Noble Metal Nanoparticles and Clusters

From Basic Concepts to Novel Functionalities

Jukka Hassinen
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Jukka Hassinen

A doctoral dissertation completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Science, at a public examination held at the lecture hall AS1 of the school on 8th April 2016 at 13.

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Abstract

Noble metal nanoparticles and clusters are some of the most fascinating materials of contemporary nano-focused science. This thesis focuses on the synthesis and functionalization of three different noble metal nanomatериал systems: atomically precise gold clusters, plasmonic gold nanoparticles, and few-atom silver clusters.

In publication I, mixed-monolayer-protected atomically precise Au$_{25}$ cluster cores having a variable amount of bulky calix[4]arene moieties were synthesized. A mixed ligand feed was used directly in the cluster synthesis to simultaneously facilitate the binding of bulky ligands and the size-focusing of cluster cores.

In publication II, thin-layer chromatography (TLC) was established as a feasible method for precise separation of binary mixtures of Au$_{25}$L$_{18}$ clusters with different ligands (L), as well as a binary mixture of different cluster sizes, protected with the same ligand. TLC even allowed a separation of mixed-monolayer-protected clusters differing only slightly in their composition.

In publication III, a novel method was developed for functionalization of plasmonic gold nanoparticles with positively charged ligands. This two-step phase-transfer method allows rapid cationization of simple citrate-capped gold nanoparticles in the size range 8–20 nm. These cationic gold nanoparticles formed electrostatic self-assemblies with virus particles, thus demonstrating their ability to bind large biomolecules.

In publication IV, cationic gold nanoparticles were combined with negatively charged cellulose nanocrystals. This mixture forms colloidal state electrostatic assemblies in which the inherent twist of the nanocrystals leads to chiral positioning of the gold nanoparticles and thus to the emergence of a chiral plasmonic signal.

In publication V, the synthesis of silver clusters was demonstrated in a solid polymer thin film by direct laser writing. Direct laser writing with a tightly focused near-infrared femtosecond laser beam produced highly photostable submicrometer-scale fluorescent structures in thin films of silver-containing poly(methacrylic acid).

The results of this thesis offer new approaches to the synthesis and functionalization of noble metal clusters and nanoparticles. One of the most significant discoveries was the realization of TLC as an efficient method for separation of gold clusters. In addition, positively charged gold nanoparticles with tunable size were successfully prepared and used in electrostatic self-assemblies that exhibit plasmonic properties. All in all, the findings of this thesis extend the knowledge of previous research and suggest applicability of these nanomaterials at various developing areas.

Keywords  noble metal clusters, gold nanoparticles, calix[4]arene, functionalization, thin-layer chromatography, cationization, electrostatic self-assembly, direct laser writing

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<th>Author</th>
<th>Jukka Hassinen</th>
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<tr>
<td>Name of the doctoral dissertation</td>
<td>Noble Metal Nanoparticles and Clusters - From Basic Concepts to Novel Functionalities</td>
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<tr>
<td>Publisher</td>
<td>School of Science</td>
</tr>
<tr>
<td>Unit</td>
<td>Department of Applied Physics</td>
</tr>
<tr>
<td>Series</td>
<td>Aalto University publication series DOCTORAL DISSERTATIONS 46/2016</td>
</tr>
<tr>
<td>Field of research</td>
<td>Engineering Physics</td>
</tr>
<tr>
<td>Manuscript submitted</td>
<td>19 January 2016</td>
</tr>
<tr>
<td>Date of the defence</td>
<td>8 April 2016</td>
</tr>
<tr>
<td>Permission to publish granted (date)</td>
<td>2 March 2016</td>
</tr>
<tr>
<td>Language</td>
<td>English</td>
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Abstract

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In publication V, the synthesis of silver clusters was demonstrated in a solid polymer thin film by direct laser writing. Direct laser writing with a tightly focused near-infrared femtosecond laser beam produced highly photostable submicrometer-scale fluorescent structures in thin films of silver-containing poly(methacrylic acid).

The results of this thesis offer new approaches to the synthesis and functionalization of noble metal clusters and nanoparticles. One of the most significant discoveries was the realization of TLC as an efficient method for separation of gold clusters. In addition, positively charged gold nanoparticles with tunable size were successfully prepared and used in electrostatic self-assemblies that exhibit plasmonic properties. All in all, the findings of this thesis extend the knowledge of previous research and suggest applicability of these nanomaterials at various developing areas.

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| ISBN (printed) | 978-952-60-6702-5 |
| ISBN (pdf) | 978-952-60-6701-8 |
| ISSN-L | 1799-4934 |
| ISSN (printed) | 1799-4934 |
| ISSN (pdf) | 1799-4942 |
| Location of publisher | Helsinki |
| Location of printing | Helsinki |
| Year | 2016 |
| Pages | 120 |
Tiivistelmä
Jalometalleista valmistetut nanohiukkaset ja ryppäät ovat yksiä modernin nanoteknologian kehittämiä materiaaleja. Tämä tiivistelmä käsittää kolme tällaista nanomateriaaliyhmää: atomaarisen tarkkoja kultaryppäitä, plasmonia kultananohiukasias ja muutamista atomeista koostuvia fluoresoivisia hopearyppäitä.

Julkaisussa I valmistettiin atomaarisen tarkkoja Au₃₅–kultaryppäitä, joiden ligandikerrokoostui yksinkertaisesta butaanidiolaatista ja vaihteelevästä määrää kookkaita, monihampaisia kaliksareeniligan järjestelmä. Binäärisen ligandiseoksen käyttäminen jo ryppäiden valmistusvaiheessa mahdollisti samanaikaisesti kookkaiden kaliksareeniligan kiinnittymisen ja kultaryppäiden koon fokusointumisen atomaarisen tarkoituksien.


Julkaisussa III kehitettiin uusi positiivisesti varautuilla ligandeilla sojuattujen plasmonisten, 8–20 nm kokoisten kultananohiukkasten valmistusmenetelmä. Tämä kaksivaiheinen faasinsirtoon perustuva menetelmä mahdollistaa yksinkertaisten negatiivisesti varattujen sitaattistabiloituja kultananohiukkasten muuntamisen kationisiksi kultananohiukkasksi. Nämä kationiset kultananohiukkaset muodostivat sähköstäädissä itsejärjestyneitä rakenteita viruspartikkelien kanssa, mikä osoittaa niiden pystyvän sitomaan suuria biomolekyylejä.

Julkaisussa IV kationisia kultananohiukkasia yhdistettiin negatiivisesti varattuun nanokiteen selluloosaan, jolloin muodostui kolloidisia sähköstäädissä itsejärjestyneitä rakenteita. Nanokiteiden synnyynnäinen kiertosyysy johti kultananohiukkasten kiraaliseen järjestymiseen näiden pinnalla, ja siten kiraalisen plasmonisen signaalin syntyyn.

Julkaisussa V valmistettiin hopearyppäitä laserin avulla polymeerimatriisissa. Hopeaa sisältävien polymetakrylihippo-ohutkalvojen laserointi fokusoidulla lähi-infrapuna-alueen femtosekuntisarilla tuotti fotostabiille, alle mikrometrin kokoisia fluoresoivia rakenteita.

Väitöskirjassa esitetyt tulokset tarjoavat uusia menetelmiä jalometallinanohiukkasten ja ryppäiden valmistukseen ja funktionaliointiin, ja tarjoavat mielenkiintoisia mahdollisuksia jatkotutkimuksiin. Yksi merkittävimmistä havainnoista oli, että ohutkerroskromatografiat pystyttävät hyödyntämään kultaryppäiden erotelussa ja puhdistamisessa. Lisäksi kehitettiin kationisten, plasmoniasta kultananohiukkasten synteesimenetelmä, joka tarjoaa uusia mahdollisuuksia plasmoniasta, sähköstäädissä itsejärjestyneiden rakenteiden valmistuksesta.

Avainsanat jalometalliryppä, kultananohiukkanen, kaliksareeni, funktionaliointi, ohutkerroskromatografia, kationisointi, sähköstäättäminen itsejärjestyminen

ISSN-L 1799-4934 ISSN (painettu) 1799-4934 ISSN (pdf) 1799-4942
Julkaisupaikka Helsinky Painopaikka Helsinky Vuosi 2016
Preface

The research presented in this thesis has been carried out at the Department of Applied Physics at Aalto University during the years 2012–2015. These four years have been extremely instructive and valuable while at the same time so enjoyable, thanks to the awesome people I got a chance to work with. However, research is never smooth sailing and at times I was not even sure if I would manage to make it to this point. Somehow pieces of the puzzle started to fit and a handful of fortunate findings in various regions of the nanoparticle research field ultimately led to the formation of this thesis. A tale of serendipity, I would say.

I wish to express my sincere gratitude to my supervisor, Professor Robin Ras, for your enthusiasm, perseverance and continuous confidence in my work, that have formed a solid basis for my research. I have learnt a lot from your abilities to write confidently and to recognize and pinpoint strengths of the new results and observations which were often just blurry thoughts in my head. This work would not have been possible without the support, supervision, and funding you have managed to acquire. With these words, I wish to thank you for everything you have done for me.

I enjoy playing volleyball, where teamwork is crucial in order to succeed. The same holds also for research—importance of cooperation cannot be overemphasized. This thesis consists of five articles, all of which have been done in collaboration with other groups. I can still remember our collaborative meeting with Professor Heikki Tenhu’s group in 2012, and the discussion afterwards with Dr. Petri Pulkkinen, that led to investigations of calixarene-protected gold clusters and nanoparticles. Thank you for all the interesting and valuable discussions and supply of materials during these years. In 2012, collaboration was also initiated with Professor Juha Toivonen and his student, Puskal Kunwar, who has proven to be a productive experimentalist. Thank you for all your efforts. I wish you all the best in the future.

I am especially grateful for having the possibility to visit the research groups of Professor Thalappil Pradeep and Professor Hannu Häkkinen during our three-year collaborative project. I have witnessed tremendous success and development occurring in both groups, and got to know great people along the way. I wish to especially thank Atanu Ghosh, a hard-working, striving researcher with whom we initiated the work on cluster separation that ended up being a great success. Special thanks also to Manoj Kumar Panwar and Avijit Baidya for their continuous hospitality at the Indian Institute of
Technology Madras. I am also thankful to Dr. Elina Kalenius for skillful mass spectrometric analyses at the University of Jyväskylä.

I would like to thank Dr. Kirsni Salorinne and Dr. Stefan Knoppe for their efforts on examining this thesis and their valuable and inspiring comments. I also greatly appreciate Professor Christopher Ackerson for accepting the invitation to act as my opponent in the upcoming thesis defence.

At Aalto, I have been surrounded by great people. I wish to thank Professor Olli Ikkala for welcoming me as a part of your group in 2012 and the inspiring discussions ever since. I would also like to thank Professor Mauri Kostiainen and Professor André Gröschel for all the valuable discussions over the years. In addition, thank you Dr. Isabel Diez for proofreading my thesis. I wish to extend my gratitude also to other colleagues from the groups of Soft Matter and Wetting and Molecular Materials for all the help and for generating a great working atmosphere: Johanna Majoinnen, Mika Latikka, Johannes Haataja, Maja Vuckovic, Panu Hiekkataipale, Henrikki Mertanen, Tomy Cherian, Dr. Tina Löbl, Dr. Marjo Kettunen, Dr. Nonappa, Dr. Angel Sanchez Sanchez... the list is nearly endless. Oh, one more, thank you Matti Toivonen especially for encouraging late-night discussions regarding work and life in general. I have also enjoyed the occasional jogs and visits to the gym with Teemu Myllymäki, Tuukka Verho, Mikko Poutanen, and the Beast of MolMat.

Last but certainly not least, it has been an absolute pleasure to inhabit office 115a in Nanotalo. Sharing the room with such an outstanding bunch of workmates has been a blast: Ville Liljestrom, Dr. Jenni Koskela, Tuukka Verho, Dr. Jason McKee, Teemu Myllymäki, Lahja Martikainen, Joona Mikkilä, Riikka Koski and Dr. Matilda Backholm. The past four years would not have been the same without you. Our witty conversations saved many days when the actual science was not working out as expected (which is quite often in experimental science!). I wish all the best in your future endeavours!


Espoo, 1 March 2016

Jukka Hassinen
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<tr>
<td>2-SPy</td>
<td>2-pyridylthiolate</td>
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<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>Ag@PMAA</td>
<td>poly(methacrylic acid)-protected silver clusters</td>
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<tr>
<td>AgNP</td>
<td>silver nanoparticle</td>
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<td>AuNP</td>
<td>gold nanoparticle</td>
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<tr>
<td>BDT</td>
<td>1,3-benzenedithiolate</td>
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<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<tr>
<td>BT</td>
<td>butanethiol, equal to BuS in publication</td>
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<tr>
<td>BuSH</td>
<td>butanethiol</td>
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<tr>
<td>c-C₆</td>
<td>cyclohexanethiolate</td>
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<td>Calix-4S</td>
<td>tetrathiolate of Calix-4SH, also equal to “Calix”</td>
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<td>Calix-4SH</td>
<td>25,26,27,28-tetrakis(4-mercapto-n-butoxy)calix[4]arene</td>
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<tr>
<td>CCMV</td>
<td>Cowpea chlorotic mottle virus</td>
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<tr>
<td>CD</td>
<td>circular dichroism</td>
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<tr>
<td>CNC</td>
<td>cellulose nanocrystal</td>
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<td>COSY</td>
<td>correlation spectroscopy</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<td>DCTB</td>
<td>trans-2-[3-(4-tertbutylphenyl)-2-methyl-2-propenylidene]-malononitrile</td>
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<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
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<td>DLW</td>
<td>direct laser writing</td>
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<tr>
<td>DMSA</td>
<td>meso-2,3-dimercaptosuccinic acid dithiolate</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
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<tr>
<td>ESI-MS</td>
<td>electrospray ionization mass spectrometry</td>
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<tr>
<td>ET</td>
<td>electron tomography</td>
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<tr>
<td>fcc</td>
<td>face-centred cubic</td>
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<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
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<tr>
<td>HAADF-STEM</td>
<td>high-angle annular dark field scanning transmission electron microscopy</td>
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<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
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HPLC high-performance liquid chromatography
HT hexanethiolate
IUPAC International Union of Pure and Applied Chemistry
LSPR localized surface plasmon resonance
LUMO lowest unoccupied molecular orbital
MAA methacrylic acid
MALDI-MS matrix-assisted laser desorption ionization mass spectrometry
MPC monolayer-protected cluster
MSA mercaptosuccinic acid thiolate
MUTAB (11-mercaptoundecyl)-N,N,N-trimethylammonium bromide
NMR nuclear magnetic resonance
NP nanoparticle
ODA octadecylamine
p-MBA para-mercaptobenzoic acid
PAGE polyacrylamide gel electrophoresis
PAMAM polyamidoamine
PET phenylethanothiolate
PhC≡C phenylalkynyl
PMAA poly(methacrylic acid)
PR₃ phosphine
PSD particle size distribution
PVP poly(vinylpyrrolidone)
QY quantum yield
RNA ribonucleic acid
ROESY rotating frame nuclear Overhauser effect spectroscopy
RSH thiol
S₂P(OiPr)₂ O,O-diisopropyl dithiophosphate
SAXS small-angle X-ray scattering
SEC size-exclusion chromatography
SEM scanning electron microscopy
SEM-EDS scanning electron microscopy with energy-dispersive X-ray spectroscopy
SG glutathione thiolate
SQUID superconducting quantum interference device
SR thiolate
StBu tert-butylthiolate
STEM scanning transmission electron microscopy
TBBT 4-tert-butylenzenthioleate
TEM transmission electron microscopy
THF  tetrahydrofuran
TGA  thermogravimetric analysis
TLC  thin-layer chromatography
TOA  tetraoctylammonium ion
TOAB  tetraoctylammonium bromide
TOCSY  total correlation spectroscopy
UV-vis  ultraviolet and visible range
XRD  X-ray diffraction

$V_A$  atomic valence
$\tau$  bleaching time constant
$k_B$  Boltzmann constant
$z$  charge
$\kappa^{-1}$  Debye screening length
$D$  diameter
$\varepsilon_r$  dielectric constant
$I_{DLW}$  direct laser writing intensity
$e$  elementary charge
$I$  ionic strength
$m$  mass
$n^*$  total number of valence electrons
$\varepsilon_0$  vacuum permittivity
$T$  virus triangulation (Caspar-Klug) number
$\lambda$  wavelength
$\zeta$  zeta potential


* These authors contributed equally to this work.
Author’s Contribution

Publication I: Mixed-Monolayer-Protected $\text{Au}_{25}$ Clusters with Bulky Calix[4]arene Functionalities

The author had the main responsibility for designing and performing the experimental work, interpretation of the results and writing the manuscript. Dr. Elina Kalenius conducted the ESI-MS measurements. Dr. Petri Pulkkinen provided the calixarene ligand used in the study.

