,000$ nm even $< 0.002$. Additionally disadvantageous are their limited thermal and photochemical stability. For some applications like fluorescence lifetime imaging increasingly used for *in vitro* and *in vivo* imaging studies, their short emission lifetimes (typically $< 2$ ns) can hamper their successful use for time-gated emission and lifetime discrimination. Semiconductor NCs with NIR and IR emission present superior systems, which possess unbeatably high PL QYs. A comprehensive comparison of organic dyes and NCs can be found in the review by Resch-Genger et al.
The optical properties of semiconductor NCs will be discussed in more detail in the following. These properties depend on the unique electronic structure of QDs. They exhibit features that lie in between those of bulk semiconductors and atoms giving rise to discrete energy levels. The separation of occupied and unoccupied states by the band gap, which is typical for semiconductors, becomes size-dependent and increases with decreasing QD diameter. Absorption of light by a QD occurs if the energy of the light exceeds the band gap. The absorption process is followed by the creation of an electron-hole pair and the formation of a so-called exciton. The absorption onset depends on the NC size, as the energy of the excited state decreases as the particle size increases. There have already been few attempts to develop calculations for the NC size from the absorption spectra. A systematic study of the dependence of the optical gap on the NC size gave an empirical relation proposed for CdTe.  

$$d = (9.8127 \cdot 10^{-7})\lambda^3 - (1.7147 \cdot 10^{-3})\lambda^2 + (1.0064)\lambda - (194.84)$$ \hspace{1cm} (2.1)$$

A revised approach by Rogach et al. is based on fitting the sizing curve for CdTe obtained from aqueous synthesis by using a calculation of the 1s-1s transition energy for spherical CdTe NCs using an extended theoretical approach. The extension over the usually applied effective mass approximation additionally considers Coulomb interactions and finite potential walls at the particle boundaries in the surrounding medium. As depicted in equation the band gap of the NC is the sum of confinement energies of electron $E_{e,1s}$ and hole $E_{h,1s}$, the Coulomb energy $E_c$ and the bulk band gap energy $E_{\text{gap, bulk}}$. The size dependence is given by the confinement energies that scale with $1/d^2$ and the Coulomb energy that scales with $1/d$.

$$E_{\text{gap}} = E_{e,1s} + E_{h,1s} + E_c + E_{\text{gap, bulk}}$$ \hspace{1cm} (2.2)$$

The extinction coefficients of colloidal CdTe QDs, which can be determined in this way, are reported to be insensitive to changes in the solvent refractive index or surfactant composition. Contrary, there are reports stating that it is important to keep in mind that the scaling law is not universal for a certain NC core material, but depends on the type of organic ligand shell used. The position of the emission band is also dependent on the QD size, its line width is influenced by spectral diffusion and the size distribution. The energies emitted are smaller than the energy necessary for excitation, the difference is called Stokes shift. After formation of the exciton, relaxation of the latter to a state with a total angular momentum of two occurs upon nonradiative loss of energy. As the transition to the ground state is forbidden, a so-called dark exciton has formed, which is reflected in the typically long emission lifetime. Still, the radiative relaxation can happen by a slight perturbation in the crystal lattice or by phonon coupling. As phonons significantly involve the motion of surface atoms, the presence of ligands strongly influences this process, which partly explains the influence of the ligand on...
Another prominent feature of QDs is their blinking. According to a first intuitive picture, blinking occurs due to Auger photoionization of the NC. In more detail, ionization of the NC by an Auger process can happen if two electron-hole pairs are present at the same time. After ionization, the NC does not emit anymore and occurs to be in the OFF state. If the NC is then neutralized by a second Auger process, the radiative emission can be restored leading to the ON state. More complex theories have also been presented. Thiolates can suppress the blinking by filling trap states. The overall emission intensity is usually evaluated by the assignment of the PL QY. The PL QY $\Phi_f$ is defined as the number of photons emitted $N_{em}(\lambda_{ex})$ relative to the number of photons absorbed $N_{abs}(\lambda_{ex})$, see equation (2.3). Its value can be close to unity if the rate of the radiationless transition to the ground state is much smaller than the radiative decay rate. Many QDs possess rather high PL QYs as the radiative decay is a highly possible relaxation pathway. Especially non-blinking NCs can have a PL QY close to 1. As for QDs with a small PL QY, the dissipation of energy occurs via heat, those NCs can easily lose their stability. The PL lifetime $\tau$ is defined as average time spent in the excited state and corresponds to the inverse of the total decay rate, which is the sum of rates that depopulate the excited state via radiative relaxation $k_r$ and nonradiative relaxation $k_{nr}$. The PL QY is defined as the ratio of the radiative to the overall relaxation rate. In this sense, the PL QY and the measured lifetime are correlated by the radiative decay or the inverse intrinsic lifetime, which is the lifetime of a fluorophore in the absence of nonradiative processes.

$$\Phi_f = \frac{N_{em}(\lambda_{ex})}{N_{abs}(\lambda_{ex})} = \frac{k_r}{k_r + k_{nr}}; \quad \tau = \frac{1}{k_r + k_{nr}} \quad (2.3)$$

Both the PL QY and the lifetime are strongly determined by the number of surface trap states. Since surface atoms have a fewer number of neighbors, they possess unsatisfied bonds, which may introduce energy levels within the band gap of the NC. These surface trap states lead to a strong carrier localization, in other words, charge carriers are likely to be trapped in these states. Consequently, the overlap of the electron and hole wavefunctions nearly vanishes, enhancing nonradiative recombination and energy dissipation in the form of heat by coupling to vibrations. Therefore, it is essential to control the surface quality and eliminate the dangling bonds by overgrowing a shell of a wider band gap semiconductor or coating the surface with suitable ligands. Vice versa, as the emission is strongly dependent on the ligand, the value of the PL QY can be utilized as a direct measure of the quality of the surface shell and passivation. The strong influence of the surface capping on the optical properties has been overviewed in chapter 1. Responsible mechanisms include (i) surface passivation, i.e., shift of the energy of surface states away from the band gap by the formed bond preventing nonradiative relaxation via these states, (ii) surface relaxation and reconstruction, meaning surfactant molecules modifying the surface free energies and thereby hindering or facilitating the reorganization of surface atoms, (iii) carrier trapping, leading to PL quenching as trapping of one carrier precludes radiative recombination of the electron-hole pair, and (iv) orbital mixing by strong electronic
coupling of the organic ligand to the delocalized states of the QD. One prominent way for surface passivation of CdTe NCs is the photochemical treatment of CdTe-TGA under inert atmosphere, which strongly increases the PL QY by formation of a shell of the larger band gap material CdS.

A variety of thiol capping ligands can be applied during the synthesis of semiconductor NCs in water. As explained in more detail in chapter 1, these ligands provide control of the kinetics of the nucleation and particle growth reaction during NC synthesis and passivate the NC surface by electronic interaction with surface sites, provide stability, solubility and functionality.

Some of the typically used thiol ligands for the aqueous synthesis of NCs are displayed in figure 2.1. 1-Thioglycerol (1-TG) and 2-mercaptoethanol (2-ME) were upon the first thiol stabilizers used for CdTe NCs. 2-ME allows the preparation of very small CdTe NCs, however, with weak and broad emission. 1-TG shows additional advantages, such as enabling the synthesis of thiol-capped CdTe NCs in nonaqueous solvents, like dimethylformamide and the preparation of highly luminescent CdHgTe NCs. Also one of the earliest thiols used and by now one of the most widely used ligands for CdTe QDs is thioglycolic acid (TGA). Mercaptopropionic acid (MPA) enables the preparation of large and bright CdTe NCs. Both, TGA and MPA allow the synthesis of very stable aqueous solutions of CdTe NCs, whereas TGA allows spectral tuning between 500 and 700 nm, MPA allows 530 - 800 nm. TGA was additionally applied for a successful one-pot synthesis of strongly emitting CdHgTe NCs. Bidentate ligands are expected to achieve even higher stability and dihydrolipoic acid (DHLA) has been reported as successful example, just as 2,3-dimercapto-1-propanol. 3-Mercaptobutyric acid was designed to foster highly emitting CdTe NCs due to a change of secondary coordination, which is typically observed for mercaptocarboxylic acids by introducing the methyl group as a side chain and optimizing the chain...
Mercaptosuccinic acid combines TGA- and MPA-like features and enables a fast growth of CdTe NCs. In contrast to the previous examples that cause negative charge at the NC surface, 2-(dimethylamino)ethanethiol and cysteamine can be used to prepare positively charged NCs. 2-(Dimethylamino)ethanethiol was used to stabilize CdTe NCs that were assembled into nanosheets. Cysteamine is potentially useful for bioconjugation as it allows for the covalent attachment via amide bond formation. Further biocompatible ligands are given by tiopronin, useful for the preparation of CdTe/CdS core/shell NCs, glutathione (GSH), which is a suitable ligand for NCs used for cell imaging, and L-cysteine enabling conjugation to biomolecules. More complex thiol-ligands are thiolated polyethylene glycol methyl ether (mPEG-SH), which create amphiphilic character of the CdTe NCs, and 5-mercaptomethyltetrazole allowing for the reversible formation of 3D networks of CdTe NCs. Depending on the ligand applied, also the growth rate differs. For CdTe it was found that MPA stabilization ensures very fast growth achieving the largest CdTe NCs, because the stability of the Cd(MPA) complex is lower than for Cd(TGA) enabling a faster release of monomers and faster growth for MPA-capped CdTe NCs. There is also an increasing use of NCs in (bio)analytical detection, because they present excellent candidates for biosensing and as biological labels for fluorescence imaging. Therefore, the need for sufficient functionalization promoted the development of several possibilities of attaching various biomolecules to the NC surface. The secondary functionalization of NCs with biomolecules is feasible via (i) covalent binding, such as covalent attachment to present ligands (e.g. via amide bond formation) or replacement of thiols by thiolated biomolecules, and (ii) non-covalent attachment, like electrostatic association of e.g. a polymer or hydrophobic interactions. In the present work, DNA was chosen as example for the functionalization with biomolecules and the appearing challenges to detect small concentrations thereof, see section 2.4.
2. STRONGLY EMITTING, WATER-SOLUBLE NANOCRYSTALS

2.2 Colloidal synthesis of CdTe nanocrystals

The tremendous development in the field of thiol-capped, water-soluble CdTe NCs is illustrated by the publication of at least a thousand of papers, dozens of patents, several books chapters and even a complete book solely on this material. In the current work, CdTe NCs were chosen as model system for the study of the surface of water-soluble NCs, not only because they have received a large amount of attention due to their strong PL in the visible region, but also because many aspects influencing their optical properties such as reaction conditions and NC core structure are well studied already. Some important properties of the material itself are given in table 2.1. The band gap of bulk CdTe of 1.53 eV limits the wavelength to which the emission of the NCs can be tuned, whereas the exciton Bohr radius provides efficient quantization within the particles smaller than this value.

Table 2.1: Relevant properties of bulk CdTe presenting the basis for NCs with size-tunable emission in the visible spectral region.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band gap energy in bulk CdTe at 300 K</td>
<td>$E_g = 1.526$ eV</td>
</tr>
<tr>
<td>Exciton Bohr radius in bulk CdTe</td>
<td>$a_B = 7.3$ nm</td>
</tr>
</tbody>
</table>

Figure 2.2 depicts the approach for the aqueous synthesis of CdTe NCs used in this work. Briefly, a Cd salt is dissolved in water together with the thiol used for NC stabilization. The variety of thiols that can be applied, was already shown in figure 2.1. Upon adjusting the pH, Cd$^{2+}$-thiolate complexes of various composition form. This is often accompanied by the appearance of a turbid solution because of the low solubility of the uncharged 1 : 1 complex of Cd$^{2+}$ and TGA$^{2-}$. By the decomposition of Al$_2$Te$_3$, H$_2$Te is formed and injected into the well deaired Cd$^{2+}$-thiolate solution leading to the formation of precursors containing Cd$^{2+}$, Te$^{2-}$ and thiolates. Nucleation and growth of the NCs then proceed upon heating to reflux.
under open air conditions. Based on the comprehensive studies on the influence of reaction conditions on the final NC quality, the optimal values were chosen, which are the molar ratio $\text{Cd} : \text{Te} : \text{thiol} = 1 : 0.5 : 1.3$ and a pH of 12. It is important to note that the ligand is present during the synthesis and can decompose or react with metal ions in several ways, which makes the assignment of the final structure of the NC surface more complicated. Especially, the potential presence of Cd-thiolate complexes also in the ligand shell will be discussed in detail in section 3.3. The formation of a CdS shell or CdTe NCs with a sulfur-enriched shell due to the decomposition of thiols with prolonged boiling is also possible, but the extent strongly depends on reaction conditions and ligands applied. However, for large NCs obtained after long growth times, the incorporation of sulfur into the NC cannot be prevented. The growth rate is also influenced by the ligand. Figure 2.3 shows the absorption and emission spectra of a set of TGA-capped CdTe NC samples with growth times ranging from 5 to 120 min giving rise to emission maxima from 529 to 575 nm. The shift of the emission maximum to larger wavelengths clearly indicates the growth of the NCs. As already described in section 2.1 it is possible to determine the size of the NCs from the wavelength of the absorption maximum. Based on the sizing curve and the fitting function including the calculation of the transition energy, the NC diameter $d$ was determined and is in the following referred to as NC size. For the exemplary set of samples shown in figure 2.3 this results in sizes of 2.1, 2.3, 2.4, 2.5, and 2.7 nm, respectively.

Figure 2.4 shows the characterization of TGA-capped CdTe NCs by transmission electron microscopy (TEM), X-ray diffraction (XRD), and combustion elemental analysis (EA). TEM images reveal CdTe NCs with high crystallinity, quasi-spherical shape, and core sizes comparable to the results obtained from absorption spectra. XRD analysis shows the good agreement of the measured pattern with expected reflections for cubic CdTe, which points to the absence of a significant amount of sulfur in the crystalline NC core. This is also supported by the determination of the elemental ratios using organic combustion, yielding a molar ratio of $\text{C} : \text{H} : \text{S}$ of approximately 2 : 3 : 1, which is expected for the deprotonated form of TGA having the
2. STRONGLY EMITTING, WATER-SOLUBLE NANOCRYSTALS

<table>
<thead>
<tr>
<th>element</th>
<th>wt%</th>
<th>molar ratio</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>7.029</td>
<td>2</td>
</tr>
<tr>
<td>H</td>
<td>0.872</td>
<td>3</td>
</tr>
<tr>
<td>S</td>
<td>8.753</td>
<td>0.9</td>
</tr>
</tbody>
</table>

table

Figure 2.4: Characterization of 2.6 nm sized CdTe-TGA NCs using TEM (left), XRD (middle), and combustion EA (right). The lines in the XRD pattern show the reflections for cubic CdTe with their relative intensities.

chemical formula of $\text{C}_2\text{H}_3\text{O}_2\text{S}$. It has to be emphasized that the investigated CdTe NCs were obtained after one hour of growth time. The short reaction time seems to permit a minimized amount of sulfur incorporated into the CdTe NCs and was therefore favorable for the subsequent studies of the influence of the ligand shell on optical properties. For samples obtained after longer boiling time it has to be kept in mind that this situation can be different. Still, in both cases strongly emitting CdTe NCs can be obtained.

As depicted in figure 2.5 the PL intensity of thiol-capped CdTe NCs strongly depends on the pH. The quenching of the emission upon decreasing pH may be caused by decomposition of metal-thiolate complexes due to protonation of the carboxylate group and agglomeration of the NCs by the change of the overall charge of the NC. In the literature different observations are presented, which point to the presence of several processes that lead to pH-induced changes on the NC surface. On the one hand a PL enhancement upon decreasing the pH is stated.\[34,62,63]\ Supposed reasons include structural changes of the ligand shell by the formation of a shell of Cd-thiol complexes\[62]\ and secondary coordination of the carboxyl oxygen to pri-

Figure 2.5: The PL QY is decreasing with lowering the pH for similarly sized MPA- and TGA- capped CdTe NCs in solutions of comparable concentrations. The PL QY values are given relative to the highest PL QY of the respective sample. The dotted lines are only a guide to the eye.

\[pH\] titrations were performed by R. Schneider (BAM Berlin).
mary thiol binding Cd,[63] both leading to PL enhancement. Another explanation is based on the high concentration of thiolates in basic solutions, which can act as hole acceptors and trap charge carriers thereby quenching the PL.[34] On the other hand several studies show the decrease of PL intensity at low pH values due to aggregation of NCs.[64-66] Explanations include the detachment of ligands at acidic conditions, i.e. the protonation of the sulfur atom at low pH values, followed by the loss of coordination to the NC, the dissociation of the ligand from the surface, the loss of stability and precipitation of the NCs.[64] This is accompanied by the formation of surface defects leading to shortened PL lifetimes at low pH.[66] The aggregation was observed to correlate with the pKₐ of the ligand[63] and the size of the aggregates increases with decreasing pH.[66] Basic conditions, however, improve the dispersion of NCs. In either case the pH-dependent PL shows the potential of the use of water-soluble NCs as pH sensors.[60] A comprehensive study on the pH-dependent PL of CdTe NCs by Xu et al. summarizes the various effects and shows the interplay between two main trends leading to a maximum PL at a certain pH.[67] Decreasing the pH starting from basic solutions leads to a decrease of electrostatic repulsion between free and bound ligand. Consequently, more free ligands can diffuse into the NC ligand shell and thereby improve the PL. By further decreasing the pH the reduced electrostatic repulsion of the NCs itself leads to aggregation and loss of stability leading to a decrease of PL intensity. The optimal pH value mainly depends on the pKₐ value of the applied thiol ligand. Our observations show a pH of 8 as optimum for the PL of MPA-capped CdTe NCs. Additional effects influencing the overall charge of the NC include secondary coordination of the ligands and counter ions that adsorb or penetrate into the ligand shell. Overall, a better stability for MPA-capped CdTe NCs than for TGA-capped ones is observed. However, from the smaller pKₐ values for TGA one would expect an influence of the pH on the PL QY to occur at lower pH values for TGA than for MPA. As the pKₐ value correlates with the amount of protonated and deprotonated form of the thiol, the disagreement suggests that not only the protonation of the ligand causes the pH-dependent PL. Additional effects can include a better secondary coordination by MPA and consequently a more stable ligand shell, which is less sensitive to the pH or the presence of a larger amount of sulfur incorporated into the CdTe NC, especially at the surface, due to minor stability of MPA against decomposition during the synthesis. This example underlines the fact that the gain of more information about the ligand shell of water-soluble NCs is necessary for the understanding of changes of optical properties. A detailed analysis of amount and structure of the ligand shell is shown in chapter[3] and [4].
2.3 Colloidal synthesis of CdHgTe nanocrystals

A comprehensive review on narrow band gap semiconductor NCs has recently been published by the group of Rogach. The most popular representatives are lead chalcogenides, which are usually prepared in organic solvents, and require either ligand exchange or encapsulation for the use in water. Currently, the only NIR emitting colloids that can be synthesized in sufficient quality directly in water are CdHgTe NCs. This, in conjunction with the possibility to tune the optical properties not only by size, yet also by material composition, renders these materials very attractive for all applications of NIR emitters in aqueous environments and for applications imposing size restrictions or requiring reporters of identical size, but with different optical properties. The development of NIR emitting water-soluble NCs is based on well-studied CdTe NCs and the idea to extend the emission of CdTe to longer wavelengths by alloying CdTe with a material possessing similar lattice parameters, as shown in table 2.2, but a narrower band gap. The tendency of HgTe/CdTe heterostructures to undergo interdiffusion has been well known. As the work on colloidal HgTe NCs showed the successful synthesis in water, however with poor stability, the work on CdHgTe NCs was initiated. The earliest work on CdHgTe NCs aimed originally for core-multishell heterostructures by cation exchange, but the authors observed alloying of the metal cations at least to some extent. The procedure is based on the incorporation of Hg$^{2+}$ ions into preformed CdTe NCs and the involvement of several steps of growing shells and alloying. However, this method yields NCs with limited tunability of the PL band and emission maxima of up to 1100 nm with PL QYs of 0.4 could be reached at maximum. In the following, a direct one-step synthesis of CdHgTe NCs was developed. Initially prepared CdHgTe NCs possessed PL maxima only up to 830 nm and showed improved photostability when capped by a CdS shell. Further developments allowed the synthesis of NCs with a PL peak wavelength tunable up to 1150 nm and narrow emission bands. The authors also investigated the internal structure of the prepared CdHgTe NCs. It is stated that for similar metal ion starting concentrations, nucleation and growth will be faster for HgTe at the beginning due to the 20 times lower solubility of HgTe compared to CdTe. In this way the final structure of the NC is not completely alloyed, but more a quasi core/shell architecture with Hg-rich cores and Cd-rich shell regions. Lesnyak et al. developed the aqueous one-pot synthesis even further and could obtain TGA-

<table>
<thead>
<tr>
<th>Lattice parameters in zinc blende crystals at room temperature and the resulting lattice mismatch.</th>
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</thead>
<tbody>
<tr>
<td>CdTe</td>
</tr>
<tr>
<td>HgTe</td>
</tr>
<tr>
<td>0.2% lattice mismatch</td>
</tr>
</tbody>
</table>

1Parts of this section have already been published - Reproduced by permission of The Royal Society of Chemistry
capped CdHgTe NCs covering the emission range of 640 - 1600 nm. Despite the addition of only small amounts of Hg precursor, a significant decrease of the band gap could be achieved. The study suggests the same growth kinetics as for CdTe NCs and shows a smaller Cd content in the final NCs than in the feed ratio, as also observed by Sun et al. accompanied by a decrease of the Cd content with NC growth due to the lower solubility of HgTe. Besides CdHgTe QDs, also the preparation of CdHgTe nanorods based on the partial cation exchange in CdTe nanorods has been reported together with the assumption that redistribution of cations leads to CdHgTe alloys rather than layered structures. The synthesis of hydrophobic CdHgTe NCs was achieved by ion exchange of organically synthesized CdTe NCs and yields NCs with reasonably narrow emission bands. The detailed analysis of the mechanism of the exchange process states a molecular ‘welding effect’, by which NC join together forming alloy NC wires with inhomogeneous distribution of cations. A similar observation of the formation of alloyed NC wires was made for the aqueous ion exchange synthesis of CdHgTe NCs followed by ligand removal by precipitation and the attachment of the NCs to chains. In addition to the study of the exact core structure, there are also publications dedicated to the importance of the surface for CdHgTe NCs. To name a few examples, Wang et al. presented CdHgTe/gelatin nanospheres with enhanced photostability and weakened dependence of the emission on pH. Another work shows the possibility to tune the dipole moment and thereby enhance the performance of a photovoltaic device by surface modification. In summary, the preparation of CdHgTe NCs by ion exchange allows band gap engineering by compositional tuning and enables a detailed investigation of composition-dependent change in optical properties, whereas the one-pot synthesis yields highly emitting, and stable NCs with emission covering both important telecommunication windows at 1330 and 1500 nm. In this respect, based on the recently reported simple one-pot reaction approach of Lesnyak et al. to the aqueous synthesis of CdHgTe, in this work CdHgTe NCs with different precursor ratios, varied growth time, and three thiol ligands, i.e. TGA, MPA, and GSH were prepared. The influence of growth time and initial Cd : Hg ratio is investigated as well as the influence of the ligand on the growth kinetics and the optical properties of the resulting colloids is assessed. In general, the preparation of CdHgTe NCs is realized analogous to the presented CdTe synthesis. Besides Cd(ClO$_4$)$_2$·6H$_2$O, Hg(ClO$_4$)$_2$·6H$_2$O is introduced to the precursor solution by which a co-precipitation of Cd and Hg with Te can occur. By the addition of only small amounts of Hg relative to the amount of Cd, it is possible to shift the emission of the NCs to significantly higher wavelengths. Figure 2.6 gives an overview of the emission of CdTe NCs and CdHgTe NCs prepared within this work. Whereas CdTe NCs show narrow emission bands located in the visible range, CdHgTe NCs emit in the NIR and exhibit slightly broader PL bands. The larger full width at half maximum (FWHM) for CdHgTe NCs points to a broader size distribution, which can be observed from TEM images as well. Besides TEM, the CdHgTe samples were characterized by elemental analysis, and optical spectroscopy. EDX measurements during TEM analysis show the presence of Cd, Hg and Te in the NC film on the grid, as well as sulfur. Notably, for ternary systems such as CdHgTe it is very challenging
Figure 2.6: Overview of the emission of CdTe and CdHgTe NCs prepared within this work covering the visible and NIR spectral region.

to obtain samples that provide reasonable comparability. Their emission properties depend on the one hand on the size and the Cd : Hg ratio. On the other hand the chemical composition of the ligand shell and the presence of a sulfur-enriched surface shell influence the stability and PL of the NCs.