Publication II: Simple and Efficient Separation of Atomically Precise Noble Metal Clusters

The author designed the experimental work together with M.Sc. Atanu Ghosh, synthesized several clusters used in the study ($\text{Au}_{25}(\text{PET})_{18}$, $\text{Au}_{25}(\text{BT})_{18}$, $\text{Au}_{144}(\text{PET})_{60}$, and $\text{Au}_{35}(\text{Calix})_{0-3}(\text{BT})_{6-18}$), performed separations of $\text{Au}_{25}(\text{Calix})_{0-3}(\text{BT})_{6-18}$, $\text{Au}_{25}(\text{PET})_{18}$ oxidation states, and $\text{Au}_{25}(\text{PET})_{18}$ synthesis intermediates with thin-layer chromatography, and analysed the separated products with UV-vis absorption spectroscopy and MALDI-MS. The manuscript was written together with M.Sc. Atanu Ghosh (50%−50%).

Publication III: Rapid Cationization of Gold Nanoparticles by Two-Step Phase Transfer

The author designed and performed all experimental work related to the synthesis of gold nanoparticles. The preparation of gold nanoparticle−virus assemblies was performed together with M.Sc. Ville Liljeström and Prof. Mauri Kostiainen, who also conducted the small-angle X-ray scattering study. The author wrote the manuscript.

Publication IV: Chiral Plasmonics Using Twisting Along Cellulose Nanocrystals as a Template for Gold Nanoparticles

The author participated in designing the experimental work together with the co-authors, synthesized the cationic gold nanoparticles (sizes 8.5 nm and 11.7 nm), and participated in analysing the results and revising the manuscript.

Publication V: Direct Laser Writing of Photostable Fluorescent Silver Nanoclusters in Polymer Films

The author designed the work together with the co-authors, prepared the thin film samples, and characterised the fluorescent microstructures by optical microscopy, SEM-EDS, AFM, Raman microscopy and spectroscopy, and absorption spectroscopy. Direct laser writing and fluorescence microscopy and
spectroscopy were performed by M.Sc. Puskal Kunwar. The author wrote the first version of the manuscript together with M.Sc. Puskal Kunwar (50%–50%) and had a major part in analysing the data and compiling the final version of the manuscript.
Other Publications

The author has also contributed to the following related publications.


1. Synopsis

Noble metal nanoparticles and nanoclusters are intriguing objects in the toolbox of today’s nano-focused materials science. These materials act as a bridge between the solid and molecular state, and therefore exhibit properties of both of these states of matter. Bulk metals are electrically conducting and highly reflective resulting from the freely moving delocalized conduction band electrons. Metal nanoparticles, on the other hand, exhibit intense colors due to localized surface plasmon resonance (LSPR), a phenomenon attributed to the collective oscillation of conduction electrons upon interaction with light. As the nuclearity of particles is further reduced to tens of atoms or less (< 2 nm), their band structure becomes discontinuous and breaks down to discrete energy levels. This gradual evolution of the band structure with decreasing particle size is called size quantization and causes, for example, these small metal clusters to show molecule-like characteristics (Figure 1).

This thesis revolves around the synthesis, characterization and surface functionalization of three different noble metal nanomaterial systems: atomically precise gold clusters, plasmonic gold nanoparticles (AuNPs), and few-atom silver clusters embedded in a polymer matrix. Chapter 2 gives an overall introduction to the materials used in the study. It includes a brief

![Figure 1. Size quantization effect: A schematic of the evolution of electronic states in differently sized metals.](image)
Synopsis

history of the development of the synthesis methods of AuNPs and clusters, as well as details of their properties, focusing on optical properties and functionalization possibilities. Next, Chapter 3 shortly describes the objectives and central research questions of the thesis.

Chapter 4 focuses on synthesis and characterization of atomically precise gold clusters. In publication I, the synthesis of atomically precise Au$_{25}$ clusters carrying a variable amount of bulky calix[4]arene moieties is presented. In addition, publication II demonstrates thin-layer chromatography (TLC) as a viable method for precise separation of these mixed-monolayer-protected clusters. TLC is shown to be a versatile tool in monitoring reactions of clusters, in cluster purification, and in separation and isolation of various cluster products.

Chapter 5 focuses on functionalization of plasmonic AuNPs and their use in electrostatic self-assemblies. Publication III demonstrates a novel method for functionalization of AuNPs with positively charged, cationic ligands. This two-step phase-transfer method allows rapid cationization of simple citrate-capped AuNPs in the size range 8–20 nm. Cationic AuNPs are intriguing due to their ability to readily interact with biologically relevant molecules which are often negatively charged. In publication IV, these cationic AuNPs are combined with negatively charged cellulose nanocrystals (CNCs). This mixture forms assemblies in which the inherent twist of the nanocrystals leads to chiral positioning of the AuNPs and thus to the emergence of a chiral plasmonic signal.

Chapter 6 describes two synthesis pathways for making poly(methacrylic acid)-protected silver clusters (Ag@PMAA). By carefully controlling their preparation conditions in aqueous solution, we observe previously unreported optical features suggesting the presence of several cluster sizes in the system. Whereas the synthesis of these clusters in solution has been known for nearly a decade, their synthesis in solid polymer films has not been previously realized. In publication V, we demonstrate the synthesis of silver clusters in a solid polymer thin film by direct laser writing (DLW). DLW with a tightly focused near-infrared femtosecond laser beam produced highly photostable submicrometer-scale fluorescent structures in a thin film sample of silver-containing PMAA.
2. Introduction to Noble Metal Nanoparticles and Clusters

As described in the synopsis, the properties of noble metal particles depend immensely on their size, particularly in the nanometer size regime. Therefore, in this thesis, a clear distinction is made between the terms “nanoparticle” and “cluster”. Though lacking an official definition from the International Union of Pure and Applied Chemistry (IUPAC), nanoparticles (NPs) are often defined as particles with size in the 1–100 nm range at least in one dimension. Due to the size quantization effect shown in Figure 1, this definition encompasses two very different classes of materials in the case of noble metal nanoparticles: plasmonic, strongly light-absorbing nanoparticles and fluorescent noble metal clusters. Therefore, in this thesis, the term “plasmonic nanoparticle” is used to describe a nanoparticle capable of supporting a LSPR. For AuNPs, this definition includes particles larger than 2.0 nm.

Metal clusters, on the other hand, are defined as a subclass of nanoparticles having a definite molecular composition and mass. Thus they are atomically precise and truly monodisperse in size. They are often protected by a distinct monolayer of passivating ligands and therefore are often referred as monolayer-protected clusters (MPCs). Clusters typically have distinct optical and electrochemical properties, caused by their discrete energy levels due to size quantization. The nuclearities of clusters generally vary from 2 atoms to roughly 150 atoms, though thiolate (SR) protected gold clusters as large as Au_{329}(SR)_{84}, Au_{520}(SR)_{130}, and Au_{940}(SR)_{160} have been recently isolated and analyzed by mass spectrometry. Clusters of this size are already plasmonic, thus generating a new special class of materials called Faradaurates, as coined by Amala Dass in 2011, honoring Michael Faraday’s early studies of colloidal gold. This thesis mostly focuses on plasmonic nanoparticles and atomically precise clusters made of gold, though clusters made of silver are also addressed in Chapter 6.

Nanoparticles and clusters made of gold have impressive physical, electronic, optical and catalytic properties compared to other metallic nanoparticles. Gold does not oxidize/corrode easily, thereby rendering it as a very stable element in the nanoscale. In addition, gold particle surfaces can be passivated by small organic molecules which enhances their stability and prevents aggregation, as further described in Chapter 2.5. Despite the vast amount of research put into developing and optimizing synthesis procedures of these
nano-objects, there are still many challenges related, for example, to their functionalization, colloidal stability in physiological conditions, shape control, synthesis yield, and reproducibility of syntheses. Nevertheless, AuNPs have shown application potential in e.g. biotechnology, materials science, electronics, electron tomography, medicine, and catalysis.\cite{12, 13} Gold clusters, on the other hand, are still in focus of a more fundamental research. Even though they have been already studied for few decades, there is still a lot to learn on their functionalization and size control. In the following chapters, I will introduce the brief history and typical preparation methods of AuNPs and clusters as well as show a glimpse of their optical properties and functionalization possibilities.

### 2.1 Brief History of Synthesis Methods

The first use of AuNPs was the production of red-colored “gold ruby glass”. The earliest notes on preparation of such glass date back to the fourth century, when the famous Lycurgus cup was manufactured by the Romans. This fascinating object possesses dichroic properties depending on the direction of the illumination, which are caused by the presence of 50−100 nm sized Au−Ag alloy particles in the glass matrix. The first mentions of aqueous solutions of colloidal AuNPs are from the 16th century, when alchemist Paracelsus claimed to have prepared a gold-containing red-colored potion, *Aurum Potabile*. The physical nature of these strongly colored solutions, or colloidal dispersions as we now better understand, was deduced by Michael Faraday in mid-1800s. He reduced gold salts with phosphorus and carbon disulfide and stated that the colors originate from small metallic particles even though he wasn’t able to see them with the microscopes of that time.\cite{10}

Another milestone of AuNP research was the synthesis of citrate-stabilized AuNPs, first described by Hauser and Lynn in 1940,\cite{14} and rigorously analyzed a decade later by Turkevich et al.\cite{15} In the procedure, an aqueous solution of sodium citrate is added to a boiling solution of HAuCl₄, which causes the reduction of gold and simultaneous stabilization of the as-formed AuNPs (Figure 2). Citrate reduction allows a facile preparation of 8−20 nm AuNPs in aqueous dispersions, though also larger particles can be made by controlling the ratios of reactants\cite{16} or via seeded growth approaches.\cite{17, 18} Though lacking nanometer-accuracy in reproducibility,\cite{20} the citrate reduction method is still highly popular due to its simplicity and was also used in this work in publications III and IV.

In 1994, Brust and Schiffrin developed another important synthesis method that allows preparation of alkanethiol-stabilized organosoluble AuNPs in 1.5−5.2 nm size range (Figure 2).\cite{21, 22} In their procedure, the gold is first transferred to an organic phase with the help of tetraoctylammonium bromide (TOAB) and then reduced by NaBH₄ in the presence of thiols. The size of AuNPs can be controlled by simply tuning the thiol-to-gold ratio: increasing the amount of thiol leads to smaller AuNPs and vice versa. These thiolated AuNPs can be handled like simple chemical compounds: they can be dried and
redispersed without apparent changes in their properties. This method can be considered as the starting point for many synthesis procedures of thiolate-protected atomically precise gold clusters. However, good quality nanoparticles larger than 5 nm are not accessible with the Brust–Schiffрин method, since further reduction of the thiol-to-gold ratio leads to AuNPs with ill-defined shapes and polydisperse sizes.22

![Image](image_url)

**Figure 2.** Examples of well-established gold nanoparticle syntheses. A) Turkevich method,15 B) Brust–Schiffрин method,21 and C) an example of Au25(SR)18 cluster synthesis by a single-phase reaction in tetrahydrofuran.23,24

The work on gold clusters started already in 1960s with studies of phosphine (PR₃) capped clusters. Clusters such as Au₅(PR₃)₄Cl,25 [Au₈(PR₃)₇]²⁺,26 Au₁₁(PR₃)₁₆Cl₉,27 Au₁₃(PR₃)₁₄(SCN)₃,28 [Au₁₃(PR₃)₁₆Cl₂]³⁺,29 [Au₃₉(PR₃)₁₄Cl₆]²⁺,30 and Au₅₅(PR₃)₁₂Cl₈3¹ were synthesized and characterized by chemical analysis and many of them also by single crystal X-ray analysis. Thereafter, core sizes Au₁₁ (undecagold) and Au₅₅ (Schmid’s cluster) have been most extensively investigated, and currently various derivatives of undecagold clusters are commercially available for labeling applications in electron microscopy.3² Due to the limited stability of phosphine-protected clusters, gold cluster research has recently been focused on thiol-protected clusters.

As mentioned above, initial approaches towards thiolate-protected AuNPs were conducted using the Brust–Schiffрин method. The particle size of the method can be controlled to certain extent by altering the synthesis conditions, such as the thiol-to-gold ratio, the reaction temperature, the reaction time, and the type of the protecting ligand. In general, smaller particles are produced by increasing the amount of thiol, lowering the reaction temperature and shortening the reaction time. However, despite rigorous optimization of these reaction parameters, atomically precise clusters cannot be directly produced by the Brust–Schiffрин method. Nevertheless, by combining strictly modified reaction conditions with post-synthetic separation techniques such as column chromatography, fractional recrystallization, or solvent extraction, a series of
alkanethiolate protected MPCs have been obtained also by the Brust–Schiffrin method.33–36 Along the same lines, a series of water-soluble glutathione thiolate (SG) protected gold clusters were produced by the group of Whetten,37,38 and their structures analyzed with electrospray ionization mass spectrometry (ESI-MS) by the group of Tsukuda.39,40

A direct, selective synthesis of MPCs requires further modification of the synthesis methods. Initial experiments with Brust–Schiffrin method indicated that Au_{25} was one of the most stable sizes among thiolate-protected clusters.41,42 Therefore, this gold cluster has been most extensively studied. The group of Jin discovered the importance of slow, low-temperature formation of Au(I):SR intermediates in the gold cluster synthesis that led to a high yield (40%) of Au_{25}(SR)_{18} clusters as compared to the room temperature preparation (8% yield).43 Following this report, they developed a facile single-phase synthesis of Au_{25}(SR)_{18} clusters in tetrahydrofuran (THF) that involved a “size-focusing” step where a polydisperse mixture of clusters was converted to Au_{25}(SR)_{18} in the presence of excess thiol, thus taking advantage of the high kinetic stability of these clusters.23 A modification of this method was used also in this work in publications I and II.

In addition to Au_{25}(SR)_{18}, many other sizes of atomically precise thiolate-protected clusters have been selectively prepared in the recent years. Crystal structures have been acquired from several thiolate-protected gold clusters including Au_{102}(p-MBA)$_{44}$,44 [Au_{25}(PET)$_{18}$]$^{-}$,45 Au_{25}(PET)$_{18}$,46 Au_{28}(PET)$_{24}$,47 Au_{36}(PET)$_{24}$,48 [Au_{23}(c-C$_6$)$_{16}$]$^{-}$,49 Au_{28}(TBBT)$_{20}$,50 Au$_{24}$(TBBT)$_{20}$,51 Au$_{30}$S(StBu)$_{18}$,52 and Au$_{33}$(TBBT)$_{32}$ (p-MBA = para-mercaptobenzoic acid, PET = phenylethanethiolate, c-C$_6$ = cyclohexanethiolate, TBBT = 4-tert-butylbenzenethiolate, StBu = tert-butyliithiolate). In a recent, impressive work by the group of Zheng, a gram-scale synthesis was developed and the crystal structures resolved for a series of alloy clusters Au$_{12}$Ag$_{32}$(SR)$_{24}$ and silver analogues Ag$_{44}$(SR)$_{24}$, each capped with three different fluorinated arylthiols.54 At the same time, similar Ag$_{44}$(p-MBA)$_{24}$ clusters were crystallized by the group of Bigioni.55 As a continuation to the synthesis of 44-atom clusters, Zheng’s group resolved the crystal structure of Au$_{24}$Ag$_{30}$(2-SPy)$_{4}$(PhC≡C)$_{20}$Cl$_2$ (2-SPy = 2-pyridylthiolate, PhC≡C = phenylalkynyl).56 In a similar fashion, Wang et al. resolved the crystal structure of [Ag$_{46}$Au$_{24}$(StBu)$_{32}$]$^{2+}$, which is the largest bimetallic cluster reported so far.57 Crystal structures have been acquired also for other bimetallic clusters, such as (AuAg)$_{25}$(PET)$_{18}$,58 (AuAg)$_{38}$(PET)$_{20}$,59 PdAu$_{24}$(PET)$_{18}$,60 and MAu$_{24}$(SR)$_{18}$ (M = Pd, Pt, Au).61,62 As illustrated by these articles, interest on alloy clusters and doping of clusters is increasing due to the highly sensitive structure–property relationships of small clusters and the ability to bring new functions to clusters. For example, a 200-fold higher fluorescence intensity was observed from Au$_{12}$Ag$_{43}$ clusters as compared to Au$_{25}$–Ag clusters having 1–12 Ag atoms.63

In addition, a myriad of atomically precise clusters have been prepared and their compositions confirmed by mass spectrometry. Au$_{20}$PET$_{16}$ was prepared by slightly modifying the Brust–Schiffrin synthesis,64 Au$_{44}$(SR)$_{60}$ clusters capped with various alkanethiols were first synthesized with a modified
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Brust−Schiffrin method,65 and later in a facile single-phase methanol-based synthesis.66 Utilization of a mild reductant, NaBH₄CN, in a methanol-based synthesis resulted in water-soluble Au₁₈(SG)₁₄ clusters.67 The group of Jin has prepared and isolated alkanethiolate-protected Au₄₀(SR)₂₄ and Au₅₅(SR)₃₁ clusters by first exposing a polydisperse mixture of Auₙ(SG)ₙ nanoparticles to an excess of alkanethiol followed by a chromatographic separation of the products.68–70 Atomically precise clusters can also be prepared in a so-called one-size-to-another approach, in which they are prepared from existing monodisperse clusters.48,50 For example, phosphine-capped Au₁₁ clusters can be used as precursors to make thiolate-protected Au₂₅(SR)₁₈ in a ligand exchange – core rearrangement reaction.71,72 In addition to tuning the core size and ligand composition of atomically precise clusters, also the elemental composition of the cluster can be altered, as shown in the synthesis of Ag₅Au₁₉(MSA)₁₀ cluster (MSA = mercaptosuccinic acid thiolate), which was prepared by reacting a mixture of mercaptosuccinic acid-protected Ag₇ and Ag₈ clusters with HAuCl₄.73

As implied in the previous paragraph, thiocarbamates (as well as phosphines) are also viable candidates to stabilize silver clusters, even though most thiols that stabilize Au clusters do not produce Ag clusters in the same manner.74 Single crystals of silver clusters have been achieved only very recently, likely due to the lower stability of clusters compared to the gold analogues. Reaching atomic precision has been difficult with silver clusters and it seems that the selection of the ligands plays a very crucial role in achieving monodispersity: reaching atomic precision seems to require rigid, bulky or multidentate ligands. In addition to the aforementioned Ag₄₄(SR)₂₄ cluster,54,55 also Ag₄₅(SR)₁₂(PR₃)₈,75 Ag₆₅(SR)₁₄(PR₃)₄,76 [Ag₁₂(SR)₂₄(PR₃)₅]²⁻,76 [Ag₂₁(S₂P(OiPr)₂)₁₂]⁺,77 Ag₂₉(BDT)₁₂(PR₃)₄,78 and Ag₃₂(SR)₁₄(PR₃)₅ have been unambiguously characterized by single-crystal X-ray crystallography (S₂P(OiPr)₂ = diisopropyl dithiophosphate, BDT = 1,3-benzenedithiolate). In addition, several clusters have been isolated and analyzed by mass spectrometry including Ag₁₄(DMSA)₄,80 Ag₁₇(MSA)₇,8¹ Ag₆₆(MSA)₈,8¹ Ag₉₉(MSA)₇,8² Ag₁₀₉(SG)₇,8₃ Ag₁₂₅(SG)₁₈,8₄,8₅ Ag₁₄₀(TBBT)₁₅₃,8₆ and Ag₂₈₀(TBBT)₁₂₀ 8⁻ (DMSA = meso-2,3-dimercaptosuccinic acid dithiolate).

Apart from thiocarbamates and other small molecules, scaffolds such as synthetic polymers,8⁸–9³ dendrimers,9⁴–9⁶ proteins,9⁷–¹₀¹ and DNA¹₀²–¹₀⁶ have been used to stabilize nanoclusters. Due to the poor stability of the clusters or the large size of these macromolecules, the precise characterization of these systems is often difficult. Synthetic polymers are also polydisperse and can form various environments for cluster formation, thus often leading to a distribution of different cluster sizes.¹⁰¹,9² For example, various stable polymer-protected “magic number” clusters are formed when reducing HAuCl₄ in the presence of poly(vinylpyrrolidone).9² These clusters can be further extracted by thiols or phenylacetylene.⁶⁸,¹⁰⁷ Polymers can also stabilize silver clusters, as will be shown in Chapter 6, which discusses the synthesis and properties of fluorescent poly(methacrylic acid)-protected silver clusters (Ag@PMAA). Dendrimers, proteins and DNA are highly monodisperse scaffolds, though
they still often lead to a distribution of cluster sizes.\textsuperscript{96,108,109} However, some templates provide atomically precise clusters directly, as shown by the formation of Au\textsubscript{8} clusters in polyamidoamine (PAMAM) dendrimers.\textsuperscript{95}

All the methods presented above can be described as “bottom-up” approaches, comprising of chemical conversion of gold salts to larger particles. In addition, there are “top-down” approaches in which noble metal nanoparticles are prepared by physical manipulation of bulk metal, such as laser ablation\textsuperscript{110,111} or sputtering\textsuperscript{112,113}. In addition, many other bottom-up methods have been developed throughout the past few decades producing nanoparticles and clusters of different sizes, shapes and surface functionalities.\textsuperscript{114–119} However, further review of these synthesis methods is outside the scope of this thesis.

2.2 Characterization Methods

In order to study the properties of nanoparticles and especially clusters, their purification and separation is crucial. Initially, fractional recrystallization and solvent extraction were used to separate specific clusters from polydisperse cluster mixtures.\textsuperscript{33–36} Thereafter, utilization of polyacrylamide gel electrophoresis (PAGE) as a separation tool has progressed studies of water-soluble clusters.\textsuperscript{37–39,120–122} Size-exclusion chromatography (SEC) has been successfully applied to the separation of Au\textsubscript{38}(SR)\textsubscript{24} and Au\textsubscript{40}(SR)\textsubscript{24}, and the isolation of Au\textsubscript{55}(SR)\textsubscript{31}, for example.\textsuperscript{68,87,123,124} In addition, high-performance liquid chromatography (HPLC) has emerged as a versatile and efficient tool for separating clusters,\textsuperscript{125–127} allowing even an enantioseparation of Au\textsubscript{38}(SR)\textsubscript{24} clusters with intrinsic chirality.\textsuperscript{128} Recently, we discovered that one of the simplest chromatographic tools, namely thin-layer chromatography (TLC) can be used to separate various cluster systems. The possibilities of TLC are demonstrated in publication \textsuperscript{II}, after which the method has been utilized in several investigations.\textsuperscript{129–132} All these efforts aim at obtaining fractions of atomically precise clusters as polydisperse cluster mixtures complicate or even completely prevent the characterization of individual cluster properties. In the following, primary structural characterization methods of nanoparticles and clusters are introduced, followed by a brief list of characterization methods of their functional properties.

**Characterization of structural properties.** In addition to particle size, important structural properties of nanoparticles include their particle size distribution (PSD), core structure, structure of the ligand layer, oxidation state, and zeta potential. In case of plasmonic nanoparticles, the particle size and PSD can be measured directly by analysing transmission electron microscopy (TEM) or scanning electron microscopy (SEM) images. In addition, ensemble methods such as dynamic light scattering (DLS) can measure the PSD of nanoparticles and confirm the result from TEM that is often based on a relatively small number of particles. The core structure of nanoparticles can be determined e.g. by X-ray diffraction (XRD), high-resolution TEM or high-angle annular dark field scanning transmission
electron microscopy (HAADF-STEM). Composition of the ligand layer can be determined by combining the results from thermogravimetric analysis (TGA) and nuclear magnetic resonance spectroscopy (NMR). In NMR, the iodine death reaction has proven to be efficient in determining the compositions of the ligand layer on mixed-ligand-protected nanoparticles. In the reaction, iodine dissolves the gold cores of the particles and detaches the ligands, thus allowing their free rotational motion and ultimately producing sharp NMR signals. Zeta potential ($\zeta$) is the potential difference of the stationary layer of fluid attached to the particle and the medium that the particle is dispersed in. Thus, zeta potential is related to the surface charge of the particles, and high zeta potential values ($|\zeta| > 30$ mV) indicate high colloidal stability. Zeta potential can be measured by methods based on DLS. It is worth noting that the structural properties determined from nanoparticle samples are often average values due to the inherent polydispersity of the particles.

Many of the aforementioned methods can also be used to characterize the structure of clusters. However, their ultrasmall size and monodispersity create additional characterization possibilities as well as new challenges, which will be discussed in the following. Ultimately, the analysis method giving the most profound structural information of clusters is the single-crystal X-ray diffraction (XRD). However, XRD requires high-quality crystals of clusters with sizes in the range of hundreds of micrometers. Obtaining large, well-ordered crystals has proven to be highly challenging in case of many clusters. In this respect, crystallization of clusters having rigid or bulky ligands has been most successful, as mentioned in Chapter 2.1. The ultrasmall size of clusters enables their study by mass spectrometry. Many thiolate-protected clusters are stable enough to ionize as intact molecular ions, thus enabling the direct determination of their $m/z$ ratio from which atomic composition can be estimated. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) has proven to be an efficient screening tool in analyzing cluster synthesis products, supported by the recognition of MALDI-MS matrices such as trans-2-[3-(4-tertbutylphenyl)-2-methyl-2-propenylidene]-malononitrile (DCTB) that are suitable for ionizing clusters. The broad $m/z$ range of MALDI-MS enables studies of large clusters, though the determination of cluster composition is hampered by the low $m/z$ resolution of the method (often $\sim 100$–$1000$ $m/\Delta m$). In order to reach better mass resolution ($m/\Delta m > 50000$), ESI-MS has been successfully applied to many cluster systems and is regarded as the golden standard in the analysis of cluster composition.

TEM analysis of clusters is rather hindered by the small size of the clusters. However, HAADF-STEM has recently enabled atomic resolution direct observations of clusters such as $\text{Au}_{38}$, $\text{Au}_{55}$, and $\text{Au}_{92}$. In addition, the 3D structure of $\text{Au}_{68}$ was recently reconstructed using a similar method. On the other hand, NMR analysis of the cluster ligand layer is more straightforward due to decreased signal broadening as compared to larger nanoparticles. In addition to 1D NMR methods, the sharpness of the signals also promotes utilization of 2D NMR techniques. Techniques based on through-bond couplings such as correlation spectroscopy (COSY) and total
correlation spectroscopy (TOCSY) can be employed to e.g. probe the distribution of ligand binding sites or studying the inherent chirality of clusters.\textsuperscript{138–140} On the other hand, methods exploiting through-space coupling such as rotating frame nuclear Overhauser effect spectroscopy (ROESY) provide information on spatial distances between nuclei and are hence well-suited for studying the conformation of ligands, as shown in publication I. In addition, diffuse-ordered NMR measurements have been utilized in non-destructive analysis of the hydrodynamic sizes of clusters.\textsuperscript{141}

\textbf{Characterization of functional properties.} As discussed in the previous chapter, the structure of nanoparticles ultimately defines their functional properties, such as optical, chiroptical, electrochemical, catalytic, and magnetic properties. These properties can be measured by conventional methods that are also used for other materials. For example, quantized core charging of clusters has been studied by typical electrochemistry methods such as cyclic voltammetry and differential pulse voltammetry,\textsuperscript{142,143} and magnetic properties of clusters by electron paramagnetic resonance (EPR) spectroscopy,\textsuperscript{144} or superconducting quantum interference device (SQUID) magnetometry.\textsuperscript{145} Optical properties are one of the most interesting functional aspects of nanoparticles and clusters, and can be determined by common spectroscopic techniques such as absorption and fluorescence spectroscopy, as well as circular dichroism (CD) spectroscopy, which describes the difference in absorption between left- and right-handed circularly polarized light. These properties will be discussed in more detail in Chapter 2.4.