The main challenge for NIR and IR emitting NCs is to reliably evaluate their PL QYs. In this spectral region, there is only a small number of reference dyes available, which possess mainly low PL QYs and thereby increase uncertainties of relative measurements. Recently there has been progress on the development of absolute PL QY measurements allowing reliable evaluation. Still, the stated values for IR emitters in literature have to be treated carefully and partly demand critical reevaluation. More details about reliable PL QY measurements are discussed in section 3.1.3.1. Measured time traces with the setup for absolute QY measurements reveals high photostability of the CdHgTe NCs and exclude photodecomposition and photobrightening for excitation with intense laser diodes. Furthermore, PL QYs of CdHgTe NCs were shown to be independent of the excitation wavelength within the present uncertainties, see figure 2.10. Importantly, H₂O absorbs strongly in the NIR and IR spectral region starting from approximately 1150 nm. To avoid interference of the solvent absorption with features of the sample, solvents with lower absorption have to be used. Here, D₂O was used as solvent for samples with emission exceeding this value.

**Influence of growth time** Figure 2.7 depicts the change of absorption and emission features of TGA-capped CdHgTe NCs with growth times ranging from 5 min to 6 h. In comparison to CdTe NCs, the absorption maxima are less distinct. For both absorption and emission bands a shift to longer wavelengths is observed with proceeding reaction time due to a decrease of the band gap. However, to assign this effect to an increase in size and/or a change in composition, the growth process of CdHgTe NCs needs to be better understood. Assuming the decomposition of the metal-thiol complexes as rate-limiting process of the growth, the different complexation constants of the respective stabilizing thiols and Cd²⁺ and Hg²⁺ become impor-
important. For example, with 11.02 the log K value of Cd\(^{2+}\)(GSH)\(^{3-}\) is significantly smaller than that of Hg\(^{2+}\)(GSH)\(^{3-}\) reaching 27.36, which underlines the strong binding of GSH and mercury and suggests different growth kinetics for GSH-capped CdTe and CdHgTe NCs.\(^{194,195}\) To further emphasize such effects, Ellman’s studies with CdTe and CdHgTe colloids were performed. The Ellman’s assay is described in detail in section 3.1.1.1. During the colorimetric test, the NCs dissolve in the presence of EDTA and Ellman’s reagent and release all thiol ligands, which are then quantified photometrically by the reaction with Ellman’s reagent yielding a yellow colored molecule with an absorption maximum at 412 nm. The process of dissolution and reaction with the analyte, i.e., the evolution of the complete absorption at 412 nm, takes a certain time. This information can be used to compare the influence of the Hg-ligand and Cd-ligand binding constants on the reaction kinetics for CdTe, CdHgTe and different thiols. The results from Ellman’s studies are displayed in figure 2.8. A comparison of CdTe and CdHgTe stabilized by MPA reveals slower kinetics for CdHgTe. The slower formation of the product of the reaction of MPA and Ellman’s reagent reflects the stronger binding of MPA to CdHgTe compared to CdTe due to the presence of Hg(II) ions. Similar effects were observed for other thiols. There was no sign for the presence of two distinguishable particle-ligand bonds (Cd-thiol and Hg-thiol) in the CdHgTe particles, as the reaction progress is not directly related to the Hg content measured by ICP-OES. Screening experiments also showed that Hg-thiol complexes alone are not the reason for the different kinetics, as addition of Hg(ClO\(_4\))\(_2\) : 6 H\(_2\)O to CdTe particles lead to completely different spectra and kinetics of the Ellman’s reaction than for CdHgTe. This implies that the overall strength of the NC-ligand bond is greater for CdHgTe.

TEM images of CdHgTe NC samples taken after different growth times during one synthesis are displayed in figure 2.9. The images show an increase in size with prolonged growth, the determined values are given in table 2.3. Additionally, high-resolution TEM images reveal high crystallinity of the NCs. Because of the close similarity of the lattice parameters for CdTe
and HgTe, as shown in table 2.2, it is not possible to elucidate the exact internal structure, i.e. core/shell vs. alloyed. In fact, the distances of the lattice planes measured from high-resolution TEM images of CdHgTe NCs yield values of approximate 0.35 nm, which is an average from the expected values for the (111) crystal lattice planes of CdTe (0.37 nm), CdS (0.32 nm), HgTe (0.37 nm), and HgS (0.34 nm). This observation suggests that a certain amount of sulfur is present in the CdHgTe NCs. Elemental analysis by ICP-OES, displayed in table 2.5, also supports this assumption. The different Te content results from the varied growth times and consequently, the different times for Te oxidation. Moreover, the Te content of all samples is relatively small. This could imply that a certain amount of sulfur is incorporated into the NCs and also, that Cd$^{2+}$ and Hg$^{2+}$ ions are part of the ligand shell coordinated by thiolates. Similar results were observed and deeply investigated in the case of equally stabilized CdTe NCs. The value for the sulfur content in table 2.5 includes the sulfur content of the NC core as well as the contribution from all ligands present at the NC surface and free in solution. Comparison of the results from ICP-OES for a series of CdHgTe NCs from one synthesis with different growth

Figure 2.9: TEM images of CdHgTe NCs obtained after different growth times as presented in table 2.3 show the increase in NC size with prolonged growth (from left to right).

†Measurements of the kinetics of the Ellman’s assay were performed by R. Schneider (BAM Berlin).
Table 2.3: Characteristics of an exemplary set of CdHgTe NC samples obtained after different growth times.

| CdHgTe-MPA, Cd : Hg : Te : MPA = 0.98 : 0.02 : 0.75 : 1.3 |
|----------------|----------------|----------|----------|--------|
| growth time    | $\lambda_{1s-1s, \text{max}}$ | size     | final Cd : Hg | PL QY  |
| 3 min          | 650 / 742 nm   | 3.5 ± 0.4 nm | 97.3 : 2.7 | 0.53   |
| 20 min         | 750 / 830 nm   | 4.1 ± 0.5 nm | 98.7 : 1.3 | 0.70   |
| 75 min         | 810 / 918 nm   | 4.8 ± 0.6 nm | 99.2 : 0.8 | 0.70   |

times, displayed in table 2.3, yields different Hg contents with a lower Hg content observed for larger NCs. One possible explanation is that Hg is preferentially incorporated into the particles during the initial stage of seed formation due to lower solubility of HgTe than CdTe in water. In this case, particle growth at later stages proceeds through incorporation of mainly cadmium ions. This is contrary to the previously reported increasing Hg content with NC growth, but is supported by the observed formation of a quasi core/shell structure for CdHgTe NCs, i.e., Hg-rich core with a Cd-rich shell. Here it is worth mentioning that ICP-OES determines the overall composition and not solely the core composition as Hg$^{2+}$ ions can also be part of the ligand shell or free in solution. The contrary findings may be caused by different sample preparation or surface coating.

Also the PL QY changes during the growth of the NCs as a result of varying size and Hg content. Results for a set of samples with different size and Hg content are displayed in figure 2.10. The PL QY was determined for the different MPA-capped CdHgTe NCs at different excitation wavelengths relatively and absolutely. The obtained values of absolute and relative measurements agree reasonably well and are independent of excitation wavelength. A trend of a decreasing PL QY with increasing size and/or Hg content can be observed. One unsettled explanation could be an increasing number of defects introduced in larger NCs or by a larger number of Hg$^{2+}$ ions, favoring emission quenching. Another reason could involve the dependence of nonradiative transitions on the energy difference. Assuming localized defect states independent of the band gap, a slower trapping rate for large band gap QDs, which are smaller in size, is expected as it was shown for PbS and PbSe QDs. Another cause may be the presence of a larger amount of Hg$^{2+}$ ions on the surface and the presence of electron transfer processes from MPA to Hg$^{2+}$ or aggregation due to striping the ligand of the surface by complexation to Hg$^{2+}$. The latter was reported for CdTe NCs used as Hg sensors, which also show quenching of the emission proportional to the Hg content. The samples presented in table 2.3 show an increase in PL QY with increasing reaction time. This observation suggests that for NCs in this size regime the effect of the NC size on the PL QY plays only a minor role and the PL increase can be explained by the decrease in Hg content.

MPA-capped CdHgTe NCs of different sizes were prepared by Dr. V. Lesnyak (Chair of Physical Chemistry, TU Dresden).
2. STRONGLY EMITTING, WATER-SOLUBLE NANOCRYSTALS

Figure 2.10: PL QY values for differently sized MPA-capped CdHgTe NCs obtained from different syntheses displaying various Cd : Hg ratios. The values were obtained via absolute (down triangle) and relative (up triangle) determination. Reproduced by permission of The Royal Society of Chemistry

In summary, a prolonged reaction time during the synthesis of CdHgTe NCs leads to an increase in size and a decrease in Hg content. This is accompanied by the red-shift of absorption and emission maxima and a change of the PL QY.

Influence of initial Cd : Hg ratio To get more insight into the influence of the Hg content and possible ways to control the amount of Hg in the NCs, the influence of the ratio of Cd : Hg applied for the synthesis is studied. Different samples were prepared using different initial Cd : Hg ratios, but keeping constant other parameters such as the choice of ligand. However, after the same reaction time the samples differed in final composition and size, see table 2.4 indicating that the different initial ratios cause different growth conditions. The left graph in figure 2.11 depicts the varied growth rates for different Cd : Hg ratios. In this case, the growth rate is defined as the change of the emission maximum. Notably, the fastest rise of long-wavelength emission is present for the highest amount of Hg applied in the synthesis. Similar absorption and emission maxima of approximate 940 and 1050 nm can be obtained.

Table 2.4: Characteristics of different CdHgTe NC samples synthesized applying varied Cd : Hg ratios but the same time of growth. The optical data were obtained from purified samples measured in D₂O.

<table>
<thead>
<tr>
<th>initial Cd : Hg</th>
<th>λ₁s−₁s,max, λem,max</th>
<th>size</th>
<th>final Cd : Hg</th>
<th>PL QY</th>
</tr>
</thead>
<tbody>
<tr>
<td>98 : 2</td>
<td>951 / 1084 nm</td>
<td>6.3 ± 0.6 nm</td>
<td>98.3 : 1.7</td>
<td>0.51</td>
</tr>
<tr>
<td>95 : 5</td>
<td>998 / 1130 nm</td>
<td>6.1 ± 0.7 nm</td>
<td>97.0 : 3.0</td>
<td>0.37</td>
</tr>
<tr>
<td>90 : 10</td>
<td>999 / 1171 nm</td>
<td>7.1 ± 1.1 nm</td>
<td>97.4 : 2.6</td>
<td>0.32</td>
</tr>
</tbody>
</table>

The PL QY measurements were performed by S. Hatami (BAM Berlin).
2. STRONGLY EMITTING, WATER-SOLUBLE NANOCRYSTALS

Figure 2.11: Growth kinetics represented by the change of emission maxima with boiling time for different initial Cd : Hg ratios (left) and different ligands applied (right). The data were obtained from as synthesized samples in H$_2$O, which were prepared using the molar ratios of (Cd+Hg) : Te : MPA = 1 : 0.5 : 1.3 (left) and Cd : Hg : Te : thiol = 0.95 : 0.05 : 0.75 : 1.3 (right). Comparison of the black curves shows a faster growth for NCs obtained from a smaller amount of Te present in the precursor solution. The dotted lines are only a guide to the eye. Reproduced by permission of The Royal Society of Chemistry.

after 1 h for an initial Cd : Hg ratio of 98 : 2, 30 min for 95 : 5, and 15 min for 90 : 10. This emphasizes the influence of the initial precursor ratio on the growth kinetics. Comparison of the samples obtained after 2 h of growth time again shows the smallest band gap for the sample obtained from the highest initial Hg content. However, similar to previous observations, the change of the band gap seems to be a complex interplay between NC size and final composition. No clear correlation of the NC size or final composition to the initial Hg content can be assigned here. TEM images again show highly crystalline QDs, however for CdHgTe NCs obtained from large initial Hg ratios with not very uniform shapes, see figure 2.12. Notably, the non-spherical shapes hamper the exact evaluation of the NC size.

Not only the initial ratio of Cd and Hg influences the growth of the NCs, but also the amount of Te applied for the synthesis. Comparison of the black curves in figure 2.11 yields the influence of the Te content. The CdHgTe NCs obtained from a smaller Te : cation ratio, shown in the left

Figure 2.12: TEM images of CdHgTe NCs prepared applying different initial Cd : Hg ratios as presented in table 2.4 with increasing initial Hg content (from left to right).
graph, exhibit a faster shift of the emission to longer wavelengths than in the case of a larger ratio, depicted in the right graph. Elemental analysis suggests that the larger final amount of Hg in the NCs is the reason for the smaller band gap.

**Influence of ligand** As the synthesis of CdHgTe NCs according to Lesnyak *et al.\(^{133}\) is very versatile, the suitability for different thiol ligands is shown for TGA, MPA, and GSH in this work. These ligands present three of the most popular stabilizers used for CdTe NCs, as shown in figure 2.1 and explained in section 2.1 in detail. TGA and MPA were chosen as these ligands yield the highest PL QY for CdTe NCs and GSH can be advantageous to improve the compatibility with biological media. Special emphasis is dedicated to the influence of the surface ligand on NC quality, PL QY, and NC stability. All synthetic key parameters such as temperature, initial molar ratios of Cd, Hg, Te, and thiol, reaction volume, and pH were kept constant to ensure identical growth conditions. However, the use of different ligands causes variations in growth mechanisms. Therefore, it is not possible to synthesize samples with different stabilizers that have exactly the same size and composition after a constant growth time. For ternary systems, many parameters influence the optical properties of the resulting material, which makes a proper comparison of differently prepared, i.e., here differently stabilized, colloids very challenging. Hence, to minimize the number of variables and to allow a proper comparison, for each ligand, a set of samples was prepared with the aim to obtain samples with similar photoluminescence features. In the right graph of figure 2.11, the evolution of the emission maxima with reaction time is compared for the three different ligands. As for CdHgTe NCs not only the growth in size, but also the incorporation of Hg has an impact on the emission maximum, the kinetics are different compared to those observed for CdTe colloids.\(^{132}\) Here, TGA-capped CdHgTe NCs give the fastest shift to long emission wavelengths. MPA-capped CdHgTe NCs can reach similar red emission maxima, although after a longer reaction time. However, complexes formed by Hg and GSH are so stable that the growth of NCs from Hg-GSH monomers is strongly slowed down and a limit of incorporation of mercury seems to be reached. Even after long reaction times, no NCs with emission maxima exceeding 850 nm could be obtained, yet the samples became unstable and aggregated.

<table>
<thead>
<tr>
<th>ligand</th>
<th>growth time</th>
<th>(\lambda_{1s-1s,max})</th>
<th>(\lambda_{em,max})</th>
<th>size</th>
<th>final Cd : Hg : Te : S</th>
<th>PL QY</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGA</td>
<td>45 min</td>
<td>864 / 1050 nm</td>
<td>4.2 ± 1.3 nm</td>
<td>97.6 : 2.4 : 35.1 : 134</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>MPA</td>
<td>75 min</td>
<td>880 / 973 nm</td>
<td>5.0 ± 1.2 nm</td>
<td>99.2 : 0.8 : 14.9 : 96</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>270 min</td>
<td>730 / 784 nm</td>
<td>5.8 ± 1.7 nm</td>
<td>99.5 : 0.5 : 6.0 : 119</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\)CdHgTe NCs with different ligands were prepared by Dr. A. Dubavik (Chair of Physical Chemistry, TU Dresden).
Taking this into account, the comparison is based on the stage of growth of the different CdHgTe NCs, as this parameter is known to be directly related to their quality and their PL QY. The samples at the beginning of the growth plateau are expected to be well comparable due to the completed phase of a pronounced change in PL and the accomplished growth. Thus, samples after the first strong rise in emission wavelength were chosen for further comparison. The samples investigated in the following are TGA-capped CdHgTe NCs taken after a reaction time of 45 min, MPA-capped CdHgTe NCs after 75 min, and GSH-capped CdHgTe NCs after 270 min, respectively, and their characteristics are summarized in table 2.5. TEM analyses reveal different sizes of the CdHgTe NCs, with the largest sizes resulting for the GSH-capped NCs. Effects influencing the Hg content may origin from the different cation-thiol binding strengths leading to an enhanced Hg incorporation for TGA-capped CdHgTe NCs compared to GSH, for which the release of Hg monomers is slowed down due to the greater stability of cation-thiol complexes. Nevertheless, for MPA- and TGA-capped NCs, these differences in size and composition result in NCs with similar absorption maxima. Importantly, also the PL QYs differ for the three samples. MPA-capped CdHgTe NCs reveal a promisingly high PL QY of 0.45, which is superior to any NIR emitting organic dye and among the highest PL QY values reported for such NCs in water.

Influence of pH The suitability of the ligands for surface stabilization of CdHgTe was additionally evaluated by the comparison of pH-dependent changes of the absorption and emission features. In the left part of figure 2.13, the changes of the PL QY with variation of pD are shown for differently capped CdHgTe NCs. In general, the pD is the analog of the pH in D₂O solutions, but differs in value due to the different equilibrium constants of H₂O and D₂O. It is determined from standard measurements with a glass electrode in D₂O solutions according to the equation below:

\[ pD = pH_{\text{reading}} + 0.40 \]  

The values are normalized to the PL QY at pD = 10 for better comparison. In general, the behavior is similar for all three ligands, which is a decrease of the PL QY at pD values smaller than 7. For TGA-capped CdHgTe the emission is completely quenched at pD = 5 and strongly diminished for MPA-capped CdHgTe. The smallest changes occur for GSH, as a slighter pD dependence is expected from the presence of both amino and acid groups. The right part of figure 2.13 shows the spectral changes of the emission upon variation of the pD for MPA-capped CdHgTe NCs. By decreasing the pD to values below 7, the emission intensity decreases strongly concomitantly with a shift of the emission maximum to longer wavelengths. Additionally, the FWHM decreases at pD values below 7. This observation suggests the precipitation of small NCs at lower pD, which is reasonable as due to the larger surface-to-volume ratio for
smaller NCs changes of the surface chemistry effect their stability to a greater extend. Changes at the NC surface may include protonation of the thiol ligand and consequent desorption from the NC surface leaving behind a destabilized ligand shell and surface traps. Absorption measurements indicate colloidally stable NCs at pD values higher than 5.7, whereas for smaller pD values, scattering becomes clearly visible indicating the onset of particle aggregation. This supports the thesis that certain NCs become instable and precipitate. A similar behavior can also be observed for the other samples as well as for CdTe NCs, as described in section 2.2.

*pD titrations were performed by R. Schneider and S. Hatami (BAM Berlin).*
2. STRONGLY EMITTING, WATER-SOLUBLE NANOCRYSTALS

2.4 DNA-modified CdTe nanocrystals

The functionalization of CdTe with single-stranded DNA molecules was used as model system for NCs with complex surface chemistry. The investigation of influence, amount and exact type of binding is especially challenging for a small number of molecules attached to the previously stabilized NCs. Because of the possibility to use DNA as building block for the precise assembly of nanostructures, we chose this specialized functionalization of CdTe NCs. NC-biomolecule architectures have attracted great attention in bioanalysis for biological imaging, labeling and sensing. Various approaches exist for biofunctionalization of NCs offering a palette of hybrid systems. Especially nucleic acid conjugated NCs have come into focus of research because of two main areas for their application. On the one hand, the general use of DNA-based nanostructures is an attractive choice for sensing and detection of biomolecules, mainly in the form of electrochemical and optical sensors. In particular, the use of QDs for sensing is advantageous compared to organic fluorophores, because QDs exhibit superior optical properties as described in detail in section 2.1. QD-DNA systems are also interesting for bioimaging and -labeling, like tumor targeting, for detection of DNA molecules, as barcodes for antibody detection, and for nucleic acid diagnostics. The other large field, for which DNA-functionalization has gathered attention, is the possibility of programmable assembly and precise positioning of NCs, which becomes especially important for the miniaturization of electronic components and the need to assemble functional building blocks precisely on the nanoscale. This has been extensively studied for metal NP-DNA hybrids and includes examples like the assembly into photonic nanolenses, chiral plasmonic nanostructures, nanoantennas, and single-electron transistors. Successful examples for programmed structures can be also given for QD-DNA structures, including electrostatically assembled chains, photoactive films, assembly into superlattices or complex aggregates. Additionally, compared to other directed self-assembly approaches DNA-mediated assembly offers the possibility to switch between ordered and non-ordered state by changing the temperature. Besides the use of DNA as template for complex structures, it is also a tunable spacer and a useful tool for the investigation of distance-dependent interactions of NPs.