\section*{2.3 Structural and Physical Properties as a Function of Size}

Gold is the most studied metal in the nanoscale due to its high stability, and therefore is in the focus of the following discussion. Bulk gold crystallizes into the face-centred cubic (fcc) structure. As bulk, it is a yellow-colored, reflective, conductive, inert metal: a seemingly endless array of close-packed Au(0) atoms. Breaking a chunk of gold to smaller pieces leads first to microparticles which exhibit similar atomic arrangement and properties as bulk gold. However, further reduction of the particle size to nanosize leads to significant changes in its structure, properties, and reactivity. When examining particles of few nanometers, their fcc core structure is gradually lost and various octa-, deca- and icosahedral structures start to emerge.\textsuperscript{136,146} Further decrease in particle size leads to the size range of atomically precise clusters, each having individual structures, many of which have been unambiguously characterized. Some specific thiolate-protected cluster sizes, such as \text{Au}_{25}(SR)_{18}, \text{Au}_{38}(SR)_{24}, \text{Au}_{102}(SR)_{44}, and \text{Au}_{144}(SR)_{60} are more stable than others and thus are often called \textquote{magic number clusters}. This unusual stability can be explained by electronic and geometric shell closings. In 2006, the group of Häkkinen conceived a divide-and-protect model, which describes the structure of thiolate-protected clusters as a core of metallic Au(0) surrounded by a layer of SR(AuSR)\textsubscript{n} “staples”, where gold is in oxidized Au(I) state as gold-thiolates. Häkkinen et al. also established a superatom complex concept\textsuperscript{147} for ligand-
protected clusters based on a previous superatom electronic theory. According to the theory, an MPC is stable when the number of valence electrons corresponds to an electron shell closure of the superatomic orbitals: \(1S^2 \left| 1P^6 \right| 1D^{10} \left| 2S^21F^{14} \left| 2P^61G^{18} \left| 2D^33S^21H^{22} \right| \ldots \right.\). This concept provides a simple electron counting rule for the valence electrons \((n^*)\) to predict the stability of \([Au_{N}(SR)_M]^z\) clusters:

\[
  n^* = NV_A - M - z
\]  

(2.1)

where \(V_A\) is the atomic valence. Thus clusters having e.g. \(n^* = 2, 8, 18, 34, 58, \ldots\) are expected to have high stability. For example, \([Au_{25}(SR)_18]^−\) consists of an icosahedral Au_{13} metallic core surrounded by six Au_{3}(SR) staples, and has an 8-electron superatom configuration, \(1S^21P^6\) (Figure 3a). Similarly, Au_{102}(SR)_{44} is comprised of a Mark’s decahedral Au_{79} core surrounded by two Au_{3}(SR)_{3} and 19 Au(SR)_{2} staples, and is a 58-electron superatom (\(1S^21P^61D^{10}2S^21F^{14}2P^61G^{18}\)). On the other hand, Au_{144}(SR)_{60} has 84 valence electrons, which does not correspond to a full superatomic shell closing. It has a 114-atom metallic core that is arranged into three concentric icosahedral shells, protected by 30 Au(SR)_{2} staples (Figure 3g). Its enhanced stability has been claimed to originate from geometric factors due to its high symmetry. However, it should be noted that crystallization of Au_{144}(SR)_{60} has not been successful so far, and therefore its structure remains elusive.

Changes in the size and structure of nanoparticles naturally lead to changes in their functional properties. These changes originate from the increase in surface-to-volume ratio of the particles as well as the quantization of the electronic states of the clusters. One of the most striking effects is the decrease of the melting temperature of nanoparticles compared to bulk. For Au, the
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melting point starts to substantially drop at sizes below 10 nm, and 2.5 nm NPs melt already at 650 °C whereas the melting point of bulk Au is 1064 °C.\textsuperscript{152}

Optically, bulk gold is yellow and highly reflective, as relativistic effects cause the 5d\to6s transition to absorb blue wavelengths while longer wavelengths are reflected. For nanoparticles, the optical properties are governed by plasmons localized on the particle surface. As the nanoparticles interact with light, the electric field of the radiation interacts with the free electrons of the nanoparticles, and irradiation at an optimal wavelength leads to localized surface plasmon resonance (LSPR), which causes strong absorption of the resonant wavelengths. As the particle size is further decreased, the size quantization phenomenon leads to further separation and discretization of energy levels, ultimately leading to fluorescence in the visible wavelengths, as well as the emergence of chirality of the atomically precise clusters. These effects will be discussed in more detail in Chapter 2.4.

Though bulk gold is highly inert, AuNPs on metal oxide or carbonaceous supports are capable of catalyzing various chemical reactions including nucleophilic addition, hydrogenation, and oxidation reactions.\textsuperscript{153–157} This curious size-dependent catalytic behavior of AuNPs is attributed to their high surface-to-volume ratio, abundance of low-coordinated surface atoms, and effects originating from size quantization. One of the most studied catalytic processes is the low-temperature oxidation of carbon monoxide, in which AuNPs supported on metal oxides have shown great promise.\textsuperscript{156} As can be expected, the efficiency and selectivity of gold catalysts are strongly affected by their particle size, support material, and particle–support contact structure.\textsuperscript{153} However, due to the polydispersity of the AuNPs and the complexity of the catalyst systems, the mechanisms behind AuNP-induced catalysis are not completely understood. Therefore, experiments with unprotected atomically precise clusters have had a pivotal role in shedding light on the structure-specific catalytic mechanisms of gold.\textsuperscript{158–160} In addition, catalytic performance of MPCs is gaining interest: [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{−} with its reversible charge state conversion to [Au\textsubscript{25}(SR)\textsubscript{18}]\textsuperscript{0} implies possibilities for redox catalysis.\textsuperscript{161} Furthermore, weakly-protected clusters such as Au@PVP (PVP = poly(vinylpyrrolidone)),\textsuperscript{162} and ligand-protected clusters having partly exposed surface atoms\textsuperscript{163} are interesting pathways to enhance the catalytic properties of protected clusters.

AuNPs can also exhibit ferromagnetic or paramagnetic properties though bulk gold is diamagnetic. The magnetic properties of AuNPs are enabled by their high surface-to-volume ratio, the ligand-induced modification of AuNP electronic states, and the strong spin–orbit coupling of gold.\textsuperscript{164} Thus, the magnetic properties are strongly influenced by the AuNP size and the Au–ligand interactions. The highest saturation magnetization values have been reported for thiolate-protected AuNPs, which can also exhibit room-temperature ferromagnetism, while weakly protected AuNPs generally show diamagnetic behavior.\textsuperscript{145,165} It must be noted that investigations on AuNP magnetism have not been consistent: despite the many variables involved in these studies, the reproducibility of the magnetic property measurements has
been remarkably poor. One possible reason for this issue is the polydispersity of the AuNP samples, which can be addressed by using atomically precise clusters. Glutathione-protected Au$_N$(SG)$_M$ clusters ($N = 10$–$39$, $M = 10$–$24$) have been shown to be paramagnetic with magnetic moment increasing with nuclearity. In another intriguing study the conversion between paramagnetic [Au$_{25}$(SR)$_{18}$]$^0$ and diamagnetic [Au$_{25}$(SR)$_{18}$]$^-$ enabled reversible chemical switching of the magnetism of clusters.

2.4 Optical Properties

Optical properties of nanosized gold have fascinated scientists already for several hundred years. Plasmonics govern the optical properties of nanoparticles with sizes above 2 nm. The interaction of AuNPs with light leads to a very strong reduction of transmitted light with extinction cross sections several orders of magnitude larger than those of organic dyes. As the nuclearity of AuNPs is reduced to tens of atoms or less, their band structure breaks down to discrete energy levels leading to sharp absorption features and fluorescence in the visible range. In the following discussion, the optical properties of nanoparticles and clusters are described in more detail.

**Plasmonic gold nanoparticles.** One of the most intriguing properties of AuNPs is the collective behavior of their conduction electrons upon interaction with electromagnetic radiation, called the localized surface plasmon resonance (LSPR). As a beam of light passes through a dispersion of AuNPs, it is strongly absorbed at the plasmon resonance wavelength ($\lambda_{\text{LSPR}}$), thus showing a sharp peak in the absorption spectrum. For AuNPs, $\lambda_{\text{LSPR}}$ typically occurs in the visible range near the green wavelength (~520 nm), thereby causing the intense red color of the dispersion. This strong absorption is caused by the interaction of the light with the free electrons of the AuNPs leading to their collective oscillations (Figure 4). When these oscillations are coherent, a resonance of surface plasmons is observed, that is localized and confined in the volume of the AuNP. In addition, light scattering around $\lambda_{\text{LSPR}}$ starts to emerge with AuNPs larger than 40 nm. LSPR can be described by Mie theory which gives an exact solution of an electromagnetic plane interacting with a homogeneous metallic sphere. In Mie theory, the electromagnetic fields are expanded in multipole contributions, and the expansion coefficients are found by applying proper boundary conditions for the system. With small particles ($D \ll \lambda_{\text{LSPR}}$, where $D$ is the particle diameter), Mie theory can be approximated by considering only the dipolar contribution, and is thus denoted as Rayleigh scattering. The position of $\lambda_{\text{LSPR}}$, and thus the color of the AuNP dispersion, is affected by the particle size, the particle size distribution, the morphology of particles, the interparticle distance, the nature of the capping agent, and the dielectric constant of the dispersion medium. Therefore, changes in the $\lambda_{\text{LSPR}}$ position can be used as a highly sensitive tool to investigate the dielectric surroundings of nanoparticles or the surface of a metal film. Consequently, this feature is utilized in surface plasmon resonance spectroscopy—a characterization method gaining in allure. In addition, the
intensity of LSPR can be utilized in determination of the AuNP concentration, if the size of AuNPs is known.\textsuperscript{170}

![Figure 4. A schematic presentation of localized surface plasmon resonance of AuNPs. Adapted from Ref. [157] with permission from The Royal Society of Chemistry.](image)

Similar to AuNPs, also silver nanoparticles (AgNPs) exhibit LSPR in the visible range with $\lambda_{\text{LSPR}}$ positioned at $\sim$400 nm in water. The differences in $\lambda_{\text{LSPR}}$ originate from the variation in the dielectric constants of metals. The LSPR of AgNPs is sharper and more intense than the LSPR of AuNPs, and therefore AgNPs are often better suited to applications related to plasmon enhancement despite their lower chemical stability as compared to AuNPs.

Even though metal NPs with size in the plasmonic region are achiral with no inherent chiroptical properties, capping them with chiral ligands can lead to plasmon-induced chiral signals in the visible range.\textsuperscript{171–173} In addition, positioning AuNPs to chiral arrangements can lead to similar CD signals due to collective Coulombic interaction of plasmonic dipoles. These signals typically exhibit positive or negative Cotton effects: bisignate dip–peak or peak–dip shapes of the spectrum, respectively, with zero-crossing at the absorption maximum.\textsuperscript{174} In chiral AuNP assemblies, the Cotton effect arises from different coupling strengths of the longitudinal and transverse plasmon modes and the random orientation of the assemblies with respect to the incident light.\textsuperscript{175} An example of this type of plasmonic coupling is given in publication \textsuperscript{IV} which demonstrates chiral AuNP assemblies on cellulose nanocrystals.

**Atomically precise gold clusters.** As the size of the nanoparticles decreases to approximately 2 nm, the electrons become more localized due to size quantization and a well-developed LSPR cannot be sustained.\textsuperscript{2} Thus, the optical properties of clusters are governed by their energy level structure, which is fundamentally related to their physical structure. The discretization of the electronic energy levels induces emergence of molecule-like electronic and optical features such as one-electron transitions and visible range fluorescence.

The absorption spectra of atomically precise clusters typically consist of multiple transitions between the energy levels. The absorption energies of small clusters manifest in the visible wavelength range, and are highly dependent on the number of atoms in the cluster as well as the metal–ligand interactions. Therefore, it is possible to distinguish different clusters based on
their absorption spectra. For example, the spectrum of Au$_{38}$(SR)$_{24}$ shows multiple well-resolved absorption peaks, whereas Au$_{40}$(SR)$_{24}$ displays only vague optical features.\textsuperscript{124} Table 1 shows absorption data of various thiolate-protected atomically precise clusters, Au$_N$(SR)$_M$. It lists up to two of the most prominent peaks as well as up to two weaker shoulder peaks of each cluster. In addition, it lists the values of optical energy gaps which correspond to the HOMO–LUMO transitions, provided that they are not forbidden. Examination of the absorption data reveals that decreasing the cluster nuclearity generally leads to more distinct optical features and an increase in the optical energy gap. However, these trends do not hold for all cluster sizes. One reason for this behavior can be related to the differences of the cluster core structures.\textsuperscript{176}

Fluorescence of MPCs has been intensively investigated due to its relevance to various applications. The fluorescence quantum yields (QY) of thiolated gold clusters are generally too low (typically from $10^{-5}$ to $10^{-3}$) for fluorescence imaging applications, though they are still more than five orders of magnitude greater than that of bulk gold (QY $\approx 10^{-10}$).\textsuperscript{177} However, there are some exceptions such as Au$_{18}$(SG)$_{14}$, Au$_{22}$(SG)$_{18}$, and Au$_{23}$(TBBT)$_{20}$, which exhibit QYs of 5.3%, 8%, and 5%, respectively.\textsuperscript{51,67,178} Doping gold clusters with other metals such as silver can drastically change their fluorescence properties. For example, a rod-shaped Au$_{12}$Ag$_{13}$ exhibits a striking QY of 40%, while similarly shaped Au$_{25}$ clusters have QY of $\approx 0.1$%.\textsuperscript{51} Silver clusters have typically higher QYs than gold clusters. The highest QY of silver clusters reported so far is 93% from Ag$_{14}$ cluster protected by a DNA oligomer containing 22 nucleobases.\textsuperscript{179}

Table 1. Optical absorption peaks and energy gaps of thiolate-protected clusters.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Absorption peak / nm (eV)*</th>
<th>Optical energy gap / nm (eV)*</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au$<em>{15}$(SR)$</em>{13}$</td>
<td>Prominent #1: 370 (3.4)</td>
<td>496 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Au$<em>{18}$(SR)$</em>{14}$</td>
<td>562 (2.2)</td>
<td>620 (2.0)</td>
<td>390 (3.2)</td>
</tr>
<tr>
<td>Au$<em>{20}$(SR)$</em>{16}$</td>
<td>485 (2.6)</td>
<td>420 (3.0)</td>
<td>390 (3.2)</td>
</tr>
<tr>
<td>Au$<em>{24}$(SR)$</em>{18}$</td>
<td>570 (2.2)</td>
<td>450 (2.8)</td>
<td>653 (1.9)</td>
</tr>
<tr>
<td>Au$<em>{28}$(SR)$</em>{20}$</td>
<td>765 (1.6)</td>
<td>855 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Au$<em>{30}$(SR)$</em>{18}$</td>
<td>680 (1.8)</td>
<td>442 (2.8)</td>
<td>800 (1.5)</td>
</tr>
<tr>
<td>Au$<em>{36}$(SR)$</em>{24}$</td>
<td>365 (3.4)</td>
<td>580 (2.1)</td>
<td>480 (2.6)</td>
</tr>
<tr>
<td>Au$<em>{38}$(SR)$</em>{24}$</td>
<td>620 (2.0)</td>
<td>704 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Au$<em>{40}$(SR)$</em>{24}$</td>
<td>570 (2.2)</td>
<td>380 (3.3)</td>
<td>729 (1.7)</td>
</tr>
<tr>
<td>Au$<em>{44}$(SR)$</em>{24}$</td>
<td>1050 (1.2)</td>
<td>630 (2.0)</td>
<td>745 (1.7)</td>
</tr>
<tr>
<td>Au$<em>{44}$(SR)$</em>{24}$</td>
<td>560 (2.2)</td>
<td>390 (3.2)</td>
<td>1240 (1)</td>
</tr>
<tr>
<td>Au$<em>{46}$(SR)$</em>{24}$</td>
<td>380 (3.3)</td>
<td>855 (1.5)</td>
<td>Vague features.</td>
</tr>
<tr>
<td>Au$<em>{48}$(SR)$</em>{24}$</td>
<td>835 (1.5)</td>
<td>760 (1.6)</td>
<td>1033 (1.2)</td>
</tr>
<tr>
<td>Au$<em>{55}$(SR)$</em>{31}$</td>
<td>968 (1.3)</td>
<td>413 (3)</td>
<td>525 (2.4)</td>
</tr>
<tr>
<td>Au$<em>{64}$(SR)$</em>{32}$</td>
<td>700 (1.8)</td>
<td>400 (3.1)</td>
<td>4592 (0.3)</td>
</tr>
<tr>
<td>Au$<em>{67}$(SR)$</em>{35}$</td>
<td>730 (1.7)</td>
<td>400 (3.1)</td>
<td>1771 (0.7)</td>
</tr>
<tr>
<td>Au$<em>{99}$(SR)$</em>{42}$</td>
<td>730 (1.7)</td>
<td>400 (3.1)</td>
<td>1771 (0.7)</td>
</tr>
<tr>
<td>Au$<em>{102}$(SR)$</em>{44}$</td>
<td>700 (1.8)</td>
<td>400 (3.1)</td>
<td></td>
</tr>
</tbody>
</table>