One prominent way to achieve direct functionalization of NCs with single-stranded DNA molecules is the incorporation into the ligand shell. There are reports on the direct use of DNA as stabilizer in a one-pot synthesis enabling the tuning of the optical properties of NCs by DNA structure and sequence. The DNA molecules applied consist of a ligand domain used for NC passivation, if necessary a linker, and a recognition domain to bind to biomolecular partners. By changing the length of the ligand domain it is possible to precisely tune the number of DNA molecules attached to one QD. However, the combination of functional and stabilizing group in one molecule that is rare on the surface might be problematic for stability and functionality. Another way includes an additional conjugation step to previously synthesized QDs, for example by using thiolated DNA molecules added to the native
Figure 2.14: Schematic of the used approach to functionalize CdTe NCs with DNA molecules. Strong attachment of the DNA molecules is achieved by the incorporation into an additional CdS shell.

ligand shell, which enables water-solubility. Here, the number of DNA molecules attached and the functionalization yield can be controlled by the conjugation conditions, such as pH, ionic strength and DNA concentration.\textsuperscript{235} Other approaches using different linker strategies\textsuperscript{212} or biotin/streptavidin interactions\textsuperscript{236} suffer from high complexity, less stability and unspecified binding sites. The following chapters will illustrate that the surface of water-soluble NCs is very dynamic and consists of a complex network of metal-thiolates, which are loosely bound. Due to this finding a functionalization route was chosen based on strong attachment of the DNA via incorporation into an additional inorganic shell.\textsuperscript{237} In particular, water-soluble CdTe NCs are coated with a CdS shell in the presence of either thiolated DNA or DNA containing a sequence of phosphorothioate modification of the DNA backbone. The chemical structures are presented in section A.2.3. Upon decomposition of CdS precursors, which consist of a Cd\textsuperscript{2+}-salt and thiols such as TGA or MPA, CdS is deposited on the CdTe surface. The DNA molecules are directly incorporated into the CdS shell. This leads to a strong attachment of the single-stranded DNA molecule to the CdTe NC, as schematically depicted in figure 2.14. Table 2.6 lists the DNA structures used for QD functionalization. A1 - C1 comprise of a sequence of five thymine linked by phosphorothioates (denoted as Z) that enable binding to the NC at the 5’ end and 20 bases to allow specific binding to the complementary DNA strand. Contrary, T1 allows binding of the QD at the 3’ end via the lipoic acid (LA) functional group at the end of the DNA molecule. T2 and A2 were chosen to vary the functionalization side and thereby to

<table>
<thead>
<tr>
<th>Table 2.6: Sequences (5’ → 3’) of the DNA molecules used for functionalization comprising of the nucleobases adenine (A), guanine (G), thymine (T), cytosine (C), thymine with a phosphorothioate linker unit (Z), and the functional group lipoic acid (LA).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A1</strong></td>
</tr>
<tr>
<td><strong>B1</strong></td>
</tr>
<tr>
<td><strong>C1</strong></td>
</tr>
<tr>
<td><strong>T1</strong></td>
</tr>
<tr>
<td><strong>T2</strong></td>
</tr>
<tr>
<td><strong>A2</strong></td>
</tr>
</tbody>
</table>
enable the formation of assemblies from differently functionalized CdTe NCs by the addition of the complementary DNA strand or the assemblies of NCs with quenching units. The diverse functionalizations will be discussed regarding the suitability for different characterization assays in section 3.2. The amount of CdS precursors added was calculated for a shell consisting of four monolayers CdS, see section A.2.3. As depicted in figure 2.15 for three sizes of TGA-capped CdTe NCs, the presence of a CdS shell is indicated by changes in absorption and emission spectra in the form of a slight shift of the absorption and emission maxima to higher wavelengths. This shift is expected due to the increase of the NC size and the formation of a CdTe/CdS core/shell structure. However, the observed shift is smaller than expected for four monolayers CdS, which indicates an incomplete shell growth. One reason could be that the thiols are not reactive enough as sulfur source upon heating to 90 °C, which may be improved by using light for thiol decomposition. Importantly, the additional conjugation with DNA molecules does not lead to changes of the QD-specific optical features. The absorption peak at 260 nm, which is specific to DNA, is not observable due to superimposition with the strong absorption of NCs at small wavelengths. Similar results were also obtained for the functionalization of MPA-capped CdTe NCs and do not depend on the DNA used for functionalization. Systematic studies were performed to probe the influence of different buffers, pH, and salt concentrations on stability and emission of the NCs modified with DNA. In general, we do not observe a different behavior compared to pure thiol-stabilized CdTe NCs. However, to reduce the amount of DNA per experiment low NC concentrations are needed. The strong dilution can lead to the decrease of stability and emission intensity, as will be shown for thiol-capped CdTe NCs in section 3.1.3.2. Consequently, a skillful choice of concentrations and purification steps is crucial for a successful preparation of highly functional, emitting CdTe NCs.
2. STRONGLY EMITTING, WATER-SOLUBLE NANOCRYSTALS

2.5 Summary

In summary, CdTe NCs present a well-studied and highly luminescent material, which is promising for a broad range of applications. The synthesis in water is a straightforward way to prepare high quality NCs without the need for high reaction temperatures. The evolving properties of the crystalline material of nanometer size are superior to those of organic fluorophores and the palette of possible functionalities introduced by various ligands is broad. Besides a widely developed understanding of the role of the ligand during the synthesis, little is known about the impact of the ligand on optical properties and the exact structure of the ligand shell. The pH-dependent PL QY underlines the strong influence of the surface ligand on the optical properties, which will be discussed in detail in the next chapter. Studies to elucidate the ligand shell structure are presented in chapter 4.

Besides NCs emitting in the visible spectral region, NIR emitting NCs have gathered a lot of attention. As cubic CdTe and HgTe have nearly identical lattice constants but very different band gap energies, the preparation of NIR emitting CdHgTe NCs is possible in a straightforward one-pot synthesis. Already small amounts of Hg incorporated into CdTe NCs allow for emission in the NIR and the possibility to cover the important low-loss fiber windows at 1300 and 1550 nm. The feasibility to synthesize CdHgTe NCs directly in water and the chance to tune the optical properties of the material not only by NC size and ligand, but also by NC composition presents special advantages of CdHgTe NCs compared to other NIR emissive QDs. Importantly, the presented study reveals insights into the three different ways how to tune the emission of CdHgTe NCs, the growth time, the initial Cd : Hg ratio and the ligand, which all influence the growth rate. With ongoing reaction, the size of the NCs increases accompanied by a decrease of Hg content. Different initial Cd : Hg ratios lead to varied growth kinetics.

However, to assign a clear correlation between changes of the emission and the Hg content or NC size demands further investigations. When comparing different ligands for CdHgTe NCs, MPA shows to yield highly emitting NCs. TGA allows a good Hg incorporation leading to strong shifts of the emission to the NIR. In contrast, GSH exhibits strong Hg complexation and the prepared NCs show a less pronounced pH dependency. The general pH dependency of the PL QY is similar as observed for CdTe NCs. The extensive study presented contributes to the goal of enabling the rational design of bright and stable NIR emissive NCs for a broad range of applications. Further studies may include the investigation of the internal distribution of Cd and Hg in one NC and its influence on the overall properties. This is especially challenging because of the small size of the NCs and the large amount of organics on the NC surface, for which elemental mapping with TEM may reach its limits. However, it would contribute to the detailed understanding of the growth mechanism of CdHgTe NCs and more synthetic control of the final properties.

As NCs are excellent candidates for bioimaging and -sensing, the biofunctionalization of QDs is of great importance. The functionalization of thiol-capped CdTe NCs with different DNA molecules is presented with the aim to develop suitable methods for the characterization of
the modified ligand shell. NCs are superior to organic dyes, but open the challenge to control the amount of DNA per NC and to achieve a reliable ratio of 1:1. Therefore, the exact understanding of binding and suitable characterization is necessary, which will be discussed in section 3.2.
3 Analysis of the amount of ligands and its influence on optical properties

The present chapter confronts the question how the amount of ligand bound to CdTe NCs can be determined, how the ligand shell reacts to dilution, purification or changes of the solvent and to which extent it thereby influences the stability and PL QY of the final NC solution. Analytic techniques such as a colorimetric test and elemental analysis (see section 3.1.1) together with theoretical calculations (see section 3.1.2) help to get a picture of the ligand shell composition for MPA- and TGA-capped CdTe NCs. Studies of the optical properties of CdTe and CdHgTe NCs upon dilution, precipitation and changes of the solvent show the influence of changes on the NC surface on the overall properties (see section 3.1.3). Additionally to the characterization of short-chain thiol ligands, the modification of the surface with DNA molecules is studied regarding the success of functionalization and the used optical and microscopic methods will be discussed in section 3.2.

3.1 Short-chain thiol molecules

3.1.1 Analytics

3.1.1.1 Ellman’s test

In a first attempt, the concentration of thiol molecules in the NC solution was determined by a colorimetric assay, the Ellman’s test. The use of a water-soluble aromatic disulfide for the straightforward quantification of sulfhydryls in biological materials from absorption measurements was introduced in 1959 by G. L. Ellman.\textsuperscript{238} Since then, the so-called photometric Ellman’s assay has become a well established and widely used method for the analysis of thiol groups in proteins, because of its speed of analysis and simplicity.\textsuperscript{239} In this work, the principle has been adapted to quantify the thiols present in the NC solution and finally to determine the amount of thiols bound to NCs.

According to figure 3.1, the Ellman’s reagent, 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB), reacts with the thiolate anion to form a mixed disulfide and the yellow colored dianion of 2-nitro-5-thiobenzoic acid (TNB\textsuperscript{2–}). As the molar absorption coefficients of the educt, DTNB, and the product, the mixed disulfide, in the visible region are very small, the absorbance at 412 nm can be directly used as a measure of the molar amount of the formed byproduct, TNB\textsuperscript{2–}. Since the\textsuperscript{50,178} Parts of this section have already been published.\textsuperscript{239} Reproduced by permission of The Royal Society of Chemistry
reaction of one thiol gives one equivalent of TNB$^{2-}$, the change of the absorbance at 412 nm correlates with the thiolate concentration in solution.

Here, an excess of DTNB was used for all measurements to assure complete reaction with thiols. Additionally, EDTA was added to protect the thiol groups from oxidation and to avoid interference from metal ions, here Cu$^{2+}$ A pH of 7.2 was used to improve the accuracy of the measurement, as suggested by Riddles et al., who showed a slower decomposition of DTNB at pH values closer to 7 and therefore, better suitability for long reaction times, which were necessary for TGA Control experiments with pH 8 did not give different results, but lead to a reduced stability of the Ellman’s reagent. At pH 8, the stock solution of the Ellman’s reagent stored at room temperature in the dark revealed a noticeable change in absorbance at 412 nm already after 24 hours, whereas solutions with a pH of 7 show similar changes only after 1 month at the earliest.

For the calibration of the Ellman’s test, freshly prepared cysteine solutions were used and measured right after mixing with Ellman’s reagent. The resulting spectra and an averaged calibration curve are shown in figure 3.1. The study of the temporal evolution of the absorbance at 412 nm revealed that for a complete reaction with TGA, longer reaction times with DTNB are necessary. Therefore, all samples were left in the dark for 1 hour prior to measurement. Taking this into account, validation of the test with aqueous TGA solutions of defined concen-
3. ANALYSIS OF THE AMOUNT OF LIGANDS

Figure 3.2: Thiol molecules tested to give reliable results in quantitative analysis by Ellman’s test.

Concentrations yielded experimental errors within 5%. Also other short-chain thiols were tested to give reliable quantitative amounts by Ellman’s test, see figure [3.2]. Notably, mercaptosuccinic acid, 2,3-dimercapto-1-propanol, and meso-2,3-dimercaptosuccinic acid yield two TNB$^2$– per molecule as they possess two thiol groups that can react with Ellman’s reagent. This has to be taken into account for the exact interpretation of the results obtained from Ellman’s test.

In a following step, the analysis was applied to NC solutions, which dissolve in solutions of EDTA and Ellman’s reagent allowing for the analysis of all thiols present. More details about the dissolution of the NCs in the presence of DTNB and EDTA are given in section A.3.3. The main challenge to overcome here is the fact that the Ellman's test determines the overall thiol concentration in solution and cannot discriminate between surface-bound and free TGA. In order to obtain the concentration of the NC-bound TGA ligands, we performed several precipitations of the NCs by addition of a non-solvent, like ethanol or iso-propanol, and subsequently separated the NCs with their surface-bound ligand shell from the free thiols. In this context, we studied the influence of ethanol and iso-propanol on the Ellman’s reaction and found that iso-propanol interferes strongly with the assay, whereas ethanol does not. Consequently, ethanol was used for the separation of NCs and free thiols. Analysis of the thiol concentration in a solution of redissolved NCs and the supernatant obtained from precipitation gave the same amount of TGA molecules as in solution prior to precipitation within the experimental error. The decrease of the ligand coverage after precipitation was measured, besides others, for 2.6 nm sized TGA-capped CdTe NCs using ethanol, yielding 2443 TGA molecules per NC for the as synthesized solution, 988 TGA per NC after one purification step and 305 TGA per NC after a second precipitation step. These results were obtained from the analysis of redissolved NCs. As follows from the determined values, by each precipitation step, approximately two thirds of the initial thiol molecules can be removed. Assuming the precipitate gives the amount of thiol, which was attached to the NC in the system before precipitation, a first purification step yields NCs with approximately 300 thiols attached. As the concentration of CdTe NCs is rather low for this example, the large excess of thiol present during the synthesis allows for the formation of a large ligand shell. Analyses of different TGA-capped CdTe NC samples were performed and the results from Ellman’s test were compared with values from elemental analysis of NCs (same batches), namely ICP-OES yielding concentrations of Cd, Te, and S. Table 3.1 summarizes the results for sulfur for four representative examples of two different
3. ANALYSIS OF THE AMOUNT OF LIGANDS

Table 3.1: Comparison of results from Ellman’s test with ICP-OES values, which are in reasonable accordenece. The data correspond to the sum of free thiols and thiols attached to the NC in a sample after one purification step. Additional structural information is obtained from ICP-OES for the TGA-capped CdTe samples yielding concentrations of Cd, Te, and S.

<table>
<thead>
<tr>
<th>CdTe-TGA sample</th>
<th>L/NC (Ellman’s test)</th>
<th>L/NC (ICP-OES)</th>
<th>Relative difference</th>
<th>Cd : Te : S c(NC)</th>
<th>c(NC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 2.6 nm</td>
<td>761</td>
<td>782</td>
<td>3 %</td>
<td>1 : 0.11 : 1.10</td>
<td>0.16 mM</td>
</tr>
<tr>
<td>2.5 nm</td>
<td>837</td>
<td>750</td>
<td>12 %</td>
<td>1 : 0.07 : 1.15</td>
<td>0.02 mM</td>
</tr>
<tr>
<td>B 2.6 nm</td>
<td>122</td>
<td>98</td>
<td>24 %</td>
<td>1 : 0.46 : 0.59</td>
<td>0.52 mM</td>
</tr>
<tr>
<td>2.4 nm</td>
<td>56</td>
<td>67</td>
<td>16 %</td>
<td>1 : 0.47 : 0.60</td>
<td>1.10 mM</td>
</tr>
</tbody>
</table>

In summary, here, it was shown that the Ellman’s assay is applicable for a first estimation of the amount of thiol ligand present in the NC surrounding. In the literature, the use of this test for a reliable quantification of protein sulfhydryls has been questioned due to steric or electrostatic inhibition. Similar effects may also be present for the analysis of NCs and may be responsible for differences with ICP-OES, but can be tolerated for a quick and easy method.

3.1.1.2 Inductively coupled plasma optical emission spectroscopy

ICP-OES is an analytical technique for the quantification of elements according to their emission intensity from excited states formed in an inductively coupled plasma. Usually, the sample preparation includes decomposition of the solid material followed by strong dilution in H2O. However, primary studies on the necessity of decomposition of precipitated CdTe-TGA NCs showed that solely dilution of the NC solution works as well. ICP-OES measurements give comparable results for simply diluted NC samples and dissolved ones using aqua regia, for which details are given in section A.3.4. Due to the straightforward sample preparation by dilution and the risk to lose material by the formation of gaseous byproducts during the treatment with aqua regia, for the subsequent studies the analysis was performed from diluted

† Samples of set B were prepared by Dr. V. Lesnyak (Chair of Physical Chemistry, TU Dresden).
3. ANALYSIS OF THE AMOUNT OF LIGANDS

samples. To ensure complete analysis, the samples were freshly prepared directly before the analysis to avoid aggregation of the NCs, which can be caused by strong dilution, as described in section 3.1.3.2.

As shown in the previous section, ICP-OES has been used for comparison with results from Ellman’s assay. Additionally, information on the structure of the ligand shell was gathered from elemental analysis, see table 3.1 here from the concentrations of Cd, Te, and S. Especially for the samples of set A a large excess of Cd and S is present. From theoretical calculations, described in section 3.1.2, we found that also for Cd-rich, faceted NCs the ratio of Cd : Te in this size regime is 1 : 0.2 maximum. The Ellman’s assay already showed good correlation of the sulfur content with the amount of thiols present. Additionally, to exclude the possibility that the presence of variably thick CdS shells causes the differences in composition, TEM investigations were performed and show similar sized NCs for samples A and B. Along with similar absorption and emission features (see figure 3.8 and discussion in section 3.1.3.2), these findings allow to conclude that variably thick CdS shells are not the reason for the compositional differences. Consequently, the excess of Cd suggests the presence of Cd-thiolate complexes in the ligand shell. Again, this is more distinct for the samples of set A. In the case of set B the elemental composition fits well to the theoretical considerations, from which the presence of large amounts of Cd-thiols can be excluded. On the contrary, the results can also point to the presence of Te on the surface. This can be explained by comparing the concentrations of NCs formed during the synthesis. The smaller the concentration of NCs, the more Cd-thiolates are present yielding a thicker ligand shell. This assumption is supported by precipitation experiments. Upon NC precipitation, the excess of Cd and S compared to the Te content decreases, thereby pointing to the loss of Cd-thiolate complexes. Table 3.2 gives values for two examples of TGA- and MPA-capped CdTe NCs. The molar ratios are given relative to the amount of Cd, whereby the decreasing concentration of Cd and thiol with the number of precipitation steps is depicted by the apparent increase of the Te : Cd ratio. The number of ligands per NC was calculated using the NC concentration determined from absorption spectra and assuming a contribution of sulfur only from the ligand. Importantly, the samples were obtained after a short time of growth, which reduces the probability of incorporation of sulfur, as shown in section 2.2. The distinct loss of Cd and S in the first precipitation step is explained by the removal of unreacted species from the solution. For both, TGA and MPA ligands, further precipitation leads to a loss of ligand, which can be assigned primarily to the separation of free and bound ligand. Additionally, the loss of Cd suggests that ligand species that are removed by precipitation also contain Cd. The effect of freeze drying as mild alternative to remove the solvent and possibly free ligands was also studied. The analysis by ICP-OES mainly yields the loss of sulfur suggesting that drying leads to the loss of volatile species from the solution like free thiol molecules. The amount of removed thiol is less for freeze drying than for precipitation, which could be explained by the presence of an equilibrium between bound and free thiol that is considerably affected by precipitation. Further discussion about the influence of precipitation on the ligand shell and optical properties will be given in section 3.1.3.3.
Table 3.2: ICP-OES analysis shows the effect of precipitation and freeze drying on the elemental composition of 2.5 nm sized CdTe-TGA and 2.3 nm sized CdTe-MPA. For the synthesis an initial ratio of Cd : Te : thiol = 1 : 0.5 : 1.3 was applied. The number of ligands per NC was calculated assuming a contribution of S only from the ligand and using the NC concentration determined from absorption spectra. The molar ratios of the freeze dried samples were obtained from dissolved NCs using aqua regia.

<table>
<thead>
<tr>
<th>Sample</th>
<th>in solution</th>
<th></th>
<th>after freeze drying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd : Te : S</td>
<td>L/NC</td>
<td>Cd : Te : S</td>
</tr>
<tr>
<td>CdTe-TGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as synthesized</td>
<td>1.00</td>
<td>0.02</td>
<td>1.54</td>
</tr>
<tr>
<td>1st precipitation</td>
<td>1.00</td>
<td>0.07</td>
<td>1.15</td>
</tr>
<tr>
<td>2nd precipitation</td>
<td>1.00</td>
<td>0.10</td>
<td>0.96</td>
</tr>
<tr>
<td>CdTe-MPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as synthesized</td>
<td>1.00</td>
<td>0.07</td>
<td>2.31</td>
</tr>
<tr>
<td>1st precipitation</td>
<td>1.00</td>
<td>0.28</td>
<td>1.36</td>
</tr>
<tr>
<td>2nd precipitation</td>
<td>1.00</td>
<td>0.34</td>
<td>1.35</td>
</tr>
</tbody>
</table>

3.1.1.3 Thermogravimetric analysis

Thermogravimetric analysis (TG) is capable of the discrimination of free ligands and those adsorbed to the NC by measuring different volatilization temperatures. Analysis of the mass loss upon heating allows the quantification of ligands bound to NCs. Additional information that can be provided by TG include the assignment of relative binding or adsorption energies for different ligands. In general, the method is based on the measurement of weight changes of bulk samples in dependence on changes in time and/or temperature. Therefore a typical TG setup consists of a high precision balance and a programmable furnace with a thermocouple to accurately measure the temperature. By this, physical and chemical transitions of the sample can be monitored, such as vaporization, sublimation, desorption, decomposition, oxidation and more. TG has found many applications in analytical chemistry and has already been used to characterize a variety of nanomaterials.

For a 3 nm sized CdTe NC, which consists of approximately 420 atoms and is capped by 300 TGA molecules, as determined by the presented methods, one third of the overall mass originates from the ligand shell. As this should be detectable with TG, measurements on CdTe-TGA NCs were performed to quantify the amount of ligand and to estimate the binding strength. Direct measurements of NC solutions were not valuable as the amount of analyzable substance after evaporation of the solvent was to small to observe reliable mass changes, even for highly

‡Freeze drying and subsequent sample preparation for elemental analysis was performed by S. Hatami (BAM Berlin).
3. ANALYSIS OF THE AMOUNT OF LIGANDS

Figure 3.3: Results of the TG-FTIR analysis of 2.7 nm sized TGA-capped CdTe NCs from dried powder (left). The black curve shows the TG signal, which corresponds to the mass loss in percent. The first derivative thereof is shown by the DTG curve, which is depicted in gray. The red and blue graphs show the intensity of the FTIR signals of H$_2$O and CO$_2$, respectively. Several proposed reaction pathways for the observed decomposition of ligand are sketched (right).\textsuperscript{1}

concentrated solutions. Therefore, the NC samples were freeze dried and the NC powder was investigated. All TG measurements were performed under inert atmosphere. The analysis of pure TGA gives a strong peak at 154 $^\circ$C accompanied by a mass loss of almost 100 $\%$, which indicates evaporation of TGA. In contrast, TG spectra of CdTe-TGA NCs show no peak at the expected temperature, which indicates that the ligands are bound in the ligand shell and cannot simply evaporate. To further analyze the curves of mass loss, TG was combined with FTIR spectroscopy for identification of the formed volatile products. The results are shown in figure 3.3 (left). The mass loss at 300 $^\circ$C correlates well with an increased signal of CO$_2$ at 2364 cm$^{-1}$ and H$_2$O at 3854 cm$^{-1}$, as can be clearly seen from the DTG signal, which is the first derivative of the TG signal. This finding indicates that prior to a possible desorption of ligand decomposition to CO$_2$ and H$_2$O occurs. Similar observations were presented for the thermal decomposition of silver(I) carboxylates.\textsuperscript{242} Figure 3.3 (right) depicts possible reaction pathways that yield CO$_2$ and H$_2$O as products. As shown, the formation of CO$_2$ and H$_2$O is possible in the presence of protonated carboxyl groups only. Assuming that two ligands react to form one CO$_2$ and one H$_2$O molecule, the observed mass loss of 13 $\%$ corresponds to 245 ligands per NC. For the investigated sample of 2.7 nm sized CdTe-TGA NCs, Ellman’s analysis yielded 313 ligands per NC obtained after precipitation. The good agreement of the obtained values underlines the assumption that decomposition of formed complexes in the ligand shell is the mechanism for the mass loss of CdTe-NCs upon heating.

\textsuperscript{1}TG-FTIR measurements were performed by Dr. U. Braun (BAM Berlin).
3. ANALYSIS OF THE AMOUNT OF LIGANDS

3.1.2 Modeling

To interpret the determined amount of ligand per NC by the use of Ellman’s test and ICP-OES, theoretical studies were performed to calculate the amount of ligand that would fit in a monolayer of covalently bound molecules on the surface of one NC. Therefore, the required space of a TGA molecule rotating with a certain kinetic energy was estimated by MD simulations, see figure 3.4. The simulations of a single fixed ligand molecule were performed using the Universal force field (UFF). The ligand molecule was bound to a cadmium atom, which itself was bound to three fixed surface chalcogenide atoms. In this way, the ligand was able to move freely. The considerations include the respective covalent radii of the atoms. From the obtained trajectory, the area \( A_{\text{Ligand}} = \pi r^2 \) covered by the ligand and the maximum height \( z_{\text{max}} \) over the particle’s surface were calculated. For this purpose, a spherical NC with radius \( r_{\text{Particle}} \) was assumed. Division of the area of the NC surface by the required space of a ligand molecule yields the number of ligands per NC assuming the formation of a ligand monolayer shell on the NC surface. The number of ligands \( N_{\text{Ligand}} \) estimated as minimum density of ligands for a fully covered NC with a monolayer ligand shell is then:

\[
N_{\text{Ligand}} = \frac{4\pi(r_{\text{Particle}} + z_{\text{max}})^2}{A_{\text{Ligand}}}
\]

In other words, \( N_{\text{Ligand}} \) reflects the maximum ligand coverage of the NC surface allowing the complete rotational freedom of the ligand. Higher ligand densities may be possible taking into account the restriction of rotational freedom and the presence of a sufficient number of binding sites. However, calculations of the number of possible coordination sites for the ligands yield comparable values.

In figure 3.5, the dependence of the theoretical ligand coverage on NC size is depicted for TGA and MPA. The larger the NC, the lower is the curvature of the NC surface, which leads to a smaller number of thiol molecules that can cover the CdTe surface per nm\(^2\). The larger thiol ligand MPA allows a smaller number of thiols on the NC surface for a full coverage in comparison to TGA. These insights are used for comparison with the experimental results of

<table>
<thead>
<tr>
<th></th>
<th>( z_{\text{max}} )</th>
<th>( A_{\text{Ligand}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGA</td>
<td>0.664 nm</td>
<td>0.4788 nm(^2)</td>
</tr>
<tr>
<td>MPA</td>
<td>0.825 nm</td>
<td>0.8121 nm(^2)</td>
</tr>
</tbody>
</table>

Figure 3.4: Results from MD simulations for rotating ligands on a surface for TGA (left) and the obtained values for TGA and MPA (right). Reproduced by permission of The Royal Society of Chemistry.

\(^{\dagger}\)Theoretical calculations were performed by T. Lorenz and Dr. J.-O. Joswig (Chair of Theoretical Chemistry, TU Dresden).
the two sets of samples presented in the previous sections. The samples of set B show the smaller number of ligands per NC, which correlate well with the theoretical data. This is in accordance with the previously discussed results for NCs containing a small excess of Cd and thus, only a small amount of Cd-thiolates on their surface. It can be assumed that the ligand shell for the samples of set B mainly consists of bound TGA molecules. In contrast, the samples of set A show a considerably larger amount of ligands present, which is not consistent with the theoretical considerations. This suggests the presence of an additional shell of complex Cd-thiol and dithiol networks around the ligands bound to the surface of the NCs. Influences of this difference in the surface shell on optical properties will be further discussed in section 3.1.3.2.

Additionally, the stoichiometric ratio of cadmium and tellurium atoms in zinc blende-based NCs with different geometries, such as cuboctahedral, tetrahedral, and truncated tetrahedral shapes was estimated. Using the crystal structure data, the structures were chosen to be Cd-rich, which means that the top layer of each facet consisted of cadmium atoms. Naturally, the ratio approaches unity for large NCs. A fit of the relation of Cd : Te ratio per diameter \( d \) resulted in a \( 1/d \) dependence with different weighting factors, with those of cuboctahedral shape exceeding that for tetrahedral shape. For truncated tetrahedrons the dependence is more complex as the ratio depends also on the initial size of the tetrahedron. All together, geometrical considerations yield values for Cd : Te ratios between 2 and 4 for NCs with a radius of 1.3 nm.

### 3.1.3 Optics

#### 3.1.3.1 Reliable determination of photoluminescence quantum yields

For a reliable comparison of the emission of different NC samples the exact determination of the PL QY is essential. There are two ways to determine the PL QY directly with optical methods, either as absolute quantity without the need for a standard or relatively to a fluorescent reference with known PL QY. The latter is obtained by comparison of the absorption-
3. ANALYSIS OF THE AMOUNT OF LIGANDS

Related to that, there is the need for standards with reliable PL QYs, whereas many dyes have been recommended for the ultraviolet (UV), visible (vis) and NIR region. However, it is still challenging to accurately determine the PL QYs of fluorophores emitting in the region > 1,000 nm, because of the lack of strongly emitting and stable standard dyes. For these cases and for strongly scattering samples, only the absolute measurement of the PL QY yields reliable values. Rhodamines are typical reference dyes used for relative measurements in the visible region. Absorption and emission spectra of Rhodamine 6G (R6G) and Rhodamine 101 (R101) are displayed in figure 3.6 regarding the determination of the PL QY of a QD sample. The optical densities of dyes and sample are adjusted to have a value < 0.1 at the excitation wavelength. The excitation wavelength $\lambda_{\text{ex}}$ is chosen according to the intersection of the absorption spectra and should ideally be located at the shoulder of the dye emission.

After recording the emission spectra for both, sample $x$ and standard dye $st$, under the same conditions, calculation of the PL QY $\Phi_f$ for the unknown sample becomes possible. The calculation is given in equation 3.2 taking into account the correction for different solvents in form of their refractive indices $n$.

$$\Phi_{f,x} = \Phi_{f,st} \cdot \frac{F_x}{F_{st}} \cdot \frac{f_{st}(\lambda_{\text{ex}})}{f_x(\lambda_{\text{ex}})} \cdot \frac{n_x^2}{n_{st}^2}$$

(3.2)

$f(\lambda_{\text{ex}})$ is the absorption factor giving the fraction of excitation light absorbed by the chromophore and is defined as $f(\lambda_{\text{ex}}) = 1 - T(\lambda_{\text{ex}}) = 1 - 10^{-A(\lambda_{\text{ex}})}$ with $T(\lambda_{\text{ex}})$ being the transmittance and $A(\lambda_{\text{ex}})$ the absorbance at the excitation wavelength. $F$ is the spectral photon flux, which equals the area under the emission spectrum measured as spectral photon radiance. It is of utmost importance to know what quantity is detected by the spectrometer. The spectral photon radiance equals the emission intensity proportional to the number of photons, whereas the spectral radiance relates to the intensity proportional to the photon energy. In the case of

Figure 3.6: Absorption (left) and emission spectra (right) of adjusted dilutions of reference dyes R6G (blue) and R101 (black) as well as a QD sample (orange) allow the relative determination of the PL QY of the QD sample.
the latter, the detected intensity has to be multiplied with the corresponding wavelength $\lambda_{\text{em}}$ prior to integration. For reliable values it is recommended to use at least two reference dyes per sample, prepare always fresh solutions and carefully chose appropriate references according to similar absorption and emission features. IR 125 and IR 26 present the most common reference dyes for the evaluation of NIR emitting NCs. The PL QY value of the latter was recently redetermined to be 10 times lower than previously assumed, which renders all PL QYs measured relative to this dye questionable. The overestimation of many PL QYs of NIR emitting NCs demonstrates the need for a critical reexamination of PL QY values. Besides the establishment of sophisticated setups for the absolute measurements, new standards could be developed including the possible use of semiconductor NCs as they possess significant PL QYs also in the IR. However, therefore some limitations that these materials bring along have to be overcome, such as the concentration dependence of the emission. To design NCs that can be used as PL QY standard material, the complete understanding of these processes and the prediction of their performance is of importance. This issue is addressed in the following section.

### 3.1.3.2 Dilution studies

Previous studies have shown that the ligand shell of water-soluble NCs is dynamic and is influenced by the concentration of the colloidal solution. Grabolle et al. observed a concentration dependence of the PL QY for CdTe-TGA NCs, which is differently pronounced for differently sized NCs. The influence of the particle size, i.e. the different surface-to-volume ratio, implies ligand adsorption-desorption as possible reason for the concentration dependence. The presence of an equilibrium between bound and free state of the ligand is schematically depicted in figure 3.7. In concentrated NC solutions the equilibrium favors the bound state of the ligand, whereas upon dilution, the equilibrium is interrupted and in the changed state the equilibrium is more shifted to the free ligand. Besides the influence of NC size, this section shows the important effect of ligand shell structure and composition on the concentration dependence of dilution.

![Figure 3.7](image-url)

Figure 3.7: According to the present state of knowledge, the dilution process of NC solutions is supposed to go along with the desorption of ligand from the surface of the NCs.

*The presented dilution curves were measured by R. Schneider and S. Hatami (BAM Berlin).*
3. ANALYSIS OF THE AMOUNT OF LIGANDS

CdTe NCs In order to gain a first insight into the influence of different ligand shell structures on the optical properties of CdTe NCs, the absorption and PL behavior of samples A and B that consist of equally sized NCs (see table 3.1) were compared. As follows from figure 3.8 depicting the absorption spectra, both colloids reveal very similar absorption features regarding the spectral shape and spectral position of the first excitonic maximum as to be expected considering their similar size. However, the emission properties are different, with the emission spectrum of sample B being red shifted by 10 nm compared to sample A. Interestingly, although both samples reveal almost comparable PL QYs for absorbances of 0.04 at the excitation wavelength, dilution of these NCs results in a considerable decrease in PL QY in the case of B whereas the PL QY of sample A is much less affected. The absorbance is used here as measure for the NC concentration. The PL QY values are normalized to the highest value obtained for the respective sample. As previously shown, both samples exhibit different ligand shell structures, which implies that the ligand coverage strongly influences the concentration-dependent emission properties. For sample B having a thin ligand shell strong dilution can almost completely quench the emission. The reason can be desorption of ligands from the NC surface upon dilution resulting in an incomplete surface coverage and the formation of surface traps, which quench the band gap emission. In contrast, sample A possesses a thick ligand shell with a large amount of Cd-thiolates present, which may also be subject to desorption processes during dilution. The large amount of the Cd-thiolate complexes, however, shields the NCs

Figure 3.8: Absorption and emission spectra of 2.6 nm sized CdTe-TGA NCs reveal no distinct difference for the two samples A and B (left). The change of the PL QY with decreasing NC concentration is, however, more pronounced for the sample with less TGA molecules on the NC surface (right). The dotted lines are only a guide to the eye. Reproduced by permission of The Royal Society of Chemistry
from complete quenching of the emission. This result underlines the strong dependence of the optical properties on the ligand coverage, which is especially important for applications that demand strong dilutions.

The same studies were performed for MPA-capped CdTe NCs, shown in figure 3.9. Similarly, a strong decrease of the PL QY is observed at NC concentrations below optical densities of 0.01. The extent, however, is slightly smaller for MPA-capped NCs than for TGA-capped ones. This could point to a higher stability of the ligand shell made of MPA. As the determined amount of ligand per NC is larger for the MPA-capped sample shown, it is difficult to conclude, whether the effect is caused by the type of ligand or by the amount of ligand. The initial value of the PL QY at high concentrations is not only determined by the amount of ligand per NC, but also by the quality of the NC core, which is influenced by the ratio of Cd : ligand present during the synthesis. The table in figure 3.9 depicts the comparison of two CdTe-TGA samples obtained from different initial Cd : TGA ratios applied for the synthesis. Notably, the different Cd : thiol ratios show the influence on the PL QY as reported earlier, but not on the final amount of ligand per NC.

**CdHgTe NCs**  
Aiming at the use of these CdHgTe NCs as fluorescent reporters for bioimaging studies, the influence of the NC environment, i.e. NC concentration on the PL QY was studied. Previous studies with TGA-stabilized CdTe colloids of different size show a size-dependent and strong decrease of the PL QY at low NC concentration due to dilution-induced ligand desorption processes, which is disadvantageous for e.g., bioimaging. In contrast, the investigated CdHgTe NCs do not show a concentration dependence of their PL QY for all three ligands applied, see figure 3.10. Even additional precipitation of the MPA-capped CdHgTe sample with iso-propanol, a non-solvent, which decreases the amount of ligands on the surface of the NC, does not cause a clear concentration dependence of the PL QY. This suggests that
the ligand shell is very stable. One reason could be that the stabilizing ligands are very tightly bound to the CdHgTe core and hence not subject to ligand adsorption-desorption equilibria any more. This finding also emphasizes the favorable influence of even small amounts of Hg\textsuperscript{2+} on the optical properties and stability of such alloy NCs. Also, the large amount of sulfur present in the NCs assists the observed good stability. Previous studies on CdTe could show that larger NCs are subject to a weaker concentration dependence than smaller NCs.\textsuperscript{49} As the size of the CdTe NC correlates with the sulfur content due to the hydrolysis of thiols,\textsuperscript{31} a highly sulfur-enriched shell at the NC surface may also be a reason for a strong emission independent on the NC concentration. A similar behavior may also be true for CdHgTe, meaning that the presence of sulfur supports the high PL and the stability upon dilution. Alternatively, the solvent D\textsubscript{2}O, forming stronger hydrogen bonds than water, may influence the bonding of the thiol ligands and the NC surface. The possible influence of the solvent will be discussed in section 3.1.3.4. This, in conjunction with their red emission maxima, high PL QY, and ease of preparation renders these CdHgTe systems especially attractive as new generation of fluorescent reporters.

### 3.1.3.3 Precipitation studies

In a simple picture, the precipitation of NCs from solution allows the separation of free and bound ligands. The precipitation of NCs is promoted by the addition of a non-solvent, such as alcohols that lead to a decreased relative dielectric constant of the solution mixture and consequently a reduced electrostatic repulsion of the NCs.\textsuperscript{245} As presented in section 3.1.1.2, precipitation influences the ligand adsorption-desorption equilibrium. In the following, more details on the influence of precipitation on stability and optical properties of CdTe NCs are given.

Figure 3.11 shows the different sample qualities before and after precipitation. The TEM images depict the as-prepared sample and the sample after one and two precipitation steps.
Figure 3.11: TEM images of 2.6 nm sized CdTe-TGA NCs show the presence of unreacted species, such as excessive Cd-TGA complexes in the as-prepared sample (left) and aggregates due to reduced stability after two precipitation steps (right). The solution obtained after one precipitation step shows well-dispersed NCs (middle).

from the left to the right. Whereas the NC size is constant, the arrows highlight the main differences. In addition to the NCs, in the as-prepared sample secondary features are present, which can be assigned to unreacted species and excessive Cd-thiolate complexes. After cleaning the as-prepared solution by one precipitation step, a stable colloidal solution of NCs is obtained. By a second precipitation step further stabilizers are removed leading to a destabilization of the NC solution. This is depicted in the TEM images by a noticeable larger amount of aggregates than those present in commonly dried TEM samples of NC solutions that were purified by one precipitation step.

Along with a decreasing stability caused by more precipitation steps, the PL QY decreases. Figure 3.12 (left) shows that the relative change of the PL QY with the number of precipitation steps is very similar to the decrease of the ligand concentration. This observations suggests that the change of the PL QY is directly linked to the amount of ligand present in the NCs.

Figure 3.12: The relative change of the PL QY upon precipitation matches the relative change of the overall ligand concentration in solution determined by Ellman’s test (left). Differently sized NCs obtained from one synthesis show similar values for relative PL QY and relative ligand density obtained after filtration and thiol quantification by Ellman’s test (right). Both observations underline the dependence of the PL QY on the amount of ligand for TGA-capped CdTe NCs.
solution, which is reduced by precipitation. Similar results were obtained for differently sized NCs, however, showing the relation of PL QY and amount of ligand attached to the NC using filtration instead of precipitation. As the previous results showed that precipitation changes the ratio of bound and free thiol, in this case filtration was used to separate the solvent with free thiol from the NCs with the attached ligands. Figure 3.12 (right) depicts the similarity of the relative number of ligands per nm$^2$ measured after filtration, which most probably equals the number of ligands bound to the NC in the parent solution, from which the PL QY was determined and is also drawn as relative value. All numbers are related to the highest value. In summary, precipitation allows a certain separation of bound and free ligand, but additionally removes ligand from the NC surface. This leads to a decreased stability of the colloidal solution and a decrease of the PL QY. Quantification of the amount of bound and free thiol without changing the equilibrium is possible by NMR and will be described in section 4.2.

3.1.3.4 Influence of the solvent

Besides the influence of pH, NC concentration, and the extent of purification on the optical properties of the NCs, which were shown to be caused by a varying amount of ligand per NC or a different structure of the ligand shell, the influence of solvent changes will be discussed below.

A variety of CdTe NC samples were prepared by separate syntheses according to the same procedure and the same initial precursor ratios, but after different growth times. All samples were purified by one precipitation step and diluted in different solvents for the PL QY measurements. Notably, independent of the absolute value of the PL QY, for all studied samples, the highest PL QY is reached in D$_2$O solutions and the smallest PL QY was measured in solutions from tap water, see figure 3.13. The values in pure and ultrapure water are similar and the differences are in the range of reported uncertainties of the measurement. By a multistep

Figure 3.13: Different PL QY values of CdTe NCs depending on the solvent show the highest values for solutions of D$_2$O.
purification from tap, over pure to ultrapure water contaminants by ions, organics, particulates, and bacteria are reduced. In the order of ultrapure H$_2$O, pure H$_2$O, 99.96% pure D$_2$O, and tap H$_2$O the specific conductance increases showing the increasing amount of ions present in the solvents. The low value of PL QY in tap water is most probably caused by the large ion concentration leading to destabilization of the colloidal solution and aggregation of the NCs causing quenching of the emission. The certain amount of ions present in pure water does not seem to influence the stability and emission of the CdTe NCs remarkably. In some cases the presence of ions may be favorable for an increase of emission intensity by increasing the stability of the ligand shell or by passivating dangling bonds, as also observed for CdTe NCs incorporated into NaCl crystals. From the chemical point of view, H$_2$O and D$_2$O are the same as they possess the same binding lengths, binding angles and dipole moments. Differences occur with regard to the molar mass, melting and boiling point, refractive index, and more importantly by different dissociation constants leading to different neutral points. MD simulations showed additional differences in the dynamics of the network of hydrogen bonds and the analog deuterium bonds. Notably, D$_2$O exhibits 25% less deuterium bond changes and longer deuterium bond lifetimes, respectively. The fact that the strength of the hydrogen bond is different for H$_2$O and D$_2$O is also the reason for the toxicity of D$_2$O for biological systems. The consequently different interaction with molecules, in this case with the thiol ligands on the NC surface, might be a reason for the different PL QY values measured. A stronger hydrogen bond may cause a more compact ligand shell and a better passivation. However, no clear correlation of the PL increase with the initial PL QY, NC size, or amount of ligand could be determined. In either case, the observation of the influence of solvent changes on the obtained PL QY underlines once more the importance of the ligand shell for the overall optical properties.

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**MD simulations to obtain information about difference of H$_2$O and D$_2$O were done by Dr. J.-O. Joswig (Chair of Theoretical Chemistry, TU Dresden).**
3.2 Modification with DNA molecules

As stated in the recent review by Ma and Kelley on QD-DNA conjugates, the understanding of DNA-QD interactions is still incomplete. DNA-modified CdTe NCs, as introduced in section 2.4, present a complex system, because the amount of DNA molecules attached is insufficient and the direct measurement is not possible. In this respect, for a thorough characterization more complex methods with high sensitivity are in demand. Within the scope of the current work, indirect measurements using the additional functionality introduced by DNA are utilized. The programmed binding can be monitored either by optical methods, which allow a high sensitivity especially for experiments involving the quenching of NC emission, or by microscopy that directly images the successful assembly of NCs. Despite the presented finding of a complex ligand structure, the ligand shell is depicted in a simplified way in the following sketches for better visibility.

3.2.1 Optics

Energy transfer has been shown to be a powerful spectroscopic tool to study hybrid nanosystems, since it is a very sensitive method to monitor interactions and conformational changes of fluorophores. Semiconductor NCs are particularly favorable for such studies because of their high stability, broad excitation range and narrow emission. NCs conjugated to biomolecules for energy transfer based assays are widely studied. Also for the proof of successful functionalization of NCs with DNA molecules quenching of the NC emission can be utilized. Therefore, a complementary DNA strand with a quencher molecule attached is used, in this case the BlackBerry® Quencher 650 (BBQ), which efficiently quenches emission maximizing at wavelengths near 650 nm. The principle of the quenching experiment is depicted in figure 3.14. The binding of BBQ in close proximity to the QD is expected to cause quenching of the QD-specific emission. In first experiments, 3.2 nm sized CdTe NCs were functionalized with T2, purified by filtration to remove unbound oligonucleotides and mixed with a complementary oligonucleotide consisting of a sequence of 18 adenine and BBQ at the 3’ end. To assist the incubation by mixing, the samples were heated to 40 °C and slowly cooled down. The heating aims for melting of the DNA and a controlled reorientation upon slow cooling to room temperature to enable successful complementary binding. Optical changes were monitored by absorption and emission spectroscopy. The emis-

![Figure 3.14](image)

Figure 3.14: The quenching experiment is based on the assembly of a complementary oligonucleotide with a quencher molecule attached to the DNA-functionalized NC.
3. ANALYSIS OF THE AMOUNT OF LIGANDS

Figure 3.15: The assembly experiment is based on the connection of differently functionalized NCs by a complementary DNA strand.

Emission intensity differs for the mixture of DNA-functionalized NCs and DNA-BBQ compared to the mixture containing DNA-BBQ and CdTe/CdS NCs without DNA attached. This points to a different interaction of the NCs with the quencher molecule and suggests successful functionalization. However, the changes are not significant enough to draw a reliable conclusion. Potential optimization includes the change of the binding site of the quencher to reduce the distance of QD and quencher, which may lead to more pronounced quenching. Further improvement of the yield of complementary binding can be achieved by adjusting the heating and cooling procedure. Still, this characterization method shows a great potential for the proof of DNA-functionalization.

Additional studies to characterize the binding of DNA to the NCs include the use of programmed assembly of NCs via the introduced DNA functionality. Differently sized NCs are functionalized by diverse DNA molecules. The addition of a DNA strand that is complementary to both NC functionalities allows the assembly of the differently sized NCs to a network, in which changes of the optical properties of the NCs are expected. Figure 3.15 shows the principle of this assembly approach. After mixing the differently sized and functionalized NCs and addition of the complementary DNA, the mixture is heated to 40 °C and slowly cooled down to allow successful complementary binding. The resulting emission spectra are depicted.

Figure 3.16: Emission spectra of differently sized DNA-functionalized CdTe/CdS NCs (dotted black lines), their mixture (solid black line), and their mixture containing complementary DNA (solid red line). Changes of emission after addition of the linking DNA strand indicate the formation of NC assemblies.
3. ANALYSIS OF THE AMOUNT OF LIGANDS

in figure 3.16. The dotted lines represent the spectra of the separate NCs, whereas the solid black curve shows the emission of the mixture without complementary DNA. The red curve is obtained after mixing with complementary DNA and incubation. A significant shift of the emission maximum accompanied by a decrease of the emission of the small NCs indicates the formation of NC assemblies. Additionally, measurements of the fluorescence lifetime of the differently sized NCs suggest the formation of assemblies. The PL lifetime of the small NCs strongly decreases upon incubation with the complementary DNA, whereas the PL lifetime of the large NCs changes only slightly.

3.2.2 Microscopy

To further investigate the programmed formation of assemblies, TEM measurements were used to image the formed structures. Figure 3.17 clearly depicts the formation of assemblies in a structure that is typical for NC gels. This observation complements the picture of programmed assembly of DNA-functionalized NCs for proving the successful functionalization of NCs with DNA.