* Values extracted from a recent review by Jin.\textsuperscript{176}
In addition, the nature of the stabilizing scaffold is crucial regarding the fluorescence properties of clusters. For example, Au\(_N\) (\(N = 5, 8, 13, 23, 31\)) clusters stabilized by PAMAM dendrimers exhibit QYs ranging from 10% of Au\(_{31}\) up to 70% of Au\(_5\), thereby being comparable to organic dyes.\(^{186}\) Also other macromolecular scaffolds, such as proteins, can stabilize clusters with a high QY. Bovine serum albumin (BSA) protected Au\(_8\), Ag\(_{15}\), and Au\(_{25}\) clusters have been reported with QYs of 6%, 11%, 6%, respectively.\(^{98,99,181}\) Lysozyme has been used to stabilize gold clusters having QYs of 6%,\(^{182}\) 16%,\(^{183}\) and 56%.\(^97\) In addition, e.g. lactotransferrin and insulin have been successful scaffolds for gold clusters with QYs ranging between 3% and 7%.\(^{184}\)

Chirality in atomically precise clusters is more versatile than in the case of larger AuNPs. Chiral signals originating from gold clusters were first observed in 2000 by Schaaff and Whetten in a study of size-separated fractions of Au–glutathione clusters in the size range of 20–40 atoms.\(^{38}\) These clusters exhibited strong chiroptical responses with multiple Cotton effects in their CD spectra. Four different reasons have been formulated to explain the optical activity in chiral MPCs: 1) protection with chiral ligands; 2) chiral adsorption geometry of ligands; 3) inherent chirality of the metal core; and 4) cis/trans isomerism in the SR(AuSR)\(_n\) staples.\(^{185}\) Achieving chiroptical responses via protecting clusters by chiral ligands is a rather obvious and perhaps the most studied way,\(^{38,138,186,187}\) though the mechanism of chirality transfer from the ligands to the metal core still remains unclear. Intrinsic chirality of clusters protected with achiral ligands has been observed from e.g. Au\(_{38}\)(PET)\(_{24}\),\(^{47}\) Au\(_{40}\)(PET)\(_{24}\),\(^{188}\) and Au\(_{102}\)(p-MBA)\(_{44}\),\(^{44}\) and their enantiomers have been separated by chiral HPLC,\(^{128,188}\) or by chiral phase transfer.\(^{189}\) In these clusters, the chirality has been suggested to mostly originate from the chiral arrangement of the SR(AuSR)\(_n\) staples around the metallic core. In addition, there are few clusters having a chiral metallic core, such as [Au\(_{20}\)(PR\(_3\))\(_4\)]Cl\(_4\),\(^{190}\) Au\(_{28}\)(TBBT)\(_{20}\),\(^{50}\) and Au\(_{133}\)(TBBT)\(_{52}\).\(^{53}\)

### 2.5 Functionalization

Functionalization of plasmonic AuNPs and clusters is of paramount importance when considering their applications. The ligand layer on the AuNPs largely defines their solubility, chemical and colloidal stability, electronic structure, and their interactions with other molecules or particles. Considering thiolate-protected AuNPs, there are three main pathways to end up with a desired functionality on the nanoparticle surface.\(^{191}\) Firstly, the most straightforward way is to directly stabilize the nanoparticles with desired ligands during the nanoparticle synthesis (Figure 5A). Secondly, when the desired ligands are not suitable for direct synthesis, they can be deposited to the surface of ready AuNPs in a process called ligand exchange (Figure 5B). In ligand exchange, an excess of incoming thiol replaces a portion of the thiol or other weakly bound ligand that is originally present at the nanoparticle surface. The third option is the covalent linking of desired functionalities to ligands that are already present at the nanoparticle surface (Figure 5C).
The ultimate aim of functionalization is often to end up with a desired number of functionalities on the surface of AuNPs. Keeping this in mind, all the aforementioned strategies have their advantages and disadvantages. Direct functionalization via e.g. Brust–Schiffrin method is fast and simple and the size of the particles can be controlled well. However, not all types of thiol ligands can be used in Brust–Schiffrin protocol, and other direct synthesis protocols utilizing e.g. water-soluble thiols can lead to poor control over the particle size and size distribution. In addition, the use of binary ligand mixtures typically leads to a distribution of compositionally different mixed-ligand protected AuNPs. Ligand exchange is a powerful method to bring desired functionalities to existing AuNPs without significant modification of their metallic core. However, also the ligand exchange reactions lead to different mixed-ligand distributions on AuNPs.126 In addition, bulky ligands do not necessarily undergo ligand exchange due to steric hindrance. The covalent linking method shown in Figure 5C, while being versatile, is restricted to end-functionalized thiols capable of further reacting with suitable small molecules.

Water solubility is essential regarding the bioconjugation of AuNPs and applications related to biological systems. Nanoparticle syntheses in aqueous environment have advantages such as good metal precursor solubility and the possibility of direct bioconjugation. However, AuNPs cannot easily be synthesized in water at high concentration due to ionic interactions, and electrostatically protected AuNPs cannot typically be dried and redispersed without aggregation. Thiolate-protected AuNPs are most commonly synthesized in organic media, which allows for high stability and high NP concentrations. Thus, functionalization pathways based on phase transfer of NPs from organic to aqueous media (and vice versa) allow exploiting the advantages of syntheses in either of these media, together with subsequent
transfer of NPs to different environments for further applications. Numerous different phase-transfer methods have been reported. For example, electrostatically protected NPs have been phase-transferred to organic solvents by acid-mediated mixing with alkanethiols and by alkylamines. In addition, transfer of water-soluble MPCs to organic media has been successful by e.g. utilizing electrostatic interactions, or changing the pH of the dispersion. On the other hand, organosoluble MPCs have been rendered water-soluble by ligand exchange reactions with acid-terminated alkylthiols or by interdigitation of surfactants to the hydrophobic capping layer of AuNPs. In this work, phase-transfer methods are extensively utilized in publication III, which demonstrates preparation of cationic, plasmonic AuNPs by functionalization of negatively charged citrate-capped AuNPs via a two-step phase-transfer procedure.
This thesis is a collection of work related to synthesis of metal clusters and nanoparticles. The goal of research during my doctoral studies was to develop novel functionalities for these materials, ultimately leading to new applications. The most central research questions and objectives of my work are listed below.

- **Can bulky ligands, or receptors, be attached on the surface of small atomically precise clusters?** Regarding the development of new functionalities, one aspect was to investigate ways to prepare metal clusters that could be used as building blocks for making cluster assemblies. Assemblies of clusters could have enhanced collective optical or electronic properties compared to the properties of single clusters. Covalent linking of clusters with e.g. dithiols easily leads to large, uncontrollable cluster networks. Therefore, we aimed at preparing controlled cluster assemblies via host–guest chemistry. Calixarene was chosen as the host molecule due to its ability to complex cationic guest molecules with a reasonably high stability. However, calixarenes are large macrocycles, and functionalizing atomically precise clusters with them is not straightforward.

- **Can the amount of calixarenes per cluster be controlled?** Regulating the amount of receptor units would enable precise control of the nature of the cluster assemblies. For example, preparation of clusters having only a single binding site would enable formation of cluster dimers, provided that suitable linking chemistry exists.

- **Can well-defined, highly plasmonic, positively charged gold nanoparticles be prepared?** The literature on preparation of large (> 5 nm) positively charged AuNPs is scarce. One of the aims of my work was to produce such AuNPs to be utilized in preparation of binary electrostatic self-assemblies by combining cationic AuNPs with anionic biological components, such as viruses and nanocelluloses.

- **Can silver clusters be prepared in a solid polymer matrix?** Brightly fluorescent and photostable silver clusters have been prepared in few solid state matrices, such as glass and zeolites. However, polymers have advantages over these inorganic materials, such as flexibility and moldability. Fluorescent clusters in solid state could have potential in applications related to data storage or sensing.
- What is the composition of silver clusters formed in poly(methacrylic acid)? Despite the numerous studies regarding poly(methacrylic acid)-protected silver clusters (Ag@PMAA), there is only little information available on the fundamental question of their composition. Identification of the composition of Ag@PMAA clusters, and separation of individual cluster sizes would be essential steps towards further understanding of structure–function relationships in the system.

These questions will be addressed in Chapters 4–7 of this dissertation, and in more detail in publications I–V.
4. Controlling the Monolayer Composition – Bulky Receptors on Gold Clusters

This chapter describes a step towards preparation of atomically precise gold clusters having a controlled amount of functional units on their surface. Moreover, the functional entity used in this work is a calix[4]arene-derivative—a bulky, tetradentate thiol. Bulky or structurally complex ligands can bring unprecedented valuable functionalities to metal clusters, enabling applications related to e.g. molecular recognition, sensing, imaging or self-assembly. Previously, such applications have been demonstrated with plasmonic AuNPs capped with receptors such as calixarenes, cyclodextrins, and oligonucleotides. In addition, there is an increasing interest on preparing MPCs capped with receptors. For example, in 2015, Guo et al., prepared a bimetallic Ag₆Au₆ cluster fully protected with 12 ethisterone ligands, which act as specific probes for estrogen receptor α. Nevertheless, synthesis of atomically precise clusters carrying a controllable amount of bulky receptors had so far not been realized, likely because ligand exchange reactions, while frequently carried out for small ligands, turn out not feasible for bulky ligands.

In publication I, we demonstrate the synthesis of atomically precise, fluorescent gold clusters with bulky, multidentate calix[4]arene-derivative. The starting point is a modified Brust–Schiffrin-type single-phase synthesis method using a mixed thiol ligand feed, thus leading to mixed-monolayer-protected clusters. We show that the ligand composition is tunable by simply varying the ratio of ligands in the synthesis feed. However, even though the ligand composition can be controlled to some extent, this procedure always yields samples with populations of slightly different ligand compositions. Subsequently, to gain further control of the system, we show in publication II that thin-layer chromatography (TLC) is a viable method for precise separation of these mixed-monolayer-protected clusters. In this work, we also demonstrate TLC separations of simple model clusters of different size, ligand composition, and charge. In addition, we demonstrate how TLC is applicable in monitoring cluster synthesis and purification of cluster samples. In general, we foresee these procedures as general strategies for incorporation of controlled amount of bulky ligands to cluster surfaces.
4.1 Calixarene-Functionalized Au$_{25}$ Clusters

Calix[N]arenes are organic macrocycles with a variable amount ($N$) of phenolic units, connected by methylene bridges (Figure 6A). Their name originates from the Latin word *calix* (chalice) as a result of their cup-like three-dimensional structure. As realized by Gutsche in the 1970s,$^{230}$ these hydrophobic cavities enable their use in host–guest chemistry. Calixarenes are able to complex non-polar molecules through hydrophobic interactions and cations through cation–π interactions. Therefore, they have been used in molecular recognition,$^{218}$ sensing, and formation of supramolecular structures.$^{203,204}$ However, it must be noted that simple, non-functionalized calixarenes can exist in different conformations. Calix[4]arenes, for example, exist as mixtures of cone, partial cone, 1,2-alternate and 1,3-alternate conformers in solution (Figure 6B). However, it is possible to lock the conformation by functionalization of the upper or lower rim of the calixarene. As can be anticipated, the conformation, functionalization, as well as the size of the cup strongly influence the complexation properties of calixarenes. The most common calixarenes are the smallest ones with $N = 4$, though calixarenes having up to ten units have been reported.$^{231}$

![Figure 6. Structures of calix[N]arene (A), the different conformations of calix[4]arene (B), and the ligands used in publication I (C). Reprinted in part with permission from publication I. © 2014 American Chemical Society.](image)

Functionalization of gold surfaces with calixarenes has lately received attention in the scientific community. The optical properties of AuNPs combined with the versatile complexation properties of calixarene has enabled colorimetric sensing possibilities for anions,$^{210,216}$ metal ions,$^{211,213,217}$ pesticides,$^{212}$ amines,$^{214,232}$ amino acids$^{233}$, and other positively charged small molecules$^{209}$. Although calixarenes have been used to functionalize plasmonic AuNPs, introducing them to atomically precise gold clusters has not been
trivial. In a recent study, the group of Katz succeeded in preparing Au$_{11}$ clusters protected by five phosphine-calixarene units which leave part of the gold surface accessible to be utilized in catalytic applications. In our approach, we aimed to control the amount functional units per cluster to create a more appealing starting point for cluster assemblies and complexation experiments.

Publication I describes the synthesis of mixed-monolayer-protected Au$_{25}$ clusters in which the thiolate monolayer consists of thiolated calixarene molecules (25,26,27,28-tetrakis(4-mercapto-n-butoxy)calix[4]arene, Calix-4SH) and butanethiol (BuSH) (Figure 6C). In our approach, we chose to use a 4-membered calixarene (cup diameter 5–6 Å) due to the increasing size mismatch with larger calixarenes and Au$_{25}$ structure (diameter of 5–6 Å and an average distance of 4–5 Å between nearest thiolate binding sites). Calix-4SH was synthesized by substituting the lower rim hydroxyls of calix[4]arene with butanethiol spacers following the recipe by Pulkkinen et al., thereby locking its conformation to cone. Ligand exchange of such a bulky receptor to alkanethiol-protected Au$_{25}$ clusters was not successful, likely due to steric hindrance. To overcome this restriction, we examined the possibility of preparing MPCs using a mixed thiol feed directly in the synthesis. BuSH was used as a secondary ligand to enable the size-focusing to Au$_{25}$ clusters and to allow a complete mixed-ligand monolayer. A known procedure for making single-ligand Au$_{25}$ clusters was used as a starting point to make the mixed-ligand clusters. In the procedure, a mixture of Calix-4SH and BuSH was added to a THF solution of HAuCl$_4$ and tetraoctylammonium bromide (TOAB). In few hours, colorless Au–thiolates were formed, and clusters were subsequently formed by reduction by NaBH$_4$. The resulting polydisperse cluster mixture was then size-focused to magic number clusters (Au$_{25}$) due to the presence of excess thiols.