Another way to precisely assemble DNA-functionalized NCs is the use of DNA origami structures as templates. DNA origami structures are formed from a single-stranded DNA scaffold and a defined set of staple strands. Due to the precise folding owing to base pairing, scaffold and staple strands form a predesigned two- or three-dimensional structure. The extension of selected staple strands with overhanging single-stranded DNA sequences, to form so-called capture strands, give rise to further functionalization. In this work, so-called six-helix bundles were used, which consist of six double helices forming a tube of a length of 414 nm. The designed structures contain ten overhangs with a distance of 40 nm that were used for the attachment of DNA-modified NCs. This is shown schematically in figure 3.18. The exact control and the versatility of the DNA origami design allows to attach a distinct sequence of different NCs on the six-helix bundle functionalized with the respective complementary DNA strand. The results for the assembly of ten equal NCs on the six-helix bundle are discussed below. Atomic force microscopy (AFM) was applied to monitor the success of the NC binding to the DNA origami surface. Figure 3.19 depicts the results for 3.2 nm sized CdTe/CdS-TGA NCs.
which were modified with the oligonucleotide T1 (see table 2.6) bound to six-helix bundles with ten overhangs consisting of a sequence of 18 adenine. The analysis of the AFM images present a yield of 84% six-helix bundles containing 9 or more NCs. In addition to the high yield, observations show some connected six-helix bundles. This can be caused by NCs with several oligonucleotides bound to the surface, which may bridge single six-helix bundles. To

![AFM images and three-dimensional profile of 3.2 nm sized T1-CdTe/CdS-TGA NCs assembled on six-helix bundles show a high yield of functionalization and moreover some origami structures that are connected by attached NCs.](image)

†† Preparation of six-helix bundles and conjugation with NCs as well as AFM analysis was performed by Dr. A. Henning (Chair of Physical Chemistry, Measurement and Sensor Technology, TU Dresden).
reduce the number of DNA molecules on the NC surface, a sequence length comprising of 20 phosphorothioate internucleotide linkages is supposed to control the functionalization with solely one DNA molecule.\textsuperscript{228}

Analysis of the height profiles obtained from the respective AFM images reveal a height of the functionalized six-helix bundles of 8-9 nm, see figure 3.20. In comparison to a reference six-helix bundle without NCs bound, the height difference of 6-7 nm can be assigned to the size of the NCs including their ligand shell. Besides the theoretical height of a six-helix bundle of 7 nm, the obtained value of 2 nm is expected for AFM measurements. Due to drying prior to the measurement, the diameter of a double helix decreases to 1 nm. Consequently, the height of a dried six-helix bundle, in which two double helices lie on top of each other, exhibits a height of 2 nm. The obtained value of the NC size matches the 3.2 nm sized CdTe core plus a ligand shell containing short-chain thiol and DNA molecules with a shell thickness of 3-4 nm. This is in good agreement to expectations that result from the analysis of the complex shell of short-chain thiols.

The successful assembly of NCs on DNA origami structures is a proof for the DNA-functionalization of NCs. Additionally, AFM allows to estimate the NC size and the yield of functionalization. However, this procedure presents a time- and material-consuming method and will therefore play a minor role for characterizing the functionalization of NCs. Instead, the precise assembly of NCs on DNA templates offers great potential for the preparation of functional structures on the nanoscale that may include antenna structures, waveguides or energy transfer cascades.
3.3 Discussion

The presented use of Ellman’s test is an especially promising and easy way to quantify the amount of thiols and was adapted for the first time for the analysis of the ligand amount in NC solutions in the current work. Complementary studies of CdTe NCs with ICP-OES allow the estimation of the quantity of ligand per NC. Additionally, ICP-OES gives information about the chemical composition of the NC-ligand system and clearly demonstrates that precipitation of the NCs from solution leads to a loss of S and Cd. Studies on the desorption of ligand upon heating by TG-FTIR show that the ligands are not loosely attached to the NC, but bound in a network on the NC surface. To evaluate the obtained ligand quantities, theoretical calculations were applied to estimate the number of ligands that fit in one monolayer on the NC surface. The comparison of experimental and theoretical results rules out the simple picture of the ligand shell of water-soluble CdTe NCs being solely a monolayer of ligands covalently bound to the NC. This is in contrast to results from literature for the ligand density of Au NCs and various semiconductor NCs with different ligands providing solubility in organic solvents. An overview of the results for ligand densities is given in table 3.3. Alkanethiols are expected to bind to Au NCs via covalent bonds in a monolayer, whereas surface coverages of approximately 5 ligands per nm$^2$ can be obtained. Similar but slightly smaller values are obtained for semiconductor NCs capped by various ligands, which are soluble in organic solvents. The smaller densities obtained for bulky ligands, such as TOPO, are expected due to steric hindrance. In comparison to the results from theoretical calculations within the scope of the present work, the values are similar and point to the presence of monolayers of ligands on the surface of NCs in organic solvents. In contrast, the surface of water-soluble NCs is weakly studied so far. First calculations of ligand densities obtained from XPS measurements yield values similar to the quantity expected for a monolayer of ligand. However, the values increase with decreasing pH and the XPS measurements from layered films may not reflect the situation in solution. The current work shows that the situation is different for water-soluble NCs in solution indeed. The experimental results yield larger ligand densities pointing to a large ligand network on the NC surface. Even if analyzed samples with a thin ligand shell exhibit similar ligand densities compared to the presented values from literature, the resulting NC emission is strongly affected by sample dilution. CdTe-TGA NC samples with a more stable emission exhibit ligand densities that are larger by a factor of 2 - 5. Interestingly, the total amount of ligand in the NC solution of water-soluble CdTe related to the NC surface area reaches values that are by more than a factor 10 larger then expected for a complete monolayer. The large amount of ligands present in the NC surrounding seems to be necessary for strong NC emission. Studies of the emission properties show the significant influence of the present amount of ligand on the concentration dependence of the emission yield of CdTe NCs. A way to reduce the amount of ligands efficiently is precipitation of NCs accompanied by the loss of emission intensity. Overall, the presented results suggest the presence of metal-thiol complexes in the ligand shell. Possible chemical structures are given in figure 3.21. The presence of disulfides as product
3. ANALYSIS OF THE AMOUNT OF LIGANDS

Table 3.3: The comparison of values for the ligand density on the NC surface from literature and from the current work show higher values for water-soluble CdTe NCs, which indicates the presence of a ligand network rather than a monolayer of ligands on the NC surface. The values are obtained from colloidal solutions in organic solvents.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>NC-ligand system</th>
<th>Density (nm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>Au-alkanethiols</td>
<td>5.3-5.5 (from atomistic simulations)</td>
</tr>
<tr>
<td>51</td>
<td>CdSe-TOPO *</td>
<td>1.3</td>
</tr>
<tr>
<td>79</td>
<td>CdSe-carboxylate *</td>
<td>4.6</td>
</tr>
<tr>
<td>80</td>
<td>CdSe-phosphonate *</td>
<td>3.5</td>
</tr>
<tr>
<td>77</td>
<td>PbSe-carboxylate *</td>
<td>4.2</td>
</tr>
<tr>
<td>251</td>
<td>PbS-carboxylate *</td>
<td>5.3</td>
</tr>
<tr>
<td>251</td>
<td>PbS-thiolate *</td>
<td>5.0</td>
</tr>
<tr>
<td>96</td>
<td>CdSe-carboxylate *</td>
<td>0.3-4.5</td>
</tr>
<tr>
<td>96</td>
<td>CdS-carboxylate *</td>
<td>0.4-1.1</td>
</tr>
<tr>
<td>96</td>
<td>PbSe-carboxylate *</td>
<td>3.1</td>
</tr>
<tr>
<td>96</td>
<td>PbS-carboxylate *</td>
<td>2.4-4.3</td>
</tr>
<tr>
<td>53</td>
<td>CdTe-TGA</td>
<td>4.8 (S atoms per nm$^2$ at pH 9)</td>
</tr>
<tr>
<td>53</td>
<td>CdTe-MPA</td>
<td>5.4 (S atoms per nm$^2$ at pH 9)</td>
</tr>
<tr>
<td>theor. results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of this work</td>
<td>CdTe-TGA</td>
<td>3.3-4.8 (for a monolayer)</td>
</tr>
<tr>
<td></td>
<td>CdTe-MPA</td>
<td>2.1-3.3 (for a monolayer)</td>
</tr>
<tr>
<td>experim. results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of this work</td>
<td>CdTe-TGA</td>
<td>11.8-17.3 (for thick ligand shells)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.7-5.7 (for thin ligand shells)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37-47 (sum of free and bound TGA)</td>
</tr>
<tr>
<td></td>
<td>CdTe-MPA</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8-55 (sum of free and bound MPA)</td>
</tr>
</tbody>
</table>

of oxidation reactions as well as Cd-mercaptocarboxylic acid complexes of varying composition is highly probable. Furthermore, there are several coordination structures available. Additional CH$_2$ groups between thiol and carboxyl function may enable better secondary coordination via the carboxyl oxygen. This could explain the slightly better stability of MPA-capped CdTe NCs than TGA-capped NCs against dilution and the accompanied decrease of PL QY. For CdHgTe NCs a very stable and concentration-independent PL QY was observed pointing to an improved stability of the ligand shell in the presence of Hg$^{2+}$ ions. The strong complexation ability of Hg$^{2+}$ is reflected in the large binding constants reported for Hg-thiol complexes. Albeit the use of MPA as example for the drawing of possible structures in figure also TGA can form the presented complexes. Five-membered rings have already been reported for Cd-thiolate coordination. Studies on the influence of the solvent on optical properties imply that solvent molecules also contribute to the ligand network as
also depicted in figure 3.21. This will be discussed in more detail in section 4.3. In summary, the presented work underlines the strong influence of the ligand shell on the optical properties of thiol-capped CdTe NCs. Especially, the dependence of the emission on NC concentration, amount of ligand and pH emphasizes the need to assign these parameters when stating a PL QY.

Besides the complex structure of the ligand shell composed of short-chain thiols, the modification of the NCs with functional biomolecules and the characterization thereof is still possible. Studies of DNA functionalized NCs show that programmed assembly is a promising way to characterize and to use the highly functional NCs. Further methods for the characterization of the NC-DNA functionalization are based on optical methods. First promising results underline the great potential of these characterization techniques to prove and estimate the degree of functionalization, but need further investigation to allow routine analysis. Additionally, the work emphasizes the need to selectively choose the characterization method depending on the ligand, the NC/ligand system and the question to be solved. For TGA-capped CdTe NCs a comprehensive characterization of the ligand shell structure is presented in the following chapter.
4 Analysis of the ligand shell structure

From the previous chapter first ideas about the composition of the ligand shell of water-soluble semiconductor NCs could be gathered. To gain a deeper insight into the exact structure of the ligand shell more advanced techniques were used and are presented below. Studies utilizing dynamic light scattering (DLS) aim for the determination of the NC size including the ligand shell and the evaluation of the surface charge. Nuclear magnetic resonance spectroscopy (NMR) allows the in situ study of the ratio of bound and free ligand and yields information on the chemical environment of the ligand in the shell. Furthermore, small-angle X-ray scattering (SAXS) was utilized to gather information on the size of core and shell at the same time. SAXS allows to conclude from different scattering contrast on the composition of core and shell. Together with quantitative analysis of CdTe-TGA NCs presented in chapter 3, these studies allow to estimate a structural model of the NC-ligand system including a chemical formula.

4.1 Dynamic light scattering

4.1.1 Theory

DLS is a simple and nondestructive technique to study the size of NPs in solution. The analysis of fluctuations in scattered light intensity from the interaction of laser light with the solution of permanently moving particles allows the determination of the particle diffusion coefficient and consequently the hydrodynamic particle size. By measuring the hydrodynamic radius, DLS complements size analysis by TEM and absorption spectroscopy, which yield the NC core size. By comparison of the obtained values, the thickness of the ligand shell can be estimated. Figure 4.1 depicts the basic setup and principle of data processing for DLS. Monochromatic and coherent light from a laser is focused on the sample. As the particles in the

\[ I(\text{size}) \]

Figure 4.1: Principle of the DLS measurement and data processing.

*The results of this section were obtained within the framework of the Bachelor thesis written by R. Weichelt under the mentoring of the author.*
A detector collects the scattered light at a given scattering angle \( \Theta \). The time-dependent scattering intensity \( I(t) \) displays the fluctuations of the scattered light due to constructive and destructive interference caused by the stochastic change of the distance of the scatterers due to particle movement. This is also represented by concentration fluctuations in the scattering volume due to the Brownian motion of the particles. The fluctuation is directly linked to the particle size as larger particles move slowly, whereas small particles move quickly. To analyze the movement a time autocorrelation function of scattered intensity \( g^{(2)}(\tau) \) is calculated from the intensity trace according to equation 4.1.

\[
g^{(2)}(\tau) = \frac{\langle I(t) \cdot I(t+\tau) \rangle}{\langle I(t) \rangle^2} \tag{4.1}
\]

The values in \( \langle \rangle \) represent the average over time and scattering volume. For further analysis of the obtained function that decays exponentially with the correlation time \( \tau \) it is useful to convert the intensity autocorrelation function \( g^{(2)}(\tau) \) (second order) to the electric field autocorrelation function \( g^{(1)}(\tau) \) (first order) via the so-called Siegert relation:

\[
g^{(2)}(\tau) = 1 + \gamma[g^{(1)}(\tau)]^2 \tag{4.2}
\]

\( \gamma \) describes a correction factor that depends on the geometry of the DLS setup. For monodisperse samples, the correlation of the scattered electric field amplitude will be a single exponential decay yielding the decay constant \( \Gamma \), as depicted in the equation below.

\[
g^{(1)}(\tau) = e^{-\Gamma \tau}, \quad \Gamma = q^2 D, \quad q = \frac{4\pi}{\lambda} \sin \left( \frac{\Theta}{2} \right) \tag{4.3}
\]

\( \Gamma \) is proportional to the translational diffusion coefficient \( D \) and a function of the scattering angle \( \Theta \), related by the magnitude of the scattering vector \( q \). \( \lambda \) is the wavelength of light in the present medium. In case of polydisperse samples, the autocorrelation function is a sum of exponential decays as shown in equation 4.4. A simple technique to characterize this sum of exponentials is the cumulant method. The logarithm of \( g^{(1)}(\tau) \) is expanded as a power series in time yielding the cumulants \( K \):

\[
g^{(1)}(\tau) = \sum_i A_i e^{-\Gamma_i \tau} \quad \ln[g^{(1)}(\tau)] = -K_1 \tau + \frac{1}{2} K_2 \tau^2 + ...
\]

The first two terms are of importance yielding the average decay rate by \( K_1 \) and thereby the diffusion coefficient as well as a measure of the dispersion by \( K_2 \). A more powerful technique utilizes an inverse Laplace transform to obtain a distribution function of relaxation rates and is implemented in the frequently used algorithm known as CONTIN. Following the analysis of the autocorrelation, the conversion of the obtained diffusion coefficient \( D \) to the hydrodynamic radius \( R_h \) is then possible via the Stokes-Einstein relation:
where $k_B$ is the Boltzmann constant, $T$ the absolute temperature and $\eta$ the viscosity of the solvent. In this way, DLS is a powerful technique for the determination of the NP size in solution, if possible uncertainties are clarified. Due to the existence of electrostatic interactions between particles DLS results show a dependence on scattering vector and concentration. Therefore, exact values should be obtained from several measurements at varied scattering angle and concentration by extrapolation to zero for both factors. However, not every instrumentation allows the variation of $q$. Additionally, the concentration-dependent changes could also give information on the degree of particle interaction.

Furthermore, based on DLS the use of electrophoretic light scattering allows to gather information about the dispersion stability of particles by measuring the zeta potential as indicator for the surface charge of NPs. Because the measurement of the surface charge of NPs is not trivial, commonly the electric potential at a location away from the particle surface is determined. A charged particle in solution is surrounded by an electric double layer due to the assembly of counter ions in the interfacial region. The surrounding layer can be divided into two parts, an inner region, at which ions are strongly bound, the so-called Stern layer and an outer diffuse region where ions are less strongly attached. When a particle moves due to Brownian motion, ions within a boundary move with the particle. This boundary is named hydrodynamic shear or slipping plane. The potential that exists at the slipping plane is defined as zeta potential and provides information on the stability of the particles in solution. If the absolute value of the zeta potential is high, strong electrostatic repulsion between the particles is present and the solution is stable, whereas small absolute values indicate a high probability of the particles to form aggregates.

The zeta potential is determined by the measurement of the electrophoretic mobility by electrophoretic light scattering. An electric field applied induces the movement of the charged particles to the electrode of opposite charge and the velocity is proportional to the charge of the particle. The measurement of the velocity is based on the Doppler effect. Irradiation of a sample with a laser beam and detection of the scattered light yields a frequency shift proportional to the speed of the particle movement. The Doppler frequency shift $\nu_D$ can be used for determination of the velocity $U$ of the particles according to equation 4.6 with $\lambda$ being the wavelength of the light in the present medium and $\Theta$ the scattering angle. As the electrophoretic mobility $\mu$ is related to the velocity $U$ via the strength of the applied field $E$, the calculation of the zeta potential $\zeta$ becomes possible according to the Henry equation:

$$\nu_D = \frac{2U}{\lambda} \sin \left( \frac{\Theta}{2} \right) , \quad U = \mu \cdot E , \quad \mu = \frac{2\zeta \epsilon}{3\eta} f(\kappa r)$$

$\epsilon$ describes the dielectric constant, $\eta$ the viscosity and $f(\kappa r)$ Henry’s function, which depends on the inverse screening length $\kappa$ and the particle radius $r$. The calculation of the electrophoretic mobility of a spherical particle is complicated and several approximations exist.
4. ANALYSIS OF THE LIGAND SHELL STRUCTURE

water-based systems usually the Smoluchowski approximation is used yielding a value for $f(\kappa r)$ of 1.5.

4.1.2 Results and discussion

DLS measurements of CdTe NCs emerged to be very challenging due to the small particle size and absorption of the laser light as well as interference of emission with the scattered light. Additionally, the removal of aggregates by filtration and a careful adjustment of the NC concentration were necessary to obtain valuable results. Still, the quality of the measurements differed strongly, which could be verified by the quality of the cumulant fit of the autocorrelation function. In this way, only a certain set of samples gave reasonable values. For 2.5 nm sized CdTe-TGA NCs the intensity distribution yielded two peaks, one with a maximum at 6.6 nm corresponding to the hydrodynamic diameter of the NCs and a second peak ranging from 50 to 500 nm with a maximum at 142 nm representing agglomerates, which could not be removed by filtration. TEM analysis indicates a core size of 2.6 nm, similar to 2.5 nm obtained from the absorption spectrum. Comparison of the values with the result from DLS yields a thickness of the ligand shell of approximately 2 nm. Together with theoretical calculations, presented in section 3.1.2 which predict a shell thickness of 0.7 nm for a monolayer of TGA ligands, the finding suggests the presence of a more complex ligand shell. In this way, DLS underlines the findings from the analytical studies explained in the previous chapter.

In the first instance, DLS yields a distribution of particle sizes weighted according to the scattering intensity of each fraction, the intensity distribution. As the scattering intensity scales with the particle size to the power of six, this result is very sensitive to the presence of large particles, but can also be misleading, because already a small amount of aggregates can dominate the distribution. The volume distribution, which describes the relative proportion of components based on the volume, can be obtained from the intensity distribution using Mie theory. This conversion is based on several assumptions including the presence of spherical and homogeneous particles, the knowledge of the refractive index of the material and the absence of errors in the intensity distribution. As the latter exhibits inherent errors, the volume distribution should not be considered absolutely. In this regard, the intensity distribution is preferably used to report the particle size. The intensity distribution of 2.5 nm sized CdTe-TGA is depicted in figure 4.2 (left) for different NC concentrations. The expected effect of dilution includes the reduction of the hydrodynamic size due to desorption of ligands as indicated by previously shown results. However, the small changes expected can not be resolved by the DLS measurement and notably, the peak at 6.6 nm stays constant upon dilution. With decreasing NC concentration a reduced scattering intensity can be observed for the NC signal. In contrast, an increased intensity is present for aggregates indicating a larger amount of aggregates caused by decreasing NC concentration. This finding suggests the decreasing stability of the NCs due to desorption of the ligand by dilution and the stronger tendency of the NCs to agglomerate. However, the amount of aggregates is still low, as the investigation of the volume distribution
yields no peak for agglomerates. Additionally, zeta potential measurements were conducted for different NC concentrations and are shown in figure 4.2 (right). The absolute value of the zeta potential decreases upon dilution indicating the reduction of charges in the NC ligand shell by the changed equilibrium towards free ligand. However, these results should be treated with care, because the low concentrations could cause a low quality of the measurement. In this case, the observed decrease of the zeta potential is due to the increasing signal contribution of aggregates, which become more apparent for strongly diluted samples. The attempt to monitor temporal changes after dilution by DLS did not give clear results, which suggests that the process of ligand desorption occurs on a fast time scale. The decrease of the absolute value of the zeta potential after two days underlines the decreasing stability of highly diluted NC samples with time.

Further studies on the stability were performed with regard to precipitation of the NCs. Zeta potentials measured after precipitation of 3.3 nm sized CdTe-TGA NCs using iso-propanol and subsequent redispersion in ultrapure water are shown in table 4.1. The decrease of the absolute value of the zeta potential indicates the decreasing stability of the colloidal solution after an increased number of precipitation steps. This observation is consistent with the findings presented in section 3.1.3.3 pointing to the loss of ligands from the NC surface.

Similar to TGA-capped, also MPA-capped CdTe NCs show the decrease of the absolute zeta potential with dilution. At similar concentrations and for similar sized NCs the zeta potentials are comparable, which indicates a very similar ligand shell consisting of either MPA or TGA. At similar concentrations, larger NCs show a better stability indicated by a larger absolute value of the zeta potential and give qualitatively better DLS signals. Additionally, CdHgTe NCs capped by TGA, MPA and GSH were characterized by DLS and zeta potential measurements. DLS measurements yield peaks only at relatively large sizes, which do not allow to estimate

Figure 4.2: The intensity distribution of 2.5 nm sized CdTe-TGA NCs obtained from DLS measurements shows the increasing content of aggregates with decreasing NC concentration (left). Complementary, the decrease of the absolute value of zeta potential underlines the decreasing stability upon dilution (right). The error bars indicate the standard deviation.
Table 4.1: With an increasing number of precipitation steps the absolute value of the zeta potential of CdTe-TGA NCs decreases, which indicates the decreasing NC stability due to the loss of ligand.

<table>
<thead>
<tr>
<th>Zeta potential</th>
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</thead>
<tbody>
<tr>
<td>1st precipitation</td>
</tr>
<tr>
<td>2nd precipitation</td>
</tr>
<tr>
<td>3rd precipitation</td>
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</table>

The size of the ligand shell. This may be caused by the strong absorption of CdHgTe NCs in the visible region interfering with the measurement and the possible presence of a significant amount of aggregates. A reduced stability compared to CdTe NCs is also reflected in a smaller average zeta potential. However, the zeta potential remains relatively stable upon dilution. The absence of a pronounced concentration dependence as observed for CdTe NCs is in good agreement with the results from dilution studies measuring the PL QY, as presented in section 3.1.3.2. The fact that the ligand shell of CdHgTe NCs shows to be less affected by changes of the NC concentration indicates the favorable effect of Hg for the ligand shell.

In summary, DLS measurements allow the determination of the hydrodynamic size and indicate the presence of aggregates in a noninvasive way. By comparison with results from TEM and absorption measurements the estimation of the thickness of the ligand shell becomes possible. Dilution and precipitation studies show the decreasing stability of the NC solutions in form of a reduced absolute zeta potential probably due to the desorption of ligand from the NC surface. However, more information about the exact structure of the ligand shell need to be gathered by other methods as shown in the following.
4. ANALYSIS OF THE LIGAND SHELL STRUCTURE

4.2 Nuclear magnetic resonance spectroscopy

4.2.1 Theory

Nuclei of isotopes with a nonzero spin exhibit an intrinsic angular momentum \( p \) and consequently a magnetic moment \( \mu \). As shown in equation 4.7, they are correlated by the gyromagnetic ratio \( \gamma \). In the presence of a homogeneous and static external magnetic field, the spin states split into \( 2I + 1 \) different energy levels depending on the spin quantum number \( I \). For a proton (\(^1\)H) \( I \) equals \( \frac{1}{2} \) and two energy levels evolve, as depicted in figure 4.3. The energy of each level depends on the strength of the external magnetic field \( B_0 \) as shown in equation 4.8 with \( m \) being the magnetic quantum number \( (m = I, I-1, ..., -I) \).

Irradiation of the sample with energy equaling the difference of the energy levels \( \Delta E \) leads to excitation of the nuclear spin to a higher energy state. The resonance frequency is determined by the magnetic field and the magnetic properties of the nuclei, see equation 4.9. Following the excitation, relaxation processes occur in the form of energy loss by heat dissipation, so-called spin-lattice relaxation and loss of coherence to other spins, known as spin-spin relaxation. The spin-lattice relaxation is also referred to as longitudinal relaxation, as the change of nuclear magnetization occurs in the same orientation as the external magnetic field with a typical relaxation time of \( T_1 \). The spin-spin relaxation, also named transversal relaxation, leads to changes of magnetization perpendicular to the external magnetic field due to interactions of neighboring nuclei and is characterized by the relaxation time \( T_2 \). In modern NMR experiments excitation is usually realized by a radio frequency (RF) pulse that allows irradiation of all frequencies in a spectral width simultaneously. The nuclear magnetic response is obtained in form of a free induction decay (FID). Using Fourier transform the final frequency domain spectrum is then calculated from the time domain signal. As shown in equation 4.9, the resonance frequency should be constant for a nuclei of a certain isotope in a defined magnetic field. However, the shielding effect of the surrounding electron shell leads to a reduced effective magnetic field at the nuclei in a certain chemical environment. As the resulting changes of the resonance frequency are very small, the NMR signal is given as relative frequency difference to a reference in the form of the chemical shift \( \delta \) in the range of ppm. In detail, \( \delta \) is defined as difference be-

\[
\begin{align*}
\mu &= \gamma \cdot p \\
E_m &= -\mu_z \cdot B_0 = -\gamma \cdot m \cdot \frac{\hbar}{2\pi} \cdot B_0 \\
\nu &= \frac{\gamma}{2\pi} \cdot B_0 \\
\delta &= \frac{\nu_i - \nu_{st}}{\nu_0}
\end{align*}
\]
ween the resonance frequency of the studied core \( \nu_i \) and that of a reference \( \nu_{st} \) related to the operating frequency of the spectrometer \( \nu_0 \), as shown in equation \( 4.10 \). In detail, the chemical shift gives direct information on the chemical environment of the probed nuclei. The line width of a NMR signal depends on field inhomogeneities, long range coupling and the relaxation time \( T_2 \), which is related to size and mobility of the molecule. Line broadening can also be caused by exchange phenomena via intermolecular processes like proton transfer or intramolecular processes such as rearrangements. The exchange rate \( k_{ex} \) directly influences the appearance of the different states in the NMR spectrum depending on the characteristic time constant of the NMR measurement \( \tau_{NMR} \). A slow exchange leads to two separated signals with the average lifetime \( \tau_{ex} \) being larger than \( \tau_{NMR} \), characterized by the condition \( 4.11 \). In contrast, a fast exchange is present for case \( 4.12 \) and yields one averaged signal as \( \tau_{ex} \) is so small that several exchange processes occur during one NMR experiment. For one-dimensional \(^1\)H-NMR experiments, \( \frac{1}{\tau_{NMR}} \) equals the inverse of the diffusion delay.

\[
\begin{align*}
\text{slow exchange} & \quad k_{ex} \ll \frac{1}{\tau_{NMR}} \\
\text{fast exchange} & \quad k_{ex} \gg \frac{1}{\tau_{NMR}}
\end{align*}
\]

(4.11)

(4.12)

Referring to ligand molecules of NCs to be probed by NMR, a slow exchange rate between free and bound ligand would lead to markedly different NMR peaks for bound and free ligands. The potential of NMR for the identification and quantification of ligands bound to the NC has already been shown in the literature. Some exemplary works are shown in table 1.1 in chapter 1. In general, NMR is an advantageous method for the identification of molecules bound to the NC surface, because it is nondestructive and more-importantly probes the situation in solution without perturbing the equilibrium between free and bound ligands. For tightly bound ligands, the free and bound state can be distinguished from line width and chemical shift. \(^1\)H-NMR has been widely used for the study of PbS, PbSe, CdS, CdSe, CdTe, InP, and ZnO QDs prepared in organic solvents capped by thiols, carboxylic acids, amines, phosphonic acids, and phosphine oxides. \(^1^3\)C-NMR is not as sensitive as proton NMR, but can also be useful for the verification of ligand binding to the NC. \(^3^1\)P-NMR was also shown to help the analysis of the ligand shell composition. In all one-dimensional NMR techniques, a resonance broadening can be observed as general feature for strongly bound ligands. This is caused by the different rotational correlation time for the ligand bound to the NC compared to free ligand molecules. The larger correlation time of bound molecules, consequently a slow tumble rate, leads to a fast relaxation rate, i.e. a short relaxation time \( T_2 \), which causes a broad signal in the NMR spectrum. As this is still no complete evidence for the binding and there are cases, in which one-dimensional NMR does not allow to distinguish between free and bound molecules, advanced two-dimensional NMR experiments are used.

One promising NMR technique is diffusion-ordered spectroscopy (DOSY), which is also known...
as pulsed magnetic field gradient NMR (PFG-NMR) method. By applying a pulsed field gradient it becomes possible to measure diffusion coefficients associated with $^1$H resonances and to differentiate species according to their diffusion coefficient. Figure 4.4 depicts a simple pulse sequence for a DOSY measurement based on spin echo. Two equal magnetic field gradient pulses of the length $\delta$ and magnitude $g$ are introduced in between the RF pulses. These identical pulses would normally cancel each other out, but due to the occurrence of diffusion during the time that separates the gradient pulses, the intensity of the resonances of the studied molecule will be attenuated. After repeating this experiment for varied gradient strengths, attenuation profiles for each chemical shift can be obtained, which follow equation 4.13.

$$\frac{I}{I_0} = e^{-D(\gamma_\chi \delta g)^2\Delta'}$$

(4.13)

The attenuation of the echo intensity $I$ is an exponential decay depending on the diffusion coefficient of the species $D$, the gyromagnetic ratio of the studied nucleus $\gamma_\chi$, the duration of the gradient pulse $\delta$ and the effective diffusion delay $\Delta'$. The latter depends on the pulse sequence and the gradient shape. By fitting the measured decay according to this equation, the diffusion coefficient for each chemical shift can be obtained. As the diffusion coefficient of molecules attached to a NC is clearly different to the one for free molecules, the two-dimensional method allows to distinguish between free and bound ligand. The successful use of DOSY for the characterization of semiconductor NCs and for the study of ligand exchange processes has been shown in detail.\textsuperscript{[68,76–80,94,98]} For NCs with tightly bound ligands it is also possible to calculate the hydrodynamic radius from the obtained diffusion coefficient by the use of the Stokes-Einstein equation, see equation 4.5 and to gather information on the size of the ligand shell.

For the study of dynamic ligands, which undergo very fast ad- and desorption processes, usually a combination of one-dimensional $^1$H, DOSY and nuclear Overhauser effect spectroscopy (NOESY) is necessary to assign molecules that are associated to the NC surface.\textsuperscript{[68,78]} Transfer NOESY (trNOESY) allows to assign resonances to bound molecules at exchange rates, at which no line broadening or separated diffusion coefficients are observable. Cross-coupling signals in trNOESY stem from dipole-dipole interactions of protons within the same mole-
It could be shown that these interactions are enhanced for molecules immobilized on NCs.\textsuperscript{68,78–80,93,98} A similar technique is rotating frame nuclear Overhauser effect spectroscopy (ROESY). Whereas NOESY involves solely longitudinal relaxation effects, ROESY additionally probes transversal relaxation and can therefore complement results from NOESY experiments.\textsuperscript{82}

Besides identification of molecules acting as NC ligands, NMR also offers the possibility of quantification of the surface coverage. \(^1\)H-NMR is capable of quantifying the concentration of bound ligands, if signals can be assigned that originate solely from bound molecules and if a standard for quantification is present, \textit{e.g.} dibromomethane as internal standard.\textsuperscript{76} Importantly, the reference added has to be inert with respect to NC and ligand molecules. Additionally, information on the nature of the ligand/NC bond and on ligand exchange kinetics could be gathered from advanced NMR experiments. The study of ligand exchange with a combination of the presented NMR methods allows the determination of the local environment of the ligand and the rate of ligand desorption from the NC surface.\textsuperscript{79,80,98,99}

### 4.2.2 Results and discussion

Despite the rising popularity of NMR for the characterization of the ligand shell, no studies on CdTe NCs synthesized in aqueous media existed so far. The present work shows the information obtained from \(^1\)H-, \(^{13}\)C- and DOSY NMR for the ligand shell of CdTe consisting of TGA molecules.

From the \(^1\)H-NMR spectrum, which is shown in figure 4.5 for 2.6 nm sized CdTe-TGA in D\textsubscript{2}O, different signals can be obtained, one for the CH\textsubscript{2} group of TGA molecules bound to the NC surface and one for free TGA in solution. In general, the spectra show only one peak for TGA, which can be assigned to the CH\textsubscript{2} group as expected, because the protons of thiol and carboxyl group exchange with deuterium and therefore do not give a signal. One peak at 4.7 ppm stems from H\textsubscript{2}O molecules and some minor peaks with very low intensity are due to degradation of.

![Figure 4.5: \(^1\)H-NMR spectrum of 2.6 nm sized CdTe-TGA NCs in D\textsubscript{2}O. The different peaks for the CH\textsubscript{2} group of TGA allow discrimination of bound and free thiol.](image-url)
TGA and can also be found in the pure TGA solution. The broad signal at a chemical shift of 3.28 ppm can be assigned to the signal of the bound TGA molecule, whereas the unbound molecule shows a resonance peak at 3.25 ppm. The signals exhibit different chemical shifts due to a different environment of the investigated protons and different line widths due to different $T_2$ relaxation times. The changed $T_2$ time originates from the fact that ligands bound to the NC are less mobile than unbound molecules. As the peaks appear separated, a slow exchange of free and bound ligand is present with respect to the NMR timescale. From the difference of the resonance frequencies, which is 18 Hz in this case, the exchange time can be estimated to be larger than 55 ms. By fitting the resonance peaks and integration, the ratio of free : bound ligand can be estimated to be 5 : 95. The same sample was also studied by Ellman’s analysis combined with precipitation to separate bound from free TGA and to quantify the amounts. This analysis yields a notably different ratio for free : bound ligand of 60 : 40. These different findings prove that precipitation interrupts the equilibrium between bound and free ligand and changes the actual ratio, which can be probed in situ by NMR. This confirms the assumptions, which were obtained from ICP-OES analysis and optical studies of the precipitation process presented in section 3.1.1.2 and 3.1.3.3. Furthermore, the small amount of free ligand indicated by the results from NMR suggests that in this case ‘bound’ does not exclusively mean covalently attached to the NC, but immobilized on the NC, e.g. by van der Waals forces.

The comparison of the $^1$H-NMR results for CdTe-TGA NCs, pure TGA and a mixture of Cd$^{2+}$ salt and TGA in D$_2$O is shown in figure 4.6 (left). At similar pD values an increasing chemical shift for the CH$_2$ group can be observed in the order of pure TGA, Cd/TGA mixture and free TGA in a CdTe NC solution. This points to a change of the chemical environment of the CH$_2$ protons in the different solutions. In the Cd/TGA mixture the formation of Cd-thiol complexes of structures as discussed in section 5.3 is highly probable. The coordination of TGA molecules to Cd$^{2+}$ ions can be the reason for the different resonance frequency of

![Figure 4.6](image-url)
the protons. Furthermore, two additional and very broad peaks can be observed in the NMR spectrum, which suggest the presence of aggregates of the Cd-thiol complexes leading to a reduced relaxation time $T_2$ of the molecules. As in the NC solution the peak for the free TGA molecule is located at even higher ppm values, unbound TGA seems not to be present as simple TGA molecule in the NC solution, but rather in species from Cd-thiol complexes, disulfides and other reaction products, as shown in the previous chapter in figure 4.21. As the typical pD values for NC samples purified after synthesis by precipitation, drying and redissolving in $D_2O$ lie in the range of 10-12 depending on the actual extent of purification and the dilution, the pD of the solutions of pure TGA and the Cd/TGA mixture was adjusted to 12 for comparison. For unknown reason, adjusting the pD of the NC solutions by using NaOD or by dissolving the dried NCs in a phosphate buffer did not yield valuable spectra. Even though slight deviations in the pD are possible by this approach, they would not explain the large differences in the spectra, which can be observed.

$^1$H-NMR spectra for differently sized CdTe NCs are shown in figure 4.6 (right). The different NC size leads to a change of the peak shape for the bound ligand. With increasing NC size, the line width of the resonance peaks increases as expected. This can be explained by the rotational correlation time $\tau_c$ for spherical particles, which is according to the Stokes-Einstein-Debye equation, shown in equation 4.14, strongly dependent on the particle size. As also shown in equation 4.14, $\tau_c$ as well as the peak width $\Delta \nu_{1/2}$ are proportional to the relaxation rate $1/T_2$. Consequently a larger size causes a larger correlation time and therefore a broader signal.

$$\tau_c = \frac{4\pi \eta r^3}{3k_BT}, \quad \tau_c \propto \frac{1}{T_2} \propto \Delta \nu_{1/2} \quad (4.14)$$

Additional to the line broadening, the signals for the different sized NCs appear at different chemical shifts with lower values for larger NCs. The absence of a peak for free TGA in the shown spectra is probably caused by the appearance of all TGA associated to the NC. The origin thereof is not completely understood and may be caused by a larger extent of purification or a changed exchange rate in these samples. Further information on the composition of the ligand shell is obtained from $^{13}$C-NMR. The values were obtained from heteronuclear single quantum coherence-insensitive nuclei enhanced by polarization transfer (HSQC-INEPT) measurements. For CdTe-TGA one resonance peak can be observed for the $CH_2$ group at higher ppm values than for the pure TGA solution. This observation is again a sign for a covalent bond of the TGA molecule to certain species and the absence of pure TGA molecules as free ligands. Together with the previous results, this may point to a covalent modification of TGA, e.g. in the form of disulfides or Cd-thiol complexes, which are immobilized on the NC surface. To further study the difference between bound and free ligand, DOSY measurements are applied to CdTe-TGA NCs. Figure 4.7 shows the DOSY spectrum for 2.6 nm sized CdTe-TGA in $D_2O$. Three $^1$H-NMR signals can be assigned to three species with different diffusion coefficients. The signal for $H_2O$ at 4.7 ppm exhibits the largest diffusion coefficient. The two signals assigned to the $CH_2$ group of free and bound TGA give clearly different diffusion coefficients.
4. ANALYSIS OF THE LIGAND SHELL STRUCTURE

Figure 4.7: The DOSY spectrum of 2.6 nm sized CdTe-TGA NCs in D$_2$O identifies three different species in solution. The peak with the large chemical shift indicates H$_2$O and the two peaks at 3.3 ppm clearly correlate to species with different diffusion coefficients, such as free and bound ligands.

In this way, DOSY proves the presence of the two TGA species. For the free ligand a diffusion coefficient of $10^{-8.86}$ m$^2$s$^{-1}$ can be obtained. The broadened signal for the bound species exhibit a diffusion coefficient of $10^{-9.67}$ m$^2$s$^{-1}$. Using the Stokes-Einstein equation (4.5) to calculate the hydrodynamic radius, this diffusion coefficient would yield a radius of 0.9 nm, see section A.3.13. This is, however, somewhat smaller than the expected NC radius of 1.3 nm obtained from absorption spectra. One possible explanation includes that the ligands are not strongly bound and do not form a compact core/shell system with the NC. Then the calculation of the particle size from the diffusion coefficient assuming a spherical particle is not valid, which can cause the error. This would suggest again that the majority of ligands is not tightly bound to the NC, but immobilized on the NC. The presence of distinct peaks for bound and free species also in DOSY measurements indicates a slow exchange between the two states. With the diffusion delay of 100 ms used in this experiment, a correlation time that is larger than this value can be suggested.

Additionally, the effect of dilution of CdTe-TGA NC solutions was studied by NMR measurements. Figure 4.8 shows the results for $^1$H-NMR (left) and the diffusion coefficients obtained from DOSY (right). With decreasing concentration shape and position of the peak for the bound ligand changes. Besides the constant peak for the free TGA, the chemical shift for the protons of the bound molecules shifts to lower ppm values. The shift towards the resonance of the free ligand suggests a change of the chemical environment upon dilution in a way that the ligand attached to the NC becomes chemically more similar to the free ligand. One possible process could be a loosening of the shell structures. The sum of the integrals for the CH$_2$ group decreases with dilution as the overall TGA concentration decreases. By fitting and integration of the signals for bound and free TGA, the relative ratios are obtained. In contrast to the ex-
3.25 3.20 3.15 3.10 ppm
-9.0 -9.5 -10.0 -10.5 log (m²/s)

Figure 4.8: The ¹H-NMR resonance peak (left) and peak for the diffusion coefficients obtained from DOSY (right) change with decreasing NC concentration. The inset shows a photography of the samples under day (left) and UV (right) light.

pectations of a decreasing ratio of bound : free TGA with dilution due to ligand desorption, a relatively constant ratio of 92 : 8 is determined. The high ratio is again a sign for the fact that in NMR all TGA molecules somehow associated to the NC contribute to the signal of bound TGA. On a first look, the constant amount of ligand is contrary to the findings from dilution experiments presented in section 3.1.3.2. The studies showed a decreasing PL QY with dilution probably caused by the desorption of ligand. However, the concentrations used for the NMR measurements are in the range of 0.6 mM and consequently much higher than the concentrations, in which observable changes of the PL QY occur. The photographs under day and UV light illustrates the large concentrations necessary for the measurements. DOSY measurements on the same samples show a slight increase of the diffusion coefficient associated to bound TGA molecules with decreasing concentration. This could be caused by changes of the diffusion coefficient due to particle interactions present at high concentrations. However, if the effect would be caused by the concentration dependence of the diffusion, a shift should also be present for the signal of free TGA, which is not the case. The increase of the diffusion coefficient is related to a decreasing hydrodynamic size, which could be caused by a loosening of the ligand shell and a smaller applicability of the calculation of the size for spherical systems. Concomitant with the increase of the diffusion coefficient, the signals progressively become more diffuse with dilution. This would suggest the shift from distinct species with a defined diffusion coefficient towards less defined structures. These observations underline the previous findings that the ligand shell of CdTe-TGA NCs is not compact and tightly bound to the NC surface as well as the structure of the organic shell changes upon dilution. DOSY measurements of pure TGA also show a broadening of the DOSY peak with time. This may point to oxidation products of the molecule giving rise to different species with slightly different diffusion coefficients. The presence of oxidation products of TGA and more complex structures probably are the reason for less distinct features in NC solutions, which would be expected for pure TGA molecules bound to the NC.
In summary, the NMR studies show the great potential of the method for the evaluation of the ligand shell structure. The presence of free and bound ligand species is identified by the use of $^1$H-NMR and DOSY experiments. The results additionally confirm the existence of modified TGA structures, such as Cd-thiol complexes. The relative amount of these species attached to the NC seems to be unaffected by dilution, but the structure of the shell is supposed to change. The comparison of the results for peak width and diffusion coefficient obtained with those theoretically expected point to a weak binding to the NC. Further studies on the binding strength could include studies on thiol-capped Au NCs, for which a covalent binding of thiol to the Au surface is expected and could serve as a reference. The use of NOESY is expected to further clarify the picture of how the ligands are immobilized on the NC surface and to which extent they exchange with species in solution. More insights into the exchange processes are expected to be obtained from titration experiments with constant NC concentration and varied TGA concentration. Also temperature-dependent NMR measurements could shed light on the chemical nature of bound ligand species. To expand the presented study, the investigation of the precipitation process, changes at different pH and dependency of the storage time by NMR would give more insights into possible ways to change the amount of ligand on the NC surface. Besides the aforementioned, one drawback of NMR studies is the need for relatively high concentrations.
4.3 Small-angle X-ray scattering

4.3.1 Theory

In general, the structural investigation of nanomaterials is enabled by the observation of quasi-elastic scattering by the material. In the case of utilizing X-rays for the experiment, scattering occurs by electrons and the contrast is a function of the difference of electron density. On the contrary, neutrons are scattered at the atomic cores and the contrast depends on the different scattering lengths of the atoms. By the choice of the angle different size regimes are probed. Small-angle scattering resolves dimensions in the nanometer regime, whereas wide-angle scattering probes interatomic distances and allows the resolution of atomic dimensions.

The principle of a small-angle X-ray scattering (SAXS) experiment is depicted in figure 4.9. A collimated X-ray beam of a certain wavelength, which is shaped by mirrors and slits, is directed to the sample. Following the elastic interaction with the sample, the scattered radiation is collected in a two-dimensional, circularly symmetric pattern at the detector. The distance between detector and sample is chosen to be in the range of meters for high resolution. In order to protect the detector, the initial beam is blocked by a beam stop, which is introduced right in front of the detector. The geometry is characterized by the scattering vector $q$ with a magnitude $q = \frac{4\pi}{\lambda} \sin \left( \frac{\Theta}{2} \right)$ for a scattering angle $\Theta$. By the use of $q < 10 \text{ nm}^{-1}$, SAXS shows to be a powerful technique to determine properties of particles in the size range of 1 to 100 nm. From the intensity recorded on the two-dimensional flat plane detector, a one-dimensional scattering curve can be extracted, which shows the function of the scattering intensity $I$ depending on the value of the scattering vector $q$. This function contains a number of structural parameters of the studied sample, such as particle sizes, volume fraction, and shapes. One usual way to extract this information is based on the development of a theoretical model for the studied system and further refinement until the calculated curve fits the experimental data. Therefore, the understanding of the scattering process is of utmost importance.

The scattering amplitude for one single electron is given by $A_e(q) = e^{i\varphi \mathbf{r}}$, which accounts for the phase shift of the scattered radiation depending on the scattering vector $q$ and the position described by $\mathbf{r}$. For coherent scattering of an assembly of scatterers, the scattering

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Figure 4.9: Schematic setup of SAXS measurements.

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†The results of this section were obtained within the cooperation with E. Gericke and T. Hendel (Chair of Physical Chemistry, HU Berlin) and are partly also subject to the Master thesis of E. Gericke. All measurements and calculations were performed by E. Gericke.
amplitude becomes the sum of all scattering contributions weighted by the scattering factors \( f_i \). The latter describes the ratio between the scattering amplitude of the object and that of one electron at identical conditions. By introducing the continuous electron density \( \rho(r) \), the scattering amplitude for coherent scattering \( A(q) \) is then described by the equation below.

\[
A(q) = \sum_{i=1}^{N} f_i e^{i\mathbf{q} \cdot \mathbf{r}_i} = \int_{V_r} \rho(r) e^{i\mathbf{q} \cdot \mathbf{r}} dV_r
\]  
(4.15)

This is true for the scattering of a single particle with the particle volume \( V_r \). In the general case of small angle scattering, \( \rho(r) \) is exchanged by the local scattering length density \( \eta(r) \).