The structure of the clusters was investigated by absorption spectroscopy, ESI-MS, and NMR spectroscopy. Absorption spectra displayed distinct features at 680, 442, 400, and 320 nm, which are prominent for the Au$_{25}$(SR)$_{18}$ cluster. The attachment of Calix-4SH to the cluster surface was confirmed by ESI-MS analysis, which showed mixtures of clusters with composition Au$_{25}$(Calix-4S)$_x$(BT)$_y$ in every sample (Calix-4S and BT are thiolates of the calixarene and BuSH, respectively). As an example, Figure 7A and B display the data from the sample with 0.36% Calix-4SH in the synthesis feed. Interestingly, the vast majority of clusters observed had an even number of BT ligands, thus indicating that the calixarene binds to Au$_{25}$ predominantly with an even number of thiolates, i.e. two or four “legs”. Binding with one or three legs would result in clusters having an odd number of BT, for example Au$_{25}$(Calix-4S)(BT)$_{15}$ and Au$_{25}$(Calix-4S)(BT)$_{17}$. However, the amount of such odd-numbered clusters was negligible. Binding with two or four thiolates also supports our recent study of Calix-4S-protected AuNPs, in which a binding with an average of three thiolates was observed.

NMR measurements were conducted to confirm the structure of the calixarenes on the Au$_{25}$ surface. To our surprise, we observed an intriguing resonance pattern in the aromatic region (Figure 7C), where the free Calix-
4SH produces only one resonance signal at 6.6 ppm. The pattern was composed of a center signal at 6.6 ppm and two doublet signals at 6.1 and 7.1 ppm symmetrically around the center resonance. To further investigate the origin of the signals, 2D NMR techniques were applied. The absence of cross peaks in COSY and TOCSY indicated that all the signals in the aromatic range originated from separate spin systems. However, cross peaks were observed between the side signals at 6.1 and 7.1 ppm in ROESY, thus indicating a short spatial distance between these nuclei.

Taking into account Calix-4S’s binding behavior with two or four thiolates, it is reasonable to suggest that the center aromatic NMR signal emerges from Calix-4S bound with four thiolates, and the side signals from species bound with two thiolates. These side signals are considered to originate from a distorted pinched cone conformation of Calix-4S. A similar conformer has...
been observed also in tetrakis(n-alkoxy)calix[4]arenes at low temperatures, as the interconversion between cone and pinched cone conformations is slowed down. Such conformational locking of calixarenes by a steric impediment, as seen now on the surface of Au$_{25}$ clusters, has not been previously reported. Contrastingly, this conformer is not observed on larger AuNPs protected by di- or tetrathiolated calix[4]arenes,	extsuperscript{204,208} thus indicating that the high surface curvature of the Au$_{25}$ is behind the distortion of the Calix-$4$S.

Importantly, the amount of calixarenes on the cluster surface could be controlled by altering the ratio of thiols in the synthesis feed (Figure 7D and E). Thus, we were able to produce cluster samples having an average of one calixarene unit per cluster up to six calixarene units per cluster. Therefore, we foresee the use of mixed-ligand feed as a general strategy for incorporation of bulky ligands to cluster surfaces. Despite the possibility of tuning the ratios of ligands on the Au$_{25}$ clusters, this synthesis method did not allow for atomically precise control of the ligand layer composition. In order to achieve clusters with a single composition, post-synthetic purification is necessary, and will be discussed in the following chapter.

4.2 Separation of Gold Clusters by Thin-Layer Chromatography

There is an urgent need for accessible purification and separation strategies of MPCs in order to promote the study of their fundamental properties. Whereas some synthesis protocols may directly produce atomically precise MPCs without the need for separation, other synthesis protocols always yield a mixture of slightly different MPCs and their separation often is a challenge. To overcome this challenge, various methods have been studied in the last decade for cluster separation. Although size-exclusion chromatography (SEC),	extsuperscript{124} high-pressure liquid chromatography (HPLC),	extsuperscript{125–127} and polyacrylamide gel electrophoresis (PAGE)	extsuperscript{38,39} have been successfully applied to separate various cluster systems, thin-layer chromatography (TLC) has unexpectedly been left unattempted in the cluster community despite its utmost simplicity. TLC is a rapid and convenient separation method, whereas e.g. HPLC and PAGE require advanced and expensive instruments, which are not always available.

TLC is a standard technique in organic chemistry for separating small molecules. In a typical TLC experiment, a sample mixture is applied to a plate of aluminium coated by a thin layer of adsorbent, such as silica, cellulose, or aluminium oxide. The sample is dried on the adsorbent layer and eluted in a shallow pool of suitable solvent or solvent mixture (Figure 8). The eluent ascends the TLC plate due to capillary forces and drags the analytes with it. The separation is based on the differences in solubility of the analytes and differences in their adsorption to the TLC plate which is mainly caused by their polarity differences. The separation greatly depends on the eluent, which needs to be tuned to obtain the best performance. After achieving sufficient separation, the separated materials can be isolated by cutting the bands off the TLC plate and immersing the pieces into a suitable solvent.
Controlling the Monolayer Composition – Bulky Receptors on Gold Clusters

Figure 8. Cluster separation using thin-layer chromatography. Butanethiolate (BT) protected Au_{25} clusters elute faster compared to phenylethanethiolate (PET) protected ones due to their lower polarity. Reprinted with permission from publication II. © 2014 American Chemical Society.

Publication II focuses on separation of MPCs with TLC. Various model clusters, such as Au_{25}(BT)_{18}, Au_{25}(HT)_{18} (HT = hexanethiolate), Au_{25}(PET)_{18} (PET = phenylethanethiolate), and Au_{144}(PET)_{60} were synthesized with known methods in order to investigate the applicability of TLC to different cluster mixtures. Initially, separations of clusters having Au_{25} nuclearity but different protecting ligands were studied. As shown in Figure 8, Au_{25}(BT)_{18} and Au_{25}(PET)_{18} clusters are easily separated due to their significant difference in polarity (DCM/hexane 60:40). To further challenge the performance of TLC regarding differences in cluster polarity, we were able to separate Au_{25}(BT)_{18} and Au_{25}(HT)_{18} having only a very small polarity difference (Figure 9I). During these experiments we observed unexpected reactivity of clusters during the TLC run. For example, eluting freshly prepared Au_{25}(PET)_{18} clusters with DCM/hexane 70:30 resulted in two bands, which were found to correspond to the two oxidation states of Au_{25}(SR)_{18} clusters, [Au_{25}(PET)_{18}]^{-} and [Au_{25}(PET)_{18}]^{0} (Figure 9II). Interestingly, the lower band ([Au_{25}(PET)_{18}]^{-}) disappeared during prolonged elution and converted to neutral Au_{25}(PET)_{18}, indicating that a silica surface can catalyze the oxidation of Au_{25} clusters.

TLC separation of a mixture of clusters with different nuclearities was demonstrated using Au_{25} and Au_{144} clusters protected by the same ligand, PET (Figure 9III). Here, the reason for separation is the size of the clusters—the van der Waals interactions are stronger in larger AuNPs compared to smaller ones, thereby causing the large clusters to be retained more on the TLC plate. In addition, TLC separation was successfully applied to a multicomponent mixture of mixed-monolayer-protected Au_{25}(Calix)_{0-3}(BT)_{0-18} clusters synthesized in publication III (Figure 9IV). By fine-tuning the eluent composition, we were able to separate clusters having only a very small compositional difference from the cluster mixture, e.g. Au_{25}(Calix)(BT)_{16} and Au_{25}(Calix)(BT)_{14}. Although some bands contained multiple cluster compositions, those could be further separated by collecting the desired band and running the TLC again with a different eluent composition.

TLC can also be used in cluster purification and monitoring reactions of clusters. Removal of the unreacted thiol from the cluster product is often a challenge and thorough purification requires, for example, time-consuming
dialysis or crystallization, which is a tedious task in case of clusters. With TLC, we were able to detect and separate excess phenylethanethiol from a thoroughly methanol-washed Au$_{25}$(PET)$_{18}$ sample by eluting the clusters with a non-polar eluent. The clusters can be subsequently cut from the TLC plate and redispersed to a desired solvent.

Figure 9. TLC separations of various cluster systems. I) UV–vis spectra of the TLC-separated Au$_{25}$(BT)$_{18}$ and Au$_{25}$(HT)$_{18}$. (A) Photograph of the TLC plate used for separation. (B) MALDI-MS data of TLC-separated materials confirming that bands 1 (red trace) and 2 (blue trace) are pure Au$_{25}$(HT)$_{18}$ and Au$_{25}$(BT)$_{18}$, respectively. The fragment Au$_{21}$(SR)$_{14}$ is shown with an asterisk. II) UV–vis spectra of the TLC-separated [Au$_{25}$(PET)$_{18}$]$^-$ and [Au$_{25}$(PET)$_{18}$]$^2_-$ (A) Photograph of the TLC plate used for separation. (B) Photograph of the extracted clusters. (C) MALDI-MS data of TLC-separated materials confirming that bands 1 (blue trace) and 2 (red trace) both have the same m/z ratio of Au$_{25}$(PET)$_{18}$. III) UV–vis spectra of TLC-separated PET-protected Au$_{25}$ and Au$_{144}$, matching with the previously reported spectra. The red trace has been vertically shifted for clarity. Inset: photograph of the TLC plate used for cluster separation. (B) MALDI-MS data of crude mixture and isolated Au$_{25}$(PET)$_{18}$ and Au$_{144}$(PET)$_{60}$ (black, blue, and red trace, respectively). IV) Photograph of the TLC plate used for separating Au$_{25}$(Calix)$_{0-3}$(BT)$_{6-18}$ clusters. (B) MALDI-MS data of Au$_{25}$(Calix)$_{0-3}$(BT)$_{6-18}$ mixture (black trace) and the separated bands 1–5. Adapted with permission from publication II. © 2014 American Chemical Society.
Figure 10. TLC monitoring of Au25(PET)18 synthesis. A) Photographs of TLC runs during the synthesis (DCM/hexane 60:40). The relative amount of Au25(PET)18 increases with time. B) MALDI-MS data of bands 1–4 after 1 h reaction time. Band 1 is composed of Au25(PET)18 (obs. 7380 m/z, theor. 7391 m/z) and band 2 is a mixture of Au38(PET)24 and Au40(PET)24 (obs. 10759 and 11152 m/z, theor. 10774 and 11168 m/z, respectively). Bands 3 and 4 are composed of various larger clusters. Apart from the signal pattern, band 3 shows a separate signal at 17975 m/z, which is interpreted as Au67(PET)35 based on previous reports (theor. 17999 m/z).237 Reprinted with permission from publication. © 2014 American Chemical Society.

We have also demonstrated the possibility of monitoring cluster synthesis with TLC. During the synthesis of Au25(PET)18, a polydisperse mixture of clusters is first formed after the reduction of Au–thiolates. The evolution of this mixture was followed by TLC (Figure 10). Therefore, it is possible to study the size-focusing mechanism and obtain information about the stability of clusters by observing the reaction intermediates. In general, the data further validates a correlation between the size and the retention factor of clusters protected with the same ligand, as observed also with Au25(PET)18 and Au144(PET)60.

We foresee that TLC separation of clusters will become a widely followed strategy for separation and purification of various cluster systems. In a very recent work,132 TLC was found to be applicable to citrate-capped nanoparticles in 13–100 nm range, thus further expanding the possibilities of the method.
5. Cationic, Plasmonic Gold Nanoparticles

As discussed in Chapter 2.5, surface functionalization of AuNPs plays a crucial role in tailoring their properties for applications. Considering AuNPs in biological and biomedical applications, properties such as water solubility, colloidal stability, shelf life, size, toxicity and charge of the AuNPs are some of the most essential parameters. One of the most crucial features of water-soluble nanoparticles is their charge, which governs their electrostatic interactions with other charged molecules or surfaces.

Cationic AuNPs are essential building blocks in the toolbox of materials science. They can be utilized, for example, in transfection applications such as gene therapy\textsuperscript{238–240} and drug delivery\textsuperscript{241} due to their high surface-to-volume ratio, low toxicity, capability of binding negatively charged molecules, and ability to internalize into mammalian cells. In addition, as many biological components, such as proteins and viruses, are negatively charged at neutral pH, their combination with cationic, plasmonic AuNPs would allow formation of plasmonic electrostatic self-assemblies.\textsuperscript{242,243} Positive charge also improves the immobilization of AuNPs on negatively charged substrates, such as glass and paper.

Considering the attractive properties of cationic AuNPs, it is remarkable that reports on preparation of good quality AuNPs with a tunable size larger than 5 nm are very scarce.\textsuperscript{240,244–248} Large nanoparticles would give a tremendously enhanced plasmonic response due to the cubic dependence of the LSPR intensity on the AuNP diameter.\textsuperscript{170} This enhanced, narrow LSPR absorption would provide a more beneficial starting point for e.g. sensing applications.

To address this lack of suitable synthesis strategies, we investigated the possibility of cationization of the well-known citrate-capped AuNPs. In publication III, we show that citrate-AuNPs with sizes ranging from 8 to 20 nm can be readily cationized using a two-step phase-transfer protocol. The strategy utilizes a hydrophobic amine ligand as an intermediate capping agent, which is subsequently substituted with a cationic thiol ligand. The procedure does not cause aggregation and the dimensions of the nanoparticles remain unaltered during the cationization process. By combining the cationic AuNPs with negatively charged Cowpea chlorotic mottle virus (CCMV) particles, we have also demonstrated their ability to bind and form assemblies with large negatively charged biomolecules.
In publication IV, we combine the cationic, plasmonic AuNPs with sulfuric acid hydrolyzed, negatively charged cellulose nanocrystals (CNCs). Due to their opposite charges, the colloids interact and form stable supracolloidal nano-objects upon dropwise addition of the CNC dispersion to the AuNP dispersion. We show that these complexes exhibit a chiral plasmonic response which originates from the internal twist of the individual CNCs.

5.1 Synthesis of Cationic Gold Nanoparticles

Two main pathways can typically be used for making cationic AuNPs. Firstly, cationic AuNPs can be prepared by reducing gold salts directly in the presence of cationic stabilization agents, such as lipids, \(^{244}\) aminoalkanethiols, \(^{240,247,249}\) polymers, \(^{246,247,250}\) or surfactants \(^{245}\). Secondly, they have been produced by attaching cationic ligands to ready-made AuNPs via ligand exchange reactions \(^{239,243,251−253}\) and encapsulation utilizing amphiphilic polymers \(^{254}\). Though direct preparation methods are often preferred due to their simplicity, making AuNPs in the presence of cationic ligands typically leads to poor particle size control and broad particle size distribution, ultimately leading to ill-defined optical properties. On the other hand, ligand exchange reactions are usually well-understood and controllable. However, they have been mainly performed to AuNPs prepared by Brust–Schiffrin method which is limited to sizes 1.5–6 nm, whereas larger AuNPs would show an enhanced plasmonic effect (Figure 11).