The product of the amplitude and its complex conjugate relates to the form factor of the particle \( F(q) \), which is determined by the shape, size and structure of the particle. Considering a particle in solution, the scattering amplitude is determined by the contrast of the electron density between particle \( \rho_p(r) \) and solvent \( \rho_s \), defined as \( \Delta \rho(r) = \rho_p(r) - \rho_s \). Additionally, due to the motion of the particle, the scattering amplitude will be a spherical average \( \langle A(q) \rangle \) with \( \langle e^{i\mathbf{q} \cdot \mathbf{r}} \rangle = \sin(qr)/qr \).

In the simplest case of a sphere with radius \( R \), \( A(q) \) is given by the following equation.

\[
A(q, R) = 4\pi \int_0^R r^2 \Delta \rho \frac{\sin(qr)}{qr} dr = \frac{4}{3} \pi R^3 \Delta \rho \frac{\sin(qR) - qR \cos(qR)}{(qR)^3}
\]  
(4.16)

For an assemble of particles, the final scattering intensity \( I(q) \) depends on the form factor \( F(q, r, \Delta \rho) \), the structure factor \( S(q, r) \) and on the number distribution of scatterers per unit volume \( N(r) \). \( F(q, r, \Delta \rho) \) was shown to be the product of scattering amplitude and its complex conjugated, as well as to be determined by the exact structure of the particle. \( S(q, r) \) additionally accounts for interference of scattered radiation and gives information on the particle interaction and in special cases on the mean particle distance. In solution, \( S(q, r) \) is expected to equal one, as the particles are randomly oriented and distributed.

A special form of the SAXS technique is anomalous small-angle X-ray scattering (ASAXS). The technique is based on the energy dependence of atomic scattering factors \( f(E) \) close to X-ray absorption edge, shown in equation 4.17.

\[
f(E) = f_0 + f'(E) + if''(E)
\]  
(4.17)

\( f_0 \) describes the scattering factor at energies far from the absorption edge and equals the number of electrons in the atom, i.e. the atomic number \( Z \). \( f'(E) \) and \( f''(E) \) are additional contributions that describe real and imaginary part of the atomic scattering and depend on the energy of the radiation. Based on this relation, element-specific contrast variation is possible in the vicinity of the absorption edge. This enables to determine elemental compositions of distinct phases in the system studied. Additionally, by the element-specific structure analysis ASAXS allows to distinguish between different structural models.

For SAXS and ASAXS measurements with high resolution, usually synchrotron radiation is
Figure 4.10: Photograph of the setup for ASAXS experiments at BESSY II (left) and the sample chamber for measurements at the Cd-K edge in air (right).

used. This is advantageous compared to other X-ray sources, because of higher brilliance, better collimation and high intensity. Figure 4.10 shows the layout of the ASAXS beamline at BESSY II, which was used for the experiments. The sample detector distance is continuously variable from 0.8 to 3.7 m. Sample chamber and vessels were changed depending on the measurement to realize the optimal sample thickness, as described in detail in section A.3.14. For ASAXS measurements at the Cd-K edge, NC solutions were probed in vials of 1 cm inner diameter, which was the necessary sample thickness for measurements at high energies.

In summary, SAXS is capable of probing the size of NC core and shell at once and directly in solution. ASAXS additionally allows to determine phase compositions.

4.3.2 Results and discussion

To complement the presented results for thickness and composition of the ligand shell of TGA-capped CdTe NCs, small-angle scattering techniques were used. The already given discussion points to the presence of Cd$^{2+}$ in the ligand shell, but did not yield a final proof and quantitative results. This, however, can be achieved by element-sensitive ASAXS measurements, as shown in the present section. Small-angle neutron scattering (SANS) additionally allows to vary the solvent contrast and thereby analyze the contribution of solvent molecules to the ligand shell. SAXS measurements of differently concentrated NC solutions indicate changes of the ligand shell structure upon dilution and allow the prediction of a structural model.

In general, the SAXS curves exhibit a typical scattering pattern for q values of 0.1 - 1 nm$^{-1}$ indicating nanostructures of 1 - 10 nm in size. An increasing scattering amplitude for q values below 0.1 nm$^{-1}$ point to aggregates with sizes larger than 100 nm. To minimize the content of aggregates and Cd-TGA structures that would give rise to strong scattering background, all samples were centrifuged prior to the measurement. For large scattering angles the scattering curves show characteristics for spherical particles. However, to further evaluate the scattering curves, a valid model has to be developed. Therefore, several models were applied and compared to the experimental data. Especially the combination of SAXS, ASAXS and SANS allows to find a structural model close to reality. ASAXS measurements were performed close to three
absorption energies, the Cd-K edge at 26711 eV, the Cd-L3 edge at 3539.5 eV and the Te-L3 edge at 4338 eV. For each case, SAXS measurements were conducted at four or five X-ray energies below the absorption edge in order to monitor changes of the scattering intensity dependent on the energy. The results are shown in figure 4.11 and depict differences for Cd and Te. The intensity differences of the scattering curves show a larger region of the scattering vector for which Cd contributes to scattering compared to Te. This suggests that Cd is a constituent of ligand shell and NC core, whereas Te can be only found in the NC core. A detailed analysis will be given below.

To fit the scattering curves from ASAXS measurements different form factors were calculated based on various models. These models include a simple sphere with a certain size distribution, a core/shell system with independent core size and shell thickness, a core/shell system with connected core size and shell thickness and a core/shell system with a constant shell thickness. Additionally, estimations for systems with a NC core and a ligand shell exponentially migrating into the solvent and a combination of linked core and shell size with an exponentially decaying shell were used. The resulting form factors are given in section A.3.14. The best results could be obtained for the model of a core/shell system with core and shell size depending on the particle size as well as for an exponentially decaying shell. The obtained fits are depicted in figure 4.12 showing the good agreement with the experimental data. The slight differences of the data points originate from the subtraction of the scattering background, which is given by incoherent scattering and contributions from aggregates. As the latter cannot be determined with high accuracy, it was kept as free parameter to be fitted, which may cause slight differences in the scattering curves.

Based on the assigned model of a core/shell system it is possible to calculate a plot of the
scattering length density, i.e. the electron density in this case, relative to the distance from the center of the NC core. This in turn allows to determine a radius of NC and ligand shell due to the different electron densities, as schematically shown in figure 4.13 (left). The results for electron density plots of the presented ASAXS measurements at different energies are depicted in figure 4.13 (right). Notably, both Cd and Te show distinct energy-dependent changes of the contrast in the region of high electron density, which can be assigned to the NC core. In consideration of the fact that the contrast of pure TGA is similar to that of H₂O, the obvious presence of a region with a contrast between that of the NC core and the solvent proves the

**Figure 4.12:** Fitting of the ASAXS data for 2.7 nm sized CdTe-TGA NCs shows the best results based on a model of a core/shell system, in which both core radius and shell size are a function of the particle size (left) and for a model of a shell exponentially migrating into the solvent (right).

**Figure 4.13:** SAXS measurements allow the determination of the core radius \( R_{\text{core}} \) and the particle radius including the ligand shell \( R_{\text{shell}} \) according to the scattering contrast (left). By applying a model of a core/shell system to the ASAXS measurements the contrast depending on the radius can be extracted from experimental data. The contrast is different for Cd and Te indicating that Cd is part of the ligand shell (right).
incorporation of a high-contrast element in the ligand shell. The fact that the energy-dependent changes in the region of the ligand shell are more pronounced for Cd, additionally evidences the presence of Cd in the ligand shell. The small changes observed for Te stem from the challenge that energies of the Cd-L3 and Te-L3 edge are close together and it is not possible to probe the Te edge completely without addressing Cd. Following this analysis SAXS allows to determine core and shell size of TGA-capped CdTe NCs. Furthermore, the calculation of the approximate number of Cd$^{2+}$ ions in the ligand shell is possible by comparison of the volume integrals of NC core and shell. For the studied sample, this calculation yields that 36 % of the total amount of Cd is located in the ligand shell. As the contrast of TGA is too small the exact amount of ligand cannot be estimated. Assuming the highly probable ratio of TGA : Cd of 2 : 1, this results in a suggested formula for the studied 2.7 nm sized CdTe-TGA NCs of $[\text{Cd}_{140}\text{Te}_{140}]_{\text{core}}[\text{Cd}_{80}\text{TGA}_{160}]_{\text{shell}}$.

The analysis of differently sized CdTe-TGA NCs is shown in figure 4.14 (left). The obtained values for the NC core from SAXS measurements agree well with data calculated from absorption spectra and TEM analysis. The thickness of the ligand shell is 1.6 nm on average, which matches the results obtained from DLS and the considerations of the quantified amount of thiols. This in conjunction with the presence of Cd$^{2+}$ in the ligand shell rules out the sole existence of a monolayer of bound TGA molecules and points again to a network of Cd-thiol complexes on the NC surface. To further investigate the structure of the ligand shell and changes upon dilution, SAXS measurements with varying NC concentration were performed. Interestingly at the first sight, an increasing size of both core and shell can be observed with decreasing concentration. Analysis of the shell thickness depending on NC concentration yields the curve drawn in figure 4.14 (right). Notably, upon decreasing the NC concentration the shell thickness initially increases to a maximum followed by a decrease at

Figure 4.14: The SAXS results for differently sized CdTe-TGA NCs are in good agreement to the particle size obtained from optical absorption measurements (left). SAXS measurements of 2.7 nm sized CdTe-TGA NCs at different NC concentrations show two trends, an initial increase of the ligand shell thickness followed by a decreasing shell thickness at very low concentrations (right). The dotted line is only a guide to the eye.
low concentrations. This observation can be explained by a loosening of the ligand shell and possibly a swelling by solvent molecules. Further dilution may cause a further loosening and the desorption of ligand species from the NC surface. In order to further study the interaction of solvent and ligand shell SANS measurements were performed at a wavelength of 6 Å. As $H_2O$ and $D_2O$ cause strongly different scattering contrast in SANS experiments, measurements of CdTe-TGA NCs in both solvents were performed. The results indicate that ligand molecules significantly contribute to the ligand shell. A revised chemical formula would then be $[Cd_{140}Te_{140}]^{\text{core}}[Cd_{80}\text{TGA}_{160}(H_2O)_x]^{\text{shell}}$.

In summary, for the first time SAXS measurements on TGA-capped CdTe NCs were performed and could be used to develop a structural model of the system. The combination of SAXS, ASAXS, and SANS yield the best model to be a crystalline CdTe core with a gradual water-swollen shell of Cd-thiol species. The thickness of the ligand shell was determined and the amount of Cd in the ligand shell can be estimated.
4.4 Summary and development of a structural model

To sum up, the evaluation of the ligand shell structure was performed by the use of three advanced analytical methods. DLS allows the estimation of the shell thickness and yields a value in the range of 2 nm, which is significantly larger than expected for a monolayer of ligand. Dilution and precipitation studies monitored with DLS underline the decreasing stability of the NC solution with decreasing NC concentration or increasing number of precipitation steps. The change of the ligand shell is suggested to be the reason for the change in stability, but further investigation of the exact structure by other methods remains necessary. In this respect, NMR was applied due to the capability to directly study the ligand shell in solution without perturbing the equilibrium between bound and free ligand. The presence of separated signals in the $^1$H-NMR and DOSY spectrum for CdTe-TGA NCs proves the presence of different species, i.e. free and bound ligand. The estimation of the molar ratio of both species indicates a large amount of TGA associated with the NC core. The investigation of the chemical environment of the ligand in the NC solution points to the presence of modified TGA structures, which supports the previously drawn assumption that Cd-thiol complexes form the ligand shell of water-soluble CdTe NCs. By comparison of the experimental results with expected values, a relatively weak binding of the ligand species is suggested. Studies of the concentration dependence using NMR indicate changes of the ligand shell structure upon dilution, however, leave the question on the exact changes open. To answer the question and to determine the exact composition and size of NC core and shell, SAXS was utilized in addition. To extract these information from the scattering curves from CdTe-TGA NCs, a model was developed by the use of element-specific SAXS. It was found that the characteristics of CdTe NCs capped by TGA are best described by a crystalline core surrounded by a water-swollen Cd-thiolate shell. The verification of this model by ASAXS and SANS measurements enables to gather knowledge of the ligand shell of water-soluble CdTe NCs by SAXS. Thereby, the presence of Cd and solvent molecules in the ligand shell can be proved and the amount of Cd in the ligand shell can be quantified.

By the combination of the methods used for the quantitative and structural analysis, the development of a structural models becomes accessible. A general chemical formula is given as follows.

$$[\text{Cd}_x\text{Te}_y]^{\text{core}}[\text{Cd}_z\text{TGA}_{2z}(\text{H}_2\text{O})_i]^{\text{shell}}\text{TGA}_n$$

To determine the variables, information obtained from different methods need to be combined. At first sight, the sum $x + y$ equals the number of atoms forming the NC core and should be ascertainable from the size of the NC core. However, as the actual geometry of the small inorganic crystals cannot be definitely assigned, this calculation can cause large errors. As an example, the volume of a cuboctaeder is 0.56 times the volume of the circumscribed sphere. Consequently, an assumption of a spherical geometry would give almost the double number of atoms as for the same sized non-spherical NC. Other possible geometries include tetrahedra...
and truncated tetrahedra with varying extent of truncation. Instead, the calculation of the composition of the NC is done using the results from Ellman’s test for the total amount of TGA in solution \( n(TGA)_{Ellman} \), the molar ratios of Cd : Te and Cd : S from ICP-OES, \( \left( \frac{n(Cd)}{n(\text{Te})} \right)_{\text{ICP-OES}} \) and \( \left( \frac{n(Cd)}{n(S)} \right)_{\text{ICP-OES}} \) respectively, and the determined amount of Cd in the shell relative to the amount of Cd in the core from ASAXS measurements \( \left( \frac{n(Cd \text{ in shell})}{n(Cd \text{ in core})} \right)_{\text{SAXS}} \).

I) \[
2z + n = n(TGA)_{Ellman}
\]

II) \[
\frac{x + z}{y} = \left( \frac{n(Cd)}{n(\text{Te})} \right)_{\text{ICP-OES}}
\]

III) \[
\frac{x + z}{2z + n} = \left( \frac{n(Cd)}{n(S)} \right)_{\text{ICP-OES}}
\]

IV) \[
\frac{z}{x} = \left( \frac{n(Cd \text{ in shell})}{n(Cd \text{ in core})} \right)_{\text{SAXS}}
\]

For a 2.7 nm sized CdTe-TGA NC sample, Ellman’s test yields 223 TGA molecules. ICP-OES resulted in a molar ratio of Cd : Te : S of 1 : 0.49 : 1.37 and ASAXS measurements suggest a molar ratio of Cd in the shell to Cd in the core of 0.57. This results in the following chemical formula for 2.7 nm sized CdTe NCs capped by TGA.

\[
[Cd_{104}Te_{80}]^{\text{core}} [Cd_{59}TGA_{118}(H_2O)_i]^{\text{shell}} TGA_{105}
\]

It has to be admitted, that the presented calculation of the composition still includes some assumptions. The amount of TGA in the ligand shell is estimated to give a molar ratio of Cd : TGA of 1 : 2, which is assumed as average for the different stoichiometries of Cd-thiol complexes, as depicted in figure [3.21]. The exact ratio of free and bound ligand was expected to be accessible by NMR studies. However, the observations showed a large percentage of the overall amount of ligand species connected to the NC. Together with the finding that solvent molecules contribute to the ligand shell, it is likely that there is no clear border of the ligand shell and Cd-thiolate complexes from solution can also assemble to the NC surface. From the NC core size and the assumption of a spherical shape the number of atoms in the NC core would equal approximately 280, which is significantly larger than the calculated value in this case. This may point to a non-spherical shape of the NCs. The excess of Cd in the NC core suggests a Cd-enriched surface of the studied CdTe NCs, which may cause the observed bright emission, additionally supported by the sufficiently large amount of ligands present.

All together, the presented characterization techniques allow to draw a picture of the ligand shell of CdTe-TGA NCs, even though some assumptions have to be proved by further studies. For example, extended NMR studies will help to resolve the type of ligand binding and exchange processes. The findings thereof could also clarify if the presented composition is dynamic.
5 Conclusion and perspectives

Water-soluble CdTe NCs have gathered considerable attention for many applications because of, amongst others, their small size and bright luminescence. The colloidal synthesis and the properties of the NC core are well studied, but the material is still subject to basic research. To allow stable and bright emission also under application-relevant conditions, a thorough knowledge about the surface chemistry of the NC is of importance. Despite the awareness of the strong influence of the ligand shell on the overall properties, a comprehensive study dedicated to the ligand shell of short chain thiol-capped CdTe NCs was in demand. The current work contributes to this goal by the use of several methods to resolve the amount of ligand present, the influence on emission properties, and the structure of the ligand shell.

It was shown that the aqueous synthesis is also capable of preparing NIR emitting CdHgTe NCs. In the presented work, the one-pot synthesis of TGA-capped CdHgTe was expanded to the use of MPA and GSH as ligands for bright and stable NCs. A detailed study about parameters that influence the optical properties showed that the emission can be tuned by the growth time, the initial Cd : Hg ratio, and the choice of ligand. These parameters influence the growth, which is reflected by changes in NC size and composition. It was shown that CdHgTe NCs exhibit a more stable ligand shell compared to CdTe NCs, since CdHgTe NCs are less prone to a concentration-dependent PL QY. In this context, the present work contributes to the design of the next generation of highly emissive and long-term stable NIR emitting NCs.

Besides the modification of the core material of water-soluble semiconductor NCs, the current work also aimed for modification of the ligand shell using highly functional DNA molecules. Biofunctionalization of CdTe NCs was achieved without the need for elaborate encapsulation strategies and significant increase of the particle’s size. The successful functionalization of thiol-capped CdTe NCs with oligonucleotides is promising for the precise and programmable assembly of NCs. Besides the large amount of studies on the DNA-functionalized metal NPs, less publications are dedicated to QD-DNA conjugates and especially the investigation of the exact binding of oligonucleotides to the NC surface. This work emphasizes the need of characterizing the success and extent of functionalization. First attempts were shown to probe the binding of DNA to the NC via optical and microscopic methods, which need to be extended to allow routine analysis. Further studies may include the design and preparation of functional assemblies of single NCs based on DNA origami templates for the use as active photonic or electronic building blocks.

A more detailed study of the surface chemistry was presented for short-chain thiol-capped CdTe NCs in this work aiming for quantification of ligands and resolving the ligand shell structure, including the specific influence on optical properties. As a quick and easy method
to quantify the amount of thiols that can be performed without advanced instrumentation, the Ellman’s test was introduced for the analysis of NCs. Accompanied by elemental analysis the investigation yields values that point to the presence of more ligands per NC than expected from theoretical calculations for a monolayer of thiol molecules on the NC surface. Additionally, thermogravimetric studies show that the ligand molecules are not loosely assembled within the ligand shell. Attempts to remove ligands from the NC surface by precipitation of the NCs from solution indicate the presence of Cd in the ligand shell of CdTe NCs. The results presented suggest the involvement of Cd-thiol complexes of various compositions and structures in the ligand shell. This is in contrast to NCs soluble in organic solvents that show typically a monolayer of organic ligands on the NC surface. The different observation may be caused by the different chemical structures of the ligands used for solubility in aqueous and organic solvents, respectively, since for the latter the stabilizing molecules consist of separated parts responsible for solubility and coordination to the NC. The presented studies on the influence of the amount of ligand on emission properties of CdTe NCs emphasize the need to pay attention to the conditions used for handling, optical measurements, and application of NCs. The current work shows the strong influence of pH, NC concentration, type and purity of the solvent, and the number of purification steps on the emission of water-soluble semiconductor NCs. Additionally, the current work presents methods that deliver a deeper insight into the structure of the ligand shell of TGA-capped CdTe NCs. DLS was utilized to estimate the shell thickness and to study the stability based on zeta potential measurements. Dilution as well as precipitation were shown to decrease the colloidal stability. NMR studies prove the presence of free and bound ligand species, which both consist of modified TGA molecules such as Cd-thiol complexes. NMR also indicates that the ligand species are not strongly bound to the NC. Further studies utilizing advanced NMR techniques could yield information on the ligand binding and possible exchange processes. The utilization of SAXS facilitates the identification of size as well as composition of core and shell of the NC. Advanced SAXS techniques allowed the development of a model for CdTe-TGA NCs that can be applied for the analysis of scattering curves, which is profitable for further studies to be performed in the future. SAXS measurements also prove the presence of Cd and solvent molecules in the ligand shell. Altogether, the thorough quantitative and structural analysis of CdTe NCs allows for the development of a structural model including information on stoichiometries. The results show the presence of Cd-thiol complexes and indicate the challenge to define a clear border between ligand shell and the solvent containing additional Cd-thiol complexes. To gather a deeper insight into probable chemical structures, theoretical calculations of the stability of different complexes may be subject to further research. All in all, the presented work contributes to the establishment of a clearer picture of the ligand shell of water-soluble semiconductor NCs and the possibility to evaluate the material for certain applications. Depending on the need suitable ligands have to be chosen. Functionalization strategies to circumvent dynamic changes of the surface coverage and the PL intensity may include encapsulation relying on amphiphilic polymers that can be cross-linked. However,
er, this would lead to a considerable increase in NC size, which can be disadvantageous for applications with size restrictions like energy transfer-based signaling strategies. To avoid the large amount of organic molecules, which may hinder the use of NCs for optoelectronics, new classes of ligands have been developed by other working groups. One prominent class is represented by inorganic ligands that include chalcogenido- metalates, lead halide perovskites and metal halide complexes, small inorganic oxoanions and polyoxymetala-
tes, as well as compact halide and pseudohalide ligands. By the use of these ligands, the preparation of all-inorganic nanomaterials becomes possible. The latter may be assembled into conductive films with short interparticle distances and superior electronic communication between the NCs. Also in this case, studies on the surface chemistry are necessary for the control of the overall properties and should be subject to prospective work.

Further studies may also include the analysis of the ligand binding strength to the NC by adsorption isotherms. However, due to the complexity of the ligand shell of water-soluble, thiol-capped NCs, the challenge to assign a signal that is directly linked to the surface coverage and a model that can be applied, needs to be addressed at first. The presented study indicates that the relationship between luminescence and the amount of ligand is more complex than the simple coverage. Additional studies can focus on the addition of ligands to solutions of constant NC concentration and constant pH to study effects of excessive ligands. Remaining challenges also imply the fact that the presented methods give mean numbers, but do not define the number and relative amount of the different species that compose a NC solution. Therefore, studies on the surface should be extended to the use of chromatographic methods or analytical ultracentrifugation.
A Experimental section

A.1 Materials

Unless otherwise noted, all chemicals used were of analytical grade or of the highest purity available and employed without additional purification. All solutions were prepared using ultrapure, Milli-Q® water (Millipore) unless otherwise specified. For certain experiments, pure water was obtained from RiOs™ Essential water purification systems (Millipore) and tap water was taken from a water tap in Dresden Südvorstadt. Cadmium perchlorate hydrate (Cd(ClO₄)₂·6H₂O) and mercury perchlorate hydrate (Hg(ClO₄)₂·6H₂O) were purchased from Alfa Aesar. Sodium hydroxide (NaOH), sulfuric acid (H₂SO₄, 95-98 %), hydrochloric acid (HCl, 37 %), nitric acid (HNO₃, 65 %) and glutathione (GSH) were obtained from Sigma-Aldrich. Aluminum telluride (Al₂Te₃) was received from Cerac Inc. Iso-propanol, thioglycolic acid (TGA), ethylenediaminetetraacetic acid (EDTA), L-cysteine, and deuterium oxide (D₂O, 99.96 %) were purchased from Merck. Mercaptopropionic acid (MPA), 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman’s reagent), and sodium deuteroxide (NaOD, 40 % in D₂O, 99.5 % D) were bought from Aldrich. Trizma® base (Tris) was obtained from Sigma. Standards for PL QY measurements Rhodamine 101 (R101) as well as Rhodamine 6G (R6G) were purchased from Radiant Dyes Laser, 1,1’,3,3,3’,3’-Hexamethylindotricarbocyanine iodide (HITCI) was obtained from Lambda Physik. DNA oligonucleotides were received in purity obtained after high-performance liquid chromatography (HPLC) as dried powder. The oligonucleotide consisting of a sequence of 18 adenine and BBQ at the 3’ end (A18-BBQ) and the complementary sequence to oligonucleotides B1 and A2 were purchased from biomers.net. All other DNA oligonucleotides (A1, B1, C1, T1, T2, A2) were bought from Life Technologies. For further use, the dried powders were dissolved in Milli-Q® water to a concentration of 100 µM and stored at -20 °C.