![Figure 11. Comparison of cationic AuNPs prepared by different methods. a) UV-vis spectra of AuNPs: 2.6 nm (red; prepared by Brust–Schiffrin method), 8.5 nm (green; prepared by two-step phase transfer), and polydisperse mixture (blue; prepared by direct synthesis). The 2.6 nm and 8.5 nm AuNPs have the roughly the same molar nanoparticle concentration to illustrate the difference in the strength of the LSPR. b) Corresponding TEM images (scale bars 20 nm). Reprinted with permission from publication III. © 2015 John Wiley and Sons.](image)

As discussed in Chapter 2.1, citrate-AuNPs are typically 10–20 nm in size and they are straightforward to make in a high yield. Therefore, they are tempting precursors for further functionalization attempts. Cationization of citrate-AuNPs has been previously attempted by removing excess citrate by centrifugation \(^{255}\) or by a lengthy process of exchanging citrate to a thioctic acid followed by another exchange to an aminoalkanethiol \(^{256}\). However, these procedures typically lead to undesired aggregation due to the electrostatic attraction between the oppositely charged ligands \(^{255,257}\).
In publication III, we demonstrate a procedure which can be used to cationize citrate-AuNPs in the size range of 8–20 nm. The key to a successful synthesis is a phase transfer followed by a second phase transfer (Figure 12). The citrate-AuNPs were synthesized with the Turkevich method refined by Puntes. These particles were phase-transferred to an organic solvent with octadecylamine (ODA). Also shorter hydrophobic amines can be used in the process, though longer aliphatic chains improve the phase-transfer efficiency, as observed also in previous literature. The ODA-particles in the organic phase were then phase-transferred back to aqueous phase by the addition of (11-mercaptoundecyl)-N,N,N-trimethylammonium bromide (MUTAB) and by decreasing the pH so that ODA becomes protonated and detaches from the AuNPs. Thus, the MUTAB-AuNPs are surrounded by quaternary ammonium groups and are positively charged in a wide pH range.

![Figure 12. Two-step phase transfer: Citrate-AuNPs are converted to cationic MUTAB-AuNPs via ODA-AuNPs. Reprinted with permission from publication III. © 2015 John Wiley and Sons.](image)

This two-step phase-transfer procedure has many benefits—it is easily scalable, rapid, and produces highly stable particles with a high zeta potential (+40–65 mV). In addition, the concentration of AuNPs can be increased during the process by simply adjusting the volumes of the phases. Purification of the final product is also simple since the solubility of ODA in water is negligible. This two-step phase transfer works for particle sizes 8–20 nm and does not alter the particle sizes of the original citrate-AuNPs (Figure 13).

We have also observed that MUTAB-AuNPs form a film with a golden lustre upon drop casting and drying on a flat substrate. Surprisingly, this golden film can be easily redispersed into water as nanoparticles without aggregation or particle loss (Figure 14). Further investigation on the structure and properties of these films is ongoing.

This synthesis procedure can be easily scaled up, with the limiting factor being the citrate-AuNP synthesis. Currently we can prepare ~150 mg of AuNPs per batch with ~10 % polydispersity. As the whole synthesis procedure is facile and fast, we foresee that this method becomes a widely followed strategy for making cationic AuNPs.
5.2 Gold Nanoparticles in Electrostatic Self-Assemblies

Self-assembly is a process in which elementary components spontaneously form ordered assemblies without human intervention. It is directed by weak noncovalent interactions such as hydrogen bonding, hydrophobic interaction, van der Waals forces, π–π interactions, and electrostatic as well as electromagnetic interactions. Self-assembly is a fundamental phenomenon in nature, and occurs at multiple length scales from the molecular level up to macroscale. Folding of proteins, formation of lipid membranes, and crystallization of molecules are examples of processes driven by self-assembly. Electrostatic self-assemblies are held together by electrostatic interactions. They are nondirectional Coulombic forces between charged objects and in solution their strength can be controlled by tuning the Debye screening length.
where \( \varepsilon_0 \) is the vacuum permittivity, \( \varepsilon_r \) is the dielectric constant of the solvent, \( N_A \) is the Avogadro constant, \( e \) is the elementary charge, and \( I \) is the ionic strength of the solution (in units mol/m³). Debye screening length describes the distance of how far the electrostatic effects persist: outside a sphere that has a radius of \( \kappa^{-1} \), charges are electrostatically screened. Self-assembly via electrostatic interactions provides a simple yet versatile pathway for making ordered nanostructures which could act as metamaterials, in which novel properties originate from the collective behavior of the constituents.²⁴²,²⁴³,²⁵⁹

5.2.1 Chiral Plasmonics with Cellulose Nanocrystals

Publication IV demonstrates an example of electrostatic supracolloidal self-assembly between two nanoscale objects, CNCs and AuNPs. CNCs are stiff, rod-like, negatively charged colloidal objects with typical lateral dimensions of ca. 5–10 nm and length of 50–300 nm. They exhibit longitudinal right-handed twisting due to their native crystalline structure. Combining them with positively charged AuNPs induces an attractive electrostatic interaction between CNCs and AuNPs. As a small amount of dilute dispersion of CNCs is slowly added to an excess of AuNPs (\( D = 8.5 \pm 1.2 \) nm, nominal molar CNC:AuNP ratio \( \approx 1:30 \)), fibrillar superstructures are formed, which consist of a few CNCs covered by AuNPs (Figure 15).

Figure 15. Electrostatic binding of cationic AuNPs onto CNCs. A) Cryo-TEM image of CNCs. B) PSDs of the three AuNP batches used in the study. C) Schematic of the preparation of CNC-AuNP fibrillar superstructures. D) Schematic of a CNC/AuNP fibrillar superstructure.
The size of these superstructures was restricted to nanoscale and their redispersibility allowed straightforward measurements of their optical properties. The LSPR of the AuNPs exhibited a 3 nm redshift upon binding to CNCs, which is likely caused by the change in the refractive index of the surrounding environment of the AuNPs (Figure 16A). The inherent twisting of the CNCs led to chiral positioning of the AuNPs on the CNC surfaces. This arrangement of AuNPs induced a chiral plasmonic signal which could be measured by circular dichroism (CD) spectroscopy. The observed CD spectrum consisted of a bisignate dip–peak in the visible range, with a zero-crossing at $\lambda_{\text{LSPR}}$ (Figure 16B), thus corresponding to a right-handed helical assembly of the AuNPs. This phenomenon is also called a positive Cotton effect. Similar plasmonic coupling has been observed earlier in e.g. AuNP-decorated DNA origami nanostructures. Although such a sophisticated DNA-based scaffold yields tunable chiroptical properties, it is worth noting that a CD signal of similar magnitude can be reached by our method with simple, affordable CNCs as the template for AuNPs.

Electron tomography (ET) of the CNC/AuNP nano-objects was performed in an effort to visualize the chiral arrangement of the AuNPs on CNCs (Figure 16D). ET also enabled us to determine the nearest neighbor distances of the AuNPs in these assemblies. The average nearest neighbor distance between AuNP surfaces was measured to be 1.5 nm, thus ensuring the possibility of plasmonic coupling. In addition, the AuNP coordinates derived from ET data could be utilized in simulating the CD response from CNC/AuNP assemblies with the coupled-dipole approach. The simulated spectra (Figure 16C) reproduce the experimental observations, thus further validating that the observed CD signal originates from the chiral arrangement of AuNPs.

Figure 16. Optical properties of AuNP/CNC nano-objects. A) UV–vis and B) CD spectra of CNCs, AuNPs and CNC/AuNP nano-objects. C) Simulated CD spectra from 4 different electron tomograms of the nano-objects containing varying amounts of AuNPs. D) An example of an ET reconstruction of 118 AuNPs (red color overlaid with the corresponding gray-scale cryo-TEM image).
The electrostatic interaction between CNCs and AuNPs can be controlled by tuning the Debye screening length in the dispersion. Increasing the ionic strength leads to screening of the charges and therefore to destabilization of the electrostatic self-assemblies. Addition of salt to the CNC/AuNP assemblies caused separation of the CNCs and AuNPs together with a gradual disappearance of the CD band at ~580 nm, which further proves that the observed CD signal originates from electrostatic binding of the AuNPs on the twisted CNCs (Figure 17).

Decreasing the AuNP concentration in the sample preparation process (CNC:AuNP ≈ 1:15) led to pronounced aggregation and precipitation due to simultaneous interaction of AuNPs with multiple CNCs. On the other hand, increasing the excess of AuNPs (CNC:AuNP ≈ 1:60) induced the formation of well-dispersible single CNCs covered with AuNPs. Interestingly, the chiral plasmonic signal was very weak in these samples, thus indicating the importance of the formation of fibrillar superstructures on the CD signal.

Use of larger nanoparticles (11.7 ± 1.7 nm) in a similar particle concentration did not produce a well-defined CD signal due to pronounced aggregation followed by precipitation. We tentatively suggest that such nanoparticles are too large in comparison to the CNCs surfaces, thus possibly causing steric hindrance, increased van der Waals interactions, or inability of the large AuNPs to sense the chiral template. Using smaller 2.6 ± 0.5 nm AuNPs led to formation of fibrillar CNC/AuNP superstructures, but no CD signal was observed as the nanoparticles were too small to sustain a strong LSPR. All in all, a strong plasmonic chiroptical response requires optimization of the AuNP size and concentration.

In summary, a simple route for chiral plasmonics was demonstrated by electrostatic self-assembly of AuNPs on chirally twisting CNCs. The studied concept is fundamentally different from the previously observed left-handed chiral plasmonic responses which have resulted from nanoparticles embedded in chiral liquid crystals of highly concentrated CNCs. There exists a wealth of other biological helical colloidal materials that could also allow templating of nanoparticles leading to chiral plasmonics, taken their concentrations, dimensions, and interaction strengths are tuned.
5.2.2 Ordered Assemblies with Cowpea Chlorotic Mottle Virus

Binary nanoparticle superlattices are periodic nanostructures with lattice constants much shorter than the wavelength of light.\textsuperscript{243} To further demonstrate the potential of MUTAB-AuNPs in these kinds of nanoscale assemblies, we combined them with negatively charged Cowpea chlorotic mottle virus (CCMV) particles (publication III). CCMV is an icosahedral protein cage with 28 nm outer diameter, formed by 180 identical coat protein subunits arranged in $T = 3$ symmetry around the central RNA strand. In these experiments, it acted as a large negatively charged model biomolecule. By carefully optimizing the experimental conditions and thus tuning the electrostatic interaction between the colloids, we were able to form ordered electrostatic $AB_3$ type fcc CCMV–AuNP self-assemblies with 8.5 nm MUTAB-AuNPs (Figure 18). The ordered structure of these self-assemblies could be verified by small-angle X-ray scattering (SAXS). It is worth noting that smaller 2.6 nm cationic AuNPs form an $AB_8$ fcc lattice with CCMV.\textsuperscript{243} Therefore, we anticipate that increased particle size range of cationic AuNPs enabled by the two-step phase-transfer procedure will promote studies of biohybrid assemblies.

\textbf{Figure 18.} Small-angle X-ray scattering (SAXS) characterization of CCMV–AuNP crystals. a) Integrated SAXS curves measured from 8.5 nm AuNPs and complexes of CCMV and AuNPs. b) Structure factor of the CCMV–AuNP sample compared to a theoretical curve calculated for an fcc structure with both tetrahedral and octahedral voids occupied by AuNPs. Lattice constant $a = 45.5$ nm is obtained from the fit (red solid line) consisting of Gaussian peaks (FWHM = 0.01 Å$^{-1}$) at the theoretical reflection positions for an fcc structure. A previously published Lorentz-corrected SAXS curve of CCMV complexed with 2.6 nm cationic AuNPs is shown in comparison (dotted line).\textsuperscript{243} c) A schematic presentation of the assigned $AB_3$ fcc crystal structure obtained from 8.5 nm AuNPs and CCMV and the previously published $AB_8$ fcc structure obtained from the 2.6 nm AuNPs.\textsuperscript{243} Two models of the unit cells are shown for both cases: AuNPs only (left) and full (right). Reprinted with permission from publication III. © 2015 John Wiley and Sons.
6. Formation of Silver Clusters in Poly(methacrylic acid)

As mentioned in Chapter 2, biopolymers such as proteins and DNA have shown promising potential as scaffolds for generating fluorescent nanoclusters. In addition, much simpler synthetic polymers can also form suitable environments for stabilizing clusters. For example, poly(N-vinylpyrrolidone) has been used to produce ultrasmall $\text{Au}_2$ and $\text{Au}_3$ clusters, as well as mixtures of larger magic number clusters ($\text{Au}_{35}$, $\text{Au}_{43}$, $\text{Au}_{58}$, …) which can be further extracted from the polymer by small molecules. Even though gold nanoclusters are generally highly stable, and their structure and chemistry are well-known, they often lack certain features that would be beneficial for many applications. One such property is a high quantum yield (QY). Overlooking few exceptions, thiolate-protected gold clusters typically exhibit QYs of $10^{-5}$–$10^{-3}$, which are typically not enough for e.g. fluorescence imaging applications. The QYs of silver clusters, on the other hand, are higher compared to gold, with some silver clusters possessing QYs approaching unity. Therefore, they are promising candidates for labelling and sensing applications. In addition, data storage applications utilizing the high photostability of silver nanoclusters have been suggested and demonstrated.

Polymers with repeating carboxylic acid groups have been identified as efficient scaffolds for stabilizing highly fluorescent silver nanoclusters. Poly(methacrylic acid) (PMAA) is a commercially available polyelectrolyte which can stabilize silver clusters that are stable for years in ambient conditions. In Chapter 6.1, we carefully investigate the formation and evolution of Ag@PMAA clusters in an aqueous solution. Four distinct emission peaks are observed from the cluster mixture, including a previously unreported emission peak that disappears after prolonged irradiation during cluster synthesis. By controlling the wavelength of light used in the cluster synthesis, it is possible to control the ratio of different types of clusters.

In Chapter 6.2, we discuss how fluorescent silver clusters are formed in a solid PMAA thin film by direct laser writing (DLW). This technique can be used to produce sub-micron scale fluorescent patterns of arbitrary shape. DLW-stabilized silver nanoclusters are fluorescent in the red region, and show excellent photostability when compared to the well-known Rhodamine 6G dye.
6.1 Formation of Silver Clusters in a Dilute Polymer Solution

Silver nanoclusters in PMAA have been extensively studied for the past decade. However, despite the numerous studies regarding this system, there is only little information available on the composition of these nanoclusters. Identification of the composition of Ag@PMAA clusters, and separation of individual cluster sizes would be essential steps towards further understanding of structure–property relationships in the system. The sizes of Ag@PMAA clusters have remained elusive for many reasons. The clusters are too small to be directly measured from electron microscopy images. In addition, the large size (~100 kDa) and polydispersity of the polymer severely hamper its mass spectroscopic characterization possibilities.

Despite the difficulties related to the characterization of the Ag@PMAA clusters, we have tried to obtain more information of the system by carefully investigating their formation and evolution with fluorescence spectroscopy. The nanoclusters were synthesized by visible light irradiation of aqueous solution containing AgNO₃ and PMAA. The ratio of reactants was chosen so that there was six Ag⁺ ions for each MAA unit (Ag/MAA = 600%). In contrast to earlier studies, the irradiation was performed by using discrete wavelength bands. With this procedure we could carefully tune the photon energy and thereby control the processes occurring in the reaction mixture.

Fluorescence measurements of the Ag@PMAA clusters revealed two interesting features that are not reported in earlier studies: 1) an emission peak at 500 nm and 2) four distinct emission peaks in total (Figure 19A). Because of the energy quantization (Figure 1), the emission energy typically increases with decreasing nuclearity. Therefore, it is straightforward to hypothesize that the 500 nm emission originates from smaller-sized silver clusters than what has been previously observed. The presence of four spectroscopically distinct peaks suggests the presence of four types of clusters that are different in nuclearity, oxidation state, or interaction with the PMAA matrix, as suggested in earlier literature. Differences in interaction with the PMAA matrix would likely lead to broadening of emission peaks and therefore it is difficult to imagine such distinct peaks originating entirely from such a reason. The oxidation state of a cluster can greatly affect the position of the emission peak as well as the emission broadness. All the fluorescence emissions are quenched when adding NaCl to the solution, thus suggesting that the clusters are positively charged. However, the exact charge states are unknown due to the lack of ESI-MS data. All in all, due to the similar broadness of the emission peaks, it is most plausible to address these four distinct emission peaks to clusters differing in their nuclearity.

In addition, by finely tuning the photon energy in the irradiation step, we can control the relative amounts of different cluster species (Figure 19B). The relative amount of clusters emitting at 500 nm is high when photon energies ≤ 2.2 eV are used in the irradiation step, while the relative amounts of clusters emitting at 640 nm and 775 nm dominate when using photon energies ≥ 2.7 eV.
Formation of Silver Clusters in Poly(methacrylic acid)

Figure 19. Distinct emission peaks of Ag@PMAA clusters. A) Fluorescence emission spectra of the clusters: $\lambda_{\text{exc}} = 370$ nm (blue trace) and $\lambda_{\text{exc}} = 510$ nm (red and black trace). The positions of the peaks are marked with vertical dashed lines for clarity. B) Relative intensities of emissions centered at 500 nm (blue squares), 640 nm (red circles), and 775 nm (black triangles) after 2 h irradiations of Ag@PMAA samples with different discrete wavelengths. C) Synthesis of Ag@PMAA clusters ($\lambda_{\text{irr}} = 500$ nm) monitored with fluorescence spectroscopy ($\lambda_{\text{exc}} = 370$ nm).

We have also investigated the evolution of the emission peaks during the irradiation. As seen in Figure 19C, the intensities of the emission peaks increase as the irradiation progresses. Furthermore, prolonged irradiation causes the emission band at 500 nm to decrease and finally disappear. One possible explanation for this observation is that the clusters emitting at 500 nm act as intermediates for clusters emitting at higher wavelengths. Ultimately, PMAA gets saturated with larger clusters, thus preventing further formation of smaller clusters. However, exact analysis of the fluorescence data is problematic due to interfering effects from e.g. photobleaching and possible energy transfer phenomena between different clusters.

All in all, the structure of Ag@PMAA clusters cannot be verified without accurate mass spectral measurements, which are still hampered by the high molecular weight and polydispersity of the polymer. We have attempted to extract the clusters from the high-molecular-weight PMAA by various small molecules including thiols, amines, and PMAA oligomers. However, such extractions are not straightforward and can easily cause changes to the structures of the cluster cores thus potentially invalidating the results. Recently, we have succeeded to synthesize clusters in short PMAA oligomers. However, MALDI-MS of these clusters was not successful due to the irreversible aggregation of clusters during drying. Therefore, the composition of the Ag@PMAA clusters still remains a mystery.
6.2 Fluorescent Silver Clusters in Thin Polymer Films

Whereas silver clusters in liquid media have gained a lot of attention due to their high fluorescence quantum yields, antibacterial activity and ability to reach atomically precise compositions, silver clusters embedded in solid matrices have shown remarkable properties of temporal stability and photostability. In the solid state, silver clusters are typically produced by photoirradiation of a matrix containing silver ions, causing photoreduction of silver and stabilization of the as-formed clusters by the surrounding media. Additionally, few thiolate- and selenolate-protected clusters have also been prepared by simple grinding of the solid reactants. Glass and zeolites have been successfully used as matrices to host silver clusters prepared by irradiation with femtosecond lasers. The exceptional stability of these clusters combined with high-resolution patterning possibilities make them promising candidates for high-capacity optical recording media.

Inorganic matrices such as glass and zeolites are robust matrices though they can be brittle. Polymers, on the other hand, are interesting matrix candidates due to their flexibility and moldability. In publication, we introduce a direct laser writing (DLW) technique for submicrometer-scale patterning and efficient stabilization of silver nanoclusters in a solid polymer thin film. The ~40 nm thin film samples were prepared by spin-coating aqueous solutions containing PMAA (1.5 w-%) and varying amounts of AgNO₃. A tightly focused near-infrared femtosecond laser beam was used to write the structures, as schematically shown in Figure 20. The laser (790 nm) produced 290 fs pulses with a repetition rate of 80 MHz. The sample could be moved by a three-axis motorized scanning stage, which was manipulated with a piezo controller, thus enabling accurate movements of the sample during writing. The sample could be simultaneously imaged by a camera utilizing a broadband light source illuminating from the opposite side of the sample.

![Figure 20](image-url) A simplified presentation of the direct laser writing procedure. Reprinted with permission from publication. © 2014 American Chemical Society.

The optical and structural properties of the laser-generated fluorescent microstructures were thoroughly investigated by optical microscopy and atomic force microscopy (AFM). Figure 21A shows the bright-field microscopy image of a DLW-produced line array and the corresponding fluorescence
microscopy image. It can be easily observed from the fluorescence intensity line profile that the fluorescent areas coincide with the written lines, whereas the fluorescence originating from the the background is negligible. The emission spectra of the fluorescent areas (Figure 21B) are characterized by a broad emission band ranging from 500 nm to 750 nm, together with a sharp peak at 510 nm ($\lambda_{\text{exc}} = 473$ nm). The broad band is the fluorescence emission from the clusters and the sharp peak is interpreted as an enhanced Raman scattering phenomenon which is further discussed in the following paragraph. The emission intensity clearly depends on the silver content in the film, further verifying that the fluorescence originates from the silver clusters. AFM was used to investigate the topographical features of the written structures. Surprisingly, we observed a formation of grooves at the location of the written structures (Figure 21C and D). As shown in Figure 21D, the breadth of the grooves could be controlled by varying the laser intensity of DLW ($I_{\text{DLW}}$), ultimately reaching sub-micron scale features. Interestingly, the fluorescence is observed as the location of the grooves even though most of the material has been ablated during the DLW.

Figure 21. Optical and structural properties of fluorescent microstructures containing silver clusters in Ag@PMAA samples (Ag/MAA = 50%). A) Top: Bright-field microscopy image of a line array produced by DLW ($I_{\text{DLW}} = 80$ GW/m$^2$, $\lambda_{\text{dlw}} = 780$ nm). Middle: A corresponding fluorescence microscopy image of the same area ($\lambda_{\text{exc}} = 473$ nm). Bottom: A fluorescence intensity profile across the marked line. B) Fluorescence emission spectra of samples with Ag/MAA ratios ranging from 0 to 75% ($\lambda_{\text{exc}} = 473$ nm). Inset shows the Ag concentration dependence of the fluorescence intensity. C) AFM images of structures written with $I_{\text{DLW}}$ of 80 GW/m$^2$, 240 GW/m$^2$, and 480 GW/m$^2$, from top to bottom, respectively. D) Topographic line profiles of the line arrays shown in (C). The line profiles for the red and blue curve have been vertically shifted by 20 and 40 nm, respectively. All the scale bars equal 5 $\mu$m. Adapted with permission from publication V. © 2014 American Chemical Society.
To further investigate the origin of the sharp peak in the fluorescence spectrum, we measured Raman spectra from the 50% Ag:MAA thin films containing microstructures written with $I_{dlw}$ of 480 GW/m² and $\lambda_{dlw}$ of 780 nm (Figure 22A). No Raman signals were detected from the areas unexposed to the writing beam, whereas two broad peaks were observed from the positions of the written structures at 1340 cm$^{-1}$ and 1590 cm$^{-1}$, corresponding to $\nu_{\text{COO}^-}$ (sym.) and $\nu_{\text{COO}^-}$ (asym.) vibrational modes, respectively. Surface (XY) mapping of the Raman spectra clearly indicated that the Raman signals originated only from the positions of the written structures (Figure 22B). This observation further supports that metal particles capable of Raman enhancement are formed during the direct laser writing process. Similar Raman enhancement by silver clusters has been observed previously and attributed to a large excited state charge separation in the clusters. The Raman mapping of written structures was performed by measuring 20 x 20 individual Raman spectra in the 10 μm x 10 μm area with a tightly focused 633 nm laser beam with an intensity below the direct laser writing threshold.

The photostability of written structures was investigated by bleaching the fluorescence of written structures with 473 nm continuous laser excitation.
(I_{exc} = 0.3 \text{ MW/m}^2) and recording emission spectra as the bleaching occurs. As seen from Figure 22C, the region without written structures bleaches rapidly, whereas the written structures retain their emission more efficiently. It must be noted that the emission spectra of the written structures were measured from a circular area sized over 3000 \mu m^2, thus containing unexposed area between and around the written line arrays (Figure 22C, inset). Therefore, the observed decrease in the emission of the written structures is mostly due to the bleaching of the unexposed area in the measurement area. As a further verification, exponential fits were performed to the data points of the photobleaching experiment (Figure 22D). A single exponential fit to the data of the unexposed area displayed a bleaching time constant of 4.3 s. The data points of the area containing written structures followed a biexponential behavior with time constants of 4.7 s and 350 s, which were attributed as bleaching of the background and written structures, respectively. We also compared the photostability of Ag@PMAA clusters to a commonly used organic fluorescent dye, Rhodamine 6G, in a similar PMAA matrix. As clearly seen in Figure 22C, the organic dye bleaches more rapidly than the Ag@PMAA clusters, thus further highlighting the superior photostability of the clusters.

All in all, we have shown that silver clusters can be prepared in a solid polymer matrix by DLW. These clusters in PMAA are fluorescent in the red region and show excellent photostability when compared to the well-known Rhodamine 6G dye. We see our work as a significant advance in the patterning of silver clusters and we anticipate that this technique can be generalized to other metal–polymer systems as well.
The work presented in this thesis has concentrated on the synthesis of metal clusters and nanoparticles. A special focus was set on their functionalization, as well as separation of gold clusters, and using plasmonic gold nanoparticles in biohybrid self-assemblies.

In publication I, we were able to synthesize Au$_{25}$ clusters with bulky calix[4]arene ligands on their surface by using binary ligand mixtures in the synthesis feed. By adjusting the ratio of the thiols in the mixture, we could control the average amount of calix[4]arene units from one to six per cluster. Despite the apparent control of the functional units, this procedure yields mixtures: clusters containing multiple slightly different ligand compositions. However, we envisage mixed-ligand feeds as a general strategy in functionalizing clusters with bulky ligands which do not readily undergo ligand exchange.

Further control of the ligand layer composition could be achieved by a post-synthetic separation, as demonstrated in publication II. For the first time, thin-layer chromatography was realized as a feasible purification and separation method of atomically precise noble metal clusters. Separations of simple model clusters of different size, ligand composition, and charge could be readily accomplished. TLC showed sufficiently high separation resolution to enable, for example, the separation of Au$_{25}$(Calix)(BT)$_{16}$ and Au$_{25}$(Calix)(BT)$_{14}$. In addition, TLC was successfully applied to cluster synthesis monitoring and rigorous purification of cluster samples. We foresee that TLC will become a widely followed method for separation of various cluster systems.

Ultimately, preparing clusters possessing only one calixarene unit was realized by TLC. However, making cluster assemblies turned out to be highly challenging due to solubility difficulties of suitable linker molecules. In addition, the observed distortion of the calix[4]arene cone on Au$_{25}$ clusters is likely to obstruct the host–guest complexation process. This was observed in preliminary complexation experiments with a cationic guest molecule, in which the complexation was weaker than in the case of calix[4]arene-protected nanoparticles. In the course of this work, we have made observations suggesting negligible collective optical properties in an analogous covalently linked cluster network, where the distance between clusters is even less than it would be in the calixarene-cluster case (data not shown). Therefore, further pursuit of host–guest mediated calixarene-cluster assemblies was deemed futile. However, calixarene-functionalized clusters could find use as molecular recognition elements in applications related to e.g. sensing.
In publication **III**, a two-step phase-transfer functionalization method was developed to prepare plasmonic, cationic AuNPs from simple citrate-capped AuNPs in the size range of 8–20 nm. Combining these cationic AuNPs with negatively charged CCMV particles led to formation of ordered assemblies, thereby demonstrating the AuNPs’ ability to bind and form assemblies with large negatively charged biomolecules. Preparation of cationic AuNPs in this size range has been challenging in the past, and thus we anticipate that this synthesis method will promote studies of biohybrid assemblies—an objective which we are currently pursuing at multiple fronts.

As another example of a biohybrid self-assembly, the cationic AuNPs were combined with negatively charged CNCs in publication **IV**. These colloids formed stable supracolloidal CNC/AuNP fibrillar superstructures which exhibited a chiral plasmonic response originating from the internal twist of the individual CNCs. These electrostatically self-assembled CNC/AuNP superstructures could be broken down by screening the charges upon addition of salt. Electron tomography of these fibrillar superstructures facilitated a theoretical simulation of the CD response using the coupled-dipole approach. The simulations corresponded well to the experimental data, thus confirming that the observed positive Cotton effect originates from the chiral arrangement of the AuNPs.

In publication **V**, fluorescent silver clusters were prepared in solid PMAA matrix by direct laser writing. DLW with a near-infrared femtosecond laser was used to produce submicrometer-scale fluorescent patterns of arbitrary shape in PMAA thin films. The patterned areas exhibited broadband fluorescence in the visible range, and groove-like topographic features were observed at the fluorescent areas indicating ablation of most of the organic material. DLW-stabilized silver nanoclusters showed excellent photostability when compared to the well-known Rhodamine 6G dye. This fabrication method could open up new possibilities for applications related to imaging or metal ion sensing, and could be further applied to prepare other types of metal nanoclusters embedded in different polymer matrices.

The research field of nanoclusters and nanoparticles has vastly expanded during the past two decades. Especially the research on clusters is booming, and advances in their synthetic methods, structure elucidation, and applications are frequently presented. This work has been an effort to offer new approaches to the synthesis and functionalization of noble metal clusters and nanoparticles for the rapidly growing research community. The concepts and results presented in this thesis demonstrate interesting ways to synthesize gold and silver clusters, to functionalize nanosized gold particles, and to self-assemble them with biological components. As a significant practical advance, the work offers a convenient method for separation and purification of gold clusters. Nevertheless, more efforts are needed in order to feasibly utilize the intriguing properties of metal clusters. Especially aspects related to scaling up the syntheses, examining the stability of clusters in various environments, and investigating their biocompatibility need to be rigorously investigated in order to take necessary steps towards real-life applications.
References


References