A.2 Syntheses

A.2.1 CdTe

A typical synthesis of CdTe NCs capped with TGA was performed as follows. In a three-necked flask fitted with a septa and valves, 0.576 g (1.37 mmol) of Cd(ClO₄)₂·6H₂O was dissolved in 60 ml of water and 0.165 g (1.79 mmol) of TGA was added under stirring, followed by adjusting the pH to 12 by dropwise addition of an appropriate amount of a 1 M NaOH

*Parts of this section have already been published [50,146,178]. Reproduced by permission of The Royal Society of Chemistry*
solution. The solution was deaerated by argon bubbling for 1 h. Under stirring, H₂Te gas, which was generated by the reaction of 0.1 g (0.23 mmol) of Al₂Te₃ lumps with an excess amount of 0.5 M H₂SO₄, was passed through the solution together with a slow argon flow for nearly 30 min to yield a molar ratio of Cd²⁺ : Te²⁻ : TGA = 1 : 0.5 : 1.3. The formation of CdTe precursors was followed by further nucleation and growth upon refluxing at 100 °C under open-air conditions with a condenser attached. If not stated otherwise, the samples were concentrated on a rotary evaporator, precipitated by the addition of iso-propanol and subsequently dissolved in ultrapure water. No special treatments allowing to narrow the size distribution or to increase fluorescence quantum yields were applied post-preparatively. For syntheses of 100 ml, 250 ml, and 500 ml the amount of Al₂Te₃ was scaled to 0.2 g, 0.4 g, and 0.8 g and the amount of other chemicals were changed respectively to the molar ratio. MPA-capped CdTe NCs are prepared according to an identical procedure.

The concentration of the NC solutions was determined by absorption and emission spectroscopy according to equation A.1. The normalized absorbance $A_{\text{norm}}$ depends on both optical density at the absorption maximum and full width at half maximum of the emission band. The extinction coefficient $\epsilon$ is related to the diameter obtained via the empirical sizing curve from the wavelength of the absorption maximum. $L$ denotes the path length of the sample, i.e. the cuvette size.

$$c(\text{NC}) = \frac{A_{\text{norm}}}{\epsilon \cdot L} \quad (A.1)$$

### A.2.2 CdHgTe

The synthesis of CdHgTe NCs is carried out according to the CdTe synthesis. Additionally to Cd(ClO₄)₂ · 6H₂O, Hg(ClO₄)₂ · 6H₂O is dissolved in the precursor solution in an amount according to the used initial Cd : Hg ratio. The procedure is identical for the applied ligands TGA, MPA, and GSH.

### A.2.3 CdTe/CdS-DNA

Modification of CdTe NCs with DNA was performed on the basis of the work by Deng et al.²³⁷ The utilized CdTe NCs were synthesized according to the procedure described above (A.2.1). For the additional CdS shell, a freshly prepared solution of 25 mM MPA was mixed with a 25 mM solution of Cd²⁺ obtained from dissolved Cd(ClO₄)₂ · 6H₂O in H₂O to achieve a molar ratio of MPA : Cd²⁺ of 2 : 1. By utilizing 1M NaOH the pH was adjusted to 12. A distinct volume is added to approximately 0.15 nmol CdTe NCs to achieve a molar ratio of NC : Cd²⁺ : MPA of 1 : 850 : 1700. After mixing and sonication for 5 minutes, 100 µM DNA solution was added to obtain a molar ratio of NC : DNA of 1 : 10 or 1 : 5 as desired. The sample was mixed, heated to 90 °C, and kept at this temperature for 40 minutes. After cooling to room temperature the sample was diluted to the final volume necessary for recording absorption and emission spectra. The shown spectra were measured utilizing a 0.3 x 1 cm quartz cuvette from
Appendix A: Experimental Section

Hellma GmbH, which allows absorption and emission measurements of volumes of 1 ml. For comparison, a reference sample upon the addition of H$_2$O instead of DNA was prepared. The calculation of the stated amount of CdS precursor necessary for four CdS monolayers was done according to the equation below:

$$V_{\text{shell}} = \frac{4}{3} \pi \left( (r_{\text{core}} + m \cdot d_{\text{ML}})^3 - r_{\text{core}}^3 \right), \quad n_{\text{shell}} = \frac{V_{\text{shell}} \cdot \delta_{\text{core}} \cdot N_A \cdot n_{\text{QD}}}{MW_{\text{core}}}$$  \hspace{1cm} (A.2)

$V_{\text{shell}}$ describes the volume of the shell material per NC, $r_{\text{core}}$ is the radius of the core, assuming a spherical geometry, $d_{\text{ML}}$ is the thickness of one monolayer of shell material. The molar amount of precursor required to deposit $m$ monolayers of shell material $n_{\text{shell}}$ is calculated using the density of the core material $\delta_{\text{core}}$, Avogadro’s number $N_A$, the molar amount of NCs in solution $n_{\text{QD}}$, and the molar weight of the core $MW_{\text{core}}$. Figure A.1 illustrates the phosphorothioate modification of the DNA backbone compared to usual phosphodiester linkage. In the case of presented thiolated DNA, the 3' end of the oligonucleotide carries lipoic acid as functional group bound to the oligonucleotide via the carboxyl group.

![Chemical structure of DNA](image)

Figure A.1: Chemical structure of DNA containing a sequence of phosphorothioate modification of the DNA backbone in comparison to the usual phosphodiester linkage.

A.3 Methods

A.3.1 X-ray diffraction and combustion elemental analysis

The XRD measurement presented was performed from a concentrated CdTe-TGA NC solution by a PANalytical X’Pert Pro using Cu-K$_\alpha$ radiation in reflection mode. The values were obtained from the addition of five measurements, which lasted 12 min each. Combustion EA measurements were performed from NC powders obtained after one purification step using a Hekatech EA 3000 Euro Vector CHNSO Elemental Analyzer.
A.3.2 Optical spectroscopy

Optical characterization of CdTe NCs was performed by UV/vis absorption spectroscopy with a Cary 50 spectrophotometer (Varian Inc., Palo Alto, CA) and fluorescence measurements with a Fluoro-Max-4 spectrophotometer (HORIBA Jobin Yvon Inc., Edison, NJ). Unless otherwise specified, all absorption and fluorescence measurements were performed with air-saturated freshly prepared NC or dye solutions using 1 x 1 cm quartz cuvettes from Hellma GmbH. The PL QY of the NCs was evaluated using R101 (PL QY = 0.915, solvent ethanol), R6G (PL QY = 0.916, solvent ethanol) as reference standards, which were lately remeasured[133]. The stated values were obtained from the average of the measurements relative to the two PL QY standards. For measurements in the NIR spectral region, a Cary 5000 spectrophotometer (Varian Inc., Palo Alto, USA) and a Fluorolog-3 spectrofluorometer (HORIBA Jobin Yvon Inc., Edison, NJ, USA) were utilized. Depending on the emission range, H$_2$O or D$_2$O was used as solvent for NC solutions. PL QY measurements of CdHgTe NCs were performed absolutely according to the recent publication[146] and/or relatively to HITCI dye (PL QY = 0.283, solvent ethanol). The emission spectra shown correspond to the spectral photon radiance. It should be noted that the spectral radiance is depicted in the publication.[178]

A.3.3 Ellman’s test

For the quantification of thiol molecules, a 1 mM stock solution of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman’s reagent) in Tris buffer (pH 7.2, adjusted with HCl) containing 0.1 M Tris and 1 mM EDTA, and L-cysteine standards (1-6 mM) in water were freshly prepared. For the assay, 2.75 ml of the buffer, 200 µl of an Ellman’s reagent solution, and 40 µl of the sample or standard solution were mixed. After 1 minute (for the standard) and 60 minutes of reaction in the dark (for the sample), the absorbance was measured. Standard curves from the absorbance at 412 nm allowed the determination of the thiol concentration. The molar amount of thiol per NC was calculated by comparison of the thiol concentration with the NC

![Emission spectra](image)

Figure A.2: The emission spectra of CdTe-TGA NCs reveal the decreasing emission intensity in the presence of EDTA and/or Ellman’s reagent, which is probably caused by the dissolution of the NCs.
concentration derived from absorption and emission spectra. Figure A.2 illustrates the dissolution of CdTe-TGA NCs shown by the diminished PL in the presence of either EDTA, which forms complexes with Cd\(^{2+}\) ions, and/or Ellman’s reagent, which reacts with thiols.

**A.3.4 Inductively coupled plasma optical emission spectroscopy**

Elemental analysis was done on a Perkin Elmer Optima 7000DV ICP-OES system with an instrumental error below 1 %. Cd quantification was performed at 214.44 nm; for Te a detection wavelength of 214.28 nm and for S 180.67 nm was used. All results were obtained from triple measurements with relative standard deviations below 1.5 % for Cd and S and below 1 % for Te.

Measurements were performed for simply diluted as well as dissolved by *aqua regia* (volume ratio H\(_{2}\)NO\(_3\) : HCl of 1 : 3) and diluted NC solutions. As shown in table A.1 the results for CdTe are similar for both sample preparations. Thus, ICP-OES of CdTe NCs was measured from diluted solutions. In contrast, for CdHgTe NCs the analysis of simply diluted solutions causes underestimation of the Hg content. Consequently the ICP-OES results for CdHgTe NCs were obtained from samples treated with *aqua regia*. Therefore, the NCs are precipitated by the addition of iso-propanol and dried in vacuum. The NC powder is then dissolved by the careful addition of *aqua regia* and diluted with ultrapure water to the desired concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cd</th>
<th>Te</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>diluted</td>
<td>14.32 ±0.17 mg/l</td>
<td>1.34 ±0.00 mg/l</td>
<td>4.77 ±0.04 mg/l</td>
</tr>
<tr>
<td>dissolved</td>
<td>14.23 ±0.31 mg/l</td>
<td>1.49 ±0.02 mg/l</td>
<td>4.90 ±0.03 mg/l</td>
</tr>
</tbody>
</table>

**A.3.5 Thermogravimetric analysis**

Thermogravimetric analysis was performed using a Mettler Toledo TGA/SDTA 851 coupled to an FTIR spectrometer in N\(_2\) atmosphere. For the presented measurement a heating rate of 20 °C/min was applied. Prior to the measurement, the sample was freeze dried and the NC powder was filled into an aluminum oxide crucible.

**A.3.6 Theoretical calculations**

For the estimation of the required space per ligand, after an NVT equilibration at 300 K for 50 ps, production runs were performed over 250 ps in an NVE ensemble. The resulting trajectory contained 10,000 frames obtained every 25 fs.
A.3.7 Dilution experiments

All diluted solutions of sample and dye were freshly prepared directly before the measurement of absorption and emission. For strongly diluted samples, absorption measurements were performed in 5 x 1 cm quartz cuvettes from Hellma GmbH and the optical density was recalculated to a path length of 1 cm for comparison. The emission spectra of differently concentrated NC solutions were compared regarding the spectral shape to rule out reabsorption effects. Control experiments with dyes show no concentration-dependent PL.

A.3.8 Precipitation experiments

For precipitation of NCs, iso-propanol or ethanol was added to the NC solution stepwise, followed by centrifugation for 90 s at 14,100 relative centrifugal force (rcf), until the supernatant shows no observable emission under the UV lamp. The supernatant is removed and the NC precipitate is redispersed in H$_2$O by the use of vigorous shaking and short sonication of 5 min at maximum. To remove insoluble aggregates, the obtained solution was centrifuged for 5 min at 14,100 rcf.

A.3.9 Quenching and assembly studies of CdTe/CdS-DNA

Prior to quenching and assembly assays the DNA-modified NCs were filtrated through a 10 kDa Nanosep centrifugal device (Pall Life Sciences) for 15 min at 2,400 rcf to remove unbound oligonucleotides. The presented spectra were measured utilizing an ultra-micro quartz cuvette from Hellma GmbH, which allows absorption and emission measurements of volumes as low as 45 µl in a 3 x 3 mm light path. For the quenching experiments, 47 µl T2-modified CdTe/CdS-MPA (NC : DNA = 1 : 5) containing 7 µM oligonucleotides were mixed with 3 µl of 100 µM A18-BBQ. Changes in emission and absorption spectra in comparison to a mixture of A18-BBQ and a NC reference without oligonucleotides attached evolve after incubation of the mixture at 40 °C for 20 min and slow cooling to room temperature for 1 h. The chemical structure of the quencher is shown in figure A.3.

![Figure A.3: Chemical structure of the BlackBerry® Quencher 650 attached to the 3’ end of an oligonucleotide consisting of a sequence of 18 adenine, which was utilized for quenching experiments.](image-url)
For the assembly experiment, 48 µl of filtrated B1-modified CdTe/CdS-MPA (NC : DNA = 1 : 5) were mixed with 48 µl of filtrated A2-modified CdTe/CdS-MPA (NC : DNA = 1 : 5) with a smaller core size. To promote the NC assembly, 4 µl of a complementary DNA strand with the sequence of 5′–AGG GGG TGA AGT GAA TGC CTGG CTA CAT CTC CCA CTC TCC–3′ were added. Besides the complementary parts to B1 and A2, the sequence consists of four free bases for more flexibility. After incubation at 40 °C for 20 min and at room temperature over night, changes of the emission compared to the mixture without the complementary DNA evolve.

A.3.10 Assembly of CdTe/CdS-DNA on DNA origami structures

To immobilize the CdTe NCs functionalized with single-stranded DNA molecules on DNA origami structures, the following experimental procedure was applied. 100 µl of 165 nM T1-modified CdTe/CdS-TGA (NC : DNA = 1 : 10) solution was filled up to 200 µl 1xTM buffer (40 mM Tris, 12.5 mM Mg(OAc)₂, pH 8.0) and filtered twice for 5 min at 5,000 rcf through a 30 kDa microcon centrifugal filter unit (Millipore) in order to remove unbound DNA molecules. The final volume was adjusted to 100 µl with 1xTM buffer. 25 µl of this brightly emitting solution was subsequently mixed with 25 µl of a 1.6 nM solution of 6-helix bundles that were purified from excess staple strands using a 100 kDa Nanosep centrifugal devices according to the manufacturer’s instructions. After overnight incubation at room temperature on a rocking shaker and filtration through a 100 kDa Nanosep centrifugal devices the solutions still exhibited the QD specific photoluminescence. Samples for AFM characterization were prepared by first, 2 min incubation of 10 µl 5 mM MgCl₂ on a freshly cleaved mica surface followed by rigorous washing with H₂O and drying under a nitrogen stream. Then, 10 µl of the sample was pipetted and after 2 min removed with a Kimwipe (Kimtech Science). The mica surface was gently washed twice with H₂O and dried under a nitrogen stream. AFM measurements were performed at room temperature using a multimode scanning probe microscope with a Nanoscope 3A controller (Digital Instruments/Veeco Probes/USA). Images were taken with TESPW AFM tips (Bruker, Inc., USA) using the tapping mode at their resonant frequency. The images were analyzed with WSxM SPIP software (Nanotec, Inc., Spain).

A.3.11 Transmission electron microscopy

Samples for transmission electron microscopy (TEM) were prepared by drop casting diluted NC solutions either onto a copper grid coated with a silicon dioxide film or carbon coated copper grids, which were freshly hydrophilized by plasma treatment (air plasma, medium energy, 15 s), and subsequent evaporation of the solvent. To further reduce aggregation of the NCs on the TEM grid, a second preparation route is based on utilizing octadecyl-p-vinylbenzyltrimethylammonium chloride (OVDAC) as a transfer agent to solutions in toluene according to a procedure described in literature. The diluted NC solutions in toluene were drop casted onto a copper grid coated with a thin Formvar-carbon film. High-resolution TEM
imaging was carried out on a Tecnai F20 microscope (from FEI Company), operating at 200 kV acceleration voltage. The images were analyzed with iTEM software (Olympus Soft Imaging Solutions GmbH).

A.3.12 Dynamic light scattering

Dynamic light scattering experiments were performed utilizing a Zetasizer Nano ZS from Malvern instruments with a HeNe laser operating at 633 nm. All samples were filtered through syringe filters (0.22 µm pore size, PVDF) prior to the measurement. Determination of the hydrodynamic size was done from NC solutions in a 1 x 1 cm quartz cuvette and in backscattering mode at an angle of 173°. This approach is advantageous, as the beam does not travel through the entire sample thereby reducing the effects of multiple scattering. A folded capillary cell was used for zeta potential measurements with detection carried out at an angle of 17°. The zeta potential values stated are the average obtained from triple measurements. For both measurements, the filled cuvettes were kept at 25 °C for 2 min prior to and during the measurement. Analysis of the results was done with the Zetasizer software 7.02. For the determination of volume and number distribution, the used refractive index and extinction coefficient of CdTe at 632.8 nm are 2.982 and 0.350, respectively.

A.3.13 Nuclear magnetic resonance spectroscopy

$^1$H-NMR, HSQC, and DOSY spectra were recorded on a Bruker Avance III-600 instrument with 600 MHz for $^1$H and 151 MHz for $^{13}$C experiments. D$_2$O (99.96 %) was used as solvent for all experiments. For $^1$H-NMR, chemical shifts are reported as δ values in parts per million (ppm) relative to internal HDO (δ = 4.700 ppm). For $^{13}$C-NMR, chemical shifts are given relative to tetramethylsilane (δ = 0.000 ppm) as external reference. DOSY spectra were acquired with the ledbpwp2s pulse program and applying magnetic z field gradients of 5.57 G/cm. For both measurements, the filled cuvettes were kept at 25 °C for 2 min prior to and during the measurement. Analysis of the results was done with the Zetasizer software 7.02. For the determination of volume and number distribution, the used refractive index and extinction coefficient of CdTe at 632.8 nm are 2.982 and 0.350, respectively.

A.3.14 Small-angle X-ray scattering

For all SAXS measurements, the measured signals were scaled by the beam intensity, sample thickness, and sample transmission. For background subtraction a water reference was mea-
sured. For high energy SAXS measurements, the monochromatic high energy beamline of the Helmholtz-Zentrum Berlin für Energie und Materialien (HZB) at synchrotron BESSY II was utilized to take measurements in the range of 25 to 27 keV. The HZB SAXS/ASAXS instrument equipped with an air sample holder was used. The beam length in air was minimized to 10 cm to reduce the scattering background caused by ambient atmosphere. For data collection a 2D multi-wire proportional counter gas detector was utilized. The sample detector distance was calibrated by silver behenate with a first order scattering vector of $q = 1.076 \text{ nm}^{-1}$. For calibration of absolute scattering intensity the secondary standard glassy carbon (1 mm sample thickness, scaled to water as primary standard) was used. Data analysis was performed using the software SASred. For low energy SAXS measurements, the monochromatic bending magnet beamline of the Physikalisch-Technische Bundesanstalt (PTB) at synchrotron BESSY II was used to take measurements in the range of 3.3 to 4.5 keV. The HZB SAXS/ASAXS instrument equipped with an in vacuum sample holder was utilized. Measurements were taken with cuvettes offering a sample thickness of 170 µm for liquid samples in ultra-high vacuum. For data collection a Pilatus M1 in vacuum detector was used. The sample detector distance was calibrated by sequential changing of the distance while measuring silver behenate and an extrapolation to zero sample detector distance. For calibration of absolute scattering intensity the secondary standard glassy carbon (90 µm sample thickness, scaled to water as primary standard) was used. Data analysis was performed using Mathlab®. For SANS measurements, the V4 SANS instrument of HZB at the neutron source BER II was used with neutrons of a wavelength of 6 Å. For data collection a 2D $^3$He detector was used. The sample detector distance was calibrated by silver behenate with a first order scattering vector of $q = 1.076 \text{ nm}^{-1}$. For calibration of absolute scattering intensity a water filled cuvette was used. For data analysis the software BerSANS was applied. For SAXS measurements of differently concentrated

![Graphs](image)

Figure A.4: Fits for ASAXS data for 2.7 nm sized CdTe-TGA NCs based on core/shell system I (left), II (middle) and a shell exponentially migrating into the solvent combined with core/shell system II (right).
samples, an instrument of the Bundesanstalt für Materialforschung und Prüfung (BAM) was used (Anton Paar Typ SANSess®, CuKα).

Figure A.4 illustrates the results from the models that do not fit the experimental data well. The models developed are based on the form factors below.

sphere  \[ F_s = K_s^2(q, R, \Delta \rho) \] (A.4)  
\[ K_s(q, R, \Delta \rho) = \frac{4}{3} \pi R^3 \Delta \rho^3 \frac{\sin(qR) - qR \cos(qR)}{(qR)^3} \] (A.5)  
core/shell I  \[ F_{csI} = [K_s(q, R_1, \Delta \rho) - K_s(q, R_2, \Delta \rho(1 - \mu))]^2 \] (A.6)  
core/shell II  \[ F_{csII} = [K_s(q, R, \Delta \rho) - K_s(q, \nu R, \Delta \rho(1 - \mu))]^2 \] (A.7)  
core/shell III  \[ F_{csIII} = [K_s(q, R + \Delta R, \Delta \rho_2) - K_s(q, \nu R, \Delta \rho_2 - \Delta \rho_1)]^2 \] (A.8)  
exponential shell  \[ F_{expshell} = \int_0^\infty 4\pi r^2 \cdot (\eta_{expshell}(r) - \eta_{matrix})^2 \cdot \frac{\sin(qR)}{qR} dr \] (A.9)  
\[ \eta_{expshell} = \begin{cases} 
\eta_c & r \leq R_c \\
\eta_{exp}(x) & R_c \leq r \leq R_c + \Delta R \\
\eta_{sol} & r \geq R_c + \Delta R 
\end{cases} \] (A.10)  
\[ x = \frac{r - R_c}{\Delta R} \] (A.11)
Bibliography


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**Versicherung**

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Datum, Unterschrift

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**Erklärung**

Die vorgelegte Dissertation wurde an der Professur für Physikalische Chemie der Fachrichtung Chemie und Lebensmittelchemie der Fakultät Mathematik und Naturwissenschaften der Technischen Universität Dresden unter der wissenschaftlichen Betreuung von Prof. Dr. rer. nat. habil. Alexander Eychmüller angefertigt.

Es existieren keine früheren erfolglosen Promotionsverfahren.

Hiermit erkenne ich die Promotionsordnung der Fakultät Mathematik und Naturwissenschaften der Technischen Universität Dresden in der Fassung vom 23.02.2011 inklusive der Änderungen vom 15.06.2011 und 18.06.2014 an.

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